SYNERGISTIC PLANT-MICROBE INTERACTION IN MODULATING MICRO/NANO PLASTIC DEGRADATION FOR SUSTAINABLE ECOSYSTEM

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in Partial Fulfillment of the Requirements for the
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DOCTOR OF PHILOSOPHY

in

BIOTECHNOLOGY

by

MEGHA
(2K21/PHDBT/01)

Under the supervision of

Prof. JAI GOPAL SHARMA
Department of Biotechnology
Delhi Technological University
Delhi

DR. DEENAN SANTHIYA
Department of Applied Chemistry
Delhi Technological University
Delhi

To the
Department of Biotechnology

DELHI TECHNOLOGICAL UNIVERSITY

(Formerly Delhi College of Engineering)
Shahbad Daulatpur, Main Bawana Road, Delhi-110042, India

December, 2024

DELHI TECHNOLOGICAL UNIVERSITY

(Formerly Delhi College of Engineering)

Shahbad Daulatpur, Main Bawana Road, Delhi- 42

CANDIDATE'S DECLARATION

I, **Megha** hereby certify that the work which is being presented in the thesis entitled **"Synergistic plant-microbe interaction in modulating micro/nano plastic degradation for sustainable ecosystem**" in partial fulfillment of the requirements for the award of the Degree of Doctor of Philosophy, submitted in the **Department of Biotechnology**, Delhi Technological University is an authentic record of my own work carried out during the period from **August, 2021 to October, 2024** under the supervision of **Prof. Jai Gopal Sharma, Department of Biotechnology, Delhi Technological University, Delhi and Co-Supervision of Dr. Deenan Santhiya, Department of Applied Chemistry, Delhi Technological University, Delhi**.

The matter presented in the thesis has not been submitted by me for the award of any other degree of this or any other Institute.

Candidate's Signature

Megha

2K21/PHDBT/01

This is to certify that the student has incorporated all the corrections suggested by the examiners in the thesis and statement made by the candidate is correct to the best of our knowledge.

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Shahbad Daulatpur, Main Bawana Road, Delhi- 42

CERTIFICATE BY THE SUPERVISOR(s)

Certified that **Megha** (2K21/PHDBT/01) has carried out her research work presented in this thesis entitled **"Synergistic plant-microbe interaction in modulating micro/nano plastic degradation for sustainable ecosystem"** for the award of **Doctor of Philosophy** from Department of Biotechnology, Delhi Technological University, Delhi, under our supervision. The thesis embodies results of original work, and studies are carried out by the student herself and the contents of the thesis do not form the basis for the award of any other degree to the candidate or anybody else from this or any other University/Institution.

Prof. Jai Gopal Sharma
Department of Biotechnology
Delhi Technological University
Delhi

Dr. Deenan Santhiya
Department of Applied Chemistry
Delhi Technological University
Delhi

Prof. Yasha Hasija
HoD & DRC Chairperson
Department of Biotechnology
Delhi Technological University
Delhi-110042

Synergistic Plant-Microbe Interaction in Modulating Micro/Nano Plastic Degradation for Sustainable Ecosystem

MEGHA

ABSTRACT

Contaminated soil is one of today's most difficult environmental issues, posing serious hazards to human health and the environment. Contaminants, particularly micro-nano plastics, have become more prevalent around the world, eventually ending up in the soil. Numerous studies have been conducted to investigate the interactions of micro-nano plastics in plants and agroecosystems. However, viable remediation of micro-nano plastics in soil remains limited. A significant amount of leftover plastic from the extensive usage of plastic film mulch and effluents from surface runoff and industrial activities has accumulated and ultimately formed microplastics (MPs) in agricultural soils. However, it is uncertain how crops would be impacted by microplastics from plastic mulch film.

In order to observe the effects of plastic fragments especially microplastics in plant and soil, the growth, physio-biochemical characteristics, and morphology of *Brassica juncea* (mustard plants) exposed to two types of HDPE microplastics – HDPE_MPs and HDPE_beads, were studied. Upon interaction with MPs and beads, the height, biomass, chlorophyll content, phenolic content and proline content of *Brassica juncea* plant were drastically lowered. This work emphasizes that MPs may have higher detrimental impacts for terrestrial ecosystems, which warrants additional investigation in future studies, and offers a fresh insight into the possible effects of MPs with varying biodegradability's on soil-plant systems.

Secondly, to observe the impacts of microplastics on wild plants, a simulated dump yard model was prepared studying impact of two different types of microplastics: high-density polyethylene (HDPE) and nylon-6,6, on tropical wild plants: *Cynodon dactylon* (L.) and *Portulaca grandiflora*. The effects of microplastics on the two plants were evaluated using confocal laser scanning microscopy for morphological inspection, antioxidant activity, chlorophyll content analysis, and biometrical parameters (root and shoot height, biomass

output). The uptake of microplastics by plant parts could be observed through the symplastic and apoplastic pathways. The morphological studies could confirm the mechanism of uptake within plant parts. The accumulation of microplastics within the root and aerial parts of leaves could provide a phytoremediation strategy by phytoextraction of microplastics. Mechanisms showing the uptake of MNPs by plants is demonstrated by explaining the apoplastic and symplastic pathways. The major accumulation occurs in the root hairs and aerial parts of leaves thereby showing a phytoextraction strategy.

Finally, synergistic plant-microbe interaction was studied to determine the capability of soil microbes in degrading microplastics and also harnessing the plant nutrition and growth. For this, the isolation of soil microorganisms was carried out using metagenomics sequencing to identify the bacterial strain that showed the most degradation efficiency. Also, two other microplastics, PP and PVC, were taken for the research study to ascertain the importance of bacterial isolate for microplastic degradation. To confirm microplastic degradation by the isolated bacterial strain, *Acinetobacter baumannii*, both the microplastics were subjected to FTIR analysis, thermogravimetric study, weight loss % for a span of 50 days and morphological characterization to observe the changes in the structure of microplastics post bacterial inoculation. The results confirmed degradation efficiency in both the microplastics stating the efficacy of microbes for microplastics elimination for sustainable ecosystem. These results conclude the effectiveness of isolated bacteria in microplastic degradation and potentially leading to the development of more effective and sustainable solutions for managing plastic waste.

This thesis is summarized in five chapters:

- Chapter 1 discusses a brief introduction about agricultural pollution of microplastics and its remediation technologies. It also talks about the sources and impacts of microplastics on human health and surrounding ecosystem.
- Chapter 2 outlines the materials and methods involved in carrying out the objectives of the research study.
- Chapter 3 focuses on results and discussion for the objectives designed for the research.

- Chapter 4 focuses on the summary, conclusion and future scope of the research study.
- Chapter 5 discusses the references that were used in the research study.

To better comprehend the finding of this research, future insights on live imaging of microplastics within plant parts could provide substantial information on phytoaccumulation of microplastics. The buildup of microplastics in soil is the last point that needs more attention. The remediation potential of soil could be determined by analyzing the amount of microplastics left over after accumulation in plants. Overall, it can help in the sustainable remediation of soil containing microplastics in nearby groundwater system for cleaner environment.

ACKNOWLEDGEMENTS

A PhD thesis is not just an academic exercise but also a testament to the researcher's perseverance, analytical skills, and intellectual curiosity. Earning a PhD requires an extended period of rigorous study, original research, and contribution to knowledge in a specific field. The journey is intellectually challenging and rewarding, however, it requires the invaluable support of numerous individuals. Foremost, I thank God for all of his blessings and for giving me the strength and fortitude to carry me through this journey.

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LIST OF ABBREVIATIONS AND SYMBOLS

MPs	Microplastics
MNPs	Micro-Nano Plastics
HDPE	High density Polyethylene
PP	Polypropylene
PVC	Poly vinyl Chloride
PG	*Portulaca grandiflora*
SG	*Cyanodon Dactylon*, Scutch Grass
FTIR	Fourier transform infrared spectroscopy
SEM	Scanning Electron Microscopy
TGA	Thermogravimetric Analysis
%	Percentage
cm	Centimeter
gm	Gram
mg	Milligram
h	Hour
min	Minutes
mL	Milliliter
nm	Nanometer
°C	Degree Celsius
v/v	Volume per volume
LOX	Lipid Oxidation
PS	Polystyrene
ROS	Reactive Oxygen Species
MDA	Malondialdehyde
MSM	Minimal Salt Medium
TCA	Trichloroacetic Acid
TBA	Thiobarbituric Acid
Wt.	Weight
w/v	Weight per volume
µg	Microgram
kg	Kilogram
CSE	Centre for Science & Environment
µm	Micrometer
mm	Millimeter

CHAPTER 1

INTRODUCTION & REVIEW OF LITERATURE

Chapter 1- Introduction & Review of Literature

1.1 Background:

The accumulation of emerging contaminants (ECs) in agricultural ecosystems is one of the main concerns of environment in today's scenario (Taheran et al., 2018). The behavior, fate and ecological impacts of ECs has led to inadequate management and loss of biodiversity (Lodeiro et al., 2019). The most influential attribute of man-made activities is the discharge of plastic (Galloway et al., 2017), a polymer used in everyday life. According to reports from the Centre for Science and Environment (CSE) of India, 79 % of the total plastic produced enters the environment in the form of waste, of which only around 9 % is recycled ("Managing Plastic Waste in India," 2020). Plastics are widely used due to their lightweight, flexibility, durability, non-rusting nature and high persistency (Lambert and Wagner, 2017). These properties of plastic that make it suitable for packaging and other engineering applications make it difficult to degrade. Globally, the production of plastic exceeds around 150 million tonnes every year. In 2015, plastic consumption per capita was highest in the US at 109kg, followed by Europe, China, Brazil and India with 65kg, 38kg, 32kg, 11kg, respectively, making a global average of approximately 28kg plastic per capita (Sharma and Mallubhotla, 2019). The data on plastic production and its fate as of 2018 is described in Figure 1.1 (cycles and Text, 2018).

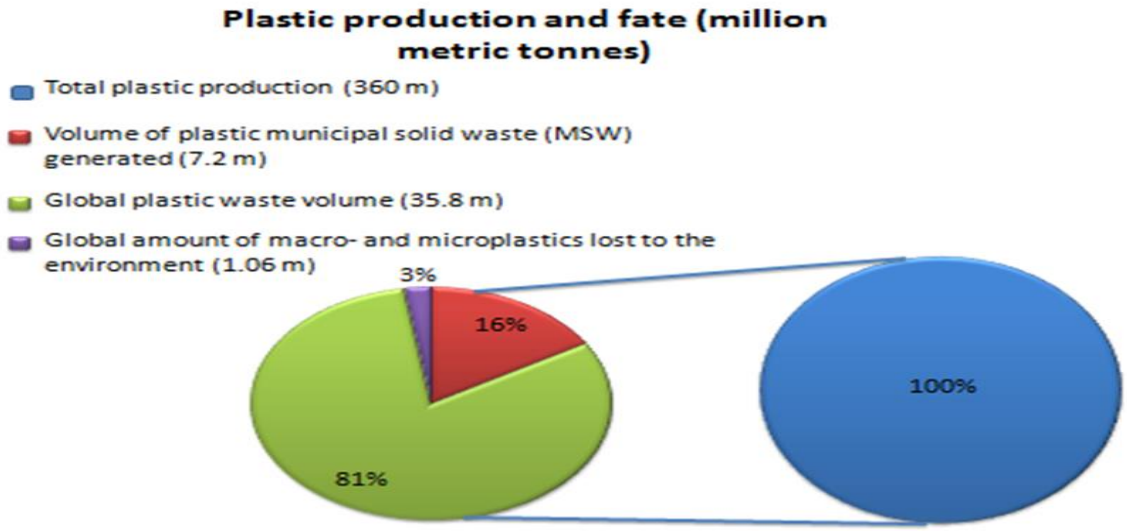


Figure 1.1: Global plastic production and its fate

The worldwide increase in production, mass consumption and waste management has led to the ubiquitous accumulation of plastics in oceans and soils (Wang et al., 2019). On entering the environment, these plastics in the form of microplastic particles pose a significant threat to human health, causing disorders such as reproductive toxicity, carcinogenicity and mutagenicity (Gasperi et al., 2018). Moreover, petrochemically derived conventional plastics are majorly responsible for ecological problems like climate change and loss of biodiversity. Furthermore, plastics that are biodegradable in laboratory conditions and waste management and natural conditions merely exist (Briassoulis and Innocenti, 2017).

Pollution with plastic particles first gained attention in the marine ecosystem, including oceans, sediments, freshwaters and coasts (Alice A. Horton et al., 2017; Windsor et al., 2019). Nonetheless, only limited knowledge and information exists on sources, sink and distribution of plastics in agricultural ecosystems. The agricultural ecosystem is affected by a myriad of plastic contaminants, and it is essential to establish better analytical methods for monitoring and toxicity assessments of soil. Reports have been published recently to demonstrate plastics contamination in different agricultural soils (table 1.1).

Table 1.1: Reports on plastic pollution in various soil samples

Type of soil	Sampling depth	Size of plastic	Method	Reference
Farmland soil	5 cm	1-5mm	FTIR	(Piehl et al., 2018)
Coastal soils	2 cm	< 5mm	Microscope and FTIR	(Xu et al., 2020)
Home garden soils	10-20 cm	10-20 µm, > 50µm	Stereo-microscopy	(Huerta Lwanga et al., 2017)
Agricultural soils	25 cm	0.97 mm	Floatation and Microscope	(Corradini et al., 2019)
Woodland soils	5 cm	10-50 mm	Organic digestion by 30% KOH:NaCl O, Density separation by $ZnCl_2$ and NaCl	(Zhou et al., 2019)
Paddy soils	10 cm	0.02- 1 mm	FTIR	(Lv et al., 2019)

Farmland soils	5 cm	1-5 mm	Attenuated total reflection (ATR)-FTIR	(Piehl et al., 2018)
Greenhouse field	0-20 cm	>1 mm	Ultrasonication, GC-MS, High throughput sequencing	(Sun et al., 2018)
Forest buffer zone	0-10 cm	1-0.05 mm	Density extraction by NaI, H_2O_2 digestion, microscope	(Zhang and Liu, 2018)
Floodplain soils	5 cm	5 mm – 2.5 cm	FTIR	(Scheurer and Bigalke, 2018)
Fruit field	3-6 cm	>0.1 cm	Floatation, Heating and Microscope	(Zhang et al., 2018)
Agricultural soils	15 cm	0 – 0.5 mm	Floatation and Metallographic	(Ding et al., 2020)

			microscope	
Horticultural soils	10 cm	28 ± 13 cm^2	Gas chromatography	(Ramos et al., 2015)
Coastal soils	2 cm	< 5 mm	Floatation, Stereo-Microscope, FTIR, SEM	(Zhou et al., 2018)
Farmland soils	3-6 cm	1.91-1.48 mm	Density extraction, H_2O_2 digestion, µFTIR	(Liu et al., 2018)
Seagrass soils	0-15 cm	2000–5000 µm	FTIR	(Dahl et al., 2021)

Various reports have claimed that most plastic materials are integrated towards breakdown compared to degradation (Bansal et al., 2021). These large plastics generate smaller fragments of size less than 5 mm, referred to as microplastics (Mammo et al., 2020). Further deterioration of these microplastic fragments results in the formation of eventually smaller particles of size less than 0.1 µm, commonly called nanoplastics (Dahl et al., 2021). Despite occurrence of microplastics by degradation, they are also incorporated as specific constituents in many products used in daily life. These microplastics bioaccumulate within the soil and have adverse effect on plant growth and development (Ding et al., 2020). For

this reason, concern on impact of microplastics on plant performance, along with soil microbes and activity in soil has been the study for research (de Souza Machado et al., 2018).

1.2 Contaminants in the Agricultural Ecosystem:

In general, contamination in agricultural soil occurs due to the build-up of toxic chemical compounds, salts, radioactive materials, and different types of micro/nano plastic particles formed by the disintegration and breakdown of plastic products (Weldeslassie et al., 2018). Herein, plastic contaminants and their effect on soil properties are briefed.

A. Physical Contaminants: Different types of physical pollutants present in agricultural soils include plastics. Various studies have observed the origin and fate of plastic fragments in the terrestrial ecosystem (Alice A Horton et al., 2017). Plastic products in the soil can be categorized as macroplastics, microplastics and nanoplastics. Direct release of plastic products in soil contains a large proportion of macroplastics of size more than 5mm (Qi et al., 2018). Most of the macroplastics particles disintegrate into smaller fragments without undergoing degradation (Scott Lambert, Chris Sinclair, 2014). The breakdown of large macroplastics into smaller pieces of size less than 5mm is referred to as microplastics (Mammo et al., 2020). Microplastics originating directly from cosmetic products and various industries are termed primary microplastics whereas disintegration of large plastic results in the formation of secondary microplastics (Iqbal et al., 2020). Increased exposure time causes further deterioration of microplastics into smaller fragments of size less than 0.1 µm, referred to as nanoplastics (Ng et al., 2018). Different characteristics of plastics, including size, shape, charge, density and surface properties (Rilling et al., 2019), influence their transport into the soil. For example, plastic fragments in soil are surrounded by various microorganisms and other substances, commonly referred to as ecocorona (Galloway et al., 2017). Thus, the development of ecocorona can have a strong influence on plastic size and shape and their migration within the soil. Therefore, the effect of plastics on soil biota and organisms requires greater understanding and transport pathways that facilitate delivery of

plastics into soil need to be monitored and controlled.

B. Chemical contaminants: Plastic fragments including microplastics and nanoplastics provide large surface area and hydrophobicity for sorption of various chemical pollutants, including polychlorinated biphenyls, dioxin-like chemicals, polybrominated diphenyl ethers, toxic metals, antibiotics and other pharmaceutical compounds (Fred-Ahmadu et al., 2020). Microplastics also contain various additives, including phthalates, bisphenol A and nonylphenols (Hermabessiere et al., 2017). Such additives are leached in the agricultural soil after the degradation of microplastics and have an adverse effect on microorganisms and plants (Wang et al., 2021). The interaction of microplastics with chemicals has significant risks to the agricultural ecosystem as compared to plastics alone. Adsorption of nonpolar and polar organic pollutants and heavy metals is highest on the surface of microplastics that affects the soil attributes (F. Wang et al., 2020). For example, the sorption rate of phenanthrene on biodegradable poly (butylene adipate co-terephthalate) microplastics was higher due to high salinity (Zuo et al., 2019). Interaction of microplastics with toxic metals such as zinc posed higher desorption of zinc in the intestine of earthworms than its adsorption in soil (Hodson et al., 2017). Most of the agricultural soil is polluted with pesticides that have more significant occurrence with microplastics. For example, glyphosate interaction with low-density polyethylene microplastics altered the volume and weight of earthworms (*L. terrestris*) (Yang et al., 2019). Bioaccumulation of dufulin on interacting with microplastics caused oxidative damage and interference in the metabolic profile of *Eisenia fetida* (W. Sun et al., 2021). The release of various additives from microplastics is one of the sources of toxicity in agricultural soils. For example, the accumulation of hexabromocyclododecanes (HBCDDs) on interaction with microplastics reached an abnormally higher concentration in earthworms (*E. fetida and M. guillelmi*) found in the soil (B. Li et al., 2019). Also, accumulation of hydrophobic organic contaminants was observed in earthworms of clean soil due to microplastics pre-contaminated with pollutants like polychlorinated biphenyls and polycyclic aromatic hydrocarbons (Rodrigues et al., 2019; J. Wang et al., 2020). Not only the adsorption is

affected by the type of chemical contaminant, but it also relies on various environmental factors, including temperature, pH and salinity (Zhang et al., 2018). For example, pesticides adhere to the surface of microplastics because of the presence of sodium ions in the soil. Also, the morphology of microplastics can be changed by weathering processes resulting in increased concentration of polar functional groups (Corcoran et al., 2015). Assessment of toxicity of microplastics with chemicals is essential to determine the ecological risk and effective management of the agricultural ecosystem.

1.3 Entry pathways for plastic in the Agricultural Ecosystem:

Soils are associated with all environmental sectors and support food production. The introductory pathway for plastics, including microplastics and nanoplastics, into agricultural soils, is via different sources, including plastic mulching, municipal solid waste and sewage sludge, compost, irrigation, littering and atmospheric deposition (figure 1.2) (Alice A. Horton et al., 2017). Plastics get introduced into the agricultural soils by applying these sources as mentioned above and cause a global change in soil properties (Kershaw and Rochman, 2015). Some of the impacts of plastic pollution in soil include structural loss and reduced soil microbes activity, increased viral diseases and soil pudding (Amare and Desta, 2021).

a) Agricultural plastic mulch: Plastic mulching is the most preferred technique for receiving greater harvest and crop quality thereby increasing soil temperatures and water efficiency (Kader et al., 2017). The widely used plastic mulch is high and low-density polyethylene (PE) (Kasirajan and Ngouajio, 2012). Though plastic mulch helps increase the productivity of field crops, it also leads to contamination of soil with adverse effects on the agricultural ecosystem. Moreover, plastic mulches contain harmful additives like phthalates with concentrations of 50-100 mg phthalates kg^{-1} in soils with plastic mulching (Wang et al., 2013). A case study by Liu et al., 2014, observed an accumulation of 50-260 kg hm^{-2} plastic particles in topsoil, affecting plant growth and altering the food chain.

b) Sewage Sludge: The highest concentration of microplastics and nanoplastics pollutants in the soil is via sewage sludge used for fertilization in fields (Corradini et al., 2019). Around 90% of plastic contaminants found in industrial wastewater are retained even after treatment and concentrate in sludge (Tagg and Labrenz, 2018). Apart from that, sewage sludge also contains synthetic polymers added by the draining and disposal of cosmetic products (Mason et al., 2016). A study by Mahon et al., 2017 observed approximately 4000 to 15000 microplastics particles kg^{-1} (dry weight) sludge and neither of the processes such as lime stabilization, thermal drying, anaerobic digestion could help remove microplastics from the soil. This makes sewage sludge one of the potent pathways for plastic input in agricultural soils.

c) Compost: Composted bio-waste application as fertilizer is a relevant entry source of plastic in agricultural soils (Guo et al., 2020a). Bio-waste of residential households is not correctly disposed of or separated and contained within plastic bags made of high-density polyethylene (HDPE) (Bläsing and Amelung, 2018). Commercial bio-waste comprising spoiled food plastic packaging is left untreated and enters the soil (Stubenrauch and Ekardt, 2020). Moreover, the degradability of biodegradable plastics depends on their type (Ohtaki and Nakasaki, 2000), and most plastics decompose at high temperatures above 50 ºC (Raubenheimer and McIlgorm, 2018). Thus, biodegradable plastics cannot be used for domestic waste as an alternative for disposing of waste or packaging for food.

d) Irrigation: The most common practice to provide water to plants is irrigation in agricultural soils. Mostly groundwater which is formed by infiltration through soil is used where larger plastics are usually gets separated and only smaller plastics like microplastics and nanoplastics pass through macropores of soil and reach groundwater (Bol et al., 2016). Thus, farms irrigated by groundwater can contain concentrations of microplastics and nanoplastics that accumulate in agricultural soils. Moreover, water scarcity, population increase and urbanization have caused such irrigation practices where direct use of untreated or wastewater is seen (Nations), 2009). According to reports, approximate 7% of total

irrigated land is fed untreated wastewater and the majority of the population consumes food produced using contaminated wastewater (Mateo-Sagasta et al., 2013). As previously discussed, untreated wastewater contains high concentrations of microplastic and nanoplastics contaminants affecting soil properties.

