Determination of total mercury and mercury species in fish and aquatic ecosystems of Moravian rivers

P. Houserova[1], V. Kuban[1], P. Spurny[2], P. Habarta[1]

[1]Department of Chemistry and Biochemistry, [2]Department of Fishery and Hydrobiology, Mendel University of Agriculture and Forestry, Brno, Czech Republic

ABSTRACT: Contents of total mercury and mercury species (methylmercury – MeHg, inorganic mercury – Hg^{2+}) were determined in four Moravian rivers – Jihlava, Becva, Loucka and Dyje (Czech Republic). Five tissues (muscle, gills, liver, kidney and skin) of chub (*Leuciscus cephalus*), zoobenthos, sediments and water samples were analyzed. Time stability of samples was also tested. The highest levels of total mercury were determined in muscle tissues of all tested fish. Relative contents of MeHg in muscle tissues of fish ranged from 83.6% to 92.0% of the total mercury contents. The relative contents of MeHg in sediments and in zoobenthos samples correlate very closely (correlation coefficient −0.83). A considerably lower content of MeHg (1.3–11.4%) was found in river sediments compared with lakes. A comparison of observed sampling sites (Vladislav, Hrubsice) proved the adverse effect of industrial contamination on the water ecosystem of Jihlava River and incomplete removal of mercury species in a sewage station.

Keywords: speciation; total mercury; methylmercury; inorganic mercury; chub (*Leuciscus cephalus*); Jihlava; Becva; Loucka; Dyje; muscle; gills; liver; kidney; skin; zoobenthos; sediment; water; sample stability

The mercury cycle in water environments has long been receiving considerable attention because of the high toxicity of its compounds, with particular respect to methylmercury (MeHg), accumulation of both the organic and inorganic forms of the element in organisms and their biotransformation and biomagnification in the aquatic food chains (Morel et al., 1998; Ikingura and Akagi, 1999; Anonymous, 2002; Fournier et al., 2002; Ipolyi et al., 2004).

The high nutritional value of fish makes it an ideal component of a healthy and balanced diet. Elevated levels of MeHg in aquatic organisms, especially fish, represent both an ecological and human health concern. Thus the ingestion of contaminated fish is the primary input of mercury to humans and piscivorous wildlife. Fish is therefore a product for which suitable measures should be taken to provide chemical monitoring of the risks deriving from its consumption. Most authors deal with mercury determination in muscle, which is consumed. Monitoring of mercury species in individual tissues is less common (Nakagawa et al., 1997; Fournier et al., 2002; Cabanero et al., 2004; Ipolyi et al., 2004; Agusa et al., 2005; Scerbo et al., 2005; Storelli et al., 2005).

Mercury concentration in fish are influenced by fish age, mercury concentration in water ecosystem, food contamination, chemical, biological and physical processes in aquatic environment and seasonal variations (Vigh et al., 1996; Boening, 2000; Anonymous, 2002; Kotnik et al., 2002; Ipolyi et al., 2004; Sunderland et al., 2004; Svobodova et al., 2004; Sarica et al., 2005).

The Czech Republic faces increasing risks of environmental pollution caused by the emissions of mercury into the environment from a number of natural, as well as anthropogenic sources. Although accurate determinations of total mercu-

ry concentrations mainly in various fish species from Bohemian rivers and reservoirs are now available (Svobodova et al., 1999, 2004; Rehulka, 2002; Spurny et al., 2002; Dusek et al., 2005; Marsalek et al., 2005) reliable mercury species data are still scarce. Sizable monitoring of mercury species in Moravian rivers hasn't been performed.

The aim of the paper was the determination of mercury species (inorganic mercury – Hg^{2+}, methylmercury – MeHg, ethylmercury – EtHg and phenylmercury – PhHg) and total mercury (T-Hg) in five different tissues (muscle, gills, kidney, liver and skin) of chub (*Leuciscus cephalus*) from four Moravian rivers (Jihlava, Becva, Loucka and Dyje). Correlations between total mercury and methylmercury contents in sediments, zoobenthos and fish muscle and the influences of industrial regions on water ecosystems were also observed.

MATERIAL AND METHODS

Study area and samples

Mercury contamination was observed in four Moravian rivers (Jihlava, Loucka, Dyje and Becva). Two sampling sites were selected in Jihlava basin – Vladislav and Hrubsice. The first sampling site was near a sewage works (Vladislav – river kms 86.1) and the second one was near the town Hrubsice (river kms 43.5). The sampling sites were detached by the hydroelectric power station Dalesice. The sampling sites are reported in Figure 1. River Jihlava springs in Bohemian and Moravian Highlands near the village of Jihlavka. The river length is 184.5 km and Oslava River is the largest affluent. The hydroelectric power station Dalesice is situated in the middle of the river, and it consists of two reservoirs

– the main reservoirs near the town of Kramolin and the detention reservoir near the town Mohelno. A serious influence of the hydroelectric installation Dalesice on a water ecosystem of Jihlava River was also evaluated in the study. The Jihlava River runs through an industrial region (the towns Jihlava and Trebic – boot, paper and engineering industry, nuclear power station – Dukovany) but also through an agricultural region (Hrubsice).

The Loucka River is an important right-hand affluent of the Svratka River (the junction is near the town of Tisnov). Svratka River runs through the largest Moravian city Brno. The water ecosystem of the Loucka River is affected namely by the spirit industry (in the village of Radesin) and uranium pits in an area of Dolni Rozinka. The sampling site was selected near the town Ujezd (river kms 6.7) and is reported in Figure 1.

