Application of the intestinal tissue of invasive Prussian carp as a bioindicator organ in the metal exposure assessment of the Ilova River

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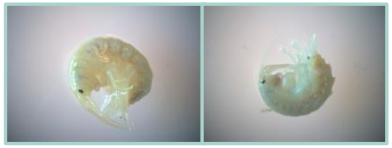
Bioindicator organisms and biomarkers

Indication of metal exposure in aquatic ecosystems

Carassius gibelio **Bloch, 1782**

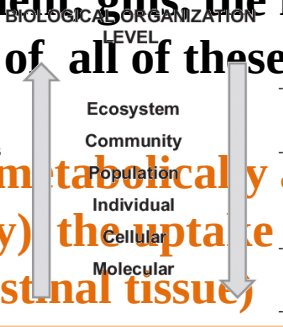
Bioindicators

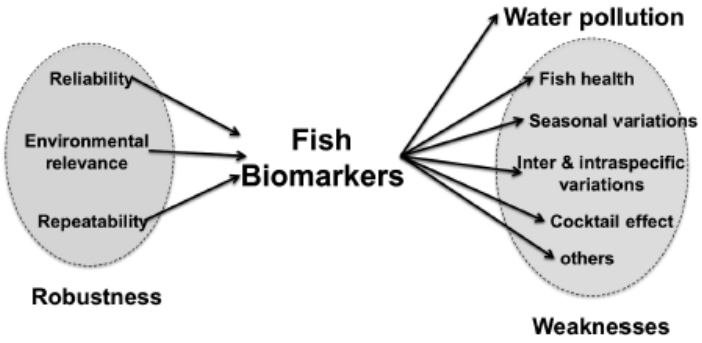


***Gammarus fossarum* Koch, 1936**
***Gammarus roeseli* Gervais, 1835**

Biomarkers

- Sites of metal uptake in the aquatic organisms - **integument, gills, the intestine or the combination of all of these ways**
- Indicator organs – **metabolically active tissues (liver, kidney), the uptake sites for toxicants (gills, intestinal tissue)**





Prussian carp (*Carassius gibelio* Bloch, 1782)

- Bioindicator:

Prussian carp (*Carassius gibelio* Bloch, 1782)

- Native in eastern Asia, widely introduced to Europe → invasive species in Croatia.
- Eastern European or wild form of the goldfish
- Spawning period: May-July (able to reproduce from unfertilized eggs (gynogenesis). Females spawn with several other species, for example *Cyprinus carpio* and *Carassius carassius*, but the eggs just develop without being actually fertilized resulting in a female only population)
- Can strongly tolerate low oxygen concentrations and pollution

- Sampling campaigns:

Autumn sampling – 5.10.2017.

Spring sampling – 3.- 4.5.2018.

- Indicator organ: **the intestine → site of dietary metal uptake.**

Main goals

Assessment of biological responses to the wastewaters impact in the Ilova River, using the dominant fish species, Prussian carp (***Carassius gibelio***) as biondicator organism:

a) application of multi-biomarker approach, which inovolved biomarkers of metal exposure (metallothioneins; MT), oxidative stress (malondialdehyde; MDA) and of antioxidative capacity (total glutathione; GSH and catalase; CAT) for the first time in the intestine of Prussian carp

b) first measurement of metal levels in the intestinal cell cytosol of Prussian carp, as bioavailable metal fraction

c) estimation of proportions of potentially toxic metal fractions from the dietary uptake route

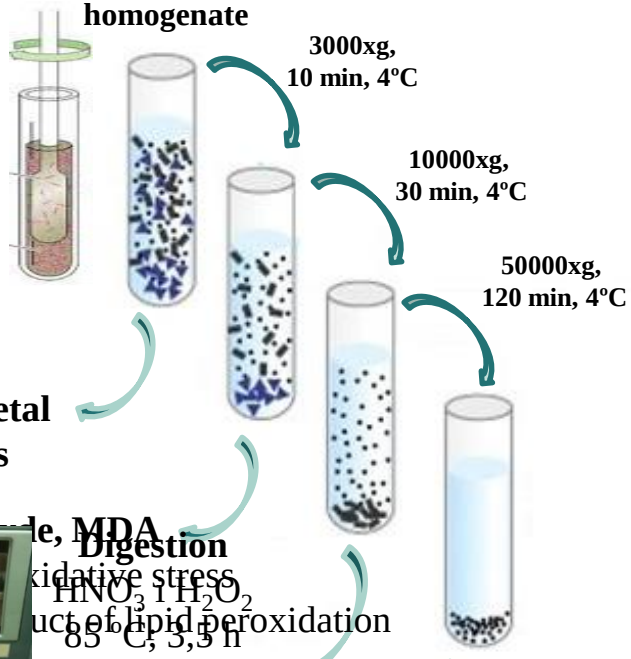
Indication of potential risks for the protected area of nearby Lonjsko Polje Nature Park

