Effect of metals, with special attention of Cd, content of the Svitava and Svratka rivers on levels of thiol compounds in fish liver and their use as biochemical markers

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Abstract

OBJECTIVES: The aim of this study was to assess the effect of metals, with special attention of cadmium (Cd), content of the Svitava and Svratka rivers (Czech Republic) on levels of thiol compounds such as metallothioneins (MT), glutathione (GSH/GSSG), and cysteine (Cys) concentrations in chub (*Leuciscus cephalus* L.) liver and their use as biomarkers.

DESIGN: Levels of thiol compounds in fish liver were determined by the differential pulse voltammetry Brdicka reaction. Cadmium content in *L. cephalus* liver was measured by atomic absorption spectometry (AAS) with electrothermal atomization. The contents of thiols and Cd were correlated with the Cd and metals content of river bottom sediments.

RESULTS: Results were inconsistent with those of experimental studies. Correlations between liver Cd content and MT, Cys, GSSG were not significant in any locality ($p > 0.05$). Cd liver content was negatively ($p< 0.05$) correlated with GSH at one (Židlochovice) of the seven tested sites. GSH and GSSG correlated negatively in five localities ($p > 0.05$). Correlations between Cd in sediment and Cys and GSSG ($p > 0.05$), and MT was negative ($p< 0.05$). The relationship between sum of metals value and MT was negative ($p< 0.05$), similarly with GSSG ($p > 0.05$).

CONCLUSION: Many factors influence content of MT, GSH, and GSSG, and data for Cd were equivocal. Monitoring the aquatic ecosystem to identify metal content using thiol compounds in fish liver as biomarkers is complex. More relevant data including laboratory experiments will be required to assess most of the factors influencing thiols and their relevance to Cd contamination.

ORIGINAL ARTICLE

Abbreviations & units

AAS	– atomic absorption spectometry
Cys	– cysteine
EROD	– ethoxyresorufin-*O*-deethylase
GSH	– reduced glutathione
GSSG	– oxidized glutathione
HMWP	– high molecular weight protein
MT	– metallothionein

INTRODUCTION

Heavy metals such as cadmium (Cd), lead (Pb) and mercury (Hg) produced in connection with human activity occur in the environment and constitute a health risk to organisms, including humans (Ionescu *et al.* 2006; Foltinova *et al.* 2007). In many aquatic ecosystems, release of metals from industrial and agricultural sources leads to concentrations above natural background levels (Lange *et al.* 2002). Cadmium is a toxicant of environmental concern ranked tenth on the European Union list of priority substances (Water Framework Directory). As a nonessential metal it has no known biological function and can cause deleterious effects (Bervoets & Blust, 2003). Although Cd toxicity is well-studied in aquatic toxicology, little is known about the mechanism of its action at low, environmentally realistic concentrations (Wang & Crowley, 2005). As a consequence, predicting toxic effects at environmental concentrations remains difficult under natural variable situations (Reynders *et al.* 2006). There is a need for reliable specific biomarkers for identifying and measuring environmental Cd content.

