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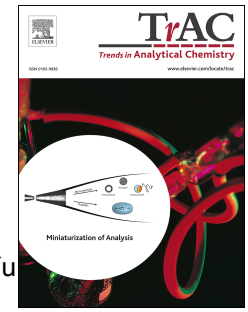

Microplastics in the environment: A review of analytical methods, distribution, and biological effects

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Microplastics in the environment: A review of analytical methods, distribution, and biological effects

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Abstract：Microplastics (MP) (<5 mm) are crucial pollutions which are widely distributes in the environment. Recently, the studies of MP have increased rapidly due to increasing awareness of the potential and growing risks of biological effects during storage and disposal. However, due to limitations in analytical methods and the methods of environmental risk assessment, the distribution and biological effects of MP are still debatable issues. To clarify the potentially environmental and biological impacts of MP in the consecutive environment, (1) analytical methods to assess MP, (2) environmental transportation and distribution of MP and (3) the effects of MP on biota, including the additives and sorption- desorption of MP in both terrestrial ecosystem and aquatic ecosystems were summarized. Based on the reviewed publications, we propose considerations for addressing the insufficiencies of analytical methods, distribution and biological effects of MP in ecosystems so we can adequately safeguard global ecosystems.

Key words: Microplastics; Plastics; Analytical method; Ocean; Sediment; Soil

1. Introduction

Plastics pollution is known to that change the global environment and causes negative effects on wildlife [1]. In recent decades, small size plastics, e.g. microplastics (MP) and nanoplastics (NP), in environment were focused by an increasing number of publications due to their potentially harmful biological effects [2,3]. MP has been defined as measuring 0.06–0.5 mm, or less than 1 mm[2]. Recent studies have termed MP as a small particle < 5 mm (one-dimensional) derived from plastics from different origins and compositions such as polyethylene (PE), polypropylene (PP), polystyrene (PS), nylon (PA), thermoplastic polyester (PET), poly(vinyl chloride) (PVC), cellulose acetate (CA), polysterol polystyrene, polyethylene terephthalate, and foamed polystyrene[3,4,5]. These particles are insoluble in water, nondegradable (according to standardized tests), and possess diverse physicochemical properties that determine bioavailability to organisms[6]. This concept of MP is widely adopted in both terrestrial and aquatic ecosystems [2,3,4,5]. In order to better understand the environmental behaviors of MP, MP can be further classified into small MP (< 1 mm) and large MP (1–5 mm) according to the European MSFD Technical Subgroup on Marine Litter [7]. The sources of MP are varied, with most coming from residential households, landfills, construction, factories,

farmland, ships, and marine platforms (Figure 1). In residential areas, washing machines can produce more than 1900 fibers (100 particles L^{-1}) in one wash cycle [2]. Personal care products (e.g., toothpaste, facial cleanser, facial scrub, bath foam, and cosmetics all contain small MP) which can collectively discharge huge amounts of MP into sewer systems after use [8,9]. Furthermore, waterborne paints, electronics, coatings, medical applications, and adhesives can also produce high amounts of MP after friction and decomposition [2,8,9]. Because of the large quantity in waste water involved, especially for wastewater treatment plant effluent[2,10], factories, and waste water can transfer high amounts of MP to soils, rivers, lakes, and oceans even with a low concentration (1 particle L^{-1}) in the effluent. In agriculture, aside from industrial sludge, plastics mulch is another important source of MP [1]. Plastics mulch has been widely adopted to increase crop yields by reducing evaporation and increasing temperature. These products can also produce high MP when the plastics cannot be recycled efficiently [1]. Greenhouse materials, soil conditioners, manure, irrigation, garbage, atmospheric deposition, and debris from the friction of plastics products are other sources of MP, which primarily accumulate in agricultural soil[11]. These compost applications can result in 0.08–6.3 kg ha^{-1} per year of visible plastics in arable soil, with annual MP input reaching $0.6–4.3\times10^5$ and $0.4–3.0\times10^5$ tons in European and North American agricultural soil, respectively, higher than that in the surface water of the ocean[12,13].

[Figure1 should be here]

Although agricultural soil could store more MP than the oceanic basins[12], most MP flows into lakes and then oceans[11]. Previous publication indicated that ten percent of plastics waste flows into the ocean[2], resulting in 60–80% of marine litter being plastics[14]. In the ocean, plastics pieces range around 0.485–5.4 trillion in number, weighing $3.54\times10^4–2.36\times10^5$ tons[13,15], which could be potentially the largest sink of plastics pollution in the future[11]. In addition, much plastics waste per year has been discharged into the ocean by ships with some MP coming from people living on ships during navigation with additional MP from plastics degradation of the ships[11]. Because MP can be transported by wind, runoff, and ocean currents[16,17], MP can be broadly distributed in the global environment. From the above sources, it can be concluded that MP sources are diverse and occur through multiple activities, across large areas, from a number of different anthropogenic activities. The level of MP contamination is high and increasing, potentially threatening biota in terrestrial and aquatic ecosystems.

The distribution and negative effects of large plastics have been the focus of research to date. Results show that birds, reptiles, and fish were directly harmed by plastics and could become

entangled in ropes and drown[18]. They could also ingest plastics fragments that may clog their stomachs and digestive tracts [18]. Recently, the vast storage, and wide distribution of fine-sized MP has been recognized, and the biological effects of MP has emerged as areas of interest. To better understand the distribution and effects of MP in the environment, many analytical methods have been developed and investigated using different soils, sediments, water and biotas. Based on available literature (N = 99), this review examines the following: (1) analytical methods for detecting and quantifying MP in the environment (N =21); (2) the transportation and distribution of MP in water, sediment, and soil (N =25); and (3) the effect of MP, MP additives, and MP absorption on biota (N =67). We also suggest several ideas for further research.