The Becva River, the largest affluent of the Morava River, has two headwaters, which have junctions in the town of Valasske Mezirici. The sampling site was selected near the town of Choryne (river kms 54.7 – Figure 1). An industrial contamination of the river prevails in the sampling site. The Dyje River, analogously to the Becva River, has two headwaters with junction near the town of Raabs (Austria). The river runs through both Austria and Czech Republic. The Jihlava and Svratka Rivers are the largest affluents. Austrian affluent Pulkau contaminates the Dyje River. The river is divided by five water reservoirs. The sampling site was selected near the town of Hevlin (river kms 81.4 – Figure 1).

Bioaccumulation of mercury species was evaluated in selected clean tissues (dorsal muscle, gills, liver, skin and kidney) of chub (*Leuciscus cephalus*), n = 7–10. The samples were collected in July 2004. Total length (TL) of fish was ranged among 23.2 to 43.7 cm. Ages of the fish were between 3–7 years.

Table 1. Characteristics of observed water ecosystems

Sampling site	Hrubsice	Becva	Loucka	Vladislav	Dyje
Water temperature (°C)	8.1	23.1	12.1	17.0	17.5
pH	8.79	8.52	8.05	7.71	7.63
O_2 concentration (mg/l)	13.35	9.52	10.70	9.00	8.20
O_2 saturation (%)	115.1	113.2	104.0	97.0	86.0
Conductivity (mS/m)	45.9	34.0	38.4	32.0	53.5
Zoobenthos abundance (pieces/m^2)	–	201	540	494	440
Zoobenthos biomass (g/m^2)	–	3.4	6.9	6.5	6.1

Figure 1. Map of sampling sites
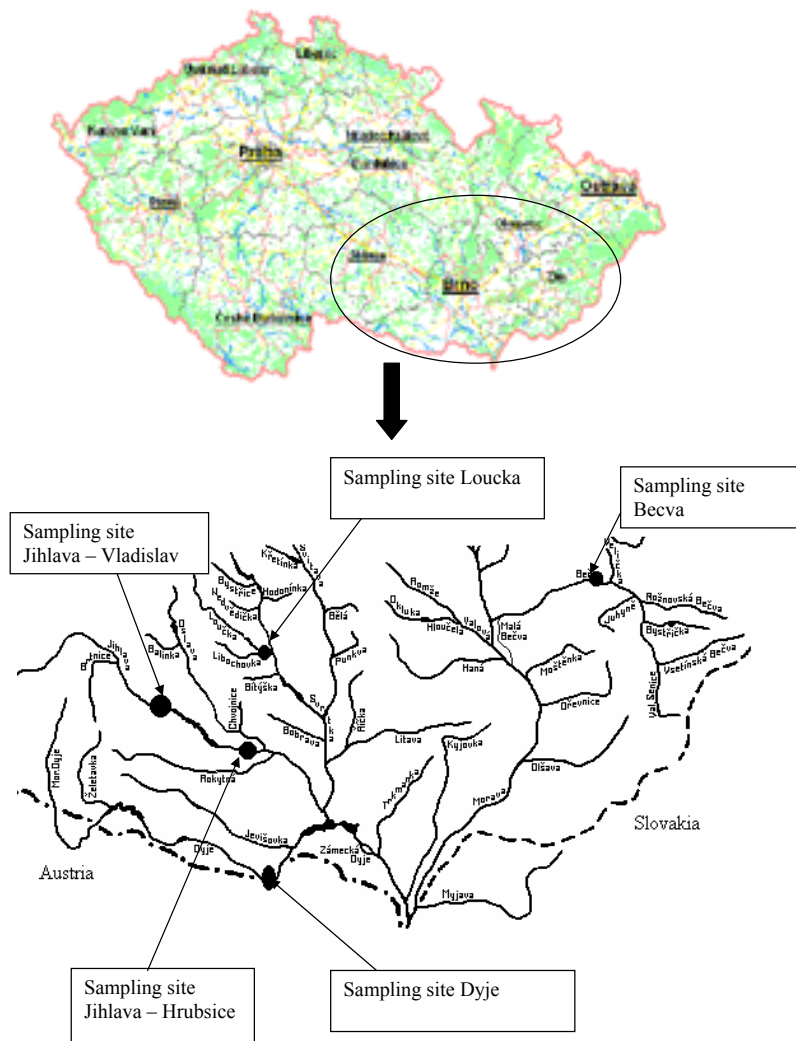
Mixed samples of zoobenthos (*n* = 10), sediments (*n* = 10) and water were obtained from the aquatic ecosystems described above. The sediments were sampled 10 cm under bottom surface. Characterizations of observed water ecosystems are presented in Table 1.

Carefully separated and cleaned tissues of the fish, the samples of sediments and zoobenthos were immediately deep frozen (–18°C), freeze-dried (–52°C, 48 hours) and homogenized by a Grindomix GM 200 mill (Retsch GmbH & Co. KG, Germany). The water samples were filtered, acidified and deep frozen.

Methods

Sample storage and preservation. Neither loss nor transformation of the mercury species was observed in the biological samples (fish, zoobenthos) during storage (6 months). A transformation of the mercury species (methylation Hg^{2+}) was observed

in the sediment samples. A slow increase in the methylmercury concentration was observed, but the total mercury contents were constant, if the samples were not stored deep frozen (Figure 2). Stability of the tested sediments was extended by freeze-drying and by deep freezing storage (Figure 2). γ-Irradiation ensures the stability of the reference material (CRM 580).

