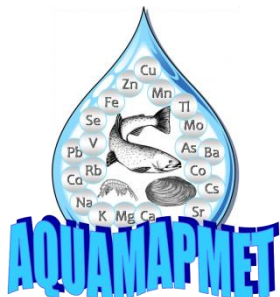


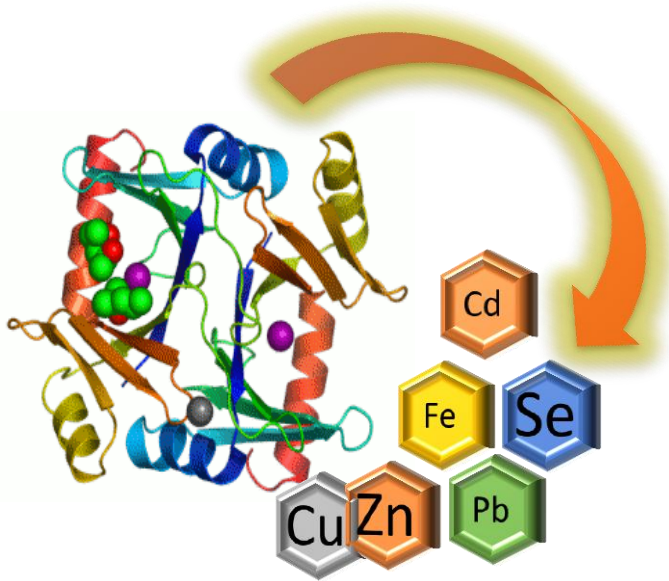
Cu, Cd, Zn, Fe and Tl distributions in liver of brown trout (*Salmo trutta* Linnaeus, 1758) and Prussian carp (*Carassius gibelio* Bloch, 1782) analyzed by use of SEC-HPLC and HR ICP-MS



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Metals in organisms

- metals play an important role in the chemistry of life
- metal ions can either be part of the structure of proteins, constituting so-called metalloproteins, or below molecular weight biomolecules (metalo-biomolecules) which are involved in numerous biological processes that occur in cells and tissues



- it is important to understand which metalloproteins and/or metallo-biomolecules are involved in the different biological processes to achieve a better understanding of the modes of transport and distribution of metals

- metal contamination is one of the major concerns in environmental chemistry and biochemistry since they are non-biodegradable primary food entering trophic chains in the aquatic ecosystem
- depending on their concentration non essential (Cd, Pb,V) but also essential trace metals and metalloids such as Cu, Co, Fe, and Zn can cause toxic effects on living organisms

Metal toxicity:

- binding of metals to essential biomolecules such as enzymes and transporters
- involvement of certain metals in the formation of radicals

→ part of metal bioaccumulated within the organism can also be detoxified



Metalomics

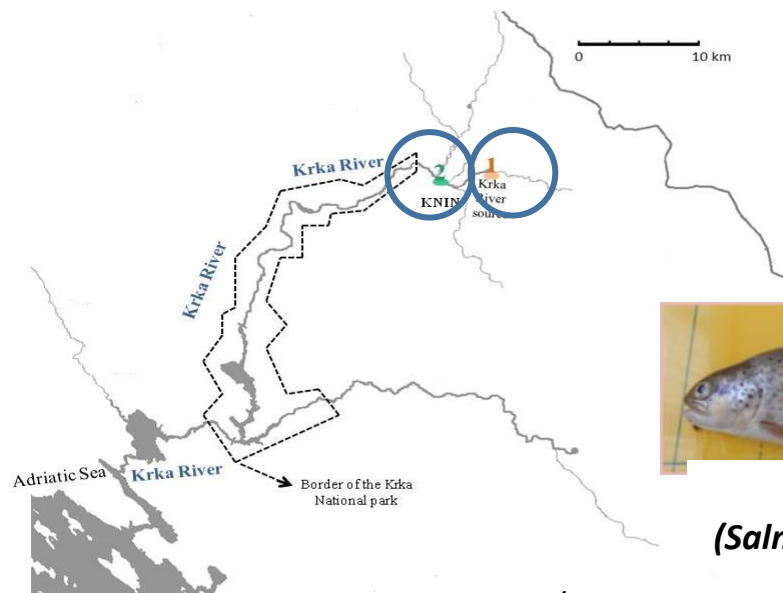
- answer questions related to the role, assimilation, transport and storage of metals in biological systems

Metalomics is defined as the analysis of the entirety of metal and metalloid species within a cell or tissues, whereas **metalloproteomics** focuses on exploration of the function of metals associated with proteins

Assessment of metal exposure in the aquatic environment

- fish are often used as **indicator species** of pollutant exposure in the aquatic environment

