BIOMARKERS OF ANTIOXIDATIVE DEFENSE AND METABOLIC TISSUE CAPACITY IN FISH INTESTINE

THIRD PROJECT MEETING

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

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BIOMARKERS

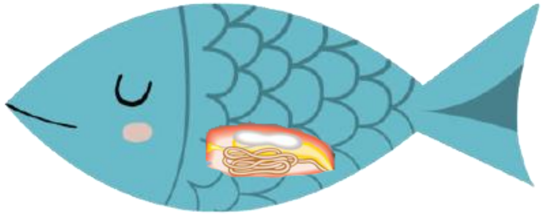
Multibiomarker approach

– **changes** in **cellular structures** or **functions** that reflect an interaction between a biological system and a potential chemical, biological or physical harmful factor

MULTIBIOMARKER APPROACH – necessary in environments exposed to **complex mixtures of contaminants** → to assess the biological responses of the organisms that inhabit them

Brown trout (*Salmo trutta* Linnaeus, 1758)

- fish **intestine** was evaluated as an indicator organ responsible for dietborne uptake

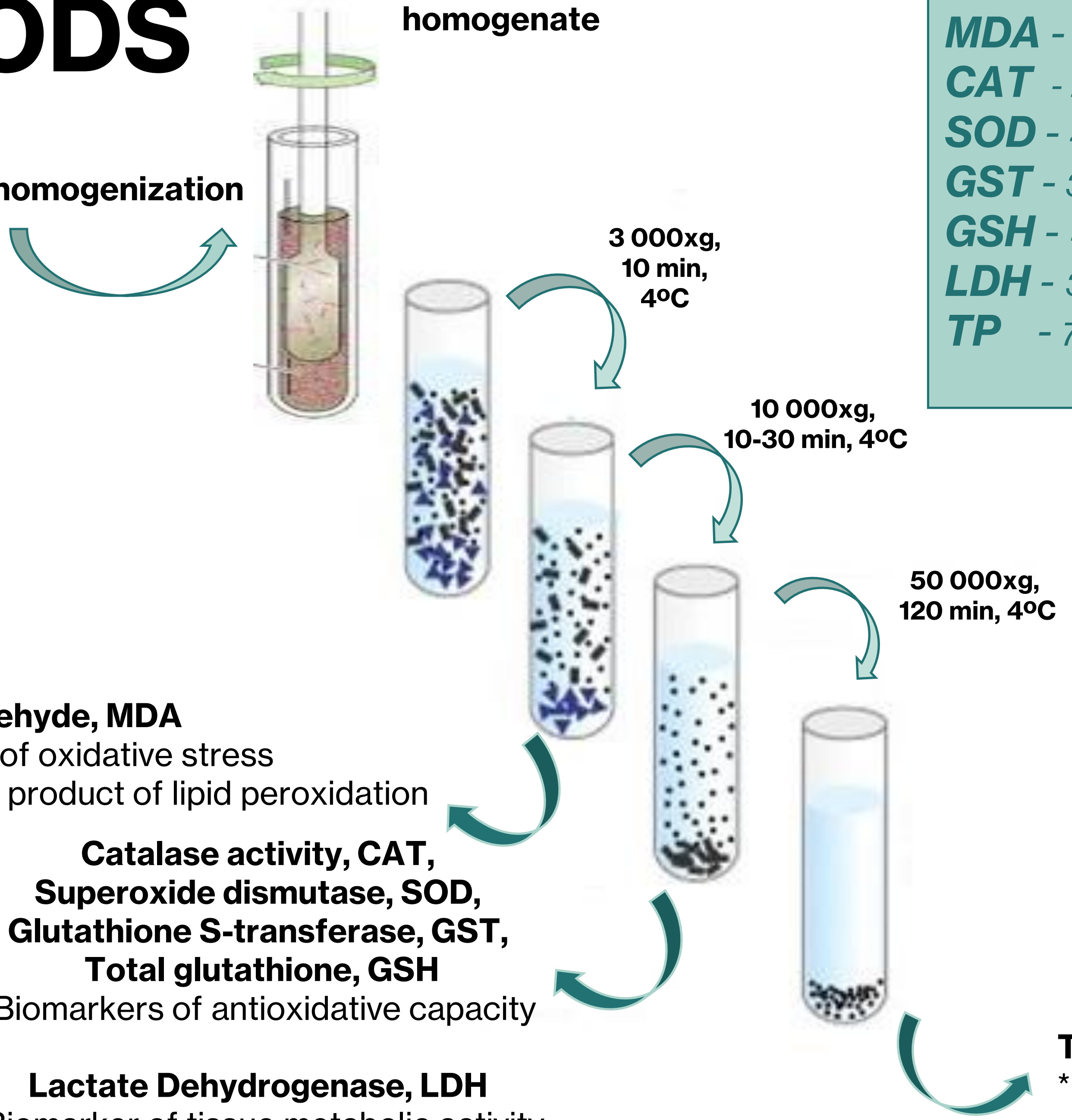


METHODS

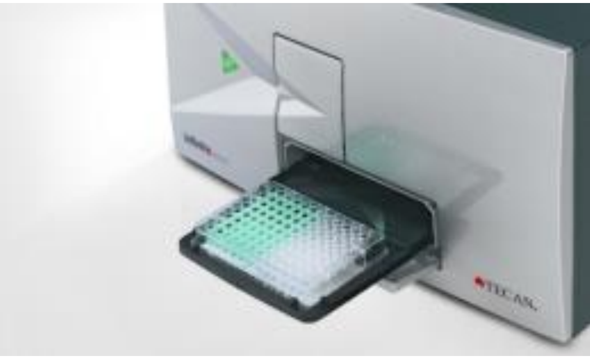
Fish intestine

homogenization

homogenate



MDA - *535 nm*
CAT - *240 nm*
SOD - *420 nm*
GST - *340 nm*
GSH - *412 nm*
LDH - *340 nm*
TP - *750 nm*



