

Metal(loid) concentrations in fish muscle, intestine and acanthocephalans with respect to different pollution and environmental conditions

Zorana Kljaković-Gašpić
Institute for Medical Research and Occupational Health (IMROH)

THIRD PROJECT MEETING

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

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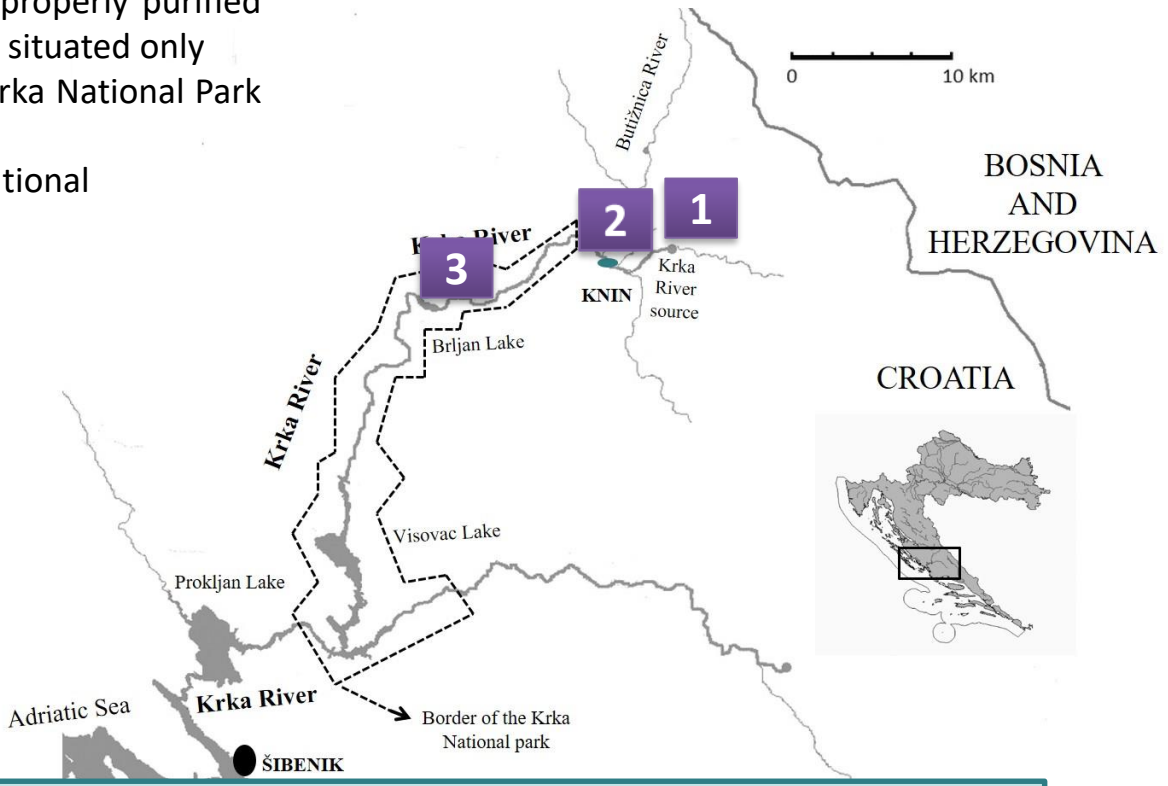
HrZZ Croatian Science Foundation

ANDRIJA STAMPAR TEACHING INSTITUTE OF PUBLIC HEALTH

BIOTOXMET

Study area and sampling locations

- Anthropogenic influence caused by unproperly purified industrial effluents from the screw factory situated only 2 km upstream of the beginning of the Krka National Park (KNP).
- Most of the watercourse proclaimed National Park in 1985.



Bioindicator organism:
brown trout (*Salmo trutta* Linnaeus, 1758)

Indicator organs/tissues:

- Muscle (as the site of Hg accumulation)
- Intestine → site of dietary metal uptake
- Intestinal parasites, acanthocephalans (as effective metal bioaccumulators)

Two seasons: spring and autumn 2021

Main goal

➢ To assess potential differences in accumulation of metal(loid)s under influence of wastewaters in the Krka River, using native fish brown trout (*Salmo trutta*) as biondicator organism

How?

- By measuring mass fractions of 25 metal(loid)s (Al, As, Ba, Ca, Cd, Co, Cr, Cs, Cu, Fe, Hg, K, Mg, Mn, Mo, Na, Ni, Pb, Rb, Se, Sr, Tl, U, V, Zn) in muscle of fish, intestine and intestinal parasites of trout
- Statistical analysis of the data

Histopathological changes in fish tissues

Multi-biomarker approach (oxidative stress: MDA; antioxidative capacity: GSH, CAT)

Indication of potential risks for the protected area of Krka National Park (KNP)

Contribution to the development of water management plans

Methods

Digestion with HNO_3 i H_2O_2 (85 °C; 3.5 h) (Laboratory for Biological Effects of Metals, IRB; in-house method):