The extract stability was controlled for two months. The extracts (6 mol/l HCl + 0.1 mol/l NaCl) of reference materials DORM-2 and CRM 580 (46.4 µg/l Hg) were stored in a brown glass bottle in a refrigerator. The extract was stable for at least 30 days. Neither loss nor transformation of the mercury species was observed during this time. Formation of relatively robust chloro-complexes ($HgCl_4^{2-}$ or $RHgCl_2^-$, R is alkyl or aryl) prevents adsorption and/or degradation of the individual mercury forms on lab-ware walls. Gradual reduction of total mercury and methylmercury concentrations was observed after 30 days (Figure 3).
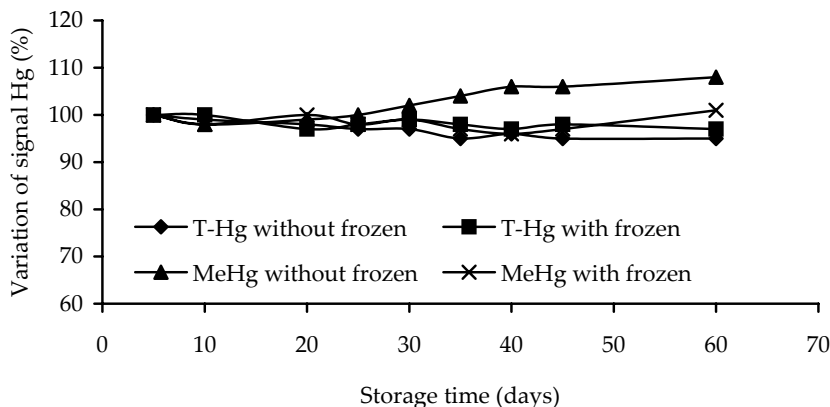
Figure 2. Stability of sediments

Analytical methods. The methods used for the determination of the total mercury and the mercury species are described elsewhere (Houserova et al., 2006a). Here, only a brief summary is mentioned.
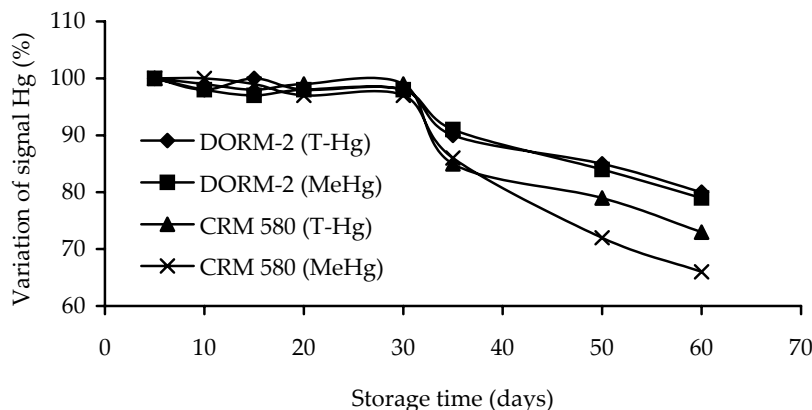
Sample extraction: The extraction agent (6 mol/l HCl + 0.1 mol/l NaCl) was added to 0.2 – 1.0 g of a sample and extracted in the high-pressure microwave digestion unit Ethos SEL (Milestone, Italy). An optimal extraction time for biological materials was 10 min, while for sediment samples only 7 min was required. Weaker bonds of mercury species in the sediments leaded to a decrease in the extraction time (Figure 4).

After filtration (filter paper No. 389, disc diameter 12.5 cm), the filtrate was diluted with acetate buffer (pH = 5) up to the final volume 25 ml. The prepared samples were injected directly into the HPLC/CV-AFS system for separation and for the determination of the mercury species.

Total mercury determination: Homogenized solid samples were directly weighed (50–100 ± 0.1 mg) into pre-cleaned combustion boats, and automatically inserted into the AMA 254 analyzer (Altec, Prague, Czech Republic). The samples were dried at 120°C for 90 s and thermally decomposed

at 550°C for 180 s under oxygen flow. The selectively trapped mercury was released from the amalgamator by a brief heat-up and finally quantified (measuring cycle, 60 s) as Hg^0 by cold-vapor AAS technique at 253.65 nm.

Mercury species determination: The extracts were analyzed by the HPLC/CV-AFS for the determination of mercury species concentration (Hg^{2+}, MeHg, EtHg, PhHg) after sample preparation steps. An isocratic elution of the mercury species was performed at a flow rate of 0.15 ml/min using a mobile phase containing of 7% (v/v) CH_3OH and 0.05% (v/v) 2-mercaptoethanol at pH 5 in an acetate buffer with increase in CH_3OH content up to 100% in the 15th minutes. The effluent from a Hypersil BDS C18 column (3 μm particle size, 2 × 125 mm, Hewlett Packard, Palo Alto, CA, USA) was merged with a stream of acidified bromide/bromate mixed solution (0.2 mol/l KBr + 0.04 mol/l $KBrO_3$ in 5% HCl, excess of Br_2 was eliminated by 0.004% (m/v) hydroxylamine hydrochloride, flow rate 2.5 ml/min) and then passed through a UV cracking reactor (PTFE tube 0.5 mm × 10 m, UV lamp power 12 W). All the mercury species in individual chromatographic zones were converted

