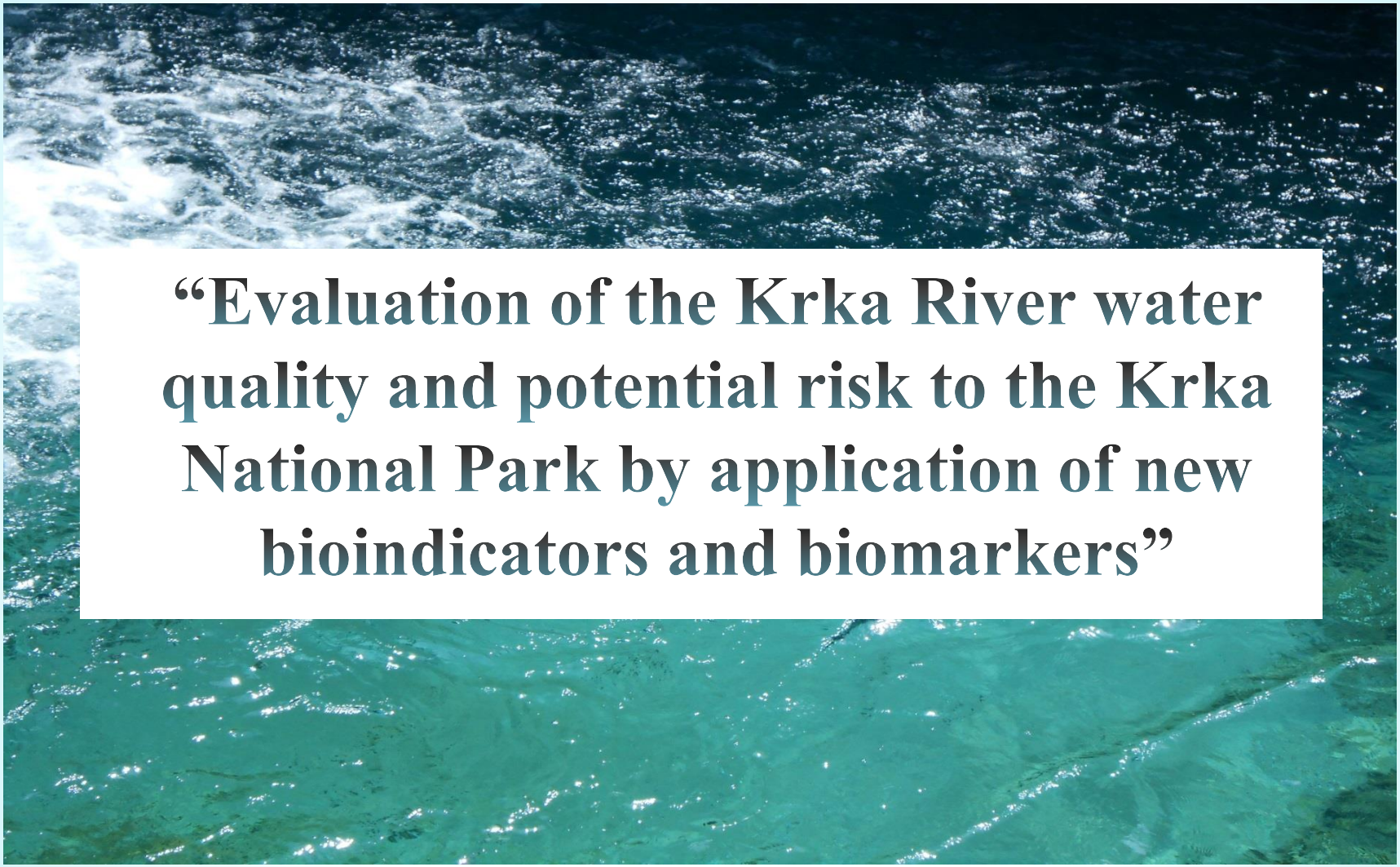


“Evaluation of the Krka River water quality and potential risk to the Krka National Park by application of new bioindicators and biomarkers”

Vlatka Filipović Marijić
Ruđer Bošković Institute

“Adris” foundation

ZAKLADADRIS

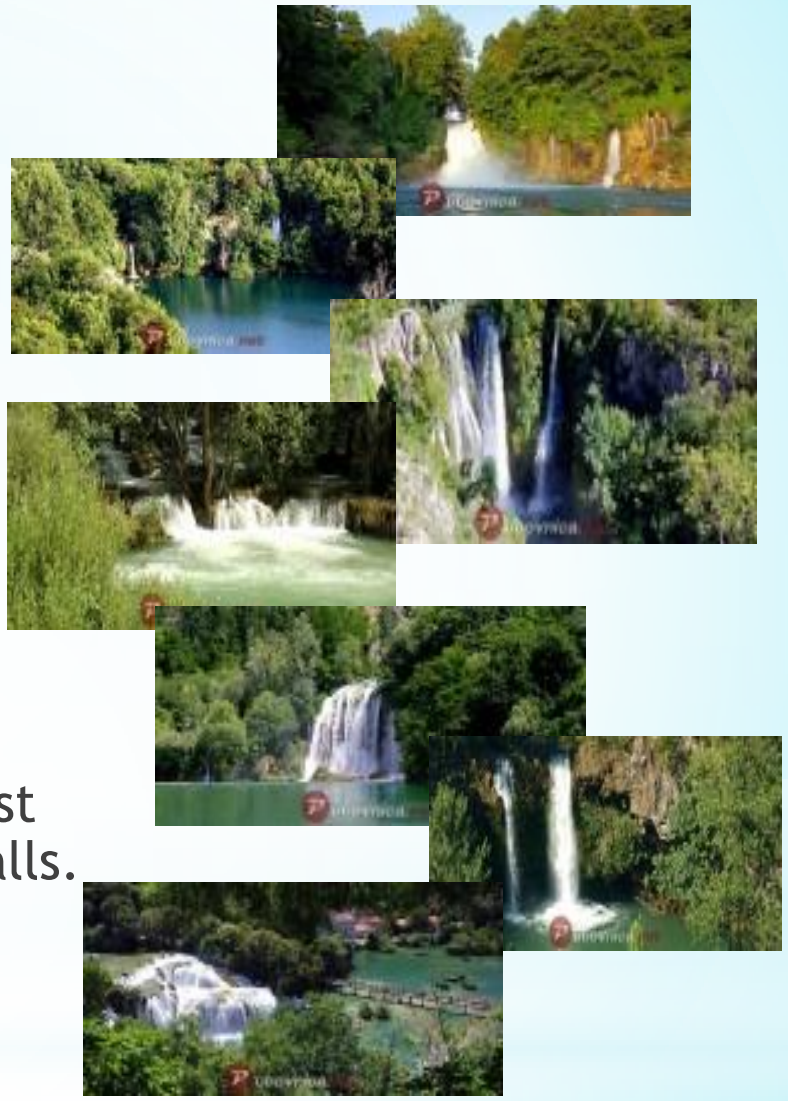
- Founded in 2007 by the Adris group, which represents one of the most successful companies in Croatia

- Objectives of the Foundation are to provide support for students, scientists and innovators, scientific and research projects, to support artistic creation and artists, to support projects that contribute to the protection and preservation of Croatian authenticity, to support humanitarian projects and to provide help for children without parental care. Over the past seven years, 26.3 million kuna has been allocated

- Laboratory for Biological Effects of Metals - research project on the Krka River (2015-2016)

ADRIS

*Krka River



*The lower watercourse is proclaimed National Park in 1985

*The present day appearance of the Krka canyon is the result of tectonic movements and surface karst-building processes in the carbonate layers.

