



REVIEW PAPER

Microplastics on the growth of plants and seed germination in aquatic and terrestrial ecosystems

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ABSTRACT

Growth of plants, apart from being complex and highly dynamic, is directly dependent on the environmental conditions, particularly the quality of soil for terrestrial plants and the water quality for aquatic plants. Presence of microplastics in the environment may affect the plant growth in numerous ways depending on the contents of the growing medium. However, increasing presence of microplastics at an alarming rate due to its pervasive usage and mismanagement of plastics have led to significant environmental problems. Several research studies have been conducted as well as reviewed to investigate the toxic effects of microplastics on aquatic systems, but studies that investigate the toxic effect of microplastics on the terrestrial systems are limited. Hence, in this review the individual and the combined effects of microplastics on the growth of plants and seed germination in both aquatic and terrestrial ecosystems are concisely discussed. At the beginning accumulation of microplastics on aquatic and terrestrial ecosystem is discussed and the reasonable solutions are highlighted that can mitigate the effects from the widespread increase of the plastic debris. Thereafter, the individual and combined effect of microplastics on seed germination and plant growth is reviewed separately while summarizing the important aspects and future perspectives. This review will provide an insight into the existing gap in the current research works and thus could offer possible implications on the effect of microplastics on plant growth and seed germination in aquatic and terrestrial ecosystem.

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INTRODUCTION

Widespread usage and mismanagement of plastic have been identified as a growing environmental effect in both aquatic (Wright *et al.*, 2013) and terrestrial ecosystems (Bläsing and Amelung 2018). Microplastics are considered to be contributing to plastic debris which are mainly categorized as primary and secondary microplastics depending on their formation (Nel and Froneman, 2015). The plastic debris whose initial particle size is less than 5 mm when manufactured are called “primary microplastics” and they are intentionally added to such as scrubbing agents, toiletries and cosmetics (Boucher and Friot 2017) or as ingredient for larger plastic productions (Nel and Froneman, 2015). The secondary microplastics is the most common source of pollution in the aquatic system which originates from fragmentation of larger plastic particles through photodegradation, biodegradation and other weathering processes such as thermo-oxidative degradation, mechanical degradation, and physical stress (Andrady, 2011). Different definitions exist for describing the range of size of microplastics

and nanoplastics, but in general microplastics is considered as particles ranging from 100 nm to 5 mm in size and nanoplastics as particles lower than 100 nm in size (Hernandez *et al.*, 2019). Fig. 1 gives an illustration of the types of sources in the terrestrial and aquatic environments, methods of degradation and fragmentation processes with the corresponding ranges in the size of plastic debris.

Several research studies regarding the effect of microplastics on plant growth have been conducted (Bhattacharya *et al.*, 2010; Jiang *et al.*, 2019; Prata *et al.*, 2018). According to the studies, the effect of microplastics on plants is directly proportional to its concentration. The presence of microplastics can delay the relative seed germination, root and shoot growth by inhibiting water uptake through short-term and transient mechanical blockage of pores in the seed capsule. Moreover, microplastics accumulate near the root hair results in a reduction of the growth rate (Bosker *et al.*, 2019). Initially, most of the investigations focused on examining the effect of microplastics on aquatic plants (Sjollema *et al.*, 2016) because of its accumulation in aquatic ecosystems

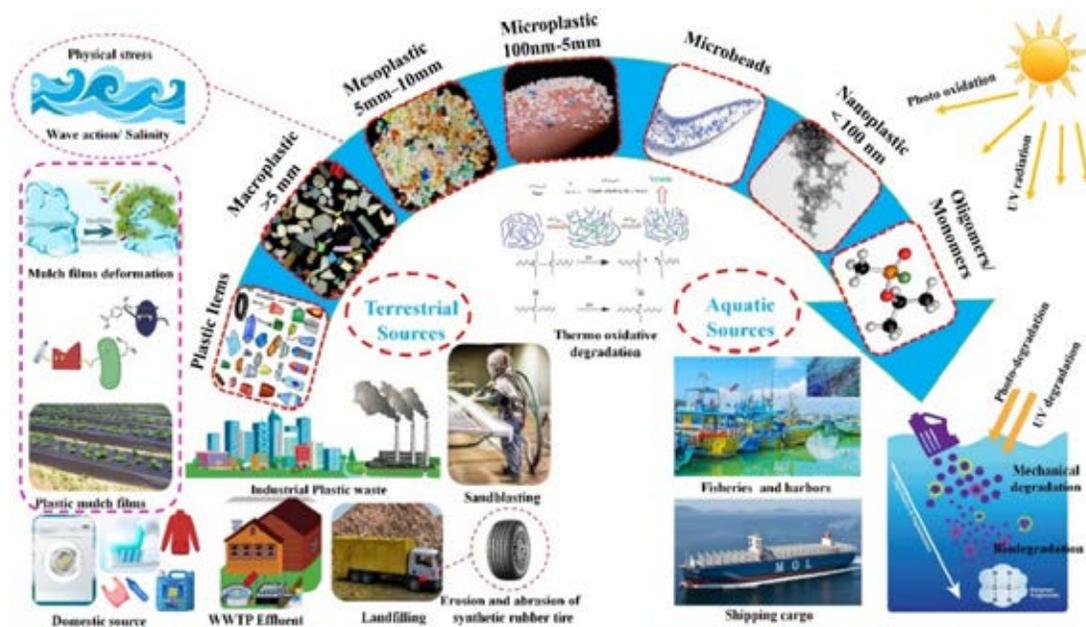


Fig. 1: Nano- and microplastic accumulation sources with the encircled (dotted lines) circle on the left showing the terrestrial sources and that on the right showing the aquatic sources. The range in the size of plastic debris resulting from the degradation and fragmentation process of plastic items to oligomers/monomers is shown across the arc in blue. Each of the squares represented in the red dotted line shows the standard size range as a result of different fragmentation cause

(Claessens *et al.*, 2011; Lechner and Ramler, 2015) in comparison to terrestrial ecosystems. Recently, attention has shifted to include effects of microplastics on terrestrial systems too. The effect of microplastics and engineered nanomaterials (Miralles *et al.*, 2012a) on plant growth varies as a function of plant species, structure and size of particles, chemical composition, and surface area. Furthermore, the effects on plant growth can be negative or positive (Rillig *et al.*, 2019). There are also reports related to the increase in the adverse effect from combination of microplastics with other chemical compounds in comparison to the effect of microplastics alone (Prata *et al.*, 2018). Consequence of expanded microplastic use becomes a threat to the human health (Cox *et al.*, 2019; Forte *et al.*, 2016; Schirinzi *et al.*, 2017) since microplastics can be transferred from prey to predators through the food chain. Furthermore, health of aquatic and terrestrial biota (Deng *et al.*, 2017; Hurley *et al.*, 2017) could be affected by nano and microplastics resulting in a number of unexplored health hazards. In highly urban areas, there exists report that a mean of 3223 and 1063 microplastic particles per year is ingested, respectively by children and adults released as road dust (Dehghani *et al.*, 2017). A few research studies have been implemented to assess the risk assessment of microplastics in food (Rainieri and Barranco, 2019) and it was found that the microplastics content of commercial salt is between 550–681 particles/kg in sea salts, 43–364 particles/kg in lake salts, and 7–204 particles/kg in rock/well salts (Yang *et al.*, 2015). It is estimated that the annual microplastic consumption of Americans' caloric intake was between 39000 to 52000 particles depending on age and sex. The estimated value could reach 74000 and 121000 from taking into consideration the effects of inhalation. Moreover, for people who drink only bottled water, they may ingest an extra 90000 microplastics annually and those who drink only tap water may be ingesting 4000 microplastics annually (Cox *et al.*, 2019). Furthermore, plastic teabags release approximately 11.6 billion microplastics and 3.1 billion nan plastics into a single cup at around 95°C temperature (Hernandez *et al.*, 2019). An abundance of microplastics was found in untreated and treated drinking water of water treatment plants (Koelmans *et al.*, 2019; Pivokonsky *et al.*, 2018). In order to mitigate the severity of the problem, a few countries have started regulating and banning the production

