

The Handbook of Environmental Chemistry 95

Series Editors: Damià Barceló · Andrey G. Kostianoy

Defu He

Yongming Luo *Editors*

Microplastics in Terrestrial Environments

Emerging Contaminants and Major
Challenges



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Microplastics in Terrestrial Environments

Emerging Contaminants and Major Challenges

Volume Editors: Defu He · Yongming Luo

With contributions by

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Series Preface

With remarkable vision, Prof. Otto Hutzinger initiated *The Handbook of Environmental Chemistry* in 1980 and became the founding Editor-in-Chief. At that time, environmental chemistry was an emerging field, aiming at a complete description of the Earth's environment, encompassing the physical, chemical, biological, and geological transformations of chemical substances occurring on a local as well as a global scale. Environmental chemistry was intended to provide an account of the impact of man's activities on the natural environment by describing observed changes.

While a considerable amount of knowledge has been accumulated over the last four decades, as reflected in the more than 150 volumes of *The Handbook of Environmental Chemistry*, there are still many scientific and policy challenges ahead due to the complexity and interdisciplinary nature of the field. The series will therefore continue to provide compilations of current knowledge. Contributions are written by leading experts with practical experience in their fields. *The Handbook of Environmental Chemistry* grows with the increases in our scientific understanding, and provides a valuable source not only for scientists but also for environmental managers and decision-makers. Today, the series covers a broad range of environmental topics from a chemical perspective, including methodological advances in environmental analytical chemistry.

In recent years, there has been a growing tendency to include subject matter of societal relevance in the broad view of environmental chemistry. Topics include life cycle analysis, environmental management, sustainable development, and socio-economic, legal and even political problems, among others. While these topics are of great importance for the development and acceptance of *The Handbook of Environmental Chemistry*, the publisher and Editors-in-Chief have decided to keep the handbook essentially a source of information on "hard sciences" with a particular emphasis on chemistry, but also covering biology, geology, hydrology and engineering as applied to environmental sciences.

The volumes of the series are written at an advanced level, addressing the needs of both researchers and graduate students, as well as of people outside the field of

“pure” chemistry, including those in industry, business, government, research establishments, and public interest groups. It would be very satisfying to see these volumes used as a basis for graduate courses in environmental chemistry. With its high standards of scientific quality and clarity, *The Handbook of Environmental Chemistry* provides a solid basis from which scientists can share their knowledge on the different aspects of environmental problems, presenting a wide spectrum of viewpoints and approaches.

The Handbook of Environmental Chemistry is available both in print and online via www.springerlink.com/content/110354/. Articles are published online as soon as they have been approved for publication. Authors, Volume Editors and Editors-in-Chief are rewarded by the broad acceptance of *The Handbook of Environmental Chemistry* by the scientific community, from whom suggestions for new topics to the Editors-in-Chief are always very welcome.

Damià Barceló
Andrey G. Kostianoy
Series Editors

Preface

In 1907, the first fully synthetic plastic was invented by Leo Baekeland, who coined the term “plastic.” Since then, plastic has gradually become an invasive part of our lives. Nowadays, plastic is ubiquitous. It is estimated that more than 8300 million metric tons of virgin plastics have been produced to date [1]. Global annual plastic production reached 348 million tonnes in 2017 [2]. The growth of plastic production in the past decades is exponential and has substantially outpaced any other manufactured materials. For instance, it is estimated that four trillion plastic bags are used worldwide annually; approximately one million plastic water bottles are used globally every minute [3].

Plastic has brought many societal benefits, but it is evident that our current approaches to plastic use and disposal have resulted in a big environmental issue. For example, single-use plastic bags or bottles might only be used one time or a few minutes, but they need to take several hundred or even more than 1,000 years to disintegrate and remain in the environments. Plastic solid waste or plastic pollution has become a serious threat to the environment and ecosystem health.

Microplastics (MPs) refer to fine plastics less than 5 mm in size and include primary source from the original production of small-sized particles and secondary source from degradation or fragmentation by large plastics. About 10 years ago, MPs were initially detected in global ocean and marine organisms. In recent years, more and more studies showed the occurrence of MPs in freshwater, soil, air, and other terrestrial environments. As one of the most widespread and long-lasting pollution on the surface of our planet, MPs are mostly derived from anthropogenic activities in terrestrial environments. It is apparent that the management and control of MPs pollution need to be primarily based on terrestrial ecosystems as well as production processes.

This book summarizes the available research progress of MPs as emerging pollutants in terrestrial environments. Multidisciplinary research reviews are covered in methodology, environmental geography and chemistry, toxicology, ecology, management, and even legislation. The content generally includes: (1) analytical methods of MPs; (2) source, fate, and distribution of MPs in terrestrial

environments; (3) ecological risks of MPs in terrestrial environments; (4) the toxicity and health risks of MPs; and (5) management and control approaches for MPs in terrestrial environments. We should point out that, at the time of writing this book, there are much less publications on MPs in terrestrial environments than in marine environments. Despite the big knowledge gap in this field, we have done our best to present a comprehensive research overview of MPs in terrestrial environments, such as the sources, fate, abundance, and impacts of MPs, as well as the suggestions on potential solutions for terrestrial pollution of MPs.

Despite a few books about MPs in ocean and aquatic systems or freshwater, as we know, this is the first book specially focusing on MPs in terrestrial environments. This book was originally proposed by the editor-in-chief of the series book “The Handbook of Environmental Chemistry,” Professor Damià Barceló, in November 2018. The book editors designed the frame system and primary outline of contents in January 2019 and then invited the corresponding authors in China and organized a special meeting for compiling this book on June 4, 2019, just during the period of “The 2nd National Conference on Environmental Pollution and Management of MPs” in Nanjing, China (Fig. 1). The majority of the corresponding authors attended this meeting and the detailed contents and outlines of each chapter in the book were determined according to the compiling principles after this meeting. A total of 91 researchers have contributed 22 chapters to achieve this book.



Fig. 1 A special meeting for this book at Nanjing, China (June 4, 2019)

The editors hope that the book will be useful for environmental researchers, ecologists, toxicologists, research students, policy-makers as well as the general public. It is expected that the contents can arouse the interest of more scientists and engineers to explore MPs and attract more administrators and public to be aware of the issue of (micro)plastic pollution and its terrestrial management importance and urgency.

Finally, we specially thank Professor Damià Barceló to recommend us to organize this book. We would express our sincere acknowledgments to all the authors for their valuable contribution and efforts to create these chapters. We would like to acknowledge the support by the National Key Research and Development Program of China (Grant No. 2016YFC1402202), the National Science and Technology Major Project for Water Pollution Control and Treatment (No. 2018ZX07208008), the Key Research Program of Frontier Sciences, CAS (QYZDJ-SSW-DQC015), the External Cooperation Program of BIC, CAS (13333KYSB20160003), etc. We also thank the responsible publishing editors Tanja Weyandt and Ramya Venkitachalam for their wonderful work for the production of this book.

Shanghai, China
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Part I
Analytical Methods of Microplastics
in Terrestrial Environments

Analytical Methods for Microplastics in Environments: Current Advances and Challenges



Menting Liu, Shibo Lu, Yingxin Chen, Chengjin Cao, Moritz Bigalke, and Defu He

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Abstract Numerous studies have shown the presence of microplastics (MPs) in the environment. As an emerging global contaminant, the concentrations of MPs need to be evaluated, to assess its impacts on ecosystems and humans. This chapter reviews the development of analytical approaches from sample collection to MP characterization and quantification. This chapter contains a critical overview and a comparative assessment of sampling and sample preparation procedures for water, soil,

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sediment, biological, and atmosphere samples. We discuss sample preparation techniques such as flotation, filtration, digestion of organic matter, and analytical techniques such as morphological and physical classification, identification, and quantification of MPs. Furthermore, we address the advantages and disadvantages of these techniques, compare MP assay methods for different environment matrices, and discuss the challenges in the establishment of standard methods. In future research, it will be important to develop efficient assay protocol, such as basing on fully or semiautomated analysis, and to improve the accuracy of identification and quantification for MPs, especially nanoplastics.

Keywords Digestion, Environment, Microplastics, Sampling, Separation

1 Introduction: Overview of Analytical Procedures

Global production of plastics has exponentially increased from 2 million metric tons in 1950 to 280 million tons in 2016. The vast majority of plastic products (79%) are discarded into the environment [1]. These plastic wastes can be fragmented and turned to smaller-sized litter. Microplastics (MPs) usually refer to plastic debris less than 5 mm in sizes. Although primitive MPs are proven to contain in the personal care products, synthetic fibers, sandblasting media, abrasive particles, and resin pellets, the major MPs are stemmed from the breakdown of large plastic products [2–5]. MPs have been widely detected in freshwater [6], benthic sediment [7], soil [8], atmospheres, seawater [9], and beach sand [10] and even distributed in remote areas [11] such as polar regions [12] and the Tibet Plateau. Moreover, MPs can be taken up by various aquatic organisms, such as fish and mussels [13–15], and terrestrial organisms, such as earthworms and snails, and even get absorbed by plants [16, 17]. Thus MPs may have wide impacts on the earth ecosystem and deserved attention.

Analyzing MPs in the environments can provide basic information of the level of pollution, changing trends of MP concentrations, and the possibility of the exposure of organisms. Laboratory experiments to study the response of organisms to MP exposure furthermore require knowledge about environmental MP concentrations and characteristics to deliver realistic results of environmental significance. Furthermore, these experiments require appropriate methods for quantifying MPs. There is an increasing number of publications about analytical methods to characterize and quantify MPs in recent years. However, there is a lack of standardization of analytical methods and protocols among researchers, and the different methods used today may under- or overestimate MP pollution and generate incomparable data [5, 18].

The analytical methods for the different environmental matrices are variable, but all include similar procedures of sampling, sieving, floatation, filtration, digestion of

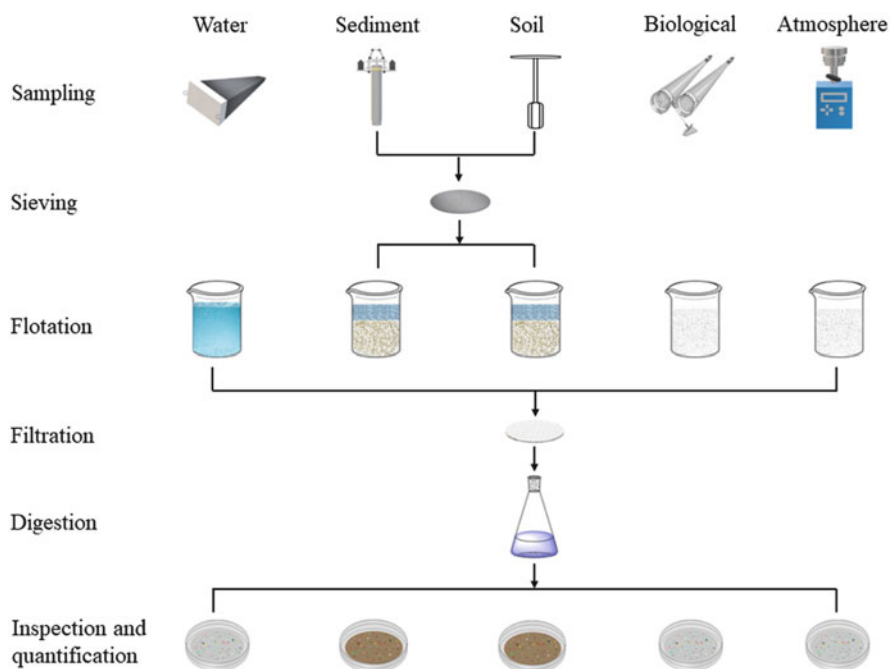


Fig. 1 The analytical methods to analyze microplastics in various environmental media

the natural organic matter, and finally identification and quantification (Fig. 1). For sampling, nets are applied for aquatic systems, while stainless steel spoons or spatulas and different kinds of corers are usually used to collect superficial sediment or soil [19–21]. Seabed sediments are collected by core and bottom trawl [22, 23], while the suspended atmospheric MPs are collected using particulate samplers [24]. Besides, for biological samplings, researchers need to dissect animals and detect gut contents [4]. After that, pretreatment is needed to remove larger impurities by manual selecting and sieving. Then, MPs are separated by density flotation using saturated salt solutions such as sodium chloride (NaCl), sodium bromide (NaBr), zinc chloride ($ZnCl_2$), or sodium iodide (NaI) [2, 25–28]. In the digestion process, different enzymes, acids, alkaline solutions, hydrogen peroxide, or Fenton reagent is employed to remove the natural organic matter [29–32]. Following these analytical preparations, MPs are relatively clean, and the sizes, numbers, and polymer types of MPs can be analyzed. These different steps in the analysis of MPs will be discussed in the following chapters in detail to make an evaluation about the best methods to analyze MPs across different environments.

However, there is one approach which fundamentally differs from the abovementioned procedure. A pressurized fluid extraction method has been suggested to extract MPs from glass beads, soil, and municipal wastes [33]. This method is based on the solubilization of plastics in hot solvents (dichloromethane at 200°C) to extract the liquid sample and recover the residue after evaporation of the

solvent. The residue is quantified by weighting, and thus the mass of the extracted plastic can be determined. The polymer type can be further analyzed by FTIR, but this process might be complicated in case those different polymers are extracted together and the spectra overlap. However, this method was never applied in any other study and will not be further discussed here.

2 Collection of Environmental Samples

2.1 Water Samples

The volume of water samples usually varies among different surveys; only a portion of the samples is of interest and needs to be preserved for further processing. These water samples are usually obtained by filtering large volumes of water through nets. Sea surface samples were mostly collected by a trawl along a certain transect, such as Manta net and Neuston net [18, 34, 35]. The Neuston net can be used in deep seas, while the Manta net in calm seawater. The most common approach is using zooplankton nets for sampling water column [4]. In addition, other devices, such as the rotating drum sampler for bulk samples of 10 L and the continuous plankton recorder, were also used by researchers [2, 36].

The depth of water column sampling depends on the objectives of the studies and is different in different studies, for example, 50–60 μm (surface microlayer, Neuston layers), 15 cm, and 25 cm noted in previous studies [36, 37]. The deepest water sample taken was up to 212 m deep in oceans [37]. When sampling in seabed, benthic nets should be attached to the bottom, while bongo nets are used for medium depth of water column [4]. Some researchers applied metal sieves to tap Neuston layers at the depth of 150–400 μm under the water surface [38]. This is a typical depth of the microlayer [39], which has the advantage of surface tension to sample the microlayer water.

The findings about MP abundance in the water column are related to the sampling nets and closely dependent on the mesh size and opening area of the net. According to previous studies, mesh sizes ranged from 0.053 to 3 mm, with the majority in the range from 0.30 to 0.39 mm [5]. The net aperture for rectangular openings of Neuston nets ranged from 0.03 to 2 m² for sea surface sampling [40]. For circular bongo nets, apertures of 0.79–1.58 m² were applied for sampling water columns [37, 41]. The length of the net usually was in the range 1–8.5 m, while most nets are of 3–4.5 m length. One study proposed the combination of two different-sized sampling devices for water column samples: one 3-m-long bongo nets and one 1-m-long epibenthic sleds to get the best results [42]. The findings about the abundance of MPs depend on the mesh size: the number of MPs increases when the smaller mesh-sized net is used, because small particles are also collected. However, the majority of researchers applied moderate mesh sizes because smaller mesh-sized nets usually result in clogging on the surface of nets.

2.2 *Soil and Sediment Samples*

Sampling is the first step for analyzing MPs in soil or sediment. He et al. pointed out that sampling sites need to be set appropriately in order to give a good indication on overall MP pollution in the soil [43]. For sediments, sampling was usually carried out along the strandline with a spoon and/or a trowel, using quadrats for a large area, and sampling different depth strata using corers [5]. Stainless steel shovels or other manual tools were applied to acquire soil samples [8, 44].

Sandy beaches are the common area for collecting nearshore sediment samples. Due to the lack of uniformity in the beach zone, sampling area and positions need to be selected in terms of specific geographical conditions [23, 45, 46]. Some studies covered the entire range of the beach, from the intertidal to the supralittoral zone. Other studies distinguished several littoral zones [2] or sample sediments across different zones [47, 48]. Some researchers focused on the flotsam deposited at the high tide line [5]. According to the characteristics of sediment, sampling units were inconsistent, including area, weight, and volume of sediment. Sediment depths were also variable among previous studies. Most researchers sampled a certain depth under the surface, such as 5 cm for surface sediments [46, 49], or more deep such as 10 cm [36, 47]. Other studies sampled sediment in the depth range up to 32 cm [45, 50]. Considering the dynamic changes of beaches and tidal zones, MPs and organic matter may interact or buried and will be trapped to a certain depth under the beach [51].

Recently, different types of soil have been studied for MPs. MPs were detected in floodplain soils, paddy soils, agriculture soils, as well as at the road surface in industrial zones or forest areas [8, 26, 29, 33, 52]. The sampling schemes differ considerably with respect to the area sampled, depth of the sampling, and replicate samplings. The area differs between 8×8 cm and 100×100 cm, while the depth differs between 2 and 10 cm, and in some studies different depth intervals were analyzed, e.g., 0–3 and 3–6 cm or 0–10 and 10–30 cm [8, 29, 53]. The depth sampled also depends on the use of the soil: in natural soils, the samples were taken close to the surface, while in agricultural soils, sampling depth often depends on the depth of ploughing.

2.3 *Biological Samples*

MPs can be taken up and ingested by aquatic and terrestrial organisms, which can transfer as food into humans, and may pose latent health risks. In order to explore MP concentrations in biota, the sampling of organisms is an important step. Owing to the diversity of biota and their habits, different instruments and approaches have been employed for acquiring samples. In the aquatic environment, zooplankton can be collected with a bongo net [54]; fish species can be obtained with the pelagic net, trawl, electricity, or purchase from the local fishermen [55, 56]. Crustaceans such as

shrimp can be captured with bottom trawls, creels, or traps [57, 58]; bivalves such as mussels and oysters can be collected directly from the water body or sampled with a mussel trawl [59]. Other studies did not report special methods in detail, for example, for gaining whales, sharks, turtles, and seals [60–63]. To terrestrial biota, the uptake of MPs has been rarely investigated. Chicken and earthworm casts were manually selected; chickens were dissected and different parts of the body were analyzed [64]. Currently, the number of MPs per weight of organisms or individuals [65] was usually used as the quantitative units of MPs. The percentage of individuals containing MPs was also utilized to indicate the occurrence rate [56].

2.4 Atmospheric Samples

According to recent literature, collection methods of atmosphere samples were mostly based on special pumps. For example, Dris et al. pumped and collected indoor air at a speed of 8 L min^{-1} through quartz fiber GF/A Whatman filters (1.6 mm, 47 mm). Sampled volumes were in the range between 2 and 5 m^3 in terms of the local population density [66]. A $500\text{-}\mu\text{m}$ -sized plankton net and $50 \mu\text{m}$ clean steel filters were used in a previous study [67], while in another study, researchers used an intelligent middle flow total suspended particulate sampler with an intake flow rate of $100 \pm 0.1 \text{ L min}^{-1}$. Collection was conducted in triplicate over 1 h and by filtering approximately 6 m^3 per air sample.

But also passive sampling devices such as rain samplers or NILU particulate fallout collectors were used to sample wet and dry deposition [68]. Air samples need to be immediately sealed and used for further observation and identification in laboratories [24].

3 Separation Techniques

After collection, the samples need to be separated in order to acquire clean MPs for subsequent identification and measurement. Separation techniques include density flotation, filtration, sieving, and digestion processes.

3.1 Flotation

Density separation is the most reliable method, which has been commonly employed for the separation of MPs from various types of environmental mediums [43]. In fact, the density of different polymer plastics is largely variable and in the range from 0.90 to 1.45 g cm^{-3} . Of them, polypropylene and polyethylene have the lowest densities ($0.92\text{--}0.97 \text{ g cm}^{-3}$). High-density MPs include polyethylene terephthalate

Table 1 Candidate MP-extraction solutions and their characteristics

Salt	Density of saturated solution (g mL ⁻¹)	Total costs of 100 mL extraction solution	Hazard statement	Environmental mediums	References
NaCl	1.19	\$0.21	No	Water, sediment, soil, fish, clams,	[2, 8, 69, 70]
CaCl ₂	1.42	\$0.59	H319	Sediment, soil	[29, 71, 72]
NaBr	1.55	\$1.63	H303-H313-H320	Sediment, soil	[28]
ZnCl ₂	1.68	\$6.21	H302-H314-H410	Sediment,	[27]
NaI	1.89	\$26.49	H315-H319-H400	Water, sediment, soil, birds	[25, 73, 74]
H ₂ O	1.0	–	No	Water, soil	[53, 75]
KBr	1.58	\$13.3	No	Water, sediment, fish	[56, 76]
Na ₂ WO ₄	1.4	\$4.51	H302	Water, sediment, earthworm	[47, 77, 78]
Oil	–	–	No	Sediment, soil, water	[71, 79]
NaCl and NaI	–	–	H315-H319-H400	Sediment, soil	[44, 80, 81]
NaCl and Na ₂ WO ₄	–	–	H302	Sediment	[82]

Note: density information for reagents came from experimental data; cost information for reagents obtained from Aladdin (China). The letter “H” for hazard statement, “3” for health hazards, and “4” for environmental hazards were from Globally Harmonized System of Classification and Labelling of Chemicals (GHS)

(1.30–1.35 g cm⁻³), polyformaldehyde (1.41 g cm⁻³), and polyvinyl chloride (1.40–1.45 g cm⁻³). Nevertheless, the density of the mineral phase of soil or sediments is up to 2.65 g cm⁻³. This difference in the density provides the possibility of separating MPs from mineral particles. When MP-contained samples are suspended into a density solution, plastic particles will float on the surface of the solution, but the more dense materials will sink to the bottom of the vial.

Multiple types of solutions have been employed to isolate MPs from environmental matrices; their cost, environmental hazard, and separation efficiency should be considered to select suitable reagents (Table 1). According to previous studies, saturated NaCl solutions were most widely used, due to the characteristics of being cheap and nonhazardous. However, some plastic particles with density higher than 1.18 g cm⁻³, such as PVC and PET, have not been fully extracted. To overcome this drawback, Nuelle et al. developed a two-step method, using NaCl solution for pre-extraction and NaI solution (1.80 g cm⁻³) for further flotation [81]. Other

researchers used CaCl_2 to replace NaCl solutions. Although the extraction effect of CaCl_2 solution is higher than NaCl solution, the divalent Ca ions may agglomerate the organic material, which results in difficulty in subsequent identification experiments [29]. Another study suggests that optimum density of extraction solution is $1.6\text{--}1.8\text{ g cm}^{-3}$, which can be achieved using ZnCl_2 or NaI [83]. However, those solutions are expensive, and ZnCl_2 is hazardous to the environment and embryotoxic to aquatic organisms [71]. In a recent study, NaBr solution was suggested for MP extraction because of its high efficiency to most polymers in soil [26] and because of its advantages of being low cost and environmentally friendly.

Some instruments have been developed to facilitate the extraction by density separation. An elutriation column and a plastic sediment separator were used for separating MPs from sediment with a recovery rate of 98–100% [84, 85]. A simple flotation separation device was designed to realize semiautomatic MP separation with a recovery rate of 90% [81]. Recently, a device for the circular extraction of soil MPs had been designed to achieve better recovery with environmentally friendly consequences [26]. This device included a separation, vacuum filtration, and solution recovery system. Therefore, the separation process can be achieved by combining appropriate flotation reagents and devices.

Besides density separation, oil extraction or the use of electrostatic charges to separate MPs from water, sediment, or beach sand had been discussed in the literature [71, 86]. However, these methods have rarely been applied in the practice for the analysis of microplastics.

3.2 Filtration

In order to remove large debris, sieving pretreatment is sometimes used before density flotation. For example, water samples can first be sieved through 500 μm mesh sieves [87]. It is also necessary to sieve dry soil and sediment before analysis. Plastics can be separated using sieves of variable mesh sizes. Five mm sieves are used to remove large macroscopic debris from beach samples; the disaggregated sediment can be sieved through the stacked sieves with 5 and 0.3 mm meshes [88]. Soil samples can pass through 5, 2, 1, or 0.5 mm sieves [29, 53, 89]. Referring to MP analyses, plastic particles larger than 5 mm need to be excluded; however 2 mm sieves were used by the majority of researchers to manually collect bigger plastic items for further analysis.

After density flotation, MPs can be separated from the supernatant over a filter, usually aided by a vacuum [2, 36], and then collected on a filter membrane for optical or spectroscopic analysis. Membrane filters are made of different materials, including alumina, ceramics, and polycarbonate. Currently, the filter membrane for MP analysis mainly includes glass fiber membrane, cellulose acetate membrane, cellulose nitrate membrane, polycarbonate membrane, nylon membrane, and alumina membrane (Table 2). The surface of the glass fiber filter membrane is very rough, is not suitable for subsequent spectrum identification by IR spectroscopy, and

Table 2 The characteristics of filter membranes in MP analysis

Filter membrane	Surface	Hydrophilicity	Fiber release	Interference on infrared signal	Chemical resistance
Glass fiber	Rough	Hydrophilic	Yes	No	No
Cellulose acetate	Smooth	Hydrophilic	No	No	Alkali
Cellulose nitrate	Smooth	Hydrophilic	No	No	Alkali
Polycarbonate	Smooth	Hydrophobic	No	Yes	No
Nylon	Smooth	Hydrophilic	No	No	No
Alumina	Smooth	Hydrophilic	No	No	No

Information from [26, 69, 90–94]

has the disadvantage of releasing fibers. The polycarbonate filter membrane is not hydrophilic, which is not conducive to the retention of MPs. Furthermore, the polycarbonate itself has a strong infrared signal, which may disturb the signal when MPs are identified by FTIR. Therefore, glass fiber and polycarbonate filter membranes are not recommended. In contrast, the surface of the cellulose or nylon filter membrane is smooth; and both types of filter membranes are soft and convenient for the practice of extraction [95]. Alumina-based filter membranes are transparent to IR above $1,250\text{ cm}^{-1}$ and are the best available choice for transmission FTIR [29]. Therefore, cellulose, nylon, or alumina membranes were widely applied in previous studies. The sizes of membrane pores vary and can select mostly dependent on types of samples. For water samples, the pore size of the sieves or filter papers varied from $1\text{ }\mu\text{m}$ to 0.5 mm [3]. For biological samples, researchers used filter membranes with pore sizes between 0.45 and $5\text{ }\mu\text{m}$ [95]. However, filter membranes with large-sized pore, ranging from 0.45 to $20\text{ }\mu\text{m}$, have been often used for soil sample analysis [29, 53], because soil samples often block filter membranes. Using a filtration cascade including multiple filters of different pore size can be considered for avoiding blockages of filter pores [96].

3.3 Digestion of the Organic Sample Matrix

Environmental samples usually contain organic matter of different kind, which cannot be separated by the density separation and filtration. Thus an extra step of removing impurities is required to purify MPs. According to previous studies, acidic, alkaline, or oxidizing treatments, enzymatic digestion, and electrostatic separation have been performed to remove impurities (Table 3).

A variety of acids have been applied to digest organic matters in different samples. Some studies have shown that a strong and hot nitric acid solution (HNO_3) was effective to remove organic matters [30, 84], but it may affect polystyrene foam spheres, nylon fibers, and polyamide. Another study proposed a protocol of 4:1 mixture of HNO_3 and perchloric acid (HClO_4) as a digestion solution. Results

Table 3 Digestion methods for separating and purifying MPs

Digestion types	Specific reagents	Suitable environment mediums	Advantages	Disadvantages	Reference
Acid treatment	HNO ₃ , HCl, HNO ₃ , HClO ₄	Digestion of organic matters in soil, sediment, or biological samples	Very effective in digesting organic materials	Digestion of some kind of plastics (e.g., PA, PET)	[27, 29, 30, 97]
Alkaline treatment	NaOH, KOH	Biological samples	Gentle method, high effectiveness in digesting biological tissues	Likely affect plastic; do not eliminate alkali-insoluble organic matters	[30, 31, 98]
Oxidation	H ₂ O ₂	Removal of organic matters in soil, marine organisms, and sediment	Gentle method, high effectiveness in digesting organic materials	Smaller effect on some plastics (PET and PP)	[81]
Fenton reaction	H ₂ O ₂ , Fe(II) salts	Marine samples, soils	Gentle method, no degradation of plastic if the samples are cooled and the pH is controlled, effective in digestion of organic matter	Wooden structures will not be digested	[99]
Enzymatic digestion	Proteinase K, protease A-01, lipase, cellulase enzymes	Wastewater samples and marine organisms	Gentle method, no degradation of plastic	Needs an additional oxidative step (e.g., H ₂ O ₂) to fully digest organic substances, costly, and very time-consuming	[31, 100]

The different digestion methods are also used in combination (e.g., KOH and H₂O₂)

showed that several types of polymers were degraded, significantly to polyamide, polyurethane, and black tire rubber elastomer and slightly to acrylonitrile butadiene styrene, polymethyl methacrylate, and PVC [101]. Collard et al. used sodium hypochlorite (NaClO) and HNO₃ rinses plus ultrasonication to digest fish stomach contents [102]. They reported an effective digestion of impurities with no obvious destruction on MPs, except for a 25% mass loss of PVC. Comparatively, low-concentration nonoxidizing acids, such as hydrochloric acid, are insufficient for digestion at room temperature, yielding a large amount of organic residues.

Nevertheless, the strong oxidizing acids, such as sulfuric acid and nitric acid, would damage multiple MPs [31].

Alkaline solutions have been demonstrated to effectively digest biogenic samples. Foekema et al. [98] used 10% KOH to digest fish digestive tracts over a 2–3-week period, but did not evaluate its impacts on MP particles. Compared with enzyme-based and acidic treatment, Cole et al. [31] tested sodium hydroxide (NaOH) as digestion solutions. They found that 10 M NaOH solutions were the optimal for alkaline digestion. However, this solution had an obvious impact on nylon, polyvinylchloride (PVC), and polyethylene particles. However, the treatment of alkalis did not impact MPs in terms of results from Catarino et al. [103] and Nuelle et al. [81].

Oxidizing treatments of hydrogen peroxide (H_2O_2) have been frequently applied for the digestion of natural organic debris. For instance, 30% H_2O_2 treatment of the dried sediment samples, residues on the filters after filtration, or the surface of MPs themselves can effectively remove large amounts of organic impurities [85]. Mixtures of H_2O_2 and other reagents, such as sulfuric acid (H_2SO_4), can rapidly eliminate the natural organic matters within the samples [104]. Considering slight or negligible effects of H_2O_2 on multiple types of MPs, the oxidizing treatment method of H_2O_2 is the most popular protocol for digesting organic matter to purify MPs.

The Fenton reaction is a combination of H_2O_2 with a Fe(II) salt and has been first suggested as a purification method for water and sediment samples [88]. However, this first version of the method included the use of elevated temperatures and was not checked for its harm to the different polymer types. In a more recent paper, the pH was controlled, and the reaction was cooled to avoid high temperatures that might degrade plastics [99]. This method gave a good digestion of organic matter but did not affect polymers.

As an emerging approach, enzymatic degradation has been recently applied to remove organic matter. According to previous studies, MP-containing samples were incubated with a mixture of technical enzymes including lipase, amylase, proteinase, chitinase, or cellulose [31, 105]. These enzymes attack very specifically certain types of organic compounds such as proteins, lipids, and carbohydrates. Cole et al. reported the application of the proteolytic enzyme proteinase K in the treatment of marine samples [31]. Results show that up to 97% of organic materials were removed in plankton-rich seawater samples, whereas MP debris was not affected. However, enzymes are usually costly, and the enzymes need extended time to digest organic compounds. The enzymes are rather specific for certain organic compounds, and the combination of enzymes needs to be adjusted for different sample types. Furthermore, the method needs to be additionally combined with an additional oxidation treatment (e.g., H_2O_2) to fully remove organic matter [32].

4 Quantification and Identification of MPs

4.1 *Morphology Characterization, Quantification, and Classification*

After extraction and purification, the suspected MPs need to be further analyzed and classified. Visual inspection is still the most common method used. It can be achieved by naked-eye observation or with the aid of an optical microscope (typically a stereomicroscope), with the help of certain selection criteria [5, 106]. Visual inspection is convenient and can be used as a first step to reduce the number of particles for subsequent spectroscopic characterization. Shapes, colors, and texture are the bases to determine whether a suspected item is MP [107]. In this process, the experience of researchers is important. MPs vary in shape from irregular to spherical and long-thin fibers. Plastic pellets present tablet-like, oblong, cylindrical, spherical, and disk shapes, mostly spherical to ovoid with rounded ends [108]. The most common colors found were white or related. Colors of special MPs can facilitate separation of MPs from a large quantity of other debris. These colorful particles have a high probability by eye picking, in favor for subsequently chemical identification. However, the plastic items with dull colors are easily overlooked; this observation bias usually results in underestimation of MP numbers [5]. In addition, visual inspection may not provide accurate information about MP abundance due to the presence of similar particles such as clay and algae. If no digestion treatment, it is very hard to visually differentiate MPs from other non-plastic particles with similar sizes and shapes. In addition, visual inspection is unreliable to distinguish MPs from the enormous variety of other inorganic particulate matters or organic fibers from cellulose and starch debris. According to experience, visual inspection and determination of MPs are strongly affected by several factors, such as personal factors, microscopy quality, and sample matrix characteristics. Furthermore, the practice of visually counting MPs suffers the drawback of size limitation of some small-sized MPs. Up to 70% error rates were reported in the visual inspection; the number of error increases with a decrease in the size of MPs [5].

Scanning electron microscopy (SEM) can provide high-resolution images of MPs by sending a high-intensity electron beam at the sample surface and scanning it in a raster scan pattern. Surface details (up to 0.5 nm resolution) of plastic items can be imaged by the electrons at very high magnifications. Potential MPs can be differentiated from other organic or inorganic impurities by examining the high-resolution images of their surface morphology [107]. SEM can also be used to analyze the weathering progress of MPs in natural environments, mostly in terms of the changes of surface textures on plastic particles, like cracks and pits [109]. Although SEM can provide morphology information of very small particles [110], it has the disadvantages of being cost-expensive and time-consuming. The technique is even more restricted (no color) in differentiating between plastics and other particles and thus has a high potential for wrong classification; furthermore it does not give any information about the type of polymer [110, 111].

In general, the methodologies of characterization, quantification, and classification are not standardized yet, and the abundance unit of MPs and the size ranges monitored are still nonuniform up to date. Therefore, researchers need to reach a consensus about the protocol of qualification of MPs.

4.2 Identification of MP Polymer

Chemical and physical identification of the potential items is pivotal to accurately analyze MPs. Micro-FTIR is a powerful and commonly used technique for MP identification. The spectrometer signal depends on the change in the permanent dipole moment of a chemical bond, making it sensitive to polar functional groups (e.g., in different plastic polymers). The FTIR signal can be obtained by reflection or transmission of the IR signal or by attenuated total reflection (ATR), while transmission is the best method for small MPs. Some FTIR spectrometers are equipped with focal plane array (FPA) detectors, which have a lower spectral quality compared to the normal mercury cadmium telluride (MCT) detectors, but allow to analyze the whole filters with a high resolution by utilizing up to 128×128 detectors [100, 112]. FTIR microscopes have spatial resolutions down to $5 \mu\text{m}$ [113]. However, FTIR requires a minimum sample thickness (150 nm) and sample deposition onto an IR-transparent substrate [105]. Because of these limitations, FTIR is best suited for items larger than $20 \mu\text{m}$. However, agglomerates or films of smaller particles may be analyzed by μ -FTIR [105].

In contrast, Raman spectroscopy is suitable to identify MPs of smaller sizes down to about $1 \mu\text{m}$. If a MP particle is exposed to monochromatic light, molecular vibrations cause the scattering of the light and allow for identification of the polymer type [114]. The main advantage of Raman spectroscopy is that identification is based on the complete wavelength spectrum and amorphous carbon can be detected. For instance, even after degradation following exposure to UV, the Raman spectra of MPs is not significantly altered [114]. The spatial resolution of Raman microscopy is approximately $1 \mu\text{m}$, so particle shape and thickness of small MPs do not influence the measurement. Therefore, Raman spectroscopy is an approach more suitable to identify small MPs in comparison with μ -FTIR [115].

Mass spectrometry-based methods are also used to analyze the polymer type of MP. For the mass spectroscopic analysis, the MP needs to be digested or burned to analyze a liquid or gas sample. Thus, the methods cannot give information like size and shape of MP particles but the polymer type and the quantity of the MPs. One mass spectroscopic method used for the quantification of MPs is thermal desorption coupled with gas chromatography-mass spectrometry (TDS-GC-MS). For the analysis the samples are placed onto thermogravimetric balances and heated up to $1,000^\circ\text{C}$ [116]. Degradation products are adsorbed onto a solid phase and then transferred to a thermal desorption unit, which desorbs the compound with specialized heating programs. These samples are then analyzed after separation of the different compounds on a chromatographic column and identification by mass

spectrometry. TDS-GC-MS technique needs relatively much sample material (up to 100 mg) [117].

Pyrolysis coupled with gas chromatography-mass spectrometry (py-GC-MS) is a similar identification approach. The sample is decomposed at high temperatures, following analysis by mass spectrometry after gas chromatographic separation. For py-GC-MS only small sample masses are possible to be analyzed, and even single MP particles can be analyzed. However, single-particle analysis is very time-consuming and not reasonable for higher particle numbers. For bulk analysis py-GC-MS might require a pretreatment similar to FTIR or Raman spectroscopy. For py-GC-MS, it is hard to get an inter-lab reproducibility, because identification results are highly dependent on sample preparation, pyrolysis type, and pyrolysate transferring. In the process of sample preparation, electrically heated filament pyrolysis was used by a resistively heated coil [118]. In furnace pyrolysis, the solid sample can be put into a preheated furnace; a carrier gas transports the pyrolysate into the gas chromatography column [118]. In fact, py-GC-MS can simultaneously identify the polymer type and contained additives in MPs.

5 Quality Assurance and Quantity Control

In order to avoid contamination, it is absolutely necessary to carry out quality assurance and quantity control (QA/QC) in the whole process. In sampling, plastic tools must not be used because of plastic leaks. Glass, steel, or glass materials are recommended as well as working with latex gloves and cotton cloths to avoid additional plastic contamination. During the extracting process, since there are some synthetic fibers in the atmospheric fallout, blank or control samples should be prepared to exclude contamination with airborne fibers, and air circulation and exposition time should be minimized. Furthermore, all reagents and distilled water need to be filtered for use, and all devices were rinsed three times with distilled water.

6 Summary

In this chapter, we reviewed analytical methods for analyzing MPs in multiple environments including water, soil, atmosphere, and biota. Most studies use similar protocols including sampling, sieving, floatation, filtration, purification, identification, and classification. Despite numerous publications about MPs, the analytical method has not been standardized yet. A result of the lack of the standardization is that the findings of different studies are not comparable. The main problems in the establishment of standard methods include the following: (1) Sampling protocols about site contributions, sample number, volume, and sampling frequency have not been clearly defined for the different environment matrices. (2) Storage and transport

of samples for MP analysis need to be standardized. For example, some MP fractions might stick to the wall of containers, resulting in losing MPs during the procedure. (3) Different studies deal with different size ranges of MPs, and thus the generated MP counts are not comparable. (4) The organic matter and other impurities cannot be effectively removed in the sample pretreatment. On the other hand, many sample pretreatment methods might affect certain polymer types. Standardized digestion protocols using environmentally friendly reagents need to be established for soil, sediment, and biota samples. (5) It remains a challenge to extract and identify small MPs. (6) Current analytical protocols are mostly based on manual handling, such as digesting and extracting samples one by one and counting and classifying MPs individually which is time-consuming and cost-expensive. Automatic separation technology might help to make MP analysis more effective. (7) The analytical techniques for MP analysis might give different results. Optical identification might cause inaccurate results due to misinterpretation and limitation to larger MPs. FTIR and Raman microscopy differ in the size range they analyze. These imaging methods will give results as particle count (number of particles kg^{-1}), size and shape, and particle-specific polymer composition, which will not be comparable with mass spectroscopic methods which will report bulk MPs and polymer-specific concentration (e.g., mg kg^{-1}).

Future research should focus on establishing standardized methodologies for sampling and extracting MPs from different types of environmental matrices; developing efficient analysis techniques, such as basing on fully or semiautomated analysis; and promoting accurate identification and quantification technology for MPs. Strict quality control criteria should be defined, and certified reference materials are needed to allow for high-quality comparable results. In addition, there is an increasing demand to develop reliable and efficient methods of detecting plastic particle at even smaller sizes down to the nanoscale.

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Identification and Characterization Methods for Microplastics Basing on Spatial Imaging in Micro-/Nanoscales



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Abstract Microplastic pollution is a global problem in recent decades due to their ubiquity in the oceans, sediment, soil, or wastewater. The bioavailability and adsorbability for toxic chemicals of MPs have detrimental effects upon living organisms. This work attempts to provide a critical overview on modern instrumentation and promising techniques for identifying and visualizing micro- or nanoplastics. Fourier transform infrared spectrum (FT-IR) and Raman spectroscopies combined with microscopies, scanning electron microscopy (SEM), hyperspectral imaging (HSI), and confocal laser scanning microscopy (CLSM) were widely used in the identification and visualization of microplastics in organisms and environments. The advantages and limitations of each identification and characterization method were indicated for MP analysis basing on spatial imaging in micro-/nanoscales. In addition, some novel methods may possibly be applied to micro-/nanoplastics identification, such as atomic force microscope (AFM), which may be used to identify and characterize the surface morphology, chemical composition, thermal and mechanical properties of MPs at the nanoscale. However, there is a need to improve and develop new methods to reduce the identification time and effort for sub- or nanom micron plastics and obtain more useful physical and chemical information in environmental MPs.

Keywords Characterization, Identification, Microplastics, Spatial imaging

1 Introduction

Microplastic pollution is a topic of increasing concern to the society [1], which has emerged as a global contaminant of serious concern to human and ecological health [2, 3]. Daily discharges of microplastics (MPs, defined as <5 mm in size) are estimated in the range of 50,000 up to 15 million particles, whereas little information on nanoplastics (NPs, <100 nm) release is available [4, 5]. Researchers generally believe that NPs, which are widespread in the environment, may be more hazardous than MPs because they can permeate biological membranes [6–8].

The main sources of MPs and NPs include (1) polymer nanoparticles manufactured for specific purposes [9]; (2) degradation of plastics by UV, mechanical forces, hydrolysis, and microbiology; and (3) wastewater treatment plant discharges [10, 11]. MPs are ingested by marine and terrestrial organisms, and there is some evidence of translocation beyond the gut and fewer evidence of transfer from one trophic level to the next [12].

MP and NP exposure has already been associated with toxic effects, including enhanced immune response, decreased growth rate, fecundity, and negative impacts on subsequent generations [13]. In parallel, these particles can transport other harmful chemicals used as additives in their fabrication or adsorb to them because of the strong adsorption capacity of MPs and NPs, thereby facilitating the entry of

Table 1 Advantages and disadvantages of currently identification and characterization methods for MPs basing on spatial imaging

Method	Advantages	Disadvantages
Micro-FT-IR	Polymer composition of major or typical MP types Chemical mapping directly	Possibility of false positive Possibility of missing small (<20 μm) and transparent MPs Poor spatial resolution (10–20 μm) Long operating time NPs can't be identified
Raman	Nondestructive identification of very small MPs (<20 μm) Wider spectral coverage Higher sensitivity to nonpolar functional groups Low water interference High spatial resolution (1 μm)	Fluorescence interference Long measurement time Can't real-time monitoring NPs can't be identified
HSI	Noninvasive and nondestructive to identify chemical compounds of each spatial pixel according to their spectral information Can directly identify and distinguish MPs (0.5–5 mm) without separation	Huge number of data and redundant information Data processing is complex NPs can't be identified
SEM	High-resolution images Two-dimension imaging The surface morphology of NPs can be identified	Expensive Sample preparation and examination are time-consuming, and complex MP's colors can't be identified
CLSM	High spatial resolution Can identify MPs or NPs in organisms or cells Three-dimensional imaging	Fluorescence interference Expensive and high technical requirements
AFM	Nanometer resolution Three-dimensional imaging AFM can together with nano IR, nano TA, and LCR to evaluate various polymer NPs can be identified	The imaging range is small The speed is slow Too much influence by the probe

these substances into organisms [14–16]. Thus, robust analytical methods are required to identify and characterize spatial imaging for MPs and NPs both in exposed organisms and the environments.

At present, the most commonly used methods to identify and characterize MPs based on micro-/nanospatial imaging are still Fourier transform infrared spectrum (FT-IR), Raman spectrum, and scanning electron microscopy (SEM). Certainly, some new recognition and characterization methods, such as a confocal laser scanning microscope (CLSM), hyperspectral imaging (HSI), and atomic force microscopy (AFM), have also attempted to be applied to the identification and characterization of MPs at the micro-/nanoscale. Each method and various combinations have its own advantages and disadvantages, as shown in Table 1. However, identification and visualization of MPs and NPs in micro-/nanoscales are still a challenge, whether from wastewater, oceans, sediment, or soil.

2 Fourier Transform Infrared (FT-IR) Spectroscopy

FT-IR microspectroscopy (micro-FT-IR) is a tool that combines FT-IR spectroscopy with microscopy, which is a common technique to characterize MPs in micron-grade [17–19]. Micro-FT-IR is particularly useful as it requires little sample preparation and can be used to identify MPs directly on membrane filters [20]. Currently, analyses of MPs can be performed in transmission [21, 22], reflectance [23], and attenuated total reflectance (ATR) modes [24]. Among them, transmission mode gives high-quality spectra but requires infrared transparent substrates [25], while reflectance mode [26] enables the rapid analysis of thick and opaque samples, and ATR mode [24] produces stable spectra from irregular microplastic surfaces. Therefore, these modes are highly suitable for detecting MPs in environmental samples.

As a vibration spectrum, different MPs exhibit different IR spectra, so that IR can be used as a fingerprint for MP identification. When the IR beam is scanned on the sample to record the fingerprint spectrum along with the position information (X–Y axis), an image can be generated from the IR intensity to map and visualize the MP's distribution [27]. A previous study by Tagg, Sapp et al. [28] shown that micro-FT-IR imaging was an effective method for the visualization of PE, PP, PS, nylon-6, and PVC in wastewater. Harrison et al. [20] eliminated the need for visual sorting of microplastics by introducing a chemical mapping technique using reflectance micro-FT-IR spectroscopy.

However, with this point-by-point-based mapping approach, scanning an entire membrane filter would take an implausible amount of time, and the use of smaller apertures would further increase the scanning time [29]. These drawbacks could be improved with the focal plane array (FPA)-based reflectance imaging method. This technology can provide information for the identification of MPs on larger surface areas, at faster times and without compromising spatial resolution compared with single beam mapping [28].

3 Raman Spectroscopy

Raman spectroscopy serves as another common method for the characterization of MPs [30–32]. Raman is a nondestructive technique requiring minimal sample preparation, with a highly specific fingerprint spectrum and negligible interference from water, which provides the possibility for direct analysis of aqueous sample [33]. Another advantage of Raman mapping is the capacity of signal enhancement [34, 35]. Scientists have implemented Raman to map MPs toward their identification and visualization, with a lateral resolution down to 1 μm . When a visible laser source (<800 nm) is employed, the lateral resolution of identification and mapping can be high [36].

It was reported that Raman could identify MPs down to 10 μm in the marine environment; at the same time, replicate Raman spectra were recommended as point

measurements on different spots of each particle toward the identification of the plastic particle [37, 38]. Usually, the Raman signal intensity depends on the surface morphology of the plastic sample and the position of the focal plane on the sample. The mapping image is generated by the Raman intensity at the selected characteristic peaks to visualize MPs, such as PS ($1,000\text{ cm}^{-1}$), PET ($1,720\text{ cm}^{-1}$), PE ($1,059\text{ cm}^{-1}$), PVC (695 cm^{-1}), and PP (402 cm^{-1}) [36]. The suspicious part from image can be further identified by extracting the full Raman spectrum at the mapping pixel. It is compared with the standard spectrum (fingerprint) of different plastics to verify and confirm whether the mapping signal is from a specific plastic or noise. However, Raman spectroscopy is sensitive to the pigment chemicals, organic matter, and the additives in the MPs, which from the surface groups that appear due to the weathering/aging/degradation and the polymer density (low or high) [28, 31, 39, 40].

4 Hyperspectral Imaging (HSI)

An innovative approach, based on hyperspectral imaging (HSI), was developed in order to set up an efficient method to directly identify and distinguish microplastic classifications and morphological and morphometric analyses from the non-polymer components [41]. HSI contains both the spectral and spatial image information, indicating that detailed spectral information can be extracted from each pixel of the image. As a rapid, noninvasive, non-destructive, and reliable technology for the characterization of the microplastic waste, HSI opens up a promising way for improving the plastic pollution monitoring [42, 43]. A common automatic classification system has been developed to recognize polypropylene (PP), polyethylene (PE), and polystyrene (PS) [41].

It has rapidly emerged and fast grown in the plastic waste sector for the identification of different polymers. Reliable information on abundance, size, shape, and polymer type for the whole ensemble of plastic particles in each sample were retrieved from single hyperspectral images [44–48]. Based on the advantages of this method, hyperspectral remote sensing has been used for large pieces of debris identification in the marine litter monitoring field [49–51]. HSI techniques combined with image processing and chemometric methods were also investigated to directly determine and visualize the MPs with particle size from 0.5 to 5 mm on soil surface, which contains different materials, including MPs, fresh leaves, wilted leaves, rocks, and dry branches [52]. However, the Hughes effect has the negative impact on the classification of HSI due to the highly correlated and redundant data, causing a decrease of the classifier's accuracy [43].

5 Electron Microscope (EM)

Scanning electron microscopy (SEM) and transmission electron microscopy (TEM) can provide extremely clear and high-magnification nanoscale images of MPs or NPs. High-resolution images of the surface texture of the particles can facilitate the discrimination of MPs or NPs from organic particles [53–55].

SEM observations enabled the detection of microbes sitting in pits and grooves, suggesting degradation of the MPs surface [56]. To visualize the composition and the spatial structure of microbial communities on MPs or NPs, this method offered a useful firsthand look at bacteria, diatoms, and other protists and small invertebrates on MPs surfaces [57–62]. While SEM offers a detailed look at life on the surface of plastic litter, it is limited in terms of the taxonomic resolution recovered from this approach. Energy-dispersive X-ray spectroscopy (EDS) combined with SEM can further differentiate MPs of other particles, such as shells, fish scales, ceramic flakes, and other materials from MPs, thanks to the elemental analysis [63–68].

However, the colors of MPs cannot be used as identifiers in SEM. At the same time, SEM-EDS is also expensive and requires substantial time and effort for sample preparation and examination, which limits the number of samples that can be handled.

6 Confocal Laser Scanning Microscopy (CLSM)

Not only SEM, TEM, and other electron microscopy techniques but also optical microscopy such as confocal laser scanning microscopy (CLSM) has been widely used in polymer science. CLSM is considered as the useful technique to characterize several types of nanosized plastic structures and particles in cell or organism [69]. For example, CLSM demonstrated that 100 nm polystyrene (PS) MPs can be accumulated in *V. faba* root and most probably blocked cell connections or cell wall pores for transport of nutrients. The application of CLSM has been provided a new insight into the toxic effects of MPs on *V. faba* and further is used to the ecological risk assessment of MPs on higher plants [70]. CLSM technique was also found to be suitable to detect and characterize the intake of NPs in simple matrices (e.g., *Daphnia magna* and *Oryzias sinensis*) by CLSM. For example, the analysis of *D. magna* by CLSM and Z-stack imaging was used to confirm the presence of NPs in the inner guts [71].

However, this technique still has some shortcomings, such as the contradiction between scanning speed and phototoxicity degree, resolution, which restrict the range of fluorescence imaging observation of living cells and tissues. In addition, its high price and high technical requirements still limit its more application.

7 Atomic Force Microscopy (AFM)

Atomic force microscopy (AFM) is able to achieve the nanoscale resolution often necessary for miscibility evaluation for polymer. However, this technique is not able to identify the chemical composition of different phases, resulting in the physical and chemical properties of polymer which are very limited. Therefore, atomic force microscopy-based infrared, thermal, and mechanical are widely used in material, pharmaceutical, and polymer analysis to obtain more information [72–82].

Standard atomic force microscopy (AFM) imaging together with nanoscale infrared spectroscopy (nano IR), nanoscale thermal analysis (nano TA), and Lorentz contact resonance (LCR) measurements were used to evaluate the miscibility behavior of various polymer ratios. These combined technologies show clear contrast between the continuous and discrete domains for phase-separated system [82]. Therefore, it may be a potential candidate for source analysis of MPs in the environment.

AFM-IR can acquire IR absorption spectra and absorption images with spatial resolutions of 50–100 nm [83], which can be used to analyze NPs in the environment [84]. However, it is difficult and time-consuming to find a nanosized single particle to focus by AFM-IR in an unknown sample.

A nano TA ramp is obtained by heating the probe linearly with time, while the extent of cantilever bending is recorded. When a thermal event occurs, the sample surface becomes softened, and then the AFM tip penetrates into the sample. The local maximum in the temperature ramp is typically defined as the onset of the thermal event [85]. This method may obtain the thermal properties of environmental MPs at the nanoscale.

Lorentz contact resonance (LCR) AFM is a type of contact resonance AFM. In contact resonance AFM, information about the viscoelastic properties of a sample in contact with an AFM probe can be evaluated at the nanoscale by measuring the contact stiffness between the probe and the sample [86, 87]. The amplitudes and peak frequencies of the oscillation are determined by the mechanical properties of the material in contact with the AFM tip. By recording the oscillation amplitude as a function of position at a certain frequency, surface images that reflect differences in the relative stiffness of each component can be obtained [82]. This technique may provide an effective way to study the mechanical properties of MPs at the nanoscale.

8 Conclusion and Prospect

Identification and characterization methods for MPs basing on spatial imaging in micro-/nanoscales remain challenging and are under development. The most commonly used analysis methods are infrared spectrum and Raman spectrum imaging,

which can identify MPs at the scale of millimeters in varied and complex environmental matrices. However, these methods are not suitable for the identification, characterization, and spatial imaging of NPs with greater impact on ecological and human health.

SEM, TEM, CLSM, and HSI, which are fully or semi-automated analytical methods, are widely used in the identification, characterization, and spatial imaging of MPs and NPs in environment and organisms, in order to obtain more physical and chemical information. However, all of these methods have some disadvantages, which limit their wide application in MPs research.

Therefore, new technologies should be developed and utilized to provide more physical and chemical information for MPs based on micro- and nanoscale spatial imaging. For example, AFM is able to provide surface morphology, chemical composition, and thermal and mechanical properties of MPs at the nanoscale or NPs. This information is important for MPs source analysis, transport, and fate analysis. However, the identification and characterization of MPs or NPs in the environment by AFM has not been reported, and therefore, environmental scientists should pay more attention to it.

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Part II
Source, Fate, Distribution of Microplastics
in Terrestrial Environments

Microplastics in Urban Environments: Sources, Pathways, and Distribution



Rong Qiu, Yang Song, Xiaoting Zhang, Bing Xie, and Defu He

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Abstract Due to high-density anthropic activity, the urban environment is regarded as one of the major sources of microplastics (MPs). MPs can be produced in the process of tire wear, landfill and sewage treatment, construction, industrial activity, household laundry, and so on. According to recent studies, MPs have been widely detected in urban atmosphere, ground surface dust or soil, and municipal rivers. Due to lightweight and low density, MPs can easily float and transform between different environmental matrices in urban ecosystems. Storm-water runoff is regarded as an important pathway of MP from land to urban rivers or coastal waters. By wind transportation, MPs on the municipal ground surface can be transferred to urban rivers or the atmosphere. After treating sewage treatment plants, concentrations of MPs can be extremely reduced in the discharged water but increased in the sludge. MPs have also been found in landfills and may leak into other environmental substrates. It can be concluded that MPs can migrate and transform among multiple urban environments through physical and biochemical drivers. Distribution and

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transformation of MPs are closely related to the urban ecological environment and also pose a potential risk on the health of urban residents. More research work needs to fully reveal the source and fate of MPs in urban environments.

Keywords Characteristics, Microplastics, Occurrence, Source, Urban

1 Introduction

Increasing urbanization is an actual threat to the surrounding environment. The urban environment is characterized by high-density residential and anthropic activity. Since plastic products are increasingly used by urban resident, plastic waste and their decomposition outcome microplastics (MPs) have become an emerging environmental issue of increasing concern. Cities are commonly regarded as one of the major sources of MPs, which mostly include packaging, textile, furniture container, transportation, electronics, and construction materials. These plastic products can be further crushed and fragmented into MPs [1–3]. MPs can be transferred among different environment matrices, such as urban atmosphere, surface grounds (dust), soil, and water body (urban rivers or lakes).

Figure 1 shows the schematic diagram of sources and transference of MPs in urban environments. MPs come from household activity, industrial production, urban runoff, atmospheric activities, sewage treatment plants, etc. [4]. Specifically, MP items largely generate from accidental loss of plastic particles during the factory

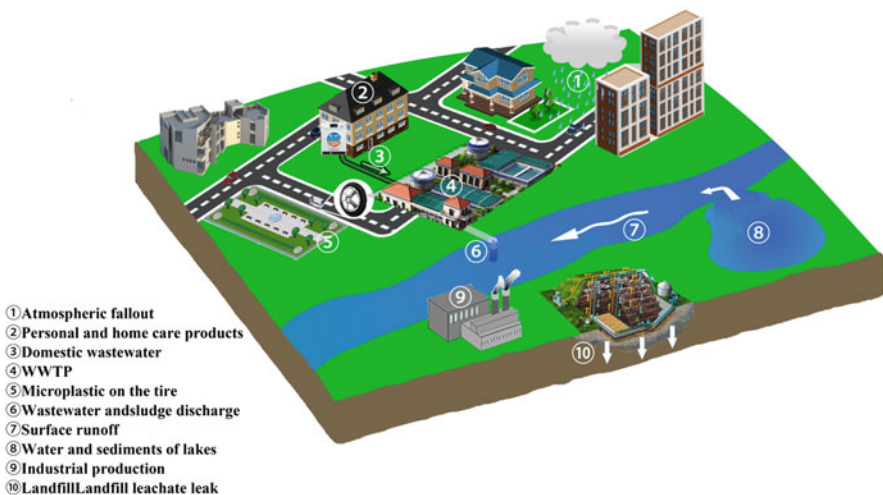


Fig. 1 schematic diagram of sources and transference of MPs in urban environments

and transportation and fiber loss during the washing process of textiles and other daily activities [5]. Browne et al. [6] considered that domestic sewage provided a possible way for the entry of MPs. In addition, there are other ways, including the fragmentation of larger items, the introduction of small particles used as abrasives in cleaning products, and overflow of plastic powder and particles. Recent studies have also shown that the high level of plastic contamination in urban freshwater systems, whose concentration is comparable to that in marine systems [7]. Actually, the presence of MPs in municipal water is considered as important transport carriers for terrestrial MPs to the coastline and the open sea environment [2]. Freshwater systems, especially urban rivers [8], are deemed as an important medium for transferring plastic fragments [9]. MPs were widely detected in multiple urban lakes, rivers, and sewage treatment plants in China [10–13]. Tire wear particles on roads and the polymer of the paint are considered as sources of MPs in urban environments [9]. Tire-derived MPs can further transfer into urban rivers or atmosphere, through surface runoff or wind. In the urban ecosystem, MPs can be randomly distributed into the atmosphere, soil, water column, and sediment, by way of precipitation, surface water erosion, sedimentation, etc. [14]. Horton et al. [9] pointed out that sewage, roads, and surface runoffs were sources of MPs in sediments of the Thames river [9]. Other studies had shown that anthropogenic influences and hydrodynamics have the potential to affect the accumulation and transport of urban MPs [8, 15]. According to the diagram in Fig. 1, MPs show complicated environmental behavior in the urban system. In this chapter, we will review the possible sources, paths, and distribution of MPs in urban environments.

2 Microplastics in Urban Atmosphere

MP appearance in the urban atmosphere is generally closely linked to intensive anthropogenic activities [15]. Nevertheless, to date, a few researchers have investigated the emerging pollutants in the atmosphere. Dris is the first scholar who pays attention to MPs in urban atmosphere [16]. He pointed out MPs were easily transported by wind and could exist in the atmosphere for a long time. Due to their small size and relatively low density, atmospheric MPs thereby can have impacts on the urban ecosystem [17]. MPs can also be potentially inhaled by animals and humans and thus pose a threat to human health [18]. According to a recent study [16], wind and atmospheric deposition can transfer MPs to remote places, eventually entering marine environments.

2.1 *Source and Characteristics of Microplastics in Urban Atmosphere*

A number of recent studies showed the presence of MPs in urban atmospheres (Table 1). According to the shape of MPs, fiber is the dominated type, but small percentages in other shapes include foam, fragment, and film [17, 19–22, 24]. So the fiber-dominated is one of the pivotal characteristics of MP pollution in urban air. MP fibers (MFs) present in the atmosphere can originate from a variety of sources on the ground. Due to wind and air flow, MFs can float and enter into the human respiratory system like other pollutants [17]. It is presumable that synthetic textiles, erosion of synthetic rubber tires, and city dust are the main sources of these MFs [18]. It was reported that more than 90 million metric tons of textile fibers were globally produced in 2011. Two thirds of the production is synthetic and plastic fibers, mostly including polyethylene terephthalate (PET), nylon (rope and woven products), and rayon [20, 21, 24]. The fibrous plastic has grown by about 6.6% per year over the past decades [25]. It is predictable that the degradation and fragmentation of these fibers produce the prevalence of fibrous MPs. It is noted that fiber-dominated MPs have also been observed in indoor space as well as in atmospheric fallout of outdoor environments [19]. In addition, the commercial use of fine diameter (1–5 mm) plastic fibers was considered to produce MFs in certain ways, such as in the sportswear industry [26]. Significant developments in the synthetic fiber industry and the widespread use of inexpensive non-woven fabrics may be helpful to explain the prevalence of microfiber in the atmospheric environment [27].

In daily life, plastic fibers are commonly produced from textiles. These microfibers may be shed and released directly or indirectly when the clothing is worn or during washing and drying [28, 29]. In addition, the physical breakdown of compounds also results in microfibers in daily activities including walking and strenuous exercising, through wearing and tearing of pants. In addition, MP fibers can be produced because of mechanical wear or damage of textile clothing and bedding including pillows, blankets, and curtains. In the sun, photooxidation and thermal effects of drying clothes can easily promote decomposition and degradation of these textiles, which causes the release of microfibers [30]. Afterward, these fibrous MPs can be broken into smaller-sized fine items through wind shear or wear and other environmental drivers [26].

In urban atmosphere, multiple shape MPs can come from waste disposal, road traffic, and so on. Actually, the majority of plastic waste is disposed by dumping into open soil landfills, which gives plastic plenty of exposure to the atmosphere. The continuous exposure can increase the chance of coarse plastic fragments breaking up, which results in the release of MPs [31, 32]. In addition, human activities on the ground, such as industrial cutting or grinding synthetic materials, mowing grass, and automobile tire wear, can produce a significant amount of MPs, which are further transferred into the atmosphere [27, 33].

A recent study showed that atmospheric MPs appear at different rates and sizes for several months [24]. It was because meteorological factors, such as weather,

Table 1 Microplastics in urban atmosphere

Survey site	Survey date	Analytical methods	Collect result		References
			Polymer type	Shape	
Urban in France	2015	Collected through a steel funnel	PET; polyamide; the mixture of PET and polyurethane; the mixture of cotton and RY	Fibers (67%, 200–400 μm and 400–600 μm are pre-dominant size ranges) Fragment (30%) Granules (3%)	110 \pm 96 particles $\text{m}^{-2} \text{day}^{-1}$ [17]
Paris indoor (air and dust), France	2015	Through a pump and a filter (1.6 μm , 47 mm)	The mixture of cotton and polyamide; polypropylene; polyamide fibers and copolymers of polypropylene and polyethylene	Fiber smaller than 3,250 mm	2,070 and 7,300 fibers $\text{m}^{-2} \text{day}^{-1}$ [19]
Paris (outdoor), France	2015	Same as above	–	Fibers smaller than 1,650 mm	0.3–1.5 fibers m^{-3} [19]
Dongguan, China	2016	Using a sampling device equipped with a glass bottle	PE (14%), PP (9%), PS (4%), RY	Fiber (mostly) foams, fragment, film	Average 36 \pm 7 particles $\text{m}^{-2} \text{day}^{-1}$ [20]
Yantai, China	2014–2015	Pre-filtered through a set of stainless steel sieves (mesh sizes of 5 and 1 mm)	PET, PE, PVC, PS	Fiber (mostly) foams, fragment, film	1.30 \times 10 ² –6.24 \times 10 ² particles $\text{m}^{-2} \text{day}^{-1}$ [21]
Sakarya province, Turkey	December 2016–May 2017	Through an air vacuum filter (50 and 500 μm) device	–	Average fragments (50%) and fibers (50%)	Average eight items 30 min^{-1} [22]
Shanghai, China	March and April in 2018	An intelligent flow collected suspended particulate sampler	PET, PE, PES, PAN, PAA, RY, EVA, EP ALK	Fiber (67%) Fragments (30%) Granules (3%) 23.07–9,555 μm	0–4.18 nm^{-3} (items per cubic meter of air) [23]

wind speed and direction, humidity, temperature, and cyclones, can affect the amount of MPs in urban air [17]. The amount of atmospheric MPs may be dependent on a number of factors. For instance, rainfall can change MP abundance [22]; however, no significant correlation had been found between MPs in atmospheric fallout and the rainfall in a study of Paris [17]. Another report showed that the deposition flux of MPs varied seasonally with lowest deposition flux in autumn, which was mostly due to the variability of meteorological conditions in different seasons [21]. In addition, consumption habits and socioeconomic status of local humans, transportation, and urbanization are also related to the amount of MPs in urban atmospheres.

In a closed or semi-closed compartment, MPs are usually produced through mechanical wear or damages of textile clothing and bedding such as pillows, blankets, and curtains. A study showed that the MP concentration, especially microfibers, in indoor air (1–60 fibers m^{-3}) was higher than that in outdoor air (0.3–1.5 fiber m^{-3}) [19]. Therefore, indoor exposure to airborne MP fibers or particles may pose a threat to human health [19, 34]. Comparatively, occupational air exposure to MPs may be of high risks than household exposure. It is noted that some special factories using high volumes of polymeric materials, and lack of efficient ventilation, may result in chronic exposure to high concentration of airborne MPs. Furthermore, indoor MPs can persistently enter outside atmospheres [19]. But only 30% of outdoor particles can penetrate the indoor rooms in terms of an estimation [35]. Therefore, indoor air is the main source of atmospheric MPs in the urban environment [18].

2.2 Fate and Distribution of Airborne MPs

Airborne MPs could become a source of contamination for terrestrial and aquatic ecosystems. Due to light and low density, dynamic exchange of MPs among various environmental systems occurs frequently [18]. Just like over the sea, atmospheric aerosols can spray and generate MP particles in the urban air [36, 37]. This process could be associated with the MP cycle in the urban environment. MPs in the urban atmosphere can be carried by wind and fall to the ground and plants on the surface and by precipitation or unstable atmospheric disturbances [38]. A recent study showed some similarities in morphological characteristics and chemical composition between marine MPs and terrestrial MPs, which indicates that marine MPs may be derived from terrestrial environments through atmospheric transportation and deposition. These MPs, major microfibers in the atmosphere, could be also transported and deposited on surfaces of ground in cities [39].

The environmental behavior of MPs in the atmosphere may be similar to other air pollutant particulate matters [18]. Influenced by density and buoyancy, atmospheric MPs present vertical distribution characteristics, usually higher concentrations near the ground. A recent study investigated the content of MPs at different altitudes of atmospheres and showed the highest MP concentration was at 1.7 m above the

ground [9]. Additionally, wind erosion should be considered as a transport driver of MPs in terrestrial environments [40]. For instance, an increase of wind speed leads to a decrease in atmospheric MP concentrations [24]. Similarly, outdoor temperature affects the migration of MPs in the atmosphere. Additionally, urban topography, like distances between buildings, and local meteorology and thermal circulation (heat islands perturbing air flow) could also affect the distribution of MPs in air [41], especially to low-density polymers such as PE and PP MPs [9, 42]. Compared with outdoor air, the migration behavior of MPs in a closed indoor environment is not subject to these restrictions; room partition, ventilation, and airflow can have impacts on the behavior of indoor MPs [35]. Of MPs, airborne nanoparticles (<100 nm) can rapidly diffuse in indoor compartments in terms of a recent analysis [43]. However, there are, to date, limited studies on atmospheric MPs especially in urban environments. Furthermore, there are no uniform method standards for analyzing atmospheric MPs. More studies are needed to investigate the environmental behavior of MPs in the urban atmosphere.

3 Microplastics on Ground Surface of Urban Environments

High density of vehicles is another characteristic in urban areas. Some studies have shown that particles released by automobile tire wear are an important part of MPs on urban ground surfaces [27]. According to Unice et al. [44], tire wear degradation caused multiple-color paint to peel off road signs and then flowed into rivers through rainfall. Tire and road wear particles (TRWP) are formed at the frictional interface of the tire and road surface and consist of polymer-containing tread with pavement mineral and binder encrustations. Some scholars are consistent to recognize TRWP as an important source of MPs on urban surface grounds [45–48]. Due to its physical properties, tire wear could be mixed with particulate matters from the pavement or road dust and change into aggregates. It was pointed out that tire materials would account for up to 70% of MP release into the urban environment [49]. TRWP and their aggregates are eventually transported off the street through surface runoff or street cleaning and can migrate into the atmosphere by suspension [50, 51]. As another way, after crushing and recycling, these tire materials [52] can be used as filling embankment materials in lawns [53], playgrounds [54], and so on. In addition, the environmental concentration of automotive tire particles can be estimated by chemical markers, such as plastic additive in tire and rubber type [55]. The other sources of ground surface MPs include road sign paint shedding [9], beads in personal care products, and household dust generated by household plastic products [56]. Totally, these MPs can further enter into water bodies or atmospheres in the urban areas.

In the urban environment, another store of MPs is dust on the surface of the grounds. The main types of municipal waste disposal are usually dumped and exposed to sunlight. In this process, plastic waste has undergone a combination of biodegradation, photodegradation, thermal oxidation, and thermal degradation

[31, 32], as well as mechanical wear, and increases the chance of coarse plastic fragments breaking up into MPs. Therefore, many factors can determine the settlement of MPs in road dust or urban topsoil. For example, the practice of mowing grass on the roadside causes littering to be decomposed by lawn mowers, which includes plastic, and produces MPs in the process [29]. Street dust is an ideal indicator of urban environmental quality, since it can reflect pollutants from different media such as urban air, water, and soil [57]. The main sources of dust pollutants include vehicle traffic, road wear, brake pad tear and wear, road paint wear, and atmospheric deposition [58]. Pollutants generated by urban street construction can also be contained in street dust pollutants. A recent study showed that street dust was dominated by spherical particles, film, fragments, and fibers, among which large amounts of type particles were detected in the road dust in the city of Iran [38]. In another city, Bushehr, the majority of MPs was found as fibers (75.87%), and was detected in all street dust samples, with an MP concentration of 210–1,658 MP items 10 g^{-1} dust [59].

Large amounts of MPs can enter into aquatic environments through runoff from urban areas. The conveyance of MPs dependent on overland runoff can be viewed as a pathway of MPs from land to sea [56]. Flowing through the road and urban pavements, MPs are washed by the rain; some will enter the artificial pipes, and others will enter the natural reservoirs, such as ponds [27]. Additionally, a recent study showed that wind erosion would be considered as a transport pathway of MPs in terrestrial environments [40]. MPs on the surface of the ground can be blown into the atmosphere by wind through buoyancy. Therefore, MPs are itinerant on urban surface; and via various pathways, MPs likely migrate into other environmental matrices, such as aquatic environments.

4 Microplastics in Aquatic Environments of Urban Areas

4.1 *Urban Natural Water Body*

Urban river systems are important sinks for the discharge of various pollutants from local residential and industrial areas. Urban rivers can receive MPs via atmosphere, surface runoff, industrial production processes, and sewage treatment plants. Natural water body could provide a temporary reservoir of MPs in the short term [8]. Subsequently, these pollutants in urban rivers could enter into mainstreams, bigger river, or even open sea [8]. Some rivers and estuary environments have been identified to have heavy MP contamination. Rivers, especially those flowing through large cities, were considered to be the main source of land-based MPs entering the ocean [60]. In recent years, increasing studies have globally explored the presence of MPs in urban lakes and rivers (Table 2). Despite the variance of MP concentration, the presence of MPs has been widely determined in urban rivers in terms of previous studies (Table 2). Of these reports, the highest abundance of MPs was $8,925 \pm 1,591\text{ nm}^{-3}$, which was found in urban lakes in Wuhan, China [65]. According to Eriksen et al. [72], the

Table 2 MP pollution in urban water environments

Sample collection	Location	Assay methods	Concentration	Forms of MPs	Size	Polymer types	Reference
Water	Urban water in Shanghai, China	μ -FTIR	80–7,400 items m^{-3}	Majority of fibers: 37–88%	Majority were 100–1,000 μm	PES: 27.7%; rayon: 14.4%; PP: 8.7%	[8]
	Ciwangke River, Majalaya, Indonesia	Raman spectroscopy	$5.850 \pm 3,280$ particles m^{-3}	Fiber: 65% Fragment or other forms: 35%	50–100 μm : 116 L^{-1} , 101–300 μm : 44 L^{-1} , 301–500 μm : 64 L^{-1} , 501–1,000 μm : 64 L^{-1} , 1,000–2000 μm : 54 L^{-1}	Polyester and nylon	[61]
	Jiaojiang, Oujiang, Minjiang estuaries, China	Micro-Raman spectroscopy	$100.0\text{--}4,100.0$ nm^{-3}	Over 90% for fiber and granule	Over 90% for 0.5–5.0 mm Over 70% for <2 mm	Total of 41 MPs: 21 particles PP, 16 particles PE, 3 particles PTFE, 1 particle PVC	[62]
	Auckland, New Zealand	FTIR	17–303 items m^{-3}	Fragment: 39% Fiber: 34% Film, foam, pellet	Majority were 63–500 μm	PHM, EEAC, PP, PE, EVA, EPDT, EVAC	[7]
	Four estuarine rivers in the Chesapeake Bay, USA	Raman microspectroscopy	<1.0–>560 $g\ km^{-2}$	Fragments (0.3–5.0 mm), synthetic fibers, extruded polystyrene	–	PE	[63]
	Raritan and Passaic rivers, New Jersey	Pyr-GC/MS, HS-SPME/GC-ITMS	28,000–3,000,000 particles km^{-2}	Fragment: 38% Foam, line (fiber, filament), film, pellet	1 mm to >4.5 mm: 71% 0.3–0.99 mm	2-Butanone, 4-(2,6-trimethyl-1-cyclohexen-1-yl)	[64]
	Urban lakes and reaches in Wuhan, China	FTIR	$1,660.0 \pm 639.1\text{--}8,925 \pm 1,591$ nm^{-3}	Fiber: 52.9–95.6%, granule, film, pellet	<2 mm: 80%	PET, PP, PE, nylon, PS	[65]
	The Pearl River along Guangzhou City, China	Microscopy	379–7,924 items m^{-3}	Fiber: 80.9% Fragments: 18.9% Films: 2.2%	0.02–1 mm: 44.8% 1–2 mm: 36.5%	PP: 35.7% PE: 28.6% PET: 28.6% Other: 7.1%	[60]
	The River Tame and its tributaries in Birmingham, UK	(FTIR)	165 particles kg^{-1} (mean)	Fragments: 49%; fibers: 22%; foams, films, irregular spheres, and commercial fragments	250 μm to 1 mm: 46%	PE: 50% PVC: 30% PMMA: 20%	[66]
	The fjord (Byfjorden) in	FTIR	12,000–200,000 particles $kg^{-1}\ dw$	Majority were fibers, next were fragments and spheres	<100 μm : over 95% <25 μm : 56.3–70.1%	PUR/Acr resins, 37.3%; R3, 16.8%; PP, 9.5%; EVA,	[67]

(continued)

Table 2 (continued)

Sample collection	Location	Assay methods	Concentration	Forms of MPs	Size	Polymer types	Reference
	western Norway					9.3%, PA, 4.5%; PEST, 4.7%; PE, 3.9%; PChl, 2.2%	
	An estuary in Tasmania, Australia	Centrifugation and filtration	Core A ($N = 7$): 211 MPs B ($N = 7$): 252 MPs	Fiber: 87% Sheet: 9% Fragment: 3% Beads: 1%	Core A: 137 in 63 μm , 49 in 100 μm , 24 in 1 mm, 1 in 4 mm Core B: 132 in 63 μm , 93 in 100 μm , 27 in 1 mm	–	[68]
	Ciwalengke River, Majalaya district, Indonesia	Raman spectroscopy	30.3 ± 15.9 MP particles kg^{-1} (dry sediments)	Fiber: 91% Fragment: 9%	50–100 μm : 12 L^{-1} , 101–300 μm : 10 L^{-1} , 301–500 μm : 36 L^{-1} , 501–1,000 μm : 52 L^{-1} , 1,000–2,000 μm : 72 L^{-1}	Polyester, nylon	[61]
	Eighteen streams in and around Auckland, New Zealand	Malvern Mastersizer 2000 laser diffraction particle analysis, FTIR	9–80 items kg^{-1}	Fragment: 79% Fiber: 20% Film, foam	Majority were 63–500 μm	PHM, EEAC, PP, PE, EVA, EPDT, EVAC	[7]
	Urban water areas in Changsha, China	Microscopy, SEM	270.17 ± 48.23 – 866.59 ± 37.96 items kg^{-1}	Fragments: 50.82% Fiber: 28.15% Film: 18.14% Foam: <10%	Majority (58.31%) were < 1 mm	PS: 29.41% PE: 19.12% PET: 14.71% Few: PP, PA, PVC	[69]
	Vembanad Lake, India	Micro-Raman spectroscopy	$252.80 \times 10^6 \pm 25.76 \times 10^6$ particles km^{-2} (mean)	Dominance: Film and foam Fragments, fiber/line, pellets	–	HDPE, LDPE, PP, PS	[70]
	Edgbaston Pool in Birmingham, UK	Microscopy	250–300 particles kg^{-1} dried sediment	Fragment, foam, film, fiber	–	–	[71]
	The River Kelvin in Glasgow, UK	SEM-EDS	161–432 MPs kg^{-1} dry sediment	Fiber: 88%, pellet: 0.25–0.7 mm, fragment	–	–	[2]
	The Pearl River along Guangzhou City, China	Microscopy	80–9,597 items kg^{-1}	Fiber: 54.7%, fragments: 43.3%, films: 1.9%	0.02–1 mm: 63.3%, 1–2 mm: 29.5%, 2–3 mm: 7.6%, 3–4 mm: 3.3%, 4–5 mm: 1.6%	PP: 26.2% PE: 47.6%	[60]

average abundance of MPs was about $43,000 \text{ km}^{-2}$ in the lakes near cities; the highest abundance is up to $466,000 \text{ km}^{-2}$. Morritt et al. [73] compared the size of MP fragments in the Thames in the UK and found that sewage treatment plants were the main source of MPs.

Abundance of MPs in urban freshwater is closely related to anthropic activities; high density of population usually causes high abundance of MPs [74]. Lasee et al. [75] demonstrated that the presence of large amounts of MPs in water bodies around densely populated urban areas. Another study also confirmed that human factors affected the abundance of MPs in urban regions [65]. Nevertheless, some researchers reported relatively high concentrations of MPs in remote freshwater environments, with extremely low population densities and low levels of industrialization [39, 76]. Even though the reason is largely unknown, we speculate that complex mechanisms can be involved in this transport process.

MPs in urban rivers might deposit into sediment and more likely to accumulate through sedimentation. On the contrary, MPs in the sediment can be released into water bodies under the action of water flushing [69]. Peng et al. [14] proved that urban river sediment might be a reservoir of land-based MPs and also a source of marine MPs. They surveyed sediment in the rivers of Shanghai, China, and found that secondary MPs accounted for the majority of the MPs in urban water environments. However, due to the lack of the practice of waste classification in China and other developing countries, most of plastic productions has not been recycled for the usage, which leads to the big possibility that primary plastic can be fragmented into MPs, which is ultimately discharged into water environments of urban areas.

4.2 *Municipal Sewage System*

In municipal sewage system, wastewater treatment plants (WWTPs) are attributed to a major pathway for MPs to enter into the aquatic environment in urban areas. To date, the presence of MPs at WWTPs has been widely reported in Australia [77], Europe [78–80], and the USA [81, 82]. In a recent study, researchers have investigated MPs at a full-scale WWTP, Eastern China, with two parallel wastewater treatment systems, including oxidation ditch and membrane bioreactor. They found that MP concentrations increased across the treatment systems and depended on the facility of the treatment process. Influent MPs were removed by 99.5% in membrane bioreactor system, while 97% in oxidation ditch system [83]. Other investigators reported that MP removal at WWTPs could reach around 99%, but the residual MPs discharged into surface water receivers were still in huge amount [81, 84]. These MPs from WWTPs will continuously be discharged into urban rivers as one of the important sources for freshwater MPs [85].

The estimation showed that China released about 209.7 trillion MP microbeads (306.9 t) per year into the water environment, 80% of which came from sewage treatment plants [86]. Primary MPs are originally derived from personal care products such as toothpaste, cleansing gels, and soap and enter the sewage treatment

plants through sewers [87]. Secondary MPs are produced by the treatment process of the facility in sewage treatment plants, mostly via photolysis, oxidation, and degradation [4]. Of these MPs in municipal sewage systems, fibers such as polyester and nylon in synthetic garments [88] are the main types [6]. Peng et al. [14] identified the amount of polyester, rayon, and other microfibers, which indicates that clothes washing lead to a large part of microfibers entering water bodies. Except for microfiber, a small number of other MPs types, such as chips and films, are available in WWTPs. Despite the variance of the composition of MPs in different sewage treatment plants, fibers were commonly reported as the dominated morphotype [8]. Mark et al. [6] conducted a comparative test of MPs in wastewater discharged from marine sediments, sewage treatment plants, and wastewater originally from washing machines. The ratio of polyester fibers in marine sediments and sewage was similar to that used for textiles; further analysis showed that a piece of clothing can fall off more than 1,900 fibers per wash, releasing up to 100 fibers per liter of discharged sewage. It is predicted that more fibers will enter the sewage treatment in winter. Dris et al. [16] collected raw water, sedimentation water, and treated conventional water from the Seine-Center wastewater treatment plant downstream of the Paris water. After assay they found high concentration of MPs, i.e., 260×10^3 – 320×10^3 particles m^{-3} , in the raw water. The majority of the MPs were fibers and in the size of mm scale. The domination of microfibers in the wastewater can be due to the washing machine. After pretreatment before cloth washing, the amount of MPs was greatly reduced to 14×10^3 – 50×10^3 particles m^{-3} , and the MP dimensions all decreased to below 1,000 μm . It indicated an effective approach of the removal of MPs in the sewage treatment plant.

The fate of MPs across the treatment system in the WWTP is also associated with the accumulation of MPs in sludge. After trapped in the sewage treatment plant, the sludge may contain a large amount of MPs, which may be applied for agriculture, and result in MP contamination in farmland soil [9, 89]. Lassen et al. [49] reported that 1.00 – 24.0×10^3 MP particles kg^{-1} ($\geq 10 \mu m$) were contained in sludge of a sewage treatment plant in Germany. According to a study in Vancouver, Canada, the wastewater treatment plant retained up to 99% of MPs and mostly accumulated in the sludge [85]. In the sludge, content of microfibers was up to 9.7 ± 3.7 fibers g^{-1} and higher than other MP shapes. Another study showed that average numbers of MPs were $22.7 \pm 12.1 \times 10^3$ particles kg^{-1} (dry sludge) in waste sewage sludge collected from 28 WWTPs in China [90]. In fact, a large proportion of MPs in the sewage tend to mix with the sludge and precipitate in WWTPs [91]. According to a survey in two sewage treatment plants in Turkey, the removal rate of MPs can be up to 73–79% [87]. Despite practical efficiency of MP removal in WWTP, a large amount of MPs can still be released into the sewage outlet. Therefore, pioneering and targeting designs need to be developed to elevate MP removal in WWTP [60].

5 Microplastics in Municipal Solid Waste

With the development of urbanization, solid waste is dramatically increasing [92]. It is estimated that about one billion tons of municipal solid waste is globally produced; less than 200 million tons are processed in waste-to-energy plants. There is a large amount of plastic waste in solid waste. In 2017, China's plastic production was 84.58 million tons [93]. It was estimated that 8.82 million tons of plastic waste were poorly managed in China, of which 133.353 million tons entered the ocean in 2010 [94]. Although some plastic wastes have been recyclable, the majority is often mixed with other types of domestic wastes [95] and burned or landfilled together with municipal solid wastes [92]. Landfill is a waste treatment strategy all around the world. Due to poor management, landfills are predicted to store 21–24% of global plastic waste [42]. A large amount of plastics are buried in landfills and are subject to relatively more severe environmental conditions, including the leachate pH (from 4.5 to 9), high salinity, temperature fluctuations, the generation of gas (such as carbon dioxide and methane), physical stress, and microbial degradation. These can cause plastics to break into smaller fragments and produce MPs or even NPs [5].

The presence of MPs in municipal solid waste was supported by a series of experiments and analysis [96]. Alimi et al. [97] proved that MPs could be intruded into soil through landfill leachates. Kilponen [98] found that MPs in a creek were stemmed from an old closed landfill leachate. He et al. [5] studied the occurrence and characteristics of MPs in different MSW landfill leachates and explored the potential of MPs as a source of MSW landfills. Seventeen different kinds of plastics were detected in landfill leachate, with leading polymer types of PE (34.94%) and PP (34.94%). They concluded that differences of plastic types in leachates might be related to regional differences in waste composition and landfill conditions. The MPs in landfill leachate were almost irregular in shape and rough in edges, which indicated the fragmentation process of larger plastic wastes in landfills [99]. The fragmentation might be the long-term process of producing MPs according to analysis [5]. Most of these plastics were in the size of 100–1,000 μm (74.88%); the number of MP particles increased with the decrease of MP sizes. The high percentage of small MPs indicated that smaller plastic fragments were more likely broken and further carried by leachate in the landfill environment. These small-sized MPs could be easily uptaken by soil biota, even microorganisms in landfill, which lead to a latent ecology risk [100–103]. Taking the typical megacity Shanghai, for example, Su et al. provided a systematic overview of MP pollution characteristics in landfill systems by investigating the MP abundances and fates in leachate and refuse over different landfill age [104]. The results indicated that abundance, size, and polymer type varied from landfill age, and the oxidative degradation of polyethylene MPs occurred in the landfill process, especially for the landfill time of more than 20 years. This study concluded that the fates of MPs in landfills were determined by the increase consumptions of plastic products and the degradation process of MPs in landfills.

Landfills are not the ultimate destination of plastics, but a potential source of MPs in other environmental matrices. The MPs in the leachate can leak into the environment through leachate leakage and discharging from the leachate collection treatment system. Foose et al. [105] demonstrated that leakage in the landfill system was a pathway of MPs entering the aquatic environment. Additionally, soil application of tiny solid components of landfills may introduce MPs into the terrestrial environment. MPs can accumulate in the soil and may be transported and redistributed by wind or flow into the aquatic environment via surface runoff [106, 107]. In addition, the MPs contained in the landfills can also be discharged through the ventilation of the aeration or closed landfill in the aerated bioreactor landfills.

6 Conclusions

In urban environments, MPs can be produced in the process of tire wear, landfill and sewage treatment, construction, industrial activity, household laundry, and so on. Due to lightweight and low density, MPs can easily float and transform within urban ecosystems. Meteorological conditions are the key factors for the migration of MPs in urban atmospheres. Wind erosion should be considered as a transport pathway of MPs, which makes MPs float in the atmosphere for a long time, and fill into different parts of a city, making it easier for humans to contact with MPs through breathing. Storm-water runoff is important for the conveyance of MP into the aquatic environment, which can be deemed as a pathway of MP from land to water bodies [56]. Rainfall also can result in atmospheric MPs falling down the surface of the ground and eventually enter into urban rivers. A large amount of MPs are produced by washing machines, cosmetics, plastic beads, and other processes and then enter into sewage treatment plants through municipal sewers. After treatment of sewage treatment plants, concentration of MPs can largely reduce in the outfall water; but MPs will be accumulated in the sludge. Additionally, MPs have also been found in landfills and can leak into other environmental matrices. We can conclude that a mass of MPs can migrate among the ground, the air, the water, and even the ocean. Distribution and transformation of MPs are closely related to urban eco-environments and human activities.

To date, knowledge about MPs in urban environments is very limited. Future researches need to focus on several aspects:

1. Standard sampling methods should be developed to facilitate the comparison of MP distribution in different cities.
2. More research work needs to investigate MP content in urban atmospheres, to further explore the joint pollution of MPs with other pollutants, and to analyze latent health risks.
3. More work is necessary to discover the source and fate of MPs in multiple urban environments.
4. New technology and methods need to be developed to control MPs, especially microfiber released from household cloth washing to WWPT and urban rivers.

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Microplastics in Agricultural Soils



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Abstract Microplastics (MPs) are widespread contaminants that produce at least 300 Mio t of plastic annually, from which a large amount ends up in the environment, where it persists over decades, harms biota, and enters the food chain. Yet, almost nothing is known about MP pollution of agricultural soils. Hence, the aim of this work is to review current knowledge on:

1. The sampling and separation methods in agricultural soils. Currently, the sampling and extraction of MPs from soil are similar to those in water and sediments.
2. Possible source input in soils, such as residual mulch degradation, compost and sewage sludge, atmospheric precipitation, and surface runoff and irrigation.
3. The spatial-temporal distribution of MPs, which may be affected by artificial tillage disturbance, irrigation infiltration, and organisms in agricultural soils.
4. Composition, shape, size, abundance, morphology, and other pollution characteristics that are discussed. However, comparison of reported microplastic abundances and other pollution characteristics are often impossible or require additional calculations based on assumptions.
5. Environmental effect of MPs in agricultural soils.

Yet, the current data based on microplastic pollution in soil is still poor. Accordingly, further research on the prevalence and fate of MPs in agricultural soils is urgently warranted. In addition, we also suggest other perspectives for future studies on microplastic pollution and soil ecotoxicity of plastic wastes, providing a direction for such research.

Keywords Agricultural soils, Environmental effect, Microplastics, Pollution characteristics, Source

1 Introduction

Research on microplastics (MPs) as an environmental contaminant is rapidly advancing. MPs refer to the plastic particle pollutants with the size less than 5 mm in the environment, including fiber, fragment, film, foam, and other shapes. In particular, the source, distribution, pollution characteristics, environmental behavior, and ecological effect of MPs in the offshore and tidal beach environment have been the concern of environmentalists [1, 2]. However, the pollution of MPs in terrestrial ecosystem, especially agricultural ecosystem, has only recently attracted environmental scientists' attention [3–5].

Relevant researches have shown that the MP abundance in terrestrial ecosystems may be 4–23 times that in the ocean. The annual input of MPs in terrestrial ecosystem is far more than that in the global ocean [6], but, to date, few studies have shown that MP content in agricultural soils is very high. Michael Scheurer et al. found that 90% of the soil samples in Switzerland flood plains had different degree

of MP pollution. They also found that MP pollution level is closely related to economic development and population density, suggesting that human activities play a vital role in MP pollution [7]. In China, there were only two reports on microplastic pollution in agricultural soils [8, 9]. There are few reports on MP pollution in agricultural soils. It may be that the research methods and modes of MPs in marine environment are not suitable for the soil. Due to the complexity of the soil environment, a large number of MPs are encased in the complex clay minerals, which precipitate rapidly during the separation and extraction process [4, 10].

MPs and various additives may also affect the physical and chemical properties of agricultural soils [11, 12]. For example, MPs and additives may have certain effects on soil pH, soil hydrophobicity, water and nutrient transport, and soil carbon source, but relevant reports are rare. MPs can still affect plant growth, for example, various additives released by the plastics degradation (such as titanate plasticizers) may enter the mesophyll through the respiration of plants, damaging chlorophyll or inhibiting the formation of chlorophyll, thereby harming the growth of the plants [13, 14]. The surface of MPs can adsorb harmful organic pollutants that existed in the environment, which is influenced by the physical and chemical properties of MPs and pollutants, as well as the environmental conditions. The biological toxicity of MPs can significantly increase after organic pollutions sorbed on them [15, 16].

Due to the complex agricultural soil environment, the investigation and research on MPs in soils are relatively weak [17]. Therefore, it is urgent to strengthen the optimization of MP separation and extraction method in agricultural soils, as well as the investigation and research on the source, distribution, size, abundance, and characteristics of MP pollution, in order to provide certain scientific basis for the risk control and treatment of MP pollution in agricultural soils.

2 Sampling and Separation of MPs

There is a lack of consistency in sampling and extraction techniques used to quantify MPs in soils, due to the rapid development of microplastic research. Currently, the sampling method of MPs in agricultural soils mainly refers to sediment sampling methods [18–20]. Selective sediment samples for plastic were taken with tweezers and tablespoons or picked up by hand. Usually, MPs have three different sampling methods [21]: (1) sampling a linear extension along the strandline with a spoon and/or a trowel [8], (2) sampling an areal extension using quadrats, and (3) sampling different depth strata using corers or trowel [9].

In general, the choice of sampling strategy and sampling approach will eventually determine the unit of observed abundances. A wide range of sampling approaches has been used to monitor MPs in soil, and as a result, the reported abundance of MPs is usually expressed in different units. For example, the studies sampling an area often report abundances per unit of surface (m^2) employed quadrats. If areal bulk samples up to a specific depth are taken, the reporting unit is m^3 , and other widely used reporting units are wet/dry weight (kg) [21].

As a result of the large variety in techniques applied, comparison of reported microplastic abundances between studies is often impossible or requires additional calculations based on assumptions. Therefore, it is clear that in order to completely understand the distribution of MPs in the agricultural soil environment, a harmonization and standardization of techniques and protocols are urgently needed to enhance microplastic research and monitoring.

After sampling, different approaches have been used to separate the microplastic fragments from the soil matrix [22]. The separation and extraction methods of MPs in soil are still similar with that in water column and beach sediments.

The separation and extraction methods of MPs mainly adopt density separation and the automatic extraction device [23, 24], based on the differences in density between plastic and soil particles [25–27]. As the density of the NaCl solution is 1.2 g cm^{-3} , only low-density plastics will float to the surface and can be extracted [28] (e.g., PE, PP, and PS). Other different salt solutions such as zinc chloride (ZnCl_2 , density $1.5\text{--}1.7 \text{ g cm}^{-3}$, NaI, density $1.6\text{--}1.8 \text{ g cm}^{-3}$) are used to obtain high-density MPs [29, 30] (e.g., PVC, PET).

In order to guarantee the reliability and quantity of the microplastic identification, acid, alkali, oxidation, enzyme, and electrostatic adsorption should be used to remove residual organic matter and organic debris after the density separation of plastic particles [10, 31]. The supernatant obtained by density flotation is first filtered through a vacuum filter (3–5 times); subsequently, the collected MPs are dried and further separated by visual identification and microscope.

3 Source and Distribution

3.1 Possible Sources of MPs in Agricultural Soils

The source of MPs in the agricultural soils is different in aquatic ecosystems; it is mainly derived from residual mulch degradation, compost and sewage sludge, atmospheric precipitation, and surface runoff and irrigation. MPs are widely distributed in the agricultural soils, which can be migrated to remote areas by wind and other external forces in surface soil. It is for the same reason that MPs were also brought into the deeper soil layer through human activities and soil organisms in the plough layer.

3.1.1 Residual Mulch Degradation

It is reported that the covered area of plastic film in Europe reaches $4,270 \text{ km}^2$ [32]. The global use of plastic film has been increasing, with an annual growth rate reaching 5.7% in 2019 [33]. In China, the plastic film for agriculture reached 1.455 million tons in 2015, accounting for about 90% of the total use of plastic film in the world, covering an area of 18.33 million hectares or more; however, the recovery

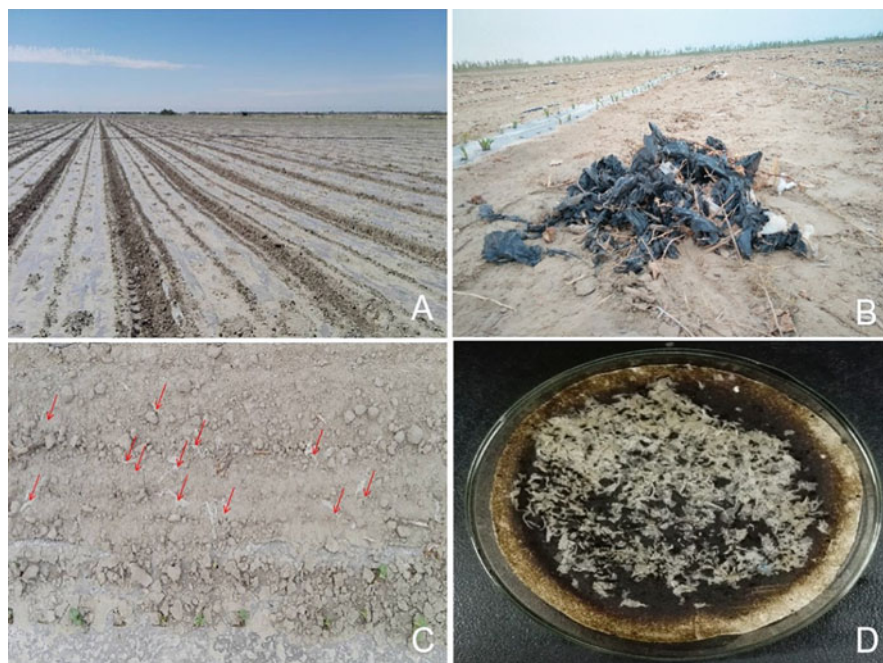


Fig. 1 Plastic film mulching field (a); residual mulch (b, c); MPs formed by degradation of residual film (d) in Xinjiang, China

rate of plastic film for farmland was less than 60% [34]. For example, by 2011, farmland residual film had reached 3.43×10^5 tons, accounting for 15.3% of the cumulative coverage, in China. Among them, the amount of residual membrane in 60.7% of the regions is higher than the national standard of residual membrane (75 kg ha^{-1}), even as high as 502.2 kg ha^{-1} in some regions, which increases with the increase of service life [35]. A large amount of residual film in agricultural soils, gradually breaking up, degrade, through a series of physical, chemical, and microbial effects such as tillage tearing, illumination, and wind erosion, and continuously form a new type of pollutant MPs in agricultural nonpoint sources, as shown in Fig. 1. Therefore, film residue decomposition is an important source of MPs in agricultural soils.

3.1.2 Compost and Sewage Sludge

There has been evidence that soils receive plastic input by application of compost and sewage sludge [36]. Compost is widely used as fertilizer in agriculture. In the EU about 18 Mio t of compost were produced in 2008, and estimations forecast a further increase of about 37% until 2020 [37]. Recommended compost application rates are typically in the range of $30\text{--}35 \text{ t ha}^{-1}$ annually [38], but, in some countries

like Germany, application is restricted to 20 or 30 t ha⁻¹ within 3 years [10]. Although most of the plastic can be removed before and after composting by, e.g., sieving and manual sorting, and biodegradable plastic might be degraded during composting, MPs are still found as the end product of compost from local composting plants. Depending on application dose, compost application can thus lead to an annual plastic input to arable fields of visible plastics that may reach 0.016 to 1.2 kg ha⁻¹ up to 0.08 to 6.3 kg ha⁻¹ [10]. Consequently, compost, especially of municipal origin, must be considered as a serious entry path of plastic in soil.

An international survey of MPs in wastewater treatment plants found that about 90% of MPs remained in the sludge after sewage treatment [39–41]. The application of sewage sludge as fertilizer is a widespread practice in agriculture. In Europe and North America, about 50% of the sludge is used in farm soil; Nizzetto et al. [6] calculated a yearly input of 6.3–43 and 4.4–30 million tons of MPs to European and North American agricultural soils, respectively, exceeding the estimated pollution of marine surface water. This alarming number makes sewage sludge as one of the most important inputs to MPs.