spectrophotometrical measurements according to the protocols

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

Catalase activity, CAT,
Superoxide dismutase, SOD,
Glutathione S-transferase, GST,
Total glutathione, GSH
*Biomarkers of antioxidative capacity

Lactate Dehydrogenase, LDH
*Biomarker of tissue metabolic activity

Total proteins, TP
*Biomarkers of general stress

LOCATIONS

- Krka River source (**KRS**) - a reference site

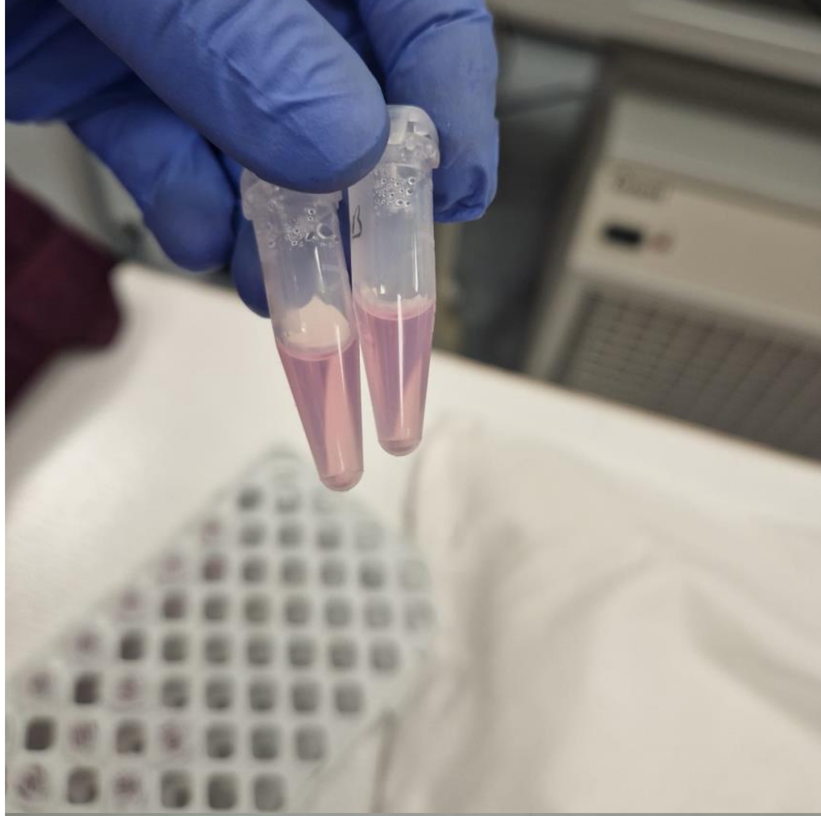
- location downstream of municipal wastewaters from the Town of Knin (**KRK**)

- location in the Krka National Park, Brljan Lake (**KBL**)

