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Evaluation of the gastropod *Radix balthica* as bioindicator of cobalt exposure in outdoor mesocosms

Maëva MARIMOUTOU¹, G. BAREILLE¹, P. BALDONI-ANDREY², A. BOULLEMANT³, C. COURRÈGES¹, C. GELBER², S. MOUNICOU¹, H. TABOURET¹, S. LE FAUCHEUR¹

¹Université de Pau et des Pays de l'Adour, E2S-UPPA, CNRS, IPREM, Pau, France; ²TotalEnergies OneTech Pole d'Études et de Recherche de Lacq, France; ³BCenv, Gardanne, France (Email: maeva.marimoutou@univ-pau.fr)

INTRODUCTION

Most studies concerning metal interactions with molluscs focus on metal accumulation in their soft bodies and the whole shell. Indeed, gastropod shell has the interesting feature to grow throughout their life by integrating some of the chemical elements present in their environment, including metals.

The present study aims to examine the kinetics of cobalt (Co) accumulation in gastropod soft tissue and shell, in order to evaluate the use of *Radix balthica* as bioindicator of metals in freshwater. Cobalt was selected due to its increasing use in recent technologies of transport or energy storage.

Cobalt in the shell was analyzed along the last growth layers by Femto-second Laser Ablation Inductively Coupled Plasma Mass Spectrometry (Fs-LA-ICPMS - micrometer scale).

MATERIALS AND METHODS

Radix balthica



Ubiquitous species in European rivers

Outdoor mesocosm experiments : 28 days-exposure in June 2021 (spring) & October 2021 (autumn)



32 adult organisms per box

4 exposure Co concentrations ($\mu\text{g}\cdot\text{L}^{-1}$)

- June : 0.7, 2.4, 24 and 56
- October : 0.4, 5.4, 31 and 64

- June : Pseudo replicates, 4 streams, 3 box per stream

Sampling: 7, 14, 21 and 28 days of exposure

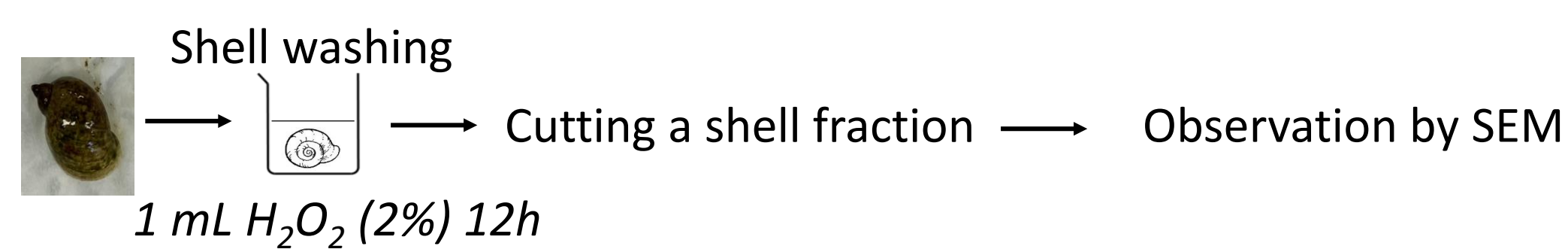
- October : Triplicates, 12 streams, 1 box per stream

Water analysis

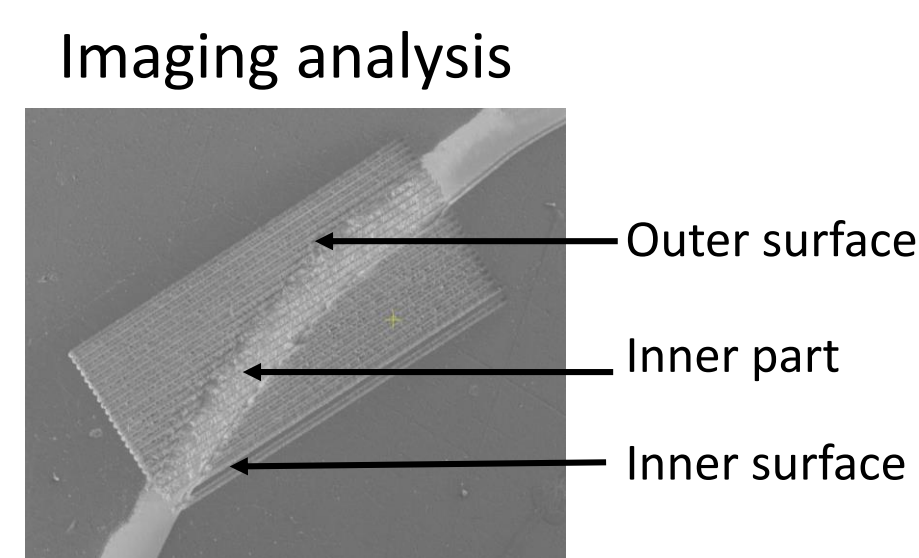
- Physico-chemical parameters (pH, T°C, conductivity, oxygen)
- Dissolved metals, cations/anions, DOC, alcalinity