1. Fish muscle: 0.3 – 0.35 g
2.50 mL HNO_3 + 1.25 mL H_2O_2

2. Fish intestine: 0.15 – 0.2 g
2.50 mL HNO_3 + 1.25 mL H_2O_2

3. Acantocephalans: 0.03 – 0.035 g
2.25 mL HNO_3 + 0.75 mL H_2O_2

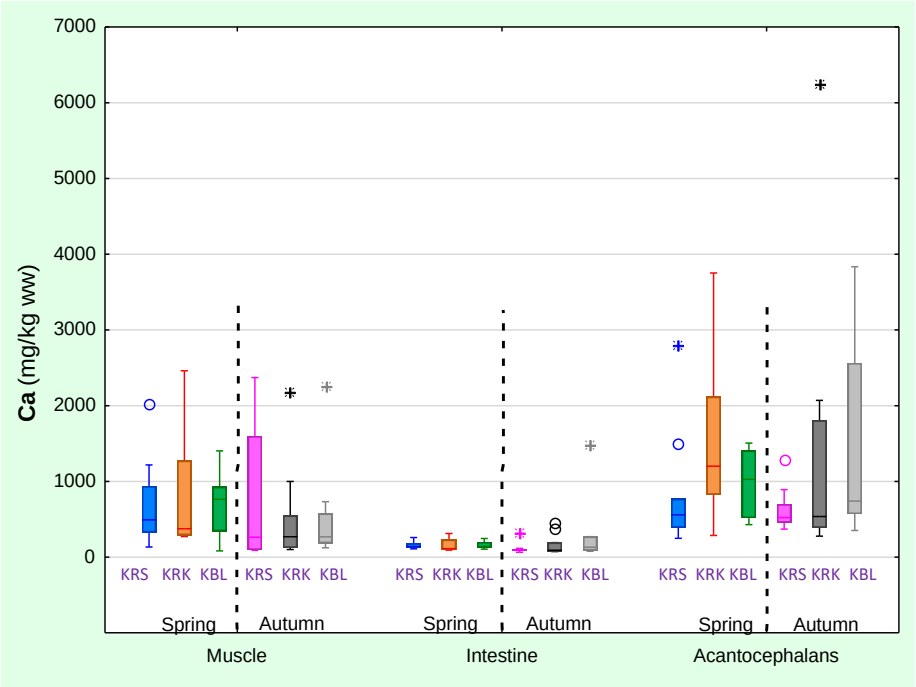
Determination of metal(loid)s (IMROH):

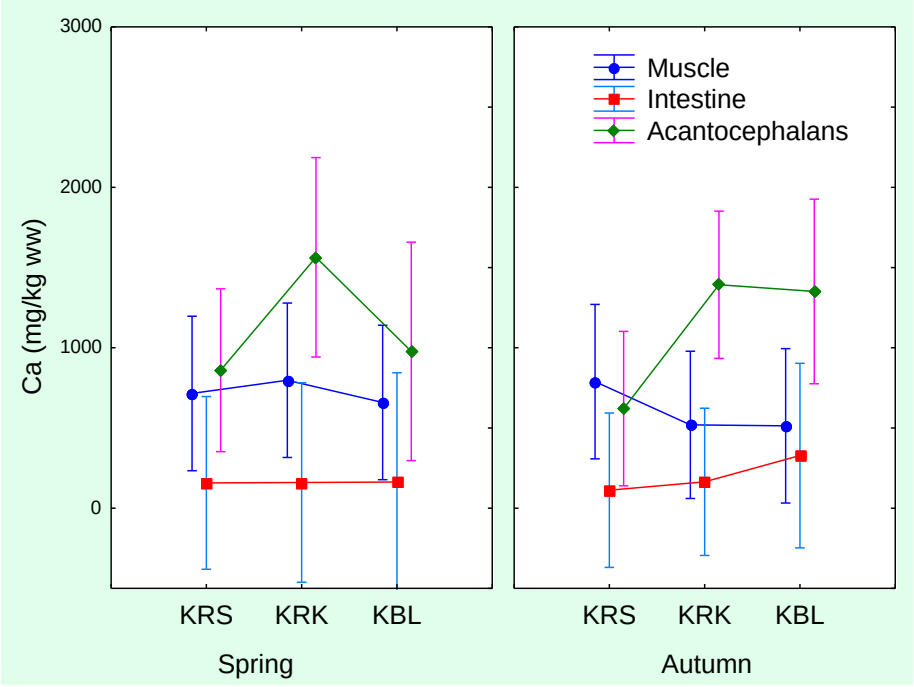
- inductively coupled plasma mass spectrometer (ICP-MS)

