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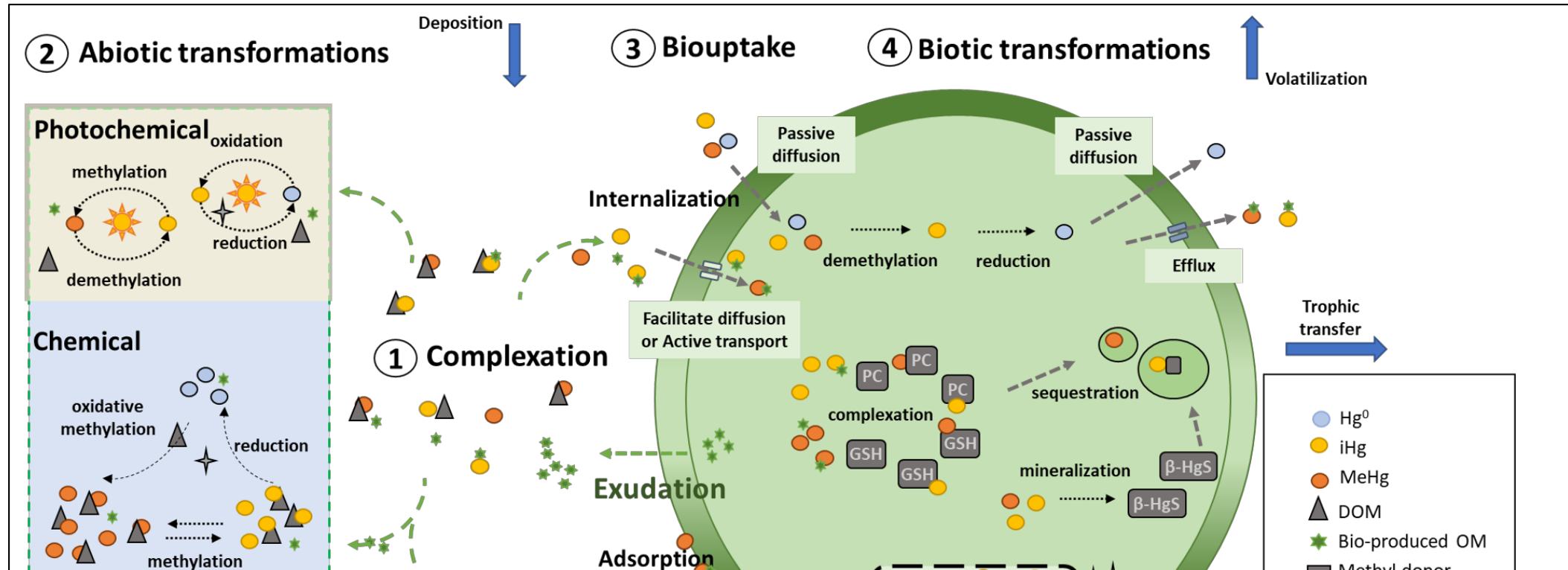


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# Beyond bioaccumulation: Probing mercury's effects in phytoplankton metabolism

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# Mercury species - phytoplankton interactions



## Research questions

- How do mercury compounds affect phytoplankton species?
- How does phytoplankton affect mercury speciation?

# Experimental approach

Unexposed control  
2h exposure  
3nM to 100nM Hg(II) = .3nM to 100nM MeHg

**Green alga**



*Chlamydomonas reinhardtii*

**Diatom**



*Cyclotella meneghiniana*



Photo: Rémy Millet

## Physiology endpoints

Oxydative stress  
Membrane permeability



Chlorophyll a  
Carotenoids

## Bioaccumulation

10 nM and 100 nM Hg(II) and MeHg

Unexposed control

Adsorbed vs intracellular via 1mM cysteine rinse



Analysis with CVAFS

## Subcellular partitioning

Lavoie et al. (2009, 2011)

## Hg(II) and MeHg transformations

Demethylation/methylation

3 nM <sup>199</sup>Hg(II) and 0.3 nM <sup>201</sup>MeHg

Reduction bioassays

GC-ICP-MS

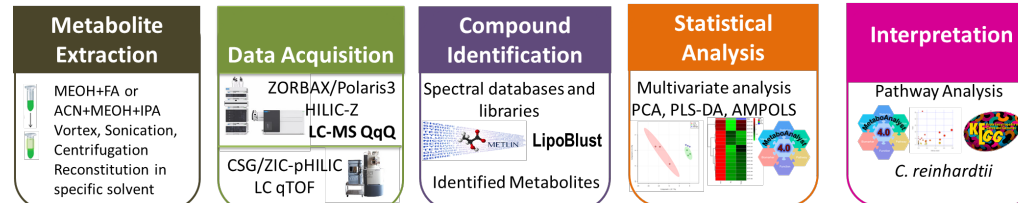


## Metabolome response / quantification of metabolite abundance

### Targeted metabolomics

Liquid chromatography mass spectrometry (LC-MS/MS)

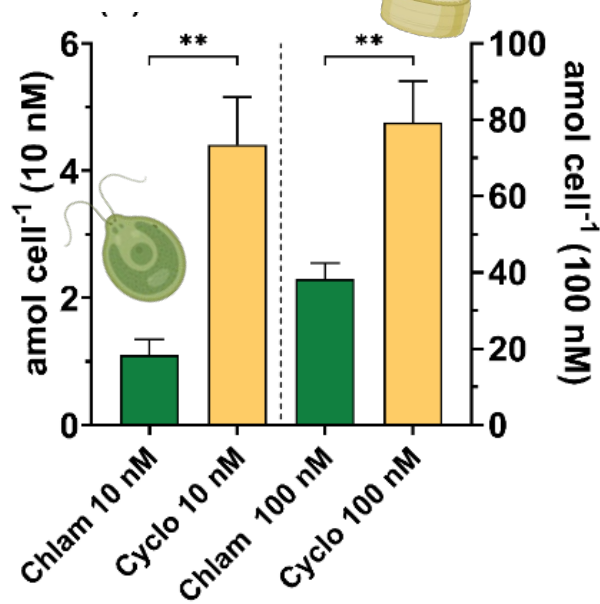
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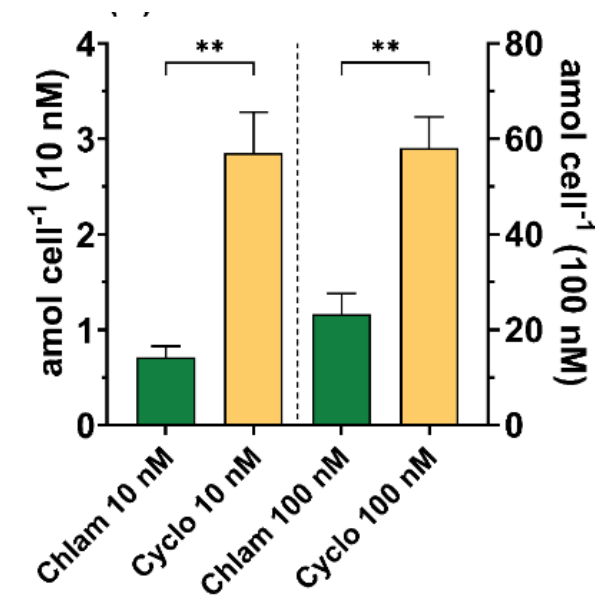
# Accumulation and sub-cellular distribution