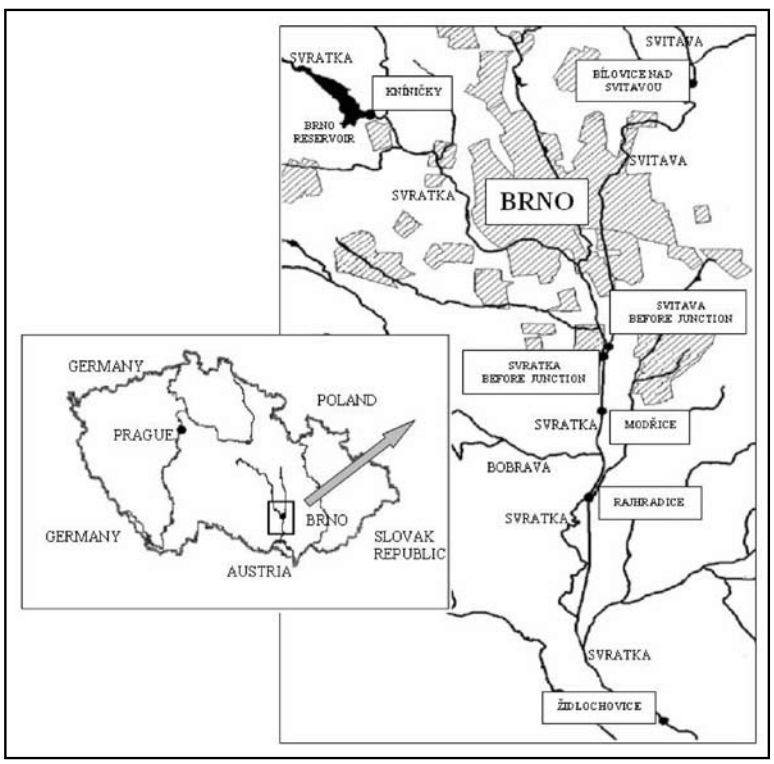

Figure 1. Locations of sample sites around Brno (Czech Republic).

Although the liver is considered a major organ for Cd accumulation in fish (Köck *et al.* 1996), the ultimate target for Cd seems to be kidney (Glynn & Olsson, 1991). Wicklund *et al.* (1988) reported that Cd is accumulated in the liver and that further transport to other organs is limited. There is little or no way to eliminate a toxic metal once assimilated into the body (Kille *et al.* 1992). It seems that chronic exposure, which may lead to steady state of hepatic Cd levels, requires long-term exposure (Yudkovski *et al.* 2008). We used liver as a sample tissue in this study.

The intracellular fate of metal ions depends strongly on thiol-containing molecules, particularly glutathione (GSH/GSSG) and metallothioneins (MT) (Eaton *et al.* 1980) that provide sulphydryl cysteine groups for binding a variety of metals, including Cd (Mason & Jenkins, 1995; Maracine & Segner, 1998).

Tripeptide glutathione (*γ*-L-glutamyl-L-cysteinyl-glycine) is the most abundant low-molecular-weight thiol-containing molecule in the cell. At physiological levels it acts as a free-radical scavenger in several important non-enzymatic antioxidant processes (Belcastro *et al.* 2009) and is involved in the cellular defence against the toxic action of metal cations (Meister & Anderson, 1983). It occurs as reduced (GSH) and oxidized (GSSG) forms, due to its function as an antioxidant resulting in the oxidation of GSH to glutathione disulphide (GSSG).

Metallothioneins are heat stable low molecular weight proteins characterized by a high content of cysteinyl residues (up to 30% of total amino acids), the absence of aromatic and hydrophobic amino acid residues, and a high affinity towards metal ions (Hamer, 1986). The in vitro affinity of single metals to MT decreases in the hierarchical sequence: $Hg^{2+} > Ag^{+} > Cu^{2+} > Cd^{2+} > Pb^{2+} > Zn^{2+} > Co^{2+}$ (Hamer, 1986; Vasak, 1991). Metallothioneins are regarded as two pools represented by basally-synthesized proteins involved in essential mineral regulation, such as Cu or Zn (Bremner, 1991), and induced proteins involved in nonessential or excessive amounts of essential metal detoxification (Kägi & Schäffer, 1988; Roesjadi, 1992; Jezierska & Witeska, 2001). It was demonstrated that different fish species has various isoforms of metallothioneins. Turbot (*Scotopthalmus maximus* L.), cod (*Gadus morhus* L.) and pike (*Esox lucidus* L.) contain one form of MT, whereas *O.mykiss*, *C.auratus,* red sea bream (*Pagrus major* T&Sch.) and channel catfish (*Ictalurus punctatus* R.) contain two forms of MT marked as MT I and MT II (Smirnov *et al.* 2004). Brouwer *et al.* (1992) concluded that one form of MT function primarily in the detoxification of metals, whereas other form plays important role in metal homeostasis. It was published

Table 2. Contents of metals in sediments from the sample sites.