Gastropod analysis

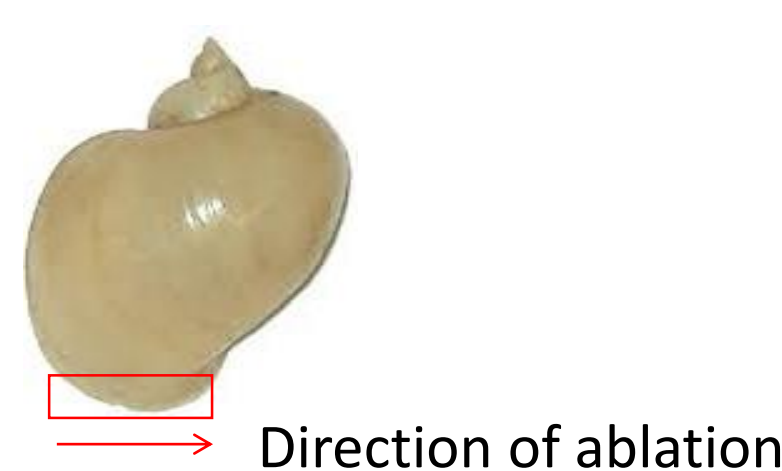
① Shell structure by SEM



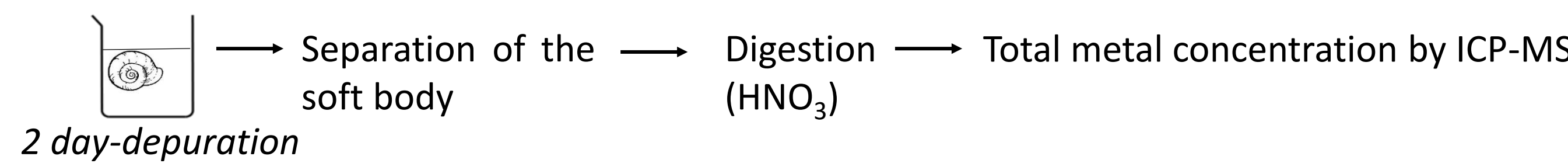
② Fs-LA-ICPMS shell Imaging analysis



Ablation at the outer surface



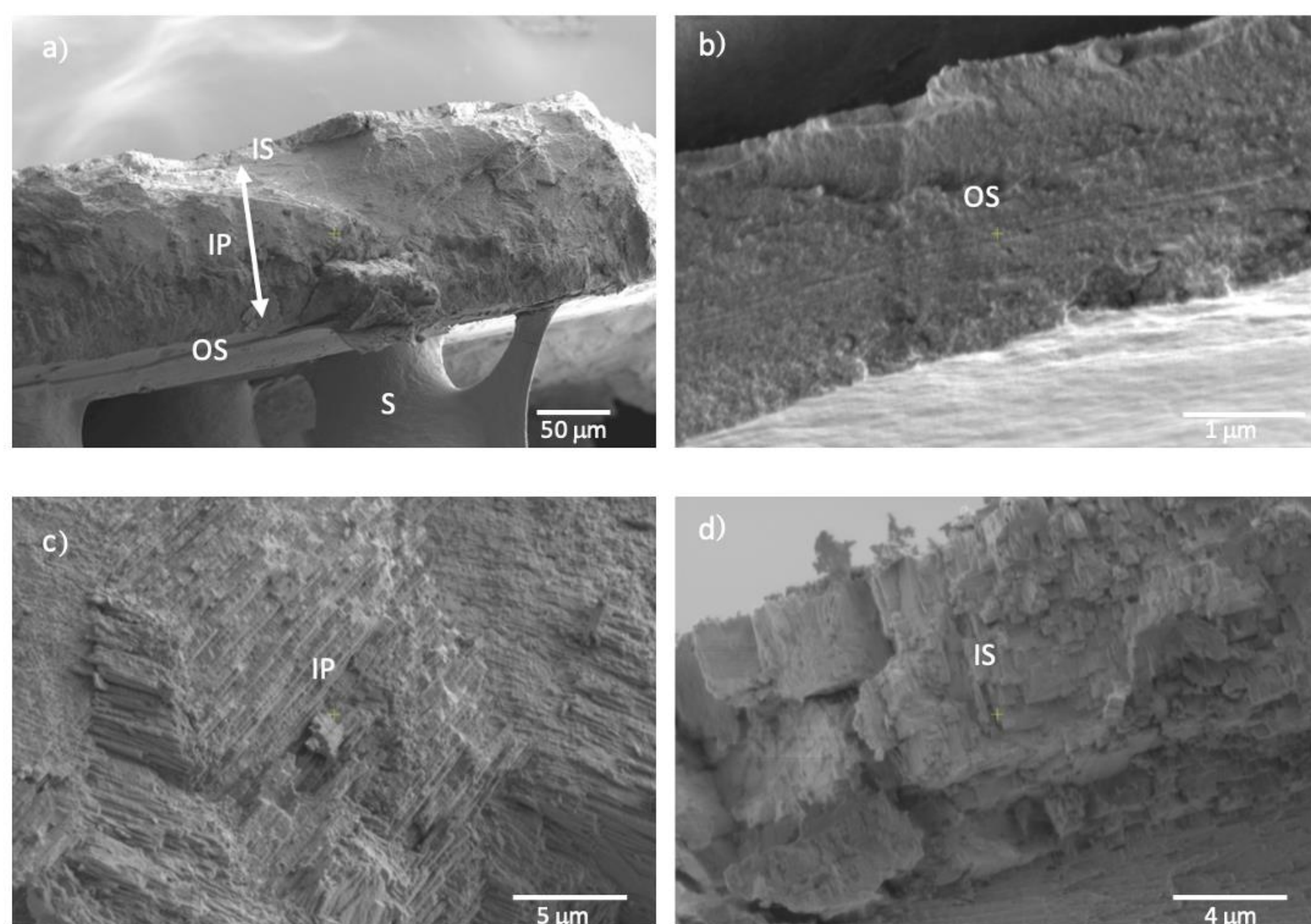
③ Soft tissues



- Mapping focused on the last growth deposit
- 1 replicate per concentration at 28 day-exposure
- Analysis within a single growth ridge in the exposed shell part
- 3 replicates per conditions and each sampling day