2. Analytical methods of MP in the environment

MP has various types and density (0.9 - 2.3 g cm^3) in the environment, and can be classified by density (light/heavy) or flexibility (hard/soft) according to its physical properties[4,6,19]. Since to the sources of plastics vary, the physiochemical properties also vary: (1) MP color vary, and include red, white, clear, blue, green, black, purple, yellowish, and brown as detected microscopically[11]; (2) MP shapes are complex, including long or short (used to describe fibers), triangular (two-dimensional [2D]), rectangular (2D), circular (2D), polygonal (2D), column-like (three-dimensional [3D]), spherical (3D), pellet-shaped (3D), cuboid (3D), cone-shaped (3D), pyramidal (3D), and other irregular shapes[20]; (3) chemical constituents of MP are complicated, and include PE, PP, PS, PA, PET, PVC, CA, polysterol, polyethylene terephthalate, and foamed polystyrene. Because MP has similar physiochemical properties (density, color, and chemical element composition) to other environmental materials such as SOM (various colors with the density of 1.4-1.8 g cm^3) [21], it is difficult to distinguish and estimate quantitatively, especially for soil samples with a high internal OM content [4,19]. Many analytical methods have been developed to measure MP in water systems, sediments, and soils. Procedures typically include separation, identification, and quantification.

2. 1 Extraction and separation methods

The extraction process is important and differs for sediment and seawater. For water, large MP can be (1) directly separated using a net in the water or (2) directly filtered by filters or sieves after a known volume water is collected, followed by visual sorting [4]. MP typically has relative low density (0.9-2.3 g cm^3)[4], so for sediment and soil having a high density (2.6-2.7 g cm^3)[21], floatation is a popular method, wherein MP is often extracted by a higher-density salt solution. The higher the solution density, the greater the density range of MP can be collected. Saturated NaCl (1.2 g cm^{-3}), $ZnCl_2$ (1.5-1.7 g cm^{-3}), NaI (1.6-1.8 g cm^{-3}), and $CaCl_2$ (1.5 g cm^{-3}) have been used as

97 solutions to float MP from sediment and soil, especially NaCl and $CaCl_2$ given their low cost and
98 lack of pollution potential[22]. In order to separate flotation (plastics and organic matter) from the
99 minerals in the mixture solution after centrifugation, a rubber disc is inserted into the middle of a
100 centrifugal tube. This can prevent mineral particles from being resuspended, which can substantially
101 reduce separation time [17]. However, a high amount of OM can be floated and is difficult to
102 separate from MP, especially in soil samples[5,6,17,23]. Acid solvents, alkali solvents, or oxidation
103 agents, including KClO (30%), NaOH (56% or 52.5 M), H_2SO_4(96%), HNO_3 (65% or 22.5 M), and
104 H_2O_2 (30% or 32.6 M), have been used to digest and remove SOM from floatation, with HNO_3 and
105 H_2O_2 adopted most often[17]. However, many of these agents decompose or disintegrate MP into
106 smaller particles, which reduce MP weight and number, as well as altering the shapes of particles.
107 For example, the digestion of HNO_3 and H_2O_2 can reduce 2–7% MP[17,23]. However, a recent study
108 confirmed that the suitability of Fenton's reagent under suitable temperatures (<40℃) and pH (<3)
109 could remove OM from MP in sludge (86.9% ± 9.87%) and soil (106% ± 13.8%) in 2 hours. There
110 were only minor alterations in the size and mass of MP decomposition (0–5.5% for size, 0.2–2.9%
111 for mass) [24]. Furthermore, despite floatation being widely adopted, several issues persist: (1) the
112 extraction process is complicated and time-consuming; (2) the extraction process is easily polluted;
113 and (3) it is difficult to obtain high precision, especially for high-density MP. Thus, professional
114 techniques and equipments of MP extraction, especially on the time-saving, low-cost, low-pollution
115 and the automatic, should be developed.

116 In agriculture, PE and PP (especially light-density PE [LDPE]) have been widely used[1,6,20],
117 such as plastics mulch. The density of PE and PP is typically less than 1.0 g cm^{-3}, lower than that of
118 distilled water at one standard atmospheric pressure. Therefore, distilled water can be used as a
119 solution to extract/float MP from field soils as indicated in the loess plateau region of northwest
120 China[6]. Recently, a new method using pressurized fluid extraction has been introduced, and the
121 solvent (e.g., methanol, hexane, and dichloromethane) has been used to extract MP from soils under
122 high-temperature (180-190℃) and high-pressure conditions (1500 PSI). After extraction, solvents
123 can be removed from extracted residues by evaporation under a stream of nitrogen [25]. This method
124 is highly efficient but there are also some insufficiencies: (1) it requires special equipment and
125 organic solvents, thus involving high costs and potential environmental pollution; (2) parts of SOM
126 may also be dissolved in the organic solvent, resulting in an inaccurately large measurement of MP
127 content; (3) MP could be pyrolysed under the high temperature and decrease the mass recovery of
128 MP; (4) this method can only be used to measure MP mass, while the shape and microstructure of
129 MP are destroyed[17,26,27].

2.2 Identification and quantification

When the collection of MP content is high enough, a weighting method is usually adopted after impurities are removed, especially when a high mass of MP with few impurities is easy captured via net in lake or ocean water or collected from sediment [4]. However, when a small amount of MP is extracted, the weight of MP is difficult to measure using balance scales[8]. Raman spectroscopy (2 mm), time gated Raman method spectroscopy ($\leqslant$ 125μm and $\geqslant$ 5 mass%)[28], Micro-Raman spectroscopy (>100 μm), Micro-FTIR spectroscopy(>100μm)[29], μ-Raman spectroscopy (>1 μm), μ-FTIR spectroscopy(>10 μm)[30], macroscopic dimensioned near-infrared (NIR) in combination with chemometrics (>10 μm and 1 mass%) [28] and hyperspectral imaging technology (0.5 -5 mm) [31] can be used to identify plastics type, but the processes are big problem is time consuming and there are problems with uncertain extrapolation [32] and it is difficult to know the MP quantification [17,31]. Sequential pyrolysis-gas chromatography coupled with mass spectrometry (PY-GC-MS) [7] and thermal extraction desorption gas chromatography mass spectrometry (TED-GC-MS) [32] can be used to simultaneously identify polymer types of MP particles and associated organic plastics additives to obtain a precise MP weight. Compared with PY-GC-MS (sample amount of 0.5 mg), TED-GC-MS can measure greater larger samples (>20 mg), and TED-GC-MS is suitable for analysis of complex environmental samples [32]. The deficiency of these methods is the expense of the equipment and the operation process is complicated. Recently, a heating method followed by photo comparison before and after heating has been used to identify low-density MP (LMP) from soils when LMP content is low (0.008 g kg^{-1})). Furthermore, LMP size (>50 μm) and shape can be observed under a stereomicroscope, and weight can be calculated by heating models[6].

