

# Tracing the sources and microbial degradation of PCBs in field sediments by a multiple-line-of-evidence approach including compound-specific stable isotope analysis

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1 **Microplastics as pollutants in agricultural soils**

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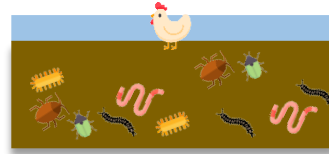
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Dissemination of MPs in the environment  
(Pharmaceuticals, Transportation,  
Agriculture, Compost)



Effect of MPs on soil environment  
(Plants, Animals and Microbes)



Isolation of MPs from the soil  
(Sieving, Density separation,  
Filtration and Extraction)



- Identification and characterization of MPs
- Assessment of ecological potential risks

## 22 **Abstract**

23       Microplastics (MPs) as emerging persistent pollutants have been a growing global concern.  
24       Although MPs are extensively studied in aquatic systems, their presence and fate in agricultural  
25       systems are not fully understood. In the agricultural soils, major causes of MPs pollution include  
26       application of biosolids and compost, wastewater irrigation, mulching film, polymer-based  
27       fertilizers and pesticides, and atmospheric deposition. The fate and dispersion of MPs in the soil  
28       environment are mainly associated with the soil characteristics, cultivation practices, and diversity  
29       of soil biota. Although there is emerging pollution of MPs in the soil environment, no standardized  
30       detection and quantification techniques are available. This study comprehensively reviews the  
31       sources, fate, and dispersion of MPs in the soil environment, discusses the interactions and effects  
32       of MPs on soil biota, and highlights the recent advancements in detection and quantification  
33       methods of MPs. The prospects for future research include biomagnification potency, cytotoxic  
34       effects on human/animals, nonlinear behavior in the soil environment, standardized analytical  
35       methods, best management practices, and global policies in the agricultural industry for the sake  
36       of sustainable development.

37       **Keywords:** Environmental pollution; Microplastics; Analytical techniques; Agricultural systems;  
38       Soil health; Sustainable development.

39

## 40 **1. Introduction**

41       In recent years, anthropogenic activities are considered as the key drivers of biodiversity loss  
42       and ecosystem functions (**de Souza Machado et al., 2018a**). A typical marker of human activity  
43       is the overwhelming amount of plastics produced and used. Plastics are chemically miscellaneous  
44       groups of synthetic polymeric materials with multiple applications in modern lifestyle (**Galloway**

45 **et al., 2017**). It has been estimated that the majority of the produced plastics (>80%) are  
46 thermoplastics, which are industrialized via polymerization to form high-molecular-weight  
47 polymers from low-molecular-weight monomers (**de Souza Machado et al., 2018a**). Their  
48 physical and chemical characteristics can be altered and strengthened by physical methods (e.g.,  
49 extrusion, melting, and palletization) and chemical methods (e.g., mixing with antioxidants,  
50 copolymer polycarbonate, plasticizers, and colorants) (**Bittner et al., 2014**). As a result, plastic  
51 materials have a strong physical structure along with complex chemical properties. Owing to low  
52 cost, efficient malleability and durability, the demand and usage of plastics have been increasing  
53 continuously in the past decades (**PlasticsEurope, 2015**), and 8300 of plastics are generated  
54 worldwide (**Geyer et al., 2017**).

55 In 2016, 27.1 million metric tons (Mt) of plastic wastes were collected in the European Union  
56 (EU), of which 31.1% were recycled, 41.6% were used for energy recovery, and the remaining  
57 27.3% were disposed of at landfill sites (**PlasticsEurope, 2018**). It was estimated that the  
58 contemporary manufacturing and waste management strategies would lead to 12,000 Mt of plastic  
59 wastes in landfills/natural setting by 2050 worldwide (**Geyer et al., 2017**). As shown in **Table 1**,  
60 the European plastic demand was ~47.8 Mt in 2014; however, only 54% and 16% (approximately)  
61 of plastics underwent the waste management process and recycling system, respectively  
62 (**PlasticsEurope, 2015**). More recently, 64.4 Mt of plastics production was reported in the EU,  
63 while 8.4 Mt of plastic wastes were collected and recycled inside/outside the EU (**PlasticsEurope,**  
64 **2018**). The worldwide plastic recovery and recycling rates might be even lower. It was found that  
65 approximately 32% of plastic wastes might be present in the soil environment (**de Souza Machado**  
66 **et al., 2018b; Jambeck et al., 2015**). Plastic wastes exposed to the natural environment can

67 undergo weathering processes such as degradation and disintegration due to the collective actions  
68 of physio-chemical and biological factors (**de Souza Machado et al., 2018a; Whitacre, 2014**).

69 Plastics waste including biodegradable plastics are actually more susceptible to physical  
70 disintegration (fragmentation) than degradation (mineralization), which result in smaller sizes of  
71 plastics (**Whitacre et al., 2014**). The generated plastics with particle size <5 mm are generally  
72 considered as microplastics (MPs) (**Li et al., 2018**). The global presence of MPs is probably due  
73 to the extensive production of microplastic particles (e.g., microbeads) for diverse applications.  
74 Natural disintegration and degradation of MPs can also generate plastic particles <0.1 µm in size,  
75 which is known as nanoplastics (NPs) (**de Souza Machado et al., 2018a**). Owing to the deleterious  
76 effect of MPs on the marine and shoreline environment, MPs have attracted a lot of attention of  
77 marine scientists (**Galloway et al., 2017; Li et al., 2018; Wang et al., 2019**). Similar effects of  
78 MPs are also observed in freshwater and estuarine ecosystems (**Horton et al., 2017a**), and there  
79 also are emerging concerns over MPs as pollutants in the aquatic environments (**Li et al., 2018**).

80 MPs pollution in the soil environment has received nominal scientific attention in comparison  
81 to that of marine environment, whereas the former might be 4-23 times larger than the latter in  
82 terms of mass (**Horton et al., 2017b**). Terrestrial soil tends to accumulate more MPs than aquatic  
83 ecosystems (**Nizzetto et al., 2016a**). The United Nations Environment Programme (UNEP)  
84 identified that large quantities of particulate plastics found within the marine environment globally  
85 result from the land-based sources (**UNEP, 2016**). According to **Jambeck et al. (2015)**, 4.8 to 12.7  
86 Mt of terrestrial plastic wastes enter the ocean annually, equivalent to 1.7 to 4.6% of the total  
87 plastic wastes generated worldwide. Sediment transfer during soil erosion is a process that allows  
88 the transport of particulate plastics from terrestrial to aquatic ecosystems. Despite this linkage to

89 terrestrial sources, most scientific investigations on plastic particles have neglected their effects  
90 **(Bolan and Bradney, 2019; Bradney et al., 2019)**.

