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Cytosolic distributions of highly toxic metals Cd and Tl and several essential elements in the liver of brown trout (*Salmo trutta* L.) analyzed by size exclusion chromatography and inductively coupled plasma mass spectrometry

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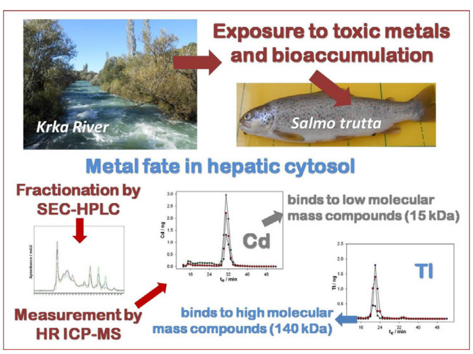
GRAPHICAL ABSTRACT



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ABSTRACT

Cytosolic distributions of nonessential metals Cd and Tl and seven essential elements among compounds of different molecular masses were studied in the liver of brown trout (*Salmo trutta*) from the karstic Krka River in Croatia. Analyses were done by size exclusion high performance liquid chromatography and high resolution inductively coupled plasma mass spectrometry. Common feature of Cd and Tl, as highly toxic elements, was their distribution within only two narrow peaks. The increase of cytosolic Cd concentrations was reflected in marked increase of Cd elution within low molecular mass peak (maximum at ~15 kDa), presumably containing metallothioneins (MTs), which indicated successful Cd detoxification in brown trout liver under studied exposure conditions. Contrary, the increase of cytosolic Tl concentrations was reflected in marked increase of Tl elution within high molecular mass peak (maximum at 140 kDa), which probably indicated incomplete Tl detoxification. Common feature of the majority of studied essential elements was their distribution within more peaks, often broad and not well resolved, which is consistent with their numerous physiological functions. Among observed associations of essential metals/nonmetal to proteins, the following could be singled out: Cu and Zn association to MTs, Fe association to storage protein ferritin, and Se association to compounds of very low molecular masses (<5 kDa). The obtained results present the first step towards identification of metal-binding compounds

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in hepatic cytosol of brown trout, and thus a significant contribution to better understanding of metal fate in the liver of that important bioindicator species.

1. Introduction

The metal pollution can affect every stage of the aquatic food chain, leading to the disturbance of the whole ecosystem (Van Campenhout et al., 2010). In highly contaminated aquatic environment, fish can accumulate metals both from surrounding water and from food (Dragun et al., 2012, 2016; Filipović Marijić and Raspor, 2012; Rajeshkumar et al., 2018). It can result with high level of metal bioaccumulation in their organs and possible development of toxic effects (Dragun et al., 2013; Qu et al., 2014). However, although the concentrations of trace metals in organs of aquatic animals are often used to assess their exposure to metals in aquatic systems (Luoma and Rainbow, 2008), such information is not sufficient to estimate if bioaccumulated quantity of metal would cause damage to exposed fish. Metal toxicity arises predominantly from the binding of metals to essential biomolecules such as enzymes and transporters and the involvement of certain metals in the formation of radicals (Mason and Jenkins, 1995), but part of metal bioaccumulated within the organism can also be detoxified. Therefore, next to basic studies on the concentrations of metals bioaccumulated in fish organs, it is essential to further investigate the fate of those metals in the cells, and to determine whether they are more likely to cause harm to the fish or to be detoxified and excreted from fish organism. In the scientific field which deals with the comprehensive analysis of the entirety of metal and metalloid species within a cell or tissue (Szpunar, 2005), it is a common first step to use the combination of different techniques of high performance liquid chromatography (HPLC) and inductively coupled plasma mass spectrometry (ICP-MS) as a powerful tool for recognizing the cytosolic ligands that bind specific elements (Goenaga Infante et al., 2006; Santiago-Rivas et al., 2007).

