

Impact of heavy metals (Cu, Fe, Pb, Zn) on carbon and nitrogen uptake of the diatom-bearing benthic foraminifera *Heterostegina depressa* d'Orbigny 1826

Mario Bubl¹, Wolfgang Wanek², Petra Heinz¹, Thilo Hofmann³ and Michael Lintner¹

¹University of Vienna, Department of Palaeontology, Vienna, Austria

²University of Vienna, Department of Microbiology and Ecosystem Science, Division of Terrestrial Ecosystem Research, Vienna, Austria

³University of Vienna, Department of Environmental Geosciences, Vienna, Austria

27th of May 2022; EGU General Assembly 2022 – Vienna, Austria

Introduction

Heterostegina depressa

- Protozoa, Foraminifera
- Alcide d'Orbigny
 - Original description in 1826
- Larger Foraminifera
- Calcareous test
- Hosting diatoms
 - Symbiotic algae
- Inshore and offshore reefs
- Tropical to temperate Atlantic, Pacific and Indian Ocean



Heterostegina depressa - living
Department of Palaeontology – University of Vienna

Hypothesis

- I. Effects in respect of increased or decreased symbiotic activity
 - Copper negative impact – iron positiv impact?
- II. Increased Lead concentration - not toxic?
 - Two selfcontained results (Lintner et al., 2021 and present study)
- III. Enrichment of Zinc is leading in lower concentrations to a higher activity – in contrast to this, a higher concentration shoud reveal in decreased symbiotic activity

Setting & preparation

Setting

Heterostegina depressa culture

- Salzburg/Austria
- Haus der Natur
- Shark tank
- 2015

Synthetic seawater

- 25 °C
- 35 PSU
- 8:16 h light/dark rhythm
- 40 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$



Heterostegina depressa culture
Department of Palaeontology – University of Vienna

Setting & preparation

Preparation

Artificial seawater preparation with synthetic marine salt

- Including essential micro,- and macronutrients
- 33PSU

Heavy metal *stock solution*

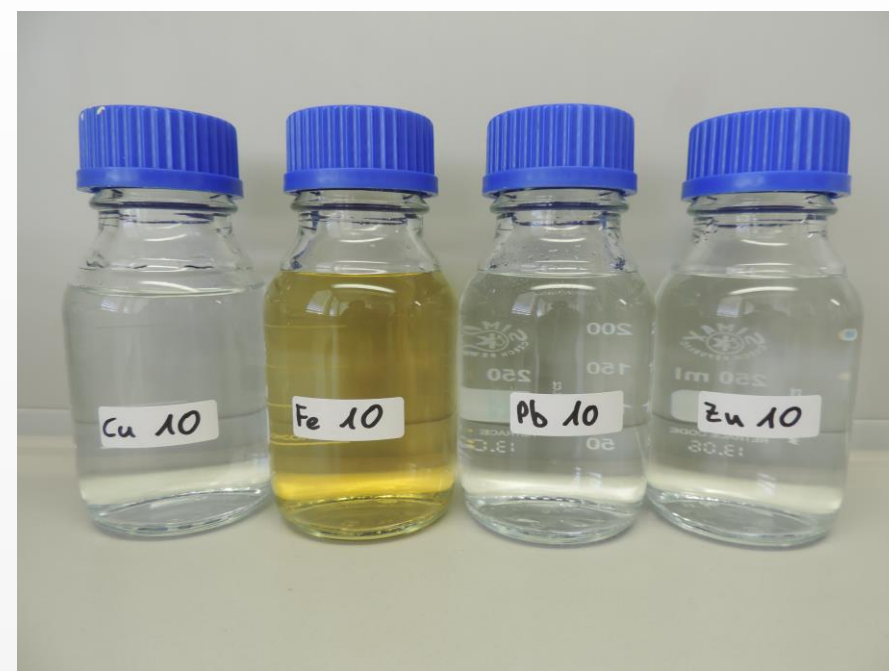
- Water-soluble salts (two chlorides, two sulfates)
- **Interference factor**
- Dissolved in distilled water
→ **c1** µg/ml & **c2** µg/ml solutions

Weighed portion of isotopes

- Sodium bicarbonate – ^{13}C
- Ammonium – ^{15}N chloride
- **Measured parameters**

ICP-MS & OES measurements of synthetic seawater

- **c0** initial value: **Cu, Fe, Zn** < 10 µg/l & **Pb** < 0.5 µg/l



Dissolved heavy-metals – **c1** [µg/ml]



Dissolved heavy-metals – **c2** [µg/ml]

Setting & preparation

Preparation

Enrichment of seawater with metal-concentration (c1 & c2)

➤ **Interference factor**

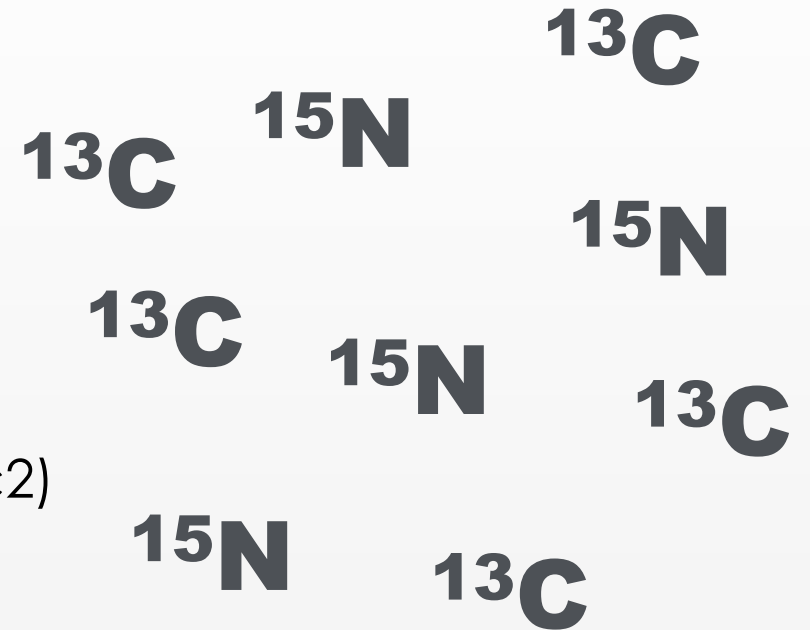
Weighed portion of isotopes

- Sodium bicarbonate – ^{13}C
- Ammonium – ^{15}N chloride

➤ **Measured parameters**

1.5 L artificial seawater enriched by 7.5 ml of heavy metals and isotopes

➤ **c1 $\mu\text{g/l}$ & c2 $\mu\text{g/l}$**



Analytical methods

Isotopic Incubation experiments

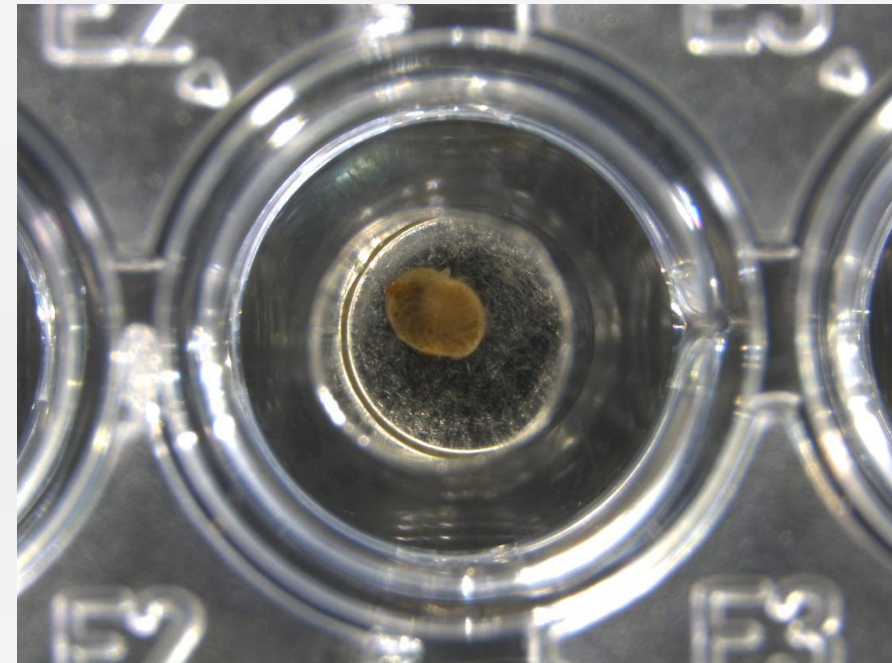
- **Crystallization dishes** with artificial seawater
 - Contact with metals & isotopes
- **7 Foraminifera**/dish (mass dependency)
- **Incubation** for several days (**1d**, **3d**, **5d** and **7d**)
- Flush-washing
- **Tin capsules** + Foraminifera
- **Air-dried** and **decalcified** (2M HCl)
- **Drying cabinet**: 50 °C for 3 days
- Weighed portion → cytoplasm

Incubator

- **25 C°**
- **33 PSU**
- **12:12 h** light/dark rhythm
- **40 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$**



Crystallization dishes - Incubator



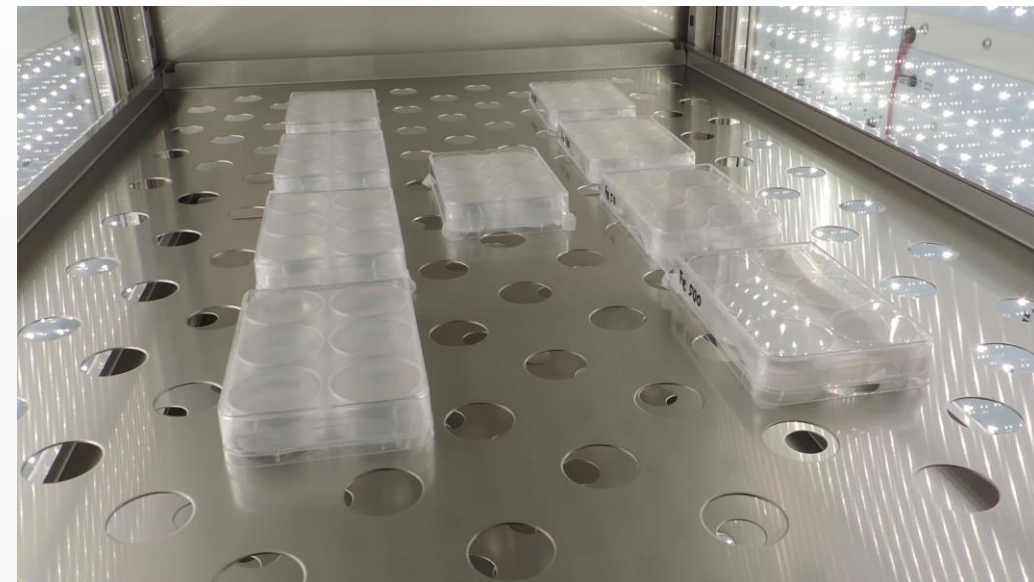
Wellplate with tin capsules + Foraminifera