## Hg(II) accumulation

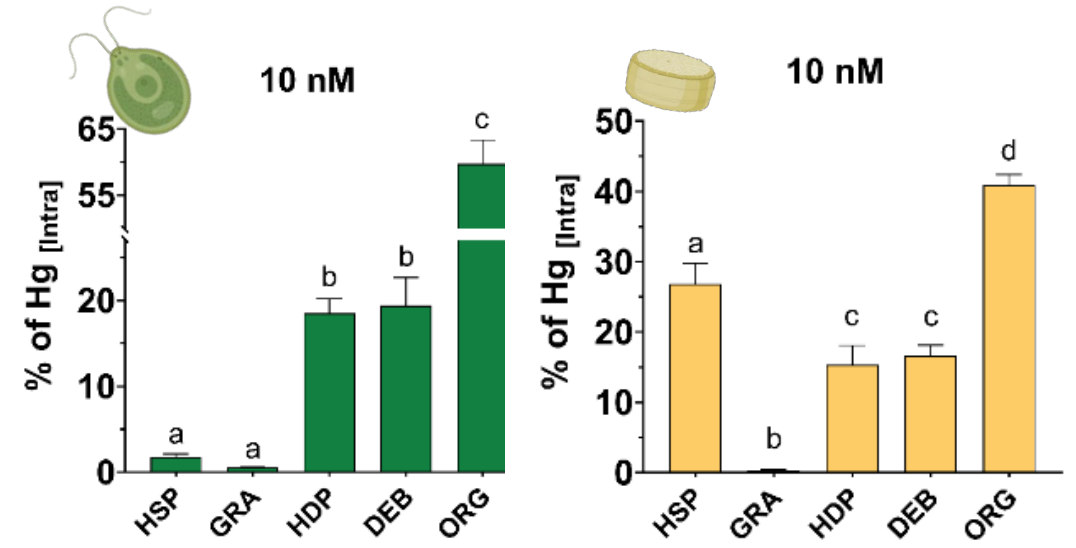
### Cellular Hg



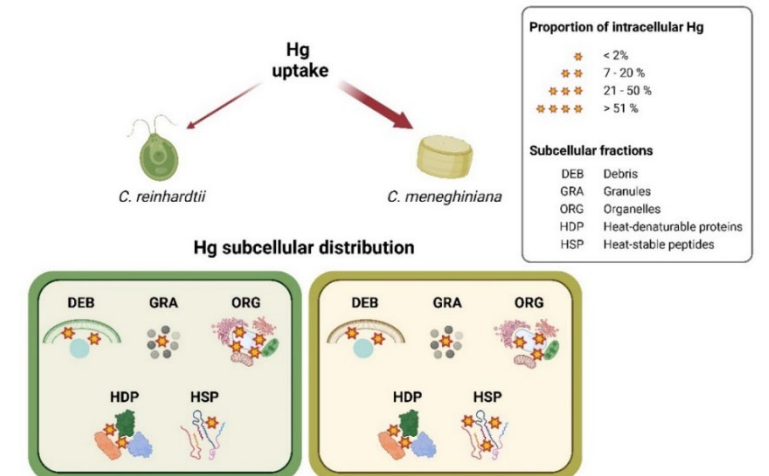
### Intracellular Hg



## Hg(II) subcellular partitioning

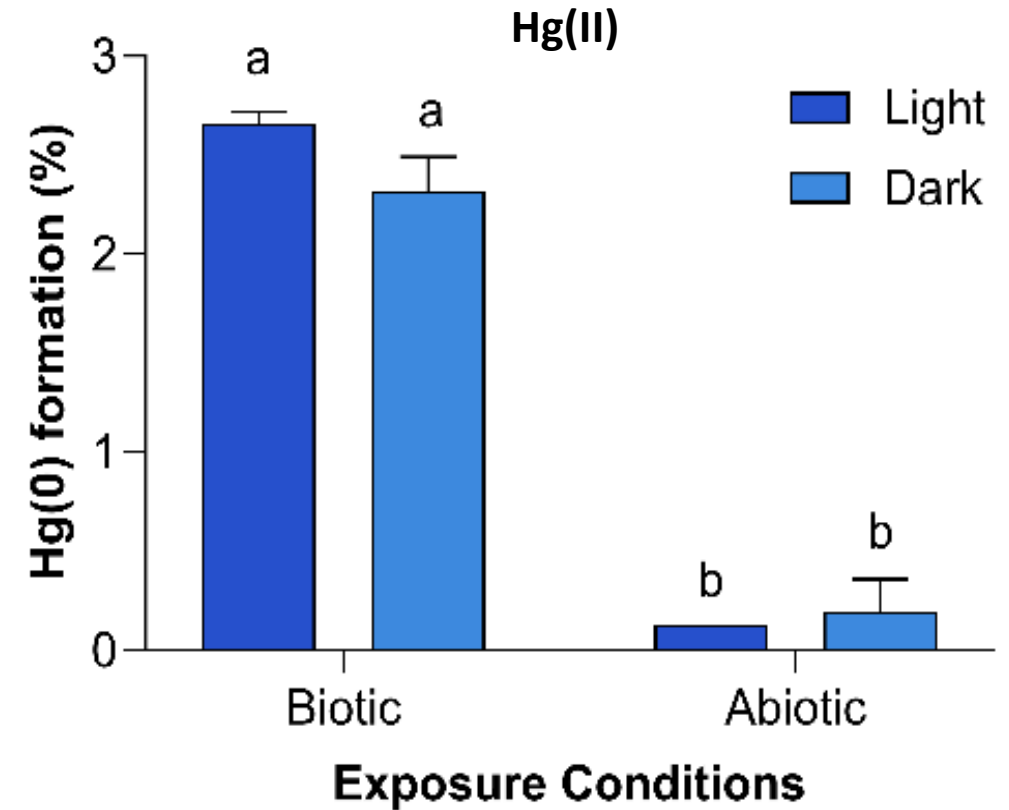
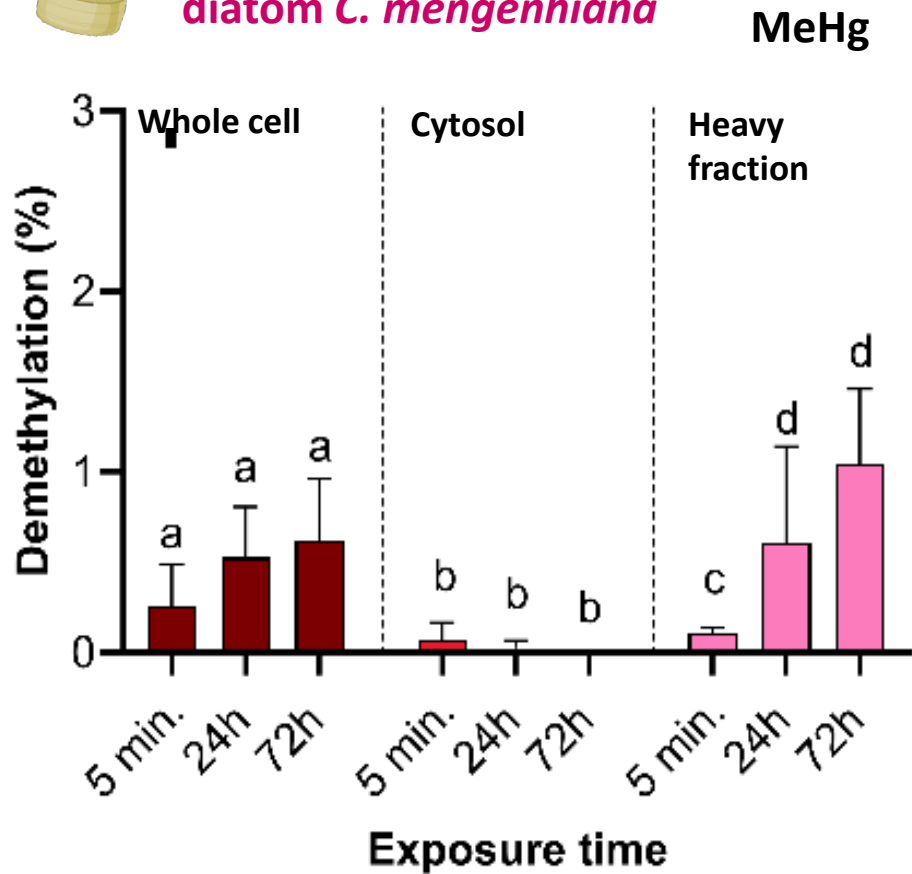


