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Effect of Metals on Metallothionein Content in Fish from Skalka and Želivka Reservoirs

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The aim of this study was to determine the metal content (Hg, Cd, Cu, Ni, Pb, Zn, and As) of muscle and liver in fish from the Skalka and Želivka (control) reservoirs and to assess the capacity of metals to induce synthesis of metallothioneins in muscle, liver, and gill under natural conditions. The content of total mercury was significantly higher ($p < 0.05$) in muscle and liver from Skalka Reservoir than in samples from Želivka Reservoir. Methylmercury represented the main form of total mercury in all muscle samples. Significant differences ($p < 0.05$) between tested sites were observed for copper, zinc, and arsenic. No significant differences were found in metallothionein content when comparing the localities. Metallothionein liver content was negatively correlated ($p < 0.05$) with total mercury at both reservoirs. The results indicated that metallothionein did not seem to be induced by high metal contamination, therefore the suitability of metallothioneins as a marker of chronic metal exposure in fish is uncertain under field conditions.

Keywords: methylmercury, bioaccumulation, environmental pollution, heavy metal, environmental electrochemistry

1. INTRODUCTION

During recent decades, metal pollution associated with human activity such as industry, mining, agriculture, household waste production, and motor traffic has increased [1-16]. Metal contamination of the aquatic environment is a long-term issue, since metals accumulate in aquatic organisms, including fish, and persist in sediments [17]. Fish are an important source of metals in human nutrition, and those from metal contaminated sites present a potential risk to human health. Aquatic organisms present, not only simple sources of accumulated metal, but can interact with metals, altering their toxicity.

Due to exposition of biosphere with metals, organisms have developed various strategies to protect themselves against adverse effects of these ions and their compounds. The homeostasis of metals in both plant and animal cells maintain by low-molecular mass compounds rich in –SH moieties. In animals, metallothioneins (MT) play a key role in the maintaining of metal homeostasis. Metallothioneins are a group of low molecular mass (2 to 16 kDa) single-chain proteins. The metal binding domain of MTs consists of 20 cysteine residues juxtaposed with basic amino acids (lysine and arginine) arranged in two thiol-rich sites [18]. Based on their affinity to metals these proteins are able to transport essential metals to place of need or detoxify toxic metals to protect cells [19].

Two isoforms of MT, MT-1 and MT-2, are described in all vertebrates, including fish [20]. However, between mammalian and fish MT, there are some small differences in structure [21]. In fish the relationship between MTs and metals was mainly demonstrated in case of cadmium, copper, mercury, zinc, and silver [22-24]. Metallothionein synthesis varies with fish species, age and an analyzed tissue [25]. External factors, such as season, temperature, and diet, can also effect MT induction [26].

There have been published numerous papers [27-36] showing level of MT in animals, including fish, as bioindicator of pollution of environment by metals as cockle [37], barbel *Barbus graellsii* [38], mussel [39,40], seal pups [41], fish *Hemiborbus mylodon* [42], puffer fish *Takifugu obscurus* [43], calm *Mytilus galloprovincialis* [44], oyster *Crassostrea gigas* [45], and *Crassostrea virginica* or medaka *Oryzias latipes* [46,47].

Various analytical techniques including spectrometry [48-50], liquid chromatography [51], capillary electrophoresis [52-58], saturation methods [59] and electrochemistry [60] can be employed for detection of MT [31,35,61,62]. Electrochemical methods belong to the most sensitive ones, mainly differential pulse voltammetry Brdicka reaction [63-69] and chronopotentiometric stripping analysis [32,70,71]. Besides electrochemical methods with high sensitivity to MT, there are utilized also immunochemical methods including enzyme-linked immunosorbent assay [65,72,73], radioimmunoassay [74,75] or blotting techniques [72] for detection of MT with convenient detection limits. In addition to the direct detection of MTs as biomarkers, expression of MT genes is also recently used in studies with fish [25,76]. The aim of the present study was to determine the metal content and to assess the effect of metals on metallothionein levels in fish tissue under natural conditions.