	Metals measured in sediments (µg/kg dry weight)												
Sample site	Cd	As	Ba	Co	Cr	Cu	Mo	Ni	Pb	Se	Zn	Al	Ti
Knínički	0.12	1.1	69	3.3	4.4	6	0.047	5.4	27	0.068	42	2800	47
Bílovice nad Svitavou	0.42	1.3	91	4.6	9,5	21	0.071	8.7	25	0.14	84	2100	41
Svitava before junction	0.59	1.1	82	3.7	10	21	0.079	7.8	33	0.093	97	1900	39
Svratka before junction	2.5	2.9	180	7.2	29	62	0.13	18	60	0.20	230	3500	72
Modřice	1.4	2.5	110	7.2	24	46	0.17	17	44	0.18	150	3000	55
Rajhradice	1.6	2.4	170	7.2	22	65	0.23	18	53	0.27	240	3700	56
Židlochovice	2.3	2.2	140	6.5	26	55	0.13	17	52	0.17	230	2800	52

that Cd detoxification mechanism rests in possibility of Cd displacing Zn in MT (Dallinger *et al.* 1996; De Smet *et al.* 2001), but it is not possible exchanging Cu in accordance to affinity line order. The total value of MT includes Cd/Zn-MT and Cu-MT (Olsvik *et al.* 2000; De Smet *et al.* 2001). From the affinity line and sum of metals determined in sediment in our study we have suggested that the most important metal for MT sequestration was Cd. Other metals engaged on MT affection were Cu, Zn and Pb. As mentioned, basally-synthesized pool of MT to carry out the physiologically functions (Roesjadi, 1992), can be topped by the induced MT synthesis. Since the induction of MT biosynthesis by metal ions is relatively slow, GSH may work as an initial defence against metal ion toxicity (Singhal *et al.* 1987) by binding to metals before the induced synthesis of MT reaches effective levels.

Cysteine, as the most important basic molecule for metal binding, plays the key role in providing SH group. This functional thiol group can join metal ion as well as reactive oxygen species and in case of GSH even other molecules. We have assumed that measuring of Cys facilitates to indicate increasing level of thiol molecules after metal exposure.

The aim of the present study was to assess the effect of metals, with special attention of Cd, content of the Svitava and Svratka rivers in the Brno conurbation, Czech Republic, on levels of thiol compounds such as metallothioneins (MT), glutathione (GSH/GSSG), and cysteine (Cys) concentrations in chub (*Leuciscus cephalus* L.) liver. A second aim was to confirm that the concentrations of these cellular stress molecules are effective indicators of this type of water pollution, and that European chub was suitable as an indicator fish.

MATERIALS AND METHODS

Animals and sampling. Investigation was conducted from June to September 2008 at seven locations on the Svitava and Svratka rivers (***Fig. 1***). The indicator species selected was the European chub, a common freshwater cyprinid that inhabits both clean and polluted rivers and has been used as an indicator species in many environmental pollution studies (Blahova *et al.* 2008; Havelkova *et al.* 2008; Randak *et al.* 2009). A total of 102 male chub were captured by electro-fishing. Fish were weighed and measured, and scales were collected for age determination (***Tab. 1.***). Livers were removed and immersed in dry ice for transport to the laboratory, where they were stored at –80 °C until analysis. At each location, composite bottom sediment samples were collected for determination of metal content.

Table 1. Characteristics of *Leuciscus cephalus* captured at the sample sites.

Sample site (River km)	Fish n	Age (years) (mean ± SD)	Weight (g) (mean ± SD)
Svratka River – Kníničky (56.2)	14	5.8 ± 1.8	307 ± 191
Svitava River -- Bílovice nad Svitavou (18.0)	15	4.8 ± 0.9	129 ± 32
Svitava River before junction (0.6)	17	4.1 ± 1.2	162 ± 66
Svratka River before junction (40.9)	17	5.6 ± 1.2	236 ± 96
Svratka River – Modřice (38.7)	7	5.4 ± 1.0	268 ± 64
Svratka River – Rajhradice (35.0)	17	5.0 ± 0.7	243 ± 75
Svratka River – Židlochovice (30.0)	15	3.8 ± 1.2	228 ± 88

Chemical monitoring was provided by the Institute of Public Health, Ostrava. Cadmium content as well as that of other metals, of sediment was measured by atomic absorption spectometry (AAS). Contents of single metals measured in sediments from sample sites are recorded in the ***Tab. 2***.