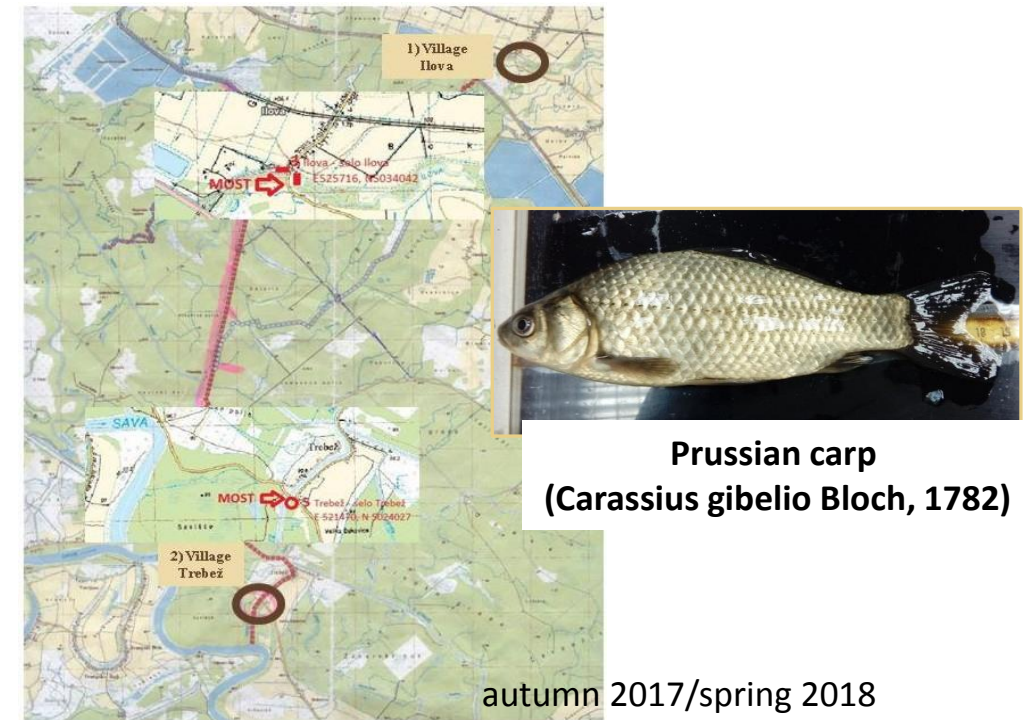
Krka River



Brown trout
(*Salmo trutta* Linnaeus, 1758)

autumn 2015/spring 2016

Ilova River



Prussian carp
(*Carassius gibelio* Bloch, 1782)

autumn 2017/spring 2018

Indicator organ → liver

-target organ because of it's importance in the detoxification of toxic substances

OBJECTIVES

To investigate and identify the molecular masses of cytosolic biomolecules that bind Cu, Cd, Zn, Fe and Tl in the **liver**

- brown trout (*Salmo trutta* Linnaeus, 1758), as a representative fish species and important bioindicator of karstic rivers
- Prussian carp (*Carassius gibelio* Bloch, 1782) in the lowland Ilova River

Experimental work

HEPATIC TISSUE HOMOGENISATION

20 mM Tris buffer, pH 8.6 at 4 °C
5 mM DTT



CENTRIFUGATION

50000 x g, 2 h, 4 °C



Obtained **S50 fraction (cytosol)**
contains metals bound to
biomolecules of different
molecular masses

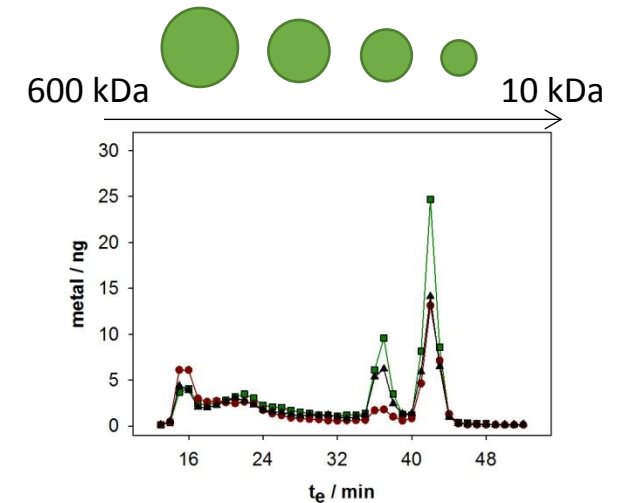
Column: Superdex™ 200 10/300 GL
Mobile phase: 20 mM Tris buffer
Flow rate: 0.5 mL/min
UV detection: 254 and 280 nm



SEC-HPLC

HR ICP-MS

Metal distribution among biomolecules of different molecular masses in the liver cytosol

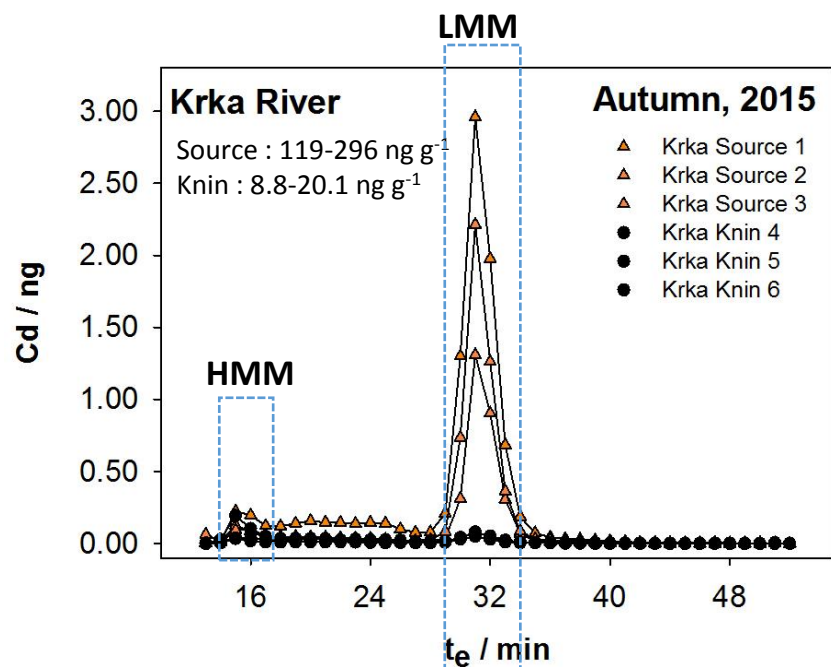


SEC-ICP-MS- offline

Protein standards used for column calibration

Thyroglobulin	669 kDa
Apoferritin	443 kDa
Amylase	200 kDa
Alcohol dehydrogenase	150 kDa
Albumin	66 kDa
Carbonic anhydrase	29 kDa
Metallothionein (MT)	13.3-6.6 kDa