2. EXPERIMENTAL PART

2.1 Sampling sites

The study was carried out at Skalka and Želivka Reservoirs. Skalka Reservoir is located in western Bohemia on the river Ohře near the border with Germany [1]. The reservoir was built in 1964 and has a surface area of 378 ha. The main purposes of the reservoir are to maintain minimum flow rates in the river Ohře, to protect the area downstream of its dam against flooding, and for generation of electricity. The reservoir is also used for recreation and water sports. Skalka Reservoir had been contaminated by sewage water effluent containing mercury from a chemical factory in Marktredwitz (Germany) since 1974 [77]. Želivka Reservoir is situated in Central Bohemia, 4 km above the confluence with the Sázava River. The reservoir was gradually filled from 1970 to 1974 to a current area of about 1600 ha. Želivka Reservoir is the main water source for Prague. A short time after filling, a high level of mercury was detected in fish tissue, although no source of mercury pollution was discovered [78]. During monitoring of mercury content in fish from 1974 to 2011, a significant decrease was observed [1]. Mercury content in fish muscle is currently low, and the reservoir is considered to be mercury uncontaminated; thus it was used as a control locality in the present study.

2.2 Materials

Sampling was performed in April 2011 by electrofishing. Forty-nine fish were captured from Skalka Reservoir and 48 from Želivka Reservoir (Table 1). Fish were weighed, and scales were collected for age determination. Samples of liver, gill, caudal kidney, and muscle were taken and stored at -18 °C for later analysis.

Table 1. The main characteristics of sampled fishes from both localities.

Locality	Skalka Reservoir			Želivka Reservoir		
Species	n	weight (kg) mean ± SD	age (years) mean ± SD	n	weight (kg) mean ± SD	age (years) mean ± SD
Asp *(Aspius aspius)*	5	1.73 ± 0.28	5.6 ± 1.1	9	1.28 ± 1.16	4.9 ± 2.1
Pikeperch *(Sander lucioperca)*	5	1.05 ± 0.90	3.6 ± 2.1	4	2.06 ± 1.10	4.5 ± 2.1
Pike *(Esox lucius)*	5	1.80 ± 0.73	3.8 ± 0.8	7	0.82 ± 0.57	3.0 ± 1.0
Perch *(Perca fluviatilis)*	5	0.45 ± 0.24	3.4 ± 0.6	7	0.19 ± 0.21	2.4 ± 0.5
Bream (*Abramis brama*)	5	0.67 ± 0.16	5.6 ± 1.5	9	0.65 ± 0.13	5.8 ± 0.8
Roach *(Rutilus rutilus)*	5	0.19 ± 0.63	4.2 ± 0.5	6	0.39 ± 0.44	4.5 ± 2.2
Chub *(Leuciscus cephalus)*	5	0.48 ± 0.55	4.2 ± 1.8	6	0.16 ± 0.10	3.3 ± 1.0
Silver bream *(Blicca bjoerkna)*	5	0.25 ± 0.79	5.6 ± 0.9	-		
Common carp *(Cyprinus carpio)*	5	0.74 ± 0.30	4.0 ± 1.1	-		
Rudd *(Scardinius erythrophthalmus)*	4	0.13 ± 0.47	3.3 ± 1.2	-		

