Mercury and Methylmercury Content in Chub from the Svitava and Svratka Rivers at Agglomeration Brno

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Abstract

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The aim of our study was to determine the total mercury and methylmercury contents in the muscle of indicator fish, to determine the total mercury contents in the sediment, and to evaluate the health risks associated with fish contamination. Chub (*Leuciscus cephalus* L.) were caught in seven localities on the Svratka and Svitava Rivers in the Brno agglomeration in 2007. The total mercury content was determined by atomic absorption spectrophotometry using an AMA 245 analyser. Methylmercury levels were determined by gas chromatography (using electron-capture detector) after acid digestion and extraction with toluene. The highest levels of total mercury and methylmercury contamination in the fish muscle (0.18 ± 0.09 mg/kg and 0.16 ± 0.09 mg/kg, respectively) were found at the Rajhradice site (the Svratka River, under the Brno city), whereas the lowest contents of mercury and methylmercury (0.08 ± 0.02 mg/kg and 0.04 ± 0.03 mg/kg) were detected at the Modřice site (the Svratka River). Total mercury content in the sediment ranged from 0.06 mg/kg to 1.38 mg/kg, the higher value having been detected in the sediment from the Svratka River at the Rajhradice site above the confluence with the Svitava River. The lowest content was discovered at Kníničky (the Svatka River). The hazard indices calculated for the selected localities showed no health risk to the common consumer or to the fishermen and their families.

Keywords: Czech river; *Leuciscus cephalus*; methylation; methylmercury; sediment; total mercury

The contamination of aquatic systems is presently at the centre of attention in the research community. Most of the xenobiotic compounds have the ability to accumulate in living organisms (for example fish), and sometimes they can be hazardous to people. Included in this group of compounds are heavy metals, polychlorinated biphenyls (PCB), dioxins, polyaromatic hydrocarbon (PAH), and others. In the Czech Republic, much attention has been directed to the monitoring of heavy metal (including mercury) contamination of surface water and the evaluation of the health hazards related to human fish consumption (Maršálek *et al.* 2006).

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Mercury is known as a global pollutant and is distributed throughout the natural environment affecting many bio-organisms. Methylmercury accumulates in fish, resulting in these fish becoming the main source of human methylmercury contamination. Inorganic forms of mercury do not participate in mercury bioaccumulation in ecosystems, therefore the determination of the organic forms is essential. Inorganic mercury is methylated in freshwater ecosystems into methylmercury (MeHg) (WHO 1990). This mercury methylation mechanism was first described by Landner (1971) and by Wood (1971). Methylation involves both biotic and abiotic pathways. The biological production of methylmercury largely depends on anaerobic sediment bacteria of the genus *Methanobacterium* (Hamasaki *et al.* 1995). Bacteria which have the abilities to reduce sulphates play an important role in the process of methylation in the water sediment. The process of methylation occurs fastest on the surface of the sediment (Compeau & Bartha 1985). The rate of methylation is a function of both the activity of methylating bacteria and total mercury concentration (Gilmour & Henry 1991).

MeHg accumulates in fish as it advances through the food chain. Methylmercury (MeHg) makes up over 95% of total mercury (THg) in the fish tissues (Mason *et al.*1995; Houserová *et al.* 2006a)

The aims of the present study are:

– to determine THg and MeHg concentrations in the muscle of chub which were used as an indicator species and were caught in 7 localities on the Svitava and the Svratka rivers at the Brno agglomeration,
– to detect total mercury concentration in the sediment from the localities in which the indicator fish were caught,
– to evaluate the health risks associated with the consumption of fish from these localities.