and use of microbeads (Kramm *et al.*, 2018; Rochman *et al.*, 2015). This study focused on the individual and the combined effects of micro/nano plastics on the growth of plants and seed germination in both aquatic and terrestrial ecosystems. Here, the main topic of microplastics in different systems was categorized into three subtopics namely, the accumulation, effect on seed germination and plant growth. Firstly, the accumulation of microplastics in plants under both aquatic and terrestrial environments is discussed in detail. Next, in relation to the effect on plant growth, studies related to the individual and the combined effects of microplastics on seed germination and growth were considered. This study currently ongoing as a part of a doctoral dissertation entitled "Application of Optical Interferometric Techniques in monitoring the synergic effect of Microplastic with heavy metals on seed germination and plant growth" at Saitama University, Japan during 2019 - 2022.

Microplastic accumulation

It is a well-known fact that the abundance of microplastics exists all around the world affecting the growth of plants and can be found in oceans including deep oceans (Claessens *et al.*, 2013), coastal waters (Moore *et al.*, 2002), pelagic zones (Doyle *et al.*, 2011), coastal sediments (Claessens *et al.*, 2013) and beaches (McDermid and McMullen, 2004). Moreover, the river (Castañeda *et al.*, 2014; Driedger *et al.*, 2015; Nizzetto *et al.*, 2016a) and other terrestrial environments are also potential sites for microplastic accumulation. In 2018, world and European plastic production respectively reached almost 359 million tones and 61.1 million tons. Particularly, in Europe, plastic had been widely used for different purposes such as packaging (39.9%), building and construction (19.8%), automotive industry (9.9%), electrical and electronic appliances (6.2%), household and sport (4.1%), agriculture (3.4%) and others (16.7%). Furthermore, under the absence of control measures to cut production rate and mismanagement of plastic disposing methods, it is predicted that by 2050, approximately plastic waste in landfills or in the environment would reach 12,000 Mt (Geyer *et al.*, 2017). The plastic waste is largely accumulated in oceans due to its overwhelming usage and mismanagement (Jambeck *et al.*, 2015; Siegfried *et al.*, 2017; Van Sebille *et al.*, 2015). Consequently, the growth rate of aquatic plant and life

expectancy of fish have been affected. The source of microplastics classified as primary is originating from synthetic fibers from clothes and houses and those due to the degradation of macroplastics classified as secondary microplastics arise from waste incineration and landfills (Dris *et al.*, 2016). Fibers including microplastics and small plastic debris generated through machining processes are transported by wind (Airborne microplastics) into the aquatic system (Dris *et al.*, 2016) or precipitated on plant surface (Chen *et al.*, 2020) leading to negative impact on both the aquatic and terrestrial ecosystems. The gravity of atmospheric fallout of the airborne microplastics has been expanded to human life arising in a number of health hazards, particularly in the respiratory system with 250 μm sized microfibers found in deeper lung regions of human (Enyoh *et al.*, 2019; Pauly *et al.*, 1998; Prata, 2018).

Microplastic accumulation in aquatic ecosystem

Recently, researchers reported different kinds of potential sources as the reason for accumulation of microplastics in aquatic system having an ecological impact. Among them, effluents from wastewater treatment plants (WWTP) contribute significantly as a potential source in urban rivers (McCormick *et al.*, 2014). Such effluents present in the domestic wastewater arise from microplastic particles that are intentionally added as ingredients for facial scrubs (Cheung and Fok, 2017; Lei *et al.*, 2017), cosmetics (Duis and Coors, 2016; Leslie, 2014), hand cleansers (Gregory, 1996) and fiber present in synthetic textiles (Browne *et al.*, 2011) and the removal of which being difficult due to their small sizes and low buoyancy (Fendall and Sewell, 2009). Thus, the domestic microplastics from household wastewater is directly transported to the rivers, and the rivers further transport that plastic and consequently to oceans. Hence, urban rivers are a potentially important source of the microplastic accumulation, particularly in the aquatic system. The plastic pollution in the Laurentian Great Lakes ecosystem was investigated in 2012 and an average count density of $43,157 \pm 115,519$ particles/ km^2 was found (Eriksen *et al.*, 2013). The ocean is playing the role as the main sink for microplastics and the accumulation rate is rapidly increasing. Estimating the exact amount has been very challenging not only because of the lack of collection methods for microplastics (MPs) and

nanoplastics (NPs) and further in characterizing them. Hence, different models have been developed to predict the transport of microplastic pollutions (Ballent *et al.*, 2013) and their accumulation (Pini *et al.*, 2019). As of 2014, estimated total amount in oceans across the world is more than 5 trillion floated plastic debris corresponding to a weight of around 250,000 tons (Eriksen *et al.*, 2014). The total accumulated microplastic amount on the water surface was estimated as 93,000–236,000 tons per year in the global ocean (Nizzetto *et al.*, 2016a). The accumulation of microplastics is enhanced through the commercial shipping process (Barnes and Milner, 2005). Moreover, microplastics are ingested (Cole *et al.*, 2013; Desforges *et al.*, 2015) and taken up (Besseling *et al.*, 2013; Ferreira *et al.*, 2016; Lu *et al.*, 2016) by marine biota leading to numerous health hazards. Sea urchin embryos (Della Torre *et al.*, 2014) and *Mytilus* (Bråte *et al.*, 2018) have been used to estimate MPs accumulation, pollution and toxicity. Furthermore, more plastics are being ingested by pelagic fish and more fibers are being ingested by benthic fish (Browne *et al.*, 2008; Lusher *et al.*, 2013; Murray and Cowie, 2011; Neves *et al.*, 2015) as a result of contamination of their natural habitat (Davison and Asch, 2011). Consequently, presence of microplastics resulted in decreased life expectancy of fish with abnormalities in their behavior possibly from the penetration of the microplastics through the blood brain barrier (BBB) into brain (Mattsson *et al.*, 2017).

