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Concentrations of metallothionein in the bivalve molluscs *Anodonta spp.* and *Unio spp.* from Latvian lakes with different anthropogenic pressure

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Abstract. Concentrations of metallothioneins (MT) were analysed in bivalve molluscs *Anodonta spp*. and *Unio spp*. from 6 lakes and watercourses of Latvia with different anthropogenic pressure. The aim of the study was to find the relationship between MT in molluscs and concentrations of heavy metals in sediments. *Unio spp*. and *Anodonta spp*. showed no significant differences in the concentrations of MT. High concentrations of heavy metals (Cd, Pb, Hg, Cu, Zn) were detected in the deepest parts of polluted water bodies, however their concentrations significantly decreased in near shore sediments inhabited by molluscs. Only Zn tends to accumulate in the molluscs, reaching 2-7 times higher concentrations in organisms than in sediments. There was statistically significant correlation between MT and Hg due to high affinity of Hg to MT-complex. Concentrations of Hg and MT in molluscs were related also to condition index of molluscs, suggesting that accumulation of heavy metals in molluscs depends on their physiological status.

Key words: biomarkers, metallothionein, molluscs, lakes, north-eastern Europe

Introduction

Metallothioneins (MT) are non-enzymatic proteins with a low molecular weight, high cysteine content, able to bind particular heavy metals. It is generally considered that these proteins play a role in the homeostatic control of essential metals (Cu, Zn) as they can act as essential metal stores ready to fulfil enzymatic and other metabolic demands (Brouwer et al., 1989; Viarengo and Nott, 1993; Roesijadi, 1996). However, MT can bind also non-essential metals like Cd and Hg thus displacing normally MT-associated essential metals (Amiard et al., 2006). Neosynthesis of MT in the presence of heavy metals suggests the potential use of MT concentration in organisms as biomarker of metal exposure.

Lakes of Latvia can be characterized as relatively shallow (average 5-9m), soft bottom water-bodies with thick layer of sediments, particularly in the deeper parts, able to accumulate heavy metals. Freshwater monitoring programs in Latvia involved the analysis of heavy metals in sediments but there never was an attempt to monitor the heavy metals or other hazardous substances in aquatic organisms or to quantify the possible biological effects of pollutants.

Bivalve molluscs are universally recognized as appropriate organism for quantification of biological effects of environmental pollution due to more or less sedentary lifestyle, high population densities and filter feeding capacities (Rainbow and Phillips, 1993). The most widely distributed bivalves in Latvian lakes are from *Anodonta* and *Unio* genera's (*A.anatina, A.cygnea, U.tumidus,* and *U.pictorum*). There are lakes where representatives of both genera are living together while others are occupied by single species. The aim of the study was to estimate the influence of heavy metals on the concentrations of MT in the *Anodonta spp*. and *Unio spp*., in order to develop the biological tool for the assessment of freshwater quality.

Materials and Methods

Molluscs were sampled in the water bodies with different anthropogenic pressure: clean, unpolluted lakes-Lizdoles, M.Baltezers and Juveris and water bodies with known pollution of heavy metals- Kisezers, Sarkandaugava, Hapaks during 2010-2011, 4 or 5 times per season at 1m depth. Molluscs were immediately dissected, foot and digestive gland placed in liquid nitrogen for further analysis.

The analysis of metallothioneins was carried out according to Viarengo et al. (1997). In short, the tissues were homogenized 1:3 (w/v) in reducing conditions (0.05 M sucrose TRIS buffer, pH 8.6, containing 0.01% β - mercaptoethanol). The homogenates were centrifuged at 30,000g at 4ºC for 20 min. The resulting supernatants were collected and ethanol/chloroform fractionation was used to obtain a partially purified metalloprotein fraction. Concentration of MT was measured by spectrophotometric determination of -SH groups using Ellman's reagent (DTNB) with GSH as a standard.

For analysis of heavy metals whole molluscs were freeze-dried and homogenized using IKA analytic mill A11 basic. Aliquots of about 500mg dried sample material were digested using a CEM high- pressure microwave reaction system MARS 5 according to EPA method 3051A. Lead, cadmium and arsenic concentrations were determined using a Varian SpectrAA -880Z atomic absorption spectrometer equipped with GTA-100 Zeeman graphite tube atomizer. Zinc, copper and nickel were determined by flame atomic absorption on a Varian SpectrAA -880. Mercury was determined with cold vapour atomic absorption spectroscopy using a Varian SpectrAA -880 equipped with vapour generation accessory VGA-77.

The sediment samples were freeze-dried and size fractioned using Retch sieve shaker. Aliquots of 1g were used for analysis as described above. Sediments and molluscs for analysis of heavy metals were collected in September 2011.

