Accumulation of metal(loid)s in fish intestine and acanthocephalans with examples of their cytosolic distribution

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FINAL PROJECT MEETING BIOTOXMET
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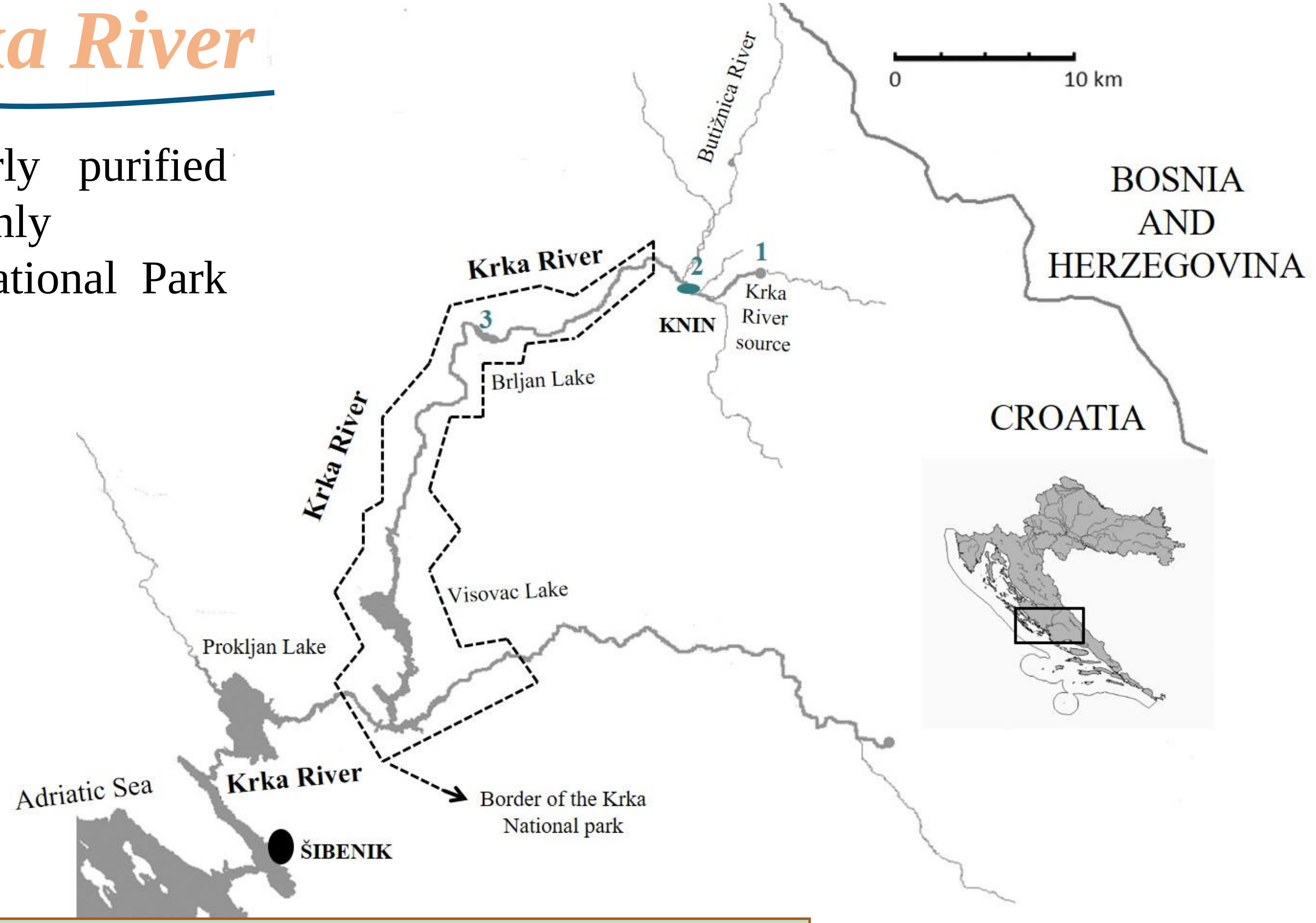
Study area and sampling– Krka River

- 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).



Sampling
1 Krka R
2 downst
3 Locatio

Bioindicator organisms:

1. brown trout (*Salmo trutta* Linnaeus, 1758.)

Indicator organ: the intestine → <u>site of dietary metal uptake</u>.

2. acanthocephalans (*Dentitruncus truttae* Sinzar, 1955)
intestinal parasites, confirmed as organisms of very effective metal accumulation capacity

Methods

Metal determination

Fish intestine
Acanthocephalans

Digestion
HNO_3 and H_2O_2
85 ºC; 3.5 h

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

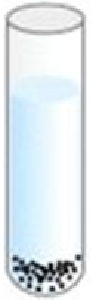
Cytosolic concentrations and distribution

Fish intestine
Acanthocephalans

homogenization

Homogenate

50000xg,
120 min, 4ºC



Cytosol
*Biologically
available and
potentially toxic
fraction

size-exclusion HPLC-ICP-MS

cytosolic
concentrations

Environmental conditions

1. Water samples

- **Fe, Mg, Mn**, **Na** and **Rb** were mostly the highest at **KRK**
- **Ca, Sr** and **V** were mostly the highest at **KNP**
- Highest concentrations of **Tl** at **KRS**
- **Cd, Cu, Se** and **Zn** did not show significant differences between sites
- Water physico-chemical parameters pointed to mostly **very good ecological status** at **KRS** and **KNP**, but pointed to ecological status **below good** at **KRK**, due to high concentrations of nutrients and COD in both seasons

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ELSEVIER

Spatial and temporal variability of dissolved metal(loid)s in water of the karst ecosystem: consequences of long-term exposure to wastewaters

Tatjana Mijošek[a], Zorana Kljaković-Gašpić[b], Tomislav Kralj[a], Damir Valić[a], Zuzana Redžović[a], Sara Šariri[a], Ivana Karamatić[a], Vlatka Filipović Marijić[a,*]

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[b] Institute for Medical Research and Occupational Health, Ksaverska c. 2, 10000 Zagreb, Croatia

2. Sediment samples

- **All trace elements, K and Na in sediments showed the highest concentrations at KNP**
- **Mg** concentrations showed the highest values at **KRS**, and **Ca** at **KRK**
- **Cu** is the only element **without significant differences** between sites

Environmental Pollution 356 (2024) 124358

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ELSEVIER

Interrelation between environmental conditions, acanthocephalan infection and metal(loid) accumulation in fish intestine: an in-depth study☆

Tatjana Mijošek[a], Sara Šariri[a], Zorana Kljaković-Gašpić[b], Željka Fiket[a], Vlatka Filipović Marijić[a,*]

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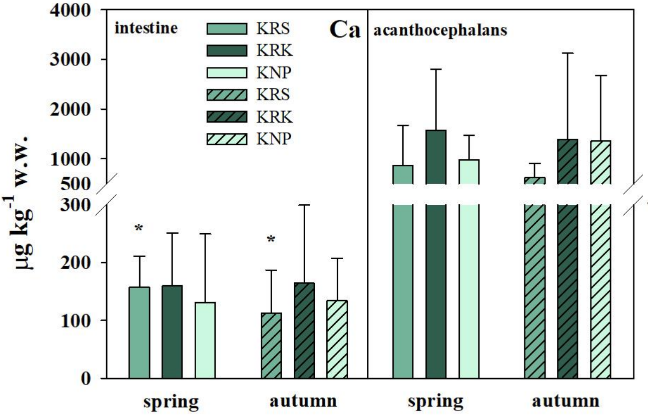
[b] Institute for Medical Research and Occupational Health, Ksaverska 2, 10000 Zagreb, Croatia

Contamination of sediments best seen in downstream lake systems starting in Brljan Lake, which serve as sink for metals due to intensive sedimentation and low flow but also help self-purification process of water

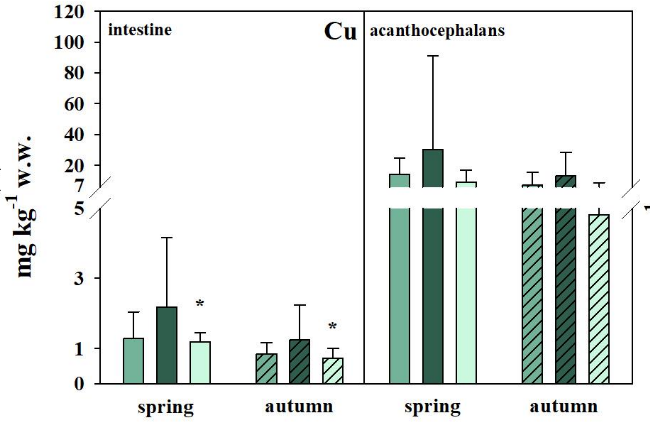
Metal(loid)s accumulation in fish intestine and acanthocephalans

a) the highest at KRK

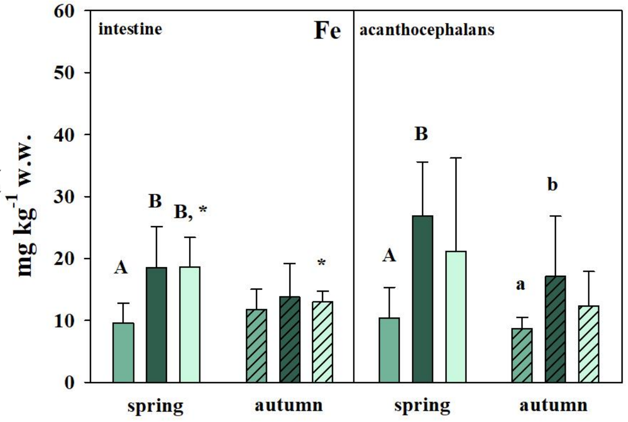
No correspondance with water and sediments



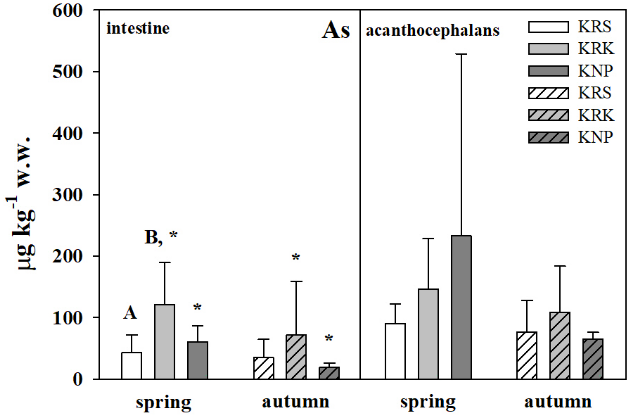
Sediment, but not water



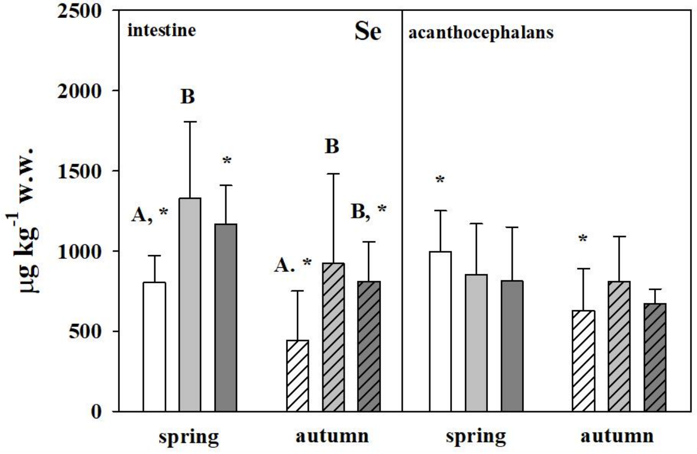
GC!!



