Plastics in Focus: The Influence of Polystyrene on The Fitness of Freshwater Algae

Komazec Bruno[1*], Balen Biljana[1], Dimitrov Nino[2], Peharec Štefanić Petra[1]

[1]Division of Molecular Biology, Department of Biology, Faculty of Science, University of Zagreb, Horvatovac 102a, 10000 Zagreb, Croatia
[2]Croatian Institute for Public Health, Rockefellerova 7, 10000 Zagreb, Croatia

*bruno.komazec@biol.pmf.unizg.hr

INTRODUCTION

Polystyrene particles are used in various industries due to their unique physicochemical properties, including increased elasticity and heat resistance. Considering the increasing production, it is necessary to study their impact on the natural ecosystem, processes of biodistribution of substances from the environment and accumulation in food chains, as they are inevitably released into various habitats. Since *Chlorella vulgaris* is one of the most abundant microalgae in aquatic ecosystems, it is often used as a model organism to assess the impact of materials of anthropogenic origin, such as polystyrene particles, on aquatic habitats.

MATERIALS & METHODS

To investigate the effects of PS particles on the freshwater algae *Chlorella vulgaris*, algae cultures were grown in a liquid BBM nutrient medium for four days and then treated with 40 mg L^{-1} PS, which is considered the upper limit for human exposure to styrene monomers. The accumulation of PS particles in the cells and on the EPS layer around the cells was measured by pyrolysis-gas chromatography coupled with mass spectrometry (Py-GC-MS), while visualization of the cells under native conditions and analysis of the cell ultrastructure was performed by TEM. To determine the fitness of the algae after treatment with PS particles, the level of reactive oxygen species (ROS), the level of lipid peroxidation and protein damage were analysed, while the antioxidant potential was investigated by measuring the activity of antioxidant enzymes.

RESULTS

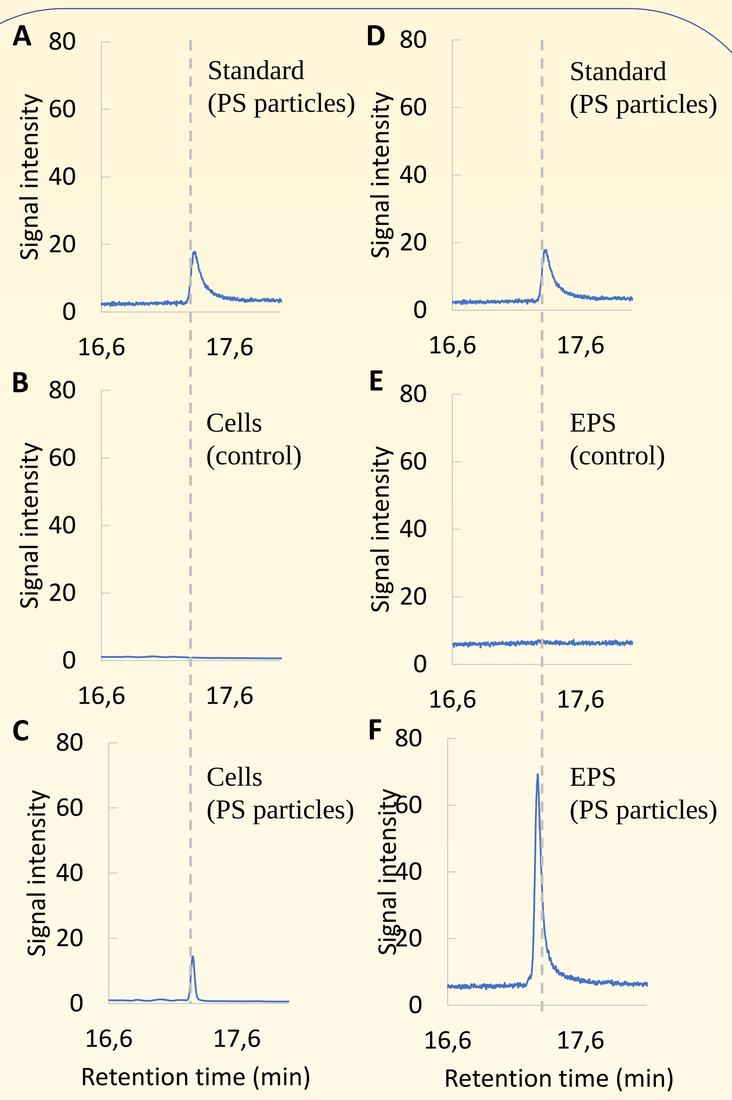

Figure 1. Ion chromatograms of polystyrene trimers in PS stock suspensions (A, D), in control cells (B) and the EPS layer (E) of *C. vulgaris* algae and in cells and the EPS layer of the algae after 72 h exposure to 40 mg L^{-1} PS (C, F), obtained by Py-GC-MS.