Analytical methods

Microscopy-PAM

- University of Vienna Biology Building
- Chlorophyll fluorescence analyses
- **Same** incubation **parameters**
 - 1 day 3 days 5 days 7 days
- **Wellplates** with artificial seawater
 - + **6** foraminifera/ metal/ concentration + **6 blank** foraminifera
- Foraminifera reacting with ultraviolet light → Intensity fluctuations
- PAM stimulates the symbionts (photosystem II in chloroplasts)

Active chloroplasts → active diatoms → healthy foraminifera



Wellplates - Incubator



Pam - University of Vienna Biology Building

Impact - Copper



Heterostegina depressa - living
Department of Palaeontology – University of Vienna - Bar scale is 500 µm

Cu_c2_day 0



Heterostegina depressa - living
Department of Palaeontology – University of Vienna - Bar scale is 500 µm

Cu_c2_day 5

Impact - Iron



Heterostegina depressa - living
Department of Palaeontology – University of Vienna - Bar scale is 500 µm

Fe_c1_day 0



Heterostegina depressa - living
Department of Palaeontology – University of Vienna - Bar scale is 500 µm

Fe_c1_day 3

Impact - Lead



Heterostegina depressa - living
Department of Palaeontology – University of Vienna - Bar scale is 500 µm

Pb_c1_day 0



Heterostegina depressa - living
Department of Palaeontology – University of Vienna - Bar scale is 500 µm

Pb_c1_day 5

Impact - Zinc



Heterostegina depressa - living
Department of Palaeontology – University of Vienna - Bar scale is 500 µm

Zn_c1_day 0



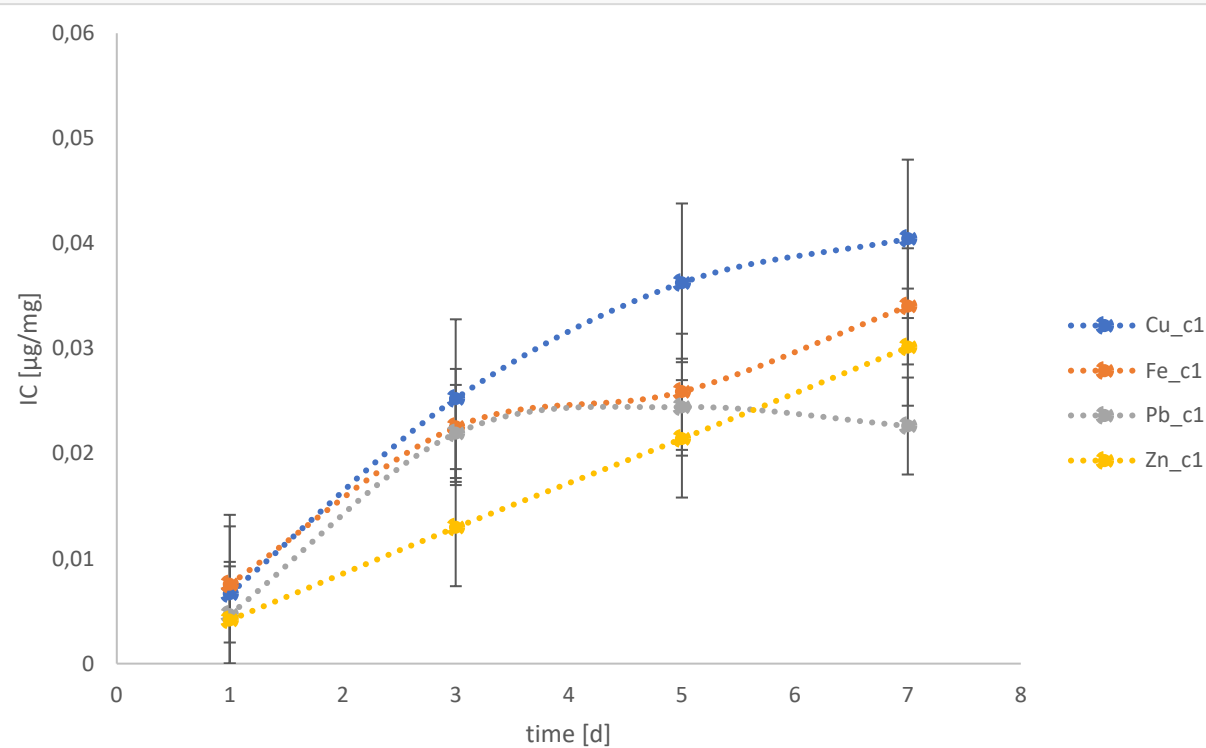
Heterostegina depressa - living
Department of Palaeontology – University of Vienna - Bar scale is 500 µm

