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Intracellular distribution of trace metals and metalloids in the digestive gland of freshwater bivalves from genus *Anodonta*

Toxic effects of metals depend not only on their total cellular concentrations, but also on their distribution within the cell ¹. The aim of this study was to compare the intracellular distribution of nine selected metals and metalloids (essential: Cu, Zn, Fe, Mo and Se; nonessential: Cd, Ag, Pb and As) in the digestive gland (DG) of freshwater bivalves *Anodonta exulcerata* from the Visovac Lake and *Anodonta anatina* from the Brljan Lake, both located in the Krka Nacional Park, protected area of the karst Krka River in Croatia. Upstream from this protected area there is a potential source of anthropogenic contamination via municipal and industrial wastewater effluents from the town of Knin. The freshwater bivalves are often applied as bioindicators of environmental contamination in the lacustrine ecosystems. The digestive gland of bivalves is a main organ for metal storage and detoxification, and therefore widely used as a target organ in biomonitoring studies ².

Metal/metalloid contents in the cytosolic tissue fraction as well as the metal/metalloid distribution among cytosolic biomolecules were assessed. Cytosolic metal/metalloid concentrations were determined by high resolution inductively coupled plasma mass spectrometry (HR ICP-MS). The separation of cytosolic biomolecules was performed by size exclusion high performance liquid chromatography (SEC-HPLC) on Superdex 200 10/300 GL column, and subsequent metal/metalloid analyses were performed off-line by HR ICP-MS.

Cytosolic concentrations of Cu, Fe, Mo, Ag and Pb were higher in DG of bivalve *A. anatina* from the Brljan Lake, while cytosolic Cd was higher in DG of *A. exulcerata* from the Visovac Lake. Analysis of the metal distribution among cytosolic biomolecules allowed the definition of molecular masses of biomolecules involved in binding of particular metals. Resulting metal/metalloid profiles were the same in both studied bivalve species for all studied elements at different levels of metal accumulation. Exception was chromatographic profile of Pb with one additional distinctive peak for DG of *A. anatina* with increased cytosolic Pb from the Brljan Lake. The results of this study represent the first data on cytosolic metal/metalloid concentrations and their intracellular distribution in the DG of bivalves from genus *Anodonta* in the Visovac and Brljan Lakes and as such can serve as a starting point for comparison in future studies of metal contamination and conservation of these species.

KEY WORDS: trace metals/metalloids, subcellular distribution, freshwater bivalves, the Visovac Lake, the Brljan Lake

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