Reflection on the utilization of microplastics as experiment material in laboratory

Yanchao Chai ( ycchai@yeah.net )
Nanjing Normal University  https://orcid.org/0000-0003-3870-3011

Research Article

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Abstract

More and more researches about microplastics (MPs) have been emerging, as the environmental MPs are detected ubiquitously. Various kinds of MPs have been added heavily as exogenous pollution in toxicological experiments. This perspective is intended to figure out utilization situation of MPs in laboratory and discrepancies between the choice of experiment material and environmental composition of MPs. Here we show, the total number of MPs utilized in 204 articles could be equivalent to that contained in $10^{15}$ kg dry wastewater treatment sludge, or comparable to the total existing mass in 600 km$^{-2}$ South Pacific subtropical gyre. This means that laboratory could be becoming a potential hotspot of MPs pollution as there is no criterion aiming at treatment of MPs-rich experiment waste. In addition, commercial Polystyrene (PS) microsphere with size less 1 µm was one popular experiment material, which is inconsistent with predominant composition of environmental MPs. That probably overestimate the adverse outcome of MPs as these purchased plastic sphere lack representativeness for actual environmental MPs. This work would shed light on the control and calibration of choice of MPs material in the future research.

1. Introduction

There are about 380 million tons plastics to be produced annually, globally already summing up to 8300 million metric tons (Geyer et al. 2017), of which almost 90% are directly abandoned into environment without recycled process (Gross 2017). Microplastics (MPs), generally referring to polymers with diameter below 5 mm, are derived from degradation of big-bulk plastics (secondary MPs) or produced as such originally (primary MPs) (Barnes et al. 2009; da Costa et al. 2016; Thompson et al. 2004), like abrasive additives in personal care productions. Environmental MPs have been getting more and more attentions, as they are bound to exhibit higher biological activities than their larger peer due to their bioavailability and penetrability benefitting from high surface-to-volume ratio (Espinosa et al. 2018). Toxicological experiments of MPs have been becoming a research hotspot in recent year, as they had been detected as exogenous pollutants in all ecosystem, including soil, groundwater, ocean, and even organism and their quantity shows inevitable increasing trend (Duis and Coors 2016; Lehner et al. 2019; Li et al. 2015; Machado et al. 2018; Panno et al. 2019). Various and numerous MPs have been utilized as experiment materials in laboratory to explore their ecological toxicity, meanwhile, which will also generate MPs-contained experimental waste. That will cause laboratory to become potential source of MPs pollution if there are no treatment measures. Unfortunately, as far as we know, there is not standard treatment protocol aimed at these MPs-contained waste. It is necessary to clarify the flux of MPs used in laboratory experiments to evaluate and underline the urgency of establishing treatments. In addition, the composition of environmental MPs is extremely complicated, which reflects in kind, shape, size, concentration and so on (Knight et al. 2020; Schellenberger et al. 2019; Xu et al. 2020). Thus, choosing proper experiment material is the first step to simulate and reappear the ecological effects of MPs in the environment. It is practical to establish and synchronize the connection between natural situation and utilization of MPs in laboratory, which can guarantee the reality and authenticity of these
experiments. It is also necessary to figure out the utilization information of MPs as experiment materials and compared with their actual environmental composition.

In this perspective, the information of MPs utilized as the toxicological materials were collected and analyzed from articles published, on the one hand, to evaluate the potential possibility of laboratory acting as MPs pollution source. Meanwhile, it is compared with situation of MPs composition found in field investigations. Then, the current status and problems of MPs research could be summarized, which will shed light on experimental design and calibrate deviation in the future laboratory research.

2. Materials And Methods

The articles were searched by the keyword “Microplastics” in Google Scholar, and chosen if MPs were added artificially as experimental materials. Finally, 204 articles were counted until 2019. Then, the information of MPs as experimental material was extracted from those articles, including total dosage (counted by mass and number of particles), sources (primary or secondary), polymer types, size ranges and shapes.

One experiment treatment was counted as one record. In this work, primary MPs are counted according to the definition the they are produced originally as the current micro-size, and secondary MPs are generated from the fraction of larger plastic materials, under natural condition or artificial stimulation. In addition, the dosage was just counted by mass in most articles. In present work, the number of particles was estimated by the transformation from mass based on density and volume.

3. Results And Discussion

3.1 Laboratory is becoming the potential hotspot of MPs pollution anthropically

There were at least 37,562.61 g MPs to be utilized as experiment materials in those 204 papers, which were equivalent to $3.80 \times 10^{18}$ particles. And total 247 usage records of MPs material were collected. Primary MPs accounted for 74.90% of total usage records with 185 kinds (Fig. 1), which hold 74.62% of total mass and 99.99% of number of particles.

