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Targeted and non-targeted metabolomic analyses of *Heterocapsa cf. bohaiensis* under nickel and iron high concentration and its photophysiology

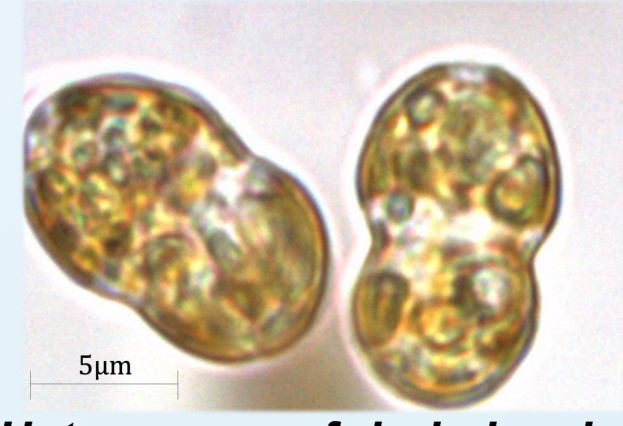
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Introduction

❑ **New Caledonian** coastal waters are naturally rich in **metallic trace elements**. (Biscéré et al. 2017; Merrot et al. 2022)

❑ An excess of metallic inputs induce an **oxidative stress**. (Pinto et al. 2016; Danouche et al. 2022)



Heterocapsa cf. bohaiensis

❑ ***Heterocapsa cf. bohaiensis***, a dinoflagellate isolated in New Caledonia, is associated with shrimp and crab mortalities. (Xiao et al. 2018)

❑ Might be a good candidate to study the production of **metabolites of interests** (e.g. carotenoids, biotoxins, fatty acids...)

❑ Does the species **adapted** to this particularly **metal-rich** environment and does it impacted the **photosystem** and **metabolome** by developing **adaptive strategies** ?

Aim: Study the impact of nickel & iron on the photosystem and metabolome

Materials & Methods

Culture in photobioreactors:

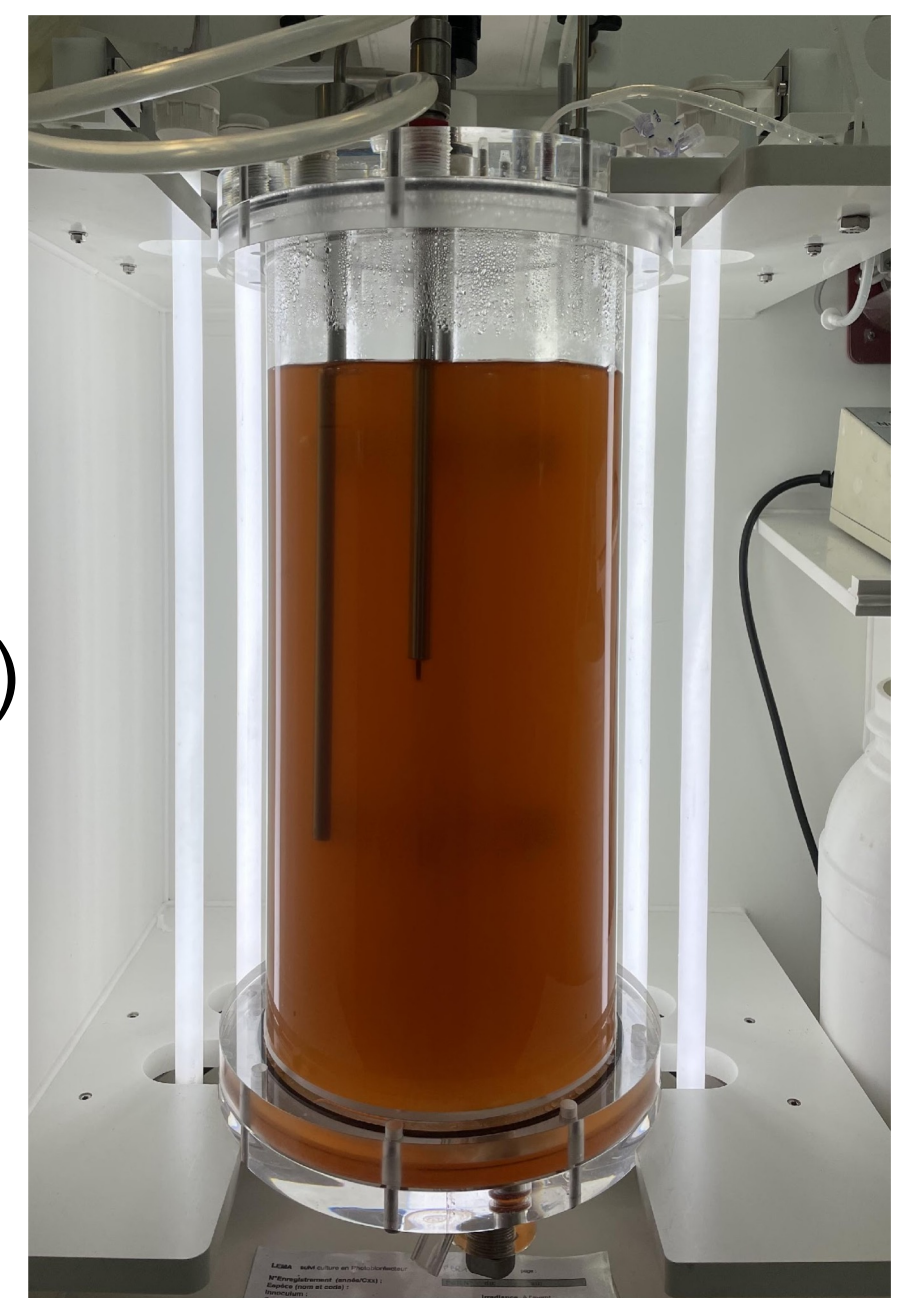
- Continuous culture of *H. cf bohaiensis*
- Addition of **nickel** and/or **iron** after steady state