3.1.3 Atmospheric Precipitation

Atmospheric deposition may also be a source of MP pollution in agricultural soils. Researchers found that atmospheric MPs in an urban environment near Paris have atmospheric fallout of 29–280 items m⁻² day⁻¹, and 90% of which were fibers [42]. For the first time, Luo Yongming research group reported a large amount of MPs in the atmospheric environment of coastal cities in China, and the sedimentation flux of the atmospheric MPs is up to 1.46×10^5 (m⁻² a), mainly consisting of fibers [43]. These MPs deposited in the atmosphere may directly enter the topsoil of farmland in the suburbs of the city and cause MP pollution to agricultural nonpoint sources. However, there is still a lack of research and comprehensive understanding of the microplastic pollution about atmospheric precipitation.

3.1.4 Wastewater Irrigation and Surface Runoff

Surface runoff and agricultural irrigation will bring a large amount of MPs into agricultural soils, which is one of the sources of MPs in soil too. Sewage contains a lot of MPs; although the sewage is discharged after treatment in sewage treatment plants, there are still a lot of MPs with small particle size, which enter the soil environment through farmland irrigation. It has been reported that the plastic concentration of 0 to 125,000 items per m³ for treated wastewater, up to 275,000,000 (cabbage), 406,250,000 (maize), and 625,000,000 (cotton) plastic items, may be applied to fields per ha and during growing season by irrigation [10, 44–46]. Zhao et al. [47] have shown that the abundance of MPs floating on the surface of the Yangtze river spit reached 4137.3 ± 2461.5 m⁻³; Di and Wang [48] also found microplastic abundance reaching $4,703 \pm 2,816$ pieces m⁻³ in the

Yangtze river from Chongqing to Yichang. Even in remote inland lakes, there is a large amount of MPs [49], which can be carried into the soil by agricultural irrigation and surface runoff.

3.2 Spatial and Temporal Distribution of MPs in Agricultural Soils

MPs were distributed evenly throughout the tillage layer, due to the large artificial tillage disturbance. The lighter and smaller MPs in farmland topsoil may be transported to other areas by wind [50]. While the heavier and smaller MPs may be brought into deeper soil layers by farming and soil organisms [51–53]. Rillig et al. [54] observed a vertical movement of PE beads in the size of 710 to 2,800 μm by earthworms down to 10 cm within only 21 days. In addition, the researchers found that the pores created by earthworm activity also facilitated MP migration into the subsoil with water [55]. Soil animals such as hoppers and mites can accelerate the migration of MPs in deep soil through surface adhesion, grasping, and pushing, as well as excavating mammals such as gopher or mole [56].

Due to the continuous degradation of residual plastic products in soil, more MP accumulation is generated, resulting in the increase of MP abundance with the increase of arable land use time [35]. However, there are still relatively few researches on the spatial-temporal distribution characteristics of MPs in agricultural soils. Therefore, environmental scientists should pay urgent attention to this problem.

4 Pollution Characteristics of MPs in Agricultural Soils

MPs are widespread contaminants, virtually present in agricultural soils. Visual identification is an essential step, which can directly and quickly obtain the surface texture and other characteristics of possible MPs such as size, shape, abundance, and color [17]. But, visual identification of MPs is sometimes inaccurate and should be combined with other physical or chemical technologies. However, very little information can be found on the composition, shape, size, abundance, and morphology of MPs in the soil. It is still a big challenge to explore pollution characteristics and ecological risk assessment of MPs in soils.

4.1 *Composition and Shape*

Currently, the most commonly used methods are first identifying and quantifying MPs through visual and microscopic inspection, followed by chemical characterization by micro-Fourier-transformed infrared (μ -FT-IR) and Raman spectroscopy. From the reviewed studies on water and sediment, 50% used Fourier-transformed infrared (FT-IR) spectroscopy-based methods, 32.5% visual inspection, and 10% Raman spectroscopy, whereas electron microscopy, staining dyes, and gas chromatography-mass spectroscopy (GC-MS) were each used in 2.5% [31].

The main types of MPs in agricultural soils include polyethylene (PE), polypropylene (PP), polystyrene (PS), polyvinyl chloride (PVC), polyester (PET), etc. The specific chemical components can be identified by FT-IR [57, 58] and Raman spectroscopy [59, 60]. At the same time, the shapes of MPs in agricultural soils can be mainly determined by visual and microscopic examination. Typically, there are seven main categories according to shape: fiber, fragment, flake, film, foam, debris, and pellet.

4.2 *Size and Abundance*

In terms of particle size, it can be divided into five intervals: <0.5 mm, $0.5\text{--}1$ mm, $1\text{--}2.5$ mm, $2.5\text{--}5$ mm, and >5 mm [47]. MP abundance is usually expressed as “item m^{-2} ,” “item m^{-3} ,” “g km^{-2} ,” “g km^{-3} ,” and so on [61]. Zhang Guosheng et al. have shown that the abundance of plastic particles ranges from 7,100 to 42,960 particles kg^{-1} (mean 18,760 particles kg^{-1}) in the soil aggregate and 95% of the size was distributed between 1 and 0.05 mm, in southwest China [8]. He Defu’s team has shown that in the shallow and deep farmland of the Shanghai area, the MP abundance was 78.00 ± 12.91 and 62.50 ± 12.97 items kg^{-1} , respectively. The MP size was mostly less than 1 mm and the category mainly with fiber, film, and fragment [9]. Up to now, no studies have been conducted on the abundance, size variation, and related pollution characteristics of MPs in agricultural soils in the arid northwest of China, especially in Xinjiang which has a serious residual film pollution.

In general, the spatial-temporal difference in the size and abundance of MPs in agricultural soils is not clear. There are few reports on the pollution characteristics of MPs in agricultural soils, and the relevant data are also not comparable. Therefore, it is very important to carry out the research on the size and abundance of MPs and establish a unified standard for measuring the size and abundance of MPs in agricultural soils.

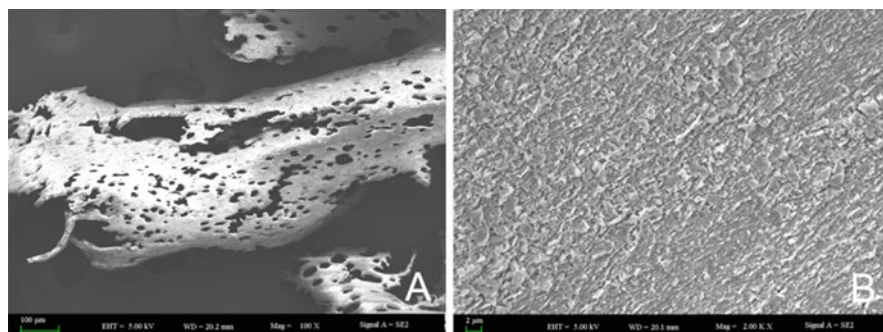


Fig. 2 SEM of MPs from the agricultural soils in Xinjiang, China (unpublished data)

4.3 Morphology

The SEM is used for the identification of MPs and provides high-magnification and clearer structural images of MPs. Generally, different types of MPs were observed with different morphological features on their surfaces using the SEM, which indicated that the surfaces of MPs in agricultural soils had been strongly weathered. Mechanical, chemical, and biological processes have presumably been involved in the weathering under the prevailing conditions in the soil conditions [62].

Figure 2 shows SEM of the partial surface of MPs from soil samples in Xinjiang, China. We found that the surface of MPs was destroyed and roughened, indicating that MPs were degraded by mechanical erosion, chemical oxidation, biological degradation, or plastic material properties. In addition, information on various minerals and microbial communities attached to the surface of MPs can be obtained by SEM-EDS [63, 64].

In the past research, the pollution status of MPs was mainly focused on aquatic ecosystems. Relatively, there are few research reports on MPs in terrestrial ecosystems, especially the investigation and research on MPs in agricultural ecosystem. As an important part of the terrestrial environment, agricultural ecosystem plays a vital role in human food security. However, there are few researches on the source, distribution, abundance, size, and pollution characteristics of MPs in agricultural soils. Therefore, the ecological risks and environmental effects of MPs on agricultural soils ecological environment are not clear.

5 Effects of MPs on Agricultural Soils

MPs may affect soil structure and physical and chemical properties when it enters agricultural soil. Rillig is one of the earliest scholars to pay attention to soil MP pollution [4], pointing out that the accumulation of MPs in soil would affect soil properties, soil functions, and biodiversity. Subsequently, some researchers

conducted relatively in-depth studies and found that MPs in soil had adverse effects on water and nutrient transport and crop growth [12, 65].

De Souza Machado et al. [66] pointed out that MPs can affect soil bulk density, hydraulic characteristics, and aggregate changes at environment-related concentrations. Liu et al. [67] believed that polypropylene had certain effects on the concentration of soluble organic carbon (DOC), soluble organic nitrogen (DON), soluble organic phosphorus (DOP), and PO_4^{3-} in soil, as well as the activity of FDA hydrolytic enzyme and phenol oxidase. In addition, MPs can change soil properties by absorbing harmful substances in soil solution, increasing porosity, changing aggregate structure, or becoming part of soil aggregates [68]. A field survey has shown that more than 70% of the microplastic particles combine with soil aggregates, especially with microaggregates, in Yunnan, thus changing soil structure and affecting soil microenvironment [69]. Generally, different types of MPs may have different environmental effects on soil properties. For example, polyester can significantly reduce soil water-stable aggregates, while polyethylene can significantly increase the number of soil water-stable aggregates [70]. However, there is still a lack of long-term and in situ studies on the interaction between plastics and soil aggregates. Therefore, the impact of plastic pollution on soil water transport and soil and water conservation is basically impossible to judge.

MPs can also be ingested by soil animals, so they may accumulate in the soil food chain, thus affecting soil animals at all nutrient levels [4]. However, these studies mainly focused on the impact of MPs on model animals and paid insufficient attention to the impact on actual soil animals. In addition, MPs also have some influence on soil microbial community, but currently, the main concern is the impact of plastic mulching on microbial community and microbial degradation of plastics [71, 72].

6 Conclusion and Prospect

MP pollution is a global problem, but the current research is relatively scattered; the future needs some systematic thinking. With regard to the impact of MP pollution on agricultural soil ecosystems, the following issues need special attention in the future:

1. It is difficult to separate microplastic particles in agricultural soils, due to soil texture, organic matter, and aggregate structure. Therefore, in view of the diversity and the complexity of agricultural soil, it is necessary to carry out methodological research on the separation and identification of different types of MPs and establish relevant technical specifications.
2. As the source of MPs is relatively complex, it is difficult to analyze the source of MPs in agricultural soils. From the perspective of source control of MPs, it is urgent to establish corresponding research methods for source analysis and formulate standards. At the same time, the spatial and temporal distribution of MPs in agricultural soils lacks research data. The gradient distribution, migration,

and environmental effects of MPs with different types, sizes, and degradation degrees are also not clear in agricultural soils, so further research is needed.

3. At present, the effects of MPs on soil structure, physical and chemical properties, and soil processes and functions have not been deeply explored. In the future, it is necessary to further explore the impact of MPs on soil properties, structures, soil nutrient cycling, and soil health. The effects of MPs on soil microbial functional groups and functional genes also need further study.

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The Distribution and Characteristics of Microplastics in Coastal Beaches and Mangrove Wetlands



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Abstract Microplastics found in coastal environments can be transported to and accumulate in different coastal environmental media by diverse driving factors and pathways. Increasing numbers of studies indicate that microplastics accumulate in

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coastal areas with highly intensive human activities. However, there are few discussions on the occurrence of microplastics in coastal sediments and coastal wetlands according to different land utilization patterns. In this chapter we investigate the distribution of shape, size, and abundance of microplastics in northern coastal beach sediments. We found that pellets, foams, fragments, flakes, films, fibers, and sponges occurred in beach sediments with different land utilization patterns. The abundances of microplastics were 344 particles kg^{-1} in tourist beaches, 1,226 particles kg^{-1} in beaches adjacent to mariculture areas, 98 particles kg^{-1} in beaches near fishing ports, and 1,302 particles kg^{-1} in undeveloped beaches. Foams were dominant in tourist beaches and beaches near fishing ports, while flakes dominated in beaches adjacent to mariculture areas and in undeveloped beaches. The differences are likely due to different anthropogenic influences in coastal zones. We then illustrate the characteristics and spatial distribution of microplastics in different mangrove sediments covering the main mangrove forest growing areas of south China. The abundance of microplastics was 1,302 particles kg^{-1} , and foams and fibers were the dominant shapes in mangrove sediments. The differences in distribution of microplastics in mangrove sediments are related to anthropocentric influences such as mariculture and fisheries and to the density of vegetation. Moreover, different potential sources of microplastic are contributors of microplastic pollution in coastal zones and need to be evaluated. They include land sources and offshore marine sources together with coastal atmospheric deposition. The results contribute to our understanding of microplastic pollution in coastal sediments with different coastal land utilization patterns, and they provide a reference for the management and control of microplastic pollution in coastal environments.

Keywords Characteristics, Coastal wetlands, Distribution, Microplastics, Sources

1 Introduction

Microplastics occur in coastal environments worldwide [1–3], especially in coastal zones with highly intensive human activities such as tourism [4, 5], mariculture [6, 7], and fishing and shipping ports [8, 9]. The spatial distributions of the abundance, composition, and size of microplastics show heterogeneity in coastal sediments due to the diversity of sources [10], variable biological and chemical effects [11, 12], complex hydrodynamics [13], and different geographical locations [9, 14]. However, few studies have focused on comparative differences in the occurrence of microplastics in coastal sediments under different coastal land utilization patterns worldwide.

There have been few studies of microplastic pollution in coastal wetland sediments. Mangroves are unique systems that play a valuable role in sequestering carbon in coastal wetlands worldwide [15]. They are capable of moderating wind

and waves, protecting embankments, promoting siltation, purifying the environment, and enhancing environmental conditions. They also provide important habitats for waterfowl, fishes, crabs, shrimps, and shellfish [16]. In recent years several studies have reported that mangrove ecosystems have been affected by microplastic pollution due to mariculture, sewage discharge, and other anthropogenic influences [12, 17]. Plastic debris retention and distribution in mangrove ecosystems caused by the density of vegetation (i.e., red mangrove or *Rhizophora mangle*) and tidal variation have been reported [18, 19]. The occurrence of microplastics in seven intertidal mangrove habitats in Singapore (collected from the top 3–4 cm at low tide, oxygenated zone) was initially investigated [17]. The abundance of microplastics was 12.0–62.7 particles kg^{-1} , and the majority of the microplastics were fibers with a diameter of $<20 \mu\text{m}$. The area of mangrove in China is 34,472 ha, accounts for about 0.4% of the global area, and is mainly distributed in Hainan, Guangdong, Guangxi, Fujian, Zhejiang, Hong Kong, and Taiwan [16, 20]. However, there have been few studies of microplastic pollution on the coast of Qinzhou Bay, Guangxi province, including the mangrove wetlands [7, 12].

Sources of microplastic pollution in the coastal environment are very complex [1]. They include land inputs such as river, sewage, and garbage dumping and marine inputs such as current transportation and shipping. The source analysis of microplastic pollution in coastal zones is regarded as an important research topic in revealing the spatial and temporal distribution of microplastics.

In this chapter we therefore introduce the characteristics and distribution in terms of shape, size, and abundance of microplastics in coastal beach sediments. The relationship between the occurrence of microplastics and intensive human activities in the coastal zone will be discussed. We will then illustrate the characteristics and spatial distribution of microplastics in different mangrove sediments, covering the main mangrove forest areas of China. Important factors and their relationships with the distribution and retention of microplastics in the mangrove sediments will be explored. Finally, different potential sources of microplastic pollution in coastal environments will be discussed. This will complement the data and provide a management reference of microplastic pollution in the coastal zone.

2 Distribution and Characteristics of Microplastics in Coastal Beaches

Intensive human activity has developed on the east coast of China, with development of mariculture, tourism, transportation, mining, salt harvesting, fishing, port construction, and land reclamation. The booming economy and population has resulted in the movement of large quantities of plastic wastes and microplastics into the coastal beaches and the waters. This section discusses the distribution of microplastics in beach sediments under different land utilization patterns along the Bohai Sea and Yellow Sea coastlines. There are tourist beaches, beaches adjacent to

mariculture areas, beaches near fishing ports, and undeveloped beaches. These are regarded as potential “hot spots” for microplastic accumulation.

2.1 Distribution and Characteristics of Microplastics in Tourist Beaches

Coastal tourist beaches are key areas of microplastic accumulation derived from anthropogenic activities [21]. Coastal tourism in China results in substantial pollution of beaches by plastics and microplastics, and descriptive statistics are shown in Table 1. The coefficient of variation (CV) of microplastic abundances of all sampling sites was 1.5. This implies that the abundance of microplastics in tourist beaches has high spatial variability. In this study the abundance of microplastics ranged from 3.3 to 2,456 particles kg^{-1} with an average of 343.9 ± 522.4 (mean \pm S.D.) particles kg^{-1} (Table 1). This is comparable to the results of other studies. For example, Yu et al. investigated microplastics in bathing beaches on the north Bohai Sea, and the abundance was around 200–400 particles kg^{-1} [22]. Retama et al. investigated tourist beaches in Huatulco Bay on the Pacific coast of southern Mexico and found a range of microplastic abundance of 0–2,300 particles kg^{-1} [4].

The morphologies of microplastics in tourist beach sediments include foams, sponges, flakes, fragments, fibers, pellets, and films. The coefficients of variation of microplastic abundances of all shape types ranged from 1.1 to 3.3 (Table 1), showing that the abundance of different shapes in the tourist beaches was highly variable. The foams had the highest abundance, 327.5 ± 513.6 particles kg^{-1} , accounting for 95.2%. Sponges (7.8 ± 12.2 particles kg^{-1} on average, accounting for 2.3%), flakes (4.1 ± 13.5 particles kg^{-1} on average, accounting for 1.2%), and fragments (2.4 ± 2.6 particles kg^{-1} on average, accounting for 0.7%) were much fewer than foams. Fibers (0.9 ± 1.1 particles kg^{-1} on average) and pellets (1.0 ± 2.1 particles kg^{-1} on average) accounted for only 0.3% of the total microplastics. Films were fewest at 0.3 ± 0.7 particles kg^{-1} , accounting for 0.1%. Foams were common in the tourist beach sediments, and this may be explained as follows. First, a very large number of foam buoys and containers are used for fishing and seafood sales. Second, a high proportion of the foam items have been discarded because they are cheap and there is little incentive for recycling. About 5.8 million tonnes of waste polystyrene foam materials have been estimated to be produced globally every year, of which about 1.8 million tonnes are manufactured each year and only about 30% are recycled in China (<http://www.plas.hc360.com>). Third, the government does not control the disposal of these foam materials. Furthermore, foam materials readily break into smaller pieces, making them difficult to collect during beach cleaning events [23]. Finally, foam pieces in coastal waters can be easily transported by water currents and can easily accumulate on the beaches as a result of tidal action. Foams were found to account for 99% of plastics on coastal beaches in Korea

Table 1 Range, average, and percentage of each microplastic type and size fraction in beaches under different land utilization patterns (unit: particles kg⁻¹)

Land use	Pellets	Foams	Fragments	Flakes	Films	Fibers	Sponges	Total
Tourist beaches (<i>N</i> = 24)	Range	0.0–8.2	0.0–9.8	0.0–65.1	0.0–3.3	0.0–3.6	0.0–40.2	3.3–2,455.6
	Average	327.5	2.4	4.1	0.3	0.9	7.8	343.9
	Percentage	95.2	0.7	1.2	0.1	0.3	2.3	100.0
Beaches adjacent to mariculture areas (<i>N</i> = 10)	Range	0.0–4.95	0.0–7.8	0.0–10,664.0	0.0–3.6	0.0–136.1	0.0–33.89	1.3–10,689.3
	Average	132.1	3.4	1,071.1	0.9	14.3	3.7	1,226.2
	Percentage	10.8	0.3	87.4	0.1	1.2	0.3	100.0
Beaches near fishing ports (<i>N</i> = 5)	Range	0.0–1.0	0.0–26.1	0.0–96.6	0.0–4.0	0.0–27.3	0.0–20.3	17.2–224.5
	Average	49.4	6.9	26.9	1.9	8.1	4.3	97.7
	Percentage	50.5	7.1	27.5	1.9	8.3	4.4	100.0
Undeveloped beaches (<i>N</i> = 14)	Range	0.0–16.9	0.0–253.9	0.0–14,705.6	0.0–22.2	0.0–73.0	0.0–56.7	13.0–14,712.5
	Average	105.5	22.1	1,151.1	2.7	13.1	5.8	1,301.6
	Percentage	8.1	1.7	88.4	0.2	1.0	0.4	100.0

Table 2 Percentage of each size fraction in beaches under different land utilization patterns (%)

	<1 mm	1–2 mm	2–3 mm	3–4 mm	4–5 mm
Tourist beaches	2.9	36.4	28.5	17.1	15.2
Beaches adjacent to mariculture areas	76.0	9.1	6.5	4.4	3.9
Beaches near fishing ports	3.8	14.3	25.4	25.3	31.2
Undeveloped beaches	77.0	8.7	6.5	4.6	3.3

[23]. However, other types of microplastics in the tourist beaches of the South China Sea include fibers, films, fragments, and granules [5, 24].

The majority of the microplastics found in this study were composed of particles within the size range 1–3 mm, accounting for 64.9% (Table 2). This is mainly due to the size fractionation of the foams which was dominated by 1–3 mm particles. The abundance of particles <1 mm was the lowest, accounting for only 2.9%. Overall, the proportion of the observed microplastics with a size range of 1–5 mm increased with decreasing plastic particle size.

2.2 *Distribution and Characteristics of Microplastics in Beaches Adjacent to Mariculture Areas*

The abundance of microplastics in beaches adjacent to mariculture areas ranged from 1.3 to 10,689 particles kg^{-1} , and the coefficient of variation (CV) of microplastic abundance was 2.7 showing very high spatial heterogeneity (Table 1). The average abundance of microplastics was $1,226 \pm 3,336$ (mean \pm S.D.) particles kg^{-1} . Most sites showed abundances of microplastics of several tens or hundreds of particles kg^{-1} . The highest abundance of microplastics (10,689 particles kg^{-1}) was found in an abandoned mariculture area (fish, shrimp, or crab) at Dongying, Shandong province, which was dominated by flakes (99.8%). The flakes were produced from the fragmentation of abandoned woven bags which were used for mariculture pools and bag dams in the coast. The lowest abundance of microplastics was found in a mariculture area at Yantai, Shandong province, with only 1.3 particles kg^{-1} , possibly attributable to the sediment texture. This sampling site was a small gravel beach with large plastic debris (>5 mm) but fewer microplastics. Furthermore, numerous sea cucumbers and abalones were cultured in this area, and a clean water environment would be required [25]. This suggests that the differences in microplastic contamination are likely also associated with different systems of coastal mariculture.

Flakes occurred in the highest percentage (87.4%) in the beaches adjacent to mariculture areas (Table 2). Foams were the second highest percentage (10.8%), while percentages of fibers (1.2%), sponges (0.3%), and fragments (0.3%) were much lower. Pellets and films were the lowest, both accounting for only 0.1%. This result differs from a report of foam accounting for 99% on coastal beaches in Korea [23]. The shape types of microplastics and their abundance in beaches adjacent to

mariculture areas differed greatly from other studies. In the Pearl River Estuary, there were large numbers of foams (>90%), fragments, and pellets, but no fibers, films, or sponges [26]. In beaches of the Hawaiian Islands of the United States, the common shape types were films and foams [23, 27].

The majority of the microplastics found in beaches adjacent to mariculture areas were composed of particles <1 mm which accounted for 76.0%. The abundance of the size fractions within 4–5 mm was lowest, accounting for only 3.9%. The proportion of the observed microplastics increased with decreasing particle size. Smaller size ranges of microplastics may have a more severe potential impact on marine biota because of their similar size range to food items of marine organisms and a high risk of ingestion [28, 29]. They may have potential impacts such as intestinal blockage, loss of nutrition, and perhaps mortality [30, 31]. In addition, smaller microplastics can act more as carriers of organic contaminants and potentially toxic metals due to their high surface area [32–35].

2.3 Distribution and Characteristics of Microplastics in Beaches Near Fishing Ports

The abundance of microplastics in beaches near fishing ports ranged from 17.2 to 224.5 particles kg^{-1} (Table 1). The average abundance of microplastics was 97.7 ± 88.7 (mean \pm S.D.) particles kg^{-1} , lower than in tourist beaches and beaches adjacent to mariculture areas. Foams, sponges, flakes, fragments, fibers, pellets, and films occurred in beaches near fishing ports. Foams were dominant at these sampling sites, accounting for 50.5% (49.4 ± 75.5 particles kg^{-1}), followed by flakes 27.5% (26.9 ± 40.9 particles kg^{-1}). Films were lowest, accounting for 1.9% (1.9 ± 1.8 particles kg^{-1}). The composition of shape types was similar to those in tourist beaches. The high percentage of foams in beaches near fishing ports is likely due to fishery activities [36].

The size fraction trend shows that the percentage of the observed microplastics decreased with decreasing particle size (Table 2). The percentage of the 4–5 mm size fraction was highest, accounting for 31.2%. The size fraction <1 mm had the lowest percentage, accounting for only 3.8%. This size distribution is different from that in tourist beaches and beaches adjacent to mariculture. However, the specific factors affecting the size distribution trends are difficult to identify. This is likely related to the length of time since the microplastics were released into the environment [37].

2.4 Distribution and Characteristics of Microplastics in Undeveloped Beaches

The undeveloped beaches (beaches which are not managed) exhibited an abundance range of 13.0–14,712 particles kg^{-1} with an average of $1,302 \pm 3,875$ particles kg^{-1} . The abundance range and coefficient of variation (3.0) of microplastic abundance showed very high spatial heterogeneity (Table 1). The abundance of flakes was highest at $1,151 \pm 3,906$ particles kg^{-1} accounting for approximately 88.4% of the microplastics. The total percentages of the remaining microplastic shapes accounted for only <10%. The average abundance and shape type composition of microplastics were similar to those at the beaches adjacent to mariculture. Clearly, the abundance and proportion of microplastics were related to coastal land utilization patterns. Large numbers of woven plastic bags have been used at undeveloped and mariculture beaches for coastal flood control, cultivation pools, or seawater transpiration pools. They are easily fragmented into small flakes within several months. They can be commonly observed at the coastal zone in Shandong province, especially near Laizhou Bay (Bohai Sea) [9].

The percentage of the observed microplastics found in undeveloped beaches showed a decreasing trend with increasing particle size (Table 2). The majority of the microplastics were composed of particles <1 mm, accounting for 77.0%. The percentage of 4–5 mm particles was lowest at 3.3%. Flakes contributed a large amount of microplastics <1 mm which were derived from abandoned plastic woven bags. These abandoned plastic woven bags may remain in the environment for a long time without local management. They readily fragment into microplastic pieces and are then released into the environment [37]. Thus, the local government needs to enhance the management and control of plastic woven bag wastes in the coastal zone.

3 Distribution and Characteristics of Microplastics in Coastal Mangrove Wetlands

Mangrove ecosystems are important in the tropical and subtropical coastal zones of China and have been impacted by microplastic contamination. However, this problem has received little attention both in China and worldwide. Up to now, only three reports have been published on microplastic pollution in Chinese mangrove wetlands [7, 12, 38], and they have focused only on Qinzhou Bay in Guangxi province. There is a lack of research on the burden of microplastic pollution in different coastal mangrove ecosystems of mainland China. We compiled information about the occurrence of microplastics in response to the lack of data. The information compiled includes the abundance of different particle shapes, polymer types, and sources present in coastal mangrove sediments collected along the Chinese tropical and subtropical bays and islands.

3.1 Abundance and Spatial Distribution of Microplastics

The abundance of microplastics in mangrove sediments showed high spatial heterogeneity (Fig. 1). The mean microplastic abundance in the mangrove sediments shows that the declining order of mean abundance in sediments among the provinces was Guangxi province (875.3 particles kg^{-1}), Fujian province (198.4 particles kg^{-1}), Hainan province (146.0 particles kg^{-1}), Zhejiang province (116.7 particles kg^{-1}), and Guangdong province (98.7 particles kg^{-1}). The abundance of microplastics in mangrove sediments ranged from 8.3 to 5,738 particles kg^{-1} . The highest abundance was detected at Jinhaiwan mangrove tourist area which is affected by a fishery, mariculture, tourism, and local direct dumping [39], followed by GX4 (501.4 particles kg^{-1}) and GX5 (274.7 particles kg^{-1}), and the lowest abundance was found at site GD7 (8.3 particles kg^{-1}). Microplastic abundances and shape types at most of the sites were much higher than in the contaminated sediments of the coastal mangrove in Singapore [17].

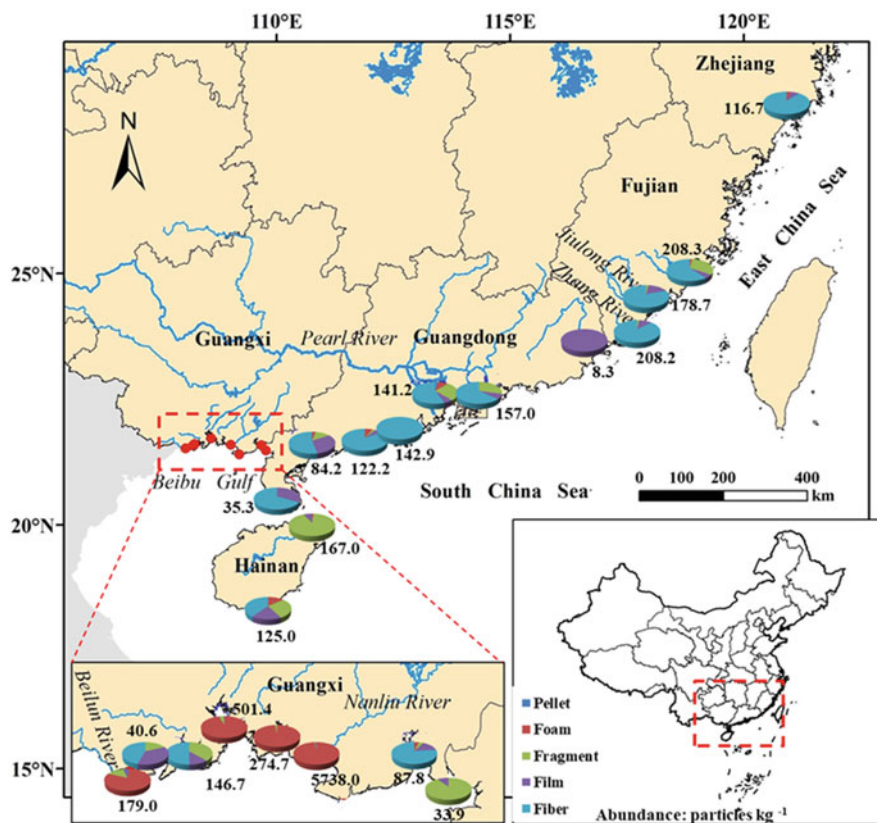


Fig. 1 Spatial abundance distribution of microplastics with different shape types along the Chinese coastal mangrove sediments

3.2 Shapes, Sizes, and Abundances of Microplastics

Microplastics of shape types including fibers, films, fragments, foams, and pellets were found in the mangrove sediments. The foams, fibers, and fragments were the dominant shapes present. This may closely be related to the extensive coastal mariculture activities, shipping activities, reclamation, and inputs of effluent waters from wastewater treatment plants near the survey site [9, 12, 23, 24]. The shape composition of microplastics differed among the mangrove locations. Foam was the main type in Guangxi province with a percentage of 93.2% and mean abundance of 743.5 particles kg^{-1} . Offshore oyster mariculture was identified as a dominant source in Guangxi province. There was an oyster (*C. hongkongensis*) culture area of around 10,133 ha in Qinzhou, the largest oyster culture area in Guangxi province [40]. Foam plastic materials are widely used as floating rafts for oyster cultivation and fish farming [12]. A large amount of foam materials was discarded and remained on the coast due to poor management. They would be further broken into small pieces under the prevailing environmental conditions. Fibers were the most common type in Guangdong (71.1%, 70.2 particles kg^{-1}), Fujian (75.9%, 150.5 particles kg^{-1}), and Zhejiang (89.3%, 104.2 particles kg^{-1}) provinces. Flakes were dominant in Hainan province (74.5%, 114.0 particles kg^{-1}). The difference in the distribution of microplastic types may be related to the differences in microplastic types used and discharged locally.

Across all samples the highest abundance of microplastics was found in the smaller size microplastics (1–2 mm). However, the spectra of the size distribution of fibers, films, fragments, foams, and pellets were different. Most fibers (57.1%) comprised particles <1 mm, and the highest percentage was of 0.5–1 mm fibers at 36.3%. The percentages of films, fragments, and foams with a size range of 1–2 mm were highest in their respective particle size fractions (films, 28.6%; fragments, 25.0%; and foams, 48.7%). However, most pellets comprised particles of 4–5 mm. Fibers were the shape type with the smallest mean size (1.36 ± 1.29 mm) and might therefore be easily ingested by most of the local organisms [41, 42] and subsequently influence the behavior of the marine organisms [43, 44].

3.3 Contribution of Human Activities and Mangrove Vegetation to Microplastic Accumulation

The results demonstrate a close relationship between the abundance of microplastics and human activities. The highest abundances of microplastics (5,738 and 501.4 particles kg^{-1} , respectively) occurred in Jinhaiwan and Qinzhou bays, possibly due to highly intensive human activities such as tourism, harbor transportation, fisheries, and local direct dumping. Offshore fisheries and oyster cultivation were the dominant sources, with a very large oyster culture area and foam plastic materials used for cultivation in Qinzhou Bay [7, 12]. The microplastic abundance at the Yingluo

mangrove reserve with limited access limitation was lowest, with only 33.9 particles kg^{-1} .

The density of vegetation is an important factor affecting the distribution of microplastics in mangrove sediments. We found that microplastic abundance at site GX1 (309.0 particles kg^{-1}) with dense vegetation (approximately 0.5 trees per m^2) was about one order of magnitude higher than at site GX1 (49.0 particles kg^{-1}) with sparse vegetation (~ 0.25 trees per m^2) in the same mangrove system. This suggests that mangrove areas with dense vegetation intercept more microplastics or plastic debris [18, 45]. Li et al. [12] reported a similar observation in which microplastic abundances in sediments outside the mangroves were much higher than those in the sediments inside the mangroves.

4 Sources of Microplastics in the Coastal Environment

4.1 *Inland-Source Input*

Large-scale discharge of sewage and garbage dumping are the main land-source input sources of microplastics in the coastal environment. Some detergents, personal care products, and industrial raw materials contain large amounts of microplastic particles [46]. They are not easily separated or removed from the sewage due to their small particle size and are then discharged into the environment with the sewage. The results of Browne et al. [1] show that $>1,900$ fibers may be released into the wastewater (>100 particles L^{-1}) in the daily cleaning process and there was one microplastic particle (<1 mm) per liter of sewage from a sewage treatment plant. Moreover, river input is another important pathway for microplastic accumulation in the coastal environment. Zhao et al. [47] found that the abundances of microplastics floating on the surface of water bodies in the Yangtze River estuary and its adjacent waters were $4,137 \pm 2,462$ and 0.167 ± 0.138 particles m^{-3} , respectively. The abundance of microplastics decreased sharply after entering the offshore sea from the Yangtze River estuary, showing clear land-source input. In addition, tourism, salt fields, mariculture, port transportation, and fisheries in coastal zones likely also produce microplastic contamination (Fig. 2). For example, coastal dock pontoons (expanded polystyrene) and mariculture facilities in Asia, Australia, Panama, and the United States are broken into thousands of microplastic pieces after damage. This has become an important source of microplastic pollution in the local coastal zone [48].

4.2 *Offshore Marine-Source Input*

Plastic pollution caused by offshore operations and ship transportation, including fishing vessels and vessels transporting industrial goods, represents a marine source



Fig. 2 Occurrence of plastic debris and microplastics on Chinese coastal beaches

of microplastics [49–51]. Microplastics may be produced by damage, dumping, or leakage during maritime transportation. On the other hand, there is a large area of millimeter-scale plastic waste enrichment. This has formed two “garbage belts” in the subtropical circulation areas of the North Pacific and North Atlantic [52, 53]. Garbage zones such as ocean vortex belts can also occur in coastal areas. They may cause microplastics to further migrate and be redistributed under the influence of ocean currents [54, 55].

4.3 Sources from the Coastal Atmosphere

Microplastics are present in the atmosphere and can be transported over long distances by atmospheric circulation [56]. Dris et al. [57] observed the presence of synthetic fibers, mixed fibers, natural polymers (artificial silk, cellulose acetate, etc.), and natural fibers (cotton, wool) from the atmosphere in Paris, France. We speculate that there are likely more microplastic types (not only fibers) in the atmosphere. These atmospheric microplastics may settle on land or be sent to the ocean through atmospheric transport. This may be an important source of microplastics in coastal environments.

We investigated the amount of microplastic sedimentation from air to land in Yantai city, Shandong province, East China. The amount of microplastic sedimentation showed seasonal differences. The daily deposition of different types of microplastics ranged from 0 to 6.02×10^2 particles m^{-2} per day. The sedimentation flux of microplastics also varied seasonally, being high in spring, summer, and winter, and the range was from 4.84×10^2 to 6.24×10^2 particles m^{-2} per day. However, deposition was lower in autumn at only 1.30×10^2 particles m^{-2} per day. In general, the amount of microplastic sedimentation from air to land reached 1.46×10^5 particles m^{-2} per year, of which the amount of fibers was 1.38×10^5 particles m^{-2} per year. Fragments, thin films, and foams were deposited at 6.29×10^3 , 7.65×10^2 , and 2.45×10^2 particles m^{-2} per year, respectively. The total amount of

microplastics obtained through atmospheric deposition in the local region each year can reach 2.33×10^{13} particles, approximately 0.9–1.4 tonnes (assuming that all microplastic types are fibers and estimated by the density of polyamide and polyester polymers which are widely used in the textile industry [58]). Dris et al. [58] estimated an amount of fiber settled in the atmosphere each year in Paris, France, with a 2,500 km² populated area, of about 3–10 tonnes. This indicates that microplastics (especially fibers) in the atmospheric environment are likely important sources of different microplastic types in coastal environment. However, atmospheric microplastic contamination has been little studied. Studies of contamination by atmospheric microplastics and its contribution to the land and ocean require further investigation.

5 Conclusions

Microplastic pollution occurs widely in different coastal beaches and wetlands and has complicated sources. Differences in the distribution of microplastics are closely related to the impact of human activities. Under the intervention of human activities, different patterns of land utilization are found in the coastal zone. This makes the distribution of microplastics in coastal zones regional and special. In the future the control and management of microplastic contamination in coastal wetland environments need to be implemented with regional specificity and accuracy.

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Microplastics in Inland Small Waterbodies



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Abstract Small waterbodies are the most numerous and widespread freshwater environments, and they play important roles in supporting freshwater biodiversity and ecosystem service delivery. There has been a considerable increase on research of environmental pollutants in small waterbodies, but only a few works have focused on microplastic (MP) occurrence and effects. MP pollution has been well documented in large freshwaters. Meanwhile, small waterbodies are also the receiving waters of MPs through stormwater runoff, atmospheric deposition, etc. In this chapter, we first introduce the definitions and characteristics of a range of small waterbodies and their ongoing threats. Next, we overview the distributions and

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characteristics of MPs in small waterbodies worldwide and offer some insights into their sources. Furthermore, we give a brief discussion about interactions of MPs with freshwater biota and describe the toxicity effects of MPs on amphibians in detail. Lastly, we demonstrate the current awareness of people about small waterbodies and provide potential approaches to minimize their MP pollution. Overall, high abundances of MPs are observed in water and sediment collected from various types of small waterbodies, and MPs pose a significant threat to the resident organisms and human health. Yet, less detailed information is available on small waterbodies' MPs at present. Therefore, we appeal more researchers and policy-makers to focus on the protection and management of small waterbodies.

Keywords Characteristics, Management, Microplastics, Risks, Small waterbodies

1 Introduction

1.1 *Characteristics of Small Waterbodies*

As an important part of the freshwater system, small waterbodies are the most numerous freshwater environments in the world and can be seen everywhere. Up to now, however, small waterbodies have no specific definition; they usually encompass ponds, small lakes, small rivers, streams, ditches, and springs [1, 2]. In general, small standing waters are defined according to the area of watershed. For example, small lakes vary in size from 1–5 ha to around 50–100 ha in area (but the Water Framework Directive (WFD) limit 50 ha) [1, 3], and the area of ponds ranges from 1 m² to about 2–5 ha [1, 4, 5]. At one extreme, the tiny puddle which forms after rain is classified into pond [1]. Small running waters such as small streams and small rivers are relatively harder to define, and the term small stream is often used interchangeably with the term headwater. Furse et al. [6] define the length of stream within 2.5 km from the source as headwater streams, while small linear headwaters are those with catchments less than 10 km² in the WFD terms [3]. In addition, other small waterbodies are distinguished mainly depending on their functions or hydrological characteristics. Ditches are man-made channels build primarily for agricultural or street drainage; normally the width of ditches is 1–3 m [7]. Spring is a strictly delimited place where the groundwater appears at the surface [8]. Furthermore, small waterbodies are naturally created or man-made and permanent or seasonal.

Small waterbodies are not only various but also numerous. The river network in Europe is more than five million kilometers long; more than 80% consist of small rivers, commonly known as headwaters, streams, creeks, or small rivers [9]. In addition, there are around 5–10 million small lakes and ponds in Europe and about 17 million ponds up to 1 ha in the United States, but the latter data omits the waters less than 1,000 m² [10]. However, half to two-thirds of all waterbodies are in the

range 25–400 m² in some area (e.g., southeast Great Plains in the United States) [11]. Accordingly, the current global number of small waterbodies may be underestimated because of omitting those smaller ones [11]. For instance, there are approximately 32,000 and 120,000 ponds ranging from 100 m² to 5 ha in area in Switzerland and Denmark, respectively [12, 13]. Furthermore, ditches constructed for agriculture are likewise widespread. In England, ditch length is about 600,000 km and in Netherlands 300,000 km [7, 14, 15]. Overall, inland small waterbodies are globally abundant freshwater habitats.

Compared to the large waterbodies, small waterbodies, especially ponds and small lakes, are areas of high biodiversity [1]. Despite small waterbodies only occupy 2% of Earth's surface area, their species richness is nearly equal to marine environments [16]. Support for this idea comes from several studies which have surprisingly found that the ponds sustain a larger proportion of freshwater species than lakes or rivers [3, 17, 18]. In addition to the common freshwater species such as macrophytes and micro- and macroinvertebrates, there are specific species such as that of frogs and toads in small waterbodies [19, 20]. It has been suggested that the patchy and comparatively isolated nature of small waterbodies may be a reason of higher speciation rates [1]. Moreover, the dominant number, physicochemical heterogeneity, and low contamination rate of small waters may be another possible explanation of their high species richness [1, 18, 21].

With the increasing concern about small waterbodies, their ecosystem services are gradually recognized by researchers and policy-makers [1, 22]. Indeed, there are three main categories of ecosystem services: (1) provision, (2) regulation and maintenance, and (3) cultural support and entertainment [1]. For example, natural and man-made stormwater ponds provide a wide range of functions, including flood control, water supply, elimination of nutrients and pollutants, enjoyment and recreation, etc. [23]. Clearly, ditches constructed near the farmlands are mainly used for land drainage and irrigation [24]. The ecosystem services of small waterbodies have been highlighted within the literature [1, 2, 5].

1.2 Environmental Pollution in Inland Small Waterbodies

Because of their patches, small volumes, and catchments, small waterbodies are much more likely to fall entirely within unpolluted areas and to be less exposed to pollutants. Once they form in regions of frequent human activities such as development and intensive agriculture, however, they are easily contaminated by a variety of pollutants [1, 2]. Because small waterbodies have a considerably small water volume, there is less potential for dilution of contaminants. Therefore, they are exceptionally vulnerable to input of even small amounts of pollutants from their surroundings [25, 26].

Lots of studies show that small waterbodies are threatened by a range of pollutants due to the acceleration of urbanization and agriculture [25, 27, 28]. A typical problem of inland small waterbodies, particularly static waters, is “eutrophication” [18, 29–31]. Forty years ago, many researchers have started to study the causes and mechanisms underlying the process of eutrophication. To date, nutrient inputs such as nitrogen and phosphorus to inland waters are considered as the main causes of eutrophication [18, 29–31]. Dodds et al. [32] analyze the total nitrogen (TN) and phosphorus (TP) concentrations of rivers, streams, and lakes in the US Environmental Protection Agency (EPA) nutrient ecoregions and find median TN and TP values in the range of 0.248–3.372 mg L⁻¹ and 0.012–0.184 mg L⁻¹, respectively, much higher than the reference median values. Likewise, the freshwater eutrophication is serious in developing countries. More than 80% of urban rivers are contaminated in China [33–35]. For instance, ammonium nitrogen concentrations of water collected from the black-odor rivers in Wenzhou are 1.17–18.51 mg L⁻¹, and TP concentrations range from 0.42 to 3.0 mg L⁻¹ [33]. These values have shown us a high nutrient concentration in freshwater systems.

In addition to nutrients, heavy metals, organic matter, pesticides, and plastics also cause inland small waterbodies to suffer from toxic pollutions [34, 36, 37]. These pollutants may enter and accumulate in small waterbodies via rainfall runoff, atmospheric deposition, mismanagement, etc. For example, stormwater ponds used extensively in stormwater management receive a variety of pollutants from rainfall runoff [27]. Weinstein et al. [37] demonstrate that polycyclic aromatic hydrocarbons (PAHs) are pervasive in the sediments of 19 stormwater ponds located in coastal South Carolina, and the high PAH levels in 5 stormwater ponds suggest that there are moderate to high risks to organisms and humans. Moreover, due to extensive application of pesticides, streams and ditches near farmlands are commonly exposed to pesticides via spray drift, edge-of-field runoff, or drainage [36]. Nearly half of European waters are at risk from pesticides [38], and 26% of 2,369 sampling sites of small streams in Germany are found to have considerable exceedances of regulatory acceptable concentrations (RAC) [36].

With increasing plastic production and usage year by year, plastic trash becomes “huge” and “ubiquitous”. Plastic pollution and risks have been gradually realized by the general public and reported by media [39]. Plastic litter entering the aquatic environment degrades to millions of smaller pieces [40–42], namely, MPs, which negatively impact waterbody ecosystems. Although MP pollution has been well documented in marine and large freshwater system, studies in small waterbodies are limited [28] (Fig. 1). So far, only a few papers have reported MP pollution in small waterbodies. Indeed, MPs can enter small waterbodies through sewage effluent and road runoff as well. Therefore, in this chapter, we will specifically introduce characteristics, risks, and management of MP pollution in inland small waterbodies.



Fig. 1 Plastic litter in a pond located in Hangzhou, China

2 Characteristics of Microplastics in Small Waterbodies

2.1 Sources of Microplastics in Small Waterbodies

Nowadays, activities involving plastics encompass packaging, textile, transportation, agriculture, electronics, and buildings and constructions, nearly covering all fields [43]. Human activities are considered as one of the major factors of MP pollution in freshwater environments [44, 45]. Particularly, plastic production, usage, and discard increase with the growing of population density, such as in Shanghai and Paris, both megacities [46, 47]. For example, Yonkos et al. [48] investigate MP contaminations in four estuarine rivers in Chesapeake Bay, USA, and find that the concentration of MPs is positively correlated with population density and proportion of urban/suburban development within the watersheds.

Illegal trash disposal and poor management may result in the increase of plastic trash from household and agricultural plastics in terrestrial environments, especially in developing countries. These plastic trashes enter small waterbodies directly or via wind transport, surface runoff, or agricultural fertilizers and then degrade into MPs, indicating a potential source of pollution for small waterbodies. For instance, lakes in tourism areas and ponds near residential areas or in parks are the recipient waters of plastic trash and MPs [28]. Another potential input of MPs to the pollution of small waterbodies may be the discharge of sewage comprising fibers from laundry wastewater [49] and microbeads from personal care products [50]. The effluent overflows into small waterbodies during storm events due to the limited treatment capacity of wastewater treatment plants (WWTPs) [51]. Some rivers in old cities are even the

direct receiving waters of effluent from residential areas and factories. People still wash their garments directly in small waterbodies in some underdeveloped areas. In recent years, atmospheric fallout has been realized as a possible source of MPs, especially microfibers [46].

2.2 Occurrence of Microplastics

Up to now, to our knowledge, there are only a few papers about MP pollution in small waterbodies (Table 1). The average MP abundance in water and sediment samples varies greatly. This difference results from some key factors such as sampling sites and methods, human activities, and features of small waterbody [60].

Among these researches, MP abundance is the lowest (0.014 ± 0.009 items L^{-1}) in water collected from fish ponds and rivers of the European Carpathian Basin [54]. The different sampling methods might explain the low concentrations of MPs. In this study, the authors use a mobile sampling system which only retains MPs between 0.1 and 2 mm in size, resulting in the loss of larger and smaller MPs [54]. Similarly, Dikareva and Simon [58] collect plastics using a phytoplankton net (63 μm mesh) and find that MP abundance is in the range of 0.02–0.3 items L^{-1} in streams in Auckland, New Zealand. However, a bulk sampling approach is used in most of other studies. Generally, water is filtered through a mesh filter in small size (e.g., 10 or 20 μm), and MPs larger than this size are supposed to be collected [28, 52, 57]. Hu et al. [28] investigate MP occurrence in the waters of six types of small waterbodies in East China and find MP concentration in the puddles up to 15.7 ± 4.6 items L^{-1} . Usually, a puddle is a downfold that has a considerably small total water volume. The rainfall runoff with MPs enters the puddles, leading to a higher MP pollution. Of course, the MP abundances are also high as well in other types of small waterbodies in the same survey area [28]. The authors suggest that the high accumulation of MPs is related to the higher population density and more anthropogenic activity [28]. For example, Shanghai is currently the most populated city in China. Shanghai has a population of 24.2 million, a primary plastic product of 3807.3 thousand tones, and a chemical fiber product of 430.0 thousand tones [61]. Furthermore, most of the sampling sites of East China locate near residential areas and textile processing plants. Surprisingly, the highest MP concentration (270 items L^{-1}) is detected in a stormwater pond in Viborg, Denmark [57]. Possible explanations for the high MP abundance are that the stormwater retention time of the stormwater pond is about 10 days and its drainage area includes production industries, retailers, building supply stores, parking lots, as well as roads.

Similarly, the trend of MP abundance in sediment is consistent with that in water (Table 1). The lowest abundance of MPs in sediment is detected in European fish ponds and rivers (0.8 ± 0.4 items kg^{-1}) [54], and the highest MP density is determined in a stormwater pond in Viborg, Denmark (9.5×10^5 items kg^{-1}) [57]. These indicate that the functions of small waterbodies probably influence their MP abundances. Stormwater retention ponds are versatile in terms of MP

Table 1 Microplastic pollution in small waterbodies worldwide

Location	Abundance	Shape	Size	Polymer type	Reference
<i>Water</i>	Items, L ⁻¹				
Creeks in Shanghai, China	0.44–4.13	88% fiber, 7% fragment, 4% film, 1% pellet	Majority: 0.1–1 mm	PES, rayon, PP	[52]
Ponds in East China	4.3 ± 7.0	88% fiber	Majority: <0.5 mm	79% PES, 7% PP	[28]
Ditches in East China	7.6 ± 7.0				
Puddles in East China	15.7 ± 4.6				
Riceland in East China	4.7 ± 3.2				
River in East China	9.0				
Lake in East China	12.5				
Riceland in Shanghai, China	0–0.7 ± 0.3	Dominant: film and fiber	Majority: 0.1–1 mm	68% PP, 32% PE	[53]
Fish ponds and rivers in the Carpathian basin, Europe	0.014 ± 0.009	–	Range: 0.1–2 mm	PP, PE, PES, PS	[54]
Stormwater ponds in Durham, USA	0.8 ± 1.0–1.7 ± 1.2	Dominant: fiber	Majority: <0.5 mm	35% PES, 30% PP	[55]
Stormwater ponds in Denmark	0.49–22.9	–	<1.03 mm	72% PP, 9% PE, 7%PVC	[56]
A stormwater pond in Viborg, Denmark	270	–	0.01–0.5 mm	PES, PP, acrylic, PA, PE, PS	[57]
Streams in Auckland, New Zealand	0.02–0.3	39% fragment, 34% fiber	Majority: <0.5 mm	PE, PP	[58]
<i>Sediment</i>	Items, kg ⁻¹				
Urban river in Shanghai, China	802 ± 594	89% pellet, 8% fiber, 3% fragment	Majority: 0.1–0.5 mm	57% PP, 17% PES, 11% rayon	[47]
Edgbaston Pool in central Birmingham, UK	250–300 (maximum)	Dominant: fiber and film	–	–	[59]
Ponds in East China	693.9 ± 1005.0	55% fragments, 43% fibers	Majority: <0.5 mm	76% PP, 11% PE, 9.1% PES	[28]
Ditches in East China	583.5 ± 961.7				
Puddles in East China	609.8 ± 70.5				
Riceland in East China	607.9 ± 344.8				
Lake in East China	148.6				
Riceland in Shanghai, China	1.7 ± 0.9–41.3 ± 15.3	Dominant: fiber	Majority: 0.1–1 mm	61% PE, 35% PP, 4% PVC	[53]
Stormwater ponds in Durham, USA	97.5 ± 85.1–274.8 ± 193.5	Dominant: fragment	Majority: <0.5 mm	61% PP, 16% PE, 14%PS	[55]
Fish ponds and rivers in the Carpathian basin, Europe	0.8 ± 0.4	–		PP, PE, PES, PS	[54]
A stormwater pond in Viborg, Denmark	9.5 × 10 ³	–	0.01–0.5 mm	PP, PE, PES, PS, acrylic, PA	[57]
Streams in Auckland, New Zealand	9–80	79% fragment, 20% fiber	Majority: <0.5 mm	PE, PP	[58]

PA polyamide, PE polyethylene, PES polyester, PP polypropylene, PS polystyrene, PVC polyvinylchloride

input pathways, and it is likely that they are important sinks for MPs. In addition, MP abundances in sediment samples collected from other types of small waterbodies are approximately hundreds per kilogram (Table 1). To sum up, MPs are abundant in small waterbodies, and such waterbodies play a role in receiving diffuse MP pollution from urban and highway areas.

2.3 Shape and Size of Microplastics

The literature in Table 1 demonstrates that MPs in small waterbodies are commonly categorized into four types according to their shapes: fiber (elongated), fragments (small irregular pieces), film (thin flat), and granule/pellet (spherical and ovoid pieces). Fibers are dominant in water. For instance, Luo et al. [52] and Hu et al. [28] find fibers to be the most abundant, accounting for 88% of the total MPs. Compared with MPs in water, the shape distribution of MPs in sediment significantly varies. Pellets are predominant in samples from urban river in Shanghai [47], fibers are prevalent in riceland [53] and Edgbaston Pool [59], while fragments are more abundant in small waterbodies in East China, United States, and New Zealand [28, 55, 58]. The morphological characteristics of MPs can be used to indicate their potential sources. For example, the pellets in urban river possibly originate from personal care products, and the potential sources of fragments in stormwater ponds are stormwater runoff containing road plastic debris.

Small size (<5 mm) is another key parameter of MPs involving their bioavailability [62]. Various sampling methods and size classifications are used in fieldworks, which make it difficult to compare data from different studies. Therefore, in this review, our concern is the main size of MPs in small waterbodies. Results show that 0.1–1 mm MPs are most abundant in small waterbodies (Table 1). On one hand, <0.1 mm MPs are hard to observe under microscopes. On the other hand, MP abundance generally increases with the decreasing of their sizes [28, 63]. Thus, these may be the reason that 0.1–1 mm MPs are dominant in these studies.

2.4 Polymer Types of Microplastics

Plastics are made from a wide range of polymers. The polymer types affect MP density, longevity, and performance and indicate their probable origins [43]. In the studies (Table 1), MPs in water and sediment samples are randomly selected to identify their polymer types using Fourier Transform Infrared spectroscopy (FT-IR). Overall, polypropylene (PP), polyester (PES), and polyethylene (PE) are the most common polymers in small waterbodies (Table 1). A larger global demand for these three types of polymer makes them more widespread in the environment [64]. Specifically, PES and PP are the dominant polymers found in water, while PP and PE are

the most abundant polymers in sediment, which is consistent with the results from studies of large freshwater environments [65, 66].

3 Occurrence of Microplastics in Freshwater Biota from Small Waterbodies

Organisms in the aquatic environment are believed to be impacted by MP ingestion [62]. Based on the above data, MP contamination is pervasive in small waterbodies of high biodiversity. Therefore, exploring MP pollution in freshwater biota in small waterbodies is necessary. Here we summarize the related researches in Table 2. Six papers investigate the uptake of MPs by fish. Results show that MPs have a relatively low detection rate and concentration, except for a high concentration of MPs (3.3 ± 0.5 items individual⁻¹) in eels [53], and a fuzzy data by Olesen et al. [57] indicate that the average MP abundance in three-spined sticklebacks and young newts is 65 items individual⁻¹ (340 items g⁻¹). In the study by Lv et al. [53], MPs found in crayfish (2.5 ± 0.6 items individual⁻¹) and loach (1.8 ± 0.5 items individual⁻¹) are likewise high. Additionally, MP contaminations are widely found in different species of tadpoles, especially *Rana limnochari* and *Microhyla ornata*, with great abundance expressed in terms of weight [28].

The difference of MPs in organisms may result from MP contamination of habitats and organismal feeding strategies [28, 70, 71]. For example, Setälä et al. [71] expose a range of animals (bivalves, free-swimming crustaceans and benthic, deposit-feeding animals) to microbeads with different concentrations (5, 50, and 250 beads mL⁻¹). Results show that the amount of microbeads in animals is concentration dependent and free-swimming crustaceans ingest more microbeads compared with benthic animals. In addition, Mizraji et al. [70] observe that herbivorous or carnivorous fish have a lower MP concentration than omnivorous ones, which have a wider range of food sources. On the other hand, the number of MPs ingested by organisms is related to the MP bioavailability as well [72], just as some invertebrate species with a wide feeding size range have been demonstrated to selectively forage on specific sizes when expose to multiple size particles.

Fibers <1 mm are the most abundant MP type in most of organisms in small waterbodies (Table 2). Furthermore, PES and rayon are the prevalent polymers, with exception of the study by Lv et al. [53]. The authors identify that PE dominate in organisms; this result is similar to that found in sediment samples. The authors deduce that the ingestion of MPs in eels, crayfish, and loach might be related to their habits. These three animals are considered as typical benthic organisms, which commonly forage and live in the bottom. Conversely, Hu et al. [28] find that the shape and polymer distributions of MPs ingested by tadpoles are most similar to that found in water, mainly resulting from tadpoles ingesting MPs when they swim through the water column.

Table 2 Microplastic pollution in organisms from small waterbodies

Species	Location	Abundance		Shape	Size	Polymer type	Reference
		Items individual ⁻¹	Items g ⁻¹				
<i>Fish</i>							
Freshwater fish	Streams within Texas, USA	Occurred in 8.2% fish		Filament, fragment, film	–	–	[67]
Eel	Riceland in Shanghai, China	3.3 ± 0.5	–	Dominant: fiber	Majority: 0.1–1 mm	91% PE, 9% PP	[53]
<i>Gambusia affinis</i>	Urban rivers in Shanghai, China	0.4–1.1	0.6–6.1	58% fragment, 41.5% fiber, 0.5% pellet	Majority: 0.02–1 mm	PES, rayon	[68]
<i>Gambusia affinis</i>	Small waterbodies in East China	0–0.2 ± 0.1	0–1.9 ± 1.0	–	–	–	[55]
<i>Gambusia holbrooki</i>	Australian urban wetlands	0.18–1.13	0.52–4.4	Dominant: fiber	Majority: <1 mm	25.7% PES, 10.1% rayon, 7.3% PA, 5.5% PP	[69]
<i>Gasterosteus aculeatus</i>	Stormwater ponds in Viborg, Denmark	65 (average)	340 (average)	Dominant: fragment	0.01–0.5 mm	PES, PP, PA, PE	[57]
<i>Newts</i>							
<i>Tadpole</i>							
<i>Rana limnochari</i>	Small waterbodies in East China	0–2.7 ± 0.8	0–168.5 ± 52.0	82.9% fiber, 24.3% fragment	Majority: <0.5 mm	71.5% PES, 9.6% PP	[28]
<i>Bufo gargarizans</i>		0.2–1.9	2.4–56.9				
<i>Microhyla ornata</i>		0.5–2.6	35.2–157.9				
<i>Pelophylax nigromaculatus</i>		1.3–1.8	3.0–4.5				
<i>Crayfish</i>	Riceland in Shanghai, China	2.5 ± 0.6	–	Dominant: fiber	Majority: 0.1–1 mm	91% PE, 9% PP	[53]
<i>Loach</i>		1.8 ± 0.5	–				

PA polyamide, PE polyethylene, PP polypropylene, PES polyester

Small waterbodies provide habitats for a variety of organisms and are areas of high biodiversity. Once they are polluted, their resident organisms will be affected by the pollutants. Here, we demonstrate that MPs are ingested by animals in small waterbodies such as amphibians which could transport MPs from aquatic to terrestrial food webs. However, the study area and species are limited. Hence, in the future, more surveys will need to be carried out in order to widely investigate MP contamination in the creatures of small waterbodies.

4 Effects of Microplastics on Freshwater Biota

Field studies have proved that MPs are widely detected in aquatic ecosystems, which increases the attention to adverse impacts on freshwater ecosystems. Scherer et al. [72] have summarized laboratory studies about the interactions of MPs with freshwater biota. They discuss biotic and abiotic factors affecting MP ingestion. Biotic factors focus on the feeding type of invertebrates and vertebrates, particularly invertebrates (e.g., flagellate, rotifers, cladocerans, blackworm, bivalves, etc.). Abiotic factors include microparticle size, shape, and taste, which affect the bioavailability of MPs. Next, the effects of MPs on freshwater organisms have been summed up by Scherer et al. [72]. A variety of physical impacts induced by MPs to algae, *Daphnia magna*, bivalves, gastropods, and fish include blockages, reduced dietary intake, and internal injuries [62]. In addition, MPs can act as carriers of chemicals and microorganisms, aggravating the adverse effects of MPs to the organisms [73–75].

Amphibians are typical animals living in small waterbodies. At present, a field work completed by Hu et al. [28] has confirmed that tadpoles can ingest MPs from their living environment. However, limited information regarding MP ingestion and its effects on amphibians is available in the laboratory. Currently, thereby, we mainly described the ingestion and effects of MPs on amphibians.

As representatives of amphibians, *Xenopus laevis/tropicalis* are always used as model organisms in the world. The tadpoles of *Xenopus laevis/tropicalis* are filter feeders which are supposed to be especially prone to MP ingestion because of their extensive feeding activities [76, 77]. Multiple studies have shown that MPs ingested by *Xenopus* tadpoles accumulated in the gills, alimentary canal, stomach, and gut, and nano-plastics are also detected in the blood, cytoplasm, nucleus, and periphery of digestive gut cells [77–80]. Moreover, micro- and nano-plastics are potential threats for the growth and development of *Xenopus* larvae. The embryos of *Xenopus laevis* exposed to 50 nm polystyrene nanoparticles display numerous malformations including disorders in pigmentation distribution; anomalies of the head, eyes, intestine, and tail; edema in ventral anterior zone; and a stunted body [79]. Additionally, embryo mortality rate exhibits a dose-dependent relationship with MP exposure [79]. However, De Felice et al. [78] expose *Xenopus* tadpoles to polystyrene MPs ($2.75 \pm 0.09 \mu\text{m}$ of diameter) at different concentrations (1×10^5 – 8.7×10^5 particles mL^{-1}) and find that neither body growth nor swimming activity of tadpoles

are affected. These differences among the researches may be induced by organismal development stage, exposure time, MP concentration, etc. Thus, further studies will need to be performed in order to fully explore MP toxic effects and risks on amphibians.

5 Management of Small Waterbodies

To sum up, small waterbodies are a critical but vulnerable part of freshwater ecosystems. Their functions are of equal importance to the larger waters, but they are more susceptible to many human activities (e.g., development and intensive agriculture, pollutant discharge) and climate change [1]. Despite this, nevertheless, small waterbodies are still the least studied part of the freshwater environment and are largely excluded from freshwater management planning, even rarely recognized by people in developing countries. In recent years, more and more countries and organizations recognize the importance of small waterbodies and pay attention to their pollution and management [1–3, 9]. For example, in 2013, a workshop organized by the European Environmental Bureau (EEB) took place in Brussels to discuss possible ways to better protect and manage small waterbodies. In 2015, a special session on “Small waterbodies – knowledge base, importance, threats, and future research priorities” was carried out at the 9th Symposium for European Freshwater Sciences (SEFS) in Geneva. This session emphasized the importance of small waterbodies and aimed to refocus research attention on these resources.

Nowadays, there is a broad consensus that small waterbodies are reservoirs of biodiversity and significantly contribute to catchment diversity and that they should be integrated into the existing legislative framework to get better protection and management [1, 2]. While there are lots of gaps in our knowledge of small waterbodies, compared to larger waterbodies. Therefore, first and foremost, more scholars should be appealed to conduct relevant researches about small waterbodies, and to reduce the gaps in knowledge. In addition, sufficient research and activity funds should be provided for environmental action. Because small waterbodies are globally abundant, this may signify a significant financial and administrative burden if they are included in the legislative framework process more extensively.

Since it is very hard to remove MPs from the environment, plastic source control and management may be an excellent way to reduce MP pollution in small waterbodies [81]. Firstly, we should take measures to reduce the use of plastics such as the ban of microbeads in personal care products, increasing the production and use of cotton clothing, replacing non-biodegradable plastics with biodegradable ones, etc. [50, 82]. Secondly, comprehensive implementation of garbage classification conduces to waste management and the mitigation of MP diffuse pollution from rainfall runoff and agricultural fertilizers [83, 84]. Finally, we should enhance the ability of wastewater treatment plants to remove MPs and avoid the release of untreated effluent directly into the receiving waters [51, 85, 86]. MP contamination in small waterbodies should be reduced through the above measures applied.

In conclusion, small waterbodies are the most numerous freshwater environments worldwide and are under all the threats affecting larger waters, but they are largely excluded from water management planning. This section introduces small waterbodies and reports their MP distribution, characteristics, and toxic effects. It suggests that MPs are prevalent in water, sediment, and resident animals, especially in the form of <1 mm fibers, probably due to human activities nearby. In addition, we discuss the potential adverse effects caused by MP exposures to resident animals, particularly amphibians. Corresponding high abundances and potential adverse effects of MPs strongly suggest the need for increasing attention and researches, reducing inputs of plastic waste, and supporting protection and effective management of small waterbodies.

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Microplastics in the Sediments: From Rivers to Lakes Investigated in the Biggest Freshwater Lake (Poyang Lake) Basin and Yangtze River in China



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Abstract In recent years, microplastic (MP) pollution is increasingly becoming a new environmental problem of global concern. Rivers and lakes are important transport channels for MPs entering the ocean, and their contribution to MP pollution can't be ignored. In this chapter, the largest freshwater lake in China, the Poyang Lake, and its typical basin wetland areas are selected as the research areas. Wetland soils or sediment samples were collected; the types, particle size, abundance, distribution, and the main sources of MPs in these samples were investigated using the flotation separation and microscopic identification methods. The results show that foams (polystyrene), fibers (low-density polyethylene), debris, and films are found in the sediments of the typical wetlands; the debris-based MPs were the major and accounted for 50–70% of the total, followed by film MPs and fiber MPs, and foam MPs accounting for the lowest proportion. MPs with size less than 1 mm occupied the dominant percentage, accounting for more than 60% of the total MPs. The distribution of MPs in the sediments of the typical wetlands in the Poyang Lake is positively related to human activities; the differences of MP abundance among

different regions are influenced by human activities, hydrological conditions, river inputting, etc. The composition of MPs among different regions is closely related to the lifestyle of people in the region and the physical and chemical properties of MPs. This chapter can further supplement the basic data of the research on MP pollution in freshwater wetlands, such as rivers and lakes in China, and provide a basis for further research on MP pollution and its ecological impact.

Keywords Microplastics, Poyang Lake, Sediments, Typical Natural Reserve wetland, Yangtze River

1 Introduction

Microplastics (MPs) refer to plastic polymers with particle size less than 5 mm, which have been confirmed as emerging pollutants and have received increasing attention. Because of small particle sizes and low density, MPs in the environment can migrate under the action of external forces, such as wind, river, and ocean current, and can be used as carriers of organic pollutants and heavy metals. Moreover, MPs can be eaten by animals by mistake and are difficult to be excluded from the body, which will cause the physical damage to organisms, blockage of the digestive tract, or pseudo-satiety and then cause the decrease of the feeding efficiency, energy shortage, injury, or death of organisms. Over the past 10 years, more and more studies had demonstrated the types, abundance, distribution, sources, and biological effects of MPs in water surface and sediments in marine and coastal environments.

In this chapter, the typical wetland areas in the Poyang Lake were selected as the research areas, which mainly include the areas seriously affected by human production and life activities, the entrance of the five major rivers of the Poyang Lake, the National Nature Reserves, and the outflow areas of the Poyang Lake flowing into the Yangtze Rivers and other important wetland areas. The soils or sediment samples in the wetlands were collected as the research materials; the flotation separation methods and microscopic identification methods were used in order to investigate the types, particle size, abundance, distribution, influencing factors, and main sources of MPs in all sediment samples.

The results show that foams (polystyrene), fibers (low-density polyethylene), debris, and films are found in the sediments of the typical wetlands, such as Poyang Lake and Le'an River section, the entrance of the five major rivers of the Poyang Lake, the outflow areas of the estuary of the Poyang Lake and the Yangtze River section, and other important wetland areas. And the debris-based MPs were the most important and accounted for 50% to 70% of the total, followed by the film-based MPs and fiber-based MPs, and foam MPs accounted for the lowest proportion. According to the analysis results of the particle size of MPs, MPs with particle

size less than 1 mm occupied the dominant position, accounting for more than 60% of the total number.

The abundance of MPs was calculated by the dry weight of sediments. The average abundance of MPs in the sediments of the Poyang Lake wetland was 1,146.17 particles kg^{-1} . And the abundance of MPs in different regions shows spatial differences. The average abundance of MPs in the Poyang Lake and the Le'an River section was 1,799.56 particles kg^{-1} ; and the average abundance of MPs in the entrance of the five major rivers of the Poyang Lake was 1,225.25 particles kg^{-1} ; the average abundance of MPs in the National Nature Reserves was 82.33 particles kg^{-1} ; and the average abundance of MPs in the outflow area of the Poyang Lake Estuary and Yangtze River section was 907.4 particles kg^{-1} . There were differences in the proportion, size, and abundance of MP particles in different study areas, but all of the samples were dominated by MPs less than 1 mm.

The surface morphology and attached materials of the MPs separated in the sediments of the Poyang Lake Basin were analyzed by scanning electron microscopy and energy dispersive spectroscopy. The results show that the surface of the MP samples in the environment is rough, with obvious weathering characteristics and some residues. There are impurities such as clay in the surface crack or attached Si, Fe, Mg, O, Al, Ca, and other elements. Therefore, the surface morphology and complexity of MPs that have existed in the environment for a long time will become carriers of persistent organic pollutants and toxic and harmful substances and form diffusion pollution through co-migration in the environment. In the future, more attention should be paid to the dynamic processes and ecological effects of MPs in the riparian environment.

The distribution of MPs in the sediments of the typical wetlands in the Poyang Lake is closely related to human activities and life activities; the differences of MP abundance among different regions are affected seriously by human activities, hydrological conditions, river inputting, etc. The composition of MPs among different regions is closely related to the lifestyle of people in the region and the physical and chemical properties of MPs.

2 Methodologies

2.1 Overview of the Study Area

Poyang Lake is the largest freshwater lake in China. It is located in the northern part of Jiangxi Province and is one of the three rivers and lakes in the middle and lower reaches of the Yangtze River. It is surrounded by mountains on three sides and lies between 28°22'–29°45' north latitude and 115°47'–116°45' east longitude. Poyang Lake is the receptor for the inland source material in its basin, and it is also an important source of agricultural irrigation and industrial and domestic water in the basin. The Poyang Lake and the five major rivers (the Ganjiang River, the Fuhe River, the Xinjiang River, the Raohe River, and the Xiushui) constitute the Poyang

Lake water system, and the Ganjiang River has four branches named the north branch, the south branch, the west branch, and the middle branch. Consequently, the lake's hydrological conditions, sediment transport, and sedimentation are affected by inputs from these five major rivers. In recent years, due to the impact of the domestic garbage discharged by nearby residents, frequent waterway transportation, and poorly regulated fishing [1], the plastic pollution problem of the five major rivers in Poyang Lake has become serious. Therefore, it is of great benefit to comprehensively and accurately evaluate the distribution characteristics of MPs in the entrance section of the five major river systems in the Poyang Lake.

2.2 Sampling Methods and Sampling Sites

In this chapter, all sample were collected in different seasons from December of 2016 to July of 2018, the typical wetlands were selected as the study areas, such as the entrances of the five major rivers in the Poyang Lake Basin, the typical wetland of the National Nature Reserves in the Poyang Lake, the estuary of the Poyang Lake and the Yangtze River section, and other important wetland areas. Five-point sampling methods with an area of each $50 \times 50 \text{ cm}^2$ were selected within each $10 \times 10 \text{ m}^2$ standard sampling quadrat to investigate and collect the sediment samples, and samples were collected by retrieving about 5 cm depth of sediments and placing them into the sealed tin foil bags. Sampling was conducted during periods of steady water flow. All samples were transported back to the laboratory and timely pretreatment in the laboratory.

2.3 Separation and Identification of Microplastics

In the laboratory, extraction of plastic particles was achieved by density separation using a saturated NaCl (1.5 g cm^{-3}) solution as the density-controlled liquid [2]. The dry samples were transferred into a glass beaker for density separation, then the salt solution was added, and the sample was magnetically stirred for 2 min. After the sediments had settled, the supernatant was carefully poured through a sieve with $2 \text{ }\mu\text{m}$ mesh size. The sediment samples were separated into the size fractions of 4.0–5.0, 3.0–4.0, 2.0–3.0, 1.0–2.0, and $< 1.0 \text{ mm}$. Materials retained on the mesh were examined by the naked eye for potential MPs or under a stereomicroscope to select suspected MPs (0.002–5 mm in size).

At present, combinations of physical (e.g., microscopy) and chemical (e.g., spectroscopy) analyses are widely used [3]. The membrane and the material retained on the sieves were dried and observed under metallographic microscope (Shanghai Precision Instrument Company, China) to select plastic debris. All particles were photographed using a Nikon digital camera DXM1200F connected to the microscope. A desktop scanning microscope (S-3400N, Hitachi Electronics, Japan) with 5–3,000 \times magnification was used to observe the morphology of MPs.

2.4 Data Processing and Analysis

The individual numbers of MPs were counted by using Nano Measurer 1.2 software, and the particle sizes were measured along the longest side of the MPs. The infrared spectrum of the separated MP particles was analyzed by using an infrared spectrometer (Nicolet 6700). All sampling site maps were plotted by using ArcGIS 10.2 software; all experimental data were processed by using Origin 9.0 software. On the other hand, the PROC UNIVARIATE procedures were also used to test the normality of the data, and PROC TTEST procedure was used to test for homogeneity in the variances. Duncan's multiple-range test was used to perform multiple comparisons and evaluate whether the MP abundance significantly differed between different sites and seasons. The level of significance was set at $P < 0.05$. All statistical analyses were performed in SPSS v.20 [4].

3 Distribution Characteristics of Microplastics in the Sediments of the Le'an River Basin of the Poyang Lake Section

3.1 Overview of the Typical Areas Disturbed and Polluted by Artificial Activities in the Poyang Lake

This section focuses on the typical areas affected by the human activities. The Le'an River of the Poyang Lake is selected as the study area which is seriously affected by mining. The Le'an River is the main tributary of the Raohe River, one of the major five rivers of the Poyang Lake Basin. It originated in the southwest foot of Wulong Mountains in the northeast of Wuyuan County in Jiangxi Province; it is 279 km long and has a basin area of 8,456 km². The upstream of the Le'an River is mainly located in the area of Dexing City in Jiangxi Province, mainly flowing through residential areas. Garbage dumped in nearby residential areas is the major source of plastic pollution. The Dawu River is the main tributary of the Le'an River, with a total length of 14 km. It runs through the largest copper mine in Asia and the third in the world, the Dexing Copper Mine. In recent years, the Dawu River has become a typical area with extremely serious pollution due to the influence of mineral exploitation and garbage dumping. The downstream of the Le'an River is located in Poyang County of Jiangxi Province, and the environmental pollution problem is not optimistic, due to the influence of the nearby residents' domestic garbage, frequent shipping in the channel transportation, and disordered fishing. It has an important ecological significance to choose the Le'an River of the Poyang Lake as the study area.

3.2 Sampling Sites in the Le'an River of the Poyang Lake

In December 2016, nine typical sampling sites were set up from the upstream to the downstream of the Le'an River (S1, S2, S3, S7, S8, S9) and included other three sites (S4, S5, S6) located in the Dawuhe River of the tributary of the Le'an River in Dexing Mine Factory (Fig. 1). The first sampling site (S1) was located in the headstream of the Le'an River in Haikou Town in Wuyuan County of Jiangxi Province, the other two sites (S2 and S3) located in the middle stream of the Le'an River in Dexing City of Jiangxi Province, and the other three sites (S7, S8, and S9) located in the downstream of the Le'an River and Poyang Lake in Poyang City of Jiangxi Province. All samples were collected by five site sampling methods and transported back to the laboratory and timely pretreatment in the laboratory.

3.3 Type and Particle Size Distribution of Microplastics in the Le'an River of the Poyang Lake

MPs were separated from the sediment samples by flotation and identified by microscope. The results of microscopic identification show that there are four types of MPs in the Le'an River of the Poyang Lake, such as debris, foams, films, and fibers as shown in Fig. 2. The color of the debris MPs is mainly milky white, blue, and yellow, the shape of which is mostly flat debris with damaged border.

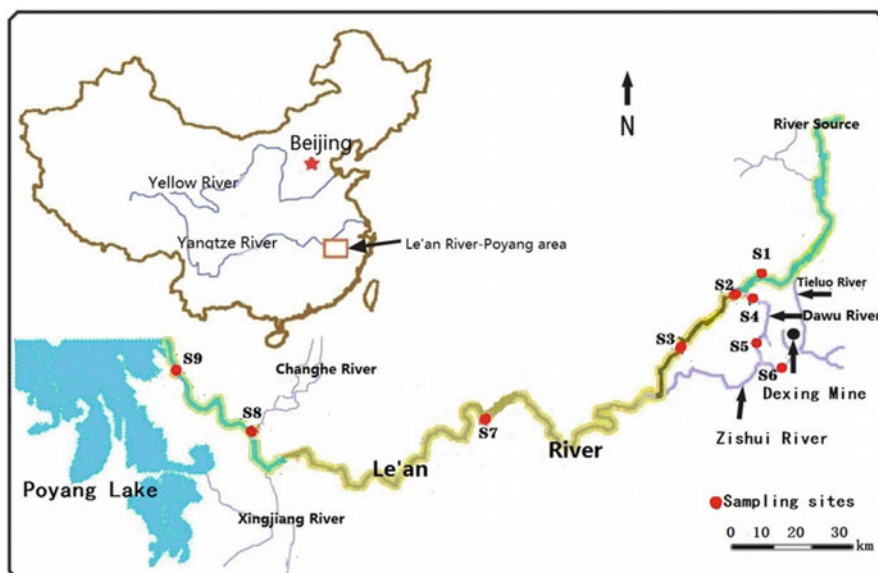


Fig. 1 Location of sampling sites in the Le'an River Basin of the Poyang Lake

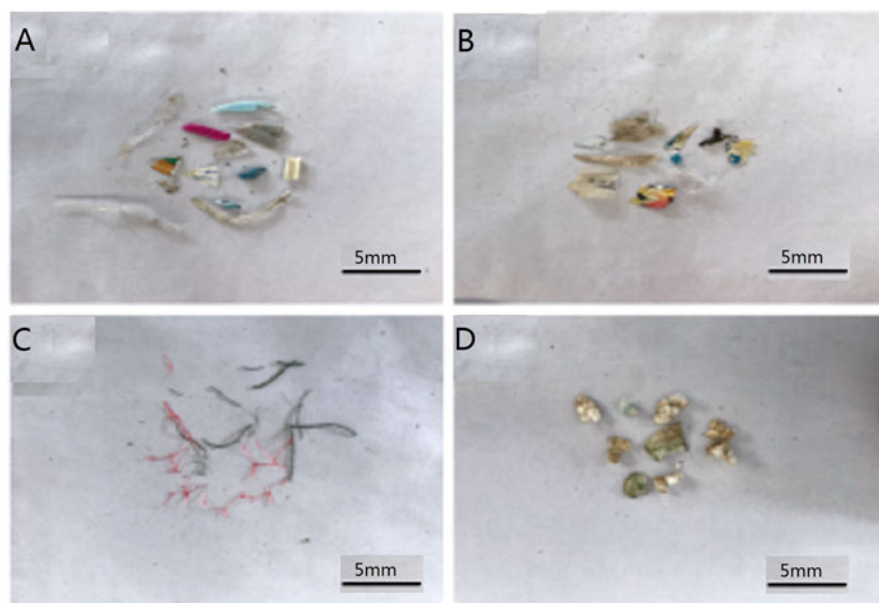


Fig. 2 Different MP samples in the sediments of the Le'an River Basin (unit: cm). (a) Debris MPs; (b) film MPs; (c) fiber MPs; (d) foam MPs

The color of film MPs is mainly milky white, red, and light blue; the shape of film MPs is mostly irregular curly. Fiber MPs are mainly blue in color and most of their shapes are curly. The color of foam MPs is mostly white, and their shape is mostly block or round.

At all the sampling sites, the proportion of debris MPs is the highest, accounting for 58.3% of the total, followed by foam MPs, accounting for 21.5%, and film MPs and fiber MPs accounting for 13.8% and 6.4%, respectively. The proportion of MPs is shown in Fig. 3.

The particle size distribution of MPs is shown in Fig. 4. MPs with the particle size <1 mm were the most abundant fraction in sediments, accounting for 62.4% of the total, and the abundance of MPs decreases with the increase of the particle size among 1–2 mm MPs, accounting for 18.7% of the total, and 2–3 mm, 3–4 mm, and 4–5 mm accounting for 8.7%, 6.9%, and 3.3%, respectively. The results of the particle size and the distribution are consistent with the results of Shandong coastal, Yangtze River Estuary, and Singapore mangrove area.

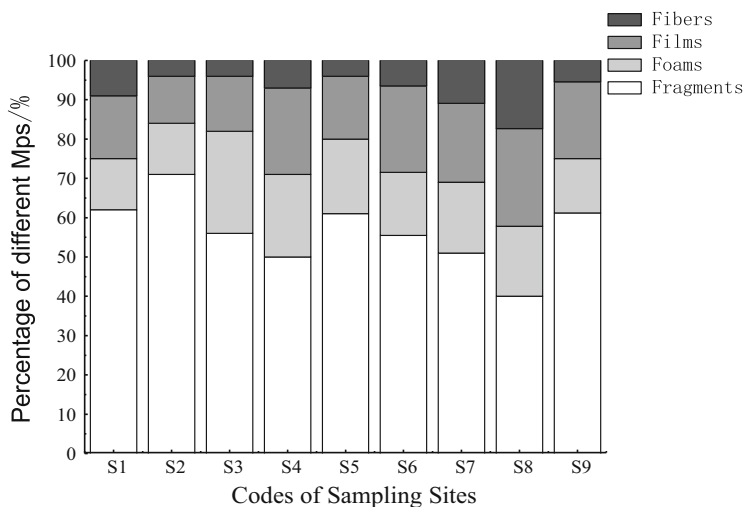


Fig. 3 Composition proportion of different forms of MPs in the Le'an River Basins

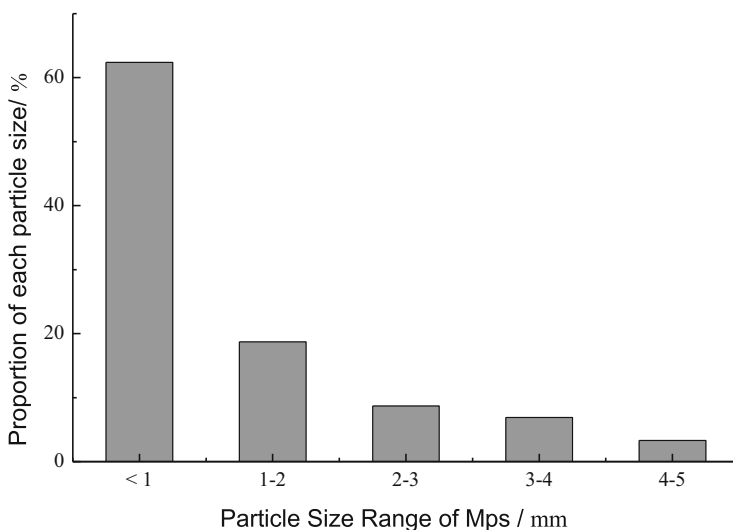


Fig. 4 Percentage chart of different sizes of MPs in the Le'an River Basins

3.4 Microplastics Abundance in the Le'an River of the Poyang Lake

This chapter carries out flotation separation methods of the sediments from the nine sampling sites in the Le'an River Basin. The average abundance value of MPs in the above nine sampling sites is 1,799.56 particles kg^{-1} . The abundance values of MPs

in different sampling sites are shown in Fig. 5. The results show that the average abundance value of MPs in the residential areas of Dexing Copper Mine (the S4 point) is 3,153 particles kg^{-1} , which is the highest in the whole study area of the Le'an River, the average value of MPs in the S5 point of Dexing middle school is 2,842 particles kg^{-1} , and the average value of MPs in the S6 point of the production area of Dexing Copper Mine is 2,619 particles kg^{-1} . The average value of MPs in the S1 point of the upstream of the Le'an River in Haikou Town is 842 particles kg^{-1} , which is the lowest in the whole study area of the Le'an River. At the same time, the nine sampling sites can be divided into three different regions including the upstreams of the Le'an River (S1, S2, and S3), the tributaries of the Le'an River (S4, S5, and S6 in the Dawu River), and the downstreams of the Le'an River (S7, S8, and S9). The average abundance value of MPs in the Dawu River is 2,871.33 particles kg^{-1} , and the values of MPs in the upstreams and downstreams of the Le'an River are 1,366.33 particles kg^{-1} and 1,121.33 particles kg^{-1} , respectively. The order of the average abundance values of MPs in the three areas were that the Dawu River, the downstreams of the Le'an River, the upstreams of the Le'an River, and the abundance values of MPs in the Dawu River are significantly higher than those in the downstreams and the upstreams of the Le'an River ($P < 0.05$). The main reason for this difference may be that the population density on both sides of the Dawu River is relatively concentrated and the domestic and industrial waste plastics are more than those of the upstream and downstream of the Le'an River, which leads to the increasing of the MP abundance. As a tributary of the Le'an River, Dawu River is the main accumulation area of the MPs because of its small surface water scouring effect. The abundance of MPs in the downstream of the Le'an River is significantly higher than that in the upstream ($P < 0.05$). The other reason may be that the MPs in

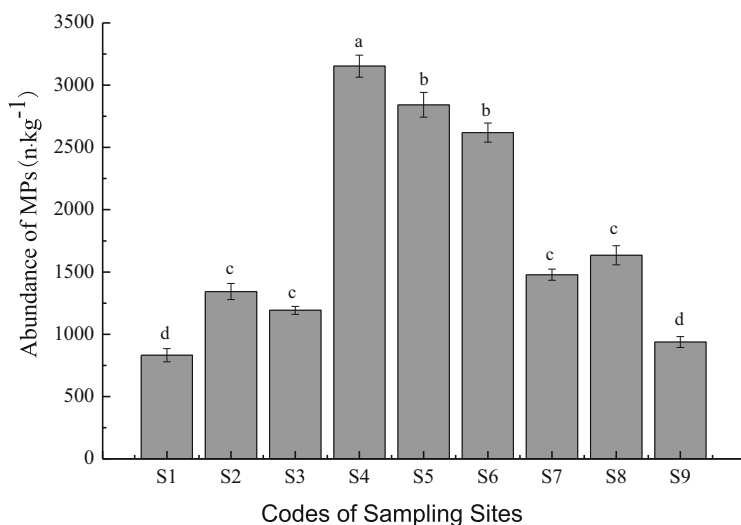


Fig. 5 Abundance value of MPs in the Le'an River Basin

Table 1 Comparison of the MP abundance in the Le'an River Basin and other reported areas

Study area	MP size	MP abundance	Regions
Mediterranean coastal beach (Venice Lagoon)	<5 mm	2,175 particles kg ⁻¹	Noam et al. [5]
Halifax Port, Nova Scotia, Canada	<5 mm	500 particles kg ⁻¹	Mathalon et al. [6]
Yangtze Estuary	<5 mm	4,137 particles kg ⁻¹	Zhao et al. [7]
Belgian coastal beach	38 μm to 5 mm	120 particles kg ⁻¹	Claessens [8]
Coastal beach of Shandong Province	0.025–5 mm	834 particles kg ⁻¹	Zhou et al. [9]
The Le'an River of the Poyang Lake	0.025–5 mm	1,799.56 particles kg ⁻¹	This study

the upstream of the Le'an River and Dawu River can be accumulated to the downstream of the Le'an River through the hydrodynamic exchange, which leads to the abundance of MPs in the area higher than those in the upstream of the Le'an River.

The results of this study are compared with those of domestic and foreign research areas, which are shown in Table 1. Noam et al. [5] found that MP abundance in Mediterranean coastal beaches (Venice lagoon) was as high as 2,175 particles kg⁻¹. Mathalon et al. [6] found that MP abundance in the Halifax Harbor, Nova Scotia, Canada, was 500 particles kg⁻¹. Zhao Shiye et al. [7] conducted a survey of MPs in the Yangtze River Estuary, and the results showed that MP abundance was as high as 4,137 particles kg⁻¹. Claessens et al. [8] took the sediment samples near the high water level in the intertidal zone on Belgian coastal beach. The results showed that MP abundance was 120 particles kg⁻¹. Zhou Qian et al. [9] surveyed the estuaries along the coast of Shandong Province. The results showed that MP abundance was 824 particles kg⁻¹. Compared with the abundance of MPs in the above regions, MP abundance in the Le'an River of the Poyang Lake was on the upper middle level.

3.5 Source Analysis of Microplastics in the Le'an River of the Poyang Lake

Based on the field investigation and the above research results, the source and abundance distribution of MPs in the Le'an River of the Poyang Lake are analyzed. The first important source is domestic waste plastic (such as plastic cases) and plastic toys discharged from surrounding residential areas, engineering plastic woven bags produced by flood control and dam filling, and fertilizer woven bags used in agricultural production. The second source is that the film plastic products produced by food packaging bags and agricultural products discharged from living areas are cracked. At the same time, the waste plastic bags produced by fishery activities in the study area are also one of the sources of film MPs in the Le'an River of the Poyang

Lake. Another important source is that aquaculture and fishing activities have led to the abandonment of some nets and fishing lines into the environment and then debris into the fine fibrous debris by long-term environmental effects. At the same time, the discharge of domestic water may also be the source of fiber MPs. Previous studies have shown that more than 2,900 fibers can be produced into the wastewater at a time in the daily laundry cleaning process, and the amount of fiber in wastewater can be up to more than 100 particles L^{-1} [10]. The other source is that the disposable plastic box produced in human daily life and plastic foam floaters in fishing boats are made up of a large number of foam plastic particles, which are easily cracked into the environment and produce MPs. In addition, some foam plastic buoys used in aquaculture are also one of the important sources of the foam MPs.

4 Distribution Characteristics of Microplastics in the Sediments of the Five Major Rivers of the Poyang Lake

4.1 Overview of the Main Five Rivers of the Poyang Lake

The five major tributaries of the Poyang Lake are the Ganjiang River, the Fuhe River, the Xinjiang River, the Raohe River, and the Xiushui River. The Ganjiang River originated from Ganzhou of Jiangxi Province, flowing through Ganzhou City, Ji'an City, and Nanchang City, and was injected into the Poyang Lake in Wucheng Town in Yongxiu County of Jiujiang City. The Ganjiang River is also the longest river among the five major rivers of the Poyang Lake. The Fuhe River originated from Guangchang County and was injected into the Poyang Lake via QinglanHu Lake, which is the second largest river in the five major rivers of the Poyang Lake. The Xinjiang River originated from the junction of the two Provinces of Jiangsu Province and Zhejiang Province and was injected into the Poyang Lake through Ruihong Town of Yugan County in Jiangxi Province. The Raohe River is formed by the confluence of the two tributaries of the Le'an River and Changjiang River, which merge into the Poyang Lake through the intersection of Longkou Wharf in Lianhu Town of Poyang County in Jiangxi Province. The Xiushui River is the smallest tributary of the main five rivers of the Poyang Lake and is also transferred to the Poyang Lake by Wucheng Town in Yongxiu County of Jiujiang City. The main five rivers of the Poyang Lake gathered the most of the surface water in Jiangxi Province and eventually flowed into the Poyang Lake.

4.2 Sampling Sites in the Entrances of the Main Five Rivers of the Poyang Lake Basin

In November of 2017, eight representative sampling sites in the entrances of the five rivers, respectively, were also selected, and the sediment samples were collected. The position of each sample is shown in Fig. 6. They are mainly the estuary of the north branch of the Ganjiang River in Wucheng Town of Jiujiang City (S1), the estuary of the middle branch of the Ganjiang River in Zhugang Town of Xinjian County (S2), the estuary of the south branch of the Ganjiang River in Jiangjunzhou Farm of Nanchang County (S3), the estuary of the Xiushui River in Wucheng Town (S4), the estuary of the Fuhe River in Dutou Town of Nanchang County (S5), the

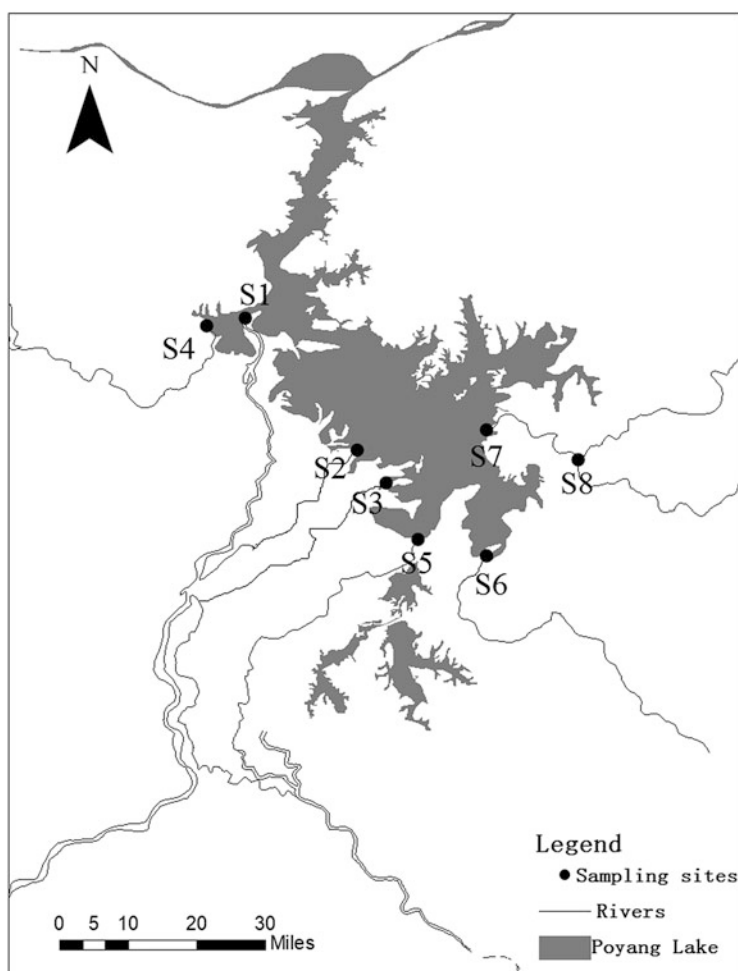


Fig. 6 Location of the sampling sites in the five major rivers of the Poyang Lake

estuary of the Xinjiang River in Ruihong Town of Yugan County (S6), the estuary of the Raohe River in Longkou Village of Poyang County (S7), and the estuary of the Le'an River in Yaogongdu of Poyang County (S8). All samples were also collected by five site sampling methods and transported back to the laboratory and timely pretreatment in the laboratory.

4.3 Shapes and Abundances of Microplastics in the Sediments of the Main Five Rivers of the Poyang Lake Basin

In this part, the shapes of MPs in the sediments of the main five rivers were identified by using the microscope, and the MP types of the five rivers are also mainly composed of debris, films, foams, and films in each point.

The composition of different forms of MPs is shown in Fig. 7. The debris MP particle size is relatively concentrated in the sediments, most of them were less than 1,000 μm , and the range of particle size was from 25 μm to 1,000 μm . But fibers, foams, and films had a wide range of particle size, and the ranges of the three types particle size were from 100 μm to 5,000 μm ; the particle size is mostly concentrated in the range from 500 μm to 3,500 μm . The composition proportion of MPs in the sediments of different sites had both consistency and some differences.

The results of MP abundance in the eight sampling sites at the entrance of the major five rivers of the Poyang Lake are shown in Fig. 8. The abundance of MPs in the sediments of the north branch of the Ganjiang River (S2) is the highest, and the

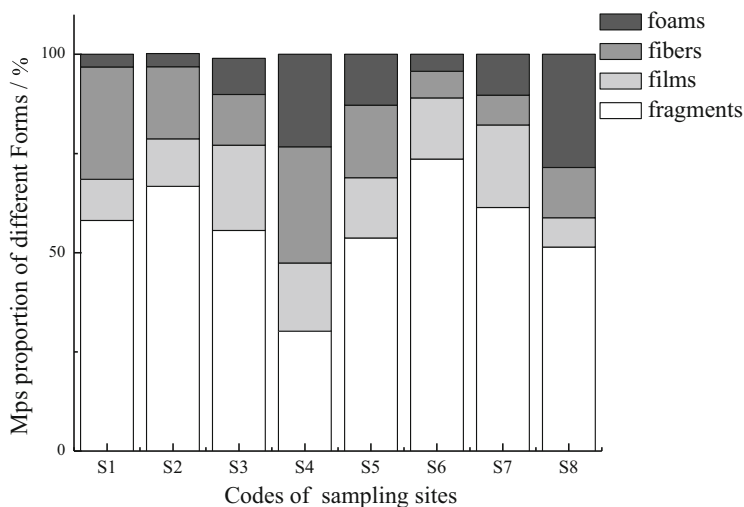


Fig. 7 Composition of MPs in the main five rivers of the Poyang Lake

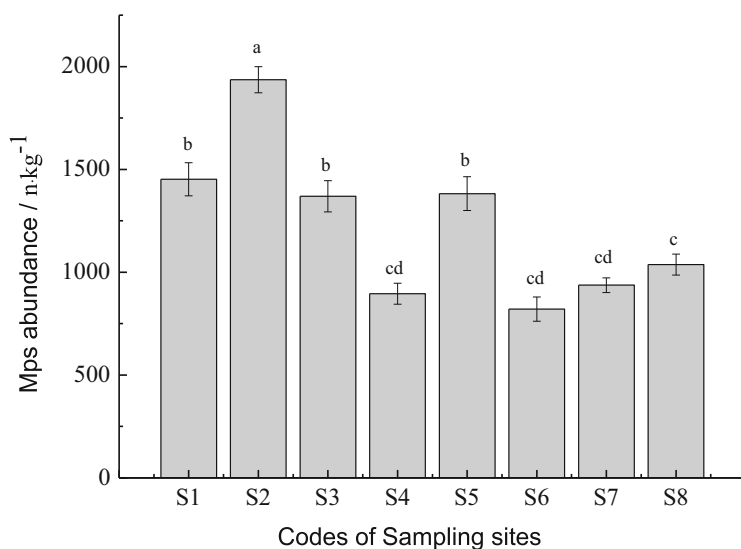


Fig. 8 MP abundance of plastic in each sampling site

value is 1,936 particles kg^{-1} , which is significantly higher than that of the entrances of other branches of the Ganjiang River (S1, S3, S5). The MP abundance in the sediments of the entrance in the Le'an River (S8) is 1,037 particles kg^{-1} , but there was no significant difference with the entrances of the Xiushui River (S4), the Xinjiang River (S6), and the Raohe River (S7).