- Hg accumulated by the cells was significantly higher in diatoms compared to green algae
- Hg subcellular distribution is algal species-dependent



# Transformations of mercury species

 diatom *C. mengenhiana*



- Biodemethylation of MeHg, but no methylation of Hg(II)

- Bioreduction of Hg (II), but negligible reduction of MeHg

# Hg(II)/MeHg induced metabolic alterations



## Metabolic response of diatom *C. mengenhiana*

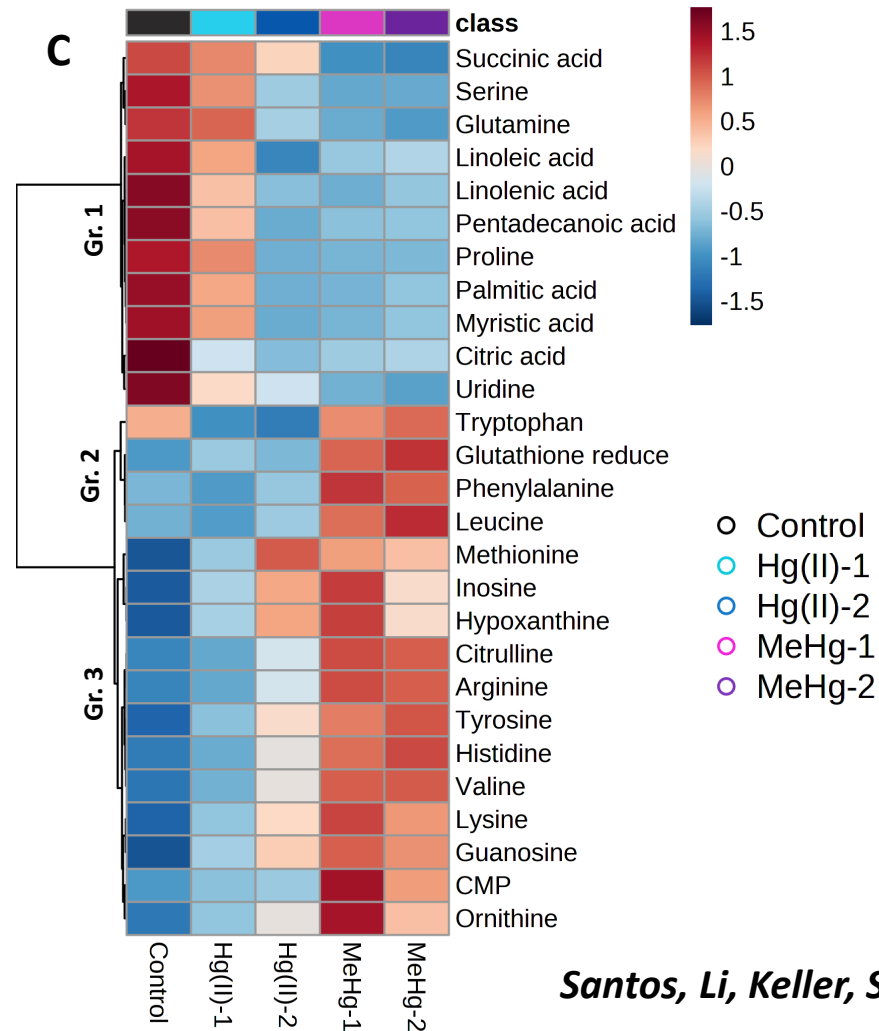
### LC- MS/MS

> 120 considered metabolites

82 primary and secondary metabolites quantified

27 responsive metabolites for Hg(II) and MeHg

- Metabolite profile significantly altered by Hg(II) and MeHg
- No much difference between low and high MeHg treatment