Figure 3. Stability of extracts in refrigerator (DORM-2, CRM 580, concentration 46.4 μg/l Hg in 6 mol/l HCl + 0.1 mol/l NaCl)
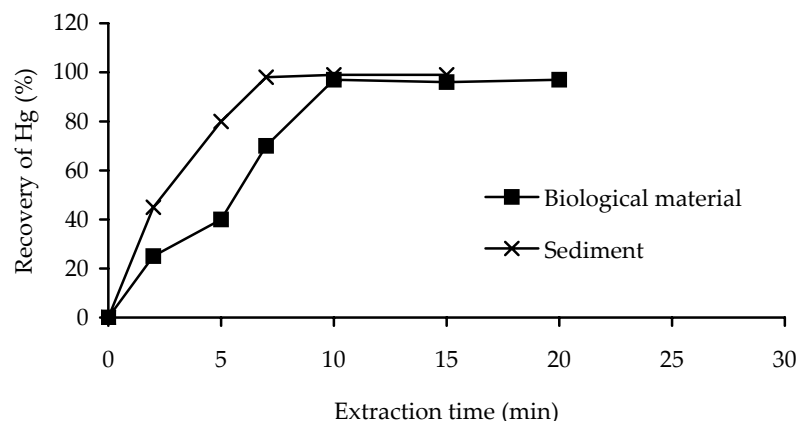
Figure 4. Dependence of extraction time on extraction recovery of Hg (extraction reagent 6 mol/l + 0.1 mol/l NaCl (10 ml), t = 60°C, DORM-2, CRM 580)

to inorganic mercury. The inorganic mercury was reduced by reaction with $SnCl_2$ (2% (m/v) $SnCl_2$ in 10% HCl, flow rate 2.5 ml/min). Elemental mercury cold vapors were purged with an argon stream, dried in a PermaPure® membrane unit and detected at 253.65 nm by a PSA Millenium Merlin atomic fluorescence spectrometric (AFS) detector controlled by an Avion software (all P.S. Analytical Ltd., Orpington, UK).

Quality assurance. The analyses of the total mercury and the mercury species in the biological samples and the sediments were performed in triplicate. Reagent blanks and the following certified reference materials were analyzed concurrently with the sediment and the biological samples to validate the method used. The instruments were calibrated with sets of standard solutions. Method calibration curves were used for results evaluation. The accuracy of the results were controlled by recovery tests and by analyses of the standard reference materials of dogfish muscle DORM-2 (T-Hg: 4.64 ± 0.25 mg/kg, MeHg: 4.47 ± 0.32 mg/kg as Hg) and the sediment CRM 580 (T-Hg: 132 ± 3 mg/kg, MeHg: 75.5 ± 3.7 µg/kg as CH_3Hg^+). The results were in the good agreement in both cases (DORM-2: THg 4.60 ± 0.13 mg/kg and MeHg 4.38 ± 0.16 mg/kg, CRM 580: T-Hg 131 ± 2 mg/kg, MeHg 75.1 ± 1.9 µg/kg as CH_3Hg^+). Limits of detection (LODs for 3 S/N criterion) were 0.05 ppb (RSD = 2.06% at 4.64 mg/kg, n = 10) for T-Hg, 0.2 ppb (RSD = 3.0% at 5 µg/l, n = 10) for MeHg, 0.07 ppb (RSD = 5.3% at 5 µg/l, n = 10) for inorganic Hg, 0.06 ppb (RSD = 3.4% at 5 µg/l, n = 10) for PhHg and 0.12 ppb (RSD = 4.4% at 5 µg/l, n = 10) for EtHg.

Statistical analysis. Statistical analyses followed standard procedures (Meloun and Militky, 1998). Data were tested for the best fit to a normal distribution using Shapiro-Wilk´s test and requirements of homogeneity of variances were determined using Bartlett's test. Parametric tests were preferred, and in some cases they were performed on logarithmically-transformed data to achieve requirements of normality and homoscedasticity. A one-way analysis of variance (ANOVA) was used for comparison of means. A linear correlation was used to follow the relation between Hg (II) and MeHg contents. The identity and accuracy of the results were verified by the t-test. Means are expressed ± S.D. considering a $P < 0.05$ to be statistically significant.

RESULTS AND DISCUSSION

Contents of total mercury in fish, zoobenthos and sediments

Chub (*Leuciscus cephalus*) was selected for monitoring biaccumulation of total mercury and mercury species in river ecosystems. Chub is omnivorous fish with the widest food web (algae, aquatic plants and terrestrial seeds, larvae, small fish and mollusc animals). Thus it is very suitable for monitoring of aquatic ecosystems (Cihar and Maly, 1978). The bioaccumulation of mercury was evaluated in selected fish tissues (see Study area and samples). The highest concentrations of the total mercury were determined in the muscle tissues of all tested fish, where mercury is bound to cysteine rich proteins (Boening, 2000; Anonymous, 2002). Also, the other authors (Anonymous, 1999, 2002; Dusek et al., 2005) observed high concentrations of total mercury in muscle tissues of various fish species. Vigh et al. (1996) found the highest mercury concentrations in the kidneys of grass carp. The lowest concentrations of total mercury were found in gills and skin of all the tested fish. The total mercury
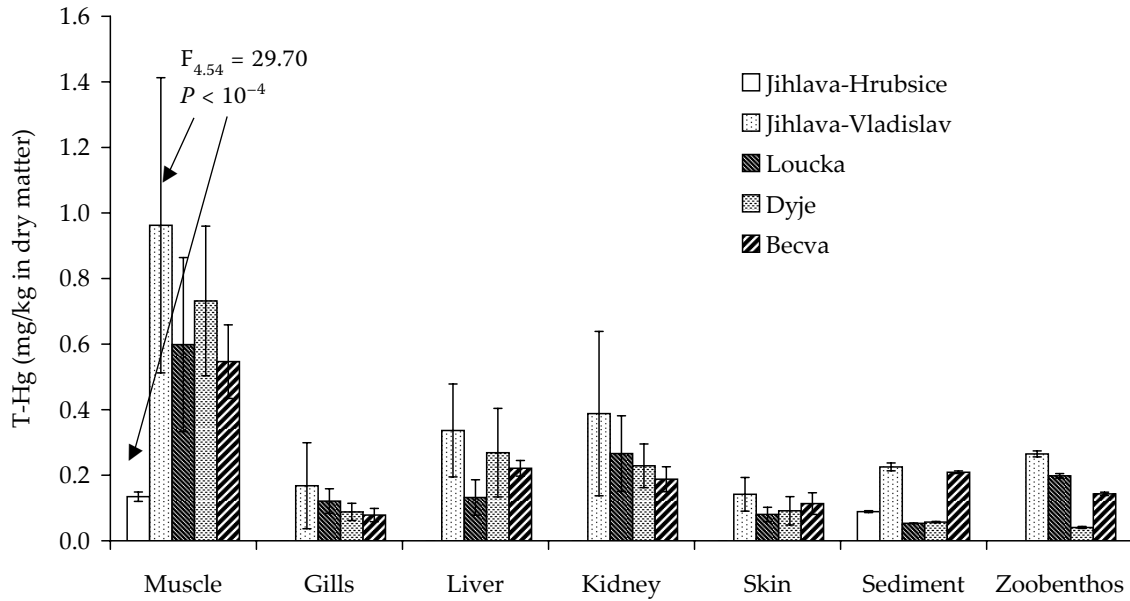
Figure 5. Dependence of T-Hg (mg/kg in dry matter) on tissues of chub. Some probabilities of statistically significant differences are noted

