METAL CONCENTRATIONS IN THE INTESTINAL FRACTIONS PREPARED BY DIFFERENTIAL CENTRIFUGATION PROCEDURE

FINAL PROJECT MEETING

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

Zagreb, 17th February 2025

Sara Šariri

The subcellular distribution of metals

- only a fraction of the total metal concentration in an organism is bioavailable (metabolic processes, toxic effects) while the rest is detoxified
- within cells, metals are distributed across different compartments - bioavailable fraction - metals bound to organelles and the cytosol
- the subcellular distribution of metals has been studied in the fish liver, gills, and gonads, while there are no studies on the intestine
- previous studies on the fish intestine have mostly reported total metal concentrations
- the aim of this study - for the first time determine the distribution of metals among subcellular fractions of the fish intestine using differential centrifugation procedure

Methods

- sampling in autumn 2021
- Intestinal tissue of 4 individuals from each location

Subcellular fractionation

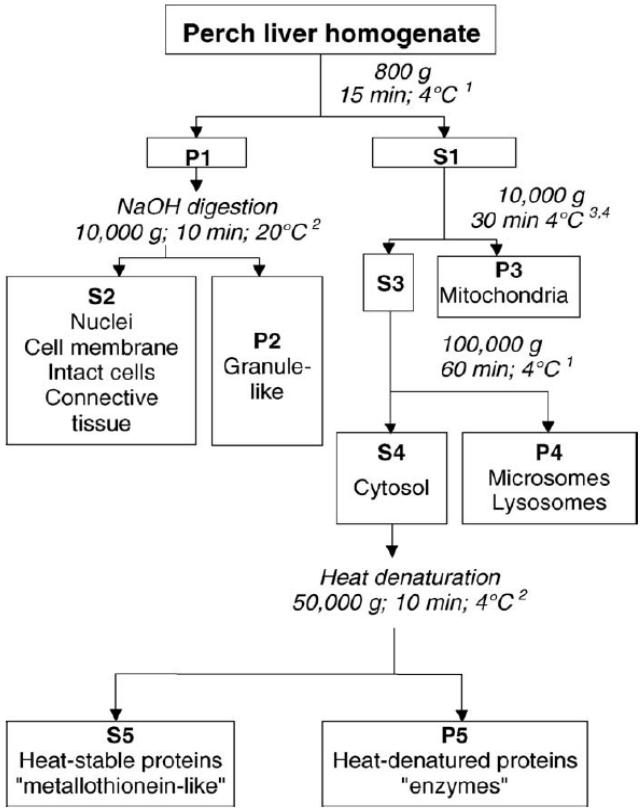


Fig. 1. Differential centrifugation method used to separate the various subcellular fractions. P: pellet, S: supernatant. (1) Durand et al. (1999), (2) Wallace et al. (2003), (3) Takeda and Shimizu (1982), and (4) Olsson and Haux (1986).

Giguere et al. 2006

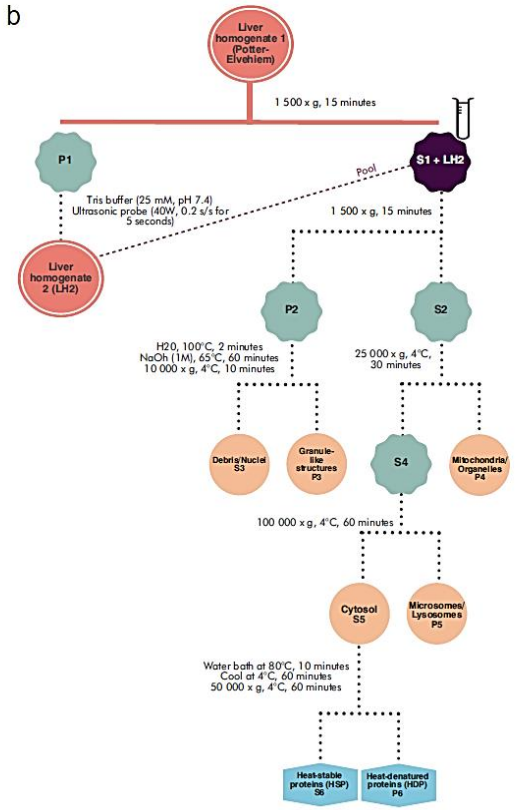


Fig. 2. Schematic illustration of the subcellular partitioning procedure used to separate Yellow Perch (a) gonads and (b) liver into operationally defined fractions. Small tubes represent the 50-µL homogenate fractions. Fractions represented by orange circles were tested with marker enzymes.