BIOMARKERS OF OXIDATIVE STRESS

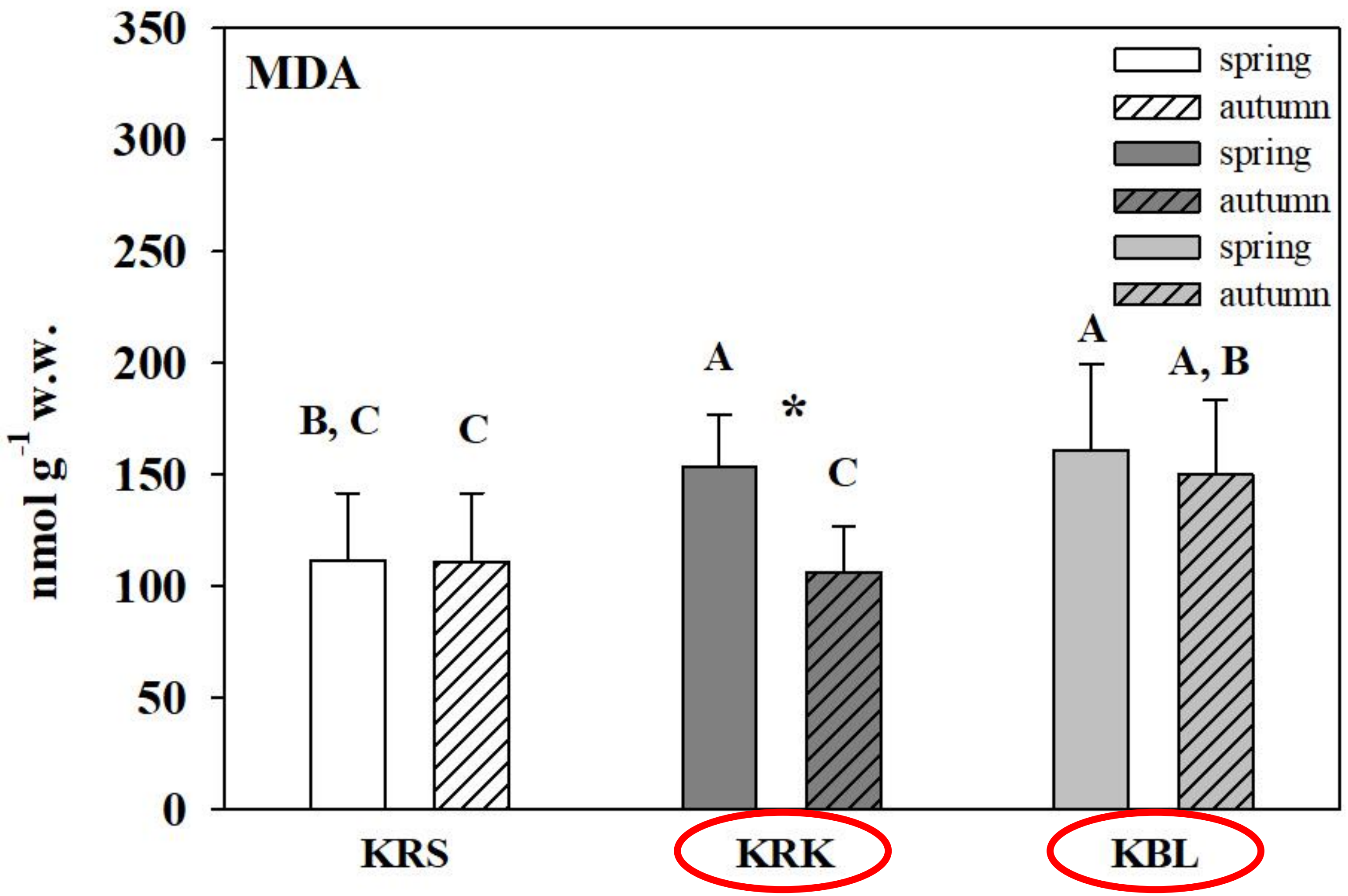
MDA – indicator of lipid peroxidation, oxidative damage

