



# The toxicity of plastic nanoparticles to green algae as influenced by surface modification, medium hardness and cellular adsorption



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## ABSTRACT

To investigate processes possibly underlying accumulation and ecological effects of plastic nano-particles we have characterized their interaction with the cell wall of green algae. More specifically, we have investigated the influence of particle surface functionality and water hardness ( $\text{Ca}^{2+}$  concentration) on particle adsorption to algae cell walls. Polystyrene nanoparticles with different functional groups (non-functionalized,  $-\text{COOH}$  and  $-\text{NH}_2$ ) as well as coated (starch and PEG) gold nanoparticles were applied in these studies. Depletion measurements and atomic force microscopy (AFM) showed that adsorption of neutral and positively charged plastic nanoparticles onto the cell wall of *P. subcapitata* was stronger than that of negatively charged plastic particles. Results indicated that binding affinity is a function of both inter-particle and particle-cell wall interactions which are in turn influenced by the medium hardness and particle concentration. Physicochemical modelling using DLVO theory was used to interpret the experimental data, using also values for interfacial surface free energies. Our study shows that material properties and medium conditions play a crucial role in the rate and state of nanoparticle bio-adsorption for green algae. The results show that the toxicity of nanoparticles can be better described and assessed by using appropriate dose metrics including material properties, complexation/agglomeration behavior and cellular attachment and adsorption. The applied methodology provides an efficient and feasible approach for evaluating potential accumulation and hazardous effects of nanoparticles to algae caused by particle interactions with the algae cell walls.

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## 1. Introduction

Nanoparticles are being used in an increasing number of applications and consumer products (Mikkelsen et al., 2015). Nanoplastics, loosely defined here as particles in the size range of 10–100 nm and with plastic-polymer cores and surface functionalization (Koelmans et al., 2015; Browne et al., 2007; EU Commission, 2011) have large use potential due to their highly versatile chemistry. They find use in applications such as material strengtheners, UV- and heat-resistant materials, antimicrobial agents, drug delivery

vehicles and doping agents (Aitken et al., 2006; Salata, 2004; Daniel and Astruc, 2004). With the number of polymer applications growing each year and the largest end use for minerals being polymer applications, it is estimated that by 2020 nanoplastics will account for the largest share of nanomaterials on the market (Manitiu et al., 2009; Fabra et al., 2013). At the same time, there is an increasing awareness of plastic pollution as an environmental problem. In general, plastic pollution stems from both the intentional production and use of small plastic particles (micron- and nanosized plastic particles) in various consumer products (Leslie, 2012) as well as unintentional release of macroplastic materials to the environment. From the degradation of brittle, weathered plastic products in the aquatic environment, micro- and nanoplastics enter marine and freshwater habitats (Lambert et al., 2013; Lambert and Wagner, 2016). The increase in surface area from macro to micro- and nano-

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sized particles upon fragmentation of plastics alters their potential for interaction with and uptake by aquatic organisms (Cooper, 2012).

Photoautotrophs, like algae and cyanobacteria, are vital to aquatic ecosystems as they are at the base of the aquatic food webs. Previously, exposure to nanoparticles has been observed to inhibit photosynthesis by algae and initiate the production of reactive oxygen species (ROS) (von Moos and Slaveykova, 2014; Ivask et al., 2014). Moreover, toxicity has been found to be a function of surface chemistry, possibly related to differences in effectiveness of physical adsorption onto the cell wall exterior (Bhattacharya et al., 2010). However, standard approaches to concentration–response testing developed for dissolved chemicals are challenged for the testing of nanoparticles in particular, as concentration is only one of multiple parameters needed to determine levels of biological effect (Baun et al., 2009). Toxic effects resulting from nanoparticle exposure have previously been successfully expressed as function of effective surface area of nanoparticles in the suspension (Van Hoecke et al., 2011). Also, the state and rate of agglomeration varies as a function of concentration and the chemical equilibrium state of functional groups, and thus include interdependent variables. The surface functionality of nanoparticles in general and nanoplastics specifically is highly versatile. At the same time, they are prone to undergo bio-functionalization, i.e. conjugation and modification by bacteria, other microorganisms and natural organic matter as well as decomposition by photo-oxidation, rendering them with different charge polarities. Due to the large variety of nanoplastics likely released in the aquatic environment (Koelmans et al., 2015; Lambert et al., 2013; Lambert and Wagner, 2016), together with the natural variability in aqueous chemistry and algal bio-productivity in marine and freshwater systems, the establishment of a coherent approach to evaluate their potential detrimental effects to green algae remains difficult.