Analysis of cadmium in fish liver. Samples were mineralized by means of nitric acid and hydrogen peroxide in laboratory autoclaves with microwave heating (ETHOS SEL, Milestone Italy). Concentrations of Cd were determined by AAS with electrothermal atomization (ZEEnit 700, Analytika Jena, Germany). The detection limits (3σ) for Cd was 0.1 µg/kg. Samples of reference materials BCR No. 278 (muscle) and IAEA

Table 3. Cadmium content, concentration of MT, Cys, GSH and GSSG in *Leuciscus cephalus* from the sample sites.

	n	Mean	Median	Minimum	Maximum	±S.D.
Cd (μg/kg) in fish						
Knínički	14	74	58	14	175	51
Bílovice nad Svitavou	15	102	99	28	249	58
Svitava before junction	17	33	29	17	56	13
Svratka before junction	17	74	59	10	203	57
Modřice	7	20	16	6	59	18
Rajhradice	16	54	48	24	89	19
Židlochovice	15	52	51	22	82	20
MT (μg/kg)						
Knínički	14	2118	24525	626	3359	934
Bílovice nad Svitavou	15	1079	1151	564	1500	305
Svitava before junction	17	1768	1681	853	2985	601
Svratka before junction	17	758	706	224	1340	365
Modřice	7	627	818	76	1045	401
Rajhradice	17	605	662	52	1144	292
Židlochovice	15	990	921	264	2392	564
GSH (μM)						
Knínički	14	12.08	8.55	2.08	38.79	10.54
Bílovice nad Svitavou	15	12.25	9.21	2.85	27.22	7.27
Svitava before junction	16	21.91	23.12	0.19	47.04	10.28
Svratka before junction	15	19.11	15.88	2.68	56.59	15.93
Modřice	7	20.55	26.26	3.03	38.83	15.13
Rajhradice	17	19.37	14.44	2.74	52.65	16.31
Židlochovice	15	15.44	9.14	1.65	53.73	15.30
GSSG (μM)						
Knínički	14	57.10	36.40	2.92	234.94	66.20
Bílovice nad Svitavou	15	103.01	71.01	1.64	281.61	100.78
Svitava before junction	16	41.97	18.14	0.98	292.12	71.14
Svratka before junction	15	117.71	32.59	0.71	786.25	203.56
Modřice	7	78.14	48.21	11.20	210.75	81.59
Rajhradice	17	34.75	13.55	2.15	241.41	59.42
Židlochovice	15	104.73	15.88	3.24	413.65	148.25
Cys (μM)						
Knínički	14	4.13	2.72	0.89	19.02	4.59
Bílovice nad Svitavou	15	3.46	2.29	0.88	13.13	3.58
Svitava before junction	16	3.53	2.45	0.83	10.45	2.96
Svratka before junction	15	6.19	4.05	0.49	29.36	7.62
Modřice	7	5.97	3.37	0.52	12.04	5.08
Rajhradice	17	2.55	1.94	0.54	10.06	2.59
Židlochovice	15	2.10	1.29	0.77	5.17	1.46

MA-B-3/TM (whole fish homogenate) were used to validate the method and check its accuracy.

Analysis of thiol compounds and cysteine in fish liver. Each liver sample (c. 0.2 g) was homogenized with exactly 1000 μL of 0.2 M phosphate buffer (pH 7.2) and transferred to a test-tube, and vortexed for 15 min at 4°C (Vortex Genie, USA). Samples were heat-treated to denature the tissue and remove high molecular weight proteins. The supernatant was held at 99 °C in a thermomixer (Eppendorf 5430, USA) for 15 min with occasional stirring, and subsequently cooled to 4 °C. The denatured homogenates were centrifuged at 15000 g for 30 min at 4 °C. (Eppendorf 5402, USA).

