

Epigenetic and phenotypic effects of two contaminants in the marine diatom *Phaeodactylum tricornutum*

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Background

Waters are often contaminated with metals, pharmaceuticals and other chemicals, which have been impacting the organisms living in them by translating into behavioral, phenotypical and genotypic alterations. The model organism to this study was the marine diatom *Phaeodactylum tricornutum*, an important microalgae in marine food chains, exposed to sublethal levels of the legacy environmental contaminant cadmium and the antibiotic ciprofloxacin, which has been recognized as an emerging contaminant.

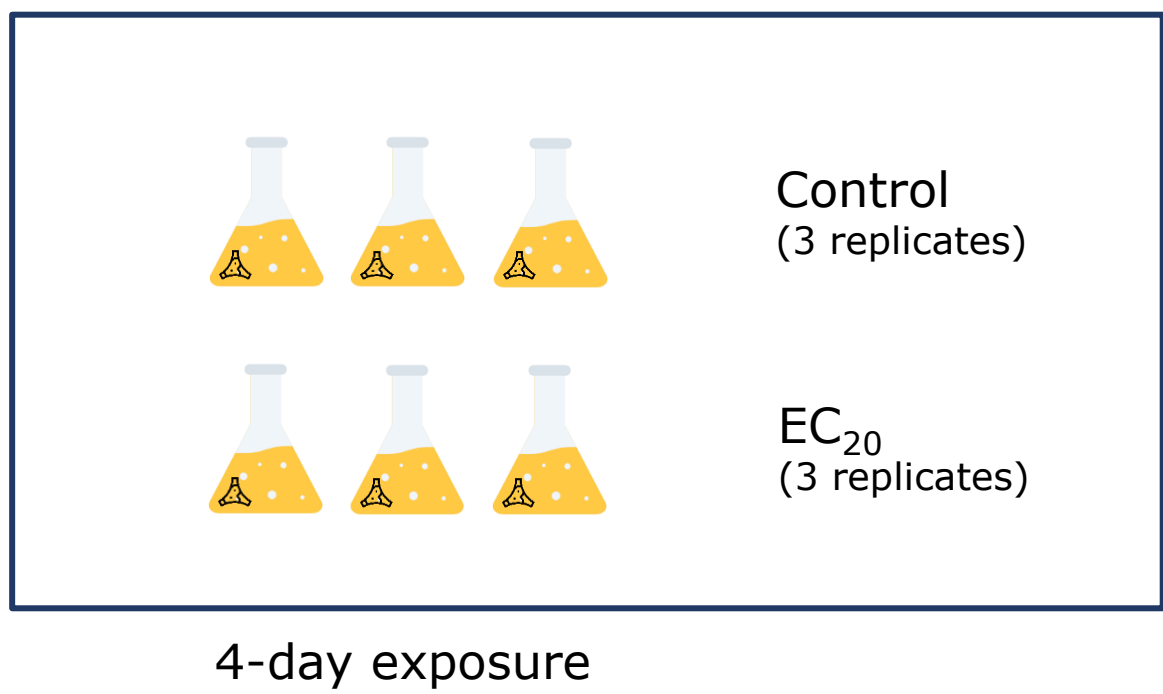
Aim

To perform a comparative study between epigenetic biomarkers (total DNA methylation) and several phenotypic endpoints, namely DNA damage (comet assay), physiological (growth, efficiency of photosystem II and oxygen production) and biochemical biomarkers (oxidative stress and damage).

Methods

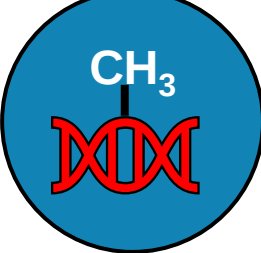
1 Exposure

ISO guideline no.	10253:2024 ^[1]
EC₂₀	
Cadmium	0.293 mg L ⁻¹
Ciprofloxacin	0.202 mg L ⁻¹