Zn_c1_day 3

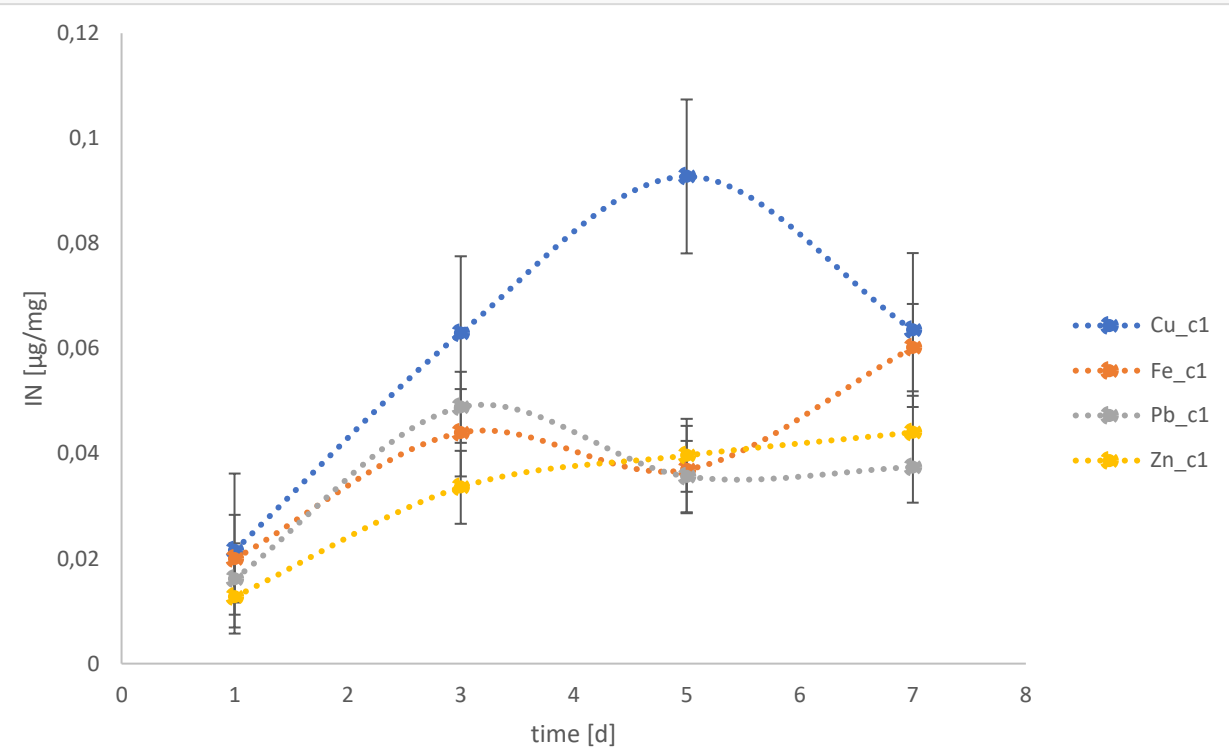
Results

Isotope uptake experience – **All Metals_c1**

^{13}C



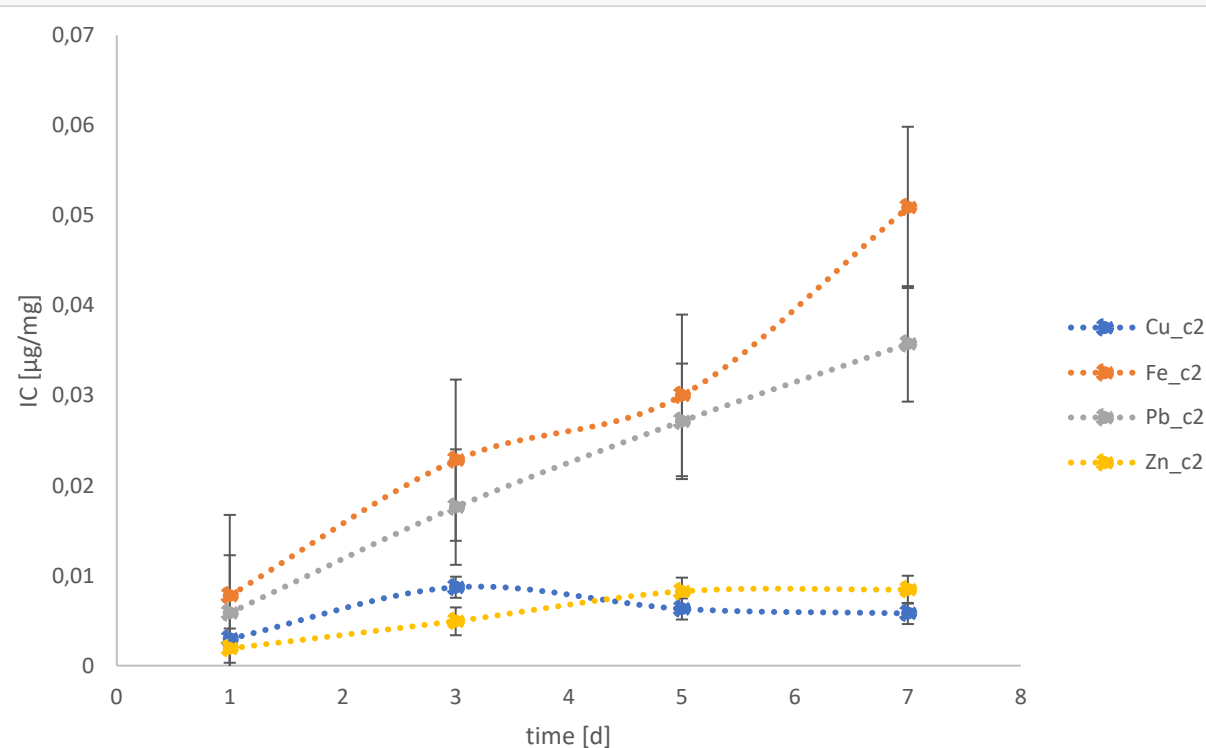
^{15}N



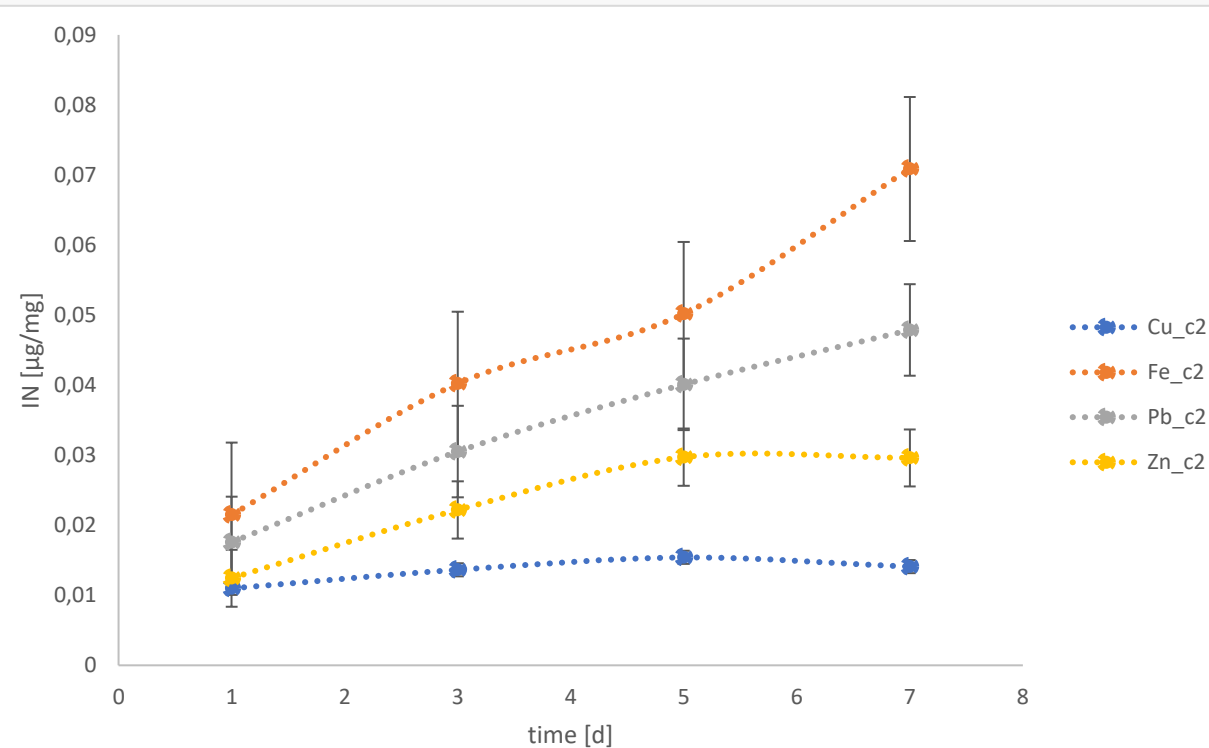
Results

Isotope uptake experience – **All Metals_c2**

^{13}C

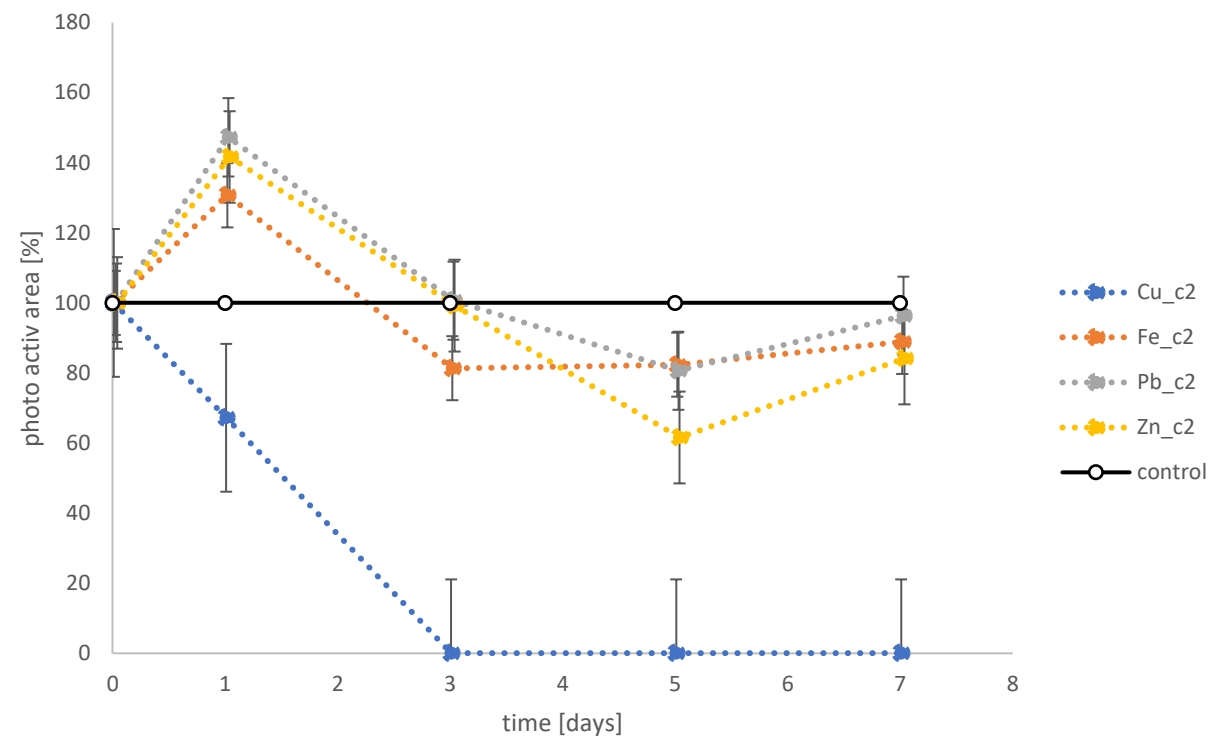
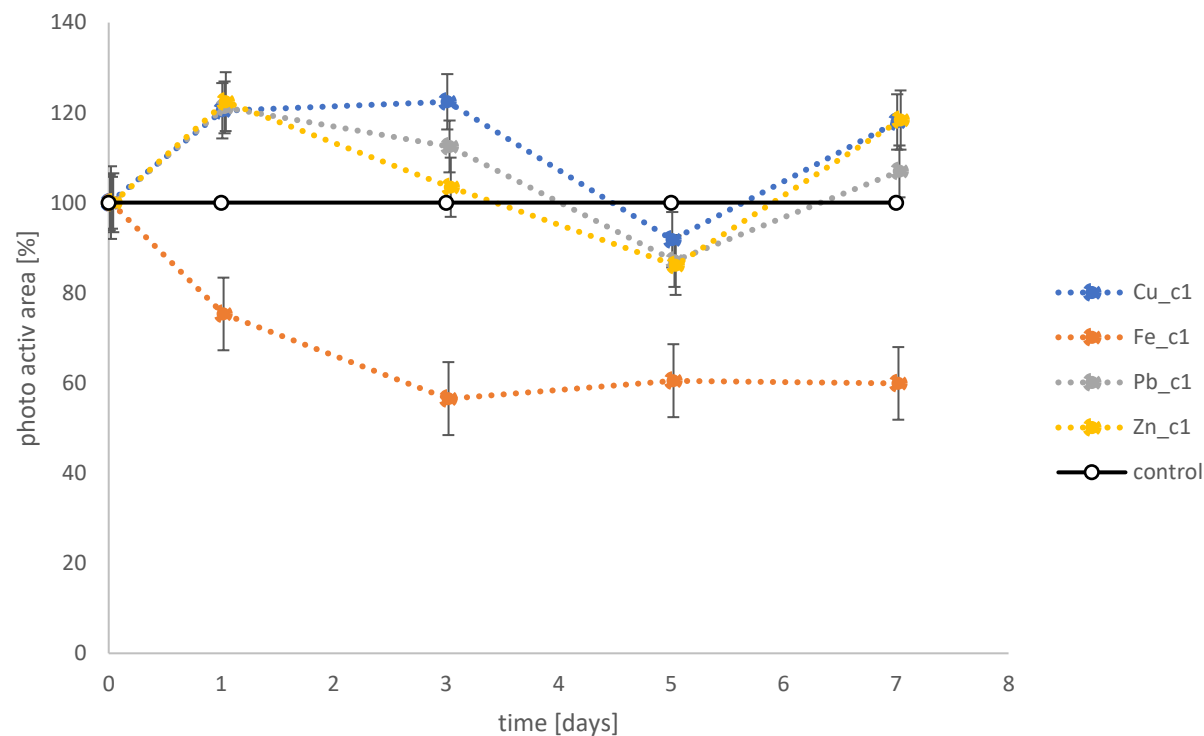


^{15}N



Results

PAM Microscopy – All Metals_c1 & c2



Conclusions

Impact of heavy metals

Copper

- Very toxic at c2 and acceptable at c1

Iron

- Reveals in increased symbiotic activity independent of concentrations c1 & c2

Lead

- Difficult to interpret at this juncture of the study however, dark, brown areas can be observed

Zinc

- Slightly elevated concentration – c1 → Serving as nutrient
- Severe elevated concentration – c2 → Toxic