Metallothioneins. Electrochemical measurements were performed with 747 VA Stand instrument connected to 746 VA Trace Analyzer and 695 Autosampler (Metrohm, Switzerland), using a standard cell with three electrodes and cooled sample holder (4 °C). 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 the reference and glassy carbon electrode was auxiliary electrode. For other experimental details see Fabrik *et al.* (2008).

Cysteine, reduced and oxidized glutathione. High performance liquid chromatography with electrochemical detection (HPLC-ED) system consisted of two solvent delivery pumps operating in the range of 0.001–9.999 $ml.min^{-1}$ (Model 582 ESA Inc., Chelmsford, MA), Zorbax Eclipse AAA Column (4.6 × 150 mm 3.5-μm particle size; Varian Inc., CA, USA) and a CoulArray electrochemical detector (Model 5600A, ESA, USA). The sample (30 μl) was injected using autosampler (Model 540 Microtiter HPLC, ESA, USA).

Statistical methods. The Shapiro–Wilk test was used to assess the normal distribution of parameters studied (Zar, 1999). Since non-normal distributions of parameters ($P < 0.05$) at some locations were identified, non-parametric tests were used. The relationship between MT, Cys, GSH and GSSG, and Cd liver content in chub, as well as between GSH and GSSG, was examined by means of Spearman rank correlation (Zar, 1999). The correlation coefficients were calculated separately for each location. The Spearman rank correlation was also used to determine the relationship between these four

Table 4. Correlations between GSH (μM) and GSSG (μM).

Sample site	N	r_s	P
Kníničky	14	–0.371	0.191
Bílovice nad Svitavou	15	–0.346	0.206
Svitava before junction	16	0.441	0.087
Svratka before junction	15	–0.025	0.930
Modřice	7	–0.500	0.253
Rajhradice	17	0.179	0.492
Židlochovice	15	–0.311	0.260

Table 5: Correlation of Cd (mg/kg) in fish and Cys (μM)

Sample site	N	r_s	P
Kníničky	14	–0.178	0.543
Bílovice nad Svitavou	15	0.021	0.940
Svitava before junction	16	0.162	0.549
Svratka before junction	15	0.093	0.742
Modřice	7	0.000	1.000
Rajhradice	16	0.447	0.083
Židlochovice	15	–0.414	0.125

markers with sediment Cd, Cu, Pb, Zn level and with metals in sediments. A single value for sum of metals and particular metal (Cd, Cu, Pb and Zn) in sediment was available from each site; therefore the median value of Cd liver content, MT, Cys, GSH and GSSG for each site was used in the analysis.

Data analyses were performed using Statistica software (StatSoft Inc., 2007).

RESULTS

Liver Cd content in fish varied from 16 to 99 μg/kg (median values), the lowest being at Modřice and highest at Bílovice nad Svitavou. Detailed information on liver Cd content, MT, GSH, GSSG and Cys in fish from different locations is recorded in the ***Tab. 3***. Correlation between liver Cd content and MT concentration, Cys and GSSG was not significant in fish from any location ($p > 0.05$ for all correlation coefficients). A negative correlation between Cd content and GSH was found at Židlochovice ($r_s = –0.539$, $p = 0.038$). The correlation of GSH with GSSG was negative in fish from five sites (***Tab. 4***.), although none were significant ($p > 0.05$). Cysteine correlated with Cd liver content negatively in fish from two sites ($p > 0.05$) (***Tab. 5***.).

