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Effects of nanoplastics and microplastics on toxicity, bioaccumulation, and environmental fate of phenanthrene in fresh water☆

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ABSTRACT

Contamination of fine plastic particles (FPs), including micrometer to millimeter plastics (MPs) and nanometer plastics (NPs), in the environment has caught great concerns. FPs are strong adsorbents for hydrophobic toxic pollutants and may affect their fate and toxicity in the environment; however, such information is still rare. We studied joint toxicity of FPs with phenanthrene to *Daphnia magna* and effects of FPs on the environmental fate and bioaccumulation of ^{14}C-phenanthrene in fresh water. Within the five sizes particles we tested (from 50 nm to 10 μm), 50-nm NPs showed significant toxicity and physical damage to *D. magna*. The joint toxicity of 50-nm NPs and phenanthrene to *D. magna* showed an additive effect. During a 14-days incubation, the presence of NPs significantly enhanced bioaccumulation of phenanthrene-derived residues in daphnid body and inhibited the dissipation and transformation of phenanthrene in the medium, while 10-μm MPs did not show significant effects on the bioaccumulation, dissipation, and transformation of phenanthrene. The differences may be attributed to higher adsorption of phenanthrene on 50-nm NPs than 10-μm MPs. Our findings underlined the high potential ecological risks of FPs, and suggested that NPs should be given more concerns, in terms of their interaction with hydrophobic pollutants in the environment.

1. Introduction

Environmental contamination caused by fine plastic particles (FPs), including micrometer to millimeter plastics (microplastics, MPs) and nanometer-scale plastics (nanoplastics, NPs), has been catching more and more concerns in recent years, especially in aquatic environment (Andrady, 2011; Browne et al., 2011; Eerkes-Medrano et al., 2015; Mattsson et al., 2015b). MPs have been widely found in water, sediments, and marine animals (Boerger et al., 2010; Browne et al., 2008; Cole et al., 2013, 2015; Davison and Asch, 2011; Murray and Cowie, 2011; von Moos et al., 2012). Environmental concentrations of FPs varied significantly among different regions. In some protected areas in the Atlantic, as high as 100 g L^{-1} of MPs was found in sediments (Baztan et al., 2014). Due to the small sizes, FPs could be easily ingested by zooplankton and zoobenthos, cause obstruction of feeding appendages, aggregate, and block the alimentary canal, limit food intake or be translocated into the circulatory system (Barnes et al., 2009; Browne et al., 2008; Murray and Cowie, 2011). Moreover, FPs accumulated in small animals could be further translocated into higher trophic level and cause more damage (Mattsson et al., 2015a). However, most studies so far were focusing on MPs. NPs, which had potentially much higher surface area and mobility in aquatic systems, were still rarely studied.

Hydrophobic organic compounds in the aquatic environment have very high tendency to be associated with suspended sediment particles (Eek et al., 2010; Xia et al., 2006). With similar physical properties and even higher surface hydrophobicity than naturally occurring suspended organic matter, plastic fragments especially FPs have even higher sorption capacity and may further influence the environmental fate of hydrophobic persistent organic pollutants (POPs) (Lee et al., 2014; Mato et al., 2001). Large amount of hydrophobic POPs, including polycyclic aromatic hydrocarbons

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(PAHs), polychlorinated biphenyls (PCBs), and polybrominated diphenyl ethers (PBDEs), can be carried by waste plastic particles (Hirai et al., 2011; Mato et al., 2001; Rios et al., 2007). Rios et al. (2007) found that total concentration of PAHs ranged from 39 to 1200 ng g^{-1}, PCBs from 27 to 980 ng g^{-1}, and DDTs from 22 to 7100 ng g^{-1} on plastic fragments and pellets. Despite of the large risks of hydrophobic POPs carried by FPs in the environment especially to biota, most studies are limited to the toxicity effects of FPs alone on marine organisms (Barnes et al., 2009; Besseling et al., 2014; Browne et al., 2008; Murray and Cowie, 2011). Researches on the joint toxicity of FPs with POPs are still very rare (Oliveira et al., 2013). In addition, several studies have shown evidences that POPs, e.g. PAHs, PBDEs, and PCBs adsorbed on MPs could be transferred into the body of different marine organisms like mussels and lugworms (Avio et al., 2015; Besseling et al., 2013; Browne et al., 2013). However, the knowledge of the impacts of FPs especially NPs on bioavailability and fate of hydrophobic organic pollutants in the aquatic environment is still very limited (Mattsson et al., 2015b).

In this study, we used radioactive tracer and chose phenanthrene (Phe) as a model compound of PAHs, which have significant carcinogenic and mutagenic toxicity to organisms (Perera, 1997; Zhang et al., 2009), to investigate the combined toxicity of FPs and PAHs, and the effects of MPs and NPs on the transformation and bioaccumulation of PAHs in fresh water, using the model fresh water filter feeding zooplankton *Daphnia magna*. Polystyrene was selected as a representative model plastic, because of the highest production volume, making up about 90% of the total plastic demand, and is therefore widely found in the environment (Andrady and Neal, 2009).