Agilent 8800

Agilent 7500

Metal(loid)s accumulation: *without statistically significant differences*



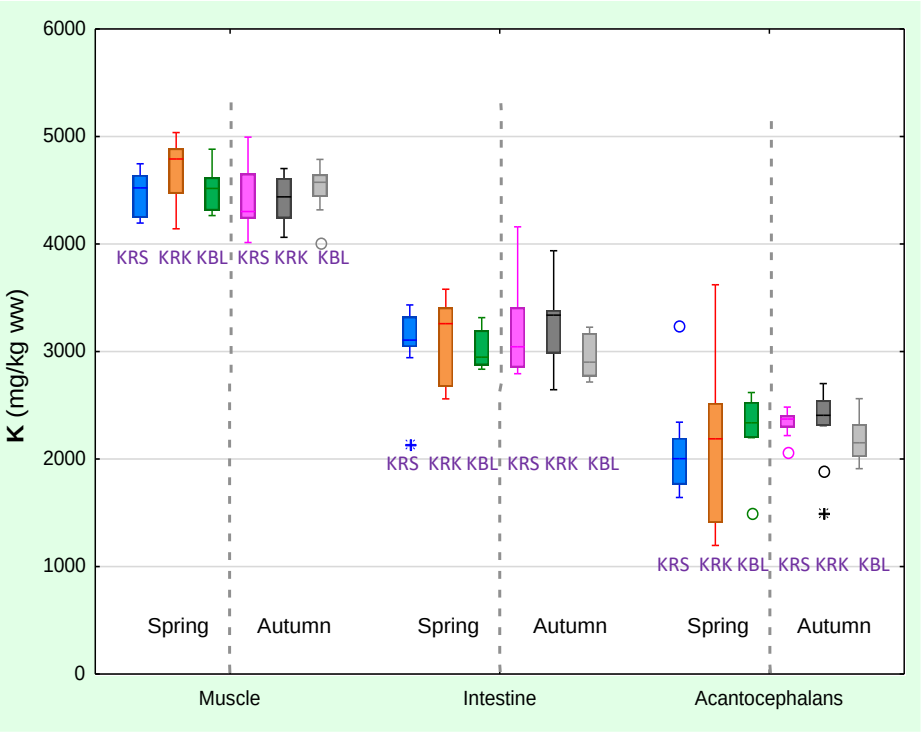


- **Ca: NO statistically significant differences:**
- between **tissues,**
- between **locations,**
- **or** between **seasons**

- **Ca did not** follow the patterns of metal concentrations in water samples, which increase downstream

Metal(loid)s accumulation: *statistically significant differences*

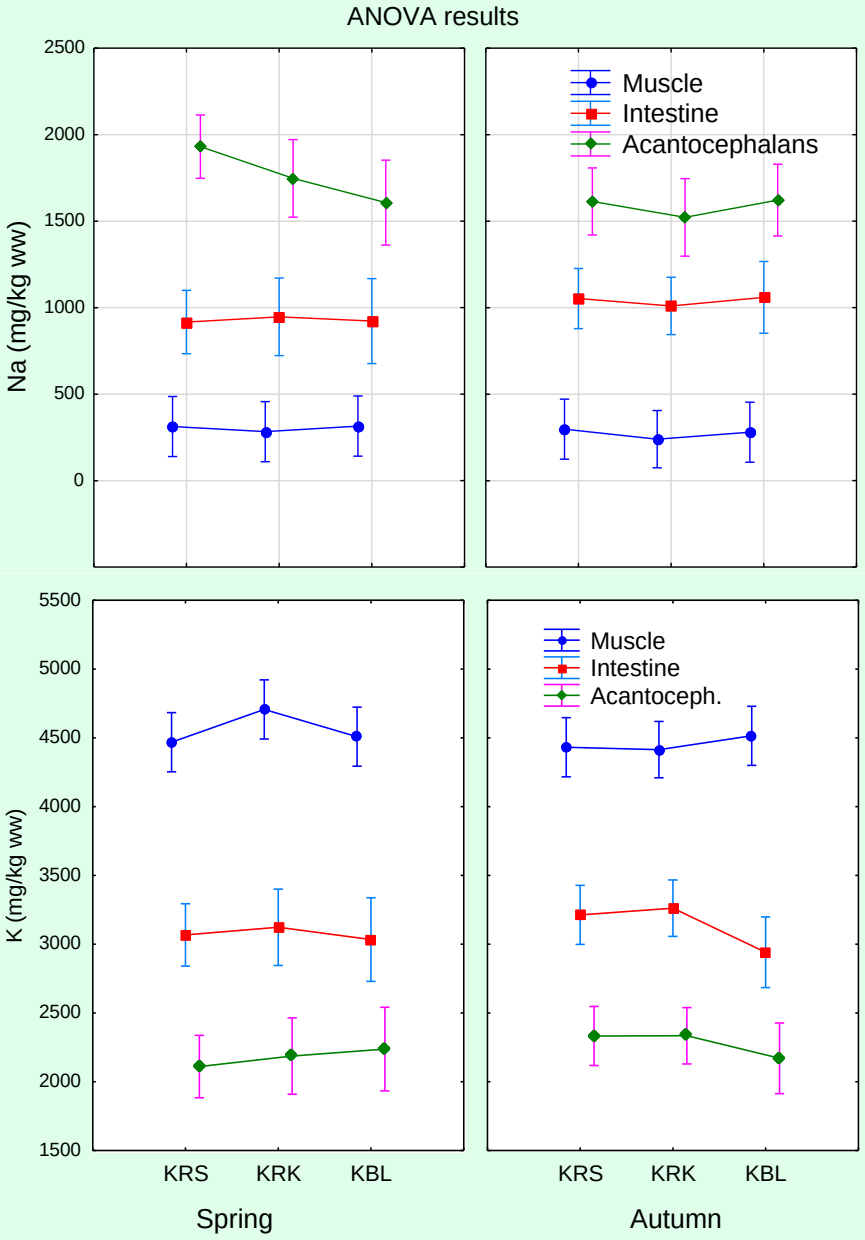
- Differences **only between tissues: K, Na**



