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Effects of mine tailing and mixed contamination on metals, trace elements accumulation and histopathology of the chub (*Squalius cephalus*) tissues: Evidence from three differently contaminated sites in Serbia

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ABSTRACT

Chub (*Squalius cephalus*) specimens were collected in Korenita River seven months after spillover from the waste water of antimony mine tailing pond and compared with chub living in Kruščica reservoir (intended for water supply) and Međuvršje reservoir (influenced by intense emission of industrial, urban and rural wastewater). Concentrations of 15 elements (Al, As, B, Ba, Cd, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Si, Sr, Zn) were determined in muscle, liver and gills of chub by inductively coupled plasma optical spectrometry (ICP-OES) and histopathological alterations in liver and gills were assessed. Chub specimens from Korenita River had higher concentrations of As, Ba and Pb in all three investigated tissues as well as higher total histopathological index values than chub from reservoirs. Specimens from Međuvršje reservoir were characterized by higher values for concentrations of Cu and Si in muscle tissue and higher values for regressive histopathological alterations in gills. Individuals of chub from Kruščica reservoir had the highest concentrations of Fe in liver, Hg in muscle and Sr and Zn in muscle while gills had the lowest value of total histopathological index. The results from the present study showed higher level of histopathological alterations as a result of mine tailing accident. As a result of mixed contamination on the Međuvršje site, histopathological index values of gills were in line with the index value from Korenita River. Increased values for Fe and Sr in chub tissue from Kruščica reservoir could be explained by geological structure of the site which is characterized by magmatic rock rich in Cu, Fe and Ni as well as dominant carbonate sediment complex of marine origin with increased level of Sr.

1. Introduction

During a four days period in May 2014 Balkan Peninsula was hit with cyclone locally named "Tamara" and internationally named "Yvette", causing heavy rainfall, above four times the norm in the area (LeComte, 2015), with highest values in 120 years since meteorological data are monitored in Serbia (Stadtherr et al., 2016). The highest level of precipitation of 213 mm in the period of three days was recorded in the town of Loznica in Western Serbia, on the border with Bosnia and Herzegovina (Tošić et al., 2017). Rainfall caused severe flooding events which subsequently led to accidents in two abandoned mining sites "Stolice" and "Zajača", near the city of Loznica. Around 100.000 m^3 of tailing slurry rich in arsenic, antimony, barium, zinc, and lead was released in the nearby stream leading to Korenita River (Vidojević et al., 2015). These types of accidents occur seldom, but usually cause devastating effect on wildlife inhabiting surrounding area (Gómez et al., 2004; Grimalt et al., 1999; Sabater, 2000).

In order to detect possible effects of such accident we compared bioaccumulation of metals and trace elements and histopathological changes in the tissues of fish as indicator organism from this and two other locations with different type and level of pollution. One was a less impacted reservoir Kruščica located within the Tara National Park in Serbia, while the other one is the eutrophic Međuvršje reservoir, a site exposed to substantial organic nutrients and heavy metals, both

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originated from anthropogenic sources (Devic et al., 2014; Lenhardt et al., 2009; Sakan et al., 2011). The species of interest in this study was the chub (*Squalius cephalus*), a widespread and the most abundant species of Serbian ichthyofauna southern of the Danube and the Sava Rivers. The chub has been used as indicator species in several field studies assessing the environmental quality of inland waters differently impacted by anthropogenic activities (Krča et al., 2007; Pavlica et al., 2011; Randák et al., 2006; Sunjog et al., 2014, 2016; Winter et al., 2005). It is a benthopelagic and omnivore species, even though adults usually prey other fish. A selected biomarker for this environmental study was histopathology of the gills and the liver. These two organs were chosen for assessment due to their vital role in fish metabolism and the impact caused by metals and trace elements (Ahmed et al., 2013; De Boeck et al., 2007; Zheng et al., 2011). Histopathology gives an insight to microscopic anatomy of vital organs and quantification of histopathological alterations represents one of the methods to determine fish health status (Ballesteros et al., 2017; Kostić et al., 2017; Kumar et al., 2017; Rašković et al., 2016; Savassi et al., 2016). Moreover, a combination of analytical and biological methods provides a powerful tool as methods of multivariate statistics can be used to determine relationships between cause and effect (Fonseca et al., 2017; Gerber et al., 2017; Giltrap et al., 2017; Lenhardt et al., 2015). In contrast to molecular or population studies, histopathology lies in the middle of the order of responses to pollutant stress within fish species (Adams, 1990; Adams et al., 2000; van der Oost et al., 2003) and is a common biomarker in the studies of polluted environment. Apart from histopathological assessment, in several environmental studies condition factor (coefficient) was chosen as a biomarker (Bervoets and Blust, 2003; Linde-Arias et al., 2008; Lohner et al., 2001). This gross morphological index is beneficial as simple, non-invasive and fast screening tool, although it is not very sensitive and other factors, such as nutritional level or disease, could also influence the obtained results, other than the level of environmental pollution (van der Oost et al., 2003).

The aim of this study was to assess bio-accumulation of metals and trace elements as well as histopathological changes in the tissues of chub from the above mentioned mine tailing polluted site and compare it with fish living in two other sites with different level of metal pollution.