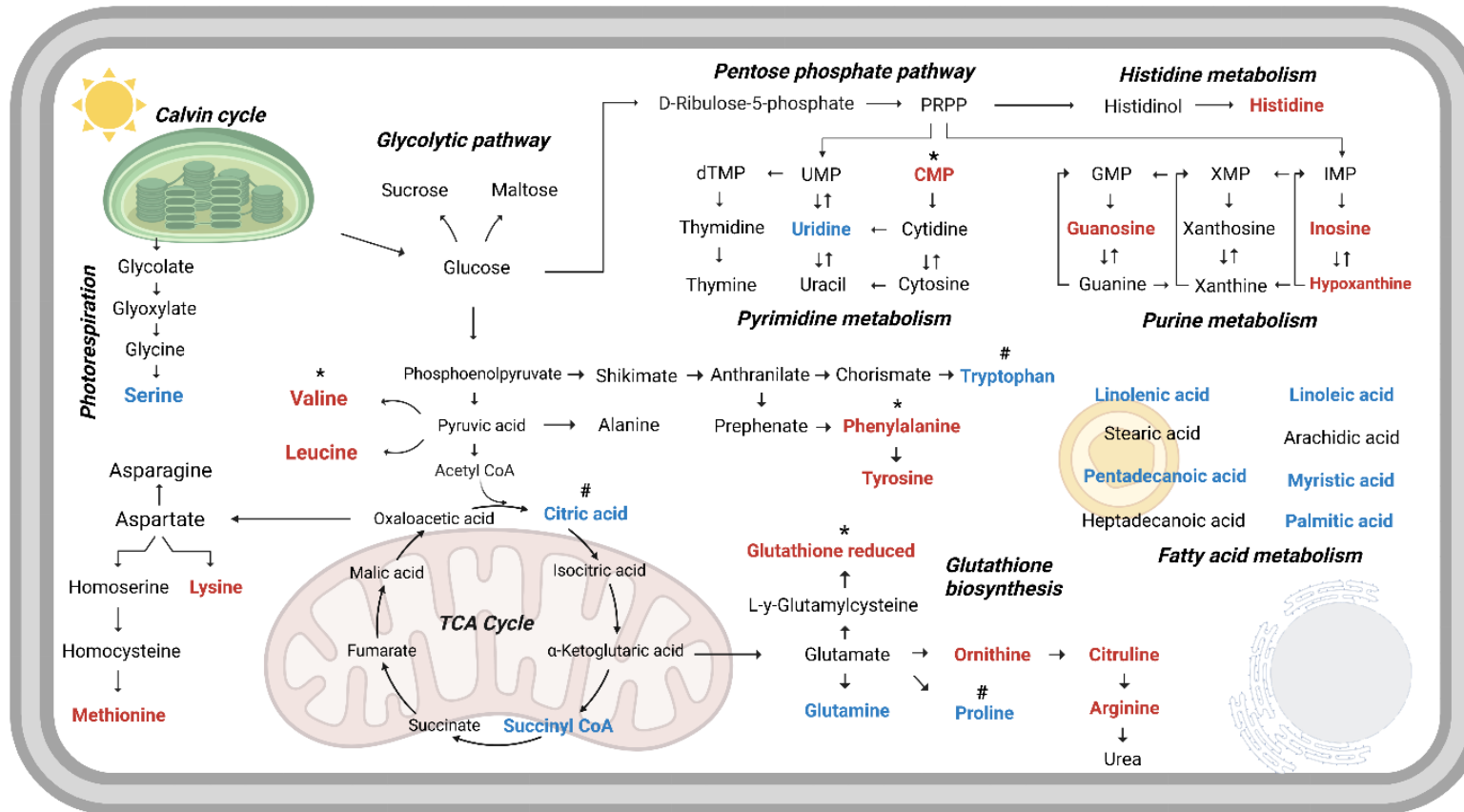
- Gr 1 metabolites decrease in Hg(II) and MeHg treatments
- Gr 2 metabolites significant increase only in MeHg treatments
- Gr 3 metabolites increase in Hg(II) and MeHg treatments

# Overview on Hg - induced metabolic pathway alteration

 *diatom C. mengenhiana*

**Accumulated metabolites**  
**Depleted metabolites**

\* only in MeHg treatments  
# only in Hg(II) treatments



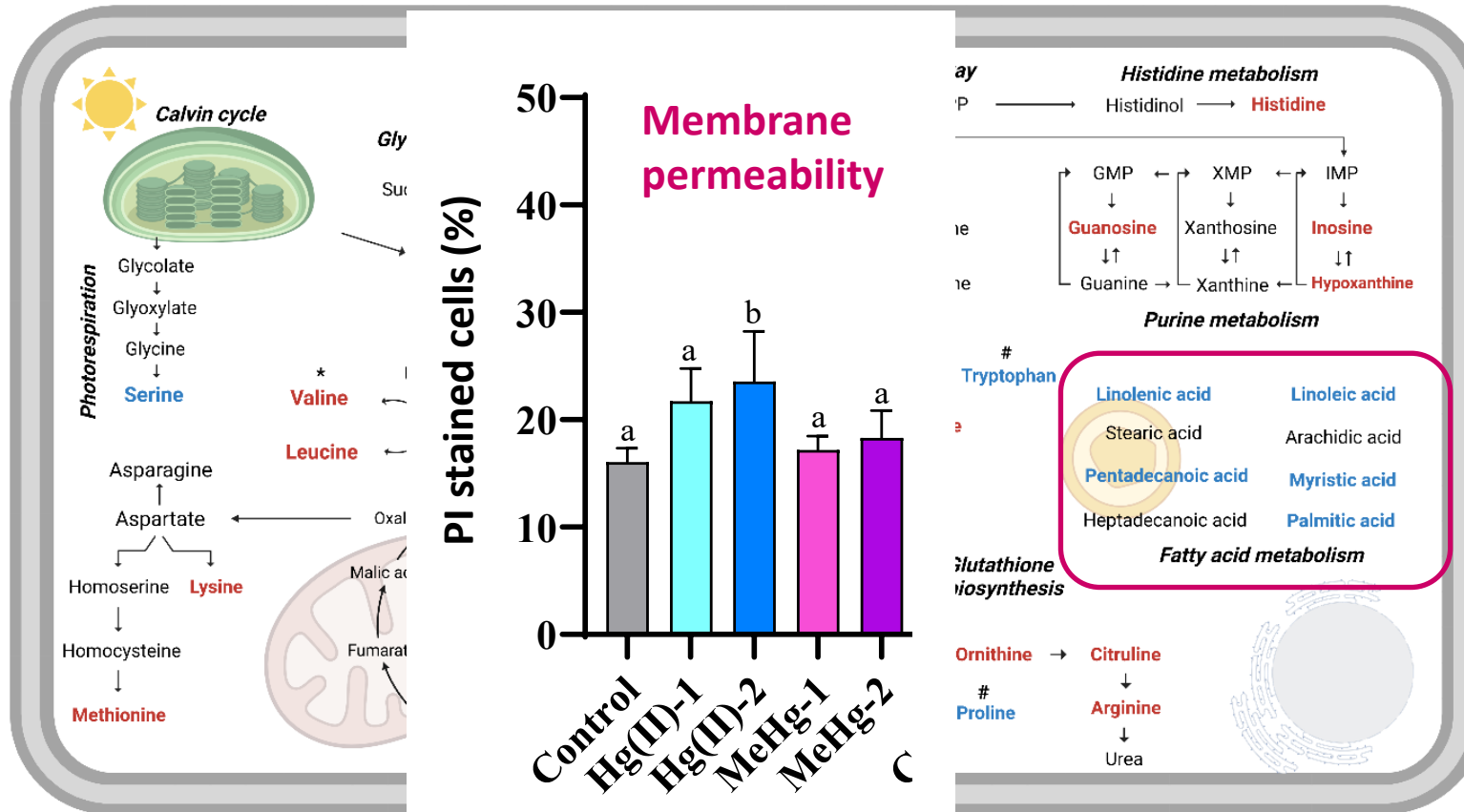
# Overview on Hg - induced metabolic pathway alteration

 *diatom C. mengenhiana*

Accumulated metabolites  
Depleted metabolites

\* only in MeHg treatments  
# only in Hg(II) treatments

## Fatty acid metabolism



- Fatty acid unsaturation and lipolysis
- Increase of the membrane fluidity
- Changes in membrane composition

Santos, Li, Keller, Slaveykova. *J. Haz. Mat.* 465, 133245 (2024)