Water, but not sediments

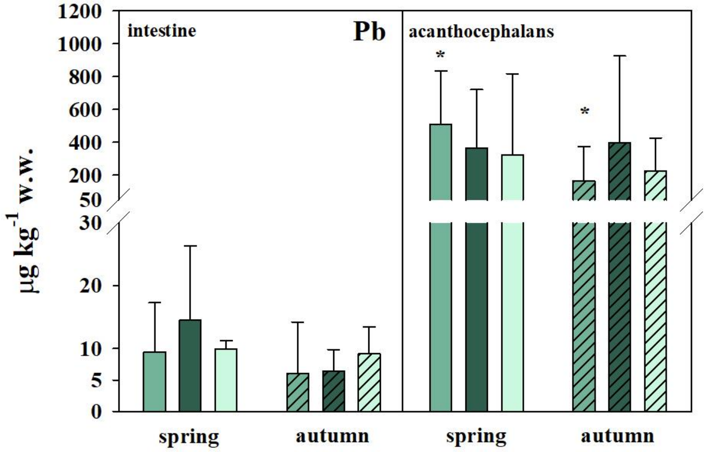


Water, but not sediments

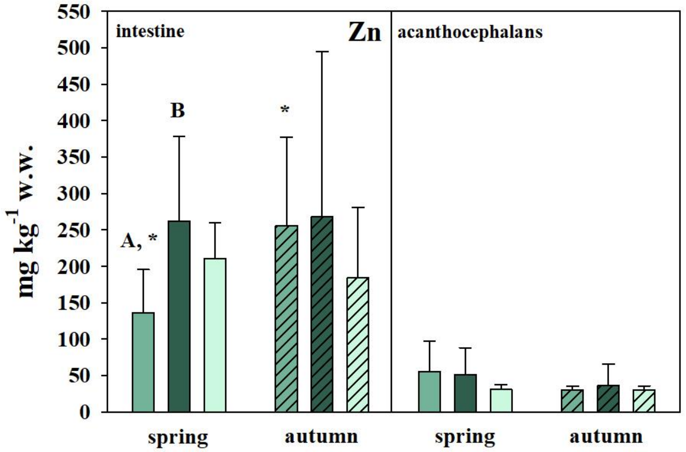


GC!!

No correspondance with water and sediments

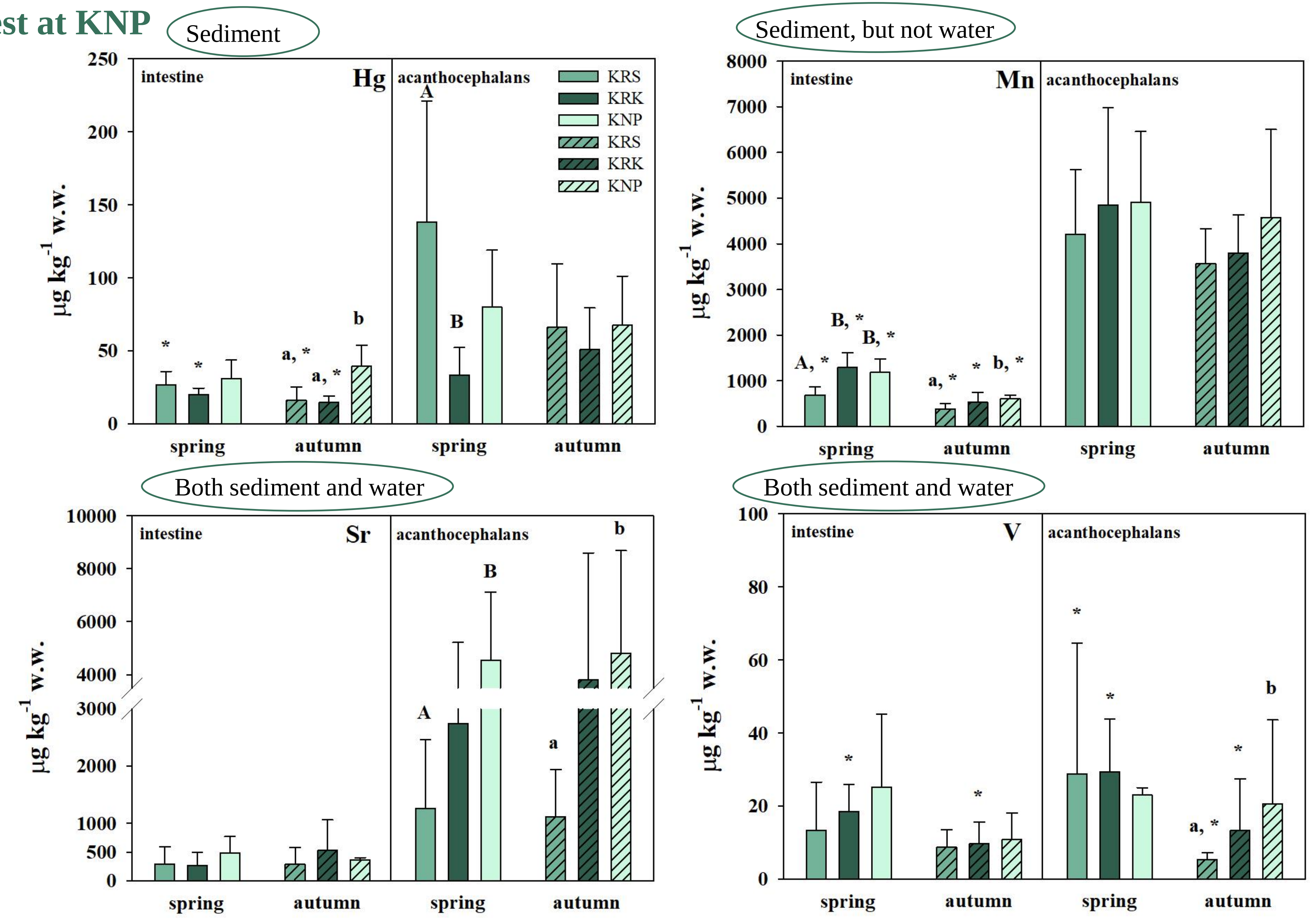


No correspondance with water and sediments

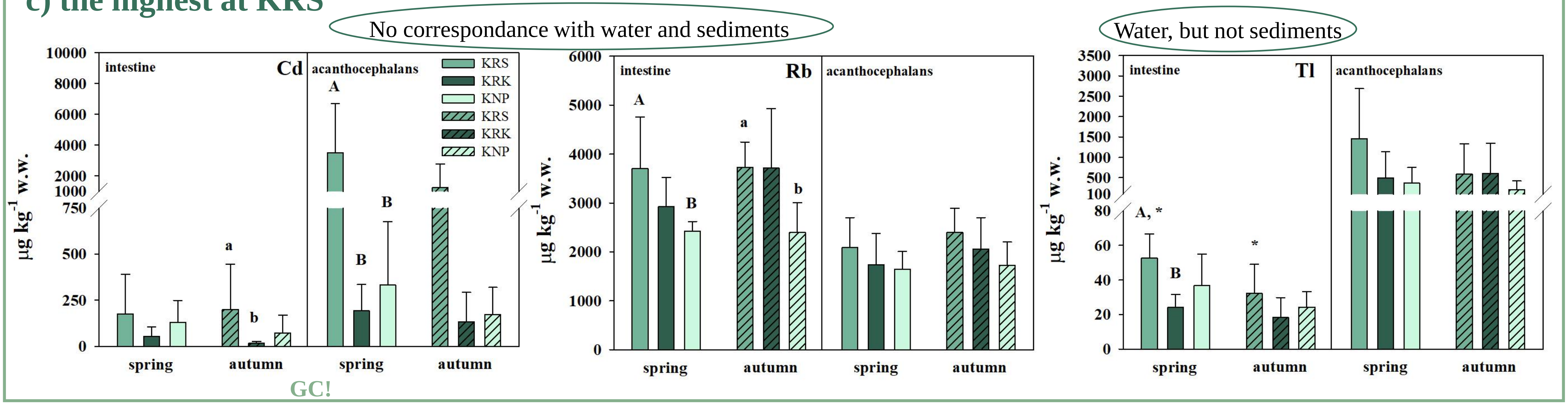


Water, but not sediments

b) the highest at KNP



c) the highest at KRS



- Patterns of many elements in fish and acanthocephalans could not be completely explained only by uptake from water or sediments, suggesting additional importance of **dietborne metal uptake** and **feeding preferences**.
- Seasonally, **metal accumulation** tended to be **higher in spring** than autumn following **enhanced fish feeding rates**.

Acanthocephalans – friendly parasites?