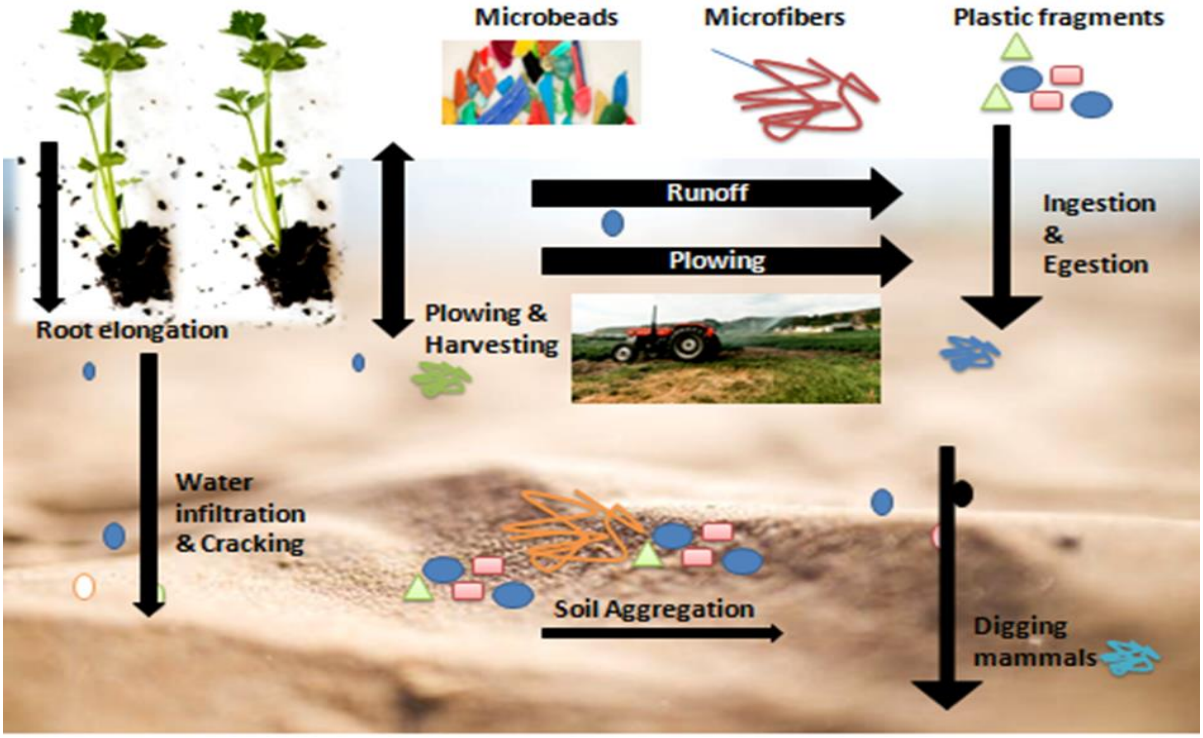


Figure 1.2: Entry pathways for microplastics in agricultural ecosystem

e) Littering: Irresponsible waste disposal near roads can also input plastic particles into the soil. Washed away litter from highways, usually caused due to storms and rain, can cause the deposition of plastic in agricultural soils (Kibblewhite, 2018). Additionally, tire abrasion on roads may introduce fine plastic particles through wash off and dust which can also harm the agricultural ecosystem (Kreider et al., 2010). Although, illegal dumping of household waste and litter accumulation on roads is restricted, yet huge concentrations of these are washed in soils.

f) Atmospheric Deposition: A notable contributor of plastic particles in the soil is through wind, blowing plastic waste from surfaces such as landfills, streets or urban areas (Rillig et al., 2017)**.** Different microplastics and nanoplastics contaminants are blown by air from suburban areas into agricultural soils, affecting their biota. A study near Paris (France) reported an atmospheric fallout of microplastics fragments which was about 29-280 items m^{-2} day^{-1} (Dris et al., 2015). Also, microplastics can be transported to long distances, including soils in remote areas, as demonstrated by Free et al., 2014.

1.4 Review of Literature:

1.4.1. MNPs and their sources:

MNPs are divided into fiber, film, pellet, powder, and fragments based on their morphology. MNPs are composed of polypropylene (PP), polyethylene (PE), polystyrene (PS), polyvinyl chloride (PVC), and polyamide (PA) as chemical ingredients. MNPs are widely classified into primary and secondary classes based on their commercial applications. Primary MNPs are manufactured for specialized uses such as cosmetics, medicine delivery carriers, industrial use, and military aids. Furthermore, synthetic textiles account for roughly 35% of total primary MNPs emitted in bodies of water (Boucher and Friot, 2017). Secondary MNPs are formed by the breakdown of large plastics through physical, chemical, and biological approaches. Mechanical forces, chemical breakdown, or microbial degradation of large plastic fragments result in the formation of secondary MNPs. Also, fishing, travelling, and catering contribute a significant proportion of secondary MNPs (Guo et al., 2020b). Vehicular transport, including tyre wear, brakes, markings is also a major source of microplastics in the ecosystem (Luo et al., 2021). Apart from major sources of MNPs in the environment, agricultural practices, recreational activities, sewage sludge application, and organic fertilizers can also contribute to MNPs pollution (Guo et al., 2020b). So, better knowledge of the potentially detrimental or deleterious consequences of these contaminants on agroecosystems is critical.

1.4.2. Impacts of MNPs to terrestrial and aquatic plants:

Size, thickness, shape, and shade of MNPs control their bioavailability, and subsequently, their toxicological impact (Haegerbaeumer et al., 2019). MNPs get adsorbed by plants, the soil rhizosphere, and soil organism thereby affecting soil physio-chemical properties (Junhao et al., 2021). Likewise, the impacts of MNPs on aquatic plants are crucial to the well-being of flora and fauna. Recently, airborne MNPs have also gained attention, suggesting that they are present both in indoor and outdoor air (Enyoh et al., 2019). Effluent discharge and surface runoff from rivers, lakes, and sediments have a direct impact on aquatic water bodies and related coastlines (Jeyavani et al., 2021). Also, MNPs particles aggregate and get ingested by marine species, thereby affecting the food chain (Prata et al., 2020). Apart from this, agricultural practices, road, and tyre wear discharge also emit MNPs on land and in the air (Luo et al., 2021). These sediments get infiltrated within the soil or discharge into water bodies, thereby affecting the ecosystem (figure 1.3).

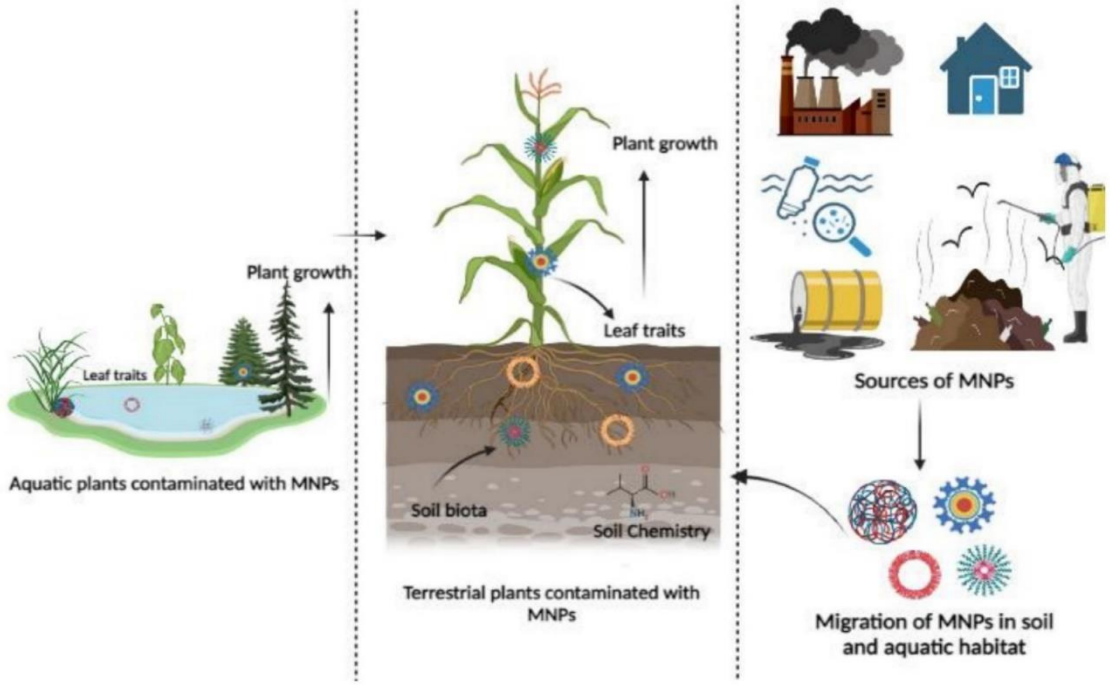


Figure 1.3: Sources of MNPs, their migration, impact, and structural changes on terrestrial and aquatic plants

a) Toxicity to terrestrial plants and soil

MNPs can physically block or clog plant tissues, such as stomata or root hairs, limiting their ability to carry out essential functions like gas exchange and nutrient uptake (Banerjee et al., 2019). MNPs contain various additives, such as plasticizers and flame retardants, which can leach out and affect plants. These chemicals may have toxic effects on plants, disrupting their metabolism, photosynthesis, and hormonal balance (Tun et al., 2022). MNPs can adsorb nutrients such as nitrogen and phosphorus, reducing their bioavailability to plants. This can lead to nutrient deficiencies and affect plant growth and productivity (Dovidat et al., 2020). MNPs can provide a surface for microbial colonization, forming biofilms. These biofilms can alter the composition and activity of soil and water microbial communities, potentially affecting the plant-microbe interactions that are crucial for nutrient uptake and disease resistance (He et al., 2022). Despite having lower MNPs emphasis than soil, air has a higher liquidity that allows for more accumulation of MNPs on leaf surfaces by means of fluid-elevated openings (de Souza Machado et al., 2019). MNPs appended to leaf surfaces might obstruct daylight and hamper photosynthesis, with comparable outcomes found in green growth (Wu et al., 2019). Due to the presence of MNPs in soil, a reduction in water-holding capacity occurs, which negatively causes a decrease in oxygen in soil aggregates (Liu et al., 2014b). Similarly, micrometer-sized polystyrene (PS) microparticles may also penetrate inside the crop plants at the site of lateral root emergence, thereby contaminating the crops (Li et al., 2020a). Seeds may be harmed as a result of plastic leachate mixed with water, which seeds absorb during germination, or as a consequence of smaller MNPs altering soil structure (Pflugmacher et al., 2020).

b) Toxicity to aquatic plants

Not only do MNPs have impacts on terrestrial plants, but recent research has found their potential to deteriorate aquatic flora. The formation of phytoplankton on the surface of water could encapsulate and entrap MNPs (Prokin et al., 2015). This sorption of MNPs may result in decreased length of principle outgrowths of established plants, repress root development,

photosynthetic action, and suitability of freshwater phytoplankton. Considering past works, microplastics can influence the photosynthesis of green plants through attachment to the outer layer of xylem and phloem tissues and consequently assemble to repress their photosynthesis (Dovidat et al., 2020). PS nanoplastics adsorption also slows down green plant photosynthetic movement while speeding up the formation of responsive oxygen, which is dependent on the physio-chemical qualities of plastics as well as the morphology and biochemical properties of green plants (Bhattacharya et al., 2010). Light is crucial for the photosynthetic process, and decreased light availability can impede plant growth and productivity (Mateos-Cárdenas et al., 2021). MNPs can induce oxidative stress in aquatic plants. These particles can generate reactive oxygen species (ROS) when interacting with plant tissues. ROS can cause cellular damage by oxidizing biomolecules, disrupting cellular processes, and impairing plant growth (van Weert et al., 2019). As higher trophic levels feed on these plants, the MNPs can be transferred up the food chain through a process called biomagnification, potentially affecting other organisms as well. The debilitating or even loss of porousness influences ordinary development and digestion cycle like the migration of compounds within and outside the cell walls of aquatic plants (Xia et al., 2015).

1.4.3. The drawbacks associated with conventional methodologies to eliminate MNPs:

The aforementioned impact of MNPs on ecosystems necessitates the utilization of various methodologies to tackle and reduce plastic contamination. Different conventional methods of eliminating plastic contaminants and their drawbacks are explained below:

a) Adsorption: In this technique, physical, chemical, or biological adsorbents such as carbon materials, zeolites, metal organic frameworks, and mesoporous materials are used to eliminate micro-nano plastic pollutants (Reineccius et al., 2021). MNPs can adhere to the surface of certain materials through physical interactions such as van der Waals forces, electrostatic interactions, and hydrophobic interactions. For example, a covalent organic framework like Tpa-H showed high adsorption energy for polyethylene, polyethylene terephthalate, and nylon-6 via molecular dynamics (Shang et al., 2022). MNPs can

chemically bind to specific functional groups on the surface of adsorbent materials through covalent or coordinate bonding (Song et al., 2023). This mechanism is often employed using modified materials with functional groups such as amino groups, carboxyl groups, or sulfonic acid groups. For example, sulphate groups of polystyrene nanoplastics were degraded under UV radiation, thereby decreasing their electrostatic potential (X. Wang et al., 2020). The major disadvantage of using this technique is the generation of additional toxic wastes, and the cost of commercial adsorbents used for the treatment of MNPs is still high (De Gisi et al., 2016).

b) Coagulation: Different types of organic and inorganic coagulants are used for MNPs removal, including ferric chloride, polyaluminium chloride, ferrous chloride, and polyamine (Zhou et al., 2021). These coagulants bind to microplastic particles by an uptake-complexation mechanism, thereby forming strong bonds with pollutants (Xu et al., 2021). For example, iron and aluminium coagulants were used for the removal of polyethylene microplastics under high polyacrylamide concentrations (Ariza-Tarazona et al., 2019). Also, metal hydroxide coagulants like iron and aluminium could help stabilize microplastics suspended in wastewater, thereby interacting via van der Waals forces to form sludge blankets (Perren et al., 2018). The major drawback of this method is its low selectivity; adsorbents are sensitive to pH, and competing ions tend to reduce the efficacy of adsorbents.

c) Membrane filtration: Dynamic membranes are utilized for influent flux and concentration of respective MNPs on the membrane during the process of filtration to enhance the removal of contaminants (Liu et al., 2021). For example, wastewater treatment plants were studied for their efficiency in removing microplastics in terms of shape, color, and dimensions using filtration processes. The microplastics removal efficiency reached approximately 90% (Ma et al., 2019). Another study showed that membrane bioreactors in combination with sand filters or disk filters, showed higher removal efficiency of microplastics when analyzed by Fourier transform infrared spectroscopy (FTIR) (Talvitie et al., 2017). The results revealed an abundance of polymers in the influent, with a high

concentration of polyethylene terephthalate and polyester. The drawback of this technique is that the initial operational cost is high, and there is a need for post-treatment mineralization.

d) Microbial remediation: The technique of microbial remediation uses microorganisms to eliminate toxic MNPs from the environment ("Advantages And Disadvantages Of Bioremediation," 2018)**.** Microbes produce enzymes, such as esterase, lipases, and proteases, that can break down the polymer chains of microplastics. These enzymes target specific chemical bonds present in plastics and initiate the process of degradation (Othman et al., 2021). Through enzymatic activity, microbes can gradually break down microplastics into smaller fragments. For example, *Phanerochaete chrysosporium*, produces an enzyme, manganese peroxidase, that could help in the degradation of polyethylene (Kang et al., 2019). Also, *Ideonella sakaiensis 201-F6* was able to degrade polyethylene terephthalate by an enzyme called polyethylene terephthalate-ase (Yoshida et al., 2016). The method of microbial remediation is restricted to compounds that can easily biodegrade and is also time-consuming.

1.4.4. Importance of Phytoremediation:

The above-mentioned techniques are associated with drawbacks that facilitates the use of phytoremediation approaches which are considered eco-friendly and effective for elimination of pollutants from environment. Conventional methods used for exclusion of plastic pollutants are energy-dependent, time consuming, and have adverse impact on ecosystem (Lourenço et al., 2019). Ability of various plants for phytoremediation is explored by numerous scientists (Rahbar et al., 2016; Rezania et al., 2016). Therefore, excessive interest is shown by researchers for improving the efficacy of conventionally-used methods by an environmentally-sound technique called Phytoremediation. It refers to efficient green technology to dispose contaminants existing in air, water and soil (Sarwar et al., 2017). Various plants owing to their characteristic property of intake of pollutants and degradation by various bacteria secreted by plant tissues (Chirakkara et al., 2016).

1.4.5. Approaches of Phytoremediation for MNPs:

Different approaches, including Phytoextraction, Phytostabilization, Phytodegradation, Phytovolatilization, and rhizosphere bioremediation, are employed by plants to facilitate the uptake of organic and inorganic pollutants from soil, thus forming the basis for Phytoremediation technology. The major plants that are utilized for phytoremediation of MNPs are described in table 1.2.

Table 1.2: Examples of different plants acting as potential sources for Phytoremediation of various contaminants

Plant Species	Accumulation Part	Contaminant for remediation	Reference
Festuca arundinacea S.	Shoots or roots	Petroleum hydrocarbon	(Steliga and Kluk, 2020)
Zea mays L.	Roots	Phenanthrene	(Baoune et al., 2019)
Chrysocoma Ciliate L.	Roots	Petroleum aromatic hydrocarbons	(Anyasi and Atagana, 2018)
Lolium multiflorum L.	Rhizosphere	Crude oil	(Hussain et al., 2018)
Lolium perenne L.	Rhizosphere microbes	Petroleum hydrocarbon	(Iqbal et al., 2019)
Suaeda glauca L.	Rhizosphere	Polycyclic aromatic hydrocarbons	(Chaudhary et al., 2021)
Iris dichotoma P.	Roots	Petroleum hydrocarbon	(Cheng et al., 2017)

a) Phytoextraction

Phytoextraction is a method to clean up contaminants from soil by absorption, accretion, and transfer of contaminants from soil to plant shoots, also referred to as phytomining (Tangahu et al., 2011). Plants act as hyper accumulators to absorb various pollutants in their shoots without any toxic effects on soil (Bian et al., 2020). An ideal hyper accumulator plant possesses the characteristic property of gathering large concentrations of MNPs within its shoots (Yu et al., 2021). For example, polystyrene microplastics and bisphenol-S showed no effect on *Pistis stratiotes* L. due to the accumulation of contaminants within the roots of plant and less translocation to other parts (L. Zhang et al., 2022). Another study showed accumulation of polystyrene microplastics within *Vicia faba* L. roots merely for around 48 hours after being exposed to microplastics (Jiang et al., 2019).

b) Phytostabilization

Phytostabilization, also referred to as phytoimmobilization, is a process of immobilizing contaminants in soil, roots, or shoots of plants thereby reducing their bioavailability in the environment (Tangahu et al., 2011). For example, polyethylene microplastics were able to adhere to aquatic macrophyte *L. minor*, due to surface stickiness and electrostatic interaction between MNPs and plant biomass (Rozman et al., 2022). Another study observed the immobilization of polystyrene microplastics in *F. vesiculosus* due to release of anionic polysaccharide on plant surface (Sundbæk et al., 2018). Also, microplastics captured within roots can reduce mobility, interaction with soil microorganisms and act as a potential source of phytostabilization. In a study by Gao et al. 2021, polyethylene microplastics adhered to root surface of *Lactuca sativa* L. without entering inside root hairs or other parts of the plant (Gao et al., 2021).

c) Phytovolatilization

Phytovolatilization is a method that uses metabolic ability of plants and soil microorganism to change toxic plastic contaminants into volatile and less toxic forms, thereby releasing them into the atmosphere (Tangahu et al., 2011). While phytovolatilization is commonly

associated with the uptake and release of organic compounds, such as volatile organic compounds (VOCs), there is limited research on its applicability to microplastics. Very few studies have investigated the potential for microplastic phytovolatilization by examining their uptake by plants as well as the subsequent release of volatile microplastic-associated compounds into the atmosphere. For example, laser confocal scanning microscopy and scanning electron microscopy provided evidence for the translocation of polystyrene nanoplastics from roots to shoots of *Triticum aestivum* L. without any effect on seed germination (Lian et al., 2020). Another study by Li et al., 2020a, observed transport of polystyrene and polymethylmethacrylate microplastics from roots to shoots of *T. aestivum* through crack-entry pathway and transpirational pull (Li et al., 2020a).

d) Phytodegradation

Phytodegradation, also known as phytotransformation, is a method to decompose inorganic pollutants in soil by the application of enzymes such as oxygenases, nitroreductases, and dehydrogenases (Ali et al., 2013). The Phytodegradation process occurs through the uptake of plastic contaminants within metabolic compartment of plants or microbes and their disintegration in soil. Degradation of pollutants occurs through two mechanisms: internal and external. In internal degradation mechanism, the MNPs are absorbed by plants that decompose through catalytic reactions by enzyme molecules, resulting in metabolic products utilized for plant growth and nutrition (Jeevanantham et al., 2019). In external degradation process, the plastic contaminants get absorbed by plant metabolic processes and hydrolyzed into smaller units (Jeevanantham et al., 2019). Formed monomer units are introduced into plant tissues for their growth and survival. For example, laccase and alkane hydrolase produced by *Staphylococcus epidermis* facilitated the depolymerization of polyethylene, forming monomer and oligomer units (Montazer et al., 2020). Also, the oxidase enzyme produced from *Pseudomonas vesicularis* PD could help in the degradation of polyvinyl chloride by oxidation of serine hydrolase active site present in polyvinyl chloride (Wilkes and Aristilde, 2017).

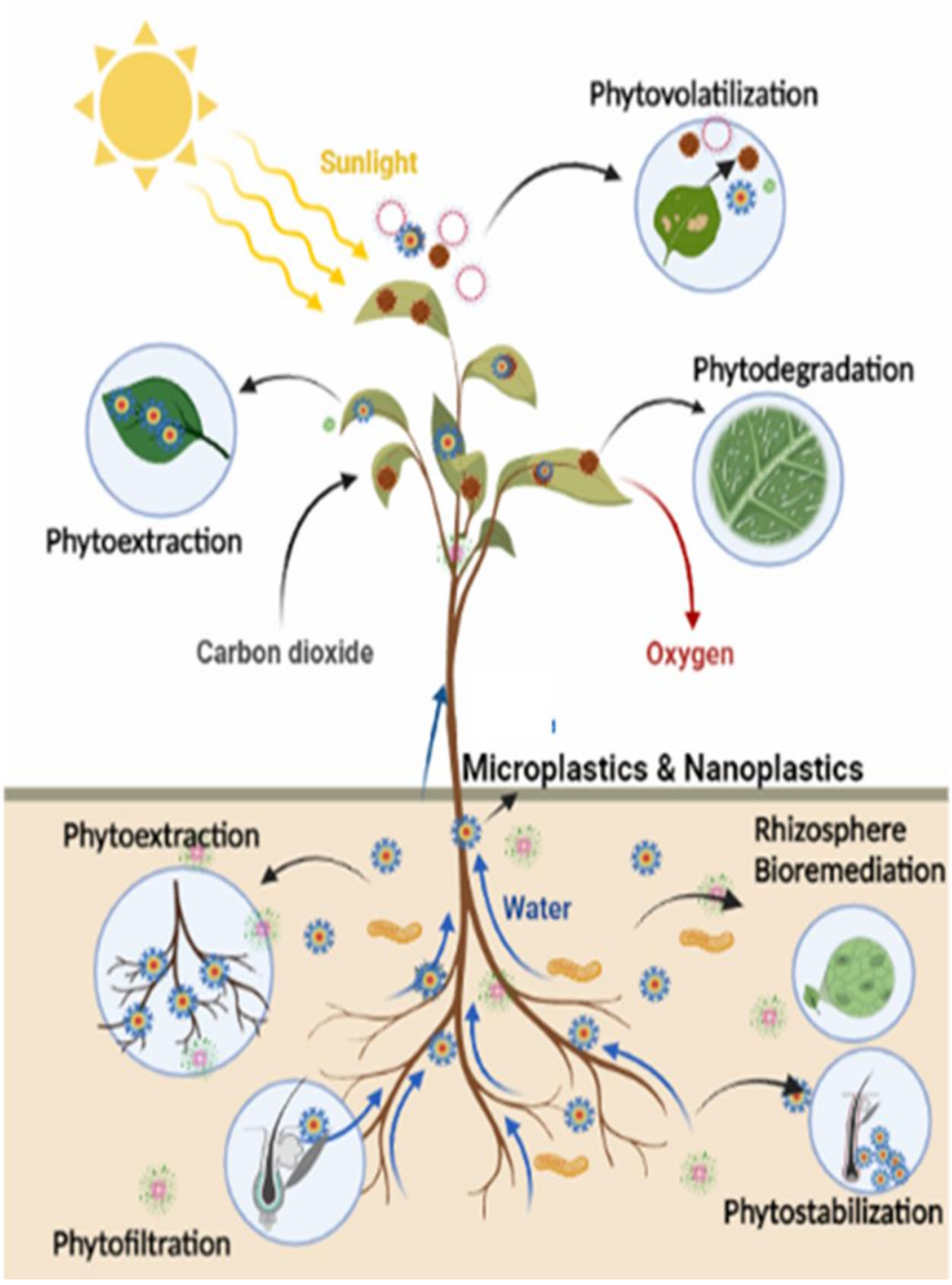


Figure 1.4: Different approaches of Phytoremediation

e) Rhizosphere Bioremediation

Rhizosphere bioremediation is a process for eliminating MNPs from soil through their degradation and breakdown under the action of plant microorganisms (Jeevanantham et al., 2019). Besides, it is also referred to Phyto stimulation, rhizosphere degradation, and plant-assisted bioremediation (Kumar et al., 2018). The growth and proliferation of soil microorganisms occur due to the presence of carbohydrates in the soil. The different microorganisms employed for remediation of contaminants in soil are listed in table 1.3. Various soil microorganisms can facilitate degradation of MNPs in soil. For example, *Bacillus cereus* and *Bacillus gottheilii* could change the structural properties of polyethylene microplastics (Auta et al., 2018). Also, *Pseudomonas capeferrum* TDA1 helped in the formation of a hydrolase enzyme that played an important role in degradation of polyurethane (Puiggené et al., 2022). Root exudates also perform as excellent contributors to improving the degradation of pollutants by increasing their activity in rhizosphere. For example, MNPs induce stress with a negative influence on the growth of *T. aestivum* and genotoxic effects on *V. faba* (Jiang et al., 2019; Qi et al., 2020). Root exudates could alleviate the stress response in plants, thereby facilitating phytoremediation of MNPs.