In the absence of direct measurement techniques (e.g. Pyr-GC/MS), we suggest the following processes: (1) when the MP size is larger than 1 mm, MP can be picked out directly; (2) when the MP size is less than 1 mm, and mixtures by flotation and filtration sample has high organic impurities, Fenton's reagent under a suitable temperature (<40℃) and pH (<3) can be used to remove OM[24]; (3) for measuring the weight of MP, a large amount of MP can be weighed by balance depending on the scale of the equipment, and few MP particles can be heated and calculated by heating models (MP density depends on the solution); and (4) microscope-combined software (e.g., Image J) can be used to measure the shape of MP, and Raman spectroscopy and FTIR spectroscopy can be used to identify plastics types[6].

Based on the reviewed publication, the unit of MP content described in the studies is not consistent and thus difficult to compare in terms of results. For example, MP weight has been

quantified as g km^{-2}, g cm^{-3}, and g L^{-1} in water systems but g kg^{-1} and g m^{-3} in soil. The MP number has been described as particle m^{-2}, particle m^{-3}, and particle L^{-1} in water systems but particle kg^{-1} and particle m^{-3} in soil[6,8]. To share research data more effectively, a standard unit of MP in water systems, sediment, and soil should be applied. We recommend (1) g L^{-1}, mg L^{-1} and particle L^{-1} in water systems; and (2) g kg^{-1}, mg kg^{-1}, and particle kg^{-1} in sediment and soil, and sampling volume should also be supplemented to transform the units.

3. Distribution of MP in the environment

MP is widely distributed across terrestrial and marine systems, from the surface down to deep layers. The distribution can be influenced by a range of processes, including; runoff, infiltration, river discharge, wind action, ocean currents, and the movement/dispersal of animals and humans across and between ecosystems (Figure 1).

3.1 Distribution of MP in sediment and water

With the increase of plastics consumption, much MP is intentionally or unintentionally produced, and continuously moves into sediments and water [8,9]. Because of oxidative degradation (photo- and thermally-initiated), friction and biodegradation, the size distribution and shapes of MP vary in sediments and water [4, 16]. MP sizes mainly range from 0.5 to 2 mm [26,33], and the shapes typically consist of pellets, fibers, and fragments. In the sediments-water systems, logically, MP only sink and accumulate in the sediment when their density exceeds seawater (>1.02 g.cm^{-3}); otherwise it tends to float on the sea surface or in the water column [26]. Thus, LMP is distributed in the surface layers of ocean water, and high-density MP (HMP) is distributed in the benthos due to sinking and biota movement[35]. However, biofouling (biomass accumulation) can increase MP density, while defouling can decrease MP density, which could result in the sinking, neutral buoyancy or floating of MP [26,35] (Figure 1). Furthermore, the distribution of HMP could also be changed by tidal fronts, high flow rate, or their large-surface area[36]. For sediments, generally, MP has the highest concentration in the upper layers (1100 pellets 0.1 m^{-3}), while only small amount of MP can be found at the 200-cm depth, translocated there by due to the preferential flow and animal movement [20].

Generally, MP found far off oceans comes from ships and sea platforms, whereas MP in nearshore areas originates mostly from the mainland by waste water, runoff, river and air flow [8,37]. For example, in the highly urbanized river in Chicago, USA, the concentrations of MP were higher upstream than that downstream, and equaled or exceeded those in the oceans and the Great Lakes [10,33]. Furthermore, plastics fragments can be easily removed by runoff and rivers as they flow from the mainland to the lower estuaries, and the highest amount of MP is typically observed in

sediment and water after precipitation [19,20]. Thus, sediments as the long-term sink of MP, the MP concentration is highly related to the terrestrial environment, and MP in sediment close to harbors and industries may be the key sources of MP pollution in the ocean [5,10,20]. For example, MP is concentrated in areas closer to the Santos estuarine channel along the beach (82 pellets, 0.036 m^{-3})[20], and MP concentration is higher in sediment near the coast versus far from the coast of China[8,38]. Similar to the ocean, MP has been found in lakes and rivers, especially in surface water and MP numbers at the 0–20 cm depth are negatively correlated with distance from the city center ($p < 0.001$) due to the influence of anthropogenic factors[31].

Although MP distribution in water and sediment has been widely reported, the distribution of MP content is not always consistent at a range of spatial scales (sites to regions) [8], presumably due to unique conditions, temporal variation, or different analytical methods. Standard methods and techniques of water sampling, sediment sampling, and MP analysis should be developed. In addition, for most sediment and water systems, MP was only monitored once. To better understand the distribution and dynamics of MP in environment (1) MP distribution should be measured in both of the horizontal and vertical directions; (2) MP distribution should be monitored at equal intervals or over certain periods in the environment to account for temporal variation associated with physical (hydrographs) and biological processes (settling and flocculation), (3) exact sources of MP in sediment, soil and water in certain areas should be ascertained; (4) samples at different depths should be considered to encapsulate a greater proportion of the aquatic environment; (5) more detail information of MP distribution should be investigated, e.g., the distribution of size, color and shape; (6) the driving mechanisms of MP distribution at the microscale should be considered, such as MP degradation from large size to small in certain environments. These factors should be investigated in subsequent research.

3.2 Distribution of MP in soil

Aside from oceans, lakes, rivers and sediment, MP has also been found in soil. There is a scarcity of relevant publications on this topic mainly due to analytical limitations associated with the removal of OM from samples. Except of sludge discharge [2,9,39], debris from plastics waste decomposition and friction are key sources of MP in soil, where MP types are varied[1,17]. At a beach, Zhou et al. [37] identified seven MP morphologies (foam, debris, snowflake, pellet, film, fiber and sponge) and found MP polymer components containing PE, PP, PS, polyether polyurethane and a polymer mixture (PE and PP) in the soil. Reports also revealed that MP content was higher in surface soil layers and the distribution was influenced by landscape patterns and land use [6,39]. It has also been reported that, compared to adjacent soils under vegetable production, the concentration

of plastics particles in buffer soil was 1.6 times lower, mainly due to wastewater irrigation and plastics mulch application[39]. Compared with farmland, MP content was even greater, varying between 0.03–7% in soil of industrial areas and along roads[6,25]. MP concentration also appears related to the population of the catchment area [17].