- Fish infected with more acanthocephalans had lower concentrations of many elements – „**biodillution effect**”
- Previous research pointed to **lower metal concentrations** in **fish infected** with acanthocephalans than in uninfected
- **Protective role** for the fish host?? ☺

Bioconcentration factors

$$BCF = c_{(acanthocephalans)} / c_{(fish\ intestine)}$$

	Krka River source (KRS)		Town of Knin (KRK)		Krka National Park (KNP)	
	Spring 2021	Autumn 2021	Spring 2021	Autumn 2021	Spring 2021	Autumn 2021
Ca	**4.3±3.0**	**6.0±1.8**	**9.3±4.8**	**9.1±9.5**	**5.9±2.1**	**6.5±4.0**
Cd	**86.0±192.2**	**8.5±10.1**	**4.9±4.6**	**16.5±28.6**	**9.4±16.2**	**15.0±21.2**
Cu	**14.6±12.8**	**7.7±7.4**	**7.3±8.9**	**10.1±11.8**	**9.2±9.4**	**7.7±7.9**
Fe	**1.1±0.5**	0.8±0.3	**1.5±0.4**	**1.4±0.9**	**1.1±0.6**	1.0±0.5
Hg	**5.5±3.0**	**3.7±1.8**	**1.3±1.1**	**3.6±2.1**	**2.9±1.8**	**1.8±0.8**
K	0.7±0.2	0.7±0.1	0.7±0.3	0.7±0.1	0.7±0.2	0.7±0.1
Mg	0.9±0.2	0.9±0.4	1.0±0.2	**1.1±0.4**	**1.1±0.3**	**1.1±0.3**
Mn	**6.3±1.8**	**9.8±2.9**	**3.7±1.0**	**8.1±2.8**	**4.6±2.3**	**7.5±3.8**
Na	**2.1±0.9**	**1.5±0.2**	**1.9±0.6**	**1.4±0.3**	**1.8±0.7**	**1.5±0.2**
Pb	**74.1±68.6**	**20.0±30.6**	**31.4±27.8**	**46.4±53.0**	**28.1±42.8**	**34.4±46.7**
Rb	0.6±0.2	0.7±0.2	0.6±0.2	0.6±0.1	0.7±0.1	0.7±0.1
Sr	**5.8±6.3**	**5.5±2.3**	**9.7±4.0**	**6.3±3.9**	**9.7±3.0**	**8.3±4.6**
Tl	**29.3±27.2**	**16.0±18.0**	**22.2±34.4**	**30.9±35.4**	**13.7±18.6**	**9.0±8.9**
V	**2.0±1.5**	0.6±0.2	**1.7±0.6**	**1.2±0.6**	**1.4±1.1**	**2.1±1.3**
Zn	0.4±0.3	0.1±0.1	0.2±0.1	0.2±0.2	0.2±0.0	0.2±0.1

Pb≥Cd≥Tl>Cu≥Ba>Mn≥Sr≥Ca>Hg>Cr≥As>Co≥Na>V>Fe

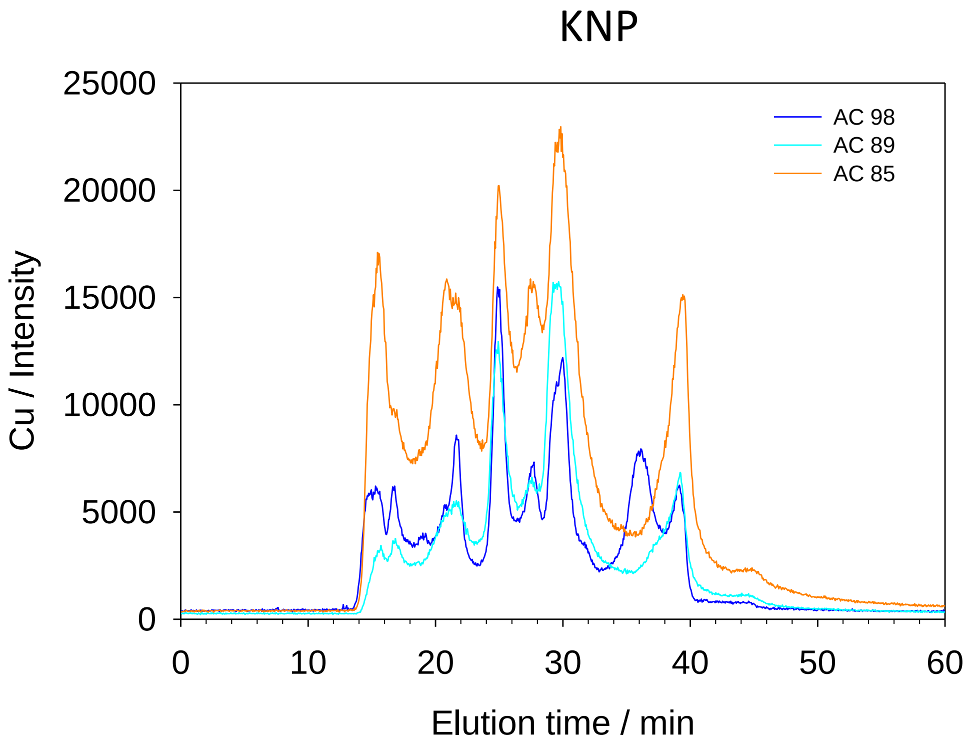
<u>Metal(loid)s distribution among cytosolic biomolecules of different molecular masses</u>

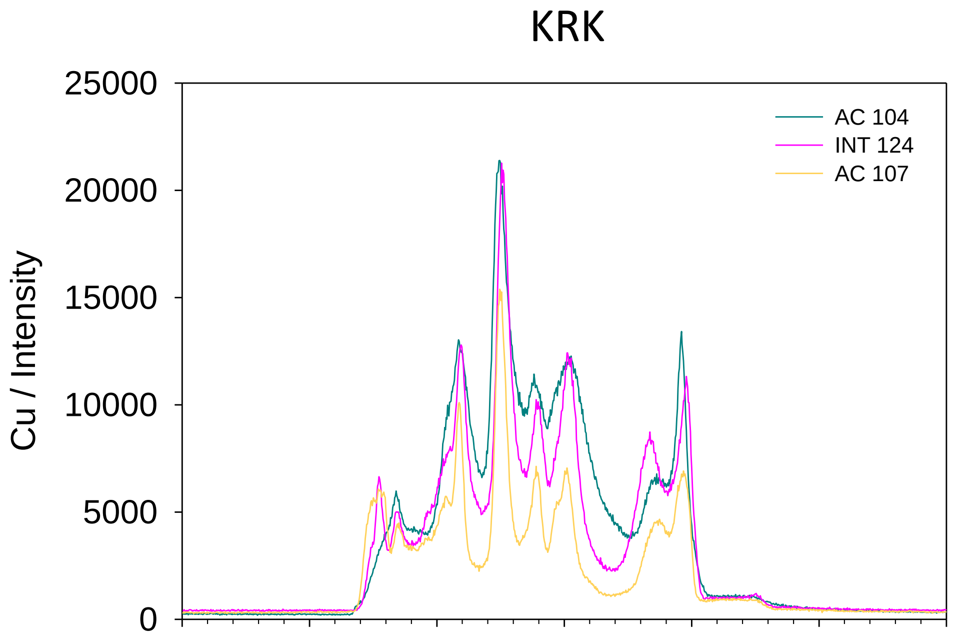
Biomolecules assigned to four categories according to their molecular masses (MM)

HMM - the high MM category (>100 kDa)
MMM - the medium MM category (30–100 kDa)
LMM - the low MM category (10–30 kDa)
VLMM - the very low MM category (<10 kDa)

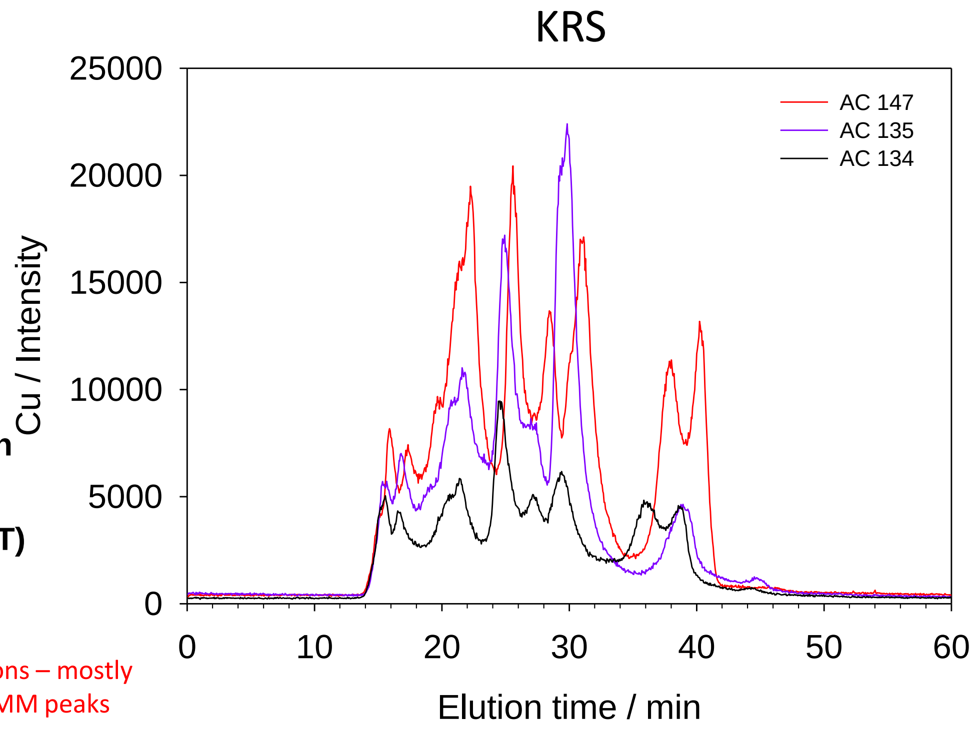
Copper

Cu	
sample	µg/kg
AC 85	1455,76
AC 98	832,722
AC 89	749,287
AC 104	1362,43
AC 107	629,552
AC 124	1146,44
AC 134	716,124
AC 135	947,562
AC 147	2008,58





Peak range		Peak maximum (min)	MM (kDa)	
HMM	HMM-1	15.3-15.9	933-770	
	HMM-2	21.4-21.9	131-112	ß amylase
MMM	MMM-1	24.6-25.0	46.9-41.2	SOD, carbonic anhydrase, albumin
	MM-2			
LMM	LMM-1	27.1-27.8	21.0-16.8	
	LMM-2	29.0-30.3	11.4-7.5	metallothionein (MT)
VLMM	VLMM-1	36.0-37.2	1.2-0.815	
	VLMM-2	39.1-40.0	0.443-0.331	