So far, there were only few studies of such kind performed on fish. Several investigations were, for example, directed to study of metal detoxification by metallothioneins (MTs) in different fish organs by application of size exclusion HPLC (SEC-HPLC) and ICP-MS in field populations of the European eel (*Anguilla anguilla*) (Van Campenhout et al., 2008) and gibel carp (*Carassius auratus gibelio*) (Van Campenhout et al., 2010). Caron et al. (2018) applied similar methodology to perform somewhat more extensive study on liver of juvenile yellow perch (*Perca flavescens*) regarding binding of several trace elements (Ag, Cd, Co, Cu, Ni, and Tl) to various cytosolic biomolecules. Our previous studies in this field included comprehensive investigation of cytosolic distributions of Cd, Co, Cu, Fe, Mn, Mo, Pb, Se, and Zn in the liver and gills of European chub (*Squalius cephalus*) from the moderately contaminated Sutla River in Croatia (Krasnići et al., 2013, 2014) and of Vardar chub (*Squalius vardarensis*) from highly contaminated Macedonian rivers (Krasnići et al., 2018).

In this study, our main aim was to identify the molecular masses of cytosolic biomolecules that bind specific trace elements in the liver of brown trout (*Salmo trutta* Linnaeus, 1758), as a representative fish species and important bioindicator of karstic rivers. We have previously characterized the water quality of the Krka River, at the locations where brown trout were sampled (Filipović Marijić et al., 2018), as well as total metal bioaccumulation in the liver of the same brown trout specimens that were used in this study (Dragun et al., 2018). The specific goals of the investigation presented in this paper included application of SEC-HPLC in combination with offline metal measurement on high resolution ICP-MS (HR ICP-MS) to separate hepatic cytosols of brown trout into fractions that contain various metal-binding biomolecules, and to define the distribution profiles among cytosolic biomolecules of different molecular masses for nine selected elements, two highly toxic metals (Cd and Tl) and seven essential elements (Co, Cu, Fe, Mn, Mo, Se, and Zn). As it is very likely that various fish species have different metal handling strategies, the additional aim of this study was to compare metal distribution profiles characteristic for brown trout liver with previously published profiles for liver of European chub (Krasnići et al., 2013) and Vardar chub (Krasnići et al., 2018), and to recognize and describe the differences between them, which could indicate different susceptibility to metal exposure in these distinct fish species.

2. Materials and methods

2.1. Study period and area

This study was a part of the comprehensive pollution study on the Croatian karstic river Krka, performed within two sampling campaigns in October 2015 and May 2016. The first results of that study, regarding water quality (Filipović Marijić et al., 2018) and metal bioaccumulation in the fish liver (Dragun et al., 2018) have been already published. The map of the sampling area, comprising of two sampling sites, was also previously published by Dragun et al. (2018). As a reference site we have chosen the Krka River source, whereas a location downstream of Knin town was chosen as a potentially contaminated site, due to known pollution sources in Knin area (e.g., industrial wastewater of screw factory and untreated municipal wastewater discharge; Filipović Marijić et al., 2018). The analyses of dissolved metals in the river water conducted in the course of this study, simultaneously with fish sampling, confirmed a slight concentration increase of several trace elements (e.g. Al, As, Co, Fe, Mn, Mo, Rb, Sr, V, and Zn) downstream of Knin town (Dragun et al., 2018; Filipović Marijić et al., 2018). The other physico-chemical parameters of the river-water, such as pH and total dissolved solids (TDS), were comparable at both sites in both seasons (Krka River source: pH 7.6–7.7, TDS ~180 mg L^{-1}; Krka Knin: pH 7.8–8.1, TDS 201–208 mg L^{-1}), and were not further considered as the significant factors in this study. The values of pH and TDS were determined *in situ* using a multiparameter water quality monitoring instruments SevenGo pro (Mettler Toledo).

2.2. Fish sampling and organ dissection

The selected bioindicator for this study was fish species brown trout (*Salmo trutta* Linnaeus, 1758). Fish samplings were carried out by electrofishing, in accordance with the Croatian standard HRN EN 14011 (2005), as described by Dragun et al. (2018). The captured fish were kept alive in aerated water tank during transportation, and in the laboratory they were anesthetized with tricaine methane sulphonate (MS 222, Sigma Aldrich) before they were sacrificed. Thereafter, we have recorded fish total masses and lengths, then isolated and weighed the liver and the gonads, and