



Short communication

Do plastic particles affect microalgal photosynthesis and growth?



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ABSTRACT

The unbridled increase in plastic pollution of the world's oceans raises concerns about potential effects these materials may have on microalgae, which are primary producers at the basis of the food chain and a major global source of oxygen. Our current understanding about the potential modes and mechanisms of toxic action that plastic particles exert on microalgae is extremely limited. How effects might vary with particle size and the physico-chemical properties of the specific plastic material in question are equally unelucidated, but may hold clues to how toxicity, if observed, is exerted. In this study we selected polystyrene particles, both negatively charged and uncharged, and three different sizes (0.05, 0.5 and 6 μm) for testing the effects of size and material properties. Microalgae were exposed to different polystyrene particle sizes and surface charges for 72 h. Effects on microalgal photosynthesis and growth were determined by pulse amplitude modulation fluorometry and flow cytometry, respectively. None of the treatments tested in these experiments had an effect on microalgal photosynthesis. Microalgal growth was negatively affected (up to 45%) by uncharged polystyrene particles, but only at high concentrations (250 mg/L). Additionally, these adverse effects were demonstrated to increase with decreasing particle size.

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Plastic particles of different types and sizes have been detected in seawater worldwide and adverse effects on several aquatic animals have been described (Wright et al., 2013; Della Torre et al., 2014; Ivar do Sul and Costa, 2014). One of the plastics commonly detected in the sea is polystyrene (PS), a high production volume material with a global market valued at over 30 billion USD (2013 data for PS and expanded PS; Transparency Market Research, 2014). Because marine microalgae are primary producers at the basis of the food chain (Kaiser et al., 2011), there is concern about the potential adverse effects of nano- and micro-sized plastic particles. It has already been demonstrated that charged nanoplastics (0.02 μm) can sorb to microalgae, inhibiting microalgal photosynthesis (Bhattacharya et al., 2010). However, the effects of uncharged and larger microplastics on microalgae have not been studied to date. The aim of this study was therefore to determine the effect

of plastic particles of different sizes (0.05, 0.5 and 6.0 μm) and surface charges (negatively and uncharged) on microalgal photosynthesis and growth using three species. It is likely that interaction between microplastics and microalgae may vary with cell characteristics like size and shape. Additionally, algal cell walls act as barriers to particle penetration and cell wall characteristics may consequently influence particle sorption. Therefore we selected the marine diatom *Thalassiosira pseudonana* with a silicate cell wall and the marine flagellate *Dunaliella tertiolecta* without a cell wall. To facilitate the comparison with the microalgal study performed by Bhattacharya et al. (2010), we also selected the freshwater green microalgae (*Chorella vulgaris*) with a polysaccharidic cell wall. As the typical algal cell wall pore size is too small (<20 nm) to transport a single (or several aggregated) PS particles through the cell wall, we hypothesised that shading (reduced access to light) would be a likely mechanism by which photosynthesis and thereby microalgal growth can be affected.

Microalgae (*D. tertiolecta*) were exposed to three different sizes of uncharged PS beads (0.05, 0.5 and 6 μm) and effects on their photosynthesis and growth were determined after 72 h (Table 1). Next, negatively charged carboxylated PS microbeads (0.5 μm) were tested with *D. tertiolecta* and the test was repeated with *C.*

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Table 1

Experimental setup including the polystyrene type, polystyrene size, test species, toxicity endpoint and test concentrations for both experiments.