Copper



Cu_c2_day 0



Cu_c2_day 5

Iron



Fe_c1_day 0



Fe_c1_day 3

Zinc



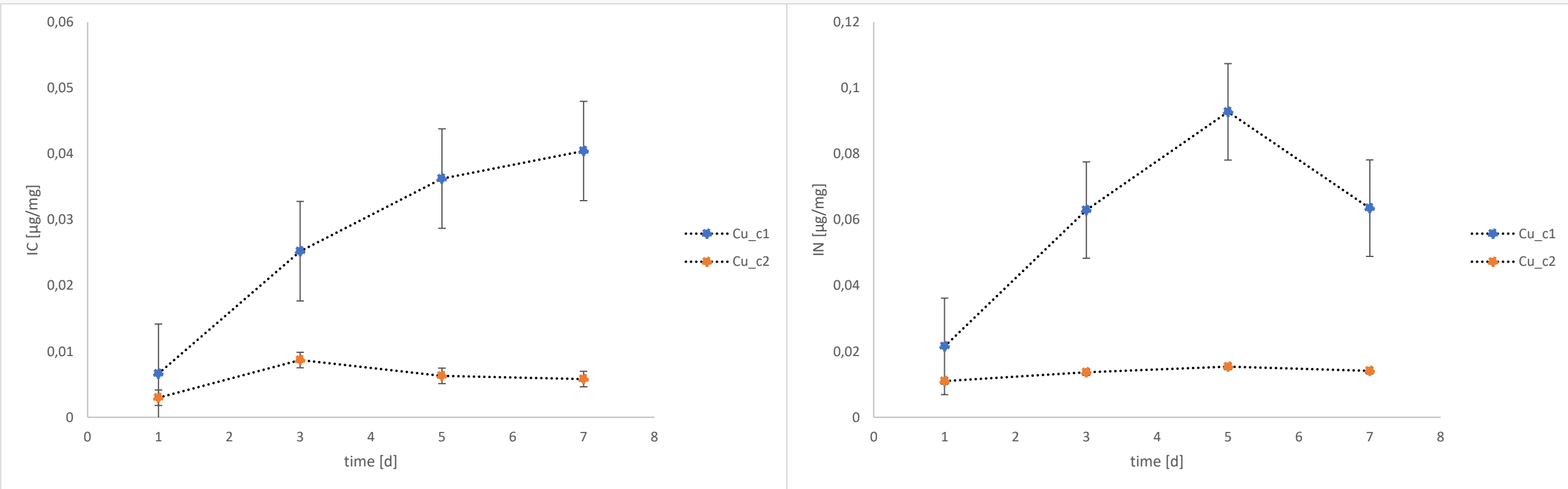
Zn_c1_day 0



Zn_c1_day 3

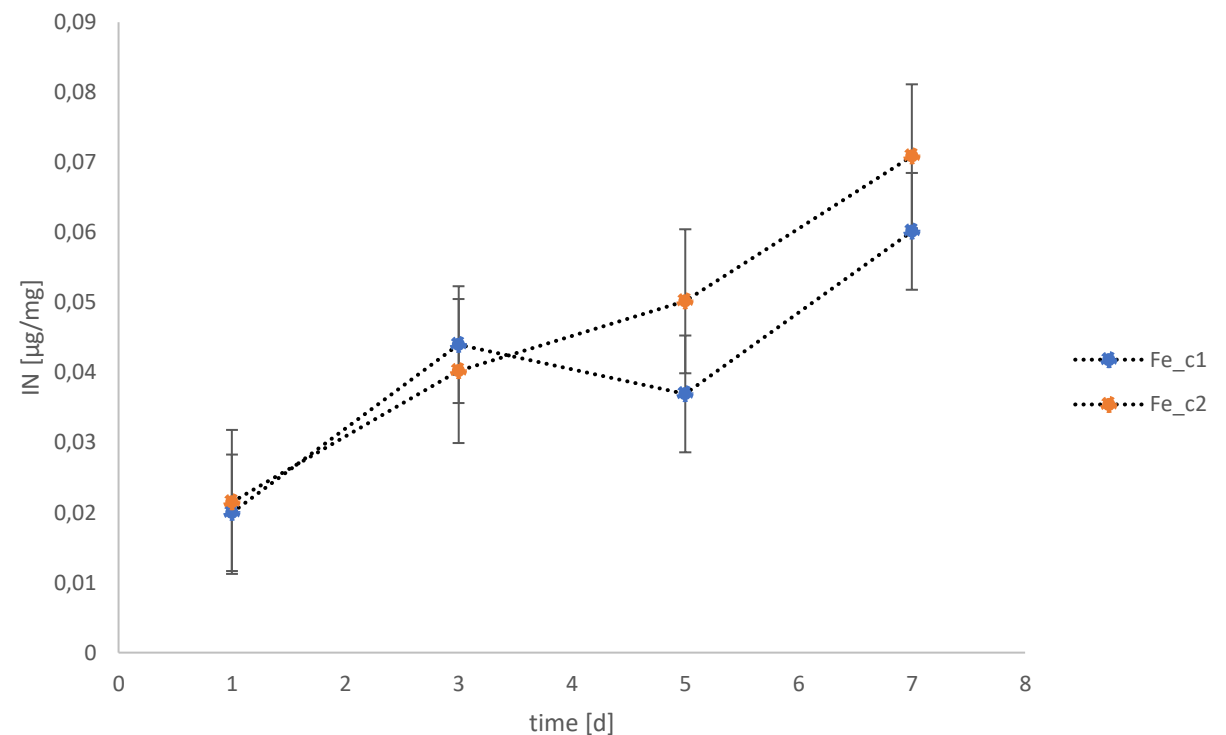
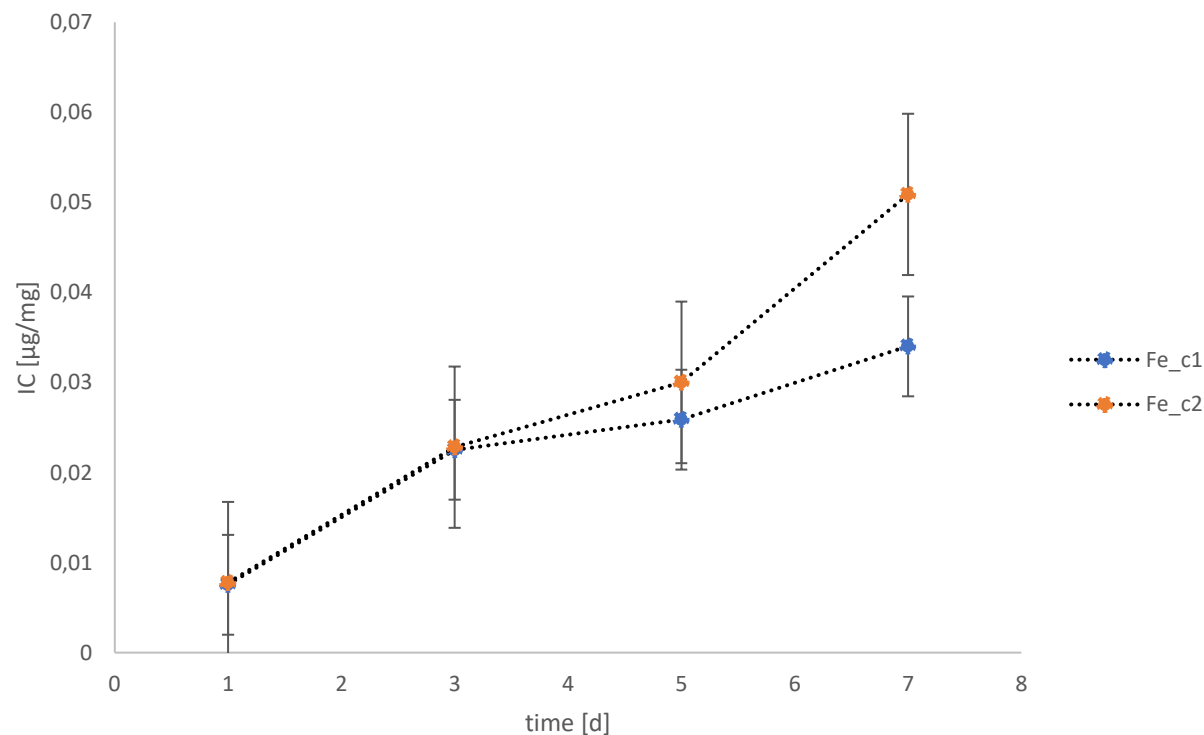
Results