91 MPs pollution threats to the aquatic environments are frequently associated with the living  
92 organisms in the aquatic environments, i.e., MPs can serve as particulate matter for ingestion  
93 **(Rehse et al., 2016)**, solid supports for pollutant transport **(Zhan et al., 2016)**, and significant  
94 chances of physical injury during the movement **(de Souza Machado et al., 2018a)**. The  
95 environmental impacts of MPs pollution in the agricultural soils may be underestimated in  
96 comparison to aquatic systems. Investigations are urgently required to draw attention to MPs in  
97 the agricultural systems. However, only a few recent studies have examined the pollution levels  
98 and the possible sources of MPs in the agricultural environment and their harmful effects on the  
99 soil biota **(Boots et al., 2019; Chae and An, 2018; Mai et al., 2018; Zhang & Liu, 2018; Zhang**  
100 **et al., 2018b)**. Recent results revealed the pervasive and persistent nature of MPs in the soil  
101 environment **(Zhang & Liu, 2018)**, and adverse effects of MPs on growth, reproduction, feeding,  
102 survival, and immunity level of the soil biota (animals, plants, and microorganisms) **(Zhu et al.,**  
103 **2018a; Zhu et al., 2019; Zhang et al., 2019)**. The latest reviews came to describe the mechanisms  
104 and behavior of MPs in the soil environment **(Qi et al., 2020)**, their biomagnification tendency via  
105 food chains, toxicological effects on soil microorganisms, and analytical methods **(Guo et al.,**  
106 **2020; Li et al., 2020)**. Nevertheless, overall research on MPs pollution in agricultural soils is still  
107 in an embryonic stage and there are many knowledge gaps in this research areas. Several key  
108 questions such as pollution levels, ecological threats, dispersion mechanisms, and development in  
109 analytical and quantification technologies still require further studies in view of the high  
110 complexity and heterogeneity of the soil environment in different agricultural systems.

111 Therefore, this review aims to offer in-depth current knowledge about sources, fate, and  
112 dispersion mechanisms of MPs in the agricultural soils. The adverse effects of MPs of the soil flora  
113 and fauna are elucidated. This review also elaborates the analytical technologies involved in  
114 detection and quantification of MPs in soil and suggests the framework for future research.

115

## 116 **2. Possible sources and dispersion mechanism of MPs in soil and agricultural environment**

117 An enormous variety and range of MPs particles are available in the soil environment due  
118 to over exploitation of plastics and their unplanned management practices (**Figure 1**). Typically,  
119 the existing literature reported that terrestrial environments are the only component of the natural  
120 systems that can act as a source and distribution pathway of MPs to the aquatic environments  
121 (**Horton et al. 2017a; Karbalaie et al., 2018; Lechner et al., 2014**). For example, the release of  
122 significant quantities of illegal commercial MPs from manufacturing plants to Reverse Danube  
123 has been reported (**Lechner et al., 2014**). Plastics near coastal zones or in surface water  
124 disintegrate due to direct physical abrasion and UV-sunlight. Nevertheless, both processes are  
125 marginal in the soil environment in which plastic disintegration and degradation could be  
126 comparatively slow (**Karbalaie et al., 2018**).

127 Previous investigations reported limited degradation of commercial polymers in the soil  
128 environment. **Arkatkar et al. (2009)** reported only 0.4% degradation of polypropylene (PP) after  
129 one-year soil incubation, while no weight loss was observed in the case of polyvinyl chloride  
130 (PVC) after soil incubation for 10-35 years. Soil texture and composition play vital roles in the  
131 degradation of synthetic polymers in the soil environment. It was reported that clayey soils showed  
132 a greater degradation of polymers in comparison to sandy soils, possibly attributed to a higher soil  
133 organic matter (SOM) (**César et al., 2009**). There were also significant impacts of MPs on soil



134 and soil-water relation including water holding capacity, bulk density of soil, microbial activities,  
135 and soil structure (**de Souza Machado et al., 2018a; 2019**). The alteration in soil structure may  
136 lead to changes in the microbial composition of the soil, although it can be difficult to predict such  
137 changes (**Rillig et al., 2019**). The decrease in bulk density of the soil due to MPs pollution may  
138 increase the rate of evaporation, thus significantly affecting plants growth (**Wan et al., 2019**).  
139 Nevertheless, MPs pollution may also have some positive impacts on the soil properties. For  
140 instance, MPs facilitated soil aeration and root penetration by reducing the bulk density of the soil  
141 (**de Souza Machado et al., 2018a, Rillig et al., 2019**) and promoted the growth of onion bulbs  
142 and roots resulting in the increase in total crop biomass (**de Souza Machado et al., 2019**).

143 There are several factors affecting the quantity of MPs deposition, retention, and transport in  
144 the soil environment, such as anthropogenic activities (e.g., inefficient waste management  
145 practices and littering), physical characteristics of plastic particles (e.g., form, size, and density),  
146 climatic conditions (e.g., rainfall intensity and wind speed), and topography (**He et al., 2018b;**  
147 **Karbalaei et al., 2018; O'Connor et al., 2019**). A substantial direct contribution of primary MPs  
148 to terrestrial systems has been introduced via areal deposition of MPs. Synthetic textile fibers, wear  
149 and tear from synthetic rubber tires, and city dust are the major atmospheric sources of particulate  
150 plastics (**Kole et al., 2017; Löhr et al., 2017**). These particulate plastics are dispersed by wind in  
151 the form of atmospheric pollution. For example, 3–7% of the particulate matter (PM<sub>2.5</sub>) in the  
152 atmosphere is estimated to consist of plastic derived from tire wear and tear (**Kole et al., 2017**).

153 Other sources of MPs in terrestrial environments include domestic wastes, personal care  
154 products, mismanaged solid waste landfills, and land application of biosolids (**Horton et al.**  
155 **2017b; Steinmetz et al. 2016; Rillig et al., 2017**). In developed countries, landfill sites are  
156 enclosed by fences and dumped wastes are typically shielded with soil cover or synthetic materials,

157 which help to restrict the run-off of MPs from the site. Nevertheless, proper waste management  
158 practices are in their infancy in developing or underdeveloped countries (**Duis & Coors, 2016**). A  
159 substantial accumulation of plastic wastes in the agricultural soils has been reported in several  
160 tropical and subtropical countries, with MPs from municipal wastes dumped in open agricultural  
161 fields, parks, or landfill sites. **Lwanga et al. (2016)** reported that approximately 1000-4000 MPs  
162 particles per kg of dry weight of biosolids were found in agrarian fields and dumping sites in  
163 Europe; **Fuller & Gautam (2016)** reported MPs found in different types of soil close to a  
164 commercial zone in Australia (**Table 2**). According to **Nizzetto et al. (2016b)**, due to the  
165 application of biosolids as fertilizer, up to 700,000 tons of MPs may enter agricultural fields  
166 annually in North America and Europe.