MATERIALS AND METHODS

Study area. In this work, attention was paid to mercury contamination in the Svitava and the Svratka Rivers. These rivers run through the city of Brno, the second largest town in the Czech Republic, with a population of 366 680. The city lies in the basin of the Svratka and Svitava Rivers. The Svratka River cuts a 29 km swath through the city and is the main water supply for the Kníničky Dam Lake, a popular recreation area in the city's

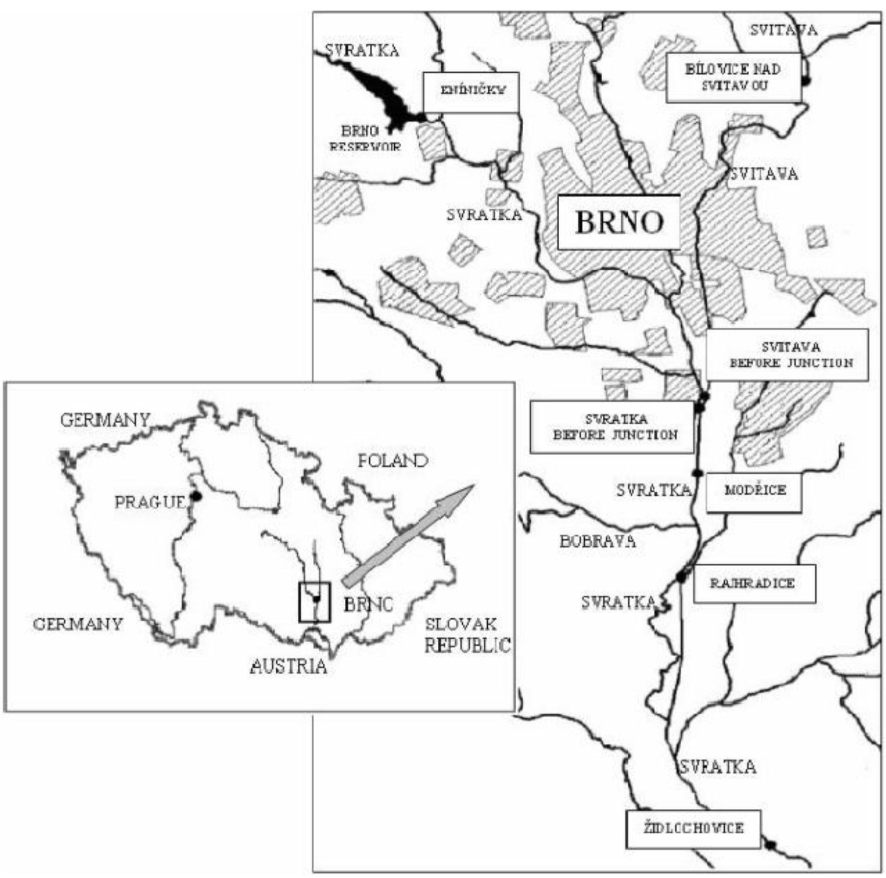

Figure 1. Map of the Czech Republic and the sampling sites

northwest corner. The Svitava River flows through the city for about 13 km. The Svitava River meets the Svratka River downstream from Brno and is the main tributary of the Svratka River. Seven sites on these rivers were chosen for assessing the influence of Brno on the mercury contamination of fish and sediments.

Two localities (Bílovice nad Svitavou; Svitava before junction) on the Svitava River and five localities (Kníničky; Svratka before the confluence; Modřice; Rajhradice; Židlochovice) on the Svratka River were chosen (Figure 1). The Bílovice nad Svitavou site characterises the river above Brno and is the control site for the Svitava River. Another site on the Svitava (Svitava before junction) is located below Brno before it flows into the Svratka.

Kníničky reservoir was built between 1938 and 1941 on the Svratka River to prevent flooding. The dam reservoir is a lake approx. 10 km long and 800 m wide in places, and contains approx. 25 million m^3 of water. The Brno Reservoir is a frequently visited recreational area with water transportation and sporting facilities. The sampling site Kníničky (56.2 river km) is located downstream from the reservoir and is above the city of Brno. One site chosen on the Svratka (Svratka before junction) (40.9 river km) is downstream from Brno before the confluence with the Svitava River. Modřice (38.7 river km) is the site of the sewage works company Brněnské vodárny a kanalizace a.s. The main activity of this company is to operate the public water works and sewage systems. The other sites are at Rajhradice (35.0 river km) and Židlochovice (30.0 river km), located after the confluence of the two rivers downstream from Brno.