Contribution to the development of water management plans

Methods

Fish intestine

homogenization



Total metal levels

Malondialdehyde, MDA
*Biomarkers of oxidative stress
*Secondary product of lipid peroxidation

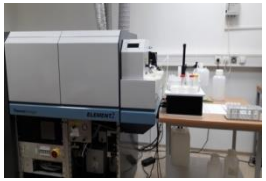
Catalase activity, CAT and total glutathione, GSH
*Biomarkers of antioxidative capacity

Digestion
HNO_3 1 H_2O_2
85 °C, 3,5 h

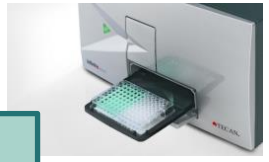
Sample dilution
20x for biomarkers
5x for trace metals

Metallothioneins, MT
*Biomarkers of metal exposure

Cytosolic metals
*Biologically available and potentially toxic fraction



Metal measurements
High resolution inductively coupled plasma mass spectrometer (HR ICP-MS)



MDA CAT GSH

spectrophotometrical measurements according to the protocols

Metallothioneins (MTs)
Low molecular mass cytosolic proteins responsible for homeostasis of essential and detoxification of toxic metals

Heat treatment

Electrochemical determination

Results

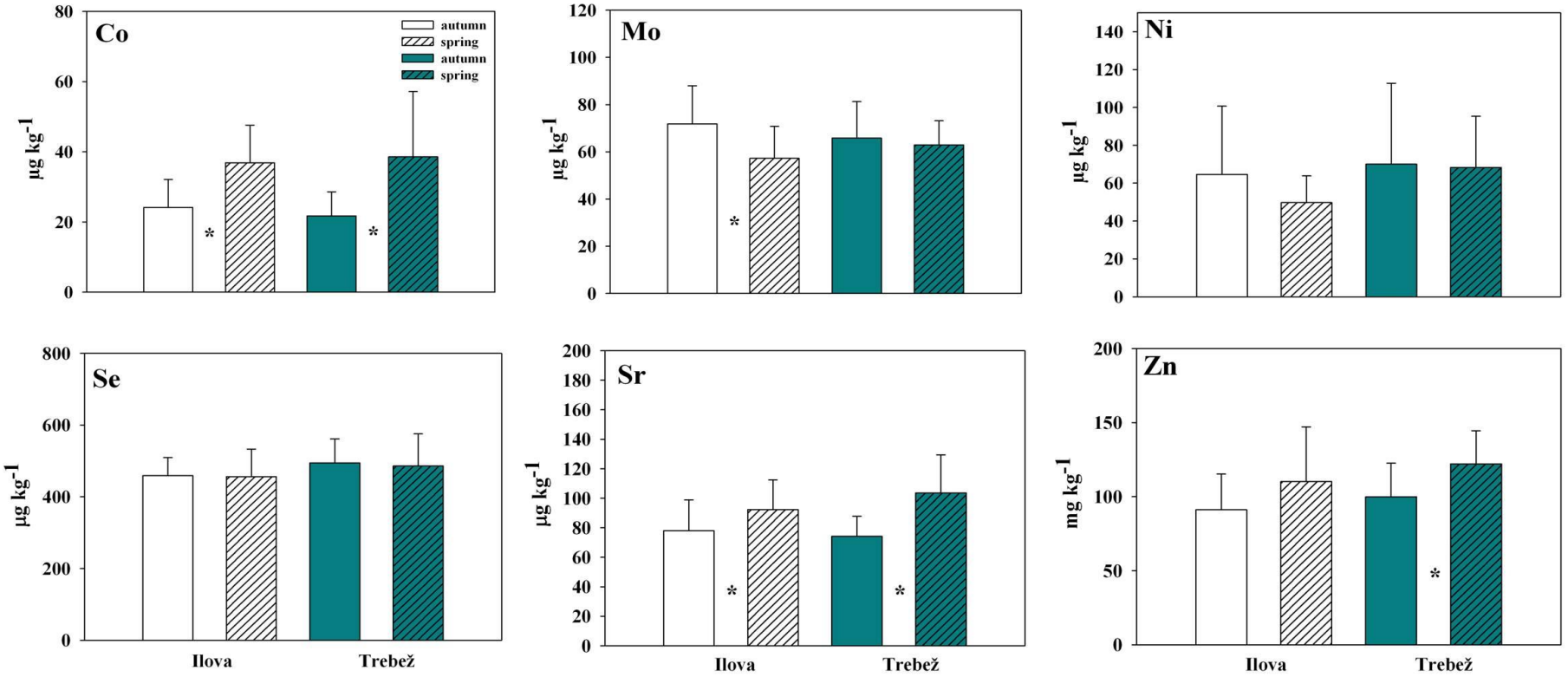
First results on metal/metalloid concentrations in the metabolically available intestinal cytosolic fractions of *C. gibelio*.