content in tested tissues of chub decreased in order muscle >> kidney ≈ liver > skin ≈ gills.

The total mercury concentration in the tested tissues, zoobenthos and sediments are presented in Figure 5. Statistically significant differences of total mercury concentration were found among the individually tested tissues with the following exceptions: the skin tissues have statistically insignificant differences compared to the gill. Statistically insignificant differences of the total mercury concentrations were also found between liver and kidney.

The muscle tissues of chub from Hrubsice contained significantly lower amount of the total mercury than the muscle tissues of fish from Vladislav and remaining three rivers. The total mercury content in the tested muscle of chub from Hrubsice (0.135 ± 0.014 mg/kg in dry matter) was 7-times lower compared to the muscle samples from Vladislav (0.962 ± 0.450 mg/kg in dry matter), 5-times lower compared to the muscle of chub from Dyje (0.732 ± 0.228 mg/kg in dry matter) and 4-times lower than the muscle samples of chub from Becva (0.547 ± 0.112 mg/kg in dry matter) and Loucka (0.598 ± 0.266 mg/kg in dry matter). Contamination of the Loucka, Dyje and Becva Rivers was approximately two times lower compared to the Jihlava River (sampling site: Vladislav).

A comparison of the evaluated sampling sites (Vladislav, Hrubsice) proved the adverse effects of

industrial contamination on the aquatic ecosystem of Jihlava River. The sewage station did not fully eliminate the adverse effects. The influence of Dalesice-Mohelno dams on aquatic fauna was observed as well. Aquatic fauna can not naturally migrate due to dams, therefore fish from the more contaminated area don't appear in the downstream water, which is not so polluted. Generally, the living environment expressively influences the T-Hg content in muscle tissues of fish (Vigh et al., 1996; Svobodova et al., 2004; Sarica et al., 2005). Because of the low range fish age (3–7 years), their age did not influence the content of total mercury in the tested tissues.

The limit of mercury concentration (0.5 mg/kg in fresh matter of fish's muscle), which is stated in the announcement of the Ministry of Health of the Czech Republic No. 305/2004, was not exceeded in any sample of the fish muscle. Only the muscle of the chub from the Hrubsice did not exceed the limit of mercury concentration (0.1 mg/kg in fresh matter of fish muscle), which was valid in the Czech Republic before becoming a European Union member.

The lowest contents of the total mercury in the sediments were observed in the Loucka and Becva Rivers. The highest content of the total mercury was found in the sediment from the Jihlava River (sampling site: Vladislav). The sediments contained 0.053–0.225 mg/kg of the total mercury

in dry matter. Any close correlation was not observed between the contents of the total mercury in the muscle tissue of the chub and in the sediments (correlation coefficient 0.39). Thus the total mercury content in the fish muscle is not related only to the mercury content in the sediments, but also to the diet composition of the fish, and to the other chemical and biological characteristics of the aquatic ecosystem.

The lowest content of the total mercury was observed in the zoobenthos from the Dyje River. The highest content of the total mercury was found in the zoobenthos from the Jihlava River (sampling site: Vladislav). The zoobenthos samples contained 0.041–0.265 mg/kg of the total mercury in dry matter. Close correlations were observed neither between the total mercury contents in the sediment samples and in the zoobenthos samples (correlation coefficient 0.54) nor between the total mercury contents in the zoobenthos and in the fish muscle (correlation coefficient 0.42). Statistically significant differences of the total mercury content were observed between the sediment samples and the zoobenthos samples in all the river ecosystems. Analyzed water samples did not exceed the limit of mercury content (0.1 μg/l) stated in the announcement of the Goverment of the Czech Republic No. 61/2003.

Bioaccumulation of the total mercury content with the increasing trophic levels was observed in all the tested aquatic ecosystems. High mercury contents in the muscle tissue of chub, but also in the sediment and zoobenthos samples prove the anthropogenic contamination of the Jihlava River.