In the vertical axis, plastics debris tends to accumulate in soil surface layers (0–30 cm) in farmland, mainly caused by the increasing use of plastics mulches and soil amendments such as organic waste like compost or sewage sludge[1,6,8,40]. Recent publications have indicated that the amount and weight of MP is 4.6 times higher at 10–30 cm than at 0–10 cm in farmland. In comparison, MP levels were higher at 0–10 cm than at 10–30 cm in both orchards and greenhouses of the Chinese loess plateau [6]. This trend may be determined by the soil tillage method as well as runoff and infiltration. Furthermore, in some cultivated areas, 95% of particles range in size from 0.05 to 1 mm, and are predominantly plastics fibers (92%); plastics films only contribute 8% to the total number of MPs. Plastics fibers are more associated with micro-aggregation, whereas plastics films and fragments are related to the macro-aggregate[39]. Animal movements can take MP from surface soils to deep soils layers [41], and preferential flow can take MP from surface soil to underground water[41,42]. Thus, MP can be moved into deep soil layers and even could be moved into underground water depending on animal movement and preferential flow pathways. Given the paucity of research on MP distribution in soil, especially agricultural soil, many issues remain unresolved related to MP distribution and dynamics in horizontal and vertical directions in different types of fields and during crop growth stages, rainy seasons, and the freezing–thawing process. MP distribution in these systems is likely influenced by (1) atmospheric deposition, water erosion, and wind erosion; (2) land use patterns, land use changes, and soil management (e.g. fertilization, irrigation, and tillage); (3) soil animal movement, interflow, and plant growth (roots); (4) and soil structure, soil properties, and microbiology. In addition, it is important to ascertain sources of soil MP in the research area, which is crucial to reducing MP pollution and better managing MP in the local region.

4. Effects of MP on biota

Based on the reviewed publications (Table 1 and Table 2)[43-76], MP invasion appears to occur across all ecosystems, from the terrestrial to marine environments in different trophic levels (Figure 1). MP has entered the food chain: (1) animals including echinoderms, mollusks, arthropods, annulatas, cnidaria, mammals, birds, amphibians, reptiles, and fish; (2) plants including alga(e) of spore-producing plants and gymnosperms and angiosperms of spermatophytes; and (3) microorganisms including bacteria and fungi and ciliophoran, protozoa and phylum

sarcomastigophora. In order to better know their biological effects, MP particles, MP additives and toxic substances adsorbed by MP were summarized separately, and the case studies of negative effects and neutral effects were represented in Table 1 and Table 2.

[Table 1 should be here]

[Table 2 should be here]

4.1 Influences of MP particles/debris on biota

Parts of publication showed MP can cause negative effective on biota (Table1). Once MPs are swallowed by aquatic animals, physical injury can follow that may could block or damage the digestive organs, contribute to the illusion of satiety, reduce ingestion, compromise nutrition and energy, and even lead to death[77,78]. Furthermore, after MP ingestion, mesopelagic fish could be harder finding it more difficult to return to deeper waters due to the buoyancy of plastics, thus leading to death [36,79]. Generally, there are three factors influencing on biota ingestion, (1) MP size: When MP size less than 1 mm, MP can be ingested by small invertebrates, but was prevented in marine isopods (*Idotea emarginata*)[12]. Even for NP, the size is also selective important, compared with small size of NP (50 nm), big size NP (180 nm) was easy ingested by fish [63]. (2) Color and shapes: MP (the proportion of 1-5 cm to 5-10 cm is 1/6) mixed with natural food sources has been found to be more easily ingested by planktivorous fishes, especially with certain colors (white, clear, and blue (87% total)) and shapes (fragments (94%), film (3%) and fishing line (2%))[79]. Furthermore, MP concentration also has an influence on the biological effect of MP. In terrestrial organisms, MP has been found in the gastrointestinal systems of the earthworm and chicken, with high-dose feeding leading to earthworm mortality due to a lack of nutrients and energy[34,78]. In water, the concentration effect of NP also happened occurs, when the concentration of NP (52 nm) was up to a 0.025 g/L, all *Daphnia* were still alive after 24 hours, while when it was above 0.075 g/L all were dead within 13 h[63]. Additional research has also shown that MP interactions with terrestrial organisms (e.g. soil-dwelling invertebrates, terrestrial fungi and plant-pollinators) can deeply influence essential ecosystem services and functions[66]. Based on the reviewed publications (Table 1), MP ranging in size from 1μm to 500 μm could cause negative effects on animals, less than 6μm could influence plant, and NP particles even could be harmful to micro biota. However, except for the concentration and size, it is not clear how shapes and colors influence the biota in cell and gene levels, although MP ingestion influenced by the size, color and shape were reported. This should be considered in the further research work, especially in combination with concentration and size.

MP, especially of a small size and NP, can invade the gut, alimentary canal, stomach, digestive gland, liver, pancreas, ovaries, gill lamella, and haemolymph and can cause physical damage[18,73]. Furthermore, MP ingested by animals can be transported into tissues and cells when the size of MP is sufficiently small, especially for NP (Table 1). Small size MP was found in the digestive organs of the earthworm (*Lumbriculs terrestris*) (29.5±26 μm) and can accumulate in mussel cells (*Mytilus edulis L.*) (<80 μm)[71]. MP has also been observed at the subcellular level at 96 h post-ingestion by blue mussels (*Mytilus edulis L.*), and notable histological changes upon uptake and a strong inflammatory response and lysosomal membrane destabilization were found after 6 h, which increased significantly with longer exposure times[48]. With longer exposure time to MP, the formation of granulocytomas and the lysosomal membrane destabilization response to strong inflammation proved that MP is taken up into cells and exerts major effects at the tissue and cellular levels[48]. Hämer et al (2014) indicated that when MP sizes smaller than 1μm, MP can translocate to the midgut gland facilitated by the complex structure of the stomach, including a fine filter system[12]. In addition, compared with larger sized of MP, small size MP and NP were shown to support a series of more severe effects, such as: (1) they (50 and 100 nm) can penetrate the cell wall of fungi and cause toxicity; (2) they (240 nm) can be transported from the alveolar space by monocytes to the tissues of rats; (3) they (50 nm) can cross the highly selective membranes of the fish brain and cause negative effects; and (4) exposure in human and non-human toxicological models resulted in gene expression changes, inflammatory and biochemical responses, and carcinogenesis. Furthermore, these negative effects on biota were influenced by surface area, size, electric charge, and hydrophobic properties and related to the adsorbed organism and cell metabolism [48,63,66,68] (Table1 and Table 2).