Cd



Four main protein categories:

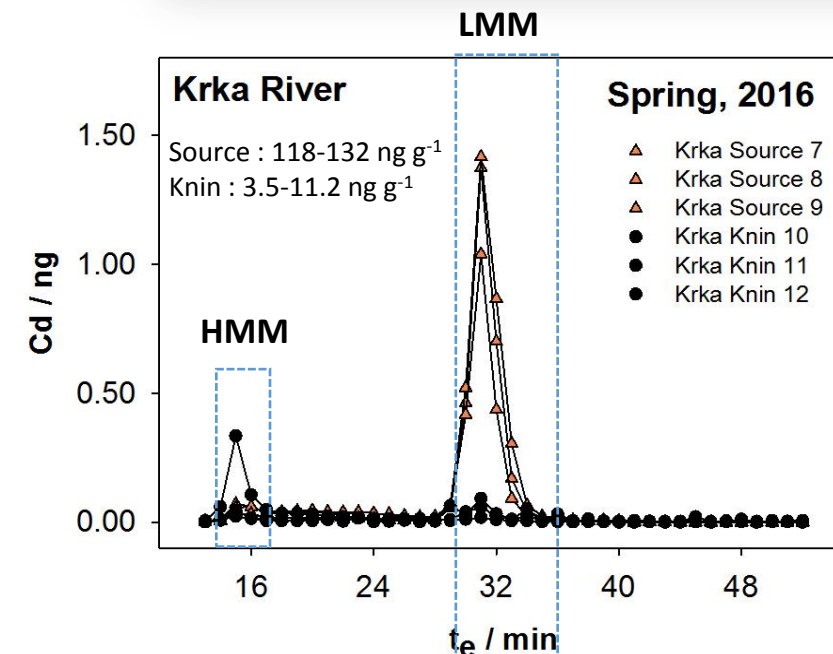
HMM (high MM proteins; >100 kDa)

MMM (medium MM proteins; 30–100 kDa)

LMM (low MM proteins; 10–30 kDa)

VLMM (very low MM proteins; <10 kDa)

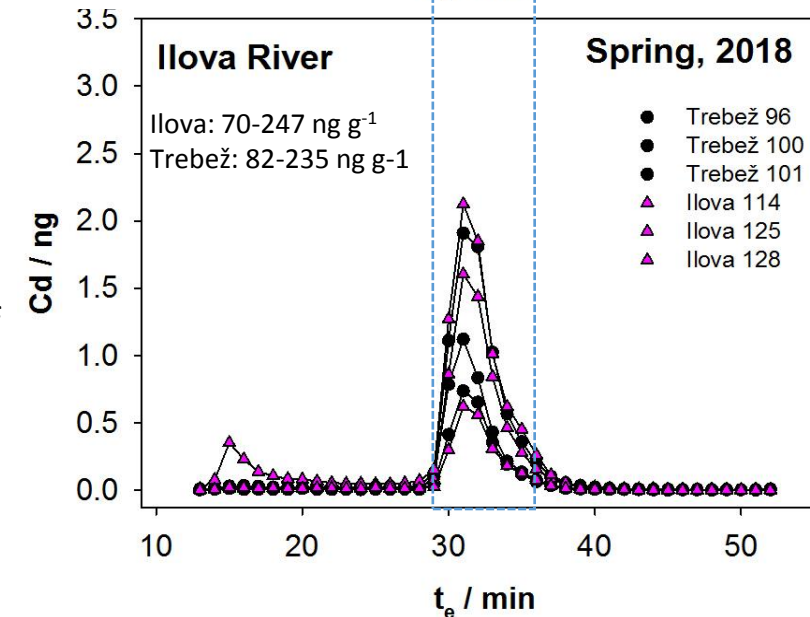
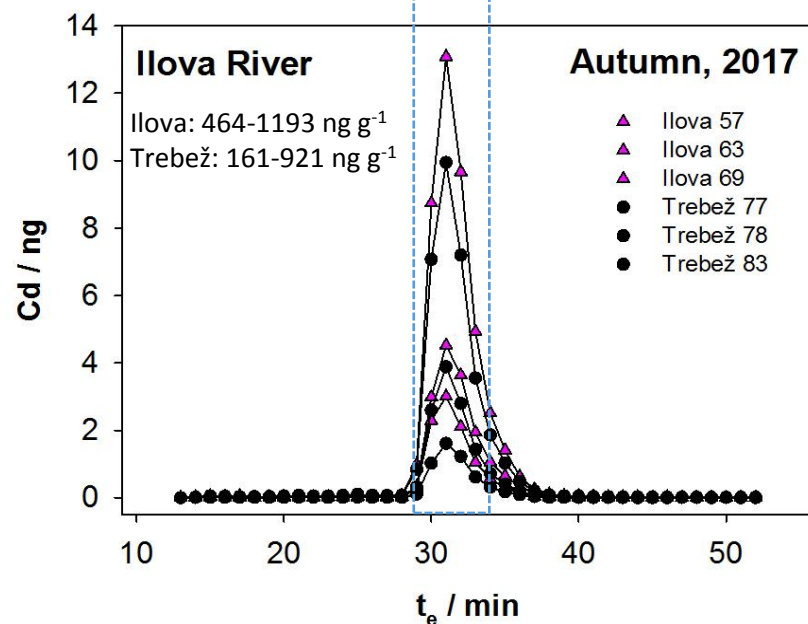
HMM biomolecule region
>500 kDa