Trace elements

Metal/metalloid levels in descending order:

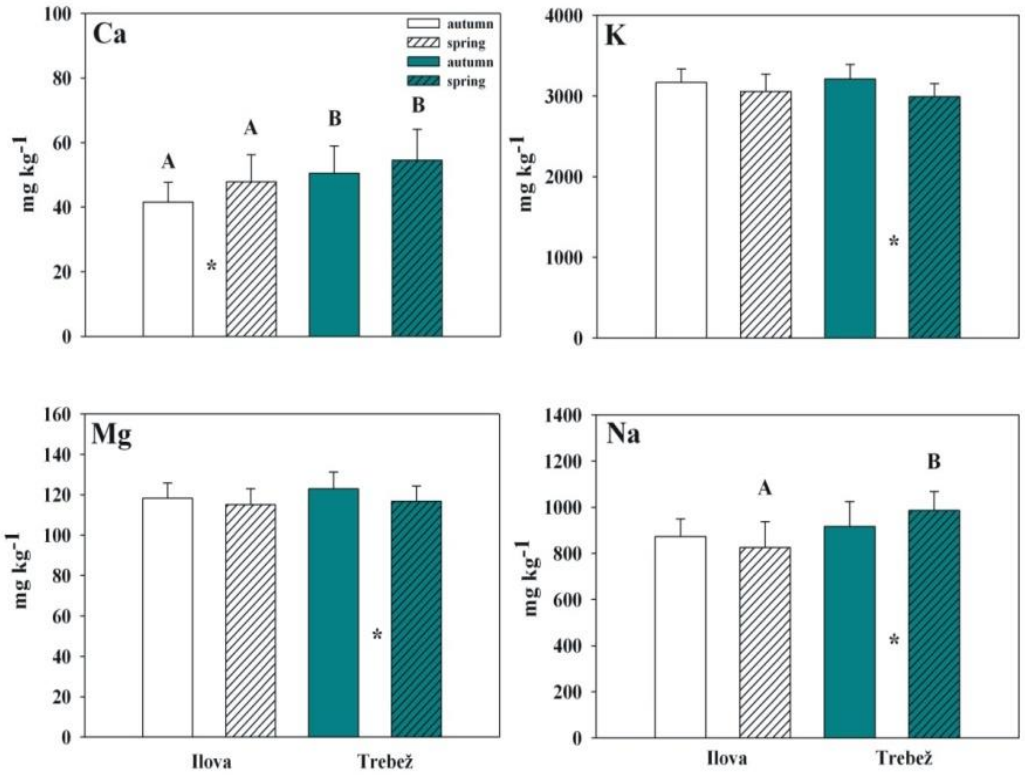
Zn>Fe>Rb>Cu≥Mn>Se>Cd>Sr>Ni>Mo≥As>Co>V≥Cs

*** Seasonal difference between the two seasons at the same location.**
A, B Spatial difference between the two locations in the same season.