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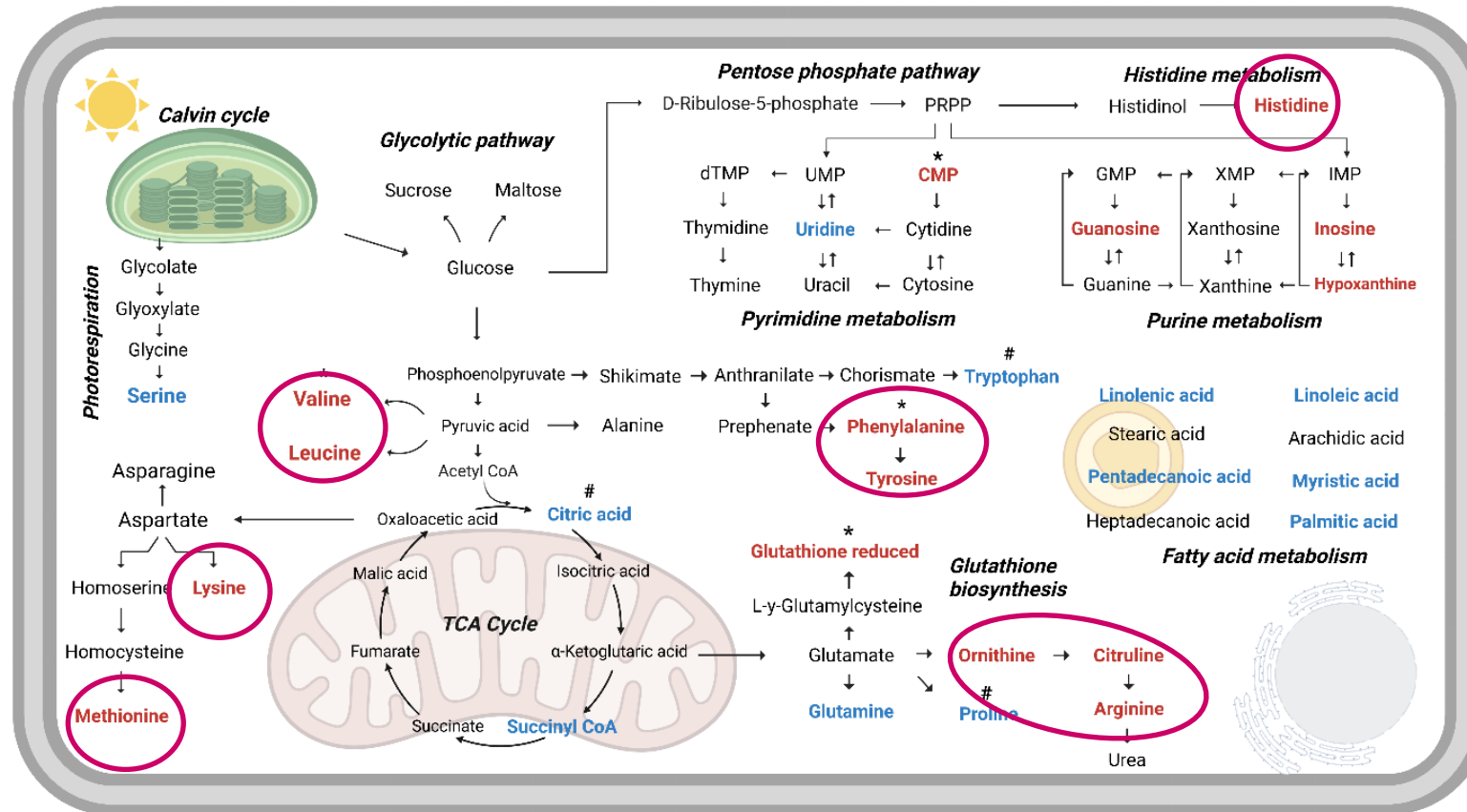
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**Accumulated metabolites**  
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# only in Hg(II) treatments

## Amino acid metabolism



General increase in the abundance of amino acids

-> alteration of the amino acid synthesis and/or potential degradation of proteins and polypeptides

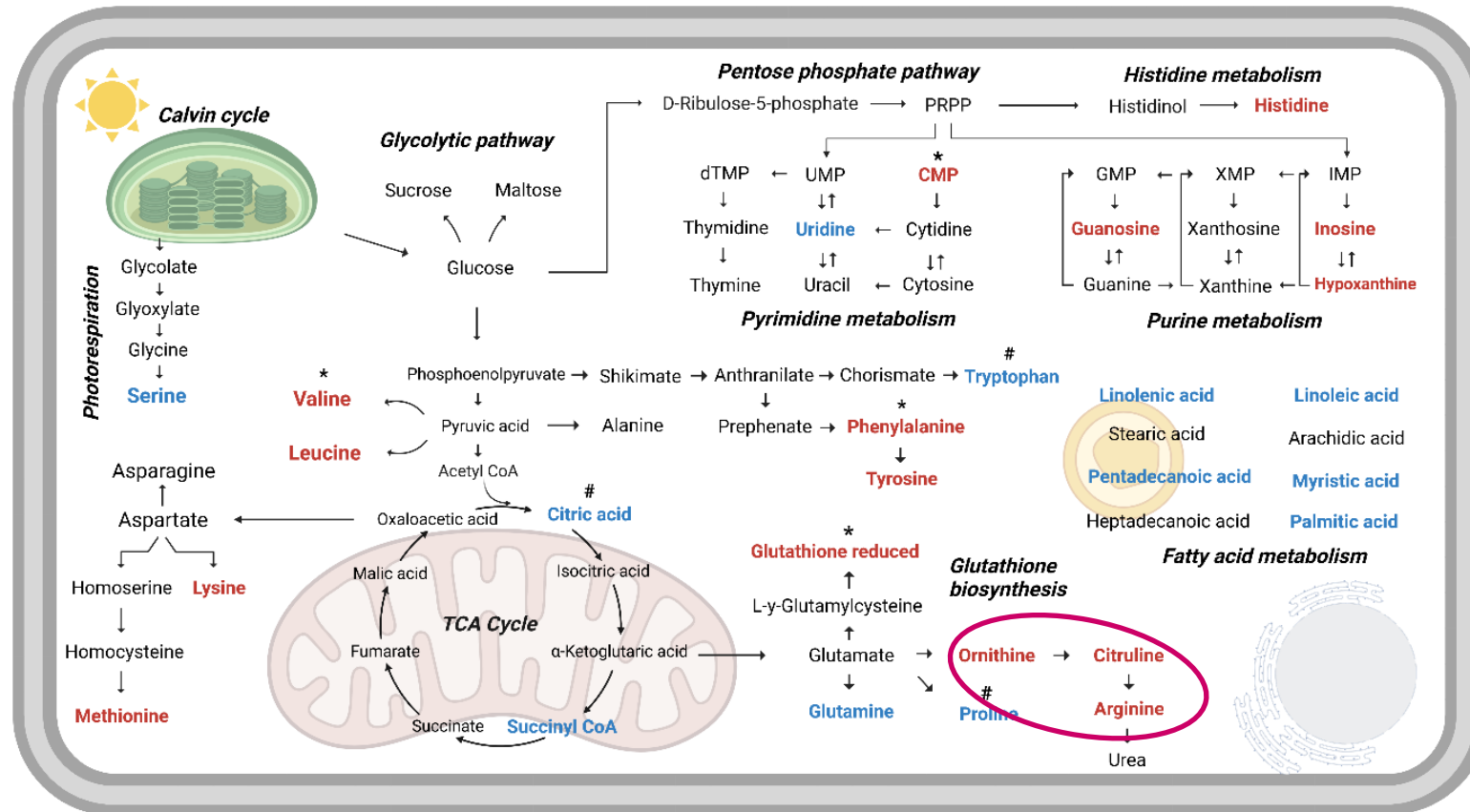
# Overview on Hg - induced metabolic pathway alteration

