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Total and cytosolic concentrations of twenty metals/metalloids in the liver of brown trout *Salmo trutta* (Linnaeus, 1758) from the karstic Croatian river Krka

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A B S T R A C T

Total and cytosolic concentrations of twenty metals/metalloids in the liver of brown trout *Salmo trutta* (Linnaeus, 1758) were studied in the period from April 2015 to May 2016 at two sampling sites on Croatian river Krka, to establish if river water contamination with metals/metalloids downstream of Knin town has influenced metal bioaccumulation in *S. trutta* liver. Differences were observed between two sites, with higher concentrations of several elements (Ag, As, Ca, Co, Na, Se, Sr, V) found downstream of Knin town, whereas few others (Cd, Cs, Mo, Tl) were, unexpectedly, increased at the Krka River spring. However, total metal/metalloid concentrations in the liver of *S. trutta* from both sites of the Krka River were still mainly below previously reported levels for pristine freshwaters worldwide. The analysis of seasonal changes of metal/metalloid concentrations in *S. trutta* liver and their association with fish sex and size mostly indicated their independence of fish physiology, making them good indicators of water contamination and exposure level. Metal/metalloid concentrations in the metabolically available hepatic cytosolic fractions reported in this study are the first data of that kind for *S. trutta* liver, and the majority of analyzed elements were present in the cytosol in the quantity higher than 50% of their total concentrations, thus indicating their possible availability for toxic effects. However, the special attention should be directed to As, Cd, Cs, and Tl, which under the conditions of increased exposure tended to accumulate more within the cytosol. Although metal/metalloid concentrations in *S. trutta* liver were still rather low, monitoring of the Krka River water quality and of the health status of its biota is essential due to a trend of higher metal/metalloid bioaccumulation downstream of Knin town, especially taking into consideration the proximity of National Park Krka and the need for its conservation.

1. Introduction

One of the major problems of aquatic systems in the world is their ever-growing contamination originating from different types of anthropogenic activities. Among many types of contaminants, metals/metalloids occupy an important place in the environmental studies. Once introduced in an aquatic system, metals/metalloids are redistributed in the water column between the particulate and dissolved phase, deposited in sediment and accumulated in the organs of various aquatic organisms, including fish, through water filtration, diet or skin absorption (Fichet et al., 1998; Kraemer et al., 2006). Such metal accumulation may leave fish populations at an increased risk of experiencing toxicity (Kraemer et al., 2006), because it has been shown to cause metabolic alterations and disturbances of biological systems (van der Oost et al., 2003).

Since total quantity of metals present in the aquatic environment is not completely bioavailable, one of the most effective ways to evaluate their potential impacts on aquatic biota is to monitor metal concentrations accumulated in an adequate and representative bioindicator organism (Kraemer et al., 2006). Brown trout *Salmo trutta* (Linnaeus, 1758) is widely present in freshwater systems in Europe and around the world. It can be found both in clean and in polluted areas and thus it

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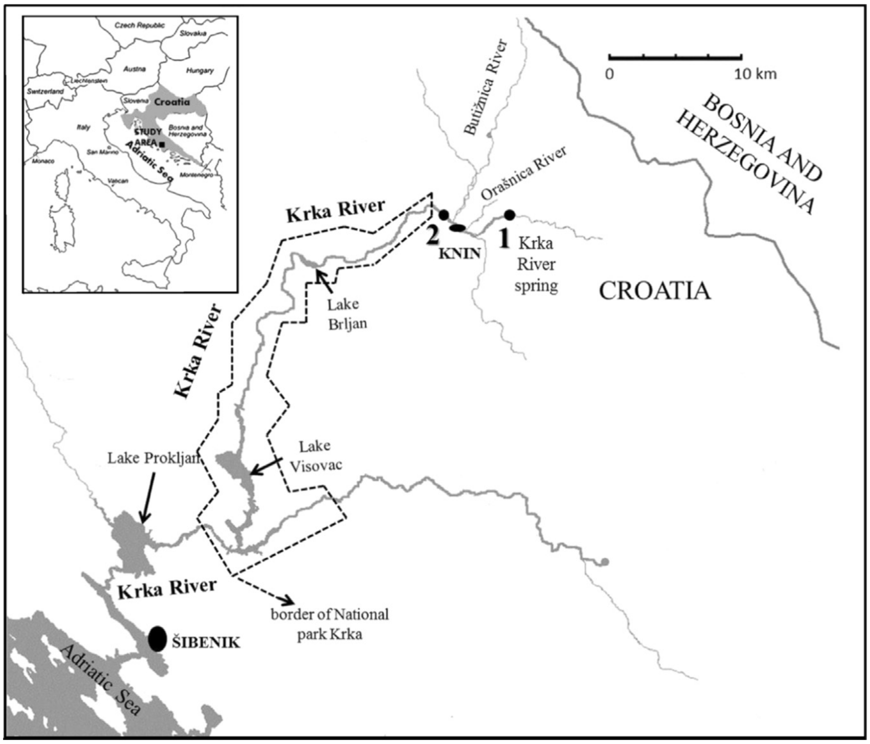

Fig. 1. Study area with marked sampling sites on the Krka River (1 – Krka River spring; 2 – Krka River downstream of Knin town), and marked position of Croatia within Europe.

represents a good species for biomonitoring (Culioli et al., 2009). For example, this species has already been proven as useful bioindicator organism for arsenic accumulation (Culioli et al., 2009). Moreover, *S. trutta* is a part of the human diet, and therefore, their contamination is also a matter of concern for human health (Culioli et al., 2009).