Isotope uptake experience - **Copper**



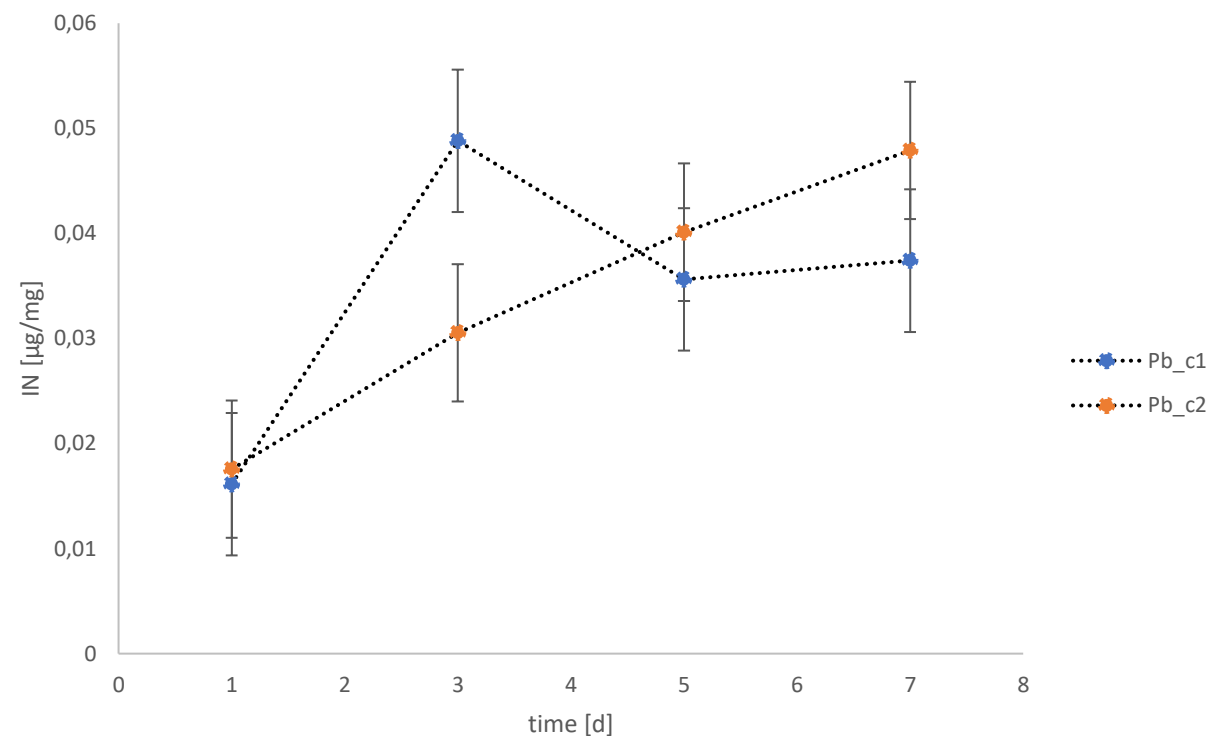
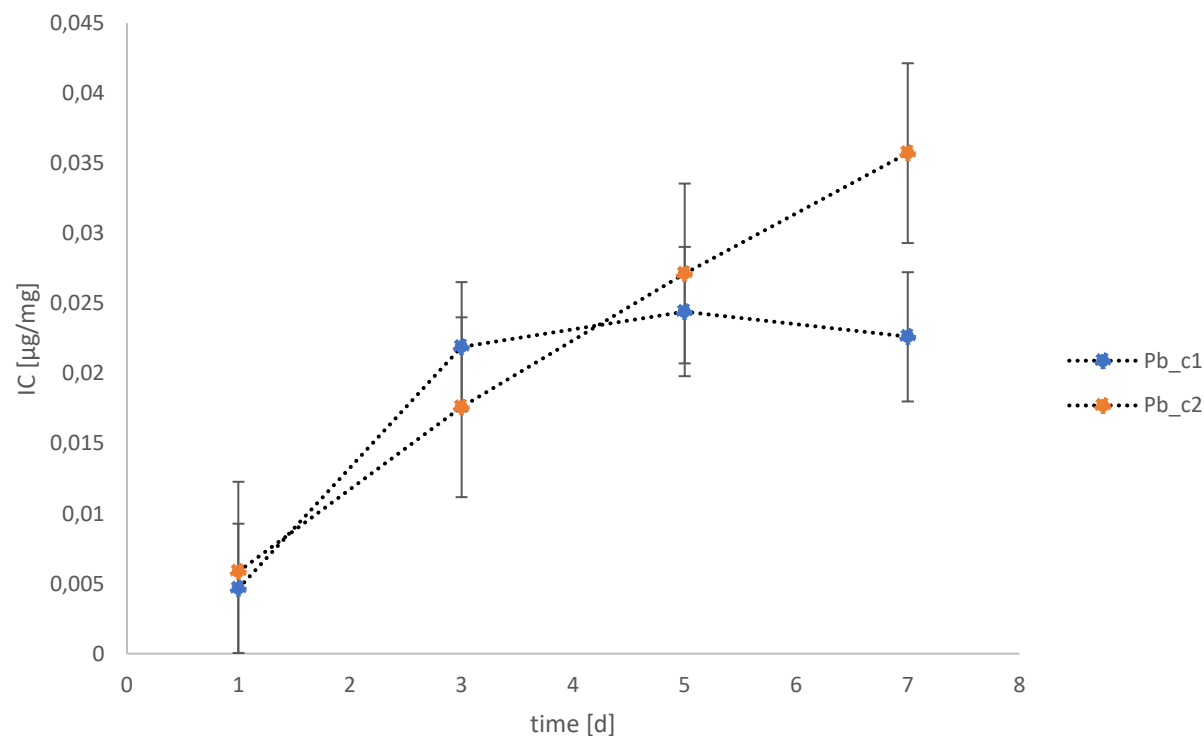
Results

Isotope uptake experience - **Iron**



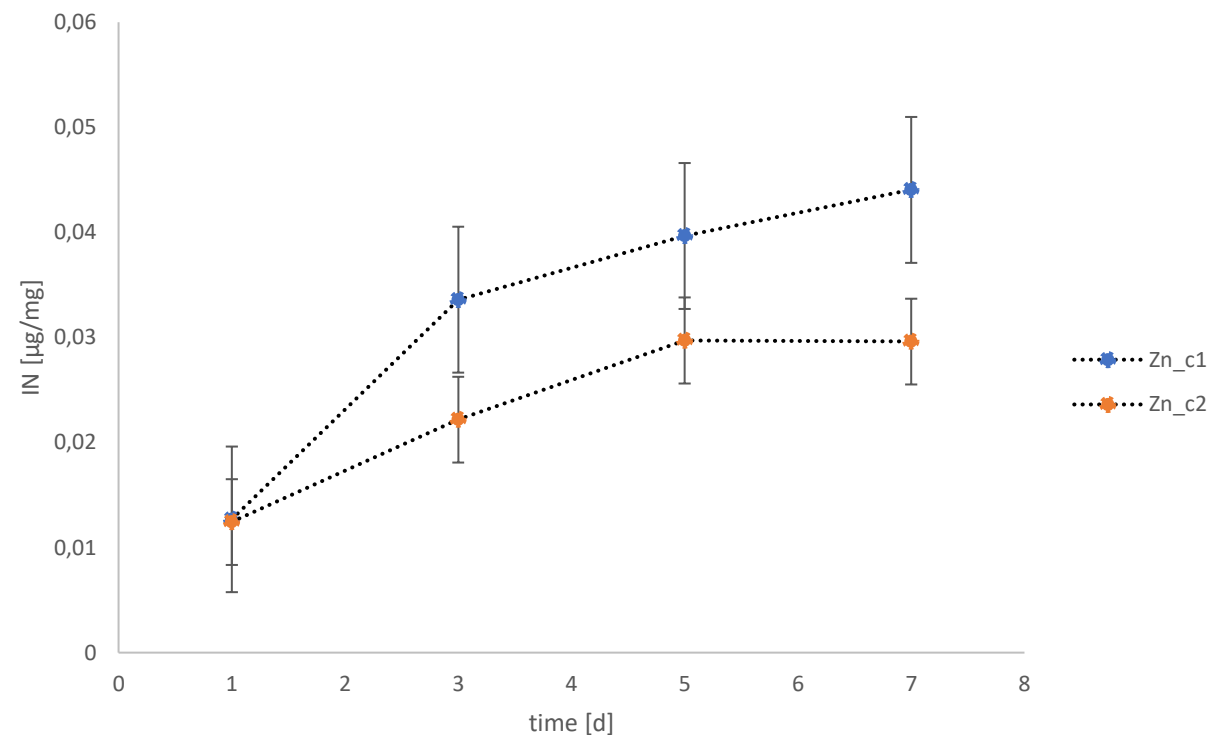
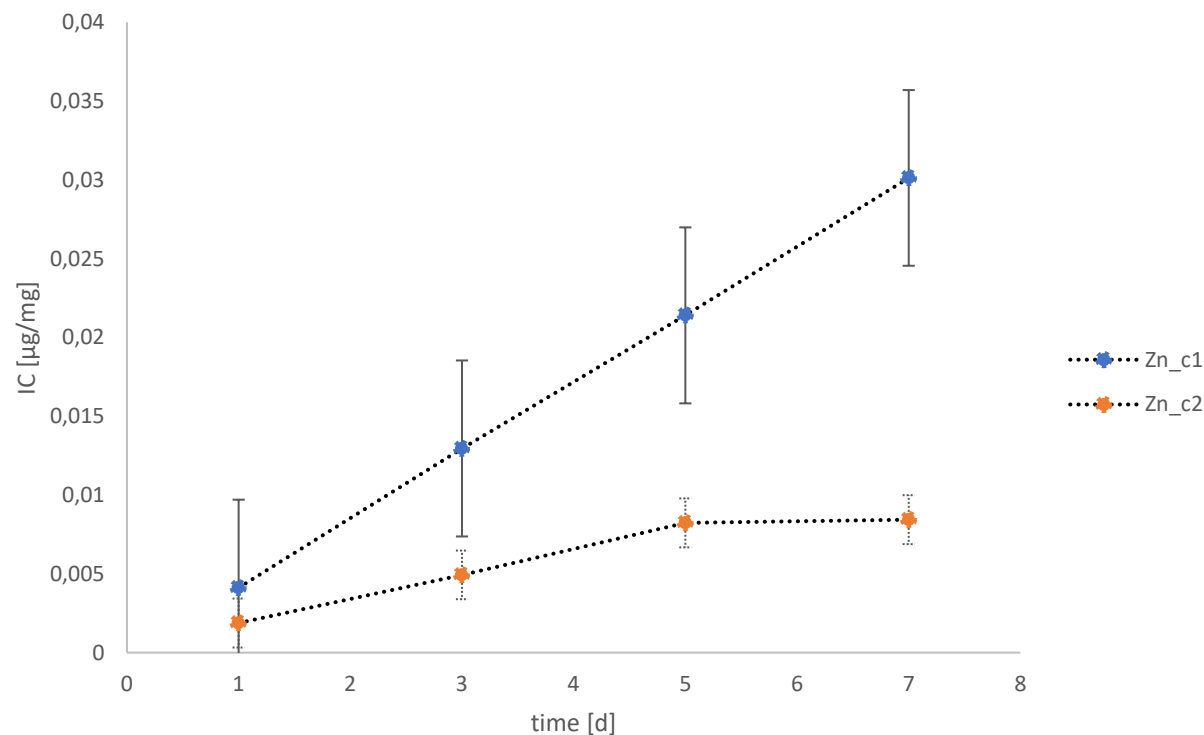
Results

