



FIELD WORK ACTIVITIES

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SECOND MEETING

Integrated evaluation of aquatic organism responses to metal exposure: gene expression, bioavailability, toxicity and biomarker responses (BIOTOXMET)

Zagreb, 16th December 2021



Study area



Sampling campaigns, locations, number of samples and planned activities

SAMPLING CAMPAIGNS		PLANNED ACTIVITIES
Sampling period	Sampling location	
January, April, July, October 2021	1. Krka spring (reference); 2. Krka influenced by the wastewater outlets; 3. Brljan lake (NPK); 4.-7. four Krka River tributaries (Krčić, Kosovčica, Orašnica, Butišnica)	<u>1.1. Water sampling</u> – 1.1.1. physico-chemical water parameters; 1.1.2. total and dissolved metal levels (7 samples in triplicates during 4 seasons, 84 in total)
April 2021		<u>1.1. Water sampling</u> – 1.1.3. ratio of $^{87}\text{Sr}/^{86}\text{Sr}$ isotopes (7 samples in triplicates during 1 season, 21 in total) <u>1.2. Sediment sampling</u> – 1.2.1. carbon content; 1.2.2. grain size distribution; 1.2.3. metal levels (7 samples, 1 season, 7 in total)
April , October 2021	1. Krka spring; 2. Krka influenced by the wastewater outlets 3. Orašnica River; 4. Industrial wastewater; 5. Municipal wastewater	<u>2.1. Water sampling</u> – toxicity testing of the river water and industrial and municipal wastewater using 2.1.1. algae; 2.1.2. crustaceans (5 samples in triplicates during 2 seasons, 30 in total)
	1. Krka spring; 2. Krka influenced by the wastewater outlets; 3. Brljan lake (NPK);	<u>2., 3., 4. Fish sampling and acanthocephalan isolation</u> – 2.2. biomarkers; 2.3. histopathologic changes (only fish); 2.4. metal levels; 3. bioavailability of dietborne metals (only fish) (around 35 individuals, 3 sites, 2 seasons, 210 in total); 4.1. metal distribution among cytosolic proteins; 4.4. gene expression (6–8 fish and parasite individuals, sites 1. and 2., season April, 12–16 in total)

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1. SAMPLING OF WATER AND SEDIMENT

The river water samples for the measurements of metals/metalloids, the $^{87}\text{Sr}/^{86}\text{Sr}$ isotope ratio, physico-chemical water parameters and toxicity testing will be collected manually in triplicates in the polyethylene plastic bottles, previously rinsed with nitric acid, and for the Hg measurements in borosilicate glass bottles, carefully following a clean protocol to avoid contamination. Water samples collected for the measurements of total metal/metalloid levels (except Hg) will be acidified with nitric acid (HNO_3) and for total Hg with the hydrochloric acid (HCl). For the dissolved metal/metalloid and $^{87}\text{Sr}/^{86}\text{Sr}$ isotope ratio measurements the samples will be additionally filtered (0,45 μm pore diameter filter) immediately in the field. All samples will be stored at 4°C (portable cooler). Sediment/soil samples will be collected with a sampling grabber or shovel, retrieving the upper 10 cm of surface sediment or soil cover and afterwards stored in plastic bags.



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2. FISH SAMPLING

The dominant fish species in the selected freshwater ecosystem is brown trout (*Salmo trutta* Linnaeus, 1758.), which will be sampled from the rubber boat by the electrofishing device (Hans Grassl, EL63 II GI, 5,0 KW, Honda GX270). As planned, 35 fish specimens will be sampled at each location, which is an optimal number for conducting all planned analyses, estimating the realistic conditions in the field, performing statistical analyses, without endangering brown trout population in the Krka River, upstream and inside the Krka National Park. Captured fish will be kept alive in a opaque plastic tank with **aerated** river water until further processing in the laboratory. Fish species will be determined according to Kottelat i Freyhof (2007).





Krka River, near municipal wastewater outlet; April - 25, October - 32



Krka River, hydropower plant Miljacka; April - 16, October - 21



Krka River, source; April - 31, October - 36

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3. *IN SITU* FIELD MEASUREMENTS

Measurements of physico-chemical parameters in water

Portable probes (Mettler Toledo) will be used for *in situ* measurements of the water temperature, concentration and saturation of dissolved oxygen, conductivity, total dissolved solids and pH at 0,1 m depth.



FIELD LABORATORY WORK



FIELD TEAM