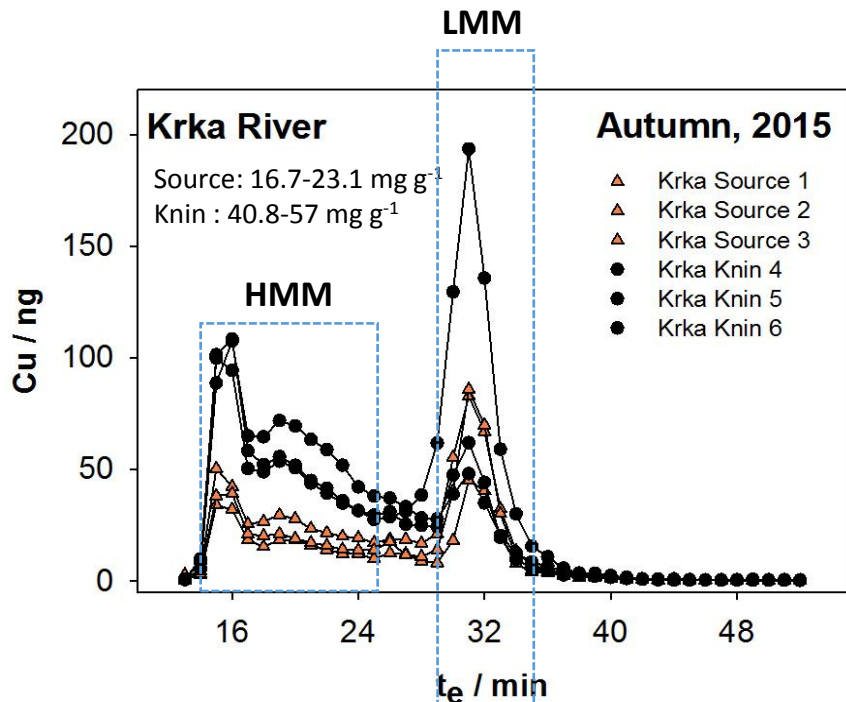
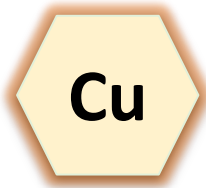


LMM biomolecule region
5- 24 kDa, maximum ~15 kDa

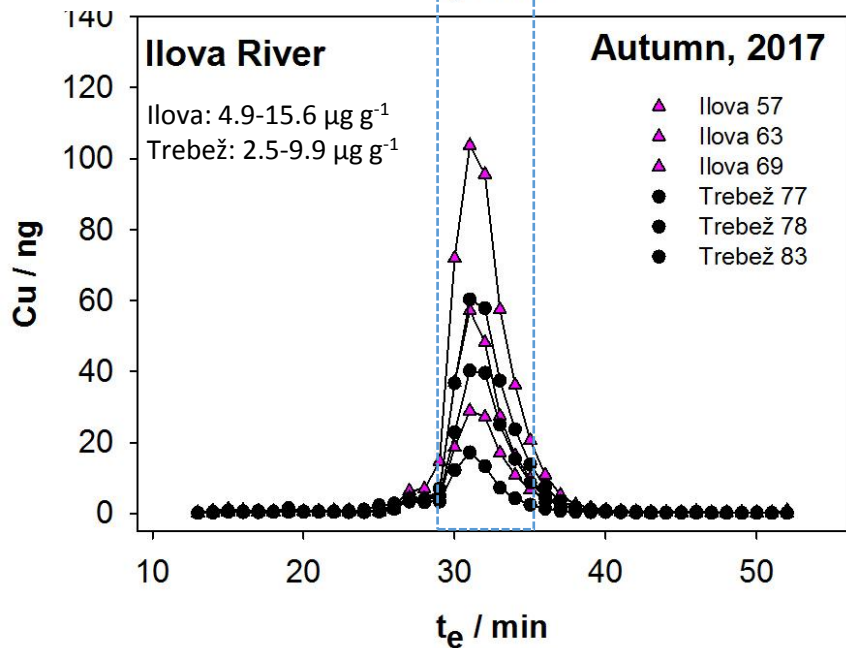
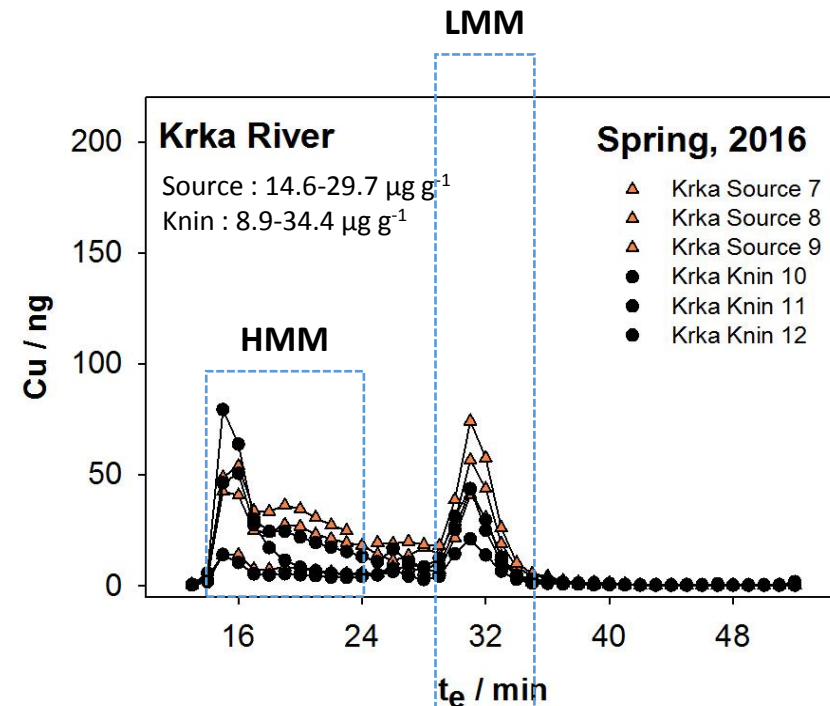
MTs (metalotionein)

low molecular mass cytosolic proteins
responsible for homeostasis of
essential (Zn, Cu) and detoxification of
toxic (Cd, Ag, Hg) metals