4.4 Microplastics Polymer Components of the Main Five Rivers of the Poyang Lake Basin

The four types typical MPs of debris, films, foams, and films in the sediments of the main five rivers of the Poyang Lake Basin were selected for identification by infrared spectroscopy. One of the hard plastic debris of the infrared spectrum is shown in Fig. 9a, which shows that the composition is polyethylene. The white typical plastic woven bag debris infrared spectrum is shown in Fig. 9b, which shows that the main composition is polypropylene. The infrared spectrum of the white foam is shown in Fig. 9c; it shows the composition is polystyrene. The infrared spectrum of the red plastic bag films MPs is shown in Fig. 9d, which is mainly composed of polypropylene. The other kind of MPs is white plastic bag film, and the infrared spectrum is shown in Fig. 9e, which is mainly composed of polypropylene. The fishing line was selected as the fiber type and analyzed by spectroscopic analysis in Fig. 9f, which shows the MP composition is polyethylene. The polymer components of the MPs can also be found in the main five rivers of Poyang Lake in the study that have certain similarities and differences compared with other studies. Zhao et al. [7]

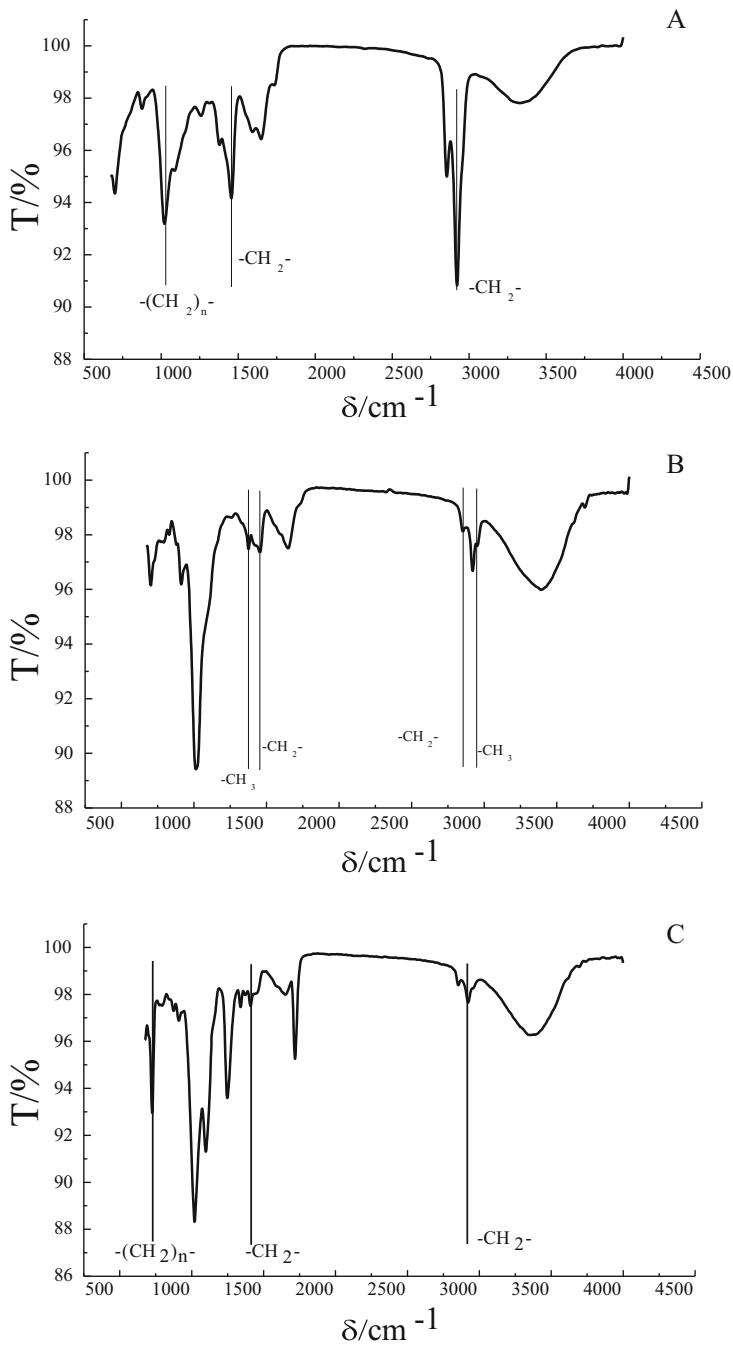


Fig. 9 FTIR spectrums of different MP samples in sediments. (a, b) Debris MPs; (c) film MPs; (d, e) fiber MPs; (f) foam MPs

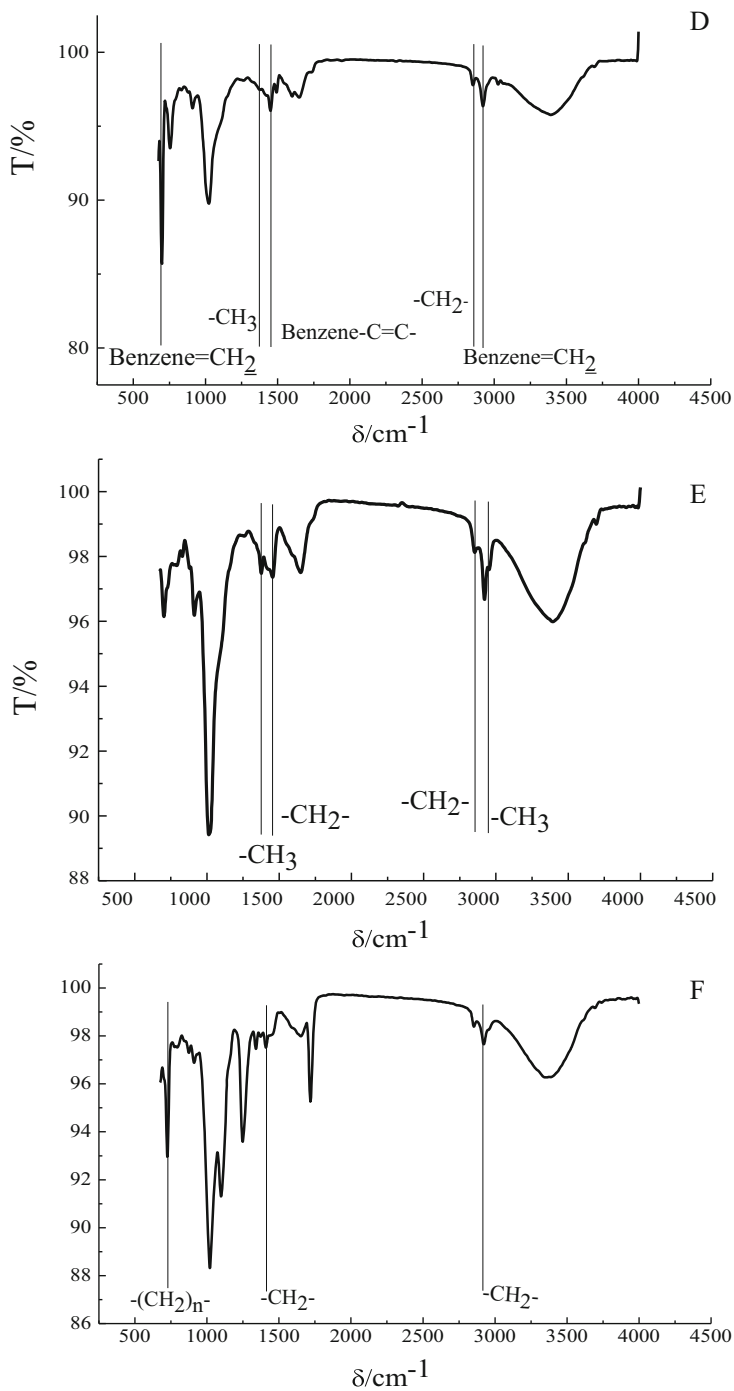


Fig. 9 (continued)

considered that the polymer composition of the MPs of the debris is polyethylene and polypropylene. The polymer components of the film are polyethylene, polypropylene, and polyphenylene, while the particles contain four polymer components of polyethylene, polypropylene, polyvinylchloride, and polytetrafluoroethylene.

The infrared spectrum of the typical MPs in the main five rivers of the Poyang Lake Basin was compared with the standard infrared spectrum of the corresponding polymer components, and the peaks of the infrared spectrum band of the MPs in the environment were found to be significantly more than the standard infrared spectrum, although the polymer components of the two are the same substance. For example, the peaks in the low-band region of the infrared spectrum of the debris MPs in the environment are significantly more than the standard, especially at the band of $750\text{--}1,500\text{ cm}^{-1}$, and the infrared spectrum is significantly different in the environment, while the standard spectrum is flat at the band range with almost no significant fluctuations. Corcoran et al. [11] considered that the surface of MPs in the environment is oxidized to a certain extent, and there are many foreign substances, which further illustrate the complexity of the MP surface in the environment. At present, more and more researchers at home and abroad try to explore the effects of MPs on aquatic organisms. Koelmans [12] concluded that micropolystyrene has direct toxic effects on the cell and molecular levels of the marine mussels. Cole et al. [13] found that the intake of MPs by zooplankton can significantly change the feeding ability of phytoplankton, and the long-term exposure to polystyrene MPs will significantly reduce the reproductive rate of *Phytophthora*. But there is no significant effect on its survival.

4.5 Comparison of Microplastics Abundance of the Main Five Rivers in Poyang Lake Basin

The order of MP abundance values of the main five rivers in the Poyang Lake Basin are as follows: the Ganjiang River>the Fuhe River>the Raohe River>the Xiushui River>the Xinjiang River. There are many reasons for these differences. The first reason is that the runoff of the river has a certain influence on the MP abundance. The greater the runoff of the river, the more it flows through residential areas, and the more plastic waste is discharged into rivers. The Ganjiang River is the largest runoff river among the five rivers in the Poyang Lake Basin; its runoff reaches 70.2 billion m^3 , which is far greater than the other four rivers. The MP abundance of the three tributaries is greater than that of other lakes. The second reason is that the amount of sand from the five rivers in the Poyang Lake Basin also affects the abundance of MPs. The most contribution of sediment transport in the Poyang Lake Basin is the Ganjiang River; its contribution rate of sediment transport is 40%, the Fuhe River and the Raohe River account for 20% respectively, the Xiushui River and the Xinjiang River account for 12% and 8% respectively.

4.6 Microplastics Types of the Main Five Rivers in the Poyang Lake Basin

The MP composition ratios of the north branch of the Ganjiang River, the middle branch of the Ganjiang River, and the south branch of the Ganjiang River have certain similarities. The proportion of debris and fibers are all the highest in the total MPs of the sediments in all branches of the Ganjiang River. The difference is that the proportion of foam MPs in the north branch of the Ganjiang River and the middle branch of the Ganjiang River is higher than that of film MPs, while the proportion of foam MPs in the south branch of the Ganjiang River is lower than that of films MPs. The reason may be due to the foam plastics in domestic waste into the entry of the main branches of the Ganjiang River into the Poyang Lake in Wucheng Town in Jiujiang City and the middle branch of the Ganjiang River into Zhugang Town in Xinjian County. In addition to the above reasons, there are also some fishing boats and shipping vessels in the main river basins of the Ganjiang River, and the plastic foam float in the hull also produces a certain amount of foam plastics in the environment. The composition of MPs in the estuary of the Xiushui River is relatively even, and the abundance values of MPs of debris, films, foams, and films account for 30.2%, 17.2%, 29.3%, and 23.3%, respectively. The reason may be that there is domestic sewage discharge around the sampling sites. Previous studies have shown that in the daily cleaning process, more than 2,900 fibers can be produced into the wastewater per cleaning. The amount of fibers per unit volume of discarded water can be more than 100 particles L^{-1} . Moreover, the abandoned fishing nets in the water are cracked to produce MPs. The proportion of MPs in the estuary of the Xinjiang River and the Raohe River is similar. The values are 73.6% and 61.4%, respectively, and the highest proportion of debris MPs, followed by the thin film MPs, with the smallest proportion of foams and films. The proportion of MPs in the estuary of the Le'an River is different from that of other sampling sites. The proportion of the film MPs is the lowest, accounting for only 7.4%, while the proportion of the fiber MPs is second only, accounting for 28.5%. The reason is similar to the estuary of the Xiushui River. There are a large number of abandoned fishing nets around the sampling sites, which produce a large number of fiber MPs after cracking. Therefore, the composition ratio of MPs in the environment is closely related to the production lifestyle around the plot.

5 Distribution Characteristics of Microplastics in the Sediments of Typical National Nature Reserves of the Poyang Lake

5.1 Overview of the Typical National Nature Reserves of the Poyang Lake Basin

In this section, the typical Nature Reserves of the Poyang Lake were focused on the Nanjishan National Nature Reserve and the Wucheng National Migratory Bird Reserve. The Nanjishan National Nature Reserve is located in the Xinjian County of Jiangxi Province. There are two small islands called Nanshan Island and Jishan Island, which are good hiding places and habitats for wintering migratory birds. The Wucheng National Migratory Bird Reserve is located in Wucheng Town of Yongxiu County in Jiujiang City of Jiangxi Province, where the main branch of the Ganjiang River and the Xiushui River merges into the intersection of the Poyang Lake. There are many kinds of rare birds such as cranes, mites, geese, ducks, and gulls in the two national reserves.

5.2 Samples Selection and Collection in the Typical Nature Reserve of the Poyang Lake

In December of 2018, the sediment samples were collected from the Nanjishan National Nature Reserve and Wucheng National Migratory Bird Nature Reserve. The five sampling sites included Nanshan (S1), Jishan (S2), Dahuchi (S3), Zhonghuchi (S4), and Banghu (S5) shown in Fig. 10. All samples were also collected by the five site sampling methods and transported back to the laboratory and timely pretreatment in the laboratory.

5.3 Morphological Characteristics and Composition of the Microplastics in the Typical Nature Reserve of the Poyang Lake

MPs were also separated from the sediment samples in the typical Nature Reserves by flotation and identified by microscope. The results of the microscopic identification show that there are also four different types of MPs, including debris, foams, fibers, and films, observed in the sample sediment. The separated debris MPs have blue and translucent colors, the shapes are flat, the edges are irregular, and there are signs of damage; the foam MPs are milky white, with no fixed shape; the fiber MPs are dark blue, with aging-like fishing line; the film MPs are more transparent, with

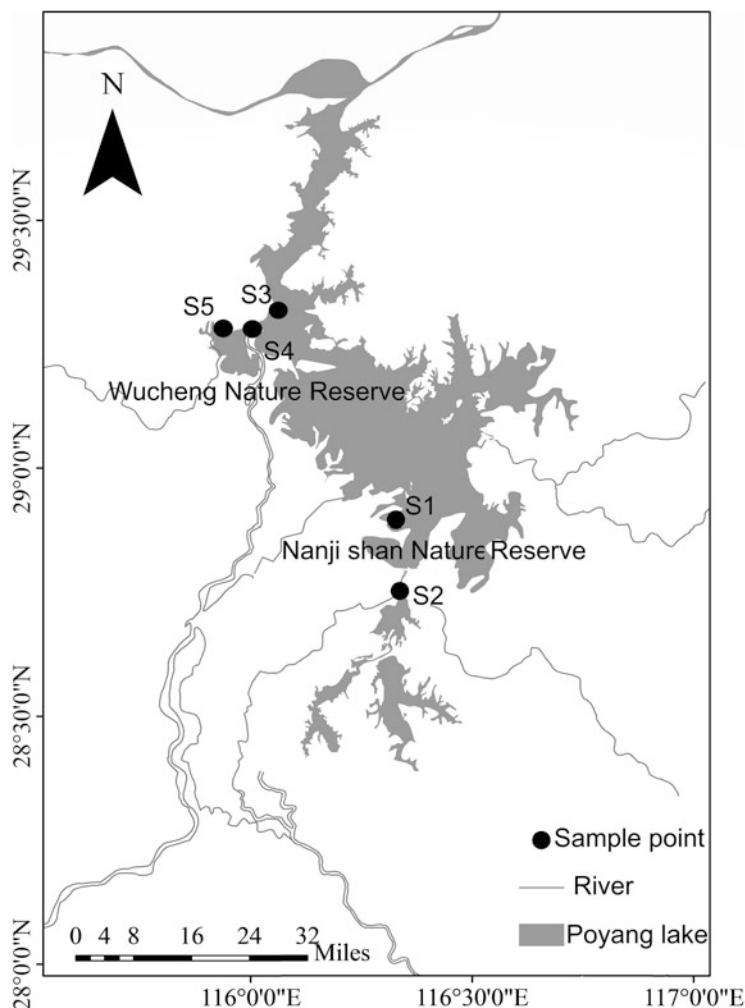


Fig. 10 Locations of sampling sites in the study area

muddy stains, also no fixed shape, and signs of damage to the surrounding edges. In the Nature Reserves, the color MPs have the highest detection rates, accounting for 61.2%. Plastics play an important role in human life, and dyeing of plastic products is an important means to improve the market competitiveness of the products. When humans use these plastic products, a large amount of plastic waste is discharged into the environment, and plastic waste left in the environment can be degraded into MPs for a long time. Colored plastic materials used in fishing lines and fishing nets in the study area may be an important source of these colored MPs. At the same time, domestic sewage discharged from the upper reaches of the Ganjiang River and food packaging waste left by human activities may also be potential sources of these

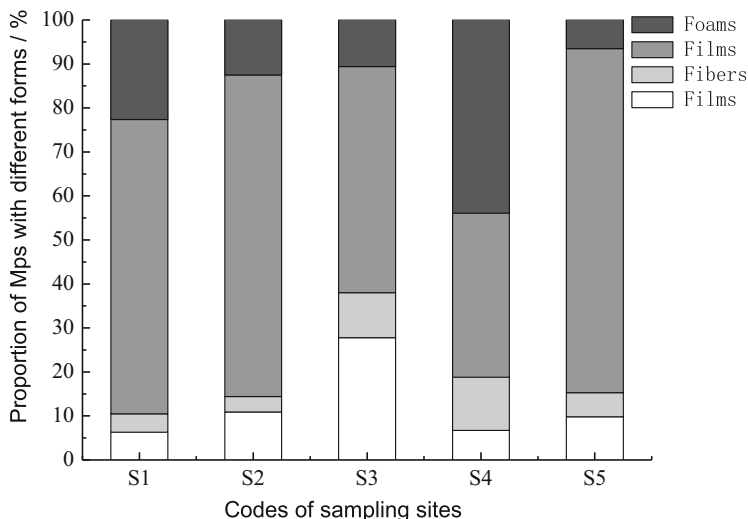


Fig. 11 Composition of microplastics in each sampling sites of the Nature Reserves

colored plastic particles. Although there are few studies on the detoxification of colored MPs by freshwater organisms, Shiber [14] in Hokkaido have shown that marine organisms have a higher probability of engulfing colored MPs than swallowing transparent MPs. Therefore, the research of colored MPs still needs to be further studied.

Among the four MPs isolated from the two National Nature Reserves of the Poyang Lake, the proportion of fiber MPs is more than half of all the samples and accounts for 53.9% (Fig. 11). A large part of the fiber MPs is derived from the degradation of fishing nets or fishing lines in fishing boats near the sampling sites. That is because the Nanjishan Nature Reserve and Wucheng Nature Reserve are both the National Nature Reserves and are less affected by human activities. In addition, household and industrial wastewater, waste slag emissions, surface runoff, agricultural production, and atmospheric deposition are also the potential sources of fiber MPs.

The types of MPs isolated in this study are compared with those of other scholars in China; there are five types of MPs in the tidal flat area of Bohai Sea which included debris MPs, fiber MPs, film MPs, granules, and foam MPs [9], while there is no particulate plastic in the Nature Reserves of the Poyang Lake. The reason for this difference is that there are a large number of transport vessels in the Bohai Sea area; the resin particles left in the transportation process are important reasons for the large number of particulate MPs in the Bohai Sea.

In some previous report, there were debris MPs, film MPs, and fiber MPs in Jiaojiang, Wujiang, and Minjiang, but no foams were mentioned, while debris, foams, and particles were found in the Pearl River beach, but no fibers and films were found [7]. The reasons for such differences are different from the study areas,

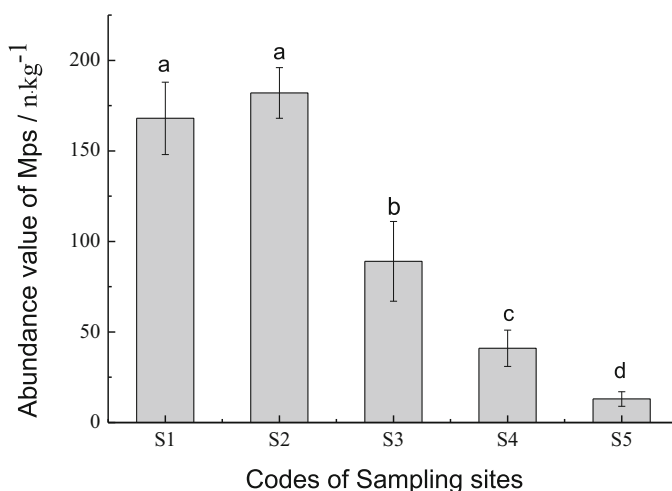


Fig. 12 MP abundance of plastic in each sampling site of the Nature Reserves

and there are differences in the classification basis. Zhao Shiye et al. [7] divided the granular MPs and resin particles into different types of MPs in the study of Jiaojiang, Wujiang, and Minjiang. Therefore, there is an urgent need to establish a systematic MPs classification standard.

5.4 Abundance of Microplastics in the Typical National Nature Reserves of the Poyang Lake

The abundances of MPs in the different five representative sampling sites in the National Nature Reserves were shown in Fig. 12. The results show that the range of the abundance values of MPs is from 13 to 182 particles kg^{-1} and the average abundance was 82.33 particles kg^{-1} . The highest abundance value of MPs is 182 particles kg^{-1} in the Jishan (S2) sampling sites of the Nature Reserves, followed by Nanshan (S1), which is 168.98 particles kg^{-1} , and then the MPs abundance values in the Dahuchi (S3) and Zhonghuchi (S4) were 89.67 particles kg^{-1} and 41 particles kg^{-1} , respectively. The lowest MP abundance value was 13 particles kg^{-1} in the Banghu sampling sites (S5).

5.5 Discussion of Microplastics Types in the Typical Nature Reserves of the Poyang Lake

There are similarities and differences in the proportion of MPs in the Nature Reserves. There is a certain similarity in the proportion of MPs in the Nanshan

(S1) and Jishan (S1) sampling sites. The proportion of fiber MPs is the highest in both sampling sites, accounting for 66.9% and 73.1%, respectively. But the difference among them is that the proportion of foam MPs is the second, accounting for 22.9% in the Nanshan, while the proportion of foam MPs is only 10.9%, and the proportion of debris MPs is 12.5% in Jishan. The reason may be that the intensity of human activities is higher in the Jishan than that in the Nanshan, and the domestic waste left into the environment by human activities made the debris MPs relatively higher in the region. The proportions of MPs in the Dahuchi (S3), the Zhonghuchi (S4), and the Banghu (S5) sampling sites accounted for 51.4%, 43.9%, and 78.2%, respectively. Among them, fiber MPs in the sampling site of Banghu (S5) accounted for a much higher proportion than other types of MPs. The reason may be that the Banghu is far away from human activities compared with the other two sampling sites, which makes the source of MPs in this area single, only MPs flowing from the upper reaches of the Ganjiang River and plastic waste left by fishing boats for fishing activities. In general, the proportion of fiber MPs is the highest MP type in the five sampling sites of the Nature Reserves, while the highest proportion of MPs in the Le'an River of the Poyang Lake section and the estuaries of the main five rivers of the Poyang Lake is debris MPs, the reason for this difference is that the Le'an River of the Poyang Lake section and the estuaries of the main five rivers are the areas with frequent human activities, and the degradation of domestic waste becomes an important part of the MP source in the region. Therefore, the composition ratio of MP in the environment is closely related to the production lifestyle around the plot.

5.6 Discussion of the Different Abundance of Microplastics in the Typical Nature Reserves of the Poyang Lake

In this part, the five sampling sites were divided into two regions according to the Nanjishan and Wucheng for significant difference analysis. It can be seen that the MP abundance of Nanshan and Jishan is significantly different from the MP abundances of the Dahuchi, Zhonghuchi, and Banghu sampling sites ($P < 0.05$). There are three main factors that cause this difference. The first is that the Nanjishan is the famous bird-viewing area in Jiangxi Province, with a large number of tourists visiting each year, and visitors will bring certain plastic garbage to the Nanjishan National Nature Reserve; the degradation of the plastic garbage discarded by tourists and nearby residents may be the source of MPs in sediments. The second is that there are fishery farming areas around the two sampling areas of the Nanshan and Jishan, and the fishing activities will also bring plastic waste, which remained in the environment. The third is that the surrounding areas of the Dahuchi, Zhonghuchi, and Banghu in the Wucheng National Nature Reserve are less affected by human activities, and only a small number of fishing boats are infested.

6 Distribution Characteristics of Microplastics in Sediments of the Estuary of the Poyang Lake and the Yangtze River

6.1 Overview of the Estuary of the Poyang Lake and Yangtze River

This section focuses on the key areas of the Poyang Lake that merge into the Yangtze River, and the estuary of the Poyang Lake into the Yangtze River section was selected as the research area. The sampling sites is located in Hukou County of Jiujiang City in Jiangxi Province, the left bank from the hillside Junction which is from the Dongsheng Dike and Puwan Village to the Meijiashoutou; the right bank is from Wenchangfu to Jiujiang Shipyard, which is the key area for water from Poyang Lake into the Yangtze River and also the throat of Jiangxi Province and the outside shipping traffic. The estuary of the Poyang Lake and the Yangtze River is the only entrance to the Poyang Lake that enters the Yangtze River, which is affected by the flow of sediments of the Poyang Lake and Yangtze River; the relationship between the rivers and lakes is very complicated. Its river channel evolution directly affects the flood control and shipping safety of the Poyang Lake. In recent years, the economic development of the estuary of the Poyang Lake and the Yangtze River has developed rapidly, continuously increasing the development of fishery resources and more and more domestic garbage from industrial and agricultural sewage and towns.

6.2 Sampling Methods in the Intersection of the Poyang Lake and Yangtze River

In this part, the samples were collected in the Hukou County of Jiangxi Province in July of 2018. The five plots are Doushan (S1), Dukou (S2), Zheji (S3), Gaoshangzhou (S4), and Meijiashou (S5) (Fig. 13). All samples were also collected by five site sampling methods and transported back to the laboratory and timely pretreatment in the laboratory.

6.3 The Distribution of Microplastics Types and Particle Size in the Estuary of the Poyang Lake and the Yangtze River

In this part, the forms of MPs in the sediments of the estuary of the Poyang Lake and the Yangtze River were also identified by using the microscope, and the main forms of MPs in sediments are mainly composed of debris, films, foams, and fibers in

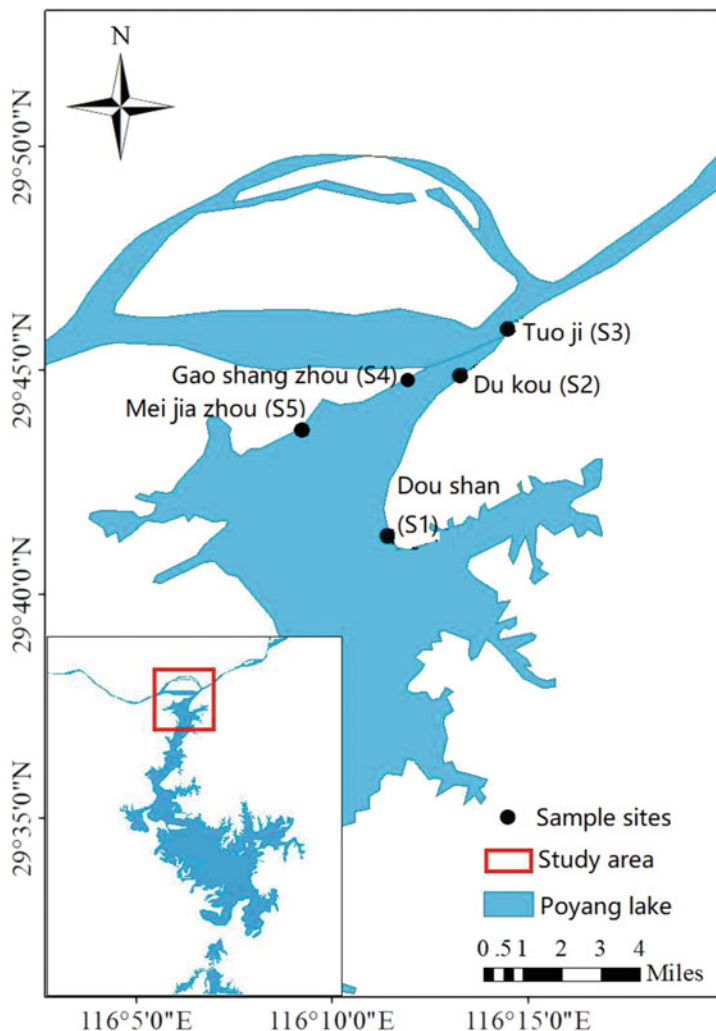
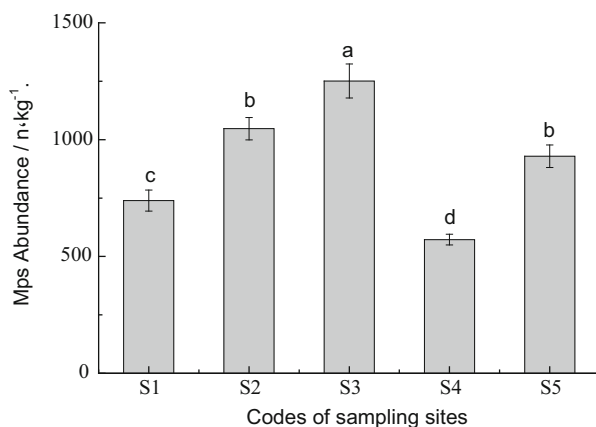


Fig. 13 Location of sampling sites in the estuary of the Poyang Lake and Yangtze River

different points. Debris MPs and film MPs accounted for the highest proportion, accounting for 50.8% and 25.1%, respectively. The main source of the debris MPs is the degradation of plastic products in daily life (such as polyethylene plastic bottles, plastic toys, etc.).

The main source of film MPs is the cracking of plastic products such as food packaging film and plastic film. The proportion of color in the study area is black (1%), transparent (23.87%), blue (13.09%), white (1.106%), red (10.60%), and other colors (2.6%), respectively. The debris MPs are mainly translucent, white, and black.

Fig. 14 MPs abundance value of plastic in each sampling site in the estuary of the Poyang Lake and the Yangtze River



The film MPs are mainly transparent and black, and the fiber MPs are mainly blue and red. The proportion of MP particle size is 0–1 mm (53.08%), 1–2 mm (27.60%), 2–3 mm (11.52%), 3–4 mm (4.72%), and 4–5 mm (3.08%). The whole trend of particle size distribution of MPs is as follows: with the decrease in the particle size of MPs, the number of MP particles in sediment samples increases, and about 80.68% of the MPs have a particle size of less than 1 mm.

6.4 Analysis of Microplastics Polymer Composition in the Estuary of the Poyang Lake and the Yangtze River

Four types of MPs were selected for Fourier transform infrared spectroscopy, and the results show that the main components are polyethylene (PE), polypropylene (PP), polystyrene (PC), and low-density polyethylene (LDPE), showing similar FTIR spectrums as shown in Fig. 9.

6.5 Microplastics Abundance in the Estuary of the Poyang Lake and the Yangtze River

MP flotation separation of the sediments in the estuary of the Poyang Lake and the Yangtze River was carried out, the abundance of the five sampling sites is shown in Fig. 14, MP abundance ranged from 572 to 1,251 particles kg⁻¹, and the average abundance value is 907.4 particles kg⁻¹. Among them, the MPs of Tuoji (S3) have the highest abundance of 1,251 particles kg⁻¹, the second highest is the Dukou (S2) with abundance of 1,047 particles kg⁻¹, Meijiazhou's (S5) MP abundance is 928.98 particles kg⁻¹, Doushan's (S1) MP abundance is 739 particles kg⁻¹, and the MPs of Gaoshangzhou (S4) have the lowest abundance of 572 particles kg⁻¹.

6.6 Analysis of Microplastics Abundance Difference in the Estuary of the Poyang Lake and the Yangtze River

The average abundance of MPs in this study area is lower than the average abundance of MPs in the entrances of the main five rivers of the Poyang Lake. The first reason may be that the waste plastic in the main five rivers has a wide range of sources. In addition to the waste plastic brought by the nearby residents' discharge and fishing activities, there are five plastic wastes discharged upstream of the river. Another reason is that the estuary of the Poyang Lake and the Yangtze River is the only entrance of the Poyang Lake which enters the Yangtze River, which affected by the Poyang Lake and the Yangtze River and sediments, the relationship of rivers and Lakes is very complicated, the river is highly fluid, causing the evacuation of MPs in the sediments. In addition, the MP abundance in the sediments of the estuary of the Poyang Lake and the Yangtze River is greater than the MP abundance of the Three Gorges Reservoir area ($192.5 \text{ particles kg}^{-1}$); this may be because from July to August every year, the Yangtze River will be poured into Poyang Lake, and the MPs of the Yangtze River will be washed by waves to the banks of the Poyang Lake. Because of the discharge of plastic waste and domestic sewage in the Yangtze River Basin, plastic waste in the Yangtze River Estuary continues to gather. Compared with the estuary of the Poyang Lake and the Yangtze River and the Brazilian Goiana ($18 \text{ particles kg}^{-1}$) [15], the Austrian Danube ($0.3168 \text{ particles kg}^{-1}$) [7], and the British Tamar estuary ($0.028 \text{ particles kg}^{-1}$) [16], the section of the estuary of the Poyang Lake and the Yangtze River is at a high level.

6.7 Analysis of Composition Differences of Microplastics Polymers in the Estuary of the Poyang Lake and the Yangtze River

The Fourier transform infrared spectrometer can measure the chemical bonds of the sample, while different chemical bonds can produce a unique spectrum, and the carbon-based polymer can be detected. The library of the Fourier transform infrared spectrometer can not only determine whether the sample is plastic but also determine its polymer type. In this chapter, MPs were selected for infrared spectroscopy analysis, and compared with the system's own library, the polymer components were identified. Debris plastics are mainly composed of polyethylene by infrared spectroscopy; the composition of thin film MPs is mainly polypropylene; the infrared spectrum of fiber MPs is mainly composed of polyethylene; the composition of foam MPs is mainly polystyrene. Comparing the infrared spectrum obtained in this study with the standard infrared spectrum of the corresponding polymer components, it is found that although the polymer components of the two are the same substance, the peaks in the infrared spectral band of the MPs in the environment are

significantly more than the peak of the band in the standard infrared spectrum, because the peaks of the debris in the environment are more complex in the band, and the standard spectrum is flat in this band, and there is almost no obvious peak.

7 Conclusions

The research areas of this chapter mainly included some typical areas disturbed and polluted by artificial activities, the entrance of the main five rivers of the Poyang Lake basins, two National Nature Reserves, and the outflow areas of the Poyang Lake flowing into the Yangtze River, and the research object is microplastics in the sediments of the wetland in the research areas. The composition and distribution characteristics of MPs were simultaneously identified by infrared spectrometer to determine the surface morphology and elemental composition of MPs in the chapter, the main sources of MPs are also analyzed and the pollution of MPs in the Poyang Lake Basins also evaluated. The main conclusions of the chapter are mainly as follows:

1. The average abundance of MPs in the sediments of the typical areas disturbed and polluted by artificial activities (the Le'an River Basin and Poyang Lake section) is $1,799.56 \text{ particles kg}^{-1}$. The MP abundance in the sediments of the middle and lower reaches of the Le'an River Basin is above-average level, compared with other areas. There are mainly four different forms of MPs including debris, foams, films, and fibers. Among the different MPs, debris have the highest abundance, accounting for 58.3% of the total, followed by foam, which accounted for 21.5%, while foams and fibers accounted for 13.8% and 6.4%, respectively. The Particle size of MPs in the area is mainly $< 1 \text{ mm}$, accounting for 62.4% of the total. With the increase of the particle size of MPs, the number of MPs tends to decrease.
2. The MPs in the sediments of the main five rivers of the Poyang Lake are mainly composed of the four types, which included debris, films, foams, and fibers. The particle size of the MPs is relatively concentrated, and most of them is not exceeding $1,000 \mu\text{m}$. Most microplastics particle size range is about from 25 to $1,000 \mu\text{m}$. The particle size range of fibers, foams, and films is from 100 to $5,000 \mu\text{m}$. The average abundance of MPs in the sediments from entrance of the five major river systems of the Poyang Lake was $1,225.25 \text{ particles kg}^{-1}$. The reason for the difference of MP abundance between samples is related to the runoff and sediment transport of the river. The main components of MPs in the study area are polyethylene (PE), polypropylene (PP), polystyrene (PC), and low-density polyethylene (LDPE).
3. The MPs in the sediments of the National Nature Reserve in the Poyang Lake are also mainly composed of four types: debris, films, foams, and fibers. The proportion of fiber in MPs samples is the most and account for about 53.9%. The average abundance of MPs in typical wetland of the National Nature Reserve is $82.33 \text{ particles kg}^{-1}$, which is the area with the lowest MP abundance value in the

whole research areas of the Poyang Lake. The main reason is that the impact of human activities in the National Nature Reserves after conservation is relatively slight. The MPs isolated from the sediments in the National Nature Reserves also have a complex surface characteristic, which is generally rough, porous, and cracked and has tears.

4. The MPs in the outflow areas of the Poyang Lake flowing into the Yangtze River are also mainly composed of four types: debris, films, foams, and fibers. The proportion of debris and films are 50.8% and 25.1%, respectively. The average abundance of MPs in the outflow areas of the Poyang Lake flowing into the Yangtze River is 907.4 particles kg^{-1} . The reason for the difference in the abundance of MPs is related to the runoff and sediment transport to the river. The main components of MPs are also polyethylene, polypropylene, and polystyrene, and the MP samples have the characteristics of rough surface, obvious weathering, and some residuals, and Si, Fe, Mg, O, Al, Ca, and other elements were observed on the surface of the MPs.
5. In this chapter, we comprehensively analyze the distribution characteristics of MPs in the different research areas and found that the distribution of MPs in the sediments of the Poyang Lake Basins is closely related to human activities. The order of the MPs abundance value of different regions is that the abundance of MPs in the sediments in the typical areas disturbed and polluted by artificial activities (The Le'an River of the Poyang Lake Section) > The entrance of the main five rivers of the Poyang Lake Basins > The outflow areas of the Poyang Lake flowing into the Yangtze River > The two National Nature Reserves in Poyang Lake. The reason for this difference is the combination of human activities, hydrological conditions, and river input impact; the difference in the proportion of MPs between different regions and the frequency of distribution is related to the local lifestyle, in addition to the physical and chemical properties of MPs.

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Sources of Microplastic in the Environment



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Abstract Generally, microplastics (MPs) in the environment come from two main types of source, which lead to different sizes of plastic particles: one is the primary source, and another is the secondary source. However, it is not easy or even impossible to identify the exact source of MPs detected in the environment. Primary

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sources of environmental MPs include plastic pellets, personal care products containing microbeads, paint, washing wastewater, sewage sludge, plastic running tracks in schools, artificial turf, rubber road in cities, and vehicle tire wear. Meanwhile, secondary sources include municipal debris such as plastic bags and bottles, fishing wastes, farming film, and other large size plastic wastes. Among these sources, vehicle tire wear is regarded as one of the most important sources of environmental microplastic due to the rapid global increase in the number of vehicles. However, available studies about the presence of rubber particles in the environment are very scarce. It is estimated that secondary sources of MPs currently account for the dominant of MPs in the environment although large plastic wastes need hundreds of years to break down into MPs under natural conditions. The appropriate management of plastic wastes and wastewater is the crucial step to prevent and control microplastic pollution in the environment in the future.

Keywords Aquatic environment, Pathway, Primary source, Secondary source, Terrestrial environment

1 Primary Source of MPs

1.1 *Plastic Pellets (Raw Materials)*

Plastic pellets are granular plastics, commonly with a diameter of 2–5 mm and a regular shape [1], which are used to make various plastic products. Generally, plastic pellets are stored, transported, and processed in the form of semifinished products. Plastic is mainly made from petroleum and coal, which are used to produce ethylene, propylene, styrene, vinyl chloride, and other materials. Plastics are divided into two groups, namely, thermoplastics and thermoset plastics. Most thermoplastics are made of virgin plastic pellets, also called preproduction pellets, beads, or nurdles. In most cases, these pellets are made during polymeric production or in recycling facilities.

Plastic particles are widely used in daily human life, including in household appliances, the clothing industry, building materials, the chemical industry, and agriculture. They are also widely used in the electrical industry, the telecommunications industry, automobile manufacturing, and medical equipment. As a persistent material, plastic pellets are very slow to degrade once they enter the environment. Additionally, due to their small particle size, they are easily eaten by fish or birds and can be easily transmitted along the food chain, which eventually endangers human health.

According to the 2019–2025 Research Report on the Development Forecast and Investment Strategy of China Plastic Granules (Particles), China's demand for plastic particles has increased significantly in recent years, from 5.11 million tons

in 2009 to 13.79 million tons in 2017. Due to the continuous increase in the demand for plastics, the production of plastic particles has increased rapidly in recent years. An unexpected result of this production is the potential transmission of plastic particles into the environment during the process of production, transportation, storage, usage and recycling of plastic particles. It is currently not possible to estimate the actual amount of plastic particles which are released into the environment from production to use; however, a recent study in the UK estimated that the country releases 5.3 billion tons of plastic particles per year into the environment [2]. Therefore, it is especially urgent for scientists to simultaneously develop alternatives to plastics and establish a sound system for the management of plastic pollution and a mechanism for the removal of plastic particles from the environment.

1.2 Personal Care Products

Microbeads are MPs that have been processed into tiny particles. Microbeads can be widely used as a replacement for the synthetic pigments that are added to personal care products to achieve cosmetic effects such as cleansing, whitening, and exfoliation, the removal of dead skin. Microbeads used in personal care products and cosmetics are mainly divided into two categories [3]: thermoplastics, which include polyethylene (PE), polypropylene (PP), polystyrene (PS), and polytetrafluoroethylene (PTFE), and thermoset plastics, which include polyurethane (PU), polyethylene terephthalate (PET), and polymethyl methacrylate (PMMA). Polyethylene microbeads account for 93% of all microplastic beads [4]. A variety of personal care products and cosmetics contain microplastic beads, such as facial cleansers, toothpaste, sunscreen, shower gel, and hair dye. Generally, microbeads enter the sewage network along with washing wastewater due to their small size, insolubility in water, and slow degradation. At present, sewage treatment equipment is unable to effectively remove plastic microbeads. As a result, these microbeads enter the environment via sewage sludge, which is widely used in agriculture.

According to statistical data from Euromonitor International, in the EU, Norway, and Switzerland, a total of 4,360 tons of plastic microbeads are used in cosmetics annually [4]. Furthermore, a preliminary study of plastic microbeads in various facial cleansers and shower gels purchased from five supermarkets in Beijing showed that about 39 tons of microbeads were released to the environment due to the use of shower gel each year in China [5]. Moreover, another recent study estimated that China releases 306.9 tons of microbeads per year into the environment [6]. In general, the amount of plastic microbeads released to the environment in different countries and regions is estimated to be as follows: South Asia (18.3%) > North America (17.2%) > Europe and Central Asia (15.9%) > China (15.8%) > East Asia and Oceania (15.0%) > South America and Africa (9.1%) > Middle East (8.7%) [7].

1.3 Paint

Paint is usually composed of pigments, fillers, solvents, and small amounts of functional additives. Paints can be divided into architectural coatings, automotive coatings, aircraft coatings, and marine coatings based on their usage, while they can be divided into natural resin paint, phenolic paint, alkyd paint, amino paint, nitro paint, epoxy paint, chlorinated rubber paint, acrylic paint, polyurethane paint, organic silicone paint, and silicone paint based on their film-forming material. Various studies have shown that the application of a paint layer to a surface may form tiny particles of plastic, which can then be released into the environment due to abrasion, aging, and erosion. Therefore, painting is one of the primary sources of environmental microplastic. Paint-related sources of environmental microplastic include architectural coatings (e.g., paint falling off during the painting of buildings), marine coatings, automotive coatings, and road-marking paint [8].

In 2015, according to the Chinese National Bureau of Statistics, the production of industrial paint in China reached 115.153 million tons, the production of architectural paint reached 5.624 million tons, the production of automotive paint was about 1,695,600 tons, the production of marine paint was about 1,373,600 tons, and the production of powder paint was about 1,717,933 tons. From 2006 to 2016, the annual growth rate of China's paint production was 14.1%. From the above data, a significant conclusion can be drawn that China's production of paint has increased rapidly in recent years. This has likely led to a consequent increase in the amount of polymer plastic particles falling off painted surfaces and subsequently being washed away by rain. Due to differences in drainage infrastructure and road conditions in urban and rural areas, the aquatic environmental pathways differ slightly between these areas. For example, in some rural areas, plastic particles enter the groundwater, while in urban areas such particles are mainly discharged into the urban drainage system through municipal pipes.

1.4 Washing Wastewater

Washing wastewater, including household laundry wastewater and washing plant wastewater, releases large amounts of plastic microfibers into the environment; these fibers are derived from shedding from various textiles. Importantly, synthetic fibers composed of polyesters and polyamides are commonly released during the washing process. It has been estimated that more than 1,900 microfibers are released to wastewater treatment plants by each piece of clothing during one washing cycle. As for sewage treatment plants, MPs cannot be effectively removed in wastewater treatment plants [6]. Therefore, the plastic microfibers that are released during the washing of clothes enter the environment with the discharging of effluent or sludge. Some studies have shown that the plastic microfibers which are found in soil, rivers, and oceans [9] represent the majority of MPs in the environment.

It has been estimated that about 69.7 million tons of fibers are used in the apparel industry each year, and synthetic fibers represent almost 60% of such fibers [10]. Additionally, studies have shown that the proportion of chemical fibers contained in clothing is greater than the global average in Asia, Africa, and the Middle East. For example, according to research on the global scale of microplastic sources [7], China's laundry washing accounts for 10.3% of global emissions of microplastic to the environment, second only to India and Southeast Asia, whose laundry washing accounts for 15.9% of global emissions of microplastic. Interestingly, studies have reported that the number of microfibers released during washing depends on the temperature, duration of washing, and type of detergent [11]. Therefore, more work should be done to determine the factors which affect the release of microfibers from textiles.

1.5 Sewage Treatment Plants

1.5.1 Effluent

Large amounts of effluents are discharged into sewage treatment plants from domestic sewage, industrial sewage, and runoff, all of which can contain various kinds of MPs from the industrial production of plastic, personal care products, chemical laundry products, automobile tire wear, and other activities. Effluents from sewage treatment plants are considered to be one of the largest sources of MPs in natural waters, since large amounts of effluents are discharged into surface water directly, although the sewage treatment process can remove more than 90% of the MPs in wastewater [12].

According to the available data, more than 30 kinds of MPs have been detected in the effluents of sewage treatment plants, including PE, PP, and PS [13]. Additionally, another study which analyzed the effluents of 17 sewage plants in the United States found that the effluent contained an average of 0.05 ± 0.024 MPs per liter [14]. Furthermore, another study found that the average concentration of MPs in the influent and effluent of the sewage from seven locations in the Netherlands ranged from 51–81 to 68–910 n/L [15]; however, due to the different sampling methods, processing treatments, and methods for the quantifying microplastic employed by different studies, the study found no significant differences between the concentration of MPs in the influent and effluent from the seven locations. Moreover, it is impossible to directly estimate the total amount of microplastic released by sewage treatment plants due to differences in economic structure, population, sewage treatment processes, and other factors.

1.5.2 Sewage Sludge

The presence of MPs in sewage sludge is mainly due to the fact that MPs physically sink in the sludge during the treatment process. Therefore, the composition of the MPs that are found in sludge is similar to that of those found in effluents. One study concluded that more than 98% of MPs in influents could sink into the sewage sludge [16]. Currently, no specific process is used to remove MPs from sewage sludge, and consequently, sludge composting and the use of sludge in agriculture lead to the release of MPs to the soil [17].

A recent study conducted sampling and analysis of the microplastic in sludge from the Gaobeidian wastewater treatment plant – the largest sewage treatment plant in Beijing which uses the traditional activated sludge process – in April, May, and June of 2018. A total of 25 kinds of plastic polymer were detected in the sludge, mainly poly(butylene adipate terephthalate) (PBA), rayon, polyamide (PA), PBMA, PE, and PET. Among these, granular MPs with an average particle size of 621.6 μm were found to be the dominant MPs in sludge, accounting for 34.07% of the total number of MPs, followed by fragmented MPs (24.72%), film-like MPs (22.53%), and fibrous MPs, which contributed the lowest amount (18.68%). The abundance of MPs in the sludge from April, May, and June 2018 was found to be $2,933 \pm 611$, $3,867 \pm 611$, and $5,333 \pm 3,501$ particles/kg dried sludge, respectively – that is, the abundance of MPs in the sludge increased each month. Accordingly, based on the output of sludge from the Gaobeidian wastewater treatment plant (37.5 t/day dry weight), it can be inferred that the total emissions of microplastic in sludge from the plant are about 1.52×10^8 n/day.

1.6 Plastic Running Tracks in Schools

In 1968, the Mexican Olympic Games officially adopted a modern running track which was largely composed of plastic, specifically polyurethane. At the same time, the ethylene propylene diene monomer (EPDM) and waste tire rubber were also added into plastics as auxiliary additives. Generally, plastic running track either contains a hybrid plastic track surface layer or a permeable plastic track surface layer. Permeable plastic running tracks are very common in locations with hot weather and heavy rainfall, while hybrid plastic running tracks are resistant against weathering and have waterproof properties. Moreover, earlier studies had shown that some plastic running tracks contained toxic chemicals. Following wear and aging, MPs can be released from the track into the environment due to wind and rainfall. However, due to the lack of data regarding the assessment factors which affect the release of MPs from plastic running tracks, it is difficult to robustly estimate the emissions of MPs from such tracks.

1.7 Artificial Turf

Artificial turf was first developed in the United States in the 1960s. It is composed of plastic fiber “turf” and filled plastic granules and has some advanced characteristics; for example, it is waterproof and nonslip, has a long service life, and is easy to maintain. Artificial turf can be used in a wide range of sporting applications, for example, on football fields, tennis courts, playgrounds, golf courses, and so on. Additionally, artificial turf is widely used in schools. Artificial turf can be divided into “artificial grass” and “rubber mat.” Artificial grass is composed of an organic polymer substance such as PP, polyamide 6 (PA6), PE, or polyurethane (PUR). The use of rubber pads in artificial turf can reduce the need for filler particles which can help the artificial grass to fix the position. Generally, the filler particles include black styrene-butadiene rubber (SBR), thermoplastic elastomer (TPE), and green rubber which is made of EPDM.

One study showed that the artificial turf of a 7,881 m² football field contained three different granular materials, including 51 tons of SBR, 61 tons of EPDM, and 87 tons of TPM [18]. Additionally, it has been shown that artificial turf in Norway contained 90% SBR and a combined 10% of EPDM and TPE. TPE is widely used in artificial turf in indoor sports halls [19]. In sports halls which contain artificial turf constructed from TPE, particles of TPE will become stuck in the tread of shoes and will thereby be released into the environment. Importantly, the filler particles in artificial turf can be regarded as a source of MPs, and the microplastic from artificial turf will also enter the environment as the turf ages and undergoes wear. For example, one study showed that artificial turf lost 0.38–0.63 kg/m³ of rubber per year [16].

1.8 Rubber Road in Cities

Rubber asphalt road is a recently developed type of advanced road material. The main raw materials that are used to produce rubber asphalt road are ordinary matrix asphalt and waste tire rubber powder. The color of rubber asphalt road is darker than that of ordinary asphalt road. Recent research has shown that rubber asphalt road has great advantages over ordinary asphalt concrete road; specifically, rubber asphalt road is resistant to both high and low temperatures, is water resistant, ages slowly, produces low levels of noise, and has a long service life.

Since waste tire rubber powder is the main raw material used in the production of rubber asphalt, the use of this material reduces the pollution caused by waste tires. However, during the use of rubber asphalt, particles of rubber plastic are released by friction between the road and vehicle tires, thus releasing MPs to the environment. Additionally, other modified asphalt roads (i.e., those are composed of PMB) often use styrene-butadiene-styrene (SBS), SBR, PE, and ethylene-vinyl acetate (EVA) plastic as modified additive components. For instance, Norway uses asphalt road

modified with thermoplastic elastomer SBS. The amount of SBS used in conventional SBS-modified asphalt roads is about 5% [21].

There has been relatively little research on the discharge of plastic particles from highways as a result of vehicle friction. The reason for this research gap may be due to the variability in the paving materials that are used and to the fact that the degree of friction is related to the tire tread of the vehicle, both of which hamper the estimation of microplastic release.

1.9 Vehicle Tire Wear

MPs released from tires by tire wear are considered to be one of the main sources of MPs in road dust. The life of motor vehicle tires is divided into three stages: manufacturing, use, and disposal. Of these three stages, the carbon emissions are the largest in the use stage.

Tires can be divided into natural rubber (NR) tires and synthetic rubber (SN) tires. Synthetic rubber is mainly composed of SBR or butadiene rubber (BR). Natural rubber is commonly used in advanced automotive tires due to the fact that its durability is better than that of synthetic rubber. At present, natural rubber is mainly derived from the Brazilian rubber tree (*Hevea brasiliensis*). The production of rubber from this tree is relatively expensive. Meanwhile, synthetic rubber is derived from the petroleum industry. In order to meet the performance requirements of motor vehicle tires, manufacturers generally add different types and different doses of chemical additives to rubber. The chemical additives for rubber tires are complex. Details of common rubber additives are given in Table 1.

In Norway and Denmark, rubber tires have been shown to be one of the main sources of environmental MPs [21]. The density of rubber particles is about 1.2–1.3 g/cm³, and accordingly they can sink into sediment when they enter an aquatic environment. Rubber particles will float in water only when washed by water [20]. Rubber particles released from the tires of motor vehicles on the road can be deposited in various environments, such as surface water, sewers, soil, and air. It is generally considered that increasing the friction of a motor vehicle tire reduces the braking distance of the vehicle and therefore increases the safety of the driver. However, the increased friction also increases the rate of emission of rubber particles from the tire. To reduce the amount of MPs generated by tire friction, drivers can avoid using winter tires during the summer; winter rubber tires are generally softer than summer rubber tires and have a better grip, which causes more tire particles to be released on dry winter roads. Additionally, in order to reduce the amount of MPs generated by tire friction, drivers should attempt to use low-emission tires, replace tires in good time, and choose public transportation [22].

Table 1 Common additives used in rubber tires

Name	Function	Characteristics	Market-weighted average (kg/tire)
Coumarone indene resins (CIR)	Tackifier, plasticizer, softener	Coumarone indene resins are divided into liquid products and solid products. Both products have good effects as tackifiers, i.e., they improve the mechanical properties and anti-aging properties of rubber, contribute to the dispersion of sulfur and carbon black, and prevent coking. The addition of coumarone to rubber leads to the thickening and reinforcing of the rubber, makes it easier to cut, increases the tensile strength of the rubber, reduces the abrasivity of the rubber, and increases the hardness of the rubber	0.081
Sulfur (S) powder	Vulcanizing agent	Sulfur powder can be used as a vulcanizing agent in crosslink rubber. Vulcanized rubber has good wear resistance. Sulfur is inexpensive, and sulfur pre-dispersion can reduce mixing time and improves storage stability	0.09
Accelerator	Reinforcing resin curing agent	Accelerators can increase the hardness of rubber and play an important role in the adhesion of the rubber to the fiber. As one of the six commonly used rubber accelerators, vulcanization accelerator can more effectively shorten the vulcanization time, lower the vulcanization temperature, reduce the amount of vulcanizing agent required, and improve the physical and mechanical properties of rubber	0.079
ZnO	Tire radiator	Zinc oxide can improve the thermal conductivity of rubber and increase the heat dissipation capacity of rubber tires. It also prevents the erosion of rubber by UV mold. Additionally, the combination of zinc oxide and stearic acid can enhance the hardness of rubber tires	0.13
Carbon black	Reinforcing agent	Carbon black is the main raw material for rubber tires. Carbon black is mainly composed of carbon, as well as small amounts of hydrogen, oxygen, and nitrogen. Carbon black generally accounts for 40–50% of the composition of rubber The presence of carbon black is the main reason why tires appear black. The finer the particle size of the carbon black used in tire manufacturing, the better the tire reinforcing performance, the higher the degree of stacking in the carbon black structure, and the higher the shear modulus of the tire. Fine-grained carbon black is mainly used to reinforce the rubber in tire treads since it imparts excellent wear resistance to the tires	1.54

2 Secondary Source of MPs

Secondary sources of microplastic are tiny fragments of plastic derived from larger plastic particles which have not been properly disposed of [23]. Over time, MPs are gradually formed by the degradation of plastic structures under the action of physical, biological, and chemical processes such as light irradiation aging, biological crushing, and mechanical grinding [24]. Secondary sources of microplastic are a significant source of environmental microplastic. Such secondary sources include the following.

2.1 *Municipal Debris*

2.1.1 Plastic Bags

Plastic bags refer to bags made of various plastic raw materials mixed with other materials (additives) which are processed by heat-sealing or bonding. Many different materials are used in the manufacturing of plastic bags. Table 2 shows the relative global demand for, and main uses of, common plastics in 2017, including PET, PE, high-density PE (HDPE), polyvinyl chloride (PVC), low-density PE (LDPE), PP, EVA, PBA, Polybutylene Succinate (PBS), polycaprolactone (PCL), polychytle, and linear low-density PE (LLDPE). Plastic bags are indispensable items in people's daily lives. They are widely used due to their low cost, extremely light weight, large capacity, and easy storage. Worldwide, up to 5 trillion plastic bags are used per year. In the United States alone, 100 million plastic bags are used by consumers every year [25]; however less than 10% of these will be recycled [26].

Since the widespread commercial use of plastic bags appeared in the 1990s, a large number of plastic bags have been disposed of in the environment, including roads, river banks, and land around cities. Due to their extremely long degradation cycle, the production and use of plastic bags are gradually being banned from production in some countries. The range and scope of interventions for reducing the use of plastic bags vary between countries; these include prohibiting the sale of lightweight bags, charging customers for lightweight bags, and taxing stores who sell lightweight bags [27].

As of January 2017, the total annual consumption of plastic bags from supermarkets in Israel was 2.7 billion. In Israel, one quarter of all bags are thrown away immediately after use, and the total weight of supermarket plastic bags in household waste is estimated to be 15,000 tons per year. However, in 2018, the State of Israel introduced a law that required supermarkets to charge customers at least 0.10 Israeli NIS (about US\$0.03) per plastic bag used. Since the introduction of this law, the use of plastic bags has dropped by 80%, and the amount of plastic-bag waste found in the sea has halved [28]. However, many countries and regions have not implemented charges or bans on the use of plastic bags, such as New Zealand and some states of

Table 2 Global demands for different types of polymers in 2017

Polymer	Density, g/cm ³	Production, %	Products
PP	0.83~0.85	19.30%	Food packaging, sweet and snack wrappers, hinged caps, microwave containers, pipes, automotive parts, bank notes, etc.
LDPE/LLDPE	0.91~0.94	17.50%	Reusable bags, trays, and containers, agricultural film, food packaging film, etc.
HDPE/MDPE	0.94~0.97	12.30%	Toys, milk bottles, shampoo bottles, pipes, houseware, etc.
PVC	1.38	10.20%	Window frames, profiles, floor and wall covering, pipes, cable insulation, garden hoses, inflatable pools, etc.
PUR	1.10~1.25	7.70%	Building insulation, pillows and mattresses, insulating foams for fridges, etc.
PET	1.37~1.40	7.40%	Bottles for water, soft drinks, juices, cleaners, etc.
PS/EPS	1.05	6.60%	Eyeglass frames, plastic cups, egg trays, packaging, building insulation, etc.
Others	/	19%	Hubcaps (ABS), optical fibers (PBT), eyeglasses lenses, roofing sheets (PC), touch-screens (PMMA), cable coating in telecommunications (PTFE), many applications in aerospace, medical implants, surgical devices, membranes, valves and seals, protective coatings, etc.

Australia [29]. Although plastic bag pollution has been reduced by the promulgation of various bans on plastic bags, there are still a large number of plastic bags left in the environment, which are likely to be a large source of environmental MPs.

2.1.2 Plastic Bottles

Plastic bottles are containers that are composed of plastic such as PET, PE, and PP. These bottles are produced by combining the constituent plastic with a corresponding organic solvent, or by heating to a high temperature, after which a plastic mold is formed through blow molding, extrusion blowing, or injection molding. Plastic bottles are mainly used as disposable containers for liquids or solids, such as beverages, pickles, honey, dried fruit, edible oils, and agricultural veterinary drugs. In some areas, plastics bags, and especially plastic bottles, are relied upon to provide clean drinking water. Additionally, due to the convenience, hygiene, low cost, and transparency of plastic bottles, most people choose to buy mineral water or other drinks which are sold in plastic bottles. It has been estimated that one million plastic bottles are sold every minute around the world at present [26]. Global sales of plastic beverage bottles reached 480 billion per year, an increase of 300 billion from 2006 to 2016 (British Guardian). Due to improvements

in waste sorting, environmental awareness, and the implementation of environmental protection policies, the recycling rate of plastic water bottles has increased in recent years. However, as of 2016, the number of plastic bottles that were properly recycled was less than 50% of sales volume. Furthermore, in 2016, only 7% of the plastic bottles that were sold were recycled and made into new bottles. Field research along the coast of China showed that plastic bottles are the most common marine plastic waste in beaches such as Liaodong Bay in Bohai Sea, Zhoushan in East Sea, and Haikou in the South Sea (data not be published). Plastic bottles may therefore be a potential source of MPs in the form of fragments or granules, both of which are detected in large quantities in the environment.

2.1.3 Disposable Plastic Tableware

Disposable plastic tableware refers to disposable utensils intended for use during meals or similar purposes which are produced by the thermoplastic molding of resin or other thermoplastic materials. Disposable plastic tableware includes lunch boxes, plates, saucers, straws, knives, forks, spoons, cups, bowls, and cans, but does not include food packaging for long-term purposes or similar purposes. Plastic tableware is widely used worldwide due to its low cost, lightness, waterproof, and durability. The results of a survey suggest that the United States uses up to 500 million plastic drinking straws per year and more than 100 million plastic containers, the large majority of which are not subsequently recycled [30]. Plastic tableware is mainly constructed from PP, PE, PS, and so on. United States generates about three million tons of disposable polystyrene utensils per year. Polystyrene (commonly known as polystyrene foam) is mainly used for the manufacture of packaging materials and food service items [31], such as foam cups, instant-noodle boxes, fast food boxes, etc. If not properly disposed of, plastic tableware may enter sewers, soil, oceans, etc.; over time, it becomes partially degraded and therefore represents a source of environmental MPs. Globally, it is estimated that plastic tableware might be responsible for 269,000 tons of plastic pollution into waterways and oceans each year.

2.1.4 Plastic Packaging

Plastic packaging refers to plastic material that envelops an item so that it maintains its original quality and value during transportation, storage, and distribution. Such packaging includes boxes, bags, films, etc. Due to its low cost, excellent oxygen/moisture barrier properties, biological inertness, and lightness [32], plastic packaging with equal or superior design is replacing packaging composed of traditional materials (glass, metal, and paper). Globally, plastic packaging accounts for about 25% of packaging materials, while plastic packaging materials account for about 39.7% of total plastic production [33].

Plastic packaging is used in all aspects of life, including clothing, food, paper towels, and so on. According to the material from which it is made, plastic packaging

can be divided into OPP, CPP, PP, PE, PVA, and EVA. In recent years, with the rise of e-commerce, in addition to the outer packaging of ordinary commodities, the use of plastic packaging has grown rapidly in the industries of express delivery and fast food. It has been estimated that, in 2016, a total of 14.7 billion plastic bags were used in the express delivery industry in China, of which about 6.8 billion were used directly in express delivery [34]. In 2017, China's express delivery industry exceeded 40 billion deliveries and produced an output of 8 million tons of wastes. In 2017, a total of 14.6 billion plastic bags were used for food and beverages in China. The world's oceans are a "plastic sink" and accept millions of tons of plastic packaging waste every year. So-called thin films of microplastic polymers that are commonly used for packaging, such as PE, PP, and PS, have been detected in large quantities in the environment [35].

2.2 Fishing Wastes

Plastic fishing waste includes buoys, floating boxes, fishing rods, fish tanks, fishing nets, fishing lines, cables, etc. The amount of commercial fishing equipment which is discarded globally each year has been estimated at between 0.13 and 135,000 tons [36]. Polystyrene foam has been detected in large quantities in marine plastic waste, for example, in the Shandong Coast, China [37–39], and Hofsgar Lake, Mongolia [40]. The presence of such waste is mainly due to the large-scale use of Styrofoam floating devices in the aquaculture industry, which has developed rapidly in recent years, and the large amount of abandoned fishing nets and foam pontoons that are lost to the sea due to natural wear and biological damage after prolonged use. Additionally, the abandonment of large quantities of feed junk bags by the aquaculture industry has also increased the amount of plastics in the marine environment. Furthermore, increasing numbers of fishing boats are using plastic fishing nets, and the renewal of fishing gear has resulted in a large number of dilapidated plastic fishing nets abandoned in the ocean. The main components of fishing nets and fishing ropes are PE or LDPE, PA (nylon), and PP monofilament [41, 42], and the difference between nets or ropes made from these different materials can be distinguished by their diameter and transparency [42]. After entering the environment, fishing nets and ropes shed fibers [43]. Additionally, maritime shipping accidents sometimes cause large quantities of plastic products to enter the ocean. Therefore, fishing wastes, and especially foams, are significant sources of MPs in the environment.

2.3 Farming Film

Another important source of environmental microplastic is agricultural plastic film. One form of agricultural mulch is a thin film composed of polyvinyl chloride,

polyethylene, and other additives which are incorporated by blow molding. About 3.4% of the total global output of plastic is used in agriculture [26]. Plastic mulch films began to be used in agriculture in the early 1950s. Such films increase soil temperature and reduce soil pollution, thus increasing crop production and increasing income, and are of great significance for ensuring food security. However, the large consumption of plastic mulch film, combined with the film's short life cycle, means that the recovery of such film is difficult, its recycling efficiency is low, and it easily releases MPs into the soil. Microplastic released from agricultural film – commonly PE, LDPE, and LLDPE – has been detected in large quantities. China uses the largest amount of plastic film mulch of any country. In 2017, 143.37 million tons of plastic film mulch was used in China, corresponding to a film area of 1,865.72 hm² [44]. When left in the soil, plastic film not only pollutes the soil and gradually destroys the agricultural productivity of the land, but may also be stored in the soil and absorbed by crops, and may thus enter the food chain and affect human health.

2.4 Others

In addition to the industries mentioned above, the construction, aerospace, and medical industries also consume significant amounts of plastic. A large number of plastic products are used in the following products: hinged covers, hub caps and other automobile parts, touch screens, pipes and fittings, foundations, roofs, floors, panels, roads, insulation, cable jackets, window frames, profiles, flooring, wall coverings, building insulation, valves, seals, and so on. Plastic products are widely used outdoors due to their resistance to environmental factors; they neither rot nor rust and require little maintenance. However, plastics can enter the solid waste stream. Plastic that has not been disposed of properly enters the environment and thus becomes a potential source of environmental microplastic pollution. The contribution of plastic products to environmental microplastic pollution depends on the effectiveness of solid waste management.

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Interaction of Microplastics and Organic Pollutants: Quantification, Environmental Fates, and Ecological Consequences



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Abstract Microplastics which act as vectors for organic pollutant transport in environment have raised increasing concerns recently. This paper provides an overview on the interaction of plastic debris or microplastics with these organic chemicals and its effects on biological receptors. Plastic additives represented one of the most important organic pollutants associated with microplastics; the types, quantification, and migration from the plastic debris or microplastics are addressed here. In addition to the chemical additives, microplastics also adsorbed hydrophobic or hydrophilic organic pollutants from the environments due to their high surface areas and affinity for these pollutants. The mechanisms of microplastic adsorption for PAHs, PCBs, and pharmaceuticals and the role of microplastic surface and solution chemistry were well discussed in the paper. The sorption affinity changed by the aging of microplastic surface was of concern in particular. The organic pollutants in the microplastics may cause toxic effects on biotas by releasing into the leachate or by contact exposure directly through microplastics ingestion. Here we reviewed the latest reports on the organic pollutant assay for the leachates from the environmental microplastics and their toxic effects on freshwater species *Daphnia magna*, brown mussel (*Perna perna*), barnacle, and microalgae using different endpoints. Bioaccumulation of organic pollutants and biological toxicology through the vector effects of microplastics were also reviewed in the paper. However, large uncertainties existed among the different studies with respect to the toxic effects of co-exposure with organic pollutants and microplastics. Therefore, further researches are recommended to be done regarding the combined effects of organic pollutants and microplastics under the different exposure scenarios.

Keywords Combined effect, Microplastics, Organic pollutant, Plastic additive, Sorption and desorption

1 Introduction

Microplastics (plastics <5 mm, including nanoplastics which are <0.1 μm) originate from the fragmentation of large plastic litter or from direct environmental emission. Their potential impacts in terrestrial ecosystems remain largely unexplored despite numerous reported effects on marine organisms [1]. Meanwhile, microplastics present in the terrestrial environment carry organic chemicals of smaller molecular size. These chemicals can penetrate into cells, chemically interact with biologically important molecules, and may disrupt the endocrine system [2]. Such organic chemicals are categorized into two groups: (1) additives, monomers, and oligomers of the component molecules of the plastics and (2) hydrophobic or hydrophilic organic compounds that are adsorbed from the surrounding environment through different mechanisms. Many of the contaminants addressed herein have known biological consequences. Furthermore, microplastics were assumed to serve as

vectors for transport of organic chemicals from environment biota and even across cell membranes to elevate intracellular stress [3]. Therefore, the objective of this paper is to review the interaction of plastic debris or microplastics with these organic chemicals and its effects on biological receptors.

2 Quantification and Environmental Fate of Plastic Additives in the Plastic Debris or Microplastics

2.1 Chemical Additives Use and Application in the Plastic Productions

Plastic additives are mainly used as plasticizers, flame retardants, stabilizers, anti-oxidants, and pigments, which are added to improve the performance and functionality of a plastic product. All the additives can be mainly divided into the following four categories based on their functional properties: (1) functional additives (stabilizers, antistatic agents, flame retardants, plasticizers, lubricants, slip agents, curing agents, foaming agents, biocides, etc.), (2) colorants (pigments, soluble azo colorants, etc.), (3) fillers (mica, talc, kaolin, clay, calcium carbonate, barium sulfate), and (4) reinforcements (e.g. glass fibers, carbon fibers).

Plasticizers are most commonly used for improving the flexibility, durability, and stretchability of polymeric films and reducing melt flow. Phthalate esters (PAEs) are the most commonly used plasticizers and include flexible vinyl, which is mainly added in PVC production, which can contain 10–60% phthalates by weight [4]. As they are not part of the chain of chemical compounds (polymers) of plastics, they can be released from these products. These plasticizers are used in various fields such as foods, toy manufacturing, electronics, pesticides, glues, paint solvents, personal care products, and pharmaceuticals [5].

Antioxidants are used as additives in many synthetic polymers including polyolefins (mainly PE and PP) which represent 60% of global demand for antioxidant additives. Arylamines are the most commonly used antioxidants in plastic food packaging. Phenolics and organophosphates (used to reduce hydroperoxides formed during oxidation to alcohols) are also used as antioxidants. Bisphenol A (BPA) and nonylphenols (NP) can also be used as an antioxidant or as a plasticizer in the PP, PE, and PVC polymers. Over three million tons of BPA was produced annually for the using in plastics [6].

Heat stabilizers are responsible for preventing thermal degradation of polymers when exposed to elevated temperatures, i.e., during the thermal processing of foods. Certain types of polymers, i.e., PVC, PVDC, vinyl chloride copolymers (e.g., vinyl chloride/vinyl acetate), and PVC blends require the addition of heat stabilizers in order to maintain their functionality. Organic chemicals such as alkyl organophosphates, epoxy compounds, and beta-diketones are the main types of secondary heat stabilizers.

Brominated flame retardants (BFRs) are a class of additives used in plastic products to reduce flammability. These BFRs are used in a variety of consumer products ranging from electronic devices to insulation foams. BFRs include a wide range of chemicals, of which polybrominated diphenyl ethers (PBDE), hexabromocyclododecane, and tetrabromobisphenol A represent the main BFRs used in the plastic industry [4]. Hexabromocyclododecane (HBCD) is the main additive chemical applied to polystyrene (PS) products, including EPS, and is especially prevalent in construction materials and electronic housings [4]. The global consumption of HBCD increased from 16,700 tons to 31,000 in 2001–2011. HBCD is not covalently bound to the polymer and can therefore be easily released from plastic products into the environment [7]. Organophosphate esters (OPEs), and in particular the triesters, are produced in high volumes and have been used as flame retardants and plasticizers for decades. The usage even increases due to the ban or restriction of brominated diphenyl ethers as flame retardants [8]. In 2011, 500,000 tons of OPEs were consumed globally, and annual consumption reached 680,000 tons by 2015 [9]. The OPEs can volatilize from a product or be lost through abrasion or dissolution, and much attention has been paid to the release of OPEs into the environment from products.

2.2 Occurrence of Chemical Additives in the Environmental Plastic Debris or Microplastics

To date, only a few studies have focused on the detection of plastic additives from MP collected in coastal beaches [7, 10–15]. A dataset summary regarding plastic additive concentrations in the plastic debris and microplastics is shown in Table 1. Mato et al. [11] detected nonylphenols in PP pellets deployed in Tokyo Bay and suggested that these compounds came from plastic additives found in the PP pellets themselves. Hirai et al. [13] observed high levels of PBDEs, BPA and nonylphenols in PE and PP fragments collected on remote or urban beaches. The source of these organic compounds was assumed to be connected with plastic additives used for the manufacture of PP and PE. A wide range of plastic additives were also identified using Pyrolysis-GC/MS with thermal desorption in MP collected from sediment of Norderney Island [10]. The identified PE, PP, PS, and polyamide-6 MP particles were associated with antioxidant additives, e.g., DEHP, DnBP, diisobutyl phthalate (DiBP), and 2,4-di-tert-butylphenol (2,4-DTBP). Moreover, Rani et al. [14] detected multiple plastic additives in plastic marine debris found on a beach in Geoje, South Korea. Indeed, the authors found BPA and phthalates in PP and PE plastic marine debris as well as antioxidants including Irganox 76 and 2,4-DTBP in PP and PE plastic marine debris. In another study which focused on ultraviolet stabilizers (UVSs) and antioxidants in the plastic debris of the beaches, it reported that

Table 1 Summary of the chemical additives measured in plastic debris or microplastic samples

Chemical additives	Sampling locations	Plastic types	Particle size	Concentration (ng/g)	References
Hexabromocyclododecane (HBDCD)	South Korean coasts	EPS bead ^a	0.99 ± 0.06 mm	70,300 ± 77,800	[10]
	South Korean coasts	EPS bead ^b	0.99 ± 0.06 mm	5.98 × 10 ⁶ ± 2.12 × 10 ⁶	
	South Korean coasts	Pre-expanded spherule ^a	3.22 ± 0.21 mm	5,810 ± 680	
	South Korean coasts	Pre-expanded spherule ^b	3.22 ± 0.21 mm	2.37 × 10 ⁶ ± 8.15 × 10 ⁴	
	South Korean coasts	EPS product ^a	n.a.	5,380 ± 1,360	
	South Korean coasts	EPS microplastics	2–3 mm	1.07 × 10 ⁶ ± 4.50 × 10 ⁵	
	South Korean coasts	Small-sized buoy (40–80 L)	Diameter 35 × 45 cm	1.45 × 10 ⁵ ± 3.79 × 10 ⁵	
	South Korean coasts	Large-sized buoy (>200 L)	Diameter 50 × 90 cm	7.11 × 10 ⁵ ± 1.25 × 10 ⁶	
	South Korean coasts	Recycled buoy (inside)	n.a.	1.94 × 10 ⁶ ± 8.89 × 10 ⁵	
	South Korean coasts	Recycled buoy (outside)	n.a.	7.51 × 10 ⁵ ± 9.69 × 10 ⁵	
	Hong Kong	Low-density EPS debris	n.a.	920 ± 1,460	
	Singapore	Low-density EPS debris	n.a.	170 ± 80	
	Brunei	Low-density EPS debris	n.a.	230 ± 140	
	Vietnam	Low-density EPS debris	n.a.	100 ± 60	
	Thailand	Low-density EPS debris	n.a.	3,510 ± 4,890	
	Taiwan	Low-density EPS debris	n.a.	2,140 ± 4,120	
	Bangladesh	Low-density EPS debris	n.a.	7,430 ± 14,300	
	Sri Lanka	Low-density EPS debris	n.a.	50 ± 20	
	Japan	Low-density EPS debris	n.a.	1.08 × 10 ⁵ ± 1.93 × 10 ⁵	
	Peru	Low-density EPS debris	n.a.	3.2 × 10 ⁶ ± 2.8 × 10 ⁶	
Canada	Low-density EPS debris	n.a.	1.47 × 10 ⁶ ± 2.27 × 10 ⁶		
USA (Hawaii)	Low-density EPS debris	n.a.	4,940 ± 5,710		
USA (California)	Low-density EPS debris	n.a.	1.21 × 10 ⁶ ± 1.98 × 10 ⁴		
USA (Alaska)	EPS fragment	n.a.	3.35 × 10 ⁶ ± 5.86 × 10 ⁶		

(continued)

Table 1 (continued)

Chemical additives	Sampling locations	Plastic types	Particle size	Concentration (ng/g)	References
PBDEs	Marbella Beach, Costa Rica	PE fragments	A few mm to few cm	0.4–5.8	[13]
	Marbella Beach, Costa Rica	PP fragments	A few mm to few cm	1.6–180	
	Seal Beach, USA	PE fragments	A few mm to few cm	12.6–41.4	
	Seal Beach, USA	PP fragments	A few mm to few cm	5.3–25.5	
	Odaiba, Japan	PE fragments	A few mm to few cm	1.1–15.5	
	Odaiba, Japan	PP fragments	A few mm to few cm	1.4–2.7	
	Kugenuma, Japan	PE fragments	A few mm to few cm	0.02–1.7	
	Kugenuma, Japan	PP fragments	A few mm to few cm	3.9–230	
	Tonkin Bay, Vietnam	PE fragments	A few mm to few cm	2.1–6.5	
	Tonkin Bay, Vietnam	PP fragments	A few mm to few cm	0.3–412	
Nonylphenols (NP)	Kasai Seaside Park in Japan	Resin pellets	0.1–0.5 cm	16	[11]
	Keihin Canal in Tokyo Bay	Resin pellets	0.1–0.5 cm	8.9	
	Kugenuma Beach	Resin pellets	0.1–0.5 cm	12	
	Shiroda Beach	Resin pellets	0.1–0.5 cm	0.13	

	Marbella Beach, Costa Rica	PE fragments	A few mm to few cm	2.4–33.4	[13]
	Marbella Beach, Costa Rica	PP fragments	A few mm to few cm	5.3–3,936	
	Seal Beach, USA	PE fragments	A few mm to few cm	0–1.1	
	Seal Beach, USA	PP fragments	A few mm to few cm	7.2–130	
	Odaiba, Japan	PE fragments	A few mm to few cm	0.3–13.4	
	Odaiba, Japan	PP fragments	A few mm to few cm	8.5–22.4	
	Kugenuma, Japan	PE fragments	A few mm to few cm	1.3–706	
	Kugenuma, Japan	PP fragments	A few mm to few cm	305–1,244	
	Tonkin Bay, Vietnam	PE fragments	A few mm to few cm	0–0.7	
	Tonkin Bay, Vietnam	PP fragments	A few mm to few cm	0–551	
	Marbella Beach, Costa Rica	PE fragments	A few mm to few cm	0–1.6	[13]
	Marbella Beach, Costa Rica	PP fragments	A few mm to few cm	0.2–14.4	
	Seal Beach, USA	PE fragments	A few mm to few cm	0–16.8	
	Seal Beach, USA	PP fragments	A few mm to few cm	0.1–4.9	
	Odaiba, Japan	PE fragments	A few mm to few cm	0–17.4	
Octylphenol (OP)					

(continued)

Table 1 (continued)

Chemical additives	Sampling locations	Plastic types	Particle size	Concentration (ng/g)	References
BPA	Odaiba, Japan	PP fragments	A few mm to few cm	0.2–2.6	
	Kugenuma, Japan	PE fragments	A few mm to few cm	0–49.2	
	Kugenuma, Japan	PP fragments	A few mm to few cm	0.8–27.6	
	Tonkin Bay, Vietnam	PE fragments	A few mm to few cm	0.8–1.5	
	Tonkin Bay, Vietnam	PP fragments	A few mm to few cm	0–154	
	Marbella Beach, Costa Rica	PE fragments	A few mm to few cm	0–729.7	[13]
	Marbella Beach, Costa Rica	PP fragments	A few mm to few cm	0.4–35.4	
	Seal Beach, USA	PE fragments	A few mm to few cm	0–6.8	
	Seal Beach, USA	PP fragments	A few mm to few cm	0–26.2	
	Odaiba, Japan	PE fragments	A few mm to few cm	0–1.4	
	Odaiba, Japan	PP fragments	A few mm to few cm	0–2.8	
	Kugenuma, Japan	PE fragments	A few mm to few cm	0–0.5	
	Kugenuma, Japan	PP fragments	A few mm to few cm	0–3.4	
Tonkin Bay, Vietnam	PE fragments	A few mm to few cm	0		

	Tonkin Bay, Vietnam	PP fragments	A few mm to few cm	0–263.6	
Irganox 1076	Geoje coast, South Korea	Plastics debris	n.a.	$1.06 \times 10^5 \pm 2.89 \times 10^5$	[14]
Irganox 1076	Geoje coast, South Korea	New plastics	n.a.	$1.50 \times 10^5 \pm 4.40 \times 10^5$	
Irganox 1010	Geoje coast, South Korea	Plastics debris	n.a.	$3.4 \times 10^4 \pm 4.9 \times 10^4$	
Irganox 1010	Geoje coast, South Korea	New plastics	n.a.	$9.5 \times 10^4 \pm 1.52 \times 10^5$	
2,4-DTBP	Geoje coast, South Korea	Plastics debris	n.a.	$2,400 \pm 3,700$	
2,4-DTBP	Geoje coast, South Korea	New plastics	n.a.	$4,100 \pm 6,500$	
BHT	Geoje coast, South Korea	Plastics debris	n.a.	$1,200 \pm 5,400$	
BHT	Geoje coast, South Korea	New plastics	n.a.	$2,400 \pm 9,600$	
UV320	Geoje coast, South Korea	Plastics debris	n.a.	$2,800 \pm 10,000$	[14]
UV320	Geoje coast, South Korea	New plastics	n.a.	$4,100 \pm 15,000$	
UV326	Geoje coast, South Korea	Plastics debris	n.a.	$4,100 \pm 15,000$	
UV326	Geoje coast, South Korea	New plastics	n.a.	$10,000 \pm 36,000$	
UV327	Geoje coast, South Korea	Plastics debris	n.a.	$1,300 \pm 4,100$	
UV327	Geoje coast, South Korea	New plastics	n.a.	$2,100 \pm 7,400$	
UV328	Geoje coast, South Korea	Plastics debris	n.a.	110 ± 320	
UV328	Geoje coast, South Korea	New plastics	n.a.	70 ± 160	[15]
Phthalic acid esters (PAEs)	Estuarine mudflats in China	PE pellets	4–5 mm	0.033 ± 0.039	
	“Wild” beach in China	PE pellets	4–5 mm	0.11 ± 0.17	
	Bathing Beach in China	PE pellets	4–5 mm	0.2 ± 0.37	
	Estuarine mudflats in China	PS foams	1–4 mm	1.43 ± 2.33	
	“Wild” beach in China	PS foams	1–4 mm	16 ± 32	
	Bathing Beach in China	PS foams	1–4 mm	4.17 ± 3.22	
	Estuarine mudflats in China	PP flakes	<1 mm	4.34 ± 2.39	
	“Wild” beach in China	PP flakes	<1 mm	15 ± 11	

(continued)

Table 1 (continued)

Chemical additives	Sampling locations	Plastic types	Particle size	Concentration (ng/g)	References
Organophosphorus esters (OPEs)	Bathing Beach in China	PP fragments	1–4 mm	1.73 ± 1.27	[15]
	“Wild” beach in China	PP fragments	1–4 mm	0.83	
	“Wild” beach in China	PE fragments	1–4 mm	27	
	Estuarine mudflats in China	PE pellets	4–5 mm	14 ± 20	
	“Wild” beach in China	PE pellets	4–5 mm	17 ± 30	
	Bathing Beach in China	PE pellets	4–5 mm	70 ± 130	
	Estuarine mudflats in China	PS foams	1–4 mm	17 ± 25	
	“Wild” beach in China	PS foams	1–4 mm	14,100 ± 34,500	
	Bathing Beach in China	PS foams	1–4 mm	970 ± 2,500	
	Estuarine mudflats in China	PP flakes	<1 mm	1,080 ± 1,250	
	“Wild” beach in China	PP flakes	<1 mm	710 ± 1,010	
	Bathing Beach in China	PP flakes	<1 mm	27 ± 18	
	“Wild” beach in China	PP fragments	1–4 mm	69	
	“Wild” beach in China	PE fragments	1–4 mm	120	
	Calblanque in Spanish	PE/PP/PS	<5 mm	385.66 ± 594.52	
Cape cope in Spanish	PE/PP	>5 mm	205.26 ± 782.72		
La Lliana in Spanish	PA	n.a.	51.39 ± 39.30		

n.d. no detected, *n.a.* data is not available

^aNon-flame retardants

^bFlame retardants

antioxidants were present at higher concentrations than UVSs in plastic debris and Irganox 1076 and Irganox 1010 were the most commonly measured antioxidants [12].

An intensive monitoring of HBCD levels was conducted for the EPS debris and microplastics collected from the marine coasts of South Korea and 12 other countries in the Asia-Pacific region. HBCD was detected extensively in EPS buoy debris and EPS microplastics stranded along the Korean coasts; the highest measured concentration was $8,670 \mu\text{g g}^{-1}$ [7]. Recycled buoys had the highest HBCD levels, followed by microplastics, large buoys, and small buoys. The *rac*-HBCD dominated diastereomeric patterns in the floating buoys as well as in the EPS microplastics. HBCD was also abundantly detected in EPS debris collected from the Asia-Pacific coastal region, with the highest concentration found in Alaskan beach of the USA. This indicated that HBCD contamination via EPS debris was a common environmental issue worldwide.

Recently, Zhang et al. [15] investigated OPEs and PAEs in the beached microplastics collected from North China, and they found that the PP flakes and PS foams contained the highest concentrations of the two additives in contrast to the PE pellets which contained the lowest. The tris(2-chloroethyl)-phosphate (TCEP), tris(1-chloro-2-propyl) phosphate (TCPP), and di(2-ethylhexyl) phthalate (DEHP) were the three predominant compounds found overall. The maximum $\Sigma 4$ OPE concentration was measured up to $84,595.9 \text{ ng g}^{-1}$ in the PS foams. The OPEs were also measured for the plastic debris sampled at three Iberian Peninsula South-eastern beaches [16]. In this study, the OPEs were the most abundant compounds in comparison to PAH, OCPs, and pesticides extracted from the plastic debris.

2.3 Migration and Release of Chemical Additives from the Plastic Debris or Microplastics

The additives can potentially migrate and be released from polymers during the exposure in environments [17]. Therefore, it is of great interest whether organic chemicals from plastic debris or microplastics cause serious environmental risks. Until now, the majority of studies on the migration and fate of plastic additives associated with plastic particles have focused on leaching of flame retardants [16], plasticizers [18], and fluorescent additives [19].

HBCD in Styrofoam buoys massively used in oyster culture farms contributed to enrichment of HBCD in surrounding sediment [20]. Paluselli et al. [18] explored the migration of PAEs from the PE plastic garbage bags and PVC cables as affected by abiotic and biotic factors. This study indicated that light and bacterial exposure increased the total amount of PAEs released from PVC cables by a factor of up to 5, whereas they had no influence in the case of PE bags. In addition to the light and microbes, the water pH, salinity, and organic matter all had impacts on the leaching of chemicals from the plastics. In another study [19], the researcher investigated the

leaching behavior of fluorescent additives from polyurethane sponge microplastics. They found that the additives amount in the water followed the order of basic water > saline water > seawater > West Lake > River > Wetland, which all showed increasing trends with solution pH and leaching time. Tris-2-(chloropropyl) phosphate which has been measured with high concentration in the microplastics was also found having a high desorption ratio from the plastic debris [16].

Nevertheless, release of plastic additives in the intestinal tracts of aquatic species seems to lower than that in environment. Koelmans et al. [21] assessed the potential of leaching of nonylphenol (NP) and bisphenol A (BPA) in the intestinal tracts of *Arenicola marina* (lugworm) and *Gadus morhua* (North Sea cod) by using a biodynamic model. They found that leaching of NP and BPA concentrations was below the lower ends of global NP and BPA concentration ranges. However, the leaching rates of various additives in environmental conditions, as well as organisms' gut conditions, need to be investigated because the amount of various additives in plastic may be very high.

3 Sorption and Desorption of Organic Pollutants in the Microplastics

3.1 Occurrence of Organic Pollutants in the Environmental Microplastics

Studies on the organic pollutants in the environmental microplastics started from monitoring of persistent organic pollutants (POPs) in the plastic resin pellets (small granules 0.1–0.5 cm in diameter). A range of organic micro-pollutants (including polychlorinated biphenyls (PCBs), DDE, and nonylphenol) have been detected in plastic resin pellets stranded on beaches [11]. There is a project named “International Pellet Watch” sponsored by Japanese scientists, which has been in operation since 2006. In the project, the collected plastic resin pellets from beaches around the world were mailed to the laboratory of Japan for POPs analysis, and a total of 30 samples of pellets from 17 countries have been analyzed by 2009 [22]. By using this dataset, the global spatial distributions of organic micro-pollutants could be mapped with extremely low-cost method. Surveys for some specific area have been carried out at Portuguese coast [23], South Atlantic [24], North China [25], and even at the remote islands in the Pacific, Atlantic, and Indian Oceans [26]. All these surveys focused on the persistent organic pollutants including PAHs, PCBs, DDTs, and HCHs. In addition to the pellets from the surface of the beaches, some authors compared the plastic pellets from the different depths of the sediment of the beaches and found that the concentration and composition of PAHs varied greatly with the depth [27].

Meanwhile, field adsorption experiments using PP virgin pellets demonstrated significant and steady increase in PCB and DDE concentrations throughout the

6-day experiment, indicating that adsorption of PCBs and DDE from the ambient environments by the pellet surfaces is the mechanism of enrichment [11]. Furthermore, a longer term of field measurement of sorption of PCBs and PAHs to five types of plastic pellets demonstrated that sorption rates and concentrations of PCBs and PAHs varied significantly among plastic types and among locations, PE and PP pellets having a higher sorption capacity than PET and PVC [28].

The concentration of organic pollutants in the plastic resin pellets was found having a relationship with the aging time of the pellets in environment. Fifty-five resin pellets from a beach in Tokyo were individually analyzed for PCBs and showed discolored (e.g., yellowing) pellets contained more PCBs than others on most of the beaches sampled [29]. Analogous to the results in Tokyo beach, aged and black pellets were also measured higher concentrations for the PCBs, PAHs, and DDT in the beaches of the Portuguese coast [23]. The increase of adsorption of POPs in the plastic pellets with the aging time may result from the increase of specific surface areas and crystallinity after a long-term exposure in environment [30, 31].

3.2 Sorption and Desorption of Hydrophobic Organic Pollutants in Microplastics

Microplastics were found having a high affinity for hydrophobic organic pollutants (HOCs) due to their high hydrophobicity and specific surface area [32, 33]. Sorption of HOCs by plastic polymers was mainly governed by hydrophobic interaction mechanism. Liu et al. [34] studied the interactions between microplastics and phthalate esters and found that the sorption of DBP was much higher than DEP on all the three tested microplastics due to the higher K_{ow} and lower solubility of DBP than DEP. For the polymers (e.g., PS) with benzene rings in their structure, π - π interaction was supposed to be one of the most important mechanisms for their strong sorption for HOCs. In the same study [34], the higher sorption of DEP and DBP by PS compared to PE could be resulted from the strong π - π interactions between PS and the two PAEs. The previous study also revealed that sorption of PAH to virgin polystyrene microplastics was higher compared to sorption to nonaromatic polyethylene, polyvinyl chloride, and polypropylene microplastics due to the strong π - π interactions between the PS and PAHs [35]. Velzeboer et al. [36] suggested that the strong sorption of PCBs to nano-PS particles in their experiment could be explained by both hydrophobic and π - π interactions. Sorption experiment with nano-PS and PAHs indicated that the adsorption isotherms were nonlinear and a high distribution coefficient up to 10^9 L/kg was obtained as a result of the π - π interactions between the planar PAH and the surface of the aromatic polymer polystyrene [37].

Sorption of HOCs to microplastics might be limited by diffusion in the plastic phase, which could be described as biphasic process, the fast sorption/desorption in the outer layer followed by slow diffusion into the inner plastic phase. This biphasic

model is frequently used for sorption of HOCs to soils or sediments [38]. A recent study showed that the sorption process of HOCs to the micro-sized PS included two stages: the fast sorption stage for the HOCs diffusing through aqueous boundary layer and the slow one for the HOCs penetrating inside the PS particle, while the mass transfer rates were extremely slow for the second sorption phase [39]. The kinetics study on the sorption of pyrene onto microplastics revealed that the sorption rates were mainly controlled by intraparticle diffusion [40]. Furthermore, the penetrating diffusion process was controlled by the molecular weight of HOCs and the polymer density. Fries and Zarfl [41] studied the sorption of PAHs to low- and high-density PE and found that the diffusion coefficients decreased while the molecular weight of the PAHs increased which indicates a hindered diffusion through the matrix as a result of a larger molecule size. Meanwhile, higher diffusion coefficients were derived for LPDE than for HDPE indicating a greater sorption velocity for LPDE according to the lower polymer density.

Several factors have impacts on the sorption of HOCs onto the microplastics. In terms of the plastic polymers, physical structures have been found to play an important role in the HOC sorption. Guo et al. [32] examined the sorption behavior of four hydrophobic organic contaminants by the different polymers and found that the organic carbon content-normalized sorption coefficients (K_{oc}) of phenanthrene, lindane, and naphthalene by PEs of same composition but distinct physical makeup of domains increased with their crystallinity reduction.

Weathering in environments of the microplastics changes their surface properties and hence alters their sorption behavior for HOCs. The weathered plastics would increase the adsorption capacity than virgin plastics [22, 29]. This might be mainly attributed to the increase in surface area due to polymer weathering that would increase the effective diffusivity and the additional sorbents attached to the plastic debris for hydrophobic contaminants [11]. An increase in the surface area due to weathering could also increase polarity of the polymer surface by introducing oxygen-containing groups, which could decrease the affinity for hydrophobic compounds [29, 42]. Sorption coefficients of naphthalene by polystyrene microplastics following aging were found up to one order of magnitude lower than for pristine particles [43].

External factors, such as temperature, salinity, and composition of the water phase (e.g., particulate and dissolved organic matter), can also influence the sorption behavior of HOCs by microplastics in waters. The temperature has an impact on the sorption of HOCs in the microplastics through changing the surface tension of solution and the solubility of HOCs in the solution. Zhan et al. [44] observed that increasing temperature decreased the sorption of PCB by PP in pure water. The salinity would impact the solubility of hydrophobic organic compounds by natural sorbents such as soils, clays, and sediments [45]. Recent experiment results revealed that the sorption capacity of PCB, phenanthrene, and PAEs in the simulated seawater is higher than those in the ultrapure water, which indicates that salinity acts as one main factor affecting sorption capacity [34, 44, 46, 47]. However, the effects were not the same for different microplastics. In a previous study, it was found that differences in salinity had no significant effects on phenanthrene sorption onto

ultrahigh molecular weight (UHMW) polyethylene microplastics [48]. Dissolved organic matter (DOM) contains abundant functional groups, which can interact with natural particles or organic pollutants and thus affect their fate and transport in the nature environment. In plastic water system, molecular sieving and pore blockage By DOM could have more important roles in the control of the sorption of HOCs by PS than by other polymers [47].

3.3 Sorption and Desorption of Hydrophilic Organic Pollutants in Microplastics

Compounds with more hydrophilic properties have been much less considered in comparison to the highly hydrophobic compounds with respect to their adsorption and desorption in the microplastics. Pharmaceuticals and personal care products (PPCPs), as emerging contaminants (ECs) in terrestrial environments, threaten the aquatic and soil resources. Most of the PPCPs have hydrophilic properties and have a high possibility of interacting with microplastics, especially aged microplastics, because of their hydrophilic, oxygen-containing functional groups [47, 49, 50]. The mechanism governed adsorption of pharmaceuticals in the microplastics included the partitioning, electrostatic interactions, intermolecular hydrogen bonding, and π - π interactions. A study regarding the adsorption of antibiotics on the different types of microplastics showed that the adsorption capacities of ciprofloxacin (CIP), trimethoprim (TMP), and sulfadiazine (SDZ) on PS are higher than those on PE [51], suggesting the π - π interactions dominated the adsorption of the three antibiotics at the aromatic surface of the PS. In another study which examined the adsorption of oxytetracycline to microplastic polystyrene, the results revealed that electrostatic interaction regulated the adsorption; meanwhile H-bonding and multivalent cationic bridging mechanisms may also have affected the adsorption [50]. Aging has a pronounced effect on the adsorption enhancement by microplastics owing to the increasing of surface areas, hydrophilic properties of the surface, and oxygen-containing functional groups after aging [49, 50].

Impacts of pH, ionic strength, and dissolved organic on the adsorption have been examined in the present studies. In contrast to the negligible effects of pH on the adsorption of HOCs by microplastics [34], the solution pH has a pronounced effect on the antibiotic adsorption by microplastics because various antibiotics will exhibit different speciation of the cation, zwitterion, and anion in a specific pH condition [49, 50, 52]. For example, the oxytetracycline was mainly in cationic form at $\text{pH} < 3.27$, predominated by a zwitterion at pH values 3.27–7.32 and dominated by anions (OTC^- and OTC^{2-}) when $\text{pH} > 7.32$. A study indicated that the maximal adsorption of oxytetracycline on the aged PS particles occurred at $\text{pH} = 5$, because the surface charge of the aged PS approached the point of zero charge (PZC 4.96) at $\text{pH} = 5$ and therefore has the maximal adsorption due to the lowest electrostatic repulsion between the oxytetracycline and PS surface [50].

Ionic strength of the solution plays an important role in the regulation of antibiotic adsorption by microplastics. Usually a reduction of adsorption will be observed with the increasing of ionic strength owing to the competing with antibiotics on the adsorption sites on the plastic surface [50–52]. However the ions of different valence state may have different influence on the adsorption. The depression of oxytetracycline sorption to the aged PS foams can be offset in the presence of Ca^{2+} through formation of ternary complexes between the cations and oxytetracycline and the surface functional groups [50].

The effects of DOM on the antibiotic adsorption were not consistent among the different studies. Xu, Liu, Brookes, and Xu [53] found the increasing concentration of fulvic acid inhibited the sorption of tetracycline on three microplastics, decreasing them by more than 90% at the fulvic acid concentration of 20 mg/L. However in the study of Zhang et al. [50], both fulvic acid and humic acid promoted the adsorption of oxytetracycline in the aged PS foams, and humic acid has more pronounced effect than fulvic acid. Such a difference is probably caused by the different microplastics used in the experiments. Further studies are recommended to elucidating the mechanisms of DOM effects on the sorption and desorption of antibiotics in the microplastics.