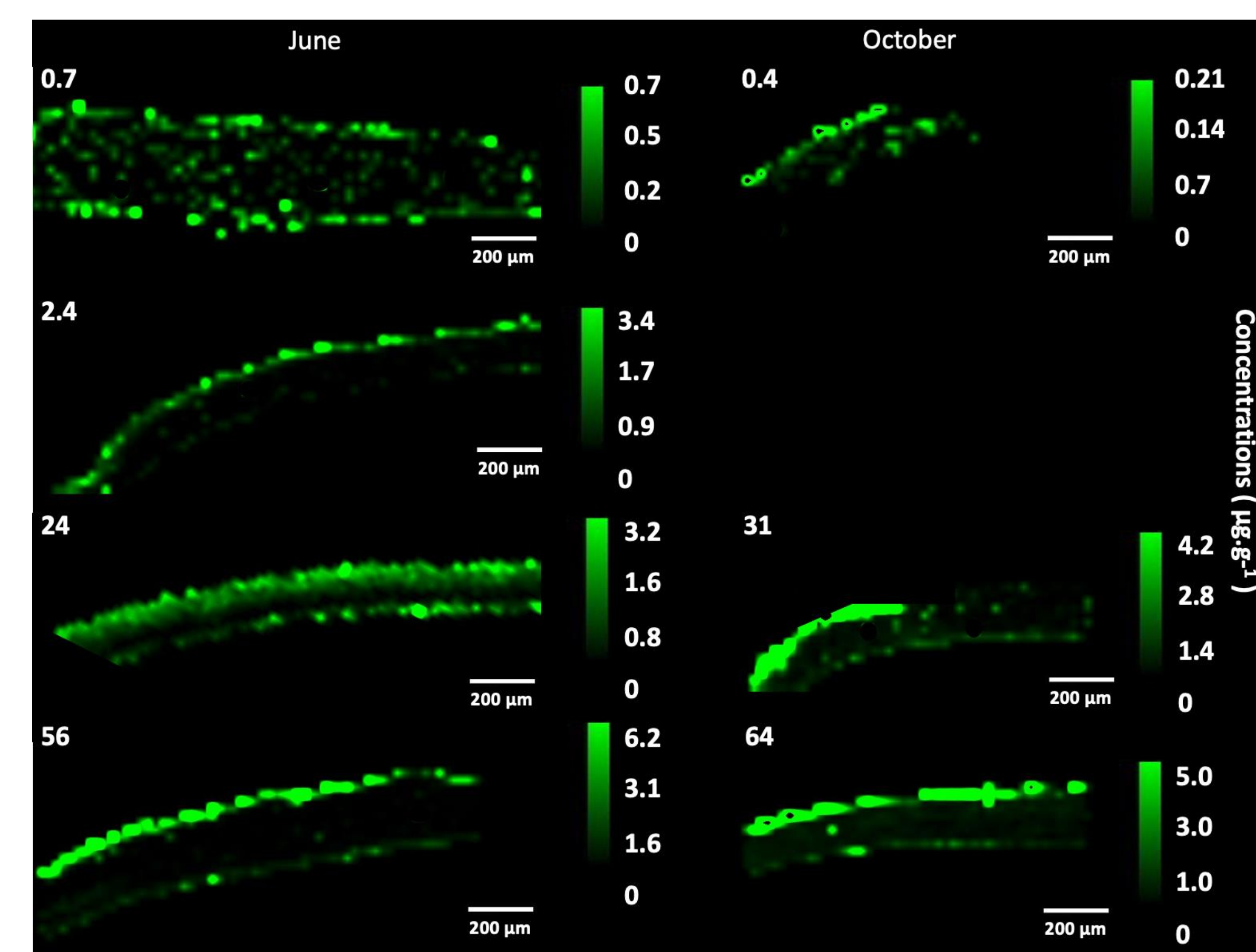
RESULTS

1) Shell gastropod structure by SEM



- Outer surface (OS) : no structure, indicative of a protein-rich layer
- Inner part (IP) : complex prismatic layer, ~ 150 μm (aragonite)
- Inner surface (IS): nacreous layer, ~ 10 μm (aragonite)
- S: support