Few publications have disclosed the biological effects of MP on plants. Sanders and Lord [80] indicated that MP could be translocated to the ovary when MP sizes were less than 6 μm and were then introduced onto transmitting tracts of various inflorescences. External adsorption of MP could harm algal species in water since high doses can inhibit photosynthesis by preventing photon transfer into algae [8, 64]. Microbeads can significantly affect root growth of duckweed (*Lemna minor*) via mechanical blocking, and sharp particles reduce the viability of root cells[65,66]. Furthermore, MP can change the physiochemical properties and biogeochemical cycling of soil as well as influence plant–soil–microorganism systems, which could exert potential impacts on key symbiotic associations in terrestrial ecosystems[66]. These research questions need to be further explored.

However, most publications showed that the negative effect stated at the highest concentrations in laboratory studies and organisms feeding, and where MP concentrations are several orders of

magnitude higher than that in natural ecosystems[81]. There are also an increasing number of some publications indicated that MP didn't cause the negative effect on biota, and the publications increasing fast with years (Table 2). They thought that biological effect of MP was influenced by MP concentration and also depend on the sensitive species, and was not a risk in marine and freshwater ecosystems aquatic environment due to the low concentrations [53,81]. For example, exposing to polystyrene MP mixed with sediment in 28 h, MP significantly reduced growth of *G. pulex* with MP concentrations, while no effects was found on the survival of *Gammarus pulex, Hyalella azteca, Asellus aquaticus, Sphaerium corneum* and *Tubifex spp*.. Furthermore, no effects were found on the reproduction of *Lumbriculus variegatus*, and no significant differences in growth were found for *H. azteca, A. aquaticus, S. corneum, L. variegatus and Tubifex spp*[53]. Foley et al. [82] using by the meta-analysis, results showed that the effects of exposure to MP are highly variable across taxa, and many of the effects summarized were neutral. Despite the fact that MP bacterial assemblages had lower taxon richness, diversity, and evenness than those on other substrates, but MP can be also the good habitat for several taxa of in rivers, e.g. decomposing organisms and pathogens, and MPs have distinct microbial habitats and may be a novel vector for the downstream transport of unique bacterial assemblages [10,33]. Thus, in order to better understand the biological effect of MP, (1) the types, size, shapes, amount and mass of MP; (2) different organisms at kinds of ages; (3) realistic exposure condition including both heterogeneity and homogeneity of MP distribution; and (4) and both of LODs and LOQs should be concerned studied in further research work. In addition, no standard classification systems have been established to assess the MP risk on biota (microbes, plants, and animals) based on MP characteristics (e.g., size, shape, color, density, and mass). This should also be addressed, and could be a long-term research work.

4.2 Influences of MP additives on biota

Additives have been widely used to improve plastics production properties, including nonylphenol, phthalates (potassium acid phthalate), bisphenol A, polybrominated biphenyls ethers and heavy metals, which are harmful to biota[8]. For example, phthalates and bisphenol A increase estrogenic activity and promote potential endocrine disruption in vertebrates and some invertebrate species, even influencing human reproductive action and causing cancer[83]. During plastics decomposition, plastics additives are continually released into the environment or directly assimilated by animals after ingestion[57]. Studies have indicated that the toxicity of leachate from plastics shows no consistent trend with irradiation time, which depends on the duration of irradiation and plastics products that threaten animals' health and can lead to death[14,84]. Actually, only parts of MP can release additives and cause negative effect in the environment[14,84]. For example,

Lithner et al. [84] found nine out of 32 tested plastic product leachates had *Daphnia* 48-h EC_{50}s ranging from 5 to 80 g plastic material L^{-1}, while the remaining 23 products had no effect on mobility was seen even at the highest test concentrations of 70–100 g plastic material L^{-1}. Similarly, Sofia et al. [14] indicated that 38% of plastics collected from the ocean (n = 21) could produce leachates that caused acute toxicity to a marine copepod (*Nitocra spinipes*) after irradiation. However, most of the studies above studies were carried in the laboratory and can't truly reflect the leachate velocity and quantity of additives from different types of MP in natural environments with complicated solar irradiation, temperature, pH, and microorganisms. Furthermore, MP additives released from water have been mainly focused on by most studies, while there is a lack of reports on the releasing and negative effect on biota in soils and sediments. Thus, in order to better understand the toxic of plastics additives, kinds of additives from different MP types under various conditions further research is needed.

4.3 Influences of toxic substances adsorbed by MP on biota (MP sorption- desorption)

Many publications indicated that plastics debris can transport contaminants and is considered a sink for persistent organic pollutants (POPs) such as polychlorinated bphenyls (PCBs), polycyclic aromatic hydrocarbon (PAHs) and dichlorodiphenyl trichloroethane (DDT) along with heavy metals (e.g., Al, Zn, Pb, Cu, Ag, and Pb)[8, 67]. In the marine environment, diethylhexyl phthalate (DEHP), dibutyl phthalate (DBP), diethyl phthalate, diisobutyl phthalate, dimethyl phthalate, benzaldehyde, and 2,4-di-tert-butylphenol were found in polymers. These POPs and heavy metals were gradually adsorbed by MP and accumulated, especially within complicated particles and larger surface areas, where the negative effects of POPs adsorbed to the surface of MPs were greater than those generated by plastics additives [17,18,37,67,85]. The sorption-desorption process of MP to organic pollutants is influenced by the molecule composition and structure of MP [86,87]. The sorption strength of phenanthrene from seawater onto plastics proceeds as PE > PP > PVC[86]. Compared with high-density PE (HDPE), light-density PE (LDPE), and PP, the sorption–desorption equilibrium of PAH and PCB to PET and PVC in the marine environment is difficult to determine, and at a lower concentrations. Thus, HDPE, LDPE, and PP have a greater risk in concentrating hazardous chemicals among marine animals[88]. Compared with virgin MP, environmental MPs and NPs have a coarser structure and higher surface area, which have the stronger sorption ability[67,87,89]. The sorption-desorption process is also influenced by a number of other factors including of pH, NaCl, and $CaCl_2$ [89]. However, except for the influences of molecule composition and structure of MP, environmental factors should also be concerned in the future, e.g. solar irradiation, temperature, pH, and microorganisms' activities, which highly relates to the process of sorption-desorption.