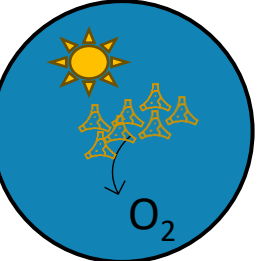
2 Biomarkers

Epigenetic



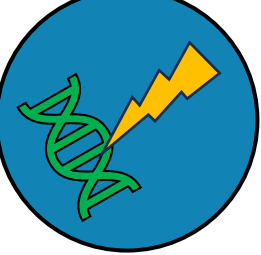
- Total 5-methylcytosine (5-mC) DNA methylation levels
EpigenTek™ MethylFlash Global DNA Methylation ELISA kit
- Whole transcriptome analysis
RNA-seq (**ongoing**)
- Assessment of DNA methylation at the cytosine level
EM-seq (**ongoing**)

Physiological



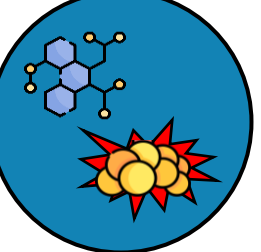
- Growth
Biomass growth – yield (**ongoing**)
- Efficiency of Photosystem II
Measurement of maximum quantum yield of PSII
- Oxygen production
Measurement of oxygen production

Genotoxicity



- DNA damage levels
Alkaline comet assay

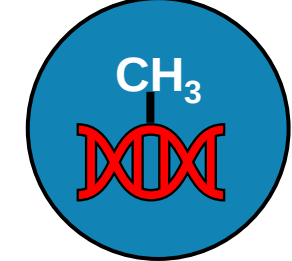
Biochemical



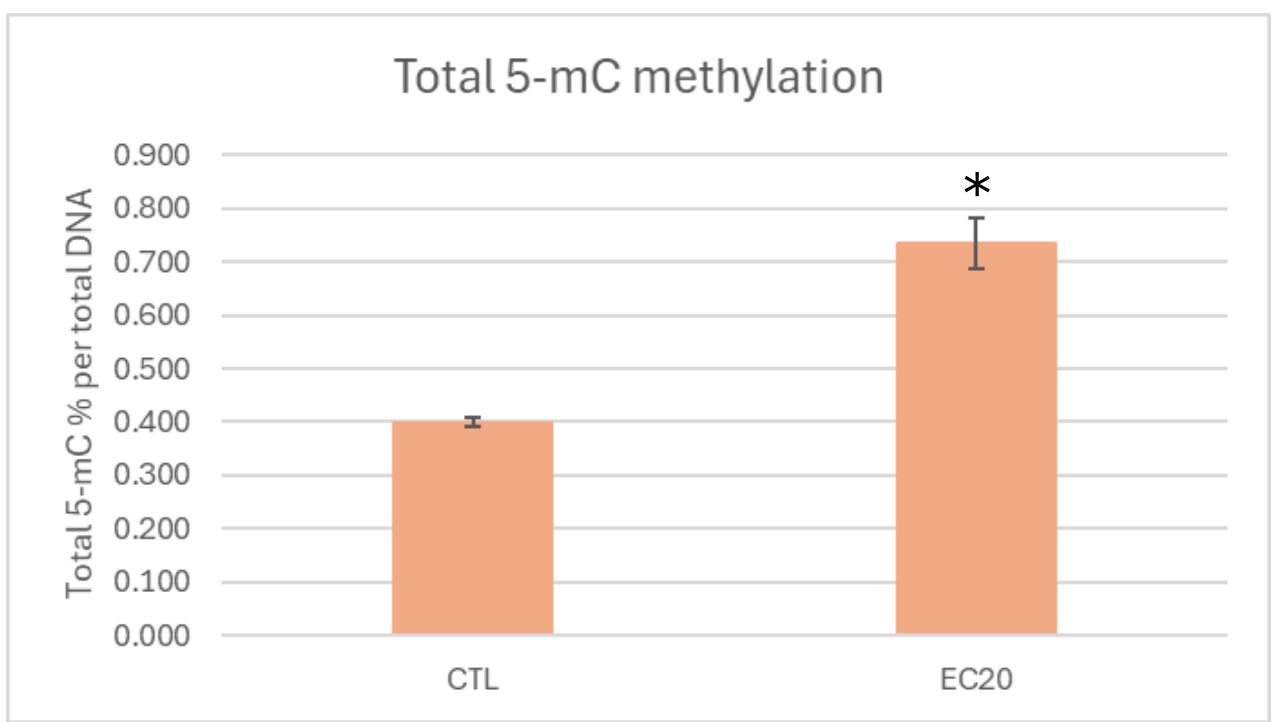
- Antioxidant enzymes activity
Colorimetric assay (**ongoing**)
- Lipid peroxidation (TBARS)
Colorimetric assay (**ongoing**)