2.3 Metal determination

Total mercury (THg) content of muscle and liver was determined by the direct method of cold vapours using an AMA 254 (Altec Ltd., Czech Republic) analyser. Methylmercury (MeHg) in the form of methylmercury chloride was determined in muscle by gas chromatography using a Shimadzu capillary gas chromatograph with an electron captured detector GC 2010A (Shimadzu Kyoto, Japan) [79-81]. Samples were prepared by acid digestion and extraction with toluene. A capillary column DB 608 (30 m × 0.53 mm × 0.83 μm; J&W Scientific Chromservis, Czech Republic) was used. Analysis was conducted with GC Solution software (Shimadzu Kyoto, Japan). Limits of detection for THg and MeHg were 1 $\mu g.kg^{-1}$ and 21 $\mu g.kg^{-1}$, respectively. The limit of detection was set to triple the standard deviation of a blank mean value. The accuracy of THg and MeHg values was validated using standard reference material BCR-CRM 464 (Tuna Fish, IRMM, Belgium). The total mercury and MeHg concentrations in fish tissue are given in $mg.kg^{-1}$ wet weight (ww). Samples of muscle and liver were used for determination of Cd, Cu, Ni, Pb, Zn, and As. Mineralization of fish tissues was carried out in laboratory autoclaves with microwave heating, using nitric acid and hydrogen peroxide (Uniclever, Plasmatronica Poland, ETHOS SEL, Milestone Italy). Samples for the determination of arsenic were processed as above and burned in a muffle oven (450 °C) with the addition of magnesium nitrate. The ash was dissolved in hydrochloric acid; As^{5+} was reduced to As^{3+}. Arsenic was determined by a hydride technique with electrothermal atomisation in iridium coated graphite tube preheated to 300 °C (Hydrae 60, Analytik Jena AG, Germany). An AAS electrothermic technique was used for determination of Pb, Cd, Cu, and Ni. Zinc was determined by an AAS flame technique. All AAS measurements were made using a high-resolution continuum source atomic adsorption spectrophotometry (HR-CS AAS, apparatus ContrAA 700, Analytik Jena AG, Germany). Accuracy of the results was validated using the following standard reference materials: CRM DORM-2 (Dogfish muscle – NRC); SRM 1566b (Oyster tissue – NIST). The following detection limits (3σ) were used: As 3.1 $\mu g.kg^{-1}$, Cd 1.6 $\mu g.kg^{-1}$, Pb 36.5 $\mu g.kg^{-1}$, Cu 12 $\mu g.kg^{-1}$, Zn 2500 $\mu g.kg^{-1}$, Ni 50 $\mu g.kg^{-1}$. The metal concentrations in fish tissues are given as $\mu g.kg^{-1}$ww.

2.4 Metallothionein determination

Levels of MT in liver, gill, and kidney were determined by the differential pulse voltammetry Brdicka reaction [3]. Differential pulse voltammetric measurements were made with the 747 VA Stand instrument connected to a 693 VA Processor and 695 Autosampler (Metrohm, Switzerland), using a standard cell with three electrodes and a cooled sample holder and measurement cell to 4°C (Julabo F25, Julabo, Germany). A hanging mercury drop electrode (HMDE) with a drop area of 0.4 mm^2 was the working electrode. An Ag/AgCl/3M KCl electrode was used as reference, and a platinum electrode was auxiliary. The VA Database 2.2 by Metrohm CH was employed for data processing. The analyzed samples were deoxygenated prior to measurements by purging with argon (99.999%) saturated with water for 120 s. The Brdicka supporting electrolyte contained 1 mM $Co(NH3)_6Cl_3$, and 1 M ammonia buffer (NH_3(aq) + NH_4Cl, pH = 9.6) was used. The supporting electrolyte was exchanged after each analysis. The parameters of the measurement were as follows: initial potential of -0.7 V, end potential of -1.75 V, modulation time 0.057 s, time interval 0.2 s, step potential 2 mV, modulation amplitude -

250 mV, Eads = 0 V, volume of injected sample 10 µl, volume of measurement cell 2 ml (10 µl of sample + 1990 µl Brdicka solution). Metallothionein concentrations in fish tissues are given as $\mu g.mg^{-1}$ of protein [63,82,83].

2.5 Statistical analysis

Metals in fish from both sites, as well as metallothioneins, were tested for normal distribution using the Saphiro–Wilk test. The majority of parameters were not normally distributed; hence non parametric statistical tests were used. The levels of metals were adjusted for age to minimize inter-species differences. The adjusted data were used for the Kruskal–Wallis test, followed by multiple comparison, to compare species between localities. The Spearman rank correlation coefficient was used for determining the relationship among analysed parameters. Significance was set at $p < 0.05$. Analysis was conducted using Statistica v. 8.0 (StatSoft).

3. RESULTS

Muscle THg concentration varied from 0.039 to 0.725 $mg.kg^{-1}$ (mean values), the lowest being in roach from Želivka Reservoir and highest in pikeperch from Skalka Reservoir. Liver THg content varied from 0.011 in rudd to 0.750 $mg.kg^{-1}$ in pikeperch, both species from Skalka Reservoir. Significantly higher content of THg in both muscle and liver was found in several species from Skalka Reservoir compared to samples from Želivka Reservoir. Methylmercury makes up the majority of THg muscle content in all fish from both localities. The proportion of MeHg in THg varied from 88 to 99 % (mean values) in predatory fish and from 51 to 84 % in non-predatory fish from Skalka Reservoir. In Želivka Reservoir, the percent of MeHg varied from 66 to 94 % in predatory fish and from 64 to 70 % non-predatory fish (Fig. 1 and 2).