Sampling of fish and sediments. In 2007, a total of 106 indicator fish (*Leuciscus cephalus* L.) were collected at the selected localities by electrofishing. The fish were immediately weighed and measured, and their gender was determined macroscopically. The scales were removed for the age determination. For THg and MeHg analysis, samples of muscle tissues were taken from the cranial parts of the fish under the lateral line, and were then put into polyethylene bags, marked, and stored in a freezer at –18°C. Table 1 shows the main biometric characteristics of the fish collected from the selected sites.

Samples of sediment were collected from the same localities during the spring, summer, autumn, and winter seasons. At each location, composite bottom sediment was collected into dark glass bottles and stored at –18°C. The sampling was validated in accordance with ISO 5667 12 norm.

Determination of THg and MeHg. The amounts of THg in muscle and in sediments were determined by cold vapour atomic absorption spectrometry on an AMA 254 analyser (Altec Ltd., Dvůr Králové n. L., Czech Republic).

Methylmercury was determined in the form of methylmercury chloride by gas chromatography (Caricchia *et al.* 1999). The samples were prepared by acid digestion and extraction with toluene (Maršálek & Svobodová 2006). A Shimadzu capillary gas chromatograph with an electron captured detector GC 2010A (Shimadzu Kyoto, Japan) was used for the analysis. A capillary column DB 608 (30 m × 0.53 mm × 0.83 µm; J&W Scientific Chromservis, Prague, Czech Republic) was used. The evaluations were made using GC Solution software (Shimadzu Kyoto, Japan) and MS Excel software.

The detection limits for THg and MeHg were 1 µg/kg and 21 µg/kg, respectively. The limit of

Table 1. Biometric characteristics (mean ± S.D.) of chub captured in the selected localities

Locality	*n*	Body length (cm)	Body weight (g)	Age (year)
Bílovice nad Svitavou*	23	20.7 ± 1.8	141± 45.8	5.0 ± 1.0
Svitava before junction*	14	21.4 ± 2.5	180 ± 69.7	4.1 ± 1.0
Kníničky**	12	24.3 ± 4.1	294 ± 175.0	5.5 ± 1.8
Svratka before junction**	15	24.4 ± 2.7	231 ± 97.0	5.3 ± 1.2
Modřice**	14	24.9 ± 2.3	296 ± 100.4	5.4 ± 1.1
Rajhradice**	14	25.8 ± 4.9	336 ± 246.7	5.5 ± 1.5
Židlochovice**	14	24.2 ± 4.3	280 ± 162.3	4.1 ± 1.2

*site at the Svitava River; **site at the Svratka River; S.D. = standard deviation detection (LOD) was set as a sum of triple the standard deviation of a blank and a blank mean value. The accuracy of the values for THg and MeHg were validated using the standard reference material BCR-CRM 464 (Tuna Fish, IRMM, Belgium).

Total mercury and MeHg concentrations in fish muscle are given in mg/kg fresh weight (FW) while THg in the sediment is given in dry weight (DW).

Statistical analysis. Statistical analysis of the data was performed using the program STATISTICA 8.0 for Windows (StatSoft CR). The data was analysed with the parametric ANOVA Tukey's HSD test.

Health hazard assessment. The hazard index was calculated according to Kannan *et al.* (1998) using the reference dose (RfD) for THg (0.3 μg/kg body weight/day) set by US EPA. To determine the maximum possible consumption of fish meat, the provisional tolerable weekly intake limit (PTWI) of 1.6 μg MeHg per kg body weight/week was used (WHO 1990).