Contents of mercury species in fish, zoobenthos and sediments

Bioaccumulation and transformation of the mercury species (Hg^{2+}, MeHg, EtHg and PhHg) was observed in the tested samples. The determination of the mercury species in gills and kidney of chub from Becva River was not performed, because of the lack of fresh tissue. Ethyl- or phenylmercury did not usually occur in biological tissues (Liang et al., 2003). The fact was proved by our observation as well.

The chub muscle tissue from the sampling site Vladislav contained statistically significant higher relative contents of MeHg (in percents of T-Hg) than the muscle of the fish from the sampling sites

Hrubsice ($F_{4.54} = 6.42$, $P = 0.02$) and Becva ($F_{4.54} = 8.28$, $P = 0.01$). The differences in the relative contents of MeHg in gills and skin tissues from the individual sampling sites were statistically insignificant ($P > 0.05$). The samples of sediments and zoobenthos from the individual sampling sites had statistically significant differences in the relative contents of MeHg.

The highest contents of MeHg were found in the muscle tissues of all the tested fish. The relative contents of MeHg in the muscle tissues of the fish were in the range from 83.6% to 92.0% MeHg of the T-Hg (Figure 6) and were in the good agreement with the literature (Boening, 2000; Anonymous, 2002; Landaluze et al., 2004; Houserova et al., 2006b).

Relative contents of MeHg in other tested tissues were following:
(i) gills 19.0–22.4%, (ii) liver 23.0–48.6%, (iii) kidney 16.7–37.5%, and (iv) skin 27.8–34.0%.

It could be concluded from the results that the methylated form of mercury is accumulated in the muscle of the fish. Significantly different results were obtained for the other tested tissues, in which contents of MeHg between 16.7–48.6% were found. The similar results were also found for the other animal species – mammalia and birds (Kim et al., 1996; Wagemann et al., 1998; Boening, 2000; Anonymous, 2002; Henny et al., 2002; Heinz and Hoffmann, 2004; Houserova et al., 2006b).

The zoobenthos samples contained 32.0–63.1% MeHg. Statistically significant differences of the MeHg content were observed among the zoobenthos samples in all the river ecosystems. Close correlations were observed between the relative MeHg contents in the sediment samples and in the zoobenthos samples (correlation coefficient –0.83), but no correlation was observed between the relative MeHg contents in the muscle samples and in the zoobenthos samples (correlation coefficient –0.05).

The river sediments contained considerably lower levels of MeHg (1.3–11.4 %) compared with the lake sediments (Houserova et al., 2006b) from the Zahlinice water reservoir (37.6 ± 5%). The sediment composition and its movement (churn, eluviation of sediments) influences the content of methylmercury in the sediments. The river sediments contain large amounts of sand components, whereas the lake sediments contain large portions of clay particles. Statistically significant differences in the MeHg content were observed between the
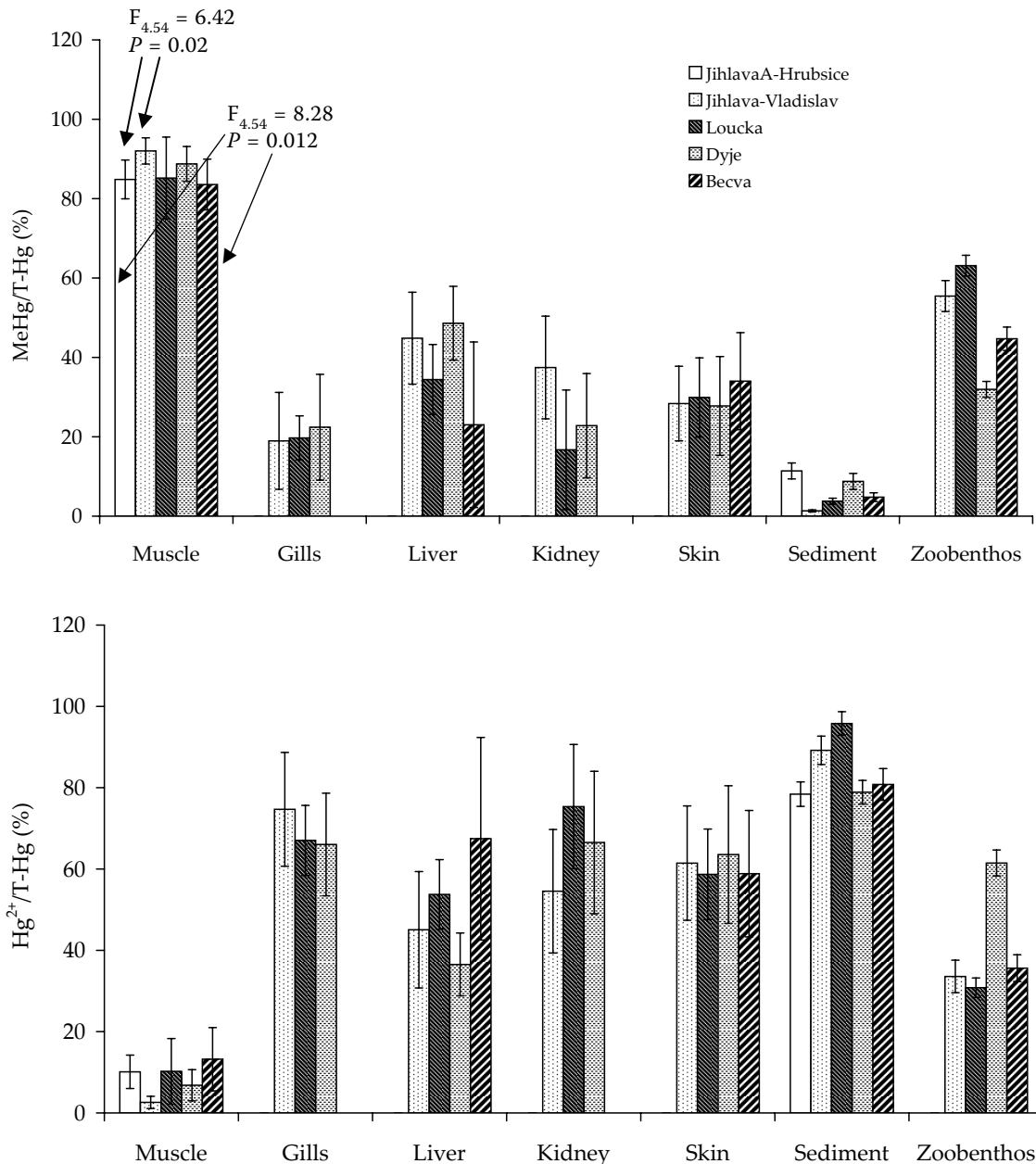
Figure 6. Dependence of MeHg/T-Hg (%) (top) and Inorg. Hg/T-Hg (%) (bottom) on tissues of chub. Some probabilities of statistically significant differences are noted