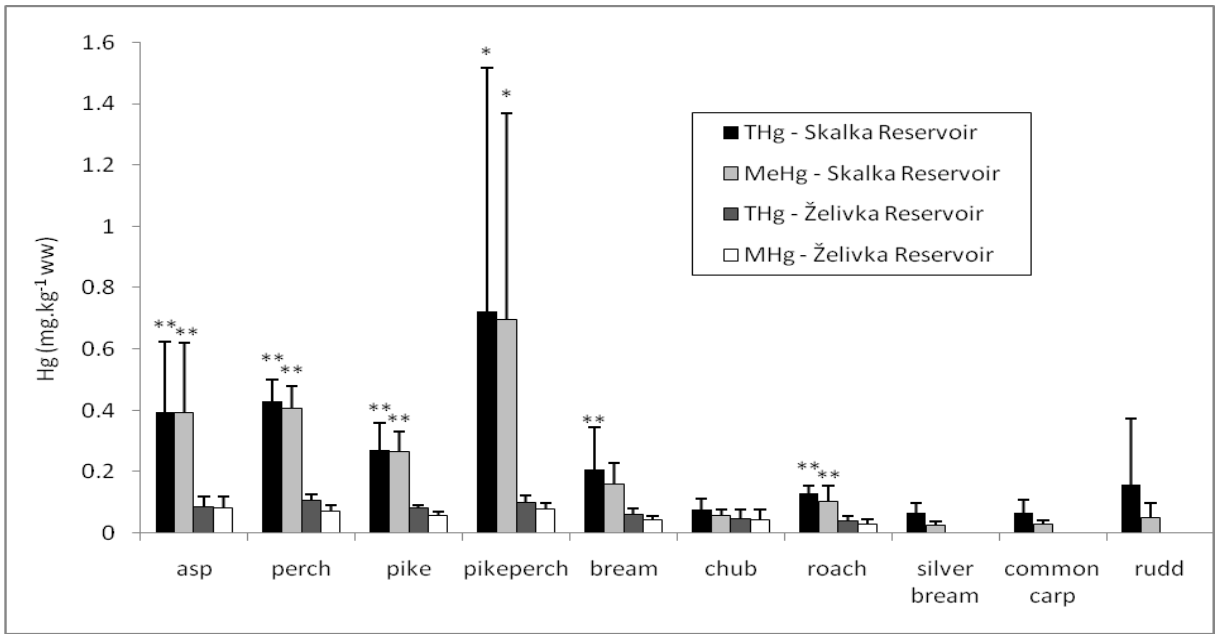

Figure 1. Total mercury (THg) and methylmercury (MeHg) ($mg.kg^{-1}$ ww) in muscle. *($p < 0.05$), **($p < 0.01$) Significant differences are indicated by asterisk. Data are adjusted for age.