Experimental conditions (in triplicate):

- **Metals:** Ni 10^{-3} M ; Fe 10^{-3} M ; Ni+Fe $0.5 \cdot 10^{-3}$ M (of each)
- **Times:** Control t_0 ; t_{24h} ; t_{72h} ; t_f (13 days)

Analyses:

- **Chlorophyll a** fluorescence using PAM (Pulse Amplitude Modulated) fluorometry
- **Bioaccumulation** capacity by ICP-MS/OES
- **Pigment** analyses through HPLC-UV (450 nm)
- **Fatty acids** - GC-MS – (Transesterification)
- **Non targeted metabolites** - NMR



H. cf. bohaiensis in 10L photobioreactor

Results

Photosynthetic efficiency

Maximum Quantum yield

- Maximum photosynthetic efficiency **decreased** by **25%** and **15%** in Fe and Ni+Fe condition, respectively

Rapid Light Curves (RLC)

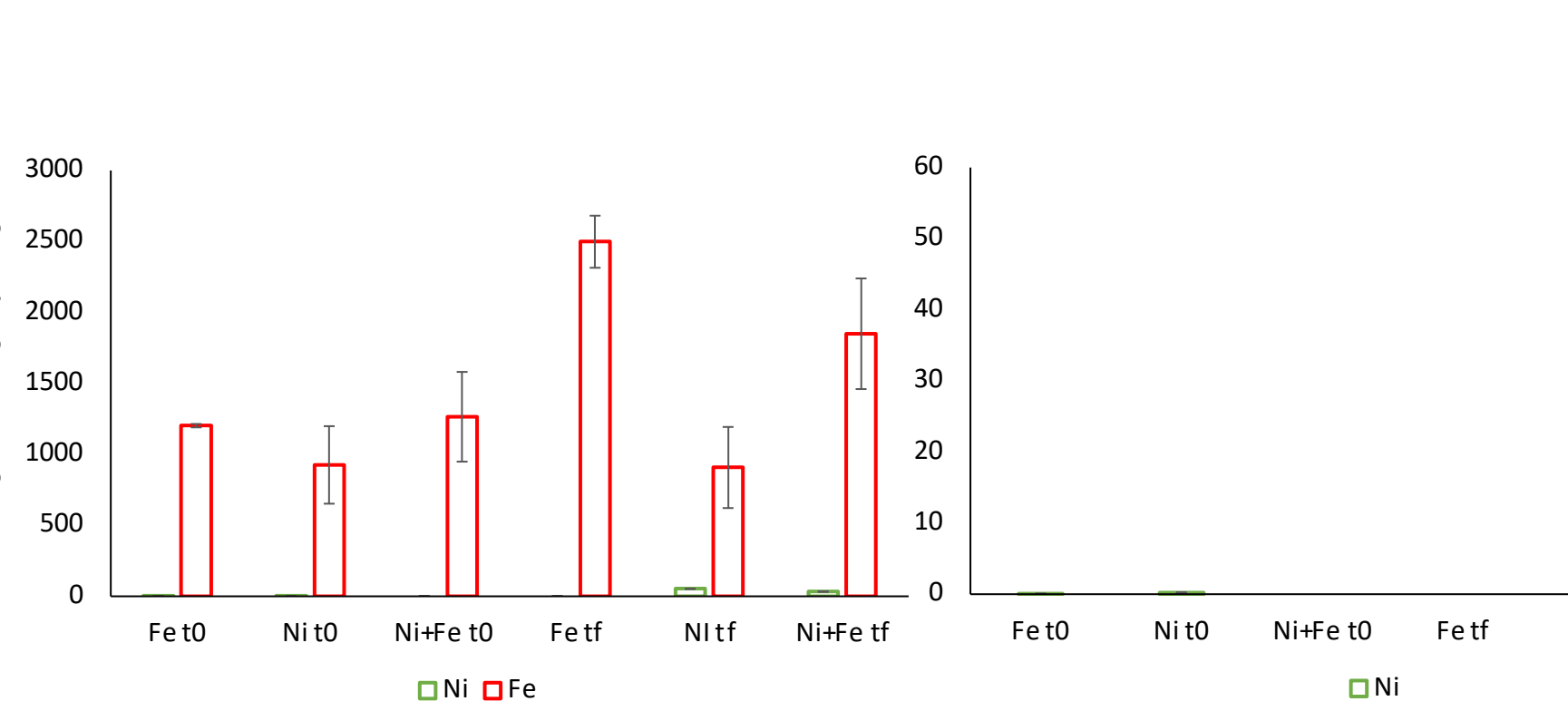
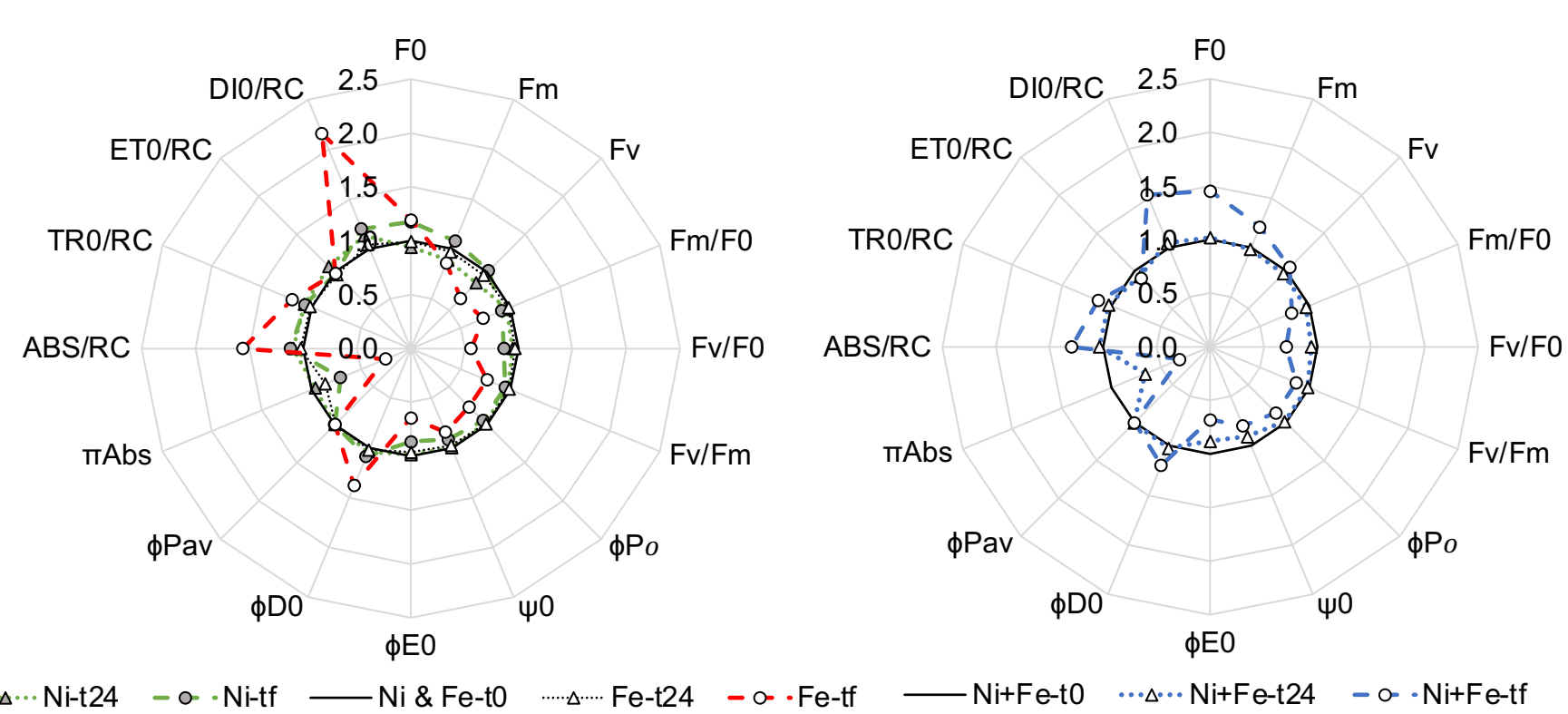
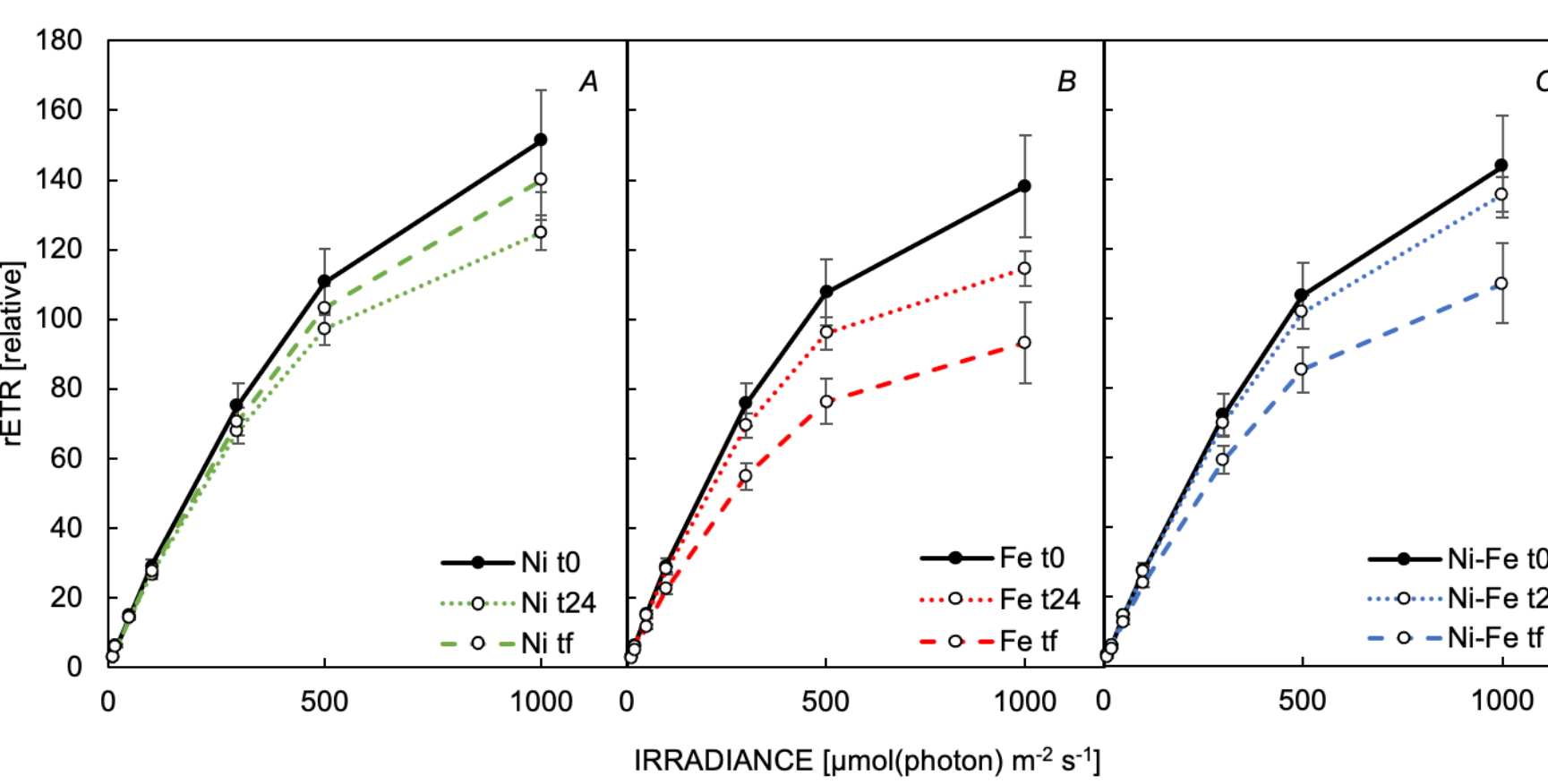
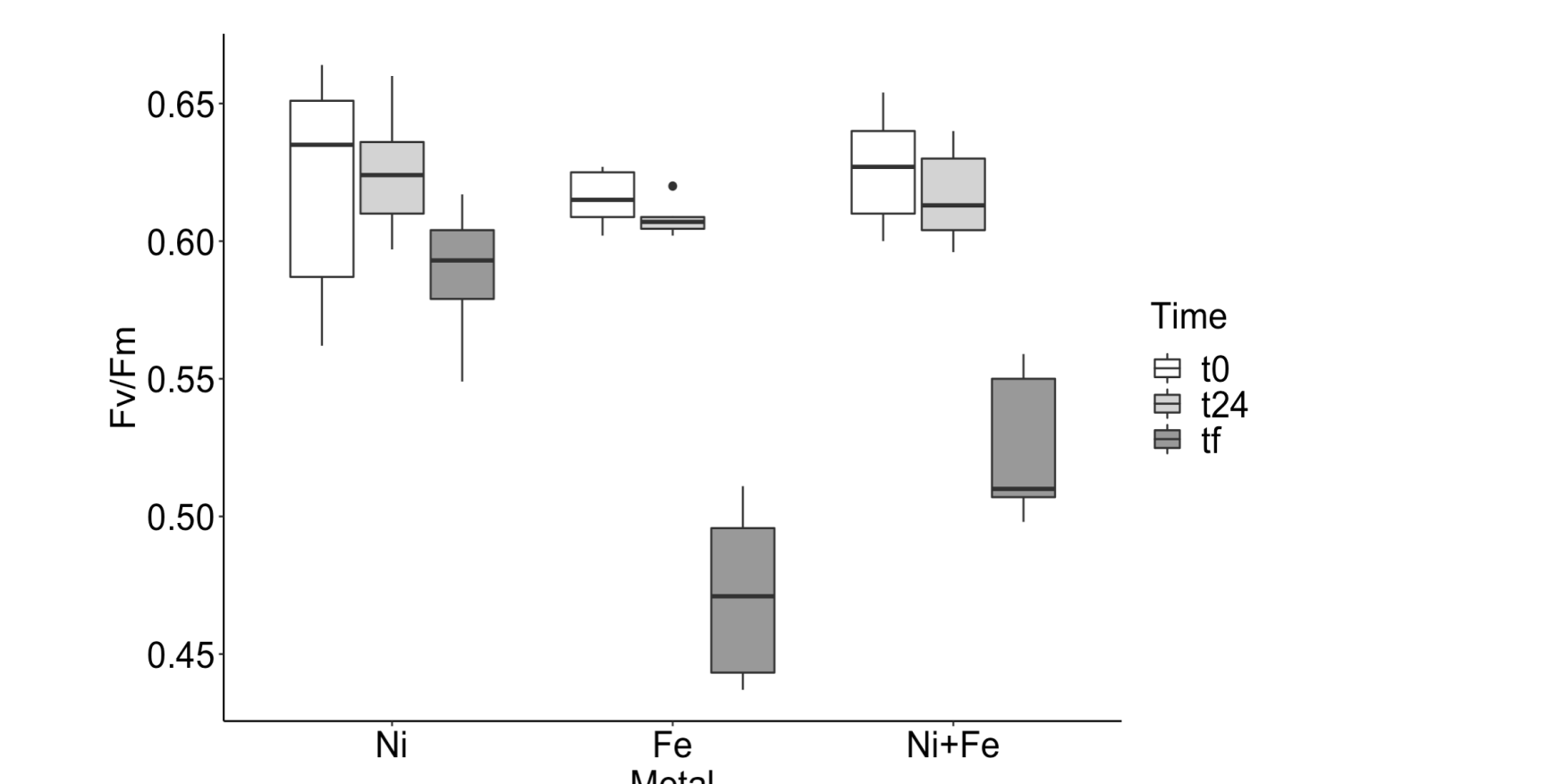
- **Negative** impact on photosynthetic activity due to high iron concentration