RESULTS AND DISCUSSION

Total mercury and methylmercury in fish

Chub (*Leuciscus cephalus* L.) was selected for analysis in the present study. It is an omnivorous fish with an extensive food web, and thus it is very suitable for the monitoring of aquatic ecosystems (Barus *et al.* 1995). Mercury was found in all 106 muscle samples of chub but MeHg was found only in 66 samples, in the remaining ones being below the limit of detection (0.02 mg/kg). The analysed fish were collected of similar age because a positive correlation between the content of mercury and the age of fish had been corroborated by a large number of studies (Maršálek & Svobodová 2006; Maršálek *et al.* 2006). No significant differences were found between the ages of fish from the studied localities. Because no significant sex-related differences in THg and MeHg contents were found, fish of both sexes were evaluated together in the rest of the study.

The results of fish tissue analyses for THg and MeHg contents are given in Figure 2. The highest mean concentrations of THg and MeHg were found in the chub from the Rajhradice site (0.18 ± 0.08 mg/kg and 0.16 ± 0.09 mg/kg, respectively). The lowest values of THg and MeHg were found at Modřice (0.08 ± 0.02 mg/kg and 0.04 ± 0.03 mg/kg, respectively).

Significantly ($P < 0.01$) higher values of THg and MeHg were found in Rajhradice in comparison with Modřice. Total mercury content in chub from Rajhradice was significantly higher ($P < 0.01$) than in