2. Materials and methods

2.1. Study area

The Chub specimens were collected between December 2014 and July 2015, on three localities on the territory of the Western Serbia: Kruščica reservoir (N 43°54″13′, E 19°23″04′), Međuvršje reservoir (N 43°54′43.07″, 20 °14′12.71″) and Korenita River (Fig. 1).

Samples were collected from the Korenita River seven months after spillover from the waste water of Zajača antimony mine tailing pond, with the assumption that fish populations from these rivers were exposed to toxic elements contained in this tailing pond. Remediation of the ground started in June 2016, after the present study was finished. Alluvium of Korenita River is feeding from four geological complexes: Carboniferous limestone, Triassic complex of limestone, dolomite, siliceous rocks, clastite and volcanic rocks, Tertiary volcanic rocks (quartz latite, dacite-andesite) and Miocene lacustrine sediments (clay, marl, tuffs, sand, and gravel). Antimony sulfide mineralization is connected with volcanic rocks on relatively big area, which is accompanied by the presence of microelements, such as Pb, Hg and As (chalcophile elements). As a consequence, concentrations of Sb, As, Pb and Hg are naturally increased in this area and due to exploitation of antimony from the end of 19th century until 1990s concentrations of these elements are even more increased in this region.

Kruščica reservoir is a part of the Tara National Park and is composed of several artificial lakes of different types. It arises from Karaklijski and Baturski Rzav and it is used as source of drinking water.

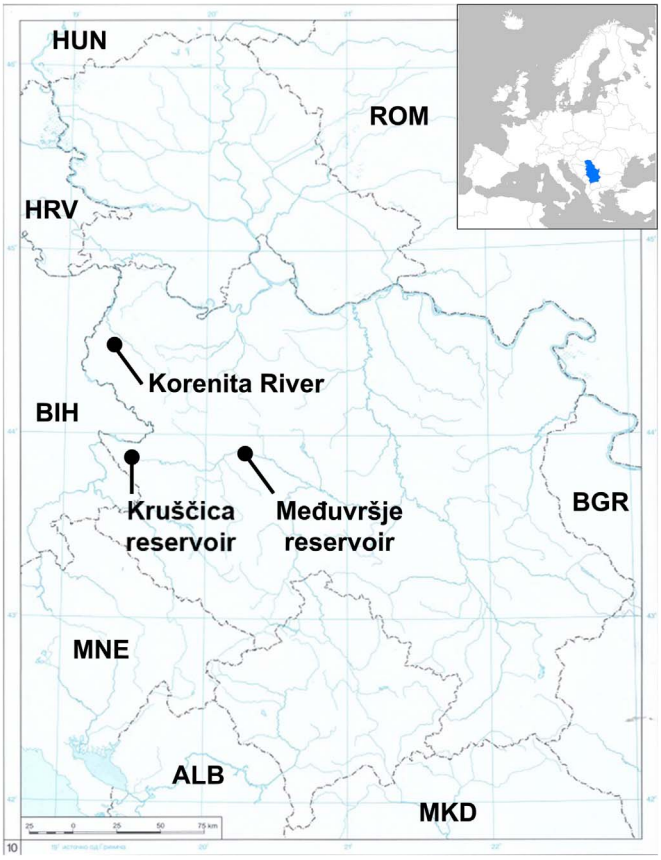

Fig. 1. Sampling locations on the territory of the Western Serbia: Kruščica reservoir, Međuvršje reservoir and Korenita River; insert: location of Serbia within Europe.

Geological surrounding of Kruščica reservoir is characterized with magmatic and sediment complex. Gabbro-diabase complex with domination of diabase and cumulate harzburgite and lherzolite (ultrabasic magmatic rocks) distinguish magmatic complex. These complexes are connected with the appearance of Cu, Fe and Ni. Cenomanian-Turonian marl and limestone, Verfen clastite and Middle Triassic limestones distinguish sediment complex. Kruščica reservoir receives petrologic materials from mentioned complexes by permanent and temporary streams after which materials are mixed and precipitate as lake deposit. Sulfide mineralization (primarily Cu sulfides) could also appear. Sediment complexes are dominantly carbonates of marine origin and due to that possess naturally increased content of Sr.

Međuvršje reservoir is the largest one on the Zapadna Morava River. It is a closed system, bordered by two dams with the water quality greatly influenced by intense emission of industrial, urban and rural wastewater. Settlements and most of the industrial facilities in the area do not have wastewater purification infrastructure. Geochemical characteristics of recent sediments in Međuvršje reservoir are a consequence of very complex geological resources that nourish Zapadna Morava River and its tributaries which are petrologically highly heterogeneous. Alluvium of Zapadna Morava River is feeding from magmatic, metamorphic and sediment complex of different age and genesis. Each of many geological units has particular geochemical status with domination of particular macro and micro elements.

2.2. Fish sampling

Fish sampling was performed with benthic gillnets (dimension 20–30 m × 1–2 m, 20–50 mm mesh size) and electrofishing (Honda 1,2κW, 6A). Gillnets were left over night. After anesthetising fish in clove oil, mass (W, g) and total body length (L, cm) of each fish were