Results (so far)

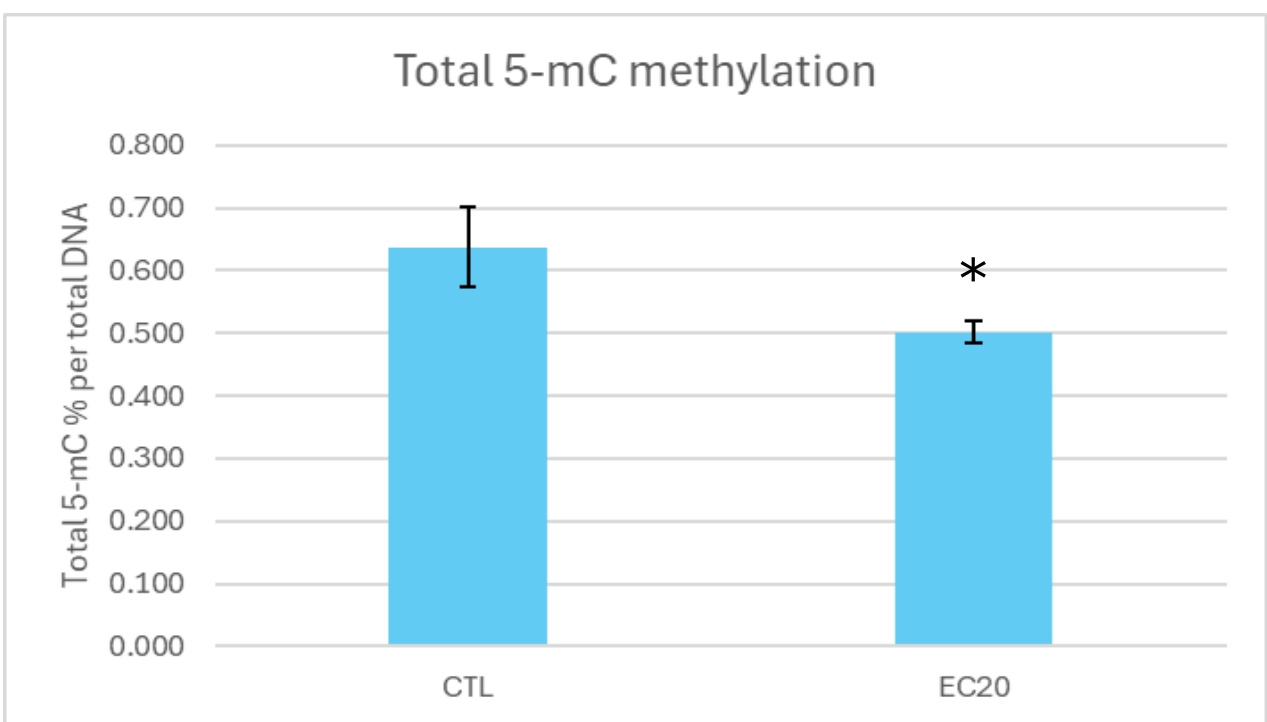
Epigenetic



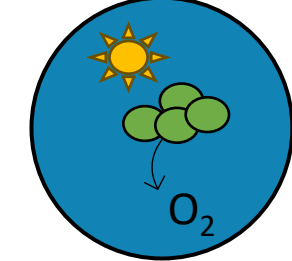
Cadmium



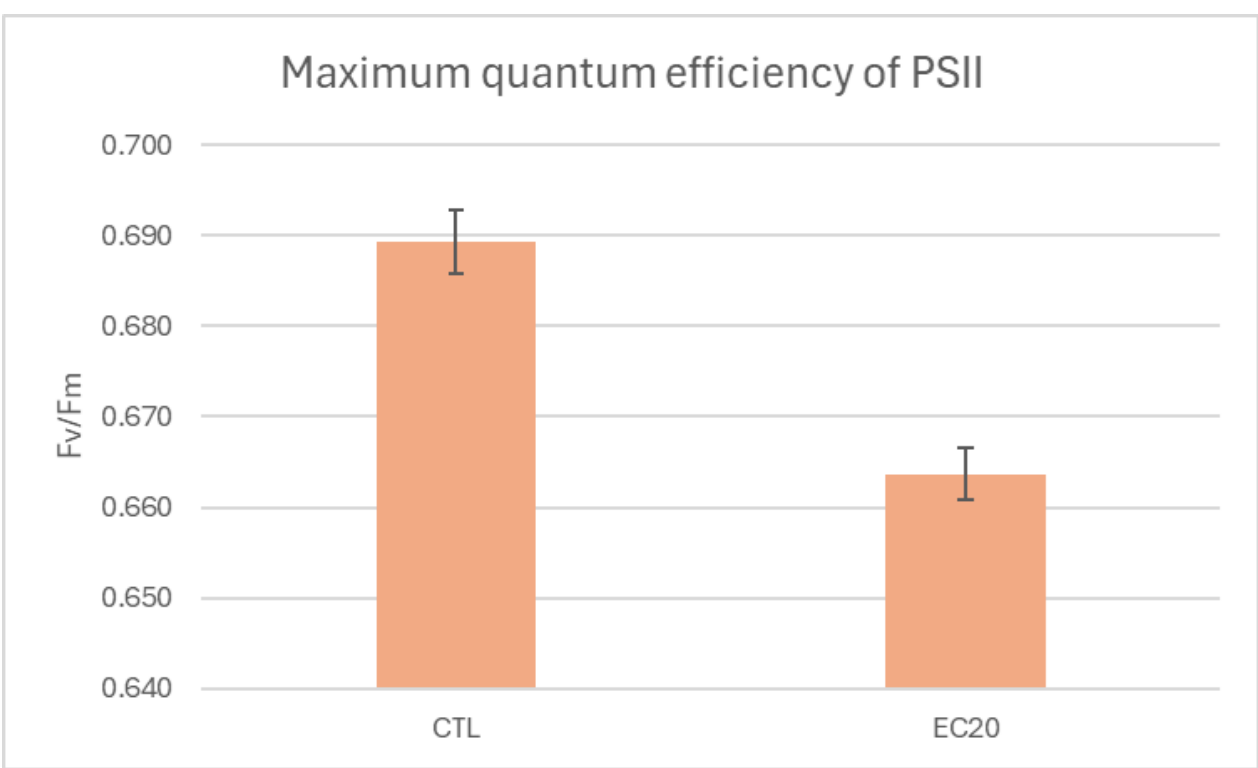
Ciprofloxacin



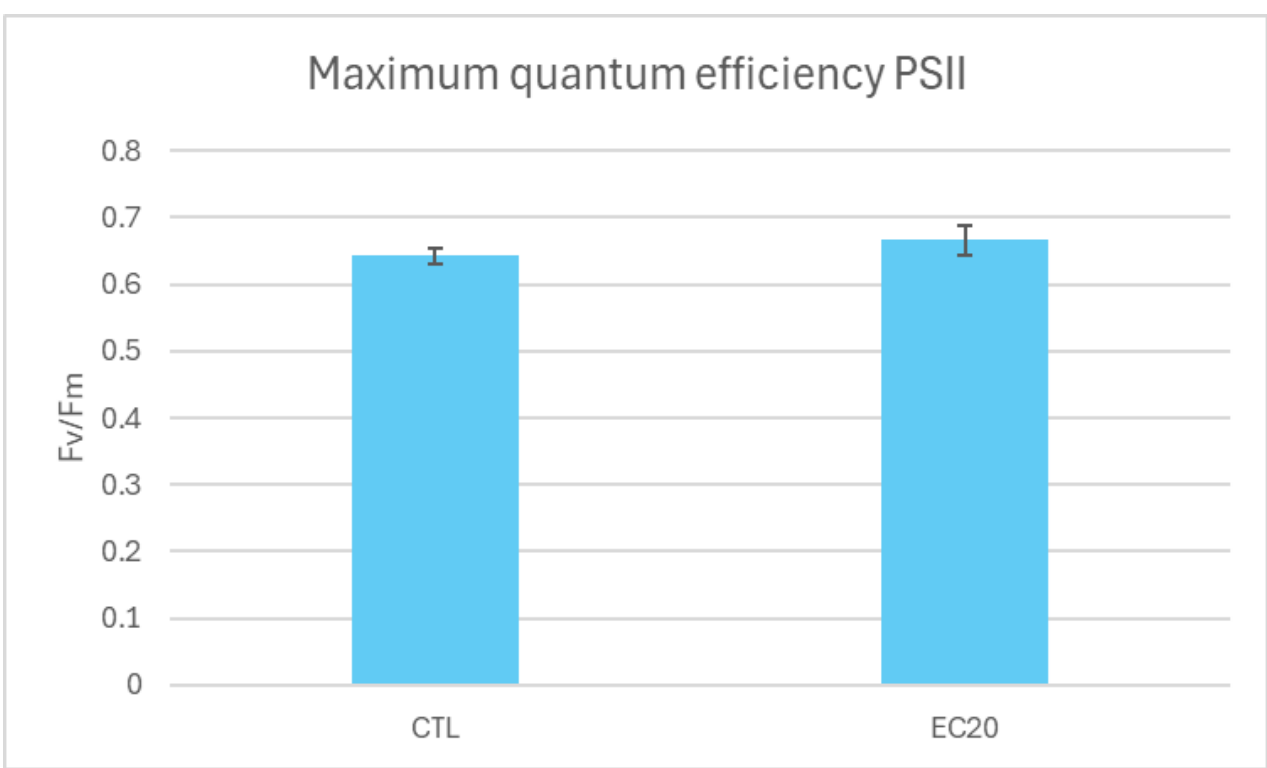
Physiological



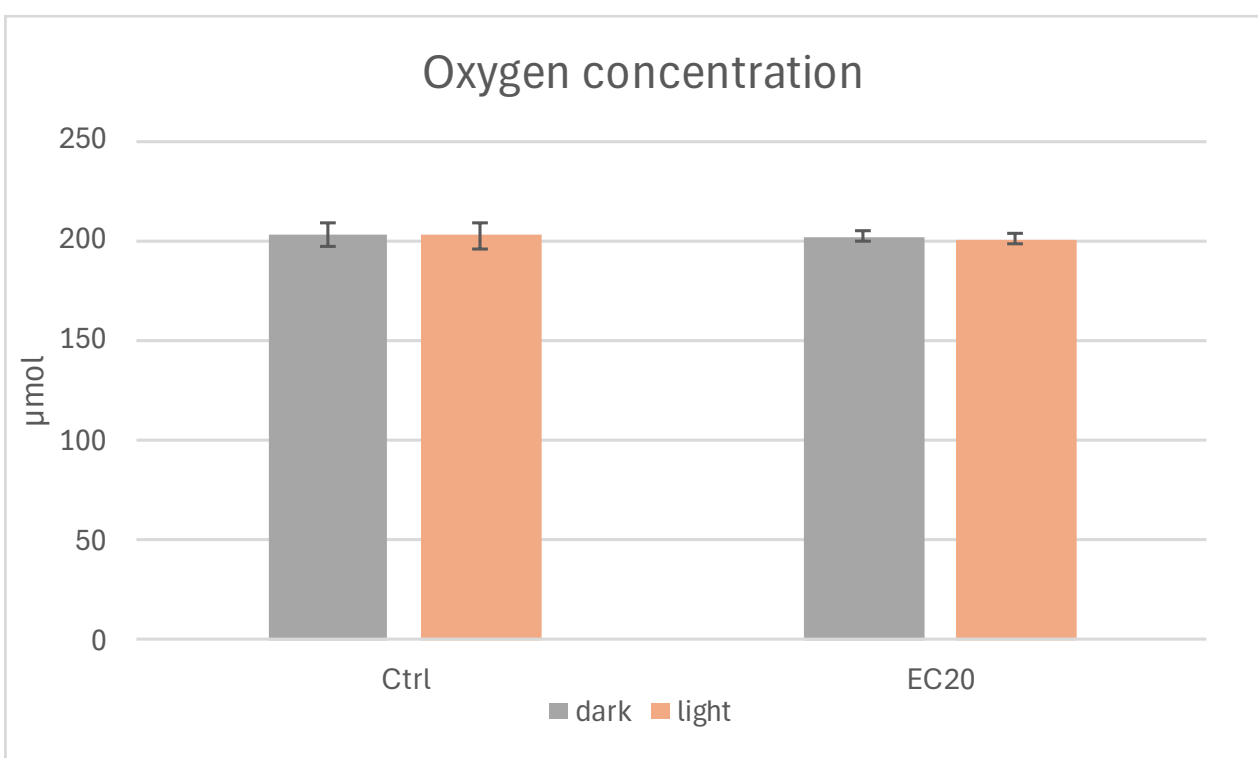
Maximum quantum efficiency of PSII



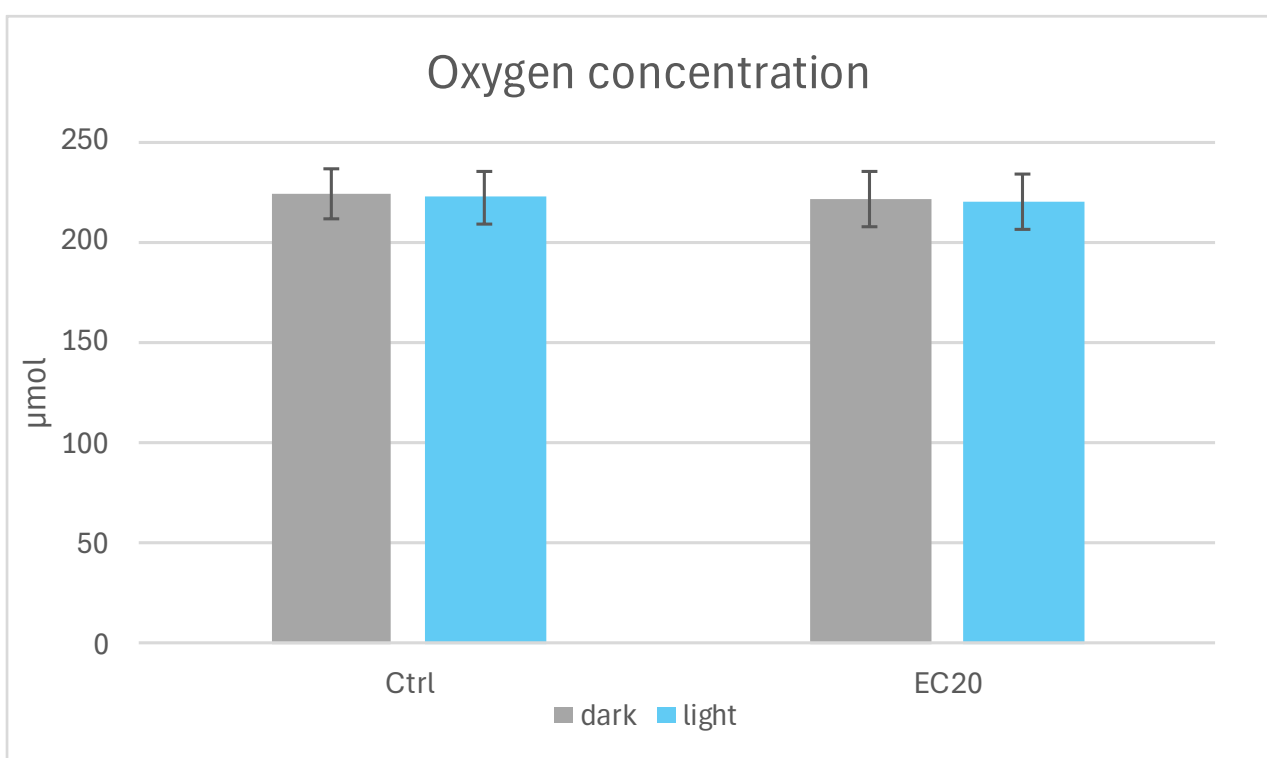
