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

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Ruđer Bošković Institute
“Adris” foundation

* 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)
*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

Only 2 km upstream of the beginning of the Krka National Park

Municipal wastewater – released directly in the Krka River, without wastewater treatment

DIV d.o.o. – svrew factory in Knin

According to the final judgment of the Šibenik County Court from 2012, town of Knin was obligated to remediate this location but the situation remained the same.

For the purposes of the mentioned trial, Institute for the Medical Research analysed the water in the so-called “Knin’s black pit”, the area in which wastewater from DIV is released, and found high concentrations of polycyclic aromatic hydrocarbon (PAH), faeces and toxic metals, especially Cd, Hg, Pb, Zn, Cu, Ni, Mo.
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 anthropogenically impacted station will be compared with the reference location, Krka spring.
Only preliminary data on metal exposure were available, collected for the purposes of the trial.
* Assessment of metal exposure in the aquatic environment

* Physico-chemical parameters
* Total dissolved metal levels in the river water

Salmo trutta L.
* 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.

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*Sampling procedure*
*Krka spring*
Krka downstream of the pollution input
<table>
<thead>
<tr>
<th>Location</th>
<th>Municipal outlet (at the moment of waste release)</th>
<th>„Knin's black pit“ - near screw factory</th>
<th>Confluence of Butižnica River in the Krka River</th>
<th>Krka River spring</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parameter</td>
<td>Turbidity /FAU</td>
<td>Conductivity /µS cm⁻¹</td>
<td>TDS /mg L⁻¹</td>
<td>Dissolved oxygen /mg O₂ L⁻¹</td>
</tr>
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<tr>
<td>Parameter</td>
<td>Turbidity /FAU</td>
<td>11</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>Location</td>
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</tr>
<tr>
<td>Parameter</td>
<td>Conductivity /µS cm⁻¹</td>
<td>1011</td>
<td>430</td>
<td>551</td>
</tr>
<tr>
<td>Location</td>
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</tr>
<tr>
<td>Parameter</td>
<td>TDS /mg L⁻¹</td>
<td>505</td>
<td>215</td>
<td>275</td>
</tr>
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</tr>
<tr>
<td>Parameter</td>
<td>Dissolved oxygen /mg O₂ L⁻¹</td>
<td>0,13</td>
<td>11,7</td>
<td>12,76</td>
</tr>
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</tr>
<tr>
<td>Parameter</td>
<td>Oxygen saturation/%</td>
<td>1,3</td>
<td>114,3</td>
<td>123,4</td>
</tr>
<tr>
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</tr>
<tr>
<td>Parameter</td>
<td>Chemical oxygen demand /mg O₂ L⁻¹</td>
<td>47</td>
<td>17</td>
<td>42</td>
</tr>
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</tr>
<tr>
<td>Parameter</td>
<td>Ammonium /mg N L⁻¹</td>
<td>0,31</td>
<td>0,19</td>
<td>0,22</td>
</tr>
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</tr>
<tr>
<td>Parameter</td>
<td>Total nitrogen /mg N L⁻¹</td>
<td>20,1</td>
<td>12,3</td>
<td>15,4</td>
</tr>
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<td>Municipal outlet</td>
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</tr>
<tr>
<td>Parameter</td>
<td>Nitrates /mg L⁻¹</td>
<td>10,8</td>
<td>1,3</td>
<td>3,0</td>
</tr>
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</tr>
<tr>
<td>Parameter</td>
<td>Nitrites /mg L⁻¹</td>
<td>0,696</td>
<td>0,399</td>
<td>0,984</td>
</tr>
<tr>
<td>Location</td>
<td>Municipal outlet</td>
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</tr>
<tr>
<td>Parameter</td>
<td>Total phosphorus /mg P L⁻¹</td>
<td>2,00</td>
<td>1,00</td>
<td>1,15</td>
</tr>
</tbody>
</table>
### Results - Microbiological Analysis