167 Properly designed and operated wastewater treatment plants (WWTPs) are able to effectively  
168 remove MPs debris up to 99.9% from the wastewater streams, but a substantial quantity of MPs  
169 remained in the biosolids (**Gies et al., 2018; Mintenig et al., 2017; Prata, 2018**). According to  
170 **Alvarenga et al. (2016)**, more than 87% of the generated biosolids from wastewater treatment was  
171 applied in the agriculture fields of Portugal in the form of composts or raw biosolids. Also, 4 to 5  
172 Mt of sludge solids are used as fertilizer in the European Union (EU) every year in agricultural  
173 lands. In some sites, commercial polymeric fibers were detected after five years of land application  
174 of biosolids. Compost application in agriculture field is also considered a significant contributor  
175 of plastics in soils. Although large- and medium-sized plastic fragments are mostly separated from  
176 the composts (**Figure 2**), minor quantity of smaller fragments formed during the process of  
177 composting (milling process) remains in the form of secondary MPs or NPs (**Bolan and Bradney,**  
178 **2019**). Polymer-based slow-release fertilizers (**Weithmann et al., 2018**) and pesticides (**Wang et**  
179 **al., 2019**) along with the weathering of plastic film mulch used over agricultural fields also result

180 in the MPs pollution in the soil environment (**Huang et al., 2020; Ramos et al., 2015; Rillig,**  
181 **2012**).

182 Personal care products (PCPs) such as gels, hand wash, shampoos, and facial cleaners are  
183 considered as the potential contributors of MPs and reach terrestrial environments via the  
184 application of biosolids and composts (**Alvarenga et al., 2016; Duis & Coors, 2016**). Polyester  
185 (PES), polystyrene (PS), and melamine are applied in different industries as abrasive materials,  
186 which are the potential sources of MPs. Other contributors of primary MPs including plastic  
187 powder and plastic resin pellets can enter the terrestrial environments due to improper handling  
188 and inefficient waste management practices (**He et al., 2018a; Karbalaee et al., 2018**). Likewise,  
189 plastic processing and recycling plants produce residues that are discharged into the environment  
190 and further transformed into MPs (**Karbalaee et al., 2018**). For example, larger quantities of raw  
191 materials utilized for manufacturing of plastics products were observed on the beaches closer to  
192 the plastic-processing and recycling plants (**Duis & Coors, 2016**).

193 Several groups of organisms such as earthworms can facilitate the conversion of primary MPs  
194 to secondary MPs and NPs by taking actions in their gizzard (**Rillig, 2012; Zhu et al., 2019**).  
195 Scraping or chewing mechanisms of Collembola or mites may lead to the generation of MPs and  
196 NPs from larger plastic debris. Likewise, burrowing mammals can also facilitate the incorporation  
197 of MPs in the soil via abrasion mechanism (**Rillig, 2012**). **Rillig et al. (2017)** reported that the  
198 activities of earthworms lead to the incorporation of PS into the soil profile from the top surface.  
199 There are various probable implications to the migration of MPs downward into deeper soil profile  
200 from the surface via existing organisms: (a) decomposition by the native microorganisms was very  
201 slow in the deeper part of the soil profile due to less microbial population (because of limited  
202 oxygen diffusion/availability), thus leading to more retention of MPs ; (b) entry of MPs in the soil

203 profile may also increase the chance of groundwater contamination with MPs and associated  
204 chemicals; (c) conversion of MPs into NPs in the soil environment via disintegration,  
205 decomposition, and abrasion may lead to further potential environmental menaces including  
206 uptake of NPs by plants (Rillig et al., 2017).

207

### 208 **3. Adverse effects of MPs in the soil environment**

209 Although MPs are defined as pure polymers of physical particles, they are often mixed with  
210 other chemical entities such as heavy metals, dioxins, and polycyclic aromatic hydrocarbons  
211 (Hong et al., 2017). Deliberate addition of extensive chemicals such flame retardants and  
212 plasticizers to the plastic products, which subsequently become MPs, can cause hazards to soil  
213 flora and fauna. A few studies demonstrated the possible pollutants transfer from MPs to beneficial  
214 soil organisms such as earthworms while others indicated the role of MPs in causing toxicity to  
215 the sludge digestive microbial flora or marine microbiota (Gaylor et al., 2013; Oliviero et al.,  
216 2019; Wei et al., 2019). During the manufacturing and processing of plastics, various chemicals  
217 and additives are used to improve the properties and the applications of the final products (Bolan  
218 et al., 2020) (Table 3). After longer exposure to the natural environment, these chemicals and  
219 additives are leached into the soil environment via slow release/desorption and photochemical  
220 degradation, causing adverse effects on soil microbial diversity and function (Bolan et al., 2020;  
221 Bolan & Bradney, 2019). Therefore, the toxicity of these chemicals associated with MPs pollution  
222 should be taken into account and carefully evaluated. A spectrum of pollutants such as pesticides  
223 (Nie et al., 2020), dyes (Kumar et al., 2019), heavy metals (Kumar et al., 2020) were found in  
224 the MPs-polluted environment. Large surface area of MPs enhances the adsorption of co-existing

225 pollutants, thus facilitating the transport and spreading of the laden contaminants that require  
226 prudent consideration and future investigations (Zhang et al., 2019).

### 227 3.1 Effects on plants

228 There are two major questions to be addressed with plants and MPs: (a) whether plants can  
229 accumulate MPs and (b) how absorbed MPs affect the plant growth and subsequently reach the  
230 food chain (Zhu et al., 2019). It is difficult to distinguish the various types of MPs in a plant tissue,  
231 which requires further studies and detailed investigations. There are different sizes of MPs  
232 identified so far including the nanoscale and microscale ones, which can get across plant's  
233 membranes and cell wall barriers and can be detected using fluorescent microbeads. Bandmann  
234 et al. (2012) observed that endocytosis assisted the entry of nano-sized (less than 100 nm)  
235 fluorescent PS beads inside a tobacco BY-2 cells, whereas edible plants were capable of  
236 incorporating micro-sized (0.2 µm) fluorescent PS beads from the environment as indicated in the  
237 whole plant culture study (Li et al. 2019). The pollution of MPs poses an additional risk to humans  
238 via trophic food chain transfer. Only few recent studies reported the adverse effects of MPs on  
239 plants (Qi et al., 2018; Rillig et al., 2019). For example, by spiking a soil sample with 1% PE  
240 plastics and biodegradable particles in the soil, both types of MPs induced negative effects on  
241 wheat plant and grain biomass, i.e., inhibition of the number and weight of the grain biomass.  
242 Although earthworms used in the same study alleviated the influence of MPs to wheat, the study  
243 did not include the examination of plant tissue containing PE particles.