2) Co distribution in shell layer by mass-spectrometry imaging at day 28

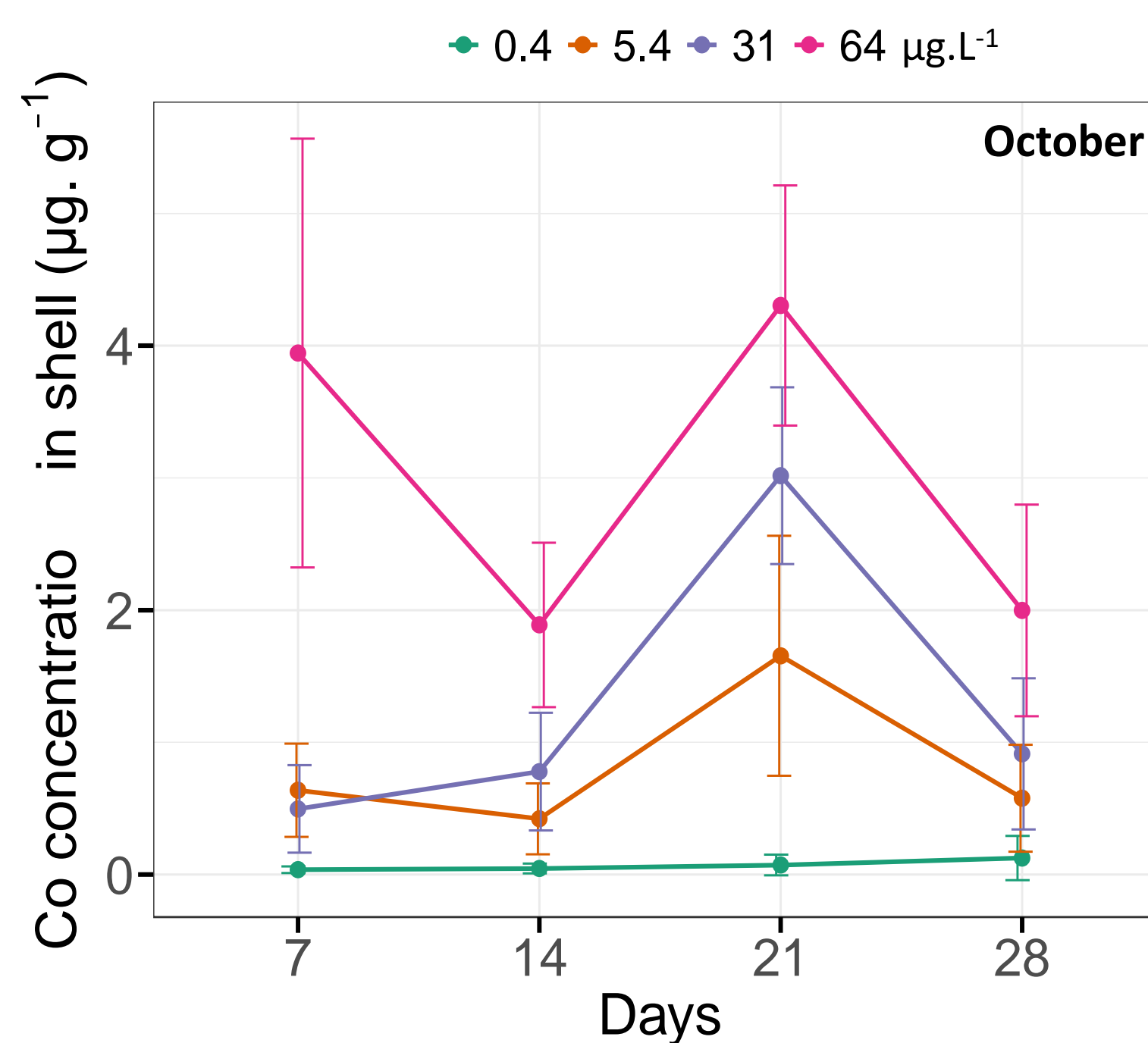


- In control (0.7 $\mu\text{g}\cdot\text{L}^{-1}$ and 0.4 $\mu\text{g}\cdot\text{L}^{-1}$) : Co distributed evenly in the shell
- High Co concentration mainly found on the shell outer surface