*Thanks to the constant process of travertine-building, the Krka River is a karst phenomenon, forming 7 travertine waterfalls.

*The flora and fauna of Krka National Park is very rich and diverse, with many endemic, rare and threatened species. This puts the Krka River among the most valuable natural entities in both Croatia and Europe. There are 1022 plant species recorded, as well as 20 fish, 221 bird and 18 bat species.

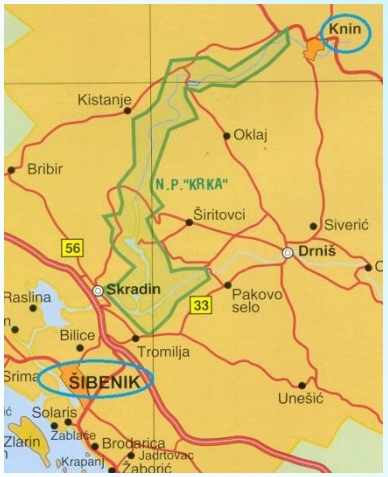
* Krka River - anthropogenic impact

inning of the Krka National Park

location but t… …me.

* For the purose… …or the Medical Research anal… …n's black pit", the area in wh… …l, and found high concentra… …arbon (PAH), faeces and tox… …, Cu, Ni, Mo

Adris project



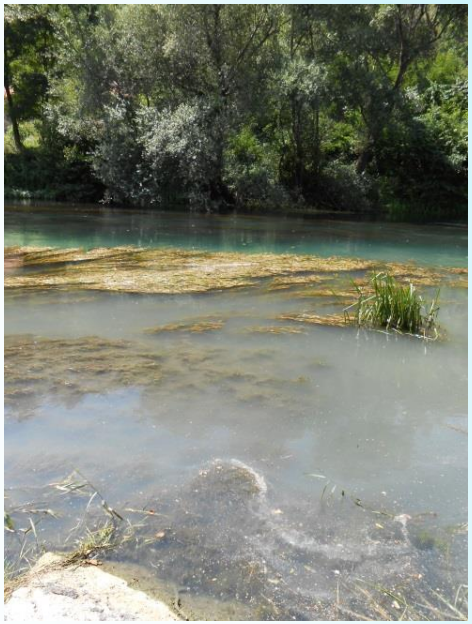
* It is of national interest for the Republic of Croatia to protect Krka National Park

* Aim:

* The project is designed to assess the water quality of the Krka River downstream of the point sources of pollution (screw factory, domestic runoff), which are situated 2 km upstream of the Krka National Park

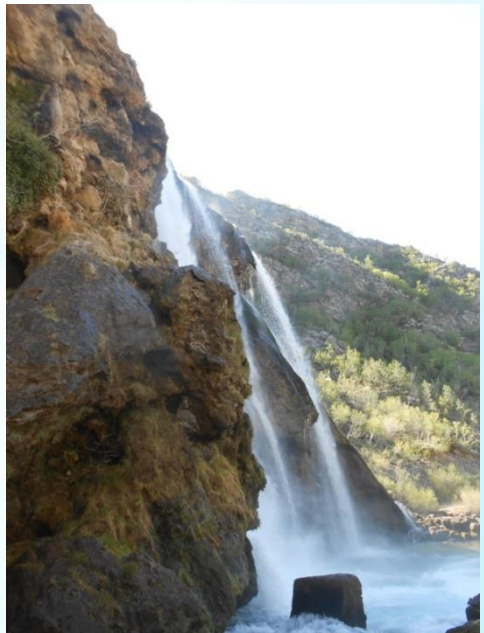
* All the data from the anthropogenicaly impacted station will be compared with the reference location, Krka spring

* Knin's black pit - polluted area



* Only preliminary data on metal exposure were available, collected for the purposes of the trial

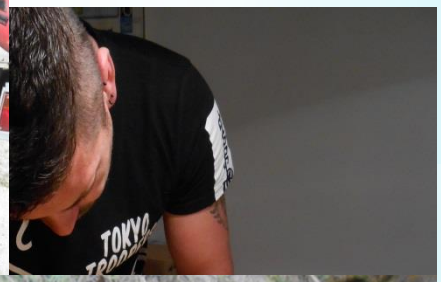
* Krka spring - reference location

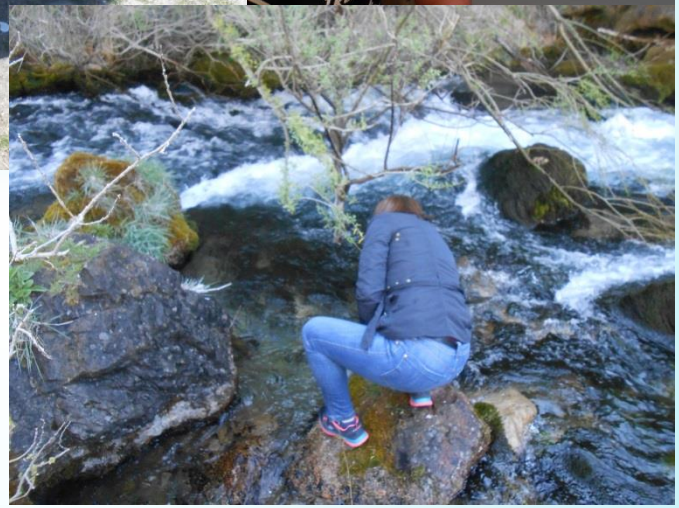


Assessment of metal exposure in the aquatic environment

* Physico-chemical parameters
* Total dissolved metal levels in the river water
* NI
  s)
* NS
  (v ue
  ar tis