Khadra et al. 2019

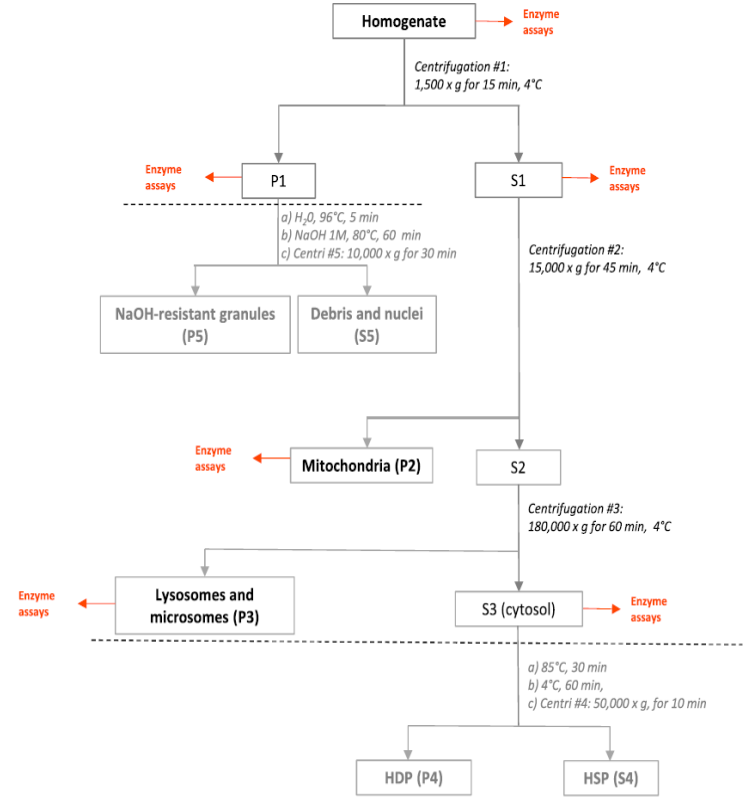
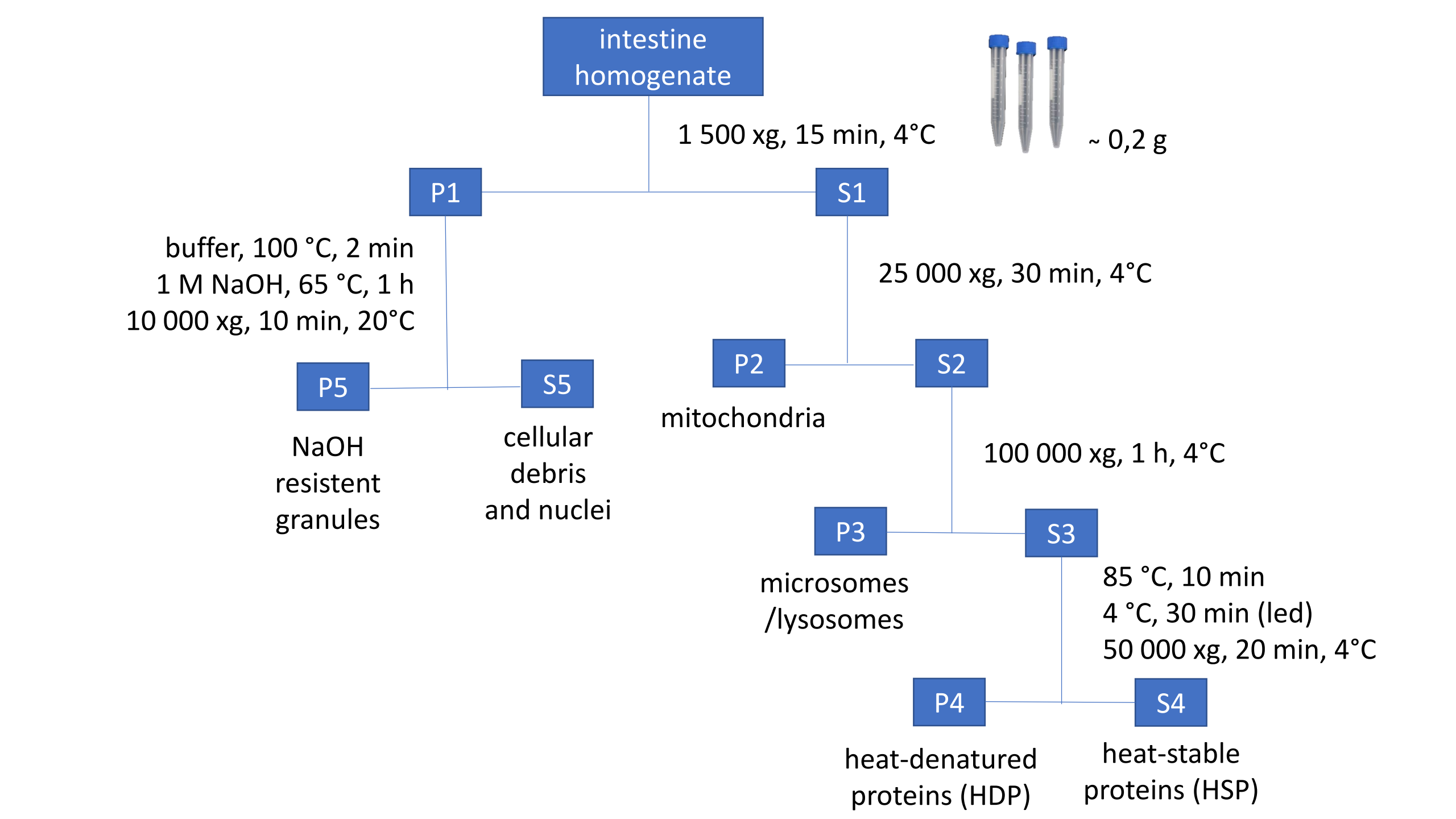


Fig. 1. Flowchart of the generic subcellular fractionation protocol for metal distribution studies. The letter P indicates pellet and S is for supernatant. The acronyms HSP and HDP mean, respectively, "heat-stable proteins" and "heat-denaturable proteins." The mention of "enzyme assays" indicates that marker enzymes can be measured in these fractions to validate the protocols. Fractions in gray and below the dotted lines were not subjected to enzyme assays as they had undergone heat treatments.