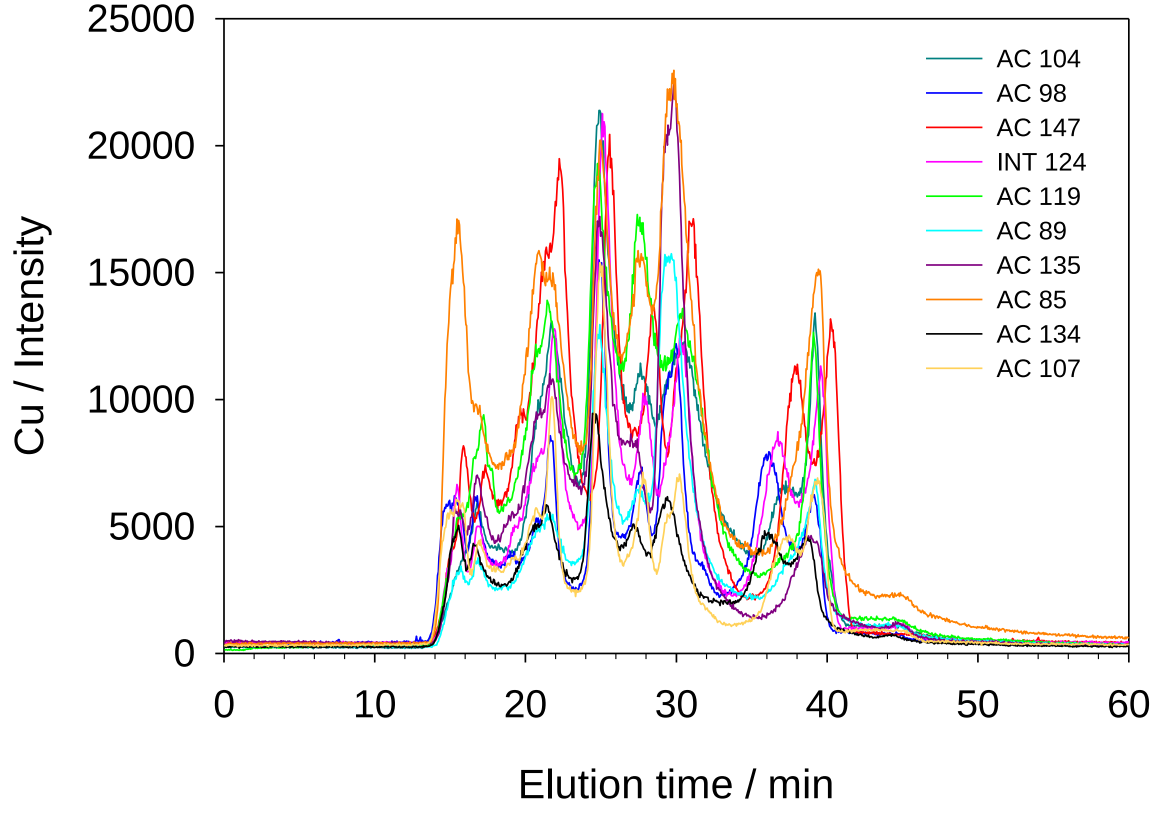


Hiigher concentrations – mostly higher MMM / (V)LMM peaks

Cu

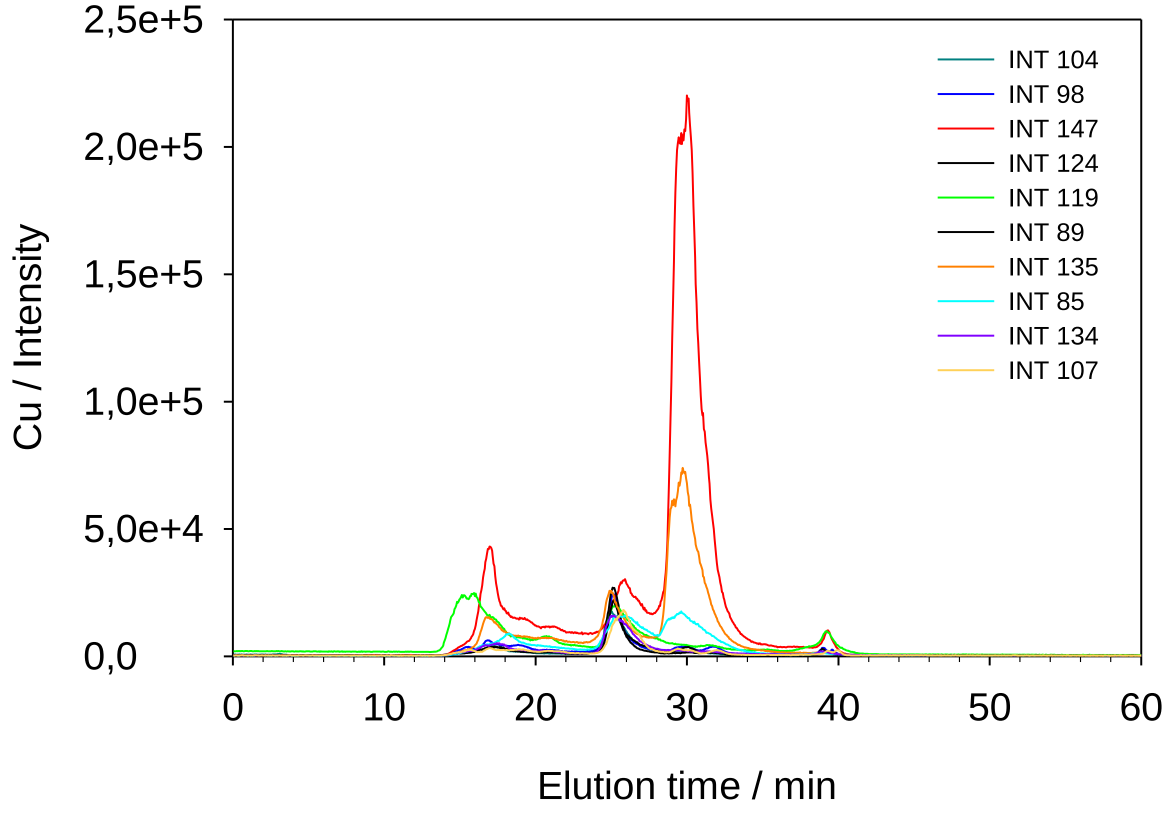
	µg/kg
A 85 A	1455,76
A 89 A	749,29
A 98 A	832,72
A 104 A	1362,43
A 107 A	629,55
A 124 A	1146,44
A 134 A	716,12
A 135 A	947,56
A 147 A	2008,58

Acanthocephalans



Fish intestine

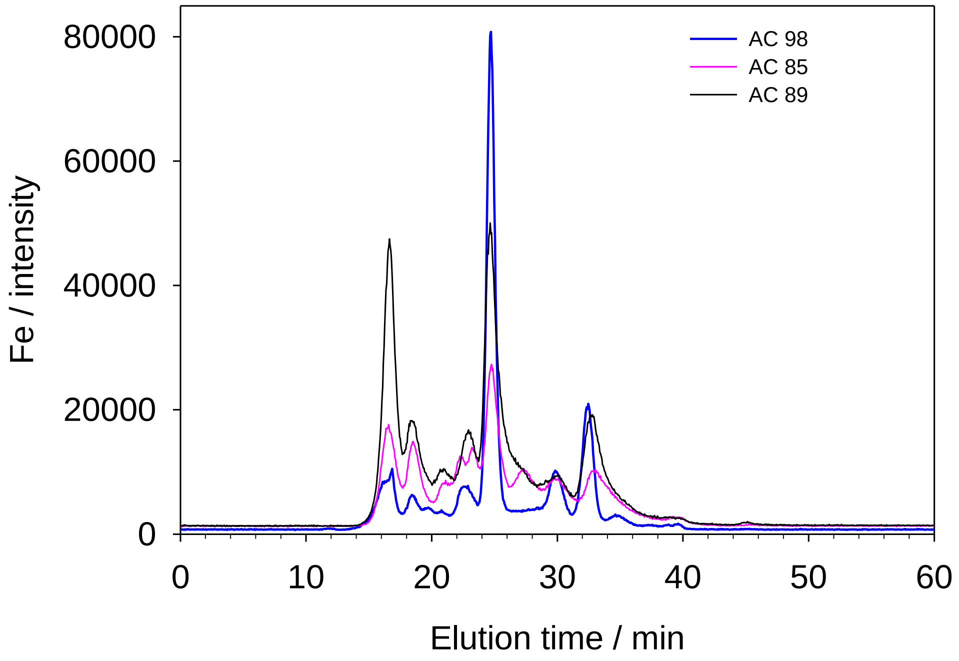
	µg/kg
I 85A	449,71
I 89 A	205,60
I 98 A	303,31
I 104 A	204,67
I 107 A	232,06
I 119 A	223,69
I 124 A	187,33
I 134 A	197,35
I 135 A	1123,72
I 147 A	2835,55



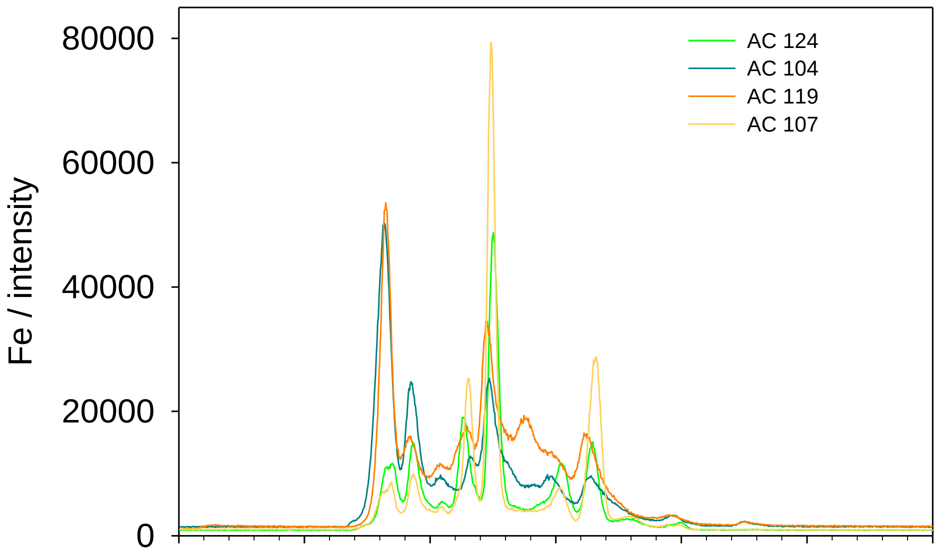
Iron

Fe	
sample	**µg/kg**
A 85 A	**2199,857**
A 89 A	**3013,637**
A 98 A	**3221,482**
A 104 A	**2213,442**
A 107 A	**3372,567**
A 124 A	**3095,147**
A 134 A	**2213,784**
A 135 A	**3397,482**
A 147 A	**1514,484**