K: Mus > Int > AC

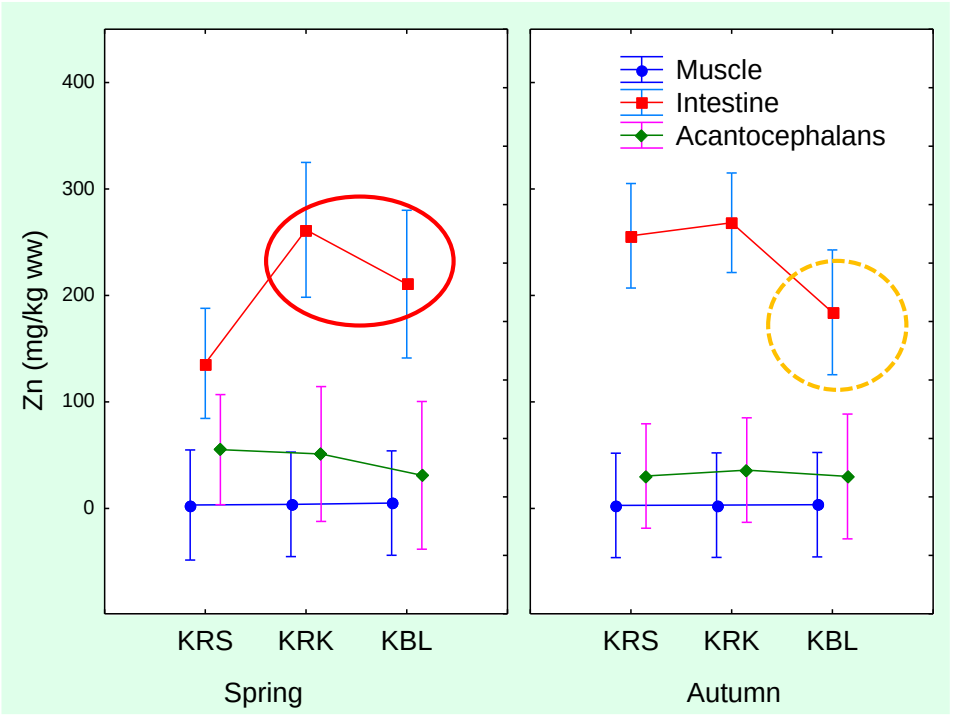
Na: AC > Int > Mus

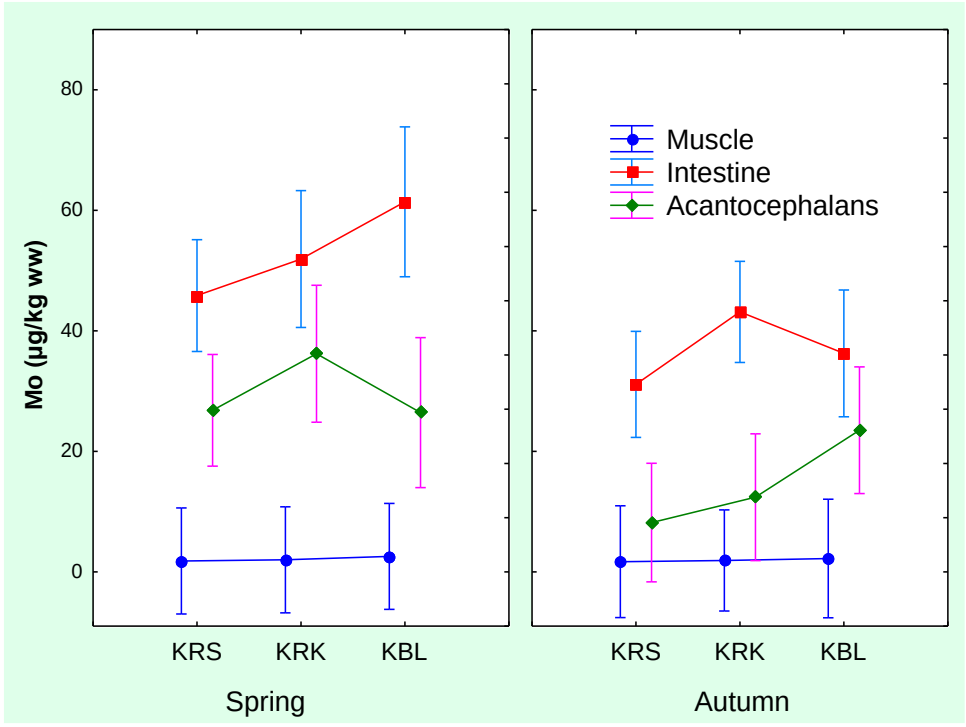
- **no spatial diffferences!!!**
- **no seasonal differences!!!**



Metal(loid)s accumulation: *statistically significant differences*

- Differences between tissues combined with season and location:
- ✓ Metal(loid)s whose concentrations in the **intestines** were **higher than in other tissues**: Zn, Mo





