Sub-lethal Effects of Cadmium and Ciprofloxacin in Freshwater Green Microalgae: Epigenetic and Phenotypic Responses

Inês P. E. Macário[1,2], Tânia Vidal[1,2], **Joana I. Santos[1,2]** ✉ jisantos@ua.pt, Albano Pinto[1,2], Fátima Brandão[1,2], Sérgio M. Marques[1,2], Silja Frankenbach[1,2], Joana Lourenço[1,2], Jana Asselman[3] and Joana Luísa Pereira[1,2]

[1] CESAM – Centre for Environmental and Marine Studies, Department of Biology, University of Aveiro, Portugal
[2] Department of Biology, University of Aveiro, Aveiro, Portugal
[3] Blue Growth Research Lab, Ghent University, Bluebridge Building, Ostend Science Park 1, 8400, Ostend, Belgium

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Background

Anthropogenic activities often lead to contamination of waters. Assuming the importance of microalgae as primary producers in freshwater food chains, we assessed the effects in *Parachlorella* sp. of exposure to sublethal levels of cadmium, a legacy environmental contaminant, and ciprofloxacin, an antibiotic that has been recognised as an emerging contaminant in freshwater. As epigenetic mechanisms shape gene-environment interactions, the study of the epigenetic alterations in these organisms can provide clues to better understand how they face environmental challenges and how to protect them.

Aim

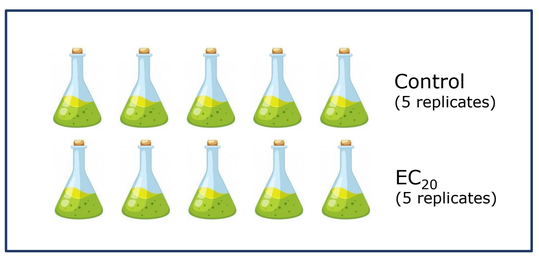
To perform a comparative study between epigenetic biomarkers (total DNA methylation) and several phenotypic endpoints, namely DNA damage (comet assay), physiological (growth, efficiency of photosystem II and oxygen production) and biochemical biomarkers (oxidative stress and damage).

Methods

1 Exposure

OECD guideline no. 201[1]

	EC_{20}
Cadmium	90.3 µg L^{-1}
Ciprofloxacin	7.68 mg L^{-1}



10-day exposure

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**)

Biochemical

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

Genotoxicity

- DNA damage levels
  Alkaline comet assay

Physiological

- Growth
  Biomass growth - yield
- Efficiency of photosynthetic activity
  Measurement of maximum quantum yield of PSII
- Oxygen production
  Measurement of oxygen concentration