Maximum quantum efficiency PSII



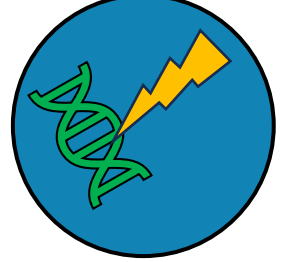
Oxygen concentration



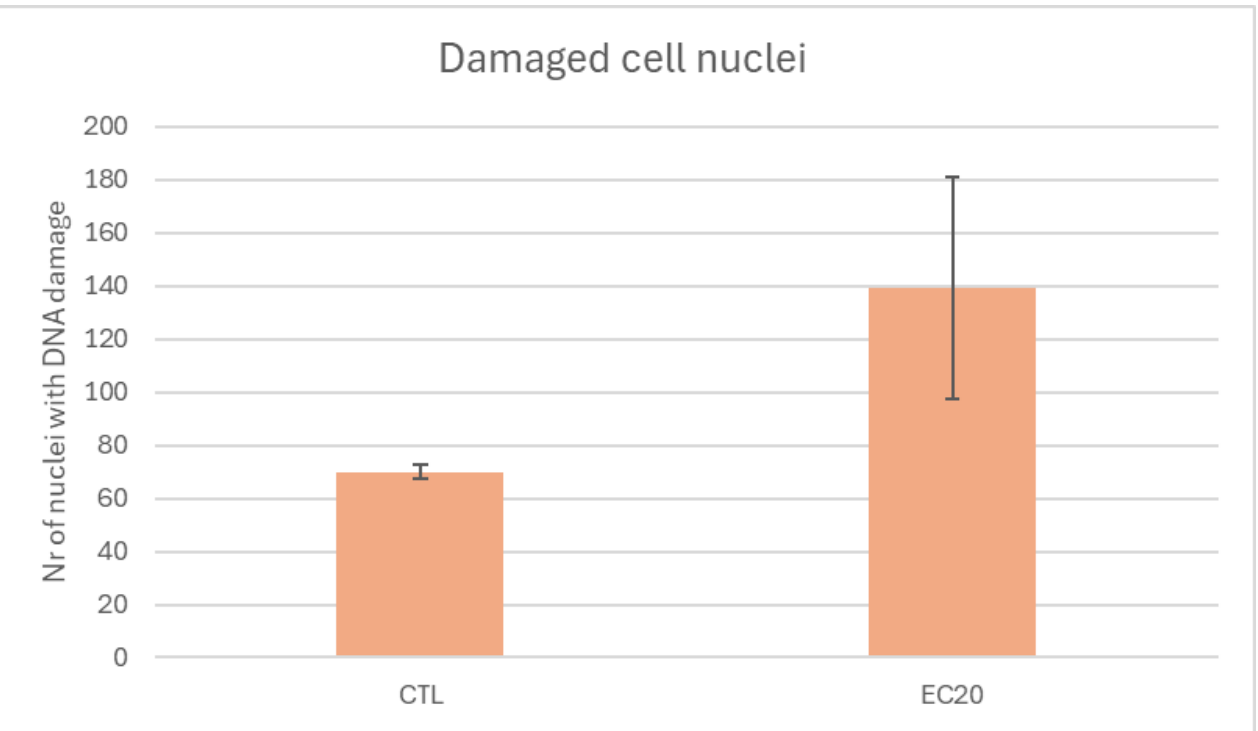
Oxygen concentration



Genotoxicity



Damaged cell nuclei



Damaged cell nuclei

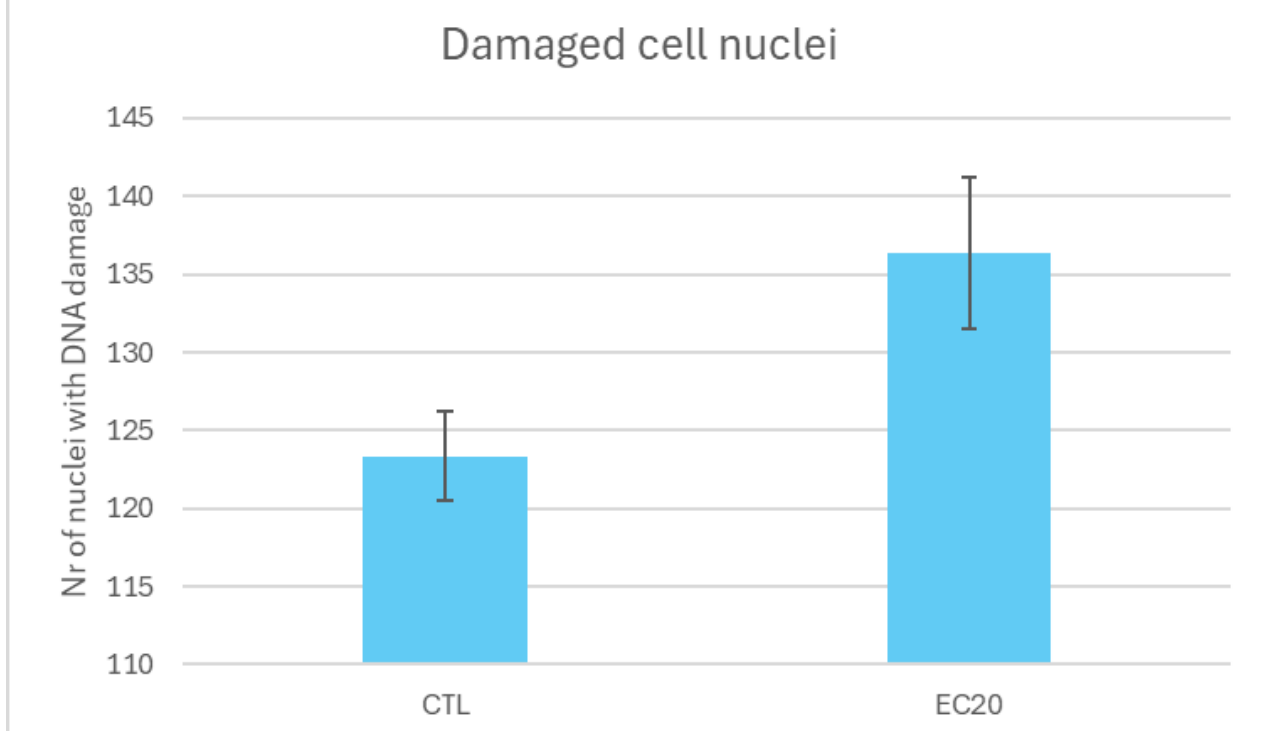


Figure 1. Biomarkers measured in *Phaeodactylum