4 Microplastics Move Additives and Organic Pollutants from Environment to Organisms

4.1 The Role of Microplastics in the Transfer and Accumulation of Chemicals from Environment to Organisms

The vector concept has been used to describe increased uptake of contaminants that adhere to microplastics by planktivores [3]. However, the relative role of microplastics as a vector for hazardous contaminants to organisms has been found negligible in comparison to natural exposure pathways in marine ecosystems [54, 55]. Hartmann et al. [56] still argued that microplastics will play a larger role as a pathway for contaminants to transfer into biota than the current estimation in some specific scenarios. Moreover, microplastics might accumulate in terrestrial and continental food webs at levels similar to or higher than in marine counterparts, although conclusive evidence is yet to be found [1]. Therefore, the process of microplastics as a carrier of contaminants from external environment to biota should not be overlooked in the terrestrial environments.

A study regarding the effects of PS on the transfer of PCBs to lugworms *Arenicola marina* (L.) indicated that a low PS dose of 0.074% increased bioaccumulation of PCBs by a factor of 1.1–3.6, an effect that was significant for Σ PCBs and several individual congeners [57]. Browne et al. [58] also found the vector effect of 5% PVC on transfer pollutants and chemical additives into the gut of

lugworms. Using the ^{14}C -labeled phenanthrene, Ma et al. [59] observed that presence of nanometer plastics significantly enhanced bioaccumulation of phenanthrene-derived residues in daphnid body. The enhanced transport of contaminants by nanoplastics was in relation to the polarity-dependent extents of desorption hysteresis, effective nonpolar and weakly polar compounds, and no effects for polar compounds [60].

Notwithstanding, latest studies showed that pharmaceutical bioaccumulation in the biota could also be enhanced in the presence of microplastics. The PS microplastics were found to enhance the bioaccumulation of roxithromycin in the tissues of freshwater fish red tilapia (*Oreochromis niloticus*) compared to roxithromycin-alone exposure [61]. Moreover, the bioaccumulation factor (BAF) of venlafaxine and O-desmethylvenlafaxine in loach tissue amplified more than ten times with microplastics present, and in liver subcellular structure, microplastic may help to transport more compounds into subtle areas and postpone the contaminant metabolism in organisms [62]. Therefore, these studies contributed to our understanding of the aquatic risks of pharmaceuticals associated with microplastics.

The promotion of contaminant bioaccumulation in the presence of microplastics probably is related with desorption rate of the adsorbed contaminants inside the biota. A laboratory gut mimic extraction using the digestive enzyme pepsin at pH 2 from the commonly-ingested plastics indicated that bisphenol A (BPA), diethylhexyl phthalate (DEHP), and butylbenzyl phthalate (BBP) concentrations were significantly increased in the gut mimic extraction relative to water extraction [63]. Another estimation using artificial gut fluid of fish indicated the microplastic ingestion might increase the total uptake rate of pentachlorobenzene and hexachlorobenzene due to their accelerated desorption from microplastics into the artificial gut solution under the scenario of extremely high intake of microplastics [64]. However, when in comparison with the biochar and wood, the plastic absorbed PCBs had a lower solubilization in the gut fluids [65]. Therefore, the contribution of the organic pollutants to aquatic organisms from microplastics still has a high uncertainty, and further studies are recommended to be carried out in this aspect.

4.2 *Effects of Leachates from the Microplastics on the Terrestrial Organisms*

One of the ecological risks associated with microplastics contamination in the environment is the release of chemicals associated with plastics to the environment shared with organisms. Most of the information available from this route of exposure comes from experiments assessing the toxicity of leachates from new plastic consumer products to aquatic invertebrates [19, 66–69]. Varied chemicals have been measured in the plastic leachates, which included HOCs [69], dioxin-like compounds [30], and endocrine-disrupting chemicals (EDCs) such as estrogens, bisphenol A, bisphenol S, octylphenol, and nonylphenol [70]. Higher concentrations of these chemicals were measured in the leachates from aged or small-sized

microplastics. In addition, the chemical concentration from the microplastics depends mainly on water environments. Luo et al. [19] found that the leached concentrations of fluorescent additives in simulated and natural water followed the order of basicwater > salinewater > seawater > West Lake > river > wetland.

The toxicity of the leachates was evaluated using the bioassay including the mortality of freshwater species *Daphnia magna* [69, 71], embryo development of the brown mussel (*Perna perna*) and sea urchin *Paracentrotus lividus* [66, 68], larval survival and settlement of the barnacle (*Amphibalanus Amphitrite*) [67], and microalgae (*Chlorella vulgaris*) physiology [19]. All these tests were observed toxic effects of the plastic leachates. Moreover, the toxicity of the leachate from beached pellets was found much higher than that of virgin pellets, which corresponds to the higher concentrations of the contaminants in the aged microplastics [30, 66, 68]. Alteration of the surface chemistry of the microplastics may also have an important impact on the toxic effects. Study of Li et al. [67] revealed that hydrophobicity of the plastic surface was negatively correlated with mortality of barnacle larval when it was tested in the plastic leachates for a short time. This indicated that increasing of hydrophilicity on the plastic surface as a result of weathering may enhance the toxic of the microplastics. However, longer-term studies are required to determine if relationships persist as plastics become weathered by environmental exposure.

4.3 Biological Consequences of Microplastic Ingestion and Chemical Transfer to Organisms

We know that microplastics are easy to be swallowed and accumulated by aquatic organisms [3] and, consequently, be transferred through food chains [72]. Therefore, the importance of combined ecological effects caused by microplastics and organic pollutants has been emphasized since microplastics can act as a vector for most of the organic pollutants [33]. The endpoint of the risk assessment involved mortality, feeding behavior, immunity, and biomarkers for oxidative stress in the bioassays. However, contrasting results have been obtained based on current literature review. A short-term experiment with large proportions of PVC (5%) showed that lugworms eating microplastics accumulated large enough concentrations of pollutants (triclosan) or additives (PBDE, nonylphenol) which enhanced the reduction of survival, feeding, and immunity of the lugworms [58]. Another study indicated that the co-effect of microplastics and venlafaxine, as well as the metabolite, might lead to more adverse effect against loach and therefore should be taken into consideration in actual environment [62].

However, more studies indicated that the toxicity of organic pollutants on the biota was not affected or even mitigated in the presence of microplastics. For example, the microplastics delayed pyrene-induced goby (*Pomatoschistus microps*) mortality and increased the concentration of bile pyrene metabolites, and simultaneous exposure to both microplastics and pyrene did not increase significantly the inhibitory effect

for acetylcholinesterase (AChE) and isocitrate dehydrogenase (IDH) activities [73]. Co-exposures and incubated exposures of microplastics and fluoranthene did not result in additive or synergistic oxidative stress response in the blue mussel, *Mytilus edulis* [74]. The combination of triclosan and PVC microplastics even has a greater reduction of their toxicity on microalgae *Skeletonema costatum* than the microplastics alone [75]. Although a significant inhibition in the activity of 7-benzyloxy-4-trifluoromethyl-coumarin O-dibenzoyloxylase (BFCOD) enzyme was observed in the co-exposure to roxithromycin (ROX) and PS microplastics compared to exposure to ROX alone, increase of SOD activity and decline of MDA content caused by co-exposure suggested that presence of microplastics might mitigate the oxidative damage [61].

In addition to the bioassay using the spiked organic contaminants with microplastics, Asmonaite et al. [76] applied the sewage or harbor effluent exposed PS microplastics which contained various environmental contaminants (e.g., PAHs, nonylphenol and alcohol ethoxylates, and others) to feed fish rainbow trout and examined the hepatic stress and lipid peroxidation in fish fillet. The results indicated that the ingestion of relatively high doses of these PS microplastics did not induce adverse hepatic stress in fish liver and the ingestion of these particles did not affect lipid peroxidation or rancid odor development, thus not affecting fillet's quality.

5 Summary

Environmental microplastics contain a variety of contaminants involved of adsorbed organic pollutants and chemical additives. Hence both microplastics and the contained organic pollutants should be considered with respect to the ecological risk caused by the microplastics. The microplastic-bound contaminants have different adsorption and desorption characterizations depending on the characteristics of the contaminants, physicochemical properties of the microplastic surface, and external environmental conditions. Aging of the microplastics in the environment would have important impacts on its surface properties and subsequently influence the adsorption for organic pollutants. Therefore, further studies are recommended to study on the interaction of the organic contaminants with environmental relevant microplastics, and fate of these contaminants in the environment. There are a lot of uncertainties regarding the organic contaminant bioaccumulation and toxic effects owing to the co-exposure of microplastics and organic pollutants to the biota. Meanwhile, researchers are concerned more about the polymer types of the microplastics than the size and shape difference in most of the studies regarding the combined effects of microplastics and organic pollutants. Hence the role of physical properties of the microplastics should also be of concern in the bioassay of co-exposure of microplastics and organic pollutants in order to provide an unbiased evaluation.

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Interaction of Microplastics and Heavy Metals: Toxicity, Mechanisms, and Environmental Implications



Xiaofeng Jiang and Mei Li

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Abstract With the wide use of plastic products in all aspects of life, more and more plastic ends up in the environment. Such plastic waste will gradually decompose, break up, and form smaller fragments through a series of physicochemical and biological processes. Among them, plastic fragments with particle size less than 5 mm are defined as microplastics (MPs). MPs have been reported to be widely distributed and to have the potential to adsorb other pollutants. Therefore, it is particularly important to evaluate the toxic effects of MPs in combination with other pollutants like metals. So far, studies on microplastic and metal toxicity have mainly focused on aquatic environments, while their impact on terrestrial ecosystems has been studied to a much lesser extent. In order to help our understanding of

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the ecological risk of MP on soil ecosystems, this chapter reviewed the interaction of MPs and metals on soil organisms.

Keywords Combined exposure, Metals, MPs, Soil ecosystem, Toxic effects

1 Introduction

Microplastics can be divided into primary and secondary MPs. Primary MPs are plastic fragments or particles whose initial particle size is less than 5 mm when they are manufactured, mainly in textiles, drugs, and personal care products [1, 2]. The secondary MPs are plastic fragments shaped by environmental forces to a particle size less than 5 mm [3]. Up to now, most studies dealing with MPs and their toxicity have focused on the marine environment. Although freshwater and terrestrial environments have been considered the origin and transport route of plastics to the sea, there is still lack of research of MPs in these environments, especially in the soil environment.

In recent years, it has been found that MPs are widely detected in the soil environment. Fuller and Gautam have investigated the concentrations of MPs in industrial soils in Sydney, Australia, and found that it varies greatly among different sites, with a minimum concentration of 300 mg/kg, and the highest concentration of 6.75×10^4 mg/kg [4]. Scheurer and Bigalke reported the MP abundance of 26 flood-plain sites in Switzerland. Their investigations showed that the highest MPs concentration could reach 55.5 mg/kg [5]. In addition, the toxicity of MPs to terrestrial organisms, such as earthworms, mice, and other, has also been conducted. It has been confirmed that MPs with particle size less than 1 mm are easily ingested by soil organisms [6]. Lwanga et al. found that MP exposure could affect the growth and movement of earthworm *Lumbricus terrestris* (*L. terrestris*). The results showed that microorganisms in the earthworm gut significantly decreased low density polyethylene (LDPE) particle size [7, 8]. Other studies have also shown the toxic effects of various MPs on other soil organisms [9, 10]. Furthermore, particle size is one of the most important characteristics of MPs toxicity [1]. For example, 1 μm is the most common size of filter food organ interception in crustaceans, so crustaceans prefer to ingest MPs with particle size less than 1 μm . Smaller particles have a greater possibility of biological intake than larger size particles, which may enter the cells through endocytosis. Although it has been assumed that the toxicity of MPs is significantly related to its particle size, there is no unified view on what kind of particle size MPs is more toxic. The toxic effects of 0.05 μm , 0.5 μm , and 6 μm MPs on rotifer *Brachionus koreanus* were compared, and it was concluded that small particle size MPs had more significant toxic effects. The antioxidant enzyme activity and mitogen-activated protein kinase (MAPK) signaling pathway in rotifer changed with different particle size of MPs [11, 12]. Likewise, another study found that MPs

with particle size larger than 50 μm had no significant toxic effect on Grass shrimp (*Palaemonetes pugio*), while the fatality rate of acute toxicity test was higher when the size less than 50 μm . It was indicated that MPs have size-dependent effects on the same species [13].

The interaction between MPs and other pollutants is present in the environment. Therefore, in order to evaluate the ecological risk of MPs, the interaction between MPs and other pollutants should be considered, and the toxic effects of combined exposure on various organisms should be addressed. However, there is still lack of research on the toxic effects of MPs in combination with other pollutants, especially MPs and heavy metals [14–17]. There are only a few articles published that deal with the combined toxic effects of MPs and heavy metals. Combined exposure of Cr^{6+} and MPs enhanced the toxicity of the juveniles of common goby – *Pomatoschistus microps* and caused strong lipid peroxidation damage in larvae [18]. By contrast, another study has shown that the combined exposure of 1 μm MPs and Cu to microalgae did not show any toxicity [19]. These studies showed that the combined exposure of MPs and heavy metals is affected not only by the particle size but also by the selected biological species. Moreover, MPs can also interact with heavy metals in the soil environment [20, 21]. Hodson et al. studied the adsorption behavior of high density polyethylene (HDPE) on Zn^{2+} in soil. They found that HDPE had stronger adsorption capacity for Zn^{2+} in soil with more abundant organic matter. The adsorption behavior was in accordance with Langmuir and Freundlich equation [20]. The aged MPs in soil also had a significant effect on the adsorption of heavy metals. Nicole et al. exposed HDPE, polyvinyl chloride (PVC), and polystyrene to artificial aging conditions (2000 h, photo-oxidation and thermal oxidation) to simulate their aging process using a column percolation test. Their results showed that the aged MPs not only significantly increased the adsorption of TOC, Cl, Ca, Cu, and Zn but also weakened the desorption and release of heavy metals, which indicated that the aged MPs had stronger fixation ability to heavy metals [21]. In addition, the functional groups in the soil are adsorbed to the surface of the MPs and may change the adsorption capacity of heavy metals. Kim et al. investigated the adsorption of Ni by the functional group-coated polystyrene. Results showed that the functional groups change the surface hydrophobicity of the polystyrene microplastic and heavy metal and then alter the adsorption of the heavy metal [22]. Turner et al. also studied the adsorption properties of polyethylene microplastics (PE-MPs) for heavy metal ions (Ag, Cd, Co, Cr, Cu, Hg, Ni, Pb, and Zn) [23]. The adsorption kinetics experiments showed that the adsorption efficiency of aged PE-MPs in river water was higher than that of original PE-MPs, which may be due to the change of the surface structure of aged PE-MPs to reach surface charge equilibrium. Holmes et al. also found that the adsorption capacity of aged polyethylene in seawater for heavy metals was stronger than that of the original polyethylene [24]. Therefore, once the MPs in the soil are weathered and aged, they can be effective carriers of heavy metal in the soil environment what can cause even greater damage to the health of the soil ecosystem.

2 Effects of Microplastics on Organisms

2.1 Individual Effects of Microplastics on Organisms

The ecotoxicity of MPs is mainly focused on smaller organisms. Organisms can directly ingest most MPs that can then cause physical damage, clog or wear ingestion organs and digestive tract, or reduce ingesting rate of organisms. Furthermore, sharp MPs can also cause damage to gills or intestinal tissues [25]. Mussel (*Mytilus edulis*) and herbivorous crab (*Carcinus maenas*) could ingest polyethylene (<80 μm) and polystyrene microspheres (10 μm) and ingested MPs could damage the intestinal tract [26, 27]. In addition, after organisms ingest MPs, they might cause the wrong sense of satiety, reduce the intake of food and act on the digestion process, resulting in energy loss, reduce growth as well as reproductive capacity, and ultimately lead to hunger and death [28].

MPs affect the individual growth, reproduction, and diversity of soil animals. Once MPs are taken up or accumulated by soil animals, in addition to causing physical damage, such as tearing of organs and tissues, the animal will also have an inflammatory response to invasive heterogenic substances [29]. In addition, the ingestion of MPs can also cause insufficient supply of nutrients and energy to soil organisms. Furthermore, the toxic substances released by MPs and the toxic effects of adsorbed pollutants can have varying degrees of adverse impacts on individual and species diversity [20, 30, 31].

The toxic effects of MPs on soil animals are related to particle size and concentration. Rillig showed that MPs with particle size less than 1 mm can be easily eaten by soil animals. After soil animals are fed with MPs, they can also remain in the body [32]. Another investigation showed that MPs are not only more likely to remain in the intestine than other ingested substances but can also pass through the intestinal wall and be transported to other tissues [33]. MPs with a particle size >1 mm remains in the intestinal tract or with the excreta while the small particles are more easily transferred, and can be accumulated by cells. It may relate to the limited space of intracellular phagocytosis of corpuscles [34]. Lwanga et al. studied the effects of different concentrations of MPs (polyethylene <150 μm) on the earthworm *Lumbricus terrestris*. It was found that the mortality rate was highest along with a negative growth rate, when the concentration of polyethylene reached 60% w/w [7, 35].

2.2 Combined Effects of Microplastics and Heavy Metals on Organisms

Heavy metals are widely distributed pollutants in the natural environment. Their toxic effects on aquatic organisms have been widely studied and they are considered to be high-risk pollutants. Heavy metals exist in various ion forms in the water

environment. It has been reported that heavy metals can be enriched by aquatic organisms and have an adverse impact on the whole ecosystem [36, 37]. MPs have the potential to act as vectors for heavy metals and may change the toxicity of other contaminants [38, 39]. Therefore, it is of great significance to explore the interaction between MPs and heavy metals for the complete evaluation of the ecological effects. Barboza et al. found that MPs could absorb mercury from surrounding water and subsequently affect the accumulation of mercury in the European seabass (*Dicentrarchus labrax*) [40]. Khan et al. reported that exposure to aged MPs could increase the bioaccumulation of Ag in the intestine tissue of zebrafish (*Danio rerio*) [41]. Luís et al. found that MP exposure could affect the toxic effects of Cr (VI) on juvenile *P. microps* [18]. Lu et al. reported that exposure to MPs and Cd resulted in increase of Cd bioaccumulation in the zebrafish (*D. rerio*) tissues and showed increased toxic effects compared to exposure to Cd alone [42]. Wen et al. investigated the single and combined toxic effects between polystyrene MPs (0, 50 or 500 mg L⁻¹) and two concentrations of Cd (0 or 50 mg L⁻¹) on the discus fish (*Symphysodon aequifasciatus*) for 30 days. The results showed that there are no obvious effects on the survival and growth of juvenile *S. aequifasciatus*, indicating that the decreasing toxicity may be due to the antagonistic effects of Cd and MPs. However, co-exposure to high concentration of MPs (500 mg L⁻¹) and Cd led to elevated protein carboxyl content, suggesting a synergistic effect of MPs and Cd on the accumulation of protein oxidation products [43]. Lu et al. investigated the biochemical markers, histopathological changes, and functional gene expression of zebrafish (*D. rerio*), showing that the presence of 5 µm polystyrene microspheres enhanced the toxicity of Cd²⁺ to zebrafish and its combined exposure with Cd²⁺ could lead to oxidative damage and inflammation of zebrafish [25]. Nevertheless, the reports regarding combined effects of MPs and heavy metals on soil organisms are still limited. Hodson et al. studied the interactions between HPDE MPs particles and zinc (Zn) to understand the effect of MPs on earthworms' metal bioavailability. Their results showed that MPs could increase Zn bioavailability; however, Zn accumulation, mortality, or earthworms weight have not changed significantly [20].

Wang et al. exposed PVC MPs to earthworm *Metaphire californica* with arsenic (As (V)), for 28 days. The total arsenic concentration and arsenic species in the soil, the gut microbiome, and the tissues of earthworm were analyzed. The findings illustrated that arsenic could be bioaccumulated in the earthworm gut and tissues. Nevertheless, total arsenic concentrations in the earthworm gut and tissues were significantly decreased when earthworms were exposed to the combination of As (V) and MPs, which may explain that MPs can alleviate the adverse effect of arsenic on the gut microbiome due to MPs possibly by inhibiting the reduction of As (V) [44].

3 Potential Mechanism of Microplastics Toxicity

3.1 Uptake, Translocation, and Accumulation of Microplastics in Organisms

Microplastic particles will be transferred to higher organisms through the food chain [45, 46]. Lwanga et al. performed one study on the trophic transfer of MPs in the terrestrial food chain, in which the concentrations of MPs in gardening soil, earthworm casts, and chicken (*Gallus gallus domesticus*) feces were analyzed. The concentrations increased along the trophic levels, and the highest concentration of MP was confirmed in chicken feces. In particular, chicken gizzards also contained MPs, and this indicated that the evidence of transfer of MPs to humans is through food because gizzards are used for human consumption [35]. Maaß et al. used two collembolan species, *Folsomia candida* and *Proisotoma minuta*, and observed the transport of urea-formaldehyde particles (200–400 µm). The transport of particles was strongly dependent on the type of particle, size of particles, and size of organisms. Nevertheless, the authors confirmed the horizontal transport of plastic particles by soil microarthropods [47]. Rillig et al. also studied the transport of PE-MP by soil organisms *L. terrestris*, which were cultured in 2.5 kg of soils covered with 750 mg of various sizes of PE-MPs particles. After 21 days of exposure, MPs were detected in the middle and bottom layers of soils, and the smallest particles (710–850 µm) reached the deepest layers of the soil. The mechanisms of plastic transport in soil were not demonstrated, but they suggested that MPs might be transported through the activities of earthworms such as ingestion/egestion, burrowing, adherence, and casts making [48].

So far, despite their ecological importance, the exposure of soil filter feeders such as nematodes, rotifers, and ciliates to MPs and nanoplastics has not yet been determined. Filter feeders in marine ecosystems have been shown to ingest micro-particles [30, 49], while filter feeders in freshwater ecosystems, *Daphnia magna* and *Thamnocephalus platyurus*, have been shown to be sensitive to nanoplastics [50]. Organisms with other feeding modes are also susceptible to microplastic ingestion. Taylor et al. found synthetic microfibers on and inside six out of nine deep-sea organisms that belong to the phyla Cnidaria, Echinodermata and Arthropoda with predatory and feeding mechanisms [51]. As such, woodlice, snails, caecilians, and other soil organisms with similar feeding mechanisms would be subjects of interest in agroecosystems. Information about the bioavailability and bioaccumulation of MPs in soil organisms is generally lacking. We know that nanoplastics can enter cells, as fluorescent nanoplastic polymers have been used as molecular probes for a wide range of biological studies with mammalian cells, for example, to measure blood flow in tissue and as tracers for phagocytic processes [34]. The translocation of a range of microparticles by mammalian gut into the lymphatic system has been demonstrated in rabbits, dogs, and rodents. There is no experimental evidence of nanoplastics being transferred from invertebrates to vertebrates. However, there is evidence of the transfer of MPs from contaminated land to vertebrates and potentially from earthworm to chicken [35].

3.2 *Molecular-Level Response*

So far, there have been published only a few papers that focused on the molecular-level response of organisms to MPs exposure [10, 52, 53]. Prendergast-Miller et al. used metallothionein (*mt-2*), heat shock protein (*hsp70*), and superoxide dismutase (*SOD-1*) as the biomarker responses to evaluate the molecular-level response in *L. terrestris* exposed to polyester-derived microfiber (MF) with 0, 0.1, and 1.0%w/w for a period of 35 days [53]. Their results showed that *hsp70* expression was downregulated at the high MF exposure, which indicated that downregulation of *hsp70* is an index of stress when *L. terrestris* is exposed to MF. However, the activity of *mt* is not completely understood. It can be explained by the shortage of metal transcription factor (*MTF-1*) in *L. terrestris* compared to other higher organisms. Therefore it is necessary to determine the transcriptional response of the earthworm's response to MF [53]. Rodriguez-Seijo et al. also studied molecular changes of earthworms (*Eisenia andrei*) exposed to PE-MPs. They concluded that multiple stress-response mechanisms of the immune system of earthworms led to, involving a wide range of molecules/enzymes, the increased content in proteins, lipids, and polysaccharides [10]. In addition, the alterations of saturation fatty acid have also been considered as a biomarker for the response of soil organisms to stress [54]. The increase in saturated fatty acids makes membranes more viscous and less permeable, while saturation reduces the susceptibility of fatty acids to free radicals [55].

4 Environmental Implications and Future Prospective

4.1 *Challenges About Toxicity Research Methodologies of Microplastics and Heavy Metals*

MPs can act as a carrier of metals and combined they can cause toxicity to various organisms. However, it is difficult to determine the contribution of MPs and metals to overall toxic activity. Furthermore, contaminants carried by MPs may be transported along the food chain [56]. Among the chemical substances present in MPs are those added during their manufacture (additives) and those present in water that are adsorbed on the surface of MPs, such as persistent organic pollutants, pharmaceuticals, pesticides or herbicides. Among pollutants that MPs can absorb, metals have been widely studied [29, 33, 56]. In addition, some metals are frequently added as catalysts, pigments, and stabilizers during plastic manufacturing [57]. The toxicity of MPs and heavy metals should not be generalized by synergistic, antagonistic, additive or independent effect. Therefore, it is important that relevant standards and rules for toxicity research on MPs should be first determined so that data from different researches can be comparable and reliable.

4.2 *Considerations for Assessing Ecological Risks of Microplastics and Heavy Metals*

The widespread distribution and accumulation of MPs in the global environment has attracted attention on its sources, migration distribution, and ecotoxicological effects. The size, quantity, and shape of MPs entering the environment are uncertain, and the related research methods and classification criteria are not unified, what causes lack of consistency in the study of environmental behavior of MPs. There is also a lack of systematic analysis of the bioaccumulation and the transfer of MPs in the food chain. In addition, the interaction mechanism of micro-plastics and heavy metals, as well as the role of the MPs in their combined toxicity needs further study.

5 Summary

This chapter reviewed the interaction of MPs and heavy metals: toxicity, mechanisms, and environmental implications. However, most of the toxicity experiments of MPs are carried out in the laboratory on single species, the exposure time is short, and the dose is higher than the environmental concentrations. Therefore, it is necessary to provide comprehensive evaluation of the MP toxic effects according to the environmental conditions. Furthermore, new molecular biological techniques such as relevant omics should also be applied to study the toxic effects of MPs. The toxic mechanism of MPs and heavy metals on organisms, as well as the toxic effects on biodiversity, community structure, and ecosystem function, also need more detailed approach, which can provide basic data support for the determination of MPs in the environment and the establishment of standards.

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Part III
Ecological Effects of Microplastics
in Terrestrial Environments

Microplastics and Their Effects on Soil Function as a Life-Supporting System



Anderson Abel de Souza Machado, Alice A. Horton, Taylor Davis,
and Stefanie Maaß

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Abstract Particles play important roles in terrestrial systems, where the natural soil environment provides a complex habitat in which the three-dimensional organization of mineral and organic matter is combined to a diverse array of water levels, microscopic life forms, and their metabolites. Soils are the foundation for most land-based life and terrestrial ecosystem services that benefit humans. When plastics

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arrive at the soil, their nonnatural structure, distinct chemical composition, and unique surface properties trigger a series of abrupt environmental changes in the soil. Indeed, the current evidence suggests changes in the fundamental physical, chemical, and microbiological properties of the soils. Consequently, water and other biogeochemical cycles, as well as plant performance and animal health, can be affected. In this chapter, we present the recent advances in understanding how microplastics can change elementary properties of soil systems, such as soil aggregation and structure. This is discussed jointly with the linked effects in the microbial activity and function. Then, we address the recent studies regarding the effects of micro- and nanoplastics on plants and animals. Finally, we elaborate the properties of the various types of microplastics, soil processes, and soil organisms that are probably influencing the observed effects. We conclude by highlighting that current scientific information is not enough to devise solid risk assessments on microplastics in soils and suggest research directions to fulfill this gap.

Keywords Biogeochemistry, Environmental change, Microbiome, Plants, Soil fauna

1 Introduction

Natural particles play important roles in terrestrial systems [1]. The very complex interaction of these particles with water, air, and natural biogeochemical cycles is what makes possible the provision of many environmental services that sustain our leisure, our food, and our health [2]. Take the example of the soil system. Soils are a collection of natural particles of various sizes and from mineral and organic origins that trap and interact with gases, liquids, and organisms. Thus, soil health is fundamental for proper function of processes affecting agriculture, global climate, or even things seemingly not related such as urban resilience to flooding [3].

We can describe the natural soil environment as a complex and highly heterogeneous habitat in which the three-dimensional organization of mineral and organic particles is combined to a diverse array of water levels, microscopic life forms, and their metabolites [3]. This structured and multifaceted physical, chemical, and biological entity is referred to as the soil biophysical environment, and it has evolved over millions of years to form the life support system touching all living things on land from the smallest microbes to the largest elephants. When water and air are combined with the inorganic matter (clay, sand, silt, minerals) and organic matter (decaying and decayed plant and animal material) within soils, the needs of the diverse array of micro-, meso-, and macrofauna can be provided. Thus, the dependency on soils is the foundation of all terrestrial ecosystems, and it is of the highest relevance to the trophic food webs as it supports directly and indirectly the incredible diversity in forms of life seen in the world [3].

While you are reading these paragraphs, innumerable physical and chemical interactions are occurring between soil particles and other soil particles, between soil particles and water, and between soil particles and air. These interactions critically affect key parts of ecosystems [3]. One example is the rhizosphere, the portion of soil that directly affects and is affected by the roots of plants. The organic material in soil is full of essential nutrients which are inaccessible to plants and must first be mineralized by microorganisms [4]. Via mineralization, complex organic molecules are transformed into inorganic nutrients which can dissociate in a water solution into electrically charged ions. These ions are then available for uptake by plant roots either directly from the soil solution or from soil colloid surfaces. Negatively charged ions (anions) such as nitrate (NO_3^-) and sulfate (SO_4^{2-}) are mostly found in the aqueous phase of the soil, the soil solution. On the other hand, inorganic components of soil such as fragments of rock and small minerals present in a water-fine particle mixture (soil colloids) attract positively charged ions (cations) such as ammonium (NH_4^+) and magnesium (Mg^{2+}). Plant roots are in intimate contact with the soil solutions and the soil colloids. Water and ions are exchanged along electrochemical gradients between the soil solution and colloids, the solution and roots, and roots and colloids in all directions [5]. Thus, all components of the soil system are involved in ion and water dances that provide plants with the necessary building blocks to perform photosynthesis.

Other physical properties of soil such as bulk density, soil moisture, and soil structural stability affect the habitability of soils by influencing water retention, aeration, and aggregation [6]. These interactions are paramount to the health of soil systems and the soil biophysical environment and, therefore, to the terrestrial components of the Earth's biosphere. It is within this context that a nonnatural foreign material like microplastics (MPs) might represent potential risks of altering the still poorly understood interactions between particles, water, chemicals, and microbes in the soil system [7].

Plastic litter arrives to soil as meso- and macroplastics (as discussed in Part 2, Chaps. 3–10). These large plastics are slowly broken down into microplastics (<5 mm) and eventually nanoplastics (<100 nm), and accompanying their decrease in size, there is an exponential increase in number, surface area [8], and likely bioavailability. The decrease in size also allows for easier integration into soil [9]. It has been shown that earthworms ingest microplastics and carry on transferring them vertically and horizontally throughout the soil column via cast excretions [10, 11]. It has been also demonstrated that collembolans can interact with micro- [12] and nanoplastic particles with consequences to their biology [13]. Thus, it is logical to assume that other soil macrofauna such as ants and termites as well as soil-dwelling animals such as moles are significant potential incorporators of plastics into soils [7]. A more well-documented integration of plastics into the rhizosphere has been taking place due to the decades-old practice of fertilizing agricultural lands with dried and pelleted sludge from human sewage [14]. This sludge, heavily laden with micro- and nanoplastics, is applied season after season directly to the crops [15]. The common practice of mass tillage further incorporates such microplastic particles into

the soil column where they have the potential to interact with soil components and are accessible to microbes and plant roots [9].

The organisms and the soils themselves change and evolve over the geological time, so that abrupt changes to soil composition on a global scale are rare. Nonetheless, plastics might be among the most sudden, pervasive, and longest-lasting forms of global anthropogenic pollution affecting soil and environmental systems more broadly. Plastics and MPs are dispersed by numerous processes in litho-, hydro-, and atmosphere and currently found in every habited and uninhabited continent on our planet. MPs swept up by wind currents are scattered down by rain or snow reaching all corners of the globe including both the Arctic Circle and Antarctica. In fact, there is a growing concern that MPs might constitute a driver of global change in terrestrial systems [7, 16]. In this chapter, we will explore some of the observed impacts of microplastic contamination of soils as a life support system and their effects for fundamental functions of the terrestrial ecosystem. We will discuss the known effects that occur to terrestrial animals when they ingest plastic particles as analogies to the aquatic environment. Nevertheless, we will mostly focus on organisms, traits, and functions that are specific to the soil and terrestrial realm, such as rhizosphere effects. In the end, some recommendations and future directions will be given.

2 The Evidence for Impacts of Microplastics on the Soil Environment

The biopersistent composition of plastics summed to their structural differences to natural matter and to the inherent association of this material with human activities yields the possibility of a myriad of hazards to the soil environment [7]. These hazards might entail relevant risks since some authors have found levels of MPs in weight of top soil up to 7% in contaminated areas [17], and counting of particles suggests that concentrations up to 40,000 microplastic particles per Kg of soils [18] might be environmentally realistic. Within soils, MPs might persist for more than 100 years due to low light and oxygen conditions [19]. Therefore, soil MPs will certainly interact with soil fauna and flora, potentially changing their behavior, their fitness, and consequently the biophysical environment and its function [7].

In fact, both conceptual and empirical evidence suggest that MPs act as drivers of environmental change in the soil environment. From passive carriers of pathogenic microorganisms to active transformers of the way soil functions, this section deals with how soil MPs are shown to change the way microbes, plants, and animals interact with their habitat.

2.1 *Microbes and Microplastics in Soil*

There are multiple mechanisms proposed as means of MPs affecting soil microbial communities, some of which are similar to the effects in aquatic microbiomes, while others might be specific to the soil habitat. For instance, within wastewater treatment plants, MPs are shown to be enriched with both pathogenic and opportunistic organisms [20]. Empirical data for the microbial communities in those particles suggests that even after environmental release, microbes in the microplastic surfaces contained higher levels of antibiotic resistance genes and other markers of microbial horizontal gene transfer [21]. If the sewage sludge is employed as a soil amendment, microplastic particles might enter soil systems and subsequently disperse such genetically diverse microbes within those systems [7]. Moreover, it is now established that, in aquatic systems, microplastic particles are surrounded by an ecocorona consisting of many proteins, organic compounds, and microbes [22]. This is relevant to soils because the majority of soil microorganisms are essentially aquatic organisms, i.e., thriving in the interstitial water on or between soil particles, known as soil pore water [23]. Thus, if MPs surface properties distinct from those of natural organic and mineral particles, it could act as a selective force to diversity of soil microbiomes [7]. The effects of MPs as vectors of pathogenic microbes or as active selective surfaces to terrestrial microbiomes remain largely unexplored and represent relevant areas for future research.

Some of the first experimental data suggesting MPs' impact on microbial function was the work from Liu et al. [24]. These authors investigated the effects of high levels of polypropylene contamination (7 and 28% of soil weight) on the cycling of soil dissolved organic matter, dissolved phosphorus, and microbial activity [24]. Their conclusion was that microplastic addition affected the decomposition of organic matter, microbial enzymatic activities, and levels of nutrients [24]. This study provided unprecedented and ecologically meaningful evidence of microplastic effects on microbial function. However, the mechanistic nature could not be formulated as information on particle size distribution and soil chemistry was missing. The only information about the polypropylene known is that particles were smaller than 180 μm , which is not accurate enough to delineate possible effects in soils, since the mode of impacts of microplastic particles is a mixture of physical and chemical effects that vary with the particle size [7, 25].

In this context, the work published by Machado et al. [26] provided additional insights into the changes in microbial function as well as the possible mechanisms underlying such as alterations of bulk density of soils. Machado et al. [26] exposed a sandy loam soil to four types of MPs varying in physical and chemical properties, namely, polyester and polyacrylic fibers, polyamide beads, and polyethylene fragments. These MPs were added to the soil at four levels of contamination (up to 2% of soil weight) and incubated under natural conditions for several weeks. Thus, these authors could associate the various changes in fundamental properties of the soils (e.g., soil bulk density, water holding capacity, and soil aggregation) with the changes in the microbial activity. The study also demonstrated that the effects

were highly dependent on the particle type and possibly not monotonically varying with concentration [26]. This implies that a simultaneous quantification of microplastic chemistry, structure, size, and concentration is essential for the assessment of the combination of impacts of various particles in the soil microbiome [26]. In other words, effects cannot be simply assigned to unspecific “microplastic” concentrations, since specific particle properties (linear vs nonlinear, size distributions, polymer, etc.) seem to matter [27]. Despite these many idiosyncrasies, their study also revealed that for most of the proxies for health of the soil biophysical environment, microplastic fibers were the ones eliciting the strongest effects. The most remarkable effect on soil microorganisms triggered by MPs was a change in microbial metabolic activity that was linked to functional changes [26]. In other words, soil microbes were not only active at different levels in soils treated with MPs. They were also impacting soil aggregation and structure in a distinct way compared to the controls.

In a follow-up experiment, Machado et al. [28] investigated the potential of MPs to trigger changes in the soil environment considering six different types of MPs added to bulk soil and rhizosphere. They reported that a broad suite of parameters of soil physical quality were affected with consequences for water dynamics. For instance, soil bulk density was decreased by the studied high-density polyethylene, polyester, polyethylene terephthalate, polypropylene, and polystyrene particles when plants were not present [28]. Also the soil structure and aggregation were affected by all microplastic treatments tested, with the intensity and direction of effects depending on the microplastic type, aggregate size fraction, and plant presence. As a result of distinct structure and density (i.e., change in total pore space), the interaction of soils with water was affected. Evapotranspiration and soil moisture were strongly affected by the presence of MPs. As an example, evaporation was increased by ~35% by polyamide beads and ~50% by polyester fibers, and smaller increases were triggered by high-density polyethylene, polyethylene terephthalate, and polystyrene fragments [28]. The observed increases in evaporation were smaller than increases in water holding capacity, which resulted in soils containing MPs retaining higher water availability. In turn, the alterations caused by MPs on structure and soil water cycling also resulted in significant changes, in general microbial metabolic activity by polyamide beads, high-density polyethylene fragments, and polyester fibers. Interestingly, microbial activity in the rhizosphere was decreased by polyamide beads and high-density polyethylene and polyethylene terephthalate fragments. Moreover, functional changes on the bulk soil and rhizosphere microbiomes were also observed, including alterations in the colonization of the roots of spring onions (*Allium fistulosum*) by arbuscular mycorrhizal fungi (AMF) (polyester fibers at 0.2% of soil weight increased eightfold the AMF presence) [28]. In fact, in addition to changes in the infection with AMF, the economics of this symbiosis was also distinct, which was evidenced by the shifts in the proportion of arbuscular, vesicular, and other AMF structures responsible for exchange and storage of metabolites. Other non-AMF infections were also increased in soils with microplastic polyamide fibers.

This potential of MPs to affect the general metabolic rate and function of the entire soil microbial communities is of great relevance [26]. Soil microbiomes are responsible for important biogeochemical cycles that affect human and environmental health [29, 30]. For instance, if MPs would negatively impact denitrification rates, it could cause problems similar to mineral fertilizers [31]. Nitrate is mobile and transferable to aquatic systems, and its accumulation causes eutrophication of surface waters and compromising aquifer potability. In fact, impacts in surface and ground water have been noticed as adverse consequences of alterations in the activity of nitrogen-cycling bacteria [32]. Moreover, the altered microbial activities observed by Machado et al. [26, 28] and Liu et al. [24] may reflect altered microbial biodiversity, while the changes in the association between microbial activity and soil aggregation [26] might represent either a shift in microbial community or a modification in the decay of soil organic matter (e.g., preferential electron donor). Indeed, feedback loops in changes of soil microbial communities and the fate of organic matter are conceivable. Moreover, the physical properties altered by MPs in Machado et al. [7, 26, 28] are known to affect microbial communities. For instance, polyester fibers can decrease water-stable aggregates, which are considered potential hotspots for microbial evolutionary processes within the soil. Thus, decreases in soil aggregation may cause habitat loss for soil microfauna, e.g., fewer surfaces for colonization or for “hiding” from predators.

It is clear that such impacts of MPs will depend on behavioral, biochemical, and physical processes taking place on the micro- and nanoscale in the soil environment, which are difficult to predict. Nevertheless, it is a vital imperative to obtain a solid understanding of the potential implications of introducing massive quantities of plastics into global soils. There are biogeochemical, ecotoxicological, and biodiversity threats associated with impacts on soil microbiomes where even small changes could have ecological and economic consequences relevant broadly for terrestrial agricultural and natural ecosystems [29]. Therefore, further studies should clarify the mechanisms of the biodiversity and functional responses of soil microbes to changes in biophysical conditions related to MPs.

2.2 Plants and Microplastics in Soil

The above-discussed effects on soil physical properties and soil microbiota suggest potential impacts of MPs on the performance of terrestrial plants [26]. There is a lack of experimental research in this area. Nevertheless, the first body of evidence sheds light into several possibly ways that MPs interact with plants [28]. One of the first studies on the interactions of MPs and plants arose from the work of Liebezeit and Liebezeit [33, 34] when it was identified that commercially available honey (both industrial and artisanal) contained MPs [34]. When tracking the sources of this contamination, these authors found that various microplastic particles were broadly present in the inflorescences of diverse plant species [33]. The bees were carrying MPs from the flowers to beehives and then to the produced honey [33]. This might

imply interference on plant-pollinator relationships. Perhaps more interestingly, Sanders and Lord [35] had detected that when 6 μm microplastic particles were introduced into transmitting tracts of styles of inflorescences of various species, these particles were actively translocated by the plants to the ovary. Thus, plastic beads compatible with sizes of pollen can travel to the ovules as do pollen tubes [7]. Environmental effects of the microplastic presence in plant inflorescences have not been demonstrated. However, other ways of the interaction plant-microplastics have been observed to cause significant effects.

The research from Qi et al. [36] was possibly the first experimental investigation of effects of macro- and microplastic residues from mulch film on the growth of wheat (*Triticum aestivum*). In this study, they reported responses of a plant-soil system exposed in a greenhouse at 1% of a sandy soil weight in low-density polyethylene or a biobased plastic (starch-based biodegradable film consisting of 37.1% pullulan, 44.6% polyethylene terephthalate, and 18.3% polybutylene terephthalate). Both macro- and microplastic films affected above- and belowground biomass of the wheat [36]. According to the authors, such alterations occurred during vegetative and reproductive growth. They also observed that the type of plastic strongly influenced the responses, with the bioplastics having the most pronounced effects.

The most comprehensive study on the effects of MPs to a single plant species currently available is from Machado et al. [28]. They contaminated a soil with one of six different types of MPs, including fibers, beads, and fragments of distinct sizes and chemistries. After a period of acclimation of soil microbiomes to the presence of MPs, they planted the spring onion seedlings. MPs affected root and leaf traits as well as total biomass and several morpho-physiological features of exposed plants [28]. For instance, polyester fibers and fragments of polystyrene, high-density polyethylene, polyethylene terephthalate, and polypropylene increased root biomass. And all the tested MPs increased root length and surface area but decreased root average diameter [28]. The effects are not always uniform, however. Polyamide beads decreased the ratio between roots and leaf dry biomass, while polyester fibers and the microplastic fragments significantly increased this ratio. Polyester fibers triggered the strongest effects on the interaction of the spring onions' roots and the surrounding microbial communities. In terms of leaf traits, polyamide beads increased onion bulb biomass, while polyester fibers nearly doubled it. Also, shifts were observed in leaf composition, including water content as well as carbon and nitrogen elemental ratios, among other traits. Considering the nature of the observed MPs' impacts on plants and soils, Machado et al. proposed a causal model (Fig. 1) in which the effects of MPs on plant performance would start with plastics altering the soil biophysical environments of bulk soil and rhizosphere [28]. These physical, chemical, and biological effects of plastics in soils would then be perceived by plants, resulting in changes of biomass allocation, tissue chemical composition, and symbioses.

A recent study by Bosker et al. [37] found that germination of seeds was significantly reduced with 8-h exposure to MPs (Fluoro-Max Green Fluorescent Polymer Microspheres), with increasing concentration and the largest particle size

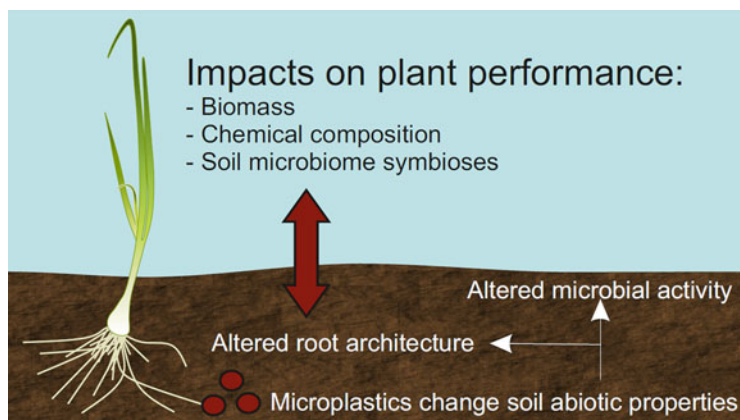


Fig. 1 Mechanisms proposed by Machado et al. [28] affecting plant performance

(4.8 μm) leading to more significant effects. However, by 24 h the effects of treatment were indistinguishable, with all treatments reaching close to 100% [37]. Root growth was also influenced by MPs which were variable depending on the particle type (50 nm particles led to a significant increase in growth, while 500 nm particles led to a significant decrease in growth) and were again only observed up to 24-h exposure after which time effects were not seen [37]. The results of this study are interesting, as studies comparing particle sizes generally show that the smaller the particle, the greater the negative effect on plants [38, 39]. Effects such as delayed germination and root growth could have implications for the timing and development of crops, although these effects were only seen over acute timescales.

Boots et al. [16] confirmed many of these previous findings when assessing the response of soil biophysical properties when the ryegrass (*Lolium perenne*) and the rosy-tipped earthworm (*Aporrectodea rosea*) were exposed to three types of microplastics (polylactic acid, high-density polyethylene, and clothing fibers). They also observed a reduction in the shoot high on polylactic acid treatment and a reduction on animal biomass by polyethylene [16]. Soil pH and water-stable aggregates were also affected. Therefore, these authors concluded that there is evidence that the tested microplastics can affect the basic, but crucial, soil properties, potentially triggering further impacts on soil ecosystem functioning [16].

Indeed, the mechanisms of intrinsic (direct) toxicity of MPs in plants remain less explored [40]. It is hypothesized that, as particle size decreases, small MPs present higher biological reactivity and, therefore, more potential for chemical-like toxic effects [7]. That is due to the fact that particles within the range of few micrometers are not expected to be taken up into the root. The opposite is true for NPs, however. The rhizodermis of roots would constitute the primary barrier for nanoplastic uptake, and although the mechanisms for nanoparticle uptake in plants are not fully described [41], it is accepted that particles within the range of few nanometers could enter into plant roots, potentially causing alteration of cell membrane and

intracellular molecules and generation of oxidative stress [42]. Such information is still to be experimentally demonstrated for NPs, but if so, it might constitute a relevant point of entrance of plastics into the constitution of continental ecosystems [40].

It is currently clear that there is a diverse array of mechanisms by which MPs can influence plant performance, i.e., from affecting biophysical environment perceived by symbionts (e.g., plant pollinators or AMF) [7, 28] up to direct intrinsic toxicity [37]. Thus, some authors have argued that effects at plant community levels cannot be discarded [40]. That is because the very properties of plant communities are often related to soil structure and composition [9]. The changes in evapotranspiration caused by microplastic films, fibers, and fragments may affect local water cycling, contributing to more pronounced drought and selection of drought-resistant plant species, not to mention the changes in soil microbiomes discussed above. Rillig et al. [40] argued that effects on plant communities are likely to occur in areas with more severe microplastic contamination, which would place natural reserves near agricultural fields or cities under higher concern. Given that plants provide the basis of nutrition for most of continental life on earth, it is essential that we work to understand possible implications of MP pollution on plant growth and diversity.

2.3 Animals and Microplastics in Soil

There are a fast-growing number of studies on effects of MPs in animals. Many studies have looked at the interaction of micro- or nanoplastics on earthworms [11, 43–48] and on isopods [49, 50] representing the macrofauna. However, fewer studies address the mesofauna, although this organism group forms a huge part of the soil's diverse community. Collembola, oribatid mites, and nematodes form the most abundant groups among the soil mesofauna. From this group, especially Collembola [12, 13, 51], mites [13], nematodes [49, 50, 52], and also enchytraeids [13] were among the studied organisms.

Soils under agricultural and other human uses can be significantly contaminated [53, 54] by various plastic types of different sizes and shapes, containing a broad range of additives that can be found in various concentrations. This might have significant effects on soil animal communities [43, 44]. For instance, earthworms and Collembola can transport particles and beads horizontally and vertically which increases in predator-prey situations [9, 12, 13, 51].

The interaction with the particles or fibers will differ depending on the body size [43–45, 52, 55] and the developmental stage of the respective organism. This means that the same particle might have negative effects on, e.g., the adult stage of an organism but not (or a different effect) on larval/nymphal stages of the same organism. Together with the highly complex food web dynamics in soil, it is very difficult to disentangle the various effects of diverse particle size and chemistries. Additionally, there are open questions on whether soil organisms ingest MPs, if they show avoidance behavior, if they can distinguish between “food” and “nonfood”

(as shown for nematodes in Kiyama et al. [56]), or if the shear presence of synthetic particles might already affect their fitness [51]. Although the knowledge about interactions in soil macro- and mesofauna with nano- and microplastic increases, many aspects still remain in the dark. Especially studies in the field have not been conducted so far. This might partly be due to nonexisting standardized methods for the quantification of the MPs but also due to a lack of soil ecotoxicological expertise and framework of relevance for detecting impacts from microplastic exposure [57].

Earthworms are well-known representatives of the soil's macrofauna, their biology is well understood, and many studies exist in regard to the effect of micro- and NPs on these organisms and vice versa. In 2017, Rillig et al. showed that *Lumbricus terrestris* can transport microplastic spheres ranging from 710 to 2,800 μm along the soil profile potentially via burrowing activity, egestion/casts, or adherence to the earthworm exterior [11]. Lwanga et al. [43] showed higher numbers of burrows as well as denser burrow walls in the presence of MPs. This vertical transport decreases the degradation of the particles, i.e., within the soils, there are suboptimal light and temperature conditions necessary to reduce the sizes of polymers before any biodegradation can occur [58]. Potential anaerobic conditions in deeper soil layers may also decrease or even inhibit oxidative degradation.

Apart from the transport by earthworms, there have been many studies on various aspects concerning the fitness of mostly two earthworm species, *Lumbricus terrestris* and *Eisenia fetida*, respectively. Lwanga et al. [44] observed increased mortality and reduction in growth of *L. terrestris* at certain microplastic concentrations in the bulk soil and an increase of microplastic particles smaller than 50 μm in size in the casts which suggests size-selective egestion as well as reduced biomass in a different study [55]. For *Eisenia fetida*, growth inhibition was reported at concentrations >1% (w/w in dry soil) [59]. A similar result was seen by Boots et al., who found high-density polyethylene particles 0.48–316 μm in size to cause a significant reduction in biomass of the earthworm *Aporrectodea rosea* [16]. However, Prendergast-Miller et al. [60] did not find lethal effects of microfibers nor active avoidance behavior. Lahive et al. exposed the small earthworm *Enchytraeus crypticus* to two different polymer types (nylon and polyvinyl chloride) and two different sizes of nylon [61]. They found smaller nylon particles (13–18 μm) to have a more significant effect on reproduction (compared to 90–150 μm), with a dose-response relationship effect: increasing concentrations led to a greater negative effect [61]. Nylon also had a significantly greater effect than polyvinyl chloride at comparable particle sizes and concentrations [61].

Another currently open question is whether plastic particles can act as vectors for pesticides or other substances. It is suspected that persistent organic and inorganic pollutants could adsorb easily to plastics and thus affect the soil community. Hodson et al. [48] found that high-density polyethylene MPs could indeed act as a vector for toxic zinc to earthworms. In contrast, Rodriguez-Seijo et al. [62] did not find similar evidence MPs sprayed with chlorpyrifos. Additionally, *Eisenia* individuals even showed avoidance behavior at the highest contamination level in this study. In contrast, *Lumbricus terrestris* did not show avoidance behavior in respect to microfibers in a study by Prendergast-Miller et al. [60]. Wang et al. [63] also

concluded from their results that MPs do not enhance the uptake of substances as they observed only very low bioaccumulation. In the same study, the authors report that although polyethylene and polystyrene were definitely ingested by *Eisenia fetida*, this did not induce oxidative stress. In 2018, Rodriguez-Seijo et al. found contrasting results: *Eisenia* individuals did indeed show an oxidative stress [64]. *Lumbricus terrestris* responded likewise to exposure to polyester fibers in a study by Prendergast-Miller et al. [60]. Despite the fact that such changes in the oxidant defense systems did not significantly affect the molecular level, they still observed lower cast production. In a study from Rodriguez-Seijo et al. [46], histopathological changes and a triggered immune response were observed in *Eisenia andrei* when exposed to MPs.

Several effects might arise from the ingestion of MPs by soil organisms. For instance, it has been shown for the earthworm *Eisenia andrei* that microplastic particles can damage the gut system and induce stress effects in the immune system [46]. To which extent this might also be true for collembolans needs to be studied in the future. In addition, it is not clear if microarthropods are able to selectively egest MPs as it has been suggested for the earthworm *Lumbricus terrestris* [44]. Collembolans regenerate the midgut epithelium together with the cuticle at each molting cycle. Thus, one can speculate that collembolan's biology might prevent them from severe damage to tissues or inflammation of gut tissues as reported for nematodes [52]. However, Zhu et al. [13] recently showed that MPs have impacts on the composition of the gut microbiome of *Folsomia candida*, resulting in a change of the isotopic signature (higher $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values). These biochemical changes were linked to the inhibition of growth and reproduction (the latter by 28.8%) and potentially also general feeding behavior. That is because higher $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values might indicate a change in the metabolic turnover and also growth rate. Under similar conditions, Yu et al. [65] reported a decreased reproduction, avoidance behavior, and an altered gut bacterial diversity at varying concentrations of polyethylene particles in artificial soils.

In addition to earthworms, PET microfibers in soils have been shown to negatively affect terrestrial snails (*Achatina fulica*), increasing oxidative stress and leading to gut inflammation after chronic exposure [66]. Additionally, this study found that the integrity of the fibers could be compromised following ingestion and egestion, with visible cracks and deterioration of the fibers. This implies that ingestion could cause them to become weak and thus more susceptible to further degradation. Similar observations have been made following ingestion of MPs by Antarctic krill, whereby ingestion led to fragmentation of MPs and the formation of NPs within the gut, suggesting that ingestion of particles could enhance breakdown of MPs to smaller MPs and NPs [67]. This is likely the result of the digestive processes – for example, it has been shown that freshwater snails especially will actively store inorganic mineral particles within their guts to aid with the grinding of food [68], while krill have mandibles specifically for grinding [69]. Lwanga et al. [45] found gut bacteria in the earthworm *L. terrestris* which were able to decay parts of ingested low-density polyethylene particles, resulting in smaller particles. Such processes are therefore designed to facilitate the breakdown of larger particles and

could feasibly lead to the degradation of plastic particles in other organisms that rely on similar mechanisms. The study by Lwanga et al. [45] is potentially significant because, if this is to be confirmed, it might imply that symbiotic gut bacteria of other organisms might also be evolving enzymatic machinery to degrade plastics. This would certainly represent an interesting topic for further detailed investigation.

The nematode *Caenorhabditis elegans* has been the subject of a number of microplastic studies, with exposure to MPs often leading to negative effects. For example, Lei et al. [70] exposed *C. elegans* to a range of differently sized micro- and nanoplastic particles (100 nm, 500 nm, 1, 2, and 5 μm , each at a concentration of 1 mg L^{-1} over 72 h). They observed significant effects on a number of endpoints including decreased growth, abnormal behaviors, increased markers of oxidative stress, and mortality. Depending on the endpoint measured, the size of the particle often made a difference to the observed response [70]. Kiyama et al. [56] demonstrated the ability of *C. elegans* to actively accumulate carboxylate microspheres of 0.5 and 1 μm in diameter. Whether consumption of such particles has negative effects on growth and health of terrestrial nematodes is not yet known.

Selonen et al. [50] conducted a very interesting experiment, analyzing the effects of polyester fibers of different lengths on enchytraeids, isopods, springtails, and oribatid mites offered in food or in soil at five concentrations (0.02–1.5% (w/w)). They only found slight effects, however, e.g., the reproduction of enchytraeids was decreased to 30% with increasing fiber concentration. This effect was only found in treatments with long fibers which were only seldomly ingested, which suggests that the observed effect might be due to changes in the environmental conditions or physical harm outside of the enchytraeid. However, short fibers were clearly ingested by enchytraeids and isopods and increased with increasing concentration in the soil treatment. Although the authors conclude that short-term exposure might not have severe effects to soil invertebrates, potential long-term effects might indeed be relevant for their fitness and would need to be tested in the future [50]. In addition, this study shows that ingestion of fibers actually happens with unknown long-term consequences.

A study with different polymers and concentrations found that polyamide, polyethylene, polypropylene, and polyvinyl chloride can have effects on growth, reproduction, and survival at concentrations as low as 0.5 mg m^{-2} on agar. Polymer type did not significantly influence the effects observed. A parallel study showed polystyrene particles to have significant size-dependent effects, with the greatest observed effects seen with the intermediate-sized particle (1 μm compared to 0.1 μm or 5 μm) [52, 70]. Further, NPs (100 nm) have been seen to cause transgenerational effects on nematodes, with a significantly reduced brood size in adults exposed to 10 $\mu\text{g L}^{-1}$ and in the resulting F1 generation, produced from adults exposed to 100 $\mu\text{g L}^{-1}$ [71]. Zhu et al. [13] found that enchytraeids which ingested NPs had a reduced gut bacterial diversity and body weight. An interesting article by Kiyama et al. [56] reports that nematodes might be able to discriminate between “food” and “nonfood” based on size, taste, and olfaction. This ability has been described so far for earthworms [46], and extrapolations to other soil organisms need to be studied in the future.

In most studies, only one species is looked at. However, especially in soils, the food web is very complex. How MPs affect the species fitness might have a strong influence on the food web's stability. For example, once a collembolan is eaten by a Mesostigmatid mite, the transfer of these particles to the next higher trophic level might be in progress. Likewise, micro- and nanoplastics in the fecal pellets, eggs, and decaying collembolan biomass could be ingested again by earthworms, constituting another unexplored pathway of plastics back into terrestrial food webs. In this sense, what happens to the next higher trophic level is not known and should be further studied as the biomagnification of plastic polymers, additives, and adsorbed substances along the food chain may severely harm the fitness of the respective organism groups. Huerta Lwanga et al. [55] found evidence for the transfer of MPs from earthworms to chickens which underlines the necessity of studying food chains not only above- or belowground separately but combined to understand the fate and also the accumulation of micro- and nanoplastic particles, which may eventually reach the human table.

In summary, there are several indications and controversies about the potential effects of MPs on soil organisms. These partly contrasting results underline the necessity of further studies taking into account several other soil organisms or even whole communities under different contamination levels, with different particle types and sizes, and in varying soil types. All these factors can presumably have very different effects on the fitness of the organisms but also on the soil health in general. For example, earthworms exposed to high microplastic contamination might suffer from increased mortality, and this might feedback to soil porosity; this has now become supported by the findings of Lwanga et al. [43, 44, 55] and Ng et al. [58].

3 Factors Accounting for the Observed Effects of Microplastics on Soil and Terrestrial Organisms

According to the currently accepted conceptual model of MPs' effects on terrestrial systems, several factors account for toxic and environmental changes derived from microplastic pollution [7]. Size, hydrophobicity, charge, density, and shape are among the properties expected to significantly affect the soil system. In fact, some evidence has been proposed for size [26] and hydrophobicity. Shape is especially important according to the work from Machado et al. [26, 28], in which fibers consistently affected more the soil biophysical environment compared to beads and fragments. Probably because fibers entangle soil particles and shape aggregates, they are expected to interact differently with soil biota.

Therefore, MPs represent an entire class of contaminants, each with their characteristic (and not necessary similar) kind of effects. The various combinations of polymer matrix, chemical makeup, additives, persistence, surface, sizes, and shapes implies that MPs might elicit a variety of environmental impacts. As previously

mentioned, polyester microfibers (at concentrations up to 0.40%) can affect soil biophysical properties more strongly than polyamide beads or polyethylene fragments (at concentrations up to 2.00%). Evidence for a particle-dependent diversity of effects has been obtained for beads, fragments, and fibers, as well as biodegradable microplastic [36], films [72], and NPs [73]. Rillig et al. [40] highlighted that foams and various other materials are still to be investigated.

3.1 *Microplastic Particle Size and Shape*

In the experiments from Machado et al. [26, 28], polyester microplastic fibers were often the type of largest physical impacts, e.g., the strongest effects on soil structure and interactions with water. Their linear shape, size, and flexibility make those particles substantially different from most natural components of soils. This, in turn, potentiates the effects on such soil biophysical properties and plant responses. For instance, rootability is inversely proportional to soil bulk density [74]. As fibers cause a remarkable effect on soil bulk density, green onions exposed to PES presented ~40% increase in root biomass associated with a decrease of ~5% in root diameter [28]. In such context, the longer and finer roots contributed to changes in leaf elemental composition (N/C) and other proxies for plant physiological status or nutrient availability.

Compared to polyester fibers, Machado et al. [28] observed less pronounced effects attributable to high-density polyethylene fragments, which can be attributed to the fact that the fragments were more similar in size and shape to the natural particles present in the tested sandy loam soil [40]. Notwithstanding, high-density polyethylene and other test fragments triggered substantial decreases in soil bulk density, changes in water dynamics, alterations of soil microbes, and consequently the response of spring onions. Therefore, future comparative studies should look at various soil types, climatic regimes, temporal scales, and microplastic properties to test whether anthropogenic particles of different types and shapes are an important driver of terrestrial global change.

When particle sizes get smaller, potential microplastic hazards might become more concerning. For fish, it has been shown that nanoplastic was able to cross the blood-brain barrier causing behavioral disorders including slower feeding rates and higher risk of being predated [75]. Although the brain of soil microarthropods is anatomically distinct from fish, neurons, receptors, functional enzymes, and molecules were very well preserved throughout evolution [7]. Thus, we still need to think about potential harmful effects on behavioral patterns in insects, especially in terms of predation risk. Micro- or nanoparticles have not been experimentally observed inside the tissue of soil microarthropods. Likewise, it needs to be tested to which extent this might affect the organisms in general besides the effects on the nervous systems or equivalents. However, a recent study shows the decrease in movement of the collembolan *Lobella sokamensis* in the soil pore system at a concentration of polystyrene and polyethylene bead and fragment of various sizes and at

concentrations of 1,000 mg/kg soil [51]. When considering the effects such as observed by Kim and An, it is important to mention that it is unclear whether the effects of MPs are of direct toxicity or an indirect response to environmental change. The abovementioned effects of MPs on the soil structure [26] might have negative effects on the feeding behavior of soil organisms. On the other hand, soil organisms also exhibit effects on the soil environment by, e.g., constructing biopores [51] in which microplastic particles can get trapped very quickly.

The potential effects of the attachment of nanoparticles to the cuticle of microarthropods should not be underestimated. The cuticle of Collembola is composed of several layers which is able to protect the individual from fouling by antibacterial and antifungal compounds but at the same time allowing gas exchange and exhibiting hydrophobic characteristics. Depending on their size and charge, plastic particles could get attached to the setae but also to the cuticle due to their hydrophobic surfaces [54] and might also pass it via the pores. The pore channel diameters range from 200 nm to 2 μm , potentially allowing a range of plastic particles to cross the cuticle.

Another, more extreme example is the oribatid mite family Damaeidae, which attaches soil particles to their cuticle and hence might act as vector of transport not only for microbes [12] but also for micro- and nanoplastic particles with unknown consequences for the individual or receiving environment. The colonization of particles by microorganisms can increase soil aggregation, meaning that the particles could potentially become bound even tighter into the soil matrix which potentially increases the resistance of plastics to degradation to more than 100 years [25, 76]. Further studies are definitely needed to clarify potential interaction between various particle sizes, the soil environment, and the inhabiting soil organisms and their behavior.

3.2 *Microplastic Particle Chemistry*

As plastics degrade, increasing in particle number and decreasing in size, soil microfauna such as bacteria and fungi may consume them [77, 78]. However, with their high C/N ratio, this new food source would leave those feeding on it lacking nitrogen and other nutrients causing them to seek it out elsewhere in the soil and leading to immobilization – the conversion of inorganic compounds to organic compounds, rendering them inaccessible to plant roots. Such immobilization could have negative impacts on plant production. Furthermore, the high C content of plastics could throw off soil organic C quantification, a method used to assess land fertility, which could negatively impact crop production [77].

Some studies revealed unexpected ways via which plastic particles directly triggered effects in soil and plant traits. Certain primary MPs (polyamide beads of 15 μm) seem to contain considerable amounts of compounds from the manufacture that are adsorbed to the particles or were loosely interacting with the polymer matrix [28]. Nitrogen (component of the amide group in polyamides) could be easily

released into soils, which supported a nearly twofold increase in leaf N content and total biomass alongside a relative decrease in the root-to-leaf ratio [28]. Thus, remaining monomers leached into the soils causing a chemical change comparable to fertilization. These primary polymer-based pellets may contain additives (e.g., lubricants) on the surface and often organic phosphite antioxidant additives in the bulk that are easily transformed to organic and inorganic phosphates. It is reasonable to hypothesize that the situation would be different in the case of aged polymer particles in real-world soils [28].

Beyond the main polymer matrix of plastic, many thousands of plastics are further processed by the addition of chemical additives before being shaped [7]. Four main groups of additives, functional additives, colorants, fillers, and reinforcements, represent a dizzying array of organic and synthetic chemical compounds which are added to the polymer backbone of plastics to imbue them with desirable characteristics such as tensile strength, reflectivity, clarity, hardness, etc. These compounds can leach from the chemical matrix of plastics as they degrade and enter the surrounding environment [7, 19, 25]. The long-term effects of leachates entering agricultural soils are unknown.

Fuller and Gautam [17] showed that in Australian top soils near roads and industrial areas, the concentration of MPs may reach up to 7% by weight. At this level, severe effects of leaching chemicals were observed. For example, nonvolatile organochlorines from polyvinyl chloride caused geochemical changes in the soil, altering soil chlorinity [17]. Another risk is the leaching of substances like bisphenol A and phthalates, which might exhibit estrogenic and other endocrine activities in vertebrates and some invertebrates [79]. Moreover, when larger plastic particles become smaller, the surface-volume ratio increases. Consequentially, the many additives bound in a physical and not chemical way to the polymer matrix might face increased probability of leaching of plasticizers and other compounds [80]. In fact, it is conceivable that the low-level toxicity associated with plastic microparticles, due to its pervasive nature, results in a selective pressure of species and species traits and unknown consequences for functional bio- but also phenotypic and genetic diversity [7], potentially creating new niches, e.g., oviposition sites, in the soil environment.

3.3 The Properties of the Soil System

Just as the various properties of MPs might account for different effects, the impacts could vary immensely with soil characteristics, i.e., natural particle origin, mineralogy, granulometry, texture, etc. [9]. At the time of writing this chapter, most of works were performed in sandy loam soil with rather simplified representations. Diverse effects should be expected if soils richer in silt and clay particles would be used [26]. Same could be said if other soil structures would be considered. For instance, pores larger than 0.08 mm (macropores) can enhance movement of soil particles, because sedimentation and sieving are not as pronounced, and they

enhance the movement of water [9]. It implies that macropores may indirectly influence how fast microplastic particles are moved in the soil.

Other soil processes similarly affecting MPs' potential fate and effects are the events of soil cracking and wet-dry cycles [9, 26, 28, 81]. Common in agricultural soils with expanding mineral types is the appearance of cracks and fissures when the soil dries. These cracks could work as potential highways for plastic particles to arrive at deeper soil layers [9]. This process could then enter positive feedback as soils with MPs are reported to present increased evapotranspiration and more intense formation of superficial cracks.

The soil biota also would play a key role in intensifying or minimizing impacts of MPs [36]. Earthworms, microarthropods, and even decomposing roots create large biopores that might contribute to the movement of MPs inside the soil in a similar fashion as macropores [9]. It has been proposed that fungal hyphae may also serve as preferential paths for movement of particles in the cm range, as has been demonstrated for the transport of bacterial cells. The cell membrane and walls of filamentous fungi has been shown to adsorb large quantities of nano- and small MPs [82]. Thus, it is conceivable that fungi might contribute to transport of NPs within soils.

Finally, certain human usage of soils might potentiate MPs' effects [81]. The plowing and harvesting of agricultural activities can be very effective for moving MPs into the deeper soil layers [9]. For instance, moldboard plowing brings about an inversion of the top soil layer. The consequence is that MPs on the surfaces will be transferred to the layer at the plowing depth whenever this moldboard is employed [9]. Likewise, the revolving of soils for the harvest of plant portions below the soil surface (e.g., potatoes, carrots) can also serve to incorporate microplastic into the various soil horizons.

4 Final Considerations and Future Directions

MPs can affect physicochemical and biological parameters of the soil. Those effects potentially have direct serious environmental consequences, such as changes in agricultural productivity and dysfunction of soil biogeochemical cycles [26, 83, 84]. In order to access the risk (i.e., the probability) of such hazards, there are some questions that need to be addressed.

Perhaps a crucial open question is what are the realistic contamination levels of MPs in soils around the globe? This will require an environmental simultaneous identification of particle size, shape, and polymer type. None of the currently available microplastic quantification performed in environmental soils analyzes these three parameters concomitantly [7, 85]. Moreover, the level of NPs in soils is unknown.

Another lack of relevant information relates to the poor design of experiments with MPs regarding their ability to provide relevant ecotoxicological information. Far too many of the studies on MPs consider a single or maximum of two exposure

levels. This renders information not useful to extract the valuable dose-response relationships of organisms to realistic MP exposures. These Dose-response relationships associate exposure to environmental effects and are fundamental to determine levels of MPs in soils that would impact important soil functions. By now, we do not know yet how these curves look like in terms of shapes and slopes [26]. In fact, it is not even clear how dose-response relationships for microplastic effects depend on the soil characteristics. Thus, it is not possible to scientifically devise at the risk current MPs might pose. The research from Machado et al. [26, 28] seem to suggest that some of these curves might not be monotonic. This needs to be confirmed by further investigations; it would imply that new ecotoxicological frameworks would need to be developed.

There is also growing concern about soil micro- and macrofauna unwittingly ingesting these particles and then transferring it upward to the higher trophic levels of terrestrial food webs [55]. Not only earthworms but another ubiquitous soil inhabitant, the nematode, has also been experimentally shown to ingest MPs [56]. These two organisms' low rank on the food chain represents a potentially significant source of microplastic contamination in the terrestrial food web. Preliminary experimental results as well suggest a high likelihood that both saprobic soil fungi and the roots of common crop plants can take up nanoplastic particles of various surface charges [82, 86]. It would be important, for example, to know if such occurrences in natural and agricultural settings could lead to both bioaccumulation and biomagnification of plastic particles and which effects this could lead to. A better understanding of the impacts of MPs on terrestrial systems thus requires special focus on soils. It is within soils that MPs could affect the natural functioning of terrestrial ecosystems in important ways other than eliciting direct lethal toxicity [7, 26, 28]. For instance, the impacts on water cycling (water holding capacity of soils and evapotranspiration rates) are relevant for numerous processes from micro-scale microbial activity to watershed-scale water management [87].

A direct area of interest between the above-discussed microplastic effects and sustainability is on food security [7]. Agriculture is an important contributor to microplastic pollution. The agricultural industry has benefited substantially from the advent of plastic greenhouses, mulches, irrigation systems, and microplastic capsules for fertilizers and pesticides [81, 88]. Some of these plastics are left out in the field, are tilled into the soil with the next rotation, and steadily increase the pool of environmental MPs. Further contamination of agricultural soils with MPs was stemmed from the application of sewage sludge as fertilizer. Nizzetto et al. [14] estimated that up to 430,000 and 300,000 tons of MPs were added per year in the form of sewage sludge to farmlands in Europe and North America, respectively. The proportion of MPs in the topsoil accumulate with further sludge amendments, and in agricultural settings where soil is amended by sewage sludge in China, for example, Zhang and Liu [18] determined that per kilogram of topsoil between 7,000 and 43,000 particles (mostly fibers) of MPs can be found.

Some effects on lab mimicking a simple agroecosystem suggests potential yield losses [16]. It remains unclear what types of impacts the increasing fraction of plastics in the soil which grows our crops on could have on global food production. The same could be stated for soil biodiversity [7, 28, 59]. The physical structure of soil and the dynamic nature of its components are crucial factors when choosing the appropriate soil for a given crop. Aspects such as the soil bulk density, water retention, texture, porosity, aggregation, and others affect the suitability for crop production. Soils need to be cohesive but not too dense or compacted as to hinder microbial and root growth throughout them or to impede water and air circulation [74]. Another important characteristic of soils, aggregate stability, or the ability of aggregates to overcome external forces and remain integrated, is a good indicator of soil organic matter as well as being important for the creation of pore spaces of various sizes which allow for their effective circulation of air and water [6]. Zhang and Liu [18] found that 72% of all MPs found in their test fields (92% fibers) were involved in soil aggregates, while only 28% were not involved. The exact properties which determine microplastic involvement in aggregate formation are yet unknown and require further observation. Also, the drying and desiccation of soils caused by MPs could lead to negative impacts on plant growth. Issues related to water retention will likely be exacerbated in the context of climate change which should increase the unpredictability of weather patterns and rainfall and cause increases in droughts [40]. Looking more closely, smaller MPs known as NPs (<0.1 μm) could potentially accumulate on soil surfaces, altering surface characteristics of various soil matrix components and influencing the normal interactions surrounding soil aggregation due to their hydrophobic properties. Little research has been conducted on potential effects of nanoplastic particles on soil structure, and further research is required to disentangle microplastic roles in soil systems.

In summary, MPs can affect soil physicochemical and biological parameters, and a current assessment of the potential risks of those particles cannot be accurately achieved because there are numerous unsolved questions. The simultaneous quantification of size, shape, and chemistry of plastic microparticles will be essential to provide insights on the realistic levels and potential environmental effects. Alongside, the provision of precise dose-response relationship is fundamental. Despite the lack of data, it is clear that MPs could affect the soil biophysical environment, triggering responses in microbes, plants, animals, and biogeochemical cycles in various terrestrial ecosystems across the globe. The extent and intensity of such change is largely unknown. Given the incipient evidence, however, it seems likely that non-negligible effects of microplastics on soil biodiversity and their environmental services might be already in place.

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Occurrence and Ecotoxicological Effects of Microplastics on Aquatic and Terrestrial Ecosystems



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Abstract The practice of using plastic for several decades now has shown its close association with us owing to its omnipresence in water and food we consume and the air we breathe. Anthropogenic activity, the most imperative cause of microplastic (MP) contamination, is evidenced in various ecosystems from land to river, oceans, and arctic to antarctic habitats. Their distribution depends on environmental factors like precipitation, wind flow, tides, waves, etc., and 75–90% plastic debris contamination comes from the terrestrial sources. Asian countries are contributing to 50% of plastic production globally, and around 18–19% of plastic is produced by Europe and North America. MP contamination has shown to pose a serious threat to different trophic levels in the terrestrial and aquatic ecosystems resulting in active ingestion, feeding impairment, stunted growth, reduced reproduction in terms of oocyte formation and decreased sperm velocity, offspring formation, changes in gene expression profiling, etc. from producer to consumer level. Owing to its

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relatively large surface area, MPs act as potent vectors in carrying persistent organic contaminants and noninvasive species to pristine water bodies. It is a wake-up call for ecotoxicologists and ecologists to study the potential adverse effects of MPs at environmentally reported particle range (since most of the effects noted and/or overestimated at relatively higher concentrations) and protection of ecosystem for sustainable development.

Keywords Aquatic ecosystem, Ecotoxicity, Microplastics, Producers and consumer, Terrestrial ecosystem

1 Introduction

The practice of using plastic for several decades has shown its close association with us owing to its omnipresence in water and food we consume and the air we breathe. As we all know, the consumption of plastic products was started at a very large scale since the 1950s, and between 1968 and 2017, the production continuously surged from 0.5 to 348 million tons per year [1, 2]. These plastic products have an extremely slow degradation process, so it always gets easily accumulated in the environment from different sources [1]. The pollution of these microparticles has been widely reported in terrestrial and aquatic ecosystems. At the initial stage, regulators and policymakers mainly focus on the large-sized plastic product management and waste disposal leaving aside the impact of degradation under the natural environment. This process culminates into the formation of smaller plastic particles called microplastics (MPs) and falls under four categories on the basis of their size: MPs (<0.5 cm), mesoplastics (0.5–5 cm), macroplastics (5–50 cm), and megaplastics (>50 cm) [3]. Basically, less than 5 mm size of plastics is eventually termed as MPs [4, 5]. MPs can also be defined on the basis of their size, shape, chemical composition, and density of particle that comes into the category of a heterogeneous group of particles due to varieties of different sources. On the basis of source of origin, it can be classified into primary and secondary MPs. Primary microplastics in the environment are mainly reported from direct sources. The sources of primary MPs in the environment are mainly from the production of plastic products in personal care products such as handwash, face cleaner, toothpaste, and various other cosmetic products that are recently exploited by human beings at larger scale [1, 6]. In several developed countries such as the USA and Canada, cosmetic products having MPs are totally banned for human use due to environmental safety point of view [7]. It has been found that 1900 polyacrylic fiber per item from washing machines is released into the aquatic and terrestrial environment [8]. Similarly, the secondary MPs are the second main contributor to plastic pollution in the environment and the major point of concern [9]. These MPs originate mainly from the anthropogenic activities of human such as littering and are released through the process of disposal of municipal

waste and their collection [5]. If we look into the figures, the estimated percentage of plastic debris in the aquatic environment is 75–90% which originates from land, and similarly, in the terrestrial ecosystem, this figure is much less, that is, 10–25% from ocean sources [10, 11]. The recent trend in the field of MP research is focussed on its occurrence, fate, and potential toxicological impacts on the environment and human health. To date, the research work on the impact of MPs on the aquatic ecosystem is focussed primarily on invertebrates and vertebrates like fish and very few studies on organisms inhabiting the vicinity of the terrestrial ecosystem. Hence, there is a need to critically evaluate the occurrence, source, fate, uptake, and potential toxic effects of MPs in aquatic and terrestrial biota.

2 Occurrence and Fate of Microplastics

Nowadays, MPs have become a topic of concern due to its persistent and hazardous nature that poses a great threat to our ecosystem and environment. There is a wide range of MPs (such as polyethylene, polypropylene, polystyrene, polyvinyl chloride, polyethylene terephthalate, etc.) found in the environment and tremendous lift in use from five million tons to three hundred million tons from the 1950s till date [12]. The occurrence of MPs in freshwater and marine ecosystems is well-known for decades, but the knowledge of their presence in the terrestrial ecosystem has been established recently [13]. The evaluation and quantification of MPs in the terrestrial environment are quite difficult to process, and recently, in 2018, the first systematic and investigative method was developed [14]. The occurrence of MPs in the environment is the consequence of direct discharge of microparticles or microbeads of plastic (known as primary MPs) that are used in the cosmetic industry or fragmentation of large plastic particles (known as secondary MPs) which are used in the manufacturing of different products that are used in day-to-day life [15, 16]. Ultraviolet (UV) radiations act as a catalyst in the photooxidation of plastics; hence, on the exposure of UV rays, plastic becomes hard and breakable which results in fragmentation into smaller particles (MPs) [17]. The two major factors that determine the distribution and occurrence of MP particles in the environment are environmental and anthropogenic factors [18] (Fig. 1).

Transportation and weathering of plastic particles start as soon as they are released into the environment and get accumulated in soil and water bodies. The fate of MPs in the aquatic environment depends on its density; MPs with lesser density than freshwater (1.0 g cm^{-3}) or marine water (1.03 g cm^{-3}) result in buoyancy and float on the surface, whereas MPs with higher density tend to be submerged [10]. It has been observed that, at the initial stage, most of the plastic particles are buoyant in nature and accumulate in the upper surface layer of the water column of approximately 20 cm. North Pacific, South Pacific, North Atlantic, South Atlantic, and Indian Ocean are some of the regions where buoyant MPs were reported [9]. It has been also reported that around 6.4 million tons of plastic per year get accumulated in the ocean through land-based sources globally [16]. It is

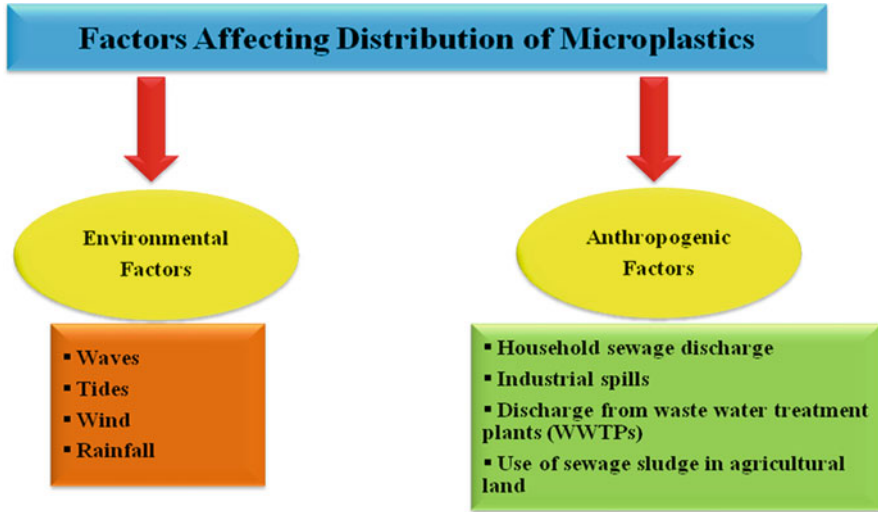


Fig. 1 Showing the factors affecting the distribution of MPs in the environment

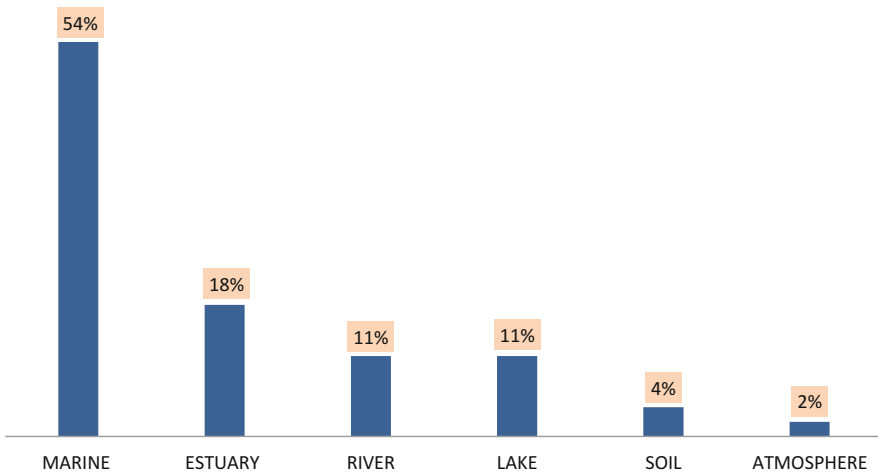


Fig. 2 Percentage of microplastics present in the environment [20]

worth noticing that Asian countries are contributing to 50% of plastic production globally, and around 18–19% of plastics are produced by Europe and North America individually [19]. Figure 2 shows the proportion of MPs in water, soil, air, etc. The marine environment has become the highest burden of MPs (54%) followed by estuary (18%), river (11%), lake (11%), soil (4%), air (2%), etc.

2.1 Occurrence and Fate of Microplastics in Terrestrial Environment

Plastic pollution in the terrestrial environment through various anthropogenic activities triggers various environmental issues. As the issue of MP contamination in the environment is increasing, there is a concern regarding the particle-induced toxicity and chemical-induced toxic manifestations during the leaching process in aquatic and terrestrial organisms. Regardless of lack of information regarding the presence of MPs in the terrestrial environment, some studies suggest the ubiquitous presence of MPs might be due to the chemical or physical disintegration of larger plastic particles into smaller ones [20]. However, it has been reported that there is production of about 275 million tons of plastic waste in 2010, out of which only 4.8–12.7 million tons of plastic were dumped into the oceans and the rest were remains in the terrestrial environment (around 262.3–270.2 million tons of plastic) which indicates larger burden on terrestrial environment compared [21].

Soil organisms play a significant role in maintaining soil fertility through the process of drilosphere. Recently, some studies have been performed which suggest that there is an interaction between soil biota and MPs which eventually results in substantial detrimental effects on behavior and the well-being of terrestrial organisms [22]. Some studies also suggested that there is an adverse effect of MPs on biological, chemical, and physical properties of soil [23, 24]. Data that are currently available give an approximation of distribution or abundance of different types of MPs (such as polyethylene, polypropylene, polystyrene, polyester, acrylic, terephthalate, polyamide, etc.) in the terrestrial environment. For example, in soil, there are $3.0 \pm 1.9 \text{ g m}^{-2}$ and $11 \pm 10 \text{ pieces m}^{-2}$ MPs present of size ranging from 25 to 100 μm ; in biota, there are $0.45 \pm 0.25\%$ w/w of MPs present in earthworms, and 364 particles of MPs were found in 16 birds with particle size ranging from less than 150 μm to 8.5 mm, whereas, in sludge, it was found to be 4,200–15,000 MP particles kg^{-1} dry weight of sludge [25].

MPs can enter the terrestrial environment through various pathways. There are various factors that affect the fate, retention, occurrence, deprivation, and conveyance of MPs in the terrestrial environment which involves inappropriate disposal of plastic waste products, dumping of sewage sludge in the landfills, anthropogenic littering, and contamination of agricultural land due to application of sewage sludge [25, 26]. It has been reported that in North America and Europe, around 40,000–300,000 tons of MPs are discharged in the soil of farmland annually through sewage sludge disposal practices [27].

Nizzetto et al. [28] reported the presence of MPs in sewage sludge generated from wastewater treatment plants (WWTPs). The wastewater from households, hospitals, and industries is directed to WWTPs which finally goes to sewage sludge. MPs with a higher density than water such as polyvinyl chloride are reported to be retained in the sludge which can be further transported [29]. It has been reported by some researchers that MP particles degrade very slowly in the soil. For instance, polyethylene is degraded only at 0.1–0.4% by weight after being in soil for around 800 days; polypropylene is degraded only at 0.4% by weight in a year of incubation, whereas

no degradation has been observed of polyvinyl chloride after being in soil for 10–35 years [30–33]. Studies have been conducted to ensure the percentage of occurrence of MPs in various ecosystems, and it has been concluded that marine water bodies receive the maximum amount of discharge, and very less is known to occur in freshwater and soil compartments [33]. However, in 2016 it was reported that the percentage of occurrence of MPs in the soil is increasing, and it was found to be 0.03–6.7% of plastic in an industrial area [34]. The findings show the inability of MPs to degrade and concluded that these particles are highly persistent and accumulate in the soil [35].

2.2 Occurrence and Fate of Microplastics in the Aquatic Environment

From the decades, plastic made its ubiquitous presence in different environmental matrices because plastic is an integral part of the human population's lifestyle across the world. The aquatic environment acts as a sink for plastic pollution. Oceans are considered as “hotspots” across the globe for the MP contamination, but this resulted from the contamination of the rivers, lakes, and ponds (freshwater bodies) of a particular geographical region. MPs have been observed on seashores, across the water column, and in surface water and also found in biota [36, 37]. In the Indian context, Nigam had found about 50–300 plastic particle m^{-2} along with high watermark in Caranzalem beach, Goa, of particle size 3–5 mm in width and 1–4 mm in length [38]. Scanning electron micrograph (SEM) and Fourier-transform infrared spectroscopy (FTIR) analysis showed 81 varieties of plastic items/kg sediment in a ship-breaking yard in Mumbai, India [39], while 68.83 plastic particles m^{-2} with 1–20 mm particle size were detected across the recreational beaches situated in Mumbai [40]. Similarly, 2–5-mm-sized plastic particles are observed from the shores of Goa and Chennai [41] and also from other geographical regions including Southern Ocean [42], Northern South China [43], Bohai Sea [44], etc. FTIR analysis confirmed the presence of polyethylene and polypropylene functional groups in biota under natural environmental conditions. It has been accepted that the proportion of MP particle contamination is proportional to its global production and usage. From the last three decades (1987–2015), it has been observed that the MP concentration was not increased at a significant level in the Baltic Sea, a marine ecosystem facing a severe surge in anthropogenic pressure [45].

The freshwater environment also has MP contamination, posing a serious threat to the biota residing in the respective niche. Recent studies revealed the occurrence of MPs in estuaries, rivers, and lakes [46]. Through the process of degradation, leaching of plastic particles may enter into the freshwater ecosystem, and it is mainly driven by the household, industrial activities, and effluent discharge from wastewater treatment plants, etc. [47]. In the Los Angeles River (USA), 1–5 mm sized was detected from 12,000 items plastic pellets m^{-3} [48]. It has been observed that MPs

occurred at higher concentration eventually in the vicinity of human habitation and tourism site near to the freshwater environment. From the Lake of Geneva and Garda, the plastic particles of more than 5 mm in size were also identified [49, 50]. In the North Pacific subtropical gyre, a mass of 32.76 particles m^{-3} and 250 mg m^{-3} were detected [51]. The surge in the human population and industries situated near to the coastal areas contribute to significant plastic contamination in the marine environment. Around 1×10^4 plastic particles m^{-3} have been observed adjacent to a polyethylene production plant near to the Swedish harbor area [52]. Population density and plastic load are significantly correlated in the 18 shores across the six continents [8] and suggested that the human population continues to increase the MP contamination in the future.

Normally, the terrestrial accumulation of plastic load is eventually released into the marine and freshwater ecosystems. Apart from this, the release of MP fibers is also reported from textile washing [8, 53]. Once in the freshwater, MPs are detrimental to aquatic organisms and perturb the eco-dynamics of a particular ecosystem. A recent study suggested the presence of MP fibers from the household washing machines [54] from various fabrics at various washing conditions and analyzed the size of fibers ranging between 11.9 and 17.7 μm in diameter and 5.0 and 7.8 mm in length. It has been shown that the average amount of polyethylene-derived MPs in fluid soap is estimated around 2.4 mg person $^{-1}$ day $^{-1}$ [55] and the probability of contaminating the freshwater environment is high due to the indiscriminate usage of personal care products. Besides 8 trillion microbeads have been shoved into the aquatic environment in the USA on a daily basis [56]. An estimate shows that plastic consumption in India will be a high level in the future and set to become the 3rd largest consumer of plastic in the world [57]. The use of plastics in daily life and its inefficient waste disposal in developing countries like India and China pose serious concerns on the pristine nature of the ecosystem and its efficient functioning.

3 Ecotoxicological Effects on Aquatic and Terrestrial Ecosystems

3.1 Effects on Terrestrial Ecosystem

The presence of MPs everywhere in the environment from ground level to sea level has received the attention of ecotoxicologists on its toxicity and safety [58] toward environmental and human health. This approach is good enough to link the relationship between the pollutants and the routes of transport which is a very serious concern toward our environment. There are two main reasons behind the environmental impact of MPs: first one is the size of the MPs which facilitates the easy transport in organisms, and the second one is their surface which acts as a vector to carry other organic pollutants through adsorption [58]. Sometimes the whole food

chain is affected because of some adverse effects of these pollutants at the primary producer level forming the basal food web.

3.1.1 Effects from Producer to Consumer Level

The effect of MPs on terrestrial organisms can only be understood on the basis of available data on plastic production, its usage, and the way they are discarded. It has been reported that the discharge of plastics in the terrestrial environment is 4–23 times more than the dumping in the marine environment [5]. MPs of varying sizes are released in the environment, but those which are less than 1 mm in diameter are more likely to be taken up by the organisms in the soil [35]. And some of the plastic congeners, such as bis (2-ethylhexyl) phthalate, are known to inhibit or hinder the microbial activities of soil microorganisms [59]. MPs are harmful contaminants of the terrestrial ecosystem as they have some hazardous properties which may cause mutation, endocrine disruption, and cancer [60, 61]. Some of the chemical entities in MPs, particularly phthalates, also possess peril on the human population owing to its uptake by plants and subsequent entry in the food chain, once being released into the soil [62, 63]. However, there is not much data available on the adverse effect of MPs on the soil microorganisms, particularly in the field of microbial transport of MPs and on the spread of antibiotic-resistant genes (ARGs). But it has been reported that polypropylene particles (7–28%) in particular have some sort of constructive effect on the activity of soil microbes, whereas destructive effect has been shown for polystyrene (PS) (1 mg kg⁻¹ of soil), polyester (0.05–0.4%), and polyacrylic (0.05–0.4%) MP particles [64, 65]. It was found that in the polystyrene particles (0.1%) when released in the terrestrial ecosystem, the withholding duration of ARGs and antibiotics seems to be increased [66]. Exposure of *Caenorhabditis elegans* to PS particles (1 mg l⁻¹) chronically with particle size ranging between 0.1 and 5.0 μm for 3 days resulted in decrease in body weight, the lowest survival rate, reduced average life span, and perturbed regulation of *unc-17* and *unc-47* gene expression resulting in cholinergic and GABAergic neuron damage in soil nematode [67]. It has been assumed that the route of exposure and possible harmful effects of MPs on soil-dwelling organisms are likely to those of sediment-dwelling organisms due to their similar feeding strategy [68]. To date, the experiment on the exposure of MPs in laboratory conditions has been conducted only on two species of earthworms (*Lumbricus terrestris* and *Eisenia andrei*) and observed the ingestion of MPs [69]. The ratio of mouth to the size of the particle to be ingested is one of the foremost factors that affect the uptake of any particle as the particles with a smaller size have more chances to get ingested by the soil organisms. Lwanga et al. [20] exposed *L. terrestris* worms to polyethylene (PE) MP particles and reported that the rate of mortality is increased by 8% at 450 g kg⁻¹ of exposure and 25% mortality was observed at the concentration of 600 g kg⁻¹ of PE exposure. Similarly, Rodrigues Seijo et al. [69] exposed *E. andrei* to PE particles at different concentrations (62.5–1,000 mg kg⁻¹ of soil) for potential adverse effects on reproduction, growth rate, mortality, etc. The size of the MP particle is in the range of

250–1,000 μm . However, no noteworthy effects were observed after 28 days of exposure. But some damages have been reported after histopathological and FTIR-ATR analysis of gut of the earthworms. Polyethylene particles are also known to act as a vector in the transference of pesticides in the agricultural fields [70]. It has been observed that MPs possess the property to bind with varying hydrophobic organic chemicals (HOCs) which are present in the environment [71]. One of the most important outcomes has been derived which suggests that leaching of MPs can result in the accumulation of various chemicals in terrestrial organisms, by exposing PBDE (polyurethane foam with $<75 \mu\text{m}$ of MP particles that contain PBDE) to *E. fetida* through the process of bioaccumulation [72]. Lwanga et al. studied the burrowing activities and distribution of MPs in *L. terrestris*. It has been concluded that MPs particles from anthropogenic littering results in high biogenic assimilation rate from surface of the ground to burrow walls of soil cause leaching of MPs due to groundwater flow which may result in exposure to soil organisms [22]. In one study *E. fetida* was chronically exposed to LDPE at different concentrations (62.5–1,000 mg kg^{-1} of soil) with size ranging from 250 to 1,000 μm . Oxidative stress was analyzed using various enzymatic biomarkers such as catalase, thiobarbituric acid, and glutathione S-transferase, lactate dehydrogenase, and modification via Fourier-transform infrared spectrometry (FTIR) and nuclear magnetic resonance (NMR) analyses. There is a significant change in thiobarbituric acid level in worms exposed between 250 and 1,000 mg kg^{-1} concentrations. The molecular response analysis using NMR reveals the difference in the proton-binding capacity to carbon atoms in the earthworms exposed to the lower concentration of MPs, whereas no significant changes at the molecular level were recorded using FTIR-ATR [73]. In 2016, the survey conducted showed the presence of anthropogenic plastic in birds with size ranging between 0.5 and 5.0 mm in China. It has been reported that 62.6% of total plastic waste was in the gut of birds [74]. Kokalj et al. reported no significant effect on body weight, rate of feeding, and energy reserves in digestive glands of *Porcellio scaber*, an isopod when exposed to MPs that are produced from plastic bag films and particles that are present in a facial cleanser for 2 weeks [75]. In southeast Mexico, at traditional Mayan home gardens where the waste management is a common practice, it has been reported the transfer of micro and macroplastic particles from soil to chickens for the first time. It was observed that the concentration of MPs is increasing from soil ($0.87 \pm 1.9 \text{ particles g}^{-1}$) to casts of earthworms ($14.8 \pm 28.8 \text{ particles g}^{-1}$) and to chicken feces ($129.8 \pm 82.3 \text{ particles g}^{-1}$), while chicken gizzard contains around $10.2 \pm 13.8 \text{ particles g}^{-1}$ which is taken by humans in many countries as food [76]. However, no evidence of the presence of MPs was found in crops. Table 1 provides the available studies on the effects of MPs on terrestrial organisms.

Table 1 Studies on microplastics and their effects on terrestrial ecosystem

Type of microplastics	Model organism	Microplastics concentration	End point	Observation	Reference
Polyurethane (PU)	<i>Eisenia fetida</i>	PU1:2000w/w Σ PBDEs83 mg/kg ⁻¹ dw	Bioaccumulation	Soil biota ingest PBDEs from soil	[72]
Light-density polyethylene (LDPE)	<i>Lumbricus terrestris</i>	1,5,10, and 15%v/v	Plastic ingestion, mortality, growth, reproduction tunnel formation, the position of the worm	Ingestion of MPs reduces the growth rate	[20]
High-density polyethylene (HDPE)	<i>Lumbricus terrestris</i>	HDPE 236, 1,261, and 4,505 mg/kg ⁻¹ Zn0.6,3,4, and 12 mg/kg ⁻¹ w	Adsorption/desorption, biomass, ingestion	No evidence of negative effect in earthworm	[76]
Light-density polyethylene (LDPE)	<i>Lumbricus terrestris</i>	7, 28, 45, and 60%w/w	Biomass, burrowing characteristics, transport of MP	Higher concentrations of MPs may, in fact, be directly related to the worm's response to stress	[77]
Urea-formaldehyde MPs	<i>Folsomia candida</i> , <i>Proisotoma minuta</i>	5 mg of the 100–200 μ m fraction and 2.5 mg of the <100 μ m fraction	Transport of MP	Increased transportation of MPs particles by <i>F. candida</i> than <i>P. minuta</i>	[78]
Polyethylene (PE)	<i>Lumbricus terrestris</i>	750 mg per 2.5 kg soil (added on the soil surface)(2, 625, 424, 203, and 75 particles per 2.5 kg soil)	Transport of MP	PE is transported through casts, egestion and sticking on the body surface of earthworm	[79]
Polyethylene (PE)	<i>Eisenia fetida</i>	62.5, 125, 250, 500, 1,000 mgMP/soil dw	Survival, number of juveniles, weight, histopathological analysis, damages to immune system response	PE damages immune system responses but has no effect on survival, reproduction, and body weight of earthworm	[69]
Plastic bag film and particles from a facial cleanser (PE)	<i>Porecllio scaber</i>	4 mg g ⁻¹ dry weight	Feeding rate, defection fate, energy reserves	No effect observe	[75]

MPs (<5 mm), macroplastic (5– 150 mm)	Soil, earthworm casts chicken feces and gizzards	Not available	Ingestion and biomagnifications	Transfer of MPs from soil (0.87 ± 1.9 particles g^{-1}) to earthworm casts (14.8 ± 28.8 particles g^{-1}) and to chicken feces (129.8 ± 82.3 particles g^{-1})	[80]
Polyethylene beads	<i>Lumbricus terrestris</i>	710–850 μm (PE-1), 1,180– 1,400 μm (PE-2), 1,700– 2,000 μm (PE-3) and 2,360– 2,800 μm (PE-4)	Ingestion and biogenic transport	Earthworms can be significant transport agents of MPs in soils	[81]
Polyvinylchloride (PVC)	<i>Hypoaspis acuteifer</i> (mite), <i>Folsomia candida</i>	5,000 MP particles	Ingestion and biomagnification	Soil microarthropods may play an important role in the disper- sion of MPs	[82]

3.2 *Ecotoxicity of Microplastics in the Aquatic Ecosystem*

MPs eventually end up in water bodies through surface runoff, WWTPs, and domestic/industrial drainage systems. Upon entry into the aquatic systems, MPs can widely disperse into various environmental matrices such as surface water, water column, and benthic sediment, which may affect their bioavailability to the aquatic biota occupying different trophic levels.