Results and Disscussion

Concentrations of MT in molluscs

During the first visit to the lakes we found that all water bodies, except Sarkandaugava, contained rich populations of bivalve molluscs from *Unio* and *Anodonta* genera's. Watercourses Sarkandaugava and Hapaks were inhabited only by *Unio spp*., while the lakes had representatives from both genera's. Comparison of MT concentrations between *Anodonta spp*. and *Unio spp*. showed no significant differences (on average 196.92 ± 48.63 and 205.79 ± 46.06 µg/g ww; ANOVA, p>0.05) in lakes Kisezers, M.Baltezers, Lizdole and Juveris. Like other researchers (Falfushynska et al., 2010; Leiniö and Lehtonen, 2005; etc.) we observed seasonal fluctuations of MT in most of the water bodies, however we could not detect similar trends for all sites. Polluted sites- Sarkandaugava, Hapaks and Kisezers showed the highest values of MT in spring (229.8, 227.9 and 282.7µg/g ww; Fig.1), suggesting that accumulation of harmful substances during the winter could influence the concentrations of MT in molluscs. The opposite trend was detected in the reference sites (at least without known pollution) - the higher MT values were registered in September - October (on average 193.1-263.8 µg/g ww; Fig.1). This could be explained by the increase of recreational activities at the end of summer. However comparing the MT concentrations at polluted sites and reference sites we could not detect the differences. On the contrast- MT concentrations at reference sites were on average higher than at polluted sites. The most polluted reference site M.Baltezers showed the lowest while the cleanest from reference sites Juveris showed the highest MT concentrations. There were small interannual variations of MT, but the differences were not statistically significant (ANOVA, p>0.05).

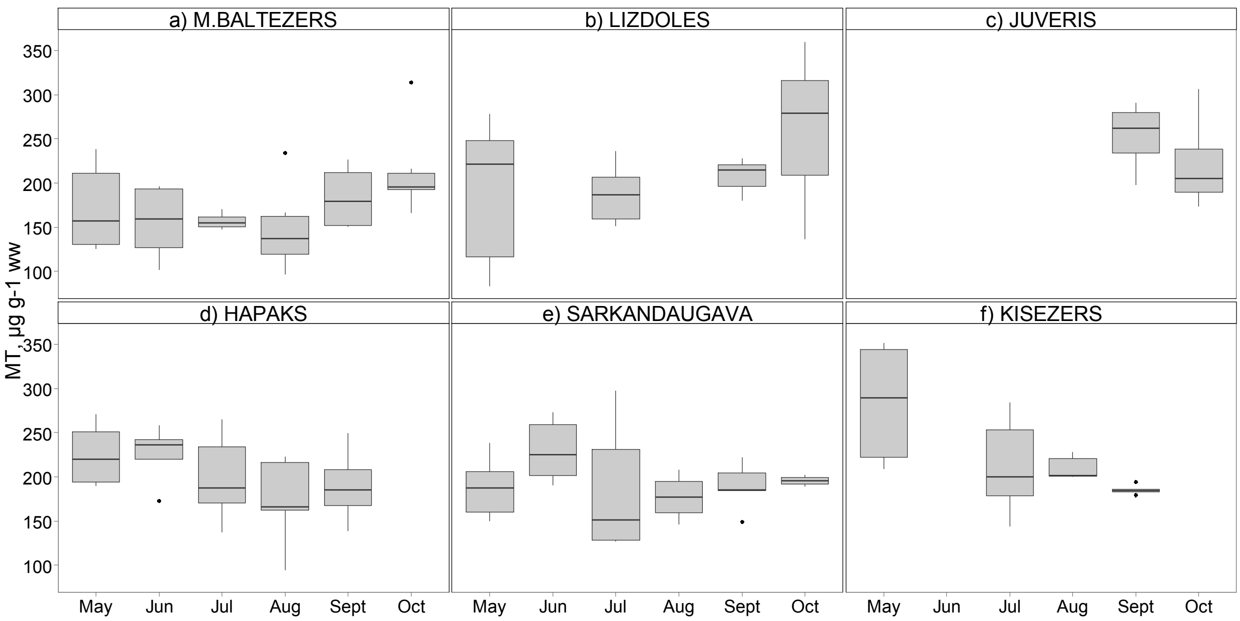

Fig.1 Concentrations of metallothioneins in the unpolluted (a,b,c) and polluted (d,e,f) lakes.

Concentrations of heavy metals in molluscs and sediments

Molluscs are universally acknowledged as organisms able to accumulate heavy metals (Amiard et al., 2006; Baudrimont et al., 2003; Falfushynska et al., 2010, Viarengo et al., 1999). However comparing the concentrations of heavy metals in the sediments and molluscs we found that only Zn tend to bio accumulate in the molluscs (Fig.2) reaching 2-7 times higher concentrations in organisms than in sediments. In few cases a small accumulation of Cu and Cd was observed but in most cases much higher concentrations of heavy metals could be found in the sediments than in the molluscs. The same tendency was found also for Pb. From our dataset we could detect the statistically significant correlation only between concentrations of MT and Hg (r=0.526, n=19) what could be explained by the high affinity of Hg to MT-complex (Hg^{2+} >Cu^{+}, Ag^{+}, Bi^{3+}>>Cd^{2+} >Pb^{2+} >Zn^{2+} >Co^{2+}; Viarengo et al., 1999; Amiard et al., 2006). Simultaneously, concentrations of Hg and MT in molluscs were related to condition index of molluscs (r=0.903, n=19), suggesting that accumulation of heavy metals in molluscs depends on their physiological status, probably- healthier animal accumulates more heavy metals.