Results

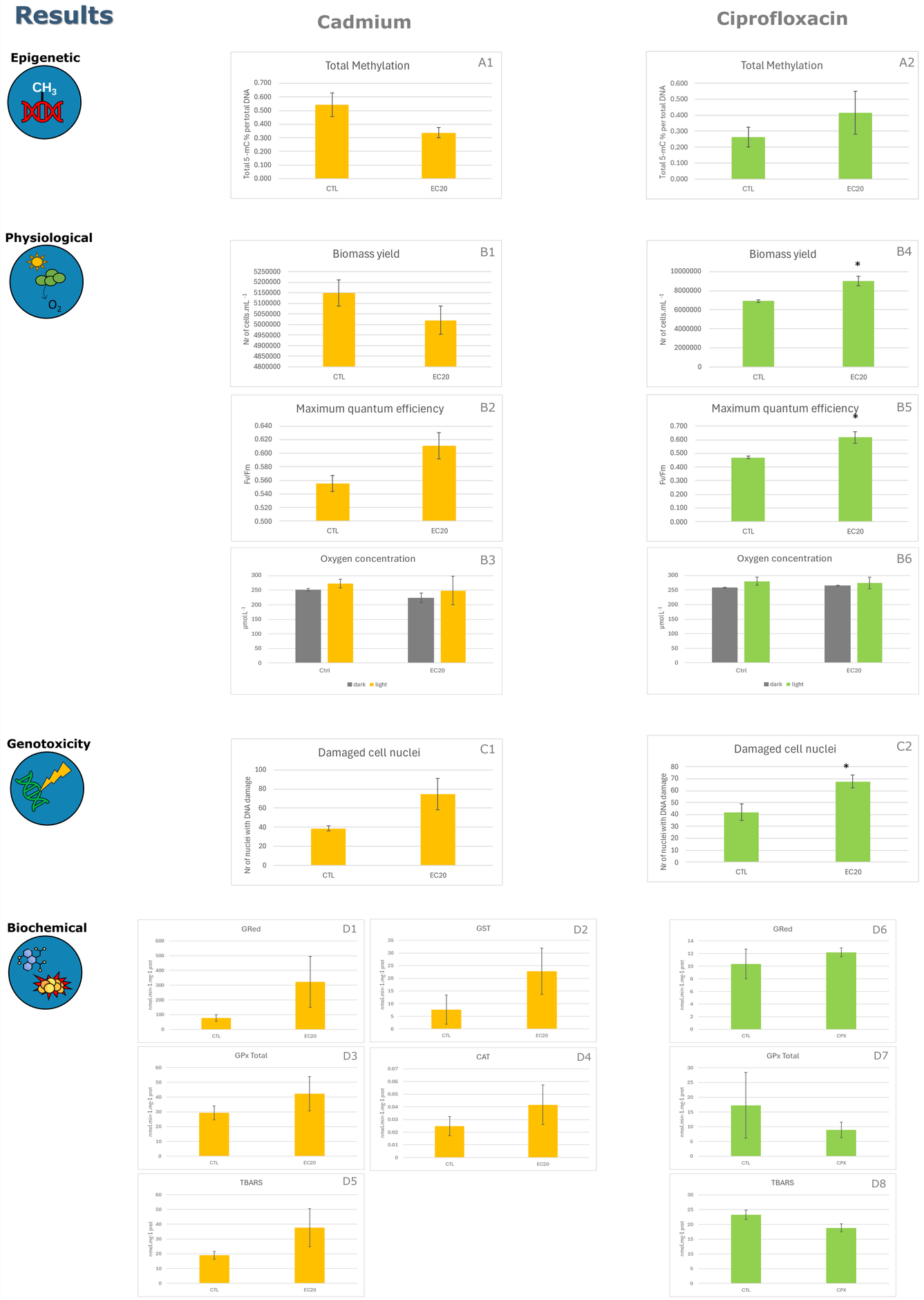


Figure 1. Biomarkers measured in *Parachlorella* sp. exposed to 90.3 µg L^{-1} of cadmium (yellow, left) and 7.68 mg L^{-1} of ciprofloxacin (green, right) for 10 days. Epigenetic biomarkers: total 5-mC levels (A1, A2); Physiological biomarkers: biomass yield (B1, B4), maximum quantum efficiency of Photosystem II (B2, B5) and oxygen concentration (B3, B6); Genotoxicity assay: DNA damage levels (C1, C2); Biochemical biomarkers: antioxidant enzymes activity (D1-D4, D6, D7) and TBARS levels (D5, D8). CTL – control group, non-exposed organisms; EC_{20} – exposed organisms. Error bars correspond to standard error of the mean values; * represents statistical differences (Independent samples T-test or Mann-Whitney U test, $p < 0.05$).

- Cadmium exposure led to non-significant global genome hypomethylation and ciprofloxacin led to hypermethylation.
- Cadmium induced a slight decrease in biomass yield, while ciprofloxacin stimulated the biomass yield, and both chemicals caused a slight stimulation in PS II efficiency.
- Both chemicals showed no significant impact in respiration and oxygen concentration in the medium.
- Both chemicals caused mild genotoxicity.
- Cadmium caused mild oxidative stress; ciprofloxacin did not show an obvious trend regarding oxidative stress.
- Whole transcriptome analysis (RNAseq) and assessment of DNA methylation at the cytosine level (EM-seq) are ongoing, which should provide evidence on the mechanistic behind the phenotypic effects noticed and changes promoted by cadmium and ciprofloxacin in DNA methylation patterns.

Takeaway message

These results, combined with ongoing work on the molecular basis, contribute to the understanding of adverse outcome pathways of toxicity caused by stressors such as cadmium and ciprofloxacin in green microalgae, allowing a feasible selection of appropriate biomarkers for improved ecological risk assessment.

References

[1] OECD (2011) Test No. 201: Freshwater Alga and Cyanobacteria, Growth Inhibition Test. OECD Guidelines for the Testing of Chemicals, Section 2, OECD Publishing, Paris, https://doi.org/10.1787/9789264069923-en.

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