Macroelements

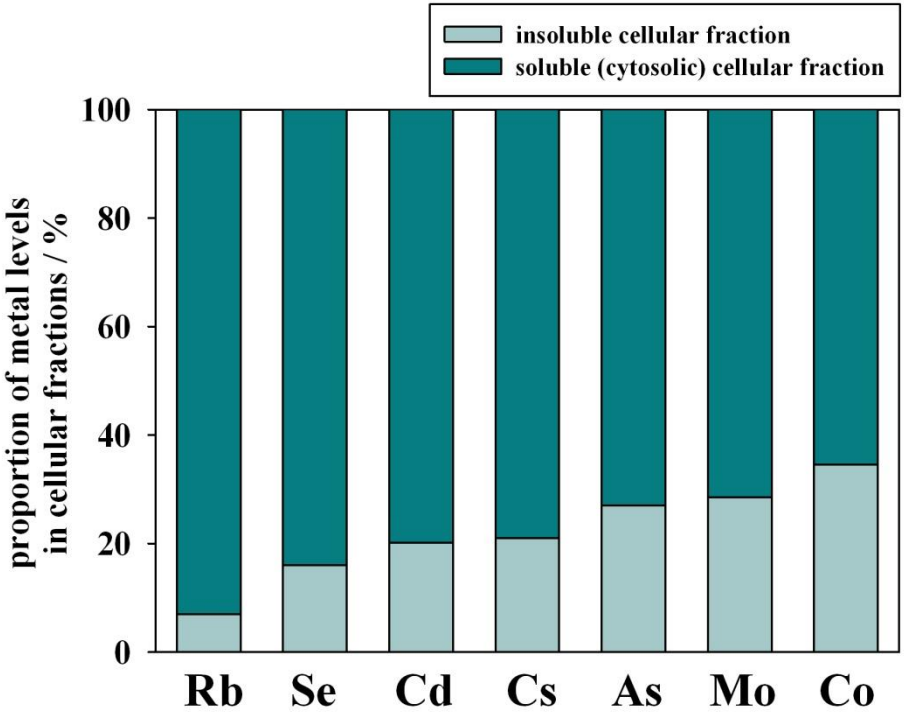
✓ **Ca** significantly higher at the contaminated site in both seasons and **Na** in spring
✓ No specific unique seasonal pattern
✓ **K>Na>Mg>Ca**

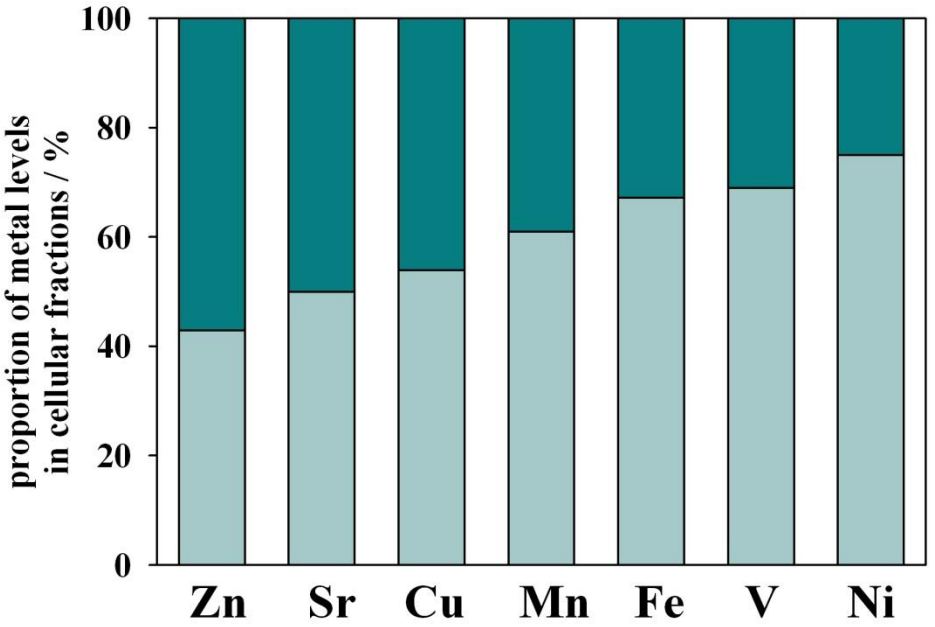


Comparison of metal levels in intestinal cytosolic fractions with:
a) Brown trout – **Zn>Fe>Rb>Se>Cu≥Mn>Ni≥Sr>Cd>Co≥Mo>As>Cs>V; K>Na>Mg>Ca**
b) European chub – **Zn>Fe>Cu>Mn>Cd; K>Na>Mg>Ca**