Polystyrene type	Polystyrene size (μm)	Test species	Toxic endpoint	Test concentration(mg/L)
Uncharged	0.05	<i>Dunaliella tertiolecta</i>	Photosynthesis, growth	25, 250
	0.5	<i>Dunaliella tertiolecta</i>	Photosynthesis, growth	25, 250
	6	<i>Dunaliella tertiolecta</i>	Photosynthesis, growth	25, 250
Negatively charged	0.5	<i>Dunaliella tertiolecta</i>	Photosynthesis, growth	25, 250
	0.5	<i>Thalassiosira pseudonana</i>	Photosynthesis	25, 250
	0.5	<i>Chlorella vulgaris</i>	Photosynthesis	25, 250

vulgaris and *T. pseudonana* (Table 1). Commercially available virgin PS beads in three different sizes (Polybead® microspheres 0.05, 0.5 and 6 μm) and both uncharged and negatively charged carboxylate (0.5 μm) were obtained from PolySciences Europe GmbH (Eppenheim, Germany). Dilutions of the original stock solution in Milli-Q® ultrapure water resulted in two test concentrations: 250 mg PS/L and 25 mg PS/L. The two PS concentrations resulted in differences in the number of particles/L for the three different particle sizes: 3.64×10^{12} and 3.64×10^{11} ; 3.64×10^9 and 3.64×10^8 ; 2.1×10^6 and 2.1×10^5 particles/L for 0.05, 0.5 and 6 μm respectively. The marine flagellate *D. tertiolecta* (Butcher, CCAP 19/27) and the marine diatom *T. pseudonana* (Hasle & Heimdal, CCMP 1335) were both cultured on f/2 medium (Sigma–Aldrich Chemie B.V., Zwijndrecht, the Netherlands), while the freshwater green microalga *C. vulgaris* (Beyerinck, UTEX 259) was cultured on BG11 medium (Sigma–Aldrich Chemie B.V., Zwijndrecht, the Netherlands). All species were cultured with a light–dark regime of 16:8 h at 16 °C at a light intensity of $50 \mu\text{mol m}^{-2} \text{s}^{-1}$ (F58W/BriteGro2084, Havells Sylvania, Raunheim, Germany). All tests were performed under identical temperature, light intensity and light regime conditions with exponentially growing cultures with a start density (1×10^5 – 1×10^6 cell/mL) allowing for exponential growth during the experiment. Tests were performed in triplicate in 50 mL glass vials with 5 mL of the microalgal suspension and the selected plastic bead treatment. Test vials were gently mixed on a shaker achieving a homogenous microbead distribution without disturbance of the microalgal suspension. Adequate homogenization was confirmed previously using visible fluorescent microbeads in the same size range. The effect on microalgal photosynthesis was determined on fresh samples after 72 h of exposure, using Pulse Amplitude Modulation (PAM) fluorometry as described by Sjollem et al. (2014). The effects of microbeads on microalgal growth were quantified by automated cell counts using a flow cytometer (BD Accuri™C6, BD Biosciences). After 72 h of exposure, three replicates per treatment were transferred to 2 mL Eppendorf tubes and fixed by adding 125 μL of a formaldehyde (18% v/v)–hexamine (10% w/v) solution to 1 mL of sample. These fixed samples were quick-frozen using liquid nitrogen and stored at -80°C until further analysis. The cell density (cells/mL) of all three replicates was determined in six-fold within a 2-week period. Effects on microalgal growth as well as photosynthesis were expressed as a percentage of the corresponding control (% control) without PS beads. The effect of the microbeads on the light availability was determined in a pilot study using a quantum sensor (LI-COR).

Negligible effects (<10% inhibition compared to control) on the photosynthetic efficiency (ΦPSII) of *D. tertiolecta* were observed upon exposure to any of the three sizes of PS bead. This indicates that even at the highest concentration of 250 mg PS/L, none of these PS beads affected microalgal photosynthesis, even though the light intensity was reduced up to 34%. However, the experiments were performed under optimal light conditions, while light limiting conditions will occur in the field. Consequently, the effect of plastic particles on microalgae photosynthesis may be different in the field. The absence of any effect on microalgal photosynthesis contrasts with previously reported effects on microalgal photosynthesis of 0.02 μm PS beads as described by Bhattacharya et al. (2010). Since