Isotope uptake experience - **Lead**



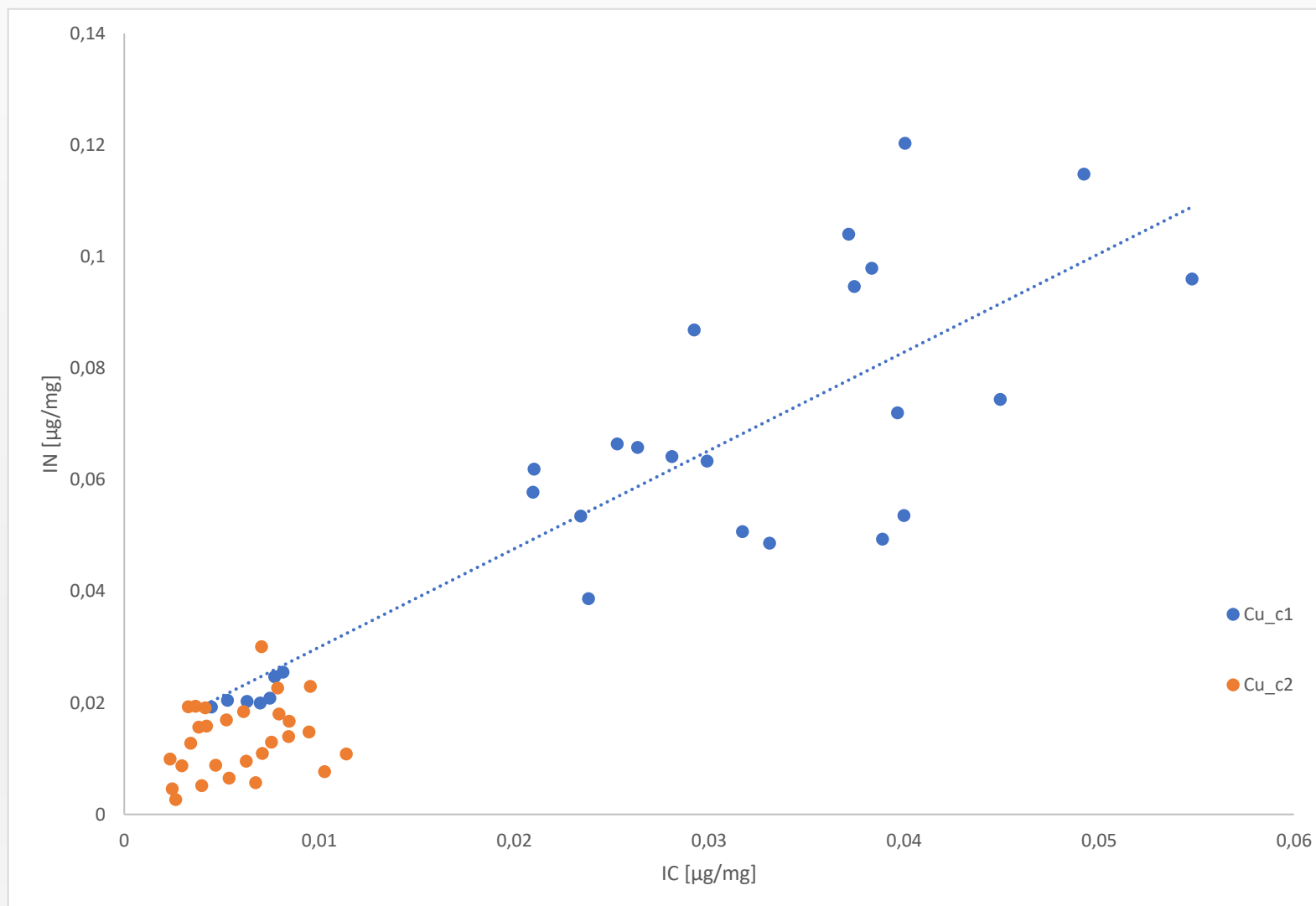
Results

Isotope uptake experience - **Zinc**



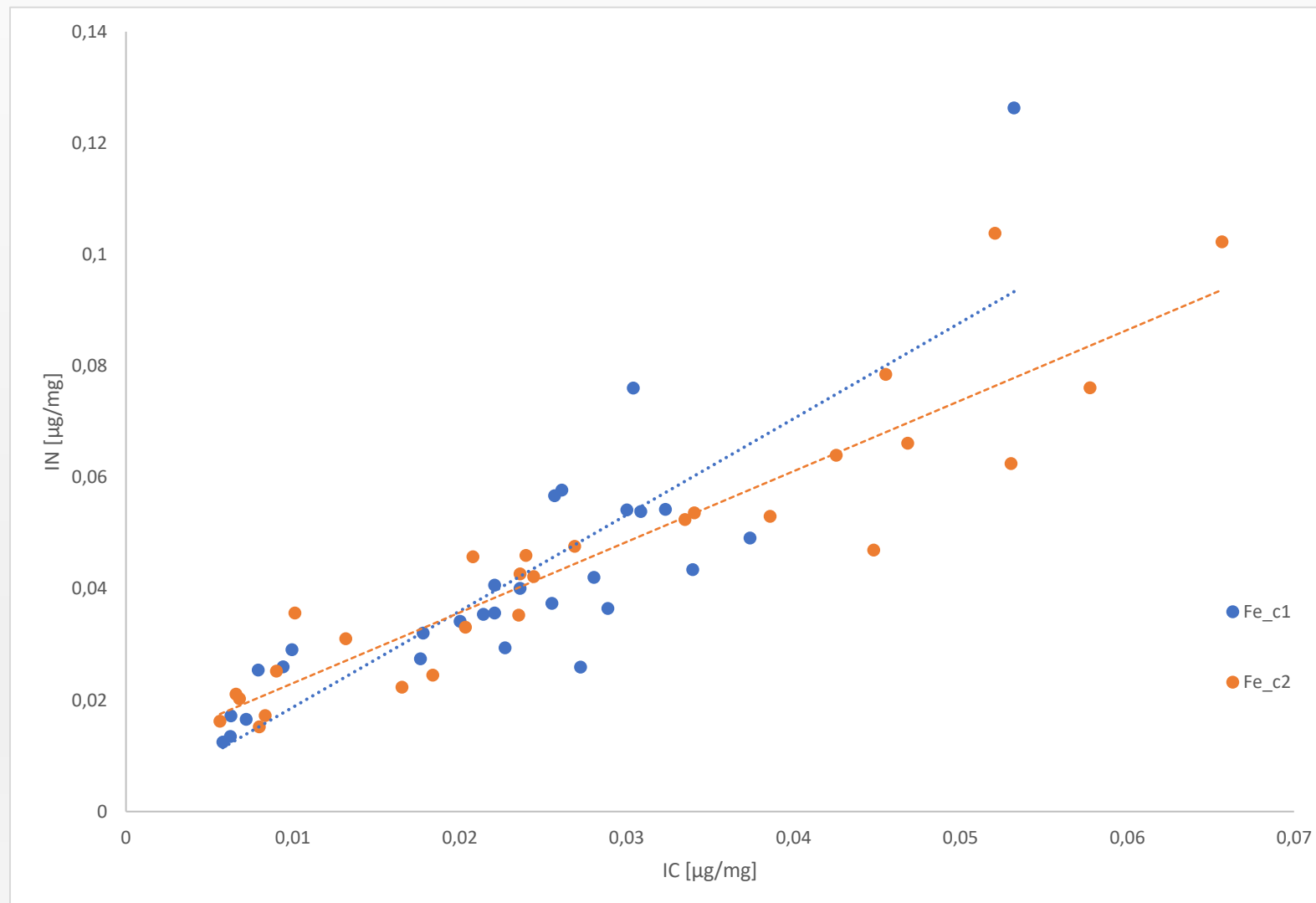
Results

Isotope uptake experience - **Copper**



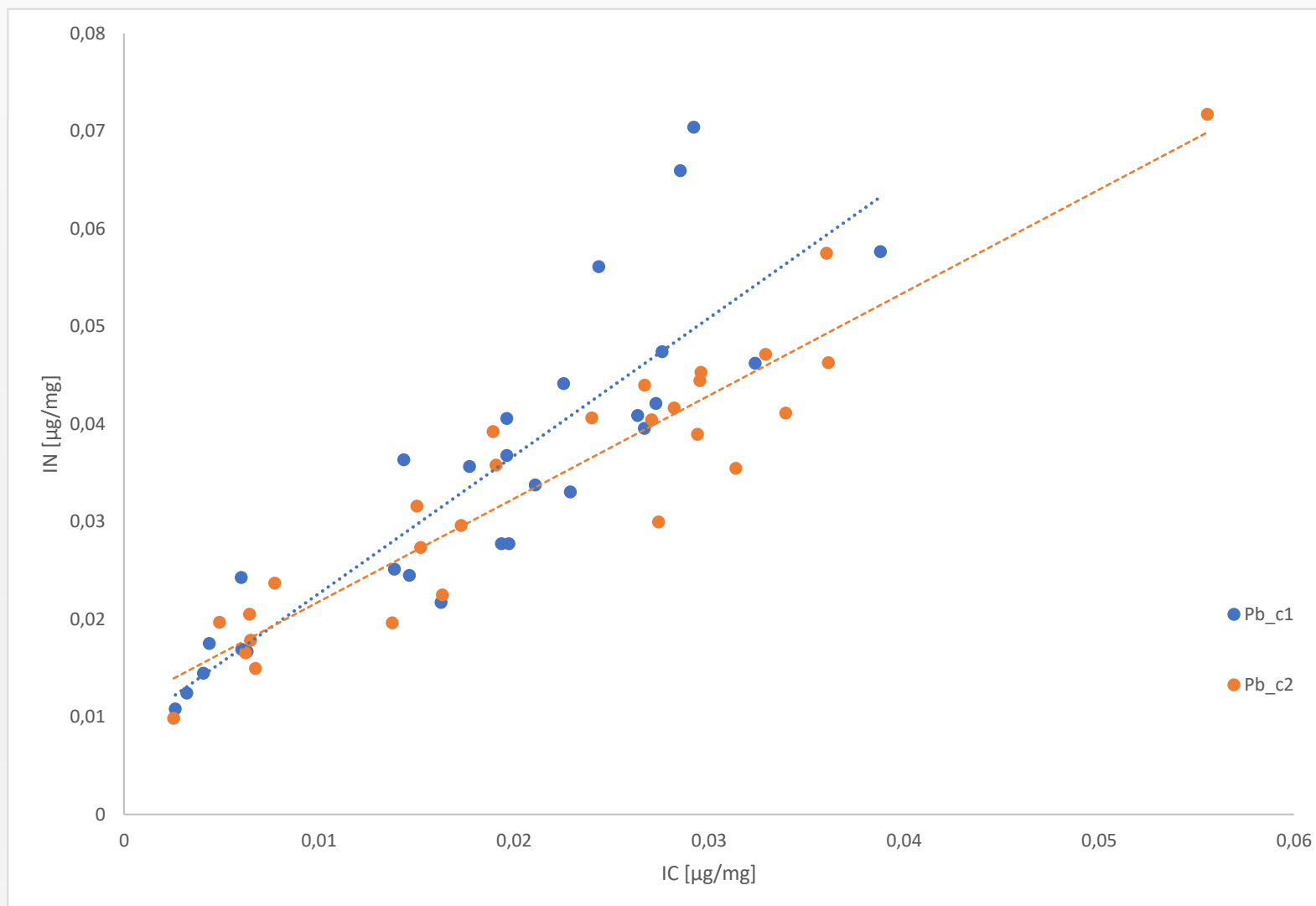