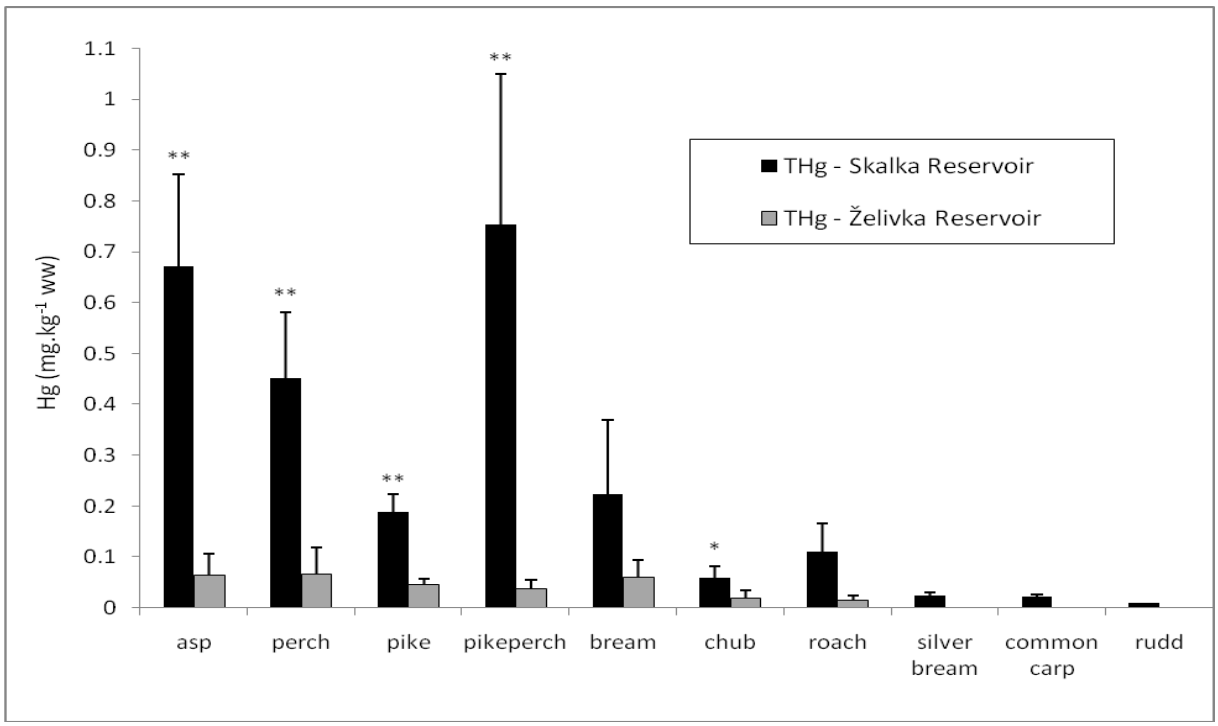

Figure 2. Total mercury (THg) (mg.kg^{-1} ww) in liver. *($p < 0.05$), **($p < 0.01$) Significant differences are indicated by asterisk. Data are adjusted for age.

The results of fish tissues analysis for Cd, Cu, Ni, Pb, Zn and As content are given in Table 2 and Table 3. Statistically significant differences ($p < 0.05$) between the sites were found for Cu, Zn, and As (Table 4). Mean copper concentrations varied from 60.4 to 304.0 μg.kg^{-1}in muscle and from 357.8 to 27056.0 μg.kg^{-1} in liver. Significantly higher Cu content was observed in muscle of pikeperch from Skalka Reservoir. Significantly higher Cu content was also found in liver of bream and perch from Skalka Reservoir. Lead content varied from 40.0 to 56.1 μg.kg^{-1} in muscle and from 43.2 to 157.5 μg.kg^{-1} in liver in fish from Želivka Reservoir, and from 39.0 to 59.6 μg.kg^{-1} in muscle and from 39.7 to 142.0 μg.kg^{-1} in liver samples from Skalka Reservoir. Lead concentrations below a limit of detection were found in several species from both localities. Zinc content varied from 4762.0 to 9885.9 μg.kg^{-1} in muscle and from 16,252.5 to 46,047.8 μg.kg^{-1} in liver in fish from Želivka Reservoir, and from 4370.2 to 15.458.3 μg.kg^{-1} in muscle and from 16,524 to 38,850.0 μg.kg^{-1} in liver samples from Skalka Reservoir. Significantly higher Zn content was found in muscle of chub and perch and also in liver of pike in samples from Želivka Reservoir. Arsenic muscle and liver content was higher in a majority of species from Želivka Reservoir. The As concentrations were significantly higher in muscle of perch, chub, and roach and in liver of perch, roach, and bream. Low Cd concentrations, near to a limit of detection, were found in muscle in both localities. Liver Cd levels varied from 24.2 to 1059.2 μg.kg^{-1} in samples from Želivka Reservoir, and from 20.1 to 469.4 μg.kg^{-1} in samples from Skalka Reservoir.

Table 2. Metal (Cd, Cu, Ni, Pb, Zn, and As) concentration in fish muscle (μg.kg^{-1} ww; mean ± SD).