Zn>Fe>Rb>Cu≥Mn>Se>Cd>Sr>Ni>Mo≥As>Co>V≥Cs; K>Na>Mg>Ca

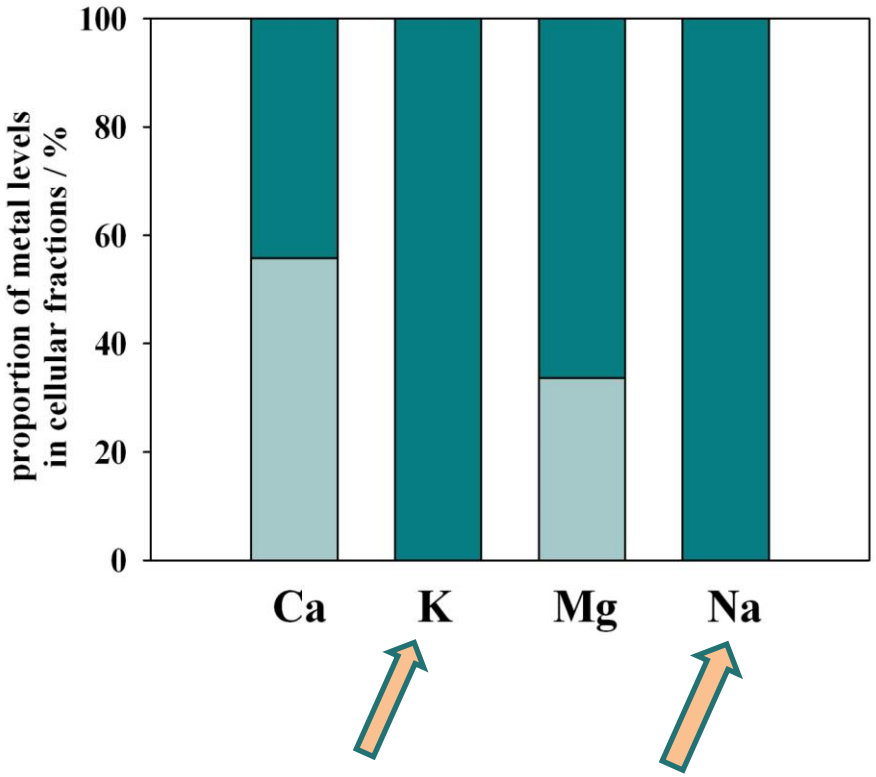
Trace elements





More than 60% of the total Rb, Se, Cd, Cs, As, Mo and Co concentrations were present in cytosols, where metals can bind to sensitive biomolecules and therefore possibly cause toxic effects → some part will still be detoxified by binding to heat-stable proteins (MTs and MT-like proteins)

Macroelements



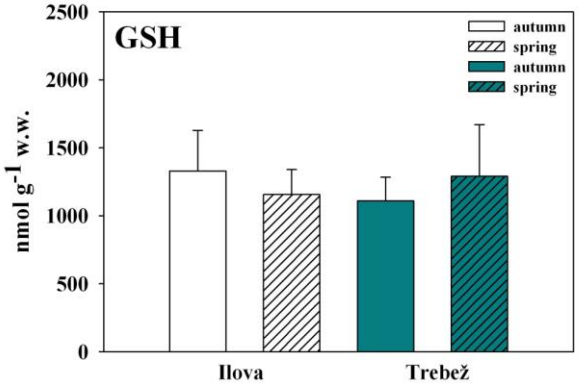
K and Na are mostly present in the cytosolic fraction as the main cations responsible for maintaining normal cytosolic osmolarity