KNP

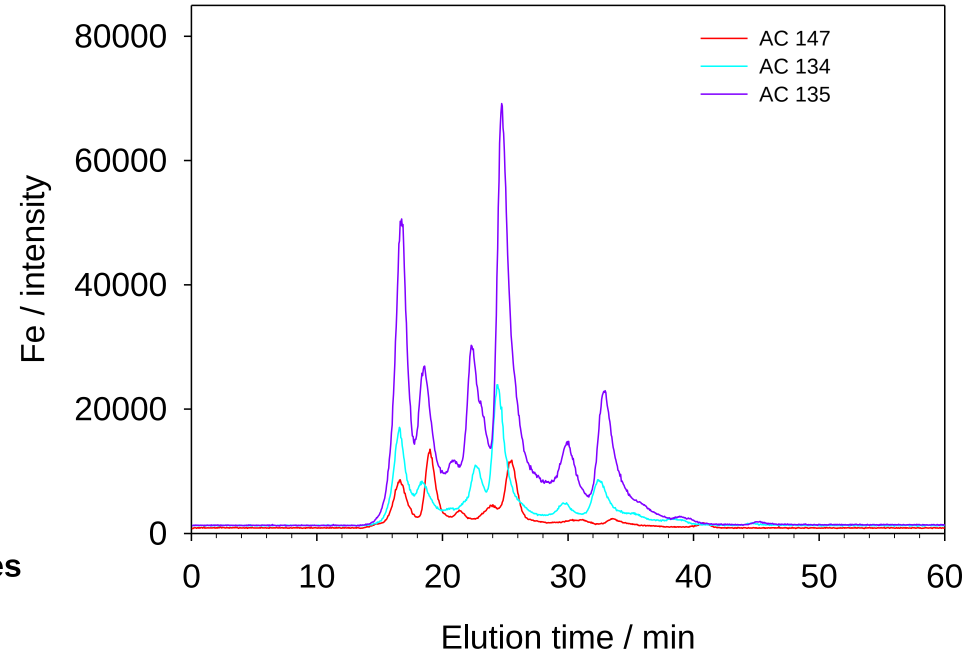


KRK



Higer concentrations –higher peaks in all regions, but especially HMM1 and MM2

KRS



Peak range		**Peak maximum (min)**	**MM (kDa)**
HMM	HMM1	16.4-17.0	655-540
	HMM2	18.1-18.7	379-313
MMM	MMM1	22.3-23.2	98.3-74.0
	MM2	24.4-24.9	51.6-44.0
LMM	LMM1		
	LMM2		
VLMM	VLMM1	29.6-30.3	9.4-7.5
	VLMM2	32.5-33.2	3.7-3.0

Ferritin

Transferrin, albumin, feroportin, catalase, hemoglobin

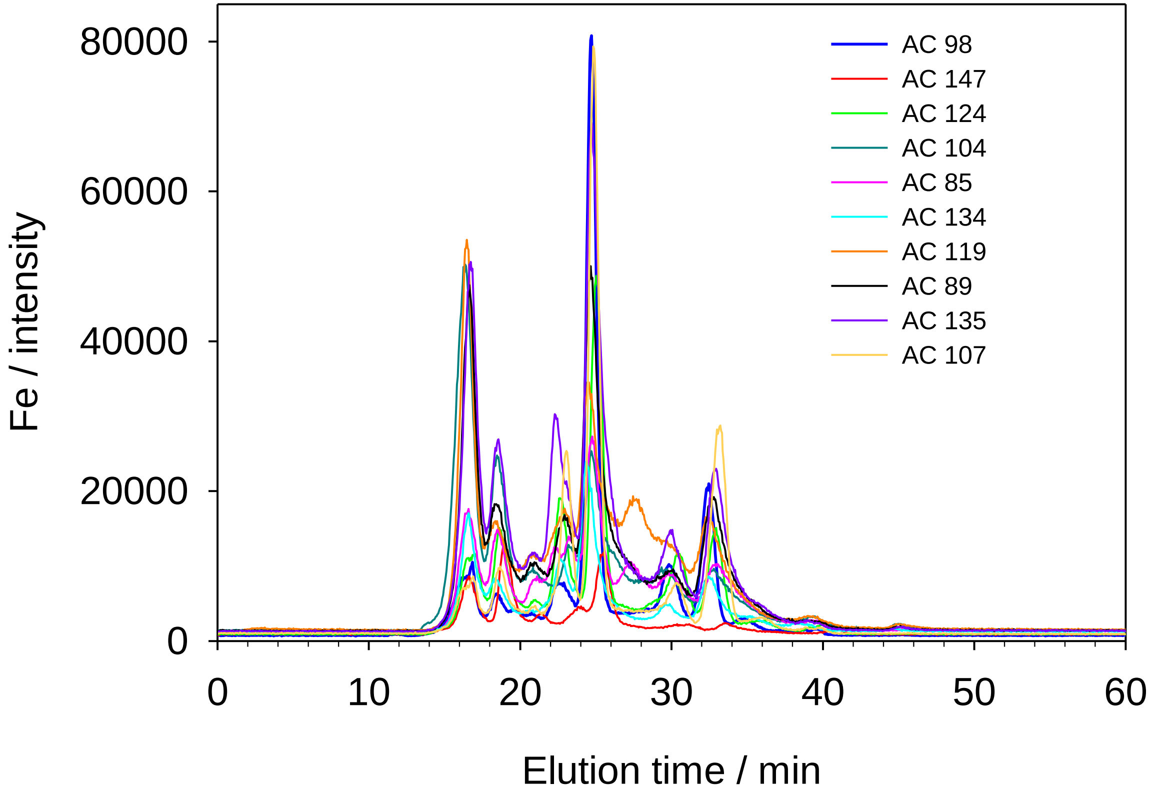
nucleotides, amino acids, pyrophosphates and Fe complexes

Fe

μg/kg

A 85 A	2199,86
A 89 A	3013,64
A 98 A	3221,48
A 104 A	2213,44
A 107 A	3372,57
A 124 A	3095,15
A 134 A	2213,78
A 135 A	3397,48
A 147 A	1514,48

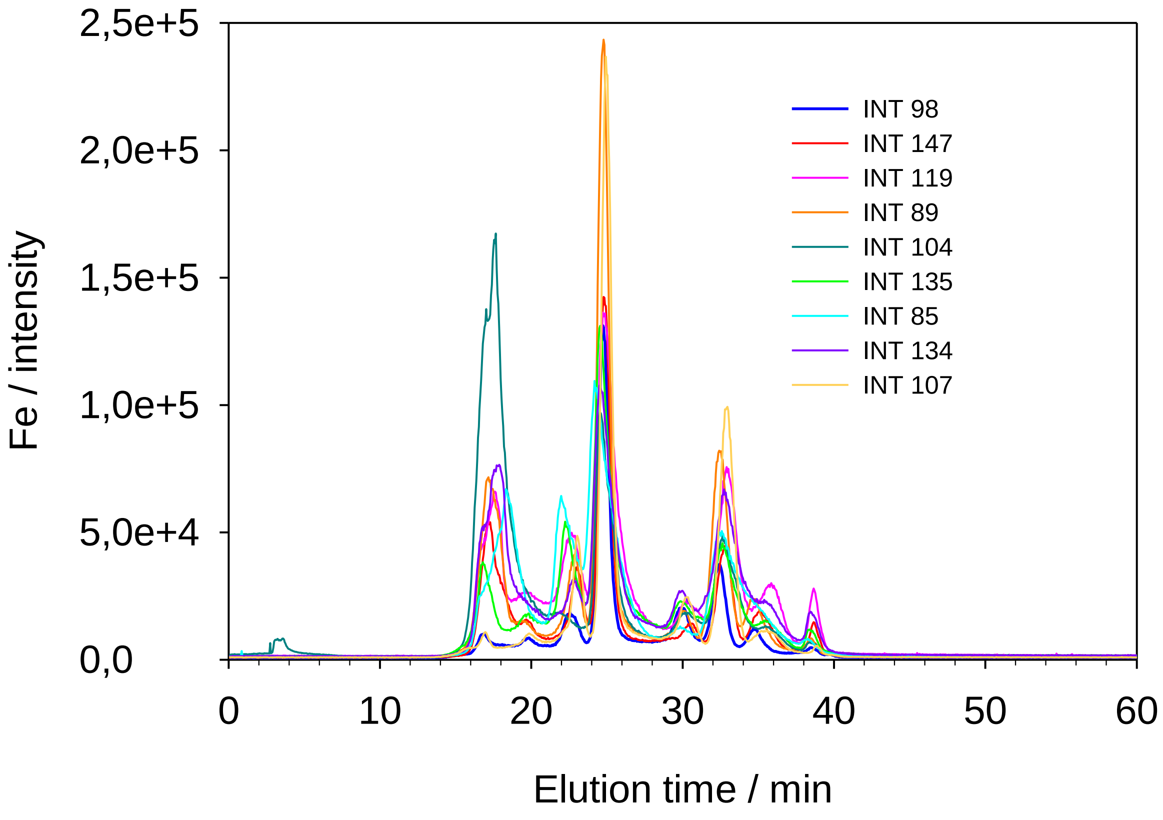
Acanthocephalans



Fish intestine

μg/kg

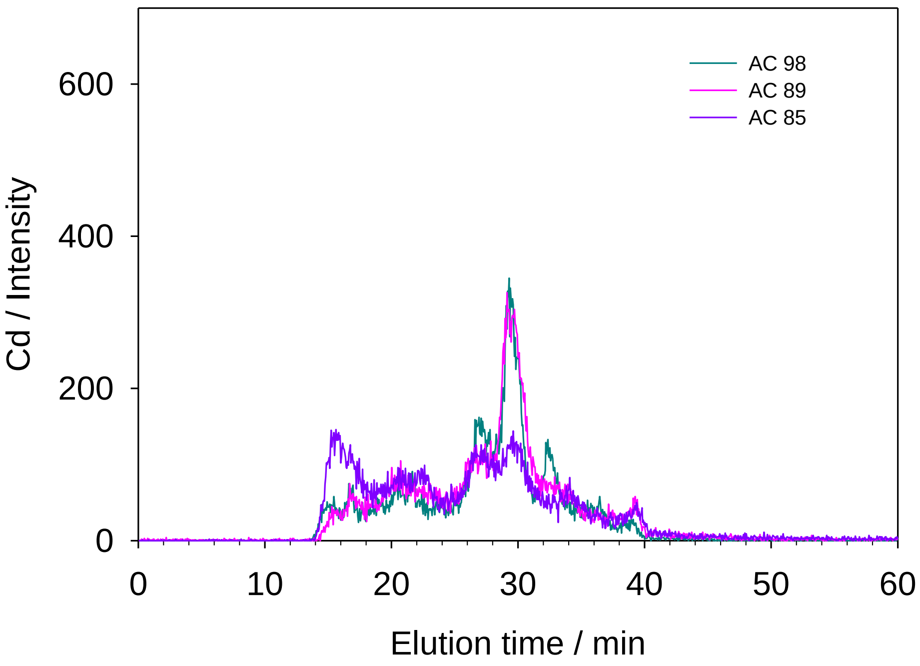
I 85A	5057,23
I 89 A	6731,05
I 98 A	3551,95
I 104 A	4488,07
I 107 A	5298,85
I 119 A	5847,28
I 124 A	6624,85
I 134 A	4943,29
I 135 A	4423,18
I 147 A	4207,42