Table 1.3: Different microorganisms employed for remediation of various contaminants that are present in the soil

Microorganisms	Contaminant Remediation	Reference
Penicillium sp.	Low-density Polyethylene (LDPE)	(Rodrigo et al., 2021)
Klebsiella pneumoniae	High-density Polyethylene (HDPE)	(Awasthi et al., 2017)
Pseudomonas sp.	Polypropylene	(Habib et al., 2020)
Penicillium sp.	Polyurethane	(Magnin et al., 2019)
Vibrio sp.	Polyvinyl chloride	(Khandare et al.,

		2021a)
Ideonella sakaiensis	Polyethylene Terephthalate	(Azubuike et al., 2016)
Lysinibacillus sp.	Polyethylene	(Jeon et al., 2021)
Halomonas sp.	Low-density Polyethylene (LDPE)	(Khandare et al., 2021b)
Cephalosporium sp.	UV-treated polystyrene	(Chaudhary et al., 2021)

1.4.6. Factors influencing phytoremediation of MNPs

Phytoremediation, the use of plants to remediate pollutants from the environment, has gained attention as a potential method for addressing microplastic pollution. While research on phytoremediation of microplastics is still in its early stages, several factors are thought to influence the effectiveness of this approach like:

a. Plant species: Different plant species possess varying abilities to take up and accumulate microplastics (Colzi et al., 2022). Some plants may have higher affinity for microplastics due to their root structures or physiological characteristics. For example, certain aquatic plants like water hyacinth (*Eichhornia crassipes* L.) and duckweed (*Lemna* spp.) have been found to effectively accumulate microplastics in water bodies (Christian and Beniah, 2019; Rozman et al., 2022).

b. Root characteristics: The morphology and structure of plant roots can influence their ability to uptake microplastics. Plants with extensive root systems, such as those with fibrous or adventitious roots, have a larger surface area for interaction with microplastics. Plants with root exudates rich in enzymes and organic compounds may also enhance microbial activity around the roots, potentially facilitating microplastic degradation (Bosker et al., 2019). For instance, maize (*Zea mays* L.) and ryegrass (*Lolium perenne* L.) have shown promise in terms of their root characteristics for microplastic phytoremediation (Ullah et al., 2021a; Wang et al., 2012).

c. Microplastic characteristics: The properties of microplastics, such as size, shape, and surface charge, can affect their interaction with plants. Smaller microplastics tend to have a larger surface area and may be more readily taken up by plants. Furthermore, the surface properties of microplastics can influence their adsorption to root surfaces and subsequent translocation within the plant (X. Wang et al., 2020; Xu et al., 2022).

d. Environmental conditions: Factors such as temperature, light intensity, and nutrient availability can impact the growth and metabolism of plants, which in turn may influence their ability to remediate microplastics (Gong et al., 2023). Certain environmental conditions may enhance plant-microplastic interactions or promote the activity of microorganisms involved in microplastic degradation (Ebere et al., 2019).

Hence, future research is necessitated to focus on phytoremediation techniques that are easy, inexpensive, and sustainable to environment. The merits and demerits of phytoremediation approaches are broadly listed in table 1.4. Also, advanced strategies must be framed for effective phytoremediation of MNPs contaminants.

Table 1.4: Various approaches of phytoremediation highlighting their merits and demerits

Phytoremediation Approach	Merits	Demerits
Phytoextraction	i.Cost-effective method compared to other strategies ii.Contaminant can be reused iii.Removal efficiency up to 95%	i.Enhanced uptake of plastic by roots ii.Leaches into groundwater iii.Phyto mass disposal is difficult
Phytovolatilization	i.Economically efficient method	i.Redeposition of the contaminant back in

	ii. Contaminant is less toxic	the soil by precipitation
Phytostabilization	i. Low cost and efficient system ii. Reduction in soil erosion iii. Tolerates high concentration of pollutants	i. Soil not rendered suitable for plant growth ii. Obligatory checks necessary for effective remediation
Phytodegradation	i. Financially and economically stable system ii. Enzymatic breakdown of pollutants feasible	i. Dependent on soil abiotic conditions and plant species ii. Contaminants may re-emerge by soil microorganisms
Rhizosphere Bioremediation	i. Microbial activity increases ii. Self-sustaining method for removal of pollutants iii. Environment friendly with low cost	i. Continuous monitoring of pH to uptake pollutants ii. Laboratory scale studies not stabilized for commercial and field purposes

1.4.7. Proposed strategies for progressive phytoremediation of MNPs

Phytoremediation mechanisms offer great potential for the removal of MNPs. However, advanced strategies can deliver greater potential for phytoremediation to be effective. Thus, various strategies have been proposed for efficient phytoremediation.

a) Selection of hyper accumulator plant species

Exploration of various hyper accumulator plant species can revolutionize the technique of phytoremediation because of their ability to absorb contaminants 100 times more as compared to natural plants (Kumar Yadav et al., 2018). For example, *L. minor* commonly used for phytoremediation of wastewater facilities, experimented with polystyrene nanoplastics to observe the impacts on accumulation and tolerance in plants. The results could provide direct evidence of no oxidative damage, unaltered chlorophyll contents, increased lipid peroxidation, and no growth suppression (Arikan et al., 2022). The polystyrene nanoplastics could only accumulate to some extent in the leaves of plants but not be translocated to other parts, thereby showing hyperaccumulation within specific plant parts (Arikan et al., 2022). Another study showed the accumulation of polystyrene nano- and microplastics within the root surface and cap cells of *A. thaliana* and *T. aestivum*. Laser confocal scanning microscopy and pyrolysis gas chromatography-mass spectrometry confirmed that polystyrene spheres accumulated only at root surface of each plant, without any evidence for internal uptake or accumulation (Taylor et al., 2020).

b) Utilization of plant-growth promoting bacteria for removal of MNPs

Plants contain diverse microbial communities residing in the rhizosphere, phyllosphere, and endosphere (Feng et al., 2017). These microorganisms participate in essential roles for plant growth, nutrition, and degradation of contaminants (Kumar Yadav et al., 2018). For example, endophytic bacteria obtained from *Oryza meridionalis* L. were found to degrade phthalates, thereby reducing their accumulation in plants and increasing yield efficiency. A culture experiment containing various endophytic strains showed that the highest degradation of di-n-butyl-phthalate occurred using *Bacillus amyloliquefaciens*. The results confirmed the ability of endophytic bacterial strain to remove phthalates and promote plant growth and development (L.-H. Liu et al., 2022). Another study showed the isolation of *Achromobacter xylosoxidans* from soil that could degrade high-density polyethylene. Attenuated total reflectance fourier transform infrared spectroscopy and scanning electron microscopy revealed degradation of microplastics by approximately 9 % (Kowalczyk et al.,

2016). Also, *Bacillus* spp. and *Rhodococcus* spp. strains isolated from mangrove sediments could help in degradation of polypropylene. Around 6 % and 4 % weight loss could be observed after 40 days of incubation in bacterial strains, which was confirmed by Fourier transform infrared spectroscopy and scanning electron microscopy analysis (Auta et al., 2018). Thus, various bacterial strains could help in degradation of MNPs and increase plant growth yield.

c) Omics-based approaches to study MNPs degradation

Plants respond differently to environmental conditions involving a range of routes, starting with changes in gene expression (transcriptomics), accumulation of protein products that help in degradation (proteomics), and formation of metabolites (metabolomics) (Forde and O'Toole, 2013). Metagenomics analysis could help in the identification of MNPs that degrade microbes and enzymes that could facilitate degradation (Staley and Sadowsky, 2016a). For example, cytochrome P450, esterase, and lipase enzymes were isolated from *Nocardioides* spp. and capable of degrading monoalkyl and dialkyl phthalate esters (Qiu et al., 2020). Apart from the identification of microbes for degradation, metabolic processes, gene identification, and expression are also essential for MNPs degradation. For example, transcriptomics was applied to identify the mechanism of degradation of polyethylene by *Rhodococcus ruber* C20 strain (Gravouil et al., 2017). Another study showed the expression of pht and pca genes isolated from *Arthrobacter* sp. ZJUTW capable of degrading dibutyl phthalate. This study revealed a combination of metagenomic and metatranscriptomic studies to ascertain the degradation of phthalate (Liu et al., 2020). Besides transcriptomics and metagenomics, metaproteomics could also facilitate the mechanisms of protein synthesis that control metabolism and obtain metabolites (Medić et al., 2019). For example, proteomic profiling of *Pseudomonas pseudoalcaligenes* helped in the identification of a PpEst enzyme that could hydrolyze polybutylene adipate terephthalate (Wallace et al., 2017).

d) Gene Editing tools to increase MNPs degradation

Phytoremediation efficiency of plants can be increased by introducing plastic accumulating

genetic determinants into the genomes of hyper accumulating species (DalCorso et al., 2019). Thus, genetic engineering tools can be explored to increase MNPs accumulation by genes accountable for plastic uptake and their decontamination (Fasani et al., 2018). For example, *Ideonella sakaiensis* 201-F6 produces a polyethylene terephthalate degrading enzyme. The genes of this bacterial strain can be genetically encoded in other bacterial strains to promote polyethylene terephthalate degradation (Anand et al., 2023). In a study by Moog et al. 2019, polyethylene terephthalate hydrolyzing enzymes were introduced into *Phaeodactylum tricornutum,* thereby showing efficient degradation (Moog et al., 2019). Apart from these genetic modifications, gene editing tools like clustered regularly interspaced palindromic repeats (CRISPR)/Cas9 could also promote manipulation of microbial species for faster degradation of MNPs. For example, *Streptomyces albogriseolus* LBX-2 could produce three different types of CRISPR sequences in which the main enzyme that helped in polyethylene degradation was oxygenase (Shao et al., 2019). Thus, genome editing could help in incorporating genes encoding MNPs degrading enzymes (figure 1.5).

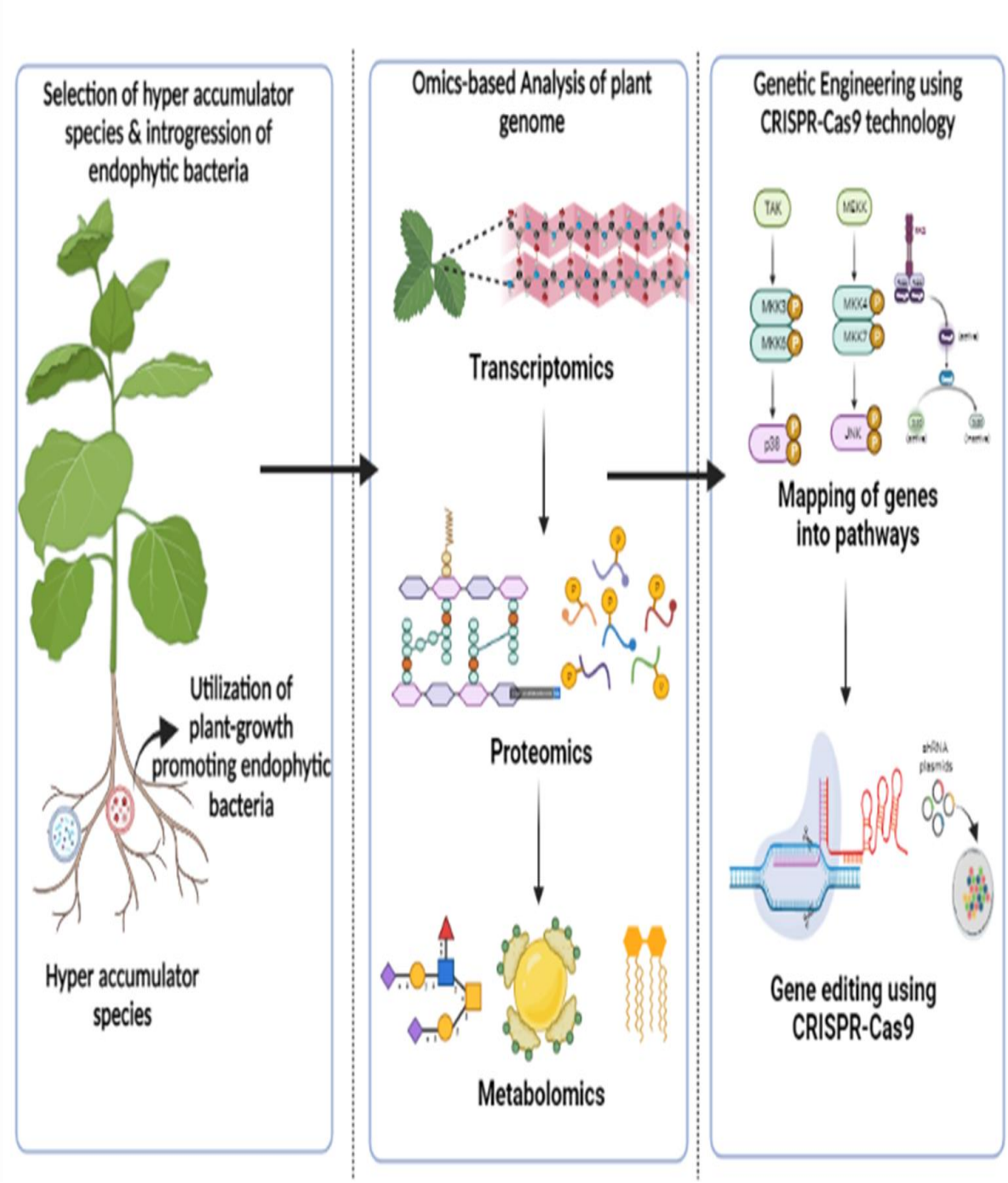


Figure 1.5: Advanced strategies for phytoremediation of MNPs: Selection of Hyper accumulator species, Omics-based analysis of plant genome, genetic engineering using Crispr-Cas9 technology

1.5 Research Gaps:

This research will highlight several major gaps in our understanding of what happens to micro/nano plastics after their migration in soil and plants.

- None of present studies have revealed knowledge on the behavior and mechanism of micro/nano plastics degradation in agroecosystem.
- No detailed methodology has been presented till today on the phytoremediation of micro/nano plastics in agroecosystem.
- Need to identify mechanisms on the migration and fate of micro/nano plastics in soil and plants
- Identification of the uncultured rhizosphere microbes acting on the most dominant polymers.

1.6. Broad Objectives of the study:

- Understanding the impact of micro/nano plastics on the growth and physiological parameters of plants.
- Describing the fate and behavior of micro/nano plastics on the biometry of wild plants.
- Understanding the phytoremediation approaches to remediate soil of micro/nano plastics.
- Synergistic plant-microbe interaction and corresponding metabolic products in modulating micro/nano plastic degradation.

CHAPTER 2

MATERIALS & METHODS

Chapter-2: Materials & Methods

2.1. OBJECTIVE 1: Understanding the impact of micro/nano plastics on the growth and physiological parameters of plants:

The major aim of present study is understanding uptake, accumulation, and translocation of high-density polyethylene (HDPE) microplastics along with their adverse effects on terrestrial plant. HDPE constitutes a large proportion of environmental pollution among all the microplastics (Awasthi et al., 2017). To facilitate this mechanism, *Brassica juncea*, commonly termed as Indian Mustard, was used as a model plant to assess sites of absorption, uptake, and accumulation within the plant. *Brassica juncea* is particularly useful for phytoremediation as it can accumulate high levels of heavy metals (lead, nickel, cadmium, mercury and selenium) in their tissues, a process called as phytoextraction (Rathore et al., 2019). *Brassica juncea* produce compounds called glucosinolates, which are broken down by enzymes to release toxic isothiocyanates. These isothiocyanates form complexes with heavy metals in the soil, where they are then absorbed by plant roots and stored in their tissues. This means they can quickly establish themselves in contaminated soil and start removing pollutants (Diwan et al., 2008). *Brassica juncea* has a high biomass, which means it can accumulate huge quantities of pollutants in tissues (Goswami and Das, 2015). Also, *Brassica juncea* has a deep root system that allows them to access pollutants that may be located deep in soil. Overall, the combination of these characteristics makes *Brassica juncea* an effective plant for phytoremediation of soil. Therefore, it is considered as the most ideal plant to study potential for microplastics remediation in soil. To our knowledge, this study is the first to observe impacts of microplastics not only on roots, but also on leaves and shoots of plants. Biochemical analysis on roots, shoots and leaves has been demonstrated to provide further evidence of microplastics intake in plants. Finally, mechanism of uptake of microplastics by plants highlighting different pathways is briefed to observe phytoaccumulation and identify the possibilities for soil remediation of microplastics.

2.1.1. Experimental design

a) Soil

To study impacts of different size microplastics on *Brassica juncea* (mustard seeds) in an open environment, a triplicate study was conducted. Pots containing equal concentrations of soil and mustard seeds with varying amounts of microplastics was used for the study. We harvested the mustard seeds after a week to determine the effects of our experiments on vegetative and reproductive growth. The sandy soil used in this study was obtained from an agricultural land in Ghaziabad, Uttar Pradesh, India, at 28° 39' 14.1588" N and 77° 26' 42.8784" E. The soil was composed of sand, silt, and clay with moderate amounts of organic matter. The air-dried soil was sieved with a 2 mm steel sieve before use.

b) Synthesis of microplastics

In this experiment, two forms of microplastics were used: (1) high-density polyethylene (HDPE) microplastics and (2) high-density polyethylene (HDPE) beads. A method described by Crespy and Landfester, 2007, was used to prepare the HDPE microplastics (HDPE_MPs) and beads (HDPE_beads). A solution composed of 1 g of HDPE powder and 20 ml of xylene was mixed using a magnetic stirrer for 1 h until completely dissolved. The HDPE solution was then gently added to 100 ml of deionized water while maintaining the sonication at 70% amplitude (Branson W450 Digital sonicator, tip size 6.5 mm) for 30 s under ice cooling. The resulting solution was centrifuged, rinsed with water and ethanol, then air-dried before storage.

c) Mustard seeds and pots

Brassica juncea (mustard seeds) was obtained from Indian Institute of Agricultural Research (IARI), Pusa, New Delhi. The seeds were surface sterilized with 0.02% sodium hypochlorite (NaOCl) before immersion in 70% ethanol (figure 2.1). After sterilization, the seeds were rinsed several times with distilled water. The seeds were grown on tissue overnight before being planted in organic soil in November 2021. Pots were irrigated twice a week at first, then once every two days during seedling emergence in January and February of 2022.

Because of the increase in temperature in February, the frequency of irrigation was increased. NPK was applied to the soil in each pot based on the mustard NPK requirement of 100, 20, and 60 kg/ha.

The pot used in the experiment was 20 cm long, 10 cm in diameter at the bottom, 13 cm in diameter at the top and had a volume of 2 l. We used a factorial experimental design. Furthermore, three control treatments with no microplastic residues were investigated. The experiment included 12 treatments performed in triplicate, as well as four independent pots containing tagged microplastics for imaging. Each treatment was replicated three times, and total 36 pots of *B. juncea* seeds were grown. The mean of three potted plants was used to describe the study's findings.

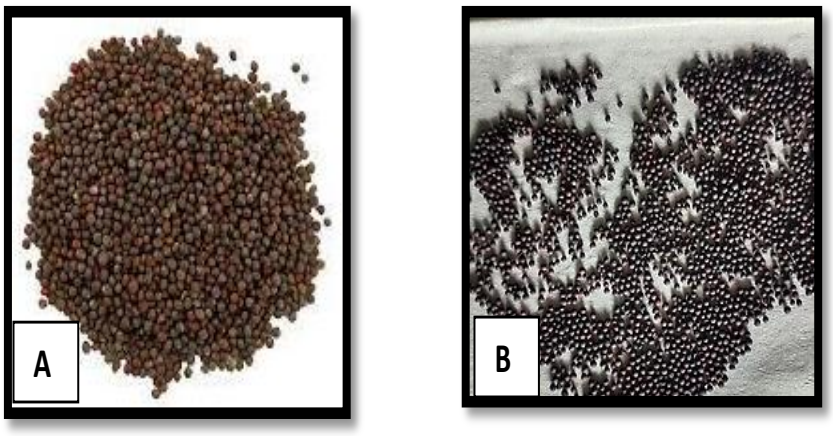


Figure 2.1: (A) *Brassica juncea* (mustard seeds) and (B) Germination done in complete dark

2.1.2. Experimental set-up and climatic conditions for growth of plant

a) Setting up

Each pot constituted 2.5 kg of sieved soil and various concentrations of microplastics (except the three control treatments without microplastics) along with 150 ml of water. Prior to filling each pot with this mixture, a piece of geotextile was placed at the bottom of each

pot to allow free circulation of air and water. After all the pots were filled, the soil moisture was uniformly set to 15%, which corresponds to the water capacity of the soil in the field. Before sowing *Brassica juncea* (mustard seeds), let sit for a week in each pot. Each container contained 12 g of litter (12.08 ± 0.06 g) and was sprayed with water to keep the litter moist.

b) Mustard Cultivation

Each pot contained 10-12 seeds, and post 2 weeks, 6-7 seedlings were selected from each pot for testing. The temperature was set at 15-16 °C during the day and 12 °C at night, with a photoperiod (14/10 h), a light intensity of 300 µmol m^{-2} s^{-1} and a relative humidity of 70 % for day and night. The pots were watered weekly with tap water and soil moisture was adjusted to between 12 % and 18 % by weight. Pots were randomly placed in the climatic chamber and rotated each after two weeks.

c) Microplastic tagging with Nile Red

For imaging, HDPE microplastic particles were labeled with Nile red as previously described (Karakolis et al., 2019). The dried microplastic particles were placed in an aqueous deionized (DI) solution of 100 µg/ml Nile red at a concentration of 50 mg of plastic particles per 10 ml of solution. This solution was prepared by dissolving 1 mg of Nile Red in 1 ml of acetone and the resulting solution was added to 10 ml of deionized water. The vials were left in the dark for two hours, rinsed three times with water, centrifuged and stored in deionized water for later use. Based on known staining procedures for microplastics, the Nile Red staining procedure was selected, including concentration of the Nile Red solution (Maes et al., 2017).