Urien et al. 2020


intestine homogenate
1 500 xg, 15 min, 4°C
~ 0,2 g
P1
S1
buffer, 100 °C, 2 min
1 M NaOH, 65 °C, 1 h
10 000 xg, 10 min, 20°C
25 000 xg, 30 min, 4°C
P5
S5
P2
S2
NaOH resistent granules
cellular debris and nuclei
mitochondria
100 000 xg, 1 h, 4°C
P3
S3
microsomes /lysosomes
85 °C, 10 min
4 °C, 30 min (led)
50 000 xg, 20 min, 4°C
P4
S4
heat-denatured proteins (HDP)
heat-stable proteins (HSP)

intestine homogenate

~ 0,2 g

1 500 xg, 15 min, 4°C

P1

S1

buffer, 100 °C, 2 min
1 M NaOH, 65 °C, 1 h
10 000 xg, 10 min, 20°C

25 000 xg, 30 min, 4°C

P5

S5

P2

S2

metal sensitive fraction

biologically detoxified metals

NaOH resistent granules

cellular debris and nuclei

mitochondria

100 000 xg, 1 h, 4°C

P3

S3

microsomes /lysosomes

85 °C, 10 min
4 °C, 30 min (led)
50 000 xg, 20 min, 4°C

P4

S4

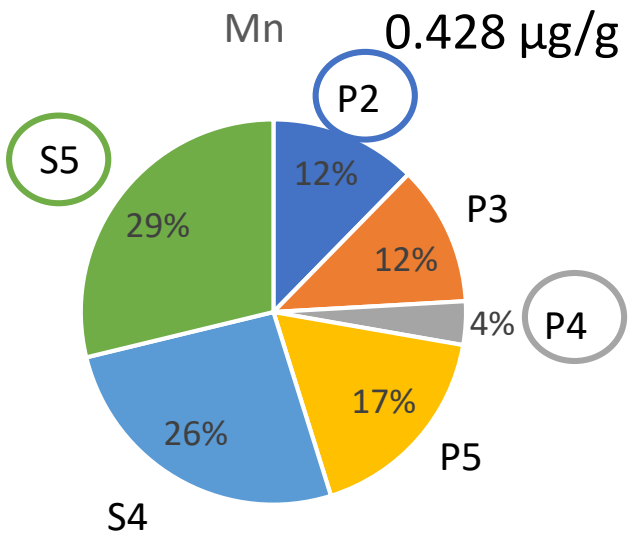
heat-denatured proteins (HDP)

heat-stable proteins (HSP)

- digestion with HNO_3 overnight, 3.5 h on 85 °C
- concentrations of Mn, Fe, Cu, Zn, Se, Rb and Sr measured in each fraction by ICP-MS

Results

Mn



n = 12

- P2 - mitochondria
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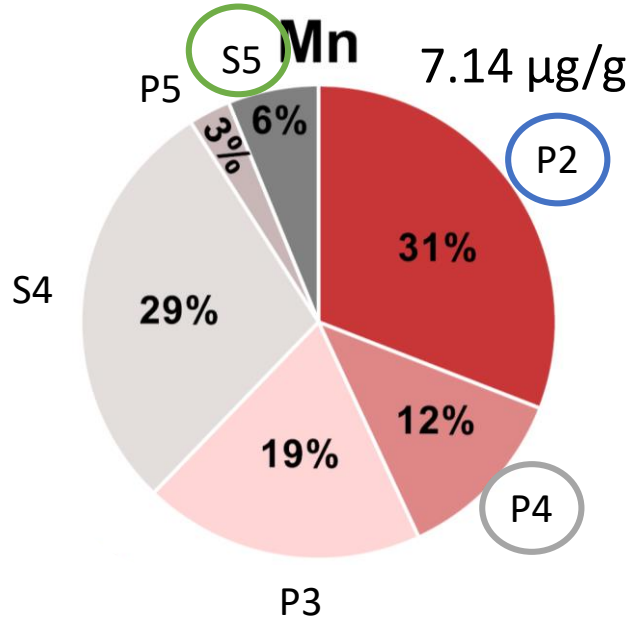
Desjardins et al. 2022
(yellow perch, liver)



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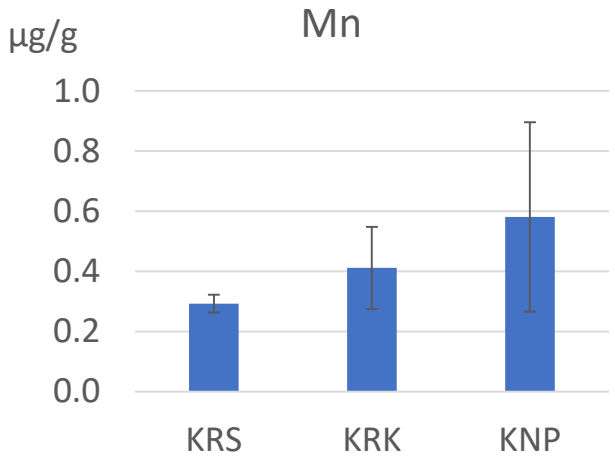

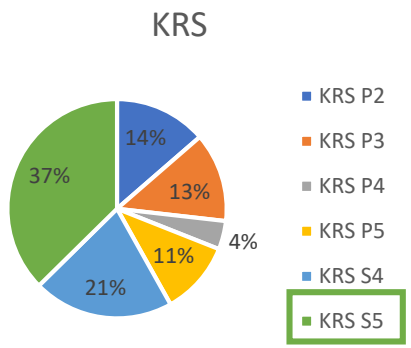

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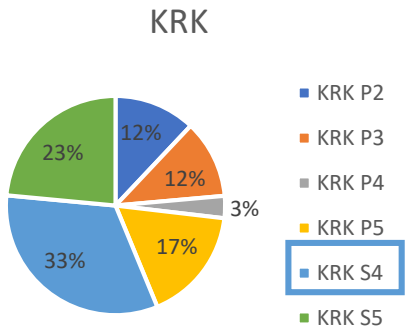