At this time, it is still debate issue whether plastics are the important agents in the transport of hydrophobic contaminants to organisms. Some of the publications showed that contaminants transferred by large MP to aquatic organisms can be neglected, while small plastics debris with novel physical and chemical properties can increase potential interaction with contaminated organisms, causing direct and indirect toxicity, especially for MPs with high surface curvature and coarse surface chemistry[66,89]. POPs adsorbed by MP could change physiological and metabolic processes. Even low-dose polluted MP can result in negative effects, for example, 1 mg of contaminated PE to a gram of sediment significantly increases phenanthrene accumulation in the lugworm (*Arenicola marina*)[86]. When MP combined with organic pollutants (PCBs, PAHs, and PBDEs) is ingested by Japanese medaka (*Oryzias latipes*), gene expression and endocrine system function can be altered for male and female adult fish[90]. Furthermore, MP and PAH mixtures could result in elevated bioavailability of these chemicals after mussel ingestion (*Mytilus galloprovincialis*), leading to toxicological implications through several molecular and cellular pathways [91]. The toxicity of chemical substances adsorbed by MP can also influence microbe growth and reproduction[27]. Furthermore, the microbiome (e.g., bacteria, virus, and microalgae) adsorbed by MP, can potentially lead to inflammation after being ingested by animals[8]. However, other reports have found that the toxicity of chemical substances could be weakened by MP[27]. Overall the flux of hydrophobic organic chemicals (HOCs) bioaccumulated from natural prey were greater than the flux from ingested MP for most habitats, and MP ingestion is not likely to increase the exposure to and thus risks of HOCs in the marine environment[89]. Fisner et al. (2013) also found that the dynamics of persistent organic pollutants (POPs) are not consistent with MP changes in deep sediment[92]. This proved that the relationship between MP and organic pollutants is complicated. Koelmans et al. (2017) also indicated that actual environmental risks of different plastics and their associated chemicals remain largely unknown due to the uncertain methods were used to assess risks of MP on biota in kinds of environment[93]. Therefore, the relationship between MP and organic pollutants and their biological effects should be supported by additional studies on (1) different organisms, (2) different MP types, sizes and shapes, and (3) in kinds of environments, and the mechanisms should be clarified at the molecule and gene level with the micro-scale.

4. 4 MP transportation in the food chain

MP has been observed in a large number of biota at a number of different trophic levels, implying that MP has been transported in the food chain over time (Table 1 and Table 2). Once MP enters aquatic ecosystems, it can be transported through the food chain, for example from small-/medium-sized to large plankton, or from *Mytilus edulis* to *Carcinus maenas*[78,82,94,95]. MP

density in the water column determine its bioavailability to planktivorous organisms and then to larger predators, possibly promoting the transfer of MP between trophic levels[19]. MP has also been found to accumulate continuously in aquatic food chains; it was detected in 86% of turtles, 43% of marine mammals, and 44% of seabirds in 1997 and in 100% of turtles, 66% of marine mammals, and 50% of seabirds in 2015[96]. MP can be found at many trophic levels in the ocean[94,95], and its scope of influence appears to be increasing[96].

Most of the aforementioned studies focused on aquatic organisms, especially fish and small animals, with only a few investigating terrestrial organisms, although MP contamination on land might be larger than in the ocean. Publications have indicated that MP was found in the digestive tract of earthworms, chickens, and birds with diverse foraging behavior[34,79,85], and agricultural pollution can be important sources in transferring MP to the guts of water birds[72]. In terrestrial ecosystems, MP concentration in chicken feces is 9 and 14 times that that in earthworm casts and soil, and MP can be transferred and accumulated in food chains [50]. For microbial species, when MP is less than 0.5 μm, it can accumulate in yeasts and filamentous fungi[66]. For plant, NPs (< 0.1 μm) can be absorbed directly by roots (based on endocytosis through the plasmodesmata or passage via ion transport channels) to enter the plant cells[66,97]. As plants are primary producers at key trophic level in food chains, it is important to clarify: (1) how MP enters plant tissues and influence plant growth and breeding; (2) how size, shape, and concentration influences MP transportation and accumulation in food chain, especially for big animals, high trophic levels and terrestrial biota; (3) how environment factors influence MP transportation and accumulation; (4) what proportion of MP can be transported in food chains; (5) how MP particles, MP additives and toxic substances are adsorbed by MP influence the various communities and population. In addition, MP can be ingested by humans in many ways, such as through seafood, beer, food wrapped in plastics, and breathing air (Figure 1) [98,99]. Despite a lack of evidence that MP has biological effects on humans, the adsorption of toxic chemicals to MP may enable the accumulation of pollutants in animals, which also threatens human health through the food chain, and which might be predicted by related models in the future[27,89].

5. Conclusions of review results

Basing on the summary above, current analytical methods, techniques, and equipment still struggle to precisely quantitatively detect MP in water, sediment, and soil with the low cost and time saving. Standardized analytical methods and techniques are urgently needed. To facilitate comparisons, units of MP quantification should be consistent, and be easy transformed. Distribution of MP concentration combed with size and shape distribution should be invested at various

457 spatiotemporal scales, especially in soils. The biological effects of MP debris, MP additives and
458 toxic substances adsorbed by MP should be clarified, and their risk of toxic assessment should refer
459 to the natural condition. Further, for biological effects, not only the individual should be focused on,
460 but also the community, population and ecosystems should be considered. More attention should be
461 paid to the influence of MP on big animals, high trophic levels and terrestrial biota. MP and NP
462 entering the tissues of plants and animals have been proved, but the mechanisms behind these
463 biological effects is still poorly known and need be clarified from the micro-perspective of cells,
464 molecules, and genes during subsequent research.