increased MDA levels → higher oxidative stress



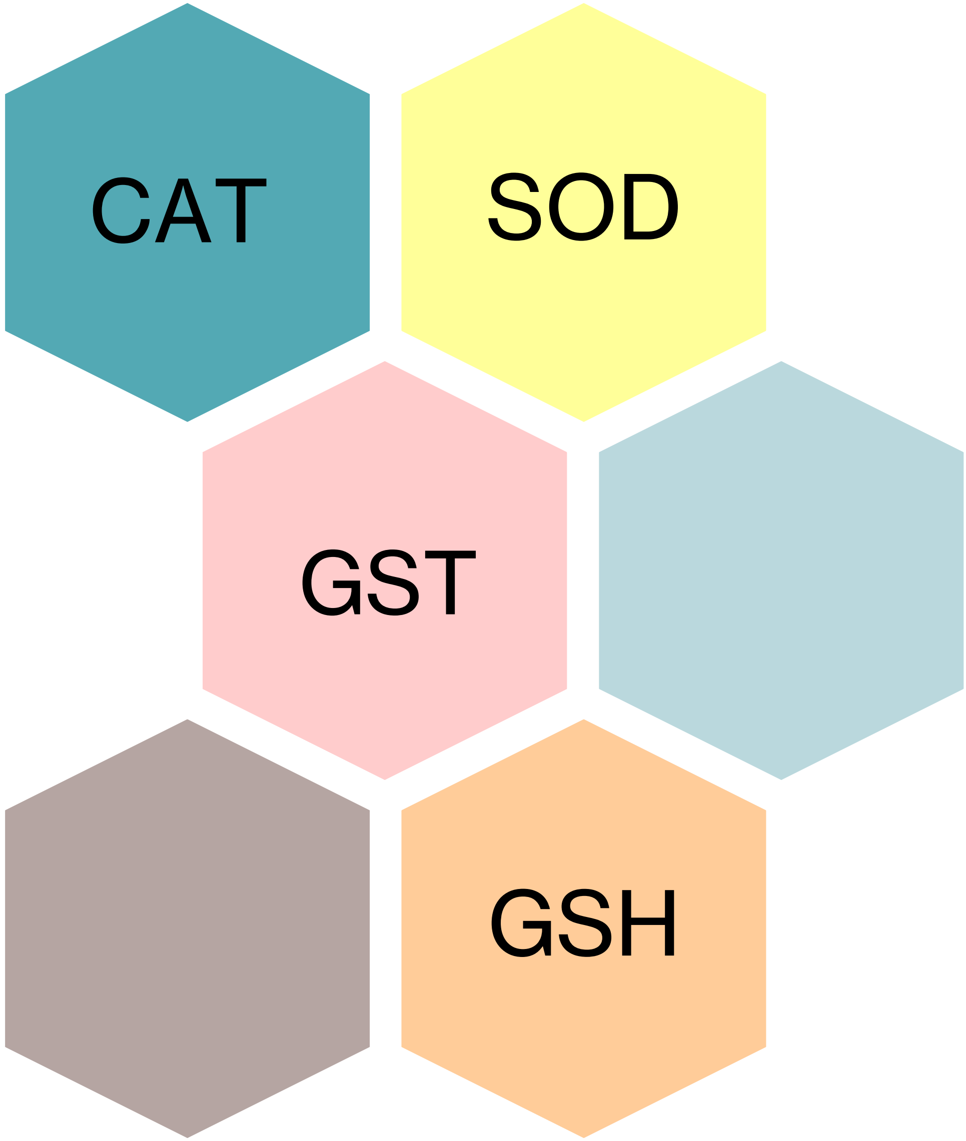
MDA

- the **highest level** of oxidative stress **in the Krka NP** in both seasons!
- significantly higher levels of MDA in KRK than KRS in spring
- higher MDA values in spring than autumn at KRK and KBL, no seasonal differences at KRS



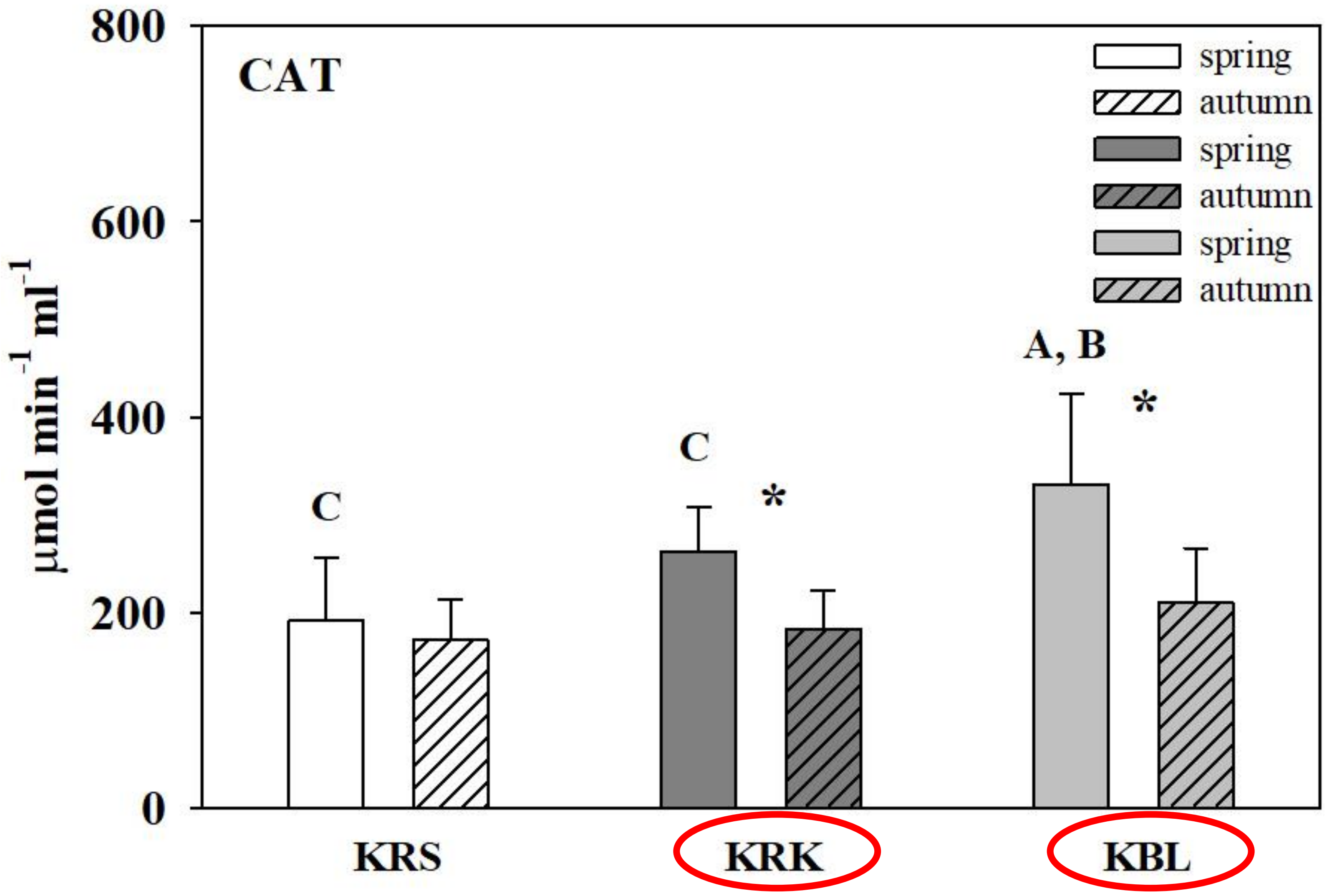
BIOMARKERS OF ANTIOXIDANT CAPACITY

- CAT and SOD – the primary defense against the toxic effects of superoxide radical in aerobic organisms
- GST and GSH – exposure to environmental chemicals, potential biomarkers for sewage water pollution monitoring