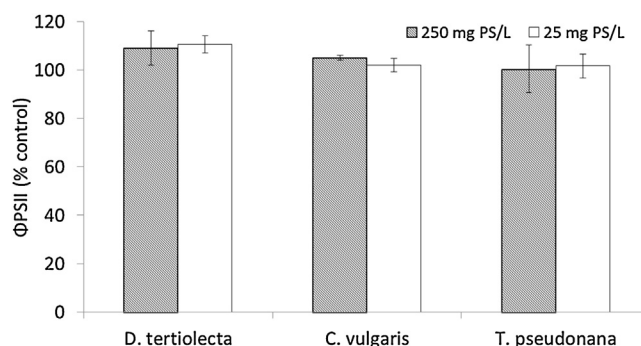


Fig. 1. Effects of 0.5 μm charged (carboxylated) polystyrene beads on photosynthesis of three microalgal species. Effects on ΦPSII of *D. tertiolecta*, *C. vulgaris*, *T. pseudonana* were determined after 72 h of exposure and expressed as percentage of control (% control). Error bars represent standard deviation. $n = 3$.

this discrepancy might have been caused by the type of plastic they used (negatively and positively charged), we additionally tested charged carboxylated beads for the middle size PS beads (0.5 μm). However, the effects of these negatively charged beads on the photosynthetic efficiency (ΦPSII) of *D. tertiolecta* also appeared to be absent (Fig. 1). Furthermore, negligible effects (<10%) of the negatively charged 0.5 μm beads on ΦPSII were observed on the other test species: *C. vulgaris* and *T. pseudonana* (Fig. 1). Our results thus suggest that the absence of an effect is not specific to *D. tertiolecta* and that these larger charged beads indeed do not affect microalgal photosynthesis under the present laboratory conditions.

In contrast to photosynthesis, a clear effect of the uncharged PS beads on the growth of *D. tertiolecta* was observed. At the highest PS concentration of 250 mg/L, the average cell density of the *D. tertiolecta* exposed to 0.05 μm PS beads was clearly reduced (45%) compared to the unexposed control (Fig. 2). Relative to controls, the algal growth rate was inhibited by 57%. Recently, Besseling et al. (2014) demonstrated that PS particles of a similar size (0.07 μm) inhibited the growth of the microalgae *Scenedesmus obliquus* by 2.5%, but similar to the present study only at very high concentrations (1 g/L). Additionally, in our study we observed a small reduction (11%) in *D. tertiolecta* growth caused by uncharged 0.5 μm PS beads, and inhibition of the algal growth rate (13%). The effect of carboxylated 0.5 μm PS beads was similar compared to the uncharged 0.5 μm PS beads (Fig. 2). With the largest PS beads (6 μm), as well as the lower PS concentration (25 mg/L), effects were <10%. These data indicate that the effect on microalgal growth increases with decreasing bead size. It must be noted that the bead concentration in the present study is based on nominal concentrations. Flow cytometric analysis of the number of particles revealed that at the highest test concentration (250 mg PS/L), the average actual concentration was up to 9 times lower than the nominal concentration. Due to technical specifications for the flow cytometer, the actual concentration could only be determined for the largest (6 μm) PS beads.

In conclusion, we observed effects of PS particles on microalgae for the smallest particles tested (0.05 μm), but only at high PS concentrations. Considering that techniques are lacking for