2.1.3. Measurements of growth parameters

a) Biometrical Analysis

Plant heights were measured using a steel tape measure on a regular basis from the 14^{th} day after seeds were sown until the 90^{th} day. Three months after planting, plants were divided

into shoots and roots. Biometrical analysis provides the overall growth, biomass, and length of roots, shoots and leaves of plants after being exposed to different concentrations of a contaminant (Pricinotto et al., 2019). In this study, different concentrations of MPs were used as contaminant to observe the growth pattern in plants. At a span of three months from sowing of seeds, root and shoot height was measured and a mean of all three triplicates were used to determine the results of study. Similarly, root and shoot biomass was weighed for each treated sample to determine mean difference with respect to control.

b) Chlorophyll content

To determine photosynthetic pigment in leaves of plant, chlorophyll estimation was done. Relative chlorophyll content in plant leaves was measured and recorded as per the process defined by Ren Hong et al., 2012 using UV-Vis spectrophotometer (Biospectrophotometer, USA). Fresh leaves were obtained from each pot to determine the chlorophyll level. For each treated and control sample, 0.5 g of leaves were weighed from each pot. 10 ml of 80 % acetone was added to chopped and homogenized leaves to make them transparent. The extract was centrifuged at 2500 rpm for five minutes. The resulting supernatant was diluted with 9 ml of 80 % acetone before being measured with a UV-Vis Spectrophotometer at 663 nm and 644 nm. Total chlorophyll content in microplastic treated and control samples was evaluated using Mackinney's work and Arnon equations –

$Chl_a = 12.7\ A_{663} – 2.69\ A_{645}$;

$Chl_b = 22.9\ A_{645} – 4.68\ A_{663}$

Total chlorophyll = $Chl_a + Chl_b$

2.1.4. Biochemical Analysis

a) Phenolic Content

A modified Folin-Ciocalteau test with gallic acid as the standard was used to assess total phenolic content (Ainsworth and Gillespie, 2007). 1 ml of plant extract was combined with 5 ml of Folin Ciocalteau's reagent after 1.5 ml of 20 % Na_2CO_3 was added (diluted 1:10 with distilled water). Color development was accomplished by incubating the test tubes in dark

for 30 minutes at room temperature, followed by measuring the absorbance at 765 nm. The total phenolic content of the sample was estimated as mg of dry mass equivalents of gallic acid (GAE) mg^{-1}.

b) Proline Content

Modified ninhydrin chromogenic techniques were used to measure the proline content (Zhang et al., 2013). A glass tube containing freshly harvested roots (0.2 g) was filled with 5 ml of 3 % sulfosalicylic acid. The glass tube was incubated for 10 minutes in a 100 ℃ water bath. 2 ml of the filtrate was digested in another glass tube after 4 ml of chromogenic solution (2 ml of 2.5 % ninhydrin and 2 ml of glacial acetic acid) was added to the filter. The glass tube was then immersed for 30 minutes in a 100 ℃ water bath. Further, to stop the reaction, glass tube was submerged in an ice bath. 5 ml of toluene were placed in the glass tube, vortexed, and then allowed to stand. Using a spectrophotometer, the toluene layer's absorbance was observed at 520 nm.

2.1.5. Morphological Analysis

a) Fluorescence Microscopy

Fresh roots and leaves were removed from *Brassica juncea* plant and cleansed with deionized water. The roots were sectioned and placed on a glass slide with a few drops of clean water. The glass slide was then gently squeezed to flatten the pure water-covered root, ensuring that no air bubbles formed between the glass slide and cover slip. A fluorescent microscope was used to view each sample.

b) Confocal Microscopy

Fresh roots and leaves with tagged microplastics were picked out and cleansed with deionized water. On a glass slide with a few droplets of distilled water, cross-sections of roots were exhibited. Furthermore, to ensure no air bubbles between glass slide and cover slip, it was gently pressed to flatten the clean water-covered root. With the use of a confocal microscope (Nikon Laser Scanning Confocal Microscope), each sample was examined to

observe the tagged microplastics. Similar observations were performed for leaf cross-sections of plant.

2.1.6. Statistical analysis

All experiments were done in triplicate. Statistical analysis of experimental data was performed using Origin2023 software, and analysis of variance (ANOVA) was performed in SPSS 21.0 with a p-value of 0.05. For triplicate samples (n = 3), all values were expressed as mean 5 % standard error.

2.2. <u>OBJECTIVE 2:</u> Describing the fate and behavior of micro/nano plastics on the biometry of wild plants:

It is possible to monitor plant development by establishing a replicated landfill system in order to determine the effects of microplastics on different types of plants. This method involves cultivating plants in mixtures or soil contaminated with microplastics or other contaminants. This framework provides a controlled environment to focus on different sections and can be used to mimic the effects of microplastic weight on plant development. It should be feasible to watch how plants grow under different ecological pressure conditions, such as openness to different concentrations of microplastics, in a simulated landfill. It is possible to evaluate the implications of varying pressure conditions for plant development, metabolic cycles, photosynthesis, and ingestion component using this methodology. In general, a simulated landfill can provide important information regarding the effects of microplastic weight on plant growth and help to lessen the ecological impact of contamination. Phytoremediation can be used to clean up contaminated soil and habitats, and the framework can be used to identify plants resistant to microplastic stress. In light of the aforementioned information, the purpose of this study is to create a simulated landfill that is exposed to two distinct groups of two microplastics: HDPE and nylon 6,6. These microplastics, which are mostly used in corporate and contemporary settings, take a long time to degrade. Discovering *Cyanodon dactylon* (L.), commonly known as Scutch grass (SG) and *Portulaca grandiflora* (PG), to diverse microplastic convergences was the driving

force behind the landfill simulation.

2.2.1. Set-up

Modern Agro Forestry provided the seeds for *Cyanodon dactylon* (L.) and *Portulaca grandiflora*. Given their ability to withstand harsh environmental conditions and thrive in temperatures and humidity levels, these wild plants were chosen for the evaluation. The soil used in this study was collected from the Shakti Khand landfill in Ghaziabad, Uttar Pradesh, India, which is located at 30° 39' 14.1588"N and 86° 26' 42.8784"E. In addition to dirt, the mud contained other elements, such as trash, small pieces, metal components, and other waste items. The effects of PE and Nylon-6,6 microplastics on the growth of *Cyanodon dactylon* (L.) and *Portulaca grandiflora* were assessed using a landfill simulation. In an open environment with daylight and sunshine, the pot test was used to determine the effects of various microplastics on *Cyanodon dactylon* (L.) and *Portulaca grandiflora*. In order to examine the effects of our research on both vegetative and regenerative development, we simultaneously collected seeds from the two plants after seven days.

2.2.2. Experimental Design

Pots that were 10 cm tall, 15 cm in diameter at the bottom, 13 cm in diameter at the top, and had a 1liter capacity were used in the experiment. It was decided to use a factorial experimental design. Three alternative approaches that left no trace of microplastics were also examined. There were four different pots containing tagged microplastics for imaging and a total of 28 treatments in triplicate throughout the experiment. There were also three copies of every treatment. The average of three potted plants was used to describe the study's findings. In each pot, 1.5 kg of dump yard soil, different amounts of microplastics (except from the four control treatments lacking microplastics), and 150 cc of water were weighed and manually combined. To enable unhindered air and water flow, a piece of geotextile was positioned at the bottom of each pot prior to the mixture being added. Twelve seeds were contained in each pot; six to seven seedlings were selected and maintained for the experiment over the duration of two weeks. The day/night photoperiod (14/10 h) was the

controlled condition that was used; the set temperatures were 10 °C at night and 15–16 °C during the day, with a light intensity of 300 μmol $m^{-2}s^{-1}$ and a relative humidity of 50 %.

According to earlier research by Karakolis et al., 2019, Nile Red was utilized to tag microplastic particles for imaging. Distilled water was mixed with a 100 μg/ml Nile Red solution and dried microplastic particles at a concentration of 50 mg per 10 ml. The solution was prepared by dissolving one milligram of Nile Red in one milliliter of acetone, and then adding the resulting liquid to ten milliliters of DI water. Vials were maintained in DI water for later use after being cleaned three times with water and two hours in the dark. We selected the Nile Red dyeing method according to accepted microplastic dyeing protocols, which included the concentration of the Nile Red solution (Maes et al., 2017).

2.2.3. Growth Parameters

With the use of a steel tape measure, plant heights were regularly measured starting on the fourteenth day following seeding and continuing until the sixtieth day. After two months of seeding, the plants were separated into shoots and roots. Using the mean of the three triplicates, the study's results were calculated. After seeding, root and shoot height were measured on a regular basis. Likewise, in order to calculate the mean deviation from the control, the biomass of the roots and shoots in each treated sample was weighed.

2.2.4. Chlorophyll Content

With a UV-Vis spectrophotometer (Eppendorf, USA) at the tip of three completely developed leaves on the 60th day for all control and treated plants in each pot, the relative chlorophyll content of plant leaves was determined and recorded, as per the protocol of (Ren Hong et al., 2012). To find out the chlorophyll concentration, fresh leaves were taken out of each pot. The treated and control samples produced 0.5 g of leaves per plant. The chopped and homogenized leaves were mixed with 10 milliliters of 80 % acetone to create a translucent texture. Five minutes at 2500 rpm were spent centrifuging the extract. After diluting the resultant supernatant with nine milliliters of 80 %, one milliliter was measured at 663 nm and 644 nm using a UV-Vis Spectrophotometer. Mackinney's research and the

Arnon equations were the sources of the formulas:

$Chl_a = 12.7\ A_{663} – 2.69\ A_{645}$

$Chl_b = 22.9\ A_{645} – 4.68\ A_{663}$

Total chlorophyll = Chla + Chlb

2.2.5. Antioxidant Activity

According to Heath and Packer, 2022, the amount of lipid peroxidation in leaves was ascertained by measuring their malondialdehyde concentration (MDA). In 10 milliliters of 0.1 % trichloroacetic acid (TCA), the leaf tissue (0.5 g) was crushed. Using an Eppendorf Centrifuge 5810 R, the homogenate was centrifuged for 15 minutes at 12,000 rpm. 2 ml of the supernatant was mixed with a 0.5 % thiobarbituric acid (TBA) solution. This combination was chilled after it had been heated to 95 °C for 30 minutes. The blend underwent a 10-minute centrifugation at 10,000 rpm. (Eppendorf UV-vis Spectrophotometer) The absorbance of the supernatant was measured at 532 nm. By utilizing its extinction coefficient, the MDA content was determined.

To ascertain the level of LOX activity 500 milligrams of leaf samples were homogenized in 0.5 milligrams EDTA-containing ice-cold phosphate buffer (7.5). The homogenate was centrifuged for fifteen minutes at four degrees Celsius at 12,000 rpm. 50 µl of the extract was added to 2.95 ml of the substrate, which was generated by mixing 35 µl of linoleic acid with 5 mL of milli Q water that included 50 µl of tween 20. The total volume was brought to 100 ml using 0.1 M phosphate buffer. The absorbance measurement was made at 234 nanometers (Doderer et al., 1992, p. 199).

2.2.6. Morphological Analysis

Confocal microscopy was used for analyzing the uptake of micro/nano plastics in both the plants. Fresh roots and leaves that had been micro plastically labelled using Nile red dye were cleaned using deionized water. On glass slides with a few distilled water drops, root cross-sections were displayed. The root was also carefully crushed with the glass slide and

cover slip while it was immersed in clean water to ensure there were no air bubbles left. A confocal microscope (Nikon Laser Scanning Confocal Microscope) was used to observe microplastics in each sample. Comparable findings were observed in the cross-sections of the leaves of both plants (*Cyanodon dactylon* and *Portulaca grandiflora*) that had been marked with distinct microplastics.

2.3. <u>OBJECTIVE 3:</u> Understanding the phytoremediation approaches to remediate soil of micro/nano plastics:

Plants have the potential to play an important role in mitigating microplastic pollution in soils by stabilizing, extracting, or enhancing microbial degradation of these pollutants. Therefore, the uptake mechanism of microplastics within plant parts could play a role in phytoremediation approaches of microplastics within the plant. The uptake of MNPs by plants is dependent on their physiological characteristics, demonstrating diverse absorption and accumulation inside plants and soil. Although very few studies have discussed the uptake mechanism of MNPs in plants, some evidence can be put forward through them. For example, one study observed the extracellular entrapment of polystyrene microbeads in the root caps of plant tissues. Further, microbeads traversed from root to leaf parts through intercellular spaces via the vascular system following the transpiration stream (L. Li et al., 2019). Another study showed the absorption of microplastics within the endodermis of plant tissues through damaged root gaps and their transport to the aerial parts of the plant by transpiration (Sun et al., 2023). The apoplast and symplast are two pathways for MNPs to go through tissues once they have entered the plant. In contrast to symplastic transport, which involves movement of water and other substances between the cytoplasm of adjacent cells through specialized structures called plasmodesmata and sieve plates, apoplastic transport occurs outside the plasma membrane through extracellular spaces, the cell walls of adjacent cells, and xylem vessels (Pérez-de-Luque, 2017). The apoplastic route is crucial for radial movement inside plant tissues and enables the passage of MNPs to vascular tissues and root central cylinder for further movement to the aerial portion (Li et al., 2020a). However, getting to the xylem through the root requires getting past a barrier to apoplastic pathway,

the Casparian strip, which must be done by taking a symplastic route via endodermal cells. Using sieve tube components in the phloem, another significant symplastic transport is also conceivable, allowing distribution to non-photosynthetic tissues and organs (Y. Liu et al., 2022). Sieve tubes in phloem also provide a passage for MNPs entry into the aerial parts of plants, as they have a thickness of approximately 0.77 µm to 1 µm, allowing small microplastics to traverse inside the plant tissues (Bussières, 2014).

The uptake mechanisms of micro/nano plastics (MNPs) by plants can share similarities with the uptake mechanisms of other nanoparticles. While research on the specific uptake mechanisms of MNPs by plants is still emerging, studies on other nanoparticles provide insights into potential similarities. Here are a few examples of how the uptake mechanisms of MNPs may share similarities with the uptake mechanisms of other nanoparticles:

a. Size-dependent uptake: Plants can take up nanoparticles through various pathways, including root uptake, foliar uptake, and uptake through plant cell walls (Ali et al., 2021). This size-dependent uptake has been reported for a range of nanoparticles, including metal-based nanoparticles and carbon-based nanoparticles (Khan et al., 2022), and it may also apply to MNPs as postulated in different studies (Huang et al., 2022; Y. Liu et al., 2022; H. Sun et al., 2021).

b. Endocytosis: Some nanoparticles can be taken up by plants through endocytosis, which involves the internalization of particles by plasma membrane (Palocci et al., 2017). This mechanism has been reported for various nanoparticles, such as metal-based nanoparticles and quantum dots (Raven, 2022). It is possible that MNPs can also be internalized by plants through endocytosis, for example, in rice seedlings exposed to polystyrene microplastics. The study observed accumulation of polystyrene microplastics in the roots of rice due to endocytosis (Wu et al., 2021).

c. Passive diffusion: Nanoparticles can also passively diffuse across plant cell membranes. This process depends on the physicochemical properties of the particles, such as their size, surface charge, and hydrophobicity (Geisler-Lee et al., 2013). MNPs may exhibit similar passive diffusion mechanisms as other nanoparticles when entering plant cells (Xu et al., 2022).

2.4. <u>OBJECTIVE 4:</u> Synergistic plant-microbe interaction and corresponding metabolic products in modulating micro/nano plastic degradation:

To study the synergistic interaction between plant and microbes, the second objective was continued and conducted over a span of 32 weeks, with plant biometrical parameters recorded at two key intervals: 16 weeks and 32 weeks. These intervals were chosen to assess both the short-term and long-term effects of microplastic exposure on both the plants, *Cyanodon dactylon* and *Portulaca grandiflora*. By comparing the data from the 16-week and 32-week intervals, it became possible to assess the progression of microplastic-induced effects on plant growth and determine whether these impacts were transient or more sustained. Also, after a prolonged period of time, impacts of microplastics were reversed stating that there might be certain microbial communities to degrade microplastics, particularly HDPE and Nylon 6,6. In view of the above facts, the present study was conducted to determine the long-term effects of microplastics exposure on both wild plants and also to ascertain the potential of microbial communities capable of microplastic degradation.

2.4.1. Soil Microbial Isolation and Identification

Following the 32-week observation period, microbial strains were isolated from the soil, and their ability to degrade microplastics was tested *in vitro*. The successful degradation of HDPE and Nylon 6,6, fragments by these isolated strains suggests that similar degradation processes may have been occurring in the soil during the experiment. The gradual breakdown of microplastics by these microbes could explain the reduced impact on plant biometrical parameters. Also, to test the efficacy of isolated microbial strain, another microplastics namely, Polypropylene (PP) and Poly vinyl Chloride (PVC), were inoculated into the microbial culture and their degradation studied (figure 2.2).

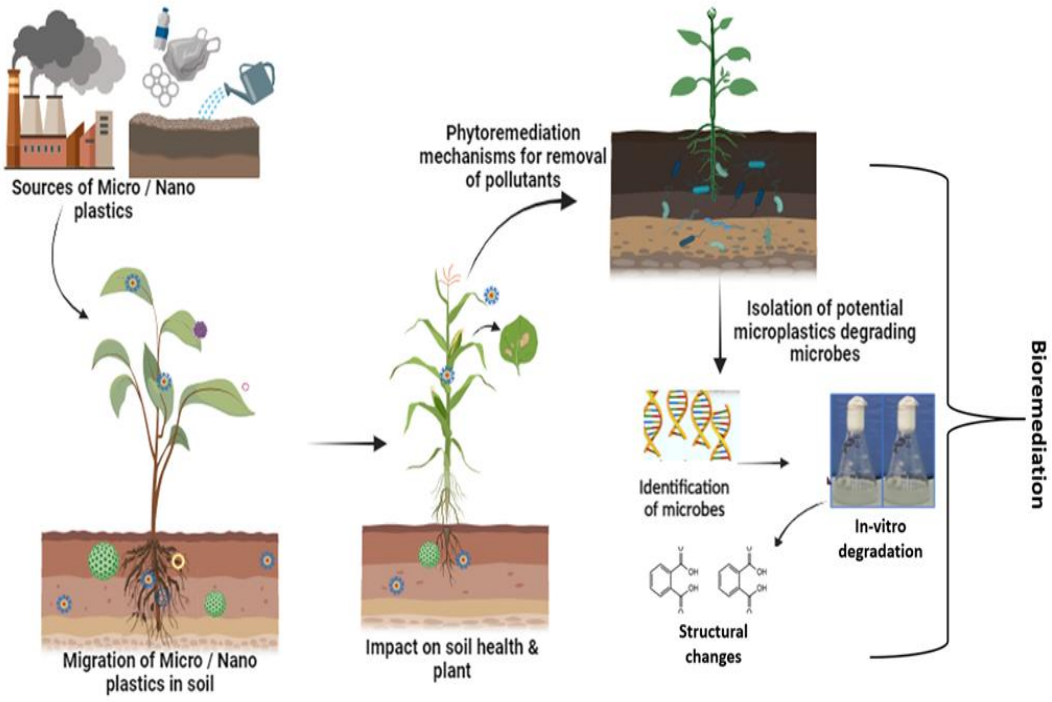


Figure 2.2: Workflow of microbial isolation and degradation study

For the purpose of creating the bacterial cultures, 4 g of sediment samples were mixed for 3 hours in an isotonic saline solution, let to settle for 40 minutes, and then the resulting supernatant was injected into LB broth medium. Following this, 48 hours were spent to grow the microbial cultures at 30 °C in a rotating incubator spinning at 140 rpm. Using LB agar plates and the pour plate technique, twelve single colonies were produced from bacterial cultures. From these bacterial strains, the ones that broke down microplastics were further selected. Analyzing the bacterial isolates' capacity to use microplastics as their only carbon source was part of the screening procedure (Staley and Sadowsky, 2016b).

2.4.2. Preparation of microbial inoculum for *in vitro* microplastics degradation

The present study used a degradation assay with Mineral Salt Medium (MSM) composition of 0.25 % $MgSO_4$, 0.025 % $CaCl_2$, 1 % KH_2PO_4, 2.3 % K_2HPO_4, 1 % NH_4Cl, 0.05 g $FeCl_3$, and 0.5 % NaCl. Additionally, 1 mL of trace element solution (20 mg L^{-1} $CoCl_2.6H_2O$, 18

mg L^{-1} $NiCl_2.6H_2O$, 24 mg L^{-1} $CuSO_4.5H_2O$, 0.5 g L^{-1} $CaCl_2$, and 1.62 g L^{-1} $FeCl_3.6H_2O$) per liter of distilled water was added, and 0.5 % (w/v) of microplastics were further supplemented. In order to start the bacterial growth process, the microorganisms were inoculated into nutritional broth and allowed to reach the stationary phase in a revolving shaker set to 32 ºC and 120 rpm. The growth of the bacterial cultures was monitored by measuring the absorbance at 600 nm using an Eppendorf UV-VIS spectrophotometer. pH and bacterial growth were continuously measured throughout the experiment. A 0.22 µm PTFE Millex filter was employed to filter the bacterial cultures after 50 days, and the filtrate and residue were then used for additional characterization.

2.4.3. FTIR analysis of microplastics

The chemical structure of microplastics was examined using FTIR spectroscopy operating at a frequency of between 2000 and 440 cm^{-1}. The Perkin Elmer 400 FTIR was employed in order to investigate the structural alterations in microplastic brought about by the interaction with microbe. The microplastic samples were dried at temperatures below 100 °C and pellets were mixed with potassium bromide to scan at a resolution of 4 cm^{-1}.

2.4.4. Thermogravimetric analysis (TGA) of microplastics

To investigate the composition and thermal stability of original microplastics and bacterially degraded microplastics, TGA was utilized. Under regulated settings, the sample had to be heated from room temperature to 900 °C for the assay. A steady nitrogen flow of 10 mL min^{-1} at a heating rate of 10 °C per minute was employed in the experiment.

2.4.5. Microplastics dry weight determination post microbial degradation

The microplastics were extracted from the broth using 0.22 µm polytetrafluorethylene (PTFE) Millex filters following a 50-day incubation period. After being cleaned with 70 % ethanol, plastic particles were dried for 12 hours at 60 °C in a hot air oven. The amount of degradation was measured using residual polymer weight. The microplastic that had been pre-incubated was weighed. The following formula was used to calculate the % weight loss

associated with the degradation of the plastic polymer:

$$\%\ weight\ loss = \frac{(Initial\ weight\ of\ polymer - Final\ weight\ of\ polymer)}{Initial\ weight\ of\ polymer} * 100$$

2.4.6. Morphological Analysis

The degradation of micro/nano plastics was examined using scanning electron microscopy. The bacterial morphology before and after treatment with microplastics was observed. This was accomplished by analyzing the bacterial cells both before and after the incubation. A 10,000 magnification and 5 kV accelerating voltage were used with a German-made SEM, the EVO18 Zeiss type. After performing a gold layer sputter-coating at 25 mA in an Ar environment at 0.3 MPa, the specimens were examined using a SEM. The samples were prepared by centrifuging the culture media and rinsing them with Milli-Q water prior to analysis. Lastly, in order to investigate the changes in bacterial morphology brought on by microplastic degradation, the produced specimens were carefully inspected using SEM.

2.4.7. Experimental Duration and Biometrical Measurements

The experiment was conducted over a total period of 32 weeks, with plant biometrical parameters recorded at two key intervals: 16 weeks and 32 weeks. At the 16-week mark, the impact of HDPE and Nylon 6,6 microplastics on the plants was measured. Plant heights were measured every two weeks using a steel tape measure. The study's findings were computed using the mean of the three triplicates. Similarly, the biomass of the roots and shoots in each treated sample was weighed in order to determine the mean deviation from the control.

At the 32-week mark, the same biometrical parameters were measured after inoculation with the bacterial isolate. Notably, an upward trend was recorded in plant height including root and shoot, and biomass, indicating potential adaptation or recovery of the plants from the initial stress caused by microplastic exposure. By comparing the data from the 16-week and 32-week intervals, it became possible to assess the progression of microplastic-induced

effects on plant growth and determine whether these impacts were transient or more sustained.

2.4.8. Chlorophyll content

The method described by Ren Hong et al., 2012 was followed in measuring and recording the relative chlorophyll content in plant leaves using a UV-Vis spectrophotometer (Eppendorf, USA) on the 16^{th} week and 32^{nd} week for both control and treated plants in each pot. To determine the chlorophyll concentration, fresh leaves were taken out of each pot. A weight of 0.5 g of leaves per pot was obtained for each treatment and control sample. Once the leaves were chopped and homogenized, 10 milliliters of 80 % acetone were added to make them translucent. The extract was centrifuged for five minutes at 2500 rpm. A UV-Vis spectrophotometer was used to measure 1 milliliter of the resultant supernatant after it had been diluted with 9 milliliters of 80 % acetone to be read by a UV-Vis Spectrophotometer at 663 nm and 644 nm. The equations used were based on Mackinney's work and Arnon equations –

$Chl_a = 12.7\ A_{663} – 2.69\ A_{645}$

$Chl_b = 22.9\ A_{645} – 4.68\ A_{663}$

Total chlorophyll = Chla + Chlb

2.4.9. Antioxidant Activity

The malondialdehyde (MDA) concentration of leaves was used to determine the degree of lipid peroxidation in leaves (Heath and Packer, 2022). 0.5 g of leaf tissue was crushed in 10 milliliters of 0.1% trichloroacetic acid (TCA). Centrifuging the homogenate for 15 minutes at 12,000 rpm was done with an Eppendorf Centrifuge 5810 R. Twenty percent thiobarbituric acid (TCA) was dissolved in two milliliters of the supernatant, and 0.5 % TBA solution was added. After heating this mixture to 95°C for thirty minutes, it was cooled down. 10,000 rpm centrifugation was applied to the mixture for 10 minutes. Spectrophotometer made by Eppendorf UV-vis using wavelength of 532 nm absorbance measurement was made of the supernatant. The MDA content was found using its extinction

coefficient.