### 244 3.2 Effects on soil microorganisms

245 Soil microorganisms, such as bacteria and fungi, can be affected by the exposure to  
246 overwhelming quantity of MPs (Bradney et al., 2019; Wijesekara et al., 2019). The effects of

247 MPs on soil microorganisms were investigated, including their effects on bacterial transport,  
248 spread of antibiotic resistant genes (ARGs), and overall microbial metabolisms. There was a  
249 threshold for plastic particles to bring positive or negative impacts to the microbial activity. For  
250 example, the addition of 0.05–0.4% polyester, 1 mg kg<sup>-1</sup> PS, and 0.05–0.4% polyacrylic particles  
251 stimulated negative impacts on the microbial activities (**Awet et al., 2018; de Souza Machado et**  
252 **al., 2018a**), while 7% and 28% of PP particles led to positive impacts on the microbial activities  
253 (**Liu et al., 2017**). Many parameters including polymer shape, type, concentration, and size of the  
254 MPs were variable in these studies, so it was difficult to generalize the toxic effects of MPs on the  
255 microbial activities with respect to individual variables. Regarding the structure of microbial  
256 community and soil structure, there was no direct evidence for deriving a generic conclusion on  
257 the MPs toxicity based on these studies. More importantly, the high concentrations of MPs under  
258 artificial spiking may be not representative of the field-relevant conditions.

259 The effects of MPs on microbial transport, metabolism, and genetic exchange are not  
260 scrutinized but a few recent studies may provide some insights. **He et al. (2018b)** observed that  
261 with increasing ionic strength, bacterial transport (*Escherichia coli*) in quartz sand was stimulated  
262 by the PS particles, while no noticeable difference was reported under a low ionic strength where  
263 both bacteria and PS particles displayed high mobility. The MPs were also found accountable for  
264 the exchange of genes between phylogenetic non-related microorganisms as they introduced  
265 additional exchange surface for genes and other metabolic products (**Huang et al., 2019; Sun et**  
266 **al., 2018**). Along with the array of potentially beneficial genes, there were many harmful genes  
267 such as ARGs that resulted in deleterious effects on human health upon transfer by MPs (**Arias-**  
268 **Andres et al., 2018; Huang et al., 2019; Imran et al., 2019**). **Sun et al. (2018)** observed that the  
269 retention times for ARGs and antibiotics were increased by the addition of 0.1% PS MPs to the

270 soil. More research should be conducted to provide additional and direct evidence on the ARGs  
271 transmission by MPs.

### 272 **3.3 Effects on soil animals**

273 There are a few reports focusing on the effects of MPs on aquatic animals, but very limited data  
274 are available for soil animals. A small subset of invertebrates was studied (including isopods,  
275 nematodes, collembolan, and oligochaeta) to examine the effects of MPs on the growth, survival,  
276 metabolism, gut microbiome, feeding pattern, and inflammatory reaction of soil animals (**Kim et**  
277 **al., 2019; Lei et al., 2018**). **Lei et al. (2018)** experimented with  $1 \text{ mg L}^{-1}$  PS particles and exposed  
278 a terrestrial nematode (*Caenorhabditis elegans*) to different sizes of PS particles (0.1, 0.5, 1.0, 2.0,  
279 and  $5.0 \mu\text{m}$ ) for 72 h. The nematode with the  $1.0 \mu\text{m}$  group demonstrated the shortest body length,  
280 low survival rate, short lifespan, and even downregulation of *unc-17* and *unc-47* genes expression,  
281 leading to irreversible damage of GABAergic and cholinergic neurons. These effects were  
282 attributed to the immediate uptake and high accumulation of  $1.0 \mu\text{m}$  particles by nematodes. The  
283 concentration of MPs was considered as a significant variable affecting the organisms' activities.  
284 **Zhu et al. (2018a)** conducted a concentration-dependent experiment with oligochaete *Enchytraeus*  
285 *crypticus*. Small concentrations of PS (0.025 wt% in oatmeal) in soil showed a positive impact on  
286 the weight of *Enchytraeus crypticus* while 0.5 wt% did not cause any significant changes. With 10  
287 wt%, a negative shift was observed in the weight of gut microbiome. Similar findings were  
288 validated by **Lwanga et al. (2016)**, who observed that the survival of *Lumbricus terrestris* was  
289 adversely influenced by the addition of PE MPs in 28–60% litter (corresponding to 0.8–1.7 wt%  
290 in soil), while PE MPs in 7% litter (0.2 wt% in soil) brought no significant change in the growth  
291 and survival. Cytotoxicity analysis of MPs and biodegradable plastics on oligochaeta can be traced  
292 through histological gut assessment. The addition of 0.0625–1 wt% PE MPs in soil did not cause

293 any deleterious effects on earthworm *Eisenia Andrei*, but induced immune response and tissue  
294 damage under the exposure (Rodriguez-Seijo et al., 2017). The starch-based biodegradable PE  
295 films led to more negative effects on the growth of an earthworm in comparison to conventional  
296 low-density PE. This might be because biodegradable plastics consisted of more toxic monomer  
297 units, including polybutylene terephthalate (PBT) and polyethylene terephthalate (PET), than the  
298 conventional PE plastics (Rodriguez-Seijo et al., 2017).

299 A wide range of soil species is sensitive to MPs including collembolan strains such as *Folsomia*  
300 *candida*. Experiments using  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  isotopes revealed that there was a substantial  
301 modification in the metabolic turnover, i.e., 28.8% reproduction impairment and 16.8% growth  
302 inhibition upon the addition of 0.1 wt% PVC MPs in soil (56-day exposure) (Zhu et al., 2018b).  
303 The impairment in reproduction and growth may be partly due to a shift in Collembolan  
304 oviposition sites and feeding habits due to MPs (Zhu et al., 2018b). Ju et al. (2019) observed  
305 similar results in the reproduction of *Folsomia* sp. when exposed to 0.1–1 wt% PE MPs in soil. In  
306 both studies, modified animal gut microbiome was observed upon the exposure. These results  
307 suggested that Collembolan species could be a potent indicator of MPs pollution and disturbance.  
308 Owing to the significant role in litter decomposition, isopods are also employed as testing  
309 organisms. Their energy reserve and feeding behavior were observed in the presence of 0.4 wt%  
310 PE MPs with food pellets. Various end points were investigated including body mass index (BMI),  
311 food assimilation, food ingestion, defecation rate, energy storage, and mortality. No effects were  
312 observed after 14 days of exposure, indicating low deleterious effect of MPs to isopod *Porcellio*  
313 *scaber* (Kokalj et al., 2018).

314 Recent research evaluated how MPs affected the bioaccumulation of organic pollutants, but  
315 bioaccumulation tendency of MPs was still not much explored. Better understanding is required



316 regarding the transfer of MPs from one trophic level to another and the bioaccumulation tendency  
317 of MPs in the soil environment. For instance, **Lwanga et al. (2017)** studied the increase in  
318 concentration of MPs in home garden soil by analyzing earthworm casts and chicken feces. The  
319 concentration of MPs increases throughout the trophic level with maximum concentration ( $129.8$   
320  $\pm 82.3$  particles  $g^{-1}$ ) in chicken feces with  $10.2 \pm 13.8$  MPs particles per gizzard of the chicken.  
321 This study predicted that the consumption of the chicken gizzard by humans can lead to the  
322 accumulation of 840 plastic particles/person/year. The potential long-term effects of MPs on soil  
323 organisms and soil animals should be further examined with future bioaccumulation and  
324 biomagnification studies.