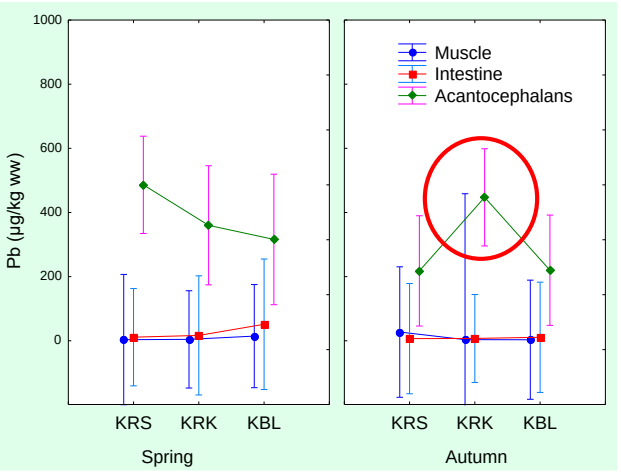
Zn: Int > Mus, AC in both seasons

- Spatial difference: KRK, KBL > KRS only in spring

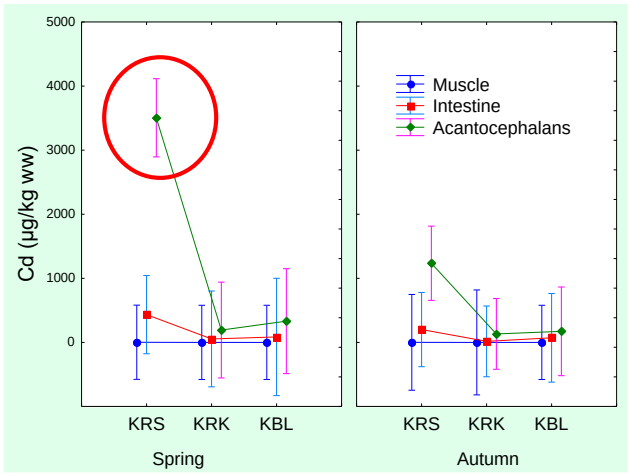
Mo: Int > AC > Mus in spring
Int > AC, Mus in autumn
no spatial differences

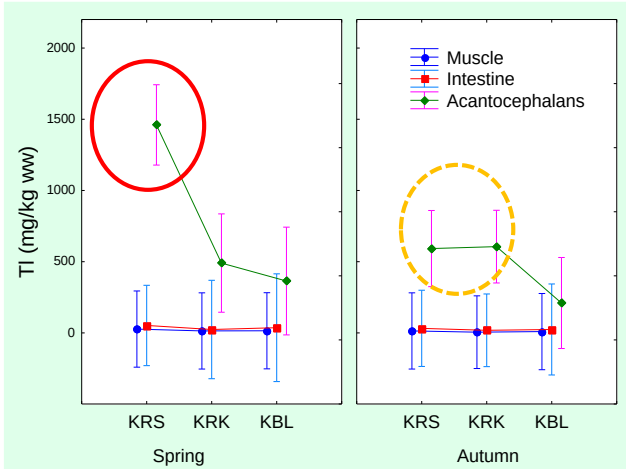
- **Zn and Mo** followed the patterns of metal concentrations in water samples

Significant differences: metal(loid)s with mass fractions **higher in parasites**

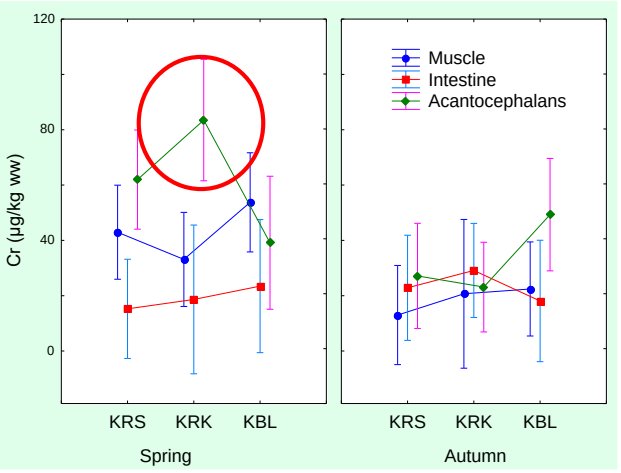


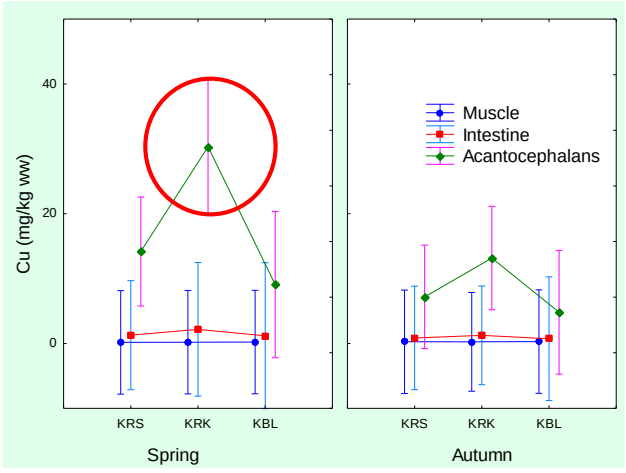
Pb: AC > Int, Mus
No spatial or seasonal differences!!!