500 milligrams of leaf samples were homogenized in 0.5 milliliters of ice-cold phosphate buffer (7.5) containing 0.5 milligrams of EDTA in order to measure the amount of LOX activity. For fifteen minutes, the homogenate was centrifuged at four degrees Celsius and 12,000 rpm. 2.95 ml of the substrate which was made by combining 35 µl of linoleic acid with 5 mL of milli Q water that included 50 µl of tween 20 were mixed with 50 µl of the extract. 0.1 M phosphate buffer was used to raise the total volume to 100 ml. A measurement of absorbance at 234 nanometers was made (Doderer et al., 1992, p. 199).

CHAPTER 3

RESULTS & DISCUSSION

Chapter-3: Results & Discussion

3.1. OBJECTIVE 1: Understanding the impact of micro/nano plastics on the growth and physiological parameters of plants:

3.1.1. Biometrical Parameters

3.1.1.1. Growth Response

On exposure of *Brassica juncea* to HDPE_MPs and HDPE_beads, significant difference in shoot and root biomass could be observed compared to the control plants (Figure 3.1 (a)). Shoot biomass for control was 3.155 ± 0.15 g compared to the treated plants showing a decreasing trend with values for 10 g MPs = 2.39 ± 0.11g, 20 g MPs = 1.738 ± 0.08 g, 10 g beads = 2.249 ± 0.11 g and 20 g beads = 1.90 ± 0.09 g. The least shoot biomass was observed for 20 g MPs in contrast to control sample indicating an adverse effect when exposed to microplastics. Similarly, root biomass also showed a declining trend on being exposed to HDPE_MPs and HDPE_beads after a span of three months (Fig 3.1 (b)). Root biomass for control was 0.85 ± 0.04 g compared to the treated plants having significant difference with values for 10 g MPs = 0.67 ± 0.03 g, 20 g MPs = 0.608 ± 0.03 g, 10 g beads = 0.45 ± 0.02 g and 20 g beads = 0.403 ± 0.02 g.

The shoot and root height also showed a declining trend on being treated with HDPE_MPs and HDPE_beads (Fig 3.1 (c) & (d)). The shoot height for control plant was 33.23 ± 1.66 cm compared to microplastics treated samples showing values with 10 g MPs = 29.8 ± 1.49 cm, 20 g MPs = 28.2 ± 1.41 cm, 10 g beads = 29.8 ± 1.49 cm and 20 g beads = 30.2 ± 1.51 cm. Also, the root height for control sample was 5.6 ± 0.28 cm compared to microplastics exposed samples showing a decreasing trend with values of 10 g MPs = 4.90 ± 0.24 cm, 20 g MPs = 3.70 ± 0.18 cm, 10 g beads = 4.30 ± 0.21 cm and 20 g beads = 4.10 ± 0.20 cm.

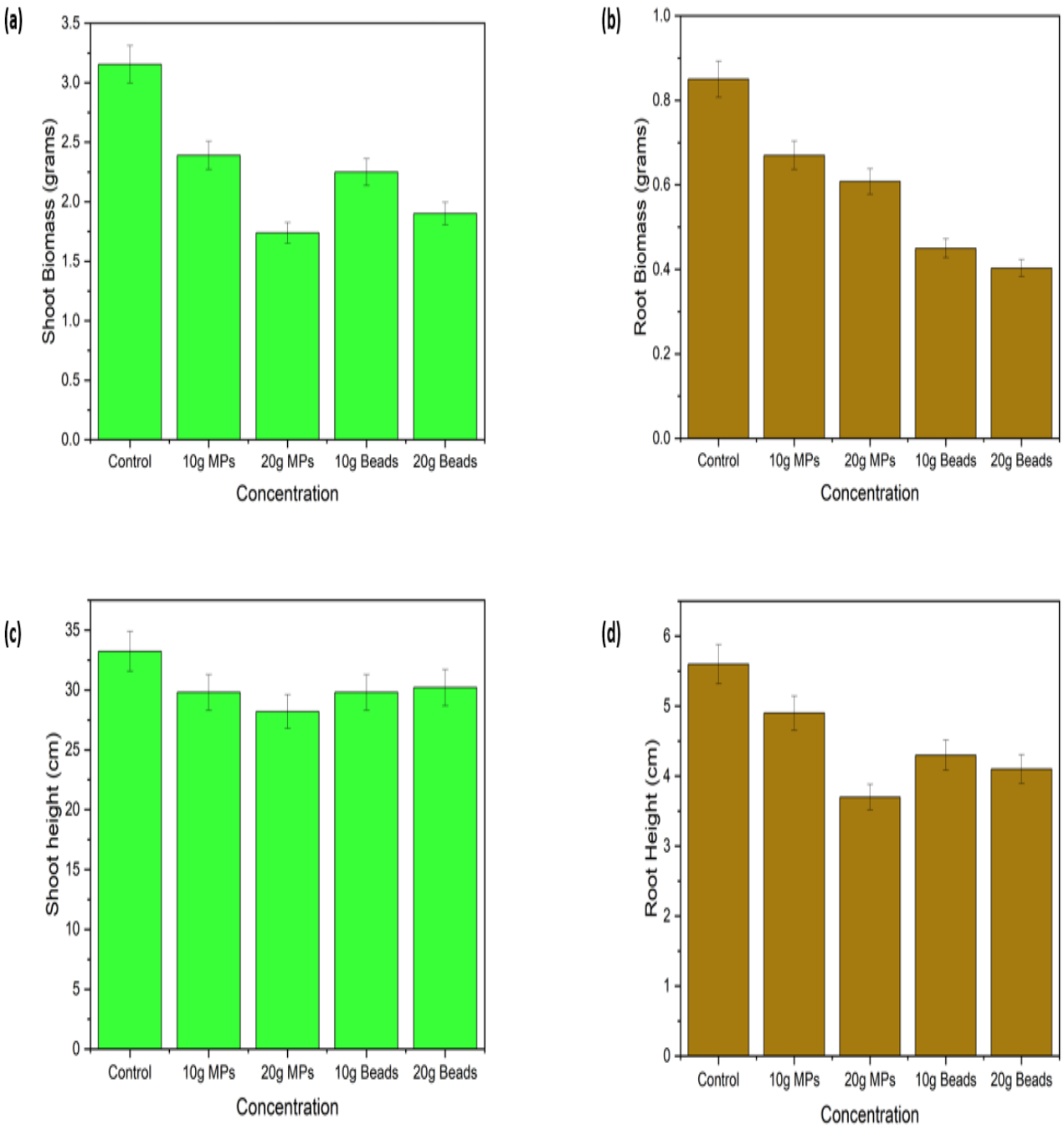


Figure 3.1: Influence of HDPE_MPs and HDPE_beads on plant samples by observing growth parameters: (a) Shoot Biomass; (b) Root Biomass; (c) Shoot Height; (d) Root Height (The values are mean of three triplicates; Error bars denote 5% standard error)

3.1.2. Chlorophyll content

The findings demonstrated that chlorophyll *a* (Chl *a*) was more readily influenced by different concentrations of microplastics than chlorophyll *b* (Chl *b*). Such significant difference in content of chl *a* and chl *b* imply that the total chlorophyll content in leaves of treated and control samples sufficiently varied (Fig 3.2 (a) & (b)). Chlorophyll *a* is the main pigment involved in process of photosynthesis whereas chlorophyll *b* is the accessory pigment that transfers energy to chlorophyll *a* (Khaleghi et al., 2013). An important metric for photosynthetic activity is the total chlorophyll content (chl *a* + chl *b*), and variations in this value are a sign of stress in plants. Thus, it could be observed that chlorophyll *a* was comparatively less for microplastics treated plant samples indicating that total chlorophyll content in leaves showed greater inhibitory effect on exposure to microplastics. Hence, the chlorophyll content in microplastic treated plant samples was eventually less compared to control.

3.1.3. Biochemical Analysis

3.1.3.1. Phenolic Content

One of the major groups of secondary metabolites found in plants, phenolics includes over 9,000 different chemicals. They serve a variety of biological purposes in plants, including defense against pathogens, protection from ultraviolet rays, pigmentation to draw pollinators, and defense against reactive oxygen species (Waśkiewicz et al., 2013). On being exposed to microplastics of different types, phenolic content in plants reduced showing a decreasing trend as observed in Fig 3.2 (c). Compared to control having phenolic content of 0.998 ± 0.04 (GAE) mg^{-1}, phenolic content in plants treated with microplastics was 10 g MPs = 0.834 ± 0.04 (GAE) mg^{-1}, 20 g MPs = 0.687 ± 0.03 (GAE) mg^{-1}, 10 g beads = 0.497 ± 0.02 (GAE) mg^{-1} and 20 g beads = 0.386 ± 0.01 (GAE) mg^{-1}. The results indicate that incorporation of microplastics decreases phenolic content in plants thereby inducing stress as compared to control plants without microplastics.

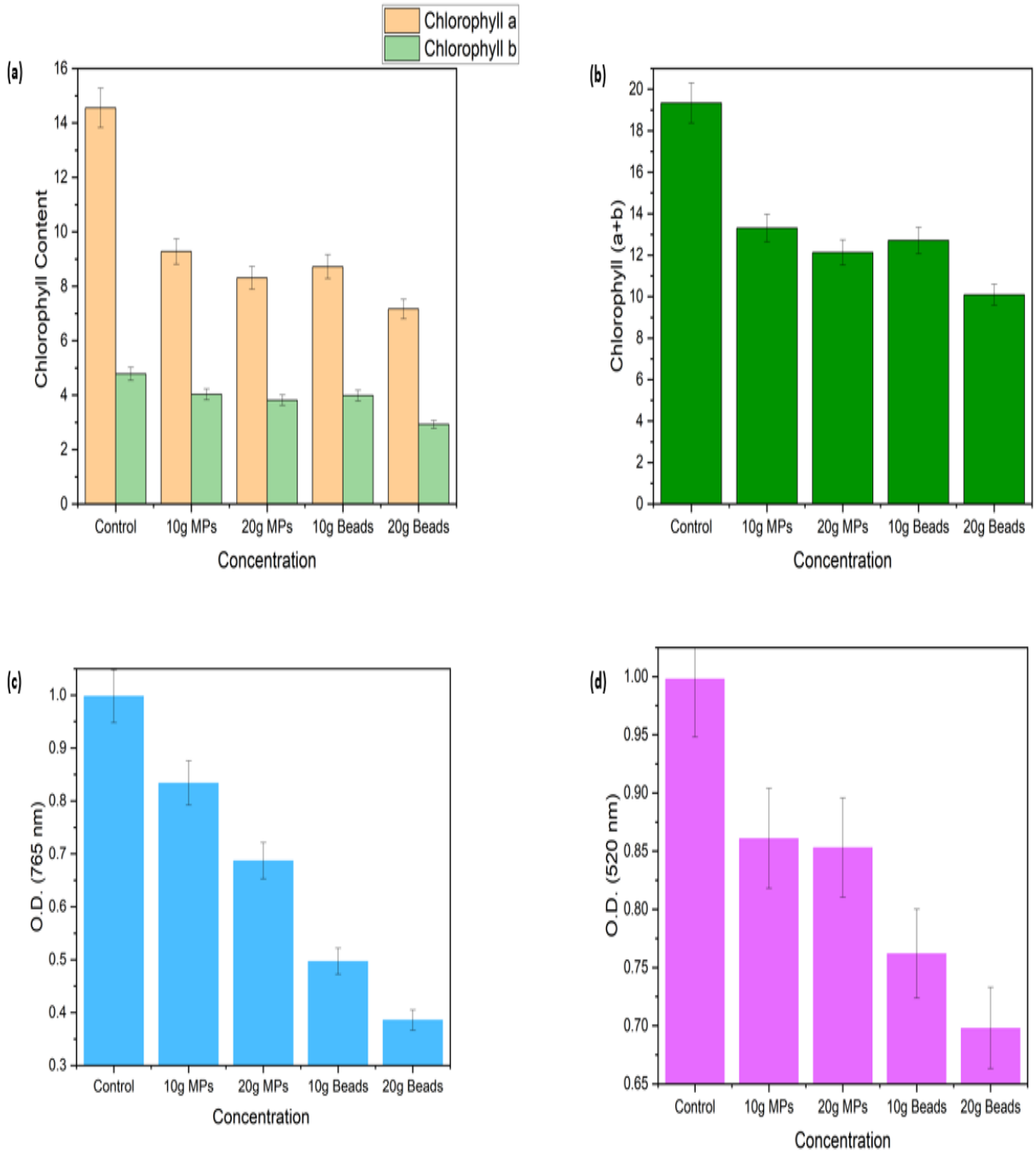


Figure 3.2: Influence of HDPE_MPs and HDPE_beads on plant samples by observing growth parameters: (a) Chlorophyll a & b; (b) Total chlorophyll; (c) Phenolic content; (d) Proline content (The values are mean of three triplicates; Error bars denote 5% standard error)

3.1.3.2. Proline Content

The current investigation demonstrated that *B. juncea* plants treated with HDPE_MPs and HDPE_beads had a lessening proline content (Fig.3.2 (d)). As proline is known to reduce oxidative stress, maintain osmotic balance, and regulate redox potential, increasing concentration of microplastics showed an opposite trend. On being exposed to MPs, content of proline gradually declined with 20 g beads showing lowest value of 0.698 ± 0.03 gg^{-1} compared to control with proline content of 0.998 ± 0.04 gg^{-1}. These values are indicative of the fact that proline is not able to reduce stress in *Brassica juncea* plants on contaminated with microplastics.

3.1.4. Morphological Analysis

3.1.4.1. Fluorescence Microscopy

In this study, we demonstrate that *Brassica juncea* plant roots may absorb individual microplastic particles with diameters between 5 and 10 µm from the surrounding soil (Fig 3.3 (a) & (b)). We were able to find and see labelled microplastic particles embedded amid root cell structures using fluorescence microscopy. Along with the tree root's inherent autofluorescence, fluorescing microplastic was also seen in the root cortex, exodermis, and vascular tissue of a lateral root, as well as in the root hairs and outer epidermal layer. (Fig 3.3(c) & (d)) provides evidence for microplastics incorporation in leaves of mustard plant as observed with red tagged MPs. Tagged microplastics could be observed in leaf sections and also induce structural changes in morphology of leaf.

3.1.4.2. Confocal Microscopy

To decipher more significant findings of the study, confocal microscopy was performed to visualize tagged microplastics within the root lateral cross-sections and leaf parts. On visualization under red and green field at 40 X and 100 X magnification, it could be observed that microplastics were embedded in root hair segments and leaf internal veins at some points (Fig 3.3 (e), (f), (g) & (h)). Because we could observe microplastics particles in

inner root and leaf structures, our results suggest that micrometer sized microplastic can easily traverse from soil to root through crack-entry and apoplastic pathway, forming basis for entry to food chain. These results confirm microplastics uptake by *Brassica juncea* plants and also provide future insights for understanding mechanism of action and impact on plants.

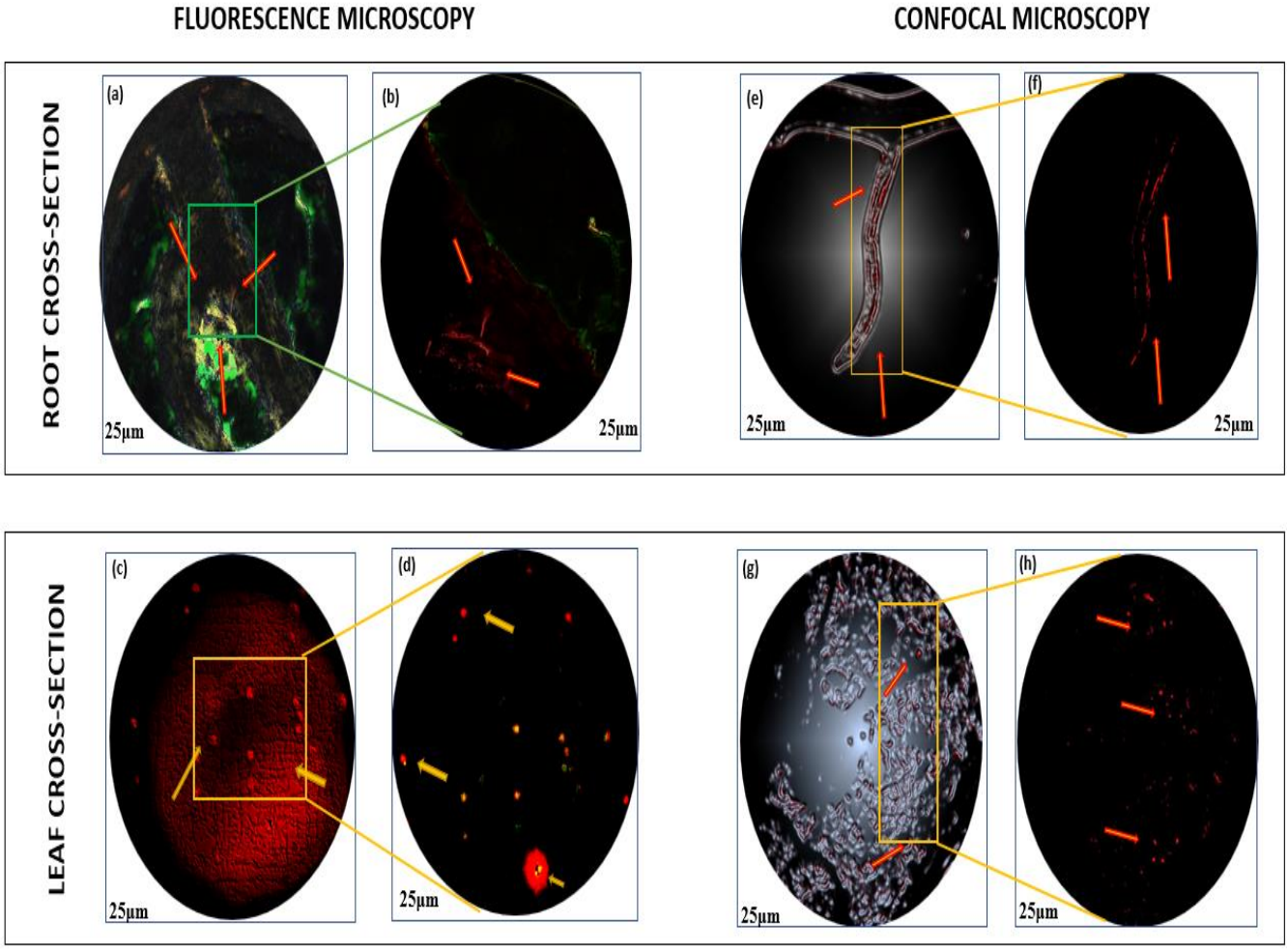


Figure 3.3: Longitudinal cross-section showing microplastic particles inside a *Brassica juncea* lateral root and leaf section in a three months old mustard plant after being exposed to tagged microplastics. Clockwise from top left shows cross sections using fluorescence microscopy, (a) & (b) show root cross-sections; (c) & (d) show leaf sections; and confocal laser scanning microscopy, (e) & (f) show root cross-sections; (g) & (h) show leaf sections. All images have been taken at a magnification of 40X and 100X.

The results from overall study provide evidence for microplastics uptake by *Brassica juncea* and its impact on growth and development. The biometrical parameters and biochemical analysis signify that microplastics are transported from root to shoot parts of the plant. Also, morphological analysis confirm uptake of microplastics from root to leaf sections of plant. The shoot biomass showed least concentration of 1.75 g on addition of 20 g MPs. Similarly, root biomass of 0.4 g was observed on addition of 20 g beads, that was least compared to all other concentrations. Also, the least shoot and root height was observed in plants treated with 20 g MPs showing shoot height at 27 cm and root height at 3.5 cm. Similar to this, least chlorophyll concentration was observed in 20 g beads thereby showing least total chlorophyll content in microplastics exposed plants. Also, the antioxidant activity depicted by phenolic content and protection of plant against stress represented by proline content was least for 20 g beads exposed plants. Morphological analysis confirmed the presence of microplastics uptake in root and leaf sections of plant. Confocal microscopy provides a rapid approach for visualization of MPs within plant parts (Li et al., 2020b). The fluorescent dyes can generate stable emission signals that are easy to distinguish from the autofluorescence generated by plant tissues (Z. Zhang et al., 2022). Thus, it is a rapid and efficient approach in detection of MPs within plant tissues (Ullah et al., 2021b). The accumulation of tagged microplastics could also be observed in Fig 3.3 (c) and (d) sections. These results portray uptake of microplastics not only by root but also by leaf sections of plant. Thus, *Brassica juncea* have been found to accumulate microplastics in their tissues, that could have significant impact on humans when consumed. This study also highlights uptake mechanism of microplastics from root to leaf sections as confirmed by morphological analysis. All the results present a significant finding providing evidence on impact on *Brassica juncea* plant after being exposed to different concentrations of microplastics.

3.2. <u>OBJECTIVE 2</u>: Describing the fate and behavior of micro/nano plastics on the biometry of wild plants:

3.2.1. Biometrical Parameters

3.2.1.1. Growth Response

Cyanodon dactylon (L.) (SG) and *Portulaca grandiflora* (PG) exposed to different concentrations of PE and nylon 6,6 showed a substantial change in shoot length and shoot biomass when compared to control plants.