3.2.1 *Microplastics Effects at the Producer Level*

Freshwater The study of negative effects of MP particles is important on the primary producer level which eventually jeopardizes the entire food web and food chain of a respective ecosystem. It has been reported that the impact of nanoplastic beads on two algal species, *Scenedesmus* spp. and *Chlorella* spp., was due to physical interaction. The electrostatic attraction initiates the adsorption of negatively charged beads on the surface by the tendency of positively charged cellulose. The ability of MP adsorption influenced by algal cells is initiated through the generation of reactive oxygen species (ROS) [83]. The exposure of polystyrene particles of size between 0.05 and 6 μm for 72 h observed no changes in the algal growth rate but reduced the photosynthesis from 2.5 to 45% in the *Chlorella vulgaris*, *Thalassiosira pseudonana*, and *Dunaliella tertiolecta*. It is suggested that the level of MP toxicity is proportional to the decreasing particle size in the primary producer level, being a major source of oxygen to higher-level biota on the ecological pyramid [84]. On the other hand, nanosized polystyrene particle (0.22 and 103 mg l^{-1}) exposure affects the algal growth and reduced the chlorophyll content leading to decreased photosynthesis in the *Scenedesmus obliquus* [71]. Exposure of *Chlamydomonas reinhardtii* (freshwater microalgae) showed some variations in the molecular response upon the MP exposure. Exposure of high density of polyethylene (HDPE) and polypropylene (PP) to *C. reinhardtii* induced aggregates that consist of 50% microalgae, 50% MPs, and exopolysaccharides. Eventually, 78-day exposure results in growth inhibition, and genes (UGD and UGE) involved in sugar biosynthesis were significantly upregulated with respect to control in the HDPE-exposed microalgae than PP exposed. The chloroplastic genes including *psaB*, *psaA*, and *rbcL* are negligibly expressed as compared to control. Similarly, it has been reported that the aggregation of microalgae with MPs was correlated to the growth inhibition [85].

Marine Water In the marine ecosystem, the effects of MPs have been observed, and most of the scientific data showed the potential effect of MPs at the producer level. The polyvinyl chloride (PVC) MP (1 μm size) exposure reduced the growth rate by 39.7% after 96 h exposure, while 1 mm PVC had no toxic effect on the *Skeletonema costatum* [86]. Contrary to this, no significant growth rate inhibition

was observed in *Tetraselmis chuii* upon exposure to fluorescent red polyethylene microspheres (1–5 μm) in the presence and absence of copper and suggested that particle size was inversely proportional to the MP toxicity [87]. Marine algae *Rhodomonas baltica* and *Oxyrrhis marina* showed that the increased uptake of the virgin (10 μm) and fluorescent polystyrene particles (1–5 μm) by *Oxyrrhis marina* than its counterpart *Rhodomonas baltica* resulted in the loss of motility and replacement of food [88]. Biofouling formation and shedding effect are the predictive mechanism of polystyrene (PS) MP-mediated toxicity that resulted in reduced chlorophyll production in the marine autotrophs and mediated the transfer of MPs from the top layer of water to the bottom of the ocean [89]. The studies suggested that the MP contamination induced by the planktonic aggregates initiates the vertical distribution in marine ecosystems. Comparably, heteroaggregates derived from the species-specific were observed in the marine *Chaetoceros neogracile*, a diatom with no adverse effect on the growth exposed to polystyrene MPs [90].

3.2.2 Microplastics Effects at the Consumer Level

In Freshwater Primary consumers of freshwater environment comprise most of the invertebrates including annelids, crustaceans, ostracods, gastropods, etc. The toxicity of MPs in the freshwater biota is limited apart from some laboratory studies that have been done on the crustaceans and cnidarians. The uptake of MPs is evidenced in freshwater consumers like *Daphnia magna*, *Gammarus pulex*, *Notodromas monacha*, and *Potamopyrgus antipodarum* under field conditions [91]. Ingestion of MPs of 0.01–1 mm size is noted in the gut epithelia and gets accumulated in the lipid storage droplets of *D. magna* indicating impairment of the filtration activity leading to life-threatening of crustaceans [92]. Additive effects of nano-plastics (50 nm) and microplastics (10 μm) with the hydrophobic compound phenanthrene have been observed in the crustacean, *D. magna*. Significant phenanthrene bioaccumulation, dissipation, and the transformation were noted suggesting the higher adsorption rate of hydrophobic contaminants on smaller-sized MP particles [93]. The accumulation of primary and secondary MPs in the gut of *Daphnia magna* increased the gut passage time [94]. It has been observed that the exposure of MPs in soil nematode *C. elegans* induced a significant reduction in body length, survival, and reproduction with increased GST enzyme activity [95], indicating particle size-induced damage is the primary effects of tiny entities in the sediment-dwelling worm.

Exposure and detrimental effects of MPs are not only confined at the producer level, but it also affects the primary and secondary consumer level through food chain-mediated exposure. MP-induced hepatic toxicity was observed in the Japanese medaka, *Oryzias latipes*, to 3 mm low-density polyethylene (LDPE) pellet exposure demonstrating the active ingestion process of MPs and its by-product-induced toxic response. Long-term exposure revealed a decreased hepatic glycogen content and

fatty vacuolar degeneration with no significant effects on *cyp 1a*, *vtg 1*, and estrogen receptor α gene expression in the female and male fish. Expression levels of choriogenin H in the male fish are unaltered, but significant downregulation has been observed in the female fish [96]. Significant uptake of MPs (1–5 mm blue nylon fragments) in different stages of the Gerreidae fish, *Eugerres brasiliianus*, *Eucinostomus elanopterus*, and *Diapterus rhombeus* from estuaries and mangroves has been reported. Differential uptake of MPs based on size and shape has been shown between 4.9 and 33.4% exposed individuals [97]. On the other hand, 12% of freshwater gudgeons (*Gobio gobio*) caught from French streams have been reported to ingest MPs [98]. Visual observation of the gastrointestinal tract revealed deposition of 12% MPs in the fish intestine, and approximately 20–30% of MP accumulation in the intestine of fish specimens were captured from the vicinity of the urban area. Recently, it has been observed that the accumulation of polystyrene MPs of different size (5 and 70 nm) in gills, liver, and the gut of *Danio rerio* [99]. Additionally, MPs act as a carrier to transfer most of the persisted organic contaminants like polychlorinated biphenyls. Injection of polystyrene MP particles induced systemic toxicity via distribution in blood vessels and accumulated in the heart of 2 dpf (day post-fertilization) embryos. The upregulation of genes *cfhl3*, *cfhl4*, *cfb*, and *c9* involved in the alternative complement pathway suggested that the co-localization of neutrophils and macrophages around the PS particles results in immunological reaction in the zebrafish larvae [100]. Thus, the exposure of MPs, to the aquatic ecosystem biota, affects the niche (functional unit of the organism) of particular trophic level leading to disturbances in the ecosystem dynamics.

Marine Water Scientific evidence clearly showed that marine biota is also exposed to MPs at a significant level and adversely modulates the function and diversity of the marine organisms, and the effects of MPs were observed in different trophic level at various level of biological organization. The potential adverse effects include feeding inhibition, energy deficiency, reduced growth and reduction in the oocyte and sperm velocity in the oysters, perturbations in prey-predator relationships, etc. [101–103].

Ingestion, translocation, and accumulation of a variety of MPs have been noted in marine mussel, *Mytilus edulis* (filter feeder) [104, 105]. Exposure of PS particles (3.0 and 9.6 μm) results in the persistence of MPs in the circulatory system after 3 days of exposure and noted till day 48 upon accumulation in digestive cavity and tubules with maximum MP abundance on day 12. Ecological impacts of MPs at cell and tissue levels were also noted in *M. edulis* after HDPE MP exposure leading to an uptake in gills and digestive gland. Histological analysis revealed a strong inflammatory response through the formation of granulocytomas after 6 h and lysosomal membrane destabilization after 96 h, considering them as prospective biomarkers of MP exposure in filter feeders [105]. Ecotoxicological effects of MPs in terrestrial and aquatic ecosystems are encapsulated in Fig. 3.

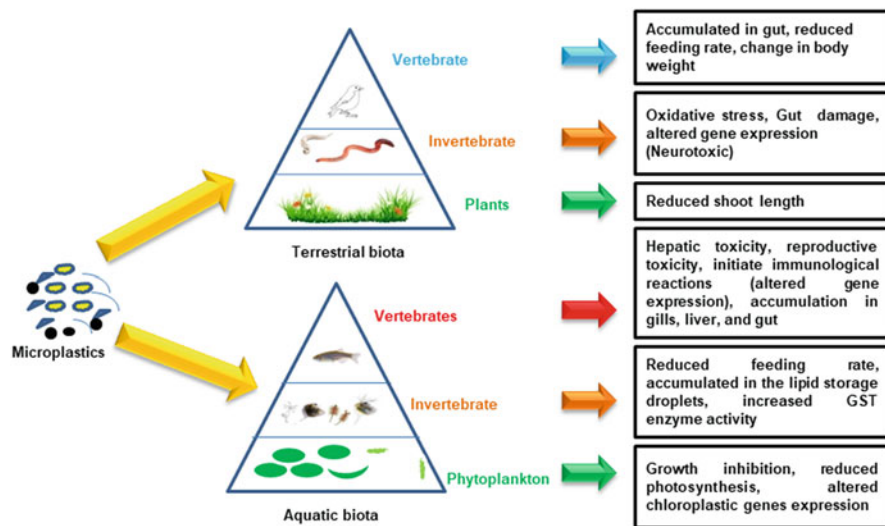


Fig. 3 Microplastics induced adverse effects on terrestrial and aquatic ecosystems (diagram drawn by Ved Prakash)

4 Conclusion

Occurrence and contamination of MPs have been recorded in various ecosystems from land to river, oceans, and arctic to antarctic habitats. Ecotoxicity data reveals that MP exposure eventually results in active ingestion, feeding impairment, stunted growth, reduced reproduction in terms of oocyte formation and decreased sperm velocity, offspring formation, perturbations in the expression profile of genes involved in vital physiological processes, etc. from producer to consumer level. Owing to its relatively large surface area, MPs act as potent vectors in carrying persistent organic contaminants and noninvasive species to pristine water bodies. However, further research is required to differentiate whether the adverse effect is due to the particle ingestion or the leaching of chemical entities from the plastic. As we have already mentioned that MPs act as vectors in carrying persistent organic pollutants in the environment. Hence, this association may have a more drastic effect on the biota inhabiting terrestrial and aquatic ecosystems. Therefore, the mechanism of action should be deciphered to reveal the combined toxic effects of MPs and other associated environmental pollutants. Hence, it is a wake-up call for ecotoxicologists and ecologists to study the potential adverse effects of MPs at environmentally reported particle range (since most of the effects noted are overestimated at relatively higher concentrations) and protection of the ecosystem for sustainable development.

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Microplastics in Soil Ecosystem: Insight on Its Fate and Impacts on Soil Quality



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Abstract Plastic film has been intensively used in (semi-)arid agricultural regions, attributing to its great benefits of improving soil productivity and crop yield in

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China. However, plastic debris, as a consequence of film mulching, remains and accumulates in soil leading to severe soil quality problems, as well as environmental concerns especially the small fragmented particles referred to as microplastics (MPs). Though increasing attention has been aroused for MPs in the aquatic environment, the knowledge of MPs' behavior and its effects on soil quality is extremely insufficient and urgently needed. In this study, we oriented the benefits of plastic film use, its contribution to agriculture productivity, and the effects of MPs on soil properties and its related soil quality indicators. Admittedly, the increasing trend of using plastic film made by light density of polyethylene would be continued in China, and the pieces of plastic particles would either be persistent and accumulated in soil layers or be slowly aging and degraded. The impacts of MPs on soil quality need more attention due to the limited studies available focusing on its fate and interactions associated with soil ecosystem services and environmental resilience. Although policies and agricultural extending services on plastic film application have been laughed for a few years, alternative materials used for producing environment-friendly film, plastic debris recycling, and solutions on pieced particle removal are the great challenges for sustainable farming. Thus, it is urgent to understand MPs' effects on soil quality which is crucial for soil-plant system and soil pollution monitoring and prevention.

Keywords Microplastics, Plastic film mulching, Risk assessment, Soil quality, Terrestrial ecosystem

1 Introduction

Plastic mulching, a promising farming technic, has been widely used attributing to its benefits for increasing crop yields in arid and semiarid areas [1, 2]. However, the presence of plastic residues has become a challenging problem for soil quality and the environment, especially small plastic particles, such as macro-, micro-, and even nanoplastic residues which are potentially harmful for agroecosystems [3] and surroundings delivered by erosion or runoff [4]. There are numbers of study that focus on microplastics in marine [5], coastal tidal flats [6], estuaries [7], lakes [8], and other water ecosystems [9] but less on the impact of soil ecosystems.

MPs in the soil environment include application of sewage sludge, flooding and street runoff, plastic litter, atmospheric fallout, landfill, and plastic film mulching [10]. The application of sewage sludge to arable land alone could add an annual MPs load to soil greater than that entering the world's oceans [11]. Although sewage sludge application has been banned in some countries, the application of compost and the use of plastic foil in agriculture become the new MPs sources to contaminate soil [10, 12]. Industrial plastics, littering, road dust, diffuse atmospheric deposition, sedimentation from water flooding, and irrigation are other sources of MPs in the

environment, but the quantity and its effects in soil are still not well-reported. Nizzetto et al. [11] estimated that around 430,000–63,000 and 300,000–44,000 tons of MPs are input annually into farmlands in Europe and North America, respectively. Landfill contributes 30.8% of 25.8 million tons of postconsumer plastics becoming airborne small particles (e.g., MPs) [13]. Furthermore, since plastic mulching is used widely in dryland area, plastic fragments from larger pieces to microparticles, as a consequence of mulching, continuously accumulate in soil and become the severe problems to soil quality and its surrounding ecological environment.

After entering into the soil, plastics will interact with pollutants in the soil, which will affect the environmental behavior and create environmental effects in the soil, as well as soil properties. MPs in soil can adsorb with other pollutants such as persistent organic pollutants (POPs) and heavy metals, which make them more harmful in the long term [14]. This adsorption includes physical adsorption and chemical adsorption. Physical adsorption is the action between adsorbate and adsorbent under van der Waals force, which mainly depends on the specific surface area [15]. The adsorption properties of MP particles is related to their own characteristics, such as material, specific surface area, amount of adsorption sites on the surface, and hydrophobicity [16]. The source and age of MPs also have a certain influence on their adsorption, and different environmental conditions, such as pH, salinity, and metal cation concentrations can also affect the adsorption properties of MPs. Polyethylene (white, diameter ~4 mm, mass ~25 mg) adsorbed with metal elements (Al, Fe, Mn) and trace metal elements (Cu, Zn, Co, Cr, Mo, Sb, Sn, Pb, Ag, Cd, U) and the adsorption mechanism may be direct adsorption of metal cation, metal ions collide with charged or neutral regions of plastic surfaces, and adsorption or co-precipitation with iron-manganese oxide [15]. Hence, MPs presence in soil would alter the elements' bioavailability affecting either soil functions or compounds' environmental behaviors.

Furthermore, MPs content in soil is likely unavoidable to affect soil organisms and decrease soil fertility and thus alter soil ecological function and global food production. Despite direct uptake of MPs by crops and transferring MPs to edible plant parts seeming unlikely, MPs could enter into the human food chain by animals and livestock [17]. Many animals are unable to digest the plastic fragments, thus preventing food from passing through the gut, but soil fauna, and especially earthworms, can digest MPs by crushing fragile plastic fragments [18]. Earthworms and other soil micro-animals are indispensable members in the soil environment, and they play an important role in transportation and transformation of MPs which, in turn, influence soil biological function to decompose organic matter. Furthermore, microbial communities on plastic debris are seen as the "plastisphere" [19, 20]. Zettler et al. (2013) found that the average plastisphere abundance was lower than that of surrounding microorganisms, while the homogeneity among communities was greater. Bacterial community on the plastic substrate has obvious discrepancies from that in the surroundings [21]. Correspondingly, the contribution of plastisphere on the surface of MPs is significant to the degradation process [22, 23]. It is reported that polycaprolactone could be degraded by impure and pure cultures of germs and *Saccharomyces* [24]. Moreover, *Comamonas acidovorans* TB-35 took advantage

of polyester polyurethane as the single carbon source and produced a polyester polyurethane-degrading enzyme [25]. As a consequence, the micromolecular water-soluble intermediates are absorbed by the cells and enter a special metabolism which might affect soil microbial communities and volatile compounds. The enhancement of microbial activity increases extracellular enzyme secretion and promotes the release of nutrients such as C, N, and P in soil, thus promoting the migration of nutrients between plants and soil [26, 27]. Meanwhile, many additives, such as stabilizers and plasticizers, added to plastic during manufacturing to increase the durability, are released during exposure and become bioavailable to soil organisms, thus threatening soil quality [28–30]. These concerns and on the presence of MPs in soil and their interaction with soil quality indicators are still a large gap in current knowledge for our understanding of MPs pollution in agricultural soil and terrestrial ecosystem.

Therefore, the scope of this chapter is to address plastic film mulching and debris of soil quality related aspects that lead to soil degradation and environmental problems. Our aim is to screen current situation of plastic film mulching and its consequences on soil quality which are not well-concerned and even not recognized. Based on the background information of plastic film mulching, implications are provided for anticipating MPs abundance that may aggravate soil quality.

2 Plastic Film Application and Its Residues

2.1 Plastic Film Application

In China, plastic film has been tremendously used in agriculture especially in dryland areas since imported from Japan in the 1970s. The quantity of plastic film application has been increased around two times from 1999 to 2016, reaching 2.60 million tons and mulching farming land 1.84×10^6 ha [31]. Great benefits of plastic film application have been achieved for crop yields and economic returns [32], and its advantages for farming can be mainly highlighted as four aspects:

1. Soil temperature and soil physical properties. After plastic film mulching, soil temperature increases [33, 34], and vapor pressure effects lead to soil porosity and soil aggregate stability increasing and bulk density declining [35, 36]. These properties are either good for seed germination, seedling emergence [37], and root growing [38] or water-heat balances inside of soil under mulching. Due to soil surface mulching, raindrop-induced soil detachment and erosion have been reduced [39], as well as avoiding soil compaction during the whole plant growing seasons.
2. Soil water conservation. Soil moisture could increase by plastic mulching [40, 41], varied by different mulching schemes shown in Fig. 1. It is reported that rain-harvesting efficiency improved significantly (65.7–82.7%) with the film fully mulched ridge-furrow water harvesting scheme in maize growing seasons

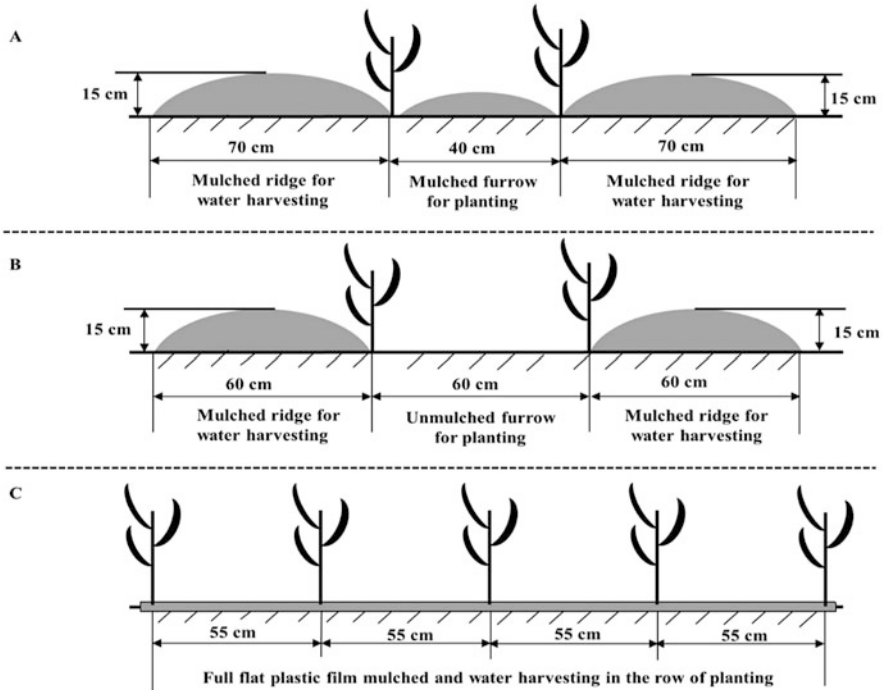


Fig. 1 The three main plastic film mulching schemes ((a) full ridge-furrow mulching; (b) half mulching; (c) full flat mulching) in the field for crop planting and water harvesting

[42]. Meanwhile, due to the plastic mulching, soil surface evaporation rate decreases [1], and average soil water storage ($750\text{--}1,500\text{ m}^3\text{ ha}^{-2}$) and the infiltration depth of soil water in dryland increased significantly, which result in high potential productivity and crop yields [43].

3. Nutrients cycling and soil microbial activities. Nutrients availability accelerates attributing to the soil temperature and water thermodynamic changes [44]. Meanwhile, the covered soil surface avoids nutrients loss by leaching, runoff, and erosion of sediment [45]. Studies indicated that soil available N, P, and K might increase [46]. Due to the positive impacts on soil temperature and soil water storage, plastic mulching is beneficial for soil microbial activities and organic matter decomposition and mineralization [47–49] which contributes to nutrients cycling and plant growth.
4. Weeds control. Concerning the large scale of farming land in NW China being covered by plastic film, weeds sprout and growing are inhibited, as well as soilborne diseases and pests due to high soil temperature. Then agrochemical products for weeds control are greatly reduced which avoids soil contamination and compounds residues threatening soil quality and food safety.
5. In addition, as a promising agriculture water-saving technique, plastic mulching combined with drip irrigation and different mulching patterns has been expanded

to the irrigation regions which used to be abandoned dryland or water-limited land, and can save water of 6,000–15,000 m³ ha⁻²: the growing degree day increasing (200–400°C), the latitude going northward (2–5°), and the altitude arising (500–1,000 m). Therefore, with great benefits achieving from plastic film application, it is no doubt that plastic film would be continuously used for water and energy consumptions in the agriculture, especially in dryland area [50].

2.2 Plastic Residues

With quantity of plastic film used continuously, plastic residue, as a consequence of plastic film application, has become the big challenge of environment problems, especially in the areas with long-term plastic film use [51]. As it is mentioned above, due to the high efficiency of harvesting water and crop yields, full ridge-furrow plastic mulching scheme in dryland regions has been widely extended [52], but plastic film is easily broken into pieces after harvesting either by weather conditions or by harvesting and plowing machines. It is reported the residues ranged from 50 to 260 kg hm⁻² in arable lands after 10 years of plastic mulching [53] and the quantity of its accumulation pieces keeps increasing in farming soil layers and field surroundings (Fig. 2).

With regard to the regulations for the production of plastic film for farming, such as its thickness and its original materials, the regulation entitled GB13735-92 has been issued which changed the standard thickness to 0.01–0.02 mm instead of 0.008 ± 0.003 [54]. However, the material used to produce plastic film is mainly polyethylene with low-density and transparent properties associated with lower costs and higher yields to farmers [55]. This type of material with additives can be strengthened films but it is fragile to be pieced physically either by plants or by harvesting machines which thousands of plastic pieces formed and left in soil after harvesting. Comparing the weight of mulch film, around 60% of plastic residue is recycled [56], but the efficiency of recycling is limited, especially recycled by machines or by farmer themselves (Fig. 3). However, machine-supported recycling



Fig. 2 Plastic debris in soil layers and field surroundings (from Tinglu Fan)



Fig. 3 Plastic collection by machine and famers (from Tinglu Fan)

or farmer manually supported recycling only can remove larger pieces of plastic film, while small pieces, such as particles less than centimeters and even invisible, remain in soil which strongly impacts soil functions in the long-term farming [57].

Concerning the materials of plastic film using in farming, the fate of plastic debris after harvesting refers to aging and degradation in soil ecosystem. However, plastic aging is a long-term process associated with weather conditions and its original materials including additives [58]. Polyethylene, the most common polymer used to produce films, seems difficult to be aged by solar radiation, temperature, precipitation, and other physically based practices. Due to the larger plastic debris recycled, the smaller residuals, such as mega-, macro-, and microplastics, are either fragmented and accumulated or slowly degraded and involved into soil physiochemical processes and microbial activities [59, 60]. Although the quantity and risks of larger pieces of plastic debris have been intensively studied in cropping system [32], the abundance, distribution, and the environmental consequences of microscopic debris are only highlighted in near few years [61]. Meanwhile, plastic particles clustered in different soil layers depend on intensive plastic film use, mulching schemes, and cropping systems. According to our recent survey, macroplastics are concentrated in 0–10 cm soil layer, while MPs were mainly detected in 20–30 cm soil layers with a 30-year history of mulching (data not published). Therefore, it is urgent to concern a broad range of plastic particle types from mega- to micro- even to nanoplastics in the soil, and understand long-term effects of plastic particles on soil functions and quality [62].

3 Impacts of Microplastics on Soil Quality

3.1 *Effects of Microplastics on Soil Physiochemical Properties*

Although MPs have been detailed in aquatic systems, few studies have been done to illustrate its impacts on soil physical and chemical properties. Regarding MPs

fragmentation and its fate in soil, it easily affects soil bulk density and water content, in accordance with the studies focusing on larger plastic residues which significantly reduced gravimetric soil water content and bulk density, decreased macropores, and altered soil water distribution [63]. Although the quantity of MPs may contribute greatly in soil, its effects on soil bulk density and porosity might be varied among different soil types [60]. Furthermore, comparing to soil without MPs contamination, soil saturated hydraulic conductivity, field capacity, and soil water repellency changed slightly but significantly increased in the treatment of relevant concentrations with 2% MPs addition [64]. If the abundance of MPs reaches a certain level, soil water characteristic curves could be shifted easily with the interaction of plastic aging and soil pore changes which potentially influence soil water availability and plant growth.

MPs significantly increased the nutrient contents of the soil dissolved organic matter, such as dissolved organic carbon (DOM), dissolved organic nitrogen, ammonium nitrogen, dissolved organic phosphorous, and phosphate [27, 65]. Liu et al. [27] found that the MPs addition led to the accumulation of high-molecular-weight humic-like materials and fulvic acid indicating that the decomposition rate of humic-like material after MPs addition was slowed and more DOM accumulated. In this case, if these compounds accumulate in soil, the sources for soil microbial activities and nutrient bioavailability for plants seem to be constrained. Meanwhile, the effect of MPs on soil iron exchanges is related to it being monovalent or multivalent. Similar as clay particles, MPs could be adsorbed with mineral and organic surfaces and surface groups but different from cations, such as Ca^{2+} , Fe^{3+} , and Al^{3+} potentially affecting the adsorption or exchange position for pollutants, such as pesticides and persistent organic pollutants [66]. Thus, further researches are needed to understand MPs effects on soil physicochemical properties, especially soil types with abundance of irons and clay particles.

3.2 Effects of Microplastics on Soil Biota

Diversity of soil animals plays an important role either for soil formation or for soil functions, especially earthworm abundance defined as a biological indicator to assess soil quality [67]. MPs integrated with soil particles could be ingested by soil meso- and microfauna and thus have the potential to bioaccumulate in the food chain [68]. Earthworms exposed to MPs showed that the growth and survival rate of earthworms were negatively affected [69] indicating that MPs in the environment potentially affect soil organisms. Earthworms acted as a transport vector of MPs in soil, incorporating material into soil via casts, burrows, and adherence to the earthworm's exterior leading to the potential risks of exposure for other soil biota communities [70, 71]. It is reported that with the MPs addition, the kinetics of glyphosate changed slightly [66] but the quantity of transport of glyphosate was influenced by the combination of glyphosate and MPs [72].

Soil microbial communities have a crucial role in nutrients cycling and influence pollutant behavior, including the mineralization, biodegradation, and detoxification of toxic compounds [73, 74]. Previous studies showed that soil microbial respiration and soil β -glucosidase, urease, and phosphatase concentrations significantly varied with the addition of high MPs content. Liu et al. [27] reported that MPs stimulated enzymatic activity and activated organic C, N, and P which were useful for the accumulation of dissolved organic C, N, and P. Furthermore, extracellular enzymes produced by micrograms are excreted and attached to the MPs surface during the degradation process. As a consequence, the micromolecular water-soluble intermediates are absorbed by the cells and enter a special metabolism which probably alters soil microbe communities [23]. In addition, concerning the polymer used for plastic film production, its residues degraded in soil might release C which can be a source for soil microbial activities and also beneficial for soil functions. Although degradation rate of plastic particles is limited and slow, the C source contribution to soil quality still needs to be studied in further research.

4 Implications and Conclusion

It is widely understood that the pressure of plastic film residues on agricultural sustainable development can be detrimental, both environmentally and to farmland productivity. However, plastic mulching combined with water harvesting technics is continuously used and extended in order to produce enough food and economic values in dryland area. With the long-term plastic film application, hence, abundance of MPs and other plastic particles increases, and they accumulate gradually in soil layers. Although it can be transported by surface runoff and leaching via soil pores, it would strongly affect soil properties and soil functions. Meanwhile, due to the interaction with soil particles and soil microbe, coupled contamination with other pollutants needs to be taken into account, and it needs more efforts to replace current low-density plastic film with alternative materials. Some bacteria isolated from worm gut could digest plastic particles [18, 75], but the efficiency and application condition remain unclear. Despite plastic debris in soil being difficult to clean, some policies can be made to prevent such “white pollution.” Unfortunately, there are a number of barriers to the design and implementation of policies to relieve these pressures and to improve the supervision and recycling systems. These barriers include the difficulties of accessing alternative materials to produce environment-friendly and cheaper plastic film and deploying the machines to recycle the residues after harvesting. Furthermore, local economic development enhanced by plastic mulching market leads to policies lack of support by local government and farmer themselves. Although subsidies have been approved for farmers or commercial companies to recycle plastic debris, labor-consuming and huge investment to reuse such debris lead to lower recycle rate. Therefore, a new approach needs to be concerned and designed involving all stakeholders to reduce or eliminate plastic pollution, especially MPs risks on soil quality.

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The Toxicity of (Nano)Microplastics on *C. elegans* and Its Mechanisms



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Abstract Microplastics (MPs) and nanoplastics (NPs) are respectively defined as plastic debris with sizes of <5 mm and <100 nm. In recent years, (nano)microplastics (N/MPs) have been widely detected in air, water, soil, and other environmental matrices. Despite knowledge gap of the risks of N/MPs, more and more researchers pay attention to the adverse effects of this type of fine plastic items on biota. *Caenorhabditis elegans* (*C. elegans*) is an ideal model organism for toxicology study on N/MPs. In this chapter, we have reviewed research progress in the toxicity of N/MPs and its mechanism basing on this model. At the individual level, N/MPs can cause lethality on nematodes and the inhibition of growth and reproduction.

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The alteration of locomotion behavior has been demonstrated in nematodes after N/MPPs exposure. Moreover, the behavioral toxicity was revealed to be involved in the especial neurotoxicity, including damages of GABAergic and cholinergic neurons. In addition, intestine damages and oxidative stress were found in nematodes exposed to N/MPPs. Several studies proved that the N/MPPs-induced effects might be closely dependent on the size and dose of N/MPPs. Recent studies showed that the toxicity of N/MPPs was mediated by the insulin signaling pathway and p38 signaling; the intestinal signaling cascade of PMK-1-ATF-7-XBP-1 and PMK-1-SKN-1-XBP-1/GST-5 could regulate the responses to nanopolystyrene particles in nematodes. Although the toxicity of N/MPPs has been largely investigated basing on *C. elegans*, the toxic mechanisms are still unclear. Moreover, current studies are most relying on a special type of pure polystyrene sphere, which might not be the representative of all N/MPPs types. Therefore, more researches on environmental (nano)microplastics with different chemical compositions and shapes need to be done in the future.

Keywords *Caenorhabditis elegans*, Microplastics, Nanoplastics, Polystyrene, Toxicity

1 Introduction

1.1 Nanoplastics

Microplastics (MPs) are usually considered as plastic debris with sizes below 5 mm, which has reached a consensus among researchers. Similarly, nanoplastics (NPs) are referred to smaller debris with the size between 1 and 100 nm, which is consistent with the European Commission nanomaterials definition [1, 2]. Despite some scholars suggested to set the upper limit of the size of NPs as 1 μm [3–6], NPs were commonly regarded as in the size of smaller than 100 nm. The sources of NPs can be mainly divided into two categories. Primary NPs are mostly stemmed from industrial plastic products including ink of 3D printers, cosmetic products used for skin exfoliators, and synthetic fibers from clothes [4, 7, 8]. In addition, the breakdown of larger debris results in secondary NPs. The fragmentation of larger plastics may be attributed to both abiotic processes such as UV radiation, thermooxidation, and mechanical crushing and biotic driving processes including microbiological activity, animal digestion, etc. [9–12]. For example, *Antarctic krill* were proved to ingest MPs (31.5 μm) and break them into NPs in the size of less than 1 μm [13].

Due to small sizes, NPs in environments cannot be accurately quantified. It is lack of effective methods for extracting, counting, and identifying NPs [14–17]. There is also no uniform standard method for sampling and analyzing NPs [18]. Some researchers have predicted that the environmentally relevant concentration of NPs is $\leq 1 \mu\text{g L}^{-1}$ in freshwater environments [19], yet it needs further support of

experimental evidence. Considering the fragmentation process in their formation, NPs have variable presence of their morphology and types in environmental compartments including water, air, soil, and sediment. Up to date, there is limited knowledge about the fate and potential toxicity of NPs [14, 20].

1.2 Toxicology of Nanoplastics

Previous studies about the toxicity of NPs are mostly based on nanoscale-sized polystyrene (PS); yet commonly used types of plastic such as polypropylene (PP), polyethylene (PE), and polyvinyl chloride (PVC) have rarely been investigated. It is mostly due to available PS products from commercial corporations [21]. Additionally, PS can technically produce into nanobeads; however, it is difficult for other types of plastics. According to a recent study, LC50 of PS-NPs on *D. pulex* was 76.69 mg L⁻¹ for 48 h exposure; PS-NPs would induce obvious inhibitions on animal growth and reproduction. In addition, a significant increase in the expression of HSP70 was demonstrated, which means the exposure of PS-NPs arouses the defense of antioxidant systems [22]. Another study showed that PS-NPs could cross cell membranes and cause tissue damages of zebra fish under conditions of laboratory exposure; however there is no considerable toxicity under natural conditions after exposure to environmentally relevant concentration of NPs [23].

Both MPs and NPs can be ingested by organisms and exert toxic effects. Some researchers have compared the potential effects between MPs and NPs and found size-dependent toxicity of N/MPs. For example, Sjollem et al. exposed three sizes, i.e., 50 nm, 500 nm, and 6 µm, of PS-M/NPs to *Dunaliella tertiolecta*. They found that smaller-sized NPs caused serious adverse effects including microalgal photosynthesis and the growth of *Dunaliella tertiolecta* [24]. Additionally, the toxicity of N/MPs may be related to size-dependent ingestion by different organisms. For example, 1–100 µm MPs can be ingested by the isopod *Idotea emarginata* [25]; while MPs with sizes of 11–700 µm MPs could be easily taken in by the marine amphipod, *Allorchestes compressa* [26]. Nevertheless, PS-NPs particles in sizes of about 1 µm can be easily taken in and accumulated in the digestive system of nematodes [27]. It is generally speculated that smaller-sized particles would be more toxic than larger-sized particles because of their larger specific surface area [28]. But there are still arguments of size-dependent toxicity of N/MPs among different research groups. For example, Lu et al. reported that 5 µm MPs induced higher activities of SOD and CAT than 70 nm NPs [29].

Actually, environmental N/MPs usually contain not only additives but also other contaminants, such as organic chemicals and inorganic salts. For instance, Besseling et al. analyzed PCB concentrations on PS-NPs after joint exposure to *Arenicola*

marina and found bioaccumulations of PCBs accompanying with the increasing toxicity such as the loss of animal weight [30]. Another study showed that PE (10–106 μm) MPs were ingested by *Danio rerio*, accompanied with silver ions; adverse effects were increased with the increasing percentage of silver found in the intestines of fish [31]. A mass of studies has demonstrated that N/MPs can play the part of transport vectors for adhesion and accumulation of other coexisting contaminants [31–33]. Compared with MPs, NPs have a larger specific surface and a higher accessibility to cross cell membranes and result in higher risks to organisms according to more researchers [24–28].

1.3 *Caenorhabditis elegans*

Caenorhabditis elegans (*C. elegans*), a free-living nematode often found in soil environments, has been established as model organism for toxicology [34]. This type of nematode is mostly hermaphrodite and self-reproductive and includes a life cycle of about 3 days, which can be divided into eggs, larva (L1, L2, L3, and L4), and adult stages [35, 36]. Germ line in nematode hermaphrodite produces male and female gametes, i.e., sperm and oocytes. Under normal circumstances, a hermaphrodite nematode can produce about 300 offspring [34, 37]. Therefore, nematodes have advantage as model organisms, such as short experimental period, easy reproduction, and convenient observation and operation.

The nematode *C. elegans* has a simple and well-defined anatomy suitable for toxicology. Normal food, bacteria *OP50*, accompanied with N/MPs particles can be ingested by the nematode, through the pharynx and transferred into the intestine [34]. Despite simple structures, the nematodes were composed of multiple types of organs, such as muscles, nervous system, gland cells, and so on. Meanwhile, researchers have fully mapped the complete cell lineages in the nematode body. *C. elegans* contains a total of about 20,000 genes, 40% of which have homology with human genes [38]. Moreover, nematode is the first multicellular animal whose genome has fully revealed. Additionally, *C. elegans* hermaphrodite has 302 neurons: 282 neurons in the somatic nervous system and 20 neurons in the pharyngeal nervous system [39, 40]. These neurons have different neurotransmitter characteristics, including cholinergic, dopaminergic, GABAergic, etc., which are comparable to higher animals [40]. A number of toxicology indicators including reproductive or developmental toxicity, behavioral toxicity, and neurotoxicity and molecule changes can be assayed, especially basing on various types of transgenic strains. As sensitive to contaminants, the nematode *C. elegans* is an ideal model organism, especially for NPs toxicology [34].

2 Toxicities of (Nano)Microplastics in *C. elegans*

2.1 Developmental and Reproductive Toxicity

2.1.1 Developmental Toxicity

Several studies have investigated effects of NPs or MPs on the nematode *C. elegans* [27, 41–44]. Most of these researchers used different-sized particles or microspheres of PS. For example, Lei et al. exposed nematodes to PS particles with five diameter sizes of 0.1, 0.5, 1.0, 2.0, and 5.0 μm , in the same concentration of 1 mg L^{-1} and for 48 h [44]. Their results demonstrated the significant inhibition of survival rate after exposure (Fig. 1a). Of five sizes, 1.0 μm MPs showed strongest lethality, i.e., an average reduction of 32.27% of survival rate. In another study, nematodes were exposed to PA, PE, PP, PVC, or PS particles on the surface of solid medium, with a

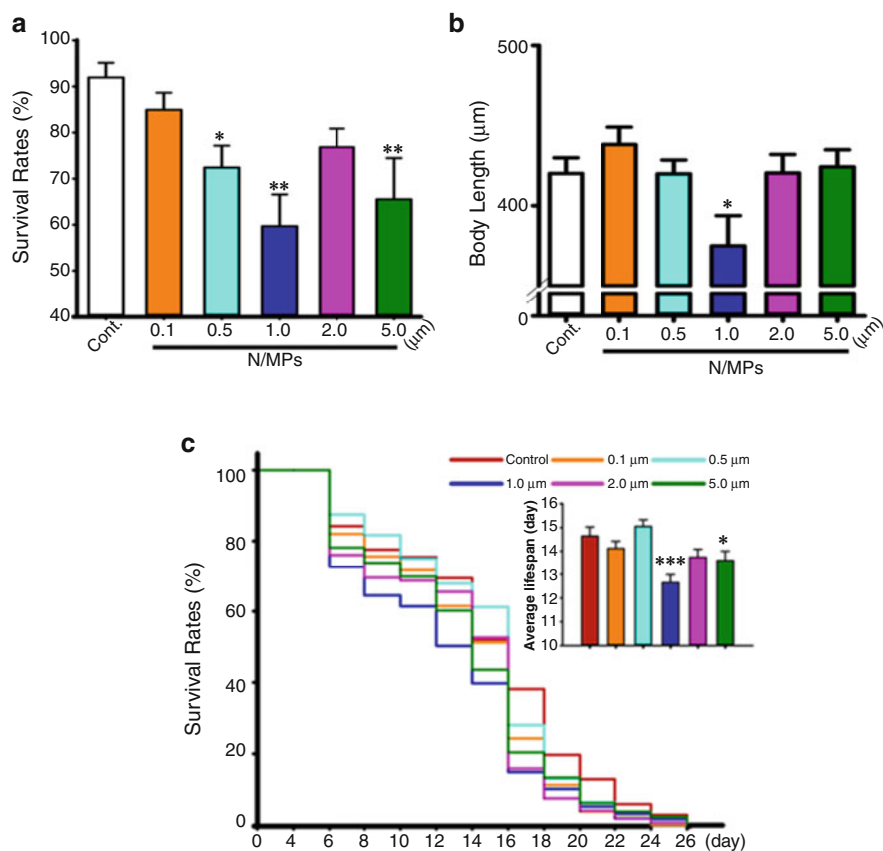


Fig. 1 Effects of different-sized PS (nano)microplastics (N/MPs) in *C. elegans* after 3-day exposure with concentration of 1 mg L^{-1} (a, survival rate; b, body length; c, life span) [44]

series concentration of 0.5, 1.0, 5.0, and 10.0 mg L⁻¹ [27]. Despite no obvious dose-effect relationship, 1.0 µm PS particles also caused the biggest reduction of survival rate of nematodes among several sizes of N/MPs. These results indicate that N/MPs can exert size-dependent lethality; 1.0 µm PS particles seem to be the most toxic to *C. elegans*. These results imply that 1 µm is an appropriate size of N/MPs to be taken in and accumulated in the digestive tract of nematodes.

Similarly, 1.0 µm PS particle exposure could induce remarkable decreases in body length of nematodes; however, there were not significant changes in other groups of 0.1, 0.5, 2.0, or 5.0 µm MPs (Fig. 1b) [44]. Lei et al. also compared the effects of different polymer types of MPs including PA, PE, PP, PVC, and PS with the same sizes [27]. They found similar toxicity of MPs on nematodes, which included slight lethality and the inhibition on the body length of nematodes. Additionally, the inhibition of nematode life span was demonstrated after exposure to PS particles in five size groups. Among them, 1.0 and 5.0 µm PS particle exposure resulted in a noteworthy decrease of average life span (Fig. 1c). In 1.0 µm PS-exposed group, nematodes presented the shortest average life span. Collectively, these studies disclosed that the toxicity of N/MPs particles was mainly dependent on the size of MPs instead of their polymer types [27].

2.1.2 Reproductive Toxicity

N/MPs exposure can result in the inhibition of reproduction of nematodes. A study investigated multiple types of N/MPs including PA, PE, PP, PVC, and PS particles on reproductive activity of nematodes [27]. Results showed five common types of MPs that induced the decrease of embryo numbers and brood size (Fig. 2). Of the exposed groups, the PP group had the lowest embryo numbers. Both the embryo numbers and brood size decreased remarkably in PE, PVC, or PS group. The biggest

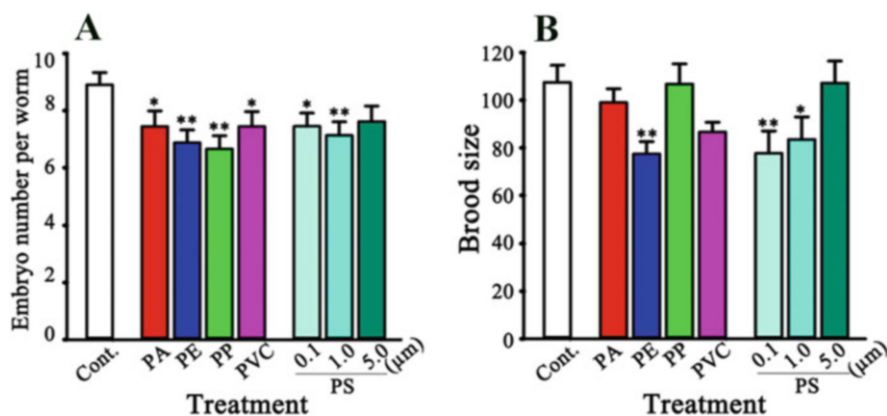


Fig. 2 Effects of PS N/MPs in *C. elegans* after exposure to 5.0 mg m⁻² different type or size particles for 2 days (a, embryo numbers; b, brood size) [27]

inhibition rates, 25.22% of embryo number and 28.02% of brood size, were found in PP and PE exposure groups. Reproductive toxicity seems to be associated with both plastic polymer and the sizes of N/MPs.

2.2 Behavioral and Neural Toxicity

2.2.1 Effects of Micro-sized PS Particles on Locomotion Behaviors

According to a recent study, exposure to 1 mg L^{-1} PS N/MPs could cause obvious changes in locomotion behaviors of the nematodes in a size-dependent manner [44]. For instance, small-sized particles (0.1 and 0.5 μm) induced the increase in the average number of head thrashes and body bends, but microscale particles of 1.0, 2.0, and 5.0 μm PS resulted in decreases of the nematodes' locomotion behavior. Furthermore, crawling movements of the nematodes were demonstrated to significantly change after exposed to PS MPs of different sizes. According to the analysis results of crawling tracks, 0.1 and 2.0 μm PS MPs induced significant increases in mean crawling speed. Moreover, angles of body bending also changed in exposed groups. Body bending angles reflect the coordination and balancing ability. A zero bending angle is an indication of no directional bias, while positive and negative body bending angles mean forward and backward bias. The results showed that 0.1 μm PS particles induced a significant decrease in body bending frequency. These results indicated that N/MPs particles could cause locomotion behavior deficits in the nematodes.

2.2.2 Effects of (Nano)Microplastics on GABAergic, Cholinergic, and Dopaminergic Neurons

Multiple types of neurons, such as GABAergic neurons, cholinergic neurons, and dopaminergic neurons, are in charge of the control of locomotion behavior in the nematode. Recently, two studies revealed the neuronal damages associated with nanopolystyrene particles in *Caenorhabditis elegans* [44, 45]. It indicates that exposure to nano-/micro-sized PS particles could be involved with neurotoxicity, which may be the mechanisms of behavioral toxicity.

In *C. elegans*, γ -aminobutyric acid (GABA) is an important inhibitory neurotransmitter, which plays an important role in motor functions. The effects of exposure to PS particles on GABAergic neurons were assayed by using the transgenic strain EG1285 (*unc-47p::gfp*), in which GABAergic neurons are visualized by the translational expression of *unc-47*. After exposure to 1.0 μm PS particles at concentration of 1 mg/L, the fluorescence intensity was significantly decreased (Fig. 3a–d), indicating the downregulated expression of *unc-47*. However, exposure to PS particles of other sizes had no impacts on the expression of *unc-47*. Besides, Qu et al. found that 100 nm PS-NPs could also induce neurodegeneration of D-type

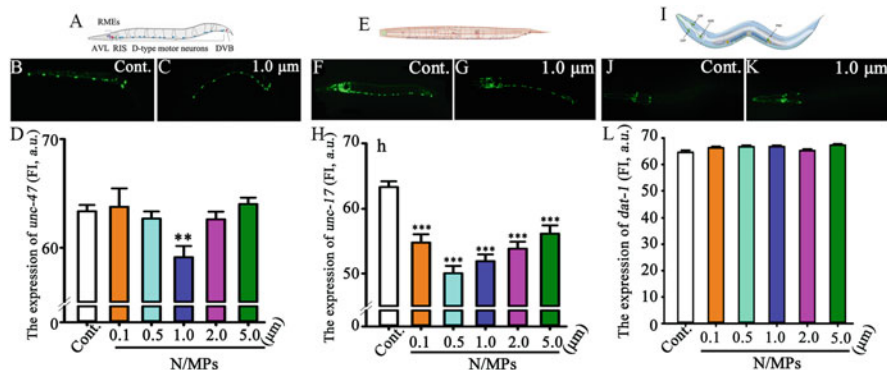


Fig. 3 Effects of PS N/MPs on *unc-47::gfp*, *unc-17::gfp*, and *dat-1::gfp* expression in EG1285, LX929, and BZ555 nematodes. The diagrammatic figure of GABAergic neurons (a), cholinergic neurons (e), and dopaminergic neurons (i). Fluorescent images of EG1285 (b, c), LX929 (f, g), and BZ555 (j, k), in the control nematodes and the nematodes exposed to 1 µm PS particles; *unc-47::gfp* (d), *unc-17::gfp* (h), and *dat-1::gfp* (l) expression pattern in control nematodes and nematodes exposed to 0.1, 0.5, 1.0, 2.0, 5.0 µm PS particles for 2 days [44]

GABAergic motor neurons in the nematodes [45]. In *C. elegans*, GABAergic neurons are comprised of RMEs, D-type neurons, RIS, AVL, and DVB. D-type neurons have responsibility for the control of ventral body and dorsal body muscles. REM neurons have control of the head. AVL and DVB control enteric movement. RIS are internuncial neurons. During the locomotion of the worm, D-type neurons suppress the contraction of ventral and dorsal body muscles. When the nematode bends its body, it will contract the muscles on the side of the body and relax the muscles on the other side of the body at the same time, enabling the nematode to keep moving in a wavy way [46–48]. The results showed that the exposure of PS particles can suppress the function of D-type GABAergic neurons. So, the special neurotoxicity may be involved with behavioral damages in crawling movement.

Cholinergic neurons can influence the posterior rhythm during the worm's forward locomotion [49]. Acetylcholine (ACh) is an important neurotransmitter in organisms, which mainly specially distributed widely in the nerve endings at neuromuscular junctions. ACh is synthesized by choline acetyltransferase (ChAT, encoded by *cha-1*) and encapsulated in synaptic vesicles by the vesicular Ach transporter (VACHT, encoded by *unc-17*) [47, 50]. In the transgenic strain LX929, cholinergic neurons can be visualized by the translation expression of green fluorescence protein (GFP) driven by the promoter of cholinergic transporter *unc-17* gene (Fig. 3e, f). After exposure to PS microparticles at the concentration of 1 mg L⁻¹, the fluorescence intensity was significantly decreased in the exposed groups [44]. It indicates the downregulated expression of *unc-17* induced by MPs. Broken and atrophied ciliated dendrites can be observed after exposure to PS particles, especially in the groups exposed to 0.5 and 1.0 µm PS particles (Fig. 3f–h). It reveals that PS particles can cause the downregulation of *unc-17* and may prevent ACh from transferring into synaptic vesicles and make the ciliated dendrites

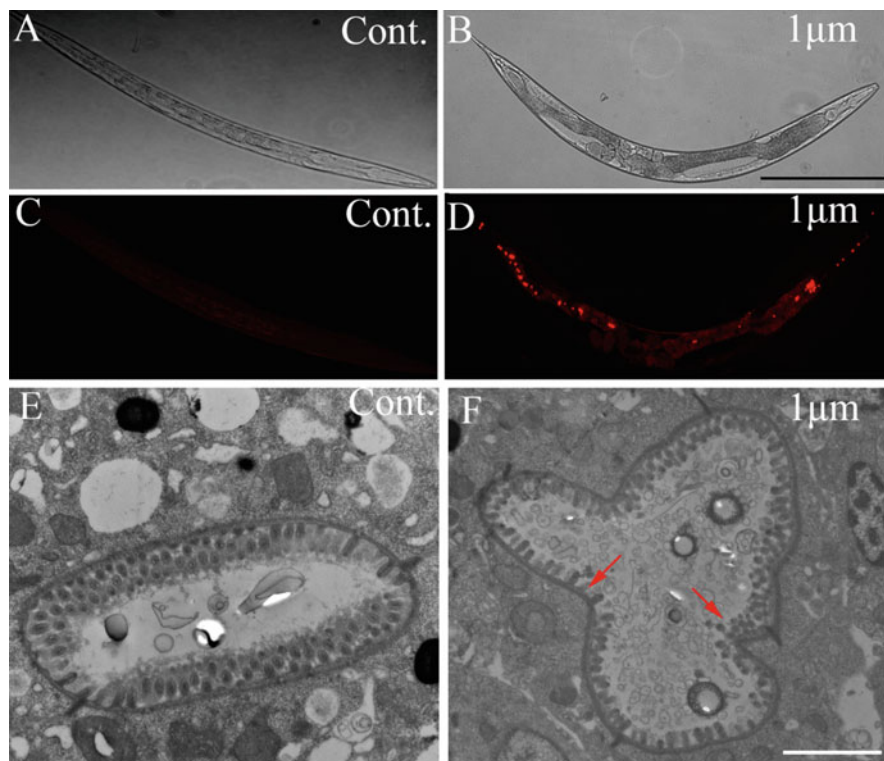


Fig. 4 Accumulation of 1 μm PS particles and intestinal damages induced by MPs exposure. (a–d) Light field (a, b) or fluorescence images (c, d) of the control and the MPs-exposed nematodes; (e–f) photomicrographs of the intestine of the control and the MPs-exposed nematodes. The red arrows indicate the intestinal damages. The black bar, 200 μm ; the white bar, 0.6 μm

broken and atrophied, causing excitatory activities in nematodes. These results support PS-induced behavior toxicity that exposure to 0.1 and 0.5 μm PS particles can induce increases in head thrashes and body.

Dopamine (DA), encoded by *dat-1*, is another important neurotransmitter regulating locomotion behavior in *C. elegans*. The neurons containing DA as the neurotransmitter are called dopaminergic neurons [36, 51]. In a recent study in our laboratory [44], the transgenic strain BZ555 (*dat-1::gfp*) was used to assay the effects of PS particles on the dopaminergic neurons. In BZ555, dopaminergic neurons are labeled by green fluorescent protein, including four cephalic (CEP) neurons, two anterior deirid (ADE) neurons, and two posterior deirid (PDE) neurons (Fig. 4i). However, after 48 h exposure to 0.1, 0.5, 1.0, 2.0, and 5.0 μm PS particles, there was no obvious change in expression of *daf-1::gfp* (Fig. 3j–l). It indicates that the N/MPs exert no or slight toxicity on dopaminergic neurons in nematodes.

2.3 Intestine Damages

2.3.1 Distribution of PS Nanoplastics in *C. elegans*

After nematodes were exposed to fluorescently labeled PS particles with sizes of 0.1, 1.0, and 5.0 μm , PS particles can be observed distributing in the digestive system, from lumen of pharynx to gut lumen and rectum. Among three sizes of N/MPs, 1.0 μm particles have the strongest fluorescence intensity in the body of nematodes. It indicates 1.0 μm PS particles can more easily accumulate in the intestine of *C. elegans* (Fig. 4a–d). So, this result supports the strongest toxicity of 1.0 μm PS particles, including developmental, reproductive, and neural toxicity. Additionally, we found noticeable damages in the nematode intestine, such as fracture of villi and the rupture of epithelial cells, especially in 1.0 μm MPs-exposed group (Fig. 4e–f). We speculate that the accumulated MPs may interact with intestinal epithelial cells through physical or chemical impacts, which further exert intestine damages.

2.3.2 Changes in Intestinal Calcium Levels of *C. elegans*

According to Lei et al., the potential effects on intestinal calcium levels were observed after exposure to PS particles (0.1, 1.0, 5.0 μm) [44]. In the KWN190 strain of *C. elegans*, the calcium indicator protein D3cpv was expressed throughout the cytoplasm of intestinal cells [27]. Results showed that 1.0 μm PS particles caused a significant decrease in intestinal calcium levels but no remarkable change in 0.1 and 5.0 μm PS groups (Fig. 5). It is consistent with size-dependent toxicity of N/MPs on intestinal damages and implies that in the activity of intestinal calcium, it is involved in the toxic mechanism.

2.4 Oxidative Stress

Oxidative stress reflects an imbalance between the production of free radicals and the ability to readily detoxify their harmful effects through neutralization by antioxidants. Oxidative damages of organisms are generally identified by assay of reactive oxygen species (ROS). The increase of ROS can cause damages in proteins, lipids, or DNA and then induce aging, diseases, or cell death [52]. Glutathione S-transferase (GST-4) is a major cellular detoxification enzyme and participates in oxidative response, which can sensitively reflect the level of oxidative stress [53]. Lei et al. used the transgenic strain CL2166 (*gst-4::gfp*) to assay the level of oxidative stress by fluorescence detection. After exposure to 0.1, 1.0, and 5.0 μm PS particles, the expression of *gst-4* was significantly increased in a size-dependent manner [27]. The expression level of *gst-4* in the 1.0 μm PS group is higher than those in the other two groups (Fig. 6). Lei et al. also investigated the oxidative stress caused by other

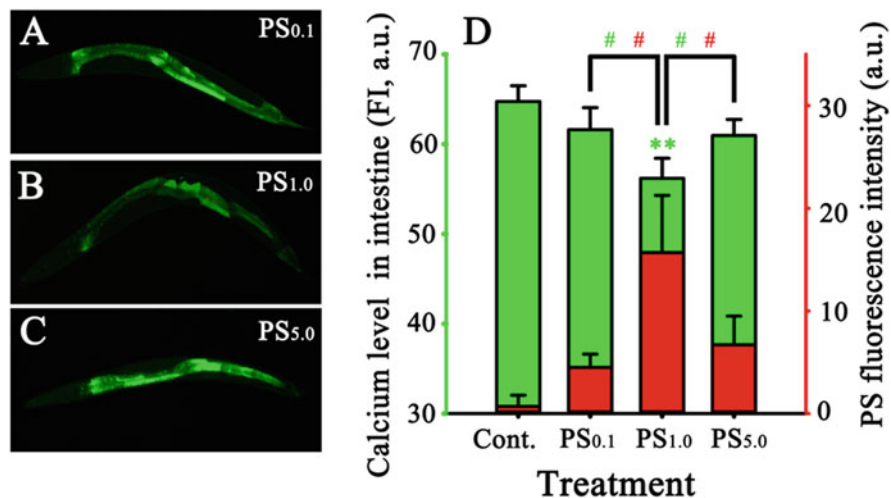


Fig. 5 Changes in calcium levels in *C. elegans*. (a–c) Calcium levels in the intestine after exposure to PS particles with different sizes. (d) Quantified values of calcium levels and PS particles accumulation in the intestine after PS exposure. Bar = 200 μm [27]

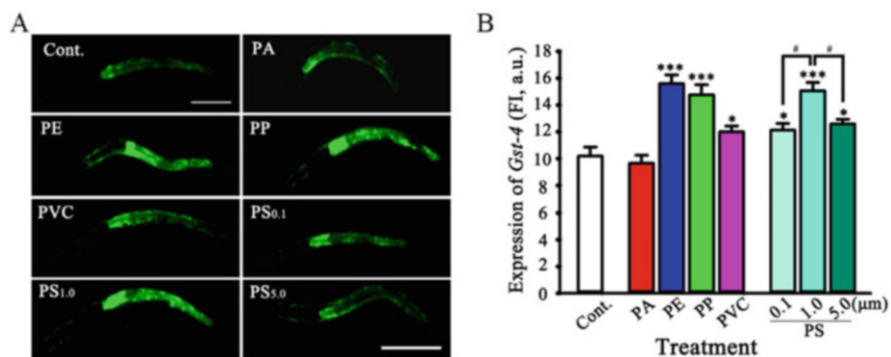


Fig. 6 Effects of MPs particles on the expression of *gst-4::gfp* in CL2166 nematodes of the control nematodes and the nematodes exposed to PA, PE, PP, PVC, and PS (0.1, 1.0, 5.0 μm) particles at concentration of 5 mg m^{-2} . (a, fluorescent images; b, expression of *gst-4::gfp*). The bar = 200 μm [27]

microplastics including PA, PE, PP, and PVC (in the size of 70 μm). The results showed that all of these MPs could cause significant increase in the expression of *gst-4*. It suggests that oxidative stress is a key characteristic of the toxicity of MPs on *C. elegans*.

Furthermore, Lei’s study showed that two natural antioxidants, curcumin and oligomeric proanthocyanidins, could decrease the elevated expression of *gst-4* induced by PS particles. Curcumin is extracted from turmeric, a traditional herbal medicine, and used as traditional medicine for curing ulceration and skin infection in

India and other countries. It was reported that curcumin could induce resistances to inflammation, oxidation, or even cancer [54]. Oligomeric proanthocyanidins are extracted from pine or other plants and have been widely used as a strong natural antioxidant [55]. The results indicate that natural antioxidants are capable of alleviating oxidative stress induced by MPs [44].

3 Mechanisms of (Nano)Microplastics' Toxicities in *C. elegans*

3.1 The Insulin Signaling Pathway

Up to now, a few studies have investigated the potential mechanisms of toxic action between N/MPs and nematodes. According to a recent study, a signal cascade of DAF-2-AGE-1-AKT-1-DAF-16-SOD-3/MTL-1/GPD-2 in the insulin signaling pathway can respond to nanopolystyrene particle exposure in *C. elegans* [56]. Insulin signaling pathway is involved in numerous life activities, such as aging, reproduction, lipid metabolism, stress response, and so on. The insulin signaling pathway contains the upstream protein DAF-2, an insulin-IGF receptor ortholog, the downstream protein DAF-16, and multiple molecules [57, 58]. Some scholars demonstrated that the depression of DAF-2 pathway can induce a resistance to heat or oxidative stress, in order to protect animals from oxidative damage [59]. Shao et al. found polystyrene NPs could induce an obvious ROS production and the decrease in locomotion behavior in wild-type nematodes [56]. These toxic actions are closely related to the decreased expressions of *daf-2*, *age-1*, and *akt-1* and the increased expression of *daf-16*. Furthermore, the expression of *daf-16* was translocated from the cytoplasm to nuclei. Mutation of *daf-2*, *age-1*, or *akt-1* could significantly suppress ROS production and behavioral deficits, after the mutant nematodes being exposed to NPs, but the mutation of *daf-16* resulted in a significant increase in ROS production. These results indicate that mutation of *daf-2*, *age-1*, or *akt-1* can induce a resistance to the toxicity of nanopolystyrene particles but mutation of *daf-16* enhances a toxic susceptibility. Moreover, the resistance induced by mutation of *daf-2*, *age-1*, or *akt-1* can be suppressed by RNAi knockdown of *daf-16*. Additionally, they found that the intestine-specific activities of DAF-2, AGE-1, AKT-1, and DAF-16 could regulate the toxicity of nanopolystyrene particles in the nematodes. These results indicate that the signaling cascade of DAF-2-AGE-1-AKT-1-DAF-16 in the insulin signaling pathway is involved in a protective response to the toxicity of nanopolystyrene particles.

In another study, researchers detected the expression of *sod-3*, *mtl-1*, and *gpd-2* gene in the intestine of nematodes and found the target gene of *daf-16* specially responded to nanopolystyrene exposure. As a superoxide dismutase, SOD-3 is involved in superoxide radical's removal in order to protect against oxidative stress [60]. MTL-1 is a metallothionein and responsible for metal detoxification and stress

adaption [61], while GPD-2 is a glyceraldehyde-3-phosphate dehydrogenase in organisms [62]. After exposure to nanopolystyrene particles, the mutation of *daf-16* could cause significantly decreases in expression of *sod-3*, *mtl-2*, and *gpd-2*; intestine-specific RNAi knockdown of these three genes could result in increase of ROS production. Furthermore, resistance to toxicity of nanopolystyrene in the transgenic strain over pressing *daf-16* could be suppressed by RNAi knockdown of these three genes. Therefore, SOD-3, MTL-1, and GPD-2 are the downstream targets of DAF-16 and play an important role in the protective response to the toxicity of NPs through the insulin signaling pathway.

3.2 The Protective Response Mediated by the Intestinal p38 Signaling

Using the model organism nematodes, Qu et al. investigated that a protective response to nanopolystyrene particles. They found the special protective response mediated by p38 mitogen-activated protein kinase (MAPK) signaling pathway, which could activate the endoplasmic reticulum unfolded protein response (UR EPR) [41]. In *C. elegans*, PMK-1 p38 MAPK signaling pathway is responsible for the regulation of oxidative stress response [63]. Stress can induce misfolding and aggregation of proteins, which will disrupt the protein homeostasis and make adverse effects on cellular viability. Eukaryotic cells have evolved specific signaling pathways known as unfolded protein responses to protect themselves from proteotoxicity, including heat shock response, endoplasmic reticulum unfolded protein response, and mitochondrial unfolded protein response [64]. So, p38 mitogen-activated protein kinase signaling pathway is an important mechanism that protects nematodes' cells from the toxic action of NPs.

Prolonged exposure to 100 nm nanopolystyrene particles ($\geq 1 \mu\text{g L}^{-1}$) resulted in severe induction of ROS production and decreases in locomotion behavior [41]. In the p38 MAPK signaling pathway, NSY-1-SEK-1-PMK-1 is a classic signaling cascade. Under conditions of NPs exposure, PMK-1 needs to be phosphorylated in order to activate the p38 MAPK signaling. According to a recent study, the expression and phosphorylation level of *pmk-1* was significantly increased in nematodes after prolonged exposure to 100 nm nanopolystyrene particles at the predicted environmentally relevant concentration ($1 \mu\text{g L}^{-1}$). In addition, elevated toxicity susceptibility to nanopolystyrene was proved in *pmk-1(km25)* mutant nematodes. Though PMK-1 can be expressed in neurons and intestine cells, only mutation of intestine-specific PMK-1 can suppress the susceptibility of NPs-induced toxicity. It indicates that intestinal PMK-1 is the regulator of the response to nanopolystyrene particles in *C. elegans*. Exposure to 100 nm polystyrene particles ($1 \mu\text{g L}^{-1}$) can also induce the increased expression of *atf-7* and *skn-1*; both genes are considered as the downstream targets of PMK-1. In *pmk-1(km25)* mutant nematodes, the NPs-induced expression of *atf-7* and *skn-1* can be significantly decreased; intestine-specific RNAi

knockdown of *atf-7* or *skn-6* can increase ROS production. When PMK-1 in the intestine of nematodes was overexpressed, the nematodes can obtain a resistance to the toxicity to NPs. RNAi knockdown of *atf-7* or *skn-1* can also suppress the resistance to NPs. These results indicate that *atf-7* and *skn-1* are downstream of *pmk-1* in the response to nanopolystyrene particles. Collectively, current studies suggest that the intestinal signaling cascade of PMK-1-ATF-7-XBP-1 and PMK-1-SKN-1-XBP-1/GST-5 can regulate the responses to nanopolystyrene particles in *C. elegans*. It may be a pivotal mechanism involved in biota's response to N/MPs; however it needs more research in higher animals.

3.3 Other Mechanisms

In recent years, several studies on the behavioral and neural toxicities of N/MPs to nematode *C. elegans* have been done. All these studies show that exposure to PS N/MPs can induce changes in locomotion behaviors and neuronal damages [44], but only a part of the mechanism of the neurotoxicity of N/MPs has been revealed. Qu et al. found that there is an association between the neurotoxicity of PS-NPs and changes in autophagy induction in nematodes [45]. Autophagy is a pathway for intracellular macromolecules degradation, which can be activated by toxicants and have the capacity to protect organism against neurotoxicity [65, 66]. Since *lgg-1* is a key regulator of autophagy [67], Qu et al. used LGG-1::GFP as the marker to investigate the effects of NPs on autophagy. The results showed that exposure to PS-NPs ($1,000 \mu\text{g L}^{-1}$) could induce a decrease in autophagy induction and could result in behavioral deficits and damages in D-type GABAergic motor neurons at the same time. Moreover, RNAi knockdown of *lgg-1* could induce a susceptibility to the neurotoxicity of PS-NPs on the development and function of D-type GABAergic motor neurons. These results imply that the damages on D-type neurons induced by exposure to PS-NPs are related with the decrease in autophagy induction.

The molecular response to nanoplastics still remains largely unknown in organisms. Qu et al. employed *C. elegans* exposed to PS-NP (100 nm, $1 \mu\text{g L}^{-1}$) to investigate the long noncoding RNAs (lncRNAs). They found that 37 lncRNAs were dysregulated, among which 22 lncRNAs were downregulated and 15 lncRNAs were upregulated [68]. Focused on the known lncRNAs (downregulated *linc-7*, *linc-50*, and *linc-169*; upregulated *linc-2*, *linc-9*, *linc-18*, *linc-32*, and *linc-61*), they examined their dynamic expression in PS-NP. Both the decreasing expression of *linc-7*, *linc-50*, and *linc-169* and increasing expression of *linc-2*, *linc-9*, *linc-18*, *linc-32*, and *linc-61* were dose-dependent in nematodes exposed to PS-NP ($1-100 \mu\text{g L}^{-1}$). Moreover, with intestinal reactive oxygen species (ROS) production and locomotion behavior sited as the endpoints, they conducted the effects of RNA interference (RNAi) knockdown of *linc-2*, *linc-7*, *linc-9*, *linc-18*, *linc-32*, *linc-50*, *linc-61*, and *linc-169* in nematodes. In results, the RNAi knockdown of *linc-2*, *linc-7*, *linc-9*, *linc-18*, *linc-32*, *linc-50*, *linc-61*, and *linc-169* in nematodes without PS-NP exposure did not induce the obvious intestinal ROS production and locomotion behavior;

however, compared with nanopolystyrene-exposed wild-type nematodes, nanopolystyrene-exposed *linc-2*, *linc-9*, or *linc-61* (all RNAi) nematodes were observed with the more severe ROS production and decreasing locomotion behavior; nanopolystyrene-exposed *linc-18* or *linc-50* (all RNAi) nematodes were observed with the inhibition of ROS production and increase of locomotion behavior. Among these five studied lincRNAs, *linc-2*, *linc-9*, *linc-50*, and *linc-61* alterations mediated a protective response to PS-NP, and the alteration of *linc-18* possibly mediated the toxicity of PS-NP, which is suggested by a further study associated with their biological processes and signaling pathways.

Qu et al. also observed the response of microRNAs (miRNAs) to PS-NP (100 nm, $1 \mu\text{g L}^{-1}$) [69]. After exposure, seven miRNAs were dysregulated by PS-NP (*mir-39*, *mir-76*, *mir-794*, and *mir-1830* downregulated; *mir-35*, *mir-38*, and *mir-354* upregulated). According to the phenotypic analysis of both transgenic strains and mutant nematodes, *mir-35*, *mir-38*, *mir-76*, *mir-354*, and *mir-794* were found to be involved in the response to PS-NP. The expression of all these seven miRNAs above was dose-dependent in nematodes exposed to PS-NP ($1\text{--}100 \mu\text{g L}^{-1}$). The previous study on the function of insulin signaling pathway has shown its response in PS-NP, and meanwhile the KEGG analysis suggested that *mir-794* could mediate in the insulin signaling pathway, which also reveals a possible molecular response pathway candidate by *mir-794* and insulin signaling. Additionally, *mir-35*, *mir-38*, and *mir-354* may influence the Wnt signaling pathway, a related pathway of controlling toxicity induction of several environmental toxicants such as graphene oxide [70]. In particular, overexpression of *mir-354* could decrease the expression of *cwn-1* which encodes a Wnt ligand. These results confirmed that *mir-354* could be an intervent to the function of Wnt signaling pathway in response to PS-NP in nematodes.

4 Summary

In this chapter, we have reviewed research progress in the toxicology of N/MPs using the model nematode *C. elegans*. Several studies have revealed that both NPs and MPs can cause multiple toxicity including lethality, reproductive and developmental toxicity, alteration of locomotion behavior, neurotoxicity, intestine damages, and ROS production in nematodes. These effects on nematodes were obviously both dose-dependent and size-dependent with (nano)microplastics. PS-MPs in the concentration ranging from $1 \mu\text{g L}^{-1}$ to $100 \mu\text{g L}^{-1}$ could induce multiple adverse effects [42, 56]. According to these studies, similar-sized MPs in different polymer types (PA, PP, PE, and PVC) showed nearly same toxicity in *C. elegans* [27, 44]. It indicates that the toxicity of MPs is closely dependent on their size, rather than their composition.

Up to date, the majority of researchers used pure PS spheres to carry out toxicology studies of NPs. However, the real environment includes various types of MPs or NPs in different chemical compositions or different shapes. Furthermore, environmental (nano)microplastics contain a variety of additives and other absorbed

pollutants such as hydrophobic organic chemicals [71]. Therefore, future toxicology researches need to focus on real environment-character N/MPs in the environmental relevant concentration level. Additionally, more studies need to reveal the toxic action mechanisms of MPs, especially about NPs' toxicity and its cellular or molecular pathway.

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Uptake of Microplastics and Their Effects on Plants



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Abstract Microplastics (MPs, 100 nm to 5 mm) may represent a risk to ecosystem and human health, and MP pollution has become a topic of global environmental

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concern. Despite many reports on the accumulation of MPs in aquatic species, research on terrestrial ecosystems is relatively scarce, and there is very little information on the uptake and accumulation of MPs by plants. In this chapter we review the published research on potential single effects and on combined effects of MPs with other pollutants such as organic and nano-sized pollutant MPs in aquatic plants including microalgae and macrophytes. We focus on recent studies on the accumulation of MPs and their potential effects on crop plants. In this chapter we also discuss the mechanisms and factors affecting MP accumulation in crop plants. Finally, we conclude by pointing to knowledge gaps and suggesting key future areas of research. This review provides a new basis for further research on MP accumulation and its potential effects on plants. Future studies are required on the accumulation and translocation of submicron and even micron-sized MPs in edible plants and their potential impacts on food safety.

Keywords Accumulation, Crop plants, Food safety, Microplastics, Uptake

Plastics are used in every stage of our lives. It is no wonder that about 330 million tons of plastics were produced worldwide in 2015 and this figure is rising [1]. As a result, contamination of our environment with plastics of all sizes is becoming one of the most widespread and long-lasting anthropogenic changes to the surface of our planet [2]. Primary microplastics (MPs, i.e., microscopic plastics of size 100 nm–5 mm) are originally manufactured in a particularly small size for specific applications, while secondary MPs originate from the fragmentation of larger plastic debris by external forces [3]. Although the oceans represent an ultimate sink for MPs, the terrestrial environment is a major recipient of plastic pollutants of all sizes owing to large amounts of anthropogenic litter from application of sewage sludge and organic fertilizers, plastic mulching, irrigation, and flooding as well as other sources such as atmospheric deposition [4–6]. Based on emission data, it is estimated that 110,000 and 730,000 t of MPs are added annually to agricultural land in Europe and North America, respectively [7]. This figure exceeds the estimated annual global burden of MPs in ocean surface waters, i.e., 93,000–236,000 t [8]. Nevertheless, plastics in the terrestrial compartment have been little studied despite the large amounts present in this compartment. Hence, there is a strong need for further work to be carried out regarding assessing the distribution, fate, and transformation of MPs in the terrestrial environment. Plastic waste is recognized as a severe global problem in agricultural soils, and the use of plastic film mulches is one example of such a large-scale application, especially in China [9–11].

There are numerous reports of MPs detected in aquatic species, suggesting that MPs are ubiquitous in the aquatic food chain, especially in marine organisms [12–15]. However, there is very little information on the accumulation and effects of MPs on higher plants. This review presents a discussion of the potential effects and the accumulation of MPs in aquatic and terrestrial plants. We conclude by pointing to knowledge gaps and suggesting key future areas of research for scientists and policy makers.

1 Toxic Effects of MPs in Aquatic Plants

1.1 MP Effects on Aquatic Plants

Knowledge on the effects of MPs on aquatic plants in both marine and freshwater ecosystems remains limited. Previous studies on this issue have been restricted to the aquatic phytoplankton (microalgae), usually focusing on the growth dynamics of phytoplankton after exposure to MPs. It seems that the toxicity of MPs to phytoplankton varies with many factors such as particle size [16], polymer type [17], concentration of MPs [18], exposure time, and target species [19].

1.1.1 Marine Microalgae

Previous studies on marine ecosystems show that MP exposure may result in a significant reduction in the growth of microalgae [20–22], and the inhibitory effects are enhanced with increasing exposure dosage [18]. Zhang et al. [16] reported that exposure to polyvinyl chloride (PVC) microspheres resulted in significant reduction in the chlorophyll content and photosynthetic efficiency of *Skeletonema costatum*. However, when the size reached the millimeter scale, MPs had no significant effects on the growth of marine microalgae [16].

1.1.2 Freshwater Microalgae

Recently it has also been found that MP exposure is able to induce toxic effects including reduction in population growth, decrease in photosynthetic activity, increase in reactive oxygen species production, and overexpression of genes involved in sugar biosynthesis in freshwater microalgae [17, 18]. It should be kept in mind that the environmental relevance and toxicity mechanisms remain unknown. Thus, considering the important role that phytoplankton plays in aquatic food webs and the rapid growth of quantities of MPs in aquatic environments, further investigation is needed to elucidate how MPs affect the survival, growth, and functioning of these aquatic primary producers.

1.1.3 Aquatic Macrophytes

The effects of MPs on freshwater species such as macroinvertebrates or fish have been investigated in some recent studies [20, 23–25], but we are aware of only very limited studies on free-floating macrophytes [26] which are very important in aquatic ecosystems. In one study there was some indication that MPs may have negative effects on the root length of *Lemna minor* under laboratory conditions [26]. In another study, van Weert et al. [27] provide the first evidence of the effects of NPs

and MPs on the growth of two sediment-rooted macrophytes, *Myriophyllum spicatum* and *Elodea* sp.

1.2 Possible Mechanisms of Toxicity

1.2.1 Physical Damage

MPs in the environment have different shapes and rough edges, and it is easy to induce physical damage to algae cells, such as cell wall damage, cell maturation cracking, etc. This destroys the integrity of the cell structure and eventually causes algae cell death. Zhang et al. [16] found that PVC with an average diameter of 1 mm can be adsorbed on *Skeletonema costatum* cells and be embedded in the cell wall, causing damage to the cell wall and cell membrane. Mao et al. [18] have also observed clearly morphological changes including unclear pyrenoid, plasma detached from the cell wall, deformed thylakoids, and cell wall thickening [18].

1.2.2 Shading Effect

Some authors have observed MPs adsorbed on the surfaces of algal cells that are associated with growth inhibition of microalgae [20, 21, 28, 29]. These observations are often attributed to shading and agglomeration. Schwab et al. [30] believe that the shading effect of particles can reduce the photosynthetic efficiency of algae. Polystyrene (PS) NPs at relatively high concentrations ($0.1\text{--}1\text{ g L}^{-1}$) have been shown to cause severe damage to freshwater microalgae in terms of growth inhibition, decreased chlorophyll levels [20], reduced photosynthetic activity, and enhanced production of reactive oxygen species (ROS) [31]. Their adhesion on algal surfaces has been observed [31–33], in particular for the positively charged amino-modified PS NPs (PS-NH₂), and recognized to be the cause of the observed toxicity. However, experiments conducted by Zhang et al. [16] show that shading has a negligible effect on the photosynthesis of algae cells. A similar result was obtained by Sjollemma [22].

1.2.3 Oxidative Stress

MPs can also cause oxidative stress in algal cells, resulting in production of reactive oxygen species (ROS), which in turn are responsible for oxidative stress [18, 31]. The increase in the intracellular ROS content will then lead to lipid peroxidation, cell membrane skeletal collapse, cell distortion, and loss of cell membrane permeability, affecting the exchange of energy inside and outside the cell.

1.3 Joint Toxicity to Aquatic Plants of Combined Exposure to MPs and Other Pollutants

1.3.1 MPs and Organic Pollutants

MPs are strong adsorbents for hydrophobic toxic pollutants and may affect their fate and toxicity in the environment. However, knowledge on the combined effects of MPs and associated pollutants on primary producers is still scant. Prata et al. [34] investigated the toxicity of procainamide and doxycycline to the marine microalga *Tetraselmis chuii* and found that toxicity increased after the addition of MPs. The reduction ratios of chlorophyll content, photosynthetic rate, and growth rate of seawater microalgae increased significantly upon the addition of MPs, as compared to treatments with the two pollutants separately [34]. PS particles reduced the toxicity of triphenyltin (TPT) to the marine diatom *Skeletonema costatum* [35]. In another report the presence of 0.55 μm PS reduced the bioavailability of triphenyltin chloride (TPTCl) but increased the toxicity of TPTCl to the green alga *Chlorella pyrenoidosa* [36]. In the case of other chemicals, the presence of MPs may decrease contaminant toxicity. Due to the strong sorption of glyphosate to PS-NH₂ nanospheres, an antagonistic effect on the growth of the alga *Microcystis aeruginosa* was observed by Zhang et al. [37]. Recently, some effort has also been made to explore the influence of di-n-butyl phthalate (DBP) on the physiology and biochemistry of lettuce in the presence of MPs and the response mechanisms of lettuce to DBP in the presence of MPs [38]. It was found that the addition of exogenous MP increased the growth inhibition rate of DBP, the degree of inhibition of photosynthesis, and the accumulation of ROS in lettuce [38].

1.3.2 MPs and Inorganic Pollutants

To date, few studies have investigated the consequences of exposure to combined MPs and metals, suggesting a general lack of knowledge on combined interactions, especially concerning NPs [39–44]. In fact, chemicals and potentially toxic metal loads on MP surfaces may be enriched up to 10⁶-fold compared to those in the surrounding seawater [45]. Due to this phenomenon, marine organisms near MPs are subjected to very high concentrations of diverse pollutants. Researchers have investigated the interaction between Cu and PS NPs and their single and combined effects on the freshwater alga *Raphidocelis subcapitata*. The findings show that Cu ions are not likely to adsorb to –COOH-functionalized PS NPs in freshwater media. Consequently, they do not alter Cu toxicity to algae. Similarly, EC₁₀, EC₂₀, and EC₅₀ of Cu in growth inhibition of the alga *Tetraselmis chuii* also did not differ from the corresponding EC_x during exposure to Cu in the presence of PE microspheres [40].

1.3.3 MPs and Nano-sized Pollutants

The marine environment has been considered as a “sink” for various pollutants, including MPs and nanomaterials. The individual and combined toxicological effects of MPs and nanomaterials in the freshwater zooplankton species *Daphnia magna* were observed [46]. However, information resulting from the simultaneous exposure of microalgae to MPs and mixtures of nanomaterials is very limited. Plain and aminated polystyrene MPs were found to enhance the toxicity of TiO₂ NPs to the marine alga *Chlorella* sp., which was further validated with oxidative stress determination studies of reactive oxygen species and lipid peroxidation assays [47]. Negatively charged carboxylated polystyrene MPs decreased the toxicity of TiO₂ NPs, likely due to hetero-aggregation between TiO₂ NPs and MPs in the system. The toxicity data obtained for the mixture were further corroborated with Abbott’s mathematical model [47]. In another laboratory bioassay, *T. chuii* cultures were exposed for 96 h to ~5-nm-diameter gold nanoparticles (AuNP) and to virgin 1–5- μ m-diameter MPs, alone and in mixture [48]. This mixture was more toxic to *T. chuii* than its individual components. Overall, the results of the study indicate that MP and AuNP have a relatively low toxicity to *T. chuii*, but toxicity increases when they are in mixtures containing high concentrations of both substances.

2 Accumulation of MPs and Potential Effects on Terrestrial Plants

2.1 Uptake and Transport of MPs in Crop Plants

MPs are not generally expected to be transported into plant tissues as it is expected that their large molecular weight would prevent them from passing through plant cell walls. However, NPs can and do get inside plant cells according to a study using tobacco plant cells which shows that 20- to 40-nm nanopolystyrene beads were taken up, but 100-nm beads were not [49]. To date, studies focusing on the bioaccumulation of MPs in crop plants are lacking, and this is surprising considering the potential impact that the bioaccumulation of MPs could have on human health (21).

In a recent study, the uptake of two sizes of PS microbeads (0.2 and 1.0 μ m, Fig. 1) and then their distribution and migration in the edible plant lettuce were firstly investigated based on laboratory experiments [50]. Li et al. [50] used fluorescent markers (Nile blue and 4-chloro-7-nitro-1, 2, 3-benzoxadiazole, which emit red and green fluorescence signals, respectively, Fig. 2) to track PS beads in plant tissues. These two fluorescent dyes produce emission signals that are distinguishable from the autofluorescence background of plant tissues, and this was found to be a sensitive and reliable detection method (Fig. 3). Sections from untreated control lettuce

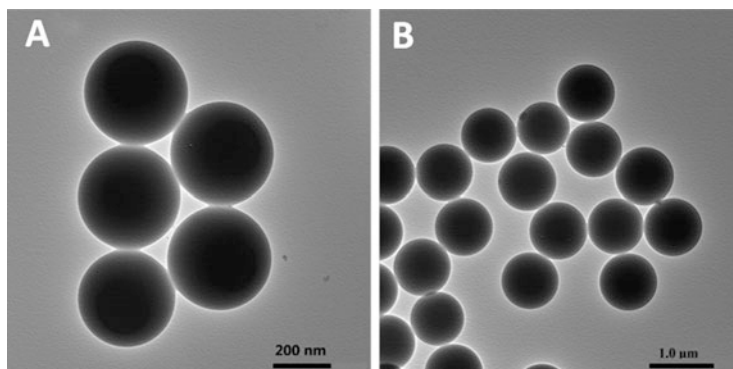


Fig. 1 Transmission electron microscope (TEM) images of two sizes of polystyrene microbeads

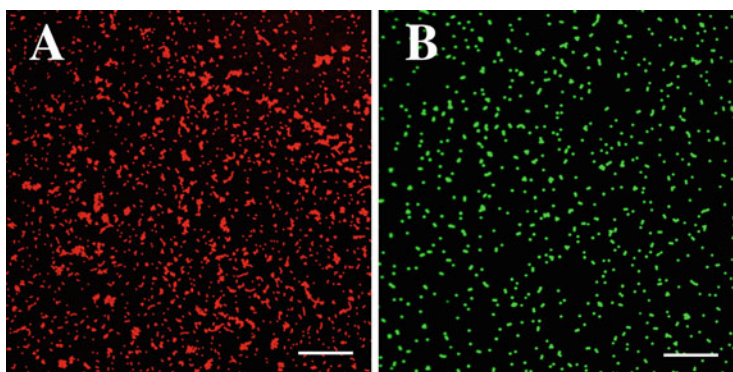


Fig. 2 Confocal images of 0.2- μm red (a) and green (b) fluorescently labeled polystyrene (PS) microbeads. Bar = 100 μm

showed no autofluorescence (Fig. 4). When roots were treated with fluorescently labeled PS microbeads, the microbeads could be identified by their fluorescence.

From the confocal images, it can be observed that the 0.2- μm PS luminescence signals were mainly located in the vascular system and on the cell walls of the cortical tissue of the roots, indicating that the beads passed through the intercellular space via the apoplastic transport system [50] (Fig. 5). Once inside the central cylinder, the 0.2- μm PS beads were transferred from the roots to the stems and leaves via the vascular system following the transpiration stream (Fig. 5). Li et al. [50] also observed that the PS beads adhered to one another and self-assembled systematically into clusters in the intercellular space of the root and stem vascular tissue of lettuce. In contrast to the root and stem, PS beads were dispersed in the leaf tissue (Fig. 6). Here, the researchers showed that higher plants can take up submicrometer-sized (0.2- μm) plastics and translocate these plastics from roots to shoots. By contrast, the uptake of 1.0- μm microbeads was found to be insignificant in lettuce roots (Fig. 7). These findings highlight the previously underappreciated

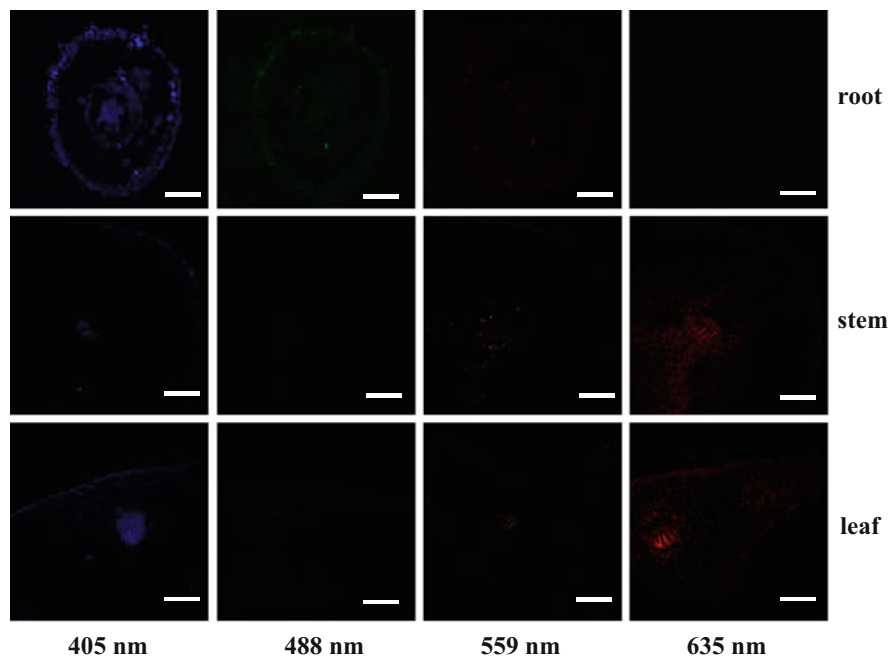


Fig. 3 Confocal images of cross sections of the lettuce root, stem, and leaf with various excitation wavelengths. Bar = 100 μ m

human exposure pathway to MPs through the consumption of contaminated crops and emphasize the need for new management strategies to control the release of MP waste products into the terrestrial environment. Ultimately, the potential impacts of low-range-sized MPs on the food safety of crop plants and human health need to be urgently considered.

2.2 *Mechanisms and Factors Affecting MP Accumulation in Crop Plants*

The mechanisms underlying the internalization of PS beads in lettuce plants remain poorly understood. Li et al. [50] observed that microbeads were extracellularly trapped in the root cap mucilage (Fig. 8), allowing the beads to adhere strongly to the root surface, as they suggest, which facilitated the internalization of particles. A “dark green tip” was usually visible to the naked eye (Fig. 9). The lettuce root cap can secrete large amounts of mucilage to defend the plant against pathogen attacks [51]. Mucilage is a highly hydrated polysaccharide, likely a pectic substance.

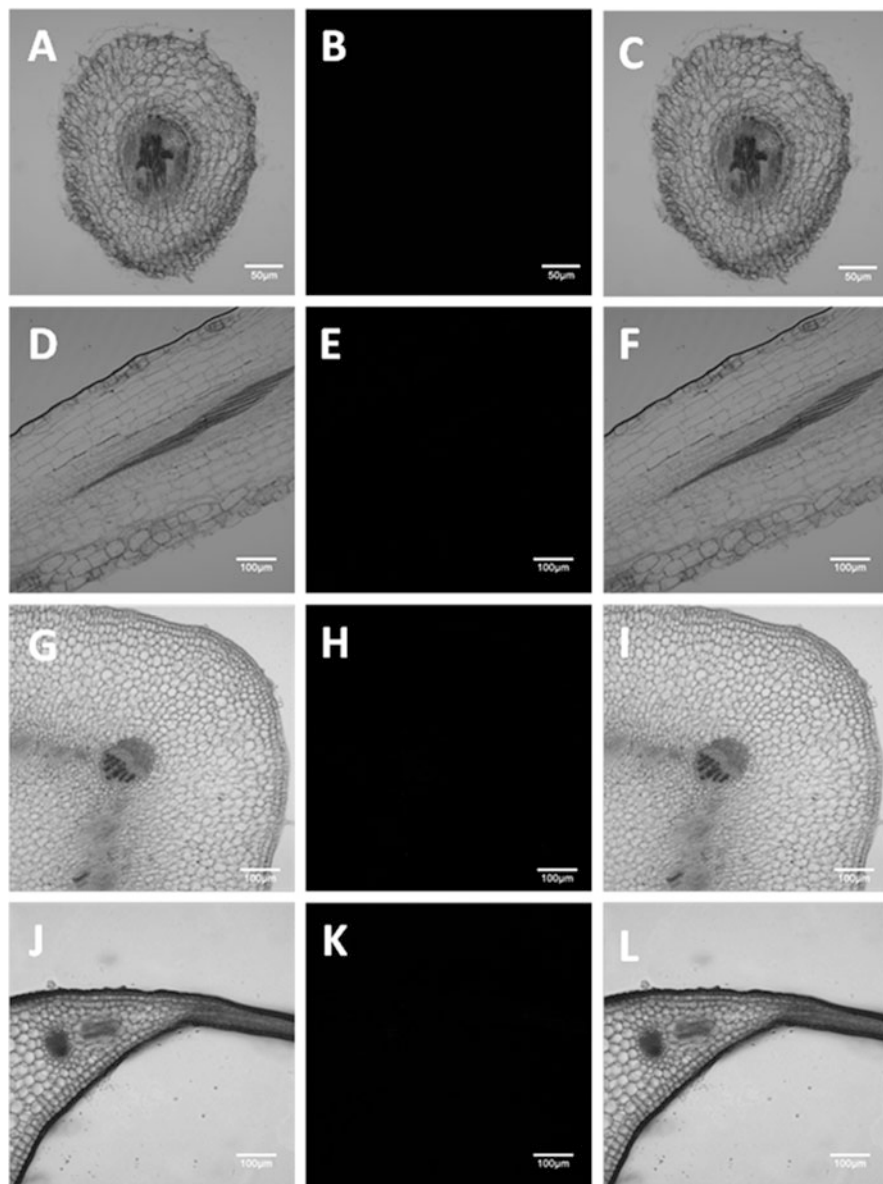


Fig. 4 Confocal images of cross and longitudinal sections of lettuce root treated for 14 days without fluorescently labeled polystyrene (PS) microbeads. Images (a), (d), (g), and (j) are the corresponding merged images of images (b) and (c), (e) and (f), (h) and (i), and (k) and (l)

However, the negative charges on the microbead surfaces did not prevent interactions with the roots, whose surface and mucilage are also likely to be negatively charged [52]. Furthermore, light microscopy and SEM observations of the plant

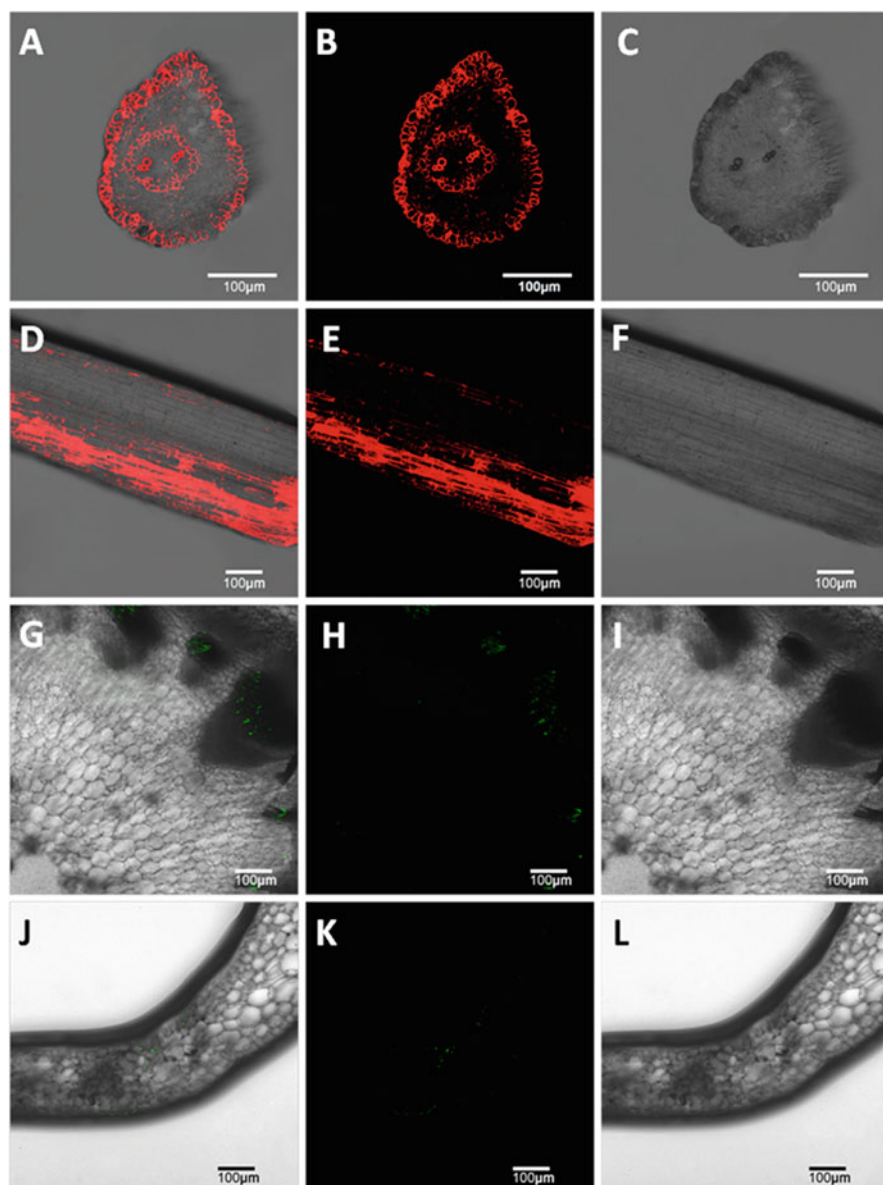


Fig. 5 Confocal images of cross (**a**, **b**, **c**) and longitudinal (**d**, **e**, **f**) sections of lettuce root, stem (**g**, **h**, **i**), and leaf (**j**, **k**, **l**) treated for 14 days with a 50 mg L^{-1} suspension of $0.2\text{-}\mu\text{m}$ fluorescently labeled polystyrene (PS) microbeads. Images (**a**), (**d**), (**g**), and (**j**) are the corresponding merged images of image (**b**) and (**c**), (**e**) and (**f**), (**h**) and (**i**), and (**k**) and (**l**)

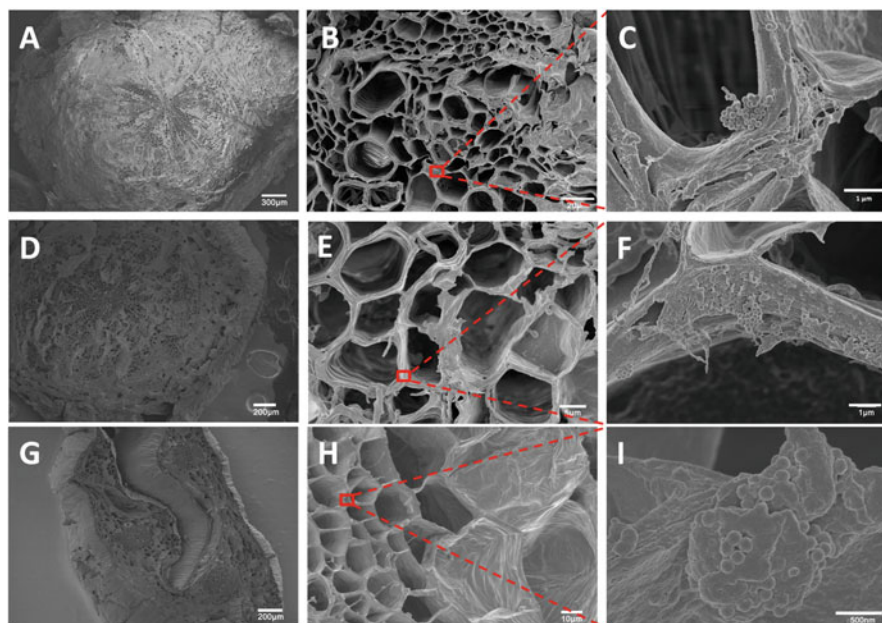


Fig. 6 Scanning electron microscope (SEM) images of 0.2- μm polystyrene microbead localization in the root and leaf of a lettuce plant. The 1-month-old lettuce plants were exposed to PS beads for 14 days. (a–f) The microbeads were detected as systematically aggregated clumps in a “grape-like” shape in the root. (i) PS microbeads were observed and dispersed in the leaf vein of the plant. Images (c), (f), and (i) show an enlargement of the pane in (b), (e), and (h), as indicated by the red square

roots (Fig. 8) reveal that the washing procedures prior to analysis did not remove all the PS beads present on the root surface, indicating that the beads adhered strongly to the root surface. Hence, in addition to the role of mucilage, it is proposed that hydrophobic interactions dominate PS microbead adhesion at the root surface owing to the high hydrophobicity of both the PS beads and the cell wall [53].