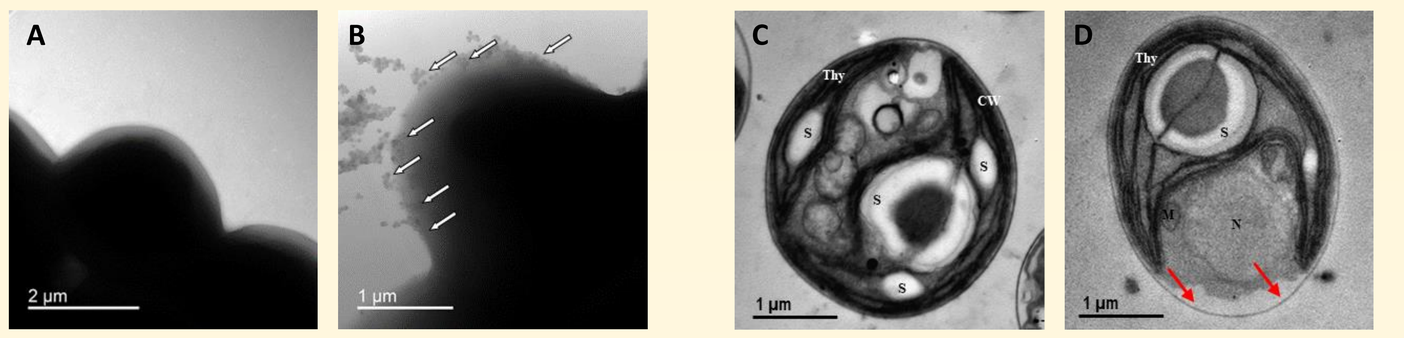

Figure 2. Microphotographs of *C. vulgaris* under native conditions (A, B) and ultrastructure (C, D) taken with a transmission electron microscope (TEM) . Control algae cell (A, C) and algae cells after 72h of exposure to 40 mg L^{-1} PS particles (B, D,. White arrows indicate probable PS particles; red arrows indicate changes in the cell wall and membrane. Thy – thylakoids; S – starch; CW – cell wall.