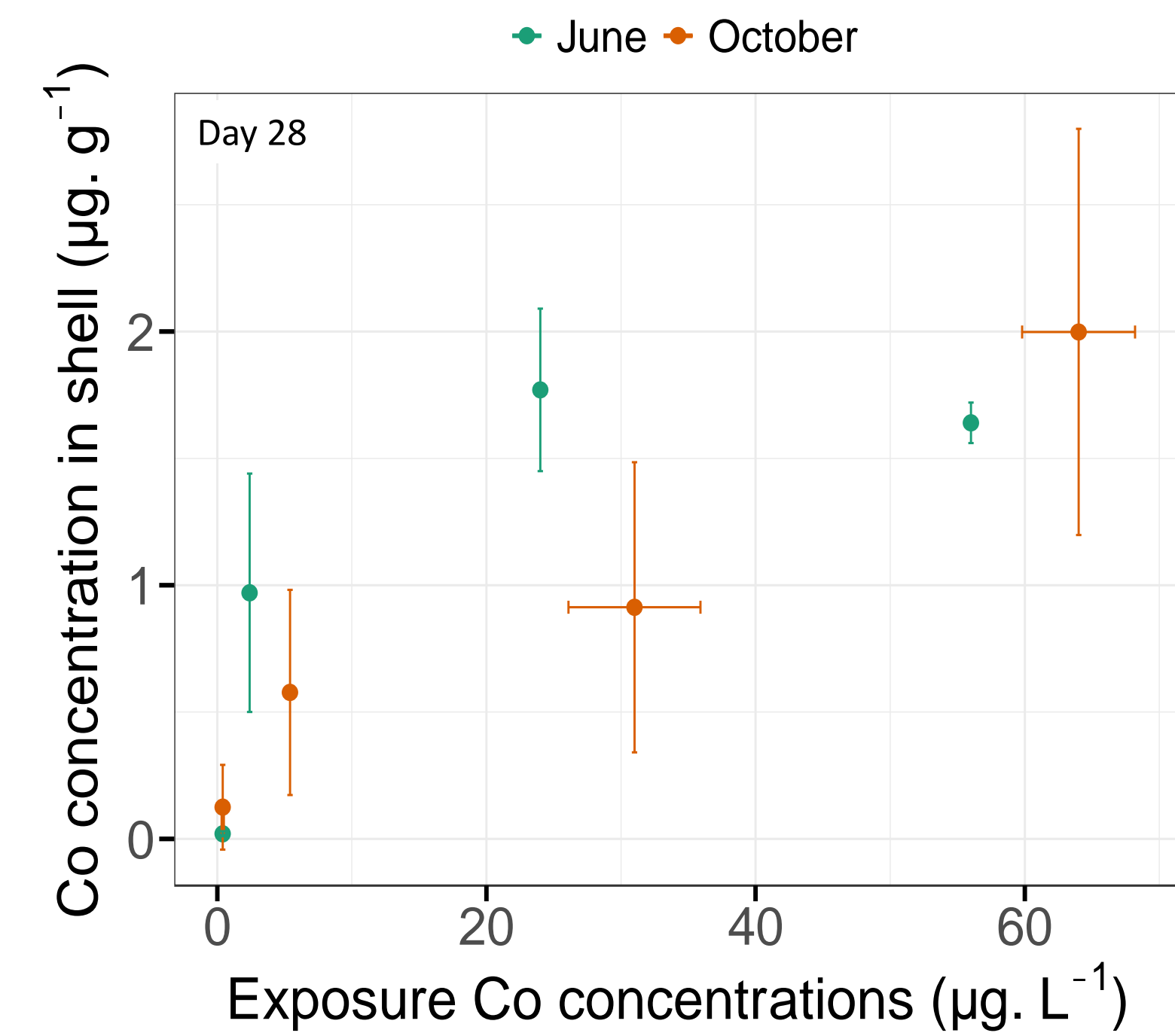
ACKNOWLEDGEMENT

We are grateful to TotalEnergies at Lacq for allowing us to perform our experiments at their facility. All members of Chair Ecotox and TotalEnergies helped with field preparation and