		Metals measured in muscle (μg.kg^{-1}) mean ± SD					
Species	n	Cd	Cu	Ni	Pb	Zn	As
Želivka Reservoir							
asp	9	5.2 ± 13.3	153.7 ± 23.0	67.1 ± 36.9	47.6 ± 22.9	9885.9 ± 15 502.0	43.4 ± 26.1
pikeperch	4	< 1.6	60.4 ± 55.0	91.4 ± 132.8	40.0 ± 2.5	4762.0 ± 3000.0	52.4 ± 13.5
perch	7	1.8 ± 2.1	154.0 ± 151.0	198.9 ± 460.0	56.1 ± 48.2	5509.7 ± 348.8	95.8 ± 32.6
pike	7	< 1.6	119.6 ± 50.2	95.5 ± 143.7	< 0.04	6637.9 ± 3479.8	64.2 ± 15.5
bream	9	2.3 ± 1.4	150.1 ± 93.2	88.9 ± 79.4	41.6 ± 36.7	6921.1 ± 6560.0	65.7 ± 21.1
roach	6	2.4 ± 1.6	304.0 ± 227.0	< 0.05	< 0.04	5789.8 ± 1510.0	140.5 ± 24.1
chub	6	< 1.6	246.3 ± 166.3	944.5 ± 2215.1	51.05 ± 27.1	7183.0 ± 1114.4	45.4 ± 33.1
Skalka Reservoir							
asp	5	1.8 ± 0.6	273.5 ± 38.3	51.1 ± 3.3	49.1 ± 20.4	6323.8 ± 4164.6	35.7 ± 40.3
pikeperch	5	< 1.6	123.7 ± 61.6	76.7 ± 115.6	55.6 ± 55.2	5992.6 ± 3429.5	18.3 ± 7.2
perch	5	1.6 ± 0.1	156.9 ± 72.0	104.5 ± 141.0	59.6 ± 77.6	9459.4 ± 10836.3	40.2 ± 8.6
pike	5	1.9 ± 0.4	152.1 ± 67.0	112.1 ± 182.0	53.4 ± 29.9	5583.8 ± 2008.9	22.9 ± 6.4
bream	5	1.9 ± 0.6	116.3 ± 42.8	59.3 ± 13.7	39.0 ± 4.8	4807.0 ± 1918.9	54.3 ± 25.0
roach	5	2.1 ± 1.3	190.5 ± 176.3	59.4 ± 21.5	40.5 ± 20.7	4960.0 ± 1555.9	41.3 ± 8.9
chub	5	1.8 ± 1.3	249.0 ± 93.7	328.2 ± 678.0	< 0.04	4625.8 ± 513.9	28.8 ± 27.5
silver bream	5	4.7 ± 5.9	194.1 ± 65.5	276.6 ± 380.2	47.2 ± 16.1	5254.4 ± 1416.8	63.2 ± 33.6
common carp	5	4.2 ± 5.4	159.6 ± 33.3	52.7 ± 6.0	< 0.04	4370.2 ± 1152.1	59.8 ± 17.8
rudd	4	1.9 ± 1.5	238.5 ± 195.6	50.5 ± 1.0	51.3 ± 46.5	15458.3 ± 14673.1	33.5 ± 24.5

Table 3. Metal (Cd, Cu, Ni, Pb, Zn, and As) concentration in fish liver (μg.kg^{-1} ww; mean ± SD).

