Unravelling the formation of a palmelloid-like phenotype in the green microalga *Raphidocelis subcapitata* when exposed to pollutants

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Introduction: Unicellular microorganisms may present different defence and survival strategies, when exposed to pollutants or adverse environments. For instance, the microalgae belonging to the genera *Chlamydomonas*, *Chlorella*, and *Dunaliella*, in response to high salinity or toxics, can form a structure (comprising non-motile cells surrounded by a matrix of polysaccharides) called "palmelloid", due to its similarity with the morphology of the alga *Palmella*.

Objectives: This work aimed to verify the formation of a palmelloid-like (multinucleated) phenotype in the freshwater microalgae *Raphidocelis subcapitata* caused by exposure to inorganic or organic pollutants. The kinetics of the formation of the palmelloid-like phenotype and its reversibility were also studied.

Methods: The microalga was incubated with heavy metals (Cd or Zn) and organic compounds [the antibiotic erythromycin (ERY) or the herbicide metolachlor (MET)] at various concentrations. After 24, 48, and 72 h the palmelloid-like phenotype formation was evaluated.

Results: In the absence of stress or when exposed to ERY or Zn, up to 200 μgL^{-1} (where growth was reduced or halted), *R. subcapitata* exhibited, primarily, a single nucleus. At sub-lethal and environmentally relevant concentrations of MET (100-200 μgL^{-1}) or Cd (200 μgL^{-1}), the alga transitioned to a multinucleated state, similar to the "palmelloid" morphology observed in different green algae. The multinucleated state is reversed when the microalgae are re-inoculated in a fresh medium (without pollutants).

Conclusions: The development of palmelloid-like phenotype in *R. subcapitata* when exposed to sub-lethal levels of inorganic or organic pollutants is not a universal defence and response strategy to stress as it depends on the pollutant, its concentration, and the duration of the exposure. The multinucleated state is a reversible process. The insights provided in this study contribute to increasing the knowledge of the strategies used by *R. subcapitata* to manage severe stress induced by pollutants.