Cadmium, Cu, Pb and Zn contents in sediment and MT concentration were negatively correlated (Cd, Cu, Pb: $p < 0.05$; Zn: $p > 0.05$) (***Tab. 6.***). Sum of metals (Cd, arsenic (As), barium (Ba), cobalt (Co), chrome (Cr), Cu, molybdenum (Mo), nickel (Ni), Pb, selenium (Se), Zn, aluminium (Al) and titanium (Ti)) content in sediments and MT concentration also showed significant negative correlation ($r_s = –0.857$; $p = 0.014$). The correlations of Cd as well as of sum of metals in sediment with liver Cd content, GSH, GSSG and Cys were not significant ($p > 0.05$ for all correlation coefficients) (***Tab. 6.***). Zinc in sediment correlated negatively with GSSG ($r_s = –0.857$; $p = 0.014$). The correlations of Cu and Pb with GSH, GSSG and Cys as well as the correlations of Zn with MT, Cys and GSH were not significant ($P > 0.05$ for these correlation coefficients).

Table 6: Correlation of sum of metals, and single metal (Cd, Cu, Pb, Zn) content in sediment and Cd liver content, MT, Cys, GSH, GSSG in fish.

	N	r_s	P
Σ metals (μg/kg) & Cd in fish	7	–0.286	0.535
Σ metals (μg/kg) & MT in fish	7	–0.857	0.014
Σ metals (μg/kg) & GSH in fish	7	0.250	0.589
Σ metals (μg/kg) & GSSG in fish	7	–0.536	0.215
Σ metals (μg/kg) & Cys in fish	7	0.143	0.760
Cd in sediment (μg/kg) & Cd in fish	7	–0.108	0.818
Cd in sediment (μg/kg) & MT in fish	7	–0.757	0.049
Cd in sediment (μg/kg) & GSH in fish	7	0.234	0.613
Cd in sediment (μg/kg) & GSSG in fish	7	–0.577	0.175
Cd in sediment (μg/kg) & Cys in fish	7	–0.108	0.818
Cu in sediment (μg/kg) & Cd in fish	7	–0.107	0.819
Cu in sediment (μg/kg) & MT in fish	7	–0.893	0.007
Cu in sediment (μg/kg) & GSH in fish	7	0.357	0.432
Cu in sediment (μg/kg) & GSSG in fish	7	–0.571	0.180
Cu in sediment (μg/kg) & Cys in fish	7	0.036	0.939
Pb in sediment (μg/kg) & Cd in fish	7	–0.179	0.702
Pb in sediment (μg/kg) & MT in fish	7	–0.821	0.023
Pb in sediment (μg/kg) & GSH in fish	7	0.286	0.535
Pb in sediment (μg/kg) & GSSG in fish	7	–0.643	0.119
Pb in sediment (μg/kg) & Cys in fish	7	0.107	0.819
Zn in sediment (μg/kg) & Cd in fish	7	–0.250	0.589
Zn in sediment (μg/kg) & MT in fish	7	–0.750	0.052
Zn in sediment (μg/kg) & GSH in fish	7	0.179	0.702
Zn in sediment (μg/kg) & GSSG in fish	7	–0.857	0.014
Zn in sediment (μg/kg) & Cys in fish	7	–0.429	0.337

Correlations among all sample values were irregular, as expected based on results of previous experimental studies.

DISCUSSION

Currently, aquatic ecosystems are monitored in long-term studies by measuring concentrations of biochemical markers such as cytochrom P450 (Havelkova *et al.* 2008), ethoxyresorufin-*O*-deethylase (Randak *et al.* 2009), vitellogenin or 1-hydroxypyrene (Blahova *et al.* 2008) in fish to assess environmental pollution by organic pollutants. The presence of metal-specific memory cells in the blood serve as biomarkers for metals in the human body (Stejskal *et al.* 2006), but are not suitable for use in fish for environmental monitoring. Several studies have concluded that MT and GSH increase after exposure to metals, particularly Cd (Thomas *et al.* 1982; Klavercamp & Duncan, 1987; Kägi & Schäffer, 1988; Olsson *et al.* 1998; Schlenk & Rice, 1998; Santovito *et al.* 2000; Kovarova *et al.* 2009). There is currently great interest in the use of specific biomarkers for analysis of environmental contamination, therefore the induction of MT (Santovito *et al.* 2000; Alvarado *et al.* 2005) as well as changes in GSH and GSSG values (Lange *et al.* 2002; Belcastro *et al.* 2009) after metal exposure is a potentially valuable tool for assessment of metals bioavailability and environmental impact. However, the results of our study indicated that employment of these biochemical indicators as markers of environmental metal pollution is problematic. This is in agreement with the statement by Lange *et al.* (2002), that the response of MT and GSH to metal exposure and the functional relationship between the two thiol pools in metal response is not understood.