Confocal images of the cross and longitudinal sections of lettuce reveal that the PS luminescence signals were mainly located in the vascular system of the roots and stems (Fig. 5). Once inside the central cylinder, nanoparticles can move toward the aerial parts of a plant through the xylem, following the transpiration stream. Nanoparticles have been shown to move with transpiratory water [54], and their uptake rates are correlated with plant transpiration [55–57].

2.2.1 Size

Lettuce plants were grown in hydroponic systems containing 50 mg L^{-1} suspensions of different sizes of microbeads to determine whether the plants were able to take up

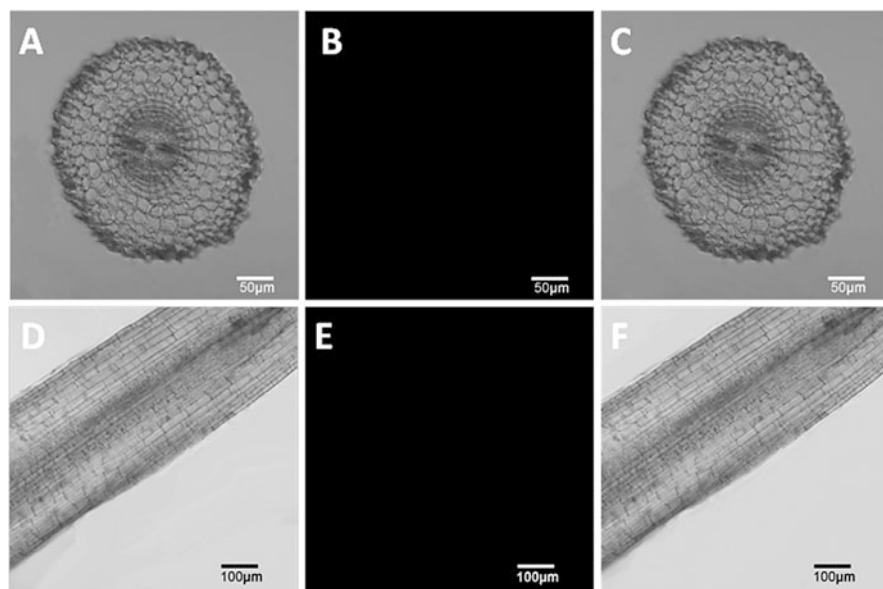


Fig. 7 Confocal images of cross and longitudinal sections of lettuce root treated for 14 days with a 50 mg L^{-1} solution of $1.0\text{-}\mu\text{m}$ fluorescently labeled polystyrene (PS) microbeads. Images (a) and (d) are the corresponding merged images of image (b) and (c) and (e) and (f). No red fluorescence was observed in cross sections or longitudinal section of the lettuce root, indicating no or very few PS beads accumulated in root tissue

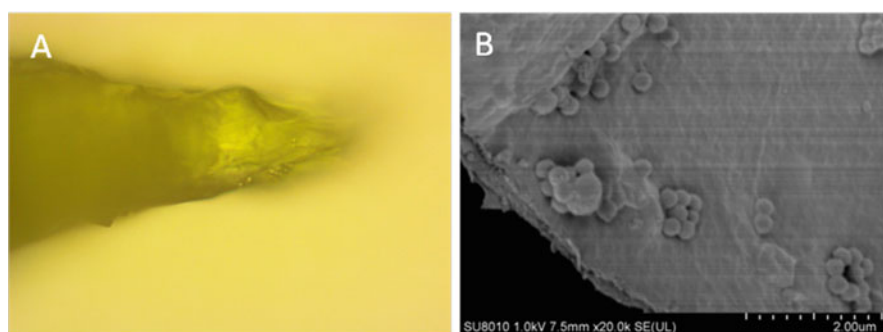


Fig. 8 Optical microscope image (a) and scanning electron microscopy image (b) of $0.2\text{-}\mu\text{m}$ polystyrene microbeads trapped by the root cap of a lettuce plant

and translocate PS beads. Fluorescence was observed in the roots, shoots, and leaves exposed to $0.2\text{-}\mu\text{m}$ beads (Fig. 5), thus providing evidence for the uptake of microbeads by terrestrial plants. Very little luminescence was observed in the vascular system or epidermis of roots exposed to $1.0\text{-}\mu\text{m}$ beads (Fig. 7). This is similar to results reported by Jiang et al. [58] as these authors found that a larger



Fig. 9 Root tips of lettuce plants treated with a 50 mg L^{-1} suspension of fluorescently labeled $0.2\text{-}\mu\text{m}$ polystyrene microbeads. The dark green tips of the lettuce plants can be clearly observed

number of 100-nm PS-MP particles entered *Vicia faba* root tips compared to $5\text{-}\mu\text{m}$ PS-MPs which were very scarce in the *V. faba* root tips.

2.2.2 Mechanical Flexibility

Despite the large body of literature on the apoplastic uptake of nanomaterials, it has been generally assumed that submicrometer-sized plastics are too large to pass through the physical barriers of intact plant tissue and, hence, cannot be internalized directly into plant tissues [59].

Results from Li et al. [50] indicate that the beads passed through the intercellular space via the apoplastic transport system (Figs. 5 and 6). Depending on their low stiffness, PS beads may be compressed and deformed upon intracellular internalization. This intrinsic property may enable them to reach the root central cylinder and the vascular tissues, resulting in their movement upward to the aerial parts of the plant. By contrast, the size exclusion limit for the penetration of plant tissues by metal- and carbon-based nanoparticles, which have a much lower conformational flexibility than PS beads, is generally $40\text{--}50 \text{ nm}$ [60, 61].

2.3 Potential Effects of MPs in Soil-Plant Systems

2.3.1 Soil Properties

With the growing accumulation of MPs in soils, a better understanding of the impacts of MPs on soil physical properties is crucial to conserve and manage soil quality. There is some evidence suggesting that MPs might cause changes in soil properties [62]. During a 5-week garden experiment, it was found that MPs affected the bulk density, water holding capacity, and functional relationship between the microbial activity and water-stable aggregates [62]. Moreover, MP-driven changes in soil properties were highly dependent on MP shape and type [62], but those particles functioned as long-term stressors on soil structure. One recent study explored the effects of polyester microfiber (PMF) concentrations (0, 0.1, and 0.3%) on bulk density, porosity, aggregation, and hydraulic conductivity of a clayey soil from a field experiment (1 year) and a pot experiment (6 wet-dry cycles) [63]. It was suggested that polyester microfibers can alter some of the physical properties of a clayey soil, e.g., pore-size distribution and aggregation. However, no effects of polyester microfibers on soil bulk density or saturated hydraulic conductivity were observed in this experiment [63]. It was also observed that polyester microfibers significantly increased the contents of water-stable large macroaggregates (>2 mm) in the 0.3% PMF (44%) and 0.1% PMF (39%) treatments compared to the control treatment (31%) in the pot experiment, but this did not hold in the field experiment [63]. The different soil aggregate distribution results between the pot experiment and the field experiment reveal the complicated consequences of MP-contaminated soil.

The results of a soil incubation experiment in a climate-controlled chamber indicate that the lower level of MP addition had a negligible effect on the contents of organic carbon, inorganic nitrogen, total phosphorus, high-molecular-weight humic-like material, and fulvic acid in dissolved organic matter (DOM) solution 30 days after the addition of the MP, but the higher level of addition significantly increased the nutrient contents of the DOM, including those of dissolved organic carbon (DOC), dissolved organic nitrogen (DON), NO_3^- , dissolved organic phosphorus (DOP), and PO_4^{3-} and high-molecular-weight humic-like material and fulvic acid [64]. In fact, the consequences of accumulation of MPs on soil properties are not well known. Further quantitative studies on terrestrial MP pollution are required to assess other deleterious effects on soil health.

2.3.2 Seed Germination

Several studies have investigated the impacts of NPs and MPs on higher plants. Most of these studies have been conducted on nonvascular plants such as phytoplankton as reviewed in Sects. 1.1.1 and 1.1.2. For example, exposure of MPs to *Skeletonema costatum*, a marine microalga, resulted in negative effects on growth and photosynthesis [16]. MP exposure to the freshwater alga *Chlorella pyrenoidosa* resulted in

physical damage to the alga and oxidative stress [18]. Bosker et al. [65] investigated the effects of three sizes of plastic particles (50, 500, and 4,800 nm) on a terrestrial plant (cress; *Lepidium sativum*) using a standardized 72-h bioassay with hydroponic exposure. Cress seeds were exposed to five different concentrations of plastics ranging from 10^3 to 10^7 particles mL^{-1} distilled water. The germination rate was significantly reduced after 8 h of exposure for all three sizes of plastics, with increased adverse effects with increasing size of the plastics. Seeds exposed to 4,800-nm MPs showed a decline in germination rate of 78% in the control to 17% at the highest exposure. No differences in germination rate occurred after 24 h of exposure, regardless of the size of the plastic used. Significant differences in root growth were observed after 24 h but not after 48 or 72 h of exposure. Impacts on germination are likely due to physical blockage of the pores in the seed capsule by MPs as shown by confocal microscopy of fluorescent MPs. At later stages the MPs accumulated especially on the root hairs. This is the first detailed study on the effects of NPs and MPs on a terrestrial vascular plant, and the results indicate short-term and transient adverse effects.

2.3.3 Plant Growth

To our knowledge, only one study has examined effects on vascular or so-called higher aquatic plants. Kalčíková et al. [26] exposed duckweed (*Lemna minor*; a freshwater plant species) to MPs, and significant reductions in root growth and cell viability were observed, but no effect was observed on leaf growth.

The impact of NPs and MPs on terrestrial systems is currently largely unexplored. Regarding the effects of MPs in a soil-plant system, Qi et al. [66] conducted a pot experiment to investigate different types (low-density polyethylene (LDPE) and starch-based biodegradable plastic) and sizes (1 mm, 500 μm , 250 μm , and 50 μm) of plastic mulch film residues present in sandy soil on wheat plants (*Triticum aestivum*) in a climate chamber. The authors report some impacts of MP films on wheat growth at both vegetative and reproductive phases. In one recent study, de Souza Machado et al. [67] investigated the effects of six different MPs (polyester fibers, polyamide beads, and four fragment types: polyethylene, polyester terephthalate, polypropylene, and polystyrene) on a broad suite of proxies for soil health and performance of the spring onion (*Allium fistulosum*). Significant changes were observed in plant biomass, tissue elemental composition, root traits, and soil microbial activities.

3 Summary

Research on the biological effects of NPs and MPs is currently at the stage of exploring possible responses and thereby investigating the interactions of plants and plastic particles. For existing studies, there is a direct and obvious link between

the uptake of NPs and MPs by plant roots and subsequent effects. Further investigations are required to unravel the mechanisms of uptake and transport of MPs in plants. In particular, the influence of particle size, particle shape, and surface ligands on transport properties needs to be investigated in more detail. Even so, knowledge on quantification of MP uptake is very limited, since the detection of small plastic particles is extremely challenging. A possible way forward might be the use of plastic particles with a metal core which are easy to measure, even at low concentrations and in small sizes, for example, by mass spectroscopy, using the same techniques as for metallic nanoparticles. The technique could be used for the quantification of NPs and MPs as well as for localization within plant tissues, although such traceable particles do not reflect naturally occurring particles that are found in the environment.

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Biofilms of Microplastics



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Abstract The occurrence of microplastics (MPs) in the terrestrial and marine environment has been gaining global attention. These microparticles carry biofilm

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communities that are distinct from the surrounding environment. MP-colonizing microorganisms are important links for the fate of MPs in different ecosystems. However, the influence of plastic-colonizing microorganisms on the fate of microplastics is largely unknown. Here we review the formation of biofilms and dynamic variation on the surfaces of microplastics together with the main research methodologies for biofilm analysis. The potential impacts of biofilm formation on the environmental fate of microplastics caused by MP-colonizing microorganisms such as weathering processes, vertical transport, sorption and release of contaminants, trophic transfer of MP particles, and potential environmental toxicity of MPs in the marine ecosystem are also reviewed. Future studies are needed on the processes and mechanisms of microplastic and biofilm interactions in the terrestrial environment.

Keywords Biodegradation, Biofilms, Extracellular polymeric substances, Microplastics, Toxicity, Vertical transport, Weathering

1 Introduction

According to the International Union of Pure and Applied Chemistry (IUPAC), biofilms are defined as aggregates of microorganisms in which cells that are frequently embedded within a self-produced matrix of extracellular polymeric substances (EPSs) adhere to each other and/or to a surface. Biofilms may form on living or nonliving surfaces and can be prevalent in the marine and terrestrial environments. Due to the large specific surface area of microplastics (MPs), many microorganisms including bacteria, fungi, algae, and protists can easily colonize the surfaces of microplastics in the form of biofilms. The formation and development of biofilms on the surfaces of microplastics may change the morphology and physicochemical properties of MPs in the environment, thus leading to diverse physical, chemical, and biological influences on the environmental fate of MPs such as weathering, vertical transportation, co-migration with chemical pollutants and pathogens, as well as biodegradation. In this chapter the methodologies and processes of biofilm formation and development on the surface of MPs are reviewed, and the different influences of biofilm formation on the properties of MPs are also investigated with the aim of better understanding the fate of MPs in the terrestrial environment.

2 Formation and Development of Biofilms on the Surfaces of Microplastics

2.1 Major Stages of Biofilm Formation

Biofilms are formed by EPS secreted by microorganisms including proteins, glycoproteins, and glycolipids which form a matrix around the microbes and enable them to attach to a variety of different biological and abiotic surfaces. Continuous changes in bacterial colonization of artificial surfaces (such as glass, stainless steel, and polycarbonate sheets) have been confirmed in seawater [1]. Different scholars divide the formation of biofilms into different stages from the core flora and time series.

Biofilm formation is divided into early stage (1–14 weeks), mid-stage (14–35 weeks), and late stage (35–45 weeks) based on changes in the core flora of the biofilm on the surface of plastic flakes exposed at the bottom of the harbor [2]. The formation process of biofilm on the surface of plastic flakes in the real marine environment is constructed. Wimpenny [3] gives a classic biofilm formation process in chronological order:

1. Rapid formation of organic molecular layers on clean solid surfaces.
2. Colonization by bacteria loosely attached to solid surfaces.
3. Colonization by bacteria more firmly attached, forming microbial communities and producing EPS.
4. Communities stretching outward to form regular and irregular structures.
5. Biofilms mature, new species enter the biofilm and grow, and organic or inorganic fragments are combined to form a solution gradient resulting in spatial heterogeneity of the biofilm.
6. Protozoa that phagocytose bacteria may prey on biofilms.
7. Mature biofilms may peel off and this cycle alternates or forms a top-level community.

Lennox [4] divides biofilm formation into five processes: (1) mucosal formation, (2) bacterial proximity and touching, (3) reversible and irreversible attachment, (4) exogenous species supplementation and growth, and (5) diffusion. Some researchers have also divided biofilm formation into four processes: (1) adsorption of dissolved organic molecules, (2) colonization by prokaryotes, (3) colonization by single-cell eukaryotes, and (4) colonization by invertebrate larvae and algal spores. These four processes may occur simultaneously or independently on the surface of the microplastic [5].

2.2 Factors Affecting Biofilm Formation on Microplastics

A conditioning layer comprising organic and inorganic materials is formed by adsorption within a few minutes of the first contact of the plastic surface with the

surrounding water. Microorganisms are in contact with the surface through repulsive or attractive interactions between cell walls and media surfaces. The initial condition layer may have the ability to control colonization by altering material-specific surface properties [6]. Biofilm formation is a multistage process mediated by a variety of factors including surface properties, nutrient solution, pH, and temperature [7]. The environment surrounding the matrix and the conditions of cell growth (such as temperature, carbon source, fluid flow, composition of nutrient media, and growth factors) are complex factors that affect the attachment of bacteria to the surfaces of MPs [8]. There are a variety of attachment mechanisms between microbes and matrices that increase the adhesion of the substrate surface through pili, bristles, flagella, and adjustment of EPS yield [9, 10]. The initial condition layer and the colonizer alter the surface properties of the material and promote the colonization of other organisms. Microbial cells can attach to the surface through specific and non-specific interactions, both depending on surface hydrophobicity/hydrophilicity, roughness, electric charge, and functional groups. The chemical properties of the condition layers are related to the roughness or hydrophobicity of the initial matrix surface and are important for biological sedimentation, indicating the importance of the first adsorption process [11]. Hook et al. [12] believe that surface hydrophobicity and polymer morphology do not affect the adhesion of bacteria to polymers. In contrast, Sanni et al. [13] propose a strong correlation between bacterial sedimentation and hydrophobicity, molecular flexibility parameters in the specific condition of poly(meth)acrylate.

3 Methodology of Microplastic-Associated Biofilm Research

3.1 Scanning Electronic Microscopy

Scanning electron microscopy (SEM) is a tool for observing the surface morphology of samples using secondary electron signal imaging [14] and is widely used in biological, medical, materials, geological, environmental, and other research fields. Energy-dispersive spectroscopy (EDS) combined with scanning electron microscopy (SEM/EDS) is a commonly used elemental microanalytical method that identifies and quantifies the target surface elements of a sample surface [15]. At present, SEM has become a common method for the study of morphology with MPs and their surface biofilms (Fig. 1). EDS is used to analyze the elemental composition of specific areas of MPs to characterize the aging and adsorption characteristics of MPs in the environment.

When observing the microplastic surface biofilm, the SEM sample preparation is usually subjected to cell fixation, dehydration, drying, and then sample analysis according to the SEM method [16, 17]. Cell fixation is an important step in sample preparation. During cell dehydration or drying, cells lose water and undergo

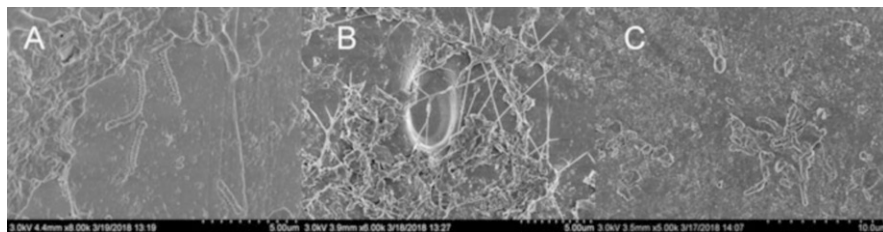


Fig. 1 Biofilm morphology on the surface of MPs at different seawater depths observed by SEM (**a**, 2 m; **b** 6 m; **c** 12 m)

structural changes, resulting in distortion of results [18]. Generally, glutaraldehyde or its combination with citric acid is used to fix microbial cells [19]. Sample drying methods generally include freeze-drying, room temperature or oven drying, and CO₂ critical point drying [17, 19, 20].

SEM can be used to visually identify microbial morphology and posture, characterizing the biodiversity on microplastic surfaces [21], or to analyze the surface morphology of MPs to understand the process of change for weathering and fragmentation of MPs in the environment [20] and helps to distinguish MPs from organic particles [22]. SEM coupled with EDS analysis can be used to identify microplastic samples, especially to distinguish carbon-dominated plastics from inorganic particles [23]. In addition, EDS is also a means of detecting harmful substances such as potentially toxic metals from the environment adsorbed on the surfaces of the MPs.

3.2 *Crystal Violet Staining*

Crystal violet is a staining solution commonly used in tissue or cell staining to stain the nucleus a deep purple color. Crystal violet is a basic dye that binds to DNA in the nucleus and binds to negatively charged surface molecules and polysaccharides in the extracellular matrix [24] while simultaneously allowing proteins to be stained. It is therefore often used as a biofilm semiquantitative method to characterize the biofilm formation process. Crystal violet staining is simple to operate, but it cannot distinguish the living status of cells. According to the mature state of the cells, potassium hydroxide is added to adjust the pH of the dye solution to 6.0–8.0. The lower pH dye solution is used for fresh cell staining, while the higher pH dye solution is suitable for matured cells. The pH can also be adjusted with aniline or pyridine to enhance the dyeing ability of the dye solution for old cells [25]. In addition, the combination of crystal violet and ammonium oxalate to view biofilms can improve the quality of protein-selective staining and enhance coloration and optical effects [26]. Cells stained with crystal violet can be decolorized with a solution such as sodium dodecyl sulfate solution, acetic acid, or ethanol [27–29], and the absorbance of the decolorizing solution is measured and can indirectly

represent the total amount of biofilm. Moreover, since the light could strongly interfere with the crystal violet staining effects, special care is needed to avoid light contamination during the preparation, storage and usage of crystal violet dye.

3.3 Laser Confocal Scanning Microscopy

Laser confocal scanning microscopy (LCSM) is a recent technique developed for the study of histomorphology. It can perform layered scanning on light-transmitting samples and is often used for the morphological study of the three-dimensional structure of bacterial biofilms [30]. LCSM is developed based on fluorescence microscopy technology and is mainly composed of a laser light source, a scanning device, a detector, a computer system, an image output device, an optical device, and a confocal system. The imaging principle is to use a laser scanning beam to form a point light source through a grating pinhole and scan the optical signal of the collecting point by point on the focal plane of the fluorescent marking specimen to reach the photomultiplier tube (PWT) through the detecting pinhole and then display the signal on the computer through signal processing. An image is formed on the screen. The term “confocal” refers to the LCSM having a pinhole light source in front of the illumination source and in front of the detector. After a series of lenses, it is finally focused on the pinhole of the light source and the pinhole [31].

LCSM can provide three-dimensional information about different cell and polymeric biofilm components such as phototrophic organisms, bacteria, and EPS [32]. In addition, the continuous development of fluorescent markers makes it possible for fluorescent dyes to target specific components of biofilms such as nucleic acids and protein residues and even to identify specific cellular physiological states, providing further description of the natural structure, composition, and cellular tissues of biofilms. According to the purpose of the research, the specific fluorescent dye to stain the sample can be selected [29], and the biofilm image along the Z-axis direction in 3D mode can be collected to obtain a complete series of stack format images. The three-dimensional structure of the biofilm can be calculated quantitatively using Imaris and ImageJ software [33]. It should be noted that the fluorescent dye should be stored at a suitable temperature according to the product description and should be protected from light during storage and use.

3.4 Flow Cytometry Combined with viSNE

Flow cytometry uses a device for automated analysis and sorting of cells. It can quickly measure, store, and display a series of important biophysical and biochemical parameters of dispersed cells suspended in a liquid. Flow cytometry and mass spectrometry flow cytometry are powerful analytical tools for simultaneously studying ten extrinsic markers in a single cell to identify rare subtypes and complex

cellular states in heterogeneous populations. These single-cell multiparametric extrinsic measurements have been used in many applications in biology and medicine [34]. Flow cytometry combined with microscopic observations reveal that micro(nano)plastics form agglomerates with mucus matter and associated microbial communities in seawater [35]. Dussud et al. [36] used 1 mmol L^{-1} pyrophosphate for cell detachment pretreatment and ultrasonication with an ultrasonic probe. The cell-separated sample was fixed with 1% (v/v) (final concentration) glutaraldehyde. The cells were then stained with a nucleic acid dye in the dark after which the cells were counted using a flow cytometer.

Visual stochastic network embedding (viSNE) is a tool for nonlinear dimensionality reduction and high-dimensional data visualization. It was originally used to visualize mass spectrometry flow cytometry data from healthy and leukemia blood samples, qualitatively distinguishing blood cell types and detecting abnormal phenotypic changes in blood cell populations. The optimized viSNE program can be used to distinguish species and different phenotypes present in biofilms. Flow cytometry is used in combination with viSNE, which quantifies the survival of large cells after cell decay and temperature stress, while in the field it detects changes in community structure driven by known environmental factors (flow conditions, dissolved organic carbon, calcium) and plastic contamination [37].

3.5 DNA Extraction and High-Throughput Sequencing

High-throughput sequencing (HTS), also known as next-generation sequencing (NGS), can sequence up to tens of millions of DNA strands in parallel at one time. It has become a common research tool in the life sciences and has been widely used in genomics, sequencing, epigenomics, and functional genomics. High-throughput sequencing can complete a variety of sequencing tasks including genome-wide, transcriptome, and macrogenome and bring new methods for functional genomics analysis.

DNA extraction is a preliminary step for high-throughput sequencing. In contrast to natural media such as water and soil, MPs are highly polymeric, and the microbial content on the surface is low. It was found that the particle size, quantity, type, and physicochemical properties of MPs affect DNA extraction [29]. Commercial kits can be selected to extract whole-genomic DNA from microplastic surfaces to increase productivity. The extracted product is subjected to purity evaluation by agarose gel electrophoresis, and its quality is evaluated by NanoDrop [38]. According to the research needs, the appropriate primer template is selected for PCR amplification, and the amplified product can be sequenced on the machine after passing the test.

Zettler et al. [46] used high-throughput sequencing technology for the first time to analyze the microbial community diversity of six microplastic surfaces, and they found that the average number of microbial species per surface exceeded 1,000. Since then, more studies have focused on the microbial community structure and

Table 1 Biofilms from the surfaces of different types of matrix

Matrix type	Environmental media	Analysis method	Reference
PE	Seawater	Stained with crystal violet	[41]
Copper, PE	Tap water	Lipid biomarkers	[42]
PS	Coastal water	–	[43]
PET	Seawater	CSLM	[44]
Stainless steel, PC	Seawater	16S rDNA, FISH, DGGE	[45]
Floating plastics	Seawater	SEM	[21]
Plastic marine debris (PMD)	Seawater	SEM and next-generation sequencing	[46]
Cylindrical pellets	Seawater	–	[47]
Glass	Lake water, Wetland sediment	DGGE	[48]
Acrylic, Glass, Steel	Seawater	T-RFLP, 16S rRNA	[49]

diversity of biofilms on MP surfaces and spatiotemporal variability of microbial community structures on biofilms on the surfaces of MPs [38–40].

Some typical biofilms formed on the surface of plastic and non-plastic materials are listed in Table 1.

4 Biofilms on Plastic Surfaces and Their Physicochemical Implications

4.1 Weathering

Plastic weathering is the process by which the physical integrity of a material is lost through the influence of abiotic and biological factors. Photooxidation is the most common non-biodegradable pathway and can be divided into three main steps: initiation (polymer chain breakage and radical formation induced by UV light), propagation (auto-oxidation), and termination (forming inert products). Weathered surfaces may exhibit changes in shape, increased surface roughness, and chemical changes (e.g., become more polar due to the formation of carbonyl groups) [6]. Over time the surface area of plastics which is available for microbial colonization increases [50], thus increasing the effects of microplastic biodegradation. On the other hand, the formation of biofilms alleviates the ultraviolet degradation by sunlight of plastics which hinders the physicochemical weathering process [51].

Biodegradation of polymers occurs in addition to physical weathering [52]. Flemming [53] reported a variety of patterns in which biofilms disrupt the structure and function of synthetic polymeric materials, namely, (1) fouling surfaces, altering surface properties, and contaminating adjacent media such as water by released microbes; (2) increased leaching of additives and monomers from the polymer matrix by microbial degradation; (3) attacking polymers and additives by

enzymes or biological groups, resulting in loss of embrittlement and mechanical stability; (4) hydration and fungal hyphal penetration of the polymer matrix, causing expansion and increasing conductivity; and (5) degradation of the polymer color by excretion of lipophilic microbial pigments. Gewert et al. [54] investigated the biodegradation pathways and products of six plastic polymers. The six plastics were divided into two categories according to the main chain components. One has a carbon chain as the main skeleton (PE, PP, PS, and PVC), and the other contains heterocyclic atoms (PET and PU). Ultraviolet radiation and oxygen are the main factors leading to the fracture of the C-C skeleton in the initial stages of microplastic degradation. The small molecular polymers after fracture may be further degraded by microbial intracellular or extracellular enzymes.

4.2 Vertical Transport

The vertical transport of MPs in the ocean is influenced by multiple physical, chemical, and biological processes [55]. Density is an important parameter to control the vertical migration of MPs. Plastic density is commonly 0.85–1.41 g cm⁻³. Low-density plastics (density less than seawater) float on the surface of seawater for migration, medium-density plastics (density close to seawater) are suspended in seawater, and high-density plastics (density greater than seawater) migrate on the seabed by suspension or mass transfer [5]. Reisser et al. [56] analyzed the distribution of low-density plastic particles below 0–5 m depth in the sea. It was found that the concentration of plastic particles decreased exponentially with increasing water depth and the smaller the particles, the easier it was for them to migrate vertically. MPs are affected by physical and biological processes during migration and by density changes. A survey of the North Atlantic found that the density of oceanic MPs increased significantly compared to nearshore MPs, mainly due to biofouling [57]. On one hand, biofilms may increase the density of MPs causing them to sink. On the other hand, biofilms may increase the buoyancy of plastic particles with higher density than water, and they more readily float [6]. With the impacts from biofilms, physical and chemical processes such as flocculation occur between the microplastic particles and the agglomerates formed settle to the seabed. Some plankton ingest MPs coated with biofilms which in turn release plastic particles with altered physical and chemical properties, increasing their sinking rate [58]. The plastic particles that converge on the bottom layer are reduced in density due to the feeding of benthic organisms on their surface biofilms, thus regaining buoyancy [59].

Numerical simulation is the main research method for studying the vertical migration of MPs in the ocean. Kukulka et al. [60] used a turbulent mixing model to simulate the migration of plastic particles in the vertical direction under buoyancy and turbulence. Isobe et al. [61] established a vertical two-dimensional particle tracking model to simulate the migration of plastic particles in coastal waters. The sediment deposition model can be used for the simulation of high-density MPs.

Ballent et al. [62] used the Mohid model (a general three-dimensional numerical calculation model) and the experimentally obtained sedimentation-resuspension parameters to simulate the migration of high-density MPs in the Nazaré canyon and found the MPs moving up and down in the canyon under tidal currents. After the model is established in the actual research, the parameters of the MP migration process need to be obtained and verified to identify the rationality of the simulation results.

4.3 Transport of Plastic-Associated Pollutants Through Biofilms

MPs have a large specific surface area and readily adsorb different pollutants including persistent organic pollutants, potentially toxic metals, and pathogens. Additives are certain chemicals added to the molecular structure of plastics to improve their properties. They have hydrophilic groups and metabolic properties and are difficult to leach with weak solvents. Plastic additives may leach and migrate as the environment changes, for example, bisphenol A and nonylphenol, which are highly hydrophilic [5]. Jang et al. [63] found the brominated flame retardant HBCD and bisphenol A on PS foam collected on the Korean coast. Plastics can adsorb persistent organic pollutants (POPs) and can act as important carriers for the transportation and diffusion of organic pollutants. Bakir et al. [64] studied the potential of microplastic transport and removal of hydrophobic organic pollutants (HOCs) in estuarine environments and found that the potential for PE transport and removal of phenanthrene and 4,4'-DDT is much greater than that for PP and PVC. Potentially toxic metals are also common contaminants adsorbed on microplastic surfaces. For example, the detection rate for Cd and Pb in the biofilms of microplastic samples was 6.9% and 7.5%, respectively, from two beaches in southwest England [65]. In addition, chemical contaminants such as drugs and antibiotics were also detected on microplastic fragments.

The distribution and diffusion of the various abovementioned pollutants in MPs and the surrounding water environment may be affected by biofilms. On one hand, biofilms may enhance the adsorption capacity of pollutants on the surface of MPs. On the other hand, specific microbes in the biofilm can metabolize and degrade organic pollutants adsorbed on the MPs [6]. Biofilms are an organic phase composed of water, lipids, and proteins, and they can adsorb water, inorganic and organic solutes, and particles [66], representing a potential barrier to the adsorption, diffusion, and release of chemicals. The viscosity of EPS contributes to the ability of biofilm-coated MPs and heteropolymers to adsorb contaminants [67]. Biofilms can increase the mass transfer resistance of pollutants to the contact with and exit from the plastic polymers [68]. Kinetic laboratory study of HOCs adsorbed onto MPs shows that when microplastic surfaces are in the presence of biofilms, the diffusion coefficient is reduced by approximately four orders of magnitude [69]. A range of

bacteria, fungi, and algae in the biofilms can degrade HOCs [70], with the additives released from MPs being used as a nutrient source to promote microbial growth [71].

5 Biofilms on Plastic Surfaces and Their Biological Effects

5.1 Microbial Community Structure

MPs have become a popular topic in microbial colonization research because of their small particle size, wide distribution, and large specific surface area. Once released into the environment, MPs are rapidly colonized by microorganisms such as fungi and bacteria and by diatoms or that form biofilms on the plastic surface [2, 72]. Because of the unique surface properties of MPs, the microbial communities colonizing the surface are different from those in the surrounding environment. MPs provide a unique microhabitat that supports the growth of some microbial consortia [73]. Thus, Zettler et al. [46] introduced the term “plastisphere” to describe the environmental niche formed by these plastics.

Microplastic surfaces in aquatic ecosystems are novel ecological habitats for marine organisms, and the composition and diversity of biofilm communities have been investigated in numerous studies [21, 46, 74]. Different methods have been used to study the bacterial composition of the plastisphere. With the development of molecular biology technology, high-throughput sequencing technology has been widely used to reveal the composition and diversity of microbial communities on the surfaces of MPs. Some studies find that microbial abundance and diversity on the surface of MPs are lower than those in the surrounding water or sediments [74, 75]. The microbial community structure of the plastisphere is largely influenced by geographical factors, spatial location, and exposure time [2, 76–78]. In addition, different types of polymers and environmental factors also have a significant impact [79, 80]. Miao et al. [81] evaluated the effects of substrate type on microbial communities and found altered metabolic pathways in microbiomes colonizing MPs. Similar results have also been found in the study of the composition and function of PE MPs communities in soil ecosystems by Huang et al. [39]. Compared to natural matrices, microbial communities colonizing the surfaces of MPs exhibit different functions and may trigger different ecological effects on the environmental fates of MPs. Further investigations are therefore needed to illustrate the potential effects of the structure and function of microorganisms colonizing the surfaces of MPs, especially the ecological effects in aquatic systems and the soil environment.

5.2 Trophic Transfer

Due to their small size and widespread presence in the marine environment, MPs can be ingested by a series of marine organisms such as zooplankton, invertebrates,

crustaceans, and fish [82, 83] and can be transmitted along the food chain through predation [83–85]. Intake of MPs may interfere with the food chain as low-nutrient organisms are predated by high-nutrient organisms and then transmitted along the food chain [86, 87]. In contrast to marine microplastic contamination, the distribution and potential impact of MPs in soil ecosystems are poorly understood. Studies show that earthworms and collembolans can transport MPs in soils and increase their mobility [88–90]. Zhu et al. [91] found that predator-prey relationships among different trophic levels can increase the migration of MPs in soils. Moreover, the movement of MPs by soil fauna may affect the bioavailability of MPs to other soil organisms [92]. In addition, most studies have focused on virgin MPs ingested by organisms along the food chain, neglecting the fact that most of the surfaces of MPs in the environment are weathered and covered by biofilms [6]. There have been few studies on the bioaccumulation of MPs and MP particles attached to biofilms at the nutritional level. Microorganisms such as bacteria and algae attached to the surface of MPs may be taken up as food by predators such as fish, thus increasing the risk of ingesting MPs [93]. In addition, the buoyancy of MPs adhering to biofilms may change, allowing them to migrate from surface waters to the bottom of the water column, thereby increasing the chance of being ingested by benthic organisms [58, 79, 94]. In summary, the formation of biofilms on the surfaces of MPs may affect the feeding preference for MPs ingested by organisms through alteration of physical and chemical properties or increasing the bioavailability of MPs [6]. Considering the actual environment, future studies should focus on the role of microorganisms and surface biofilms in the effects of MPs on nutrient transfer.

5.3 Toxicity and Adverse Effects

MPs are usually made from highly hydrophobic materials and chemical additives and are thus susceptible to contamination by a number of chemical pollutants such as POPs, potentially toxic elements, antibiotic resistance genes (ARGs), and pathogens [46, 73, 95–97]. MPs are colonized by diverse and metabolically complex microbial consortia and can be regarded as a novel microbial niche and may serve as a vector for chemical pollutants which may increase the environmental risk from the adsorbed chemical pollutants [98–100]. Environmental MPs are available to every level of the food web from primary producers to higher trophic-level organisms [101]. After a long process from source to sink, MPs are colonized by microorganisms and wrapped by biofilms [102]. The migration of hydrophobic organic pollutants (HOCs) between plastic debris and water may be affected by biofilms which have the ability to metabolize HOCs [6]. MPs have been reported to exhibit concentrations of POPs up to six orders of magnitude greater than the background concentration in the surrounding seawater [103]. Gong et al. found potentially pathogenic bacteria on LDPE MPs exposed in lake water and considered that MPs could serve as transfer vectors for harmful microorganisms in water [104]. Similarly, Wu et al. [73] compared biofilms on MPs with two natural substrates (rocks and

leaves), finding that specific ARG subtypes and several pathogenic bacterial hosts were selectively enriched by MP biofilms. Diffusion of specific microorganisms (especially pathogenic microorganisms) in MP biofilms may increase the risk of disease to other organisms including humans. However, the link between the toxicity and adverse effects on MPs and biofilms is still not fully understood. In conclusion, MPs and their associated biofilms represent ecological risks and potentially adverse effects on the environmental safety and health. Future studies are required to clarify the mechanisms of interactions among MPs, biofilm-colonizing microorganisms, and chemical pollutants.

5.4 Biodegradation

Plastics exposed to the environment may undergo either weathering or biodegradation processes under the complex influences of physical, chemical, and biological factors. The biodegradation of plastics is driven mainly by multiple degradation pathways [55]. Biodegradation of long-chain polymers is usually limited due to their large molecular weight and lack of efficient microorganisms for degradation. The biodegradation process of petroleum-sourced plastics usually includes [105, 106] (1) biofilm formation on the plastic surface, (2) depolymerization, (3) catabolism of the depolymerization by-products, and (4) biomineralization of organic matter.

The biodegradation of plastics has been reported in several studies over the last 30 years. However, there is general agreement that the process is extremely slow under normal conditions [107–110]. Biodegradation requires a crucial initial step that is the formation and development of a microbial biofilm either at the surfaces or directly into the cracks in the MPs [111]. MPs act as a novel, functionally important microhabitat in aquatic and terrestrial ecosystems and exhibit a distinctive microbial community structure which is markedly different from the surrounding environment [75, 76, 78]. Compared with planktonic bacteria, plastic-related bacterial biofilms have stronger ability to degrade plastics [112]. Delacuvellerie et al. [72] found several genera of hydrocarbon-degrading bacteria enriched on several plastics, and these bacteria are potential players in plastic degradation. Yoshida et al. [113] screened a novel bacterium, *Ideonella sakaiensis* 201-F6, that is able to biodegrade poly(ethylene terephthalate). More plastic-degrading microorganisms have subsequently been found in the environment [111, 114–118]. Although several microorganisms are involved in the degradation of plastics, it remains a challenge to obtain a strain suitable for commercial exploitation. Moreover, efficient screening techniques are a prerequisite for the isolation of highly efficient MP-degrading bacterial strains or consortia. To date, few studies have focused on the degradation of MPs by microbial consortia.

Given the importance of biofilms in changing the physicochemical properties and environmental fate of MPs, further studies are needed to investigate the biofilm-mediated sorption of hazardous chemical contaminants, pathogens, and ARGs. Studies on mechanisms of interaction, combined biological toxicities, and ecological

risks between MPs and their associated biofilms are also needed. In addition, biofilm maturity (dynamic formation processes) may have a great influence on these aspects. Moreover, the screening, isolation, and characterization of high-efficiency plastic-degrading microorganisms from biofilms, together with their enzymatic and molecular mechanisms for plastic biodegradation, are needed toward a better understanding of microplastic pollution and bioremediation in the terrestrial environment.

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Part IV
Health Risks of Microplastics
in Terrestrial Environments

Tissue Accumulation of Microplastics and Toxic Effects: Widespread Health Risks of Microplastics Exposure



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Abstract Currently, ecological risks induced by small plastic particles, fibers, and debris with size less than 5 μm (microplastics, MPs) have attracted worldwide

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attention. Notably, terrestrial mammals and even human beings would ingest MPs through feeding, breathing, or other routes. However, the potential health risks of MPs for human and other terrestrial mammals were still largely unknown. Herein, we introduced recent studies about the accumulation of MPs in different human cell lines and tissues of terrestrial mammals. Additionally, the related toxic effects of MPs were also summarized. Importantly, priorities and challenges of future health risk research about MPs, such as the key factors influencing the entry of MPs into organisms, toxic mechanisms, and interaction between MPs and other contaminants, have also been discussed.

Keywords Health risks, Human cells, Microplastics, Terrestrial mammals, Toxicities

1 Introduction

Small plastic particles, fibers, and debris with size less than 5 μm (microplastics, MPs), as a new class of environmental pollutants, have been frequently detected in various environmental metrics [1–3]. For example, MPs with different sizes, shapes, and materials have been identified in various waterbodies (surface water, seawater, wastewater, and groundwater) [4–6]. MPs also have been widely detected in soil environments [7, 8]. Basically, the concentrations of MPs in the environment are highly related to human activities [9]. Nevertheless, MPs have been detected even in sparsely populated places including polar region, deep seabed, and upper atmosphere [10–12].

Due to the wide spread of MPs in the environment, wildlife is inevitably exposed to MPs. MPs have been detected in various aquatic organisms [2, 13]. For instance, MPs are identified in many kinds of shellfish (*Scapharca*, *Tegillarca*, and *Mytilus*, etc.) [14] and fishes (*red mullet*, *Mullus surmuletus*, *Salmo trutta*, etc.) [15, 16]. In the laboratory, adverse effects were observed in these aquatic animals after ingestion of MPs [17–19]. For these effects, oxidative stress and immune responses were frequently identified at both molecular and cellular levels [15, 20]. Simultaneously, the metabolic disorders related to energy, lipid, and amino acid metabolism were observed in those toxicological experiments [17, 21, 22]. Additionally, physical damages including intestinal scratch and blocking due to MPs exposure were also observed in these animals [1]. Therefore, the ecological risks of MPs have been the hotspot in the field of MPs research.

Human and other terrestrial mammals have the potential to ingest MPs through breathing, drinking, and eating [23]. Although increasing efforts have been made in evaluating the potential toxic effects of MPs on different human cell lines and mice models [24–27], the information about health risks of MPs for human and other terrestrial mammals is still largely unknown so far. In terms of the toxic effects of

MPs, it is necessary to clarify the distribution, accumulation, and excretion of MPs in organisms. The studies of nanoparticles (NPs) could give us some useful information [28, 29]; however, the sizes of NPs are much smaller than MPs. Particle size significantly influences the environmental behavior and toxic effects of particulate matter [30, 31]. Therefore, the conclusion based on NPs cannot be directly applied to MPs.

To shed light on the potential health risks of MPs on terrestrial mammals, we reviewed recent published studies about the accumulation of MPs in different human cell lines and terrestrial mammals in this chapter. Moreover, the corresponding toxic effects were also summarized. More importantly, we suggested some priority issues and big challenges in this field. We hope this chapter would arouse more exploring on the health risks of MPs to terrestrial mammals and help to narrow knowledge gaps.

2 Effects of MPs on Cells

Human cell lines have been successfully applied in evaluating the toxicity of drugs and environmental chemicals in past decades [32, 33]. On the one hand, these cell lines are commercially available, and using cell lines can also avoid overuse of live animals [34]. On the other hand, cell experiments are time-saving compared with *in vivo* experiments [35]. Due to the low cost and short testing time, cell lines and related high-throughput detection platform have been viewed as the potential alternative to animal models [36]. Increasing researchers choose using different cell lines to evaluate the potential adverse effects of emerging pollutants [37, 38], including particulate matter [39, 40]. The accumulation and excretion of NPs in animal cells have been well demonstrated [41–43]. However, scientific evidence about the accumulation and toxicity of MPs in human cells is still in its infancy.

2.1 Accumulation of MPs in Human Cells

2.1.1 MPs in Gastrointestinal Epithelial Cells

Increasing evidences suggest that MPs can enter the digestive tract of terrestrial mammals through the food and drinking water contaminated by MPs [23]. Therefore, it is particularly important to select typical human intestinal cells to evaluate whether MPs can be transported by these cells and what toxic effects can be caused on these cells. Caco-2 cell line is a continuous line of heterogeneous human epithelial colorectal adenocarcinoma cells [44]. Although derived from a colon (large intestine) carcinoma, the phenotype of Caco-2 cells can be differentiated and polarized under specific conditions making them similar with the small intestinal cells on the morphology and function, such as tight junctions, microvilli, and function of

enzymes and transporters [45]. Therefore, Caco-2 cells, as an in vitro model, have been widely used for drug and toxicology research [46].

Recently, Caco-2 cells have been used to investigate the cellular uptake of polystyrene (PS) MPs with different sizes (0.1 and 5 μm) (Table 1) [24]. The results show that both 0.1 μm and 5 μm MPs could enter Caco-2 cells after exposed to 20 $\mu\text{g}/\text{mL}$ MPs for 12 h. The smaller size of MPs could even localize to lysosomes. Stock et al. have also conducted a similar study using 1, 4, and 10 μm PS MPs (Table 1) [47]. The order of three sizes of MPs accumulated in the cells was 4 μm > 1 μm > 10 μm .

Notably, the gastrointestinal epithelium of mammals is made of various cell types including enterocytes, goblet cells, enteroendocrine cells, Paneth cells, microfold cells (M cells), cup cells, and tuft cells. M cells and goblet cells have been used to evaluate the penetration, accumulation, and adverse effects of MPs in the gastrointestinal tract (Table 1) [47]. Consistent with the results of Caco-2 cells, MPs could also enter M cells and goblet cells, but the influences of particle sizes have not been fully elucidated, and further studies are warranted.

2.1.2 MPs in Human Dermal Fibroblasts

Airborne MPs have also aroused some attention [23]. Inhalable MPs could be a potential exposure route for human and other terrestrial animals. Nevertheless, the health risks induced by expiratory MPs are still largely scarce. According to previous toxicological studies, different exposure pathways may cause distinct outcomes [48, 49]. Therefore, the airborne MPs should not be ignored for the health risk assessment of MPs for human and other terrestrial mammals.

Human-derived fibroblasts (HDFs) have been used to evaluate the toxicity of polypropylene (PP) MPs with different sizes (~ 20 and 25–200 μm) [50]. The HDFs come from stromal tissue, which provides a protective barrier against the absorption of exogenous particles and plays a key role in repairing and healing of damaged tissues. However, the accumulation of MPs in HDFs has not been detected in that study. So far, no solid evidences are available about MPs entering the respiratory histiocytes. Therefore, future studies are needed to investigate whether MPs could be uptaken by human dermal fibroblasts. Moreover, the accumulation of MPs with different sizes, shapes, charges, and polymer types in other human cells deserves more comprehensive studies (Fig. 1).

2.2 Toxic Effects of MPs on Different Human Cell Lines

Adverse effects have been observed in different human cell lines due to MPs exposure (Table 1). These adverse effects include cell viability inhibition, induction of oxidative stress, and immune responses [24, 50]. However, consistent conclusion about the toxic effects has not been reached because the lack of standardized

Table 1 Accumulation of MPs in different cells

Species	Type	Material	Size	Shape	Exposure way	Accumulation of MPs in cells	Effects of MPs	Reference
Human	Caco-2 cells	PS	0.1 µm; 5 µm	Sphere	By adding different doses of MPs solution	<ul style="list-style-type: none"> Both 0.1 and 5 µm MPs could enter into cells Two sizes of MPs were localized to the lysosomes Size effect 	<ul style="list-style-type: none"> MPs significantly increased intracellular ROS MPs inhibited the cell viability MPs damaged the cell membrane. 	Wu et al. [24]
Human	Caco-2 cells; M cells; goblet cells; Raji B-lymphocytes; THP-1 cells	PS	Mix MPs (1, 4, and 10 µm)	Sphere	By adding different doses of MPs mixed solution (1, 4, and 10 µm)	<ul style="list-style-type: none"> All three sizes of MPs can enter into Caco-2, M, and goblet cells No size effect 	<ul style="list-style-type: none"> MPs with the high dose inhibited the cell viability MPs didn't induce immune responses of THP-1 cells 	Stock et al. [47]
Human and mouse	Dermal fibroblasts (HDFs); murine macrophages	PP	~20 µm; 25–200 µm	Irregular shape	By adding different doses of MPs solution	<ul style="list-style-type: none"> No information 	<ul style="list-style-type: none"> MPs decreased cell viability MPs significantly stimulated the cell immune system MPs increased ROS levels 	Hwang et al. [50]

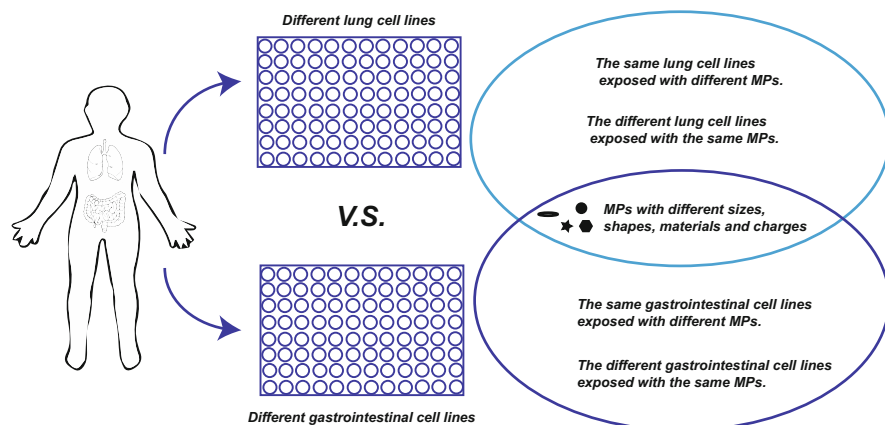


Fig. 1 Future work recommended for evaluating the health risks of MPs by using human cell lines

methods for health risk assessment of MPs makes it difficult to compare outcomes of different studies.

2.2.1 Cell Viability

So far, the effect of MPs on cell viability is still controversial. Some *in vivo* toxicological tests revealed that PS MPs could inhibit the viability of Caco-2 cells (Table 1) [24, 47], while some other studies found that MPs exposure did not induce obvious changes in the cell viability of different human cell lines. For example, polyethylene (PE) MPs (3–16 μm diameter) exposure showed no effects on the viability of human cerebral cell (T98G cells) and ovary epithelial cells (HeLa cells) [51].

2.2.2 Oxidative Stress

Oxidative stress is one of the most important mechanisms of toxic effects induced by NPs [52], which have been demonstrated by both *in vitro* and *in vivo* tests [53]. Similarly, increasing studies try to investigate the intracellular level of oxidative stress after exposure to MPs. Notably, these studies have revealed that MPs could cause the imbalance of intracellular redox state no matter whether MPs could enter the cells or not (Table 1) [24, 51]. Furthermore, the oxidative stress induced by MPs has obvious dose-response relationship, but these effects are not significantly affected by particle size [54, 55].

2.2.3 Immune Response

Intestinal epithelium is a critical tissue for the body's immunity [56]. Correspondingly, human intestinal immune-related cells such as macrophages have been used to assess the potential adverse effects of MPs. The intestinal macrophages would trigger pro-inflammatory responses by respiratory burst activity and generate nitro oxide or inflammatory cytokines [57]. Moreover, the human cell line THP-1 has been used to evaluate the potential immune responses induced by MPs (Table 1). However, no significant immune response was found in the THP-1 cells after exposed to MPs [47]. On the contrary, 20 μm PP MPs could stimulate the alterations of immune system in multiple cell lines [50].

2.2.4 Other Adverse Effects

In addition to the abovementioned toxic effects, cytotoxicity such as disruption of mitochondrial membrane potential and membrane damage induced by MPs has also been detected in Caco-2 cells (Table 1) [24]. The possible mechanism is that MPs may act as ABC transporter substrates and inhibit the activity of transmembrane ABC transporter, which finally trigger the membrane damage. To better reveal the toxic effects of MPs on cells, more typical human cell lines including gastrointestinal epithelial cells and dermal fibroblasts should be tested. Additionally, the exposure conditions of MPs, which could greatly improve the comparability of adverse outcomes induced by MPs, should be fully considered in toxicity experiments (Fig. 1).

3 Effects of MPs on Human and Other Terrestrial Mammals

Previous studies have demonstrated the ingestion of MPs in different wild aquatic animals. Although human is likely to ingest MPs through multiple routes (diet, drinking water, and respiration), the direct evidences of MPs ingestion in human and other terrestrial mammals are scarce.

3.1 *Ingestion of MPs in Human and Terrestrial Animals*

3.1.1 Wild Terrestrial Animals

MPs have been observed in seabird, which is the first public evidence of MPs in wild terrestrial animals [58, 59]. Based on these studies, it is easy to infer that higher trophic animals may also ingest MPs by preying on food contaminated with MPs or mistaking MPs for natural food items. Previous studies reported that 29% of

individual seabirds had plastics in their gut during 1962 to 2012; however, up to 90% of seabirds to date were estimated to ingest plastics. If these trends continue, this proportion will be reached 99% by 2050 [60]. Although these seabirds may live on land for more than half of their life, it is difficult to strictly classify them as typical terrestrial animals. Therefore, the question about the health risk of MPs for higher trophic terrestrial animals still exists. To narrow the knowledge gap of this question, Zhao et al. firstly investigated the accumulation of MPs in 17 terrestrial birds (Table 2) [61]. As a result, 364 plastic items (plastics sizes range from 0.5 to 8.5 mm) from 16 bird gastrointestinal tracts were identified as microscopic plastic litter under a stereomicroscope based on the plastic physical properties and melting tests. Among these plastic items, MPs account for more than 90% of the total number of pollutant items. However, in that study, the smaller MPs (<0.5 mm) were not identified in bird bodies due to the limitation of the testing instruments. These results indicated that birds as one kind of terrestrial animals are invaded by plastic debris including MPs to a certain extent.

Furthermore, MPs are also identified in chickens (Table 2). Lwanga et al. firstly found the transfer of MPs and macroplastics (5–150 mm) from soil to chickens in the traditional Mayan home gardens in southeast Mexico where the mismanagement of plastic waste is unsupervised [62]. In that study, MPs and macroplastics were identified in chicken feces and gizzards. Notably, the chicken gizzards were used for human consumption. In addition, the concentration of MPs significantly increased along the food chain, like soil (0.87 ± 1.9 particles/g) > earthworm casts (14.8 ± 28.8 particles/g) > chicken (129.8 ± 82.3 particles/g). These results indicate that MPs have obvious biological amplification and bioaccumulation through the food chain. Chickens and many other terrestrial animals are human food, which means that human beings are facing much higher health risks of MPs when consuming these foods contaminated with MPs.

3.1.2 Terrestrial Model Animals

Compared with wild terrestrial animals, there are much more studies about the interactions of MPs and terrestrial model animals (Table 2). As early as 1970, it has been reported that plastic particles could enter the model animals, although MPs have not been defined at that time [63]. Volkheimer has reported that plastic particles with sizes of 5–110 μm could enter the blood circulatory system of various model animals, including rats, dogs, and pigs, and these particles could also disseminate to other tissues [63]. It is the first scientific report showing that PE plastic particles could accumulate in terrestrial mammals. After that, Eldridge et al. found that poly (DL-lactide-co-glycolide) particles with size less than 10 μm could be taken up into the Peyer's patches of gut-associated lymphoid tissues of mouse after oral administration [64]. Especially, the smaller MPs (<5 μm) accumulated in the macrophages were able to disseminate to the mesenteric lymph nodes, blood circulation, and spleen. Moreover, LeFevre et al. reported that 1.8 μm plastic particles were able to enter the mouse liver and other solubilized tissues [65]. Recent studies have reported

Table 2 Accumulation of MPs in different species and tissues

Species	Tissue	Material	Size	Shape	Exposure route	Accumulation of MPs in tissues	Effects of MPs	Reference
Human	Lung	No information	No information	Fiber	Inhaled	Lung	No information	Pauly et al. [66]
Human	Feces	9 plastic types; PP, PE, and terephthalate with highest detection rate	50–500 µm	Fiber	No information	No information	No information	Schwabl et al. [68]
Terrestrial birds	Gastrointestinal tracts	No information	0.5–8.5 mm	Fiber; fragment	No information	Esophagus; stomach; intestine	No information	Zhao et al. [61]
Chicken	Gizzards, feces	No information	10–50 µm; 5–150 mm (macroplastic)	No information	No information	MPs in gizzards; macroplastic in gizzards	No information	Lwanga et al. [62]
Rat, dog, pig	Blood, circulatory system, digestive system	Polyvinyl chloride (PVC)	5–110 µm	Particle	Oral administration	Body fluids (blood, bile, urine, cerebrospinal fluid, etc.); subepithelial layer and lymph; liver	No information	Volkheimer [63]
Mouse	Blood, circulatory system, digestive system	Poly(DL-lactide-co-glycolide)	<10 µm	Spheres	By oral administration	MPs (<5 µm) in lymph nodes, blood circulation, spleen	No information	Eldridge et al. [64]
Mouse	Blood, circulatory system, digestive system	PS latex particle	1.8 µm	Spheres	By oral administration	Intestine piece; lung; liver; lymph nodes	No information	Lefevre et al. [65]
Mouse	Liver, kidney, gut	PS	5 µm; 20 µm	Spheres	By oral administration	Liver; kidney; gut	<ul style="list-style-type: none"> MPs induced immune response in body MPs induced liver oxidative stress MPs disordered the energy metabolism, lipid metabolism 	Deng et al. [25]
Mouse	Serum, gut, liver, gut microbiota	PS	0.5 µm; 50 µm	Spheres	Drinking water	No information	<ul style="list-style-type: none"> MPs decreased the secretion of mucin in gut 	Lu et al. [26]

(continued)

Table 2 (continued)

Species	Tissue	Material	Size	Shape	Exposure route	Accumulation of MPs in tissues	Effects of MPs	Reference
Mouse	Serum, gut, liver, gut microbiota	PS	5 μm	Spheres	Oral administration	Gut	<ul style="list-style-type: none"> MPs induced gut microbiota dysbiosis MPs induced hepatic lipid metabolism disorder MPs induced intestinal barrier dysfunction MPs induced gut microbiota dysbiosis MPs induced bile acid metabolism disorder 	Jin et al. [27]

that 5 and 20 μm MPs could enter the mouse liver, kidney, and gut after oral exposure [25, 27]. These studies also demonstrate that gut has the highest accumulation of MPs.

3.1.3 Human

Even though it is possible for human to ingest MPs through multiple routes, solid data about the ingestion of MPs in human is still rare. In the meanwhile, the potential accumulation and distribution of MPs in human tissues are largely unknown. The first observation of MPs in human body was published in 1998, which reported that plastic fibers were identified in the lung tissues (Table 2) [66]. Based on the results of that study, inhaled plastic fibers were present in 83% (67/81) of nonneoplastic lung specimens, whereas these were present in 97% (32/33) of malignant lung specimens. Finally, plastic fibers were identified in 87% (99 of 114) human lung specimens. These observations demonstrated that MPs could enter human body by breathing MPs-contaminated air. More importantly, these observations also suggested that MPs including plastic fibers with particular sizes could penetrate human lung and stay in tissues for a long time.

As described above, MPs have been detected in various foods like seafood [23] and chicken [62]. Besides, MPs have also been detected in salt, honey, sugar, and bottled drinking water [4, 67]. Thus, our daily life is full of MPs and it is inevitable for human to ingest MPs via food webs. In addition to respiratory exposure pathway, more attention should be paid to the gastrointestinal tract, because most MPs have been observed in the gastrointestinal tract of various animals. According to the latest report, MPs have been identified in eight human stools from different countries (Table 2) [68]. Although the sample size is small, this is the first report about the existence of MPs in human stool, which demonstrates the ingestion of MPs in human. However, the distribution and quantity of MPs in different human tissues are still largely unknown. Previous studies have reported the existence of plastic particles in human tissues, but these particles come from some plastic medical devices. For example, PE wear particles up to 50 μm have been identified in the liver, spleen, and abdominal lymph nodes of patients with hip or knee plastic replacements [69].

3.2 Toxic Effects of MPs on Human and Terrestrial Animals

In the past decade, many studies have well stated that MPs could not only induce physical damage but also induce many adverse effects in different biological levels

[2]. However, most of these studies focus on aquatic organisms. The potential toxic effects of MPs on terrestrial animals and humans are largely unknown, and the related studies are still in their infancy. Although, the cytotoxicity of MPs on human cell lines have been investigated in recent studies, the observations from in vitro test cannot fully illustrate the toxic effects of MPs, which means that in vivo studies are inevitable. Fortunately, increasing researchers have tried to investigate the potential adverse effects of MPs on terrestrial mammals.

3.2.1 Physical Damage

MPs-induced physical damages including internal abrasions and blockages in gastrointestinal tract were firstly observed in aquatic animals [1]. These residual MPs in the gastrointestinal tract may trigger a series of consequences, such as suppressed feeding activity, prolonged residence time, reduced energy reserves, and intestinal inflammation, which finally affect the growth, reproduction, and even survival of animals [22]. Although a large amount of MPs have not been observed in terrestrial animals, the health risk should not be ignored with the increasing accumulation of plastic waste in the environment and ubiquitous MPs in the daily life. It is important to figure out whether similar adverse effects like in aquatic organisms can also be caused in terrestrial animals or even human beings.

3.2.2 Biological Responses

More studies for comprehensively assessing the potential toxicities of MPs on terrestrial animals, especially terrestrial mammals, are advocated. More species should be used for the health risk assessment, because previous studies have demonstrated that the same pollutant could induce different biological toxic effects on different animals with different living habits [70]. Some typical terrestrial mammal models have been used to investigate the potential toxic effects of MPs. At the same time, more epidemiological investigation of MPs needs to be carried out.

Although the biological adverse effects of plastic fibers on human lung tissues have been reported in the 1990s, the health risks of MPs including plastic fiber, debris, and particles didn't attract enough attention [71–73]. Studies on occupational workers have shown that about 4% of workers from nylon flock plants in the USA and Canada suffered from interstitial lung disease (Table 2) [71, 72]. As we all know, interstitial lung disease can induce coughing and dyspnea (breathlessness) and reduce lung capacity. Similarly, symptoms have been also observed in the workers from a factory processing para-aramid, polyester, and polyamide (PA) fibers in the Netherlands (Table 2) [73]. These symptoms in occupational workers are closely related to the intake of plastic fibers in their lungs, based on the results of

histopathological analysis. But, so far, it is not clear whether MPs can cause cancer. On the one hand, studies among nylon flock workers suggested that there was no evidence of increased cancer risk, although the workers had a higher prevalence of respiratory irritation [74]. On the other hand, the detection frequency of MPs in lung tissue of lung cancer patients (97%) was significantly higher than that (83%) in nonneoplastic lung specimens [66]. Maybe, MPs are candidate pollutant agents contributing to the risk of lung cancer. More epidemiological studies about health risks of MPs are urged.

Except these epidemiological studies, increasing toxic data from laboratory terrestrial mammal models especially mouse models have also observed many adverse effects on terrestrial mammals caused by MPs (Table 2 and Fig. 2). These adverse effects from mouse models are observed not only at molecular levels but also at cellular levels. Lu et al. have shown that MPs could decrease the relative mRNA levels of some key genes related to lipogenesis and triglyceride synthesis in mouse liver and epididymal fat [26]. Meanwhile, Jin et al. have found that the transcriptional levels of several functional genes (*Cyp7a1* and *Abcb11*) related to bile acid transportation, synthesis, and signaling in the liver were significantly changed by MPs exposure [27]. Notably, previous studies have stated that bile acid metabolism plays an important role in lipid metabolism [75]. The abnormal lipid metabolism in mice is also observed at cellular levels by a few of physiological and biochemical indicators. Deng et al. have investigated the levels of triglyceride (TG) and total cholesterol (TCHO) in mouse liver after exposure to different sizes of MPs [25]. Additionally, the levels of mouse serum TG, TCHO, glucose (GLU), and total bile acids (TBA) after exposure to different MPs have also been detected in recent studies [26, 27]. Based on the results of these studies, it could be concluded that MPs could decrease the levels of TG and TCHO in mouse liver and blood, which can also confirm the imbalance of lipid metabolism in mice induced by MPs exposure. More importantly, the imbalance of lipid metabolism is also directly demonstrated by the metabolomic alterations. MPs can alter not only the levels of metabolites involved in lipid metabolism but also the pathways related to lipid metabolism in mice [25].

Oxidative stress is another major toxic effect caused by MPs. From different human cell line experiments, it's well stated that MPs could cause the imbalance of intracellular oxidative stress no matter whether MPs could enter the cells or not [24, 50]. Importantly, oxidative stress has been confirmed by some studies using mouse models. Deng et al. have observed oxidative stress in mouse liver after exposure to MPs based on the biomarkers of catalase (CAT), glutathione peroxidase (GSH-Px), and superoxide dismutase (SOD) [25]. Furthermore, the metabolites related to oxidative stress are also identified in the serum of mice [25]. Previous studies have shown that oxidative stress could aggravate the abnormal lipid metabolism [77]. As mentioned above, MPs can disturb the lipid metabolism in terrestrial mammals. Seriously, oxidative stress can also result in many other adverse effects, such as neurodegenerative diseases and cardiovascular disease [53, 78, 79].

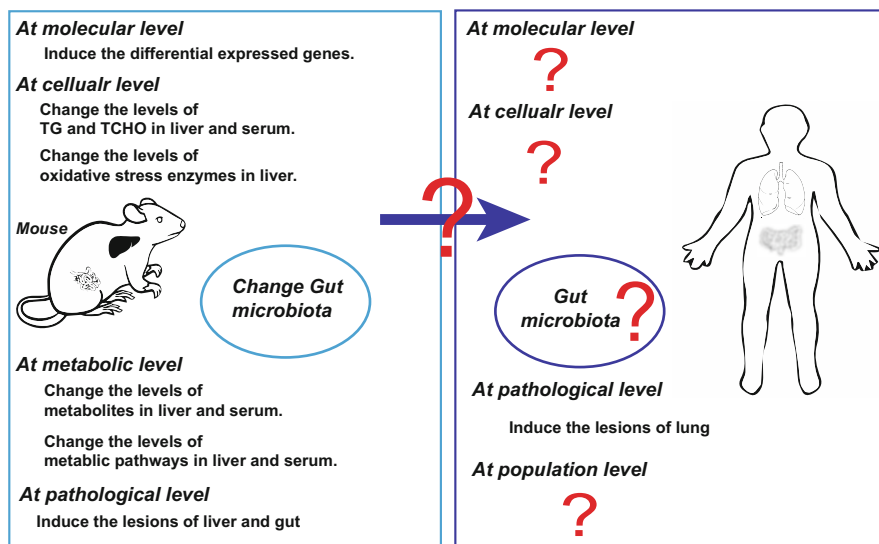


Fig. 2 The potential adverse effects of MPs on terrestrial mammals. Notably, the imbalance of lipid metabolism in mice has also been supported by the alterations of gut microbiota due to MPs exposure. It has been demonstrated that some gut microbiota related to lipid metabolism are significantly altered after exposed to MPs. For example, MPs exposure decreased the relative abundances of *Firmicutes* and α -*Proteobacteria* at the phylum level in the gut of mice [26]. On the other hand, some immune-related microbiota are also affected by MPs in our latest study (unpublished). The underlying reason is that MPs exposure may disturb the normal functions of mice intestine, which play vital roles in absorption and utilization of nutrients and keeping immune system functional. In addition, the intestinal dysfunction caused by MPs is also confirmed by histological analysis. Lu et al. have found that the volume of mucus significantly decreased after MPs exposure in the mice gut [26]. As we all know, the surface of gut lumen has a mucus layer in contact with a large number of microbiota, and the secreted mucin can protect the intestinal mucosa and resist the penetration by bacteria [76]

4 Priorities and Challenges of Future Health Risk Research of MPs

As mentioned above, terrestrial mammals or human beings face big potential to MPs exposure, and the related health risks have been a focus of research. Here, we try to put forward some key problems and challenges in realm of MPs and hope to provide some ideas and directions for the follow-up research.

4.1 Key Factors Affecting MPs Entry into Organisms

MPs size may be one of the key factors affecting the entry of MPs into the body. Nevertheless, there is no convincing conclusion about what sizes of MPs could enter mammals. Volkheimer et al. have reported that MPs with the size of 5–110 μm could enter blood circulation and then disseminate to multiple tissues in pigs, dogs, and rats after oral administration of MPs [63]. These big sizes of MPs (5–110 μm) may enter other tissues from the gastrointestinal tract by paracellular translocation [80]. Therefore, future studies should continue to address this issue by testing MPs with more range of sizes and using multiple mammal models.

On the other hand, almost all laboratory experiments use regular microsphere MPs. However, in the actual environment or in the body of wild animals, MPs are dominated by irregular shapes including fiber, fragment, film, and foam [2, 61]. Moreover, the surface characteristics and polymer types of MPs also play a key role in the process of MPs entering into the body. Therefore, the future studies are encouraged to use MPs of different sizes, shapes, surface charges, and compositions to identify the key factors affecting the entry of MPs into the body.

4.2 Key Factors Affecting MPs Toxicity

Dose-effect response is a key scientific issue and inevitable in traditional toxicology studies. Similarly, dose-effect response has been clearly demonstrated for MPs toxicities. However, the size, shape, surface charge, and material of MPs are also significantly associated with their toxicity. As mentioned above, these factors would significantly affect the entry of MPs to the body. Therefore, further studies about MPs toxicity should comprehensively consider these factors and identify the key factors affecting the toxicities of MPs.

4.3 Environmentally Relevant Concentrations and Long-Term Exposure

Most of concentrations of MPs used in current studies are much higher than that in actual environment. It is difficult to reveal the real exposure level of MPs and evaluate their precise health risks. Therefore, environmentally relevant concentrations are strongly recommended for future MPs research. Moreover, the exposure time of current studies is relatively short [81], and longer exposure is valuable for future toxicological studies of MPs on terrestrial animals.

4.4 *Combined Toxicity of MPs and Other Pollutants on Terrestrial Mammals*

During the manufacture of plastic products, many chemical additives were added to reinforce the original features or give them some new features. Seriously, these additives may leach out with the decomposition of plastics, which would induce combined toxicity of plastic debris (such as MPs) and chemical additives [23]. On the other hand, increasing studies have reported that MPs could act as carriers and transport other environmental pollutants into aquatic animals [82]. The presence of MPs may even aggravate the toxicity of other environmental pollutants [20, 83, 84]. Nevertheless, huge knowledge gaps about MPs releasing toxic chemicals into terrestrial mammals and the corresponding joint toxicity are still remaining to be filled. Therefore, more efforts should be made to assess the combined toxic effects of MPs and other pollutants on terrestrial mammals.

5 Summary

MPs have large potential to be ingested by terrestrial mammals and humans, which will bring much health risk and ecological threat. After all some toxic effects including abnormal lipid metabolism and oxidative stress have been observed in model animals. However, the potential health risks of MPs in human and other terrestrial mammals are still largely unknown. What are the key factors affecting the entry of MPs into the body, and what corresponding toxic effects will be caused? Whether MPs at the environmentally relevant concentrations could cause adverse effects on human and other terrestrial mammals? What is the combined toxicity of MPs and other pollutants on terrestrial mammals? These knowledge gaps and challenges in health risk research of MPs still need to be addressed in future studies.

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Microplastics in Food: Health Risks



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Abstract The presence and ecological risks of microplastics (MPs) are increasingly reported, whereas the impacts of MPs on human health remain largely unknown. Recent studies have confirmed the MP contamination in food items, including seafood, table salt, drinking water, etc. Dietary exposure is one of the inevitable exposure pathways of MPs, which causes concern about the potential human health risks. Whether we assess health risks or try to reduce food MP contamination, the prerequisites are to figure out the contamination pathways of MPs and their actual level in food items. At present, territorial system is facing serious environmental problems, with soil, freshwater, and air suffering from MP pollution. This leads to diversity and complexity of MP sources in food items. Therefore, we should not be

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confined to the food itself when considering MPs in food, but should take all pollution possibilities into account. In this chapter, we reviewed the literature concerning MPs in seafood, table salt, drinking water, and other food items. The potential MP sources of food items during the whole process from food acquisition to human ingestion were analyzed, with related human intake of MPs estimated. We also discussed possible translocation and accumulation pathways of MPs within human body. Given the lifetime inevitable exposure to MPs through multiple pathways, we urgently call for a better understanding of the potential MP sources in food items and a comprehensive assessment of human intake.

Keywords Drinking water, Health risks, Microplastics, Seafood, Table salts

1 Introduction

Since the concept of “microplastic” was introduced in 2004 [1], microplastics (MPs) have been found in various environmental media and organisms globally [2–6]. More recently, the threats of MPs to human health have gained increasing public interests and media attention due to the wide detection of MPs in human-related food.

As the marine environment attracted much attention, seafood has become the most studied food [7–9]. More than 690 marine species have been reported to be contaminated by MPs, including edible and nonedible ones [10, 11]. Since inedible species do not normally enter human body, their contribution to MP intake by human is negligible. Until now, MPs have been found in 202 edible species, including 201 marine species and 1 terrestrial species [12]. Subsequently, MPs are also observed in table salt and other food such as honey, sugar, beer, and drinking water [13–16]. Consumption of these food products will undoubtedly cause MP exposure through human digestive tract. Numerous experiments have demonstrated toxic effects of MPs, such as growth inhibition, oxidative damage, and immune stress [17, 18]. A recent study shows that MPs of high concentration may have caused evolutionary adaptations of some species (e.g., *D. magna* and *G. pulex*) [19]. Mammal experiments have proved that polystyrene microplastic can induce gut microbiota dysbiosis and hepatic lipid metabolism disorder in mice [20]. MP particles can also accumulate in marine organisms and transfer through the food chain to higher trophic levels including humans [10]. Despite that ample evidence suggests the MP contamination of human-related food and the related toxicological effects on animals and cells, there is, however, large unknown fields.

Food is indispensable energy supplier for human survival. Therefore, it is necessary to survey our current knowledge on direct human exposure concentration to MPs via food consumption. Related topics have been discussed previously. For example, seafood, as a carrier of MPs, should be treated cautiously considering the

influence on human health through biological accumulation and biological amplification [21, 22]. At the same time, some believe that the risk to higher trophic levels is negligible due to the rapid depuration of MPs [23]. In fact, the issue of MPs in food items is no longer about single investigation of MPs in food itself due to inevitable MP contamination in water and air for human consumption in terrestrial environment. During the whole process from food acquisition, production, packaging, and transportation to food intake, extra MPs may be introduced in any link [16]. This is a complex problem that needs to be dissected in depth. When all factors are taken into consideration, we will have more realistic data for risk assessment. Only in this way can we put forward more effective measures to control the main links of food contamination.

2 Microplastics in Seafood

Various foods are summarized in terms of contamination levels of MPs. Since these data have been reported in detail in previous studies [12, 24–26], partial data are listed in Table 1.

MPs have been found in fish from many countries and regions, ranging from 0 to 48 items/individual [40–42]. The reason that “items/individual” is used instead of “MPs/individual” is that MPs in seafood are usually characterized by their sizes, shapes, and colors, whereas compositions are not universally identified. Among these studies that have completed particle identification, relatively high concentrations occurred in China (13.54–22.21 items/individual) [43], Turkey (9.4 items/individual) [44], and Malaysia (14 items/individual) [45]. However, different methods among studies lead to poor comparability of the results. Therefore, direct comparisons and accurate conclusion cannot be made, and such situation occurs in shellfish and all the other food items.

MP abundance in shellfish (0–57.2 items/individual, 0–75 items/g) is generally higher than that in fish, with blue mussels being the most studied species [46, 47]. The largest numbers occurred in mussels from Canada (34–75 items/g) [29], followed by China (0.9–4.6 items/g) [48] and Equatorial mid-Atlantic area (2 items/g) [49]. In addition to wild mussels, some ones from fishery farms, as well as supermarket, have also been confirmed to be contaminated by MPs. Li et al. have investigated commercial bivalves from fishery farms and supermarket and found that all mussels were contaminated by MPs [27, 28].

3 Microplastics in Table Salt

MPs have been widely identified in table salt of more than 100 brands all over the world [16, 33, 36, 50], with their concentrations varying widely. The highest concentration was reported in Croatia (1.35×10^4 – 1.98×10^4 MPs/kg salt),

Table 1 Microplastics in food items

Food type	Abundance	Location	Reference
Shellfish (items/g)	2.1–10.5	China	[27]
	0.2 ± 0.3	France	[5]
	0.7–2.9 (coastal), 0.9–1.4 (supermarket)	UK	[28]
Fish (items/individual)	0.3–1.1 (GIT)	Indonesia	[29]
	0.57–1.85 (muscle)	Iran	[30]
	1.9 (liver)	Spain	[31]
Table salt (MPs/kg)	7–681	China	[16]
	13,500–19,800	Croatia	[32]
	98–232	Korea	[33]
	5–280	Spain	[34]
Drinking water (MPs/L)	338–628 (DWTP)	Czech	[35]
	3.66–13 (tap water)	England	[36]
	2,649–6,292 (bottled water)	Germany	[37]
	58.2–1,410 (bottled water)	USA	[38]
Honey	166 ± 147 fibers/kg, 9 ± 9 fragments/kg	Germany, France, Italy, Spain, Mexico	[13]
Sugar	217 ± 123 fibers/kg, 32 ± 7 fragments/kg	Germany, France, Italy, Spain, Mexico	[13]
Beer (MPs/L)	2–89 fibers/L, 12–109 fragments/L	Germany	[14]
	0–14.3	USA	[36]
Canned sardines and sprats	–	Canada, Germany, Iran, Japan, Latvia, Malaysia, Morocco, Poland, Portugal, Russia, Scotland, Thailand, Vietnam	[39]

followed by Indonesia (1.36×10^4 MPs/kg), Italy (1.57×10^3 – 8.23×10^3 MPs/kg) [32, 33], the USA (0.47×10^2 – 8.1×10^2 MPs/kg), and China (5.5×10^2 – 6.8×10^2 MPs/kg) [16, 36]. A recent study compared MP concentrations in table salts collected from different regions, using sea salt as a seawater MP pollution indicator, which indicated a significant higher MP concentration in Asia than in other

continents [38]. The lowest concentrations of MPs were reported by Karami et al. [51]. This was probably due to its filters with larger pores (149 μm), which allowed smaller-sized MPs to escape in the filtration process and thus underestimated the MP abundance.

4 Microplastics in Drinking Water

Compared to seafood and table salt, relatively few studies reported MP contamination in drinking water. The available data cover raw and treated water from drinking water treatment plants (DWTP), tap water, and bottled water [37, 38, 52]. According to the MP abundance in raw and treated water, particles larger than 50 μm can be removed from water at the treatment plants. The removal rate of MPs by traditional drinking water treatment processes varied from 25 to 90%, depending on treatment technologies [35]. For tap water samples, the lowest abundance was observed in Italy and Denmark (0 MPs/L), while the highest abundance (9.24 MPs/L) was found in the tap water of the USA [36]. The abundance of MPs in bottled water varied from 0 to 5.4×10^7 MPs/L [37, 38, 52]. Water in returnable-used plastic containers had significantly more MPs compared with that in single-used bottles [37].

The difference of detection limits among studies in the drinking water field is obvious. MPs in tap water were often analyzed by micro-Fourier transform infrared spectroscopy (μ -FTIR), capturing MPs $> 20 \mu\text{m}$ [45]. All bottled water and the water from Czech DWTP were analyzed using non-FTIR or μ -FTIR combined with other method [43, 47]. These methods included μ -Raman, dyeing method combined with μ -FTIR, and “method for the extraction and determination of MPs in organic and inorganic matrix samples,” making “small-sized MPs ($< 10 \mu\text{m}$)” detectable. The MP concentration in tap water samples may be underestimated due to the non-detectable smaller MPs using μ -FTIR, which leads to the biased result that the higher concentrations of MPs were detected in bottled water than in tap water. The identification of “small-sized MPs ($< 10 \mu\text{m}$)” is in an urgent need for accurate recognition of MPs and the relative risk assessment, regarding not only drinking water but other food items [37].

5 Microplastics in Other Food Items

MP contamination also occurred in other food products according to the literature. These rarely reported food types include beer, sugar, honey, chicken, tea, as well as canned sardines and sprats [13–15, 39, 53–55]. Besides, we have found MPs in edible seaweed (unpublished data). However, the data of these food items are so limited that more investigations are needed on a broader range, covering more regions and food types.

In recent years, MP pollution has been widely recognized in soil, where the crops and edible vegetables we eat grow. This undoubtedly poses a risk to land plants. Although there is no evidence from field investigations, laboratory studies have shown that polystyrene microsphere (0.2 μm) can be absorbed by lettuce roots and then migrate to shoots and accumulate in edible stems and leaves [56]. Although larger MPs are difficult to enter root cortex, they may adhere to plant surface and be ingested by human [57].

6 Source, Human Burden, and Potential Health Risks

6.1 Source Diagnostics

To date, MPs are ubiquitous in terrestrial environment. Together with the whole process from food acquisition to ingestion, the sources of MPs in food become diverse and complex. Figure 1 shows the potential MP sources during the whole process of seafood consumption, and other food items experience similar processes to seafood.

In the beginning, the main factor affecting food contamination is the pollution degree of the surrounding environment. Typical examples are table salt and seafood. MPs in the surrounding environment can fuse into, adhere to, or be ingested by marine animals [58, 59]. Several studies found that the abundance of MPs in sea salt was higher than that in rock salt or lake salt, which could be explained by the high level of MP pollution in coastal zones [16]. Besides, both mussel and sea salt are proposed as indicators of MP pollution in marine environment [6, 33]. There are also some plastic appliances and ropes acting as pollution sources of MPs in cultured seafood. Another MP source of farmed seafood is the feeding materials produced from MP-contaminated fish or other animals [21].

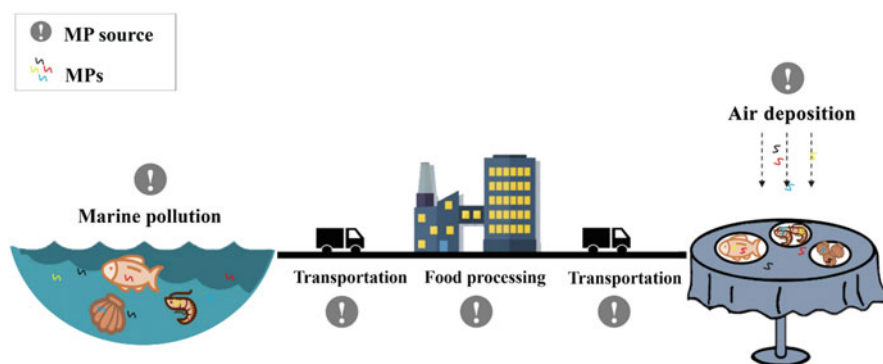


Fig. 1 Potential microplastic sources of seafood from marine environment to the table

After acquisition, food items commonly experience food processing. The presence of MPs in table salt suggests that MPs might be introduced during collection, transportation to surface water, concentration, drying, crystallization, or refinement processes [16]. Some food items are processed with additional substances such as salt or water. If these additives have been already contaminated, MPs will be introduced. Moreover, food packaging materials are often made of plastics, causing the possibility of food contamination. According to the latest research, a single plastic tea bag can release approximately 11.6 billion MPs and 3.1 billion nanoplastics into a single cup of the beverage [55]. The staggering data reminds us to pay more attention to food packaging and all external factors that may have similar effects. For instance, takeout food industry is booming in today's quick living pace, resulting in more plastic packaging for food. Such situation makes food more likely to be contaminated.

There is also an important source that needs special emphasis, which is airborne MPs. Air contact exists almost throughout the entire process of food consumption, from food acquisition to human ingestion. To date, atmospheric MPs have been discovered in many countries and regions, both indoor and outdoor environments [60, 61]. Airborne MPs may have greater contribution to food MP pollution than other sources. The risk of plastic exposure caused by mussel has been confirmed to be minimal compared to fiber ingestion through air fallout during a meal [62].

6.2 *Human Body Burden*

Contaminated food items are undoubtedly sources of gastrointestinal exposure for human. A preliminary estimate on the body burden of MPs was made based on detected MP concentrations in table salt, seafood, and drinking water. MP intake through other food items cannot be estimated due to scarce data. The abundance of MPs in table salt ranges widely from 0 to 2.0×10^4 MPs/kg. Considering the global mean intake of table salt of 10.06 g/day [63], the intake of MPs ranges from 0 to 198 MPs per day, equivalent to 0 to 7.3×10^4 MPs per year. The highest value is calculated according to the data of salt from Croatia [32]. The actual MP exposure through salt intake depends on the types and brands of table salts, as well as the study regions.

The presence of MPs in seafood has been widely recognized [27, 28, 48]. In 2014, van Cauwenberghe and Janssen first estimated the potential MP intake through seafood consumption. It showed that in Europe, where shellfish consumption was high, an adult may ingest up to 11,000 MPs per year [22]. In countries with low shellfish consumption, consumers ingest an average of 1800 MPs per year, which is still a considerable exposure. The annual intake of MPs through seafood consumption worldwide has been estimated by Hantoro et al., ranging from 11 to 3.5×10^4 particles per person [64]. Since MPs are mostly detected in gastrointestinal tracts (GITs), which are normally removed before seafood consumption, species that we eat whole pose greater threats than gutted ones. However, MPs in the eviscerated

flesh of two commonly consumed dried fish species were significantly more than in excised organs, evidencing that the evisceration does not necessarily eliminate the risk of MP intake [45]. Moreover, MPs were also detected in the muscle of commercial fish [30]. These findings raise concerns about possible implications for human consumers.

With around 1.4 L water intake per day [65], the annual MP ingestion through drinking water, including tap water and bottled water, could be in the range of $0\text{--}2.8 \times 10^{10}$ MPs. However, the MPs detected in bottled water have different size fractions ($<10 \mu\text{m}$) from those detected in tap water, seafood, and table salt. The abundance, as well as the potential human risk of particles with different size ranges, is not comparable. Therefore, when estimating the MPs in the three media, we only calculated human MP intake through drinking tap water ($0\text{--}4.7 \times 10^3$ MPs per year), without considering the data of bottled water.

Despite that we have estimated the intake of MPs by human through food consumption, these data cannot represent the real situation. When calculating it, we mostly take MP level of food itself into consideration but ignore other contamination possibilities (from food processing, air, package, etc.). Thus, more attention needs to be paid to these areas. When all factors are taken into consideration, the total amount of MPs ingested by human through food is likely to rise by orders of magnitude. However, excessive panic is uncalled-for before there is sufficient toxicological evidence related to human body. Our body is in a process of dynamic metabolism, and the unabsorbed MPs will be discharged with feces. Therefore, the absorbed MPs and the amount of pollutants (organic pollutants and heavy metals) released during MP metabolism are needed to make clear.

6.3 *Translocation and Accumulation in Human Body and Health Risks*

After ingestion, MPs are capable of translocating and accumulating in different organs and tissues. MPs have been found to be internalized in the gastrointestinal tract, and the unabsorbed portion is excreted with human feces [66]. The studies on other nano-sized particles provided evidence of penetration in the blood-brain barrier and placenta and even crossing the cell membrane [67, 68]. However, there is still no direct evidence showing the exact distribution and accumulation of MPs in human organs such as the liver and kidney or in human blood.

Our current knowledge is very poor about whether MPs will reach human organs and cause adverse health impacts. The available animal experiment results may have some implications for human health effects of MPs. Ingestion of MPs caused inflammatory responses in the digestive system of *Mytilus* [69]. The immune system of fish was the target of MP attack [70]. Inflammations including chemokine expression and pulmonary hypertension were induced by intrajugular injection of polystyrene (PS) microspheres in rats, probably due to the increased blood

coagulability or vascular occlusions [71, 72]. In vivo experiments showed that PS could be internalized in macrophages, erythrocytes, as well as rat alveolar epithelial cells, showing damages to intracellular structures [73, 74]. Moreover, persistent organic pollutants, metals, and pathogenic microorganisms can be adsorbed on MPs, and the leaching of chemical additives can also aggravate the toxic effects of MPs [75–78]. MPs have been verified to be transport vectors for hydrophobic organic chemicals (HOCs) in aqueous environments [79, 80]. Apparent enrichment coefficients of HOCs on MPs might be up to five or six orders of magnitude higher than the background concentration in the surrounding seawater. MPs may then transport HOCs over long distances and affect the environmental and biological systems [80]. The debate on the harmfulness of MPs to human health remains. Some researchers emphasized the danger posed by food chain transfer, while others claimed no adverse effect caused by MPs or MP additives [78, 81]. The controversies mostly lie in the uncertainty of MP intake estimate, which calls for more precise MP intake measurements or stimulating analysis. More research is urged to quantify the concentrations of MPs in the tissue and to understand the mechanisms of the induced human symptoms [82].

7 Conclusions

Food safety is an important issue closely related to human health. MP-contaminated food poses a global concern, and humans are vulnerable to MP exposure through consumption of these food items. The related information is scarce, and there may be more kinds of food contaminated by MPs. What's more, broader range of research subjects and the detection technologies of small-sized particles are required in addition to the uniformity of methods when we are assessing food safety. Besides, we should take all pollution sources and possibilities into consideration. On this basis, the human intake we calculate will be closer to the actual value.

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Microplastics in Aquaculture Systems and Their Transfer in the Food Chain



Weiwei Lyu, Qiang Chen, Lin Cheng, and Wenzong Zhou

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Abstract As emerging pollutants, microplastics (MPs) are ubiquitous in aquatic environments. However, our knowledge concerning microplastic pollution in aquaculture systems is limited. Aquaculture systems are designed specifically to rear aquatic animals that serve as a food source for humans. Aquaculture accounts for almost half of the fish used for human consumption worldwide. Therefore, we need to pay more attention to ecological and food safety issues caused by MP pollution in the aquaculture environment. In this chapter, we discuss the sources and distribution characteristics of MPs in aquaculture systems and explore the relationship between

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MPs and aquatic organisms. Moreover, we summarize the behavioral, morphological, and physiological responses of aquatic animals to MPs. Ultimately, we analyze the migration and potential biomagnification of MPs among trophic levels in the food chain. Based on above analysis, MP pollution needs to be effectively alleviated by developing degradable plastics and reducing the entrance of MPs into aquaculture systems.

Keywords Aquaculture, Distribution, Food chain/web, Microplastic, Stress response

1 Sources of Microplastics in Aquaculture Systems

In aquaculture systems, microplastics (MPs) are usually derived from damaging and aging of commercial fishing gear, including net cages, plastic lines, and trammel nets. In addition, MPs can also appear because of indirect transfer by surface runoff, rivers, wind, and rainfall. Plastic gears, such as fishnets, buoyant materials, and net cages, are necessary tools in marine aquaculture. These plastic gear materials break into MPs during prolonged ultraviolet (UV) light exposure and mechanical abrasion (sand and waves) [1]. The concentration of MPs in cultured mussels is higher than that of wild mussels, which might be related to the use of plastic polypropylene carrier lines that generate MPs in the aquaculture environment by fragmentation [2]. In the Xiangshan Bay of China, 55.7% and 36.8% of the MPs in the seawater and sediment come from fishing nets, buoyant material, and net cage derivatives used in mariculture. In particular, the high concentration of polyethylene (PE) foam MPs is closely related to the porous structure and the high usage rate of PE foam [3]. In shellfish culture, the use of antipredator netting does not protect shellfish, but becomes the source of MPs [4]. In marine bottom trawling and small-scale (trammel) fishery operations, wear and tear on bottom trawling and aging of fishing nets lead to MP ingestion and diffusion in the ocean [5–7]. In freshwater, MPs produced by the breakage of fishing gear are distributed in fishponds [8], lakes [9], and rivers.

Wind and atmospheric fallout contribute to the distribution of MPs in aquaculture areas. In the Gulf region, because of its semi-closed geographical conditions, typhoons increase the concentration of MPs in the Gulf, increasing the concentration of MPs in cultured oysters [10]. At the same time, storms and rainfall also increase the concentration of marine MPs [11–13]. In freshwater lakes, wind can change the distribution of MPs [14, 15].

Rivers and runoff help to transfer MPs to aquaculture areas. The MPs contained in facial care products are discharged directly into the river through the sewers, resulting in their accumulation in aquaculture systems. At the entrance of a fishpond, the concentration of MPs is higher than that at the outlet [8], because MPs are deposited in the fishpond, and the presence of MPs found in the sediment reduces the

MP concentration at the outlet. The MPs contained in soil can enter a river directly through runoff. The main source of MPs in rivers is treated sewage from wastewater treatment plants. Although 98% of the MP particles can be removed from treated sewage, the remaining 6.5×10^7 particles are still discharged into the receiving water every day [16].

Thus, the sources of MPs in aquaculture systems are both direct and indirect. The direct sources are the plastic tools used for aquaculture that are exposed to sunlight and under the mechanical action of wind, waves, and sand, which break them into MPs. Indirect sources deliver MPs into aquaculture systems in various ways. Current research has pointed to a variety of sources of aquaculture MPs; however, more research is needed on methods to block their delivery into aquaculture systems.

2 Distribution Characteristics of MPs and Their Relationship with Aquatic Organisms

The distribution of MPs is strongly linked to human activities: the more human activities, the higher concentration of MPs. Many studies have detected the presence of MPs in rivers, lakes, oceans, soils, and even in the air. In the end, water bodies are the final sink of accumulated MPs in the environment. Aquatic animals ingest MPs after exposure. Therefore, the distribution of MPs is closely related to aquatic animals.

2.1 MP Distribution and Composition

With the use of plastic products in fisheries, more and more MPs are distributed in lakes, rivers, oceans, and soils, because of damage and loss of fishing gear. Studies have shown the presence of MPs in diverse environments (detailed in Table 1).

MP debris is usually transported by wind or direct runoff after rain, and a large portion of it inevitably enters the freshwater aquatic ecosystem, where it accumulates. Therefore, the distribution of MPs in freshwater bodies also needs to be investigated. In Dongting Lake and Hong Lake in China, fishery activity was determined as an important source of MPs in both lakes [17]. Taihu Lake, one of the five major lakes in China, was found to have MPs distributed in its sediment and surface water [18]. In the Lakes Superior, Huron, and Erie of Great Lakes in the USA, massive amounts of polypropylene (PP), PE, and polystyrene (PS) were found [19]. MPs have also been found in sediments of an Indian lake where fishing, agriculture, and farming activities are very intense [20].

Many river-related studies have also shown the presence of MPs in surface water. Moreover, some MPs exist in stagnant water, which lead MPs to subside into sediments or deep-water areas. In the river Thames of London, there is an abundance

Table 1 List of studies related to microplastic contamination of lakes, rivers, oceans, and soils

Environment	Site	Compartment(s) studied	Identification	Size	Reference
Lakes	Dongting Lake Hong Lake	Surface waters	PE, PS, PVC, PS	0.05–5 mm	[17]
	Taihu Lake (China)	Lake water Lake sediments	PT, PET, PS PE, TA, PP	5–5,000 µm	[18]
	Lakes Superior, Huron, and Erie (USA, Canada)	Lake water	PP, PE, PS	0.355–4.75 mm, >4.75 mm	[19]
	Vembanad Lake (India)	Lake sediments	PE, PS, PP	<5 mm	[20]
Rivers	Rivers in Greater London (UK)	Lake water	PE, PP	No data	[21]
	Urban river (China)	River sediments	PP, PE, PR, PVS, rayon, cotton + viscose	<100 µm, 100 µm–5 mm, >5 mm	[22]
	Los Angeles and San Gabriel Rivers (USA)	River water	PS	1–4.75 mm, >4.75 mm	[23]
	St. Lawrence (Canada)	River sediments	PE	0.4–2.16 mm	[24]
	The North Shore Channel (USA)	River water	No data	No data	[25]
	Rhine River (Europe)	River water	PS, PE, PMMA, PP, PVC, acrylate	300–1,000 µm	[26]
	Rhine and Main River (Germany)	River sediments	PE, PP, PS, PA	63–5,000 µm	[27]
Oceans	Jurujuba Cove (Brazil)	Surface water	PE, PP	<1 to ≥5 mm	[28]
	Xiangshan Bay (China)	Surface water Sediments	PE, PP, PS, PET, rubber	1.53 ± 1.54 1.33 ± 1.69 mm	[3]
	Lagoon (Italy)	Sediments	PE, PP	30–500 µm	[29]
	Bohai Sea Yellow Sea	Sediments	PE, PET, PP, PA, PVC	8–5,000 µm	[30]
Soils	Switzerland	Floodplains	PE, PS, PVC, PP, Latex, SBR	500–5,000 µm	[31]
	Southwestern China	Cropped soils Riparian forest buffer soils	No data	0.05–10 mm	[32]
	Shanghai (China)	Farmland soils	PP, PE	<1 to >5 mm	[33]
	Sementa Mangrove Area (Malaysia)	Coast soils	No data	No data	[34]
	Bohai Sea and Yellow Sea (China)	Coastal soils	PE, PP, PS, PEU	<1 mm	[35]

PE polyethylene, *PP* polypropylene, *PET* polyethylene terephthalate, *PS* polystyrene, *PA* polyamide, *PVC* polyvinyl chloride, *PT* plain transparent cellophane, *TA* terephthalic acid, *PMMA* polymethyl methacrylate, *PR* phenoxy resin, *PVS* poly (vinyl stearate), *PVA* polyvinyl alcohol, *PMA* polymethyl acrylate, *PA 6* nylon 6, *ABS* acrylonitrile butadiene styrene, *SBR* styrene butadiene, *PET* polyethylene terephthalate, *PU* polyurethane

of PE and PP floating on the surface water [21]; however, there were fewer types of MPs than in an urban river of Shanghai [22], which might be related to the population size and living habits. In Los Angeles and San Gabriel Rivers, the main MPs are PS [23]. However, in the St. Lawrence River, the majority of MPs in sediments are PE [24]. In the North Shore Channel of the USA, MPs were detected in the surface water, because the treated wastewater effluent from a water reclamation plant flows into the river [25]. In the whole basin of the Rhine River, there are more types of MPs than in the Rhine River main area in Germany [26, 27].

The marine environment is the largest water body containing MPs, which are present in different sizes, shapes, and types in the surface water and sediments. In Jurujuba Cove, an area of mussel farming, Castro found high concentrations and diverse shapes and types of MPs [28]. In the surface seawater and sediments of Xiangshan Bay, a long-term intensive mariculture area, the majority of MPs are fibrous, including PP, polyethylene terephthalate (PET), and cellulose [3]. Vianello pointed out that the presence of MPs in the Lagoon of Venice has been affected by the development of aquaculture [29]. The Bohai Sea and Yellow Sea, which are the major farming sites of sea cucumber in China, contain 20–1,040 particles kg^{-1} in their dry sediment [30]. MPs in marine aquaculture environments will have a negative impact on farmed animals. Consequently, we should focus on the impact of MPs on offshore aquaculture.

In terrestrial ecosystems, MPs are also present in soil and farmland because of human activities. A study has shown that MPs in terrestrial ecosystems may be 4–23-fold higher than that in the ocean [36]. The MPs in soil are more buried, which perhaps leads to the notion that there is not a lot of plastic in soil. In developed countries, sewage sludge is commonly used as economical fertilizer for agricultural applications. In Europe and North America, approximately 50% of sewage sludge is used for agricultural purposes [37]; however, there are a lot of MPs in sewage sludge. There have been a few studies on the distribution of soil MPs. In the floodplains of Switzerland, various sizes and types of MPs are distributed [31], probably caused by deposition from rivers. In southwestern China and Shanghai, MPs have been detected in farmland because of the use of mulch and other agricultural plastics [32, 33]. Previous studies have shown that the use of mulch could result in MP pollution [38]. MPs have also been detected in the soil of mangroves [34]. Although studies have shown that MPs exist in soil, their effects on terrestrial animals or plants require further study.