sediment samples in all the river ecosystems, except in the sediments from the Loucka and Becva Rivers. In any sedimentary compartment, the MeHg concentration is a balance between methylation and demethylation processes (Trombini et al., 2003). In this case, the MeHg is bioaccumulated by aquatic animals; therefore the MeHg concentration in the sediments are relatively low.

Organisms at lower trophic levels contained the lowest proportion of the total mercury, such as MeHg. This observation is in agreement with the literature (Boening, 2000; Anonymous, 2002; Gray, 2002).

Close correlations were obtained between the contents of methylmercury (mg/kg) and the inorganic mercury (mg/kg) in the gills of the chub from the sampling site Jihlava-Vladislav (correlation coefficient 0.95), in the liver of the fish from the Loucka, Dyje and Becva (correlation coefficients 0.81; 0.89; 0.89), in the muscle of the chub from the Dyje (correlation coefficient 0.87), in the sediments from the Jihlava-Hrubsice, Loucka, Dyje and Becva (correlation coefficients 0.98; 0.90; 0.66; 0.93) and in the zoobenthos from the Dyje and Becva (correlation coefficients 0.62; 0.99) Rivers.

CONCLUSIONS

Chub (*Leuciscus cephalus*) was selected for the monitoring of the mercury species in the rivers. The muscle tissues of chubs from the Hrubsice contained significantly lower amounts of the total mercury then the muscle tissues of the fish from the Vladislav and of the other three rivers. Close correlations were observed neither between the total mercury contents in the sediment samples and in the zoobenthos samples nor between the total mercury contents in the zoobenthos and in the fish muscle. Close correlations were observed between the relative MeHg contents in the sediment samples and in the zoobenthos samples. The limit of mercury content, which is stated in the announcement of the Ministry of Health of the Czech Republic No. 305/2004 was not exceeded in any sample. The adverse effect of industrial contamination (towns – Trebic, Jihlava) on the aquatic ecosystem of the Jihlava River was proven. The sewage stations did not eliminate fully the adverse effects.

Our results help us to understand better the accumulation of the individual mercury species in the selected tissues of the fish, and show statistically significant differences between the contents of MeHg in the selected tissues.

REFERENCES

Anonymous (1999):Toxicological Profile for Mercury – U.S. Department of Health and Human Services. Public Health Service Agency for Toxic Substances and Disease Registry.

Anonymous (2002): Ecosystem Health – Science Based Solution. Canadian Tissue Residue Guidelines for the Protection of Consumers of Aquatic Life: Methylmercury. National Guidelines and Standard Office Environmental Quality Branch Environment Canada, Ottawa.

Agusa T., Kunito T., Iwata H., Monirith I., Tana T.S., Subramanian A., Tanabe S. (2005): Mercury contamination in human hair and fish from Cambodia: levels, specific accumulation and risk assessment. Environmental Pollution, 134, 79–86.

Boening D.W. (2000): Ecological effects, transport, and fate of mercury: a general review. Chemosphere, 40, 1335–1351.

Cabanero A.I., Madrid Y., Camara C. (2004): Selenium and mercury bioaccessibility in fish samples: an *in vitro* digestion method. Analytica Chimica Acta, 526, 51–61.

Cihar J. Maly J. (1978): Fresh Water Fish (in Czech). SZN, Prague, 98 pp.

Dusek L., Svobodova Z., Janouskova D., Vykusova B., Jarkovsky J., Smid R., Pavlis P. (2005): Bioaccumulation of mercury in muscle tissue of fish in the Elbe River (Czech Republic): multispecies monitoring study 1991–1996. Ecotoxicology and Environmental Safety, 61, 256–267.

Fournier F., Karasov W.H., Kenow K.P., Meyer M.W., Hines R.K. (2002): The oral bioavailability and toxicokinetics of methylmercury in common loon (*Gavia immer*) chicks. Comparative Biochemistry and Physiology, Part A, 133, 703–714.

Gray J.S. (2002): Biomagnification in marine systems: the perspective of an ecologist. Marine Pollution Bulletin 45, 46–52.

Heinz G.H., Hoffmann D.J. (2004): Mercury accumulation and loss in mallards. Environmental Toxicology and Chemistry, 23, 222–224.

Henny C.J., Hill E.F., Hoffman D.J., Spalding M.G., Grove R.A. (2002): Nineteenth century mercury: Hazard to wading birds and cormorants of the Carson River, Nevada. Ecotoxicology, 11, 213–231.