In our study, overall GSH values were decreased according to GSSG, perhaps due to its variety of functions. Glutathione plays an important role in the intermediary metabolism in non-enzymatic or enzyme mediated reactions (Mason & Jenkins, 1995), in conjugation with various endogenous or exogenous compounds to produce more water soluble end products that may be easily excreted from the organism. Glutathione is active in the capture of free radicals and binding of toxic compounds, including metals. Maracine & Segner (1998) reported that this sulphydryl-rich tripeptide can interfere with toxic metals in various ways. Reduction of enzymatic –SH groups or binding of metals involves oxidation of these groups in glutathione and promotes the transformation of GSH to GSH disulphide (GSSG) (Jezierska & Witeska, 2001). Therefore, the GSH/GSSG ratio is often considered as an indicator of the intracellular redox state, with enhanced values of GSSG/GSH pointing to oxidative stress. Lange *et al.* (2002) found no significant changes in GSH/GSSG ratio in rainbow trout (*Oncorhynchus mykiss* W.) following exposure to Cd. In our field based study, GSSG values were higher than GSH in fish from all sites and correlation between GSH and GSSG was negative in five localities, although not significantly. This is in accordance with the suggestion of Maracine & Segner (1998) and Ionescu *et al.* (2006) that an indirect protective effect of GSH against metal toxicity may be the reduction of metal-induced oxidative stress.

Conversely, fish metallothioneins have attracted special attention in the past 10–15 years, and have been studied in detail mainly under experimental conditions (Smirnov *et al.* 2004). Results indicate that MT has two interrelated roles. First, it can provide a non-toxic reservoir of Cu and Zn for a number of potentially rate-limiting Cu and Zn metalloenzymes. Secondly, it can limit the nonspecific binding of (potentially) toxic metals within the cell and thereby reduce their ability for inducing metal toxicity and protect against reactive oxygen species (Olsson, 1993; Suzuki *et al.* 1993). Frequently, metals are in the environment presented in combinations, because of their concurrent release from mining activities or industrial uses, and, hence, the total MT value is not suitable for biomonitoring of a specific metal but it can assess the incidence of certain metal mixture presented in the moment at the sample site. From the data received from chemical monitoring we have determined metals which can notably influenced level of MT. It was Cd, Cu, Pb and Zn. Although Cu has stronger affinity to MT, our point of view were focused on Cd. This resolution we have done base on fact that bioavailability of Cu in the natural surface waters is low. Cu performs complexes with naturally occurring organic ligands and reducing Cu overall toxicity (Campbell, 1995). According to the affinity line it stands to reason that Cd played the most important role in the MT induction in our study. MT total values were elevated in fish liver in correlation to physiologically occurred levels (Kovarova *et al.* 2009), but as mentioned, it is not possible to come to a definite conclusion about the influence of Cd. From correlations between single metals (Cu, Cd, Pb, Zn) and MT value we have obtained unusual results. All relationships were negative. One possible reason explaining this reality is that too many factors influence level of MT. Total MT value is enhanced upon starvation, exertion, under stress caused by different factors in connection with increase of steroid hormones (Smirnov *et al.* 2004). It was also reported (De Boeck *et al.* 2003) that induction of MT protein concentration by heavy metal is species- and tissue-specific in three freshwater fishes: rainbow trout, common carp (*Cyprinus carpio* L.) and gibel carp. There are also differences in MT content in relation to age (Bonwick *et al.* 1991) and sex (Hylland *et al.* 1992a) of fish. The response of MT mRNA provided information about MT *de novo* synthesis (Lange *et al.* 2002; Bae *et al.* 2005; Woo *et al.* 2006) varied with the metal or metal mixture, target organ, exposure time, and concentration of heavy metal (Szebedinszky *et al.* 2001). MT transcript induction appeared only upon exposure to relatively high levels of Cd, not generally existing in the coastal