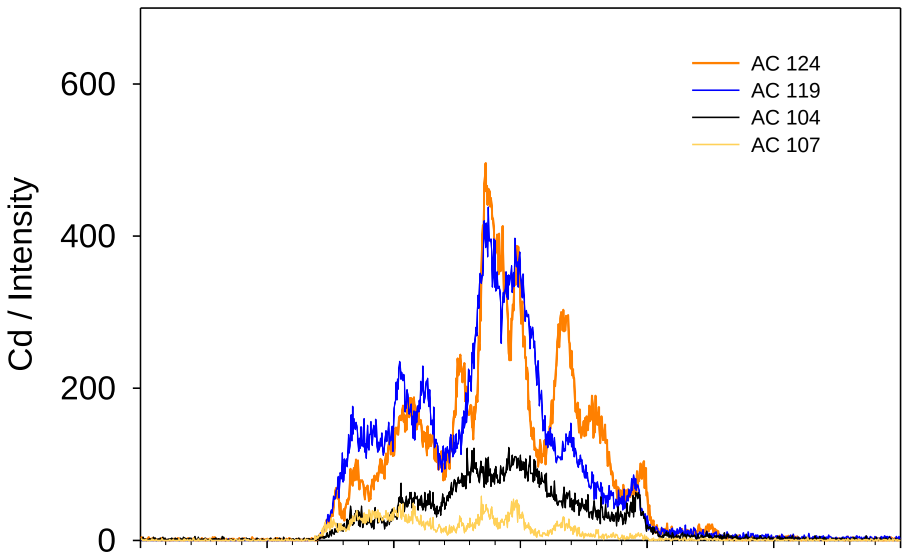
Cadmium

Cd	
sample	**µg/kg**
A 85 A	**37,389**
A 89 A	**54,329**
A 98 A	**60,214**
A 104 A	**37,499**
A 107 A	**15,719**
A 124 A	**129,019**
A 134 A	**103,248**
A 135 A	**191,059**
A 147 A	**114,948**

KNP

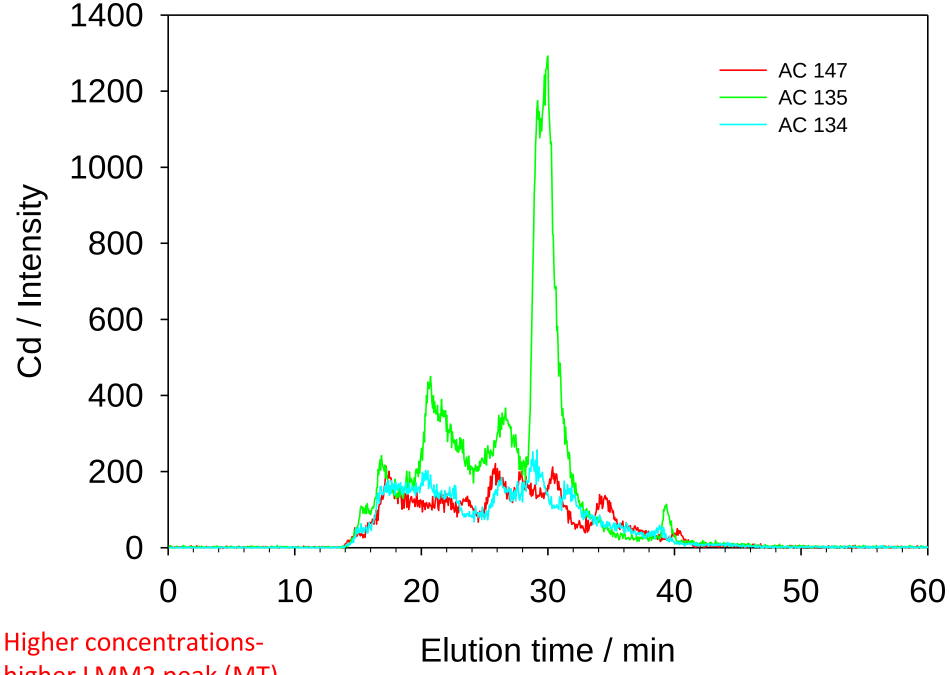


KRK



Peak range		**Peak maximum (min)**	**Molecular mass (kDa)**	
HMM	HMM1	16.4-17.2	655-507	
	HMM2	20.6-21.2	170-140	
MMM	MMM1	22.4-22.8	95.2-83.7	
	MMM2			
LMM	**LMM1**	**27.0-27.9**	**21.7-16.2**	
	LMM2	**29.3-30.5**	**10.3-7.0**	**metallothionein (MT)**
VLMM	VLMM1	33.3-34.7	2.86-1.82	
	VLMM2	39.1-39.7	0.443-0.365	**GSH**

KRS



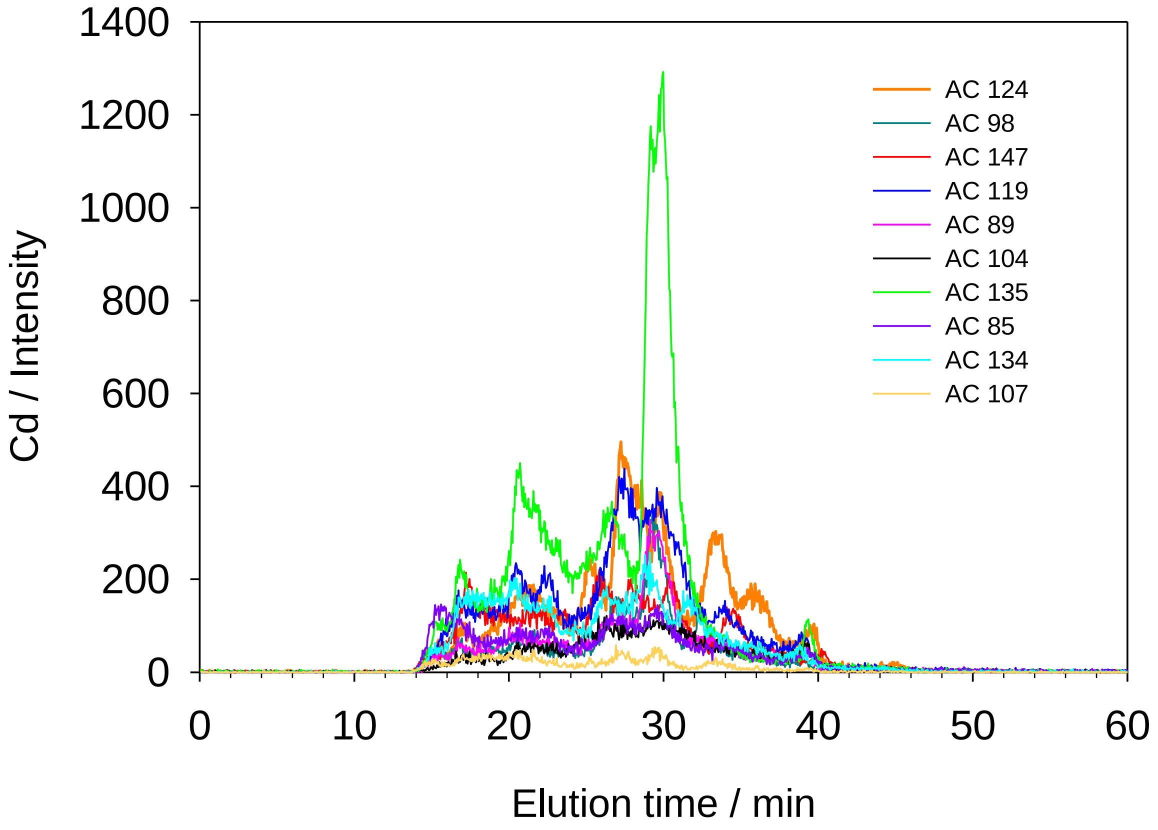
Higher concentrations-
higher LMM2 peak (MT)

Cd

µg/kg

A 85 A	37,39
A 89 A	54,33
A 98 A	60,21
A 104 A	37,50
A 107 A	15,72
A 124 A	129,02
A 134 A	103,25
A 135 A	191,06
A 147 A	114,95

