From invisible marine exopolymers to hetero-aggregates: plastics sinking?

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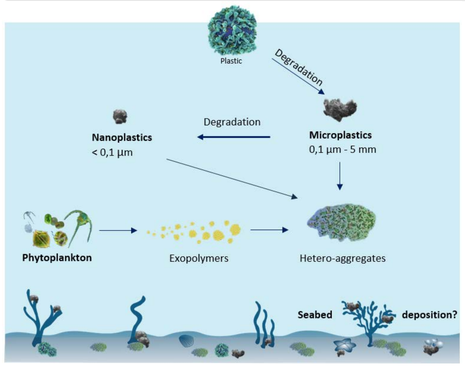
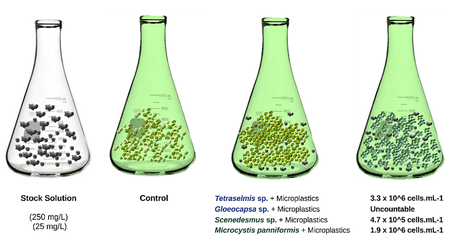
Graphical Abstract



Objectives

The objective of this work is to show the potential of microalgae as compelling biocompatible solutions to natural or urban water treatment, with regards to its exopolymer production and hetero-aggregation potential, in the presence of different ranging sizes, densities and concentration of microplastics (MP).

Experimental



Microplastics:

- Fluorescent **Blue** PS (<106 µm)
- Fluorescent **Yellow** PS (106-250 µm)
- Fluorescent **Purple** PMMA (<106 µm)
- Fluorescent **Green** PMMA (106-250 µm)

Hetero-aggregates

Fluorescence microscopy

Scanning electron microscope

Microalgae

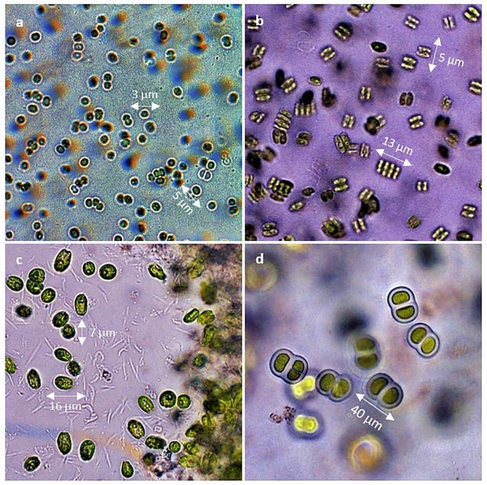


Figure 1- Bright field micrographs of *Microcystis panniformis* (x20) (**a**), *Scenedesmus* sp. (x40) (**b**), *Tetraselmis* sp. (x40) (**c**) and *Gloeocapsa* sp. (x40) (**d**).

- This study was performed with two marine microalgae: ***Gloeocapsa* sp.** *and* ***Tetraselmis* sp.** and two freshwater species: ***Scenedesmus* sp.** and ***Microcystis panniformis***.
- ***Scenedesmus* sp.** and ***Microcystis panniformis*** were obtained from the Spanish Algae Bank (BEA).
- ***Tetraselmis* sp.** *was* obtained from the collection of Mariculture Center of Calheta (Madeira).

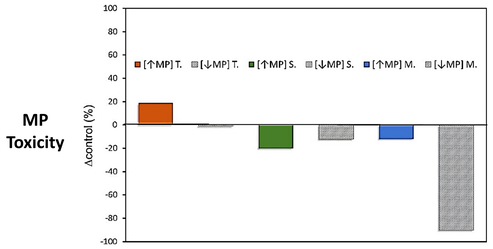


Figure 3 – MP effect on microalgal growth: T. (*Tetraselmis* sp.), S. (*Scenedesmus* sp.) and M. (*Microcystis panniformis*). These results represent the variation in cell density of the experimental groups, with microplastics, when compared with the control group.

Summary

Hetero-aggregates demonstrate to be an important pathway for the vertical transportation of microplastics, with the confirmation of its aggregation and deposition. The formed aggregates were composed by microalgae and exopolymers or microalgae, exopolymers and microplastics. The formed hetero-aggregation is dependent on the size, strength, viscosity and production of exopolymers, which is very specie specific.

Growth Curves

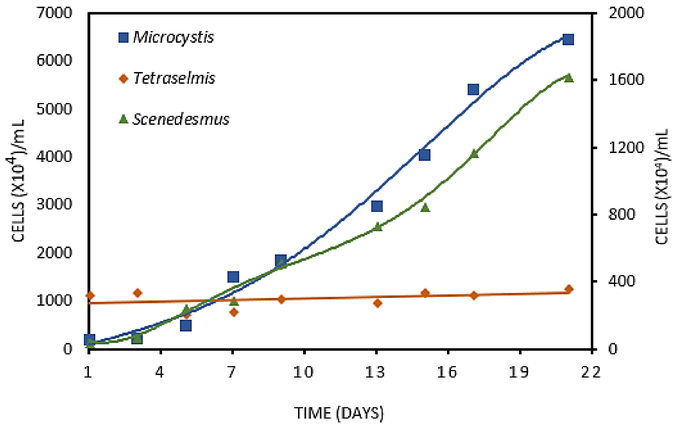


Figure 2- Growth curves for control condition of each countable microalgae. Initial concentration was 3.3 x 10^6 cells.mL^{-1} for *Tetraselmis* sp., 1.9 x 10^6 cells.mL^{-1} for *Microcystis panniformis* and 4.7 x 10^5 cells.mL^{-1} for *Scenedesmus* sp..