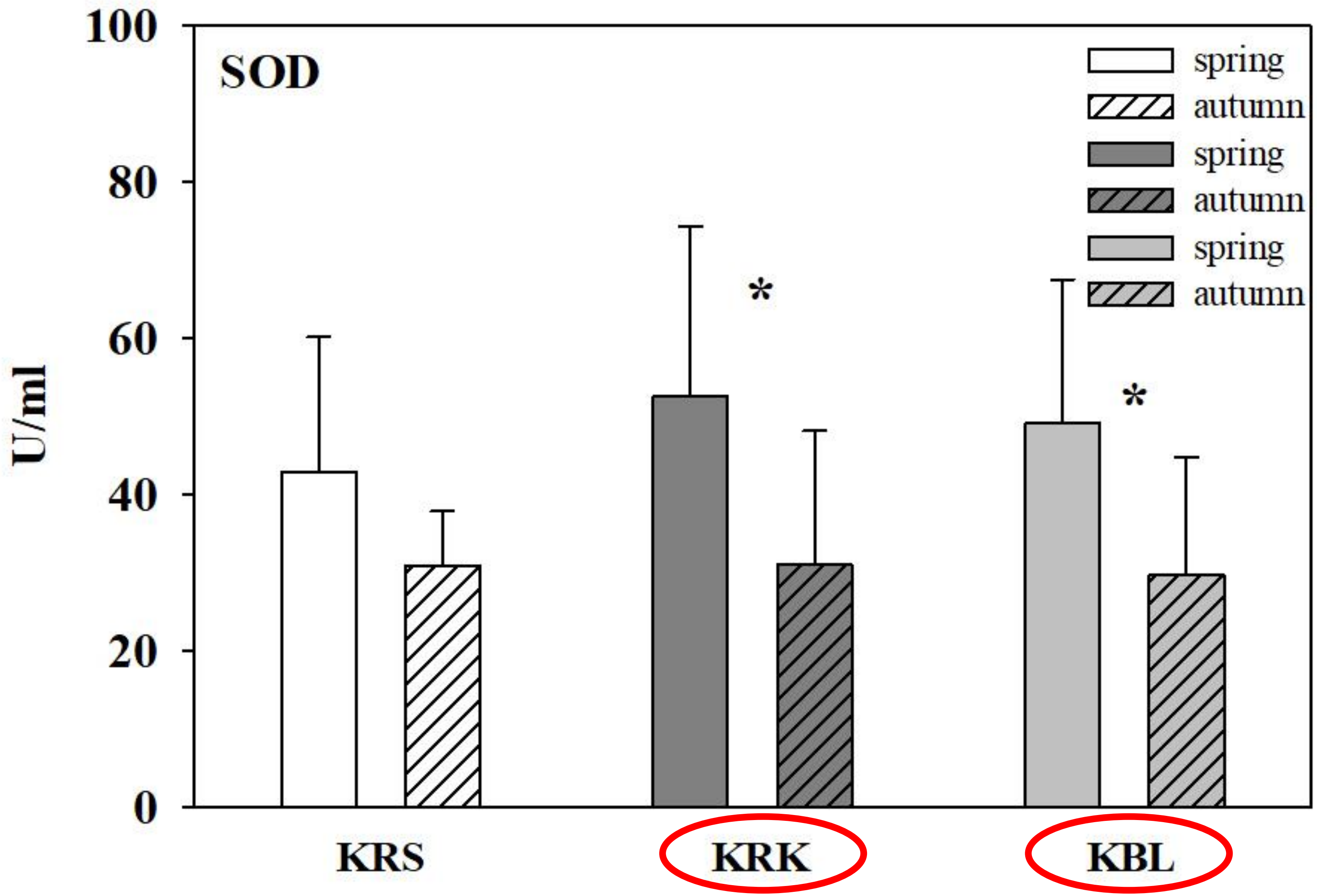
CAT

- significantly higher levels of oxidative stress in spring at KBL comparing to KRK and KRS
- trend of higher CAT levels at KRK comparing to KRS in spring
- no differences between locations in autumn
- CAT activity was higher in spring than autumn, significantly at KRK and KBL



SOD

- higher levels of oxidative stress in spring at KRK and KBL comparing to KRS (not significantly)
- no evident differences between locations in autumn
- SOD activity was higher in spring than autumn, significantly at KRK and KBL

