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Review

Gaps in aquatic toxicological studies of microplastics

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HIGHLIGHTS

- No standardized bioassay is available for assessing MP toxicity.
- Routine bioassays should be modified before employing them for MP toxicity testing.
- Density is the main factor determining the fate of MPs in aquatic environments.

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ABSTRACT

The contamination of aquatic environments with microplastics (MPs) has spurred an unprecedented interest among scientific communities to investigate their impacts on biota. Despite the rapid growth in the number of studies on the aquatic toxicology of MPs, controversy over the fate and biological impacts of MPs is increasingly growing mainly due to the absence of standardized laboratory bioassays. Given the complex features of MPs, such as the diversity of constituent polymers, additives, shapes and sizes, as well as continuous changes in the particle buoyancy as a result of fouling and defouling processes, it is necessary to modify conventional bioassay protocols before employing them for MP toxicity testings. Moreover, several considerations including quantification of chemicals on/in the MP particles, choice of test organisms, approaches for renewing the test solution, aggregation prevention, stock solution preparation, and units used to report MP concentration in the test solution should be taken into account. This critical review suggests some important strategies to help conduct environmentally-relevant MP bioassays.

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1. Introduction

Plastic is undoubtedly the most widely used synthetic polymer by modern humans. The continuous fragmentation of dumped plastic items in the aquatic environment has resulted in the generation of tiny plastic particles called microplastics (MPs). Progressive fragmentation (Klemchuk, 1990; dynamic alteration in size and shape), ability to absorb/adsorb and release other groups of chemicals (Moore et al., 2005; dynamic change of chemical composition), as well as complex interactions with a broad range of organisms (i.e. biofouling and defouling, Ye and Andrady, 1991; dynamic change in density) complicate predicting the fate and biological impacts of plastics/MPs. Standard bioassay protocols such as those developed by the Organization for Economic Cooperation and Development (OECD) and Environmental Protection Agency (EPA) are routinely used to evaluate the effect of major classes of contaminants like pesticides, heavy metals, and endocrine disrupting compounds on aquatic organisms. To reduce the chance of committing type I or II errors and assist with improving the environmental compatibility of laboratory bioassays, this critical review discusses some of the scarcely addressed features of MPs and suggests approaches for improving bioassay protocols for MP studies.

2. Density

Density, size, and shape are the primary characteristics that govern the buoyancy and transport mechanisms of MPs in the aquatic bodies (Filella, 2015; Vermeiren et al., 2016). In addition to density, wind and water currents influence the position of MPs within the water column. Due to the absence of most of these mechanical forces in laboratory-based studies, density remains the chief factor affecting the distribution of MPs in the water column. Polypropylene (PP, density 0.85–0.92 g/cm^3), polyethylene [PE; low-density (LDPE) and high-density (HDPE), density 0.89–0.98 g/cm^3], polystyrene (PS, density 1.02–1.08 g/cm^3), polyvinylchloride (PVC, density 1.30–1.58 g/cm^3), and polyethylene terephthalate (PET, density 1.29–1.40 g/cm^3) are the major manufactured polymers that satisfy 73% of European plastic demands (Plastics Europe, 2015). Polyamide (Nylon, density 1.11–1.24 g/cm^3) is another main polymer that is commonly used in the fishing industry (Pruter, 1987). With respect to their buoyancy in freshwater (density $\approx$ 1 g/cm^3) or seawater (Feezor, 1999), plastic polymers fall into three groups: positively buoyant polymers such as PP (Fig. 1a) and PE, neutrally buoyant like PS (Fig. 1b), and negatively buoyant like PVC, PET (Fig. 1c), and nylon. The density of PS is comparable or slightly higher than seawater, and depending on the particle size, they would either remain suspended or slowly sink in the water column. Based on numerical models, it was estimated that PS particles larger than 100 μm in seawater would sink, while particles of 10 μm would be suspended (Enders et al., 2015).