Initially the water bodies for present study were chosen according to data on the accumulation of heavy metals in deep water sediments. This could be called "an inherited pollution" while nova-days the concentrations of heavy metals in water of all polluted sites are close to 0, except in Sarkandaugava where Hg is slightly exceeding the MAC (Maximum Allowable Concentration) for water (0.05µg/l). However the molluscs *Anodonta spp*. and *Unio spp*. are living only in the near shore area until 2m depth where concentrations of heavy metals in sediments are 2-13 times lower than in deeper parts (Fig.2). This can be explained by the different granulometric characteristics of deepwater sediments and near shore sediments. Deepwater sediments tend to have more fine size (<63µm) particles able to absorb heavy metals, than near shore sediments. Thus we conclude that molluscs do not suffer from the heavy metals sequestered in deeper parts of water bodies unless there are any actual point sources of heavy metals. This seems to be true also for clean lakes, for example, Juveris which is recognized as a reference lake for WFD from point of view of phytoplankton, macrophytes and zoobenthos. It has limited access from the roads and the only road leads to a private country estate at which the molluscs were collected. However even the private country estate can be a significant point source of pollution what is reflected in the high concentrations of heavy metals in the molluscs and consequently high concentrations of MT.

Conclusions

We conclude a) that *Unio spp*. and *Anodonta spp*. are similar in their ability to synthesize MT in response to environmental factors and representatives of both genera can be used for assessment of environmental quality; b) in this study molluscs from moderately exposed lake M.Baltezers showed lower concentrations of MT and heavy metals than molluscs from clean reference lakes, suggesting that physiological adaptations to clean environment makes its inhabitants more prone to accumulate heavy metals when available; c) care should be taken when collecting the molluscs from reference sites, for even the single private house can be a

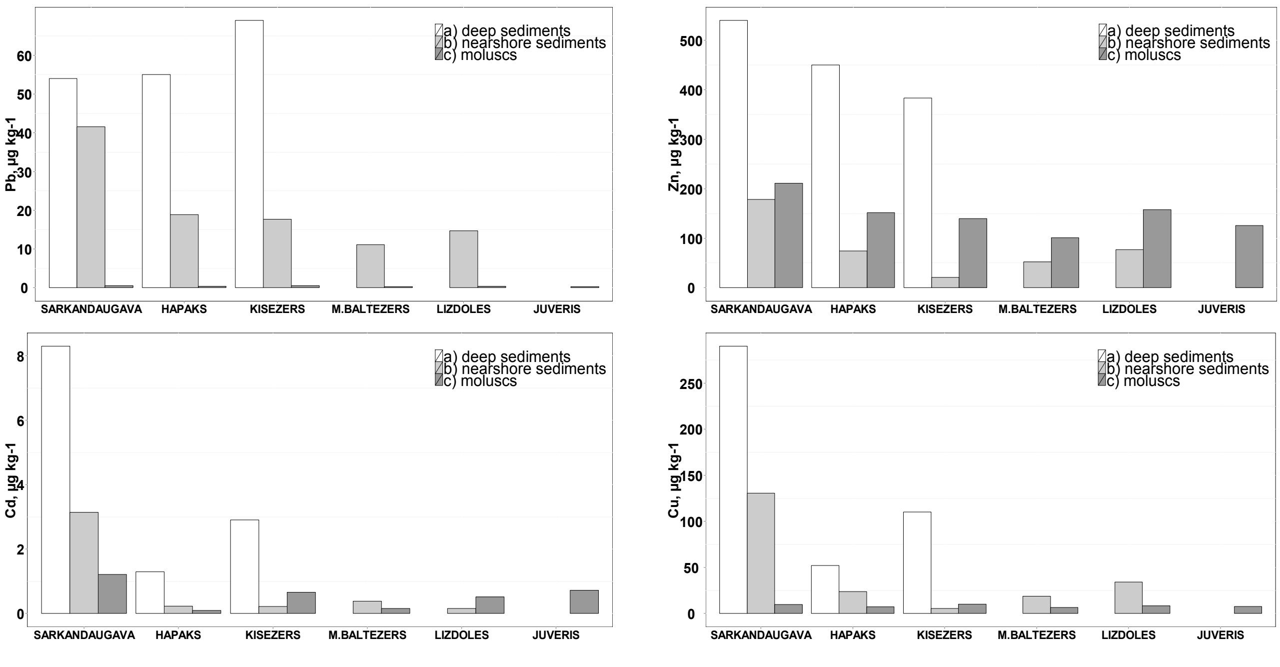

Fig. 2. Concentrations of heavy metals in Latvian waterbodies. In lakes M.Baltezers, Lizdoles, Juveris deep water sediments were not analysed. Heavy metals in the water were under detection limit.

significant point source of pollution.

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