325

#### 326 **4. Methods of extraction, detection, and characterization of MPs in agricultural systems**

327 The ubiquitous presence of MPs was studied in aquatic ecosystems (freshwater and marine)  
328 using a variety of analytical methods (**Mai et al., 2018; Zhang et al., 2018a**). However, the  
329 research maturity is still insufficient to develop the standardized methods for MPs quantification  
330 in the soil and sediment ecosystems (**He et al., 2018a**). Soil is the uppermost layer of earth and  
331 home to a wide array of organisms, where MPs get accumulated along with impurities and organic  
332 matter. The soil composition plays an important role in the detection of MPs via floatation,  
333 separation, and interference with infrared signaling (**von Sperber et al., 2017**). The development  
334 of cost-effective, time-efficient, and accurate methods to analyze and quantify MPs in the  
335 agricultural soils is an immediate need of the hour.

336 In order to assess the MPs pollution, proper selection of the sampling sites is required before  
337 analysis and quantification can help us assess the actual status of the site. Collection of the MPs

338 samples is a crucial step in the analytical procedures. Different layers of top and bottom soil should  
339 be collected depending on the soil characteristics (Liu et al., 2018; Zhou et al., 2018). Then,  
340 density separation should be performed depending on the percentage of organic matter and clay  
341 on the dried, sieved, dispersed, filtered, and separated soil sample (He et al., 2018a). Density  
342 extraction and SOM digestion are carried out after which the extracted MPs can be visualized with  
343 an optical microscope. Raman and micro-Fourier transformed infrared ( $\mu$ -FT-IR) spectroscopy are  
344 used for fingerprinting the types and distribution of MPs (Liu et al., 2018; Peng et al., 2017). The  
345 above protocol involving repetitive sieving and density separation is appropriate for analyzing the  
346 soil samples but requires future standardization of each procedure.

#### 347 **4.1 Extraction procedure**

348 Variable sizes of sieves are used in consideration of the sample type and requirement.  
349 According to NOAA (2015) procedures, dry samples are initially sieved through a 5 mm size  
350 sieve, followed by stacked 5 mm and 0.3 mm sieves to segregate the disaggregated sediments as  
351 shown in **Figure 3**. Unlike sediment and water column samples, the soil samples are primarily  
352 segregated through a 2 mm size sieve after density separation of MPs (Zhang & Liu, 2018). MPs  
353 elements can be separated from the soil sample matrix using salt solutions with known  
354 concentrations as plastic particles float over high-density solution. The concentration of salt  
355 solution should be based on the density of the MPs as the concentration of  $1.18 \text{ g cm}^{-3}$  NaCl  
356 solutions is not enough to separate high-density plastics such as PVC and PET (NOAA, 2015).

357 A feasible and low-cost method for the extraction of light-density plastic particles including  
358 PP and PE from the soil was developed using distilled water with plastic recovery rates of almost  
359 90% (Zhang et al., 2018b). The soil sample underwent heat treatment (3–5 s at  $130 \text{ }^\circ\text{C}$ ), which  
360 made the MPs present in the sample melt and converted into circular transparent particles that can

361 float on the water surface and be separated easily while other components, such as organic matter  
362 and minerals, remain in their native form. **Liu et al. (2018)** used NaCl for MPs separation from  
363 the agriculture soil sample with the ultrasonic treatment over prolonged floatation time. Seven out  
364 of nine spiked MPs types were successfully extracted, including polymethyl methacrylate  
365 (PMMA), acrylonitrile butadiene styrene (ABS), polycarbonate (PC), polyamide (PA), PE, PP,  
366 and PS. The method was designed for PVC and PET but was found to be ineffective (**Liu et al.,**  
367 **2018**). Different solutions were then proposed such as CaCl<sub>2</sub>, which was efficient in the extraction  
368 but led to agglomeration of organic matter and hindered the MPs identification (**Scheurer &**  
369 **Bigalke, 2018**).

370 **Van Cauwenberghe et al. (2015)** suggested that the extraction solution would be efficient for  
371 density range of 1.6–1.8 g cm<sup>-3</sup>, which was attainable through the usage of NaI or ZnCl<sub>2</sub> along  
372 with the addition of acid solution. However, these solutions are not environmentally friendly. Also,  
373 acid solution can alter the characteristics of MPs in the soil samples. Other methods utilizing  
374 oleophilic properties and electrostatic behavior of MPs were proposed. The oil extraction protocol  
375 was efficient for achieving 90% recovery of MPs (**Crichton et al., 2017**), while the electrostatic  
376 behavior was 100% efficient in recovering plastics from multiple environmental matrices  
377 including beach sand, water, and sediments (**Felsing et al., 2018**). However, their applicability for  
378 a large-scale extraction of MPs from the soil samples is uncertain.

#### 379 **4.2 Removal of SOM**

380 Density fractionization was not efficient enough to separate SOM from the soil samples  
381 because they have a similar density (1.0 and 1.4 g cm<sup>-3</sup>) to several plastics such as nylon and PET  
382 (**Bläsing & Amelung, 2018**). Therefore, earlier studies utilized acids, alkalis, enzymatic digestion,  
383 or oxidizing treatment (**Dehaut et al., 2016; Jabeen et a., 2017**). **Hurley et al. (2018)** tested the

384 effectiveness of different reagents, including 30% H<sub>2</sub>O<sub>2</sub> solution, 10% KOH, 10 M NaOH, and  
385 Fenton's reagent, of which Fenton's reagent in combination with density separation was found to  
386 be the most suitable for SOM removal in the soil MPs assessment. However, these reagents were  
387 likely to damage or modify the MPs. For instance, HNO<sub>3</sub> solution removed organic matter in a  
388 short span but also damaged MPs of PET, PA, and ABS (Dehaut et al., 2016; Rillig et al., 2017),  
389 while alkali treatment led to plastic abrasion (Hurley et al., 2018). Meanwhile, H<sub>2</sub>O<sub>2</sub> would change  
390 the structural morphology of PP and PE plastic but the change was minimal when it was used for  
391 digestion at 70 °C. Therefore, H<sub>2</sub>O<sub>2</sub> was considered as the most favorable oxidizing agent for the  
392 removal of organic matter from the environment matrices (He et al., 2018a; Hurley et al., 2018;  
393 Jabeen et al., 2017; Zhang et al., 2018b). Liu et al. (2018) confirmed that the oxidation by H<sub>2</sub>O<sub>2</sub>  
394 was efficient for SOM removal from the agricultural soils.