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Table 1 Negative effect of Microplastic (MP) on biota

			Negative effect	MP Sizes	MPtypes/Encounter path	Drive	Biological point	Cases	Time	References
Animal	Invertebrate	Echinoderms	Fertilization (insemination)	PS 6 μm HDPE 0-80 μm	PS microspheres； HDPE fluff Exposure	MP	Zygotes	Sea urchin (*Paracentrotus lividus)*	2017	[43]；
		Mollusks	ChE activity inhibition	Average diameter of 2 μm	Red fluorescent polymer	Only or with florfenicol, Mixtures caused feeding inhibition ChE inhibition and of isocitrate dehydrogenase activity, and increased anti-oxidant enzymes activity and lipid peroxidation levels	Gut, lumen of the digestive gland, connective tissue, hemolymphatic sinuses, and gills surface	*Corbicula fluminea*；	2018	[44]；
			Indirect neurotoxic effect	1-10 μm	Virgin polystyrene, microbeads	MP	Gum hemolymph	zebra mussel (*Dreissena polymorpha*)	2018	[45]；
			Energy Balance and Gametogenesis	6-10 μm	PS Exposure	MP	Gameto	Pearl Oyster (*Pinctada margaritifera*)	2018	[46]；
			Reduce the energy intake	63 -250 μm	PS Exposure	MP	-	*Atactodea striata*	2017	[47]；
			Lysosomal membrane destabilization	0−80 μm	HDPE Exposure	MP	Cells and Tissue	Blue Mussel (*Mytilus edulis* L.)	2012	[48]；
		Arthropods	Immobilisation	1 μm	PE Exposure	MP	-	*Daphnia magna*	2016	[49]；
		Annulatas	Weight reduction Higher mortality	<150 μm	LDPE Exposure	MP	Guts	*Lumbricus terrestris*；	2017	[50]；
		Cnidaria	Bleaching	37 -163 μm	PE	MP	-	*Acropora humilis,*	2018	[51]；
			Bleaching	37 -163 μm	PE	MP	-	*Acropora millepora,*	2018	[51]；
			Tissue necrosis	37 -163 μm	PE	MP	-	*Pocillopora verrucosa,*	2018	[51]；
			Tissue necrosis	37 -163 μm	PE	MP	-	*Pocillopora damicornis,*	2018	[51]；
			Bleaching	37 -163 μm	PE	MP	-	*Porites cylindrica*	2018	[51]；
			Significant impact the feeding	<400 μm	PE flakes ingesting Exposure	MP	Gastric cavities.	*Hydra attenuata*	2018	[52]；
			Growth and ingest	20−500 μm	PS	MP	-	Gammarus pule	2018	[53]；

	Vertebrate	Mammals	Compositive	< 150 μm	PS and PVC Feeding，drinking，inhalation;	Compositive Small MP can move from the cavity to the lymph	Gut，lymph and circulatory system	Human；	2018 [54]；
			Potential toxicological effect	-	Compositive Feeding	MP	Exposed to leached additives--polybrominated diphenyl ethers, phthalates and bisphen- olA	Baleen whales；	2012 [55]；
			Modify the gut microbiota composition and induce hepatic lipid disorder	0.5 - 50 μm	PS exposed	MP	Gum and hepatic	Mice；	2018 [56]；
		Birds	Contaminants	-	Natural PE resin pellets Feeding	Significant amounts of PCBs contained By PE	Gum	*Streaked shearwater;*	2009 [57；
		Amphibians	Reproduction and development	-	Almost all commercially available plastic products Exposed	MP extract	Estrogenic Chemicals & using gonads	*Xenopus laevis*	2011 2017 [58]&[59]
		Fish	Immune gene expression	0.2μm, 1μm, 20μm, 40μm and 90μm	PS Exposed	MP	Gill	Rainbow trout；	2018 [60]；
			Behavioural responses and reduction of swimming velocity and resistance time	1-5 μm	Fluorescent red polymer microspheres Exposed	Only or with Hg Mix- changes in behavioural responses	-	European seabass；*Dicentrarchus labrax*	2018 [61]；
			Localized thickening of the mucosal epithelium and histology and protease activity	0.100 - 1000 μm	Pristine PVC fragments	MP only	Whole body	*Barbodes gonionotus*	2017 [62]；
			Brain damage and behavioural disorders	52 53 57 58 120 180 330 nm	PAO2N	NP	Brain	Crucian carp, Carassius	2017 [63];
Plant	Spore producing plant	Alga(e)	Hindered algal photosynthesis	20nm	PS adsorption	MP	-	*Chlorella* ；*Scenedesmus obliquus*；	2010 [64]；
	Spermatophyte	Angiosperm	Affected the root growth by mechanical blocking and reduced partial the viability of cell	From cosmetic products	PE microbeads	MP particles，Sharp particles	Root or root cells	*Lemna minor*	2017 [65]；
		Gymnosperm	Possibleplant-pollinators plant-pollinators	6 μm	Polyester beads	MP particles similar to pollen	Pollen	bollum plants	2018 [66]；

Microorganism	Bacteria	-	Inhibited the growth（nano） Interrupt the ecological function	50 nm，55 nm,1 μm	PS beads	NP	-	*Halomonas alkaliphila*	2018	[67] ;
	Fungus		Not uniform	Nano	-	NP	-	*Aspergillus oryzae;*	2016	[68] ;
			Lethal effects	50 and 100 nm	PS	NP	Yeast cells	*Saccharomyces cerevisiae*	2016	[68] ;
			Not uniform	Nano	-	NP	-	*Aspergillus nidulans;*	2016	[68] ;

Note::PE represents polyethylene; PP represents propene polymer; PS represents Poly Styrene; PVC represents polyvinyl chloride.

Table 2 Neutral effect of Microplastic (MP) on animals

		Neutral effect	MP Sizes	MP types	Biological point	Cases	Time	References
Invertebrate	Echinoderms	Englobe	0.25 - 15mm	Nylon and PVC Feeding	Gut	Sea cucumber （*Thyonella gemmata;Holothuria floridana ;Holothuria grisea;Cucumaria frondosa*）.		[69]；
	Arthropods	Ingest	-	Nylon-strand ball Feeding	Gut	*Nephrops norvegicus;*	2013	[35]；
		Ingest	20−500 μm	PS Additives have been removed In sediments	Gut	Gammarus pule	2018	[52]；
		None	20−500 μm		Gut	Asellus aquaticus,	2018	[52]；
		ingest	20−500 μm		Gut	Hyalella azteca	2018	[52]；
		None	20−500 μm		Gut	Tubifex spp.	2018	[52]；
		None	20−500 μm		Gut	Sphaerium corneum	2018	[52]；
		None	20−500 μm		Gut	Tubifex spp.	2018	[52]；
		No Reproduction	20−500 μm		Gut	Lumbriculus variegatus.	2018	[52]；
	Cnidaria	NONE	Particles ranged from 37 mm to 163 mm with a mean diameter of 112.7 ± 11.1 mm (mean ± SD).	PE	-	*Porites lutea,*	2018	[50]；
	Nemathelminthes	Ingest	0.5 and 1 μm	PS Feeding	Stomach	*Caenorhabditis elegans*	2012	[70]
Vertebrate	Mammals	Ingest	1mm to 17cm	PE, PP, PVC, polyethylene terephthalate, nylon Feeding	Gum	Humpback whales；	2015	[71]；