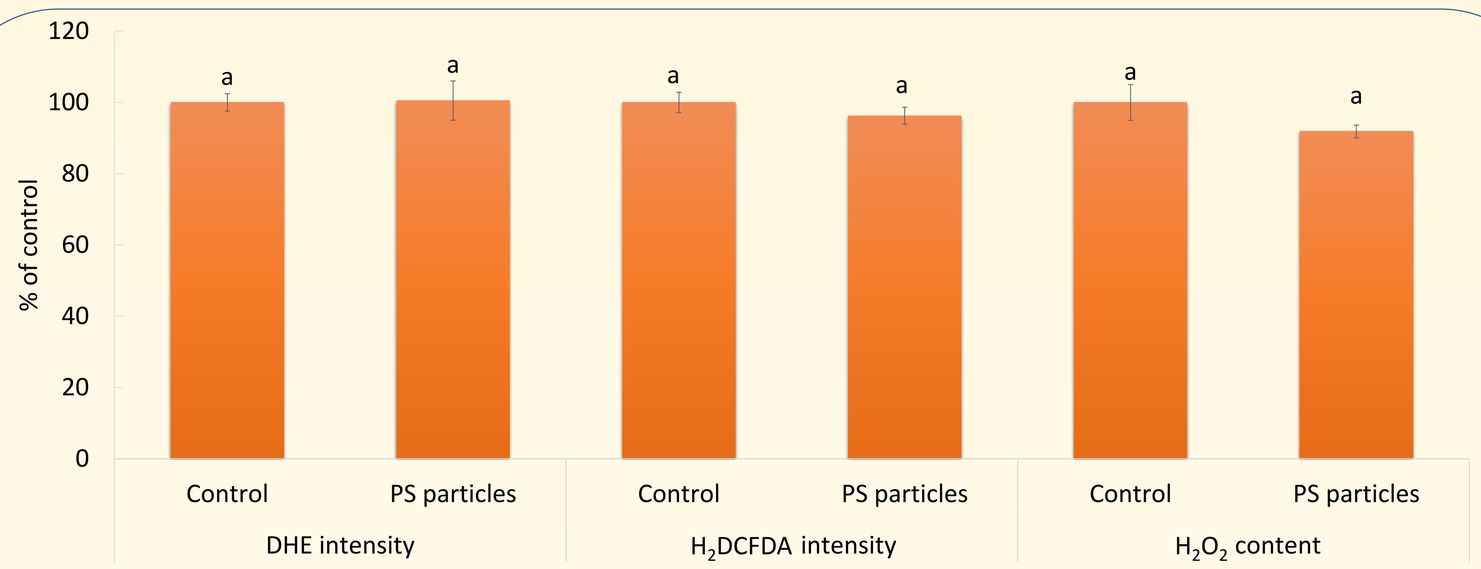

Figure 3. The content of reactive oxygen species (ROS) determined *in situ* using fluorescent probes dihydroethidium (DHE) and 2,7 dichlorodihydrofluorescein diacetate (H_2DCFDA) and the content of hydrogen peroxide (H_2O_2) measured in extracts of *C. vulgaris* control cells and cells after 72 hours of exposure to 40 mg L^{-1} PS particles.