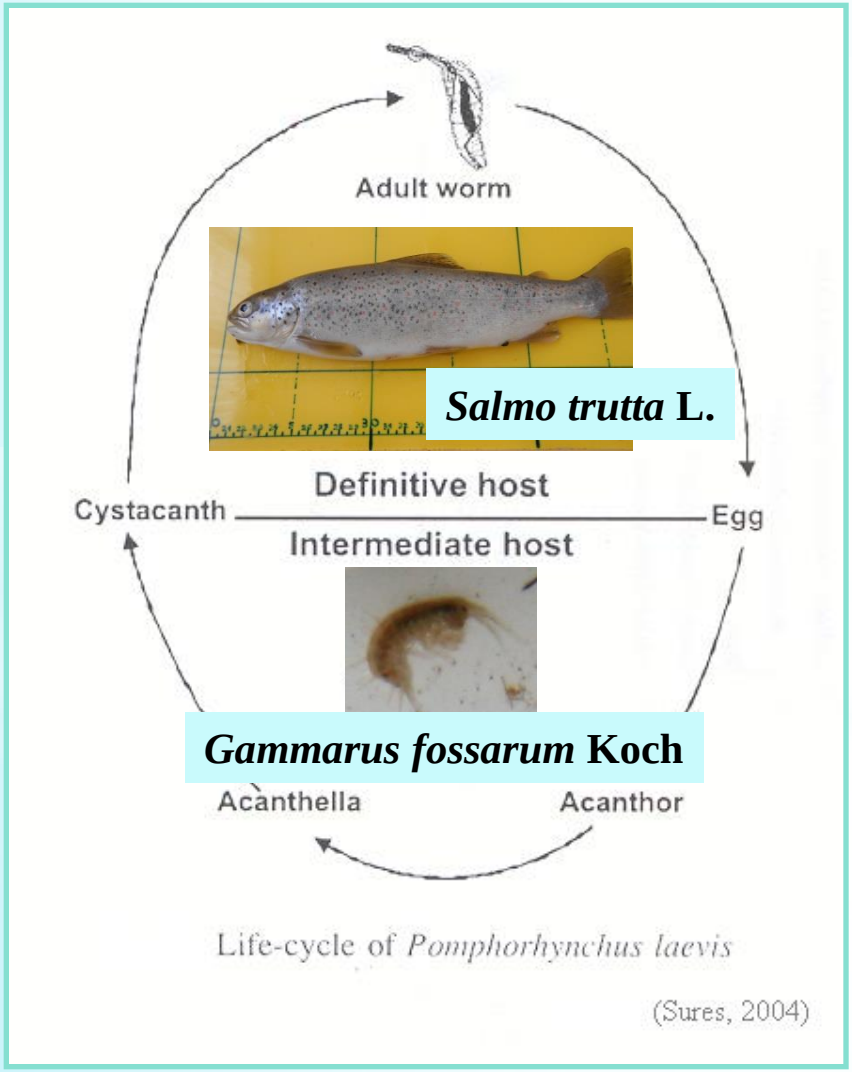
arasites



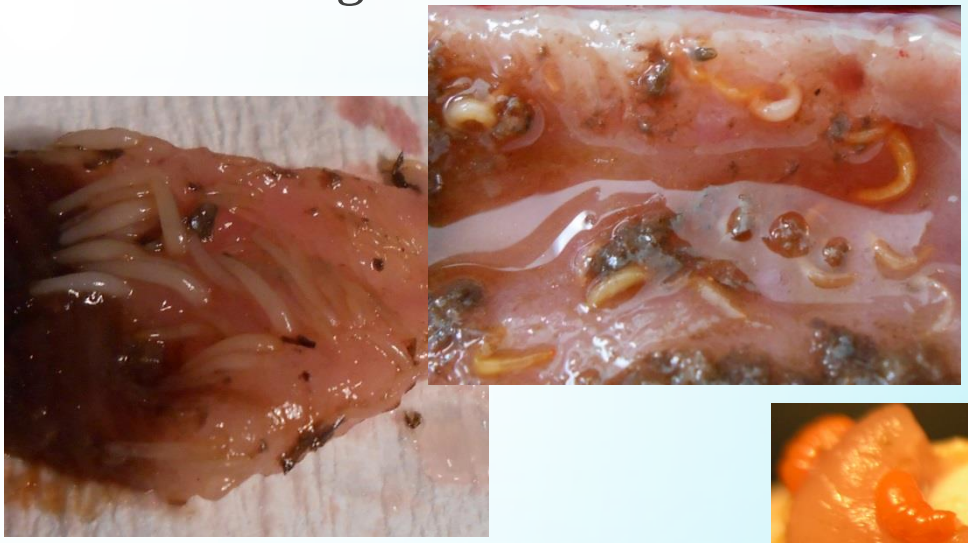


Salmo trutta L.

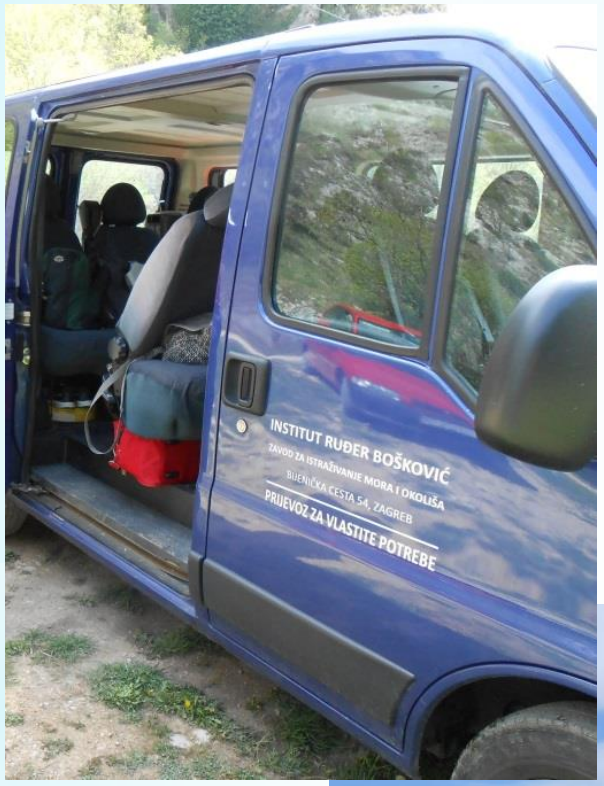
Acanthocephalans



* In the last decade fish intestinal parasites Acanthocephala are recognised as potential indicators of metal exposure since they accumulate metals, especially toxic ones, more effectively than the tissues of commonly used indicator organisms