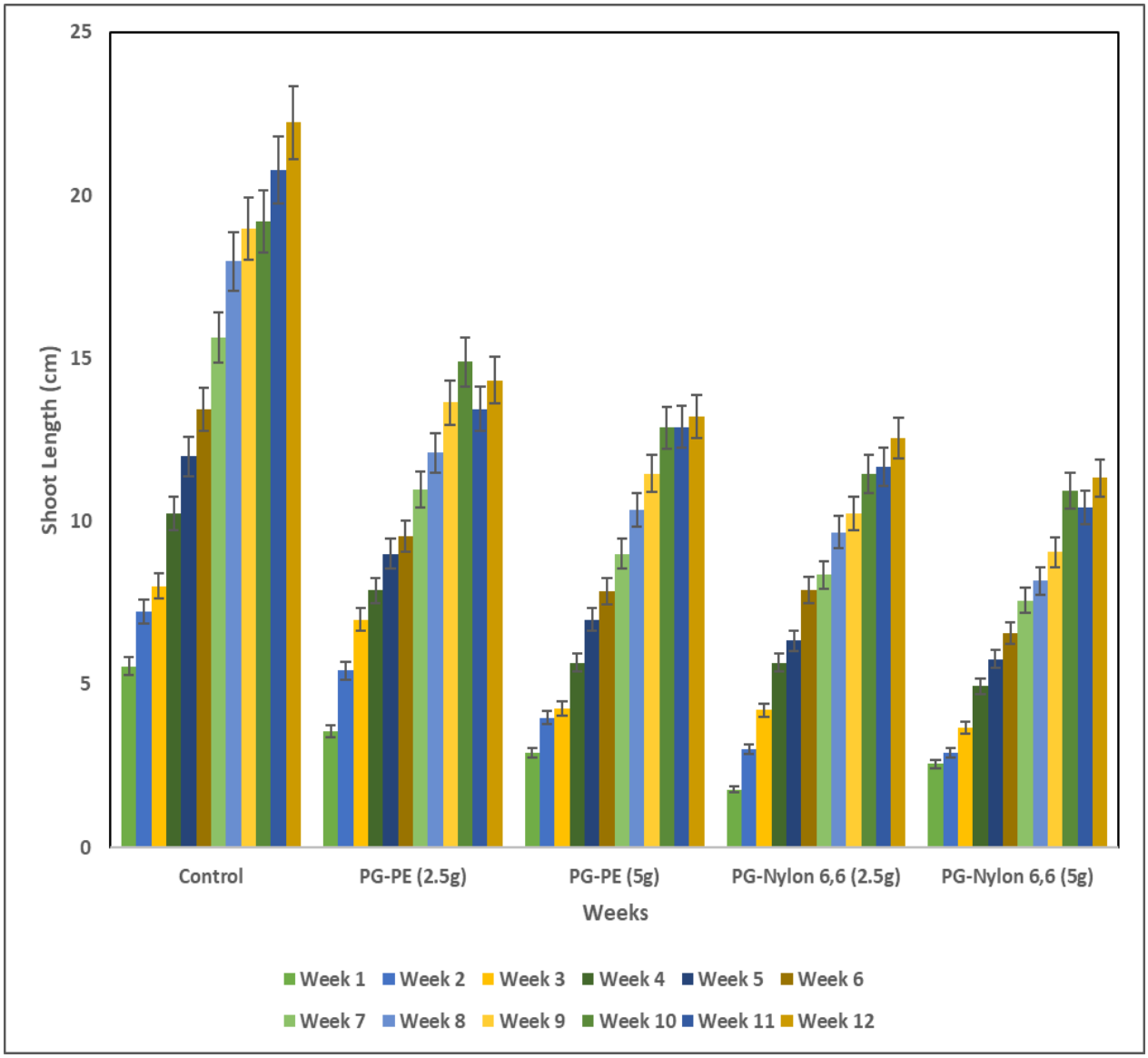


Figure 3.4: Influence of HDPE microplastics on *Portulaca grandiflora* (PG) samples by observing growth parameters: Shoot length for *Portulaca grandiflora* (PG) (The values are mean of three triplicates; Error bars denote 5% standard error)

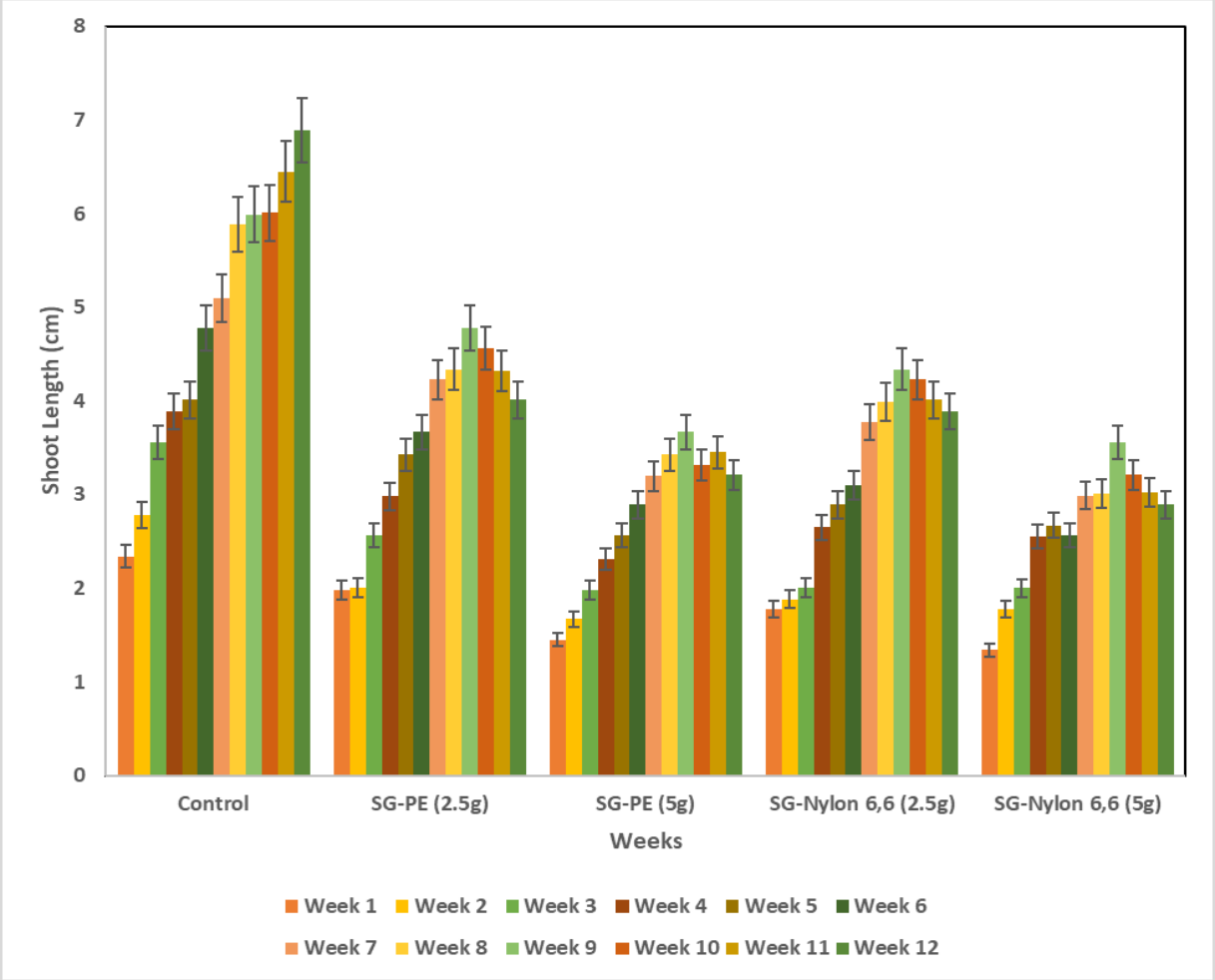


Figure 3.5: Influence of HDPE microplastics on *Cyanodon dactylon* (L.) (SG) plant samples by observing growth parameters: Shoot length for *Cyanodon dactylon* (L.) (SG); (The values are mean of three triplicates; Error bars denote 5% standard error)

The control shoot length for *Portulaca grandiflora* (PG) plant was 5.55 ± 0.27753 cm for 1st week and gradually increased to 22.23 ± 1.1115 cm for 12th week, while treated plants showed a declining trend for 1st week with values for PG - PE (2.5g) = 6.98 ± 0.349 cm, PG - PE (5g) = 4.25 ± 0.2125 cm, PG - Nylon 6,6 (2.5g) = 4.21 ± 0.2105 cm, and PG - Nylon 6,6 (5g) = 3.67 ± 0.1835 cm. Similarly, after a span of 12 weeks, in contrast to control, the treated samples of *Portulaca grandiflora* (PG) showed less growth exhibiting values for PG - PE (2.5g) = 14.32 ± 0.7166 cm, PG - PE (5g) = 13.23 ± 0.6615 cm, PG - Nylon 6,6 (2.5g) = 12.56 ± 0.628 cm, and PG - Nylon 6,6 (5g) = 11.34 ± 0.567 cm.

For microplastics treated to *Cyanodon dactylon* (L.) (SG) plants, shoot length for the control sample exhibited values of 2.34 ± 0.117 cm for 1st week with increasing trend by 12th week 6.89 ± 0.3445 cm. While the microplastics accumulated samples showed a lessening graphical analysis at 1st week with concentrations for SG - PE (2.5g) = 1.98 ± 0.099 cm, SG - PE (5g) = 1.45 ± 0.0725 cm, SG - Nylon 6,6 (2.5g) = 1.78 ± 0.089 cm, and SG - Nylon 6,6 (5g) = 1.34 ± 0.067 cm. Similarly, after a span of 12 weeks, in contrast to control, the treated samples of *Cyanodon dactylon* (L.) (SG) showed less growth exhibiting values for SG - PE (2.5g) = 4.01 ± 0.2005 cm, SG - PE (5g) = 3.21 ± 0.1605 cm, SG - Nylon 6,6 (2.5g) = 3.89 ± 0.1945 cm, and SG - Nylon 6,6 (5g) = 2.89 ± 0.1445 cm.

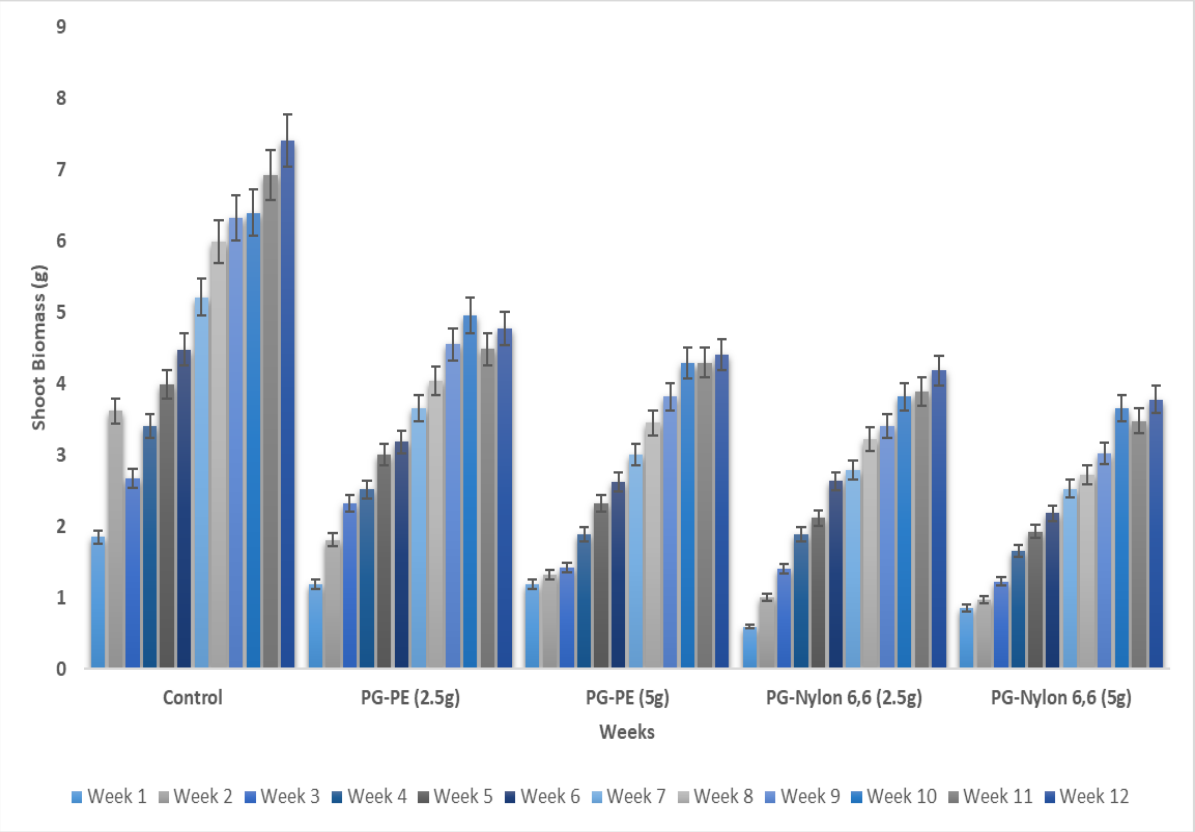


Figure 3.6: Influence of HDPE microplastics on *Portulaca grandiflora* (PG) samples by observing growth parameters: Shoot Biomass for *Portulaca grandiflora* (PG) (The values are mean of three triplicates; Error bars denote 5% standard error)

With reference to shoot biomass, in contrast to the control sample, microplastics treated samples had the lowest shoot biomass, demonstrating that exposure to microplastics had a negative impact. In case of *Portulaca grandiflora* (PG) plants, control plant displayed values of 1.85 ± 0.0925 g in 1st week followed by 7.41 ± 0.3705 g by 12th week. For microplastics treated samples, in 1st week, *Portulaca grandiflora* (PG) plants showed values of PG - PE (2.5g) = 1.18667 ± 0.05933 g, PG - PE (5g) = 1.18667 ± 0.05933 g, PG - Nylon 6,6 (2.5g) = 0.59333 ± 0.02967 g, and PG - Nylon 6,6 (5g) = 0.85333 ± 0.04267 g. Similarly, after a span of 12 weeks, in contrast to control, the treated samples of *Portulaca grandiflora* (PG) showed less biomass exhibiting values for PG - PE (2.5g) = 4.77333 ± 0.23867 g, PG - PE (5g) = 4.41 ± 0.2205 g, PG - Nylon 6,6 (2.5g) = 4.18667 ± 0.20933 g, and PG - Nylon 6,6 (5g) = 3.78 ± 0.189 g.

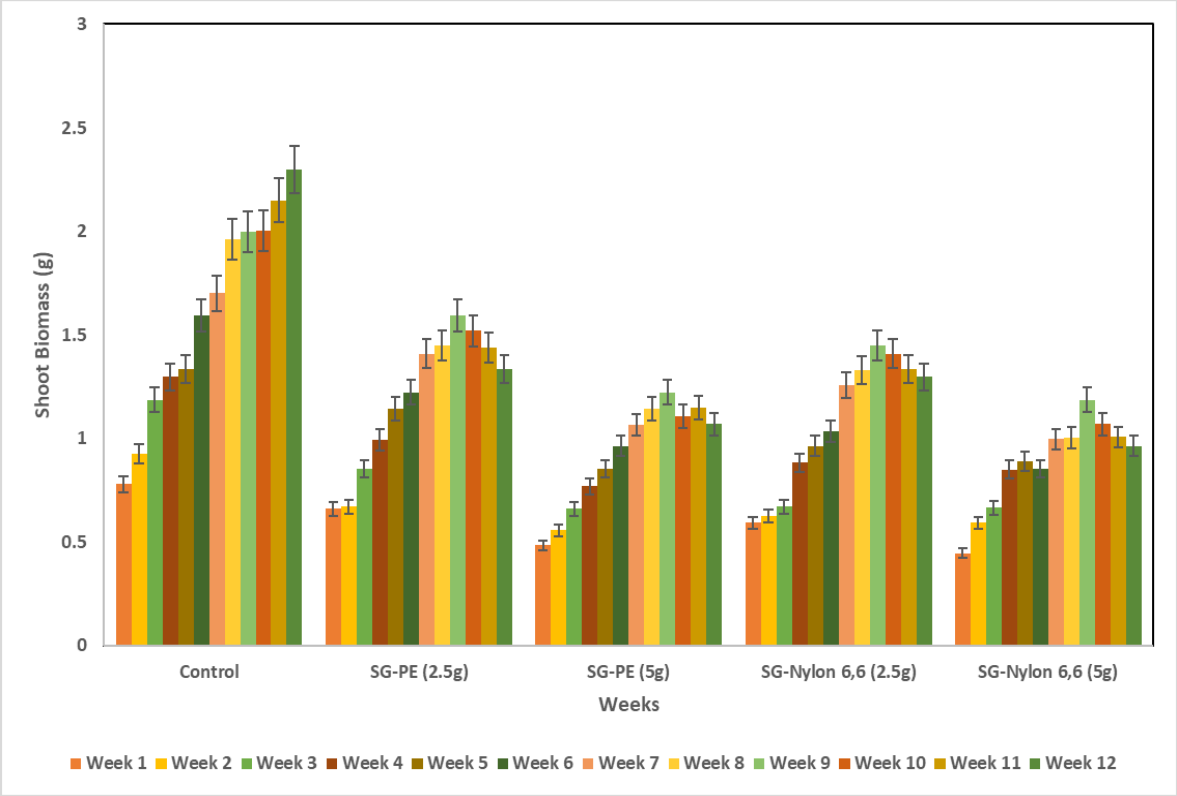


Figure 3.7: Influence of HDPE microplastics on *Cyanodon dactylon* (L.) (SG) plant samples by observing growth parameters: Shoot Biomass for *Cyanodon dactylon* (L.) (SG); (The values are mean of three triplicates; Error bars denote 5% standard error)

For *Cyanodon dactylon* (L.) (SG) plants, shoot biomass for control sample at 1st week was 0.78 ± 0.039 g and approximately 2.29667 ± 0.11483 g observed by 12th week. The plants treated with microplastics showed a declining trend in 1st week with respect to control containing shoot biomass of SG - PE (2.5g) = 0.66 ± 0.033 g, SG - PE (5g) = 0.48333 ± 0.02417 g, SG - Nylon 6,6 (2.5g) = 0.59333 ± 0.02967 g, and SG - Nylon 6,6 (5g) = 0.44667 ± 0.02233 g. Similarly, after a span of 12 weeks, in contrast to control, the treated samples of *Cyanodon dactylon* (L.) (SG) showed less growth exhibiting values for SG - PE (2.5g) = 1.33667 ± 0.06683 g, SG - PE (5g) = 1.07 ± 0.0535 g, SG - Nylon 6,6 (2.5g) = 1.29667 ± 0.06483 g, and SG - Nylon 6,6 (5g) = 0.96333 ± 0.04817 g.

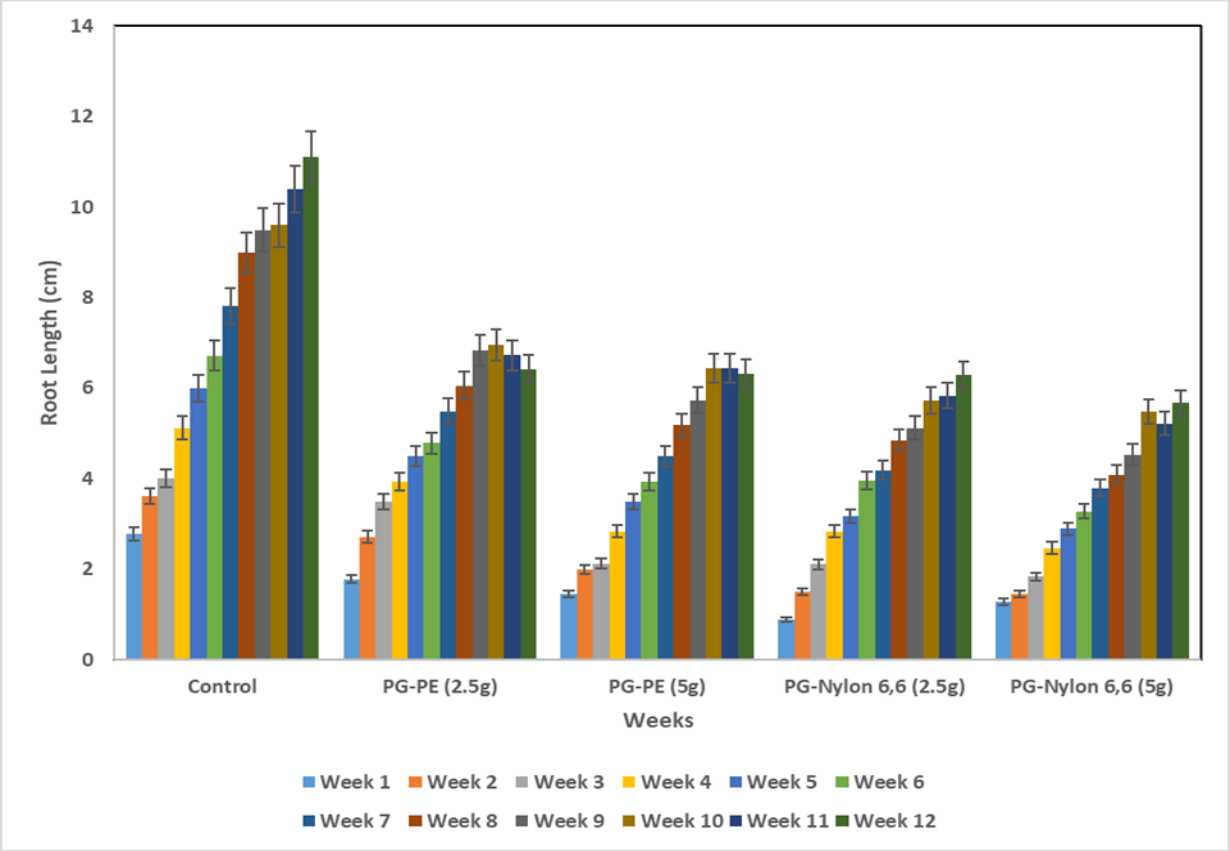


Figure 3.8: Influence of HDPE microplastics on *Portulaca grandiflora* (PG) samples by observing growth parameters: Root Length for *Portulaca grandiflora* (PG) (The values are mean of three triplicates; Error bars denote 5% standard error)

As the shoot length and biomass showed adverse impact on adding microplastics, a similar trend was observed for root length and biomass. *Cyanodon dactylon* (L.) (SG) and *Portulaca grandiflora* (PG) exposed to different concentrations of PE and nylon 6,6 showed a substantial change in root length and root biomass when compared to control plants. The control root length for *Portulaca grandiflora* (PG) plant was 2.775 ± 0.13875 cm for 1st week and gradually increased to 11.115 ± 0.55575 cm for 12th week, while treated plants showed a declining trend for 1st week with values for PG - PE (2.5g) = 1.78 ± 0.089 cm, PG - PE (5g) = 1.445 ± 0.07225 cm, PG - Nylon 6,6 (2.5g) = 0.89 ± 0.0445 cm, and PG - Nylon 6,6 (5g) = 1.28 ± 0.064 cm. Similarly, after a span of 12 weeks, in contrast to control, the treated samples of *Portulaca grandiflora* (PG) showed less growth exhibiting values for PG - PE (2.5g) = 6.42 ± 0.321 cm, PG - PE (5g) = 6.32 ± 0.316 cm, PG - Nylon 6,6 (2.5g) = 6.28 ± 0.314 cm, and PG - Nylon 6,6 (5g) = 5.67 ± 0.2835 cm.

For microplastics treated to *Cyanodon dactylon* (L.) (SG) plants, root length for the control sample exhibited values of 1.17 ± 0.0585 cm for 1st week with increasing trend by 12th week 3.445 ± 0.17225 cm. While the microplastics accumulated samples showed a lessening graphical analysis at 1st week with concentrations for SG - PE (2.5g) = 0.99 ± 0.0495 cm, SG - PE (5g) = 0.725 ± 0.03625 cm, SG - Nylon 6,6 (2.5g) = 0.89 ± 0.0445 cm, and SG - Nylon 6,6 (5g) = 0.67 ± 0.0335 cm. Similarly, after a span of 12 weeks, in contrast to control, the treated samples of *Cyanodon dactylon* (L.) (SG) showed less growth exhibiting values for SG - PE (2.5g) = 2.005 ± 0.10025 cm, SG - PE (5g) = 1.605 ± 0.08025 cm, SG - Nylon 6,6 (2.5g) = 1.945 ± 0.09725 cm, and SG - Nylon 6,6 (5g) = 1.445 ± 0.07225 cm.