environment of the Mediterranean (Yudkovski *et al.* 2008) or in rivers of the Czech Republic. A Cd level of 0.49 μg/g wet weight of striped sea bream (*Lithognathus mormyrus* L.) liver is much closer to actual levels in fish from streams, and it is not associated with significant induced MT transcript levels. Significantly induced MT transcript was related to a minimum of 7.4 μg Cd/ g wet weight of liver (Yudkovski *et al.* 2008), 100–200–fold the levels of Cd determined in our study. On the other hand, induction of MT synthesis is not linearly related to Cd level in either the liver or kidney (Bae *et al.* 2005). Santovito *et al.* (2000) reported that hepatic MT and Cu, Cd and Zn concentrations correlated positively in a teleost containing hemoglobin (*Trematomus bernacchi* B.), whereas, in a haemoglobin-free icefish (*Chionodraco hamatus* L.) hepatic MT showed a positive correlation only with Cd. Some metals, for example Ni and Cr, negatively affected the levels of MT mRNA in Japanese medaka (*Oryzias latipes* T&Sch.), in contrast to Ag, Cd, Cu, and Zn (Woo *et al.* 2006). Lange *et al.* (2002) reported inconsistent results among the responses of the two thiol pools**,** GSH and MT, to Cd exposure. GSH and MT showed comparable sensitivity to the hepatic accumulation of Cd but Bae *et al.* (2005) observed that heavy metals including Cd, Hg and Pb are found to bind in preference to the high molecular weight proteins (HMWP) in gills as well as in liver and kidney.

Other factors also play important role in metal uptake, particularly parameters of ambient water such as pH (Wögrath & Psenner, 1995), salinity (Luoma & Rainbow, 2008) temperature (Köck *et al.* 1996), and concentrations of other metals, ions (Spry *et al.* 1991), and chemicals (Mason & Jenkins, 1995; McGeer *et al.* 2007). Fish characteristics such as species (Santovito *et al.* 2000), age (Olsvik *et al.* 2000), sex (Hylland *et al.* 1992b) and behaviour can also influence metal uptake. Santovito *et al.* (2000) reported that *C. hamatus* accumulated more Cd in liver in comparison to *T. bernacchi*. The finding was suggested to be related to the feeding habits of the two species – *T. bernacchi* is a bottom-dwelling benthic species, feeding on polychaetes, molluscs and epibenthic crustaceans, all species known to accumulate large amounts of metals, whereas, the mesopelagic *C. hamatus* feeds on small fish and krill and thus has significantly lower Cd contents. The sentinel fish used for our determination was chub, because it is commonly presented fish in Czech rivers, but it inhabits the water column, where, regardless of sediment, Cd and other metals levels are low.

CONCLUSION

The present study confirms the difficulty of using thiol compounds in fish to monitor the aquatic ecosystem with respect to metal pollution. Many factors influenced values of MT. Data on GSH and GSSG levels did not provide conclusive results. Little information is available on the response of GSH and MT patterns to metal combinations, which are unique and not simply the cumulative effects of exposure to single metals. We conclude that the chub is not a suitable fish species for metal monitoring because of its open water habitat. Recently, increased numbers of studies of thiol compounds have used crustaceans and other benthic invertebrates (Amiard *et al.* 2006; Won *et al.* 2008) for assessment of metal contamination, due to their permanent *in situ* benthic habitat and high absorption of metal. However chemical monitoring of aquatic sediments is technically and financially demanding, and there is a serious requirement for measuring specific biomarkers. Eliciting details about these compounds will require further studies with actual environmental concentrations.

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