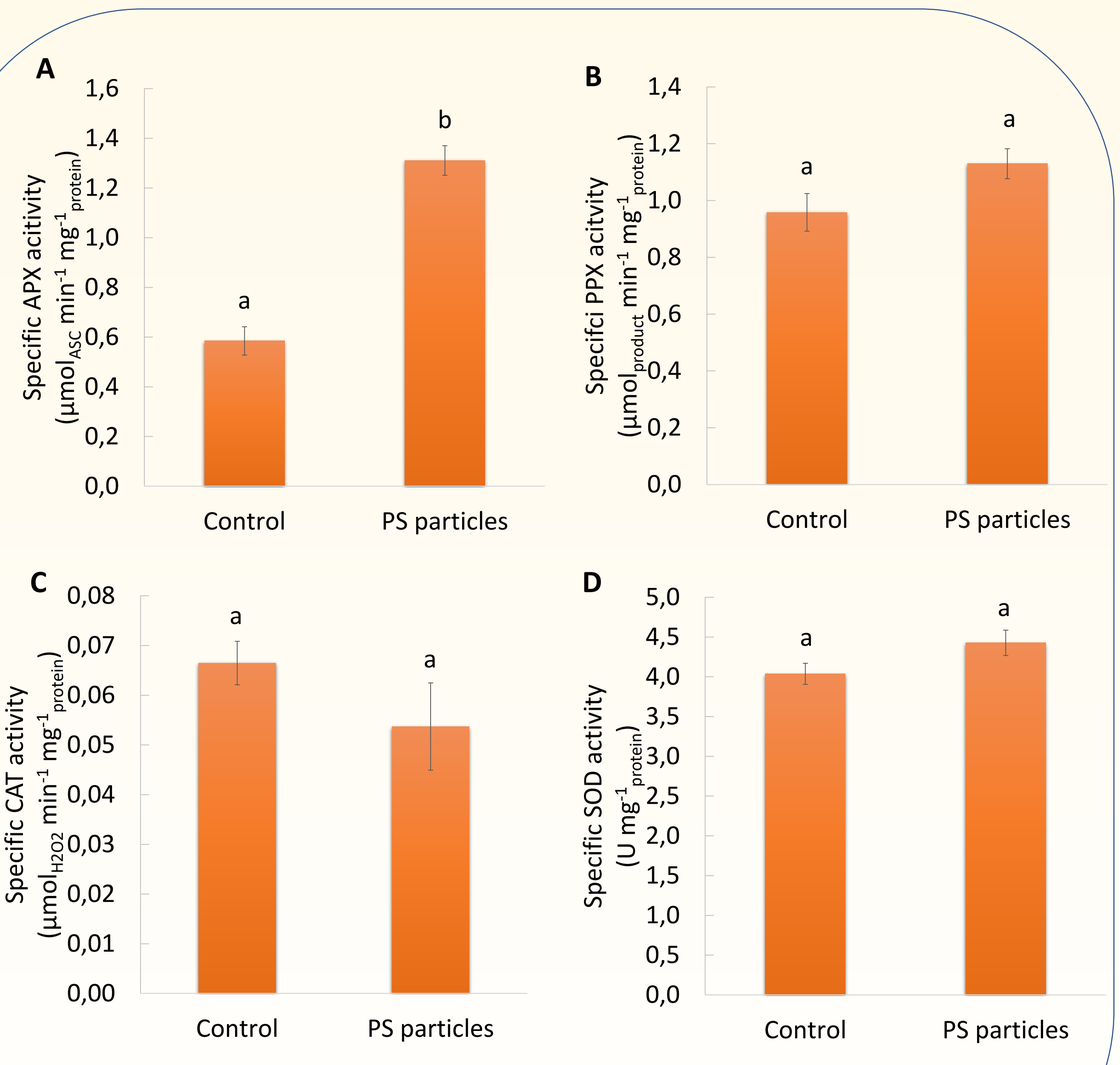

Figure 4. Activity of the antioxidant enzyme ascorbate peroxidase (APX) (A), pyrogallol peroxidase (PPX) (B), catalase (CAT) (C) and superoxide dismutase (SOD) (D) in *C. vulgaris* control cells and algal cells after 72 hours exposure to 40 mg L^{-1} PS particles.

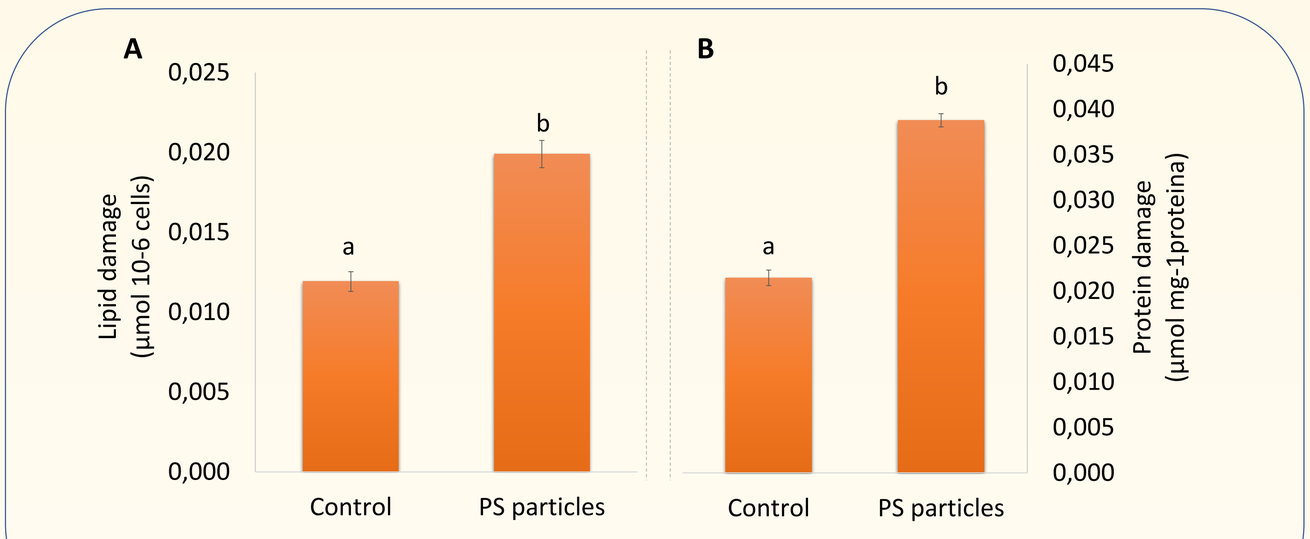

Figure 5. Content of malondialdehyde (MDA) (A) and protein carbonyls (B) in *C. vulgaris* control cells and algal cells after 72 hours of exposure to 40 mg L^{-1} PS particles.

CONCLUSIONS

- TEM analysis showed that PS particles are mostly bound to the EPS layer around the algal cells, although internalization of PS in the cells was also observed.
- No increase in ROS content was observed after treatment with PS particles, which is consistent with an increased activity of antioxidant enzymes, especially peroxidases.
- PS particles caused significant lipid peroxidation, which is consistent with the observed ultrastructural damage, manifested mainly by plasmolysis.
- PS particles caused significant carbonylation of proteins, probably due to protein absorption at the surface of the particles.

ACKNOWLEDGMENT

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