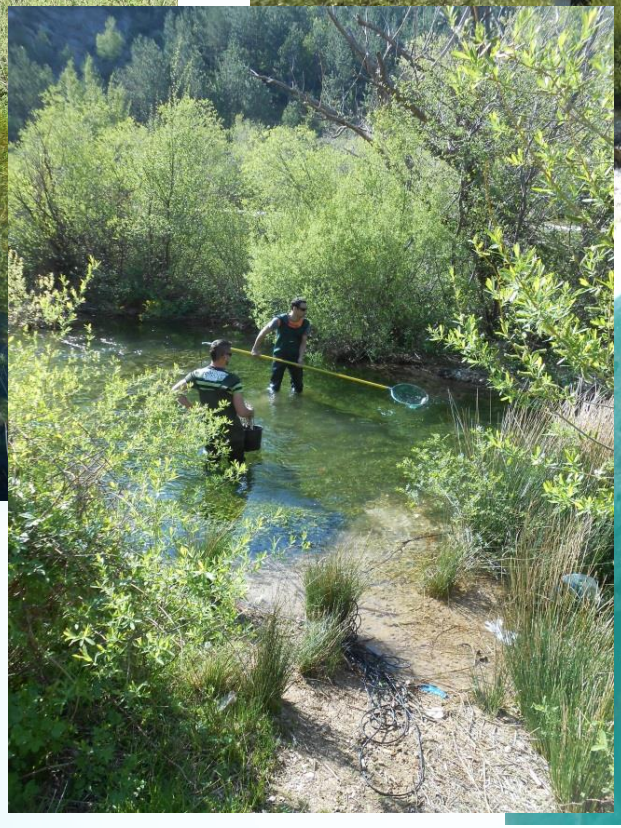


Sampling procedure

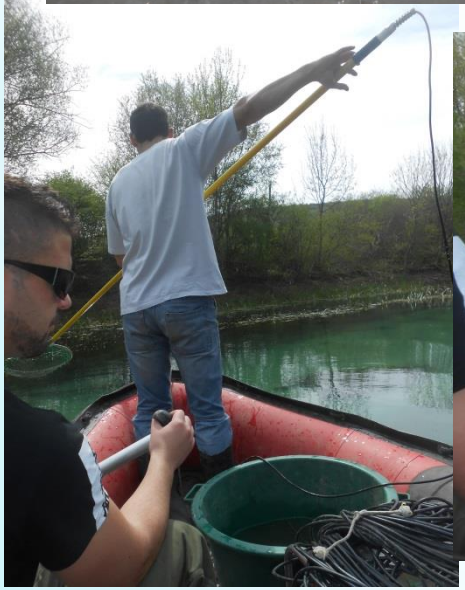


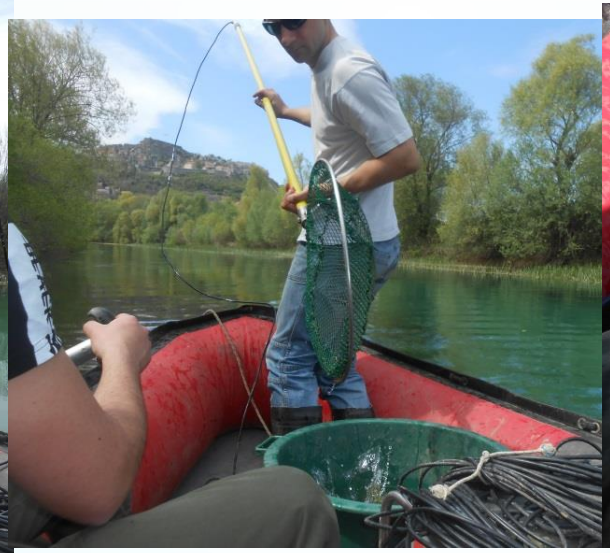
Sampling procedure

Krka spring



Krka downstream of the pollution input





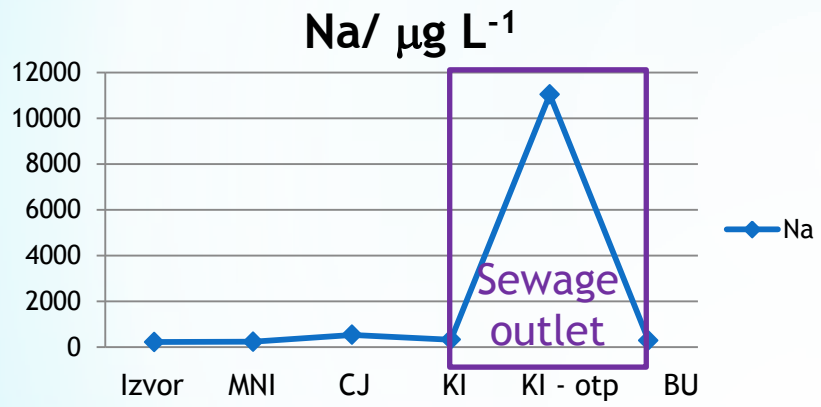
* Results - physico-chemical parameters

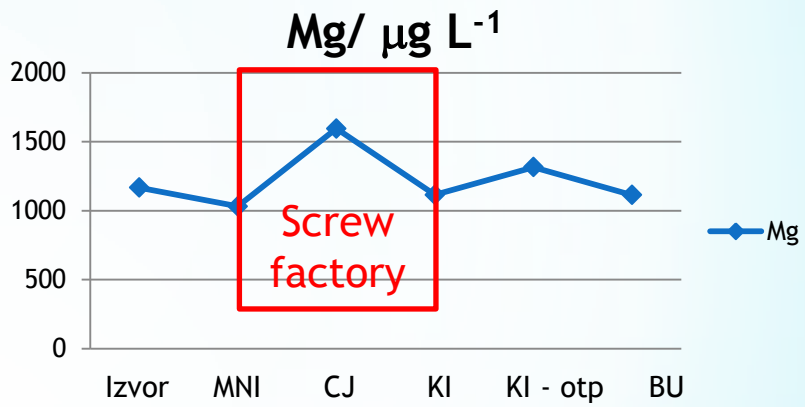
Location / Parameter	Municipial outlet (at the moment of waste release)	Municipial outlet	„Knin's black pit“ - near screw factory	Confluence of Butižnica River in the Krka River	Krka River spring
Turbidity /FAU	11	3	6	2	0
Conductivity /µS cm^{-1}	1011 IV	430 I	551 II	423 I	357 I
TDS /mg L^{-1}	505	215	275	212	179,6
Dissolved oxygen /mg O_2 L^{-1}	0,13 V	11,7 I	12,76 I	13,9 I	10,43 I
Oxygen saturation/ %	1,3 V	114,3 I	123,4 II	120,6 II	96,6 I
Chemical oxygen demand /mg O_2 L^{-1}	47 V	17 IV	42 V	8,1 III	3,5 I
Ammonium /mg N L^{-1}	0,31 III	0,19 II	0,22 III	0,14 II	<0,1 I
Total nitrogen /mg N L^{-1}	20,1 V	12,3 IV	15,4 IV	3,0 II	0,3 I
Nitrates /mg L^{-1}	10,8 V	1,3 III	3,0 III	2,5 III	0,1 I
Nitrites /mg L^{-1}	0,696 V	0,399 V	0,984 V	0,010 II	0,006 I
Total phosphorus /mg P L^{-1}	2,00 V	1,00 IV	1,15 IV	0,52 III	<0,01 I