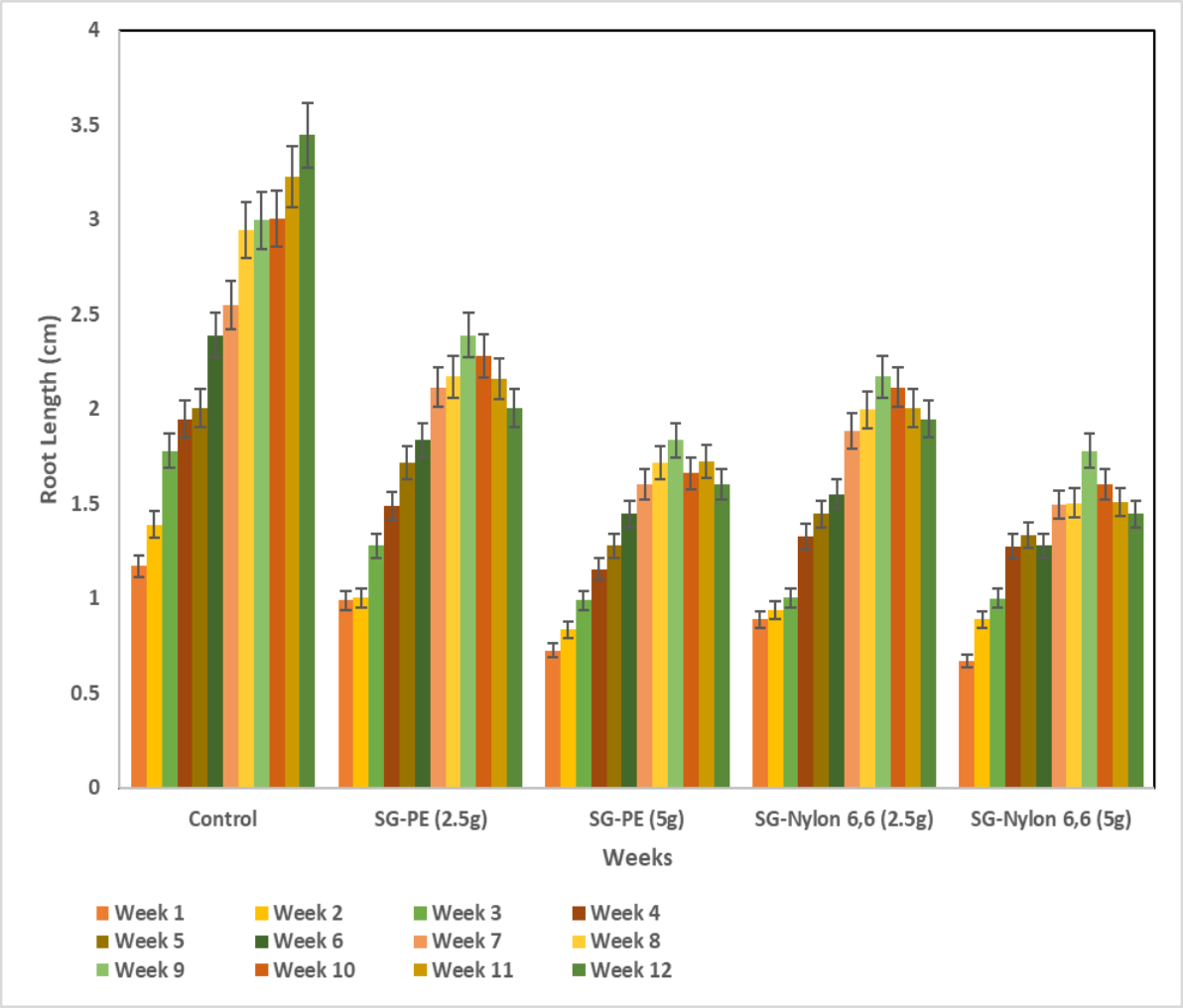


Figure 3.9: Influence of HDPE microplastics on *Cyanodon dactylon* (L.) (SG) plant samples by observing growth parameters: Root Length for *Cyanodon dactylon* (L.) (SG); (The values are mean of three triplicates; Error bars denote 5% standard)

With reference to root biomass, in contrast to the control sample, microplastics treated samples had the lowest root biomass, demonstrating that exposure to microplastics had a negative impact. In case of *Portulaca grandiflora* (PG) plants, control plant displayed values of 0.925 ± 0.04625 g in 1st week followed by 3.705 ± 0.18525 g by 12th week. For microplastics treated samples, in 1st week, *Portulaca grandiflora* (PG) plants showed values of PG - PE (2.5g) = 0.59333 ± 0.02967 g, PG - PE (5g) = 0.48167 ± 0.02408 g, PG - Nylon 6,6 (2.5g) = 0.29667 ± 0.01483 g, and PG - Nylon 6,6 (5g) = 0.42667 ± 0.02133 g.

Similarly, after a span of 12 weeks, in contrast to control, the treated samples of *Portulaca grandiflora* (PG) showed less biomass exhibiting values for PG - PE (2.5g) = 4.77333 ± 0.23867 g, PG - PE (5g) = 4.41 ± 0.2205 g, PG - Nylon 6,6 (2.5g) = 4.18667 ± 0.20933 g, and PG - Nylon 6,6 (5g) = 3.78 ± 0.189 g.

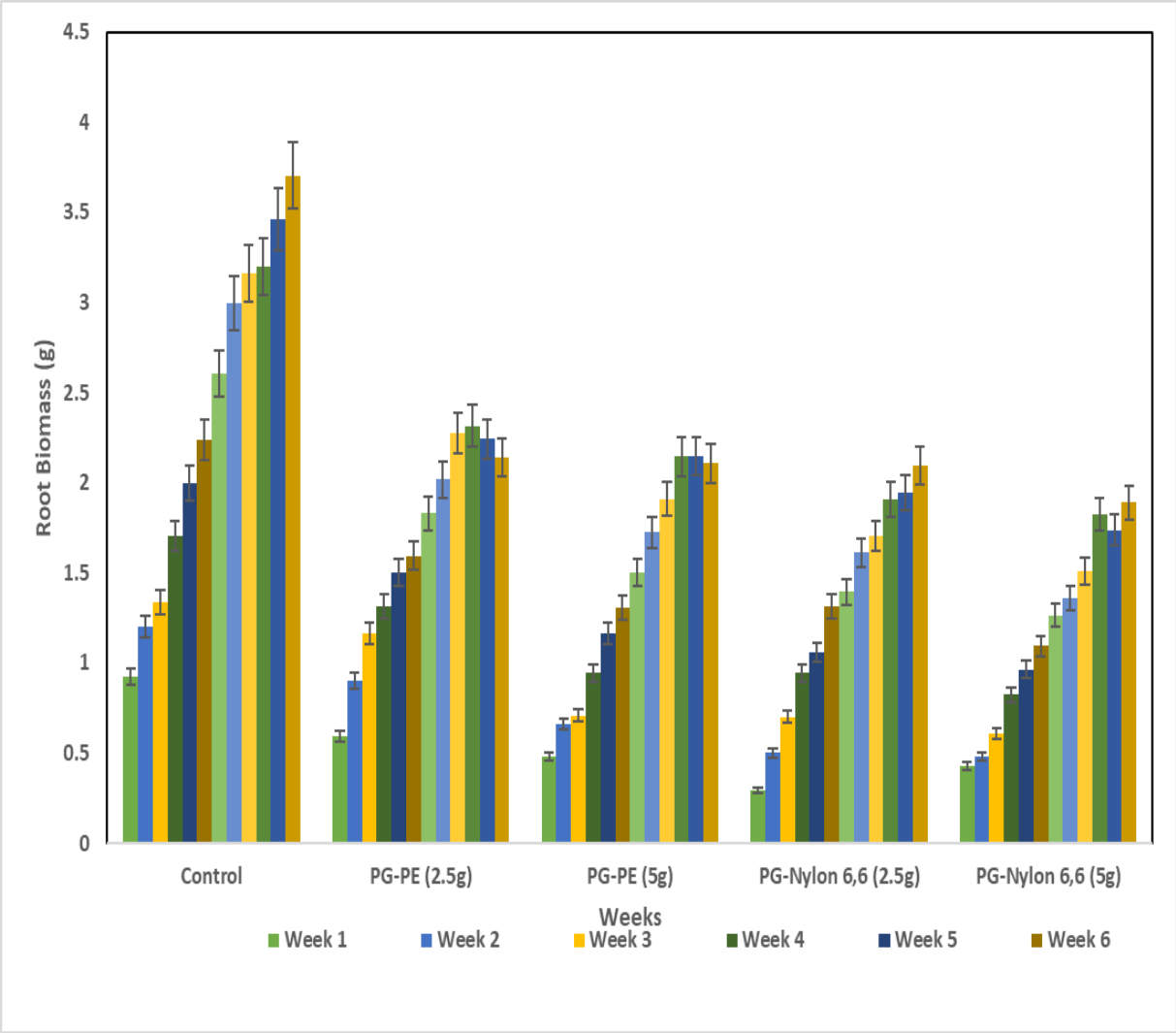


Figure 3.10: Influence of HDPE microplastics on *Portulaca grandiflora* (PG) samples by observing growth parameters: Root Biomass for *Portulaca grandiflora* (PG) (The values are mean of three triplicates; Error bars denote 5% standard error)

For *Cyanodon dactylon* (L.) (SG) plants, root biomass for control sample at 1st week was 0.39 ± 0.0195 g and approximately 1.14833 ± 0.05742 g observed by 12th week. The plants

treated with microplastics showed a declining trend in 1st week with respect to control containing root biomass of SG - PE (2.5g) = 0.33 ± 0.0165 g, SG - PE (5g) = 0.24167 ± 0.01208 g, SG - Nylon 6,6 (2.5g) = 0.29667 ± 0.01483 g, and SG - Nylon 6,6 (5g) = 0.22333 ± 0.01117 g. Similarly, after a span of 12 weeks, in contrast to control, the treated samples of *Cyanodon dactylon* (L.) (SG) showed less growth exhibiting values for SG - PE (2.5g) = 0.66833 ± 0.03342 g, SG - PE (5g) = 0.535 ± 0.02675 g, SG - Nylon 6,6 (2.5g) = 0.64833 ± 0.03242 g, and SG - Nylon 6,6 (5g) = 0.48167 ± 0.02408.

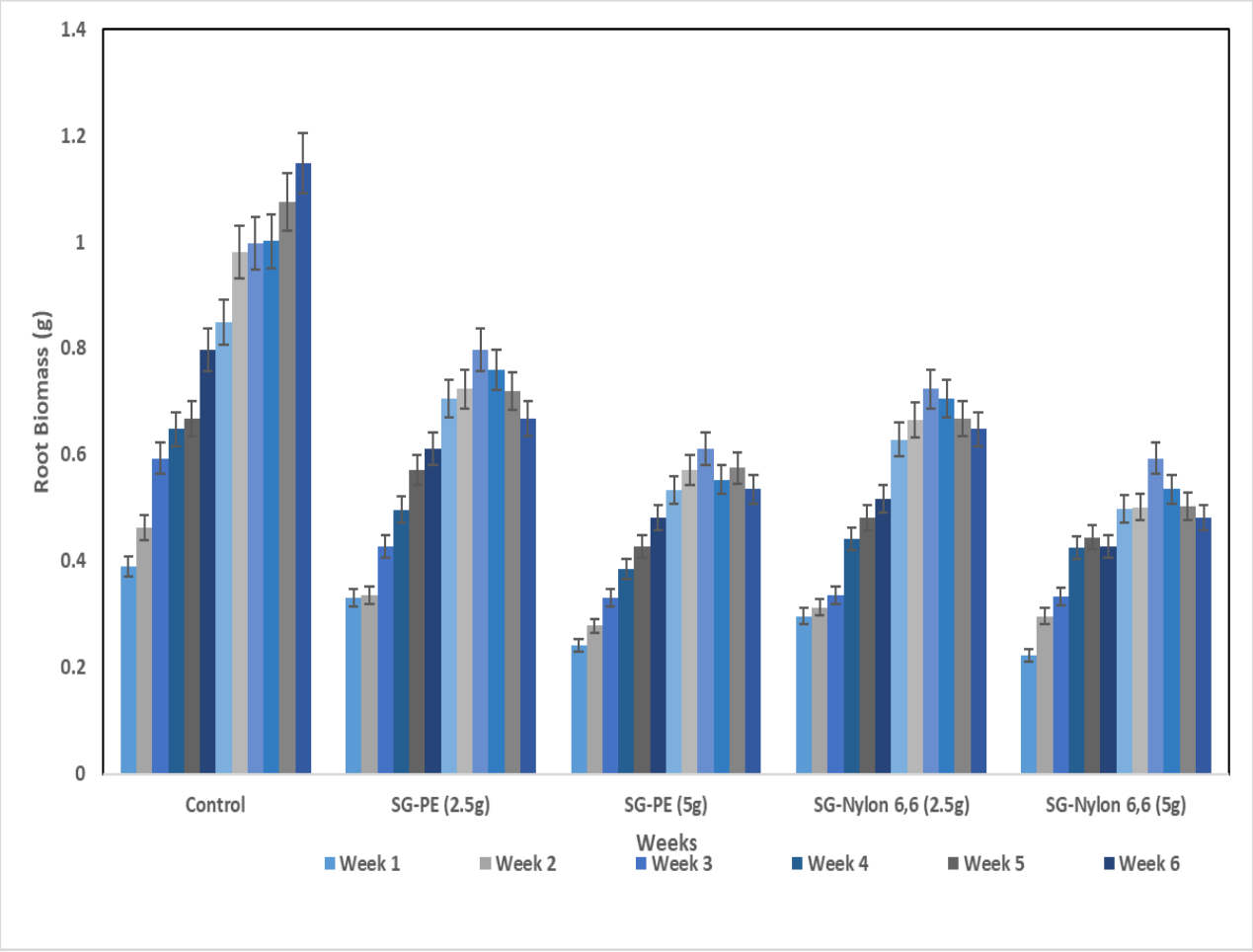


Figure 3.11: Influence of HDPE microplastics on *Cyanodon dactylon* (L.) (SG) plant samples by observing growth parameters: Root Biomass for *Cyanodon dactylon* (L.) (SG); (The values are mean of three triplicates; Error bars denote 5% standard)

3.2.2. Chlorophyll content

The results showed that varied concentrations of microplastics had a greater effect on chlorophyll a (Chl a) than on chlorophyll b (Chl b). The considerable variation in chl a and chl b content indicates that the total chlorophyll content in leaves of treated and control samples differed sufficiently. Chlorophyll a is the major pigment engaged in photosynthesis, while chlorophyll b is an accessory pigment that delivers energy to chlorophyll a (Khaleghi et al., 2013). Total chlorophyll concentration (chl a + chl b) is an important indicator for photosynthetic activity, and fluctuations in this value indicate plant stress. Thus, chlorophyll a was found to be significantly lower in microplastics-treated *Portulaca grandiflora* (PG) and *Cyanodon dactylon* (L.) (SG) plant samples, showing that total chlorophyll concentration in leaves had a larger inhibitory effect on microplastics exposure. The control plant observed the content of *chl a* as 4.783 ± 0.23915 in *Portulaca grandiflora* (PG) whereas 10.654 ± 0.5327 was observed in *Cyanodon dactylon* (L.) (SG). On a contrary, microplastics treated samples exhibited *chl a* value of PG-PE (2.5g) = 8.267 ± 0.41335, PG-PE (5g) = 8.103 ± 0.40515, PG-Nylon 6,6 (2.5g) = 8.234 ± 0.4117, PG-Nylon 6,6 (5g) = 7.1654 ± 0.35827. Similarly, for *Cyanodon dactylon* (L.) (SG) treated plants, *chl a* concentrations were SG-PE (2.5g) = 9.642 ± 0.4821, SG-PE (5g) = 8.203 ± 0.41015, SG-Nylon 6,6 (2.5g) = 7.686 ± 0.3843, SG-Nylon 6,6 (5g) = 7.1654 ± 0.35827.

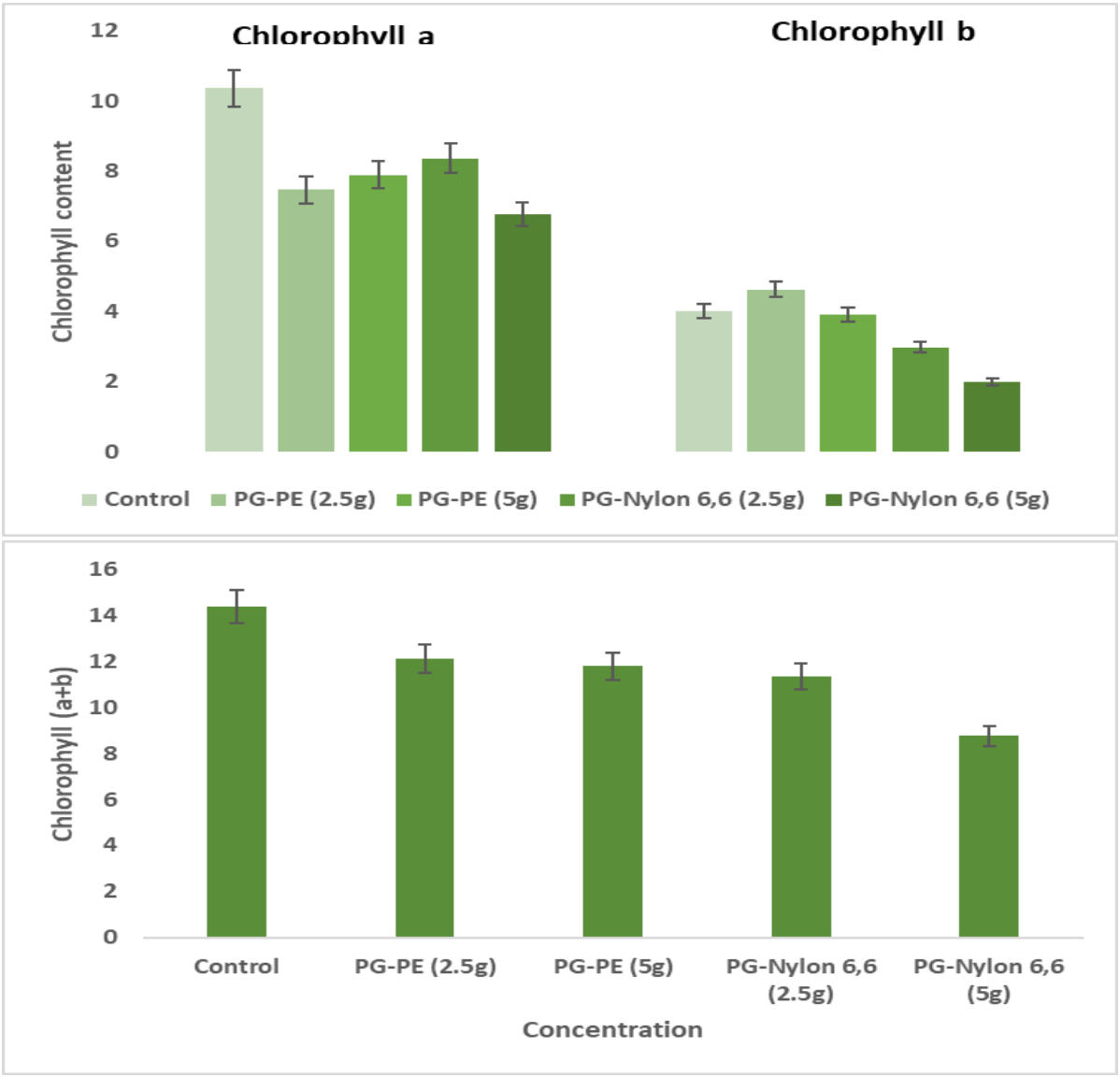


Figure 3.12: Chlorophyll content in leaves of *Portulaca grandiflora* (PG) plant samples exposed to HDPE and Nylon 6,6 microplastics: Chlorophyll content in *Portulaca grandiflora* (PG); (The values are mean of three triplicates; Error bars denote 5% standard error)

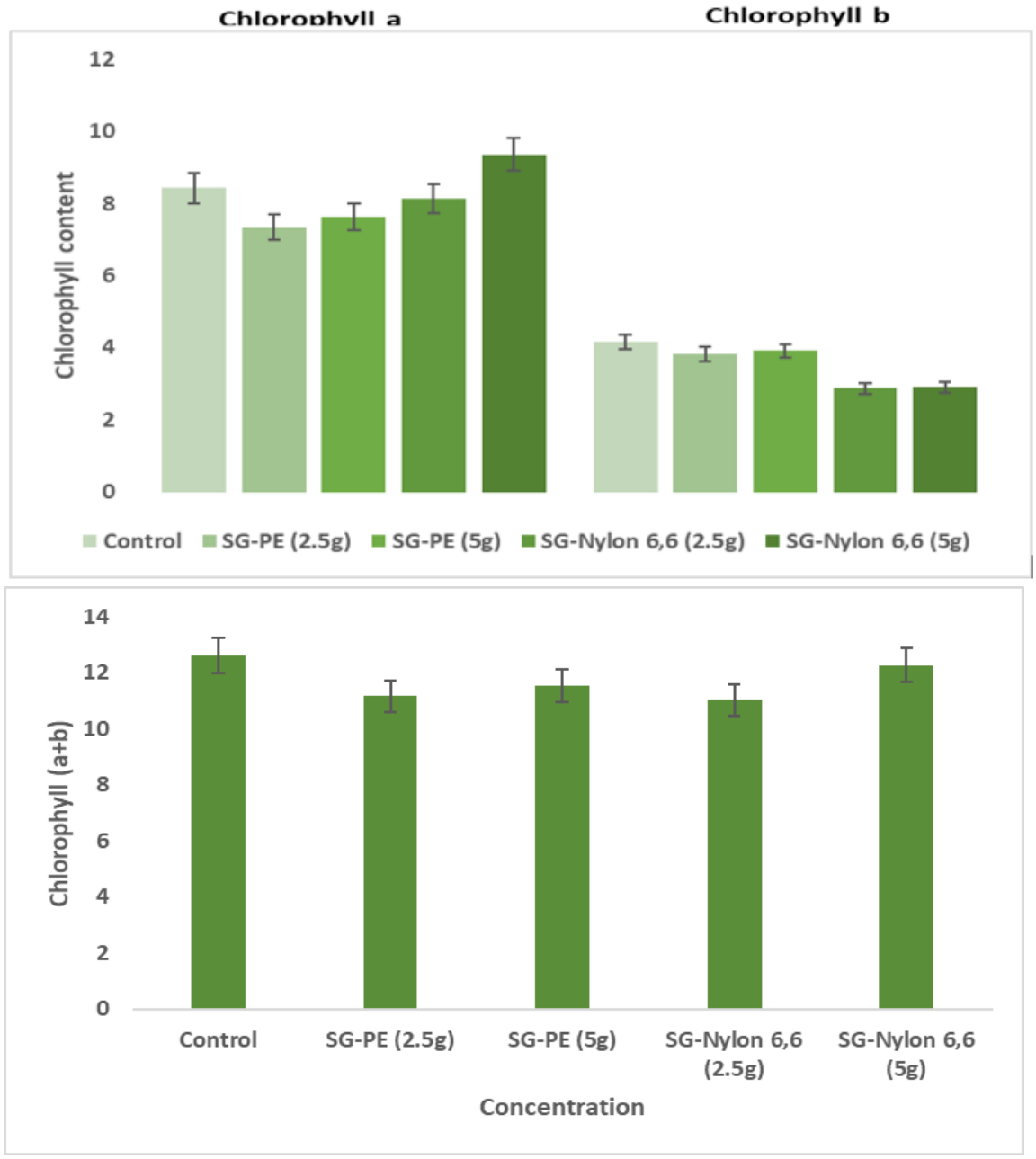


Figure 3.13: Chlorophyll content in leaves of *Cyanodon dactylon* (L.) (SG) plant samples exposed to HDPE and Nylon 6,6 microplastics: Chlorophyll content in *Cyanodon dactylon* (L.) (SG) (The values are mean of three triplicates; Error bars denote 5% standard error))

3.2.3. Antioxidant Activity

MDA levels in plants exposed to microplastics provide critical insight into the oxidative stress response and the extent of lipid peroxidation. Elevated MDA levels after microplastic treatment confirm the occurrence of stress. Malondialdehyde (MDA) is a biomarker for oxidative stress and lipid peroxidation in plants, often used to assess damage caused by environmental stressors, including pollutants like microplastics. When plants are exposed to microplastic particles, they may experience oxidative stress due to the production of reactive oxygen species (ROS). Elevated levels of ROS can damage cell membranes by causing lipid peroxidation, and MDA is one of the end products of this process.