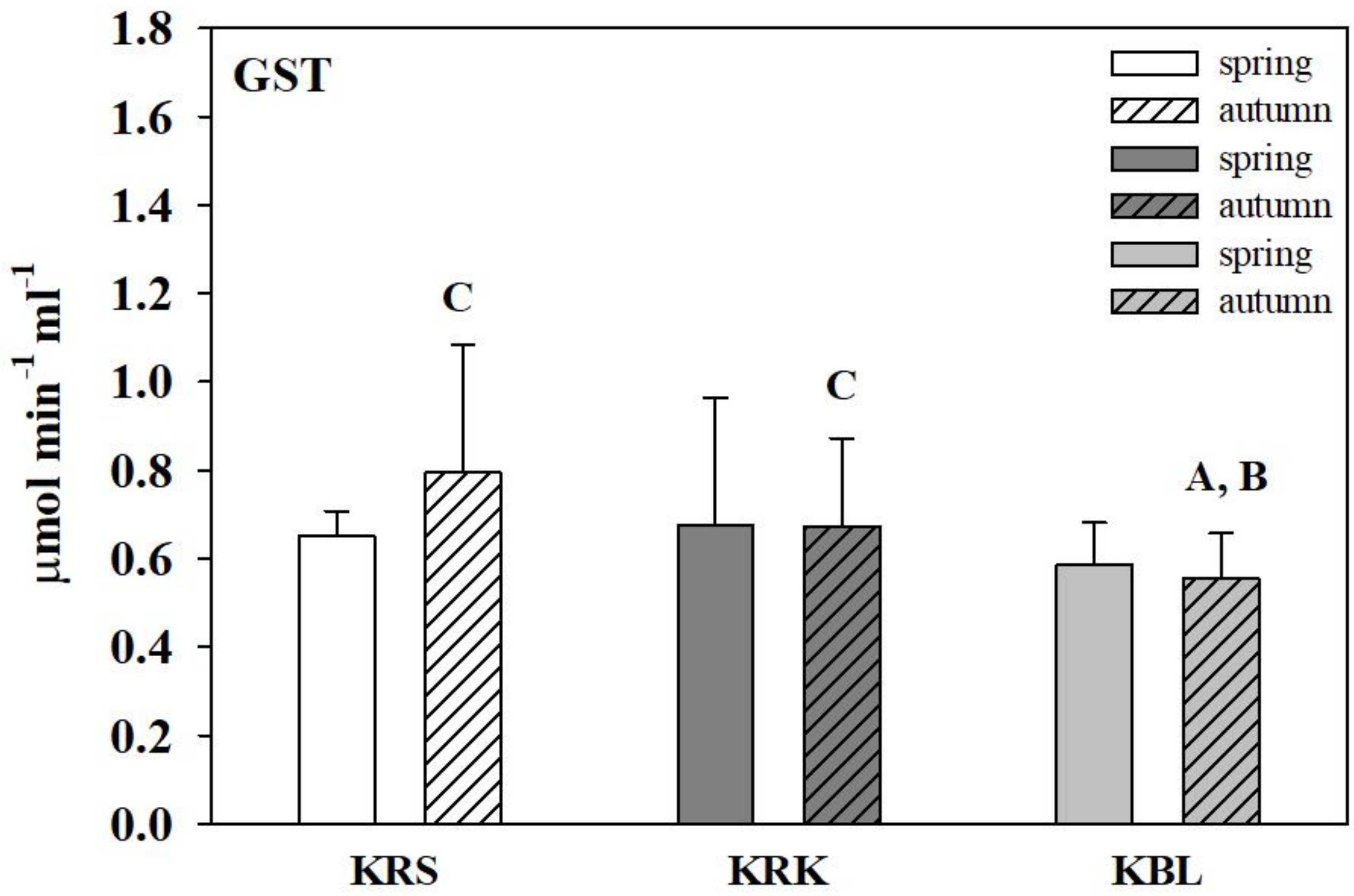


GST

- the highest level at KRS and the lowest at KBL in autumn

- comparable levels in spring between locations

- no unique seasonal trend

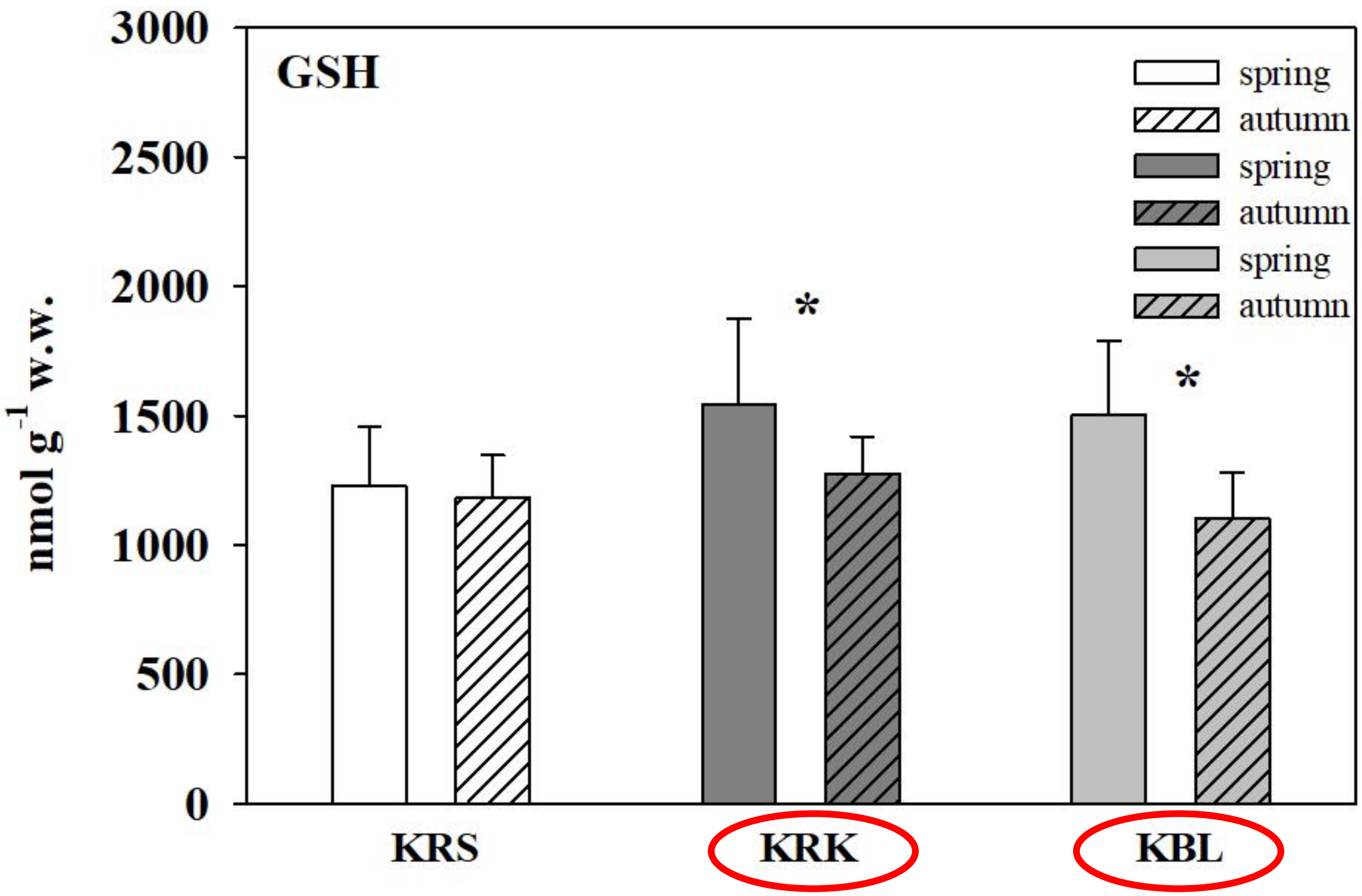