Microplastic accumulation in terrestrial ecosystem

There is growing evidence that a considerable amount of microplastics is accumulating in the terrestrial environment through different potential sources such as mulch films (He *et al.*, 2018), greenhouse materials, road dust microplastics (Dehghani *et al.*, 2017), sewage sludge (Mahon *et al.*, 2017), land filling and atmospheric deposition (Klein and Fischer, 2019). Because of the anthropogenic activities, microplastics have become an emerging threat to the terrestrial system impacting the soil environment (De Souza Machado *et al.*, 2018) and eventually might become persistent organic pollutant (Lohmann, 2017) affecting the biodiversity of the soil (Rillig, 2012). The plastic mulch films (smallest size below 80 μm) widely used in farmlands reduce soil quality, thus producing microplastics on the

terrestrial environment. Annual growth of the plastic mulch film is estimated to be around 5.7% in 2019 (Steinmetz *et al.*, 2016). China plays a key role in this aspect where 80% (20 million hectares) of agricultural land is enveloped by mulch film and in Europe that value is around 427,000 hectares. It was estimated that in China, the growth rate per year of mulch film was around 25% corresponding to 700,000 tones/year (Espí *et al.*, 2006) that reached up to 1.25 million tons in 2011 (Boucher and Friot, 2017). The emerging threat of microplastic accumulation in the terrestrial environment is the road dust that contains microplastics. In such cases, automobile vehicle tire, road making paint, construction and building materials are considered as the main sources. In general, more than 50% of tires are manufactured from artificial rubber, the abrasion of which release the microplastic particles into the terrestrial environment (Sommer *et al.*, 2018). The annual emission owing to the abrasion of tires in Norwegian roads was calculated to be around 4,300 – 5,700 tonnes/year for microplastics (Vogelsang *et al.*, 2019). Moreover, the municipal wastewater treatment plants (WWTPs) sludge (biosolids) contributes highly to the accumulation of microplastics in agricultural soil leading to adverse effects of soil biota (Mason *et al.*, 2016). The sewage sludge adopted as a fertilizer to enhance the production of agricultural crop in turn increases the rate of microplastic accumulation in the soil at an alarming rate (Horton and Dixon, 2018; Saruhan *et al.*, 2010; Singh and Agrawal, 2008). In some countries, around 50% to 80% of biosolids were processed for agricultural purpose and more than 10 million tons of sewage sludges were generated through WWTPs in Europe Union (EU) in 2010 (Mahon *et al.*, 2017). Furthermore, generated microplastics from WWTP has a very high retention rate (99%) (Magnusson and Norén, 2014). A rough extrapolation of the existing data suggests that in European and North American farmlands microplastic accumulation through biosolids are in between 63,000 - 430,000 and 44,000 – 300,000 tons per year, respectively (Nizzetto *et al.*, 2016b). In Australian agroecosystem, that value is between 2800 – 19,000 tons per year (Ng *et al.*, 2018). Furthermore, tremendous amount of microplastics is accumulating in the terrestrial environment, enhancing the ecological impact on soil (Chae and An, 2018; Rillig *et al.*, 2017a; Zhu *et al.*, 2019). Consequently, soil biota, especially earthworms

and gut microbiota are exposed to high microplastic concentration with significant reduction in their weight and reproduction (Huerta Lwanga *et al.*, 2016; Zhu *et al.*, 2018a; 2018b). Thus, the immune system of earthworms can be damaged by accumulated microplastics (Rodriguez-Seijo *et al.*, 2017). The effects are not restricted to the surface, as the earthworm exposed to microplastics could transport microplastics from the surface to groundwater level thus contaminating the groundwater as well exposing other soil biota (Rillig *et al.*, 2017b).

Regulating microplastic pollution

Microplastic pollution has been identified as an emerging threat for both aquatic and terrestrial environments. Recent research works have documented conversion or degradation of plastic waste through a combination of biodegradation, photo-degradation, and thermo-oxidative and thermal degradation processes over a long duration of approximately more than 100 years (Karan *et al.*, 2019). Hence, it is important to take prompt measures to prevent this long-lasting problem. One of the most reasonable solutions for this problem is to reduce, reuse and recycle plastics. Most of the countries have formulated different policies to reduce the plastic usage. For example, Scotland from banning the use of plastic bags has prevented 650 million bags entering into the waste system. Moreover, Ireland has successfully reduced the usage of plastic bags by 90% through increasing the taxes and introducing fine for plastic bag users. USA has also reduced plastic bags usage within the country in the range of 60% to 90% (Sharma and Chatterjee, 2017). Furthermore, most countries banned the use of microbeads in different domestic products such as facial scrubs, washing detergents, creams and toothpaste (Jiang, 2018). The microplastic accumulation rate could be reduced to a considerable amount through the reuse and recycle process. Plastic has been recycled within and outside of EU by 81% and EU 19% respectively in 2018. Especially in EU countries, plastic bottle collection machines installed close to the supermarket encourages the recycling and reusing process through providing monetary benefits for every returned bottle. One practical solution toward reducing the use of plastics could be to switch to the biodegradable materials such as polylactide (PLA) and polyhydroxyalkanoates (PHA)

(Wu et al., 2017). Biodegradable microplastics offers the same advantages as conventional plastic in terms of usage (Shruti and Kutralam-Muniasamy, 2019) while the bioplastic degradation or fragmentation was approximately estimated to be lower than 48 days (Accinelli et al., 2019), thus becoming a viable alternative. Furthermore, the particle separation efficiency of WWTP can be enhanced through new technology development that could capture even small microplastic debris avoiding pollution of terrestrial as well as aquatic environments.

Effect of microplastics on seed germination

New technologies have been incorporated in seed germination process to enhance seedling health, vegetative growth and curing plant injuries from many diseases and insect pests in which bioplastic are added as nano coating for seed coat with some active ingredients such as fertilizer or pesticide (Accinelli et al., 2016). On the other hand, such techniques directly contribute to the longtime accumulation of microplastics in the terrestrial environment (Shruti and Kutralam-Muniasamy, 2019). Moreover, the presence of microplastics could delay the relative seed germination, relative root growth and relative shoot growth by inhibiting water uptake through short-term and transient mechanical blockage of pores in the seed capsule. The germination rate was significantly reduced after 8h exposure and gradually increased with passage time and the effect was dose-dependent and size-dependent (Bosker et al., 2019). In this experiment three different sized plastic particles (50 nm, 500 nm, 4800 nm) were used with five different concentrations ranging from 10^3 to 10^7 particles/mL to observe the effect of MPs on seed germination. The decline of seed germination was observed from lowest to highest concentration for each MPs size as 67% - 38% for 50 nm, 50% - 30% for 500 nm and 55% to 17% for 4800 nm MPs emphasizing the dose-dependent adverse effect. Moreover, a clear size-dependent adverse effect was observed in highest MPs concentration resulting the reduction of seed germination up to 38% for 50 nm, 30% for 500 nm and 17% for 4800 nm. Interestingly, same tendency could be observed in the reduction of seed germination for lower concentrations as well. Thus, adverse effects on germination could increase with increase in the size of MP and its concentration. Moreover, microplastics accumulated near the root

hair resulted in a reduction of growth rate (Bosker et al., 2019). A significant difference was found in root growth after 24 h of exposure for 50 nm and 500 nm with respective rates of 16% and 21% for highest concentration in comparison to control. In contrast, 15% reduction of root growth was observed for 4800 nm for the highest concentration whereas the effect was not significant as other conditions. Furthermore, after 72h exposure to 500 nm particles, a significant difference in shoot length was observed with respective reduction rate of 19% for 10^6 particles/mL concentration compared to the control. Polystyrene nanoplastics (PSNPs) (100 nm) adsorbed and accumulated onto the spore of the plant surface (*Ceratopteris Pteridoides*) cause reduction of final spore size by 2.3% – 22.4% and inhibit water uptake reducing germination ratio by 10.4% – 88.0%. This is believed to be due to the physical blockage (Yuan et al., 2019). Moreover, seed germination and seedling growth of wheat (*Triticum aestivum L.*) were examined and significant increment of root elongation (by 88.6% - 122.6%) was recorded with respect to control condition after exposure to polystyrene nanoplastics (PSNPs). Consequently, reduction of shoot to root biomass ratio (S.R ratio) was observed during the experiment after 5 days of exposure. Macronutrient such as C and N and plant biomass increased while micronutrients Fe, Mn, Cu and Zn accumulation decreased in varying degrees. Finally, it was found that PSNPs were taken up by root tips and transported to shoot across the xylem tissue (Lian et al., 2019). Furthermore, synthetic fiber and biodegradable polylactic acid (PLA) can hinder seed germination of perennial ryegrass (*Lolium perenne*) (Boots et al., 2019). The summary of microplastic effects for seed germination in aquatic and terrestrial ecosystem is shown in Table 1.