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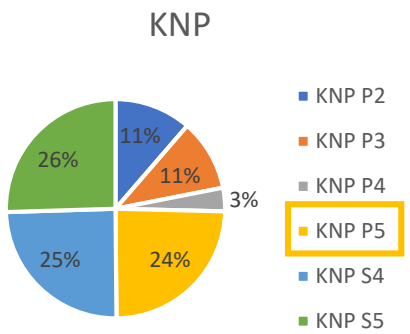

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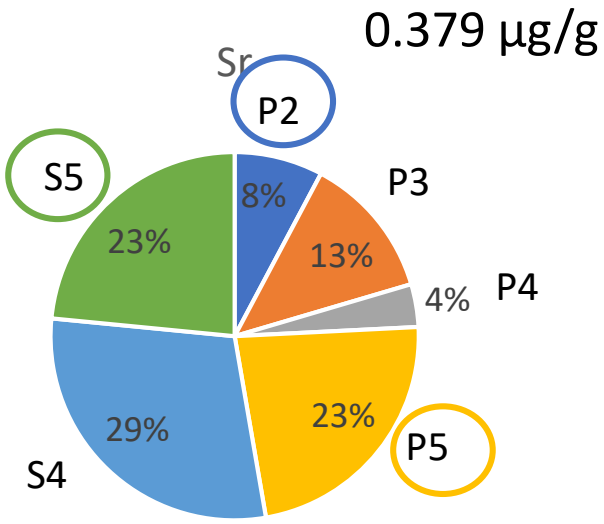
Sr



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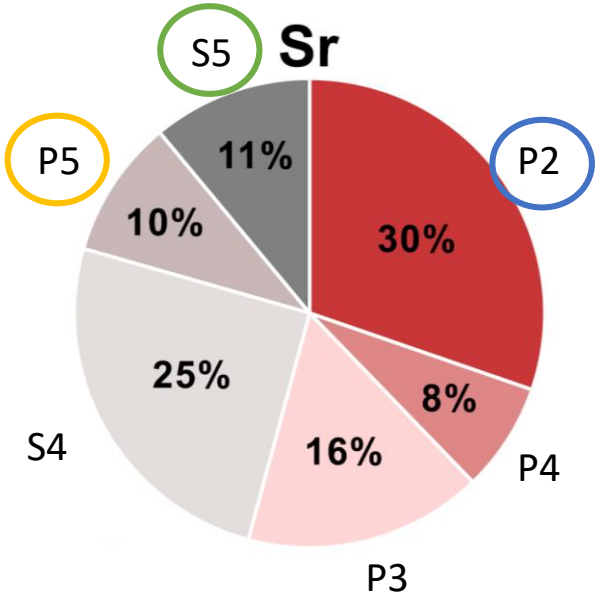
Desjardins et al. 2022
(yellow perch, liver)



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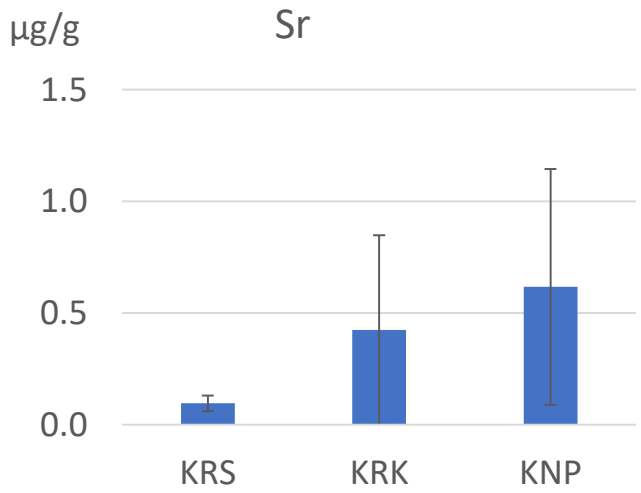

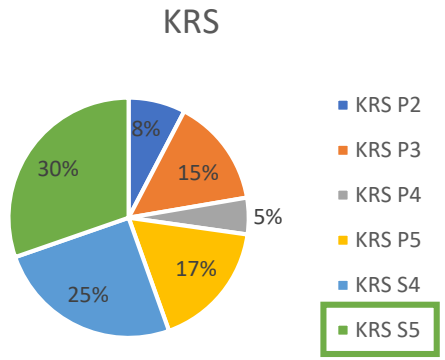

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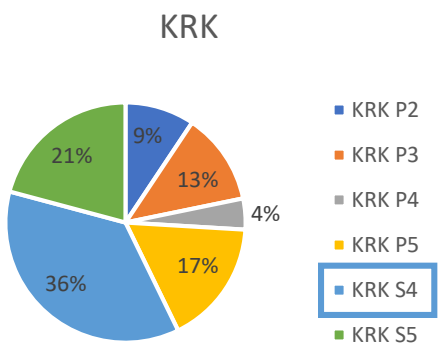

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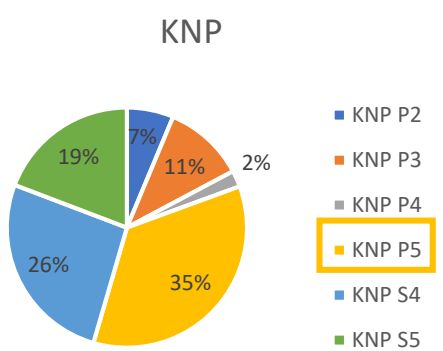