For plants, including algae, the complex cellulose-rich matrix of the cell walls is the first barrier for uptake encountered by any substance including nanoparticles (Khowala et al., 2008). Even if plastic nanoparticles are not entering the algal cells (likely if their size is larger than the cell wall pore size (Navarro et al., 2008a; Zemke-White et al., 2000)) but merely attach to the surface, ecological effects may occur higher up in the food web with algae-particle interaction as the first step in the biomagnification. It seems plausible that accumulation and toxic effects can be linked to colloidal forces in the particle-algae system and nanoparticle properties. It is therefore of crucial importance to document and understand the particle-cell wall interaction.

In this study, we investigated the influence of nanoparticle surface functionalization on their physico-chemical interaction with green algae *Pseudokirchneriella subcapitata*. Polystyrene (PS) nanoparticles with different functional groups (non-functionalized, –COOH and –NH<sub>2</sub>), as well as Au nanoparticles with hydrophilic starch and PEG coatings have been applied. We determined the effect of medium hardness (Ca<sup>2+</sup> concentration) on nanoparticle surface charge density and agglomeration. Furthermore, the functionalized PS particles were used in adsorption studies: with these particles it is possible to quantify adsorption by measuring nanoparticle depletion from the solution using UV/Vis spectroscopy. AFM imaging was used to visualize the adsorption of PS particles on the algal cell. Since only for the PS-COOH particles the physicochemical properties were significantly affected by Ca<sup>2+</sup>, we used these particles in algal growth inhibition tests as a function of medium hardness during incubation. This approach was taken to be able to distinguish between the relative influences of agglomeration and adsorption on the algal growth. To quantify particle-cell interaction forces and relate to the levels of adsorption of PS-COOH, we measured force interaction curves using colloidal probe AFM (CP-AFM). The combination of methods chosen allowed

for detailed evaluation of the potential modes of algal toxicities by plastic nanoparticles.

## 2. Materials and methods

### 2.1. Nanoplastics

Carboxylate-modified polystyrene particles (average hydrodynamic diameter  $d_H = 110$  nm) were purchased from Bangs Laboratories Inc. (indicated by the vendor as P(S)/V-COOH)) whereas amidine-modified polystyrene particles ( $d_H = 20$  nm) were purchased from Molecular Probes. Polystyrene particles ( $50 < d < 500$  nm) were synthesized by a simple polymerization reaction in ethanol using the radical initiator ammonium persulfate. After completion, the reaction medium was removed using rotary evaporation after which a stock suspension was made using acetone. This mixture of particle sizes was considered appropriate because, in relative order of magnitude, half of the size range falls in the nanoparticle definition range ( $10 < d < 100$  nm). The synthesis of hydrophilic 'bioplastic' starch-Au and PEG-Au particles is described in the Supplementary Information (SI). Sizes as well as stock concentrations of all suspensions are given in Table 1.

### 2.2. Particle characterization

All particles (except for the non-functionalized PS particles) were characterized by hydrodynamic size and electrokinetic potential in de-ionized water (unmodified pH) and relevant media (see Media preparation) using a Zetasizer Nano ZS. Dynamic light scattering (DLS) and electrokinetic measurements were performed for concentrations of 10 mg/L in de-ionized water and freshwater algal medium. UV/Vis absorption spectra were obtained using a Cary Bio50 UV/vis spectrophotometer. Calibration curves for UV/Vis absorption were constructed for varying levels of hardness (added as Ca<sup>2+</sup>) according to Beer's law assuming both absorption and scattering intensity are proportional to concentration. The particles were further described by their surface energy components in water obtained from literature. Lifshitz-van der Waals surface energies of all the polymers are approximately equal and unaffected by electrolyte concentrations (Van Oss, 2003; Hamley, 2007). In contrast, multivalent cations/anions can significantly alter the polar surface tension components (acid/base,  $\gamma^+/\gamma^-$ , surface energies in Table 1). In our study, the Ca<sup>2+</sup> ions neutralize the electron-donor monopolar energy ( $\gamma^-$ ) of the PS-COOH particles. Using this information, in combination with the electrokinetic measurements, it is possible to derive values for the polar surface components as function of complexation state (SI).

### 2.3. Medium preparation

Algal test media were prepared according to ISO guidelines (ISO, 2012). The ionic strength of the unmodified freshwater media was 2.17 mM, while the total concentration of Ca<sup>2+</sup> and Mg<sup>2+</sup> cations (water hardness) was 0.20 mM. All experiments were carried out in freshwater medium which was adjusted by appropriate additions of 1 M NaOH and HCl to pH 7.4 unless mentioned otherwise. It was verified by calculation that the addition of small amounts of NaOH and HCl for pH adjustments did not significantly alter the ionic strength of the medium. For experiments performed under varying levels of water hardness, the electrolyte concentrations of the media was varied using CaCl<sub>2</sub>.

### 2.4. Behavior of nanoplastics in test media

Inter-particle forces were evaluated by measuring the agglomeration kinetics of the particles in media with varying levels of

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