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1	Microplastics as pollutants in agricultural soils
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Dissemination of MPs in the environment (Pharmaceuticals, Transportation, Agriculture, Compost)

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



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

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

22 Abstract

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

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

39

40 **1. Introduction**

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

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

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

67 undergo weathering processes such as degradation and disintegration due to the collective actions

of physio-chemical and biological factors (de Souza Machado et al., 2018a; Whitacre, 2014).

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

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

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

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

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

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

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

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

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

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

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

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

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

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

207

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

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

227 **3.1 Effects on plants**

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

244 **3.2 Effects on soil microorganisms**

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

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

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 with increasing ionic strength, bacterial transport (Escherichia coli) in quartz sand was stimulated 261 262 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 additional exchange surface for genes and other metabolic products (Huang et al., 2019; Sun et 265 al., 2018). Along with the array of potentially beneficial genes, there were many harmful genes 266 267 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 retention times for ARGs and antibiotics were increased by the addition of 0.1% PS MPs to the 269

soil. More research should be conducted to provide additional and direct evidence on the ARGstransmission by MPs.

272 **3.3 Effects on soil animals**

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

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

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

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

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

325

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

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

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

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

347 **4.1 Extraction procedure**

Variable sizes of sieves are used in consideration of the sample type and requirement. 348 According to NOAA (2015) procedures, dry samples are initially sieved through a 5 mm size 349 350 sieve, followed by stacked 5 mm and 0.3 mm sieves to segregate the disaggregated sediments as shown in Figure 3. Unlike sediment and water column samples, the soil samples are primarily 351 segregated through a 2 mm size sieve after density separation of MPs (Zhang & Liu, 2018). MPs 352 353 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 solution should be based on the density of the MPs as the concentration of 1.18 g cm⁻³ NaCl 355 356 solutions is not enough to separate high-density plastics such as PVC and PET (NOAA, 2015).

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

float on the water surface and be separated easily while other components, such as organic matter 361 and minerals, remain in their native form. Liu et al. (2018) used NaCl for MPs separation from 362 the agriculture soil sample with the ultrasonic treatment over prolonged floatation time. Seven out 363 of nine spiked MPs types were successfully extracted, including polymethyl methacrylate 364 (PMMA), acrylonitrile butadiene styrene (ABS), polycarbonate (PC), polyamide (PA), PE, PP, 365 and PS. The method was designed for PVC and PET but was found to be ineffective (Liu et al., 366 2018). Different solutions were then proposed such as CaCl₂, which was efficient in the extraction 367 368 but led to agglomeration of organic matter and hindered the MPs identification (Scheurer & **Bigalke**, 2018). 369

Van Cauwenberghe et al. (2015) suggested that the extraction solution would be efficient for 370 density range of 1.6–1.8 g cm⁻³, which was attainable through the usage of NaI or ZnCl₂ along 371 with the addition of acid solution. However, these solutions are not environmentally friendly. Also, 372 acid solution can alter the characteristics of MPs in the soil samples. Other methods utilizing 373 oleophilic properties and electrostatic behavior of MPs were proposed. The oil extraction protocol 374 was efficient for achieving 90% recovery of MPs (Crichton et al., 2017), while the electrostatic 375 behavior was 100% efficient in recovering plastics from multiple environmental matrices 376 including beach sand, water, and sediments (Felsing et al., 2018). However, their applicability for 377 a large-scale extraction of MPs from the soil samples is uncertain. 378

379 4.2 Removal of SOM

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

effectiveness of different reagents, including 30% H₂O₂ solution, 10% KOH, 10 M NaOH, and 384 Fenton's reagent, of which Fenton's reagent in combination with density separation was found to 385 be the most suitable for SOM removal in the soil MPs assessment. However, these reagents were 386 likely to damage or modify the MPs. For instance, HNO₃ solution removed organic matter in a 387 388 short span but also damaged MPs of PET, PA, and ABS (Dehaut et al., 2016; Rillig et al., 2017), while alkali treatment led to plastic abrasion (Hurley et al., 2018). Meanwhile, H₂O₂ would change 389 the structural morphology of PP and PE plastic but the change was minimal when it was used for 390 391 digestion at 70 °C. Therefore, H₂O₂ was considered as the most favorable oxidizing agent for the removal of organic matter from the environment matrices (He et al., 2018a; Hurley et al., 2018; 392 393 Jabeen et a., 2017; Zhang et al., 2018b). Liu et al. (2018) confirmed that the oxidation by H_2O_2 394 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 applied for the identification, quantification, and characterization of MPs as listed in Table 4. The 397 samples are first visually identified for their surface texture after the classification based on their 398 399 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 MPs through a confirmation by SEM. Lenz et al. (2015) reported that nearly 70% of the particles 405 406 were erroneously labeled as MPs using FTIR and 32% of visually labelled MPs were not identified by μ-Raman spectroscopy. There was a need for combining visual identification with other
confirmatory physical and chemical techniques in the classification of MPs. SEM provides high
magnification image of the sample but requires longer time and higher costs (sample preparation,
coating, analytical cost). The sample coatings may disturb the surface texture and result in
inaccuracies in the detection of MPs (Bläsing & Amelung, 2018; Shim et al., 2017; Zhoa et al.,
2018).