tricornutum* exposed to 0.293 mg L⁻¹ of Cadmium and 0.202 mg L⁻¹ of ciprofloxacin – Epigenetic biomarkers: Total 5-mC levels (A1-A2); Physiological biomarkers: Efficiency of Photosystem II (B1, B3) and Oxygen production (B2, B4); Genotoxicity assay: DNA damage levels (C1-C2); CTL – control group, non-exposed organisms; EC₂₀ – exposed organisms. Error bars correspond to standard error of the mean values; * represents statistically differences (Independent samples T-test, p < 0.05) between the exposed and control organisms.

- Cadmium exposure led to global genome hypermethylation and ciprofloxacin led to hypomethylation
- Cadmium induced inhibition in PS II but no significant effects on oxygen production, whereas ciprofloxacin caused no significant effects on both the efficiency of PS II and oxygen production
- Both chemicals caused observable genotoxicity

References

[1] ISO (2024) ISO 10253:2024, Water quality — Marine algal growth inhibition test with *Skeletonema* sp. and *Phaeodactylum tricornutum*.

Acknowledgements

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Takeaway

- The results of this work are providing valuable insights on the effects of stressors like cadmium and ciprofloxacin in diatoms genome and concur to the adequate selection of biomarkers for an improved ecological risk assessment leading to better protection of marine microalgae.