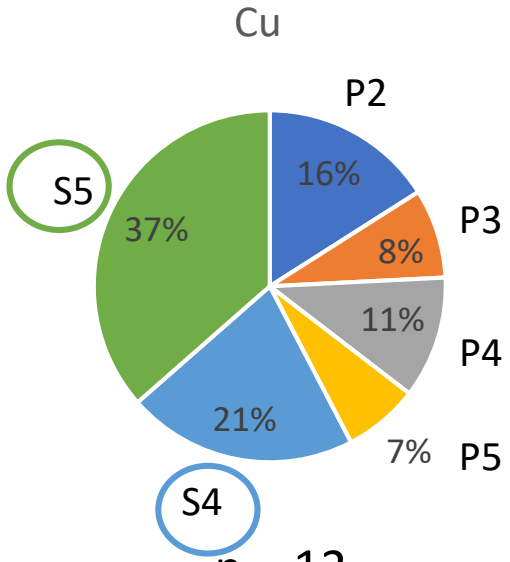
n = 4

Cu

- Giguere et al. 2006 (yellow perch, liver): on average 52% ± S.D. 8% in cytosol (P4 and S4)

Desjardins et al. 2022 (yellow perch, liver)

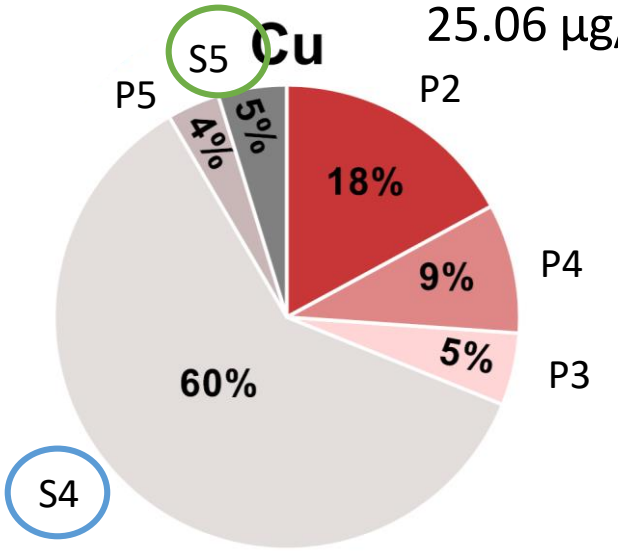
0.736 µg/g



n = 12

- P2 - mitochondria
- P3 - microsomes/lysosomes
- P4 - heat-denatured proteins (HDP)
- P5 - NaOH resistent granules
- S4 - heat-stable proteins (HSP)
- S5 - cellular debris and nuclei

25.06 µg/g



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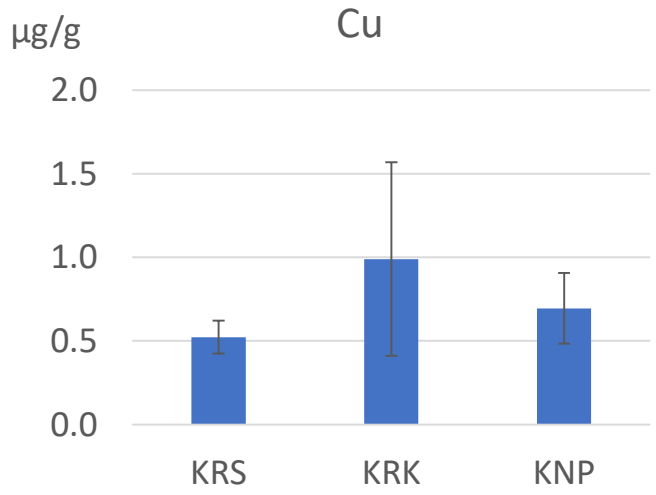

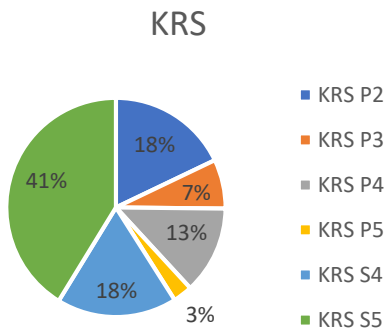

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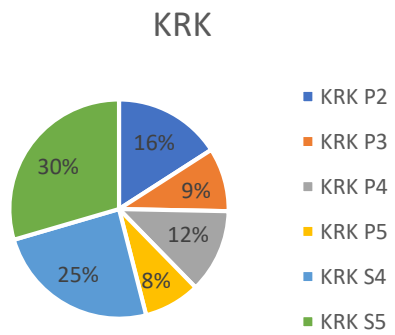

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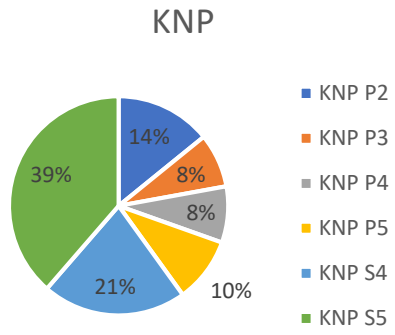
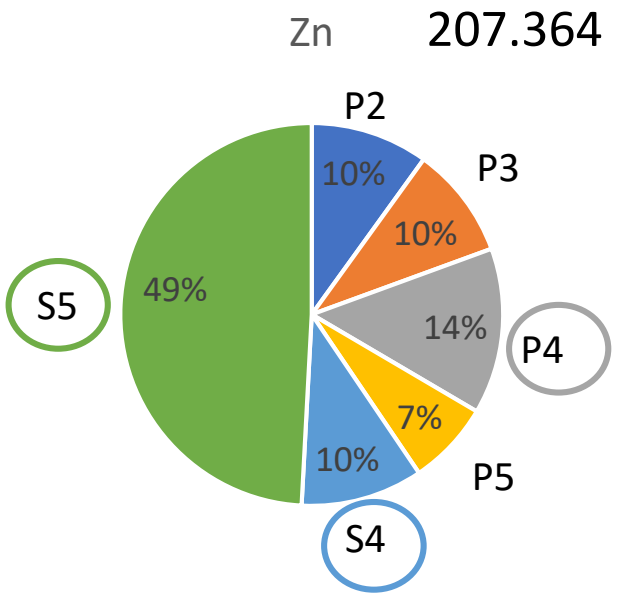