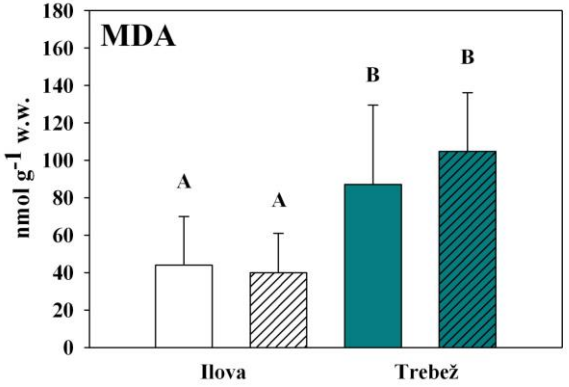
Total metal levels

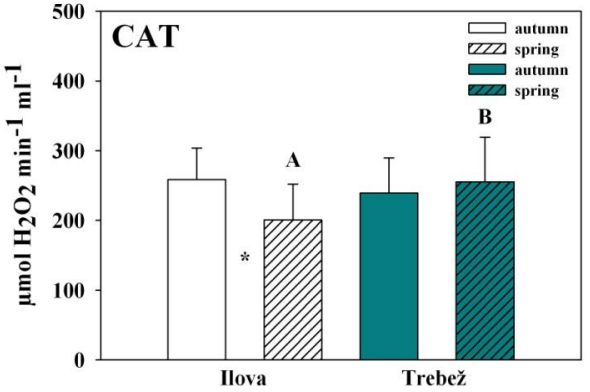
✓ mostly followed the trend of cytosolic metals, significantly **higher** levels of **Cd, Cs** and **Cu** at the **contaminated site** in both seasons; **Cd** and **Cs** significantly **higher** in **autumn** in both locations and **As, Co, Sr** and **V** in **spring**

✓ the same descending order of metal/metalloid concentrations as for the cytosolic concentrations

Biomarkers of oxidative stress and of antioxidative capacity







✓significantly higher MDA concentrations found in fish from the contaminated site, pointing to **oxidative stress**, possibly linked with mostly higher metal levels at the same site.

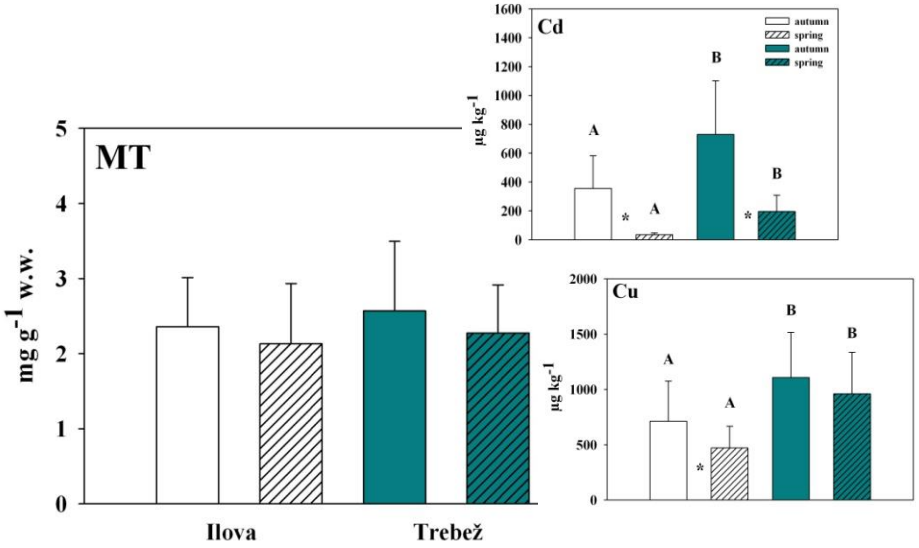
✓GSH did not show any clear trend, elevated CAT activity at the contaminated site in spring

Biomarkers of metal exposure (MTs)

✓ higher MT levels indicated higher fish **exposure to metals** at the contaminated site, but not significantly

✓ slightly higher induction in autumn at both locations

✓ levels of Cd and Cu, as one of the main MT inducers, significantly higher in autumn and at contaminated site



Conclusions

Prussian carp was shown as a suitable bioindicator species and the intestine as suitable bioindicator organ in aquatic environmental pollution assessment → example of using invasive, instead of native fish species in ecotoxicological studies.

- Metal concentrations mostly pointed to more disturbed environmental conditions at the contaminated site with significantly higher **cytosolic** concentrations of **Ca, Cd, Cs, Cu, Fe** and **Rb**, and **total Cd, Cs** and **Cu** concentrations, in **both seasons**.
- Most metals present in **cytosols** with **over 50%** of their total levels → **possible toxic effects.**
- **MTs** also indicated higher fish exposure to metals at the contaminated site compared to the reference site, but without significant differences.
- **MDA** values showed that fish at the contaminated site are exposed to higher levels of **oxidative stress** → **cell antioxidant system**, however, probably still works **effectively** in both locations (similar GSH levels and elevated activity of CAT).

The impact of wastewaters on the Ilova River still seems to be mostly moderate but it is of growing concern that both metals and MDA levels indicated the potential risk for the protected area of nearby Nature Park

Need of continuous monitoring of the region in order to protect the biota of the Ilova River itself and for a protection of nearby Lonjsko Polje Nature Park

HRZZ
Hrvatska zaklada za znanost

Croatian science foundation project: Accumulation, Subcellular Mapping and Effects of Trace Metals in Aquatic Organisms (AQUAMAPMET), project number: 4255