Metals measured in liver (μg.kg^{-1}) mean ± SD							
Species	n	Cd	Cu	Ni	Pb	Zn	As
Želivka Reservoir							
asp	9	156.4 ± 131.0	19037.4 ± 18095.9	79.7 ± 70.3	43.2 ± 21.9	38010.0 ± 15328.8	289.5 ± 309.8
pikeperch	4	63.5 ± 22.8	357.8 ± 195.0	61.9 ± 73.8	157.5 ± 257.2	16252.5 ± 3660.7	232.3 ± 128.0
perch	7	355.9 ± 125.5	5495.0 ± 4492.3	< 0.05	153.9 ± 198.2	32473.3 ± 6287.1	694.0 ± 537.4
pike	7	59.1 ± 94.1	8847.2 ± 10854.0	140.3 ± 257.9	58.7 ± 51.8	42244.0 ± 19740.3	543.0 ± 988.3
bream	9	1059.2 ± 546.9	16308.8 ± 7867.6	61.4 ± 35.4	61.7 ± 62.0	46047.8 ± 12423.3	291.9 ± 98.0
roach	6	286.7 ± 224.0	9138.5 ± 3809.7	< 0.05	79.6 ± 71.1	33467.5 ± 8239.7	332.5 ± 122.8
chub	6	24.2 ± 19.5	1735.2 ± 2166.4	< 0.05	< 0.04	30265.0 ± 17783.7	71.8 ± 7.3
Skalka Reservoir							
asp	5	73.0 ± 20.4	19660.0 ± 4380.4	< 0.05	< 0.04	33092.0 ± 4322.6	100.6 ± 22.8
pikeperch	5	73.3 ± 67.1	633.9 ± 388.2	107.2 ± 183.8	42.5 ± 5.6	16524 ± 1356.1	71.8 ± 31.8
perch	5	340.6 ± 216.1	1176.1 ± 415.6	< 0.05	< 0.04	22836.0 ± 2717.9	218.0 ± 99.6
pike	5	20.1 ± 13.6	8071.8 ± 7280.8	< 0.05	45.9 ± 30.0	30402.5 ± 6853.1	57.3 ± 26.4
bream	5	469.4 ± 84.8	27056.0 ± 9470.5	51.2 ± 6.1	39.7 ± 3.7	38850.0 ± 9931.7	122.5 ± 31.9
roach	5	60.1 ± 19.7	4235.4 ± 1698.1	193.8 ± 237.1	68.9 ± 32.7	24674.0 ± 6132.4	133.5 ± 94.3
chub	5	47.4 ± 27.3	6398.6 ± 4348.7	1790.7 ± 3915.0	103.4 ± 115.6	25146.0 ± 4874.2	63.7 ± 43.6
silver bream	5	211.9 ± 150.6	2411.5 ± 2037.5	101.7 ± 134.4	136.4 ± 84.8	22974.0 ± 6044.6	103.4 ± 40.1
common carp	5	91.3 ± 79.6	5258.4 ± 4632.2	< 0.05	86.0 ± 94.0	29863.3 ± 6536.2	54.7 ± 16.6
rudd	4	41.8 ± 18.4	10630.5 ± 10925.4	732.6 ± 1306.6	142.0 ± 199.4	38835.0 ± 10902.7	59.5 ± 22.6

Table 4. Significant differences between localities in metal concentration (Cu, Zn, and As) in fish tissues. * = $p < 0.05$; a = higher level of single metal in fish from Želivka Reservoir; b =higher level of single metal in fish from Skalka Reservoir. Data adjusted for age were used for statistical analysis.

Metal	Tissue	Fish species					
		pikeperch	perch	pike	bream	chub	roach
Cu	muscle	*b	-	-	-	-	-
	liver	-	*b	-	*b	-	-
Zn	muscle	-	*a	-	-	*a	-
	liver	-	-	*a	-	-	-
As	muscle	-	*a	-	-	*a	*a
	liver	-	*a	-	*a	-	*a

Metallothionein content varied from 1.3 to 18.1 $\mu g.mg^{-1}$of protein in fish tissue (Table 5). No significant differences were observed between localities. Significant correlations between MT concentration and single metal content are shown in Table 6.

Table 5. Concentration of MT ($\mu g.mg^{-1}$ of protein) in fish tissue.

Species	Locality	n	MT (liver) $\mu g.mg^{-1}$ mean ± SD	MT (gills) $\mu g.mg^{-1}$ mean ± SD	MT (kidney) $\mu g.mg^{-1}$ mean ± SD
asp	Želivka Reservoir	9	7.4 ± 3.8	3.9 ± 0.7	1.4 ± 0.6
	Skalka Reservoir	5	7.5 ± 1.3	3.6 ± 2.3	2.3 ± 0.9
pikeperch	Želivka Reservoir	4	6.4 ± 1.8	5.0 ± 3.2	3.4 ± 0.7
	Skalka Reservoir	5	7.0 ± 2.6	3.9 ± 1.5	9.4 ± 6.2
perch	Želivka Reservoir	7	8.3 ± 1.8	5.5 ± 0.3	2.8 ± 1.7
	Skalka Reservoir	5	4.8 ± 2.1	4.0 ± 1.7	1.3 ± 0.7
pike	Želivka Reservoir	7	18.1 ± 11.3	2.4 ± 1.1	6.8 ± 3.9
	Skalka Reservoir	5	11.0 ± 3.5	5.4 ± 1.4	3.3 ± 0.5
bream	Želivka Reservoir	9	5.3 ± 2.6	4.7 ± 1.3	2.5 ± 0.9
	Skalka Reservoir	5	10.1 ± 9.7	4.0 ± 1.5	2.5 ± 0.8
chub	Želivka Reservoir	6	4.8 ± 2.3	2.9 ± 1.4	2.9 ± 3.3
	Skalka Reservoir	5	7.1 ± 1.9	2.0 ± 1.7	4.4 ± 2.9
roach	Želivka Reservoir	6	12.3 ± 5.6	3.4 ± 1.7	1.7 ± 1.2
	Skalka Reservoir	5	5.7 ± 0.2	4.3 ± 0.9	2.3 ± 0.9
silver bream	Skalka Reservoir	5	7.5 ± 5.2	4.5 ± 1.6	8.9 ± 11.1
common carp	Skalka Reservoir	5	8.5 ± 3.7	4.1 ± 2.9	5.0 ± 3.2
rudd	Skalka Reservoir	4	9.6 ± 0.5	6.5 ± 0.7	10.3 ± 2.1