n = 4

Zn



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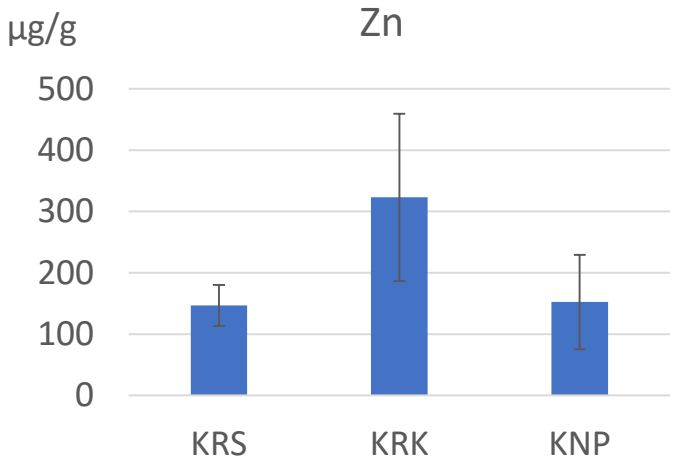
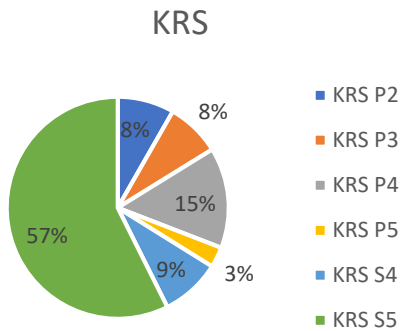
- Giguere et al. 2006 (yellow perch, liver): on average 51% ± S.D. 8% in cytosol (P4 and S4)

n = 48





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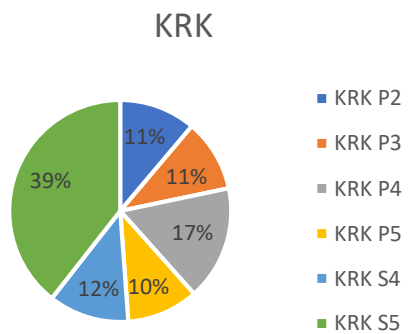

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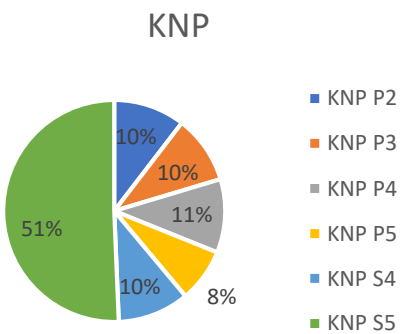

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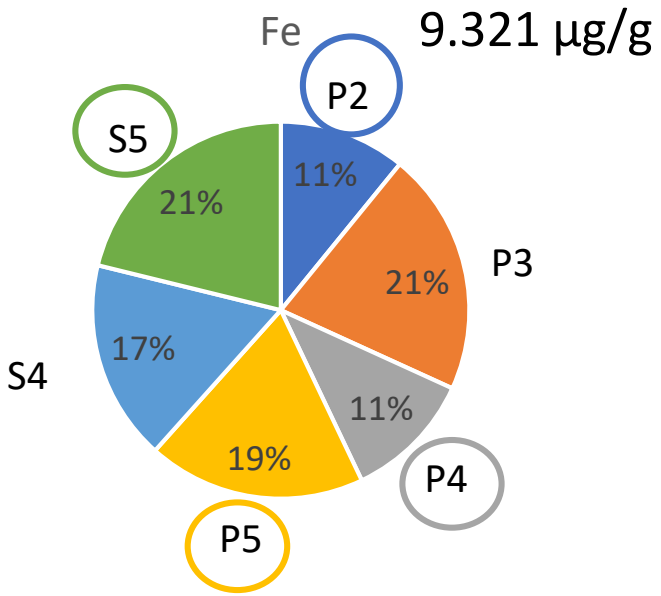
Fe

Desjardins et al. 2022
(yellow perch, liver)



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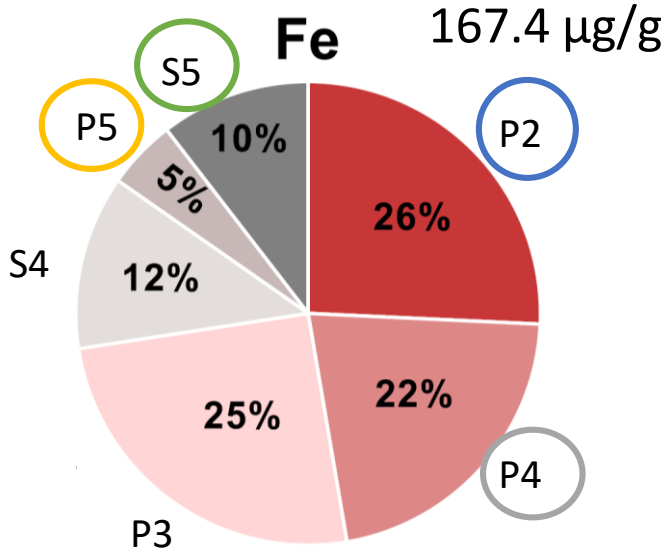