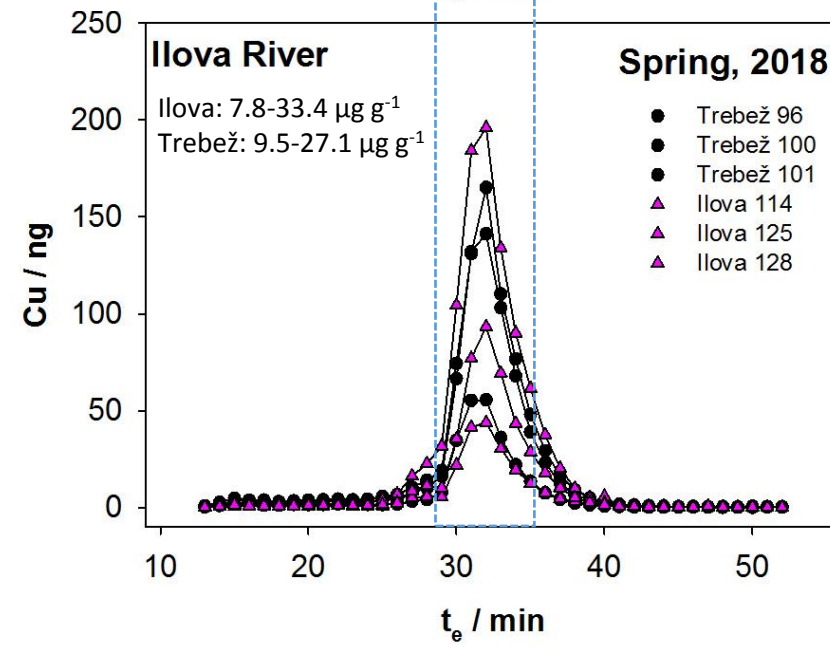




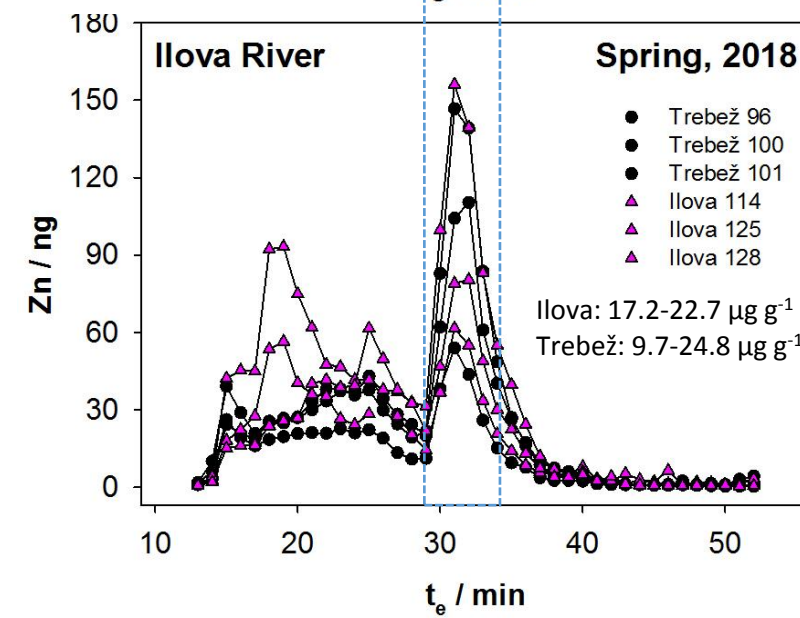
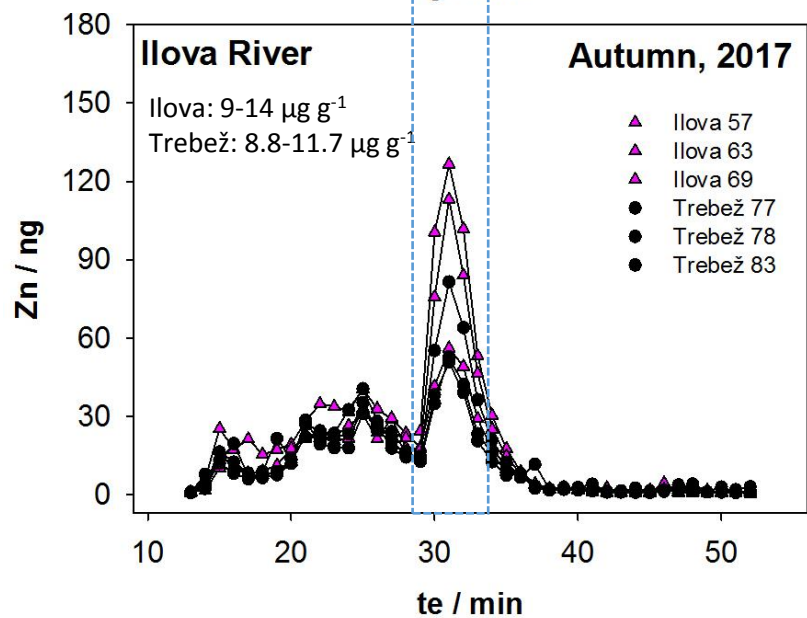
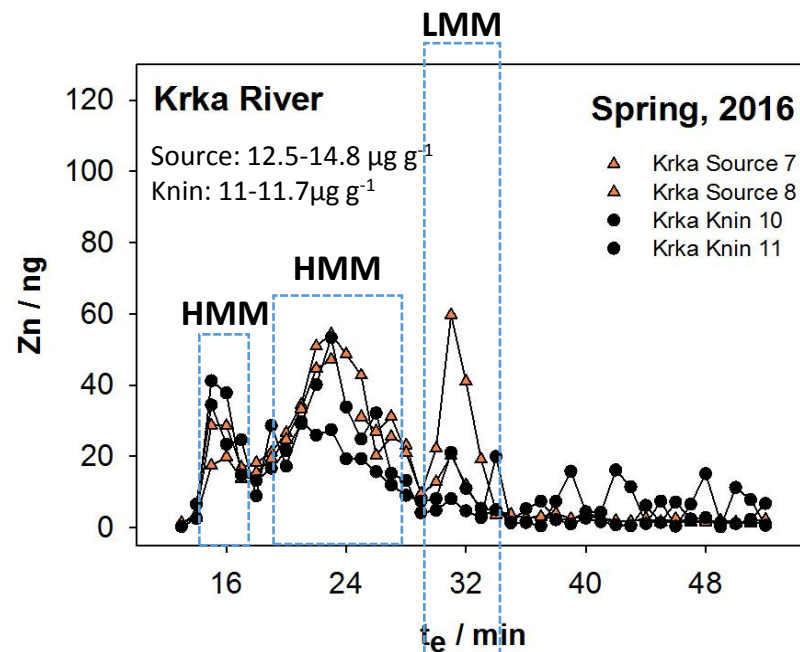
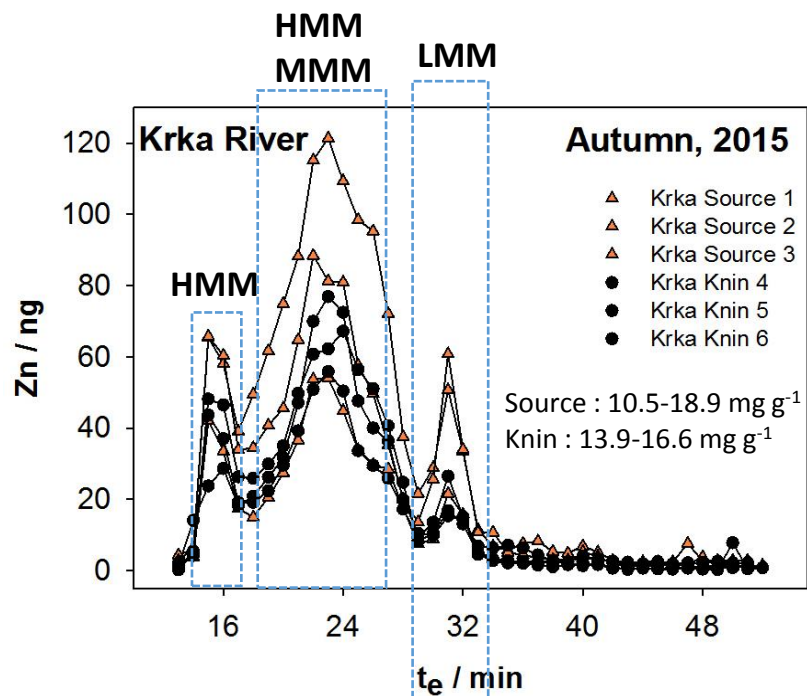
HMM biomolecule regions:
500 to 600 kDa
85 to 400 kDa
only in trout



