
Assessment of Graphene toxicity on a benthic freshwater diatom *Nitzschia palea* Interaction of carbon nanoparticles with algal biofilm

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Abstract

The use of carbon-based nanoparticles such as Few Layer Graphene (FLG) is processing in several fields such as materials engineering, owing to their nanoscale and physicochemical properties. Carbon-based nanoparticles are thus likely to be found in the environment and especially in rivers. In this study, the toxicity of FLG on the freshwater benthic diatom *Nitzschia palea* was assessed. The experimental design used allowed to distinguish the shading effect from the total effect of FLG exposure on *N. palea*, where diatoms are directly exposed to nanoparticles. To assess the effect of FLG on diatoms, a range of concentrations was tested (0.1, 1, 10 and 50 mg.L⁻¹) and several toxicity end-points were evaluated, such as the growth rate, mortality and photosynthetic activity. A growth rate inhibition of diatoms culture exposed to 50 mg.L⁻¹ of FLG was observed 48 h after the beginning of the exposure, revealing a significant effect in both conditions tested (Total and Shading test). In total exposure conditions, the growth inhibition was associated with an increase in diatoms mortality (cell viability) and photosynthesis alteration. For total exposure condition, after 144 h, the toxicity disappeared and the growth rate was recovered, suggesting a decline in diatoms mortality. Microscopy analysis showed a strong interaction between FLG and exo-polymeric substances (EPS) produced by *N. palea*. In addition, optical density measurements of the culture medium in presence or absence of diatoms allowed to demonstrate that EPS secretion contributed to clarify the water column and reduced the physical interaction between FLG and diatoms. Overall, these results suggest that graphene toxicity could be a combination of direct and shading effect, in relation with a strong interaction between biofilm and nanoparticles. These results also highlight the potential mechanisms of clarification of the water column by trapping and sticking FLG at the bottom of wells even at high concentrations.

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