Table 6. Significant correlations ($p < 0.05$) between MT content and single metal (THg, Cd) in fish tissues.

Skalka reservoir			Želivka reservoir		
	n	r_s		n	r_s
MT in liver and THg in liver	49	-0.336	MT liver and THg in liver	48	-0.493
MT in kidney and THg in liver	49	-0.589	MT liver and Cd in liver	48	-0.417
MT in kidney and THg in muscle	49	-0.546			

4. DISCUSSION

The results of THg and MeHg content confirmed our expectation about persisting high contamination of Skalka Reservoir [84]. Higher Hg levels were found in predatory fish from both localities, corresponding to their top position in the aquatic environment food chain. Consumption, especially of predatory fish from Skalka Reservoir, can be a serious health risk to humans. We also confirmed the Hg unpolluted status of Želivka Reservoir [1]. Although significant differences were found in Cu levels in various fish species among the localities, it is hard to say that fish from Skalka Reservoir are Cu contaminated, because the results are ambiguous. A possible source of copper could be the application of an algicide containing copper sulphate widely used in 1970s at the locality [85]. Copper levels in sediment need to be determined for better understanding of the issue. Arsenic levels are higher in fish from Želivka Reservoir than Skalka Reservoir, but are comparable to levels from arsenic-uncontaminated localities [86].

Currently, contamination of aquatic ecosystems is monitored using specific biomarkers. In the case of metal pollution, MT has been suggested to be suitable biochemical indicator [30]. Response of MT to heavy metals has been observed in several studies [87-89]. Although Cd is the metal most frequently connected with induction of MT synthesis [90-93], several laboratory and field studies have reported elevated MT content after mercury exposure [25,94,95]. In the present study, we did not find elevated Hg to induce MT synthesis in fish tissue. On the contrary, a significant negative correlation between Hg content and MT level was observed. The use of MTs as markers of environmental pollution is problematic, since their levels are influenced by multiple factors [96]. Total MT level can varied with species, sex, age, and size of fish [26,97], metal combinations, exposure time, water characteristics [89], and season [98,99]. Metallothionein synthesis in fish is associated with organs involved in metal uptake, metabolism, and excretion, such as gill, liver, kidney, and intestine. In our study the highest concentration of MT was seen in liver, in accordance with other authors [95,100]. An important factor is metal form. Gonzales et al. [76] conducted a study focusing on MT gene expression after MeHg exposure to zebrafish *(Danio rerio)*. No significant MT gene expression was found in fish brain, although it contained the highest MeHg concentration. Miero et al. [76] investigated the relationship between MT and THg in a lagoon contaminated by mercury discharges. No significant correlation between MT and THg levels was found. Although that study dealt only with THg, we can assume that Hg in form of MeHg was present, since the study was conducted under natural conditions. More studies dealing with the response of MT to MeHg exposure have been done in mammals [101].

Overall, organic Hg forms probably have low affinity to MT. The majority of THg in fish tissue was present as MeHg (51-99 %) in our study, which could be a reason for the lack of a positive response of MT synthesis.

5. CONCLUSION

The results showed that high mercury content does not demonstrably influence metallothionein level, therefore the suitability of metallothioneins in fish as markers of chronic mercury exposure in field conditions remains uncertain, and further well designed studies need to be conducted.

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