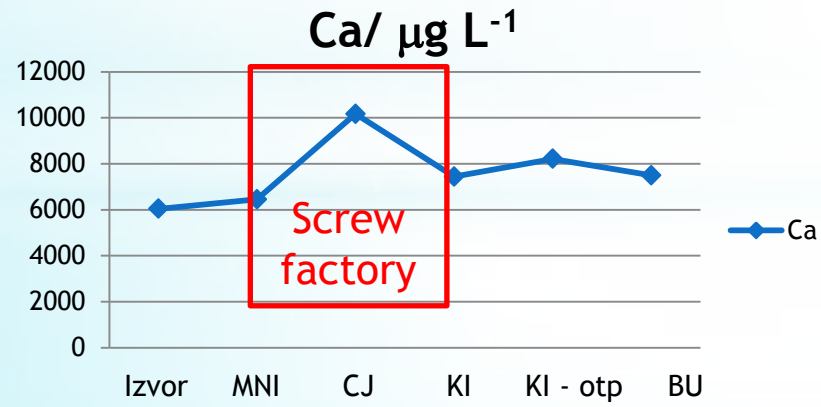
Results – microbiological analysis

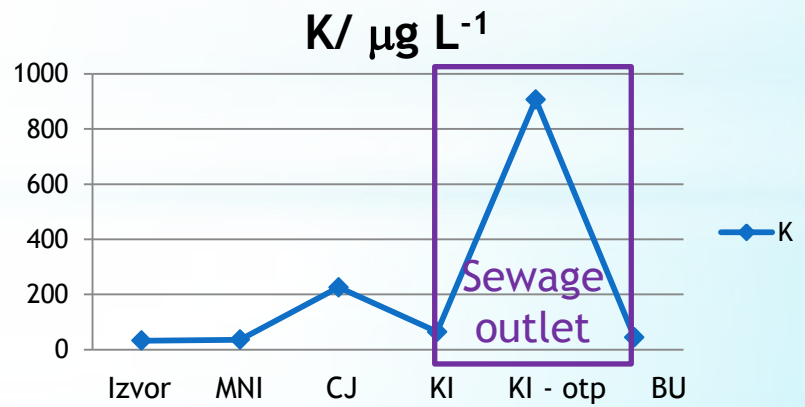
Location / Type of bacteria	Krka River spring	„Knin's black pit“ - near screw factory	Municipial outlet (at the moment of waste release)	Municipial outlet	Butižnica River confluence with the Krka River
Total coliforms (MPN/100mL)	1,5	4443,25	17328900,0	447905,0	**963,15**
Escherichia coli (MPN/100mL)	1,0	395,85	2571285,0	222075	**86,0**
Enterococcus (MPN/100mL)	< 1,0	68,85	648820,0	11990,0	**10,0**
Pseudomonas aeruginosa (MPN/100mL)	< 1,0	16,65	12285,05	346,65	**1,0**
Total amount of bacteria at 22 °C (CFU/mL)	12	19×10^4	$53{,}7 \times 10^5$	$19{,}4 \times 10^5$	**96×10^2**
Total amount of bacteria at 35 °C (CFU/mL)	**1**	**10×10^1**	**$58{,}0 \times 10^3$**	**$17{,}2 \times 10^3$**	**50**

Results - total dissolved metal concentrations (macroelements)

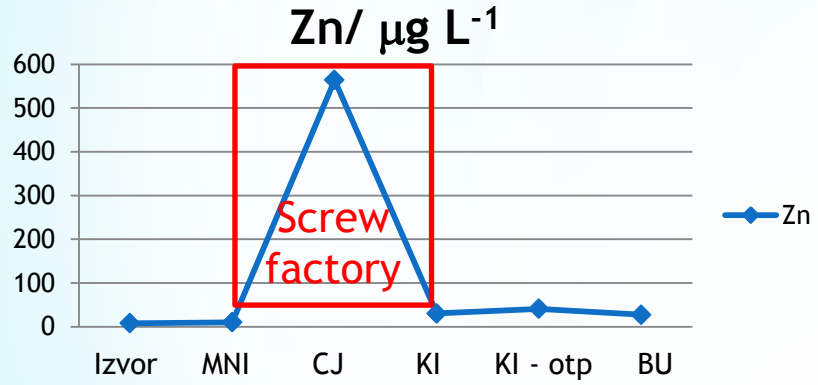


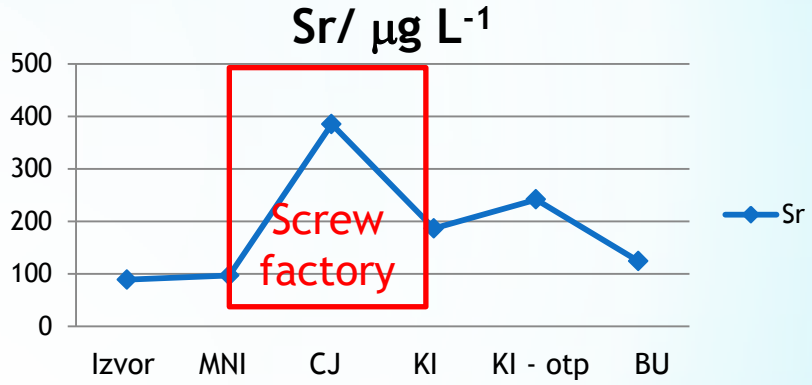


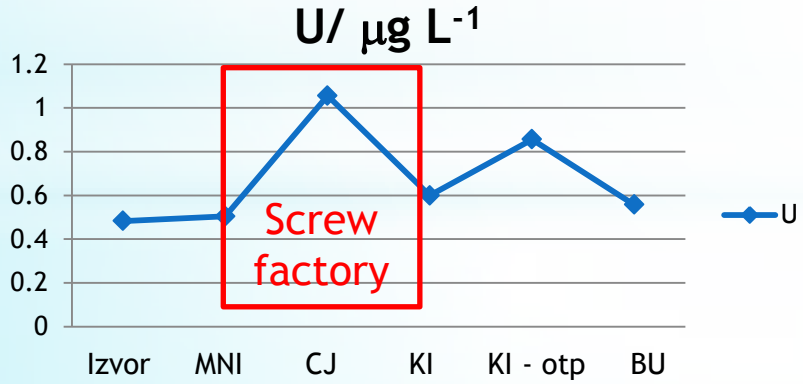


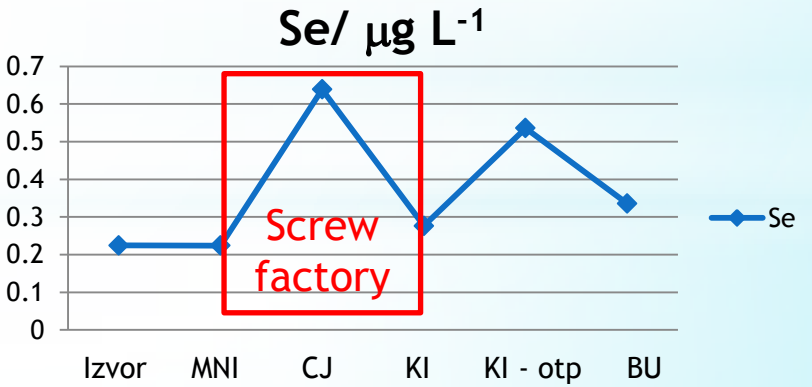


Results - total dissolved metal concentrations (microelements)