#### 395 4.3 Identification and characterization

396 Subsequent to the isolation of MPs from environmental samples, several techniques are  
397 applied for the identification, quantification, and characterization of MPs as listed in **Table 4**. The  
398 samples are first visually identified for their surface texture after the classification based on their  
399 shape, size, and color (He et al., 2018a). Chemical classification is performed using gas  
400 chromatography mass spectroscopy, Raman, or infrared spectroscopy (Li et al., 2018). MPs are  
401 sorted and grouped according to their sizes. In addition, MPs are sorted in terms of their shapes,  
402 namely fragment, fiber, foam, bead, and film. Although visual identification is a feasible step to  
403 start classifying an enormous variety of MPs, it possesses its own limitations with human errors.  
404 Eriksen et al. (2013) reported 20% of aluminum silicate particles being visually misinterpreted as  
405 MPs through a confirmation by SEM. Lenz et al. (2015) reported that nearly 70% of the particles  
406 were erroneously labeled as MPs using FTIR and 32% of visually labelled MPs were not identified

407 by  $\mu$ -Raman spectroscopy. There was a need for combining visual identification with other  
408 confirmatory physical and chemical techniques in the classification of MPs. SEM provides high  
409 magnification image of the sample but requires longer time and higher costs (sample preparation,  
410 coating, analytical cost). The sample coatings may disturb the surface texture and result in  
411 inaccuracies in the detection of MPs (**Bläsing & Amelung, 2018; Shim et al., 2017; Zhou et al.,**  
412 **2018**).

413 One of the most popular non-destructive chemical techniques to identify MPs is infrared  
414 microscopy along with  $\mu$ -Raman, attenuated total reflectance (ATR), and  $\mu$ -FT-IR spectroscopy,  
415 which offer an advantage of microspectrometry coupled with automated scanning (**Bläsing &**  
416 **Amelung, 2018**).  $\mu$ -Raman is more sensitive than  $\mu$ -FTIR as it can detect MPs size as small as 1  
417  $\mu\text{m}$  while  $\mu$ -FTIR is effective for particles size  $>10\text{-}20\ \mu\text{m}$  (**Cai et al., 2017**). The presence of  
418 organic matter interferes with the signaling, which can be avoided using a fluorescent background  
419 in the case of Raman spectroscopy (**Silva et al., 2018; von Sperber et al., 2017**). Both techniques  
420 are time-consuming and costly but provide reliable information on MPs. There are a few  
421 alternative techniques available such as hyperspectral imaging which can visualize 0.5 to 5  $\mu\text{m}$   
422 particles on the soil surface, and macroscopic dimensioned near-infrared spectroscopy combined  
423 with chemometrics which is rapid and does not require any chemical pretreatment (**Paul et al.,**  
424 **2019; Shan et al., 2018**). Another technique of thermal extraction desorption-gas chromatography-  
425 mass spectrometry (TED-GC-MS) can achieve accurate and specific quantification of PP, PE, PS,  
426 and PET (**Dümichen et al., 2017**).

427 Many parameters remain largely variable even after classifications by means of environmental  
428 factors, temporal and spatial variability patterns, reporting units, etc. It is difficult to characterize  
429 MPs in the organic matter-rich agricultural soils, and there are disparities to compare the data and

430 generate reproducible results owing to the use of different techniques. Therefore, standardization  
431 of methodological protocols is essential for effective comparison and monitoring.

432

## 433 **5. Challenges and future directions**

434 The pollution of MPs is worsening globally and the related hazards in agricultural soils need  
435 proper attention. This review describes the analytical techniques, ecological risks, and pollution  
436 characteristics of MPs in the soil environment. Scientific knowledge of MPs encompassing the  
437 sources, fate, environmental concentrations, analytical techniques, and ecological consequence are  
438 reviewed. There are noticeable knowledge gaps that demand more concerted efforts in future  
439 research, and the following challenges are of high priority:

- 440 • There is no standard protocol to isolate, quantify and characterize MPs from soil  
441 environment. It is vital to develop a precise, feasible, and efficient assay for multiple MPs  
442 identification and characterization. Future research should focus on devising a testing  
443 protocol that takes into account the variable environmental soil conditions and the  
444 heterogeneity of MPs. Depending on the origin, shape, size, and composition of MPs, it is  
445 necessary to standardize specific methods for sample collection, isolation, identification,  
446 and analysis of MPs in the organic matter-rich agricultural soils.
- 447 • There is only a limited size of database concerning the sources and fate of MPs in the soil  
448 environment and their interactions with microbes, food crops, and soil animals. We need  
449 to evaluate the distribution, transport, and degradation of MPs to holistically reveal their  
450 environmental effects and implications in the agricultural field. With time MPs are  
451 degraded partially by a range of physicochemical and microbiological drivers. It is crucial

452 to identify the individual roles of relevant natural and anthropogenic activities to elucidate  
453 the long-term fate of MPs in the soil ecosystem.

454 • Currently, the global and regional data inventory (types, concentration, composition, and  
455 types of MPs) for the pollution status of MPs in the agricultural systems and soil  
456 environment are very limited and require substantial expansion. Future research should be  
457 extended to the qualitative characterization and quantitative assessment of the MPs in  
458 different types of agricultural soils with different cropping systems under variable  
459 climates, as well as their interactions and transformations in the rhizosphere.

460 • MPs are recognized as emerging persistent pollutants that may transfer across different  
461 trophic levels along a food web. It is crucial to determine its potential cytotoxic effects on  
462 soil flora and animals/humans and evaluate the apparent transgenerational effects. It is also  
463 necessary to investigate both natural and engineered ecosystems to study the behavioral  
464 responses of plants, animals, and other microbial assemblages to widespread MPs pollution  
465 in the agricultural soils.

466 • MPs as vectors of a broad range of environmental pollutants may facilitate or inhibit the  
467 mobility and bioavailability of these environmentally persistent and potentially hazardous  
468 pollutants in the agricultural soils. Although this area has been extensively examined in the  
469 aquatic ecosystems, limited knowledge is available for different terrestrial ecosystems and  
470 needs to be properly addressed by future research studies.

471 • With the increasing recognition of the hazardous effects of MPs, behavioral change of  
472 consumers and plastics manufacturers would be necessary for attaining sustainable plastic  
473 waste management. Well-aligned initiatives, best management practices, more stringent  
474 policies, and joint efforts of citizens and government officials are urgently needed to reduce

475 illegal disposal of plastic wastes and improper use of plastic products in the agricultural  
476 industry for the sake of food safety and sustainable development.