Monitoring of metal/metalloid accumulation in bioindicators is usually carried out by measuring their total concentrations in relevant target organs. Liver and kidney are considered to be the best indicator organs for evaluating long term, chronic exposure to metals (Miller et al., 1992). This is especially true for liver, because it is the main site for metal metabolism and detoxification (Linde et al., 1998), and also has the most effective accumulation ability (Sindayigaya et al., 1994; Papagiannis et al., 2004; Vukosav et al., 2014). As a defence mechanism, hepatocytes, the main cell type in the liver, are equipped with high levels of intracellular binding proteins and peptides, which aid in the metal/metalloid sequestration, thus preventing their interaction with potentially sensitive sites (Di Giulio and Hinton, 2008; Sigel et al., 2009).

Therefore, in addition to measuring total accumulated metal/metalloid concentrations in fish liver, useful information about how aquatic organisms deal with both essential and non-essential metals can be obtained by determining metal/metalloid concentrations at the subcellular level (Barst et al., 2016). After entering the organism, trace metals usually undergo a series of metabolic processes and are subsequently incorporated into various cellular components (Mason and Jenkins, 1995; Wang and Rainbow, 2005; Goto and Wallace, 2010). They might be bound by a variety of biomolecules for metabolic function, storage, detoxification, toxicity, or excretion (Klaassen et al., 1999; Rainbow, 2002). Some metals are sequestered by metal-binding proteins (e.g., metallothioneins) or granular concretions in detoxified forms (Langston et al., 1998; Goto and Wallace, 2007). The others may be incorporated into non-detoxifying cellular components (e.g., enzymes and organelles), which could ultimately result in toxicological effects at various levels of biological organization (Wallace et al., 2003; Sigel et al., 2009; Goto and Wallace, 2010).

This study was performed on *S. trutta* from the Croatian river Krka. The Krka River is a natural karst phenomenon, and a large part of its watercourse was proclaimed a national park in 1985 (web 1). An increase in trace metal concentrations in the upper flow region, as the result of the untreated municipal and industrial waste-water discharge downstream of Knin town (Cukrov et al., 2008), presents a potential threat for its conservation, especially considering that the northern border of National Park Krka is situated only 2 km downstream of Knin town. Although *S. trutta*, which is a representative species in the Krka River, is fish widely used as a bioindicator organism for monitoring metals in freshwater ecosystems, there is only limited number of elements that have been monitored in its organs. For example, so far there is only information on Al, As, Cd, Co, Cu, Se and Zn concentrations in trout liver from different parts of the world (Karlsson-Norrgren et al., 1986; Brotheridge et al., 1998; Linde et al., 1998; Olsvik et al., 2000; Dussault et al., 2004; Vítek et al., 2007; Arribére et al., 2008; Has-Schön et al., 2008; Foata et al., 2009; Can et al., 2012; Herrmann et al., 2016). With the general aim to broaden the existing data pool on metal/metalloid levels in *S. trutta* organs which could be used in the future monitoring as the basis for comparison, we have measured total and cytosolic concentrations of twenty elements (Ag, Al, As, Ca, Cd, Co, Cs, Cu, Fe, K, Mg, Mn, Mo, Na, Rb, Se, Sr, Tl, V, Zn) in the liver of *S. trutta*. Our specific goal was to compare those concentrations at two sampling sites of the Krka River, the Krka River spring as a reference site and the location downstream of Knin town as a contaminated site. We wanted to determine if contamination of the river water have influenced metal/metalloid accumulation in *S. trutta* liver. Since the relationship between metal/metalloid concentrations and several intrinsic factors of the fish can present a confounding factor when using aquatic animals as biomonitors of metal pollution (Linde et al., 1998), we have also tested the seasonal changes of metal/metalloid concentrations in *S. trutta* liver, as well as their association with *S. trutta* sex and size. Additionally, with the aim to assess metabolically available and potentially toxic fractions of metals/metalloids in *S. trutta* liver, we have calculated the proportions of each metal/metalloid present in the cytosolic hepatic fractions, which contain heat-stable and heat-sensitive biomolecules, lysosomes and microsomes (Bonneris et al., 2005; Dragun et al., 2013a). Finally, our overall aim was to evaluate, based on the all gathered information within this study, the current quality status of the Krka River, and the