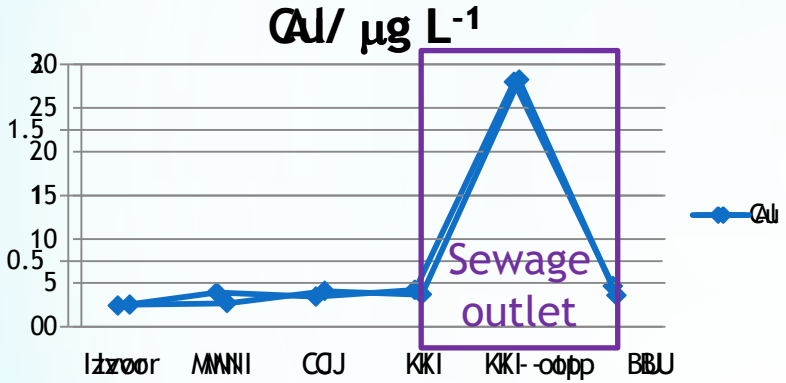


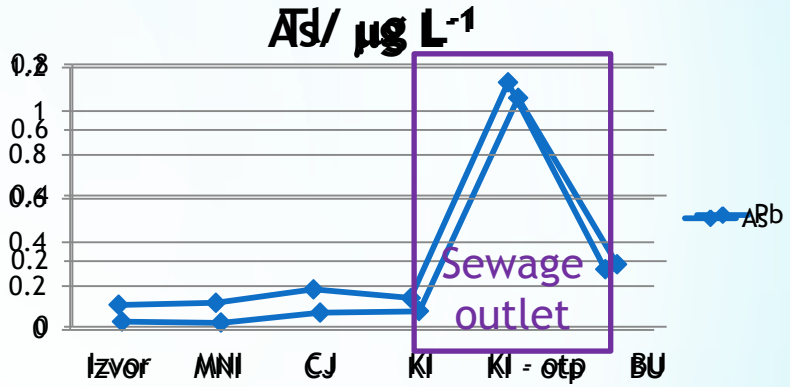


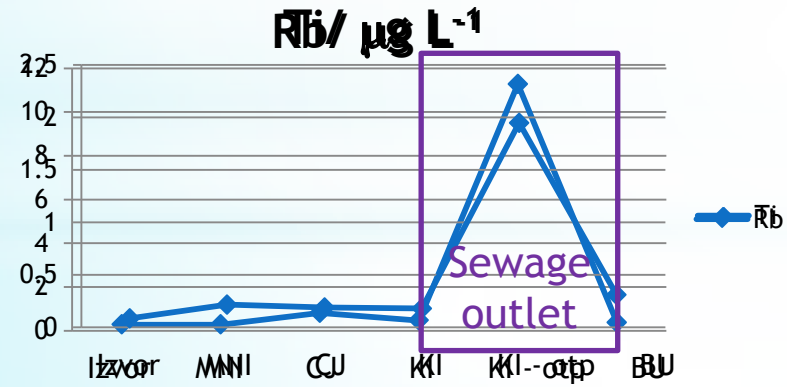


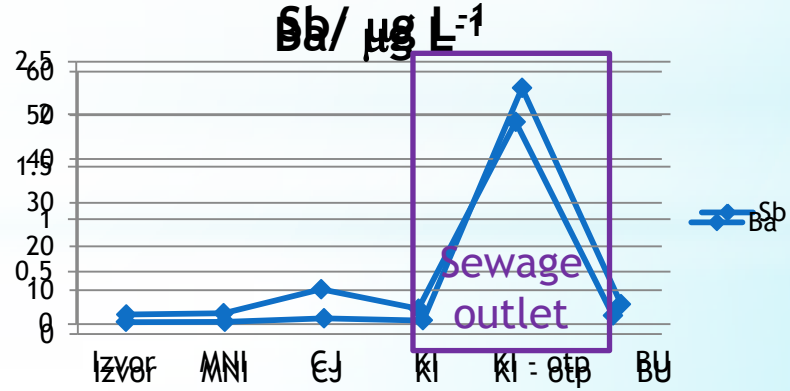


Results - total dissolved metal concentrations (microelements)

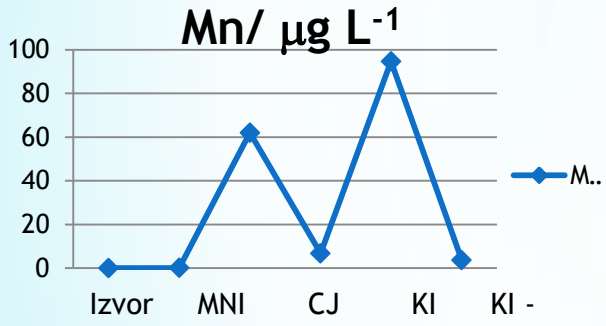


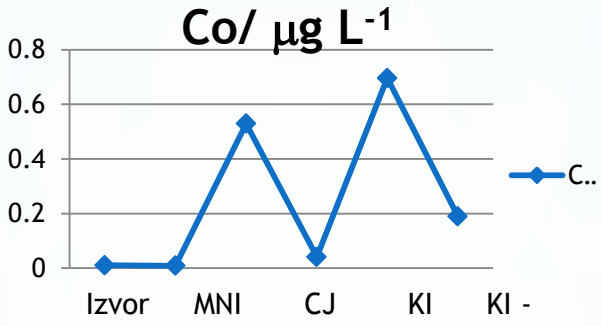


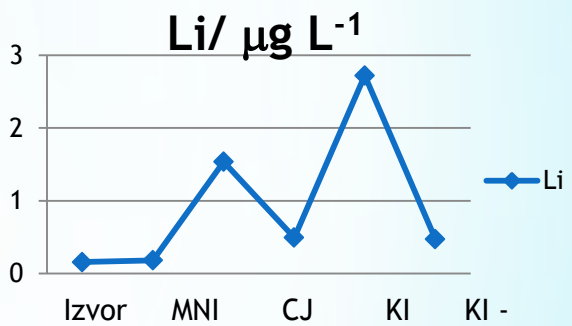


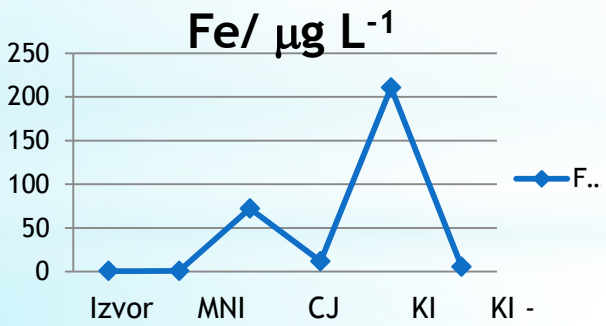


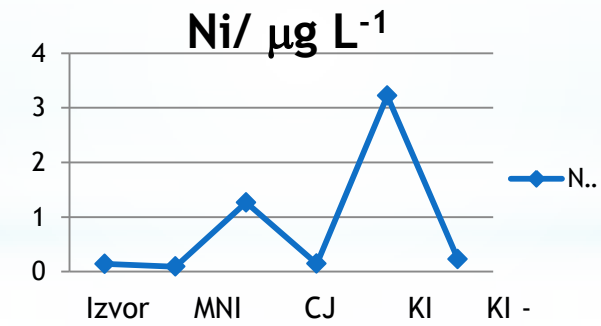
Results - total dissolved metal concentrations (microelements)