GSH

- higher levels in spring at KRK and KBL comparing to KRS (not significantly) → similar trends as for SOD and CAT

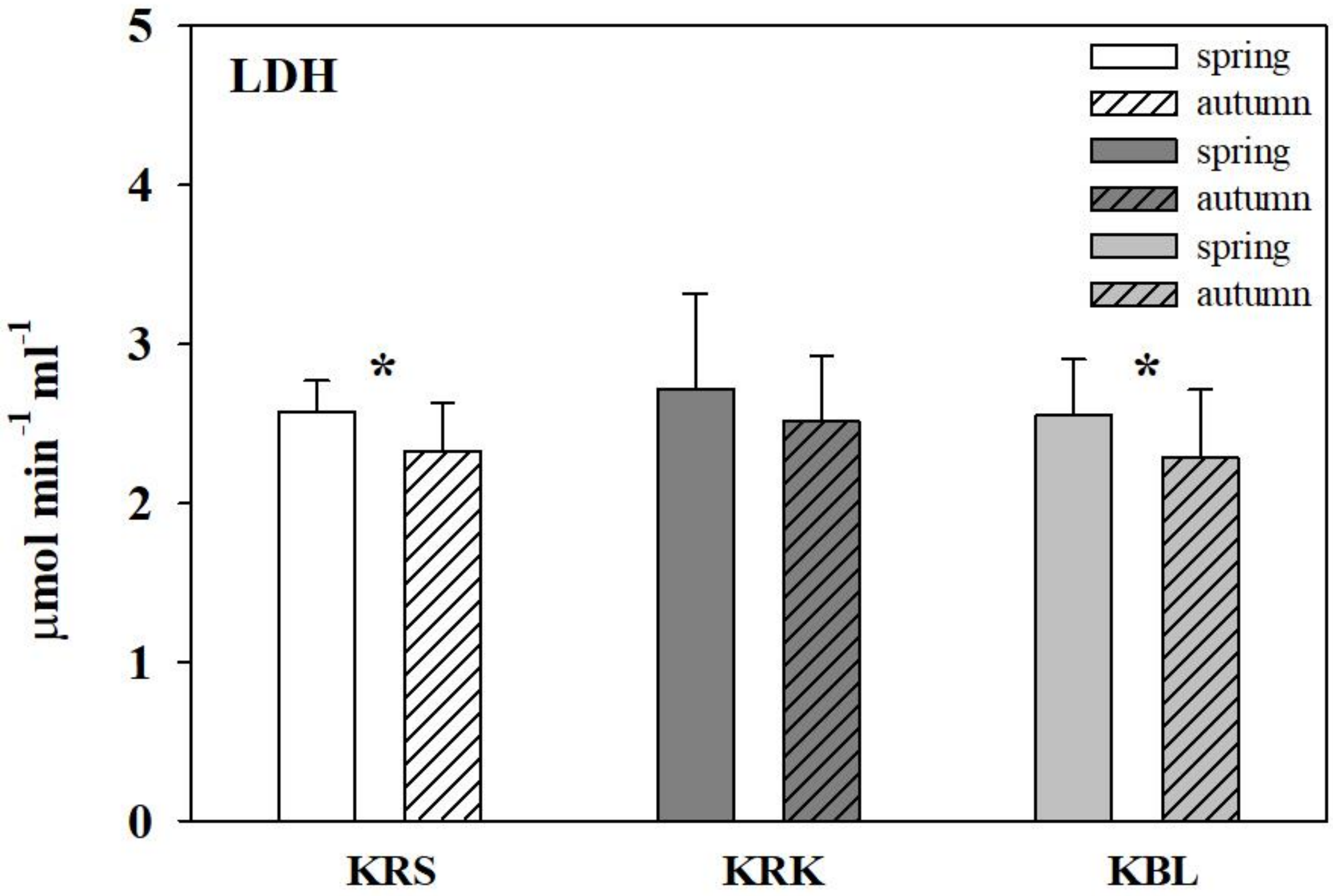
- no differences between locations in autumn