LMM biomolecule region
7 to 24 kDa
maximum ~15 kDa
-coincided with the elution time
of MT standard



Zn

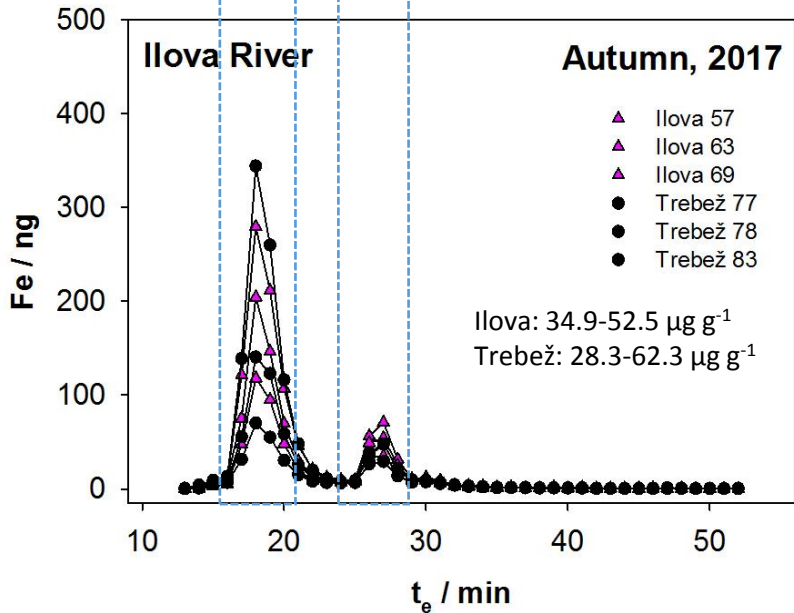
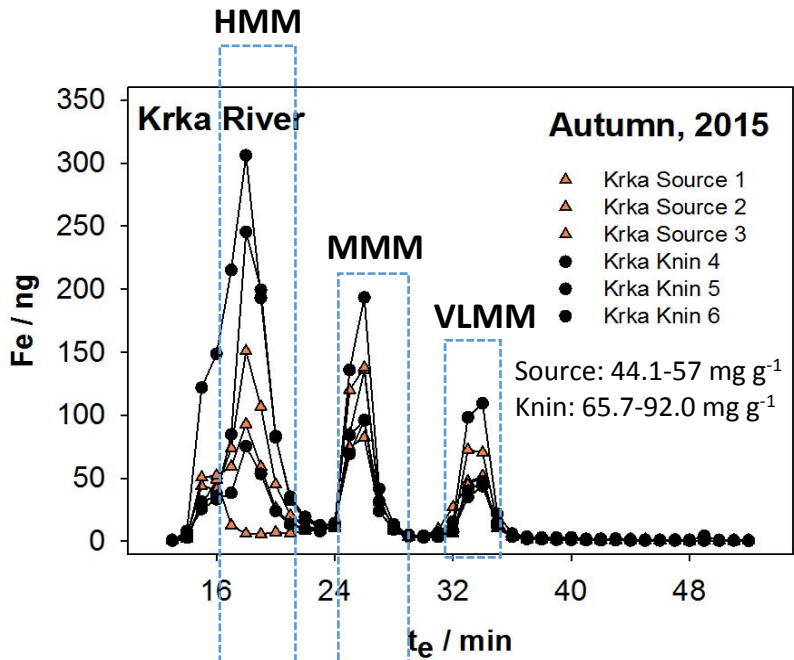


