Common barbel (*Barbus barbus*) as a bioindicator of river sediment pollution with metals

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Introduction: Metals are listed among the most important pollutants in aquatic ecosystems due to their stability and mobility through the food webs. River sediments can contain high concentrations of metals, and various aquatic organisms use sediments for various requirements in different stages of their life cycle.

Methods: In this research, total and bioavailable concentrations of 15 elements in surface sediments of three rivers – the Danube, the Zapadna Morava, and the Južna Morava, were analyzed during three seasons in 2012 (April, July, November), at three different sediment depths (0-5 cm, 5-10 cm, 10-15 cm), using inductively coupled plasma optical emission spectrometry (ICP-OES). Element concentrations were also determined in 4 tissues of the common barbel (*Barbus barbus*) sampled from all three rivers (muscle, gills, intestine, and liver). Element analysis was further carried out on barbel intestinal parasite *Pomphorhynchus laevis* (Acanthocephala), collected from the Danube River. Histological analyses of gills and liver from fish collected from the Danube and the Južna Morava was performed with the purpose of determining any tissue alterations. Scanning electron microscopy/energy dispersive X-ray spectroscopy (SEM-EDS) was used for in-depth grain analysis of the Zapadna Morava sediments, as well as tissue and parasite *P. laevis* analyses of elements in barbel sampled from the Danube River.

Results: Statistical analysis and three-way MANOVA showed significant differences in sediment grain size, average element concentration, and seasonal element concentration in the three rivers. Concentrations of Cu and Ni in the Zapadna Morava River and the Danube River were above the maximum allowable concentrations (MAC) of elements in river sediments for the Republic of Serbia, while Hg concentration was above the MAC in all three observed rivers. Concentrations of Al, Ba, Cu, Fe, Mn, and Zn varied between seasons in the Zapadna Morava and the Danube, with the highest values during the summer season. Concentrations of Al, As, Fe, Mn, and Zn varied between seasons in the Južna Morava, with the lowest values during the summer season. Highest percentage of fine sediment particles was observed in the Zapadna Morava, while the highest percentage of sand was observed in the Južna Morava sediment. SEM-EDS analysis of the Zapadna Morava sediment pointed to the presence of anthropogenic source of the higher Cu concentration in this river compared to the other two rivers. Barbel collected from the Zapadna Morava River had higher concentrations of Cu and Ni in the liver and intestine, and Zn in the muscle tissue, which corresponded with the concentrations of these elements in the sediment. SEM-EDS tissue analysis of barbel specimens from the Danube River pointed to possible anthropogenic activities causing pollution in this river. Barbel intestinal parasite *P. laevis* had high concentrations of Cu, Zn, and Pb, and in this case SEM-EDS analysis confirmed possible anthropogenic sources of contamination. Histological analysis of barbel liver and gills collected from the Danube and the Južna Morava indicated statistically significant tissue alterations in fish collected from the Južna Morava River, most probably as a result of a high organic contamination of this river.

Discussion: Total metal concentration pointed at the Zapadna Morava River as the pollution source of the Danube River. High Cu concentration in this river is consequence of anthropogenic activity. River morphology and sediment granulation influence downstream metal transport in this river. Bioavailable metal content was similar for total metal content in all three rivers. Barbel liver was identified as a organ with highest Cu concentration, while highest concentration of Ba, Mn and Sr were in the gills, and highest Hg concentration was in muscle tissue. Cu was observed in form of Cu^{2+} in the intestine of barbel collected from the Zapadna Morava River and Zn^{2+} in the gills from the barbells collected from the Danube River was confirmed using SEM-EDS analysis. ICP-OES analysis of barbel intestinal parasite *P. laevis* showd that this parasite accumulates higher concentrations of Cu, Zn and Pb. Cu most probably enters barbel through the food consumption, while Zn could enter barbel through food and gills. Histology analysis showed that despite lowest concentration of elements in sediment of the Južna Morava River barbels from this river had statistically significant alternations in gills and liver, most probably originating from high organic pollution in this river.