Results

Isotope uptake experience - **Iron**



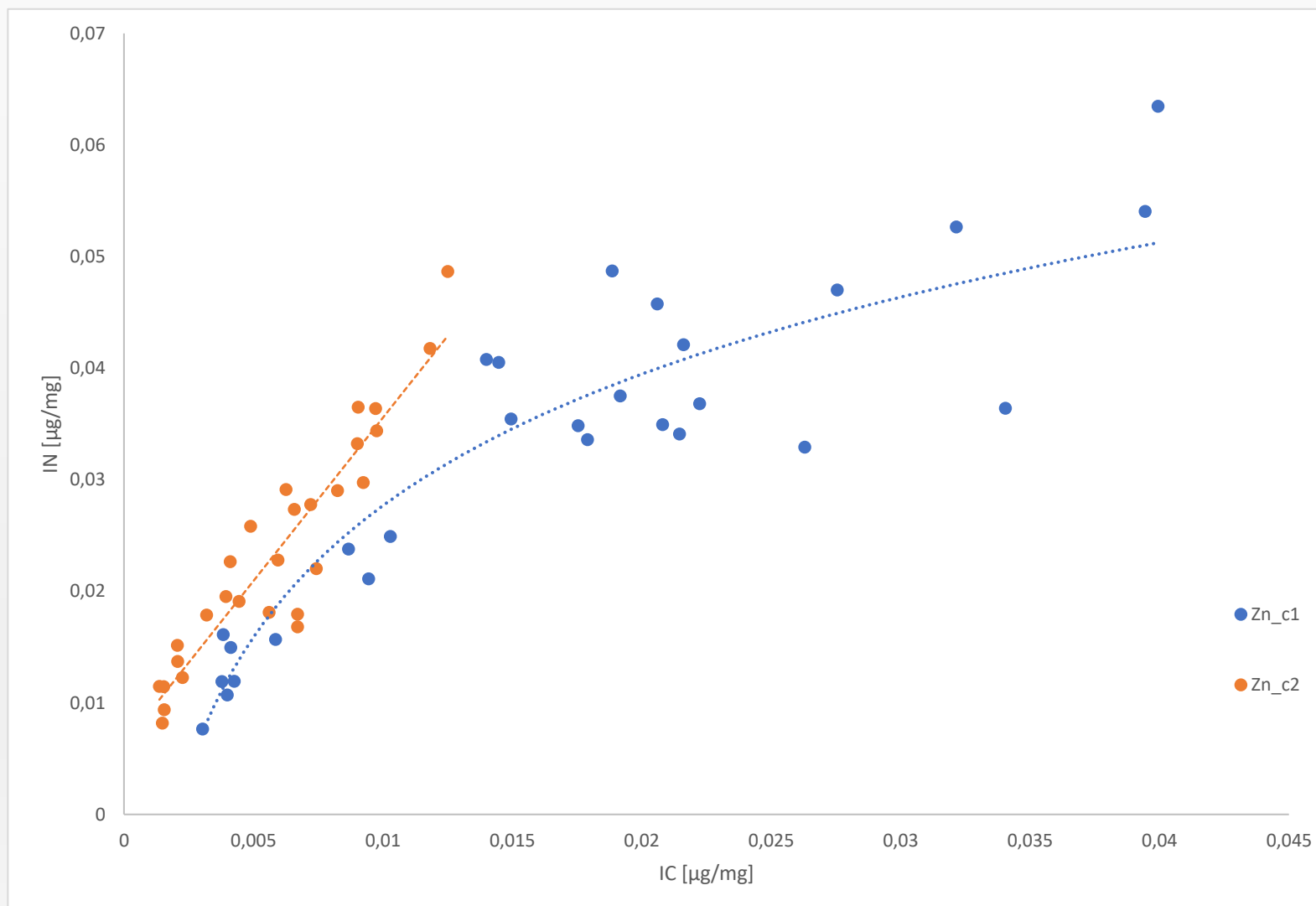
Results

Isotope uptake experience - **Lead**



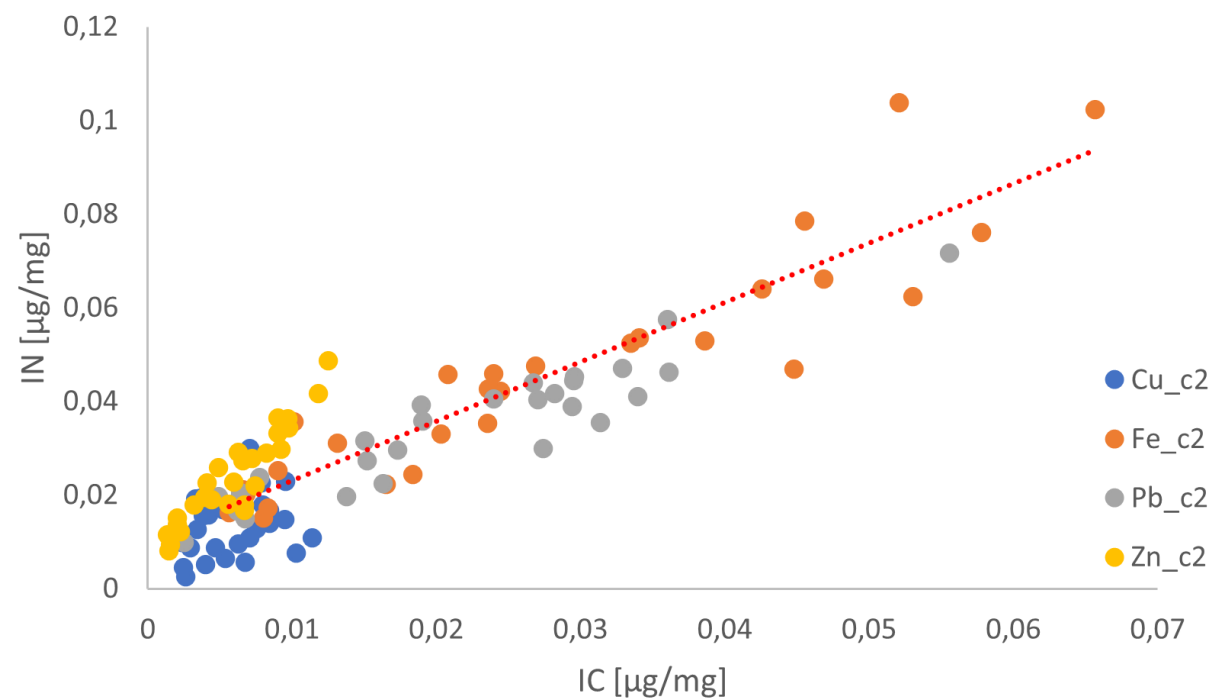
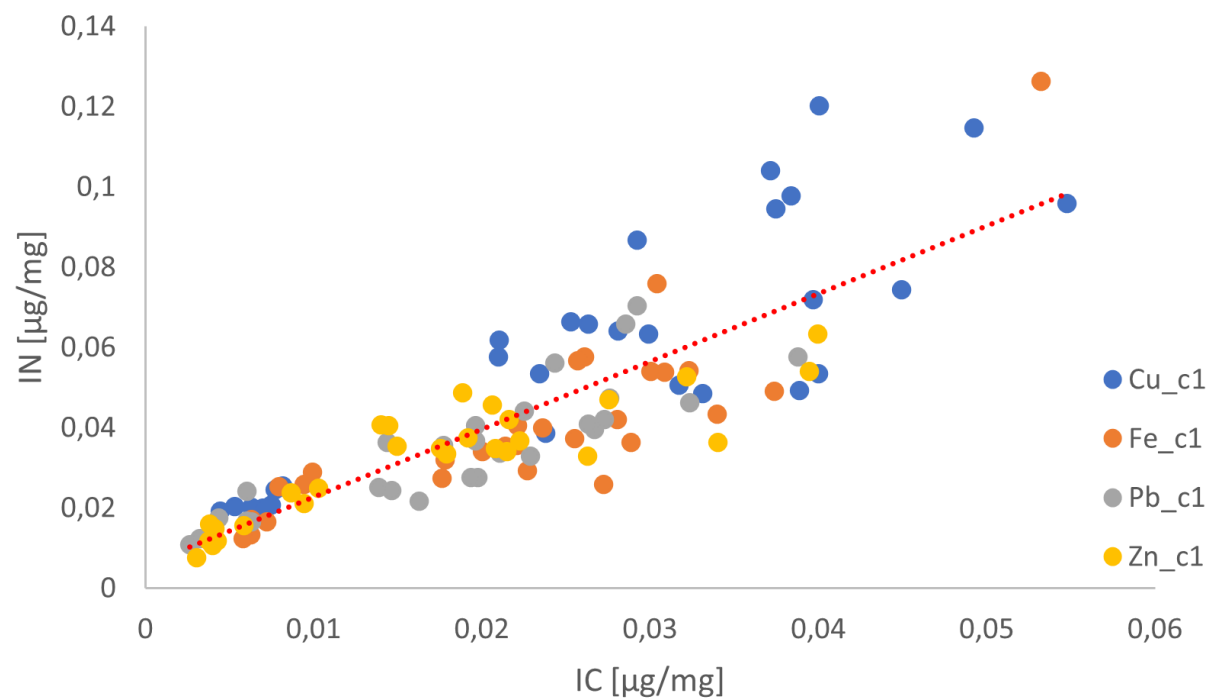
Results