MPs exist in all known environments, including soils, oceans, and freshwater, which are closely related to human activities. Therefore, we need to pay more attention to the negative impact of MPs on human daily life.

2.2 *Effects of Bioturbation on Microplastics*

A process in which animals change their habitat by affecting the structure of sediments is called biological disturbance (bioturbation) [39]. The process by

which animals transport MPs to sediments is also a form of bioturbation. Sources of MPs in the ocean include those from rivers, wind, runoff, tides, and plastic fragmentation from human marine activities [40]. There is also evidence that MPs tend to accumulate in areas with low hydrodynamics in freshwater bodies [29]. MPs in the soil come from various human activities or the rupture of discarded plastic products. Bioturbation is an important reason for the widespread distribution of MPs in the environment.

In the upper ocean water, the vertical distribution of MPs is caused by the wind hybrid drive [15], whereas the presence of MPs in sediments or at different depths of seawater is the result of bioturbation. In deep ocean trenches from the Pacific Rim (depths ranging from 7,000 to 10,890 m), MPs were detected in the hindguts of *Lysianassoidea* amphipod populations [41]. The reason is probably related to the marine snow (MPs in the surface associate with marine snow and then sink to the deep sea) [42]. Experimental research has proven that benthic animals can transport MPs deeper from the sediment surface [43], and the intensity of bioturbation is related to the species composition [44, 45]. The ingestion and egestion of the benthos are a chain reaction that results in the transfer and transport of MPs to deeper deposits [42], for example, mussel (*Mytilus edulis*) and lugworm (*Arenicola marina*)-ingested MPs were also observed in natural habitats [46]. However, in freshwater ecosystems, bioturbation has not been reported to affect the distribution of MPs; further study is required to investigate the effects of bioturbation in freshwater on the distribution of MPs.

In terrestrial ecosystems, experimental studies have proved that earthworms (*Lumbricus terrestris*) incorporate MPs from the soil surface into their burrows [47, 48]. In other animal experiments, two collembolan species could move microplastic particles and significant differences in the distance moved, and the size of the MPs between the two species was observed [49]. MPs interacted with the collembolans and altered their gut microbiome to increase bacterial diversity [50]. At the same time, MPs were found in honey, which might be related to the presence of MPs in the atmosphere. These MPs are usually from dry soil, sewage, and sludge carried by the wind and from human activities [51]. Existing research indicates that the bioturbation in the soil comprises soil organisms transferring the MPs at the soil surface to deeper soils, which might be the reason for the presence of MPs in soils of different depths and different pollution degrees.

2.3 Degradation of Microplastics

Plastic products bring convenience to human life because of their durability; however, they are also difficult to degrade, and discarded plastic waste is problematic [52, 53]. Some plastic will not be degraded after 32 years of burial in soil [54], and some petrochemicals may never degrade. However, under laboratory conditions, polystyrene degrades into nanoplastics under UV irradiation [55]. In an intertidal salt marsh habitat, plastics degrade relatively quickly [56]. Hence, different materials of

plastics degrade at different rates in different environments, and degradation is a slow and long-term process, thus requiring more long-term observation and research.

Typical manifestations of plastic degradation are embrittlement, fracture, softening, hardening, and loss of mechanical strength. The aging and deterioration of plastics are a degradation phenomenon. However, in the general sense, the degradation is only that the plastic becomes smaller, until we cannot see it with the naked eye; however, it does not disappear. Degradation is generally classified according to the agency causing it: (a) biodegradation, action of living organisms, usually microbes, and (b) photodegradation, the action of light (usually sunlight, especially UV light.).

For common plastics, such as PE, PP, and nylon, UV radiation initiates photo-oxidative degradation. Once started, it can be thermo-oxidative at moderate temperatures without the need for UV irradiation. In addition, as long as oxygen exists in the degradation system, the autocatalytic chain reaction can progress. However, there is a significant difference in the degradation rate of plastics in air and seawater [57–60] because of the lower temperatures and oxygen concentration in water environments. Other varieties of plastics on the beach or in water also undergo similar degradations. For instance, the degradation of fishing gear, such as the weathering of specific gear-related plastics, including polyethylene netting and nylon monofilament exposed in air at marine sites and twine, has been reported [61–64].

Among the currently known plastic degradation processes, non-biodegradation simply breaks large plastic fragments into small pieces (MPs) and then smaller pieces (nanoplastics); however, they still exist in the environment. Complete degradation is the process of complex interactions between abiotic and biological factors, the final products of which are carbon dioxide and water. Photooxidative degradation plays a major role in incomplete and complete degradation (the initiation of plastic degradation). However, in the deep sea or in the depths of the soil, further study is required as to whether some plastics can be degraded without light.

3 Response of Aquatic Organisms to Microplastics

MPs dispersed in the aquatic environment (water and sediment) have been reported to trigger a wide variety of toxic insults to aquatic organisms at different trophic levels, including disturbances in behavior, morphology, physiological function, as well as synergistic and/or antagonistic action of other hydrophobic organic contaminants, and thus might pose a risk to human health [65–67]. Once ingested, MPs could be considered bio-inert compounds because they cannot be digested or absorbed for degradation into synthetic polymers [68]. Moreover, MPs can absorb and accumulate metals and persistent organic pollutants (POPs) on their surfaces from the surrounding environment [69, 70]. To date, there is a knowledge gap concerning the response of aquatic organisms to MPs in aquaculture systems.

3.1 Behavioral Responses

Ingested MPs can affect the feeding habits of aquatic organisms, particularly the mechanism of ingestion and the structure of digestive organs. For example, in the pelagic copepod *Calanus helgolandicus*, the ingestion of 20 μm of polystyrene beads significantly altered their feeding capacity [71], and the feeding rate of crustacean *Nephrops norvegicus* kept decreasing with increasing addition of MPs [72]. Similar results were observed in shore crab *Carcinus maenas* [73] and brine shrimp *Artemia parthenogenetica* [74]. *Artemia* larvae are highly vulnerable to prey species; therefore, MPs become more accessible to higher trophic level organisms [75, 76], and exacerbated ingestion of MPs has been found in oligotrophic habitats [77]. In addition, the size of the ingested MPs by sea cucumbers *Apostichopus japonicus* affects their ability to fit into the cucumbers' mouth or the ability of the tentacles to grasp them [30]. Overall, these results indicated that the ingestion of MPs by organisms, which depends on the MPs concentration and encounter rates, feeding modes, and exposure time [78], could pose a threat to the aquatic food web.

3.2 Morphological Responses

As aquatic primary producers, microalgae are essential to the function of aquatic ecosystems [79], and small disruptions of the microalgal population might have serious repercussions for the aquatic food web [80]. Exposure to MPs could cause a significant reduction in the growth of microalgae [81–83], and the inhibitory effects are enhanced with increasing exposure dosage [84]. In addition, a variety of detrimental effects, such as reduced body weight of langoustine (*N. norvegicus*) [72], growth inhibition of shore crab (*Carcinus maenas*) [73], and even mortality of the Asian green mussel (*Perna viridis*) [85], were reported. Meanwhile, exposure and ingestion of MPs can also induce significant damages in the digestive tract, including the intestines of zebrafish (*Danio rerio*) [86] and brine shrimp (*A. parthenogenetica*) [74], and ultrastructural changes of epithelial cells lining the intestines in *A. parthenogenetica* [87]. Moreover, particle uptake could result in histological alterations in the digestive gland of blue mussel (*M. edulis* L) to high-density (HD)-PE exposure [88], and plastic fragments ingested by animals have even triggered ulcerations and rupture of the digestive tract and suffocation [89].

3.3 Physiological Response

High concentrations of MPs in ecosystems could damage physiological functions in living organisms [90]. Subsequently, efforts have been made to explore the physiological and biochemical response of aquatic organisms to MPs. Recently, studies have demonstrated that exposure to MPs could not only cause a large variety of

physical damage and oxidative stress to aquatic organisms but could also affect the expression of genes involved in certain metabolic pathways [84, 91].

3.3.1 Material and Energy Metabolism

Energy metabolism homeostasis encompasses both energy intake and energy expenditure, and stress can affect both energy expenditure and energy intake. Acute and chronic stress exposure can elicit physiological responses that modify the energy balance [92]. Generally, biomarkers are considered to be sensitive indicators of early stress effects of environmental contaminants in exposed organisms and represent useful tools to monitor the adverse effects of pollution in aquatic environments [93]. In addition, the RNA to DNA ratio can be used to estimate energy metabolic conditions [94].

An increase in plasma glucose has been considered as a common response to stress for the maintenance of homeostasis, and the mobilization of energy reserves through endocrine pathways can be considered an adaptive mechanism to increase energy demand [95]. High concentrations of MPs in ecosystems can damage the physiological functions in living organisms [96]. For example, mussels exposed to MPs showed increased energy consumption (respiration) compared with that of the control organisms [46], which was linked to increase stress as the organisms tried to retain their physiological homeostasis [95]. While in *N. norvegicus*, exposure to MPs caused a reduction in the metabolic rate and the catabolism of stored lipids [72]. Moreover, dynamic energy budget modeling using transcriptomic profiles showed a significant shift in energy allocation from reproduction to structural growth and elevated maintenance costs in Pacific oysters, suggesting feeding modifications and reproductive disruption, which would have significant impacts on their offspring [97].

3.3.2 Antioxidation and Immunity

Oxidative stress is defined as a disturbed balance between oxidation and antioxidant systems, and the production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) reflects an organism's capacity to deal with such stresses [98, 99]. The components of antioxidative system (AOS), which is involved in the processes of detoxification and elimination of harmful toxicants from the body, have been used to assess the adverse effects of MPs [100]. In general, superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px) are considered as enzymes of the first line of defense that directly eliminate ROS, and glutathione, as a nonenzymatic component, is involved in scavenging a wide variety of free radicals through the GSH-dependent system (including GSH-Px, GST, and glutathione reductase (GR)) [100, 101]. In addition, lipid peroxidation (LPO) has been viewed

as a self-sustaining chain reaction of molecular events resulting in oxidative damage to cell membranes, lipoproteins, and other lipid-containing structures [102]. Peroxidation of membrane lipids can alter the structure and function of lipid bilayers, change membrane permeability, and promote penetration of cells by toxic agents [103]. Similarly, nuclear alterations, such as the frequency of micronuclei, the olive tail moment, and even genotoxicity, and the malondialdehyde, the product of LPO, have also been widely used as biomarkers of exposure to environmental contaminants [98]. Exposure of MPs can induce an injury-type inflammatory response, accompanied by oxidative stress, active species elevation, and an antioxidative response. Meanwhile, as a nonspecific biomarker, the assessment of oxidative stress should be carried out by measuring LPO together with other enzymatic and nonenzymatic oxidative stress parameters [104].

Most research has focused on the toxicity of MPs to invertebrates (mussels, clams, and crabs) and vertebrates (fishes) in laboratory conditions, in which the biomarkers of the antioxidative system and oxidative damage system have been examined after exposure to MPs. In these studies, the levels of these biomarkers were observed to be significantly affected (Table 2). For example, significant inhibitory effects on Se-dependent glutathione peroxidases and catalase were observed, as well as significant enhancement of DNA strand breaks and nuclear anomalies in hemocytes of mussels (*M. galloprovincialis*) treated with MPs [105]. Exposure to MPs alone and in combination with pyrene could inhibit acetylcholinesterase (AChE) activity, and MPs could increase the GST concentration in fish bile of the common goby (*Pomatoschistus microps*) exposed to pyrene; the mixture of pyrene and MPs also reduced isocitrate dehydrogenase (IDH) activity [106]. In freshwater zebra mussel (*Dreissena polymorpha*), polystyrene microbeads exposure did not produce oxidative stress and genetic damage; however, a significant modulation of catalase and glutathione peroxidase activities was noted in mussels exposed to a mixture of polystyrene microbeads of 10 μm and 1 μm in size [109]. When Chinese mitten crab (*Eriocheir sinensis*) was exposed to fluorescent microplastic particles (diameter = 0.5 μm), the activities of AChE and alanine aminotransferase (GPT) decreased. In the same study, the aspartate aminotransferase (GOT) activity increased significantly after exposure to a low concentration of MPs and then decreased continuously with increasing MPs concentration, while the activities of SOD, GOT, GSH, and GSH-Px increased under low concentrations of MPs and decreased under high concentrations [110]. Additionally, in the freshwater bivalve *Corbicula fluminea* exposed to MPs, LPO levels increased significantly after exposure to MPs, mercury, and their mixture, and MPs alone significantly reduced the adductor muscle cholinesterase enzyme (ChE) activity, indicating neurotoxicity; antagonism between MPs and mercury was also found in *Scrobicularia plana* of ChE activity, GST activity, and LPO levels [108].

In addition, size-dependent oxidative stress responses and associated cellular damage have been demonstrated in the copepod *Paracyclops nana* after exposure to 0.5 and 6 mm polystyrene microbeads, suggesting that activation of the p38

Table 2 Effects of MPs on antioxidative biomarkers and oxidative damage biomarkers

Organism	Type	Concentration	Tissue	Biomarker	Reference
Mussel (<i>Mytilus galloprovincialis</i>)	PE, PS, PYR	20 g/L	Hemolymph, digestive tissues	CAT, GST, GSH-Px, GR, GSH, LPO, DNA strands breaks, FMN, NA	[105]
Juvenile of goby (<i>Pomatoschistus microps</i>)	PE, PYR	18.4 µg/L, 184 µg/L	Gallbladder bile	GST, ACHE, LPO	[106]
Red mullet (<i>Mullus surmuletus</i>)	N/A	0.42 ± 0.04 MPs/individual	Liver	SOD, CAT, GST, LPO	[5]
Marine mussels (<i>Mytilus</i> spp.)	PE, PS	32 µg/L	Digestive tissues	SOD, CAT, GR, GST, LPO	[107]
Peppery furrow shell (<i>Scrobicularia plana</i>)	PS	1 mg/L	Gills, digestive tissues, hemolymph	SOD, CAT, GST-Px, GST, LPO	[108]
Freshwater zebra mussel (<i>Dreissena polymorpha</i>)	PS	5 × 10 ⁵ to 2 × 10 ⁶ of 1 µm size mixed with 5 × 10 ⁵ of 10 µm size	Soft tissues, hemolymph	SOD, CAT, GSH-Px, GST, LPO, FMN	[109]
Chinese mitten crab (<i>Eriocheir sinensis</i>)	PE	40, 400, 4,000, and 4,000 µg/L	Intestine, liver	AChE, GPT, GOT, SOD, GOT, GSH, GSH-Px,	[110]
Freshwater bivalve (<i>Corbicula fluminea</i>)	N/A	0.13 mg/L	Gills	CAT, GR, GSH-Px, GST, LPO	[111]
Juvenile seabass (<i>Dicentrarchus labrax</i>)	N/A	0.25 mg/L, 0.69 mg/L	Brain, muscle	LPO	[112]

Note: superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px), glutathione S-transferase (GST), glutathione reductase (GR), glutathione (GSH), acetylcholinesterase (ACHE), alanine aminotransferase (GPT), aspartate aminotransferase (GOT), lipid peroxidation (LPO), nuclear alterations (NA), frequency of micronuclei frequency (FMN), olive tail moment (OTM), polyethylene (PE), polystyrene (PS), pyrene (PYR)

(mitogen-activated protein kinase (MAPK)14) and extracellular signal-regulated kinase (ERK) pathways in *P. nana* involve the defense mechanism against microplastic-induced oxidative stress via the MAPK/nuclear factor, erythroid 2 like 2 (NRF2) pathway, in which NRF2 is considered a key regulatory transcription factor for genes encoding defensive enzymes, including GR, GPX, and SOD, in response to oxidative stress [113]. Furthermore, particle uptake was found to influence the inflammatory response in the digestive gland of the blue mussel

(*M. edulis*) [88] and disturb the immune system in mussels (*M. galloprovincialis*) [105]. Additionally, MPs translocated in the hemolymph and hemocytes of blue mussel resulted in an early granulocytoma formation (inflammation), an increase in hemocytes, and an important decrease in lysosomal membrane stability (LMS) [114]. Furthermore, the quantity of energy assigned to immune functions could be reduced, resulting in harm to all normal physiological processes [115]. In fact, some plastics labeled as food-safe have been considered highly toxic to aquatic animals and could pose a greater threat to humans than previously realized [116]. Moreover, tiny MPs in aquatic systems have been reported to penetrate into phagocytic cells of organisms because of long-term retention of MPs in their body, which would also facilitate the transfer of MPs to higher trophic predators [75, 114]. Therefore, transcriptional approaches, including “omic” techniques and subsequently quantitative real-time PCR-targeted analyses, which are regarded as the most powerful methods to explore cellular and molecular mechanisms and the sublethal effects of MPs on aquatic organisms, have revealed the main pathways of the interactions of immune-related responses, stress responses (including antioxidants), cell signaling, and cell energy homeostasis.

3.3.3 Reproductive Behavior

Chronic exposure to MPs may result in reproductive and developmental effects in aquatic organisms and the formation of reactive oxygen species (ROS) and adverse outcome caused by MPs increased reproduction failure [117]. In previous studies, the prolonged exposure of the pelagic copepod *C. helgolandicus* to 20 μm polystyrene beads significantly decreased their reproductive output [118]. Primary and secondary MPs have been shown to affect the reproduction of three different Cladoceran species (*Daphnia magna*, *Daphnia pulex*, and *Ceriodaphnia dubia*) in a dose-dependent manner. The no observed effect concentration (NOEC) was less than the lowest tested concentration (10^2 p/mL) for at least one toxic endpoint for all species, and primary MPs appeared to have greater toxic potential when compared with secondary MPs [119]. Chronic exposure to PS microbeads resulted in reduced survival and fecundity and developmental delays in the offspring of *Tigriopus japonicus* nauplii [120]. Moreover, exposure to MPs could delay gonad maturation, decrease fecundity, and decrease the concentrations of 17β -estradiol (E2) and testosterone (T) in the plasma of female medaka (*Oryzias melastigma*). MPs also had a significantly negative regulatory impact on the female hypothalamus-pituitary-gonadal axis, as assessed using gene transcription analysis [121]. However, a significant increase in the mean number of offspring for the cladoceran *D. magna* was observed after exposure to the highest polystyrene MP concentrations of different sizes [122]. Hence, exposure to MPs could affect reproductive behavior and even offspring development of aquatic organisms.

4 Transfer of Microplastics in Food Chain of Aquaculture Systems

4.1 *Microplastic Distribution in Different Trophic Levels*

According to the classification of nutrition levels [123], the first nutrition level (producer) converts inorganic matter into organic matter, the second nutrition level (herbivores) feeds on producers, and the third nutrition level (carnivores) feeds on herbivores. By analogy, there will be a fourth and fifth nutrition level, consisting of each nutrition level to form a food chain, and each food chain to form a complex food web.

Under laboratory conditions, polystyrene beads with charged groups could bind to algae (first nutrient grade), *Chlorella* and *Scenedesmus*, and inhibit their photosynthesis [124]. At the same time, the growth of algae *Skeletonema costatum* was inhibited after exposure to MPs [125]. Zooplankton, as the second nutritional level, ingests MPs into the body which significantly decreased algal feeding [126] and causes oxidative stress reactions [127]. Low-density polyethylene MPs were also detected in wild zooplankton by spectroscopic and digestion method [128, 129]. Studies have shown that in higher trophic levels, organisms such as coelenterates (jellyfish), crustaceans (shrimp, crab), mollusks (bivalves, sea cucumbers), fish, amphibians (tadpole), reptile (turtle), and mammal (whale) will ingest MPs [130–138]. At the top of the food chain, it has been reported that MPs have been found in human feces [139].

The results of various experiments show that MPs can enter various nutrition grades, from the bottom to the top, and the whole food chain is at risk of contamination by MPs, which also threatens human health. However, under current laboratory conditions, the concentration of MPs used is much higher than the actual concentration in the environment, while the concentration of MPs in the organisms found under field conditions is lower and has not reached the level of damage to individuals. Moreover, MPs in natural environments are usually mixed in different sizes and types. Therefore, different biological effects of these MPs and the effects of joint exposure on organisms require further research.

4.2 *Biomagnification of Microplastics Through the Trophic Cascade*

Many pollutants, e.g., heavy metals, persistent organic pollutants (POPs), and MPs, can be transmitted and magnified through the food chain [140]. Under natural conditions, it is difficult to determine whether MPs are ingested directly or indirectly (nutrition level transfer). Therefore, current research is based on laboratory conditions that attempt to simulate the natural environment as much as possible the nutrient level transfer of MPs. Medium-sized zooplankton (prey), copepods, and

Marenzelleria spp., which had ingested fluorescent MPs, were incubated with the large-scale predator mysid shrimp and medium-sized zooplankton, and fluorescent signals appeared in the mysid shrimp [141]. After exposure to fluorescent MPs, mussels (*M. edulis*) were fed to crabs *C. maenas*, and high concentrations of MPs were found in the hemolymph of the crabs; however, the MPs in the hemolymph almost disappeared after 21 days of exposure [75]. In simulated natural conditions, wild-captured Atlantic mackerel (*Scomber scombrus*) were fed to captive gray seals (*Halichoerus grypus*), and the presence of MPs was demonstrated in mackerel and gray seal feces [142]; captive gray seals try to avoid the direct intake of MPs, so the MPs in the feces must have been derived from the mackerel.

After the intake of MPs by aquatic animals, the fate of MPs will vary depending on their size and shape and will be retained in the intestinal tract of the animal or even transferred from the intestinal tract to other tissues. MPs at 5 μm will be transferred to the liver of zebrafish and the hepatopancreas of *E. sinensis*, but MPs at 20 μm will not be transferred to the liver of zebrafish [132, 143]. In addition, MPs were found in the hemolymph of mussel *M. edulis* after exposure to MPs at 3 or 9 μm [114]. Most bivalves capture and retain 3–4 μm particles at 100% efficiency. They can retain particles as small as 1 μm in diameter; however, the efficiency is reduced by about 50% [144]. MPs were fed to the crab *C. maenas* and then detected in the crab hemolymph [75]. MPs with diameters ranging from 124 to 438 μm were found in the liver of wild anchovies [145]. Similar observations were also made in wild fish and shrimp muscles [146, 147].

The biomagnification of MPs during nutrient grade transfer is closely related to the retention time of MPs in animal tissues, which in turn is closely related to the size and shape of the MPs. In the copepod *C. helgolandicus*, hunger prolongs the retention time of MPs in the intestines [126]. MPs entering mussel (*M. edulis*) hemolymph can be retained for more than 48 days [114]. Some scholars have predicted the size of MPs and the probability of their biomagnification in the food chain. The longer MPs remain in animals, the higher risk of MPs passing through the food chain to higher levels of nutrition.

4.3 Microplastic Exposure in Human Food Through Aquatic Product Consumption

When MPs are exposed to commercial aquatic products consumed by humans, they are indirectly, rather than directly, ingested. It has been observed that a large number of aquatic animals consumed as human food can ingest MPs, including fish (e.g., Atlantic cod, Atlantic horse mackerel, European pilchard, red mullet, and European sea bass), bivalves (e.g., mussels, oysters), and crustaceans (e.g., shrimp, crab). The relevant research is listed in detail in Table 3.

Currently, it is beyond doubt that MPs can enter into human body through food chain transfer [142]. However, many questions still need to be answered via further

Table 3 Distribution of microplastics in human edible aquatic products and analytical methods

Food type	Microplastic average content (SD)	Method of analysis	Reference
<i>Fish</i>			
Mesopelagic (five species) and epipelagic (one species) fish, North Pacific Central Gyre	2.1 (5.8) particles/fish ($n = 235$) Size: >10,000 μm (cm range, 1–10 cm)	Stomach contents, microscopic detection Method blanks not indicated	[148]
Pelagic and demersal fish, English Channel	1.90 (0.10) particles/fish ($n = 184$), of 504 fish, 184 had microplastics Size: 130 to >5,000 μm	Digestive tract contents, naked eye detection, microplastics removed with tweezers, confirmation with FT-IR Method blanks not indicated	[135]
Commercial fish, 26 species, Portuguese coast, seven locations	1.40 \pm 0.66 particle/fish ($n = 52$; 17 out of 26 species sampled) Size: 220–4,800 μm	Stomach contents, microscopic detection, microplastics removed with tweezers, confirmation (subset) with FT-IR Method blanks not indicated	[149]
Commercial fish from fish markets in California (USA) (12 species) and Sulawesi (Indonesia) (11 species)	California: 2.03 (2.71) ^a particles/fish, mainly fibers Sulawesi: 5.03 (6.43) ^a particles/fish, mainly fragments, film, foam Size: average 6,300 (SD 6,700) μm	Digestive tract contents, extraction/digestion with KOH, microscopy (detection limit: >500 μm) Method blanks used	[150]
Pelagic (two species) and demersal (three species) fish, North Sea, Baltic Sea	1–7 particles/fish ($n = 16$) Size: <5,000 μm	Gastrointestinal tract contents, filtered through sieve (500 μm), microscopy, confirmation with FT-IR Method blanks not indicated	[151]
Commercial fish (three species) Mediterranean Sea, North Sea, and English Channel	80% of fish had microplastics in liver, mainly polyethylene Size: 124–438 μm	Digestion with NaClO, polarized light microscopy, Raman spectroscopy	[145]
Commercial fish (three species), Mondego estuary (Portugal)	38% of three species had microplastics, 1.67 \pm 0.27 (SD) microplastics/fish ($n = 46$), 96% fibers	Extraction/digestion with KOH, filtered through sieve (1.2 μm), stereomicroscopy, analyzed by μ -FT-IR	[152]
<i>Shrimp</i>			
Norway lobster (<i>Nephrops norvegicus</i>), north Clyde Sea, six locations	83% animals contained microplastics, mainly polyethylene, predominantly filaments	Formaldehyde immersed, microscopy, scanning electron microscopy, micro-Raman spectroscopy	[153]

(continued)

Table 3 (continued)

Food type	Microplastic average content (SD)	Method of analysis	Reference
Brown shrimp (<i>Crangon crangon</i>), Southern North Sea, English Channel, 16 locations	0.75 (0.53) ^a particles/g wet weight ($n = 165$) Size: 200–1,000 μm	Extraction/digestion with $\text{HNO}_3/\text{HClO}_4$, detection/counting microscopy, confirmation with hot point test Method blanks used	[131]
<i>Aristeus antennatus</i> Balearic Basin (north-western Mediterranean Sea), three locations	39.2% samples contained microplastics ($n = 58$) Majority were fibers Size: >5 mm	Formalin fixed, and stained with Alizarin-Red, take images	[154]
<i>Crab</i>			
Velvet crabs (<i>Necora puber</i>), Bay of Brest (France)	Microplastics in the intestinal tract	Three different protocols for digestion, Raman micro-spectrophotometer, microscopy	[155]
<i>Bivalves</i>			
<i>Mytilus edulis</i> , commercial mussels and wild mussels from Belgian	0.37 (0.22) ^a particles/g wet weight ($n = 9$) Size: 200–1,500 μm	Extraction/digestion with $\text{HNO}_3/\text{HClO}_4$, detection/counting microscopy, confirmation with hot point test Method blanks used	[156]
Commercial bivalves: <i>Mytilus edulis</i> , from one location (mussel farm), <i>Crassostrea gigas</i> , from one location (supermarket)	<i>M. edulis</i> : 0.36 (0.07) particles/g wet weight ($n = 72$), <i>C. gigas</i> : 0.47 (0.16) particles/g wet weight ($n = 21$) Size: 5–25 μm (55–100%), >25 μm (0–45%)	Extraction/digestion with HNO_3 , detection/counting microscopy, confirmation (subset) with Raman Method blanks used	[133]
Oysters (<i>Crassostrea gigas</i>) commercial, from fish markets in California (USA)	1.8 (1.72) ^a particles/oyster ($n = 4$) Size (mainly fibers): average 5,500 (SD 5,800) μm	Extraction/digestion with KOH, microscopy (detection limit: >500 μm) Method blanks used	[150]
<i>Mytilus edulis</i> , French-Belgian-Dutch coastline, six locations	0.2 \pm 0.3 particles/g (size range 20–90 μm) Size: 20–90 μm	Extraction/digestion with HNO_3 , detection/counting microscopy, confirmation (subset) with Raman Method blanks not indicated	[46]

n number of samples containing microplastics, *FT-IR* Fourier-transform infrared spectrometry

^aValue calculated from the paper

studied, such as follows: (1) Does the existence of a small amount of MPs cause human diseases? (2) What concentration of MPs would definitely lead to the occurrence of disease? (3) Is the occurrence of human diseases associated with the digestion of MPs alone or in combination with heavy metals and organic pollutants?

(4) Is the toxicity of MPs related to the polymer components and sizes of MPs? Therefore, further efforts should be made to fully explore the potential effects of MPs on humans.

5 Conclusions

Currently, we have limited knowledge concerning MPs in aquaculture systems. Unlike the wild environment, the ecological health of aquaculture systems is affected greatly by human factors. Therefore, in theory, sources of MPs in aquaculture area are diverse, but controllable. However, the use of plastic tools has caused the ubiquity of MPs in aquaculture environments. The degradation of plastics in the natural environment is extremely slow. Moreover, the definition of this degradation is only fragmentation – the cracking of large plastics into small pieces. The input of plastic debris changes the habitat environment of aquatic organisms, resulting in various stress reactions.

The aquaculture system is designed specifically to rear aquatic products to serve as food sources for humans. Through ingestion, MPs in aquatic products may eventually enter into the human food chain and threaten food safety. Therefore, we hereby call for the development of truly degradable plastic products. Moreover, precautionary measures should be applied to reduce the entry of MPs at the level of MP sources. Ultimately, coordinated actions to mitigate MP emissions will also benefit the ecological environment and ensure human food safety.

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Part V
Control Approaches for Microplastics
in Terrestrial Environments

Biodegradation of Plastics in *Tenebrio* Genus (Mealworms)



Shan-Shan Yang and Wei-Min Wu

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Abstract Most petroleum-based plastics are resistant to biodegradation in the environment. Observation of damage, penetration, and ingestion of plastics by insects and their larvae lead to research on biodegradation of plastics by insects. The larvae of darkling beetles (Coleoptera: Tenebrionidae), especially *Tenebrio molitor* and *Tenebrio obscurus* larvae, showed the capacity of rapid gut microbe-dependent degradation of polystyrene (PS). *T. molitor* larvae also degrade low-density polyethylene (LDPE). The biodegradation was evaluated on the basis of plastic mass balance, modification of ingested polymers, formation of biodegraded intermediates, as well as ^{13}C isotopic tracer tests. Ingested PS or LDPE polymer can be depolymerized by up 60–70% within 12–24 h after 1- or 2-week adaptation. Ingested PS or PE supports the larvae with energy for life activities but not growth. Co-feeding normal diet (e.g., bran) enhances PS and PE consumption rate significantly. Gut microbial communities shifted after the larvae were fed with PS or PE. A few plastic-degrading gut bacterial strains have been isolated from gut of *T. molitor*, but they grow on plastics slowly. The rapid biodegradation of PS and PE is likely a result of synergistic effects of intestinal microbial activities and host digestive system, and further research is needed to understand the mechanisms.

Keywords Biodegradation, Mealworms, Microbial community, Plastics, *Tenebrio* genus

1 Introduction

1.1 Major Plastic Wastes in Environment

Ever since the first industrial-scale production of synthetic polymers (plastics) took place in the 1940s, the production, consumption, and waste generation rate of plastic solid waste (PSW) has increased considerably [1]. The global annual plastic production accounts for more than 300 million tonnes [2]. The growth of plastic production in the past decades has substantially outpaced any other manufactured materials. The same properties that make plastics so versatile in innumerable applications – durability and resistance to degradation – make these materials difficult or impossible for nature to assimilate.

Today, there is a growing scientific consensus demonstrating that PSW is a major environmental concern of increasing global significance. In 2010, the total amount of PSW produced by 192 coastal countries in the world was 275×10^6 t, of which $4.8\text{--}12.7 \times 10^6$ t finally entered the ocean, while China imported $1.32\text{--}3.53 \times 10^6$ t of PSW into the ocean, ranking first in the world. In the USA, PSW generation found in municipal solid waste (MSW) has increased from 11% in 2002 [3] to 12.1% in 2007 [4]. In China, over 59.5×10^6 t of PSW is produced in 2015, accounting for 22.9% of MSW generation. More and more generation of PSW has raised enormous

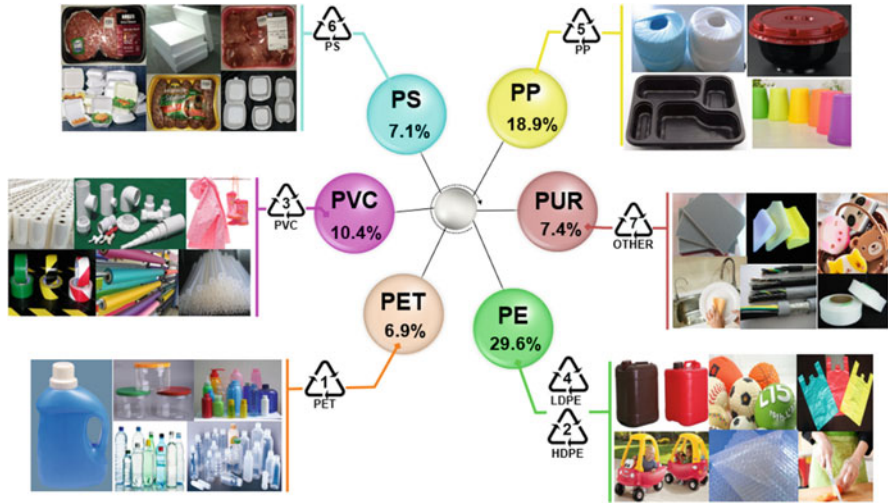


Fig. 1 The most widely used plastics on the market (figure from Yang et al. [8])

questions and challenges to the society regardless of their sustainability awareness and technological advances [1]. Moreover, the annual plastic production has been and will continue increasing in the foreseeable future [5, 6]. It is predicted that by 2025, the annual import of plastic wastes into the ocean will increase by ten times [7]. However, among the generated PSW, less than half of it was confined to discard and either contain in a managed system, such as sanitary landfills and open dumps. Major plastic polymers produced include polyethylene (PE) 29.6%, polypropylene (PP) 18.9%, polyvinyl chloride (PVC) 10.4%, polyurethane (PUR) 7.4%, polystyrene (PS) 7.1%, and polyethylene terephthalate (PET) 6.9% (Fig. 1) [9]. Thus, without a well-designed and tailor-made management strategy for end-of-life plastics, it is only reasonable to find a considerable amount of plastics wastes in the final stream of municipal solid waste.

Of particular concern, plastic pollution has the potential to poison animals and pose serious threats to human health. According to a hazard-ranking model based on the United Nations' Globally Harmonized System of Classification and Labeling of Chemicals, the chemical ingredients of more than 50% of plastics are hazardous [10]. These harmful chemicals leached from the plastic wastes or in the form of small or microplastic debris are more likely to infiltrate food webs [11] and potentially impact ecologically important species including mussels, salt-marsh grasses, and corals [11, 12]. Humans and mussels that ingested the chemicals from plastics and small or microplastic debris could accumulate in the body and harm the cells and other tissues [11, 13]. The disadvantages of plastic pollution must be carefully considered to design the best solutions to the environmental challenges posed by the enormous and sustained global growth in plastic production and use.

1.2 Biodegradation of Plastics by Microorganisms

Natural degradation is hard to get rid of plastic waste. The majority of plastics is resistant to decomposition by microorganisms [14] due to high-molecular-weight structural complexity and hydrophobic surfaces [15]. These properties make the polymer inaccessible to the microbial enzymes. The potential to decompose and degrade plastics in various environments has been studied for decades, in order to investigate the fate of plastics in the environment and to find solution to increasing accumulation of plastic wastes [16–20]. However, most of these plastics are recalcitrant to biodegradation by microorganisms, and the degradation rate is a generally very slow [21, 22]. For instance, Ohtake et al. [21] examined plastic polymer products buried under soil for 32 years and did not find any evidence of biodegradation of PS and PVC but found extremely slow biodegradation of low-density polyethylene (LDPE) film and bottle [19]. To date, slow biodegradation of LDPE, PP, and PET polymers by mixed and single microbial cultures has been reported. The mass removal or degradation is measured in periods of weeks, months, or years. Table 1 summarizes some research results of microbial degradation of major plastics, which has proved that the plastics can be degraded by several bacteria or flora from various environments, especially from soil, sludge, landfill, and other contaminated sites. The challenges to microbial biodegradation of plastics are summarized as follows:

1. Extremely poor biodegradation efficiency. The majority of previous studies focus on isolation and characterization of microbial strains in the ability of degrading PE, PS, PP, and PE (Table 1). But the isolated cultures performed poorly in both microbial growth and metabolism of target plastics.
2. Unclear mechanism of biodegradation of plastics. Most reports mainly focused on the colonization on plastic materials as well as mass loss of plastic materials added. The key metabolic genes and enzymes are rarely revealed. Therefore, searching for effective key genes and enzymatic systems for biodegrading plastics and explaining the degradation mechanisms are the key scientific questions needed to be answered.
3. Unknown intermediates and the impacts and fate of additives. The metabolic pathways and intermediates of biodegradation of plastics (PE, PS, and PP) are still unknown. The potential hazards of these degradation products have also not been investigated. In the biodegradation processes, the impacts and the fates of various additives should be addressed.

1.3 Plastic Damaging/Degradation by Insects

Since the 1950s, as plastic materials had been rapidly developed and widely applied, plastic degradation received attentions, and some research had been performed about plastic films of PE, PP, and PVC in pest insects. Most of these insects belong to

Table 1 Reported tests on microbial degradation of major plastic materials

References	Culture source	Results	Test period
Guillet et al. [23]	Activated sludge	0.7% of PS mineralized	75 days
Sielicki et al. [24]	Soil and liquids	1.5–3.0% of PS degraded	4 months
Kaplan et al. [25]	17 fungi, 5 soil invertebrates, 5 groups of microbial flora (sludge, soil, feces, garbage, corrupt plastics); 5 groups of mixed microbial flora	0–0.24% of PS degraded	35 days
		0.04–0.57% of PS degraded	5–11 months
Mor and Sivan [26]	<i>Rhodococcus ruber</i> C208	0.5% and 0.8% of PS weight loss	4–8 weeks
Atiq et al. [27]	<i>Paenibacillus urinalis</i> NA26, <i>Bacillus</i> sp. NB6, and <i>Pseudomonas aeruginosa</i> NB26	Colonization on PS. But no PS weight loss was confirmed	8 weeks
Albertsson [28]	Three <i>Phellinus ribis</i>	0.36–0.39% of PE mineralized and 0.02% of PE assimilated	2 years
Albertsson et al. [29]	Mixed culture of fungus <i>Japonica</i> and <i>Fusarium</i>	0.5% of PE mineralized	498 days
Sivan et al. [30]	<i>Rhodococcus</i> C208	0.86% of PE degraded	7 days
Tribedi and Sil [31]	<i>Pseudomonas</i> AKS2	4–6% of PE degraded	45 days
Balasubramanian et al. [32]	<i>Arthrobacter</i> GMB5 and <i>Pseudomonas</i> GMB7	12% and 15% of PE degraded	30 days
Kyaw et al. [33]	Four <i>Pseudomonas</i> strains	20%, 11%, 9%, and 1.75% of PE degraded	120 days
Harshvardhan and Jha [34]	<i>Kocuria palustris</i> M16, <i>Bacillus pumilus</i> M27, and <i>Bacillus subtilis</i> H1584	1%, 1.5%, and 1.75% of PE degraded	30 days
Yamada-Onodera et al. [35]	<i>Penicillium</i> YK	Increase of average molecular weight of PE	3 months
Cacciari et al. [36]	Microbial flora	Small molecular products of PP increased	6 months
Arkatkar et al. [37]	Soil mixed culture	0.4% of PP weight loss	1 year
Arkatkar et al. [38]	<i>Pseudomonas azotoformans</i> , <i>Pseudomonas stutzeri</i> , <i>Bacillus subtilis</i> , <i>Bacillus flexus</i>	2.5% of PP weight loss. Ultraviolet treatment improved biological accessibility of PP	12 months
Jeyakumar et al. [39]	Two fungi (F1 and F2)	Pretreatment and modification of PP effectively improved the degradation	1 year

PE polyethylene, PP polypropylene, PS polystyrene

moths in the family Pyralidae of the order Lepidoptera and darkling beetles in the family Tenebrionidae of the order Coleoptera [40–42]. Darkling beetles (*Tribolium castaneum*, *Rhizopertha*, *Lasioderma serricorne*, *Tenebrioides mauritanicus*,

Zophobas morio, etc.) in Tenebrionidae and several moths and their larvae (*Plodia interpunctella*, *Galleria mellonella*, *Ephestia cautella*) in the family Pyralidae were investigated and known to penetrate and/or consume PE, PVC, and PP films, but no efforts were made to assess the fate or biodegradation of ingested plastics [40–42]. In 2014, researchers in China reported isolation of PE-degrading bacterial strains from LDPE-eating Indian meal moth, i.e., *P. interpunctella* larvae [43], indicating that the larvae could have the capacity of degrading LDPE. Since 2017, biodegradation of PE in Pyralidae larvae has been reported in greater wax worms (*Galleria mellonella*) [44, 45] and lesser wax worms (*Achroia grisella*) [46]. Biodegradation of PS and PE in larvae of darkling beetles (Coleoptera: Tenebrionidae) has been confirmed since 2015 [47–52].

The research on plastic degradation in Tenebrionidae started as the observation of consumption of Styrofoam (or expanded PS foam) by yellow mealworms (*Tenebrio molitor* larvae) was reported by students competing in high school science fairs in the early 2000s: in 2003, Ms. Chong-Guan Chen raised yellow mealworms fed with PS foam and hypothesized that PS was biodegraded [53]; in 2009, Ms. I-Ching Tseng claimed isolation of bacterial strains from yellow mealworm gut using PS as the sole carbon source [54]. Both larvae of *Tenebrio molitor* Linnaeus 1758, commonly referred to as yellow mealworms, and *Tenebrio obscurus* Fabricius 1792, referred to as dark mealworms, belong to *Tenebrio* genus of Coleoptera within the cosmopolitan family Tenebrionidae, which is comprised of more than 20,000 species. Convincing academic evidence of PS degradation in *Tenebrio* genus was reported using *T. molitor* larvae from Beijing, China, in 2015 [47, 48]; then in the larvae from California, USA [49]; and 12 sources from China, the USA, and the UK [50]. PS degradation in *T. obscurus* larvae was reported in 2019 [51]. Ingestion and biodegradation of LDPE in *T. molitor* were also reported in 2018 [52]. In addition, *Zophobas atratus* Fabricius 1775 (Coleoptera: Tenebrionidae) larvae (commonly named as superworms, King Worms or Morio Worms) have been tested for eating PS foams by high school students at science fairs and posted on web sites for years. PS-biodegrading capability of *Z. atratus* larvae has been confirmed recently [55]. In 2010, Miao and Zhang [56] tested *Z. morio* larvae fed with LDPE, linear low-density polyethylene (LLDPE), ethylene-vinyl acetate (EVA), and PVC microplastics and Styrofoam but did not provide solid data on biodegradation. They fed the larvae with respective plastic material versus bran with a ratio of 1:1 (w/w) and then 0.5:1, 0.2:1, and finally 0:1 each week as well as with Styrofoam (PS). The larvae consumed 2.4 g PS per kg larvae per day. Based on analysis of frass egested using thermogravimetry-differential thermal synchronous analyzer (TGA-SDTA), no changes in physical properties of LDPE and EVA but changes in physical properties of residual PVC and PS were observed.

2 Biodegradation of Polystyrene (PS) and Polyethylene (PE)

2.1 Polystyrene Degradation

Industrial production of PS began around 1930 [57]. PS polymer, which is made from styrene monomers containing C=C bonds, possesses long hydrocarbon backbone with a benzene ring linked to every other carbon atom [14]. On the basis of structure, PS can be classified into three forms (Fig. 2a). PS containing all of the phenyl groups on one side is termed as isotactic PS. If the phenyl groups are randomly distributed, then it is called atactic PS. Syndiotactic PS is a new type of PS. The phenyl groups on the polymer chain are attached to alternating sides of the polymer backbone chain. The only commercially important form of polystyrene is atactic, in which the phenyl groups are randomly distributed on both sides of the polymer chain. This random positioning prevents the chains from aligning with sufficient regularity to achieve any crystallinity.

The PS products include (a) expanded PS (EPS), trade name Styrofoam, which is widely used for building insulation and packing; (b) extruded PS used for food containers, coffee cups, food trays, etc.; and (c) high-density PS products which commonly used as liquid containers, toys, etc. In 2014, the global market for PS materials was valued at \$32 billion with a projected 2020 market valued at \$42 billion [58]. Although PS is considered a durable plastic, PS products are often designed for a short service time and one-time use as a result of the low cost of this material. The sharp contrast between the remarkable durability of PS and the short service time of PS products has led to the increasing accumulation of PS waste in our environment. PS wastes are major pollutants of soils, rivers, lakes, and oceans [59] and are among the major microplastics (<5 mm) accumulating in the environment including ocean, surface water, and wastewater [9, 60].

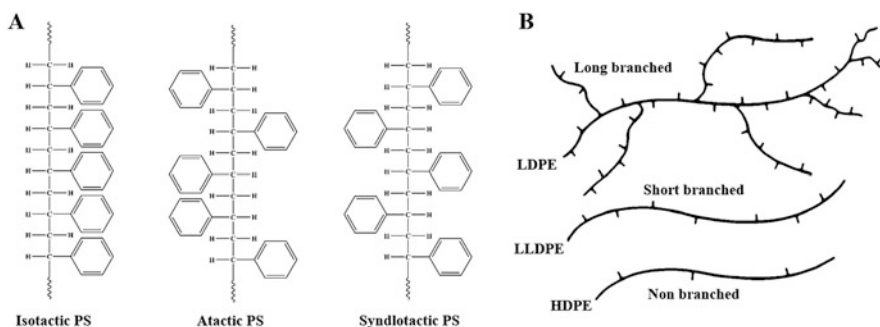


Fig. 2 The major different PS and PE polymers based on structure. (a) PS polymers (left) isotactic PS, (middle) atactic PS, and (right) syndiotactic PS. Commonly used PS products are atactic PS. (b) PE polymers. HDPE, LDPE, and LLDPE

Attempts to investigate biodegradation of petroleum-based PS pollutants can be traced back to the 1970s. Researchers have studied feasibility of PS biodegradation with microbes from soils, seawater, landfill sediment, activate sludge, and compost. Some of these studies included use of ^{14}C -labeled PS [16, 24–26, 61]. However, it has been thought that PS is not subject to efficient and rapid biodegradation by microorganisms and soil invertebrates [21, 62]. The scientific consensus was that rapid PS degradation would require photolytic or thermolytic cleavage of $-\text{C}-\text{C}-$ bonds prior to biodegradation [57, 63, 64].

2.2 Polyethylene (PE) Degradation

PE is the most used polymers around the world, and is utilized in packaging, representing $\sim 40\%$ of total demand for plastic products (www.plasticseurope.org) with over a trillion plastic bags used every year [65]. As the most common petroleum-based plastic, PE is expressed as “ $[\text{CH}_2-\text{CH}_2]_n$ ” and comprises a linear backbone of carbon atoms, which is resistant to degradation [43, 44]. Commercial PE polymers include HDPE (high-density polyethylene), LLDPE, and LDPE (Fig. 2b). HDPE is composed of linear chains which are packed closely together, with a very low level of short-chain branching, and has a high degree of crystallinity (70–95%) [66]. LDPE is characterized by a significant level of long-chain branching (typical branch length of several hundred carbon atoms) as well as short-chain branching (2–6 carbon atoms long). The short branches of LDPE hinder close packing and result in a relatively low crystallinity (45–60%). LLDPE is a linear molecule with higher level of short-chain branching than HDPE but without long chains with a middle crystallinity. The structure and physical properties of PE polymers certainly impact biodegradability. Since the early 1970s, tests on the biodegradation of virgin PE (unpretreated and without any additives), mainly LDPE, had been performed under natural environmental conditions, including soils, seawater, sludge, and compost, which harbor a multitude of diverse microbial communities [16–21, 28]. These studies concluded that the biodegradation of virgin PE was extremely slow and limited in mixtures of some microbial communities [43]. One of the well-known tests was that Ohtake et al. [19, 21] found extremely slow biodegradation of LDPE film and bottle after they were buried under soil for more than 32 years using GPC and FTIR analyses. Biodegradation of PE in the environment occurred mainly through the biological activity of microorganisms after photo- or thermo-oxidation [67, 68]. Slow (in periods of weeks/months) PE biodegradation has been observed, given appropriate conditions. For example, modest degradation of PE was observed after nitric acid treatment and incubation for 3 months in a liquid culture of the fungus *Penicillium simplicissimum* [35]. Slow PE degradation was also recorded after 4–7 months exposure to the bacterium *Nocardia asteroides* [69]. Besides, almost no biodegradation of PE through the biological activities of select microorganisms can be observed without pretreatments

[67]. However, recently, much more rapid biodegradation of PE has been found in plastic-eating insect larvae of two moth larvae [44–46] and yellow mealworms [52].

2.3 *Tenebrio* Genus in Darkling Beetles

To date, most published research results on plastic degradation by insects are reported using *Tenebrio* larvae, especially *T. molitor*. Currently, there are three extant *Tenebrio* species reported [70]; two of them, *Tenebrio molitor* and *Tenebrio obscurus*, have been observed worldwide and commercially available in China, in the USA, as well as around the world [51], while *T. opacus* Duftschmid, 1812, is only found in France [70]. Observations of *T. molitor* larvae chewing and ingesting Styrofoam (the trade marker of expanded polystyrene foam) by teenage students and then researchers lead to investigating biodegradation of PS by *T. motor* larvae. As described previously, convincing evidence of rapid PS biodegradation in *T. molitor* larvae has been reported since 2015 [47–50]. Based on the recent survey from collaborators, yellow mealworms in all 25 locations consumed PS foam, including North America (Canada, Mexico, USA), South America (Chile, Costa Rica), Asia (Cambodia, China, Japan, Indonesia, India, Iran, Israel, South Korea, Thailand), Europe (Finland, France, Germany, Poland, Slovenia, Spain, Turkey, UK), Africa (Nigeria, South Africa), and Australia [50, 71]. Detailed studies confirmed the ubiquity of PS digestion and biodegradation by 12 sources of *T. molitor* larvae: five from the USA, six from China, and one from the UK according to the study of Yang et al. [50]. As depicted in Fig. 3, 12 strains of mealworms were able to chew and burrow into block EPS. These *T. molitor* larvae were also able to chew and burrow into PE foam (Fig. 4).

3 Characterization of Plastic Biodegradation

Characterization of plastic polymer biodegradation by *Tenebrio* larvae and other insects is primarily based on (a) mass loss or mass removal of polymer fed; (b) supporting life activities by ingesting polymer as sole diet; (c) the modification of mechanical, chemical, and physical properties of egested residues (in the frass or fecula); and (d) production of CO₂ or biodegraded functional organic groups and/or intermediates. Stable isotopic tracer ¹³C and radioisotopic tracer ¹⁴C are also effective tools to prove biodegradation. These procedures were described in several review articles on microbial plastic degradation and also established in the research work on biodegradation of PS and PE in *Tenebrio* larvae [47–52, 72, 73].

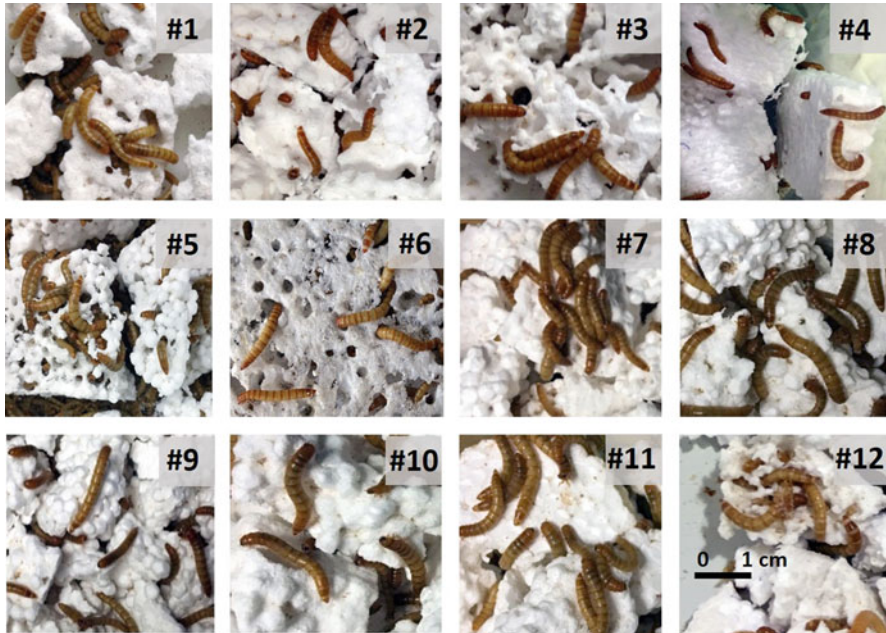


Fig. 3 *Tenebrio molitor* larvae from 12 sources have the capacity of degrading PS foam. Sources #1–#5 are from the USA; source #6 is from the Belfast, UK; and sources #7–#12 were from China (figure from Yang et al. [50])

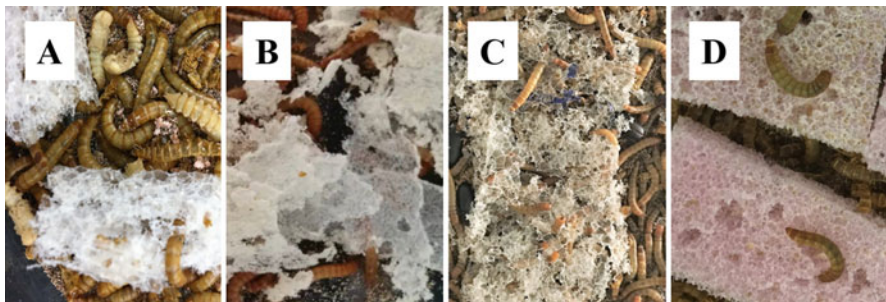


Fig. 4 *Tenebrio molitor* larvae have the capacity of ingesting and degrading LDPE foam. (a), (c), and (d) *T. molitor* larvae source from the USA. (b) *T. molitor* larvae from Harbin, China

3.1 Survival Rate and PS Consumption

Survival rate (SR) of *Tenebrio* larvae fed with PS or other plastic materials as sole diet versus that fed with normal diet bran is used as an indication to test the possibility of digestion or biodegradation of plastics [47–52] and also used for the evaluation of the effect of PE and beeswax as sole diet for greater wax worms (*Galleria mellonella*) [45] and lesser wax worms (*Achroia grisella*) [46].

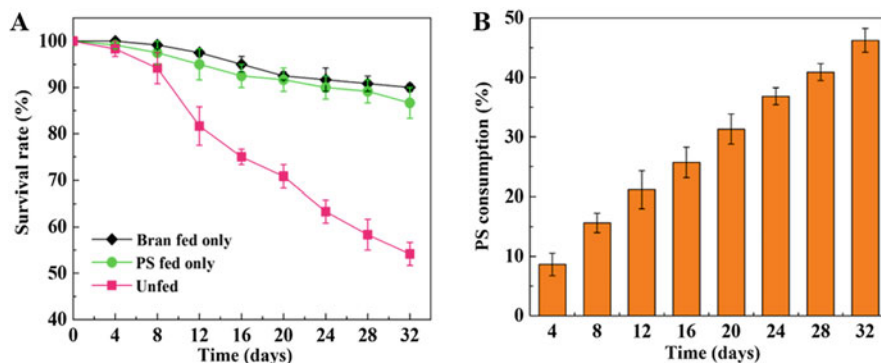


Fig. 5 *Tenebrio molitor* larvae chewed and ate PS foam for living. (a) Survival rates of *T. molitor* larvae fed with normal diet bran and PS only and unfed. (b) Accumulated PS consumption (%) over time. This test was conducted in duplicate with 120 larvae in each incubator over a 32-day period (figures from Yang et al. [49])

Most researchers use short-term SR to evaluate the effectiveness of EPS as energy source to support the life activities of *T. molitor* larvae. Yang et al. [47] reported the results for the determination of the SR of *T. molitor* larvae over 1-month period and showed that the difference between of the SR of Styrofoam-feeding larvae and the SR of conventional diet (bran)-feeding larvae was not significant (average 85%). During the 1-month rearing period, the larvae (500 in total) obtained in Beijing, China, consumed $31.0 \pm 1.7\%$ of Styrofoam with an initial weight of 5.8 g as the sole diet. In their studies, results found that almost half of the ingested PS carbon was converted into CO_2 in the mealworm gut [47]. Afterward, similar results were observed using different sources of *T. molitor* larvae around the world [49, 50]. Results showed that the SRs of *T. molitor* larvae fed with PS foam were similar to that fed with normal diet bran but significantly higher than that unfed (Fig. 5a); the PS consumption progressively increased over a 32-day rearing period with PS as the only feedstock as shown in Fig. 5b [49]. A total consumption of 0.83 ± 0.04 g PS by the end of the test was observed according to 120 mealworms. The percentage of undigested PS residue in the frass (w/w, %) decreased from $66.2 \pm 2.3\%$ on day 4 to $35.2 \pm 1.2\%$ by day 24, stabilizing at values up to 65% in the short (12–15 h) residence time of the mealworm gut. At the end of the 32-day test at 25°C, the SR of the larvae fed with EPS alone was $86.7 \pm 3.3\%$, significantly greater than that of unfed controls ($54.2 \pm 2.5\%$) and not significantly less than bran-fed mealworms ($90.0 \pm 0.8\%$). Over the 32-day period of the test, starved mealworms lost $2.6 \pm 0.2\%$ of their average weight; the larvae fed with PS alone maintained a stable weight; and bran-fed larvae experienced a $32.0 \pm 1.5\%$ weight gain. Consumption of PS, PVC, and polylactide (PLA) by *T. molitor* larvae was also tested for 21 days by Božek et al. [74]. They found that the larvae consumed respective polymers by 9%, 12%, and 3%; the larvae fed with plastics decreased

their weight by 18%, 15%, and 19%, respectively, while the weight of those fed with bran increased by 45%. This suggested that the polymers did not support larval growth.

In the long term, the change in SR is different. A test was performed with *T. molitor* larvae from the UK for 98 days under three feeding conditions: unfed, EPS alone, and EPS plus bran. The SRs of the larvae fed with EPS alone matched those fed with EPS plus bran during the initial 35 days (95.5% versus 98.0%) and then dropped to low levels, like those of unfed controls. Further investigation revealed that both the unfed larvae and larvae fed with PS alone engaged in cannibalism. The 98-day SR was 11.8% for unfed larvae and 11.5% for larvae fed with PS alone. By contrast, the 98-day SR for mealworms fed with bran plus PS was 81.5% [50]. Because PS contains only hydrogen and carbon, it does not provide adequate nutrition (N, P, Na, K, trace elements, amino acids, etc.) for a long-term survival and growth. The positive effect of PS on SR does not last for a long time due to the lack of nitrogen sources and other nutrients. The addition of bran relieved this constraint. In the absence of added bran, however, PS-fed mealworms survived by consuming dead mealworms and their molts [50].

3.2 Factors Influencing Plastic Consumption by *T. molitor* Larvae

Physical and chemical properties are essential factors influencing the consumption and digestibility of the polymers by *T. molitor* larvae. Till now, most research has been done using Styrofoam or EPS [47, 49–52, 71, 74]. More research is needed to test different materials with various additives and polymer structures.

Studies indicated that supplementation of nutrient-containing co-diet can enhance PS consumption and degradation by mealworms (Fig. 6). Wheat bran (WB) is a normal feedstock for *T. molitor* larvae and can be obtained from agricultural or food processing industries. Soy protein is a widely used food additive for humans and animals. When the larvae were fed with soy protein or WB in the presence of PS, they first ate the protein or WB and then PS. All feed conditions resulted in higher SR values than the unfed control (60.8%). SR values were similar for larvae fed with PS alone (87.5%) and for mealworms fed with PS plus soy protein (89.2%) or WB (90.8%) (Fig. 6a). Adding soy protein or WB significantly increased rates of PS degradation compared to PS alone. The 32-day PS consumption rate was 39.1% for PS alone, 76.8% for PS plus soy protein, and 67.6% PS plus WB (Fig. 6b). The weight gain of larvae fed with PS plus soy protein was 6.3% greater than that of mealworms fed with PS alone, and the weight gain of larvae fed with PS plus bran was 33.5% greater than that of larvae fed with PS alone. A long-term test over 1 year performed at Stanford University further indicated that when *T. molitor* larvae were fed with PS plus co-diets, WB could provide all nutrients for mealworms to complete their life cycle, but soy protein did not.

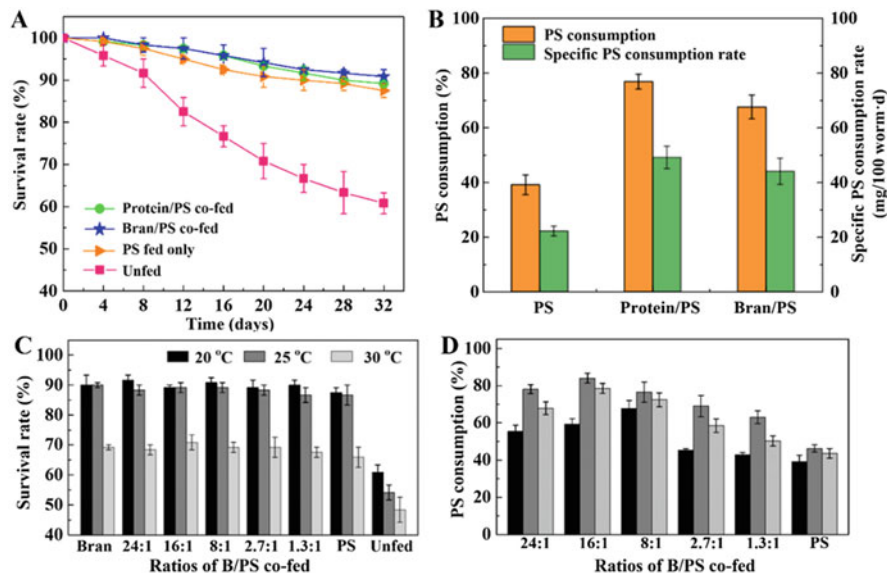


Fig. 6 Effects of co-diets and temperature on PS consumption by *T. molitor* larvae. (a) Comparison of SRs for larvae with different diets. (b) PS consumption (%) and specific PS consumption rates for unfed larvae compared to the larvae fed with soy protein plus PS or bran plus PS over a 32-day period. (c) SRs for the larvae fed with various ratios of bran versus PS at 20°C, 25°C, and 30°C over 32 days. (d) PS consumption (%) for the larvae fed with PS alone and various ratios of bran versus PS (w/w) at 20°C, 25°C, and 30°C over 32 days (mean \pm standard deviation) (figures from Yang et al. [49])

The combined effects of rearing temperature (20, 25, and 30°C) and WB/PS ratios on SR values and PS consumption rates were evaluated over a 32-day period (Figs. 6c, d). Highest 32-day percentages of PS consumed were 84.0% at 25°C for a WB/PS ratio of 16:1, 78.5% at 30°C for a WB/PS ratio of 16:1, and 67.6% at 20°C for a WB/PS ratio of 8:1. Visibly less PS residue remained in incubators fed with WB plus PS than in incubators fed with PS alone. Besides, rearing temperature had a significant impact on SR values. For the same WB:PS ratio, SRs were significantly lower at 30°C than at 20°C or 25°C. At 20°C and 25°C, SRs were similar regardless of feed ratio, but sensitive to temperature. The effects of temperature on SR and PS degradation rates are best explained by the known constraints of temperature on mealworm physiology, with a reported optimal range of 25–28°C, and by their inability to tolerate temperatures greater than 30°C [75]. The effect of temperature is likely strain-dependent since *T. molitor* larvae in Indonesia grow well at above 30°C [71].

Similarly, Brandon et al. [52] found that at the end of the 32-day experiment, the SR of the larvae fed with PE was 98.3%, a value that was not significantly different ($p = 0.92$) from that of the bran-fed controls (96.3%). There was also no significant difference in SR of mealworms fed with PE alone and mealworms fed with PE plus bran (95.0%). This indicated that PE supported the life activities during the 32-day

experiment. Consumption of PE and PS increased throughout the experiment. From the initial 1.80 g PE, the total mass loss at the end of the experiment was 0.87 g by mealworms fed with PE. For mealworms fed with PS, the total PS mass loss was 0.57 g. For both PE- and PS-fed mealworms, the mass loss was significantly greater when the mealworms received bran as a co-feed. For PE plus bran, the mass loss was 1.10 g, and for PS plus bran, the mass loss was 0.98 g. Specific rates of plastic consumption (mg plastic consumed per 100 worms per day) followed the same pattern.

3.3 Reproduction of *T. molitor* Fed with PS

Long-term tests indicated that provision of added nutrition (wheat bran) enabled *T. molitor* to reproduce and mate and could therefore enable selective breeding [49]. The first generation of mealworms fed with PS plus WB completed their life cycle (Fig. 7), developing into pupae and then beetles in 2 weeks at 28°C, and produced a second generation of yellow mealworms. A new generation of mealworms was then reared for 3 months with PS and WB; this generation appeared to have a higher affinity for PS materials. Rearing at 25°C, 120 s generation juvenile mealworms weighing ~30 mg per mealworm had a specific PS consumption rate of 16.9 mg PS/100 larvae per day or 5.6 mg PS/1,000 mg mealworms per day on a weight basis. These values fall within the range of values measured for the mature first-generation PS-degrading *T. molitor* larvae that weighed 75–85 mg per larva. Rearing with PS plus WB as their diets, the second-generation juveniles' mealworms



Fig. 7 The first generation of *T. molitor* larvae fed with PS plus wheat bran completed their life cycle and can digest various PS foam products (figure from Yang et al. [49])

eventually grew to be mature larvae (weighing 90 mg or higher, like the first generation) and then developed into pupae and beetles. The larvae fed with WB and PS completed all their life cycle stages (larvae, pupae, beetles, egg), and the second generation had a favorable PS degradation, opening the door for selective breeding. Further tests indicated that both generations of *T. molitor* larvae have similar capacity of ingesting and biodegrading various PS foams (Fig. 7).

3.4 PS Degradation by *Tenebrio obscurus* Larvae

Another member of *Tenebrio* genus, *Tenebrio obscurus* larvae (dark mealworms), also has the capacity of ingesting and biodegrading PS foam. A comparison study demonstrated the ability for PS degradation within the gut of *T. obscurus* larvae even at greater rates than *T. molitor* larvae from the same source [51]. *T. obscurus*, Fabricius 1792 larvae, obtained from Shandong, Sichuan, and Henan provinces, China, and Colorado, USA, chewed and ingested PS foam (Fig. 8). It is speculated that the chewing and ingestion of PS foam is likely an adaptive behavior intrinsic to *T. obscurus*. *T. obscurus* larvae behaved similarly to each other but differently from *T. molitor* larvae. They were all sensitive to light and mostly hid below PS foam in clusters. The larvae of *T. molitor* were less sensitive to light and spread themselves on the foam surface or penetrated the inside matrix. *T. obscurus* larvae like corn flour but do not prefer bran diet, while *T. molitor* prefer both. A test was performed to compare the PS consumption performance of *T. obscurus* versus *T. molitor* larvae; initial larvae (410) were randomly selected and placed in a food grade polypropylene container (volume of 3,300 mL) under controlled conditions ($25 \pm 1^\circ\text{C}$, $70 \pm 5\%$ humidity, and dark environment). To assess the capacity of consuming PS initially, PS blocks (7.2 g) were added. Co-diet treatments were PS plus bran (1.2 g) for *T. molitor* larvae and PS plus corn flour (1.2 g) for *T. obscurus* larvae. An additional 1.2 g of the co-diet was supplemented every 5 days to reach a final ratio of PS to co-diet of 1.0:1.0 at the end of the test. During the 31-day test with PS as the only diet, the PS mass consumption by the *T. obscurus* larvae was 55.4%, while that by *T. molitor* was 41.5% (Table 2). The PS consumption increased when co-diets were added, i.e., the *T. obscurus* consumed 67.1% of PS and *T. molitor* consumed $56.8 \pm 1.9\%$. At the end of the 31-day test at 25°C , the SRs of both species fed with EPS alone were 91.5% and 89.3%, respectively, significantly greater than those of unfed controls (67.6% and 62.0%) and not significantly less than corn flour-fed and bran-fed larvae (95.0% and 93.2%). Results showed that the *T. obscurus* were capable of rapid PS consumption at rates which were even greater than those of *T. molitor*.

In addition, both *T. obscurus* larvae from China and the USA chewed and ate LDPE foam. However, their capacity of biodegrading LDPE has not been examined.



Fig. 8 *T. obscurus* larvae from various sources can chew and ingest PS foam. The larvae from (a) *T. obscurus* from Harbin, China (14 days rearing period). (b) *T. obscurus* from Harbin, China (28-day rearing period). (c) *T. obscurus* from Shandong, China. (d) *T. obscurus* from Sichuan, China. (e) *T. obscurus* from Henan, China. (f) *T. obscurus* from Colorado, USA (figures (c)–(f) from Peng et al. [51])

4 Methods for Evaluation of Plastic Biodegradation

4.1 Residual Polymers in Frass

An effective approach to assess plastic biodegradation is to examine modification of polymers after passage of insect gut using a solvent to extract residual polymer from insect frass or fecula of the insects or larvae fed with plastic. The frass of *T. molitor* larvae contained remaining PS particles, modified PS polymers, and other residues, such as undigested exoskeletons (Fig. 9a). Tetrahydrofuran (THF) is commonly used to extract PS (or PVC) polymer from the frass of *T. molitor* larvae (Fig. 9b). In one study, *T. molitor* larvae were fed with PS foam as sole diet for 32 days. The percentage of undigested PS residue in the frass (w/w, %) decreased from 66.2% on day 4 to 35.2% by day 24, stabilizing thereafter (Fig. 9c). The polymer residue remaining after evaporation was weighed to determine the THF extractable fraction,

Table 2 Summary of PS biodegradation by *T. obscurus* and *T. molitor* larvae (data from Peng et al. [51])

Mealworm source	Initial weight, mg larva ⁻¹	Feed	Weight change (%) at the end of test	Survival rate, %	Specific PS consumption rate ^a	
					mg PS 100 larvae ⁻¹ day ⁻¹	mg PS g larvae ⁻¹ day ⁻¹
<i>T. obscurus</i>	66.15 ± 2.13	PS + C	+15.9 ± 4.1	94.9 ± 1.7	39.24 ± 1.73	5.94 ± 0.26
		PS	-8.1 ± 3.7	91.5 ± 1.5	32.44 ± 0.51	5.06 ± 0.08
		Unfed	-13.2 ± 2.7	67.6 ± 2.2	Nd	Nd
<i>T. molitor</i>	62.41 ± 1.72	PS + B	+14.6 ± 1.8	93.2 ± 1.0	33.23 ± 0.80	5.33 ± 0.13
		PS	-8.6 ± 1.2	89.3 ± 2.7	24.30 ± 1.34	3.89 ± 0.47
		Unfed	-18.2 ± 6.0	62.0 ± 2.9	Nd	Nd

^aSpecific PS consumption rates were calculated on the basis of the mass of PS consumed over the test period (31 days). PS polystyrene, C corn flour, B bran, Nd not determined. Test temperature at 25°C

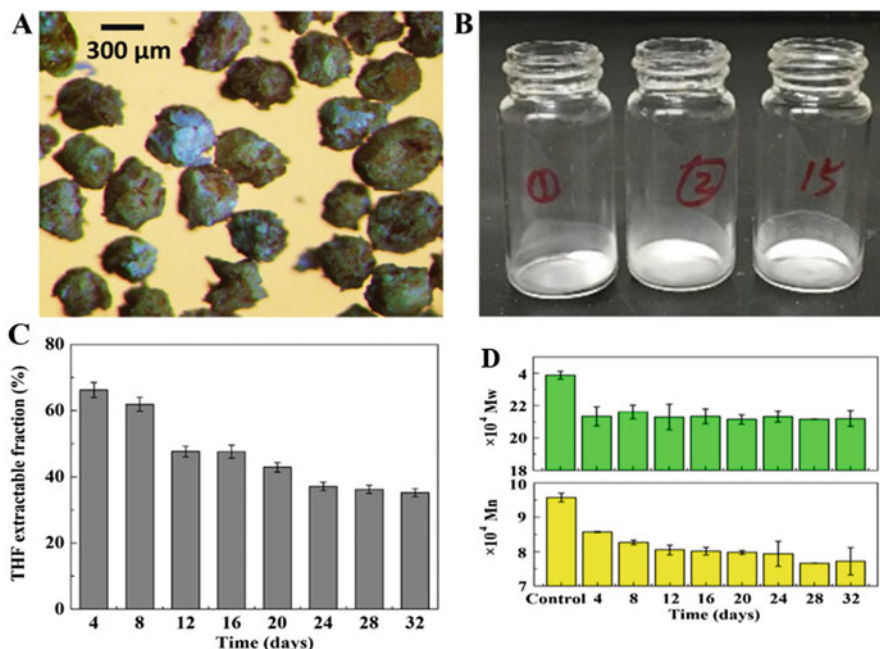


Fig. 9 (a) Frass (black) with embedded white polymer residuals. (b) Residual polymer extracted from frass. (c) Progressive decrease of THF extractable fraction of the frass of *T. molitor* larvae fed with PS foam as sole diet over a 32-day period. (d) GPC analysis shows decrease in M_w and M_n of residual PS polymer extracted from the frass during the 32-day period (figures from Yang et al. [49])

a measure of residual PS in the frass (Fig. 9b). The results suggested that the PS degradation activity increased gradually and stabilized after a 16- to 24-day adaptation period [49]. The residual PS polymers were further analyzed using GPC to examine the molecular weights (Fig. 9d). When the larvae were fed with PS plus other co-diets, the THF extract may contain other extractable components except for PS residue. Pre-extraction with ethanol and/or even water to remove impurities may be needed.

For the larvae fed with PE, the extraction of residual PE is performed with dichloromethane (DCM) solvent. The procedure of extraction of PE from the frass of *T. molitor* larvae fed with PE foam was similar but slightly different from that fed with PS as described by Brandon et al. [52]. In tests, the results showed that less than 40% of residual LDPE polymer was detected in the frass after *T. molitor* larvae were fed with LDPE for 2 weeks, indicating a rapid depolymerization and biodegradation occurred [52].

4.2 Major Analytical Methods

The evidences of biodegradation of PS and PE can be provided via analysis of egested residues of PS in the frass characterized by GPC, TGA, FTIR, solid-state ^{13}C cross-polarization/magic angle spinning nuclear magnetic resonance (^{13}C -CP/MS NMR), liquid-state ^1H NMR (^1H -NMR) analysis, differential scanning calorimetry (DSC), as well as other methods.

GPC analysis provides the information of the number-average molecular weight (M_n) and the weight-average molecular weight (M_w), which have been considered as a major indication of depolymerization and degradation of polymers [76]. GPC analysis provides information on three key indicators of depolymerization and degradation of plastic materials: M_n , M_w , and molecular weight distribution (MWD). The changes in M_n and M_w of the residual polymer in frass generally decreased significantly from those of the original PS material, indicating depolymerization or modification occurs. A typical example is illustrated in Fig. 9d. *T. molitor* larvae were fed with PS as sole diet for 32 days. The M_w in frass was much lower than that in PS feedstock. The M_n showed progressive decreasing trend. The MWD of residual polymer shifts to lower molecular weight, indicates significant and broad depolymerization [47, 48]. The ubiquity of PS biodegradation in *T. molitor* larvae was supported by the evidence of PS depolymerization within the guts of 12 sources from China, the USA, and Northern Ireland, with significant decreases in M_n and M_w accompanied by shifts in MWD to lower molecular weights (in Table 3). These results suggested that depolymerization/cleavage of the long-chain structure of PS took place and lower molecular weight fragments were newly formed in the mealworm gut. Effects of nutrient supplements and impacts of temperature on PS degradation by a *T. molitor* strain from the USA are exhibited in Fig. 10. Residue PS within frass fed with PS only and PS plus bran showed significant decrease in M_w and M_n in comparison with control (PS feedstock); and differences between different temperatures and co-feeding PS were also not statistically significant (Figs. 10a, b). All samples exhibited similar changes in MWD, with shifts to lower molecular weights than those of PS feed (Fig. 10c).

Similar results were observed during PS degradation in *T. obscurus* larvae. Peng et al. [51] compared *T. molitor* larvae from the same location; frass samples from *T. obscurus* larvae fed with PS only contained polymer extracts with M_n values that were 26.0% lower than the feedstock and M_w values that were 59.2% lower than the feedstock (PS feedstock with M_n of 107,000; M_w of 345,000). Frass samples from *T. molitor* had M_n values that were 11.7% lower and M_w values that were 29.8% lower than the feedstock. These decreases in M_n and M_w were significant for all sources (*t* test, $p < 0.05$), indicating depolymerization and degradation of PS feedstock were ubiquitous across both species. The result also suggested that *T. obscurus* larvae tested had superior PS depolymerization and biodegradation than *T. molitor* larvae test. In addition, except for the macromolecular peak, some low-molecular-weight peaks (molecular weights between 200 and 1,400) were also

Table 3 The decrease in average molecular weights (M_n and M_w) of the residual PS polymers in the frass of the mealworms fed with bran plus PS (data from Yang et al. [50])

Mealworms	M_n	M_w	M_n reduction compared with control PS (%)	M_w reduction compared with control PS (%)
PetCo Pet Store Chain, Mountain View, California (#1)	81,535 ± 1,588	211,190 ± 512	9.40 ± 0.72	7.19 ± 0.48
PetSmart Pet Store Chain, Sunnyvale, California (#2)	77,738 ± 2,040	203,006 ± 5,928	13.58 ± 3.53	10.78 ± 2.57
Timberline Fisheries, Marion, Illinois (#3)	77,945 ± 2,979	202,813 ± 8,199	13.34 ± 4.61	10.88 ± 3.24
Exotic Nutrition Pet Company, Newport News, Virginia (#4)	75,894 ± 3,836	205,549 ± 3,977	15.64 ± 4.73	9.66 ± 2.14
Rainbow Mealworms, Compton, California (#5)	77,151 ± 1,512	204,113 ± 5,533	14.27 ± 0.13	10.29 ± 2.73
A pet store in Belfast, Northern Ireland, UK (#6)	83,958 ± 4,584	182,105 ± 9,327	5.37 ± 0.19	12.10 ± 4.51
A pet store in Beijing (#7)	78,397 ± 3,770	214,922 ± 3,164	12.92 ± 2.51	5.55 ± 1.12
A store in Harbin, Heilongjiang Province (#8)	77,800 ± 2,062	212,239 ± 1,133	13.50 ± 4.08	6.72 ± 0.79
A mealworm farm in Tai'an County, Shandong Province (#9)	81,849 ± 1,535	212,780 ± 5,798	9.05 ± 0.18	6.50 ± 2.06
A pet store in Xi'an City, Shaanxi Province (#10)	81,448 ± 3,553	214,145 ± 2,717	9.53 ± 2.17	5.89 ± 1.28
A pet store in Shanghai (#11)	77,325 ± 2,279	215,754 ± 3,410	14.08 ± 1.48	5.18 ± 1.72
A pet store in Shenzhen City, Guangdong Province (#12)	82,531 ± 1,512	230,797 ± 1,960	11.19 ± 1.48	9.02 ± 1.09

Mealworms #1–#5, #7–#11, PS feedstock $M_n = 89,996 \pm 1,855$, $M_w = 227,545 \pm 1,180$; mealworms #6, PS feedstock $M_n = 88,725 \pm 19,710$, $M_w = 207,155 \pm 2,437$; mealworms #12, PS feedstock $M_n = 92,949 \pm 2,534$, $M_w = 253,675 \pm 914$

detected in the frass samples from *T. obscurus* and *T. molitor* fed with PS only, suggesting that some oligomer products might be generated. However, further confirmation test is needed to determine whether the difference of the performance by the two larvae was case-specific or generically different.

To evaluate PE depolymerization in *T. molitor* larvae, the residual PE in frass was extracted with DCM, and the samples were analyzed using high-temperature GPC

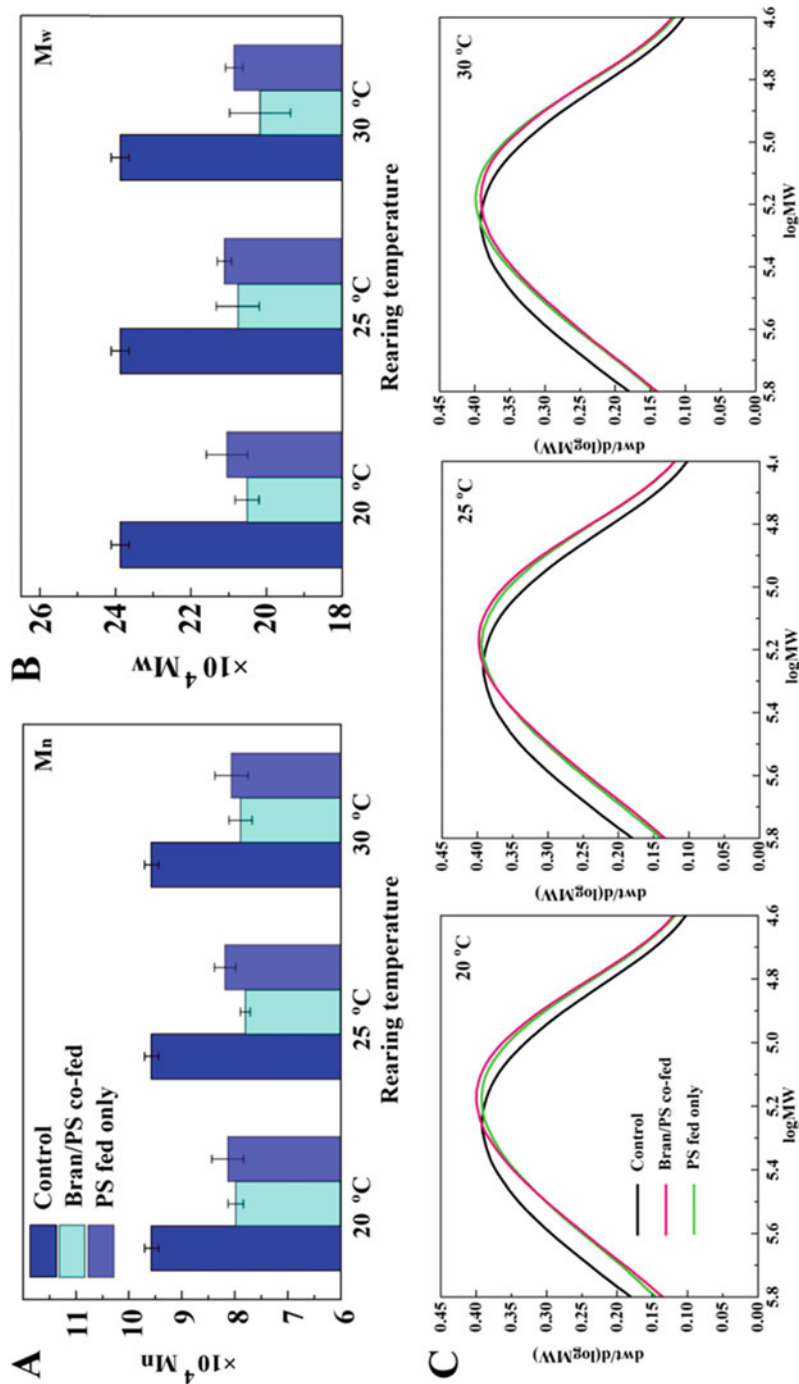


Fig. 10 Effects of nutrient supplements and impacts of temperature on PS degradation by *T. molitor* strain CA. Comparison of (a) M_n and (b) M_w of PS feedstock (control) and THF extracts of frass samples fed with PS plus bran over 32 days. (c) Shifts in MWD of THF extracts of frass from mealworms fed with PS alone and PS plus bran versus PS feedstock (control). (Data and figure from Yang et al. [49])

(HT-GPC) [52]. HT-GPC analysis of the residual polymers from the larvae fed with PE and PE plus bran showed a significant decrease in weight-averaged (M_w) and number-averaged (M_n) molecular weight compared to the PE feedstock (M_w 184,600, M_n 27,500). The residual polymer from PE-fed mealworms showed an average reduction in M_w of 61.3% and reduction in M_n of 40.15%. The residual polymer from mealworms fed with PE plus bran showed an average reduction in M_w of 51.8% and reduction in M_n of 47.6%, indicating significant depolymerization of PE occurred within the gut of the mealworms fed with PE and PE plus bran. Limited depolymerization patterns were also found during plastic biodegradation e.g. a decrease in M_w and increase in M_n occurred during PUR degradation by a mixed microbial culture [77] and increases in both M_w and M_n was observed during PS degradation by *Galleria mellonella* larvae [78]. The mechanisms of the limited depolymerization remains unknown. More studies are needed to understand the factors influencing and/or controlling the pattern of depolymerization.

Analysis of frass extracts by FTIR and ^1H NMR is another approach to confirm modification of egested PS associated with degradation [47, 49]. FTIR spectra provide useful information of formation of new functional groups as evidence of biodegraded intermediates. Figure 11a is an example. At the end of a test with 120 mealworms at 25°C, incorporation of oxygen was seen in the increase in signals associated with carbonyl groups in residual PS from the frass [50]. By comparing the FTIR spectra of the feed PS and PS in egested frass, it revealed bond changes and the incorporation of oxygen previously associated with plastic degradation via aging, irradiation, and biotransformation [79–81]. The intensities of the peaks at 625–970 cm^{-1} (ring-bending vibration) were strong in PS feedstock but much weaker in frass samples. Characteristic peaks known to represent the PS benzene ring (C=C stretch, 1,550–1,610 and 1,800–2,000 cm^{-1}) were dampened in frass samples, providing evidence of ring cleavage. Further evidence of degradation was the decrease in intensities of peak characteristic for PS [81] and the appearance of carbonyl groups (C=O stretch, 1,700 cm^{-1}) [43]. PS oxidation was most extensive for frass from mealworms co-fed with bran. The broadening of peaks at 2,500–3,500 cm^{-1} in all FTIR spectra of frass samples is associated with the hydrogen bond of hydroxyl groups and/or carboxylic acid groups, suggesting a shift from hydrophobic to more hydrophilic surface properties.

^1H NMR spectra also provide information on biodegradation. Comparison of ^1H NMR spectra for PS to the spectra of frass extracts revealed new peaks in the frass from mealworms fed with PS only and PS plus bran (Fig. 11b). These peaks were detected in regions of chemical shift associated with $-\text{CH}=\text{CH}-$, carbonyl ($\text{H}_2\text{C}=\text{O}$), and hydroxyl ($-\text{OH}$) groups. Their presence in PS residues of frass, but not in the control PS, is evidence of transformations and modifications to the PS within the mealworm gut.

Thermal analysis characterized by using a TGA coupling with the FTIR spectroscopy method was used for characterization of PS biodegradation by *T. molitor* larvae [47]. A typical analysis was identification of PS degradation by *T. obscurus* versus *T. molitor* larvae [51]. Thermal modifications of ingested PS in *T. molitor* and *T. obscurus* larvae fed with PS as sole diet were detected using TGA to compare the

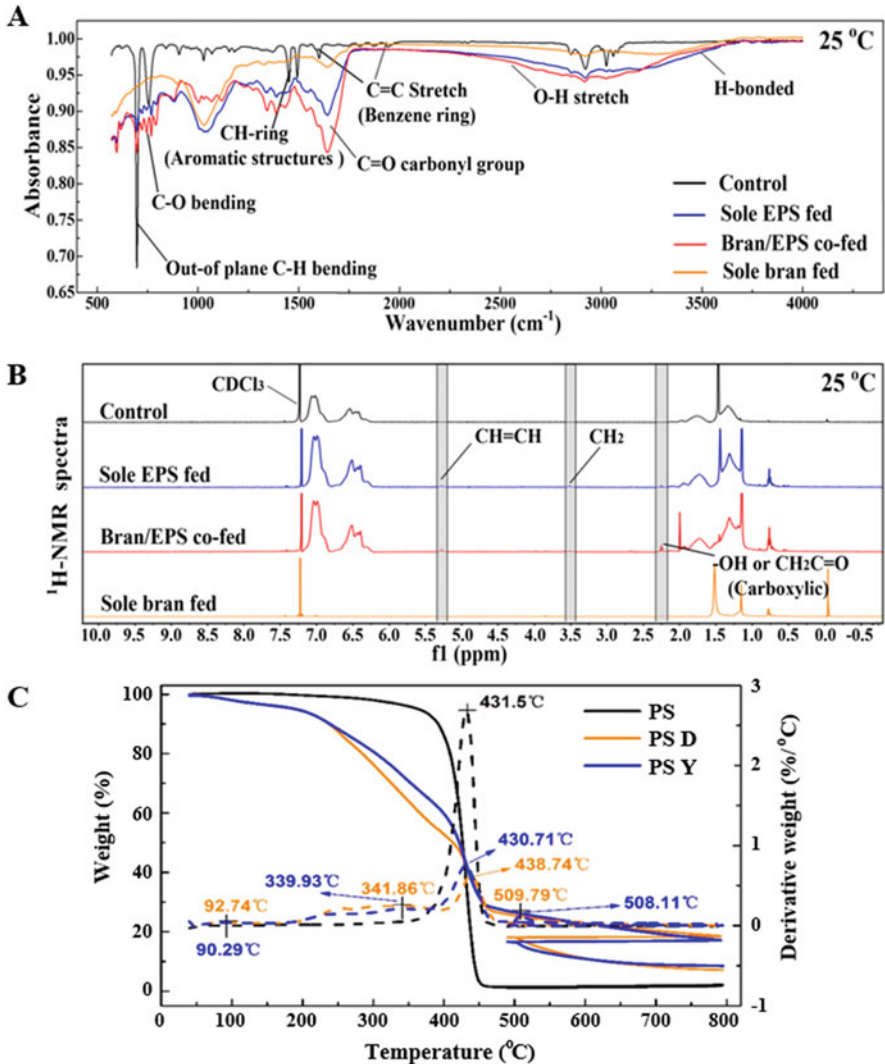


Fig. 11 Spectral analysis for the evaluation of PS degradation. (a) FTIR spectra and (b) ^1H NMR spectra of control (feedstock) and frass samples for mealworms fed with PS, bran plus PS, and bran alone. Samples were obtained on day 32. During the test, a final B:PS ratio was 16:1 g/g with 120 mealworms at $25\text{ }^{\circ}\text{C}$ (figures from Yang et al. [48]). (c) TGA spectra of PS feedstock and frass of *T. molitor* and *T. obscurus* larvae fed with PS only. Weight curve in solid line (left axis). Derivative weight curve in dash line (right axis). *Y. T. molitor*, *D. T. obscurus*, *AF* antibiotics, *B* bran, *CF* corn flour, *PS* polystyrene (figure from Peng et al. [51])

PS feedstock and residual PS in frass (Fig. 11c). Only one maximum decomposition rate (about 435°C) was detected in the PS sample. In contrast, for frass from *T. obscurus* fed with PS only (PS D) and from *T. molitor* (PS Y), four maximum decomposition rates (three under N₂ ambience and one under air ambience) appeared at 92.74°C, 341.86°C, 438.74°C, and 509.79°C, respectively (Fig. 11c). The decomposed part under 100°C was possibly classified as volatile organics (gut secretion, carboxylic acids compounds from PS biodegradation, etc.), while decomposed parts from 100°C to 360°C might be attributed to other biological wastes and biodegradation residue. The frass from both species decomposed in the same way, suggesting production of new organic intermediates with different thermal properties in the guts of the larvae. On the other hand, the mass loss ratio of the frass of *T. obscurus* larvae in the stage of 360°C to 480°C was 35.15%, while that of *T. molitor* larvae was 41.03%, in comparison with the PS feedstock of 96.32%. This result implied that the PS polymer structure deteriorated as it passed via the guts and that more PS was depleted or biodegraded in *T. obscurus*, suggesting that larvae of *T. obscurus* worked more efficiently in PS biodegradation than larvae of *T. molitor*.

The biodegradation of LDPE foam in *T. molitor* was confirmed using FTIR and ¹H NMR analyses [52]. Evidence of chemical modifications in the residual PE polymer was obtained by FTIR analysis. FTIR spectra from the residual polymers from the larvae fed with PE and PE plus bran revealed incorporation of oxygen as indicated by the appearance of peaks associated with C–O stretching (1,000–1,200 cm⁻¹) and alcohol groups (R–OH bend, 1,300–1,450 cm⁻¹; R–OH stretching, 3,000–3,500 cm⁻¹). Using ¹H-NMR analysis, comparison of the control PE spectra to the spectra of the residual polymer from the larvae fed with PE and PE plus bran revealed a new peak around 5.3 ppm in a region associated with alkene bonds (C=C–H). The results indicated formation of intermediates due to biodegradation.

In addition, due to limited reports on the research on biodegradation of PVC, PP, and PET, the analytical methods for evaluation of their biodegradation are still under development.

4.3 Stable Isotopic Tracer

Stable isotopic ¹³C tracer is a useful tool to investigate biodegradation. Isotopic studies using ¹³C-labeled PS materials have shown that PS was mineralized to ¹³CO₂ and incorporated into lipids [47]. The *T. molitor* larvae were continuously fed a 3% solidified jelly containing each of two ¹³C-labeled PS (0.4 mg/mL) and bran (0.2 mg/mL) over a 16-day period. The mean δ ¹³C values of CO₂ released by mealworms fed with α and β ¹³C-labeled PS diets were 3.3% and 3.9%, respectively. The released ¹³C-labeled CO₂ from the mealworms fed with α ¹³C-labeled and β ¹³C-labeled PS further confirmed the partially biodegradation and mineralization of PS at the end of the 16-day period. Analysis of the ¹³C CP/MAS NMR, which is usually applied to identify directly the native composition of the solid substrate without separation of

components [82, 83], was further conducted to identify functional groups indicative of depolymerization and oxidation. Compared with the spectrum of the PS feedstock, the new aromatic C (δ 140, 154, and 160) resonance signals could be ascribed to phenyl derivatives, as reported by Gilardi et al. [83]. The phenyl derivatives are possible proxies for the fragments or smaller molecules produced during depolymerization/oxidation of PS [62].

^{14}C tracer was widely used for the research on biodegradation of plastics during the 1970s–1980s [62]. However, this technique has been limited by the availability of radioisotope materials such as ^{14}C PE and ^{14}C PS.

5 Plastic-Degrading Microbial Communities and Functional Bacteria

5.1 *The Role of Gut Microbes in Plastic Degradation*

It is important to understand whether gut microflora play an indispensable role in the biodegradation of plastics, i.e., gut microbe-dependent or gut microbe-independent biodegradation. Antibiotics, such as gentamicin, nystatin, and ampicillin, suppress gut microbiota in mealworms and provide insight into the role that gut microbiota play in digestive processes, such as the digestion of cell walls and glucoside detoxification [84]. In the studies to test PS degradation, gentamicin has been used to depress or inhibit gut microbes in *T. molitor* and *T. obscurus* larvae [48–51]. Gentamicin is effective to treat mostly Gram-negative bacteria and some Gram-positive bacteria. The results showed that the microbial communities were inhibited and depressed by 10^2 – 10^3 in the presence of gentamicin (Fig. 12a), and the larvae fed with antibiotic gentamicin almost lost their ability to depolymerize PS (Fig. 12b), indicating that the gut bacteria impaired the ability of the mealworm to depolymerize long-chain PS molecules. GPC analyses indicated inhibition of depolymerization when *T. molitor* larvae from five sources in the USA were fed with gentamicin-containing WB, but depolymerization remained elevated in the control treatment (without gentamicin addition). No statistically significant differences were observed in M_w and M_n values between PS feedstock and residual polymers extracted from frass samples of mealworms receiving the gentamicin treatment for all five US sources (Fig. 12b, c). On the other hand, significant differences were observed in M_w and M_n values between PS feedstock and residual polymers extracted from control and gentamicin treatments. In tests to investigate PS degradation by *T. obscurus* larvae versus *T. molitor* larvae (Fig. 12d), gentamicin depression of gut microbes also inhibited PS depolymerization in *Tenebrio obscurus* larvae [51]. This is evidence that gentamicin suppressed gut microbiota and inhibited PS depolymerization. The depolymerization of PS is likely gut microbe dependent.

The effect of antibiotics on PE degradation in *Tenebrio* larvae has not yet been reported. However, addition of antibiotics did not inhibit the metabolism of beeswax

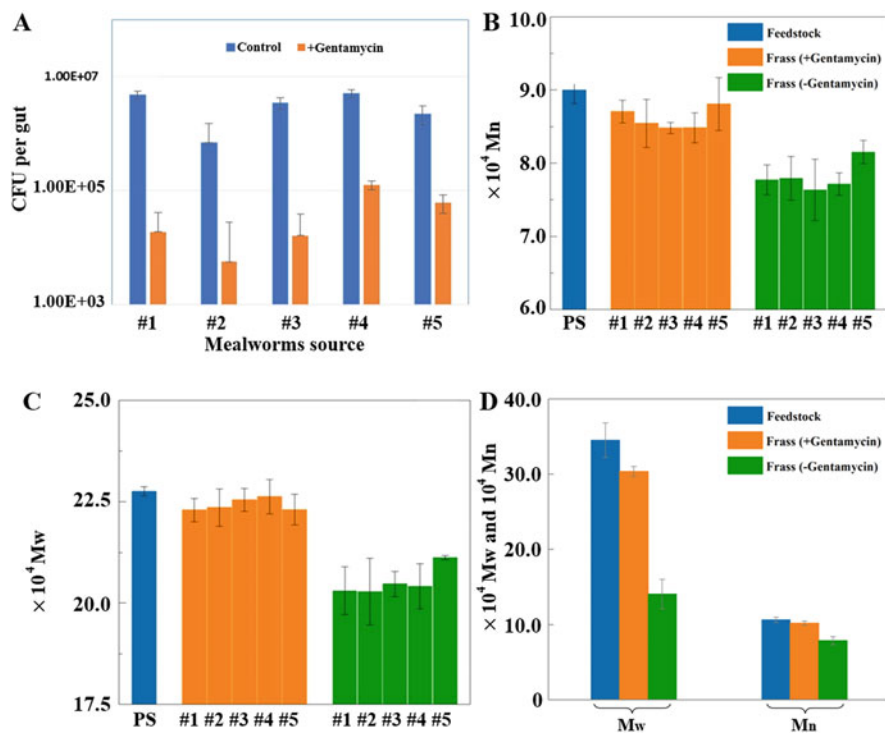


Fig. 12 Effect of antibiotics gentamicin on depolymerization of PS by *Tenebrio* larvae. (a) Depression of gut microbes by counting CFU in guts of five *T. molitor* larvae fed with gentamicin for 7 days in comparison with control. (b) Comparison of M_n in the PS residues from frass of five *T. molitor* larvae. (c) Comparison of M_w in the PS residues from frass of five *T. molitor* larvae. The five *T. molitor* larval sources are described in Table 3 (#1–#5). (d) Comparison of M_w and M_n of PS feedstock, PS polymers extracted from frass of *T. obscurus* fed with PS only without and with gentamicin. The larval source was from Shandong, China (data from Peng et al. [51])

and LDPE film by greater wax moth larvae (*Galleria mellonella*), indicating gut microbe independence [45]. Research is needed to identify whether biodegradation of PE in *Tenebrio* larvae is dependent or independent on gut microbes.