Culture Observations

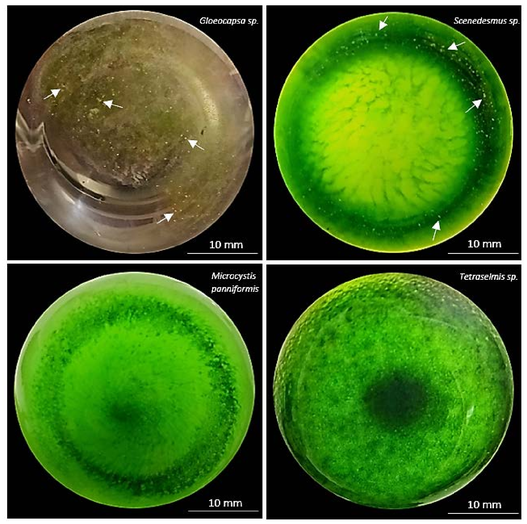


Figure 4- Photographs of each microalgae + high bead concentration condition culture, as observed from below. Each culture was photographed at the end of the 3-week long experiment, using a digital camera.

Conclusions

- *Microcystis panniformis* and *Scenedesmus* sp. exhibited a considerable amount of exopolymer production, but with lesser capabilities to aggregate microplastics.
- *Tetraselmis* sp. displays an excellent ability to aggregate both low and high-density beads, being only semi-limited by the size of the MP.
- *Gloeocapsa* sp. is a novel microalgae with outstanding exopolymer production and microplastic aggregation capabilities.

Fluorescence microscopy

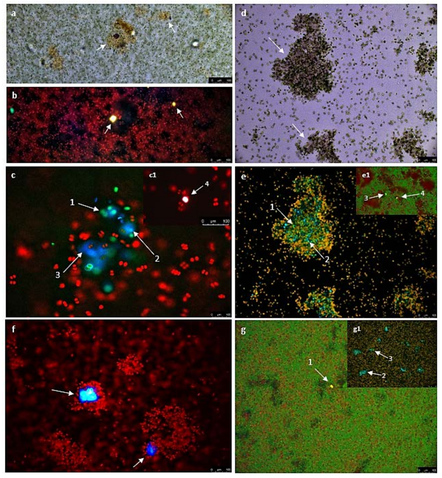


Figure 5- **a:** Bright field micrograph of *Microcystis panniformis* (high bead concentration) hetero-aggregates (x40). **b:** Same micrograph seen in **a**, but under DAPI filters (Excitation 340/80 nm, Emission 425 nm), being visible the incorporation of the fluorescent beads in the hetero-aggregates. **c/c1:** Micrographs of beads trapped in *Gloeocapsa's sp.* (low bead concentration) thick exopolymers, under DAPI filters (x20). **d:** Bright field micrograph of *Tetraselmis sp.* (low bead concentration) considerable sized hetero-aggregates (x10). **e:** Same micrograph seen in **d**, but under DAPI filters, showing hetero-aggregates composed of microalgae (orange), exopolymer (green) and beads (blue) (x10). **e1**: Micrograph of Tetraselmis' sp. hetero-aggregates (high bead concentration), under I3 filters (Excitation 450/90 nm, Emission 515 nm). **f:** Micrograph of *Tetraselmis sp.* (high bead concentration) showing colonization of beads by the microalgae, under DAPI filters. **g:** Micrograph of *Scenedesmus sp.* (high bead concentration), under I3 filters, showing the abundance of exopolymers and the aggregation of the beads (x10). **g1**: Micrograph of *Scenedesmus sp*. (low bead concentration) under DAPI filters, exhibiting aggregation of different beads.

SEM

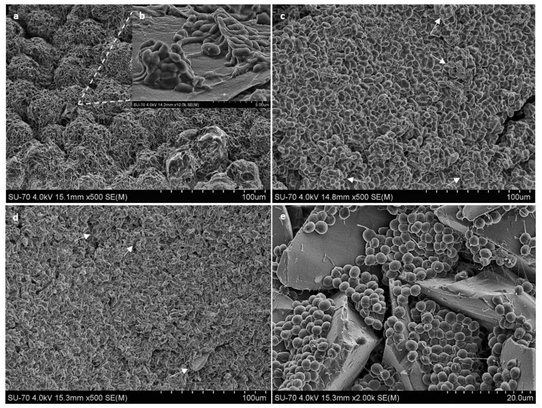


Figure 6- Scanning electron microscopy (SEM) images of microalgae hetero-aggregates. **a:** SEM image of *Gloeocapsa sp.* exopolymeric fibrous mesh (x500). **b:** Amplified SEM image of a bead trapped on the surface of the exopolymeric substances, in image **a**, showing entanglement and colonization of the beads (x10000). **c:** SEM image of *Tetraselmis sp.* cells forming aggregates (arrows) (x500). **d:** SEM image of *Scenedesmus sp.* cells, exopolymers and beads trapped in hetero-aggregates (arrows) (x500). **e:** SEM image of beads colonized by *Microcystis panniformis* cells (x2000).

Acknowledgments

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