	Birds	Ingest	Natural state	Feeding	Gum	*Fulica atra*；	2017	[72] ;
		Ingest	Natural state	Feeding	Gum	*Anas platyrhynchos*；	2017	[72] ;
		Ingest	Natural state	Feeding	Gum	*Tadorna tadorna*；	2017	[72] ;
	Reptiles	Ingest	-	Litter ingestion	-	*Caretta Caretta*	2018	[35] ;
	Fish	Ingest	PET; 36.36% Cellophane 30.30% ； Polyacrylate 15.15%	Natural state Feeding	livers Digestive tract	Red mullet（*Mullus surmuletus*）；	2017	[73] ;
		Ingest	Natural state	Natural state Feeding	Stomach	*Dia- phus metopoclampus;*	2017	[74] ;
		Ingest	Natural state	Natural state Feeding	Stomach	*Hygophum benoiti;*	2015	[74] ;
		Ingest	Natural state	Natural state Feeding	Stomach	*Myctophum punctatum*；	2015	[74] ;
		Ingest	Natural state	Natural state Feeding	Stomach	*Engraulis encrasicolus*；	2015	[74] ;
		Ingest	>3.60mm	Natural state Feeding	Stomach	*Thunnus alalunga* ；	2015	[75] ;
		Ingest	>3.69mm	Natural state Feeding	Stomach	*Xiphias gladius*	2015	[75] ;
		Ingest	>0.63mm	Natural state Feeding	Stomach	*Thunnus thynnus*	2015	[75] ;
Protozoa		Ingest	A few micrometers	PS latex beads	-	Amoebae	2018	[76] ;
Phylum Sarcomastigophora	Class Zoomastigophorea	Ingest	Nano	PS latex beads	-	Flagellates	2018	[76] ;

Note: Publications related to neutral effect of Microplastic (MP) was only found on animals. Natural state means that MP collected from natural environment, and size distribution is wide range.

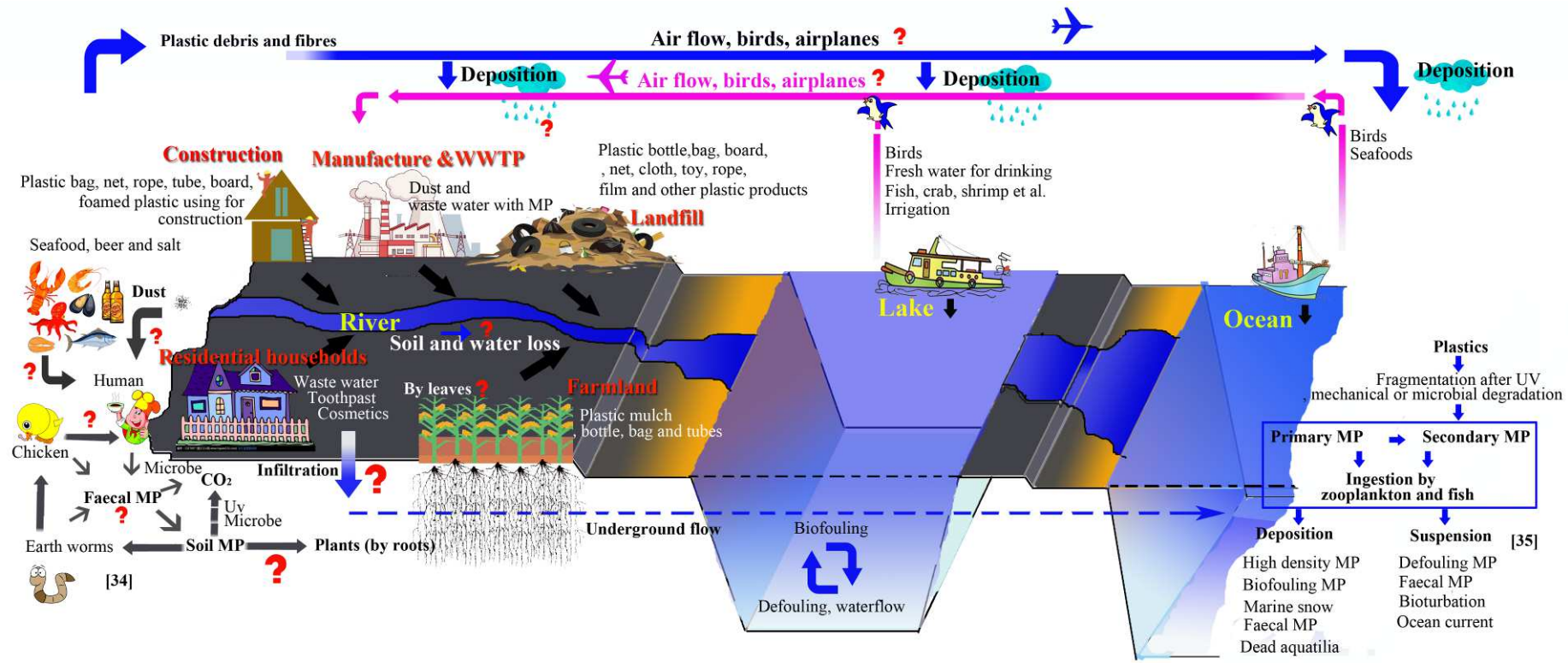

Figure 1 MP cycling in the ecosystems (Part of terrestrial ecosystem refers to [34], and part of marine ecosystem refers [35])

Highlights

- Convenient analytical method and equipment of MP should be developed.
- Transportation and distribution of MP remains large gap in environment
- Effects of MP on biota need more new evidences
- Biological effects of MP at molecule and gene level should be focused on.