In consideration of repeated testing or operation failure, the actual usage amount is definitely more than that. What do these numbers mean? According to the estimation of MPs content in sewage sludge from 28 wastewater treatment plants in China, the total number are equivalent to that contained by $10^{15}$ kg dry wastewater treatment sludge (Li et al. 2018). Wastewater treatment plant can be seen as main linkage between anthropogenic MPs and environment, from which sewage sludge have concentrated MPs content and are source of MPs pollution in soil, water and air (Habib et al. 2020). In terms of the total mass, they are comparable to MPs in more than 600 km$^{-2}$ South Pacific subtropical gyre (Erikson et al. 2013), which is hardest hit area of ocean MPs pollution. It is estimated that there are over $4.75 \times 10^{12}$ plastic particles (size range of 0.3 – 4.5 mm) floating in the global ocean (Eriksen et al. 2014), which is still lower several orders of magnitude than that contained by experiment waste from those articles we investigate. That would worsen the current MPs pollution situation once those MPs-contained experimental waste get pathway into environment.
Among all MPs materials, primary microplastics, just designed and added as detergent exclusively for experiment but not daily existing, account for 75% of all researches and 62% of total mass, which will be a direct pollution source just from scientific experiments. In addition, 3.11% of total mass contain fluorescent additions in order to tracing, which could pose more ecological threats. However, none of these 204 articles involved and underlined the treatment of MP-contained experiment waste, which will make laboratory be prone to the potential pollution hotspot of MPs. Especially, nearly 40% of these researches involved MPs with size less 100 µm that account for almost 100% of total number of particles (Fig. 2b). It is well known that the smaller the size of MPs, the more difficult they are captured and removed during wastewater treatment process (Habib et al. 2020). It is necessary and urgent to underline and execute relevant criterion about the special treatment of MP-rich experiment waste.

3.2 Discrepancies between laboratory material choice and composition of environmental MPs

More than 20 material types of MPs were used in those published articles. PS, PE, PVC and PP occupied the more than 80% of total mass, number of particles and usage records. Among them, PS was the most popular material, which shared 31.69%, 96.60% and 44.94% of total mass, number of practices and usage records, respectively (Fig. 2a). The usage frequency and dosage decreased with the increase of material size. The size range of 0–1 µm covered the nearly 100% and 35% of total number of particles and usage records. The materials with size less 500 µm contributed to 65% of total mass (Fig. 2b). MPs with shape bead took absolute advantage among the shapes of MPs material, accounting for more than 50%, 99% and 60% of total mass, number of particles and usage records, which was followed by granule and fiber (Fig. 2c).

Seen from the statistics information, the popular MPs chosen as experiment material were characterized by PS with small size (≤ 1 µm) and bead shape, which is obviously deviated from the prevalent composition of environmental MPs. Although the smaller MPs with regular bead shape that are easy to be swallowed could bring out positive ecotoxicity, and PS could be more toxic because of styrene and functional group of benzene, they are not mainstream of environmental MPs. In the sludge from wastewater treatment plants, MPs were predominated by PBA (Poly(11-bromoundecyl acrylate)), nylon, PE and PP with fiber/fragment shape and average size 50–100 µm (Habib et al. 2020; Li et al. 2018; Xu et al. 2020). Fiber shedding from clothes during laundry is main sources of MPs in the domestic wastewater (Napper and Thompson 2016). In the South Pacific subtropical gyre, the MPs with fragment shape and size range 1000–2790 µm accounted for almost 50% of total number (Eriksen et al. 2013). Zhang and Liu (2018) analyzed the distribution of MPs in soil of southwestern China, and found that 95% of the sampled plastic particles are in the size range 50-1000 µm, meanwhile, predominant form is fiber, making up on average 92% of each sample followed by fragments and films. Although MPs with bead shape is used commonly in personal care products, they probably transform into other form in short time once they enter into environment and suffer from breakdown and biofouling.

In addition, the quantity of MPs in most toxicological experiments were measured by the mass, which is opposite to that in field investigation, more by the number. This inconsistency will cause gap and confusion in refer to comparing different researches. As high bioavailability of MPs benefits from high surface-to-volume ratio due to small size, MPs with less mass may pose more threats compared to their
big-bulk peer. So, MPs exhibit more strong individual effect and the number will be more representative than their mass.

3.3 Purchased experiment material lack representativeness for environmental MPs

In those published articles, more than half took the purchased primary plastic sphere as experiment materials. On the one hand, those commercial MPs are mainly produced just as chemical reagent, rather than the dominant source from human daily activity. One the other hand, in contrast to their peer in actual environment, the plastic microsphere has too smooth appearance and regular shape without mechanical breakdown, organic corona from biofouling and aging process, due to which the microplastics will exhibit different biochemical behaviors (Fadare et al. 2020; Pflugmacher et al. 2020; Wang et al. 2020), including palatability, toxicity and absorption ability. In addition, the environmental MPs contain various kinds of chemical additives, and could cause ecological risk through leaching process (Luo et al. 2019), which is rarely possessed by the plastic sphere purchased as pure reagent. Thus, the current choice of experiment material cannot represent fully the effects and behaviors of environmental MPs, meanwhile, could confuse the actual ecological effect of MPs and should be calibrated in the future research.

**Declarations**

**Ethics approval and consent to participate**
Not applicable

**Consent for publication**
Not applicable

**Availability of data and materials**
The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

**Competing interests**
The authors declare that they have no competing interests

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**Authors' contributions**
Yanchao Chai did all work in this study

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**Figures**

![Figure 1](image)

**Figure 1**

Usage record number and percentage of primary and secondary MPs. One record means one treatment in the experiment design.
Figure 2

Percentage of different (a) types, (b) size ranges, (c) shape counted by mass, number of particles and record, respectively. One record means one treatment in the experiment design.

Supplementary Files
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