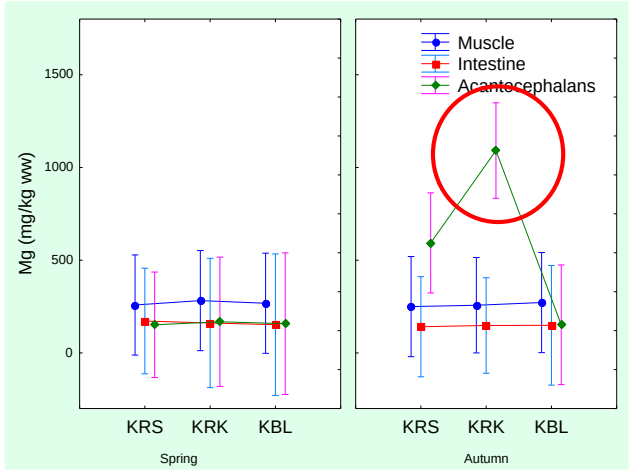


Tl, Cd: in Acantocephalans - KRS >KRK, KBL only in spring





Cr, Cu: in AC - KRK > KRS, KBL only in spring

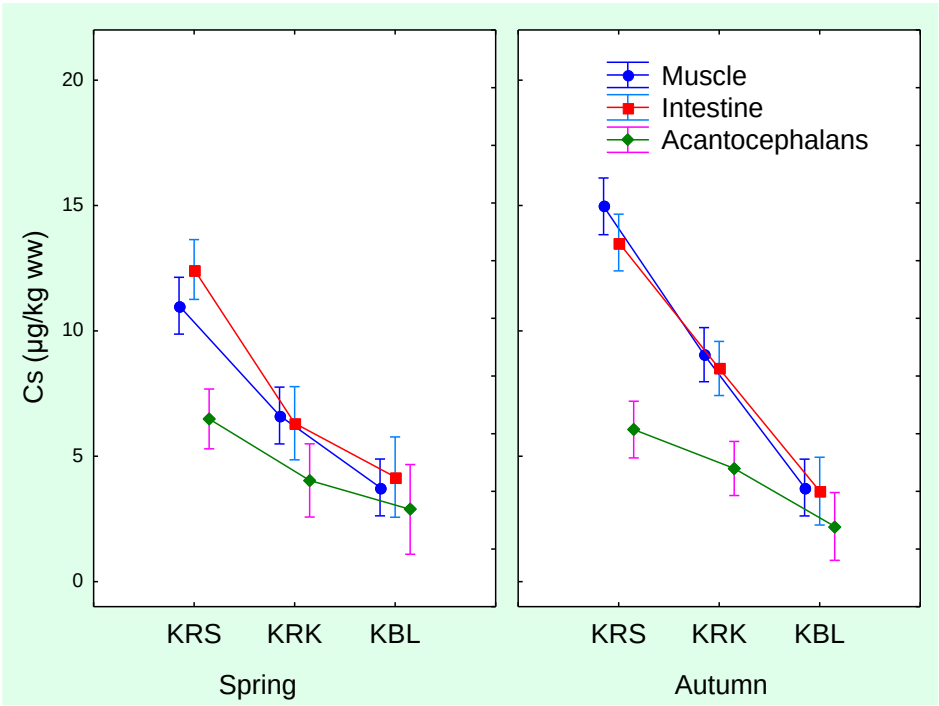


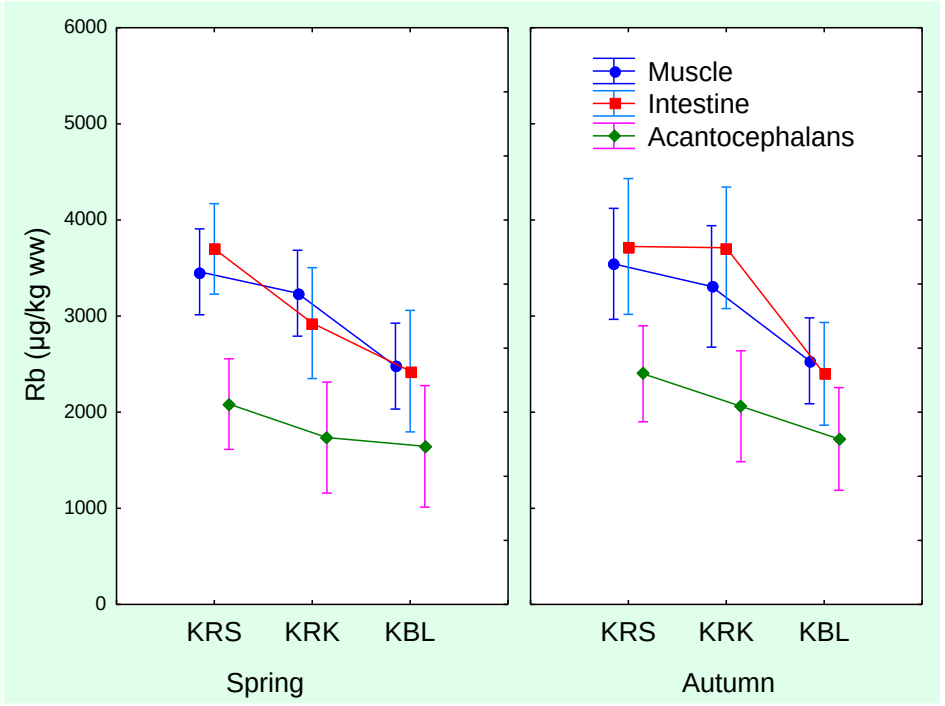
Mg: in AC - KRK > KRS, KBL only in autumn

Cd, Tl: mass fractions followed the pattern of concentrations in water
Cr, Cu, Mg: Mass fractions **did not follow** the patterns of metal concentrations in water samples