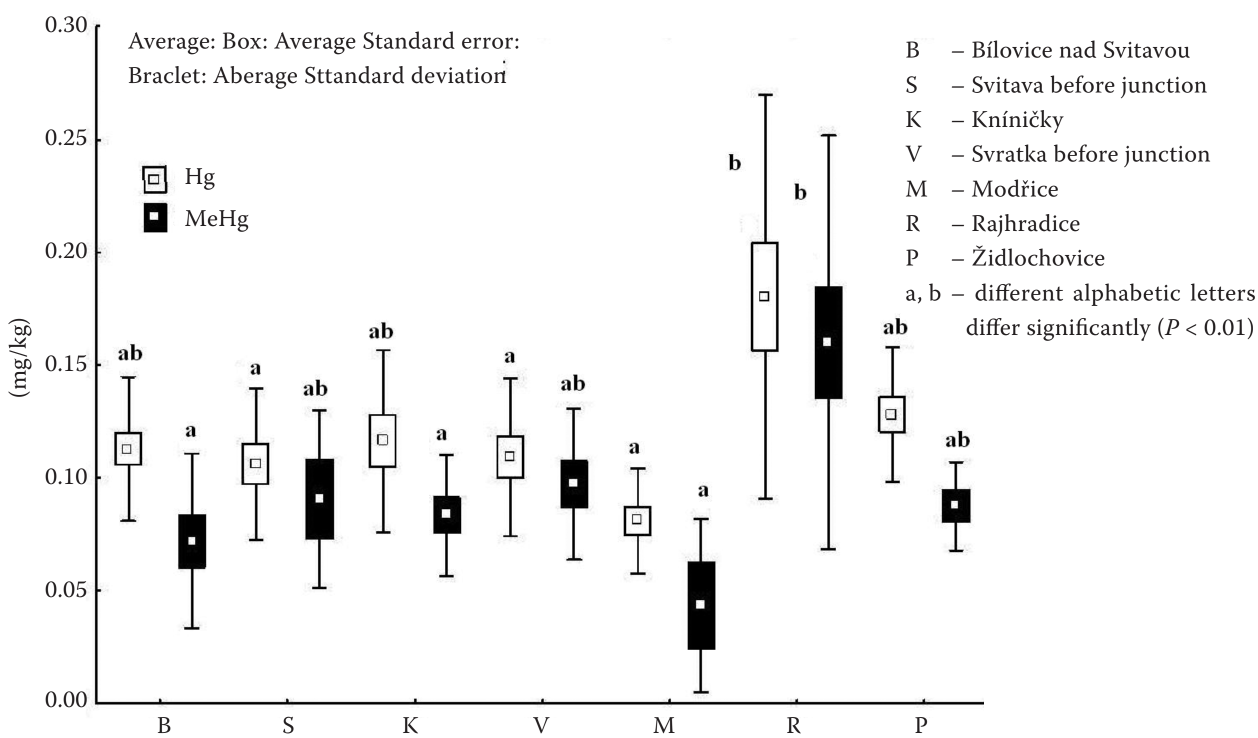

Figure 2. Mercury and methylmercury content of fish muscle

those coming from the Svitava before junction and the Svratka before junction. The amount of MeHg was significantly ($P < 0.01$) higher at the Rajhradice site in comparison with those at the Bílovice nad Svitavou and Kníničky sites. Kružíková *et al.* (2008b) monitored mercury contamination in fish from the major rivers of the Czech Republic in 2007, a similar amount of THg (0.12 mg/kg) having been found in the muscle of fish from the Svratka River (site Židlochovice; 23 river km).

The mean values of THg found in the study performed by Kružíková *et al* (2008a) in the Czech rivers (Lužnice, Otava, Sázava, Berounka, Vltava, Labe, Ohře, Dyje, Morava, Odra, Svratka) ranged from 0.07 mg/kg to 0.25 mg/kg, with the highest one being in the Labe River. That is 1.5 times higher than the greatest value of THg which was found in our study in Rajhradice site. Long-term bio-monitoring confirmed an extensive mercury contamination of fish in the Labe River (Žlábek *et al.* 2005; Dusek *et al.* 2005; Maršálek & Svobodová 2006). Another study (Kružíková *et al.* 2008b) monitored THg and MeHg in chub muscle from other rivers (Orlice, Chrudimka, Cidlina, Jizera, Vltava, Ohře, Bílina) and showed THg and MeHg amounts achieving the values between 0.07 mg/kg to 0.17 mg/kg, with the exception of the mercury content in that coming from the Jizera (0.27 mg/kg). This demonstrated that the Czech rivers, in general, do not exceed the 0.5 mg Hg/kg fresh weight limit provided by the Commission regulation (EC) No. 1881/2006 which sets the maximum levels for certain contaminants in foodstuffs in chub muscle. According to our results, it seems that the Brno agglomeration does not contribute to the mercury contamination in the Svitava and the Svratka Rivers at all. The amount of mercury in fish below the sewage works (Modřice site) is the lowest. The mercury content in fish at the Rajhradice site (4 km from the Modřice site) was significantly higher than in that at Modřice, therefore it must be affected by other sources of contamination; for example the Bobrava River, which runs through several villages and agricultural areas before entering the Svratka River.

Total mercury in sediments

Table 2 shows the amounts of THg in the analysed sediments coming from the selected sites. The higher mean values of THg were found in the sediments from two sites on the Svratka River (Svratka before junction and Rajhradice), whereas Rajhradice is the site where we found higher values of THg and MeHg in fish. The lowest concentrations of THg were found in the Svratka River (Kníničky) and in the Svitava River (Bílovice nad Svitavou). The amounts of THg collected at different times were similar and showed that the content of THg in the sediment did not vary widely during a single year. An exception was the value of THg taken in the spring from the Svratka River (Židlochovice site) and another one that taken in the autumn from the Svratka River (Modřice site) which may reflect particular local situations because the values were not similar. The contents of THg in the sediments from the Svratka River (at Kníničky) and the Svitava River (at Bílovice nad Svitavou sites) were much lower than those from the other sites. These sediments were very

Table 2. Mercury content of the sediments from sampling sites

Locality	THg in sediment (mg/kg dry weight)				
	spring	summer	autumn	winter	mean ± S.D.
Bílovice nad Svitavou*	0.146	0.100	0.073	0.128	0.11 ± 0.03
Svitava before junction*	0.371	0.424	0.778	0.817	0.59 ± 0.23
Kníničky**	–	0.070	0.077	0.051	0.06 ± 0.01
Svratka before junction**	1.220	0.907	1.380	1.340	1.21 ± 0.214
Modřice**	0.770	0.912	0.480	0.657	0.71 ± 0.18
Rajhradice**	1.267	0.890	1.280	1.15	1.15 ± 0.18
Židlochovice**	0.806	0.443	0.364	0.429	0.51 ± 0.19