Individual effect of microplastics for plant growth

Microplastics accumulation and contamination have become an emerging problem since mass production of plastic begun in 1940 (Cole et al., 2011). The adverse effect of microplastics has been increasing at an astonishing rate, giving negative impact to both aquatic (Capozzi et al., 2018) and terrestrial biota (Jiang et al., 2019) in which the microplastics debris are accumulated near the seed coat or root hair inhibiting imbibition, causing reduction of the seed germination rate and plant

Table 1: Microplastic effects on seed germination in both aquatic and terrestrial ecosystem

Seed type	Scientific name	Plastic type	Size of plastic	Tested concentration	Exposure time	Ecosystem	Effects for the germination	Reference
Vascular	<i>Lepidium sativum</i>	Nano and Microplastics	50nm, 500nm, 4800nm	10^3 , 10^4 , 10^5 , 10^6 , 10^7 particles/mL	8 h, 24 h, 48 h, 72 h	Terrestrial	Seed germination was temporally delayed through mechanical blockage of pores in seed capsule.	Bosker et al. (2019)
Plants	<i>Ceratopteris pteridoides</i>	Polystyrene nanoplastics (PSNPs)	100 nm	0, 0.16, 0.8, 4, 20, 100 µg/mL	28 days	Aquatic	PSNPs were adsorbed and accumulated on the spore surface while reducing spore size and germination ratio.	Yuan et al. (2019)
Wheat	<i>Triticum aestivum</i> L.	Polystyrene nanoplastics (PSNPs)	100 nm	0.01–10 mg/L	1, 5, 7, 14, 21 days	Terrestrial	Reduced shoot to root biomass ratio and micronutrient adsorption. PSNPs were taken up and transported to shoot via the xylem.	Lian et al. (2019)

growth by considerable amounts (Kalčíková *et al.*, 2017). The literature available so far suggests that most of the time, the effect has dose-dependent and size-dependent response. Moreover, the adverse effect of MPs on the seed germination is enhanced with the increase of particle size as large plastic particles can inhibit imbibition of water and nutrient through physical blockage compared to small particles. In contrast the adverse effect of MPs on plant growth is enhanced with decrease of particle size inducing genotoxic and cytotoxic effect. Based on existing research works, nano scale plastic particles could enter the roots and probably block cell connections or cell wall pores and disrupt the nutrient uptake inducing observed toxic effect. Furthermore, the cell damage was observed from internalized plastic particles leading to reduced root growth and shoot growth. Hence, the nano scale plastic particle gives adverse effect on root growth while reducing growth of plants. However, there is a lack of a clear evidence for nano and microplastic uptake by plants and toxicity effect on plants. Nevertheless, a few studies suggest that, the uptake and translocation ability depend on different parameters such as the organic and geometric property of plastic debris (material, size, shape), root and xylem properties (surface

area, volume) and plasma membrane potential (Trapp, 2000). Consequently, *Nicotiana tabacum* BY-2 cells can uptake 20 nm and 40 nm nanobeads through endocytic internalization into turgescient and plasmolyzed cells and 100 nm beads are accumulated or adhere near the cell wall. The large nano beads from 20 nm to 1000 nm excluding 2000 nm can be internalized in protoplasts (Bandmann *et al.*, 2012). The hypothesized that the mechanism of uptake and translocation of MPs can be the same as reported for nanoparticles uptake by plants. Nanoparticles are adsorbed to plant surfaces and taken up through natural nano or micrometer scale plant openings (Dietz and Herth, 2011). Moreover, newly developed roots have small cracks through which the small particle can enter. Those microplastics can then travel from the roots up to the edible parts of the crop along the xylem. There are several pathways exist or are predicted for nanoparticles association and uptake in plants as illustrated in Fig. 2. This type of nanoparticles uptake by plant is inversely proportional to the particle size and can provide adverse effect on plant growth and crop yield. More recently, presence of micro- and nano-plastics in edible fruit and vegetables were examined emphasizing the great risk for human health (Conti *et al.*, 2020). In that experiment apple

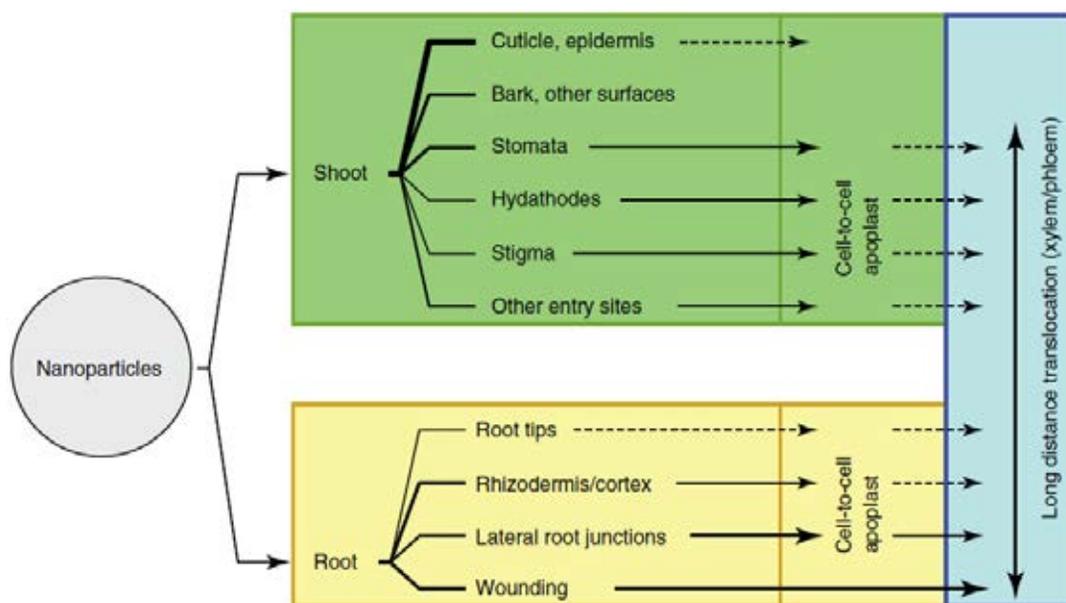


Fig. 2: Pathways of nanoparticle association, uptake, and translocation in plants. The assumed significance of the pathways is represented by the line thickness and the assumption of very low rates of transport is indicated by broken line (Dietz and Herth, 2011)

and carrot were identified as the most contaminated fruit and vegetable, respectively. The smallest size of MPs was found in the carrot samples (1.51 μm), while the biggest ones were found in the lettuce (2.52 μm).

