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Long-term toxicity of surface-charged polystyrene nanoplastics to marine planktonic species *Dunaliella tertiolecta* and *Artemia franciscana*

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ABSTRACT

Plastic pollution has been globally recognized as a critical issue for marine ecosystems and nanoplastics constitute one of the last unexplored areas to understand the magnitude of this threat. However, current difficulties in sampling and identifying nano-sized debris make hard to assess their occurrence in marine environment. Polystyrene nanoparticles (PS NPs) are largely used as nanoplastics in ecotoxicological studies and although acute exposures have been already investigated, long-term toxicity on marine organisms is unknown. Our study aims at evaluating the effects of 40 nm PS anionic carboxylated (PS-COOH) and 50 nm cationic amino-modified (PS-NH_2) NPs in two planktonic species, the green microalga *Dunaliella tertiolecta* and the brine shrimp *Artemia franciscana*, respectively prey and predator. PS NP behaviour in exposure media was determined through DLS, while their toxicity to microalgae and brine shrimps evaluated through 72 h growth inhibition test and 14 d long-term toxicity test respectively. Moreover, the expression of target genes (i.e. *clap* and *cstb*), having a role in brine shrimp larval growth and molting, was measured in 48 h brine shrimp larvae. A different behaviour of the two PS NPs in exposure media as well as diverse toxicity to the two planktonic species was observed. PS-COOH formed micro-scale aggregates (Z-Average $>$ 1 μm) and did not affect the growth of microalgae up to 50 μg/ml or that of brine shrimps up to 10 μg/ml. However, these negatively charged NPs were adsorbed on microalgae and accumulated (and excreted) in brine shrimps, suggesting a potential trophic transfer from prey to predator. On the opposite, PS-NH_2-formed nano-scale aggregates (Z-Average $<$ 200 nm), caused inhibition of algal growth (EC_{50} = 12.97 μg/ml) and mortality in brine shrimps at 14 d (LC_{50} = 0.83 μg/ml). Moreover, 1 μg/ml PS-NH_2 significantly induced *clap* and *cstb* genes, explaining the physiological alterations (e.g. increase in molting) previously observed in 48 h larvae, but also suggesting an apoptotic pathway triggered by cathepsin L-like protease in brine shrimps upon PS-NH_2 exposure. These findings provide a first insight into long-term toxicity of nanoplastics to marine plankton, underlining the role of the surface chemistry in determining the behaviour and effects of PS NPs, in terms of adsorption, growth inhibition, accumulation, gene modulation and mortality. The use of long-term end-point has been identified as valuable tool for assessing the impact of nanoplastics on marine planktonic species, being more predictable of real exposure scenarios for risk assessment purposes.

1. Introduction

Plastic debris have been globally recognized as a menace for marine ecosystems (Andrady, 2011). It is the major portion (between 60 and 80% and up to 96.87%) of the marine litter found during monitoring surveys (Gregory and Ryan, 1997; Ruiz-Orejón, 2016).

The evaluation of the impacts of the smallest fraction of plastic debris, defined as nanoplastics (< 1 μm) (Hartmann et al., 2015), constitutes one of the last unexplored areas to fully understand the importance of this emerging threat for the marine environment.

The amount of nanoplastics spread in the oceans is currently unexplored, since conventional sampling methods (i.e. neuston nets

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