One of the most popular non-destructive chemical techniques to identify MPs is infrared 413 414 microscopy along with μ -Raman, attenuated total reflectance (ATR), and μ -FT-IR spectroscopy, which offer an advantage of microspectrometry coupled with automated scanning (Bläsing & 415 Amelung, 2018). µ-Raman is more sensitive than µ-FTIR as it can detect MPs size as small as 1 416 417 μ m while μ -FTIR is effective for particles size >10-20 μ m (Cai et al., 2017). The presence of organic matter interferes with the signaling, which can be avoided using a fluorescent background 418 419 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 alternative techniques available such as hyperspectral imaging which can visualize 0.5 to 5 µm 421 422 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, and PET (Dümichen et al., 2017). 426

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

- generate reproducible results owing to the use of different techniques. Therefore, standardizationof methodological protocols is essential for effective comparison and monitoring.
- 432
- 433 **5.** Challenges and future directions

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

There is no standard protocol to isolate, quantify and characterize MPs from soil
 environment. It is vital to develop a precise, feasible, and efficient assay for multiple MPs
 identification and characterization. Future research should focus on devising a testing
 protocol that takes into account the variable environmental soil conditions and the
 heterogeneity of MPs. Depending on the origin, shape, size, and composition of MPs, it is
 necessary to standardize specific methods for sample collection, isolation, identification,
 and analysis of MPs in the organic matter-rich agricultural soils.

There is only a limited size of database concerning the sources and fate of MPs in the soil
 environment and their interactions with microbes, food crops, and soil animals. We need
 to evaluate the distribution, transport, and degradation of MPs to holistically reveal their
 environmental effects and implications in the agricultural field. With time MPs are
 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 elucidate453 the long-term fate of MPs in the soil ecosystem.

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

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

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

With the increasing recognition of the hazardous effects of MPs, behavioral change of
 consumers and plastics manufacturers would be necessary for attaining sustainable plastic
 waste management. Well-aligned initiatives, best management practices, more stringent
 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 agricultural476 industry for the sake of food safety and sustainable development.

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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)



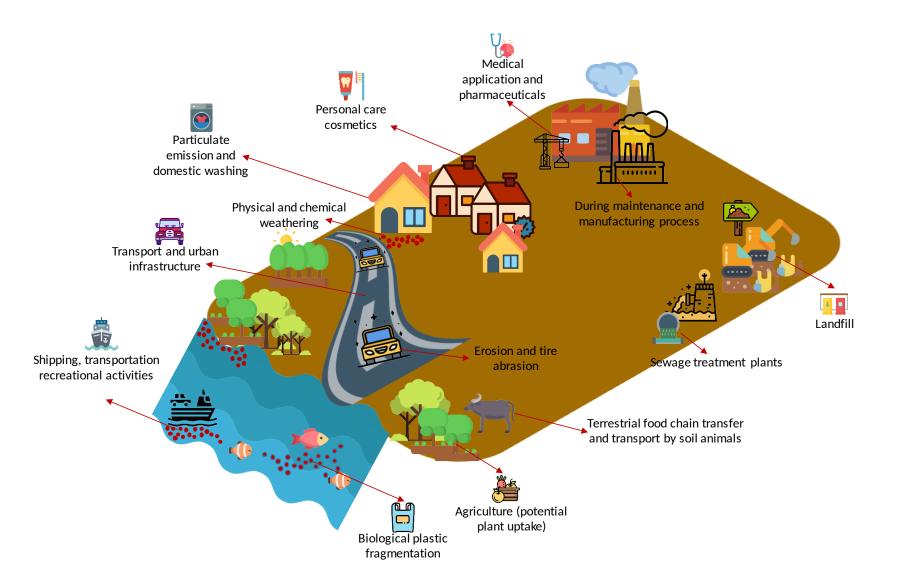


Figure.2:

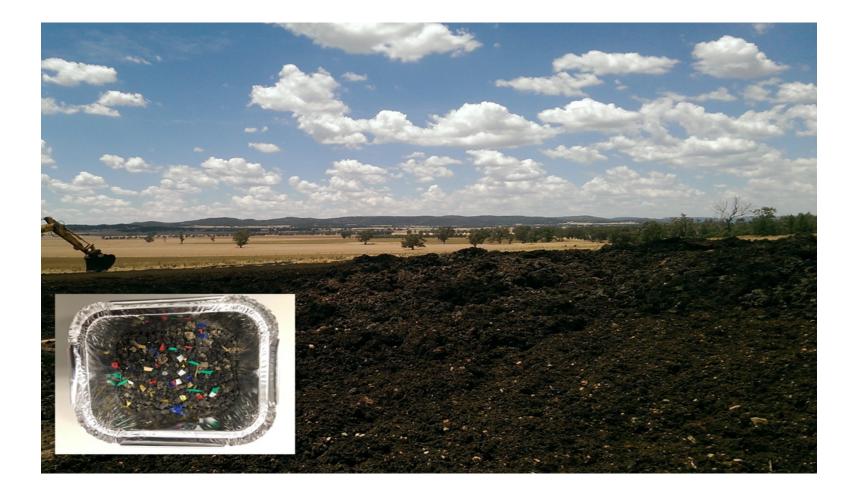


Figure.3:



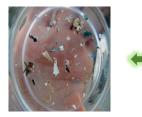
Collection of sample



Sieving after air drying the soil



Density separation with stirring to aerate (repeat 3-4 times)



Filtration to identify the microplastics in the sample



Digestion of the filtrate



Filtration and extraction

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:

Plastic (handling/disposal)	Plastic (million	Reference
Tastic (nandning/disposar)	metric tons/year)	
Plastic production (EU total, 2014)	59	(PlasticsEurope,
Thashe production (LO total, 2014)	57	2015)
Plastic waste (EU total, 2014)	25.8	(PlasticsEurope,
	20.0	2015)
Managed plastic waste (-2% mismanaged waste)	25.28	(Nizzetto et al.,
		2016b)
Mismanaged plastic waste (2% of plastic waste in		(Horton et al.,
the EU)	0.52	2017b; Jambeck
		et al., 2015)
Total mismanaged plastic waste outstanding in	0.47–0.91	(Horton et al.,
continental environments (EU)		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	Country			Size		
source/Soil	and	Abundance	Composition	range	Shape	Reference
type	location			(mm)		
Rice-fish coculture ecosystems	China (Shanghai)	10.3 ± 2.2 item kg ⁻¹	PE, PP	< 1	Mainly fibers	Lv et al., 2019
Vegetable fields	China (Shanghai)	$78.00 \pm$ 12.91 62.50 ± 12.97 item kg ⁻¹	PE, PP, Polyethersulfone (PES)	0.03- 16	Fiber, film, fragment	Liu et al., 2018
Agricultural field	China (Loess plateau)	<0.54 mg kg ⁻¹	PE, PP	> 0.1	-	Zhang et al., 2018b
Tree planted soils	China (Yunnan)	7100- 42,960 item kg ⁻¹	-	0.05- 10	Mainly fibers	Zhang and Liu, (2018)

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

Tabl	e.3:
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Types of additives/chemicals	Example	Function	Reference
Lubricants	Molybdenum disulfide and graphite	Flexible plastic manufacturing used in squeeze bottles and fiber	(Biron, 2003)
Flame retardants	Decabromodiphenyl ether	Improve the safety index of cultured marble and cable coverings	(Strååt & Nilsson, 2018)
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	(Hansen et al., 2013; Hahladakis et al., 2018)
Anti-statics	Glycerol monostearate, Indium tin oxide	Generate static electricity attraction (reduce dust collection)	(Gächter et al., 1993)

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

Table.4:

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

SEM	Even microscale	Generated electron	Generate high	Samples required	(Bläsing &
	particles can be	interacts with the	resolution images	coating, less	Amelung, 2018;
	-		resolution inages	informative.	
	analyzed	sample which		momative.	Zhou et al., 2018;
		eventually			de Souza Machado
		measures the			et al., 2018a)
		secondary ions.			
Chromatographi	c				
Pyrolysis GC-	Works well with	The GC column	It is quite	The plastics	(Dümichen et al.,
MS	particles size	coupled to a	sensitive, easier	database is limited,	2017; Ivleva et al.,
	> 500 µm.	quadrupole – MS.	and reliable	single sample at a	2017)
		the generated	method which	time with certain	
		spectra are	avoids possible	weight. single	
		identified	background	sample at a time	
		comparing with	contamination	with certain	
		available common		weight.	
		plastic database.			
Liquid	Applied for large	Samples are	Selected	Restricted to specific	(Hintersteiner et
Chromatography	sample large	prepared in	polymers	polymers (PE and	al., 2015; Elert et
(LC)	sample size.	selective for	displayed better	PET), not	al., 2017)
		analysis.	recovery.	recommended for	
				environmental	
				samples.	

Visual					
Microscopic	Stereomicroscope	The	Cost effective,	It cannot determine	(He et al., 2018a)
Counting	can identify	particles are	faster and easier.,	the nature of the	
	particles size	counted directly		sample.	
	down to µm.	and identified.			
Tagging	Microscale MPs	MPs are irradiated	It is a quick,	Impurities in the	(Shim et al., 2016;
	can be counted	with blue light and	inexpensive, and	samples may leads	He et al., 2018a)
	and visualized.	adsorption of	easier.	to overestimation	
		hydrophobic dye is		of MPs.	
		done which			
		renders them			
		fluorescent.			