477

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484

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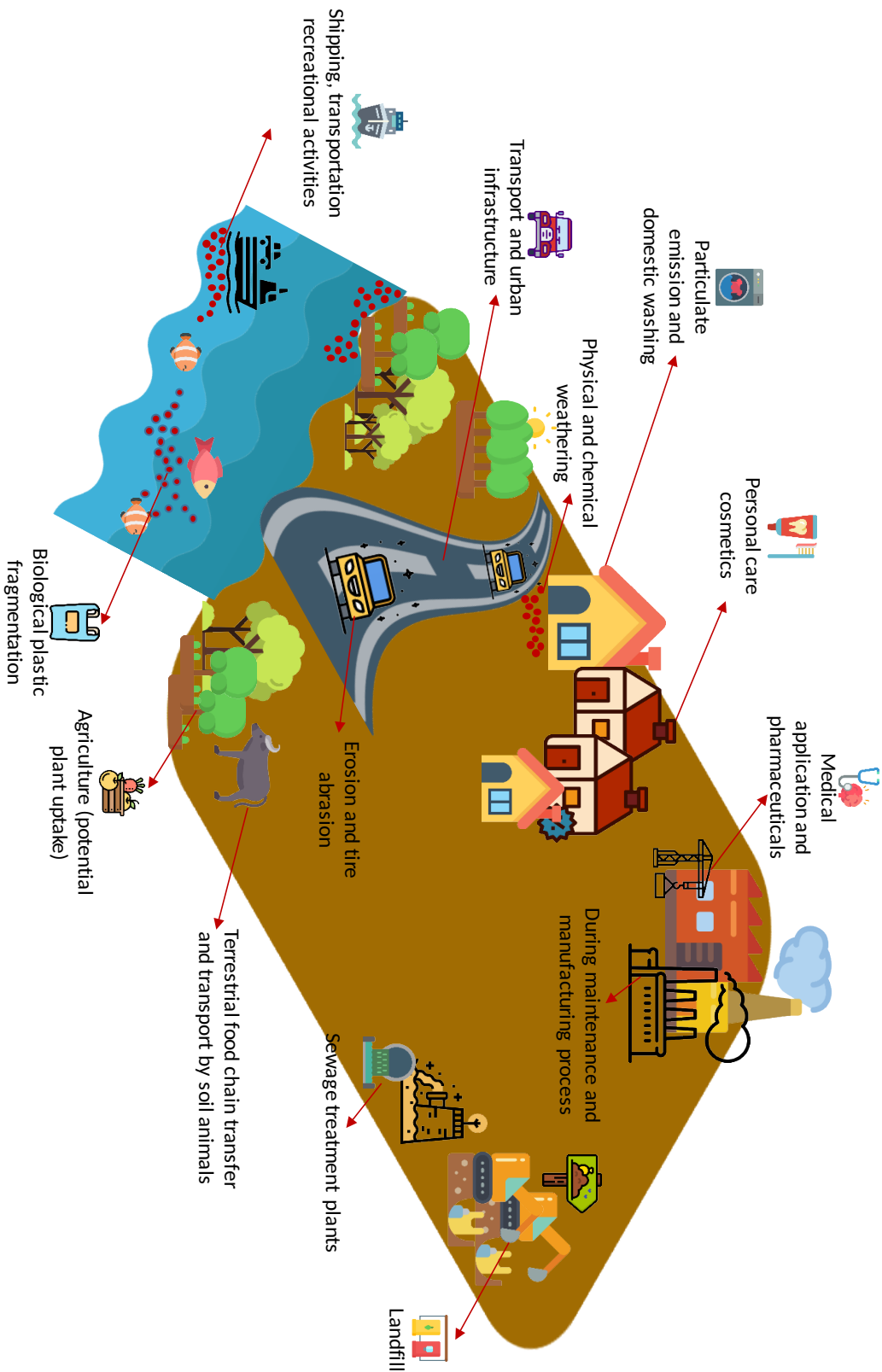
**Figure.1:** Generation and dispersion of microplastics in terrestrial environments. (adapted and modified from **Karbalaei et al., 2018**)

**Figure.2:** Compost is one of the major sources of MPs and NPs input to agricultural soils (picture taken in Australia by the authors).

**Figure.3:** Schematic representation of microplastics extraction from soil sample (adapted and modified from **He et al., 2018a**)



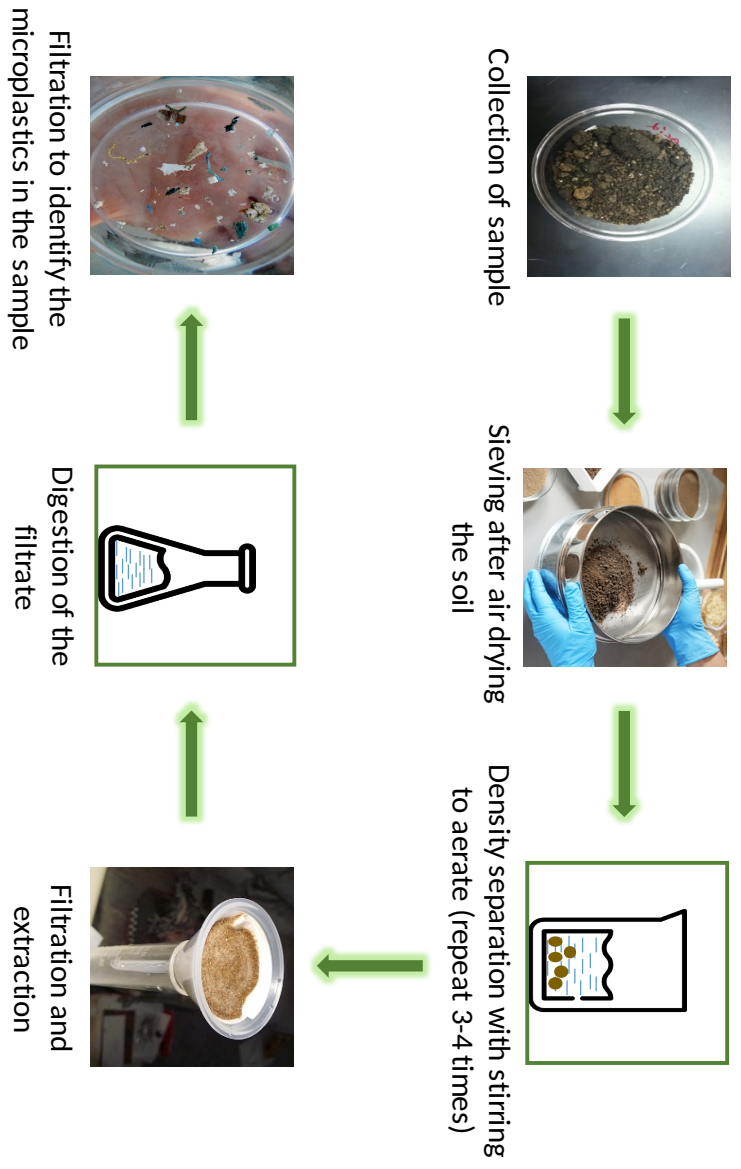
Figure.1:



**Figure.2:**



**Figure.3:**



**Table.1:** Representation of waste management statistics and plastic waste estimates released to freshwater (continental) and terrestrial environments [based on figures for European Union (EU)].