OJIP tests

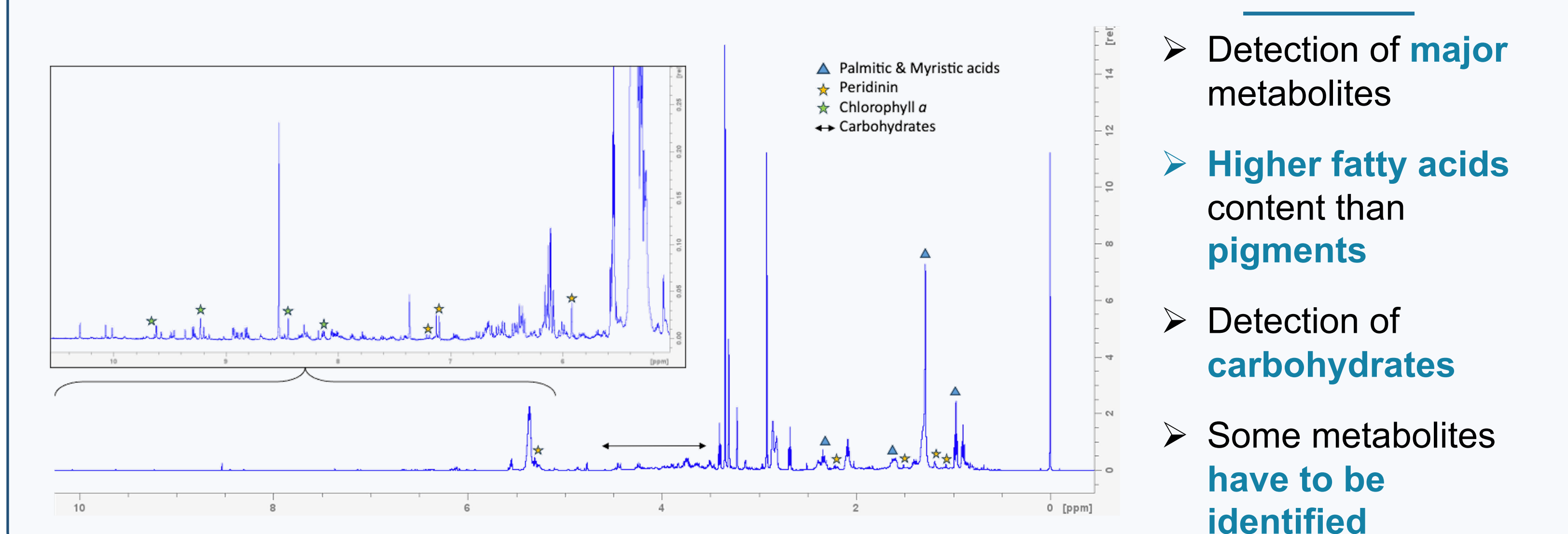
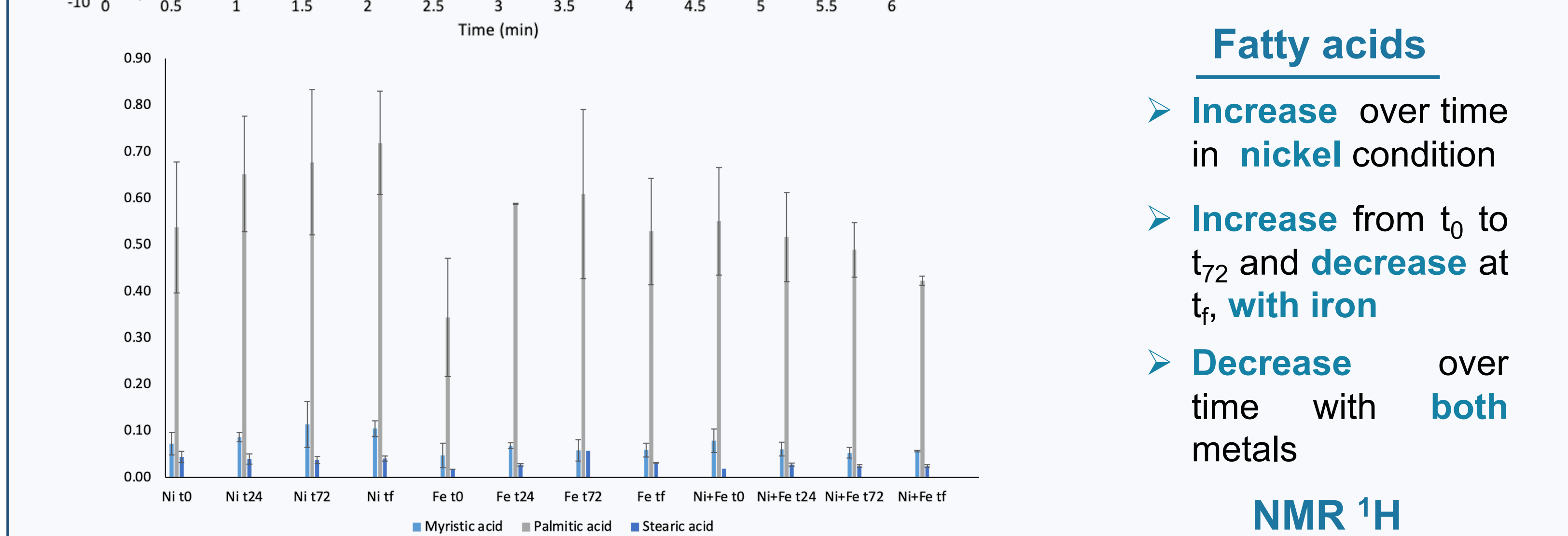
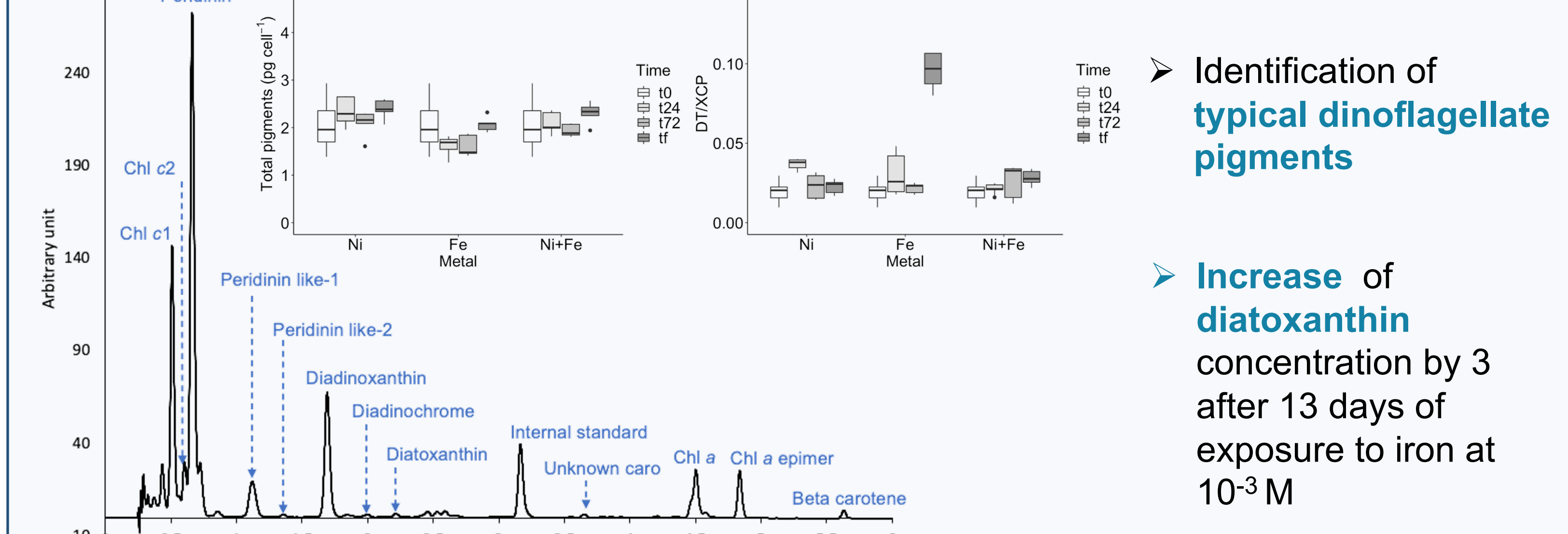
- **Decrease** of quantum efficiency parameters after 13 days with iron
- **Absorption, trapping and dissipation** were **higher** with addition of iron (concentration dependant) after 13 days