 *diatom C. menziesiana*

**Accumulated metabolites**  
**Depleted metabolites**

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# only in Hg(II) treatments

## Amino acid metabolism



an acceleration of the urea cycle  
reallocation of intracellular C and N; and the formation of carbonaceous and nitrogenous compounds necessary for growth

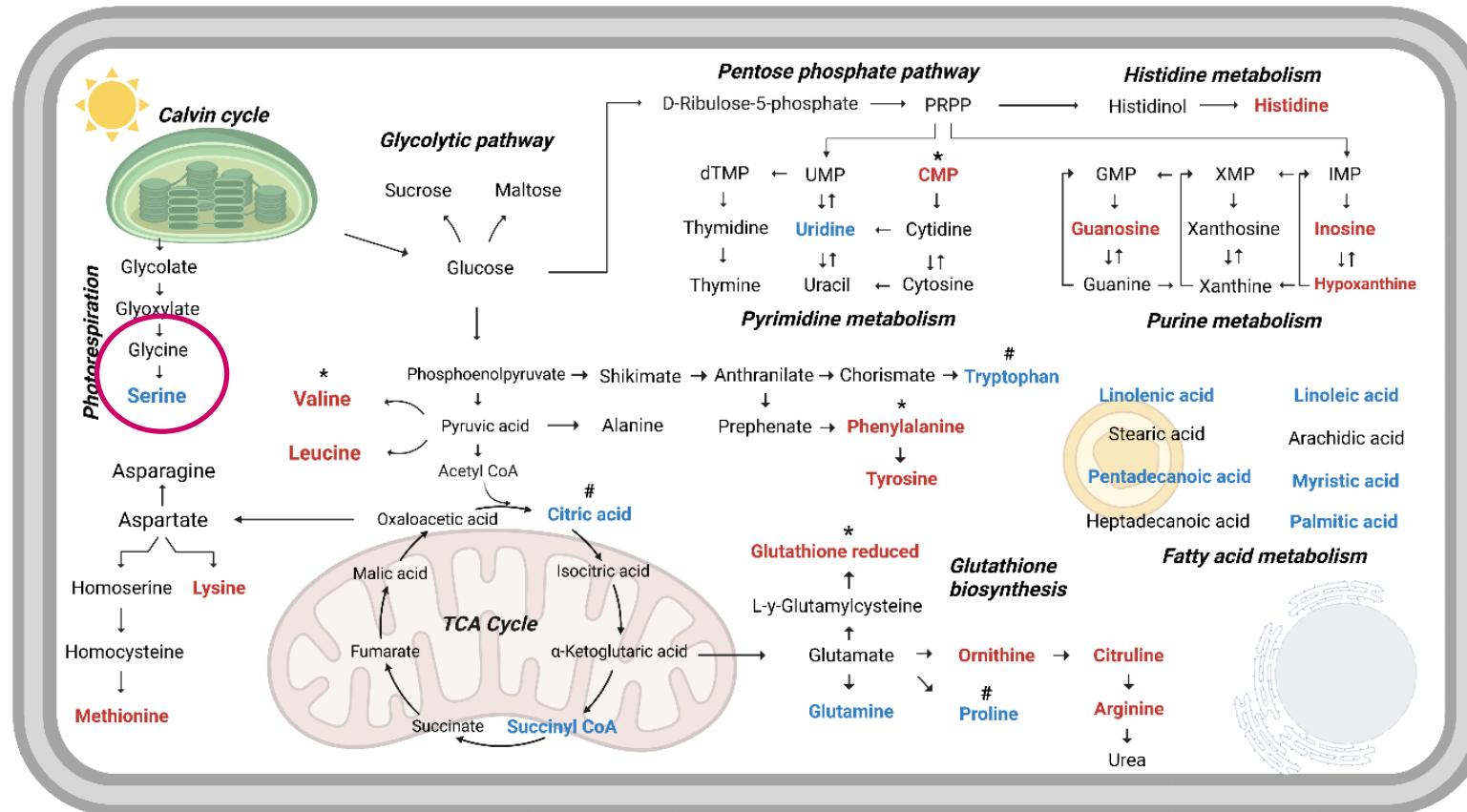
# Overview on Hg - induced metabolic pathway alteration

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**Accumulated metabolites**  
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\* only in MeHg treatments  
# only in Hg(II) treatments