**HMM and MMM
biomolecule region**

**>500 kDa
~20 to 400 kDa**

**LMM biomolecule region
9 to 19 kDa, maximum ~15 kDa**

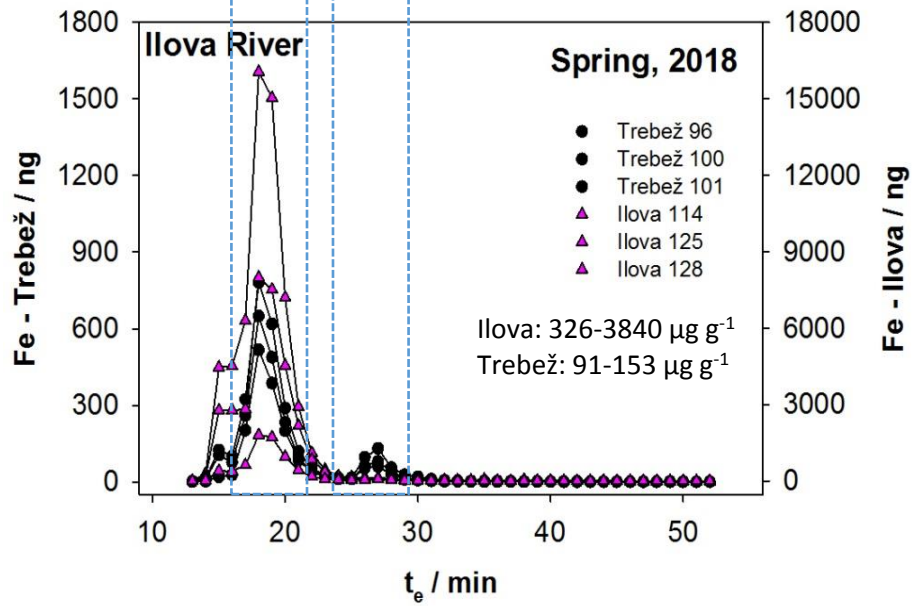
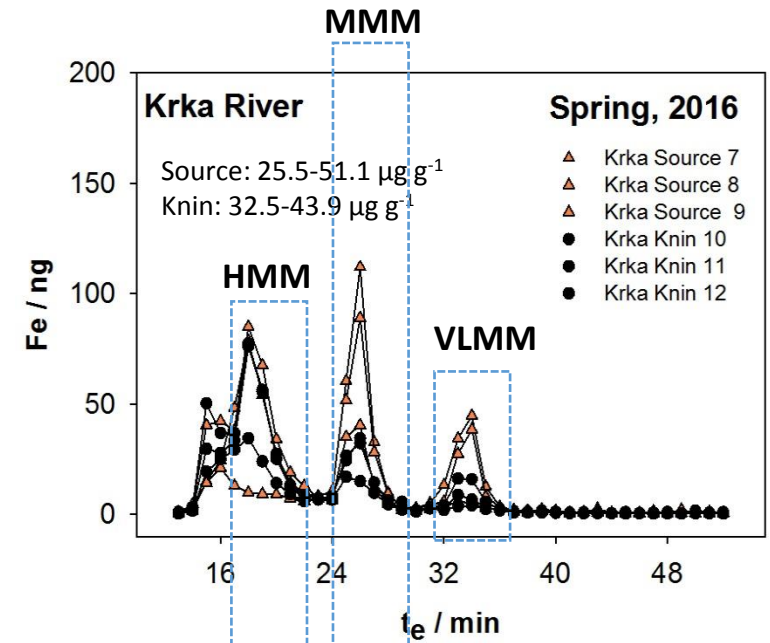
- comparable to Cd and Cu, the elution time of LMM
- coincided with the elution time of MT standard
- liver of European and Vardar chub, European eel, gibel carp