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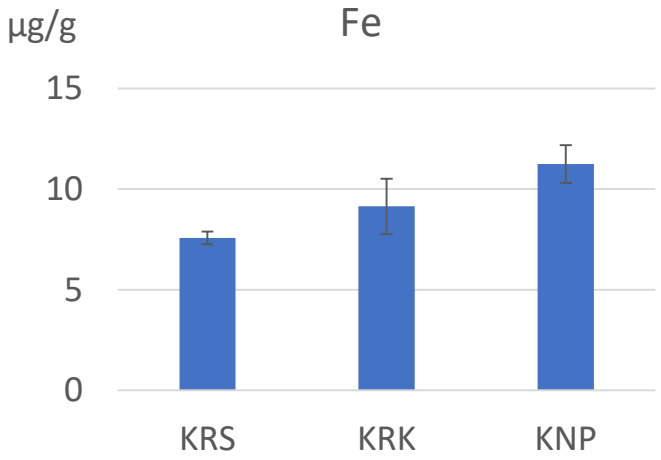

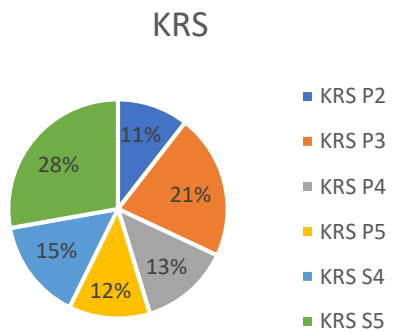

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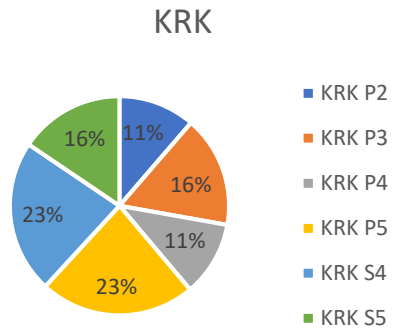

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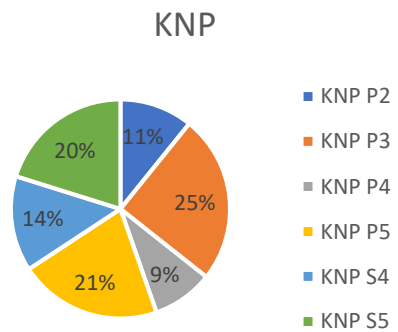
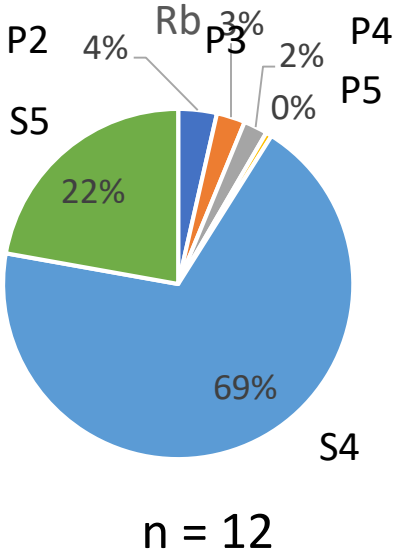


n = 4

Rb



n = 12

2.826 µg/g

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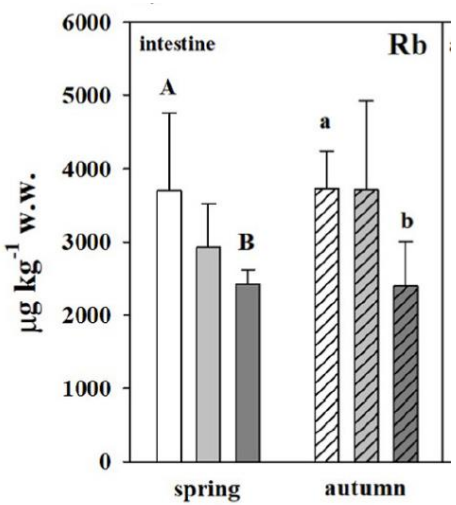

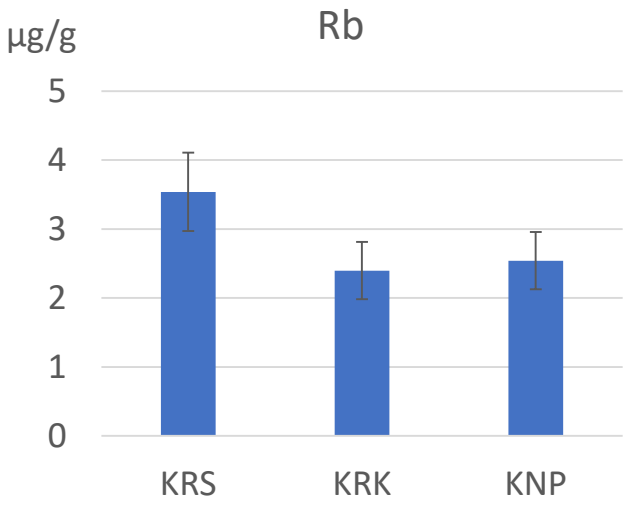

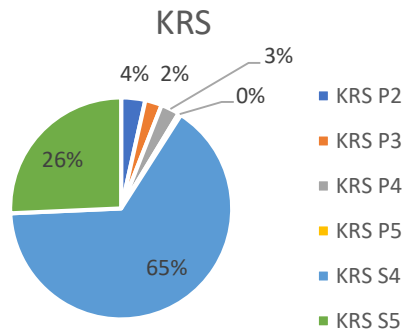

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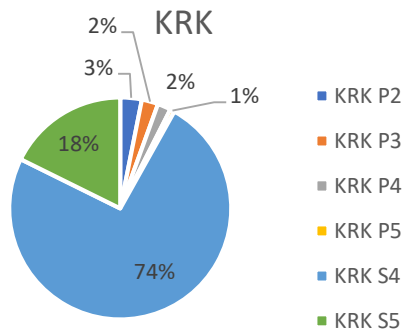