Any changes in the polymer buoyancy could largely influence their transportation and fate in aquatic environments. Nevertheless, some studies have tested the toxicity of MPs that have densities far from the standard ranges known for that polymer. For instance, although PE is a positively buoyant polymer, several studied have assessed the toxicity of negatively buoyant PE microbeads (e.g., Au et al., 2015; Ferreira et al., 2016; Fonte et al., 2016). Negatively buoyant PE could probably have a different bioavailability and toxicity to organisms than the more common positively buoyant PE. Therefore, toxicity testings are urged to use MPs that have a density close to the standard range of that particular polymer. Slight changes in the density following the incorporation of additives (see section *Chemicals in/on microplastics*) may be acceptable only if the position of MPs in the water column is not affected (i.e. no significant change in density).

Sections 2.1–2.5 revolves around other issues that are directly or indirectly related to polymer density.

2.1. Choice of polymer

As discussed earlier, positively buoyant polymers float on the water surface while negatively buoyant polymers sink. For example, in the surface waters of Tamar Estuary, Southwest England, the majority of the identified polymers were the negatively/neutrally buoyant polymers [PE (40%), PS (25%) and PP (19%)] while high-density polymers comprised the minority [PVC (7%), polyester (6%) and nylon (3%)] (Sadri and Thompson, 2014). It would be expected that pelagic organisms are mainly exposed to positively buoyant polymers while the negatively buoyant ones are mainly taken up by benthic communities (Wright et al., 2013b). For example, low-density polymers were only found in fish feeding in pelagic waters but were not detected in those feeding in demersal waters (Lusher et al., 2013). Neves et al. (2015) speculated that the higher prevalence of rayon and polyester, acrylic, and nylon 6 fibers in benthic fish might be due to the high density of these polymers. In another study, negatively buoyant polyester, polyamides, and acetate microfibers were the most prevalent type of MPs in deep-sea sediments (Woodall et al., 2014). Nonetheless, some studies have tested the impacts of positively buoyant MPs on benthic organisms like mollusks, crustaceans, and polychaetes, while others exposed pelagic organisms to negatively buoyant MPs. Obviously, these scenarios are unlikely to represent normal conditions of MP exposure in the environment. In some circumstances, environmental forces like surface circulation (Shaw and Mapes, 1979) and vertical water currents (Colton et al., 1974) could translocate the positively buoyant plastics into the subsurface layers. Such translocations, however, are temporary owing to the lower density of polymers, which results in transient and incidental exposure of benthic communities to positively buoyant MPs.

Many aspects of MP sedimentation are still unclear (Filella, 2015). Colonization by organisms (Andrady, 1998; Muthukumar et al., 2011) or abiotic factors (e.g., sediments) (Barnes et al., 2009) are among the main mechanisms causing positively buoyant MPs to sink. In a study by Morét-Ferguson et al. (2010), biofouling was suggested as the reason for the higher densities of PE and PP debris found in the western North Atlantic Ocean waters. Negatively buoyant polymers, however, are expected to deposit within the sediments unless they are transported to the surface layers with the underlying currents. Therefore, one may hypothesize that bioassays employing positively buoyant MPs on benthic organisms would resemble exposure to fouled MPs. It should be noted, however, that the physical and chemical characteristics of fouled MP could be different from the unfouled or defouled ones rendering such hypotheses less accurate. Since this is likely to be an important distinction when assessing the impacts of MP, the rest of this section discusses the potential differences in the behavior and fate of unfouled, fouled, and defouled MPs in the aquatic environments.

It has been postulated that the sharp edges of MPs (particularly fragments) could exert their toxicity by causing intestinal abrasions or inflammatory responses (Karami et al., 2016; Pedà et al., 2016; von Moos et al., 2012; Wright et al., 2013b). Biofilm formation and biofouling by macrofoulants could alter the shape and topography of MPs (Fazey and Ryan, 2016). For example, spherical PS microbeads were changed to prolate spheroid after aggregating to microalgae (*Chaetoceros neogracile* and *Rhodomonas salina*) (Long et al., 2015). Any morphological modification, however, may eventually impact the toxicity of these particles. For example, biofouling reduced the ingestion of PE microspheres by sea urchin