## Amino acid metabolism



Gly/Ser ratio increase

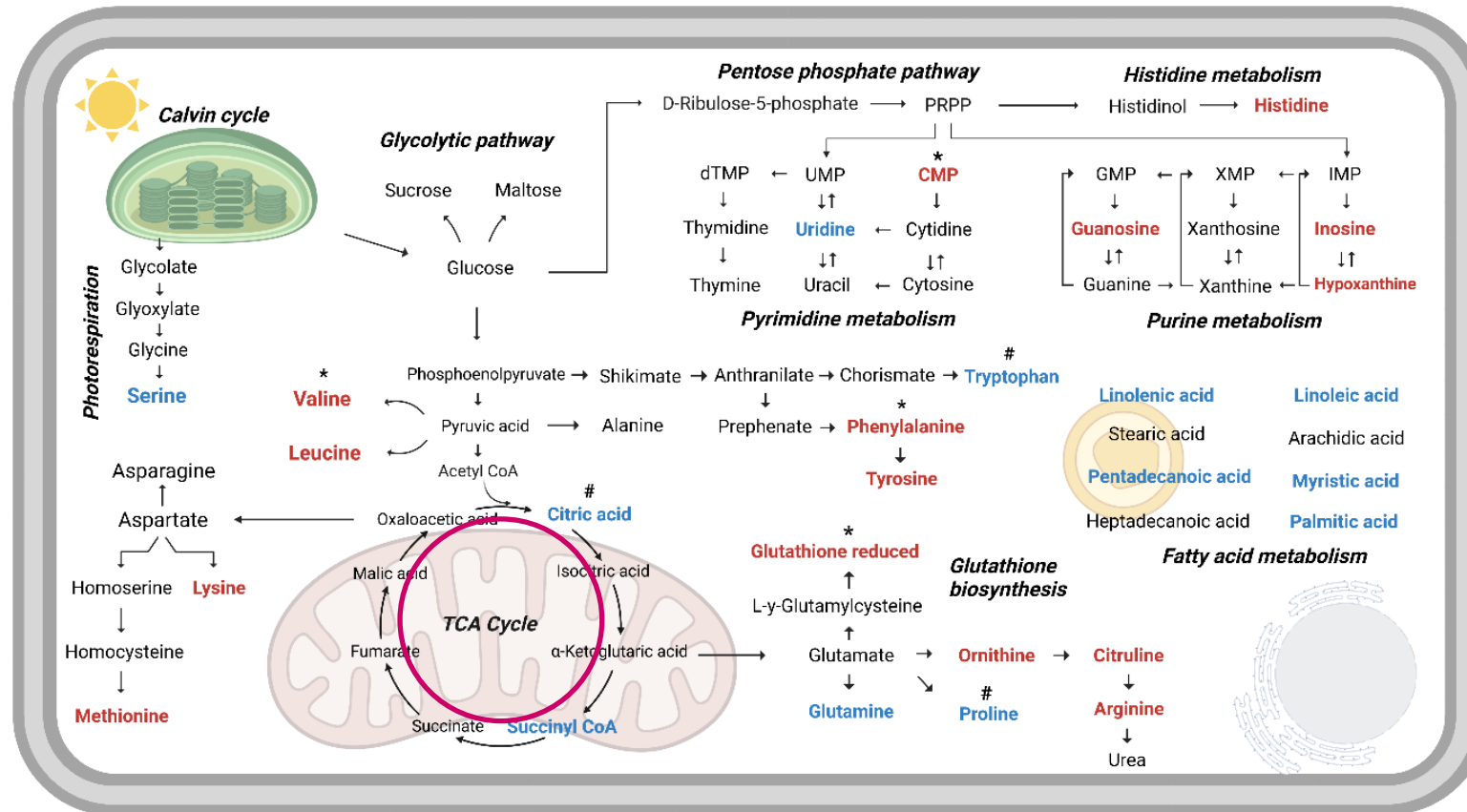
-> upregulation of the photorespiration likely provides additional energy for synthesizing various defense components to cope with oxidative stress

# Overview on Hg - induced metabolic pathway alteration

 *diatom C. menziesiana*

**Accumulated metabolites**  
**Depleted metabolites**

\* only in MeHg treatments  
# only in Hg(II) treatments



Consumption to produce the energy required for manufacturing defense compounds needed to cope with the stress induced by Hg

# Overview on Hg - induced metabolic pathway alteration

 *diatom C. menziesiana*

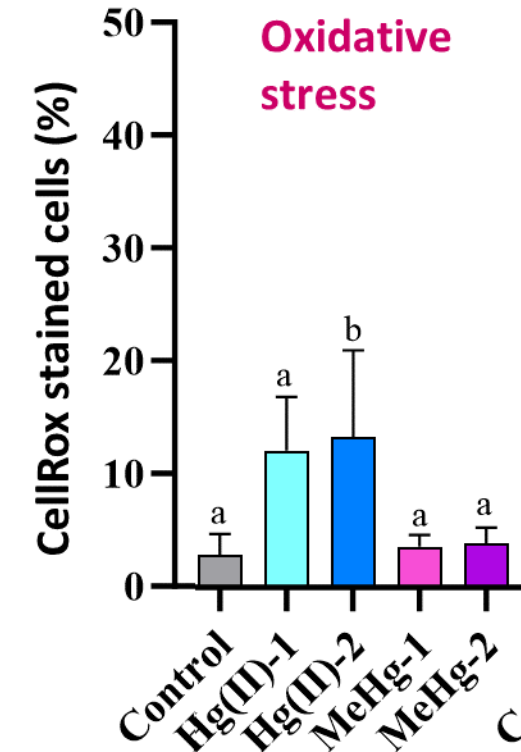
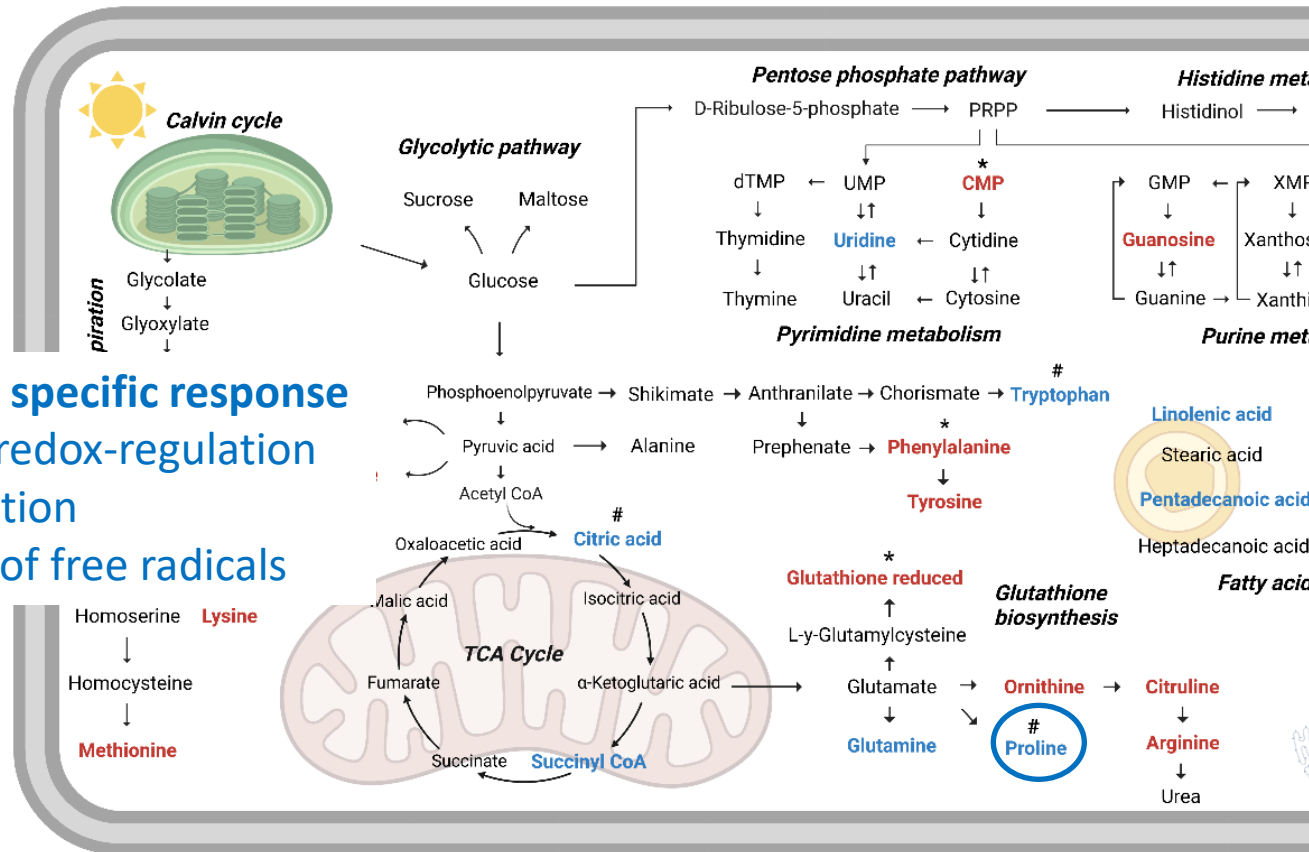
**Accumulated metabolites**  
**Depleted metabolites**