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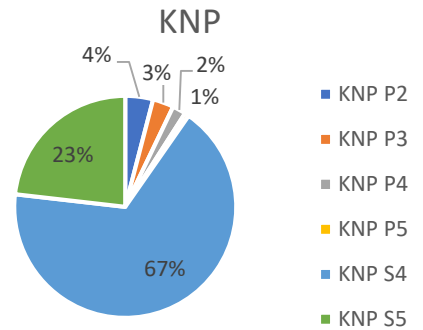

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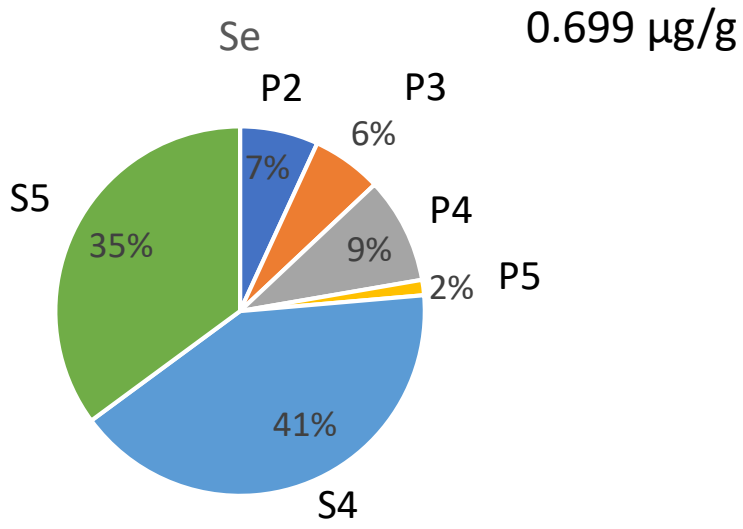

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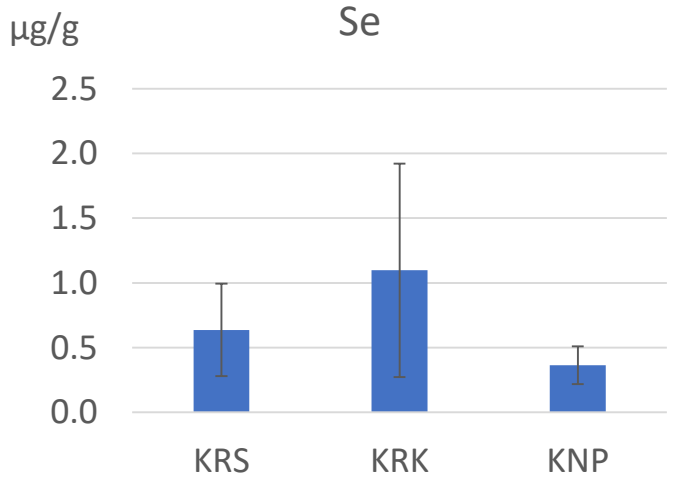

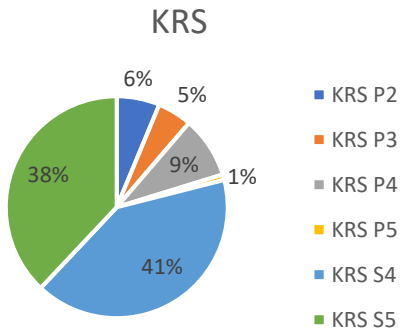

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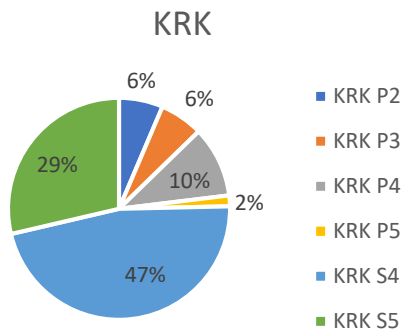

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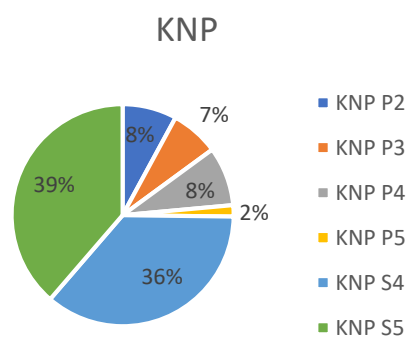

n = 4

Conclusions

- the first data for the intestinal tissue of brown trout
- no significant differences between sampling sites
- all elements - higher percentages in S5 fraction (cellular debris and nuclei) compared to the literature data – tissue specificity, incomplete homogenization

Thank you for your attention!

- Croatian Science Foundation project: Integrated evaluation of aquatic organism responses to metal exposure: gene expression, bioavailability, toxicity and biomarker responses (BIOTOXMET) project number: IP-2020-02-8502