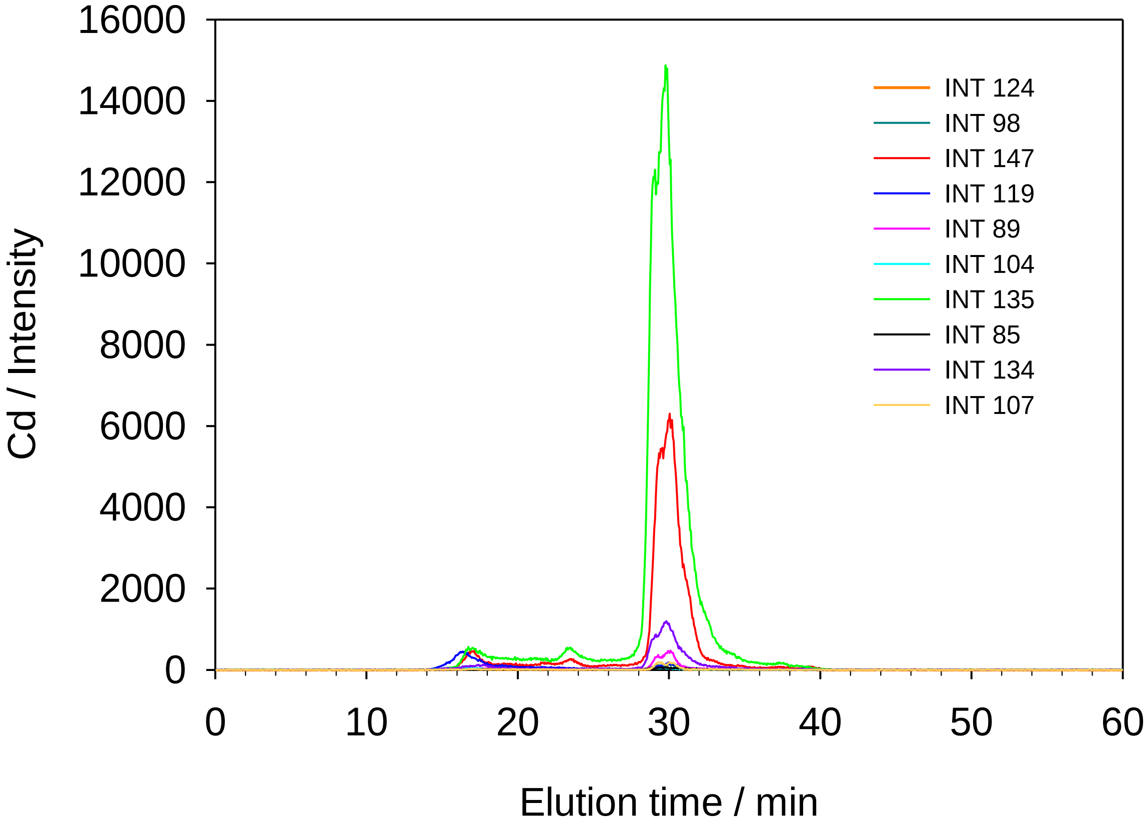
Acanthocephalans



Fish intestine

µg/kg

I 85A	2,81
I 89 A	17,27
I 98 A	7,07
I 104 A	2,09
I 107 A	8,42
I 119 A	6,92
I 124 A	5,21
I 134 A	49,13
I 135 A	586,19
I 147 A	243,41

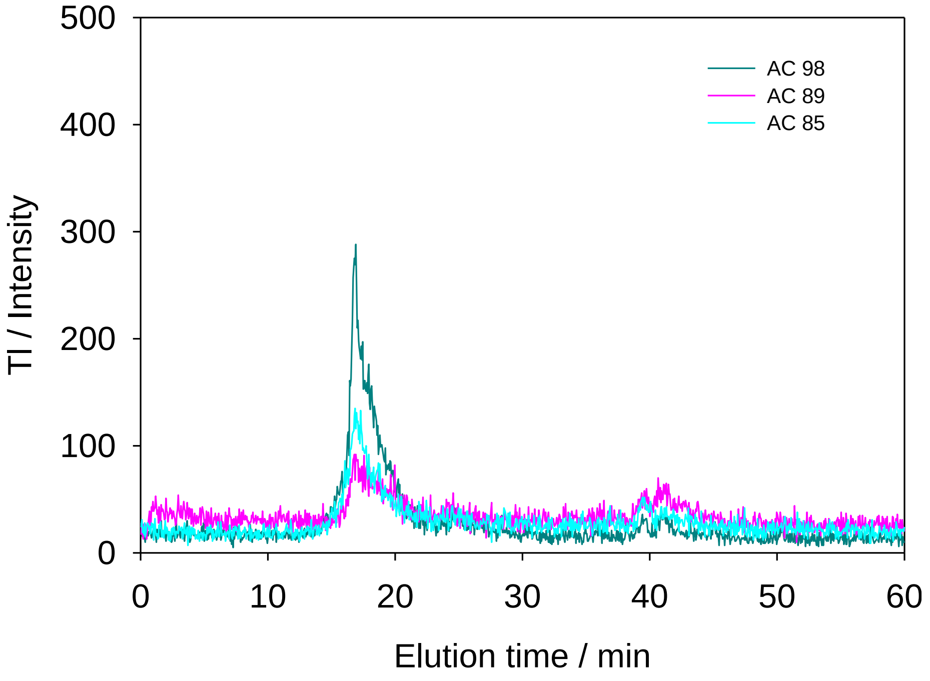


Cadmium elution in HMM and MMM protein category, was an indication that increased Cd accumulation in acanthocephalans could result with incomplete Cd detoxification and binding to proteins of higher MM than MTs.

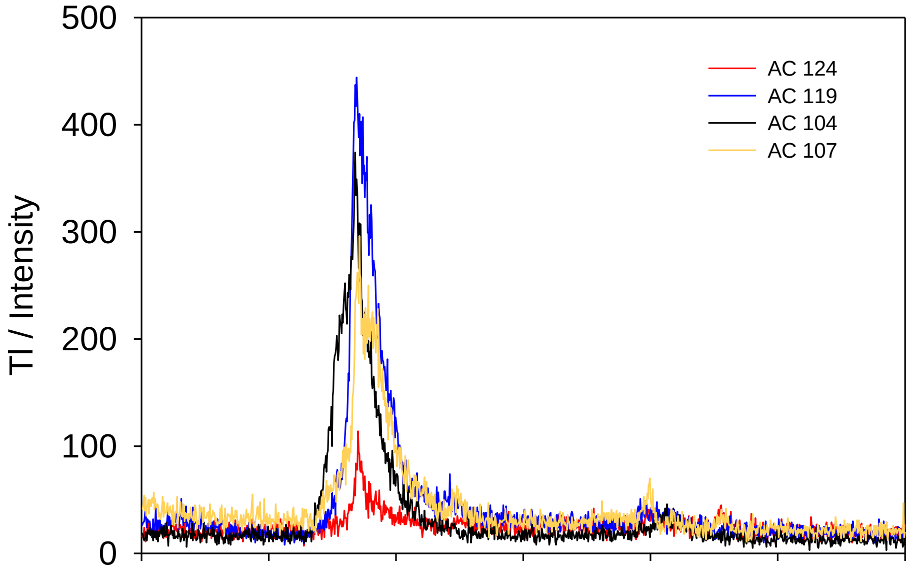
Thallium

Tl	
sample	µg/kg
A 85 A	4,125
A 89 A	6,215
A 98 A	8,085
A 104 A	6,105
A 107 A	12,375
A 124 A	2,53
A 134 A	8,58
A 135 A	29,975
A 147 A	28,08

KNP

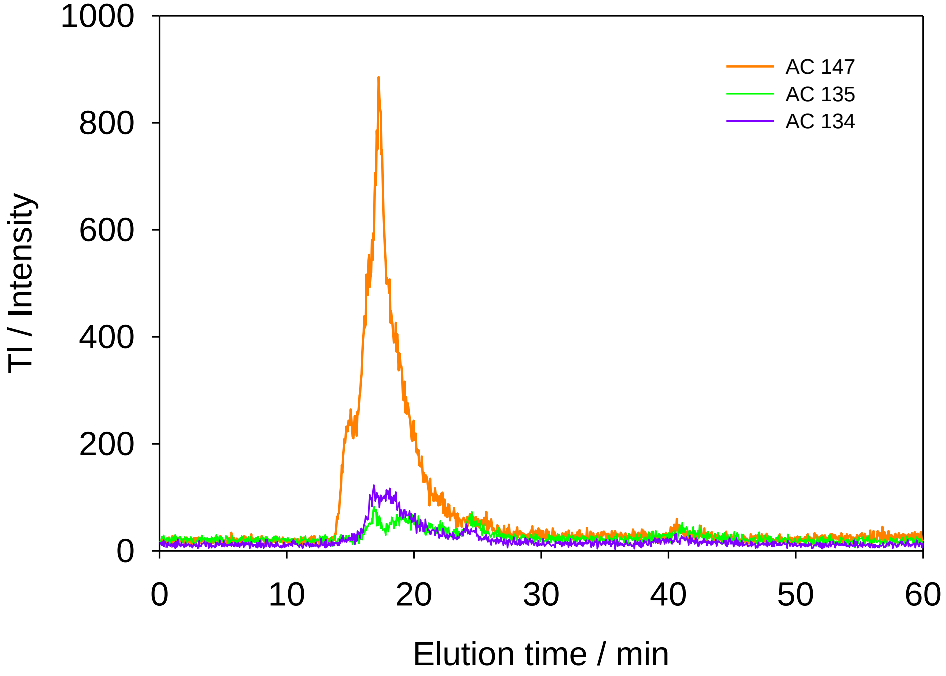


KRK



Peak range		Peak maximum (min)	MM (kDa)	
HMM	HMM-1	16.8-17.2	576-507	**protein complexes and aggregates**
	HMM-2			
MMM	MMM-1			
	MM-2			
LMM	LMM-1			
	LMM-2			
VLMM	VLMM-1	39.4-40.6	0.402-0.273	**GSH**
	VLMM-2			