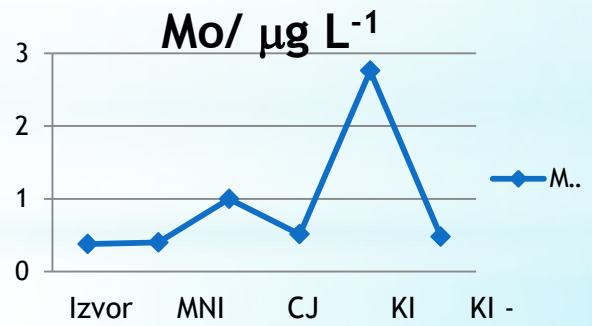




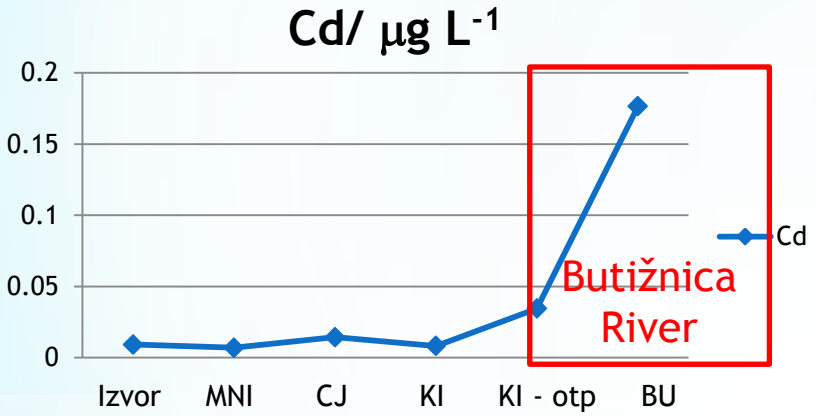


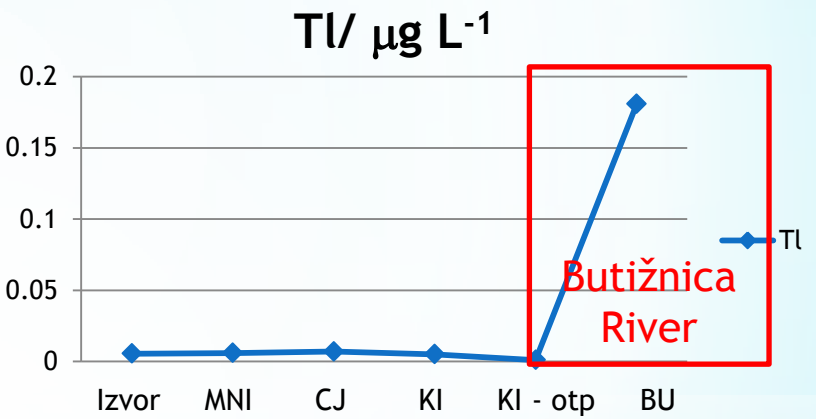


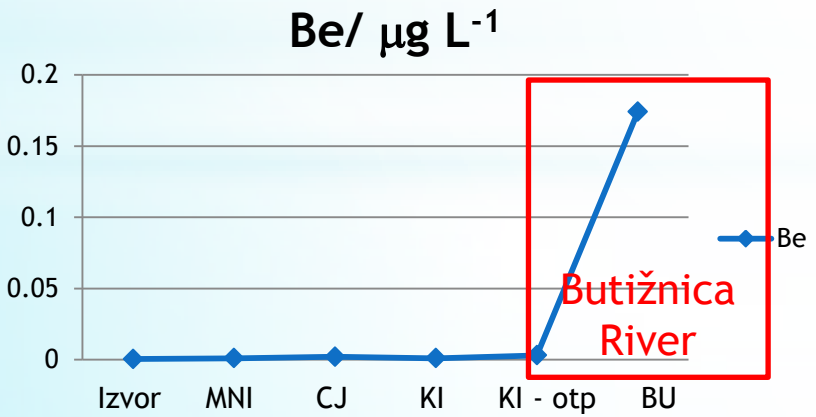


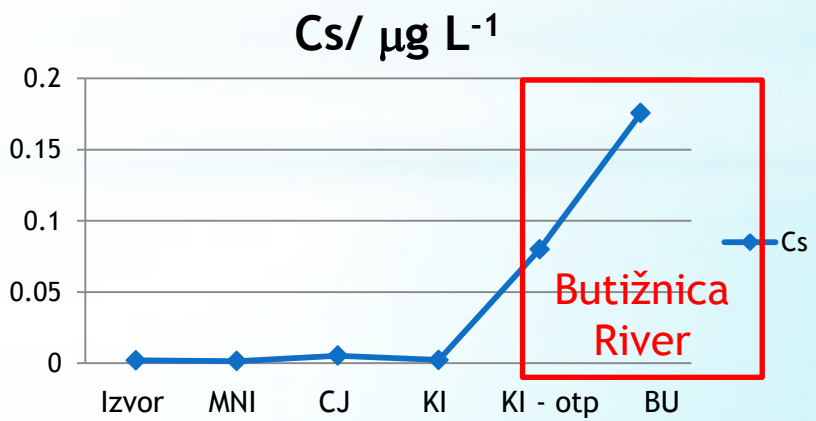


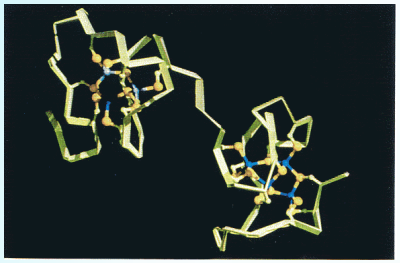
Results - total dissolved metal concentrations (microelements)