Microplastic effects on the aquatic plant growth

It is a well-known fact that, oceans, and rivers act as the main sink for the accumulation of microplastics. Hence, the adverse effect of microplastics for aquatic plant is comparatively high. MPs effects can be categorized in to three main parts as adsorption, uptake, and toxicity. Recent research studies document that, charged nano and microplastics tend to be physically adsorbed by algae species (*Chlorella* and *Scenedesmus*) causing reduction of photosynthesis activity and growth rate, inhibiting air flow and light through physical blockage. Moreover, the adsorption process of MPs may enhance the production of reactive oxygen species (ROS) in algae (Bhattacharya *et al.*, 2010). The negatively or positively charged nano plastic at a high concentration (> 50 $\mu\text{g}/\text{mL}$) reduce microalgal growth. This is due to the adsorption of nano particles onto microalgal surface (Bergami *et al.*, 2017). The microalgal growth can be reduced by the uncharged polystyrene (PS) microplastics up to 45% at the highest concentration of 250 mg/L and 72 h bioassay, compared to the control. The increasing adverse effect was observed when the particle size was decreased. The experiment was implemented by deploying three different sizes of uncharged and negatively charged microplastics (0.05, 0.5, 6 μm) to investigate small plastic particle effect for photosynthesis and growth of microalgal. However, the experimental results do not imply any obvious change in photosynthesis effect (Sjollema *et al.*, 2016). A few measurements done with atomic force microscopy (AFM) showed that adsorption capacity of neutral or positively charged microplastics onto algae cell wall (*P. subcapitata*) is comparatively higher than that for negatively charged plastic particle (Bhattacharya *et al.*, 2010; Nolte *et al.*, 2017). The root growth of the floating plant, such as duckweed species (*Lemna minor*, *Spirodela polyrhiza*) was negatively affected by nanoplastics and microbeads through mechanical blocking as a result of microplastic adsorption. Here again, the chlorophyll content and photosynthesis activities were not affected (Dovidat *et al.*, 2019; Kalčíková *et al.*, 2017). The nano and microplastic

effects on macrophytes species (*Myriophyllum spicatum*, *Elodea sp*) have been studied and shoot to root ratio (S.R) reduction was examined in both macrophytes for nanoplastics whereas root length and shoot length of *M. spicatum* were reduced by microplastics in high concentration owing to the reduction of nutrient imbibition (van Weert *et al.*, 2019). The growth rate and photosynthetic activity of algae (*Chlorella pyrenoidosa*) was reduced by PS microplastics (0.1, 1 μm) under three different concentrations (10, 50, 100 mg/L), inducing dose-dependent adverse effects. In contrast, the distortion and damages of thylakoids and cell membrane were observed after 13 days exposure to confirm physical damage and oxidative stress. The acute toxicity effect was found due to biological adaptation of algae and, cell structure recovered to its normal stage after 25 days exposure while enhancing the growth rate (Mao *et al.*, 2018). Furthermore, PSNPs can reduce chlorophyll concentration and population growth in green algae (*Scenedesmus obliquus*) after 72h bioassay (Besseling *et al.*, 2014). Zhang *et al.* (2017), examined the toxic effect of microplastics (mPVC, 1 μm) and bulk plastic debris (bPVC, 1mm) on algae (*Skeletonema costatum*) at respective concentrations of 5mg/L and 50 mg/L and found that mPVC reduced growth rate of algae with the inhibition ratio reaching a maximum of 39.7% after 92 h bioassay. Besides, at high plastic concentrations, chlorophyll content and photosynthesis efficiency decreased. However, bPVC does not have any adverse effect on both the growth rate and the chlorophyll content. Moreover, moss species (*Sphagnum palustre* L) can be used as biomonitors to examine nano and microparticle pollution in the aquatic environment. Large and small plastic debris accumulated more on dead moss materials than living moss. This is because of the increased accumulation damaged cell membrane of dead moss (Capozzi *et al.*, 2018). Table 2 illustrates the individual effects of microplastic for plant growth in the aquatic environment.

Microplastic effects on the terrestrial plant growth

The knowledge about the impact of microplastics for the terrestrial environment is very limited compared to the aquatic ecosystem (Horton *et al.*, 2017). Nonetheless, the terrestrial ecosystem has been subjected to a high microplastic accumulation due to widespread usage and mismanagement of

Table 2: Individual effect of microplastics for plant growth in aquatic ecosystem

Plant species	Scientific name	Plastic type	Size of plastic	Tested concentration	Exposure time	Effect for the plant growth	Reference
Mosses	<i>Sphagnum palustre</i>	Fluorescent unmodified PSNPs	50 nm	3×10^{11} NPs/mL (18 mg/L)	7, 14, 21 Days	More NPs were accumulated in devitalized moss than in living moss and number of particles increase with exposure time.	Capozzi et al. (2018)
Algae	<i>Chlorella pyrenoidosa</i>	Polystyrene (PS)	0.1 μ m, 1 μ m	10, 50, 100 mg/L	30 Days	Adverse effects (physical damage and oxidative stress) were observed at the beginning and enhance the growth with passage of time.	Mao et al. (2018)
Algae	<i>Skeletonema costatum</i>	Microplastics and Bulk plastic	1 μ m, 1 mm	5, 50 mg/L	1, 24, 48, 72, 96 h	There is a size and dose-dependent effect. At high concentration, microplastics had adverse effect on chlorophyll content and photosynthetic efficiency. The adverse effect decreased with time.	Zhang et al. (2017)
Algae	<i>Scenedesmus obliquus</i>	Polystyrene nanoplastic	~70 nm	44 - 1100 mg nano-PS/L	72 h	Polystyrene NPs reduce the population growth and chlorophyll concentration of algae.	Besseling et al. (2014)
Algae	<i>Chlorella and Scenedesmus</i>	polystyrene (PS) beads	20 nm	0.08-0.8	2, 24 h	Inhibited photosynthesis activity.	Bhattacharya et al. (2010)
Algae	<i>P. Subcapitta</i>	PSNPs	110 nm	0-100 μ g/mL	72 h	NPs are adsorbed onto algae cell wall and inhibited algal growth	Nolte et al. (2017)
Algae	<i>D.tertiolecta</i> ,	Polystyrene nanoparticles	40, 50 nm	0.5-50 μ g/mL	2, 3 and 14 Days	NPs were adsorbed on microalgal and diminished microalgal growth at high concentration (> 50 μ g/mL).	Bergami et al. (2017)
Microalgal	<i>D.tertiolecta</i> , <i>T. pseudonana</i> , <i>C. vulgaris</i>	Polystyrene MPs	0.05, 0.5 and 6 μ m	25, 250 μ g/mL	72 h	Growth was reduced up to 45% compared to the control at the highest concentration (250 mg/L) and the adverse effects increased with decreasing particle size.	Sjollema et al. (2016)
Duckweed	<i>Spirodela polyrrhiza</i>	Red fluorescent	50, 500 nm	10^2 , 10^4 , 10^6 particles/mL	120 h	MPs were adsorbed to the roots. No significant effect was observed on plant growth and chlorophyll production.	Dovidat et al. (2019)
Duckweed	<i>Lemna minor</i>	polyethylene microbeads	30 to 600 μ m /40 to 400 μ m	0, 50, 10, 100 mg/L	7 Days	MPs were adsorbed to the roots and root growth were significantly affected by mechanical blocking	Kalčíková et al. (2017)
Macrophytes	<i>Myriophyllum spicatum</i>	Polystyrene NPs and MPs	50 - 190 nm,	Polystyrene NPs and MPs	21 Days	The shoot to root ratio was significantly reduced by the NPs for both macrophytes due to reduction of nutrient accumulation.	van Weert et al. (2019)
Plant	<i>Nicotiana tabacum</i> BY-2 cells	Nano beads	20, 40, 100, 1000 nm	10, 30 μ m	30 min	Small nano beads (20 and 40 nm) were internalized rapidly and accumulated partially.	Bandmann et al. (2012)