**Table.2:** Occurrence and characteristics of microplastics in terrestrial environments.

**Table 3.** Additives/chemicals used in manufacturing and processing of the plastics.

**Table.4:** Analytical techniques for Identification and characterization of MPs and its advantages and disadvantages.

**Table.1:**

<b>Plastic (handling/disposal)</b>	<b>Plastic (million metric tons/year)</b>	<b>Reference</b>
Plastic production (EU total, 2014)	59	(PlasticsEurope, 2015)
Plastic waste (EU total, 2014)	25.8	(PlasticsEurope, 2015)
Managed plastic waste (-2% mismanaged waste)	25.28	(Nizzetto et al., 2016b)
Mismanaged plastic waste (2% of plastic waste in the EU)	0.52	(Horton et al., 2017b; Jambeck et al., 2015)
Total mismanaged plastic waste outstanding in continental environments (EU)	0.47–0.91	(Horton et al., 2017b)
Landfill (EU total)	8	(PlasticsEurope, 2015)
Recycling (EU total)	7.6	(PlasticsEurope, 2015)
Energy recovery (EU total)	10.2	(PlasticsEurope, 2015)
Plastic in sewage sludge (EU total)	0.063–0.43	(Nizzetto et al., 2016b)
Ocean input (EU total)	0.04–0.11	(Jambeck et al., 2015)

**Table.2:**

Soil source/Soil type	Country and location	Abundance	Composition	Size		Reference
				range (mm)	Shape	
Rice-fish coculture ecosystems	China (Shanghai)	10.3 ± 2.2 item kg <sup>-1</sup>	PE, PP	< 1	Mainly fibers	<b>Lv et al., 2019</b>
Vegetable fields	China (Shanghai)	78.00 ± 12.91 62.50 ± 12.97 item kg <sup>-1</sup>	PE, PP, Polyethersulfone (PES)	0.03-16	Fiber, film, fragment	<b>Liu et al., 2018</b>
Agricultural field	China (Loess plateau)	<0.54 mg kg <sup>-1</sup>	PE, PP	> 0.1	-	<b>Zhang et al., 2018b</b>
Tree planted soils	China (Yunnan)	7100-42,960 item kg <sup>-1</sup>	-	0.05-10	Mainly fibers	<b>Zhang and Liu, (2018)</b>

Beach soil	China (Hebei)	317 item/500 g (average)	-	1.56 ± 0.63	Fragments, granules, fibers and films	<b>Zhou et al., 2016</b>
Coastline soil	China (Shandong)	1.3- 14,712.5 item kg <sup>-1</sup>	PP, PE, PES, polyether urethane	60% in size of <1 mm	Foams, fibers, pellets, flakes, fragments, films and sponges	<b>Zhou et al., 2018</b>
Industrial soil	Australia (Sydney)	300-67,500 mg kg <sup>-1</sup>	PE, PS, PVC	-	-	<b>Fuller and Gautam, (2016)</b>
Floodplain soil	Switzerland	up to 55.5 mg kg <sup>-1</sup> or 593 item kg <sup>-1</sup>	PE, PS, PVC	< 0.5	-	<b>Scheurer and Bigalke, (2018)</b>

**Table.3:**

<b>Types of additives/chemicals</b>	<b>Example</b>	<b>Function</b>	<b>Reference</b>
Lubricants	Molybdenum disulfide and graphite	Flexible plastic manufacturing used in squeeze bottles and fiber	<b>(Biron, 2003)</b>
Flame retardants	Decabromodiphenyl ether	Improve the safety index of cultured marble and cable coverings	<b>(Strååt &amp; Nilsson, 2018)</b>
Antioxidants	Tris(2,4-di-tert-butylphenyl) phosphite, Pentaerythritol tetrakis (3,5-di-tert-butyl-4-hydroxyhydrocinnamate)	Deal with resistance against weathering and useful in plastic processing	<b>(Hansen et al., 2013; Hahladakis et al., 2018)</b>
Anti-statics	Glycerol monostearate, Indium tin oxide	Generate static electricity attraction (reduce dust collection)	<b>(Gächter et al., 1993)</b>



Foaming agents	Isocyanate, Chlorofluorocarbons	Useful in the production of building board, polystyrene cups and polyurethane carpet underlayment	<b>(Gächter et al., 1993)</b>
Antimicrobials	2,4-dichloro- 6-(3,5-dichloro- 2-hydroxyphenyl) sulfanylphenol	(Bithionol) and shower curtains; control formation of biofilm	<b>(Gächter et al., 1993)</b>
Plasticizers	Bis(2-ethylhexyl) phthalate	Utilized in gutters, wire insulation and flooring owing to slow decomposition from light	<b>Bhunia et al., 2013; Sablani &amp; Rahman, 2007)</b>
Colorants	Sudan stain, Diarylide pigment	Useful in coloring plastic products	<b>(Biron, 2003; Hahladakis et al., 2018)</b>

**Table.4:**

<b>Technique</b>	<b>Particle size range</b>	<b>Methodology</b>	<b>Advantages</b>	<b>Limitations</b>	<b>Reference</b>
<b>Spectroscopic</b>					
$\mu$ -FTIR	Particles > 500 $\mu\text{m}$ and smaller to 20 $\mu\text{m}$ can be investigated by ATR-FTIR and microscopy coupled FTIR respectively.	Depending on the molecular structure and composition of the substance, samples are exposed to defined range of IR-radiation.	Emerging, quick, and reliable nondestructive method.	Only for IR active sample, difficult to analyze nontransparent particle and particles below 20 $\mu\text{m}$ , pretreatment required expensive.	<b>(Li et al., 2018; He et al., 2018a)</b>
$\mu$ -Raman	Particles size > 1 $\mu\text{m}$ but also works for 1 to 20 $\mu\text{m}$	The shift in Raman spectra can be measured, that provided substance specific spectra.	Analyzed particle size 1-20 $\mu\text{m}$ . Nontransparent, dark particles can be analyzed.	Samples required refinements before analysis, time intensive procedure.	<b>(Li et al., 2018; He et al., 2018a)</b>

SEM	Even microscale particles can be analyzed	Generated electron interacts with the sample which eventually measures the secondary ions.	Generate high resolution images	Samples required coating, less informative.	<b>(Bläsing &amp; Amelung, 2018; Zhou et al., 2018; de Souza Machado et al., 2018a)</b>
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### Chromatographic

Pyrolysis GC-MS	Works well with particles size > 500 µm.	The GC column coupled to a quadrupole – MS. the generated spectra are identified comparing with available common plastic database.	It is quite sensitive, easier and reliable method which avoids possible background contamination	The plastics database is limited, single sample at a time with certain weight. single sample at a time with certain weight.	<b>(Dümichen et al., 2017; Ivleva et al., 2017)</b>
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Liquid Chromatography (LC)	Applied for large sample large sample size.	Samples are prepared in selective for analysis.	Selected polymers displayed better recovery.	Restricted to specific polymers (PE and PET), not recommended for environmental samples.	<b>(Hintersteiner et al., 2015; Elert et al., 2017)</b>
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<b>Visual</b>					
Microscopic Counting	Stereomicroscope	The particles are counted directly and identified.	Cost effective, faster and easier.,	It cannot determine the nature of the sample.	<b>(He et al., 2018a)</b>
Tagging	Microscale MPs	MPs are irradiated with blue light and adsorption of hydrophobic dye is done which renders them fluorescent.	It is a quick, inexpensive, and easier.	Impurities in the samples may leads to overestimation of MPs.	<b>(Shim et al., 2016; He et al., 2018a)</b>