\* only in MeHg treatments  
# only in Hg(II) treatments

Proline only in Hg(II) exposure

## Proline : Hg(II) specific response

- osmo- and redox-regulation
- metal chelation
- scavenging of free radicals



**Oxidative stress**

# Overview on Hg - induced metabolic pathway alteration

 *diatom C. mengenhiana*

Accumulated m  
Depleted meta

GS-MeHg complexes

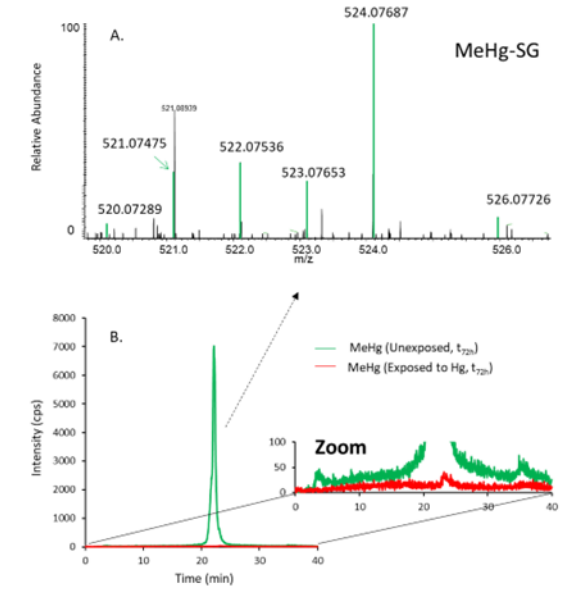
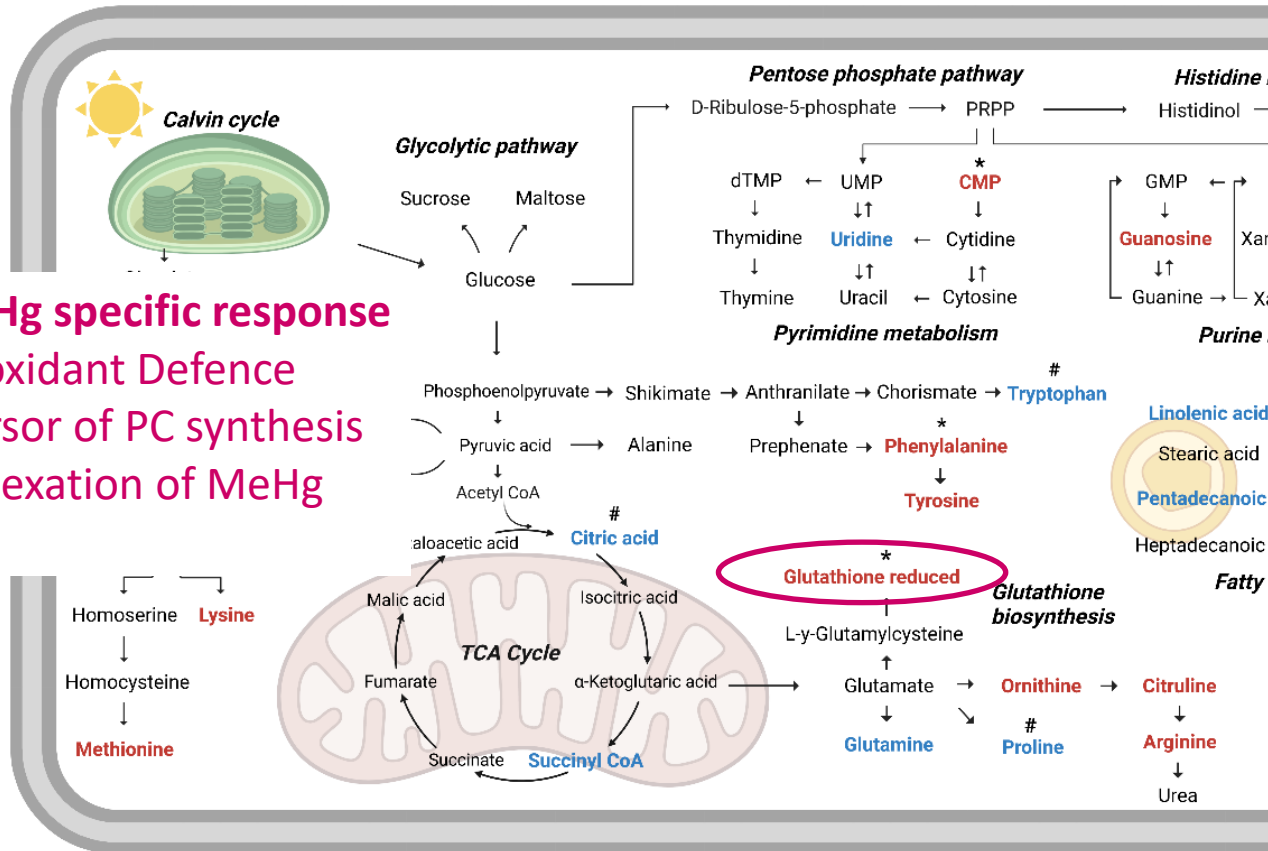


treatments  
treatments

Reduced glutathione only in MeHg exposure

## GSH: MeHg specific response

- Antioxidant Defence
- Precursor of PC synthesis
- Complexation of MeHg



identified  
by HILIC ICPMS and ESI MS



Garcia-Calleja et al. 2024 submitted

# Take-home messages

- Combination of metallomic and metabolomic approaches is important for comprehending mercury toxicity in aquatic primary producers and its potential transfer in trophic chains, especially in highly contaminated environments.



## diatom *C. meneghiniana*

- Hg bioaccumulation is algal species - and Hg species - dependent
  - More Hg(II) in diatom than green alga; More MeHg in diatom and algae than Hg(II)
- Hg subcellular distribution is algal species-dependent
- Diatom and green algae demethylate MeHg and reduce Hg(II) to Hg(0)
- Hg(II) and MeHg affected amino acids, nucleotides, fatty and carboxylic acids, and antioxidant metabolism. At equivalent cellular concentrations, MeHg alters diatom metabolism stronger than Hg(II)



## green alga *C. reinhardtii*

### Metabolomics :

Slaveykova, Majumdar, Regier, Li, Keller *Environ Sci Technol* 55 (2021) 3876.

### Transcriptomics and physiology:

Beauvais-Flück, Slaveykova, Cosio, *Sci Rep* 7(2017), 8034.



# Thank you for your attention!

Questions ? Comments?  
Discussion welcome!

## Acknowledgement



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