2. Materials and methods

2.1. Chemicals and micro-/nanoplastics

[9-^{14}C]-labelled Phe (^{14}C-Phe) in ethanol with 99% radiochemical purity and 2.02 GBq $mmol^{-1}$ specific radioactivity was purchased from American Radiolabeled Chemical Inc. (St. Louis, USA). Phe was purchased from Tokyo Chemical Industry Co. Ltd (Tokyo, Japan) with >98.5% purity. NPs and MPs suspensions made of polystyrene with concentrations of 2.5% w/v, with particle size of 50 nm, 500 nm, 5 μm, 10 μm, and 15 μm, were purchased from BaseLine ChromTech Research Center (Tianjin, China). The size distributions of the plastic particles in the experiment medium were measured by transmission electron microscopy (TEM) as follows: 50 nm (25–75 nm), 500 nm (480–520 nm), 5 μm (5–7 μm), 10 μm (8–12 μm), and 15 μm (11–16 μm). Images of the plastic particles and size distributions are shown in Fig. S1.

2.2. Organisms and culturing conditions

The cladoceran *D. magna* and the green alga *Chlamydomonas reinhardtii* on which *D. magna* feeds were both obtained from the Institute of Hydrobiology, Chinese Academy of Science. These daphnids were raised in The Hong Kong University of Science and Technology (HKUST) laboratory for more than 10 years using GF/C membrane-filtered pond water, then were raised in Nanjing University laboratory for about five years using aerated tap water at 23.5 ± 1 °C on a 14:10 h light-dark cycle with an irradiance of 50 mmol photons m^{-2} s^{-1}. Daphnids were acclimated in M4 medium (Table S1) (Elendt and Bias, 1990) for one week before experiments. The alga *C. reinhardtii* was cultivated in WC medium (Table S2) (Guillard, 1975) under the same environmental conditions as the daphnids.

2.3. Acute toxicity test for D. magna

Acute toxicity caused by test compounds (Phe and FPs) after 48 h of exposure was tested according to the United States EPA guidelines (Agency, U.S.E.P, 1987). Daphnid neonates were exposed to test compounds at six concentrations (including control) in M4 medium. The tests were performed in three replicates in 50-mL glass beakers containing 40 mL of M4 medium and five daphnid neonates (less than 24 h after birth). All beakers were covered with watch glasses, and randomly distributed in a climate incubator (20 ± 1 °C) with a 16:8 h light-dark cycle (light irradiance of 50 mmol photons m^{-2} s^{-1}). The temperature and light were controlled automatically by the incubator and monitored periodically during the experiments. The daphnids were randomly distributed over the test vessels and not fed during the experiments. After 48 h of incubation, the daphnids were checked for immobilization. Daphnids were considered immobilized when they were not able to swim after 15 s of gentle stirring, according to the United States EPA guidelines (Agency, U.S.E.P, 1987). Different combination of MPs (0, 2.5, 5, 10, and 50 mg L^{-1}) or NPs (0, 2.5, 5, 8.5, 11, and 14.5 mg L^{-1}) and Phe (0, 0.05, 0.1, 0.2, 0.4, 0.8, and 1.2 mg L^{-1}) were chosen, according to their individual EC_{50} values, to test the joint toxicity of FPs and Phe to *D. Magna.* The concentrations of FPs were obtained by diluting stock solutions provided by the company after ultra-sonication and vortexing. In all tests, Phe was dissolved in ethanol and added to the testing medium at 0.1% volumetric ratio of solvent to medium. Solvent control (0.1% ethanol) was also set during the tests and no immobilization was observed for the control.

2.4. Degradation and accumulation of phenanthrene in D. magna

Phe degradation and bioaccumulation with or without the presence of 5 mg L^{-1} NPs (50 nm) or MPs (10 μm) were investigated with <24-h old (small) and 10-days old (large) daphnids in the M4 medium. The tests were performed with ten daphnids each in three replicates. The experiment lasted for 14 days and daphnids were fed once everyday with *C. reinhardtii* at concentrations between 5 and 20 million cells per beaker. ^{14}C-Phe was used to trace the fate of Phe in the system. Briefly, 12 μL of ^{14}C-Phe (1.48 kBq μL^{-1}, 324 mg L^{-1}) were added to 40 mL of the M4 medium in 50-mL beaker to give a final Phe concentration of 0.1 mg L^{-1} and radioactivity of 17.6 kBq. MPs or NPs were added to each beaker and ultra-sonicated for 20 min for better dispense of the plastics in the beaker. Preliminary experiment proved that the sonication process did not cause degradation of ^{14}C-Phe and no metabolites were detected after sonication process. On days 2, 7, and 14, three beakers were taken out, and the daphnids were rinsed three times in deionized water for 20 min each time (Oberdorster et al., 2006) and then frozen at −20 °C before further analysis. After rinsing, no adhesion of the plastic particles on daphnid carapace was observed under optical microscope. ^{14}C-Phe and its transformation products that remained in daphnid gut and adsorbed tightly on daphnid skinafter rinsing were also taken into account for calculating bioaccumulation. The remaining medium was extracted three times with ethyl acetate and the extracts were concentrated on a rotary evaporation at 40 °C for further analysis.

2.5. Adsorption experiments

The applied dialysis technique (Li et al., 2011) in a polytetrafluoroethene (Teflon®) chamber consisting of two half-cells (each 9 mL), separated by a dialysis membrane (regenerated cellulose, cut-off 1000 Da). The detailed description of the apparatus was published elsewhere (Höllrigl-Rosta et al., 2005). At the beginning,