plastic waste causing growth reduction and toxicity effect for plants. Thus, a significant growth reduction of the higher plant (*Vicia faba*) was observed after 48 h exposure to microplastics only at the highest concentration (50,100 mg/L). Furthermore, the biomass weight and catalase (CAT) of plant root decreased by a considerable amount for 5 µm plastic debris whereas peroxidase (POD) and superoxide dismutase (SOD) enzyme activities were enhanced. The experimental results imply that the genotoxic (micronucleus test) and oxidative damage (enzymes activity) to *V. faba* is inversely proportional to particle size and thus the toxic effect of microplastics increased with decreased particle size. Same as in aquatic plants, microplastics can be accumulated near the root tip of terrestrial plants (*V. faba*) in which water and nutrient imbibition would be inhibited through the mechanical blockage of cell wall pores (Jiang *et al.*, 2019). Further, polystyrene (PS) nanoplastics induced cytotoxic, genotoxic, and oxidative damages on treated root of *allium cepa* due to external mechanical contact of nano PS with the root surface. The effect was dose-dependent and the internalization of nano PS occurred in different cellular compartments increasing the possibility of entering microplastics into the food chain (Giorgetti *et al.*, 2020). There exists limited evidence regarding microplastic uptake and translocation in terrestrial plants. However, some research studies have provided evidence about the uptake of microbeads by wheat (*Triticum aestivum*) under three different concentrations (Table 1). There was size-dependent effect where the plants ability to uptake microbeads is higher for smaller microbeads (0.2 µm) than that of the large ones. Hence, 0.2 µm microbeads were easily transported to stem and leaves across the vascular system through apoplastic pathway (Li *et al.*, 2019). Moreover, for wheat, microplastics were found to affect both the vegetative and reproductive growth. Thus, the above-ground and below-ground parts of wheat were affected by small plastic particles (Qi *et al.*, 2018). The material of plastic mulch film used for the farming process also has a significant effect on wheat growth. Moreover, the effects of microplastic in both the above and below ground soil ecosystem was observed by (Boots *et al.*, 2019), using grown grass (*Lolium perenne*) in earthworms (*Aporrectodea rosea*) under three different microplastic types (biodegradable polylactic acid (PLA), conventional

high-density polyethylene (HDPE), and microplastic clothing fibers). PLA reduced both shoot height and seed germination rate in which fiber was able to decrease only seed germination rate (6% – 7%). Furthermore, HDPE reduced the soil pH value by a significant amount. The root biomass differed significantly between treatments and shoot biomass did not exhibit much difference. As a result of that, dry shoot to root ratio differed significantly under different treatments. Chlorophyll-a, chlorophyll-b content did not show a clear difference. The individual effect of microplastics on plant growth in the terrestrial environment is given in Table 3. So far, the knowledge about uptake, translocation, and toxic effect of nano and microplastics for terrestrial plant species are very limited. Some studies documented about uptake (De La Torre-Roche *et al.*, 2013), translocation (Zhang *et al.*, 2019), and bioaccumulation (Lee *et al.*, 2008) of carbon nanoparticle into the whole plant of rice (Lin *et al.*, 2009), maize and soybean (Zhao *et al.*, 2017). Plants could take up ENPs through root tips and transport it to shoot and leaves with the help of vascular system (Ma *et al.*, 2010). Consequently, cell damage may occur causing significant cell death at a high concentration ENPs (Shen *et al.*, 2010). Furthermore, ENPs accumulated on root tips of alfalfa and wheat plants while taking up some particles into other plant parts through the vascular system (Miralles *et al.*, 2012b).

Combined effects of microplastic for plants growth

Recent studies report that the combined effect of microplastics with different persistent organic pollutants (POPs) that contain pharmaceutical, chemical, and heavy metal can give high effect on plant growth compared with the effect of microplastics alone. Existing knowledge regarding the combined effect of microplastic on plant growth is very limited and more research studies are needed to estimate the risk. The combined effect of pharmaceutical (procainamide, doxycycline) and microplastics have been studied under specific growth rate and chlorophyll-a concentration as observation parameters. The significant adverse effect of microplastics alone on microalgae (*Tetraselmis chuii*) growth was observed only at a high concentration (41.5 mg/L). On the other hand, reduction of chlorophyll was observed only in low concentrations (0.9, 2.1 mg/L). Nevertheless, even in low concentrations,

Table 3: Individual effect of microplastics for plant growth in the terrestrial ecosystem

Plant species	Scientific name	Plastic type	Size of plastic	Tested concentration	Exposure time	Effect for the plant growth	Reference
Bean	<i>Vicia faba</i>	Polystyrene Fluorescent Microplastics	100 nm, 5 µm	10, 50, 100 mg/L	48 h	Microplastics induce higher genotoxic and oxidative damage. A significant decrease of growth was observed.	Jiang et al. (2019)
Onion	<i>Allium cepa</i>	Polystyrene nanoplastics	50 nm	0.01, 0.1, 1 g/L	72 h	The cytotoxic, genotoxic, and oxidative damages were observed. Nano PS were internalized in cellular compartment.	Giorgetti et al. (2020)
Grass	<i>Lolium perenne</i>	PLA, HDPE, Microplastics clothing fibers	>2000 µm, 2000–1000 µm, 1000–250 µm, 250–63 µm, <63 µm	0.1% (w/w) (HDPE and PLA) (1 g/kg), 0.001% (w/w) synthetic fibers (10 mg/kg)	30 days	Reduced both shoot height and seed germination. The root biomass and shoot to root ratio differed significantly between treatments.	Boots et al. (2019)
Wheat	<i>Triticum aestivum</i>	Polyethylene and biodegradable plastic debris	50, 250, 500, 1000 µm	1% (w/w)	2, 4 months	Above-ground and below-ground parts of wheat plant were affected during vegetative and reproductive growth.	Qi et al. (2018)
Wheat	<i>Triticum aestivum</i>	PS microbeads	0.2, 2, 7 µm	0.5, 5.0, 50 mg/L	10 days	PS microbeads uptake by roots and transported to stem and leaves.	Li et al. (2019)