Isotope uptake experience - **Zinc**



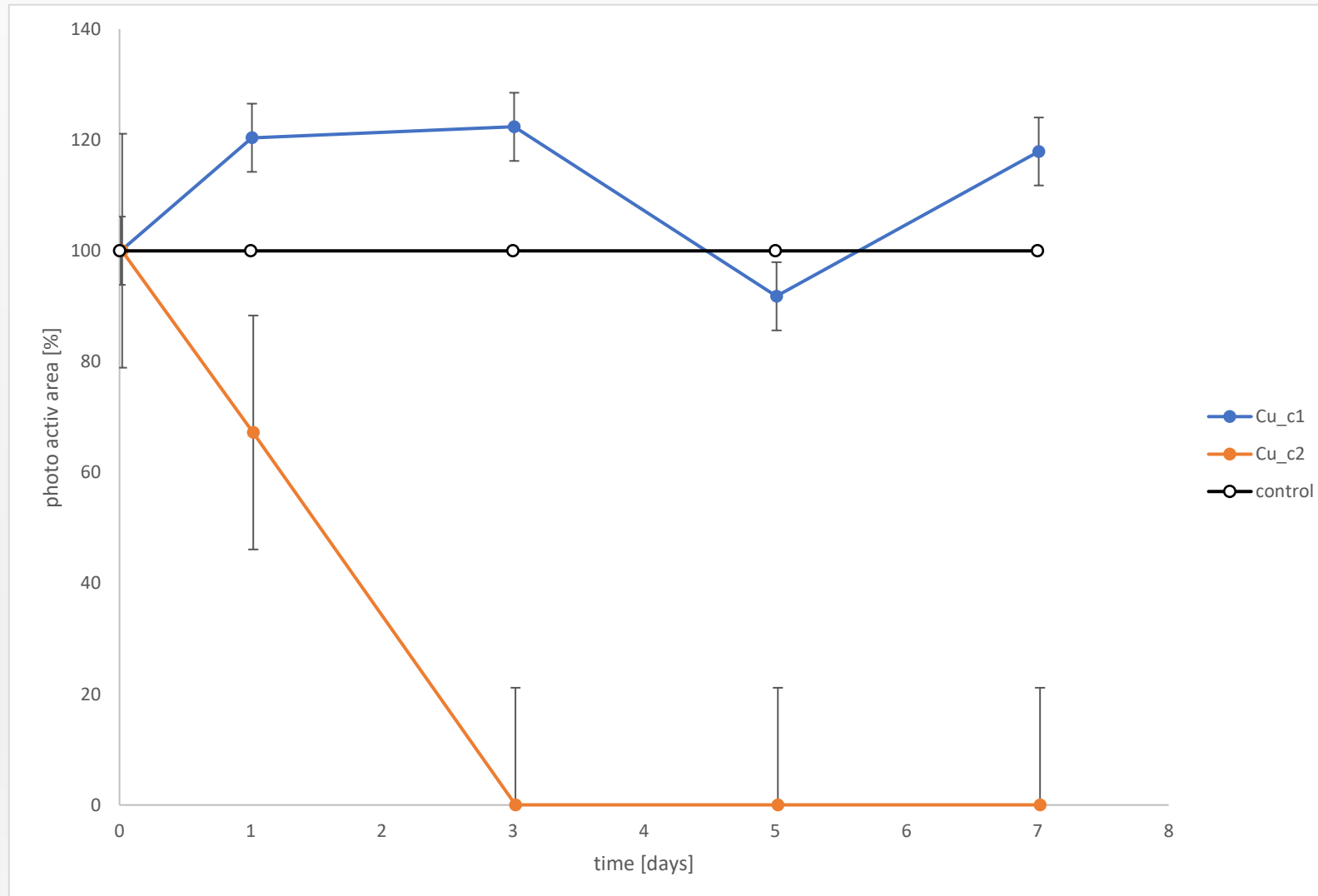
Results

Isotope uptake experience – **All Metals_C:N**



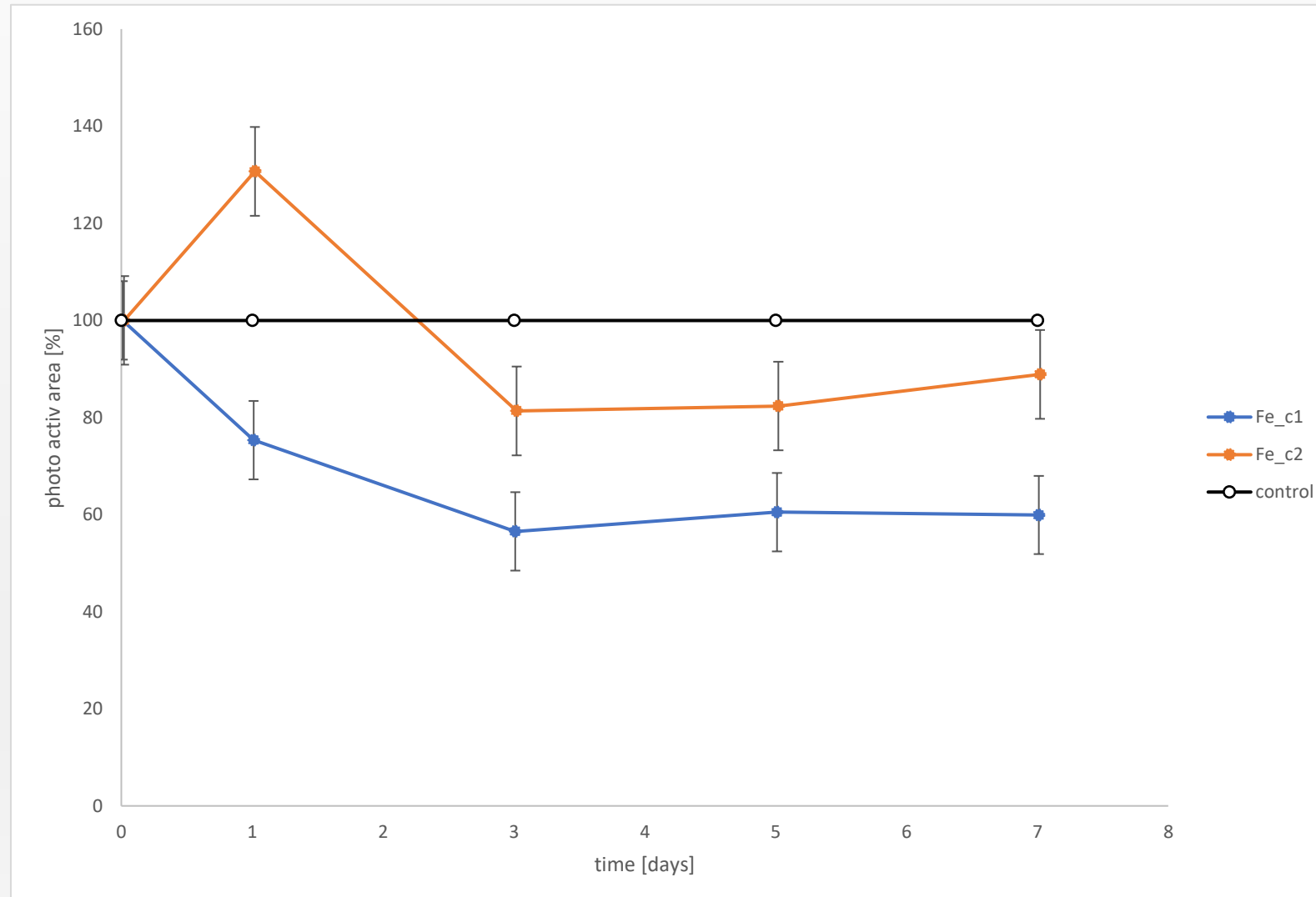
Results

PAM Microscopy - **Copper**



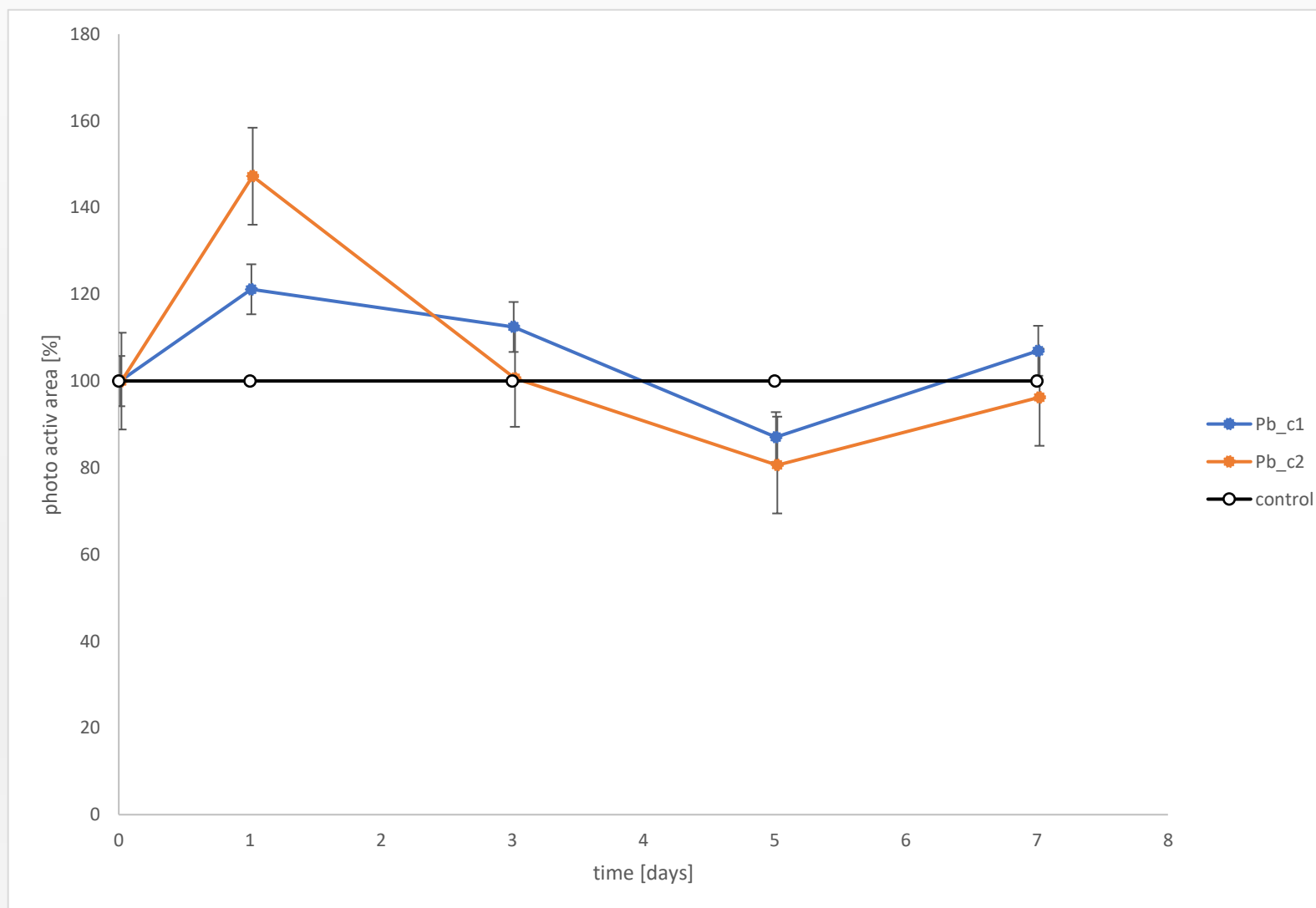
Results

PAM Microscopy - Iron



Results

PAM Microscopy - **Lead**



Results

PAM Microscopy - **Zinc**