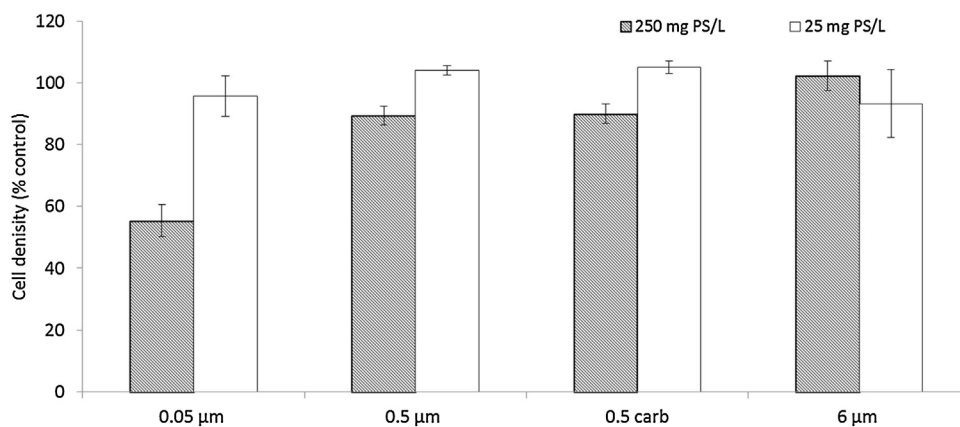


Fig. 2. Effects of uncharged polystyrene beads on *D. tertiolecta* growth. Effects on cell density after 72 h of exposure to three sizes (0.05, 0.5 and 6 µm) of uncharged and carboxylated (carb.) PS were determined and expressed as percentage of control (% control). Error bars represent standard deviation. $n = 3$.

robust measurement of field concentrations of plastic particles at this lower end of the size spectrum, shown here to be toxic to microalgae at high concentrations, method development in this area is urgently needed for research, monitoring and risk assessment purposes. Quantities of microplastics in the marine environment are likely to increase in the future as a result of continuing degradation and fragmentation (GESAMP, 2015) and of direct discharge. Hence, a full risk assessment of plastic particles with different physical–chemical properties in both the micro and nano-size ranges for microalgae, considering their fundamental role in ecosystem functioning, is required.

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References

Bhattacharya, P., Lin, S., Turner, J.P., Ke, P.C., 2010. Physical adsorption of charged plastic nanoparticles affects algal photosynthesis. *J. Phys. Chem. C* 114, 16556–16561, <http://dx.doi.org/10.1021/jp1054759>.

- Besseling, E., Wang, B., Lurling, M., Koelmans, A.A., 2014. Nanoplastic affects growth of *S. obliquus* and reproduction of *D. magna*. *Environ. Sci. Technol.* 48 (20), 12336–12343.
- Della Torre, C., Bergami, E., Salvati, A., Faleri, C., Cirino, P., Dawson, K.A., Corsi, I., 2014. Accumulation and embryotoxicity of polystyrene nanoparticles at early stage of development of sea urchin embryos *Paracentrotus lividus*. *Environ. Sci. Technol.* 48 (20), 12302–12311.
- GESAMP, 2015. Sources, fate and effects of microplastics in the marine environment: a global assessment. In: Kershaw, P.J. (Ed.), Rep. Stud. GESAMP No. 90. IMO/FAO/UNESCO-IOC/UNIDO/WMO/IAEA/UN/UNEP/UNDP Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection, 96 p.
- Ivar do Sul, J.A., Costa, M.F., 2014. The present and future of microplastic pollution in the marine environment. *Environ. Pollut.* 185, 352–364, <http://dx.doi.org/10.1016/j.envpol.2013.10.036>.
- Kaiser, M.J., Attrill, M.J., Jennings, S., Thomas, D.N., Barnes, D.K.A., Brierley, A.S., Hiddink, J.G., Kaartokallio, H., Polunin, N.V.C., Rafaelli, D.G., 2011. *Marine Ecology: Processes, Systems, and Impacts*. Oxford University Press, Oxford, UK.
- Sjollema, S.B., MartínezGarcía, G., van der Geest, H.G., Kraak, M.H.S., Booij, P., Vethaak, A.D., Admiraal, W., 2014. Hazard and risk of herbicides for marine microalgae. *Environ. Pollut.* 187, 106–111, <http://dx.doi.org/10.1016/j.envpol.2013.12.019>.
- Transparency Market Research, Polystyrene & Expandable Polystyrene (EPS) Market—Global Industry Analysis, Size, Share, Growth, Trends and Forecast, 2014–2020 (2014) <http://www.transparencymarketresearch.com/polystyrene-eps-market.html>.
- Wright, S.L., Thompson, R.C., Galloway, T.S., 2013. The physical impacts of microplastics on marine organisms: a review. *Environ. Pollut.* 178, 483–492, <http://dx.doi.org/10.1016/j.envpol.2013.02.031>.