*site at the Svitava River; **site at the Svratka River; S.D. = standard deviation different, possibly due to their sandy characteristics (this was confirmed by the particle-size analysis) and the lack of organic compounds. This could be the reason why the mercury contents were so low. The characteristics of the sediment and the natural hydrologic regimen in the Svratka River (at the Kníničky site) are influenced by the manipulation of the reservoir.

Based on our results, the characteristics of the sediment in one river is different from another, and the contents of THg in the sediments are varied as well. It is possible that THg concentration is dependent on the nature of the particular sediment present.

Varied results were also found by Houserova *et al.* (2006b) for the amount of THg in the sediments from four Czech rivers (Jihlava, Loucka, Dyje, Becva). This study showed that mercury levels ranged from 0.053 mg/kg to 0.225 mg/kg with the highest value found in the sediment from the Jihlava. The difference between our results and those found by Houserova *et al.* (2006b) could be due to the dissimilar sediments and other aquatic conditions.

In our study, no correlations were found between the content of THg in fish and THg in the sediment (correlation coefficient 0.17). This confirms the hypothesis that the THg in the muscle of fish is related not only to mercury levels in the sediment, but also to the composition of the fish diet and other biological characteristics and chemicals in the aquatic environment (Houserova *et al.* 2006b).

Health hazard assessment

Potential hazard indices were calculated for all the sites tested, our calculations having been designed according to the method of Kannan *et al.* (1998). The hazard index below 1 indicates no hazard for the consumer. The average consumption of freshwater fish in the Czech Republic was used, i.e. 1 kg per capita per year and 10 kg per member of fisherman's household per year (Ministry of Agriculture CR 2007). Table 3 shows the hazard indices. The calculated hazard indices (for the common consumer and for the fisherman's family) in the tested sites were several times lower than hazard index 1. A very low hazard index indicates no significant health risk attached to the consumption of fish from the localities followed in this study. The highest values of the hazard index for both the standard consumer and the member of the fisherman's family were found in the fish from the Svratka River (at Rajhradice site), however, these were also much lower than the limit 1, thus the potential health risk is negligible.

For the maximum tolerable weekly intake calculated for the sites tested see Table 3. The maximum tolerable weekly intake is the amount that can be eaten by a consumer at a specific site. The provisional Tolerable Weekly Intake – PTWI (set by WHO; 1.6 µg MeHg/kg body weight/week) was used for this calculation. Thus, in view of MeHg contamination, the safest site of those monitored in this study was the Modřice site, while the least safe was the Rajhradice site. The recommended

Table 3. Hazard indices for a standard consumer and a member of a fisherman's family and maximum tolerable weekly intake of chub meat from monitored sites

Locality	Hazard index[1]		Maximum weekly tolerable intake[2] (kg)
	for standard consumer	for fisherman's amily	
Bílovice nad Svitavou*	0.017	0.169	1.41
Svitava before junction*	0.014	0.139	1.24
Kníničky**	0.015	0.152	1.34
Svratka before junction**	0.014	0.142	1.15
Modřice**	0.011	0.106	2.57
Rajhradice**	0.024	0.235	0.70
Židlochovice**	0.017	0.167	1.28

*site at the Svitava River **site at the Svratka River; [1]calculation of THg according to Kannan *et al.* (1998); [2]calculation of MeHg according to WHO (1990) maximum consumption of fish from the Svitava and the Svratka Rivers ranged from 0.70 kg/week to 2.57 kg/week which is similar to that for other rivers in the Czech Republic and confirmed the results of other studies (Kružíková *et al.* 2008a, b). In general, the fish from the rivers in the Czech Republic have a very low hazard indices and their consumption poses no heath risk arising from THg and MeHg contamination.

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