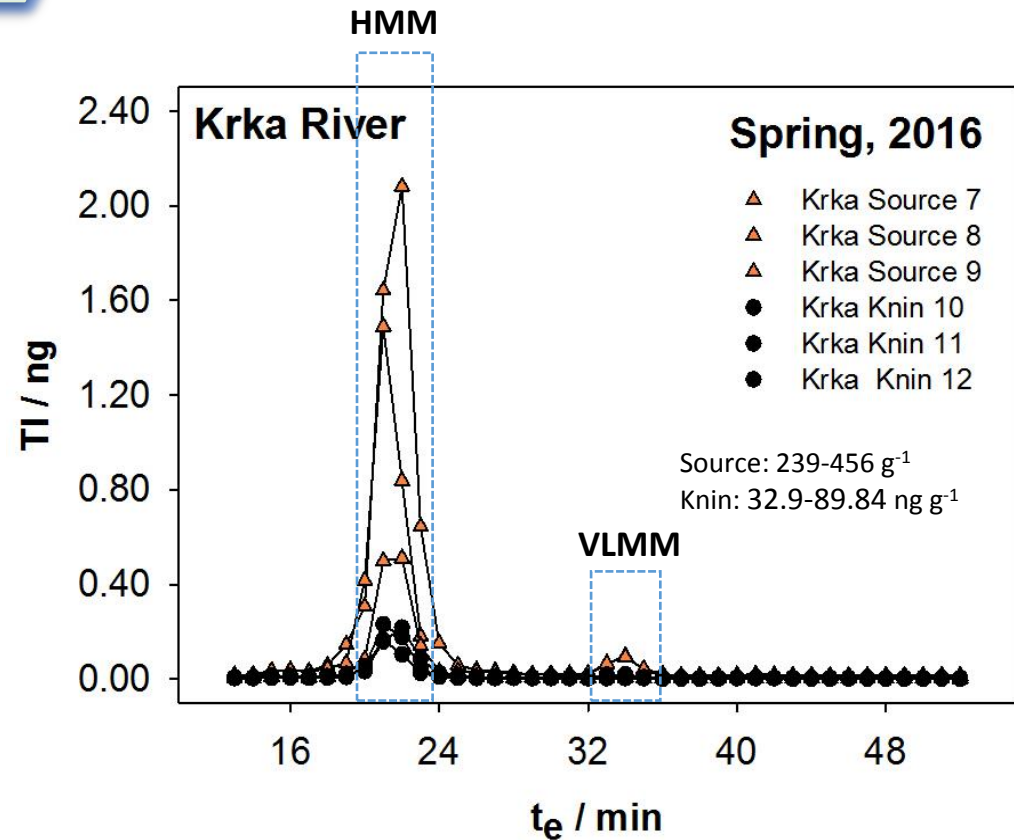
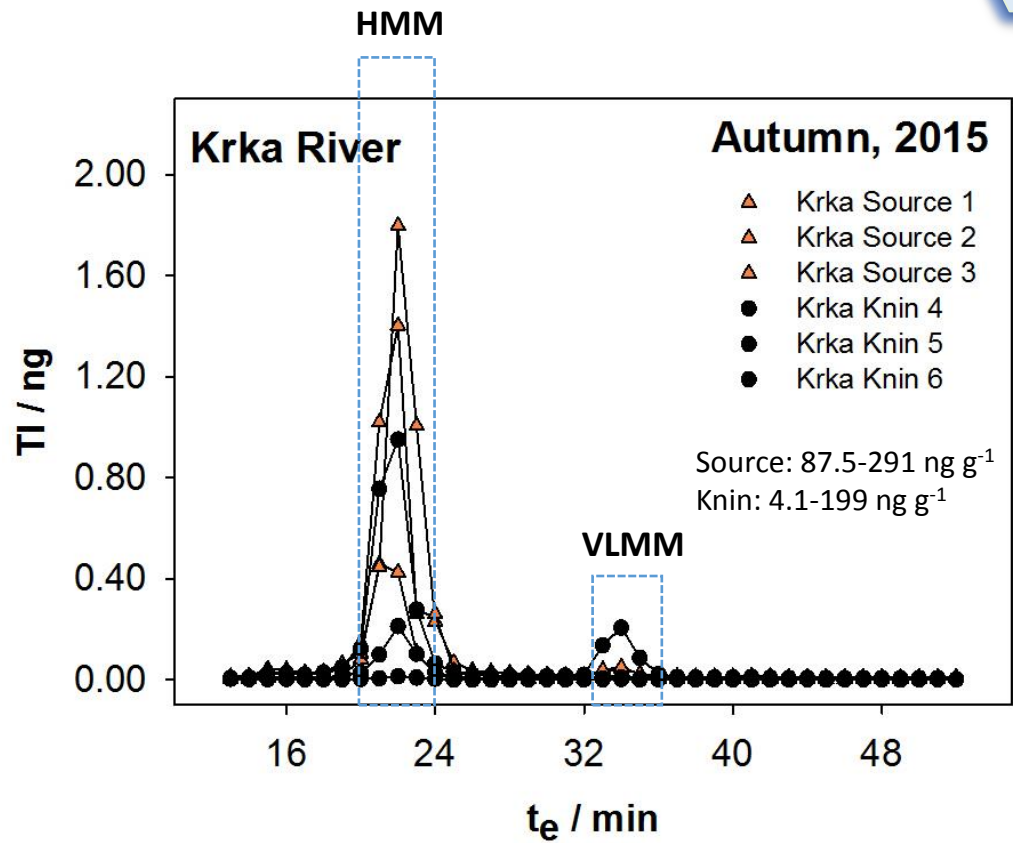
HMM biomolecule region
~200 to 600 kDa
Fe-storage protein ferritin (450 kDa)
European and Vardar chub

MMM biomolecule region
~30 to 85 kDa
blood protein haemoglobin (65 kDa)
enzyme catalase (60 kDa)
European and Vardar chub

VLMM biomolecule region
<10 kDa
only in trout



TI



HMM biomolecule region (60-300 kDa):

-aldehyde dehydrogenase (187 kDa)

- (NappKp)-ATP-ase (tetramer with MM of 274-280 kDa)

VLMM biomolecule region (4 to 11 kDa)

Conclusions

- Association of Cu, Cd and part of Zn to biomolecules of LMM (MTs) and Fe association to storage protein ferritin were observed in both studied species, brown trout from Krka River and Prussian carp from Ilova River
- Tl association to compounds of very low molecular masses (4-11 kDa) in brown trout from Krka River
- Certain differences in metal distribution were observed between two species:
 - Cd and Cu were associated to high molecular mass biomolecules (>100 kDa) only in brown trout
 - Fe binding to very low molecular mass biomolecules (<10 kDa) was observed only in brown trout from Krka River

Thank you for your attention!