Significant differences: clear **spatial differences**

Cs, Rb: **KRS > KRK >KBL** → mass fractions **decrease** downstream

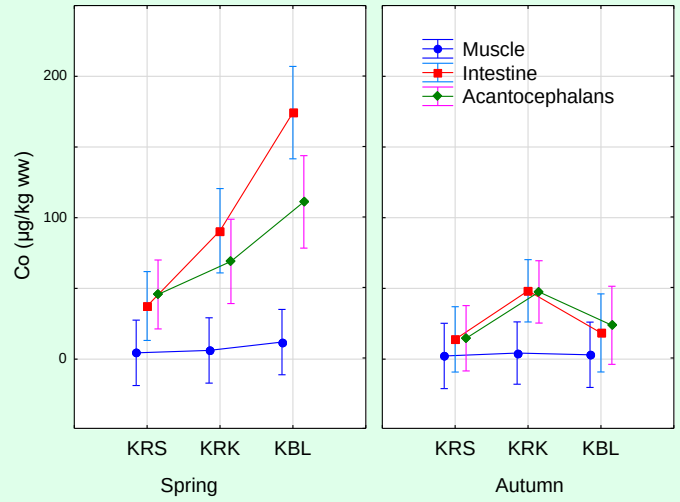


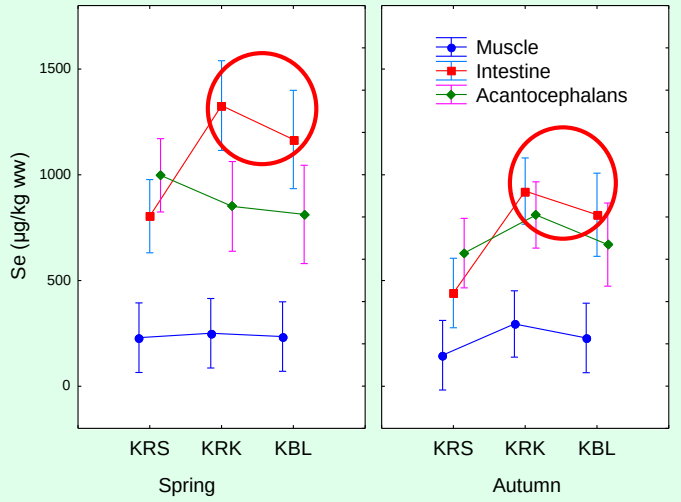


- We should compare them with water and sediment values

Significant differences: metal(loid)s whose mass fractions **increase** downstream

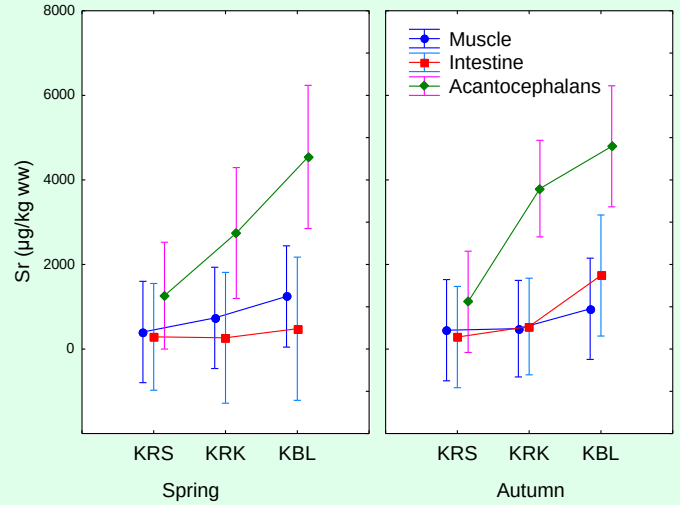
Co: Int, AC
KRS < KRK < KBL
→ **increase**
downstream
only in spring