<table>
<thead>
<tr>
<th>Location</th>
<th>Krka River spring</th>
<th>„Knin’s black pit“ - near screw factory</th>
<th>Municipal outlet (at the moment of waste release)</th>
<th>Municipal outlet</th>
<th>Butižnica River confluence with the Krka River</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Type of bacteria</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total coliforms (MPN/100mL)</td>
<td>1,5</td>
<td>4443,25</td>
<td>17328900,0</td>
<td>447905,0</td>
<td>963,15</td>
</tr>
<tr>
<td>Escherichia coli (MPN/100mL)</td>
<td>1,0</td>
<td>395,85</td>
<td>2571285,0</td>
<td>222075</td>
<td>86,0</td>
</tr>
<tr>
<td>Enterococcus (MPN/100mL)</td>
<td>&lt; 1,0</td>
<td>68,85</td>
<td>648820,0</td>
<td>11990,0</td>
<td>10,0</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa (MPN/100mL)</td>
<td>&lt; 1,0</td>
<td>16,65</td>
<td>12285,05</td>
<td>346,65</td>
<td>1,0</td>
</tr>
<tr>
<td>Total amount of bacteria at 22 °C (CFU/mL)</td>
<td>12</td>
<td>$19 \times 10^4$</td>
<td>$53,7 \times 10^5$</td>
<td>$19,4 \times 10^5$</td>
<td>$96 \times 10^2$</td>
</tr>
<tr>
<td>Total amount of bacteria at 35 °C (CFU/mL)</td>
<td>1</td>
<td>$10 \times 10^1$</td>
<td>$58,0 \times 10^3$</td>
<td>$17,2 \times 10^3$</td>
<td>50</td>
</tr>
</tbody>
</table>
Results - total dissolved metal concentrations (macroelements)

- **Na/ μg L⁻¹**
  - Izvor: 0, MNI: 0, CJ: 0, KI: 0, KI - otp: 0, BU: 0
  - Screw factory: 12000
  - Sewage outlet: 12000

- **Mg/ μg L⁻¹**
  - Izvor: 0, MNI: 0, CJ: 0, KI: 0, KI - otp: 0, BU: 0
  - Screw factory: 1500
  - Sewage outlet: 1500

- **Ca/ μg L⁻¹**
  - Izvor: 0, MNI: 0, CJ: 0, KI: 0, KI - otp: 0, BU: 0
  - Screw factory: 1000

- **K/ μg L⁻¹**
  - Izvor: 0, MNI: 0, CJ: 0, KI: 0, KI - otp: 0, BU: 0
  - Sewage outlet: 1000
Results - total dissolved metal concentrations (microelements)

- **Zn/μg L⁻¹**: Screw factory
- **Sr/μg L⁻¹**: Screw factory
- **U/μg L⁻¹**: Screw factory
- **Se/μg L⁻¹**: Screw factory
Results - total dissolved metal concentrations (microelements)

**Rb/ µg L⁻¹**

- **Izvor**, **MNI**, **CJ**, **KI**, **KI - otp**, **BU**

**Sewage outlet**

**Ba/ µg L⁻¹**

- **Izvor**, **MNI**, **CJ**, **KI**, **KI - otp**, **BU**

**Sewage outlet**

**Cu/ µg L⁻¹**

- **Izvor**, **MNI**, **CJ**, **KI**, **KI - otp**, **BU**

**Sewage outlet**

**Al/ µg L⁻¹**

- **Izvor**, **MNI**, **CJ**, **KI**, **KI - otp**, **BU**

**Sewage outlet**

**Ag/ µg L⁻¹**

- **Izvor**, **MNI**, **CJ**, **KI**, **KI - otp**, **BU**

**Sewage outlet**

**As/ µg L⁻¹**

- **Izvor**, **MNI**, **CJ**, **KI**, **KI - otp**, **BU**

**Sewage outlet**
Results - total dissolved metal concentrations (microelements)
Results - total dissolved metal concentrations (microelements)

- **Cd/ µg L⁻¹**
  - Butičnica River
  - Izvor, MNI, CJ, KI, KI - otp, BU

- **Tl/ µg L⁻¹**
  - Butičnica River
  - Izvor, MNI, CJ, KI, KI - otp, BU

- **Be/ µg L⁻¹**
  - Butičnica River
  - Izvor, MNI, CJ, KI, KI - otp, BU

- **Cs/ µg L⁻¹**
  - Butičnica River
  - Izvor, MNI, CJ, KI, KI - otp, BU
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
Thank you for your attention

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

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