5.2 Plastic-Degrading Microbial Communities

Microbial communities of *T. molitor* in relation to PS degradation have been investigated [50, 52]. Comparing the great variations in different original bacterial communities between source populations due to differences in diet at different geographic locations (China, UK, and USA) and strain-specific properties, the microbial community analyses demonstrated significant taxonomic shifts for mealworms fed with diets of PS alone and WB plus PS [50]. The dominant bacterial

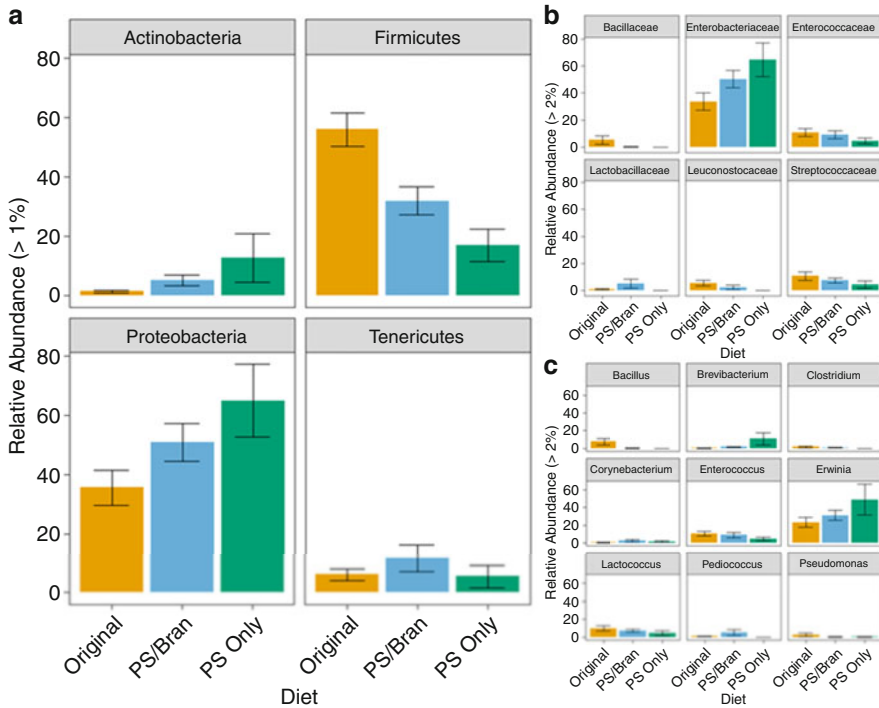


Fig. 13 Relative abundances of dominant (a) phyla, (b) families, and (c) genera in internal microbial communities from original mealworms versus those fed with PS and bran plus PS. Error bars indicate standard deviations for multiple *T. molitor* larval samples from China, the UK, and the USA (data and figures from Yang et al. [50])

phyla across mealworm samples fed with PS and WB plus PS, representing greater than 99% average relative abundance in the measured bacterial communities, were *Actinobacteria*, *Firmicutes*, *Proteobacteria*, and *Tenericutes* (Fig. 13a). The six most abundant families (>98% total relative abundance on average) were *Bacillaceae*, *Enterobacteriaceae*, *Enterococcaceae*, *Lactobacillaceae*, *Leuconostocaceae*, and *Streptococcaceae*, representing a diverse range of mostly aerobic and/or facultative bacteria (Fig. 13b). At the genus level (Fig. 13c), when the diet of the mealworms shifted from bran to PS only or PS plus bran, the gut microbiota shifted to a community with improved capabilities for PS degradation.

Brandon et al. [52] performed differential abundance analysis of the gut microbiome in *T. molitor* larvae and found that several minority OTUs strongly associated with the plastic diets (Fig. 14). The larvae were fed with respective diets, i.e., PE, PS, bran, and PE plus bran, for 32 days. This analysis revealed that two OTUs were strongly associated ($p < 0.05$) with both the plastic diets (PE and PS): *Citrobacter* sp. and *Kosakonia* sp. Both OTUs are members of the *Enterobacteriaceae*, a family known to contain PE-degrading member *Enterobacter*

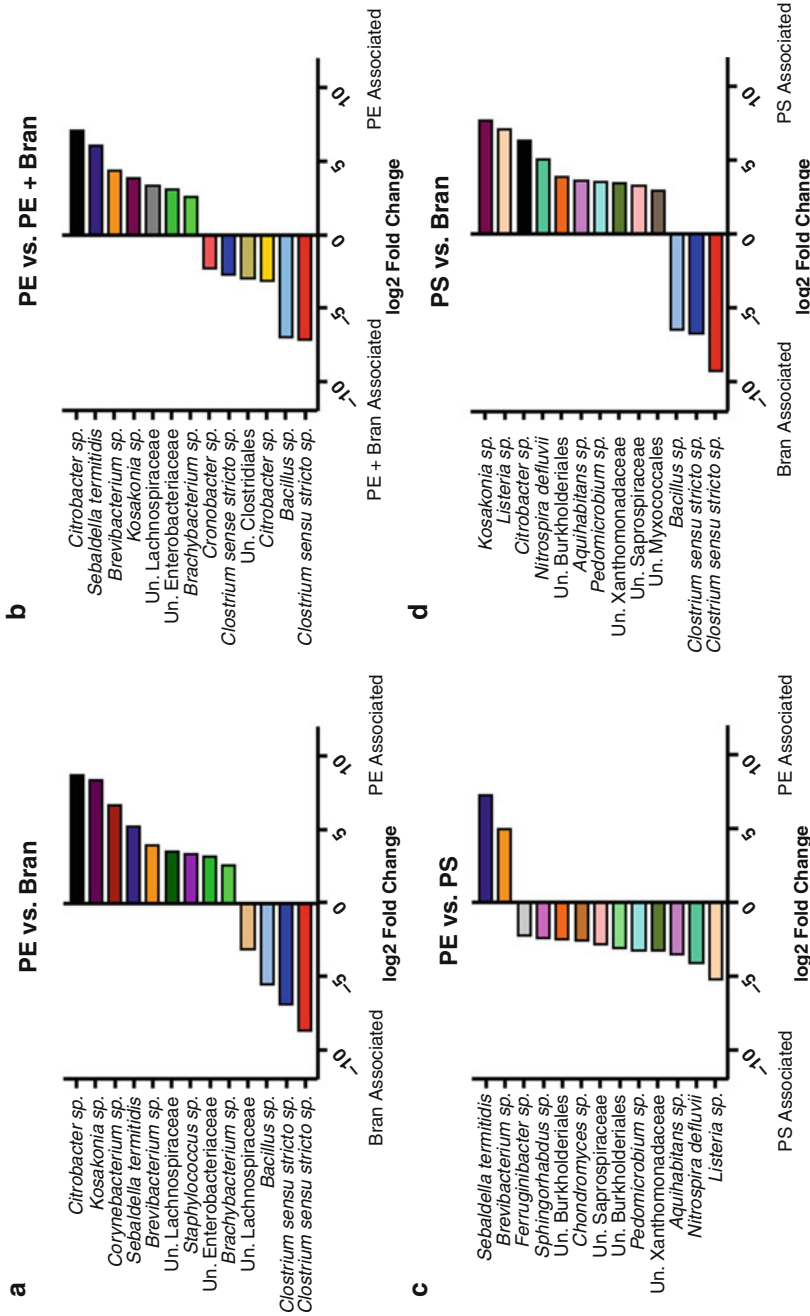


Fig. 14 Microbial community analysis of gut microbiome in different diets. (a) PE-fed microbiome versus bran-fed microbiome. (b) PE-fed microbiome versus PE + bran-fed microbiome. (c) PE-fed microbiome versus PS-fed microbiome. (d) PS-fed microbiome versus bran-fed microbiome. OTUs shown significantly ($p < 0.05$) differed between diets. Direction of fold change (\log_2) indicates which diet each OTU is more strongly associated (labeled below x-axis) (figures from Brandon et al. [52])

asburiae YT1 isolated from the gut of the larvae of Indian meal moth [43, 85]. Both OTUs can utilize oxygen (*Citrobacter* sp. are aerobic; *Kosakonia* sp. are facultative anaerobic), which could be further evidenced for their involvement in plastic degradation, as incorporation of oxygen is key in the accelerated biodegradation of both PE and PS [22, 57, 68, 72]. Both *Citrobacter* sp. and *Kosakonia* sp. were more abundant (based on relative abundance) in both of the plastic-only diets than the plastic plus bran fed diets and were also more abundant than the other OTUs identified via differential abundance analysis.

Two OTUs, both minority members of the microbial community, were significantly associated ($p < 0.05$) with PE-fed microbiomes: *Sebaldella termitidis* and *Brevibacterium* sp. (Figs. 14a–c). *Sebaldella termitidis* is phylogenetically isolated within the phylum *Fusobacteria*, is anaerobic, and is a known inhabitant of the posterior end of the termite gut track. *Brevibacterium* sp. is aerobic bacteria known to be associated with hydrocarbon degradation, including n-alkanes.

Seven OTUs, all minority members of the microbial community, were significantly associated ($p < 0.05$) with the PS-fed gut microbiome: *Listeria* sp., *Nitrospira defluvii*, *Pedomicrobium* sp., *Aquihabitans* sp., unclassified *Xanthomonadaceae*, unclassified *Saprosiraceae*, and unclassified *Burkholderiales* (Figs. 14c, d). Most of these PS-associated OTUs are aerobic, which is important when considering their possible role in the degradation of polystyrene. The increase in OTUs associated with the PS microbial community could be indicative of a more diverse suite of daughter products created in PS degradation, likely due to the more complex chemical composition of PS and the presence of benzene rings that could degrade into a variety of daughter products. It is still unknown that the changes in the PS microbial community were also affected by the presence of trace amounts (<1%) of a chemical flame retardant which is present in most commercially available PS products.

The gut microbial community of *T. obscurus* that shifted to that of high PS degradation capacity compared to *T. molitor* was proved in a recent study [51]. A ternary analysis suggested that the families *Enterococcaceae*, *Spiroplasmataceae*, and *Enterobacteriaceae* were strongly associated with the PS diet in *T. obscurus*, which was consistent with the result in relative abundance distributions. At present, however, it is difficult to prove which microbial genera or families are responsible for enhanced PS degradation because only a few PS-degrading bacteria have been isolated.

5.3 Plastic-Degrading Gut Microbes

PS-degrading bacterial strains have been isolated from *T. molitor* larval gut. A PS-degrading bacterial strain (*Exiguobacterium* sp. strain YT2, phylum: *Firmicutes*) was isolated from the larvae fed with PS as diet and was verified to degrade PS polymer [48]. *Exiguobacterium* is a genus of bacilli and a member of the low GC phyla of *Firmicutes*. This bacterial strain YT2 grew on the PS film by forming

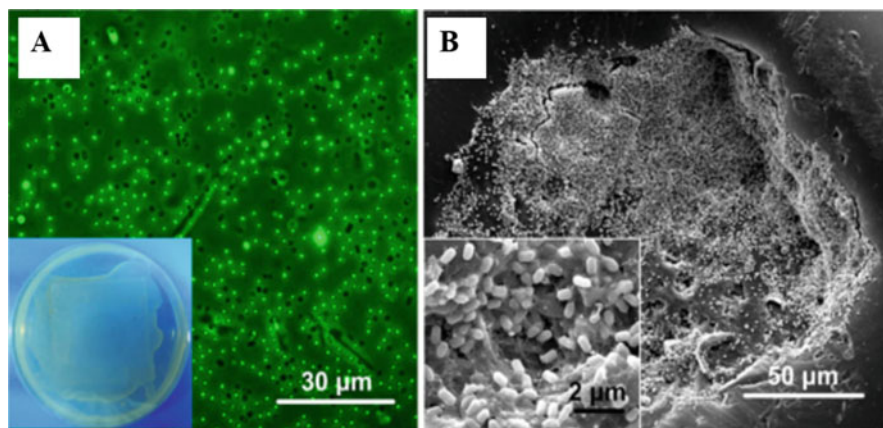


Fig. 15 Biofilm formation and deterioration of PS film surface topography after a 28-day incubation with strain YT2. (a) Fluorescent microscopic image of biofilm showed the presence of active cells after a 28-day incubation. Live cells are green, and dead cells are red. (b) SEM observations of the physical surface topography of the PS film incubated with strain YT2 after 28 days (figures from Yang et al. [48])

opaque colonies visible, but did not grow on the agar medium (Fig. 15). After a 28-day incubation, the molecular weight of the residual PS pieces was decreased based on GPC analysis, and the release of unknown water-soluble daughter products was detected on gas chromatography/mass spectrometry (GC/MS). The single culture of strain YT2 (10^8 cells/mL) removed PS by $7.4 \pm 0.4\%$ in liquid medium containing 2,500 mg/L PS pieces during a 60-day period. Analyses of GPC, X-ray photoelectron spectroscopy (XPS) scanning, and C 1s spectra of residual PS confirmed PS depolymerization by strain YT2. This proved that PS-degrading microorganisms are present in the gut *T. molitor* larvae and supported the gut microbe dependence of PS biodegradation. However, the isolated bacterial strain YT2 outside the living host appears to show much lower PS degradation efficiency than that demonstrated in the gut system. This suggests that the mechanism of PS degradation in mealworms' gut could be more complicated than gut microbial activities.

Isolation of plastic-degrading bacteria from *T. molitor* larvae was also reported by Suh and Lee [86]. They examined colonization on four different plastic (PS, PET, PP, and PVC) films as indication of plastic biodegradation and claimed ten isolated bacterial strains belonging to *Escherichia fergusonii*, *Bacillus toyonensis*, and *Klebsiella oxytoca*. Tang et al. [87] isolated unknown aerobic strains TM1 and ZM1 from *T. molitor* and *Z. morio* using yeast extract on agar plates and claimed their growth on PS plates which were prepared by adding PS emulsion (in chloroform solvent) into agar basal medium.

6 Mechanism on Biodegradation of Plastics in Insects and Research Prospects

The mechanism of rapid biodegradation of PS and PE in *Tenebrio* larva is still under investigation. Recent studies indicated that *T. molitor* larvae also have unique capacity of degrading lignin material in wheat straw, rice straw, and corn straw [88, 89]. Lignin is a class of complex cross-linked phenolic polymers and resistant to biodegradation. The mechanism of biodegradation of lignin in *T. molitor* larvae is also unknown since we do not know if the gut microbes or the larvae or both could secrete ligninolytic enzymes (heme peroxidases, laccase, etc.). Through the action of unidentified enzymes in the termite gut, lignocellulose polymers are broken down into sugars and are transformed into hydrogen. The bacteria within the gut turn the sugar and hydrogen into cellulose acetate, an acetate ester of cellulose on which termites rely for energy. It is not clear whether the capacity of degrading lignocellulose in the larvae also works on various plastics.

Based on the research results on biodegradation of plastics in insects reported and the conceptual model proposed by Yang et al. [47], a primary schematic diagram for symbiotic degradation of plastics (PS and PE) in *Tenebrio* larvae is proposed in Fig. 16 as follows. First, plastic materials (foam, film, powders, or fragments) are chewed into small particles and ingested into the gut. Chewing reduces the size of plastic particles and increases the contact surface area of particle exposure to microbes, bacterial extracellular enzymes, and digestive enzymes. During the ingestion, oxygen in air also enters the intestinal tract and then serves as electron acceptor for aerobic and facultative microbes as well as enzymatic reactions. In the gut, the ingested plastic particles are further fragmented due to mixing, stirring, and moving in the intestinal tract. The fragmented particles are further mixed with gut microbiota that excretes extracellular enzymes and digestive enzymes from the insect to catalyze the depolymerization of the particles into small molecule products. Biodegraded intermediates are further produced via various enzymatic oxidations after depolymerization. Some biodegraded intermediates were further mineralized into CO₂ and H₂O by multiple functional microbes and/or the mealworm host. Limited carbons of the intermediates are further incorporated into biomass. A part of H₂O produced is utilized by *T. molitor* larvae as water source. The biodegraded products including intermediates and CO₂ and undigested residual plastic polymers with some gut microbes are egested as frass. The frass contains plastic-degrading microbes and can be recycled back to the intestinal tract again.

The discovery of biodegradation of plastics, especially PS and PE in insects and their larva, has opened a new door to reach the fate of plastic wastes in the environment and the potential solutions to plastic pollution. To date, the published results of plastics degradation are still limited to PS and LDPE. The feasibility of biodegradation of other recalcitrant plastics such as PP, PVC, and PET in insects should be investigated. The fate of additives in plastics such as plasticizer and flame retardant should also be addressed. Fundamental research topics on biodegradation of plastics with *Tenebrio* larvae and other insects should be considered including

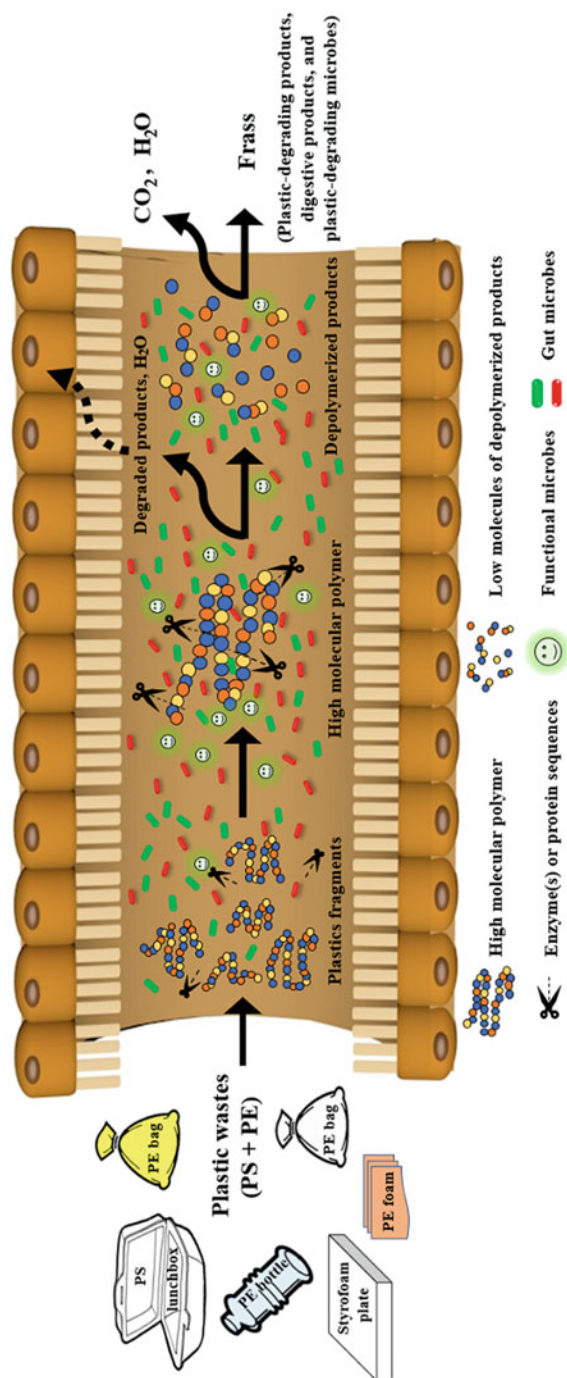


Fig. 16 Conceptual model for biodegradation of plastics in insect intestinal tract

(a) factors impacting and limiting biodegradation of plastics (polymer types, molecular weight, and structure); (b) the enzyme(s), protein sequences, and genes of functional microbes related to plastic degradation; (c) the interaction or synergistic effect between host intestinal tract and gut microbes; and (d) the plastic degradation-related or assistant digestive enzyme(s), protein sequences, cofactor(s), and genes of the insects. Understanding the mechanisms of the insect-related plastic biodegradation could greatly benefit to management of plastic wastes and recovery of resource from used plastics, production of new generation of plastics products, as well as development of innovative technologies for bioremediation of existing plastics pollution sites.

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Plastics of the Future? The Impact of Biodegradable Polymers on the Environment



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Abstract With the increasing reports on the environmental distribution and ecological risks of petrochemical plastics and microplastics, degradable plastics are considered as the optimal alternative to the traditional plastics. Compared with the traditional petrochemical plastics, the market of biodegradable plastics is still small but growing rapidly. At the same time, knowledge on the environmental distribution and ecological risks of biodegradable plastics is still limited, although their production and application continue to improve. Biodegradable plastics are divided into semi-biodegradable plastics and fully biodegradable plastics. Their ecological risks may show significant differences. In the soil environment, the particle size, shape, molecular weight of plastics, the type of functional groups in the molecular structure, and the additives added to plastics may play different roles in the biodegradation of biodegradable plastics. In current chapter, the available information of current researches on biodegradable plastics in the environment is reviewed. The environmental risk and future development of degradable plastics are also discussed.

Keywords Biodegradable plastics, Biodegradation, Ecological risks, Environmental distribution

1 The Concept, Composition, Application, and Output of Biodegradable Plastics

1.1 Definition and Classification of Biodegradable Plastics

Biodegradable plastics are defined as degradable plastics in which the degradation results from the action of naturally occurring microorganisms such as bacteria, fungi, and algae by the American Society for Testing and Materials [1]. Ammala et al. define biodegradable plastics as plastics that can be decomposed by the action of living organisms, usually microbes, into water, carbon dioxide, and biomass [2]. The national standard of the People's Republic of China defines biodegradable plastics as plastics degraded by the action of microorganisms in nature and eventually degraded into carbon dioxide and/or methane, water, and mineralized inorganic salts containing elements and new biomass [3].

According to the degradation degree of plastics under the action of microorganisms, biodegradable plastics can be divided into completely degradable plastics and semi-degradable plastics. The former can be completely degraded. In comparison, the semi-degradable plastics are a blend of non-biodegradable plastics with degradable polyesters or starch [4]. The purpose of destroying the structure of the copolymer is achieved by biodegradation of natural components.

Biodegradable plastics can also be classified into bio-based plastics and fossil-based plastics depending on the raw materials [5, 6]. Bio-based biodegradable plastics derived from renewable resources can be used in medical and pharmaceutical industries, such as polyhydroxyalkanoate (PHA) and polylactic acid (PLA). Fossil-based biodegradable plastics have been widely employed in the packaging

Table 1 Full name and abbreviation of target plastics

Full name	Abbreviation
Polyhydroxyalkanoate	PHA
Polylactic acid	PLA
Polyhydroxybutyrate	PHB
Polybutylene succinate	PBS
Polycaprolactone	PCL
Polyethersulfone resin	PES
Poly(butylene adipate-co-terephthalate)	PBAT
Polypropylene	PP
Polystyrene	PS
Polyethylene	PE
Polyvinyl chloride	PVC
Polyethylene terephthalate	PET
Polyurethane	PUR
Polyamide	PA
Polyurethane	PUR
Polyethylene furanoate	PEF
Acrylonitrile butadiene styrene	ABS
Polybutylene succinate-co-adipate	PBSA
Polyvinyl chloride	PVC
Polyoxymethylene (polyformaldehyde)	POM
Phthalate esters	PAEs
Hyperbranched aliphatic polyester and cellulose	HAPE-cell
Differential scanning calorimeter	DSC

industry, such as polyethersulfone resin (PES) and polycaprolactone (PCL). Abbreviations for common types of plastic polymers are shown in Table 1.

1.2 Representative Materials and Their Applications

Representative biodegradable plastics include PLA, PHA, polybutylene succinate (PBS), PCL, etc. The chemical structure and application of these plastics are shown in Table 2.

1.3 Production and Environmental Flux

Based on the latest market data compiled by *European Bioplastics* in cooperation with the research institute nova-Institute, the global production capacity of biodegradable plastics is 912 kt in 2018. The production capacities of biodegradable

Table 2 Representative biodegradable plastics and their applications

Plastics	Chemical structure	Application [6–8]
Poly(lactic acid) (PLA)	$\left[\text{O}-\underset{\text{CH}_3}{\underset{\text{O}}{\parallel}}{\text{C}}-\text{CH} \right]_n$	Loose-fill packaging, compost bags, food packaging, disposable tableware, upholstery, disposable garments
Poly(hydroxybutyrate) (PHB)	$\left[\text{O}-\underset{\text{CH}_3}{\underset{\text{O}}{\parallel}}{\text{C}}-\text{CH}_2-\text{C} \right]_n$	Disposable tableware, packaging materials, agricultural laminating films, coatings, fibers
Poly(butylene succinate) (PBS)	$\left[\text{O}-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{O}-\underset{\text{O}}{\parallel}{\text{C}}-\text{CH}_2-\text{CH}_2-\text{C} \right]_n$	Packaging, tableware, cosmetic bottles, pharmaceutical bottles, disposable medical supplies, agricultural films, biomedical applications
Poly(ε-caprolactone) (PCL)	$\left[\text{O}-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{C} \right]_n$	Drug carrier, cell or tissue culture rack, surgical suture, medical modeling materials, art modeling materials, toys, organic colorants

plastics with good development and industrial scale are PLA of ~218 kt, PBS of ~97 kt, and PHA of ~30 kt [9].

In China, the production capacity of biodegradable plastics has expanded rapidly in recent years. In 2018, China's biodegradable plastic industry was about 5.44 billion RMB (7.75 million \$) with an increase of 21.13% compared with that in 2017 (4.941 billion RMB or 7.03 million \$), which included 1.584 billion RMB for completely biodegradable plastics and 3.856 billion RMB for semi-degradable plastics. In 2018, China's biodegradable plastic industry produced about 650,000 tons, including 95,000 tons for completely biodegradable plastics and 555,000 tons for semi-degradable plastics.

2 The Occurrence, Degradation Efficiency, and Environmental Impact of Semi-biodegradable Plastics

2.1 Environmental Distribution of Semi-Biodegradable Plastics

Most of the fossil-based and bio-based plastics, used nowadays, are non-biodegradable, such as polypropylene (PP), polystyrene (PS), polyethylene (PE), polyvinyl chloride (PVC), polyethylene terephthalate (PET), and polyurethane (PUR) [7]. These plastics are highly stable and do not readily enter into the degradation cycles of the biosphere. Most of the employed plastics are either non-biodegradable or their degradation rate is too slow to be disintegrated completely [10]. Therefore, these non-biodegradable plastics were accumulated in the soil environment in large quantities because of improper waste management and uncontrolled littering, posing a serious threat to our planet eventually [6].

The semi-biodegradable plastics are a blend of non-biodegradable plastics with biodegradable polyesters or starch [4]. The blending of biodegradable polymers is one approach of reducing the overall cost of the material and modifying the desired properties and apparent decomposition rate. Compared to the copolymerization method, blending may be a more efficient way to achieve the properties of plastic degradability. Former study has reported blend plastics by combining PCL with conventional plastics (such as low-density PE, PP, PET, and PS) [8]. The blends of PCL and low-density PE, PCL, and PP both retained the high biodegradability of PCL. On the contrary, the degradability of the PCL part in the blends of PCL and PS, PCL and PET both dropped off remarkably. In the case of blends of PCL and PS, the biodegradability of PCL did not change significantly [4].

The global distribution of bioplastics in 2014 and 2018 is shown in Fig. 1. Bio-based non-biodegradable plastics (semi-biodegradable plastics), including the drop-in solutions bio-based PE and bio-based PET and bio-based PA, account for around 53% (0.9 million tons) of the global bioplastic production capacities in 2014

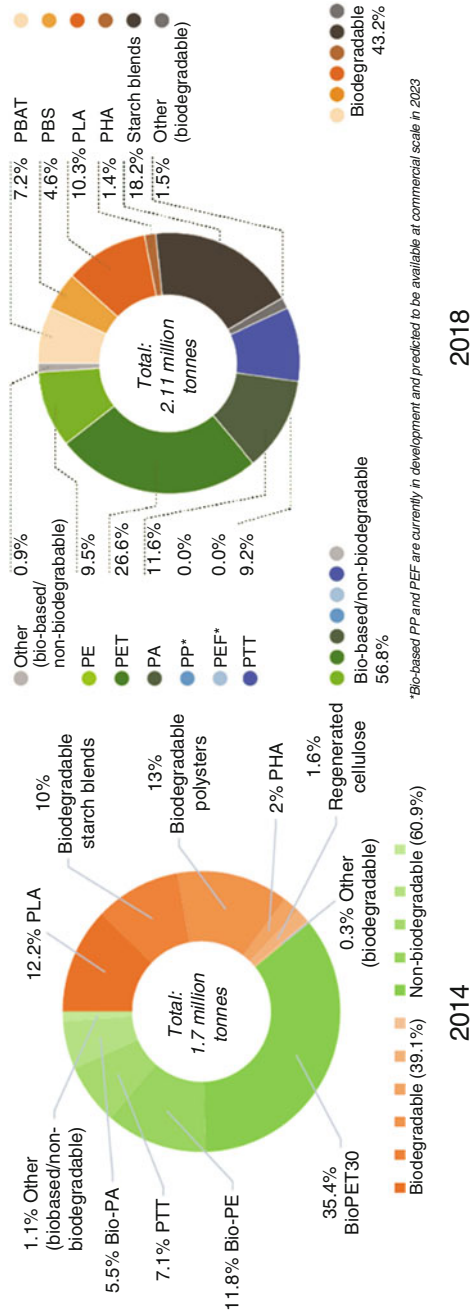


Fig. 1 Global distribution of bioplastics in 2014 [11, 12] and 2018 [9]

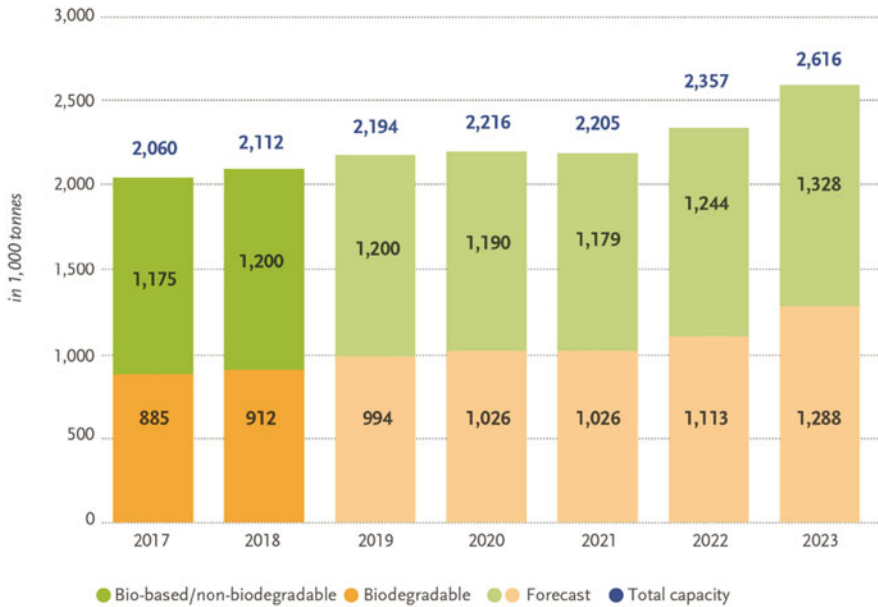


Fig. 2 Global production capacities of bioplastics from 2018 to 2023 [9]. Source: European Bioplastics, nova-Institute (2018). More information: www.european-bioplastics.org/market and www.bio-based.eu/markets

and 48% (1 million tons) in 2018, respectively. The proportion of semi-biodegradable plastic in the world was reduced from 60.9% in 2014 to 56.8% in 2018, indicating the demand for completely biodegradable plastics is gradually increased today.

The global production capacities of bioplastics from 2018 to 2023 are shown in Fig. 2. Currently, bioplastics represent roughly 1% of the 335 million tons of plastic produced annually. The global semi-biodegradable plastic production capacity is predicted to increase gradually from 2018 to 2023, increasing from around 1.20 million tons in 2018 to approximately 1.33 million tons in 2023 [9]. Specifically, the production of bio-based PE is predicted to continue to grow as new capacities are planned to come online in Europe in the coming years. The intention to increase production capacities for bio-based PET, however, has not been realized at the rate predicted in previous years [6]. Polyethylene furanoate (PEF), a new polymer, is expected to enter the market in 2023. PEF is 100% bio-based and is said to feature superior barrier and thermal properties, making it an ideal material for the packaging of drinks, food, and nonfood products. In 2023, bio-based PP is expected to enter the market at a commercial scale with a strong growth potential due to the widespread application of PP in a wide range of sectors. Additionally, bio-based PUR is another important group of polymers that have huge production capacities with a well-established market and is expected to grow faster than the conventional PUR market due to their versatility.

2.2 Degradation Characteristics of Semi-Biodegradable Plastics in the Environments

Non-biodegradable plastics mainly consist of conventional synthetic plastics such as PE, PS, PP, PET, and PVC, which have accumulated massively in the soil environment because of the randomly littering and poor waste management [6]. The biodegradation of major synthetic plastics in the soil environment is a very slow process that includes many environmental factors [2]. The basic mechanism for biodegradation of the high molecular weight plastics is the hydrolysis or oxidation by enzyme. Therefore, the main chains of plastics are biodegraded into polymer with feeble mechanical properties and low molecular weight, making it more convenient for further microbial assimilation [7]. The backbone of synthetic plastics is consisted of only long carbon chains. The characteristic structure makes polyolefins non-susceptible to degradation by microorganisms. However, a comprehensive study of polyolefin biodegradation has shown that some microorganisms could utilize polyolefins with low molecular weight [13].

PE is one of the non-biodegradable plastics with high hydrophobicity and high molecular weight. Hydro-biodegradation and oxo-biodegradation are two mechanisms of biodegradation of PE. These two mechanisms coincident with the modifications owing to starch and prooxidant are used as the two additives in the synthesis of biodegradable PE. Starch blend PE has a continuous starch phase that contributes to the hydrophilic of plastics, so it can be catalyzed by amylase enzymes. Microorganisms can easily access, attack, and remove the starch blend PE. Consequently, the hydrophilic PE with matrix is considered to be hydro-biodegraded [14]. Additionally, compatibilizer can also enhance the biodegradability of low molecular weight PE/starch blends. Generally, the blending of PE with additives enhances auto-oxidation and reduces the molecular weight of the plastics, leading microorganisms to degrade the low molecular weight plastics more easily. Although all these approaches can improve the biodegradation of PE blends, the biodegradability of PE part is still relatively low [2].

2.3 The Ecological Effect of Semi-biodegradable Plastics

Due to the growing volumes of semi-biodegradable plastics, a strong concern of the public opinion is about the environmental impact of persistent substances possibly released during the process of degradation and composting. Only the biodegradable components could be degraded in the environment. Therefore, the non-biodegradable components are broken up into smaller particles and diffuse into the environment [15]. Their ecological risk assessment can refer to that of non-degradable plastics. In addition, toxic degradation products or harmful compounds such as the additives in semi-biodegradable plastics will also release to the environment. Soil health is a key component of agroecosystem sustainability; thus

there is a need to understand the effects of semi-biodegradable plastic on both crop productivity and soils [16]. Accordingly, more research about the ecological effects of semi-biodegradable should be done in the future.

3 The Occurrence, Degradation Efficiency, and Environmental Impact of Completely Biodegradable Plastics

3.1 Environmental Distribution of Completely Biodegradable Plastics

Completely biodegradable plastics mainly include bio-based and fossil-based polymers, which are advantageous in modern industrial applications because of their high degree of biodegradability and microbial assimilation [13]. Owing to maintain the advantages conferred through using plastic products without having the serious pollution burden of waste plastics, the attention of completely biodegradable plastics is continuously growing [6]. Compared to the majority of industrial plastics, completely biodegradable plastics are supposed to convert into carbon dioxide, water, and biomass once they end up in the environment. With the increasing awareness of plastic pollution, the demand for completely biodegradable plastics is urgent nowadays [17].

Figure 2 shows the global production capacity distribution of bioplastics. Based on the market data in 2018 compiled by European Bioplastics, the production capacity of global bioplastics is predicted to increase from 2.11 million tons in 2018 to approximately 2.62 million tons in 2023 [9]. Nevertheless, completely biodegradable plastics (0.91 million tons in 2018) are only accounted for less than 0.3% of the total plastic production (335 million tons in 2018) [9].

Completely biodegradable plastics mainly include bio-based and petroleum-based biodegradable plastics [7]. Bio-based completely biodegradable plastics are consisted of PHA and PLA. Petroleum-based completely biodegradable plastics mainly include PBS, PCL, and PBAT [13]. PLA and starch blends are two most contributors of the completely biodegradable plastics, accounting for 23.8 and 42.1% of the completely biodegradable plastics, respectively (Fig. 3). Compared to that in 2018, the production capacity of PLA is predicted to grow by 60% by 2023. It's well known that PLA is a very versatile material that features significant barrier properties and is available in high-performance PLA grades that are one of significant replacements for PP, acrylonitrile butadiene styrene (ABS), and PS in more demanding applications [8]. Furthermore, polyhydroxybutyrate (PHB) as one of the completely biodegradable plastics has gradually attracted significant attention because of their biodegradation under both anaerobic and aerobic environments without releasing toxic contaminants into the environment [18].

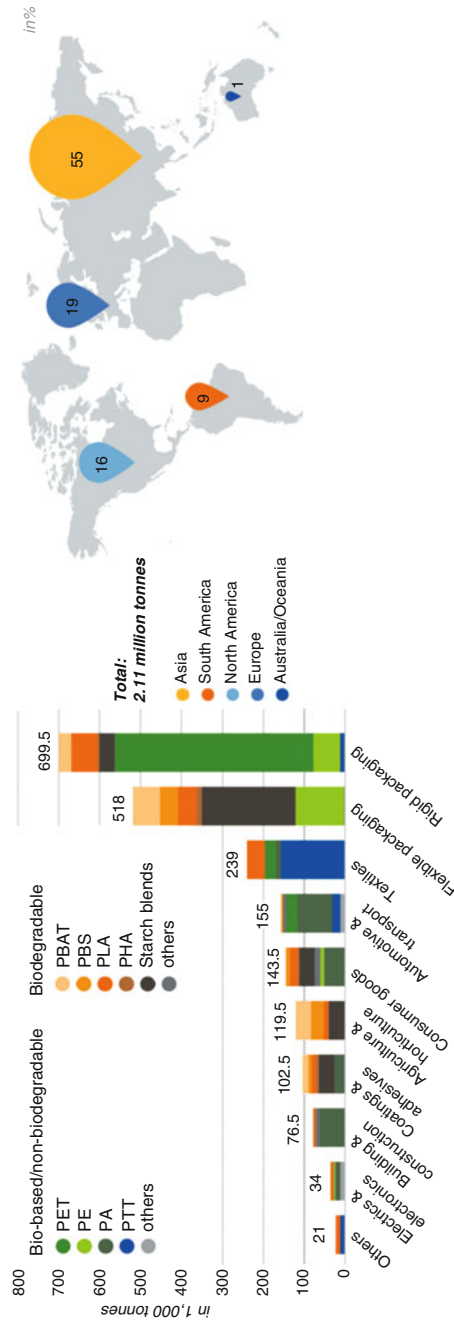


Fig. 3 The market and region distribution of completely biodegradable plastics in the environment [9]. Source: European Bioplastics, nova-Institute (2018). More information: www.european-bioplastics.org/market and www.bio-based.eu/markets

The market and region distribution of completely biodegradable plastics in the environment is provided in Fig. 3. Completely biodegradable plastics are applied to several application markets, mainly including flexible packaging, adhesives, agriculture, coatings, and textiles. Particularly in agriculture, demand for completely biodegradable plastics accounts for more than 60% of the total bioplastics. More than 50% of bioplastics were produced in Asia in 2018. In comparison, only approximately 20% of the global bioplastic production capacity is located in Europe.

3.2 Degradation Characteristic of Completely Biodegradable Plastics Entering the Environment

Biodegradation is the process of organic substances broken down by living organisms. Organic substances can degrade aerobically with oxygen or anaerobically without oxygen [7]. CO₂ and H₂O are released during aerobic biodegradation, while CO₂, H₂O, and CH₄ are produced accordingly during anaerobic biodegradation [19].

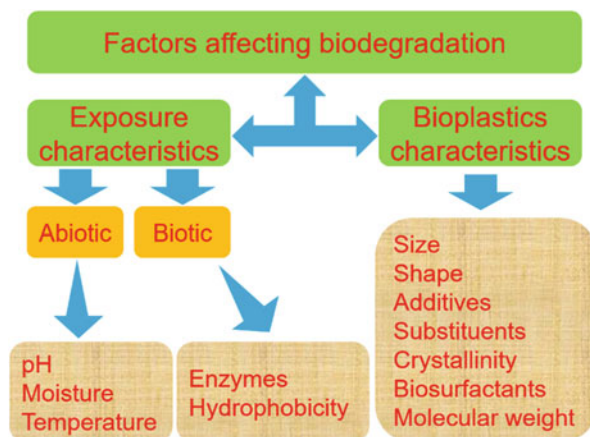
Biodegradation of bioplastics requires microorganisms to metabolize all organic components of bioplastics [20]. Specifically, the process of plastic biodegradation can be divided into three steps: (1) biodeterioration, the colonization of the polymer surfaces by soil microorganisms; (2) depolymerization, depolymerize the polymer into low molecular weight compounds by the secretion of extracellular microbial enzymes; and (3) bioassimilation, microbial uptake and utilization of these compounds, incorporating bioplastics carbon into biomass or releasing CO₂ [21–23].

Step 1: Microbial colonization of plastic surfaces. In this step, the formation of a microbial biofilm contributes to superficial degradation, fragmenting the polymeric material into smaller particles. Microbial colonization appears on the bioplastic surface through degrading soil fungi and bacteria. Factors that facilitate colonization can increase the contact area between bioplastics and microbial degraders, thus improving the biodegradation efficiency of plastics eventually [24].

Step 2: Enzymatic depolymerization of plastics. The microorganisms of the biofilm secrete extracellular enzymes, catalyzing the depolymerization of the bioplastic chain into low molecular weight oligomers, dimers, and monomers [25]. Ordinarily, the abiotic hydrolysis of these bonds is generally slower than enzymatic hydrolysis on the condition of pH and temperature that prevail in soil [21, 26]. Enzymatic depolymerization plays a role in limiting the rate of plastic biodegradation in soil. This is supported by much faster microbial utilization of oligomers, dimers, and monomers when directly added to soil than of the corresponding bioplastics in the same soil [23].

Step 3: Microbial utilization of plastic carbon. The last step in bioplastic biodegradation is the microbial assimilation and utilization of oligomers, dimers, and monomers released from bioplastics through enzymatic hydrolysis. The uptake of the small molecules produced into microbial cells and the following production of

Fig. 4 Factors affecting the biodegradation efficiency of biodegradable plastics



primary and secondary metabolites is a process recognized as assimilation. Microorganisms utilize the hydrolysis products as substrates for both respiration and synthesis of biomolecules. The most immediate solution to comprehend utilization is to follow the conversion of plastic-derived carbon into CO_2 and into microbial biomass [2]. Concomitantly, several simple and complex metabolites may be excreted and reach the extracellular surroundings (e.g., aldehydes, organic acids, and antibiotics). These metabolites are mineralized, and end products such as CO_2 , H_2O , CH_4 , and N_2 are formed and released into the soil environment eventually.

Figure 4 demonstrates several factors that influence the efficiency of biodegradation, mainly including plastic characteristics, type of organism, and nature of pretreatment [11]. The plastic characteristics such as size, shape, molecular weight, type of functional groups in molecular structure, and additives added to the bioplastics all play significant roles in plastic biodegradation [27].

Moisture: Moisture can influence the biodegradation of plastics in different ways because of the fundamental requirement of water for growth and the multiplication of microbes. Abundant moisture can increase the swift action of microbial; thus the efficiency of biodegradation is increased. Additionally, abundant moisture conditions can also influence the process of hydrolysis by generating more chain scission reactions [13].

pH: pH can modify the rate of hydrolysis reactions through controlling the acidic or basic conditions. For instance, the efficiency of hydrolysis of PLA capsules is optimal when the pH is controlled at 5. The pH conditions are altered during degradation products of various plastics, changing the rate of the degradation process and microbial growth eventually [11].

Temperature: Similarly, temperature can also have a significant influence on enzymatic biodegradation through the softening of bioplastics. Plastics with a higher melting point have less possibility of biodegradation, and potential enzymatic degradability decreases with the increase of temperature [11]. Furthermore, the

efficiency of PHA biodegradation was not constant in various periods of the year from 1999 to 2000 owing to various weather temperatures [28].

Enzyme characteristics: Different enzymes possess unique active sites and have the ability to biodegrade various types of bioplastics. Depolymerases were obtained from bioplastic-degrading microorganisms, playing a significant role in controlling biodegradation of bioplastics [29]. Moreover, it was also shown that the extracellular enzymes were involved in the depolymerization of PHB, and the specific microbially produced depolymerase can influence the distinct mechanisms of degrading PHB [6].

Molecular weight: Molecular weight plays a significant role in controlling the efficiency of biodegradation of many bioplastics owing to it can influence many physical properties of bioplastics. Ordinarily, bioplastic degradability by microorganisms is a decline with the increase of the molecular weight of bioplastics. The degradability of higher molecular weight PCL (>4,000) by lipase of a strain R. Delmar was lower than that of the low molecular weight bioplastics [4]. It is convenient for microbial enzymes to attack a substrate low in molecular weight; this is maybe the reason for this phenomenon [30]. Furthermore, high molecular weights can lead to a sharp decrease in solubility, making them unfavorable for microbial attack owing to the substrate was required for the assimilation of bacteria, and then further degraded by cellular enzymes.

Shape and size: The shape and size of the bioplastics play a significant role in altering the biodegradation process [11]. The bioplastics having large surface areas can be degraded rapidly when compared to those with a small surface area [31]. It was reported that the PHA films are degraded faster than PHA pellets owing to their larger surface area. Additionally, a larger polymer/water interface can enhance the attachment of microorganisms to the surface of bioplastics.

Additives: The structure and the composition of bioplastics can significantly influence the efficiency of biodegradation. Modifying the composition of bioplastics, including the addition of additives with high soluble sugar content, and biodegradability may be enhanced accordingly [11]. Although bio-composite production from bioplastics may have some improved mechanical properties such as high tensile strength, the biodegradation process may not be favorable under certain circumstances or become interrupted at the same stage. Consequently, the optimization of the bio-composite additives can engender a more applicable and biodegradable product [6].

Biosurfactants: Biosurfactants are amphiphilic substances and mainly adhered to the living surfaces. The low toxicity and high biodegradability of biosurfactants can enhance the biodegradation of bioplastics [32]. Moreover, the presence of specific functional groups on biosurfactants can improve the biodegradation of bioplastics and can also enhance their activities even in the extreme pH, temperature, and salinity conditions as well [33].

3.3 *The Ecological Effect of Completely Biodegradable Plastics*¹

In order to assess the ecological effect of completely biodegradable plastics, ecotoxicity tests have been conducted under controlled laboratory conditions using model organisms [21]. The choice of the test organisms depends on the specific ecosystem. The most commonly used test species for terrestrial ecosystems are soil microorganisms, soil fauna, and terrestrial plant. For aquatic ecosystems, algae, crustaceans, and fish are generally investigated for their response to completely biodegradable plastics. From a toxicology standpoint, the fragments of completely biodegradable plastics incorporated into the soil are generally considered to be safe [16]. In theory, completely biodegradable plastics should be completely catabolized by soil microorganisms, converted to microbial biomass, CO₂, and water. However, complete breakdown in a reasonable amount of time is not always observed in practice [34].

Table 3 shows the reported ecological effects of completely biodegradable plastics. Most completely biodegradable plastics did not show adverse effects on the selected organisms except PLA and PBAT. Souza et al. found cytotoxic and genotoxic effects of PLA degradation products after 76 days of incubation in the compost on the common onion (*Allium cepa*) [37]. Likewise, the negative effect on the activities of both ammonium and nitrite-oxidizing bacteria caused by PLA mulch films after 84 days of incubation in the soil was also detected [39]. PLA granules can affect the health and behavior of lugworms and directly or indirectly reduce primary productivity of these habitats after 31 days of incubation in the sand [40]. Zhang et al. have shown that the four kinds of field-weathered biodegradable plastic mulch (PLA/PHA, Organix, BioAgri, Naturecycle) could be dragged into the burrows of earthworms when earthworms are foraging for food [44].

Although these studies measured the effects of degradation products at a specific time, they did not provide enough information on the components of the product, which are responsible for the toxicity. Identification of toxic degradation products can help to further understand the toxic mechanisms and produce safe biodegradable plastics.

4 Interaction of Biodegradable Plastics with Other Contaminants

4.1 *Interaction with Heavy Metals*

Some polymers are designed as sorbents. In order to improve the adsorption capacity of polymers, the surface of many polymer matrix composites can interact with the target chemicals [45]. For example, on a degradable polymer made of hyperbranched aliphatic polyester and cellulose (HAPE-Cell) [46], the adsorption capacity of

¹Parts of this text are reused with permission from [21].

Table 3 Ecological effects of completely biodegradable plastics. Reused with permission from [21]

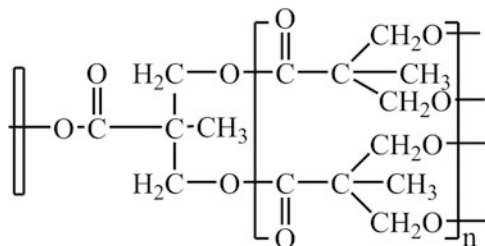
Polymer	Test material preparation	Test medium	Test system	Effect	Reference
Mater-BiDF04P (starch blend)	Powdered mulch film in the soil (10 g/800 g), incubation for 528 days	Aerobic agricultural soil	Nitrification test based on ISO 14238	No inhibition of the nitrification potential; biodegradable carbon source in soil potentially elicited microbial growth	[35]
Mater-Bi, DF04A, EF04P, AF05S0 (starch blend)	Powdered films in the soil (10 g/800 g), incubation for 6 months	Soil aqueous extracts	Set of acute and chronic bioassays with bacteria, protozoa, algae, plants, crustaceans, and earthworms	No adverse effects	[36]
PLA	PLA, nanocomposites of PLA and organoclays Cloisite 20A and Cloisite 30B in compost (50 g/300 g), incubation for 76 days Plastic films (0.3 mm thickness, 1 cm ²) in the soil, incubation for 8 months	Compost aqueous extracts Soil aqueous extracts	<i>Allium cepa</i> (cytotoxicity, genotoxicity, mutagenicity) <i>Allium cepa</i> (phytotoxicity, cytotoxicity, genotoxicity, mutagenicity)	Cytotoxic and genotoxic effects of PLA degradation products (not for the organoclays) No adverse effects	[37] [38]
	Plastic films (3 cm × 3 cm) in agricultural soil, incubation for 84 days	Agricultural soil	Microbial activity (nitrogen circulation activity)	Negative effect on the activities of both ammonium and nitrite-oxidizing bacteria	[39]
	Plastic granules (1.4–707 µm) in sand, incubation for 31 days	Sand sediments	Lugworms (biological activity, nutrient cycling, and primary productivity)	Affect the health and behavior of lugworms and directly or indirectly reduce primary productivity of these habitats	[40]
PBAT (Ecoflex®)	Plastic films (0.3 mm thickness, 1 cm ²) in the soil, incubation for 8 months Polymer granules (100–250 µm) in aqueous medium (350 mg/80 mL) with the actinomycete	Soil aqueous extracts Aqueous medium	<i>Allium cepa</i> (phytotoxicity, cytotoxicity, genotoxicity, mutagenicity) Luminescent bacteria (light emission), crustacean <i>Daphnia magna</i> (mobility)	No adverse effects No adverse effects	[38] [41]

(continued)

Table 3 (continued)

Polymer	Test material preparation	Test medium	Test system	Effect	Reference
	<i>Thermomonospora fusca</i> , incubation for 21 days				
	Plastic granules (2 mm × 20 mm) in sandy soil (1,000 mg/kg), incubation for 4, 10, 16, and 22 months	Sandy soil	Plant growth tests	No adverse effects	[42]
	Plastic films (3 cm × 3 cm) in agricultural soil (1.8 g/300 g), incubation for 7 months	Andosol soil	Soil microbiota and plant growth	Affected the growth of specific fungal species; little influence on the growth of <i>Brassica rapa</i> var. <i>Chinensis</i>	
PBS	Plastic films (3 cm × 3 cm) in agricultural soil, incubation for 84 days	Agricultural soil	Microbial activity (nitrogen circulation activity)	No adverse effects	[39]
PBS-starch	Plastic films (3 cm × 3 cm) in agricultural soil, incubation for 84 days	Agricultural soil	Microbial activity (nitrogen circulation activity)	No adverse effects	[15]
Modified starch-cellulose fiber composites	Microbial activity (nitrogen circulation activity)	Aqueous medium	Luminescent bacteria (light emission)	No adverse effects	[43]
PLA/PHA, Organix, BioAgri, Naturecycle	Plastic films (1.5 cm × 1.5 cm) in agricultural soil, incubation for 2 weeks	Aqueous medium; Skagit silt loam	Earthworms (<i>Lumbricus terrestris</i>) interaction with plastic mulches	Dragged plastic mulch into their burrows; plastic mulch was partially ingested, but only after weathering occurred	[44]

Fig. 5 The chemical structure of HAPE-Cell [47]



HAPE-Cell to Cu^{2+} , Hg^{2+} , Zn^{2+} , and Cd^{2+} was much higher when compared with cellulose. Gao et al. measured the adsorption capability of PVC, PP, PA, PE, and POM to Cu^{2+} and Cd^{2+} . The adsorption capacities of those non-degradable plastics to Cu^{2+} and Cd^{2+} are below 1 mg/g, which were much lower than that of HAPE-Cell (Fig. 5) [47]. Compared with commercial synthetic polymers, HAPE-Cell is degradable and will not cause secondary pollution. Thus HAPE-Cell is proposed as a promising sorbent for removal of the heavy metals.

PLA has been recognized as an eco-friendly alternative polymer for packing, clothing, and biomedical [48]. The degradation of the PLA is divided into two steps. Firstly, under suitable temperature and humidity, the PLA would become oligomers due to the hydrolysis of ester group. Then the smaller fragments could be degraded by microorganisms [49]. The elements of Bi, Pb, Zn, and Cd are often used as catalysts in PLA synthesis [50, 51]. When the polymers are degraded, these heavy metals may be transferred to soils, which could cause some ecological risks.

4.2 Interaction with Organic Contaminants

Many researches showed that PLA fibers had higher sorption capacity to dyes since they had more D-lactide units. Yang et al. used different disperse dyes to dye PLA and PET. PLA had higher color strength compared to PET since PLA had a lower refractive index under similar dyeing conditions [52]. Many studies have investigated the influence factor about the sorption of dyes on PLA. Karst et al. studied the effects of the structure of dyes on their sorption onto PLA [53]. The interaction energies between dyes and PLA showed a negative correlation with the percentage sorption of dyes on PLA. The functional groups $-\text{N}(\text{C}_2\text{H}_4\text{OCOCH}_3)_2$ and $(\text{CO})_2\text{NC}_3\text{H}_6\text{OCH}_3$ could form stronger interaction with PLA, while the functional groups $-\text{Br}$ and $-\text{Cl}$ could form weaker interaction with PLA. An investigation on the effects of dyeing on melting behavior of PLA using differential scanning calorimeter (DSC) was conducted. It was concluded that dyeing progress could decrease the restricting force and the crystallites became more perfect in the dyeing progress [54]. It was also reported that the rate of dyeing PLA was positively correlated with temperature, and the percentage exhaustion of dye reached 90% at 100°C [55].

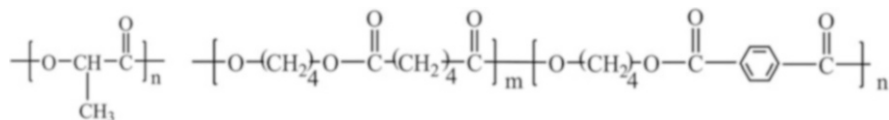


Fig. 6 The chemical structure of PLA (left) and PBAT (right)

PBAT was another typical biodegradable plastic (Fig. 6). When the sorption behavior of phenanthrene on PBAT, PE, and PS was investigated [56], the K_d values on PBAT, PE, and PS were measured to be 54,800, 15,600 and 1,340 L/kg, respectively. Much higher K_d of phenanthrene on PBAT mainly attributes to the rubbery subfraction of PBAT [56]. More researches need to be conducted since biodegradable plastics had various functional groups and their interaction with pollutants may be complicated.

4.3 Additives and Functional Monomers

Phthalate esters (PAEs) are plasticizers and additives that are also widely used in degradable plastic production [57, 58]. Polyolefins such as polyethylene and polypropylene are usually not accessible to direct microbial attack. Starch, as a natural polymer, can be degraded by microorganisms. When starch was mixed with polyolefins, the blends were easier to be degraded, if microorganisms can contact starch.

On the research of Siotto et al., aerobic biodegradation efficiency of ten biodegradable plastic monomer, adipic acid, azelaic acid, 1,4-butanediol, 1,2-ethanediol, 1,6-hexanediol, lactic acid, glucose, sebacic acid, succinic acid, and terephthalic acid, in soil was tested according to standard respirometric test, by measuring the carbon dioxide evolution [59]. During the 27–45 days of experiment, it was found that 1,4-butanediol, lactic acid, succinic acid, and glucose were completely biodegraded and the degradation efficiency of terephthalic acid was only 60%. The results showed that the degradation efficiency of the plastic monomer had a positive correlation with the percent of carbon converted to biomass.

5 Conclusions

5.1 Ecological Risks of Biodegradable Plastics

Plastics with very high molecular weights are not directly available to the living cells and therefore difficult to be harmless generally. However, low molecular weight additives can be toxic. Intermediates formed during incomplete biodegradation can accumulate in the surrounding soil, temporarily or permanently. These degradation intermediates can be monomers, oligomers, or metabolic derivatives and can interact

with the living organisms. It is, therefore, important to assess the possible ecotoxic effects of the biodegradable plastics introduced into the soil.

To date, biodegradable plastics are a promising alternative to conventional plastic. Although there are a few studies on the effects of biodegradable plastic on the soil ecosystem, considerable gaps in our understanding of biodegradable plastics and their ecological risks on soil ecosystems are still present. First, while several studies have focused on short-term effects or acute toxicity of biodegradable plastics, and their long-term effects are unexplored. Second, the relationship between plastic composition and soil organism responses needs to be identified, because the parent polymer composition and breakdown products may lead to different risk. Third, biodegradable plastic effects on soil nutrient biogeochemistry are largely unexplored [16].

5.2 Development Prospect of Biodegradable Plastics

Currently, the biodegradable polymers have offered a possible solution to the disposal of plastic waste produced from various sources associated with traditional petroleum-derived plastics. Most biodegradable plastics are used in the packaging industry, agriculture, and specialized biomedical applications. Among these biodegradable plastics, PLA is the most promising candidate to replace current plastics, because of its good mechanical strength and low toxicity [60]. Nevertheless, biodegradable plastic represents just a tiny market as compared with the conventional petrochemical plastics, and their production has not reached the level of conventional plastics [61]. Although degradable plastics meet the environmental requirements, they have some limitations in heat resistance, barrier, and mechanical properties.

Next-generation biodegradable plastics should be biodegraded and recycled in a balanced way to make their reuse possible [61]. Consequently, we must understand the degradation mechanism and degradation products of biodegradable plastics under real environmental conditions. The effect of additives also needs to be considered in a life cycle assessment of biodegradable alternatives. Researchers related to different disciplines (chemistry, engineering, materials science, biogeochemistry, and climate science) should design more environment-friendly biodegradable plastics and develop more application individually or in collaboration, to make the society more sustainable [21, 61].

Similar to the plastics we currently use, the production of new materials must take into account their raw materials and service life, as well as the basic standards of production scalability and material performance. It will take time and the key multidisciplinary developments will be required. However, biodegradable plastics are the only known choice for the future development of plastics.

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Countermeasures on Plastic and Microplastic Garbage Management



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Abstract In recent years, microplastic (MP), a new environmental pollutant, has got widespread attention around the world, and the discussions for (micro)plastic management have been increasing. In this chapter, the laws and actions concerning (micro)plastics management at international, regional, and national levels were presented. The possibility of alternatives for (micro)plastics was analyzed, and it shows that the market promotion of alternatives for single-use plastics is faced with a

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lot of difficulties, such as the cost of production, the performances of the alternatives, and so on. The alternatives of plastic microbeads, such as core-shell powder and mineral powder, can serve as a practical option to reduce plastic microbeads. Many laws are relative to garbage management in China presently, but there are still no special legislations for plastic garbage management until now. To improve the management for (micro)plastic management in China, some policy proposals are given for Chinese context, including the restrictions to add or sale MPs in personal cosmetic and care products (PCCPs), reduction of other sources of MPs such as textile and tire dust, principle of extended producer responsibility, standardization of the analytical methods of microplastic, reduction of marine litters via international and regional cooperation, establishment of public involvement mechanism, and so on.

Keywords China, Countermeasures, Garbage management, Microplastic, Plastic

1 Introduction

China is one of the biggest producers and consumers of the plastics in the world. The yield of common plastic materials such as PVC and amino molding plastic has ranked top worldwide, while the potential for further growth of these types of plastics is still huge [1]. Some of plastic garbages are discarded into the environment directly due to insufficient management countermeasures. The abandoned plastic garbages subsequently start to be broken down into debris by natural force such as ultraviolet light, wind, and hydraulic. The plastic debris with the diameter less than 5 mm is called as microplastics (MPs) [2]. In addition, the MPs can also come from the direct discharge of primary plastic particles, including microbeads used in cosmetics, raw plastic powder, etc.

In recent years, the MPs have become a hot topic in both natural and social science areas due to their ubiquitous occurrence in the environment and frequently ingestion by natural animals. For example, microplastic particles were found in the digestive tract of several marine species, such as 100% for sea turtles, 36% for sea dogs, 59% for whales, 92 types of sea fishes, and 6 types of marine invertebrates, respectively [3]. In addition, a report released by UNEP revealed that by 2050 approximately 99% of birds would ingest microplastic if measures for microplastic pollution prevention were put on the shelf. Moreover, a study in 2018 reported the MPs in human stools for the first time and meanwhile showed that these MPs could derive from seafood [4]. This result intensified a common consensus that the MPs might be transferred to human bodies through the food chain.

The MPs widely exist in freshwater, ocean, sediments, and even air according to domestic microplastic monitoring surveys; the overall level of microplastic in China was not optimistic. It is indicated in *2018 Bulletin on the State of China's Marine*

Ecological Environment that the mean abundance of marine MPs in surface water of Bohai Sea, Yellow Sea, and South China Sea was $0.42 \text{ particles m}^{-3}$ [5]. Source reduction, as a policy priority in the world, is a fundamental measure to eradicate the pathway of the MPs into environment. To improve the (micro)plastic management in China, the latest countermeasures for (micro)plastic management were reviewed around the world, and then the problems existed in the present (micro)plastic management in China were analyzed. Furthermore, the status of alternative countermeasures for plastic materials were summarized. Finally, policy suggestions to enhance (micro)plastic management in China were put forward based on the above analysis.

2 Legal and Management System to Control Microplastic Pollution

2.1 International Treaties and Actions

As the MPs pollution has been reported by the scientists, it becomes a hot international environment problem in the world. The UNEP listed plastic waste as one of the environmental issues worthy of constant concern from 2015. Marine waste was recognized as one of three themes contained in *2011 UNEP Yearbook*, which identified MPs as an emerging pollutant [6]. *Resolution on Marine Debris and MPs* was published in the first *United Nations Environment Assembly (UNEA-1)* in 2014, which also published *2014 UNEP Yearbook* and *Evaluation of Plastic Value*. The two reports totally referred to three major aspects related to MPs pollution: (1) plenty of plastic garbage in the ocean is increasingly threatening marine biota; (2) the economic loss caused by plastic waste per year was worth up to 13 billion based on conservative estimation; and (3) marine plastic pollution would be one of the top ten urgent environmental issues that deserved attention in recent 10 years [7]. The report, released by *Group of Experts on the Scientific Aspects of Marine Pollution (GESAMP)* in April 2015, equaled “the degree of damage to marine organism caused by MPs” to “the degree of damage to marine organism caused by plastic garbage.” Furthermore, the special resolutions related to MPs management were formulated continuously in UNEA-2, UNEA-3, and UNEA-4, respectively. The contents of different resolutions illustrated the deeper awareness of actions toward MP prevention. Firstly, UNEA-1 encouraged all member countries to work together to confront microplastic pollution, improve domestic legislation, and implement existing international treaties [8]. In addition, the resolution was also emphasized on addressing MPs at sources [8]. Secondly, the resolution released in UNEA-2 urged all member countries to develop necessary policies that conformed to waste categories and to encourage manufacturers of products containing MPs to seek substitutes without toxicity and harm [9]. Thirdly, the microplastic resolution of UNEA-3 set ambitious goals that prevent and reduce all types of marine pollutants,

especially pollutants from terrestrial activities [10]. UNEA-3 also decided to convene ad hoc open-ended working group meetings and meanwhile provided the secretariat for the meeting, thereby censoring barriers and alternative options confronted possibly in the course of beating MPs [10]. Finally, in view of the latest action against MPs taken by international society, UNEA-4 invited member countries to cooperate closely with private sectors in a bid to prevent primary, especially raw plastic particles (e.g., film and powder), from releasing into environment throughout production and supply chain [11].

In addition to the actions taken by *UNEP*, the other international organizations, such as *G7* and *G20*, also have released joint statements focusing on MPs. In June 2015, *G7* countries initiated *G7 Action Plan to Combat Marine Litter*. Four key areas, i.e., removal, education, research, and outreach, were counted as prior pointcuts to effectively reduce marine litters for both land-based sources and sea-based sources [12]. One action pertinent to microbead was illustrated below: “encouraging industry to develop sustainable packaging and remove ingredients from products to gain environmental benefits, such as by a voluntary phase-out of microbeads” [12]. Furthermore, five of the *G7* nations except the USA put their names on the *Ocean Plastics Charter* in 2018, which outlined a “resource-efficient lifecycle management approach to plastics in the economy” including promotion recyclable plastics by 2030, reduction of single-use plastics, and so on [13]. A joint statement, known as *Osaka Blue Ocean Vision* released in *G20* summit held in June 2019, encourages member countries to cut out marine plastic pollution by taking the comprehensive life cycle approach and innovative solutions and to reduce additional marine plastic pollution to zero by 2050 [14]. In general, these seminars illustrated the escalation of concern to microplastic pollution at international level, but resolutions derived from the seminars were lack of mandatory clauses, thereby only served as guidance for microplastic management actions.

A variety of legal conventions or agreements already existed and were kept revising in the past years. The Annex I of 1972 *London Convention (LC)* listed plastic garbage as material category that was deliberately prohibited dumping into the ocean [15]. The *International Convention for the Prevention of Pollution from Ships 73/78, MARPOL 73/78* for short and developed by *International Maritime Organization (IMO)*, aims at limiting illegal discharge of garbage from vessels and ships; plastic garbage was also listed into the no dumping list of Annex V [16]. *MARPOL 73/78*, as an important international law, has mitigated marine plastic garbage from sea-based activities, which is one of the main sources of marine MPs. As for land-based garbage, the *United Nations Convention on the Law of the Sea (UNCLOS, 1982)* prohibited member states from dumping pollutants from land-based environment into the ocean, although the plastic waste was not explicitly listed in the articles [17]. *The Basel Convention*, signed by 186 contracting parties to date, provided a legal framework for limiting illegal transboundary transfer of hazardous waste amid contracting nations and stipulated that exporters need to notify importers before planning to export waste [18]. The original *Basel Convention* did not include plastic waste into substance lists for restricting illegal transfer. Fortunately, *the 17th Conference of Parties on the Basel Convention*, held in May 2019, added the plastic

garbage to the list in Annex II of the convention, which means that exporters need to get consent of importers before transferring plastic garbage [19]. The updated *Basel Convention* will establish a mechanism for restricting illegal transboundary transfer of plastic garbage across the world, which provides a powerful legal basis for *Ban on import of foreign garbage* implemented by China in 2018.

The above international treaties with legally binding and agreements without legally binding all referred to the countermeasures against MPs from both land-based and sea-based MPs. These treaties and agreements have provided policy references for member countries that commit to taking measures to alleviate MPs pollution. The microplastic monitoring data worldwide showed widespread existence of microplastic in inland water, soil, ocean, and even air. However, international conventions, as well as workshops usually placed emphasis on marine MPs, terrestrial and freshwater MPs were seldomly selected as a topic under discussion in environmental meetings.

2.2 Regional Treaties and Actions

Until now, some regional organizations, with the European Union (EU) being the forefront, have taken an important step to deal with MPs pollution. The details of different MPs policies that applied to EU are shown in Table 1. *OSPAR*, signed by 15 governments from EU and non-EU member countries, set an *Ecological Quality Objective (EcoQO)* to judge the level of MPs pollution through analysis of the MPs intake in organisms. The aim of *EcoQO* is to protect west coasts and watersheds of Europe. It also provides a new idea for countries that intended to formulate indicators evaluating the adverse effects of MPs on ecology. *OSPAR* Commission also cooperated with all stakeholders throughout industrial chain of plastics to phase out the usage of plastic microbeads in cosmetics [20]. The *REACH regulation (EC 1907/2006)* aims to identification of adverse impacts of chemical materials on human early, thereby assessment of the feasibility of market access of chemical materials to EU [21]. *REACH* stipulated the threshold value of polymer-monomers (primary microplastic) to reduce MPs on source. The related articles were shown in Table 1. In February 2019, *European Chemicals Agency (ECHA)* proposed *REACH* to phase out the products that contain MPs equal to or greater than 0.01% w/w, MPs as a substance on its own [22]. In addition, some products, such as medicinal products, would be exempted from restriction lists according to the proposals [22]. *Marine Strategy Framework Directive (MSFD)* established a regional framework for the cooperation of member countries to achieve *Good Environmental Status (GES)* by 2020 [23]. The indicator of revised *GES* in April 2017 was that micro-litter would not cause adverse harm to the maritime and marine environment; member countries shall formulate their own threshold value in line with *GES* [24]. Denmark enacted “the Danish Marine Strategy” under the framework of *MSFD* in 2012, the objectives thereof included the monitor of the litter in beach and seabed, the analysis of MPs in sediments, and the survey of the ingestion of

Table 1 Laws and actions related to microplastic prevention applicable to EU

Policies in EU	Specific contents
OSPAR Convention	Ecological Quality Objective (EcoQO): <10% of northern fulmars should have >0.1 g plastics particles in the stomach samples of 50–100 beach fulmars from each of the North Sea over a period of ≥ 5 years [31, 32]
REACH	The polymer importers and/or producer must register the monomer with the European Chemicals Agency (ECHA) if “the polymer is made up of more than 2% or more of the monomer substance(s), and the total quantity usage of the monomer shall exceed more than 1t per year” [21, 24]
Marine Strategy Framework Directive (MSFD)	Marine strategies shall be developed and implemented in order to: (a) Protect and preserve the marine environment, prevent its deterioration, or, where practicable, restore marine ecosystems in areas where they have been adversely affected (b) Prevent and reduce inputs in the marine environment, with a view to phasing out pollution as defined in Article 3(8), so as to ensure that there are no significant impacts on or risks to marine biodiversity, marine ecosystems, human health, or legitimate uses of the sea
EU Plastics Strategy	Actions to curb MPs pollution: <ul style="list-style-type: none"> • Start the process to restrict the intentional addition of MPs, to products via REACH • Examination of policy options for reducing unintentional release of MPs from tires, textiles, and paint (e.g., including minimum requirements for tire design (tire abrasion and durability if appropriate) and/or information requirement (including labeling if appropriate), methods to assess microplastic losses from textiles and tires, combined with information (including possibly labeling)/minimum requirements, targeted research, and development funding) • Development of measures to reduce plastic pellet spillage (e.g., certification scheme along the plastic supply chain and/or Best Available Techniques reference document under the Industrial Emissions Directive) • Evaluation of the Urban Waste Water Treatment Directive: assessing effectiveness as regards MPs capture and removal
Industrial Emissions Directive	Member states issued permits for industrial installation; operators must set threshold value for the listed substance in Annex I of the Directive below: “4.1. Production of organic chemicals, such as: (h) plastic materials (polymers, synthetic fibres and cellulose-based fibres)”

animals to marine garbage [25, 26]. To the best of our knowledge, the primary MPs that end up in aquatic environment could partly be attributed to emission of sewage from plastic industries. *Industrial Emissions Directive (2010/75/EU)* is the main



Fig. 1 The EU legislative agenda related to microplastics prevention

instrument restricting pollutant emission from industrial installations, among which plastic materials were listed as restricted emission lists in *Annex I of the Directive* (Table 1). In addition, *Directive 2010/75/EU* also requires member countries to establish their own thresholds for industrial emission of plastic materials [27]. *The EU Plastic Strategy*, enacted by European Commission on 16 January 2018, contains a wide range of measures concerning plastic pollution, such as single-use plastic, marine litter, and microplastic pollution [28]. Tire abrasion has been identified as a main origin of MPs according to recent researches [29, 30]. The detailed contents in *EU Plastic Strategy* were shown in Table 1.

In addition, although EU has not officially enacted the bill to prohibit the MPs in cosmetics, some member countries such as the UK and France have enacted legislation prohibiting MPs in cosmetics. The EU legislative agenda related to microplastics is summarized in Fig. 1.

In addition to ongoing agreements or actions in EU outlined above, some other regional organizations, such as *HELCOM*, *Nordic Council of Ministers*, and *SPREP*, also have taken actions or formulate agenda to phase out the use of plastic microbeads. *HELCOM*, governed by *Helsinki Commission-Baltic Marine Environment Protection*, drew up a *Regional Action Plan for Marine Litter* that contains articles toward MPs to urge contracting parties to reduce the use of microbeads in cosmetics by applying substitutes [33]. *The Nordic Council of Ministers*, as a forum for official cooperation amid member states, launched a *Nordic program* to eradicate the environmental effects of plastic materials by seeking for harmless substitutes [34], with microbeads being quoted as an example. *The Secretariat of the Pacific Regional Environment Programme (SPREP)* endorsed a *Call for Action on Plastic*

Microbeads, which was proposed by French Government at 28th SPREP meeting of official in April 2017, with an aim to call on members to beat the abuse of microbeads [35].

According to strategies of different regional organizations, the phaseout of microbeads in personal care products was the most popular actions for microplastic reduction. The other products such as textiles and paints were paid attention only in *EU Plastic Strategy*, while it had no concrete guidance for action. The revision of laws and escalation of action in the future should keep synchronized with the latest scientific achievements in the MPs research.

2.3 National and Local Management System

Recently, many countries have seriously considered the international or regional proposals over MPs mitigation. The management mechanism imposed by different countries mainly includes legally binding or non-binding policies of plastic microbeads. Statistically, eight countries enacted microbead bans or regulations in the world in 2018 [33]. Furthermore, some cities or regions such as Illinois (America), Ontario (Canada), Taiwan (China), enacted microbeads ban earlier than the national level [33]. For instance, phased bans on plastic microbeads, agreed by state legislatures of Colorado, Maine, and New Jersey in October 2015, entered into force from 2017 and 2019, respectively [36]. In order to formulate laws on the federal level, US Congress enacted the law entitled “Microbead-Free Waters Act of 2015.” This act established deadlines for two types of rinse-off cosmetics, i.e., cosmetics such as toothpaste and cosmetics of no prescription drugs. For the former, the deadlines of ban on manufacturing and sale were 1 July 2017, and 1 July 2018, respectively. For the latter, the deadlines of ban on manufacturing and sale were 1 July 2018 and 1 July 2019, respectively. So enough time was given for the producers to change the production technologies and for importers/retailers to sell their inventory products [37].

The Canadian government was the first country to list microbeads as toxic substances under the *Canadian Environmental Protection Act (CEPA) 1999* on 1 August 2015 [38]. *Microbeads in Toiletries Regulations* entered into force in Canada on 1 January 2018. This bill in Canada also provided different deadlines for the production/import and sale of toiletries containing microbeads used for cleaning or hygiene. Detailed articles related to the deadline for different products are provided below.

- “A person must not manufacture or import any toiletries that contain microbeads, unless the toiletries are also natural health products or non-prescription drugs, in which case the prohibition applies on or after July 1, 2018.”
- “A person must not sell any toiletries that contain microbeads on or after July 1, 2018, unless the toiletries are also natural health products or non-prescription drugs, in which case the prohibition applies on or after July 1, 2019.”

It is worth noting that prescription drugs are exempted from the bill [12]. Although the bill covered a large range of toiletries such as personal hair, teeth, skin, and so on, other products that contain microbeads (e.g., cleaning products, printer toners, and abrasive media) are not included in the control list. This is a common shortcoming of the laws over microbeads enacted currently around the world; thus the law concerning all types of products containing microbeads which could be easily replaced with harmless materials, should be formulated by countries which are intended to take actions against MPs.

The USA and Canada are similar in the legislation on the ban of MPs. Both countries prohibit the manufacturing, import, and sale of products which contain microbeads. Other six of eight countries, who have officially ban the use of plastic microbeads, only focus on the sale or manufacturing of microbead-containing products, while the import was not mentioned [33]. As the types of products banned, New Zealand is the most comprehensive among the eight countries. For example, abrasive cleaning products, including household, car, or industrial cleaning products that contain plastic microbeads, were all banned under *Section 23 of the Waste Minimisation Act 2008* [39]. Instead, other national regulations only focus on personal care product containing plastic microbeads for the purpose of exfoliating or cleaning.

It should be noted that biodegradable materials were exempted from the regulations on microbeads in France and Sweden. According to the Ordinance in Sweden, biodegradable materials were defined as “Plastic microbeads that only consist of naturally occurring polymers” [33, 40]. However, the regional law, enacted by Taiwan, China, clarified the biodegradable materials as the category of plastic material and added biodegradable materials to the list of banned materials [41]. In addition, biodegradable materials were also not exempted from other national and subnational laws over microbeads such as Italy, Scotland, and so on [33]. The difference on restriction on biodegradable materials indicated the nonuniform standards in the field of MPs policymaking across the world.

In addition to the legally binding regulations against plastic microbeads, several countries have selected voluntary approaches as alternative option to combat the microplastic pollution. Australia hosted an official *Meeting of Environment Ministers (MEM)* from federal, state, and territory level across Australia to endorse a voluntary industry phaseout of microbeads by 1 July 2018 [42]. Australia government also stressed that the mandatory ban would be legislated if the stakeholders had not effectively removed the products from the market in time [42]. According to the investigation commissioned by *Department of the Environment and Energy*, the official institution in Australia, 94% of 4,400 supermarket, pharmacy, and cosmetic stores under surveys have removed the products containing plastic microbeads from the market [43]. The voluntary accord provides companies the autonomous rights to arrange their own schedule for phasing out microbeads and have achieved good effects in MPs reduction. It is therefore hopeful that the voluntary action would shift to fabric microfibers and tire wear particles, which are also the primary contributors for the MPs in the environment.

3 Legal and Management System to Control Plastic Pollution

3.1 *International Treaties and Actions*

Reportedly, approximately over 250,000 tons of plastic garbage are floating on the surface water of the oceans in the world [44]. Plastic garbage, known as secondary source of microplastic, can be broken into MPs by natural force such as wind, ultraviolet light, tides, and so on. Currently, the conventions, agreements, and resolutions against plastic pollution at international level mainly concentrate upon the management of single-use plastics. The fourth session of the *UN Environment Assembly (UNEA-4)* on 15 March 2019 adopted a resolution called “Addressing single-use plastic products pollution,” which encourages key actors as regards single-use plastic products, such as plastic producers, retailers, consumer goods industry, importers, packaging firms, transporters, and recyclers, to adopt innovative approaches to address the environmental impact of single-use plastics, such as extended producer responsibility schemes and deposit refund schemes [45]. The resolution indicated that the concern on plastic pollution has shifted to single-use plastics from marine plastic.

The theme of 2018 World Environment Day was “Beat Plastic Pollution,” and UNEP published a book entitled *SINGLE-USE PLASTICS: A Roadmap for Sustainability*, which provided a ten-step roadmap for governments that wish to take measures against the overuse of plastic items [46]. The ten-step roadmap referred to all stakeholders throughout the life cycle of plastic products and provided different detailed descriptions the stakeholders need to implement. For instance, in order to ensure the effectiveness of policy, the roadmap suggests that policy maker should conduct comprehensive impact evaluation of preferred instrument on stakeholders getting profits from plastic product manufacturing and sale. Overall, the ten-step roadmap provides significant reference for effective implementation and detailed formulation of policy over plastic pollution in different nations.

3.2 *Regional Treaties and Actions*

Early in 1994, EU adopted “Directive 94/62/EC” as regards reducing the impacts of packaging on the environment. This Directive also put forward a concept of “waste hierarchy, prevention comes first.” Subsequently, the Directive was revised several times in a bid to cover more types of plastic products; the corresponding amending articles in different revised bills were shown in Table 2. With regard to these bills, one of the major purposes to the amending version “Directive (EU) 2015/720” is to formulate specific measures to reduce consumption of plastic carrier bags, which were defined as packaging under the definition of “Directive 94/62/EC” but lack of detailed articles [47]. The contents revised in different versions show that the EU has

Table 2 The evolution lists of the EU packaging waste directive

Date	Bill	Partial amending contents
28 February 2004	<i>Directive 2004/12/EC</i>	<ul style="list-style-type: none"> • Providing the illustrative examples for the definition of “packaging” • Member states shall take measures to attain the recovery and recycling targets set by <i>Directive 2004/12/EC</i> For example, <i>no later than 31 December 2008 between 55% as a minimum and 80% as a maximum by weight of packing waste will be recycled</i> [50]
5 April 2005	<i>Directive 2005/20/EC</i>	New member states may postpone the deadline of attainment of the recycling and recovery targets. For example, the deadline shall not be later than 31 December 2012 for the Czech Republic, Estonia, Cyprus, Lithuania, Hungary, Slovenia, and Slovakia; 31 December 2014 for Poland; 31 December 2015 for Latvia [51]
28 February 2013	<i>Directive 2013/2/EU</i>	The items for the illustrative examples of packaging listed in Annex I were amended to clarify additional cases where the borderline between what is packaging and what is not [52] <i>For example, CD spindles (sold with CDs, not intended to be used as storage) belong to the categories of packaging, but CD spindles (sold empty, intended to be used as storage) belong to the categories of non-packaging</i>
29 April 2015	<i>Directive 2015/720</i>	<ul style="list-style-type: none"> • “Lightweight plastic carrier bags” and “very lightweight plastic carrier bags” shall mean plastic carrier bags with a wall thickness below 50 µm and below 15 µm, respectively • “Oxo-degradable plastic carrier bags” shall mean plastic carrier bags made of plastic materials that include additives which catalyze the fragmentation of the plastic material into micro-fragments • Member states shall take measures, such economic instruments, to achieve reduction goals, one of which indicates that the annual consumption level does not exceed 90 lightweight plastic carrier bags per person by 31 December 2019 and 40 lightweight plastic carrier bags per person by 31 December 2025 • By 27 May 2017, the commission shall adopt an implementing act for the specifications of labels or marks to unify union-wide recognition of biodegradable and compostable plastic carrier bags [47]
30 May 2018	<i>Directive (EU) 2018/852</i>	<ul style="list-style-type: none"> • “Member states shall take measures to encourage the increase in the share of reusable packaging placed on the market. . .Such measures may include, inter alia: <ol style="list-style-type: none"> (a) the use of deposit-return schemes; (b) the setting of qualitative or quantitative targets; (c) the use of economic incentives; (d) the setting up of a minimum percentage of reusable packaging placed on the market every year for each packaging stream” • The targets of packaging waste were amended For example, <i>no later than 31 December 2025 a minimum of 65% by weight of all packaging waste will be recycled</i> [53]

been trying its best to ensure sustained effects of the Directive. The latest bill, entitled as *Directive (EU) 2018/852*, has come into effect since 4 July 2018. It will be outreached into national legislation before 5 July 2020 [48]. Three highlights could be found in this bill. Firstly, Directive (EU) 2018/852 as existing law covers all types of packaging used for different fields, such as industrial, commercial, office, shop, and so on. Secondly, the Directive indicates that the oxo-degradable plastic packaging can't be classified as biodegradable items, which are hopeful to substitute plastic products. Thirdly, *Directive (EU) 2018/852* indicates that *producer responsibility schemes* for all packaging should be established in EU countries by 2025 [48].

The EU Plastic Strategy not only mentioned MPs pollution (see Sect. 2.2) but also formulated explicit strategies in other fields, such as marine litter, compostable and biodegradable plastics, and single-use plastics. Taking single-use plastics, for example, the actions include “analytical work that including the launch of a public consultation, to determine the scope of a legislative initiative on single-use plastics” [28]. In addition, on 27 March 2019, European Parliament approved a new law that intended to prohibit anyone from throwing away plastics by 2021 [49]. The highlights thereof were divided into four parts. Firstly, ten types of plastic products, which can be replaced with other environment-friendly alternatives, to be banned by 2021. Ten types of mentioned plastic products are shown below [49]:

- Single-use plastic cutlery (forks, knives, spoons, and chopsticks)
- Single-use plastic plates
- Plastic straws
- Cotton bud sticks made of plastic
- Plastic balloon sticks
- Oxo-degradable plastics and food containers and expanded polystyrene cups

Secondly, explicit recycling and reuse targets applicable to member countries were formulated in legislation, namely, the recycling rate of plastic bottles would reach up to 90% by 2029, and new produced plastic bottles must contain 25% recycled material by 2025 and 30% recycled material by 2030, respectively.

Thirdly, other plastic products exempted from ban must comply with new provisions for product design and labeling. For instance, the packaging of wet tissue must be labeled with the information involving plastic, informing consumers the impacts of throwing away wet tissue on environment.

Lastly, the law also underlines the *polluter pays principle* and stipulates that the recycle cost of some plastic items, including tobacco, fishing gears, and so on, shall be bearded by the consumers.

In addition to source reduction tactics against plastic garbage in EU, some countries also issued bilateral and multilateral joint statement or taken cooperative measures to cope with plastic garbage. China and Canada released a joint declaration on 14 November 2018; both countries agreed to take source-efficient measures to manage plastic products throughout life cycle [54]. These efforts include (1) reducing the use of unnecessary plastic products and considering comprehensively the adverse impacts of alternatives on the environment; (2) improving the

capacity of preventing plastic garbage from discharging into marine environment at source and enhancing the collection, reuse, recycling, and/or nonhazardous treatment of plastic garbage; (3) enhancing the information sharing, promoting public awareness, conducting education campaign, and reducing the use of single-use plastics and the generation of plastic garbage; and (4) guiding the development and reasonable utilization of novel plastic as well as alternatives while ensuring that these materials are harmless to health and environment. Furthermore, both sides also agreed to establish comprehensive strategic partnership of cooperation and to cope with marine plastic garbage via several ways covering many aspects, including exchanging of experience about marine plastic prevention between bilateral coastal cities, developing technical research for marine plastic garbage reduction, implementing demonstrative projects, and so on.

The bilateral treaty, known as the *Río de la Plata Bilateral Treaty* and signed by Argentina and Uruguay, aims to protect trans-boundary river *Rio de la Plata* [55]. The dumping of hydrocarbon compound was prohibited in the treaty. The common institution – *Technical Commission of the Maritime Front* (TCMF) – to regulate anthropogenic environmental damage and ensure sustainability of resources was established [55]. In general, the treaty provided an effective solution for transboundary environment problem.

3.3 National and Local Management System

By the end of June 2018, 127 countries have enacted policies on single-use plastics. The policies and actions can be categorized by four types, i.e., taxation/charge, voluntary, ban, and coordinated measures (ban and taxation/charge). Twenty-seven countries restrict the market circulation of single-use plastic products by legislation, especially on the products such as tableware, straw, packaging, and so on.

3.3.1 Taxation/Charge

Ireland is the first country to tax on plastic bags in the world [56]. The law enacted in 2002 stipulates that consumers must pay 0.15 Euros tax per plastic bag. Meanwhile, the law also gave some exempted cases, such as plastic bag used for shipping fresh food, pre-sealed packaging for fresh food, and the reusable plastic bag above 0.7 Euro. Hereafter, in order to keep the consumption of plastic bag at low level, the taxation on single plastic bag was increased to 0.22 Euro in 2007. In 2011, Ireland government enacted a legislation to explain the tax on plastic bag would be revised annually based on the fluctuation of plastic bag consumption with tax upper bound of 0.7 Euro for each plastic bag [46]. The consumption of plastic bag per person is shown in Table 3.

Some states and local governments in the USA took different incentive measures to combat the overuse of plastic bags on their own contexts. The city of Boulder,

Table 3 Number of plastic bags consumed per capita in Ireland after taxation [57]

Year	Number of plastic bags consumed per capita
Before 2002	328
2003	21
Before 2007	33
After 2007	26
2010	18
2012	14
2014	13
2015	12

Table 4 Contents of the external cost of a disposable plastic bag [58]

Composition	Cost (\$)
Contamination of recycling stream	0.014
Contamination of compostable	0.008
Collection and disposal of bags	0.072
Litter clean up	0.052
Processing in landfills	0.024

Colorado, estimated that the price of a plastic bag was \$0.198 according to the principle of *Pigovian tax* [58]. The price of a plastic bag estimated by San Francisco was \$0.17; San Francisco also refined the composition of price (Table 4). The tax of each plastic bag was set at \$0.05 in Montgomery and would be paid to government when the total amount of tax reaches up to \$100. In addition, the law of Montgomery also stipulated that the detail of the tax must be presented on the consumer's shopping receipt.

3.3.2 Voluntary

Voluntary agreements between governments and producers/retailers as another option for reducing plastic bags have been paid attention in several countries and proven to be an effective measure on plastic reduction. *Federal Ministry of Agriculture, Forestry, Environment and Water Management* (BMLFUW) of Austria signed a voluntary agreement with large-scale retailers and some environmental protection organizations in 2016, the majority of supermarkets promised to stop providing consumers free plastic bags, and the government was responsible for analysis of the documents provided by retailers and further supervision measures [59]. In 2017, New Zealand government reached a voluntary agreement with the largest chain supermarkets in New Zealand to completely phase out single-use plastic bags [60]. In general, voluntary agreement has become a novel mechanism for plastic garbage prevention, which provides enough space for companies to arrange their own schedule for phasing out plastic products.

3.3.3 Ban

African countries are almost developing economies, but some countries have enacted management systems against single-use plastics. On 1 January 2017, the ban on plastic bags enacted by Aruba came into effect and hereafter dramatically reduced plastic litter in coastal areas. Currently, the revised ban in Aruba, which covers more scope of plastic items, such as plastic cups, straws, and styrofoam boxes, will take effect in 2020 [61]. Kenya government introduced the strictest plastic bag restriction policy on 28 August 2017 by 4-year imprisonment or fines of \$40,000 for anyone or organization who produced, sell, or even used plastic bags [62]. The expensive illegal cost reduced the plastic bag usage soon, but in the long run, the adoption of affordable alternatives with the same or better performance of plastic products would be a more practical option. Furthermore, some developed economies improve the restriction on plastics continuously. France, for instance, enacted a new law in September 2016, which would ban plastic plates, cups, and utensils officially from 2020. The items made of compostable, bio-sourced materials will be exempted from ban [63]. In addition, the *Decree 2017-291* published by France on 6 March 2017 will prohibit plastic cotton buds from 1 January 2020.

3.3.4 Coordinated Measures (Ban and Taxation/Charge)

South Africa is a representative country that implements coordinated measures in the world. In 2003, South Africa government prohibited the market circulation of plastic bags with the thickness less than 30 μm and imposed tax on plastic bags with the thickness greater than 30 μm [64]. But this incentive measure didn't succeed due to insufficient public engagement. Another example is China, plastic bags with the thickness less than 0.025 mm were banned on manufacturing, and plastic bags with the thickness beyond this limit were permitted under the condition that they could not provided free [65]. Briefly, the purpose of coordinated measure is to reduce the use of the thin plastic bags and encourage reuse of thick ones.

4 China Management System Relative to the Plastics and Microplastics Mitigation

4.1 *The Current Status of Polices About Plastic and Microplastic*

In recent years, due to the rapid development of economy in China, the amount of garbage containing plastics has been increasing continually. China has been constantly improving the legislation and management on both sea-based and land-based garbages. As to laws about sea-based garbage, *Marine Environmental Protection*

Law of the People's Republic of China [66], a fundamental law of marine environmental protection, provides some principle provisions for the formulation of special marine laws, such as *Administrative Rules of Waste-dumping to the Ocean of the P.C.C. (Waste-dumping Rules)*, the *Regulations Concerning the Prevention of Pollution of Sea Areas by Vessels of the P.C.C. (Sea Areas Regulation)*, and so on. *Waste-dumping Rules* outlaw the knowingly dumping of solid waste into sea; Annex I in *Waste-dumping Rules* lists the substances that are banned on dumping into the sea, including fishing nets, ropes, plastic products, and synthetic substance that easily float or suspend in seawater [67]. Article 39 of *Sea Areas Regulation* stipulates that plastic products prohibited from throwing into sea [68]. Other laws, such as the *Regulations Concerning the Prevention and Cure of Pollution Damage of Marine Environment by Pollutants from Land*, *Regulations on the prevention and control of pollution damage to marine environment in marine engineering construction projects*, and so on, mainly focus on the solid waste but not explicitly referred to plastic garbage.

For land-based garbage, *Law of the People's Republic of China on Prevention and Control of Environmental Pollution by Solid Waste* prohibits anyone and organizations from illegally dumping solid waste into interior aquatic environment, thereby controlling the migration of waste in rivers and reducing waste that ended up in marine environment [69]. In addition, macroscopic laws, such as *Cleaner Production Promotion Law* and *Law of the People's Republic of China on Prevention and Control of Environmental Pollution by Solid Waste*, all refer to plastic pollution via some unenforceable articles, such as stressing the principle of *extended producer responsibility* to encourage producers to recycle plastic garbage. Several ministries and local governments also introduced normative documents or action plans to deal with deteriorating environmental problem. *General Office of the State Council* issued *notice on restricting the manufacturing, sale, and use of plastic bags (plastic bag ban)* in the end of 2017, which prohibited anyone from manufacturing, selling, and using plastic bags with the thickness less than 0.025 mm from 1 June 2008 nationwide and required the retailers in supermarkets, markets, and agriculture markets to charge on plastic bags instead of free supply [70]. The difference between *plastic bag ban* in China and taxation system in some western countries was the revenue from plastic bags is owned by the merchants and cannot be used to compensate the recycling cost of the plastic waste conducted by government. But the regulations did not operate effectively to reduce plastic bags over the past years; the consumption amount of plastic bags reached up to 14.7 billion just in the express industries in 2016. In addition, the *General Office of the State Council* officially banned the imports of 24 types of solid wastes in 2017, including plastics, textile, and so on, to prevent the environmental degradation caused by the disposition of foreign garbage in China [71].

The implementation of *Waste Classification System* in some cities, including Shanghai, Beijing, Xiamen, and Xian, is another action against plastic pollution. The plastic garbage commonly is classified into the categories of recyclable materials and non-recyclable materials in different regulations. The *Waste Classification*

System is expected to promote the resource recycle of the plastic materials and reduce the plastic pollution in the environment.

4.2 Mega Trend? Microplastic and Plastic Alternatives

The measures to deal with plastic pollution should be different from ban or tax and should not cause drastic change in the market. In recent years, the biodegradable plastics that are used to replace single-use plastic products have accounted over 50% of the total consumption of the biodegradable plastics [72]. Biodegradable plastics mostly consist of carbon-neutral materials, and extra carbon emission would not be generated throughout total life cycle [73]. Biodegradable plastics could be partially or completely decomposed into carbon, hydrogen, and oxygen by microorganisms, so this material could theoretically be an ideal material to replace the plastics based on petroleum. Unfortunately, biodegradable plastic products on the market can only be completely decomposed under strict conditions (e.g., enough humidity and temperature, etc.), so they are really partially biodegradable under natural condition and a long time may also be needed. Moreover, the decomposition process might increase the microplastic debris in environment. A report entitled *Biodegradable Plastics and Marine Waste*, released by EU in 2015, indicated that a kind of biodegradable plastic bag could be completely degraded into water, carbon dioxide, and other products only under industrial conditions of 50°C, but it is difficult to achieve the same effect in the natural environment [74]. Currently, biodegradable plastics accounted for relatively low proportion, for instance, 1% in 2015, of total production of plastics in the world due to high production cost and low performance. Therefore biodegradable plastic remains a conservative option to replace plastics.

Another alternative material of plastics is paper. For example, Glendale, California, enforced a ban on plastic bags while charged \$0.1 per paper bag. San Mateo, California, also implemented the similar law like Glendale, California [75]. The measures taken by both cities effectively reduced the overuse of plastic bags, but the increase of paper bags needed more natural resources and produced more environmental pollutants. It is estimated that the pollution introduced in the manufacturing of a single paper bag is 50 times than that of a similar plastic bag and the more fossil fuel is also needed [76]. In terms of resource consumption, the paper substitute only transfers external resource loss caused by plastics to other environmental territory and is still not an environment-friendly option.

However, alternative to microplastics in cosmetic products seems more successful from the experience of L'Oreal, Unilever, and so on. The function of plastic microbeads in toiletries is to exfoliate dead skin. A lot of other natural substances, such as core-shell powder, mineral powder, walnut shell, and so on, can also achieve similar effects as plastic microbeads without more production cost. Thus, natural alternatives to plastic microbeads will not hurt the benefits of toiletry industries and can serve as a feasible option to cope with microplastic pollution.

Comprehensively, higher production cost is a critical barrier limiting the popularization of biodegradable plastics. In addition, it is very difficult to isolate biodegradable plastic effectively from mixed municipal garbage so they are likely to be disposed in the same way. So the biodegradable plastic alternatives need to be further improved to enlarge its application.

4.3 Policy Proposals Against Plastic and Microplastic Pollution in China

There are certain similarities and differences in legislation about plastic and microplastic garbage management domestically and abroad. For sea-based and land-based garbage, several countries and international organizations have enacted related laws, such as marine dumping, ship-generated garbage, coast garbage, and so on. However, legislation special for plastic garbage is still missing. In this section, some proposals to reduce plastic and microplastic garbage are put forward with the international and national experience in consideration.

4.3.1 Ban on the Plastic Microbeads in PCCPs

A lot of countries enacted ban on plastic microbeads in the past years. It is imperative for China to investigate the microplastic beads in the personal care products in the market and analyze the environmental effects. With the successful international experience, the plastic microbeads should be phased out step by step on the basis of legislation.

4.3.2 Comprehensive Mitigation Strategies of the Plastic Pollution

The worldwide monitoring results show that a large portion of microplastic is microfibers and one of its primary sources is textile [77, 78]. Some simulation studies show the dominance of plastic debris is from automobile tires [30]. These results indicate that mitigation strategies against microplastic pollution should be formulated comprehensively. The EU has given a schedule to prevent tire plastic, and it is a very good example. Comprehensive measures, such as reducing the transportation plastic pollution and encouraging consumers to use fabric softener during laundry, should be formulated.

4.3.3 Extended Producer Responsibility

As the cost of plastic reclamation is still not efficiently covered, it is perhaps helpful to impose plastic environmental tax. Other countermeasures to strengthen the extended producer responsibility should also be encouraged, such as subsidies for the plastic reclamation and incentives for the producers to use environment-friendly design and recyclable materials. Totally, cost for reclamation and processing of the plastic garbage should be paid by the polluters.

4.3.4 Improvement of Public Participation System

Measures for Public Participation in Environmental Protection, formulated by the former Ministry of Environment Protection in 2015, is the basic law for public participation in environmental protection. The establishment of a quick public feedback mechanism and accurate publication of pollution information will promote public understanding of microplastic pollution. The communication between the government and the public under the *Public Participation Mechanism for Microplastic Pollution* presents a long-term solution to reduce microplastic pollution. In addition, the role of environmental public service organizations in (micro) plastic management should be underlined, especially the involvement of mechanisms for social organizations and nongovernmental organizations.

4.3.5 Enhancement of International and Regional Cooperation

There are significant diversities on microplastic monitoring results carried by the scientists in different countries presently due to the difference in the microplastic sampling and analysis methods. In addition, marine plastic pollution is an international and transboundary environmental problem, so the international and regional cooperation is very necessary. It is suggested that China should actively participate in the international and regional cooperation to assess the ecological hazards of marine MPs and find an effective way to mitigate the plastic and microplastic pollution.

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Correction to: Plastics of the Future? The Impact of Biodegradable Polymers on the Environment



Leicheng Zhao, Lili Rong, Longfei Zhao, Jintao Yang, Lei Wang, and Hongwen Sun

Correction to:
Chapter “Plastics of the Future? The Impact of Biodegradable Polymers on the Environment”
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This chapter was inadvertently published with three errors, which have now been corrected as follows:

- Caption of Table 3 has been updated to read as: “Ecological effects of completely biodegradable plastics. Reused with permission from [21]”
- Footnote has been added to the heading of Sect. 3.3 (“The Ecological Effect to . . .”) as follows: “Parts of this text are reused with permission from [21]”
- The references [21] (English version) and [34] (German version) refer to the same article. Therefore, now the reference [34] has been updated as reference [21] throughout the chapter, and the other succeeding references starting from reference [35] have been renumbered accordingly.

The updated online version of this chapter can be found at:
https://doi.org/10.1007/698_2020_462