*Biomarkers

METALLOTHIONEINS - biomarkers of metal exposure

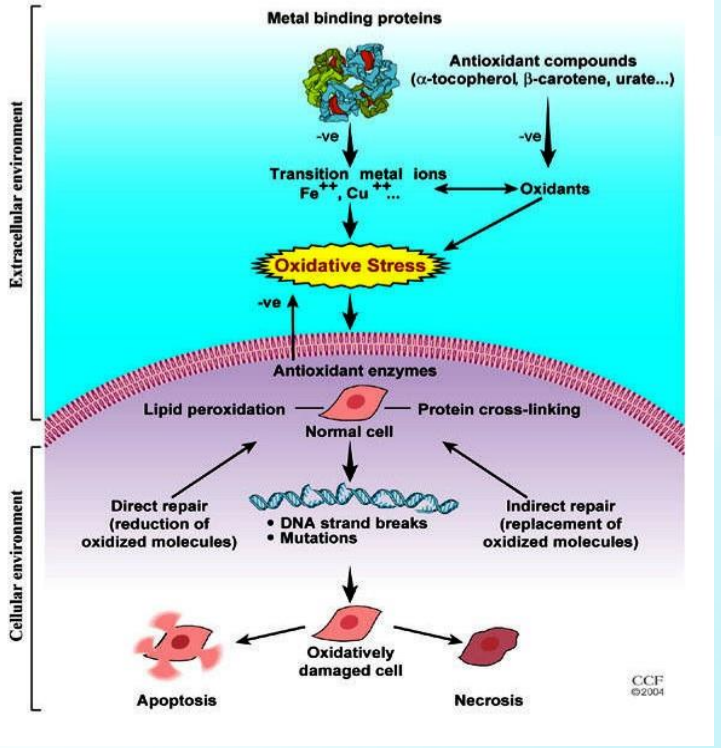
* low molecular mass cytosolic proteins responsible for homeostasis of essential (**Zn, Cu**) and detoxification of toxic (**Cd, Ag, Hg**) metals
* the harmful effect of metals is reflected as the induced and measurable amount of metallothionein in the presence of metals in the environment

MALONDIALDEHYDE

* biomarker of oxidative stress
* it results from lipid peroxidation of polyunsaturated fatty acids. Reactive oxygen species degrade polyunsaturated lipids, forming malondialdehyde

TOTAL CYTOSOLIC PROTEINS

* biomarker of general stress of the organism
* the cellular stress causes changes in synthesis of a diverse suite of proteins in response to environmental stress

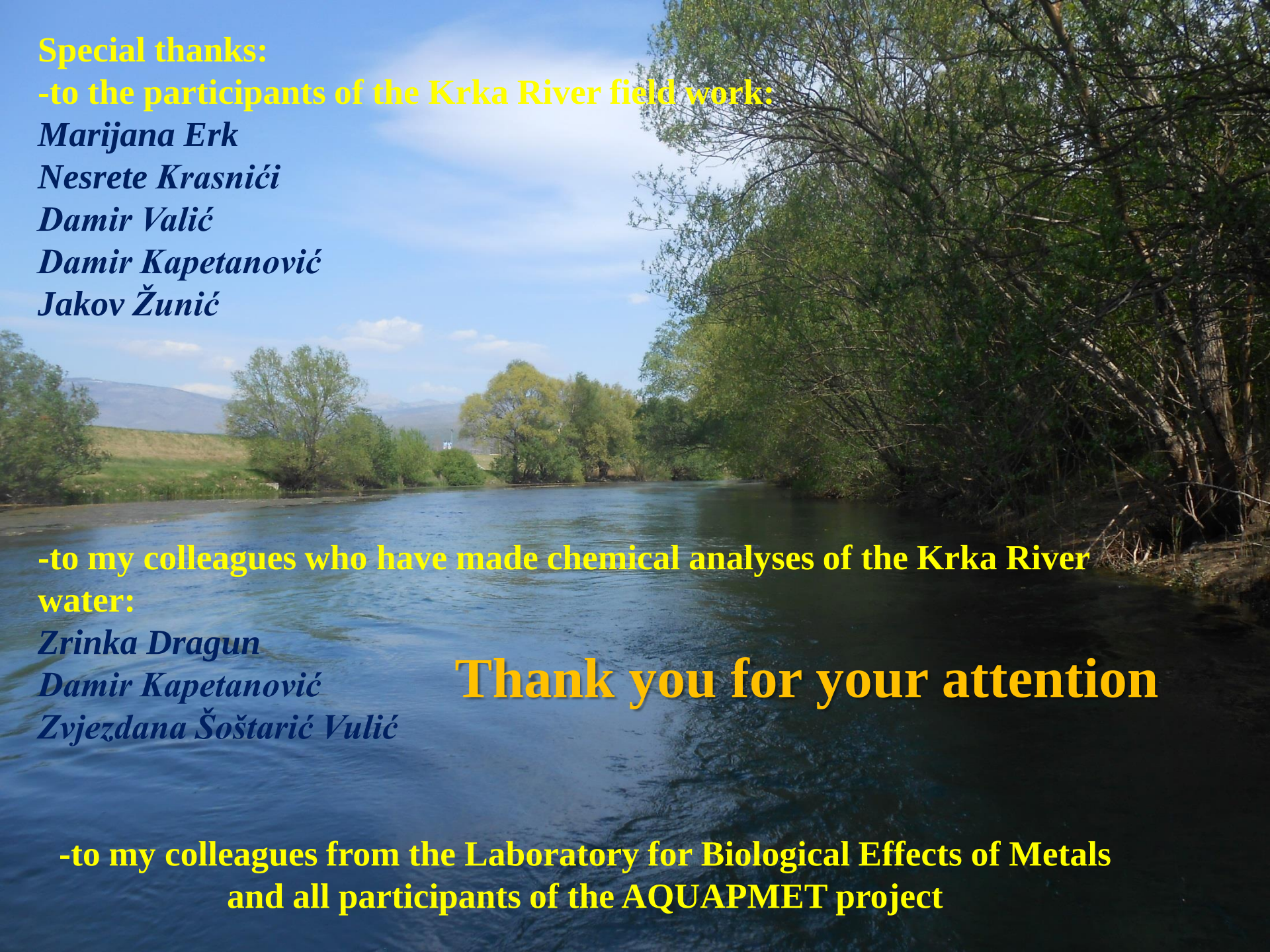


Conclusions

* ***Physico-chemical water parameters indicated the Krka River water downstream of the pollution impact of bad, poor or moderate water quality according to the Environmental Quality Standards (EQS)*** *for the physico-chemical parameters found in the Directive on water quality status of the Government of the Republic of Croatia*

* ***Total dissolved metal concentrations were increased for most of the analysed metals downstream of the screw factory or sewage outlet*** *in comparison to the reference location, Krka spring*

* *The responses of biological parameters will show us whether the toxic effect of contaminant exposure is seen on biomarker level in indicator organisms, brown trout and acanthocephalans*



Special thanks:

-to the participants of the Krka River field work:

Marijana Erk

Nesrete Krasnići

Damir Valić

Damir Kapetanović

Jakov Žunić

-to my colleagues who have made chemical analyses of the Krka River water:

Zrinka Dragun

Damir Kapetanović

Zvjezdana Šoštarić Vulić

Thank you for your attention

-to my colleagues from the Laboratory for Biological Effects of Metals and all participants of the AQUAPMET project