both pharmaceuticals were toxic to *T. chuii*, and the mixtures of microplastics-pharmaceutical were more toxic than the pharmaceuticals alone (Prata *et al.*, 2018). These results imply that the combined adverse effect is significantly high compared to the effect of microplastics alone or the pharmaceutical alone, owing to the toxicity effect of fragmented product through the degradation process. Microplastics are capable of adsorbing POPs (Bakir *et al.*, 2012) and trace metal (Holmes *et al.*, 2012) leading to a combined effect that could be either negative or positive to aquatic and terrestrial biota. The heavy metal adsorption ability of MPs is directly depending on the characteristics such as specific surface, porosity, and morphology. The adsorption isotherms were better described by Langmuir model,

which indicates that the main adsorption mechanism might be chemical adsorption (Godoy *et al.*, 2019). However, the interaction between heavy metal and aged MPs are always greater than virgin MPs owing to their long-term pre-modification through photooxidation and attrition of charged material (Turner and Holmes, 2015). Moreover, different polymer type of MPs exhibits different adsorption capability, being the adsorption order of PE > PVC > PS > PP > PET. Especially on a significant adsorption of lead, chromium, and zinc on polyethylene and polyvinyl chloride a significant adsorption of lead, chromium, and zinc on microplastics was observed. The adsorption of Cd was quite rapid initially, and the equilibrium time was approximately 90 min. An increase in the pH of the Cd solution led to an increase

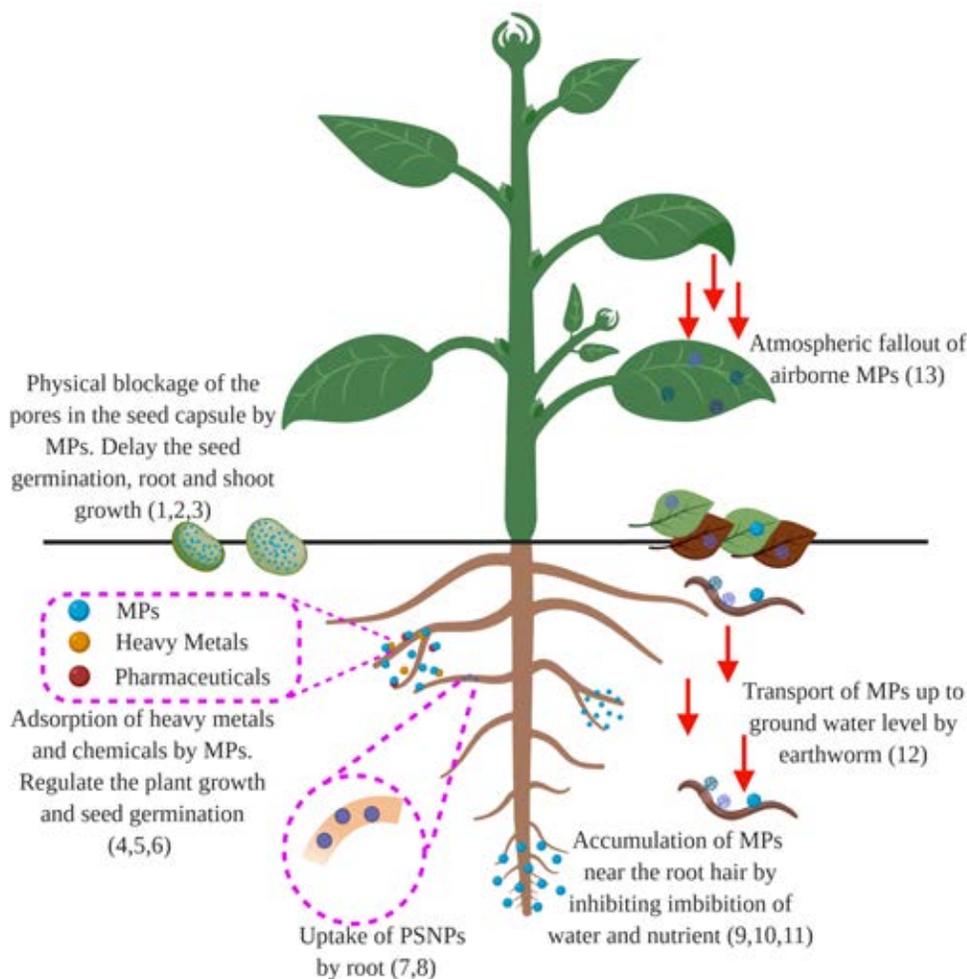


Table 3: Individual effect of microplastics for plant growth in the terrestrial ecosystem

Table 4. Combined effects of microplastic on plant growth in the aquatic ecosystem

Plant species	Scientific name	Plastic type	Size of plastic	Plastic concentrations			Chemicals concentrations		Exposure time	Effect on plant growth	Reference
				Alone	With chemical	Used chemical	Alone	With plastic			
Microalgae	<i>Tetraselmis chuii</i>	Red fluorescent polymer microspheres	1–5 µm	0.75, 1.5, 3, 6, 12, 24 and 48 mg/L	1.5 mg/L	Procainamide	104 and 143 mg/L	125 and 31 mg/L	96 h	Toxicity of combined solutions is higher than individual solutions. Growth rate and chlorophyll content were significantly reduced.	Prata et al. (2018)
Algae	<i>Microcystis aeruginosa</i>	Nanoplastic (nPS-NH ₂)	200 nm	-	1, 5, 9, 11, and 15 mg/L	Glyphosate	-	5 mg/L	96 h	Glyphosate adverse effect was significantly reduced by nPS-NH ₂ owing to high adsorption ability.	Zhang et al. (2018)
Microalgae	<i>Tetraselmis chuii</i>	Fluorescent red	1 - 5 µm	0.046 to 1.472 mg/L	0.184 mg/L	Copper (Cu)	0.02 to 0.64 mg/L	0.02 to 0.64 mg/L	96 h	Considerable combined adverse effect was not observed for virgin MPs.	Davarpanah and Guilhaermino (2015)
Fungi	<i>arbuscular mycorrhizal fungi</i>	PE and PLA	100-154 µm	-	0, 0.1%, 1% and 10% (w/w)	Cadmium (Cd)	-	5 mg/kg Cd Soil	-	Synergetic effect of MPs and Cd can alter plant growth emphasizing the risk for agroecosystems.	Wang et al. (2020)

in Cd adsorption (Wang *et al.*, 2019). Furthermore, increasing pH heavy metal solution resulted in an increase in adsorption of Ag, Co, Ni, Pb and Zn, a reduction in adsorption of Cr and no clear trend for Cu or Hg. Moreover, due to the high adsorption ability microplastics facilitated the transport of toxic chemicals such as metal or POPs, particularly in the aquatic environment (Bakir *et al.*, 2014; Verla *et al.*, 2019). The dioxin-like chemical could be adsorbed on to microplastics, enhancing the risk associated with microplastics affecting plant growth causing increased health hazards (Chen *et al.*, 2019). Zhang *et al.* (2018), demonstrated such combined effect of nanoplastics (nPS-NH₂) with glyphosate on algae (*Microcystis aeruginosa*) growth. Based on the above study, nPS-NH₂ did not exhibit a significant effect for algae growth while the glyphosate alone exhibited an extremely high adverse effect on algae growth. Nonetheless, clear reduction of glyphosate adverse effects was observed when the combined effect of nPS-NH₂ and glyphosate was considered because of the high adsorption ability of NPs. In addition, the combined effects of microplastic (1-5 µm) with heavy metal (Cu) on microalgae (*Tetraselmis chuii*) growth was investigated and a significant negative effect was observed for copper (Cu) alone whereas negligible effect was observed for microplastics alone. Furthermore, no significant adverse effect was observed after considering the combined effect of virgin microplastics and Cu due to low adsorption ability of virgin microplastics (Davaranah and Guilhermino, 2015). The experimental results indicated that no significant difference in the toxicity curves of copper in the presence and absence of virgin microplastics for the tested concentrations. Nevertheless, author emphasized that the toxic effect may be enhanced for nano-sized aged microplastics than virgin one as the aged microplastics have a high tendency to interact with metals and other chemicals. Consequently, polystyrene (PS) beads and aged polyvinyl chloride (PVC) fragments adsorbed considerable amount of heavy metals, such as copper (Cu) and zinc (Zn) in an aquatic ecosystem that was exposed to plastic debris just for 14 days (Brennecke *et al.*, 2016). Further studies are required to examine the combine effect of aged microplastics with heavy metal under long term exposure and smaller plastic debris. The effects of nano and microplastics for plant growth and seed germination is shown in Fig.