- GSH values were higher in spring than autumn, significantly at KRK and KBL



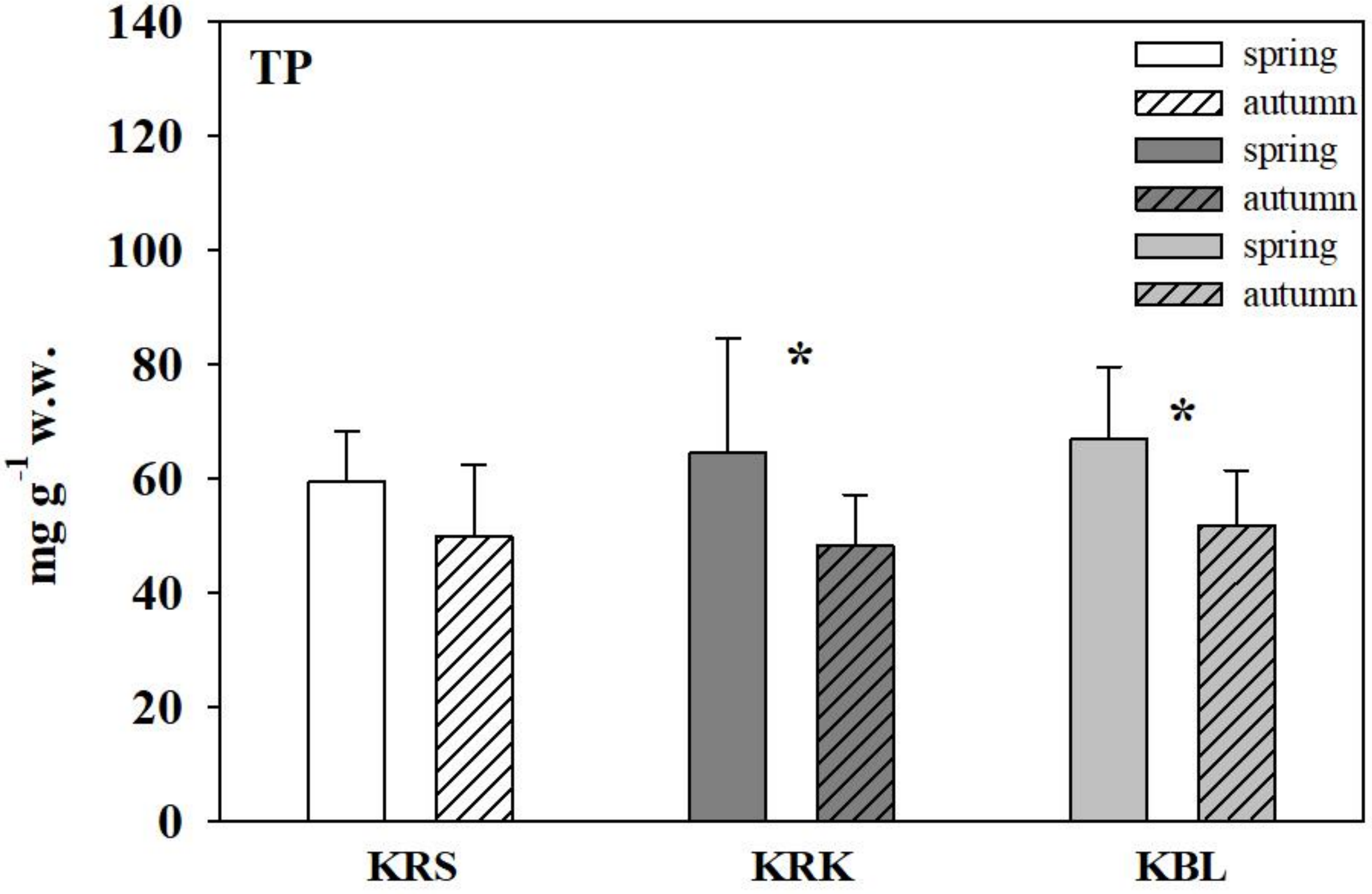
BIOMARKER OF TISSUE METABOLIC ACTIVITY

- similar values at all 3 locations in both seasons
- LDH activity was higher in spring than autumn, significantly at KRS and KBL
- no significant metabolic changes in LDH at any location (usually, elevated LDH expression in response to pollutants → possible indicator for the screening of a chemicals)



BIOMARKERS OF THE GENERAL STRESS

- no significant spatial differences observed
- TP levels were higher in spring than autumn, significantly at KRK and KBL
- the most general marker influenced by a number of biotic and abiotic factors → couldn't be directly connected to pollution



CONCLUSIONS

intestinal biomarkers pointed to rising need of strict monitoring of water quality

higher concentrations of MDA confirmed **higher level** of **oxidative stress**, even at the KBL location → **negative influence** of pollution in the Krka NP

the antioxidant system (CAT, SOD, GSH) is still active

no significant metabolic damage according to LDH activity

QUESTIONS?