Bioaccumulation capacity

- **Higher** accumulation of the specific metal when added at high concentration in the culture



Pigment analyses



➢ Identification of **typical dinoflagellate pigments**

➢ **Increase** of **diatoxanthin** concentration by 3 after 13 days of exposure to iron at 10^{-3} M

Fatty acids

- **Increase** over time in **nickel** condition
- **Increase** from t_0 to t_{72} and **decrease** at t_f **with iron**
- **Decrease** over time with **both** metals

NMR 1H

- Detection of **major metabolites**
- **Higher fatty acids** content than **pigments**
- Detection of **carbohydrates**
- Some metabolites **have to be identified**

Discussion

Photosynthetic efficiency

- *H. cf. bohaiensis* is **tolerant** to **nickel** at high concentrations
- In Iron conditions:**
 - **Fv/Fm** - *H. cf. bohaiensis* is impacted after a long term exposure
 - **RLC** - Iron reduced the ability to use light
 - **OJIP** - Iron stimulates capture and absorption of light energy & slows down the electron transport rate between photosystem II & I
 - **Increase of specific fluxes** (absorption, trapping and dissipation)

Pigment analyses

- Increase of **diatoxanthin** in iron condition
- Responsible of **dissipation increase**
- **Photosystem protection** against iron induced ROS (Huang et al. 2017)

Fatty acids

- Majority of 3 fatty acids more or less affected by the presence of metals
- NMR 1H**
 - Majority of **fatty acids**
 - Detection of **other metabolites** than in targeted methods

Bioaccumulation

- Greater bioaccumulation of iron than nickel
- **Transfer in the food chain** (Hédouin et al. 2010)

Conclusion

Aim: Study the impact of nickel & iron on the photosystem and metabolome

- ✓ **Toxic** effect of iron dependant of the concentration **on the photosystem**
 - ✓ Iron stress **adaptive strategies** to protect against ROS
 - ✓ Risk of **metal bioaccumulation** in the food chain
- ✓ Identification of specific metabolites by **targeted metabolomics**
- ✓ NMR results show metabolites previously metabolites and new ones
- ❑ **Untargeted metabolomic data** have to be statically analysed to evaluate the metabolome response to metals (NMR & UPLC-HRMS)

References

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