WATER QUALITY AND METAL EXPOSURE ASSESSMENT IN THE KRKA RIVER, KARSTIC PHENOMENON AND NATIONAL PARK IN CROATIA

Ruder Bošković Institute, Center for Marine and Environmental Research, Zagreb, Croatia

vfilip@irb.hr

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Introduction

Thanks to the constant process of travertine-building, the Krka River is a karst phenomenon which is proclaimed National Park in 1985. Its geographic position and the large number of different habitat types resulted in very rich and diverse flora and fauna, with many endemic, rare and threatened species. This puts the Krka River among the most valuable natural entities in both Croatia and Europe. The aim of the present study was to evaluate the anthropogenic influence on the Krka River in the watercourse near town of Knin, situated only 2 km upstream of the beginning of the Krka National Park. There are two potential point sources of contamination, industrial wastewater of the screw factory and untreated wastewater discharge. Contaminant exposure is evaluated in the water samples (total dissolved metal concentrations, physico-chemical and microbiological parameters) and in the indicator organism, brown trout (Salmo trutta L.), as typical representative of the Krka River ichthyofauna. Biological responses were measured in fish liver and included concentrations of cytosolic metals and biomarkers (metallothionein (MT), malondialdehyde (MDA) and total cytosolic proteins). MT was chosen as biomarker of metal exposure, MDA of oxidative stress and total cytosolic proteins of general stress.

Methods

Sampling was performed in April 2015 at two locations in the Krka River, anthropogenically impacted near town of Knin and the reference site, Krka spring. Physico-chemical water parameters were measured by Standard Methods for the Examination of Water & Wastewater (2012), while microbiological analyses were performed using the Colilert® and the Quanti-Tray/2000 (IDEXX). Metal levels (24 micro- and 4 macroelements) in water and liver cytosol of brown trout were measured by HR ICP-MS (Element 2, Thermo Finnigan). Biomarker analyses in liver of 12 trout specimens from each sampling locations were performed spectrophotometrically (Tecan Infinite M200) at 412 nm for MT by Ellman’s method, at 535 nm for MDA by Botsoglou’s method and at 750 nm for total cytosolic proteins by Lowry’s procedure.

Results

Physico-chemical water and microbiological parameters indicated Krka River water near Knin of poor quality (III-V class according to GRC, 2010). At the same location higher concentrations of total dissolved metals in water and cytosolic metals in trout liver were found for Al, As, Ba, Co, Cu, Fe, Li, Mn, Mo, Na, Ni, Sb, Se, Sr, Ti, U, V, Zn compared to the Krka spring. Levels of MDA and total cytosolic proteins were higher at anthropogenically impacted site, although not statistically significant (Fig. 1). MT levels were significantly higher at Krka spring (Fig. 1), probably following the pattern of cytosolic Cd, which is the main MT inducer. Increased Cd levels in hepatic cytosol of trout form the Krka spring might originate from dolomite rocks (Perica et al., 2007), which are enriched with Cd but additional detailed investigations of metal levels in soils and dietary metal exposure, in addition to water exposure is needed.
Conclusion
Chemical and microbiological water analyses, as well as increased cytosolic metal levels in fish liver pointed to disturbed ecological conditions in the Krka River near town of Knin. Observed anthropogenic impact was not reflected on the cellular level in fish liver (comparable MDA and total cytosolic proteins), except MT which followed increased cytosolic Cd in fish from Krka spring, probably from natural origin.

References