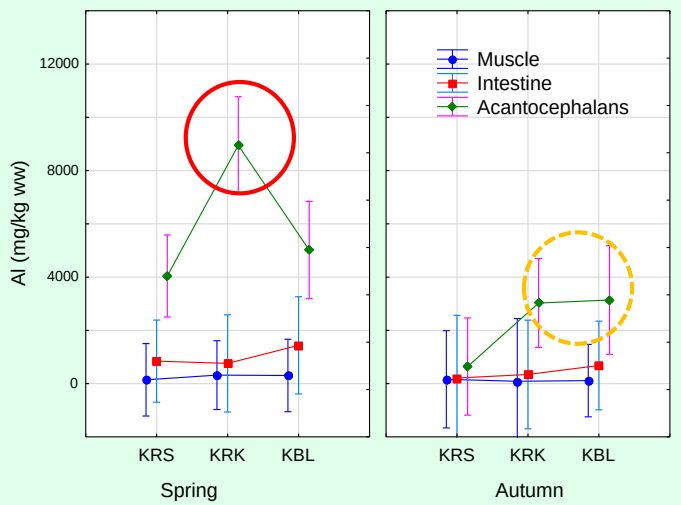




Se: Intestine
KRS < KRK, KBL
→ **Downstream**
increase

Sr: AC
KRS < KRK < KBL
→ **increase**
downstream in
both seasons

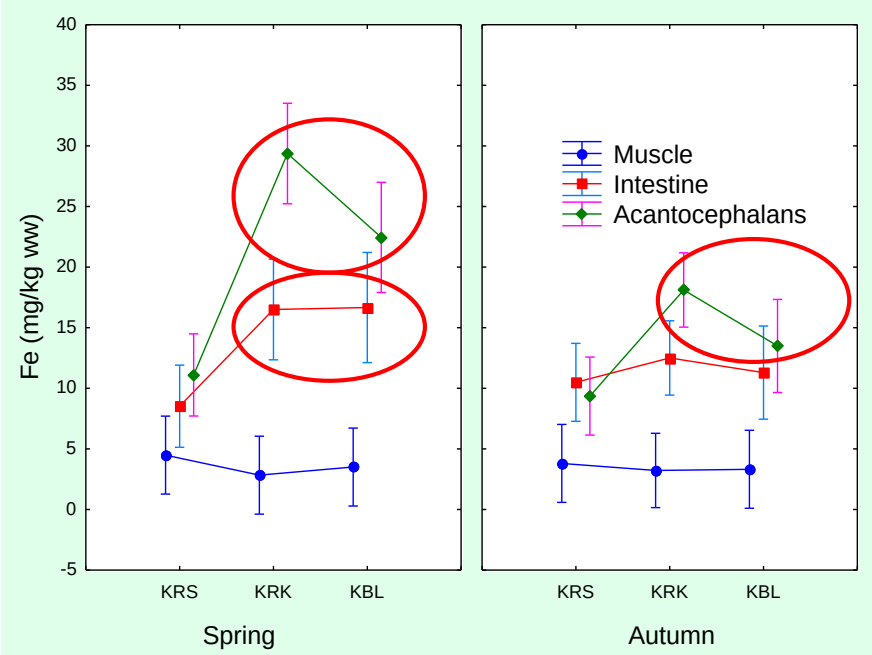




Al: AC
KRS < KRK > KBL
only in spring!!

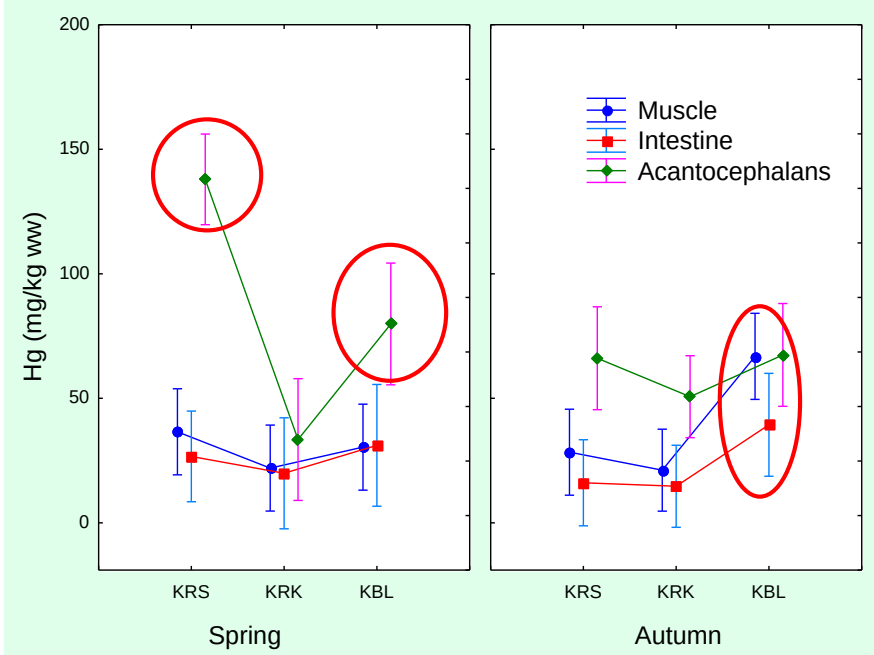
Sr: Mass fractions followed the pattern of concentrations in water
Co: Mass fractions **did not follow** the patterns of metal concentrations in water samples

Significant differences: metal(loid)s whose mass fractions **increase** downstream



Spring: KRK, KBL > KRS in Intest and AC
Autumn: KRK, KBL > KRS only in AC

Fe: Mass fractions mostly followed the pattern of concentrations in water, especially in spring



Spring: KRS > KBL > KRS in AC (similar to Cd and Tl)
Autumn: KBL> KRS, KRK in muscle!!! and intestine

Hg: we could not compare the values with concentrations in water since they were close to or below the DL of the method

- Mass fractions in tissues/organisms followed the pattern of concentrations in sediment

Conclusions

- Metal mass fractions in Acantocephalans and intestine in lot of cases pointed to more disturbed environmental conditions in fish from the contaminated site (KRK) with significantly higher concentrations of **Al**, **Co**, **Cr**, **Cu**, **Fe**, **Se, Sr** and **Zn** than in fish from KRS → wastewater impact!
- Values clearly showed that fish at **KBL** are also exposed to higher levels of greater part of metal(loid)s → impact on biota even in the Krka National Park.

↓

The impact of wastewaters still seems to be mostly moderate but it is of growing concern that some metal(loid)s indicated the potential risk for the protected area of Krka National Park

↓

Need of continuous monitoring of the region in order to protect the biota of the Krka River itself and for a protection of KNP