Houserova P., Matejicek D., Kuban V., Pavlickova J., Komarek J. (2006a): Liquid Chromatographic – Cold Vapor Atomic Fluorescence Spectrometric (HPLC/CV-AFS). Determination of Mercury Species. Journal of Separation Science, 29, 248–255.

Houserova P., Kuban V., Kracmar S., Sitko J. (2006b): Determination of total mercury and mercury species in bird and fish tissues-bioaccumulation of mercury in the aquatic ecosystem Zahlinice. Environmental Pollution, in print.

Ikingura J.R., Akagi H. (1999): Methylmercury production and distribution in aquatic systems. Science and the Total Environment, 234, 109–118.

Ipolyi I., Massanisso P., Sposato S., Fodor P., Morabito R. (2004): Concentration levels of total and methylmercury in mussel samples collected along the coasts of Sardinia Island (Italy). Analytica Chimica Acta, 505, 145–151.

Kim E.Y., Saeki K., Tanabe S., Tanaka H., Tatsukawa R. (1996): Specific accumulation of mercury and selenium in seabirds. Environmental Pollution, 94, 261–265.

Kotnik J., Horvat M., Jereb V. (2002): Modelling of mercury geochemical cycle in Lake Velenje, Slovenia. Environmental Modelling & Software, 17, 593–611.

Landaluze J.S., Diego A., Raposo J.C., Madariaga J.M. (2004): Methylmercury determination in sediments and fish tissues from the Nerbioi-Ibaizabal estuary (Basque Country, Spain). Analytica Chimica Acta, 508, 107–117.

Liang L.-N., Jiang G.-B., Liu J.-F., Hu J.-T. (2003): Speciation analysis of mercury in seafood by using high performance liquid chromatography on-line coupled with cold-vapour atomic fluorescence spectrometry via a post column microwave digestion. Analytica Chimica Acta, 477, 131–137.

Marsalek P., Svobodova Z., Randak T., Svehla J. (2005): Mercury and methylmercury contamination of fish from the Skalka reservoir: A case study. Acta Veterinaria Brno, 74, 427–434.

Meloun M., Militky J. (1998): Statistical Treatment of Experimental Data. East Publishing, Prague.

Morel F.M.M., Kraepiel A.M.L., Amyot M. (1998): The chemical cycle and bioaccumulation of mercury. Annual Review of Ecological Systeme, 29, 543–566.

Nakagawa R., Yumita Y., Hiromoto M. (1997): Total mercury intake from fish and shellfish by Japanese people. Chemosphere, 35, 2909–2913.

Rehulka J. (2002): Content of inorganic and organic pollutants in the fish from the Slezska Harta reservoir. Czech Journal of Animal Science, 47, 30–44.

Sarica J., Amyot M., Hare L., Blanchfield P., Bodaly R.A., Hintelmann H., Lucotte M. (2005): Mercury transfer from fish carcasses to scavengers in boreal lakes: the use of stable isotopes of mercury. Environmental Pollution, 134, 13–22.

Scerbo R., Ristori T., Stefanini B., De Ranieri S., Barghigiani C. (2005): Mercury assessment and evaluation of its impact on fish in the Cecina River basin (Tuscany, Italy). Environmental Pollution, 135, 179–186.

Spurny P., Mares J., Hedbavny J., Sukop I. (2002): Heavy metal distribution in the ecosystems of the upper course of the Jihlava River. Czech Journal of Animal Science, 47, 160–167.

Storelli M.M., Storelli A., Giacominelli-Stuffler R., Marcotrigiano G.O. (2005): Mercury speciation in the muscle of two commercially important fish, hake (*Merluccius merluccius*) and striped mullet (*Mullus barbatus*) from the Mediterranean Sea: estimated weekly intake. Food Chemistry, 89, 295–300.

Sunderland E.M., Gobas F.A.P.C., Heyes A., Branfireun B.A., Bayer A.K., Cranston R.E., Parsons M.B. (2004): Speciation and bioavailability of mercury in well-mixed estuarine sediments. Marine Chemistry, 90, 91–105.

Svobodova Z., Dusek L., Hejtmanek M., Vykusova B., Smíd R. (1999): Bioaccumulation of mercury in various fish species from Orlik and Kamyk water reservoirs in the Czech Republic. Ecotoxicology and Environmental Safety, 43, 231–240.

Svobodova Z., Celechovska O., Kolarova J., Randak T., Zlabek V. (2004): Assessment of metal contamination in the upper reaches of the Tichá Orlice River. Czech Journal of Animal Science, 49, 458–464.

Trombini C., Fabbri D., Lombardo M., Vasura I., Zavoli E., Horvat M. (2003): Mercury and methylmercury contamination in surficial sediments and clams of a coastal lagoon (Pialassa Baiona, Ravenna, Italy). Continental Shelf Research, 23, 1821–1831.

Vigh P., Mastala Z., Balogh K.V. (1996): Comparison of heavy metal concentration of grass carp (*Ctenopharyngodon idella*) in a shallow eutrophic lake and a fish pond (possible effects of food contamination). Chemosphere, 32, 691–701.

Wagemann R., Trebacz E., Boila G., Lockhart W.L. (1998): Methylmercury and total mercury in tissues of arctic marine mammals. Science of the Total Environment, 218, 19–31.

Corresponding Author:

Prof. RNDr. Vlastimil Kuban, DrSc., Mendel University of Agriculture and Forestry, Department of Chemistry and Biochemistry, Zemedelska 1, 613 00 Brno, Czech Republic
Tel. +420 545 133 285, fax +420 545 212 044, e-mail: kuban@mendelu.cz