sampling, especially J.M Dalens and C. Pillitteri.

3) Co concentration in external shell surface (Fs-LA-ICPMS)

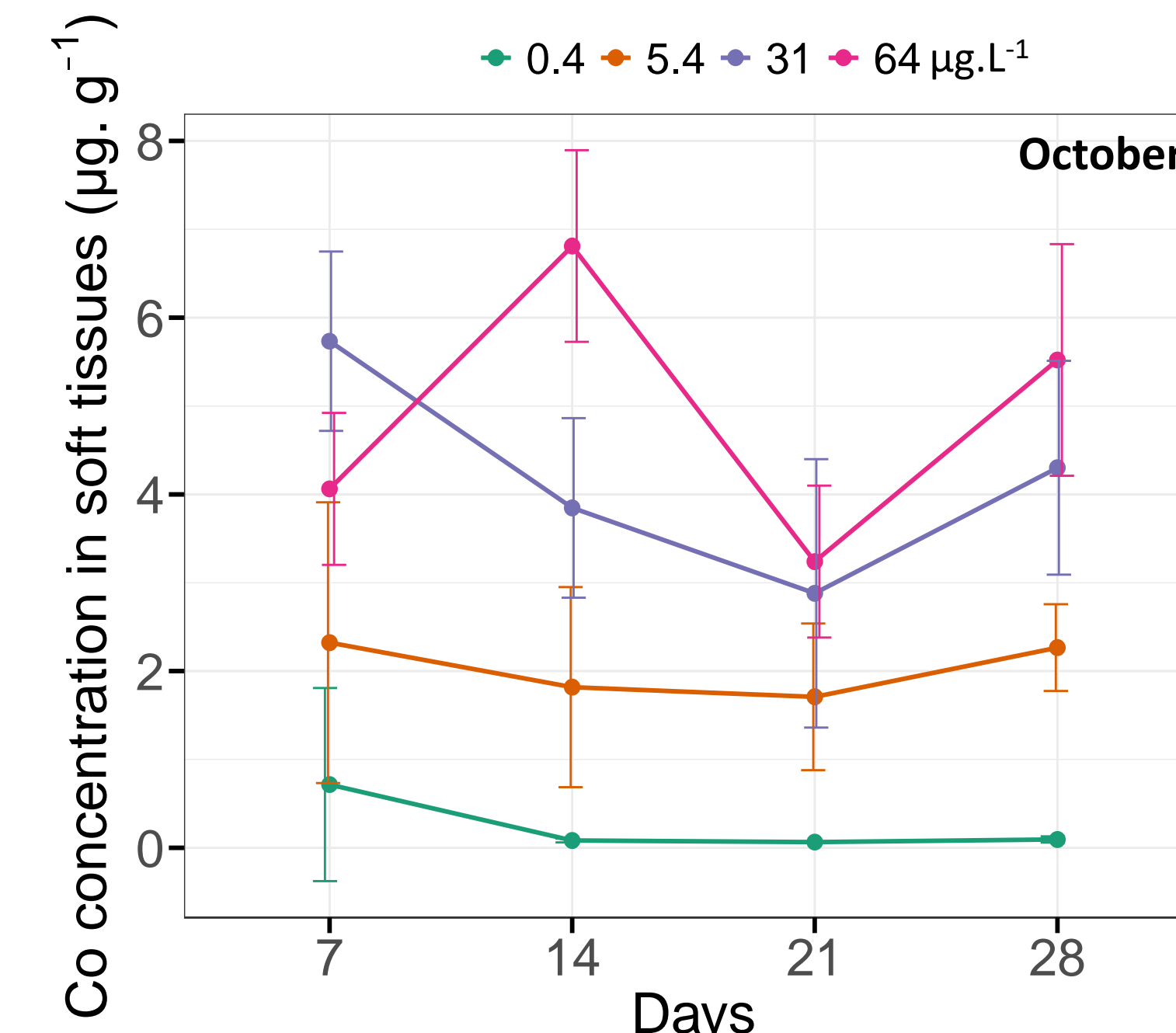


- Co accumulation observed already at day 7 and at a similar level for all exposure times