3. The combined effect of two types of microplastics (polyethylene (PE) and polylactic acid (PLA)) with cadmium (Cd) was examined by deploying arbuscular mycorrhizal fungi (AMF) community in an agricultural soil. The results of that study suggested that the combined effect of microplastics and Cd can alter the plant performance and root symbiosis while raising the risk for agroecosystems and soil biodiversity (Wang *et al.*, 2020). PLA alone showed phytotoxicity for highest concentration, reducing chlorophyll content and biomass in leaves, whereas the phytotoxic effects due to PE were considerable small. For root biomass, the combined interactive effect of PE and Cd was significant whereas such effect due to PLA and Cd combination was insignificant. Moreover, soil pH and DTPA-extractable Cd concentrations increased by both PE and PLA, but no considerable accumulation of Cd was observed. Furthermore, PLA resulted in stronger adverse effect on soil properties, plant growth and AMF community than PE due to a higher degradation ability of PLA than PE. The fragments from the PLA degradation process can interact with metals giving stronger impact.

The combined harmful health effects of microplastic and chemicals were also reported for animal and human. The effect is significant especially for aquatic biota owing to the abundance of microplastic accumulation in aquatic medium. Zebrafish (*Danio rerio*) after three weeks of exposure to the combined effects of microplastics containing chemical contaminants resulted in the combination having a significantly higher effect in comparison with either the microplastics or the chemical contaminants alone (Rainieri *et al.*, 2018). The combined effects of nickel and microplastics on *Daphnia magna* was investigated for two different microplastic types. A clear difference was observed for the combination than just for the individual cases (Kim *et al.*, 2017). Furthermore, the combined effects of microplastic with pyrene was able to delay the death of fish induced by pyrene and the pyrene concentration of fish bile was enhanced (Oliveira *et al.*, 2013). Table 4 shows the combined effects of microplastic for plant growth in the aquatic ecosystem.

CONCLUSION AND FUTURE PERSPECTIVES

This review discussed about up to date existing knowledge of the effects of microplastics on the growth of plants in the aquatic and terrestrial

ecosystem including seed germination. Limited research studies were found for the effect on terrestrial plant compared to that for aquatic ecosystem. Therefore, more research studies need to be implemented to examine the effect of microplastics on the growth of terrestrial plants. Further studies are required to monitor the effect of microplastics for aquatic and terrestrial biota and how does it affect edible plant growth, biomass accumulation and crop yield. It is a well-known fact that microplastics serve as a vector for chemical transportation; thus microplastics can adsorb heavy metal in the environment increasing the possibility of combining microplastics with different metals or chemicals. Hence, more research studies are needed to observe the combined effect of microplastics with chemicals on plant growth. Individual effect of microplastics on seed germination has been studied. However, no research evidence has been presented so far that examines the combined effect of microplastics with chemicals and heavy metal on seed germination. Thus, it is important to observe the combined effects of microplastic for seed germination while selecting different seeds having different germination rates. Moreover, airborne microplastics are transported by wind or precipitated onto the plant surface giving a negative impact to the aquatic and terrestrial environments. It can be combined with different toxic chemical and might give rise to a high negative impact to plant growth. Therefore, more research studies are required to investigate the combined effect of airborne microplastics on plant growth. A few research studies have documented that nano and microplastic accumulation, translocation and uptake are dependent on plant species, chemical and geometrical properties of plastic debris. Thus, further studies are required to evaluate how chemical and geometric properties of plastic debris affect for the growth of plants. The toxic effect might be high for nano-sized aged microplastics than virgin ones as the aged microplastics have a high tendency to interact with metals and chemicals. Hence, further studies are required to examine the combine effect of aged microplastics with heavy metal under long term exposure and smaller plastic debris. Therefore, future works are required to investigate the effect of microplastics on the growth of plants and seed germination in the aquatic and terrestrial

ecosystem to evaluate and mitigate the effects of ever increasing plastics usage of current pandemic times.

AUTHOR CONTRIBUTIONS

Y.S.K. De Silva was responsible for searching the bibliography, selecting the relevant references, revising the final version of manuscript, writing and original draft preparation. R. Uma Maheswari was responsible for conceptualization, supervision, revising the final version of manuscript, reviewing and editing. H. Kadono was responsible for conceptualization, supervision, investigation and reviewing.

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CONFLICT OF INTEREST

The authors declare that there is no potential conflict of interest regarding the publication of this work. In addition, the ethical issues, including plagiarism, informed consent, misconduct, data fabrication and, or falsification, double publication and, or submission, and redundancy have been completely witnessed by the authors.

ABBREVIATIONS

<i>AFM</i>	Atomic force microscopy
<i>AMF</i>	Arbuscular mycorrhizal fungi
<i>BBB</i>	Blood brain barrier
<i>bPVC</i>	Bulk plastic (pure polyvinyl chlorid)
<i>C</i>	Carbon
<i>CAT</i>	Catalase
<i>Cd</i>	Cadmium
<i>Cu</i>	Copper
<i>ENPs</i>	Engineered nanoparticles
<i>EU</i>	Europe union
<i>Fe</i>	Iron
<i>h</i>	Hours
<i>HDPE</i>	High-density polyethylene
<i>mg/mL</i>	Milligram per milliliter
<i>min</i>	Minutes
<i>mm</i>	Millimeter

Mn	Manganese
MPS	Microplastics
mPVC	Microplastics (pure polyvinyl chlorid)
N	Nitrogen
nm	Nanometer
NPs	Nanoplastics
nPS-NH2	Polystyrene cationic amino-modified nanoparticles
PE	Polyethylene
PET	polyethylene terephthalate
PHA	Polyhydroxyalkanoates
PLA	Polylatic acid
POD	Peroxidase
POPs	Persistent organic pollutants
PP	polypropylene
PS	Polystyrene
PSNPs	Polystyrene nanoparticles
PVC	Polyvinyl chloride
ROS	Reactive oxygen species
SOD	Super oxide dismutase
S.R	Shoot to root ratio
WWTPs	Wastewater treatment plants
Zn	Zinc
µg/mL	Microgram per milliliter
µm	Micrometer
°C	Degrees Celsius
%	Percent
%(w/w)	Percentage weight/weight
>	Greater than
<	Less than

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