KRS



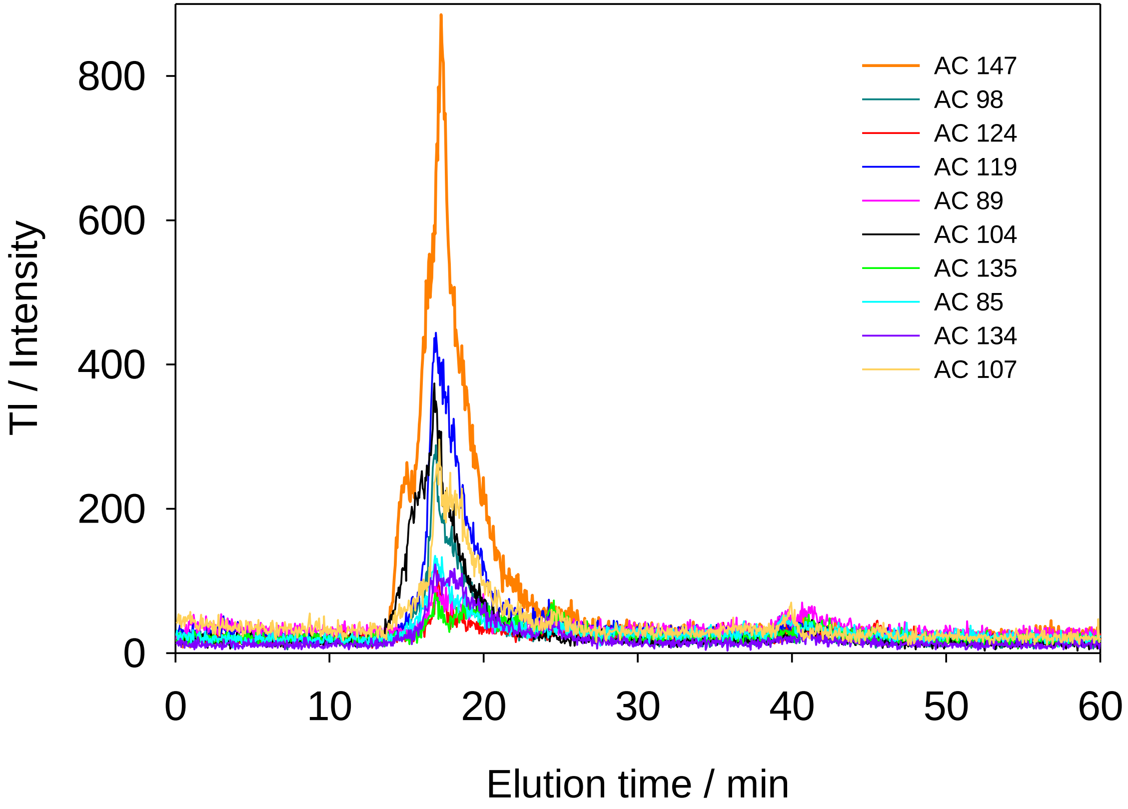
Tl

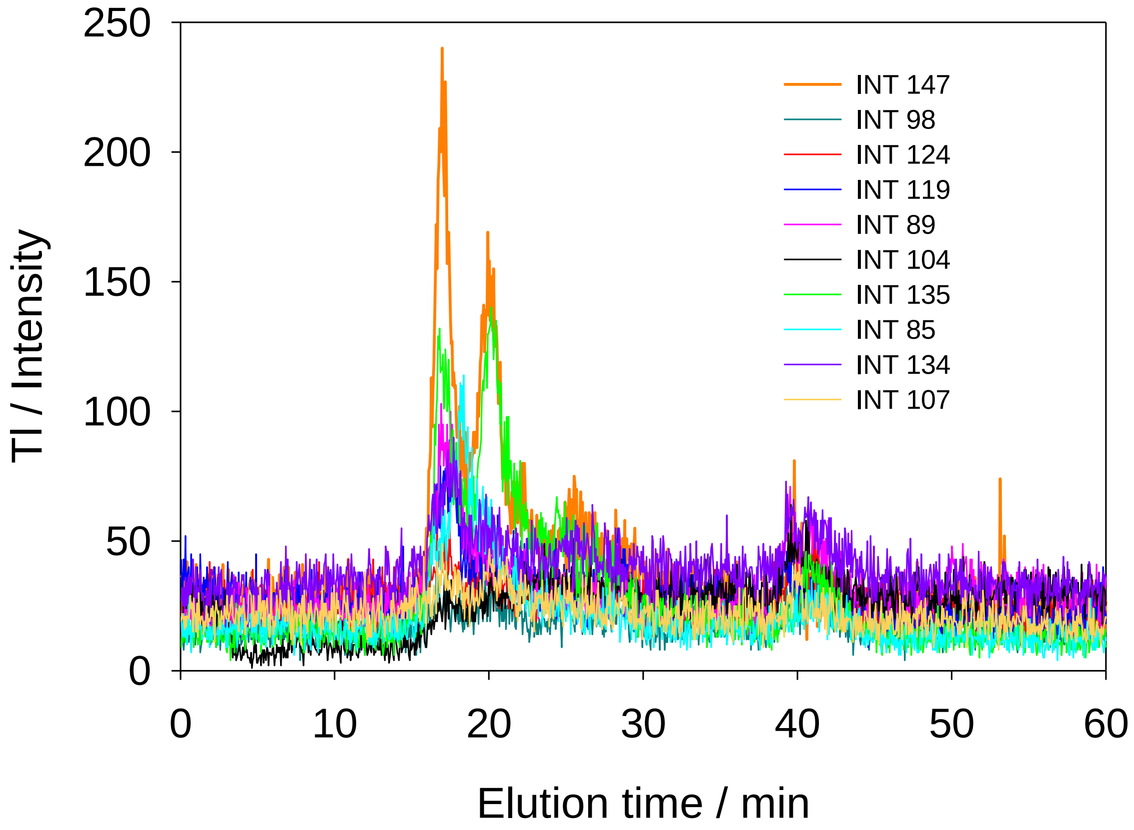
	µg/kg
A 85 A	4,13
A 89 A	6,22
A 98 A	8,09
A 104 A	6,11
A 107 A	12,38
A 124 A	2,53
A 134 A	8,58
A 135 A	29,98
A 147 A	28,08

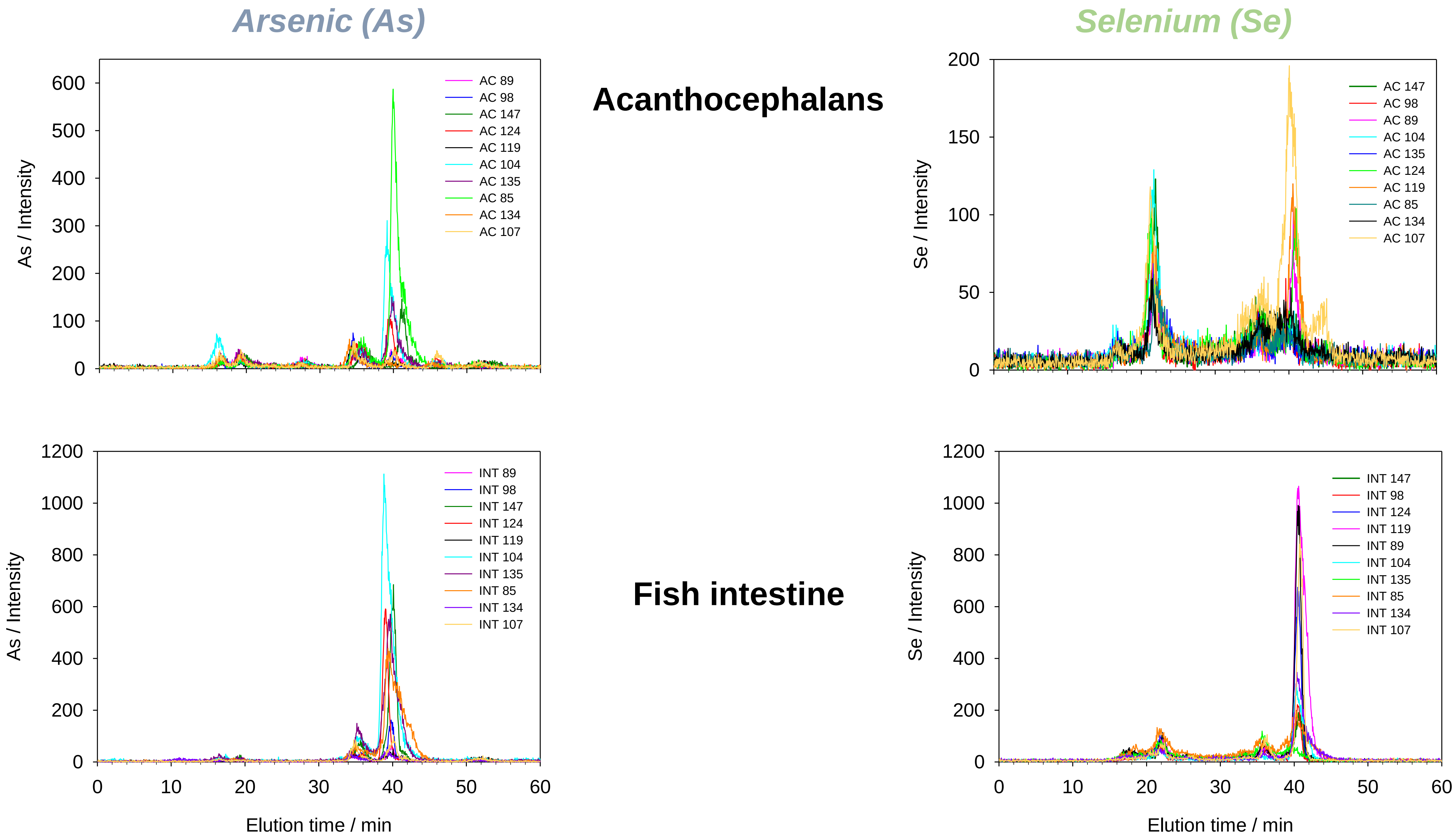
Acanthocephalans

Fish intestine

	µg/kg
I 85A	16,53
I 89 A	12,81
I 98 A	12,33
I 104 A	4,23
I 107 A	11,61
I 119 A	14,31
I 124 A	5,34
I 134 A	24,87
I 135 A	23,67
I 147 A	27,57







Arsenic (As)
Selenium (Se)
Acanthocephalans
Fish intestine
As / Intensity
Se / Intensity
Elution time / min
0
100
200
300
400
500
600
50
150
800
1000
1200
10
20
30
40
60
AC 89
AC 98
AC 147
AC 124
AC 119
AC 104
AC 135
AC 85
AC 134
AC 107
INT 89
INT 98
INT 147
INT 124
INT 119
INT 104
INT 135
INT 85
INT 134
INT 107

Conclusions

- Fish and acanthocephalans reflected environmental differences at three locations of the Krka River and with few exceptions, concentrations of **As, Ca, Cu, Fe, Se, Pb**, and **Zn** tended to be the highest at **KRK**, of **Cd**, **Rb** and **Tl** at **KRS**, and of **Hg, Mn, Sr** and **V** at **KNP**, which partially corresponded to the exposure from water and sediments, but also indicated connection with **ecology** of host and parasite species and **dietary habits**.

- **Great efficiency** of metal accumulation in **acanthocephalans** was confirmed for many elements, especially potentially toxic elements such as **Pb, Cd and Tl.**

- Higher number of acanthocephalans in the intestine seem to cause **biodilution effect** and lower metal concentrations in fish → further research needed!

- Mostly similar element cytosolic distribution in acanthocephalans in all sites.

- Some differences in cytosolic distribution between acanthocephalans and fish intestine observed ⇨ small specific differences in detoxification mechanisms.

- **Fish intestine and acanthocephalan *D. truttae* shown as sensitive indicators of low environmental metal concentrations.**

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Thank you!