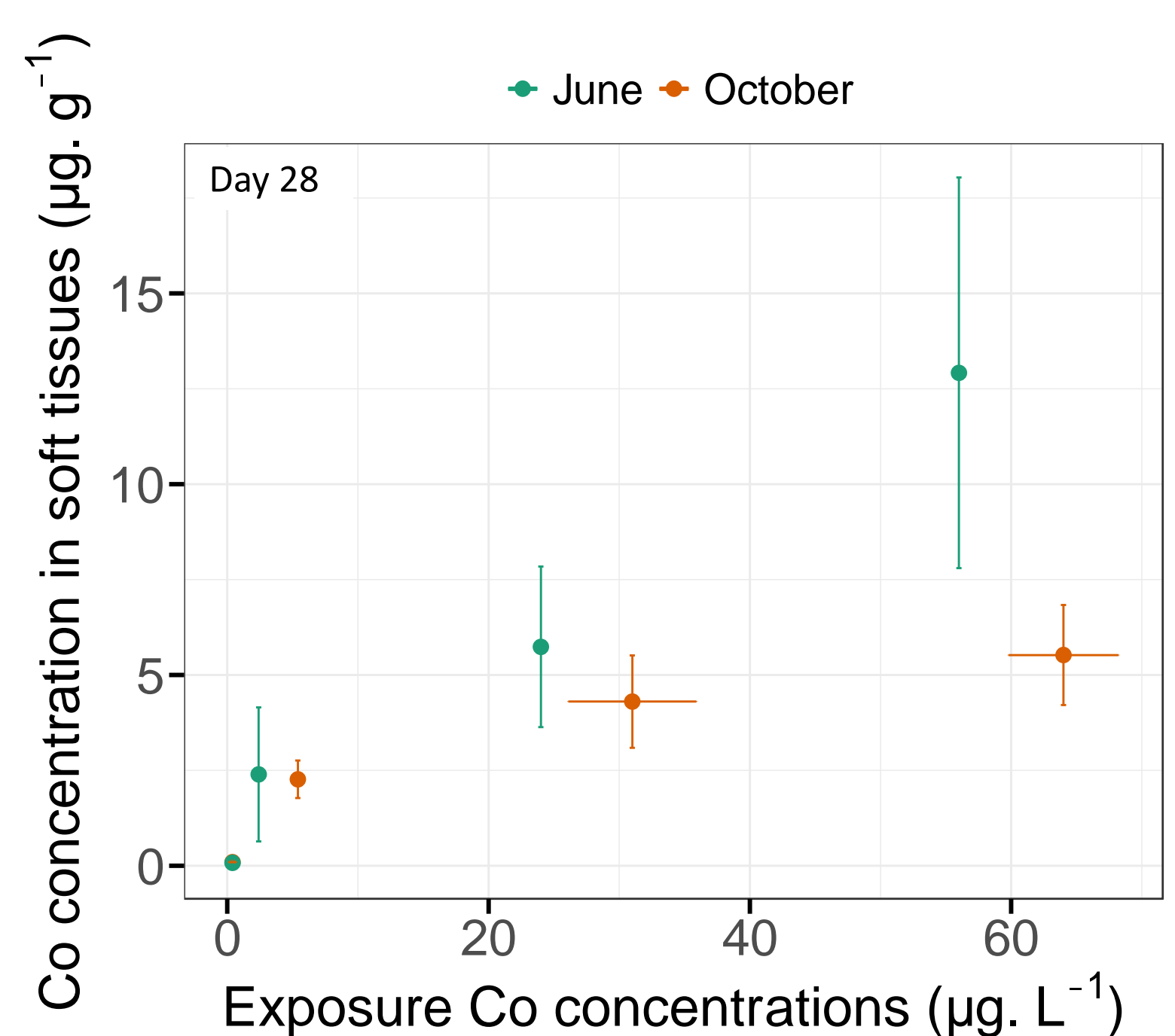


- Co accumulation increased with ambient Co exposure concentration

4) Co concentration in soft tissues

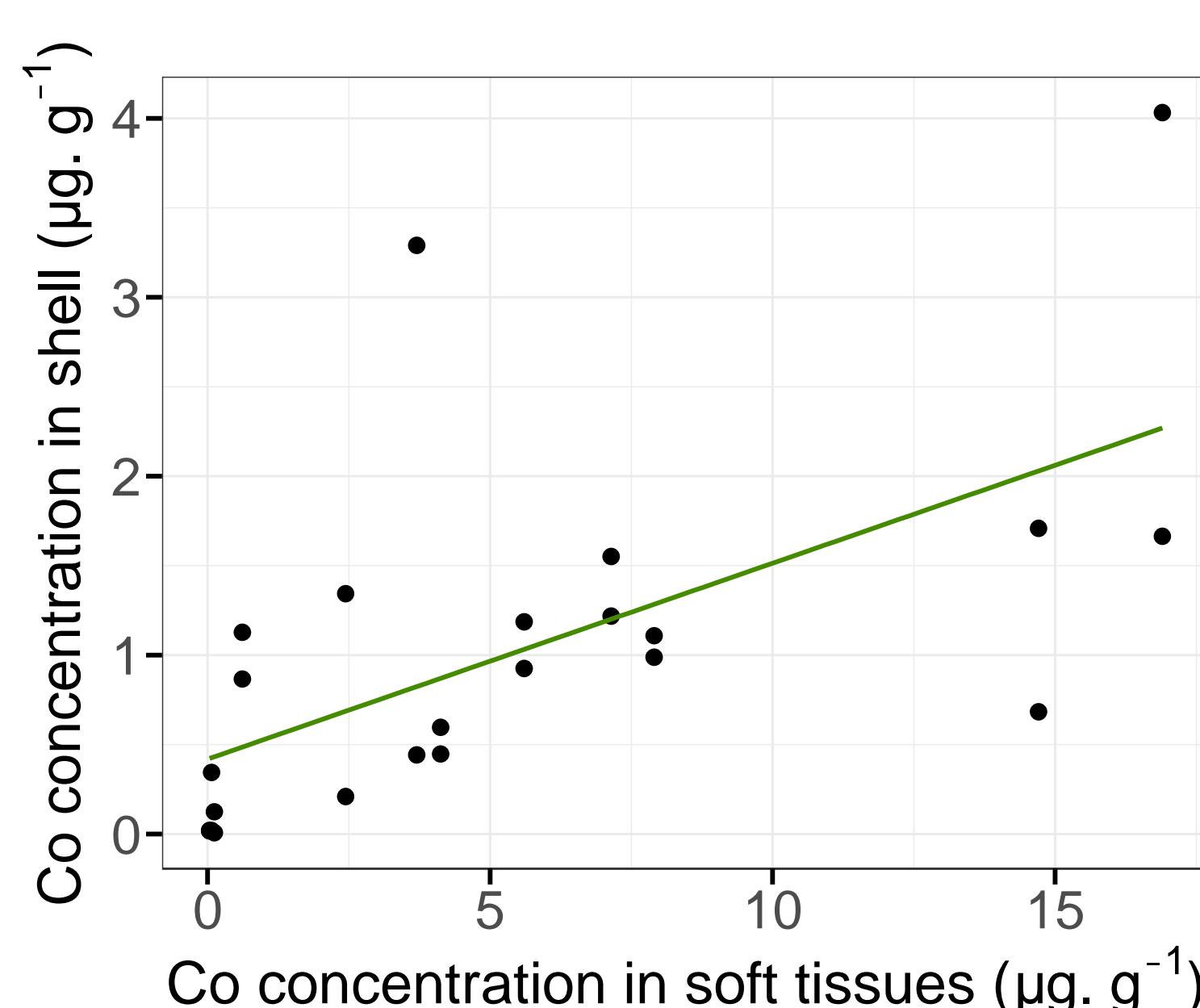


- For the two lowest Co exposure concentrations: similar Co concentrations in soft tissues for all exposure times
- For the two highest Co exposure concentrations: More variable Co concentrations in soft tissues as a function of exposure time



- In June, Co accumulation increased with ambient Co concentrations
- In October, Co accumulation reached a plateau at 31 $\mu\text{g}\cdot\text{L}^{-1}$

5) Relationship between Co accumulation in the soft tissues and the shell



- Co concentration in shell increased as a function of Co concentrations in the soft tissues

CONCLUSION

- Shell layers of *Radix balthica* are heterogeneous with different carbonate structure characteristics of aragonite.
- Cobalt is mainly located in the outer surface, which corresponds to the periostracum.
- Cobalt accumulation in shell and soft tissues took place within the first 7 days of exposure.

➔ Soft tissues and shells were both representatives of ambient Co concentrations but their relationships are not straightforward.

➔ Further analyses will focus on Co distribution in the soft tissues to better understand the relationships between Co accumulation in shells and soft tissues.