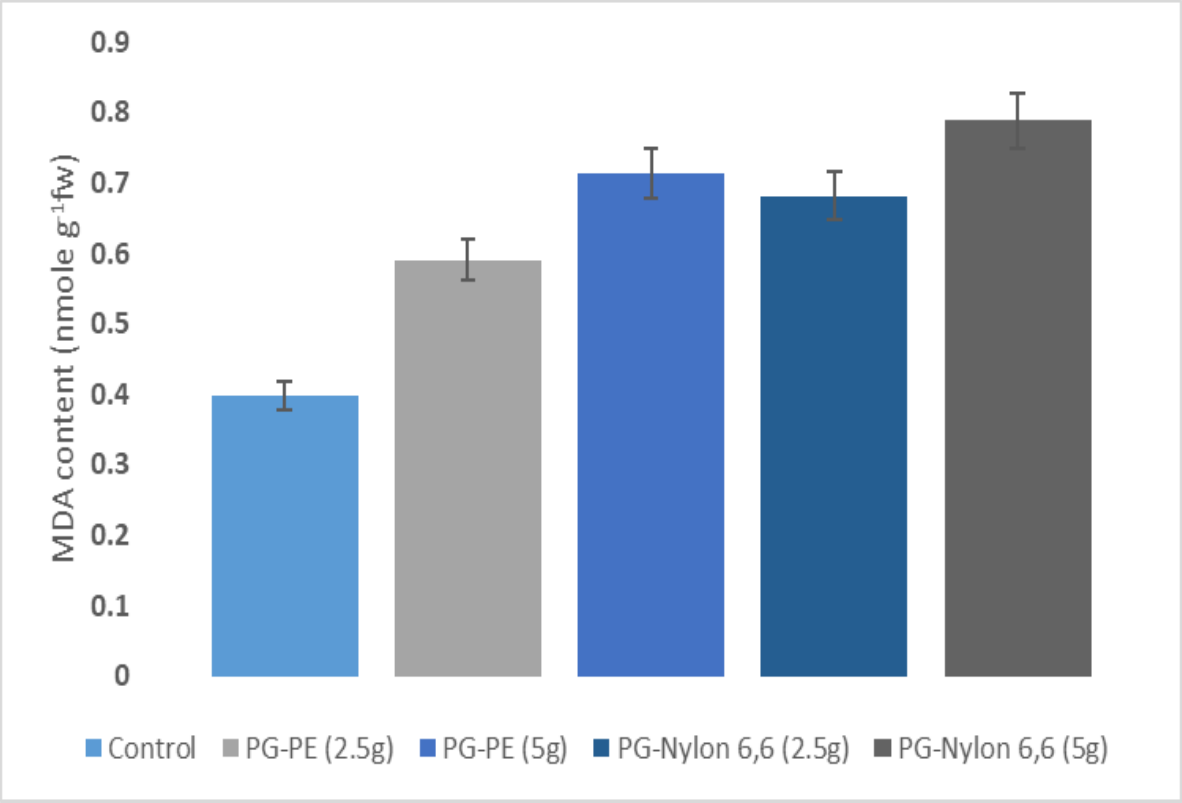


Figure 3.14: MDA content in leaves of *Portulaca grandiflora* (PG) plant samples exposed to HDPE and Nylon 6,6 microplastics: MDA content in *Portulaca grandiflora* (PG); (The values are mean of three triplicates; Error bars denote 5% standard error)

Portulaca grandiflora (PG) showed varied malondialdehyde content with control (0.399 ± 0.0454 (nmole g^{-1}fw); PG - PE (2.5g) (0.591 ± 0.0412 nmole g^{-1}fw); PG - PE (5g) (0.713 ± 0.0342 nmole g^{-1}fw); PG - Nylon 6,6 (2.5g) (0.682 ± 0.02485 nmole g^{-1}fw); PG - Nylon 6,6 (5g) (0.789 ± 0.017810 nmole g^{-1}fw). *Cyanodon dactylon* (SG) also showed a similar trend with control (0.321 ± 0.04935 nmole g^{-1}fw); SG - PE (2.5g) (0.414 ± 0.03945 nmole g^{-1}fw); SG - PE (5g) (0.603 ± 0.03375 nmole g^{-1}fw); SG - Nylon 6,6 (2.5g) (0.714 ± 0.0248 nmole g^{-1}fw); SG - Nylon 6,6 (5g) (0.876 ± 0.0195 nmole g^{-1}fw). The findings suggest that incorporating microplastics increases MDA content in plants, causing stress.

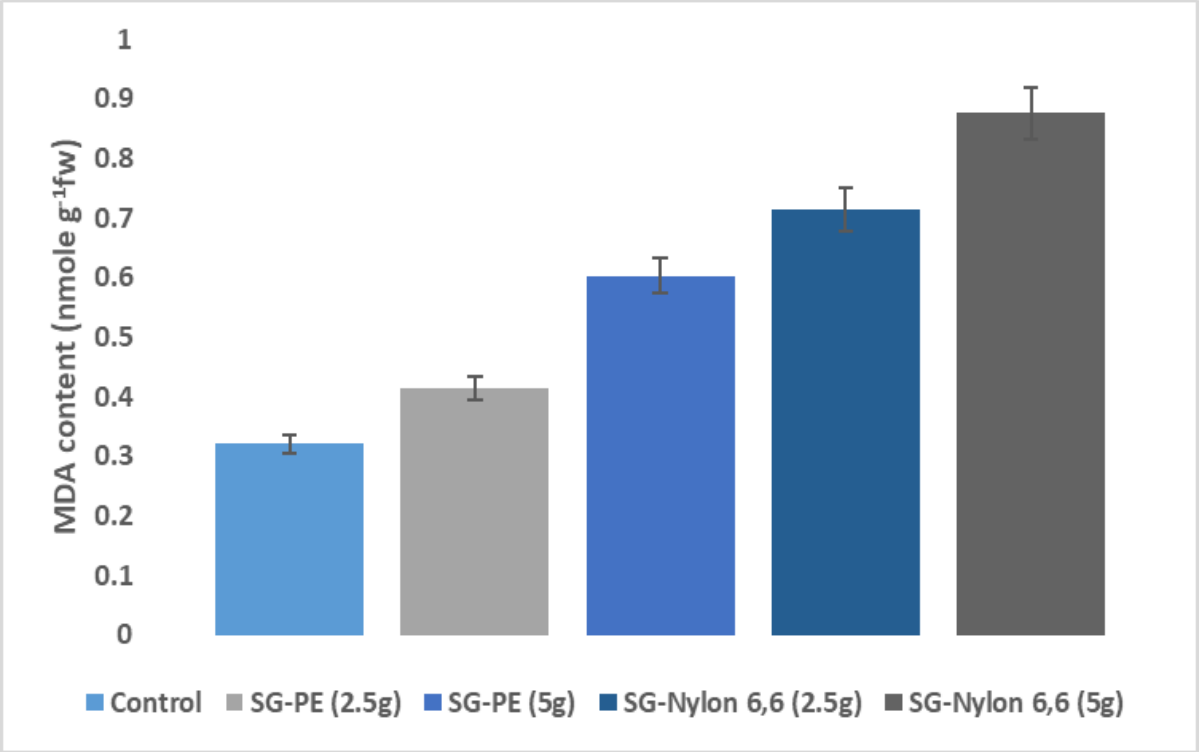


Figure 3.15: MDA content in leaves of *Cyanodon dactylon* (L.) (SG) plant samples exposed to HDPE and Nylon 6,6 microplastics: MDA content in *Cyanodon dactylon* (L.) (SG) (The values are mean of three triplicates; Error bars denote 5% standard error))

Microplastics, especially in nano- and micro-sized forms, can induce stress in plants, leading to the production of reactive oxygen species (ROS). ROS can damage cellular membranes and activate enzymatic systems such as LOX, which catalyzes the oxidation of membrane

lipids (lipid peroxidation). This process can result in:

a) Cell membrane damage: LOX mediates the breakdown of membrane lipids, causing further cell damage.

b) Inflammatory signaling: LOX-generated lipid metabolites can act as signaling molecules, triggering defense responses in plants.

The current study found that *Portulaca grandiflora* (PG) and *Cyanodon dactylon* (SG) plants treated with different concentrations of microplastics have higher LOX content.

After exposure to MPs, the concentration of LOX gradually increased, with PG - Nylon 6,6 (5g) showing the highest value of 0.870 ± 0.022 mg $^{-1}$ compared to the control with 0.421 ± 0.04915 mg $^{-1}$.

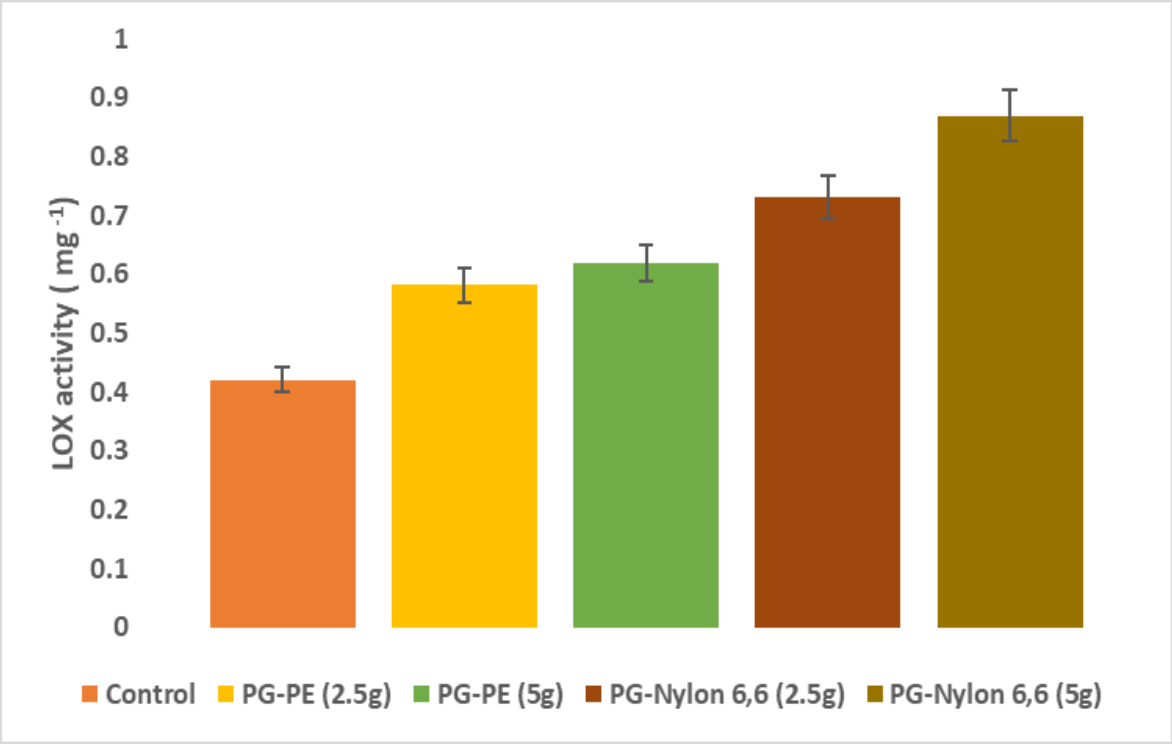


Figure 3.16: LOX content in leaves of *Portulaca grandiflora* (PG) plant samples exposed to HDPE and Nylon 6,6 microplastics: LOX content in *Portulaca grandiflora* (PG); (The values are mean of three triplicates; Error bars denote 5% standard error))

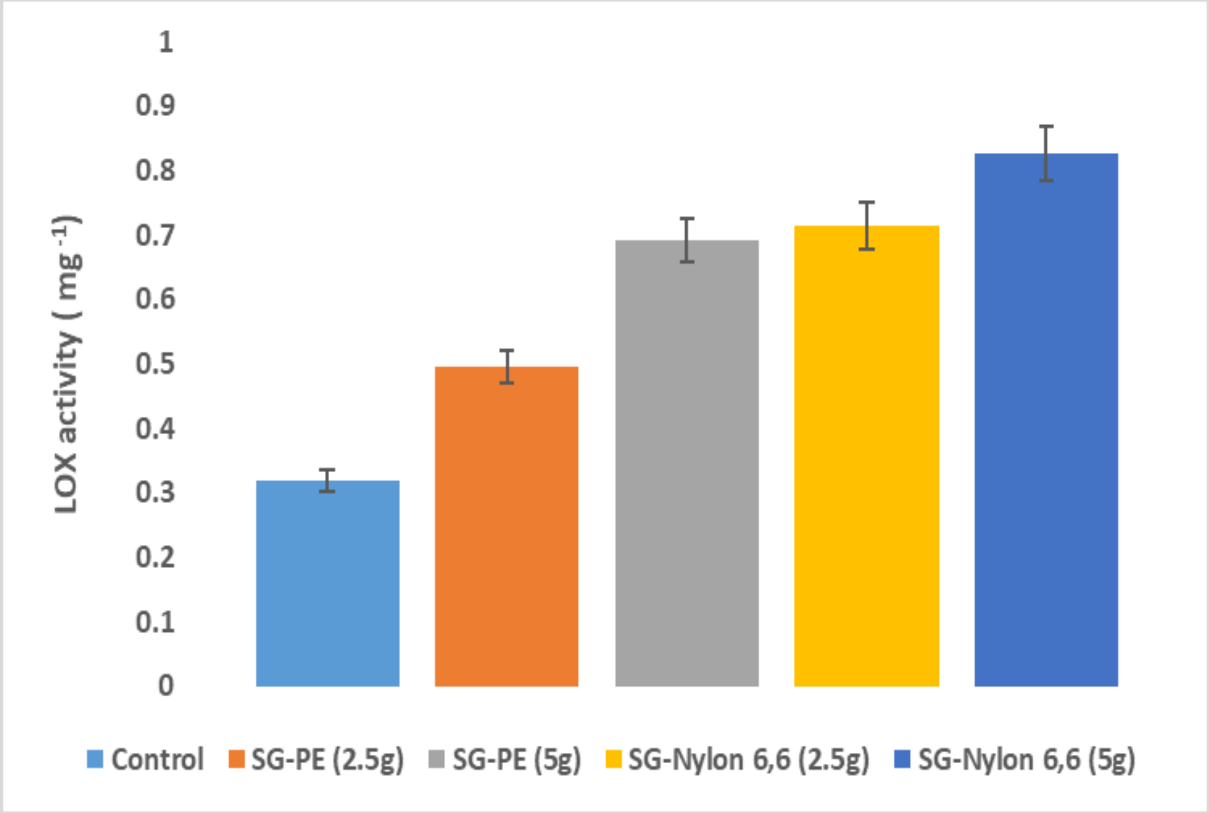


Figure 3.17: LOX content in leaves of *Cyanodon dactylon* (L.) (SG) plant samples exposed to HDPE and Nylon 6,6 microplastics: LOX content in *Cyanodon dactylon* (L.) (SG) (The values are mean of three triplicates; Error bars denote 5% standard error))

Also, the control for SG treated plants was 0.319 ± 0.0488 mg $^{-1}$, and the highest content of LOX was observed at 0.826 ± 0.0216 mg $^{-1}$ for SG - Nylon 6,6 (5g). These data indicate that LOX is not able to decrease stress in *Portulaca grandiflora* (PG) and *Cyanodon dactylon* (SG) plants infected with microplastics.

3.2.4. Confocal Microscopy

Confocal microscopy was used to visualize tagged microplastics within the root lateral cross-sections and leaf portions in order to comprehend the study's most relevant findings. At some points, microplastics were embedded in root hair segments and leaf internal veins when viewed under red and green fields at 40 X and 100 X magnification. Because we

found microplastic particles in inner root and leaf structures, our findings indicate that micrometer-sized microplastic can easily go from soil to root via crack-entry and apoplastic pathways, laying the groundwork for entry into the food chain. These findings corroborate the uptake of microplastics by *Portulaca grandiflora* (PG) and *Cyanodon dactylon* (SG) plants and provide future insights into the mechanism of action and plant response.

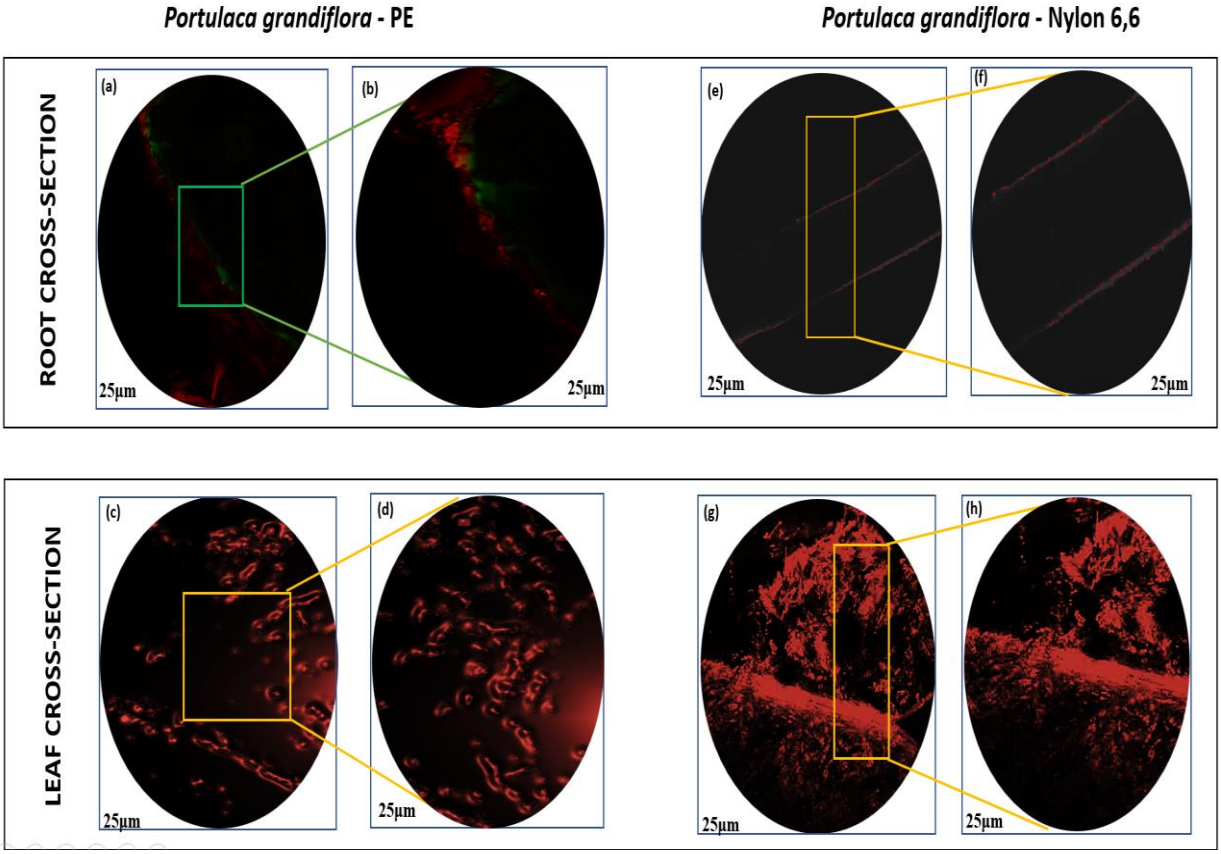


Figure 3.18: Longitudinal cross-section showing microplastic particles inside *Portulaca grandiflora* (PG) and lateral root and leaf section in a two months old plants after being exposed to tagged microplastics. Clockwise from top left shows cross sections using confocal microscopy, (a) & (b) show root cross-sections of PG-PE; (c) & (d) show leaf sections of PG-PE; (e) & (f) show root cross-sections of PG-Nylon 6,6; (g) & (h) show leaf sections of Nylon 6,6. Arrows point to fluorescing microplastic particles. All images have been enhanced using a contrast and brightness correction and taken at a magnification of 40X and 100X. Scales are shown in the bottom left corner of the respective image.

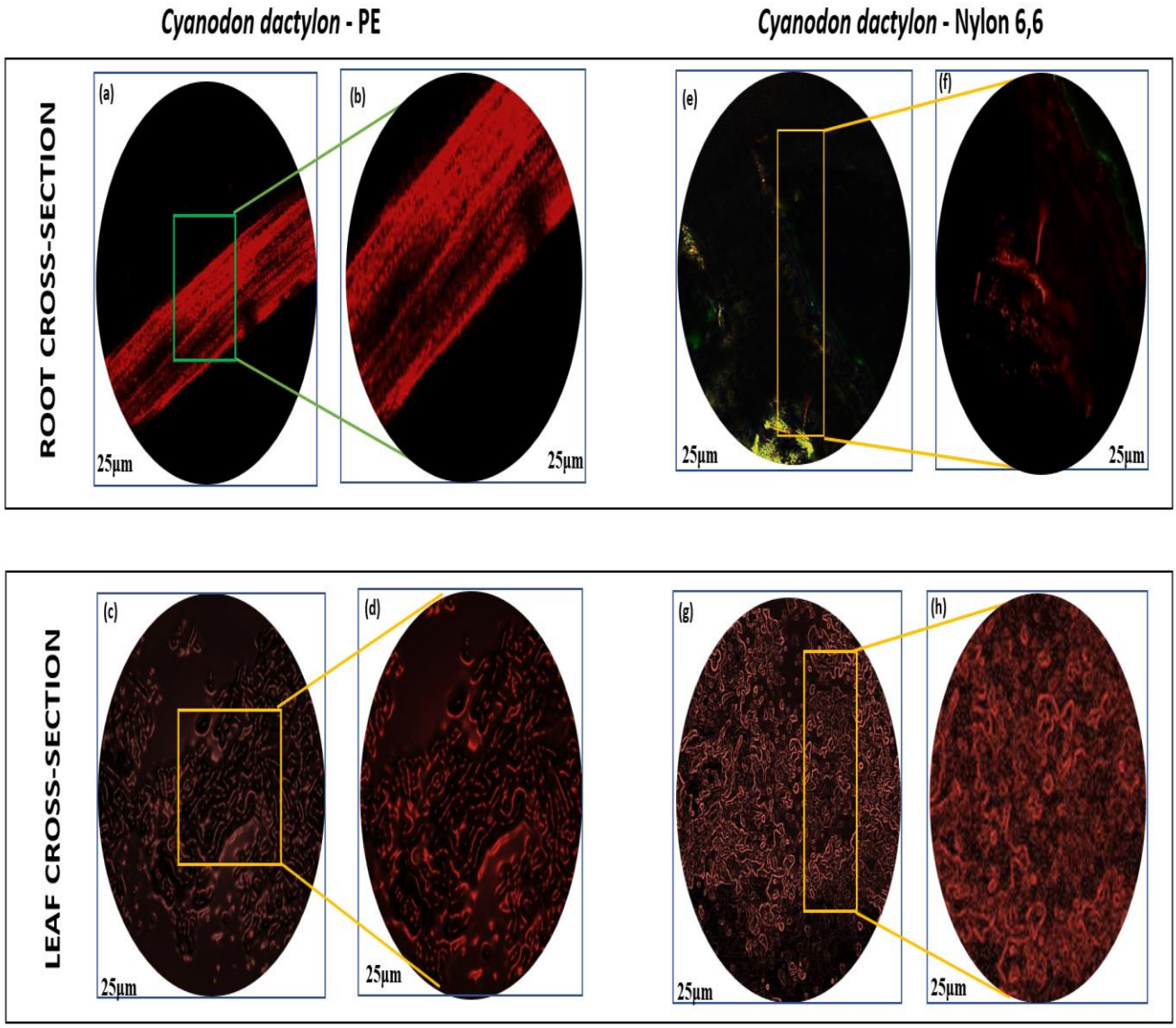


Figure 3.19: Longitudinal cross-section showing microplastic particles inside *Cyanodon dactylon* (L.) (SG) lateral root and leaf section in a two months old plants after being exposed to tagged microplastics. Clockwise from top left shows cross sections using confocal microscopy, (a) & (b) show root cross-sections of SG-PE; (c) & (d) show leaf sections of SG-PE; (e) & (f) show root cross-sections of SG-Nylon 6,6; (g) & (h) show leaf sections of SG-Nylon 6,6. Arrows point to fluorescing microplastic particles. All images have been enhanced using a contrast and brightness correction and taken at a magnification of 40X and 100X.

This study found that microplastics move from root hair sections to upper plant components such as leaves and shoots. The findings indicate that microplastics may have an effect on both plants following exposure. However, the process for transporting microplastics from soil to plant components is still in its early stages and requires further investigation. Microplastics can pass through the plant from the roots to the leaves via two routes: the apoplast and symplast (Bansal et al., 2024, 2023).

This study is the first to show microplastic absorption, uptake, and phytotoxicity in wild plants, *Portulaca grandiflora* (PG) and *Cyanodon dactylon* (SG). Physiological and biochemical investigations show that MPs can be transferred from root to leaf portions. Morphological investigation confirms the existence of MPs in various plant locations, including vascular tissues in leaf sections and root hairs. Based on these findings, the mechanism of MPs transport from roots to leaf sections via apoplast and symplast pathways appears to be relevant.

3.3. <u>OBJECTIVE 3</u>: Understanding the phytoremediation approaches to remediate soil of micro/nano plastics:

The uptake of microplastics by plant parts could help in phytoremediation by use of its different approaches. Through the above study, it could be observed that microplastics traverse from root hair sections to upper parts of plant like leaves and shoots. The results signify that microplastics have possible implications on *Brassica juncea* plant after being exposed. However, the mechanism of transport of microplastics from soil to plant parts is still in its infancy and needs more research. The apoplast and symplast routes are two pathways that microplastics can take to move through the plant from the roots to the leaves (Su et al., 2019). The apoplast is the space outside the plant cells, consisting of cell walls, intercellular spaces, and extracellular fluid. Microplastics can move through this space via diffusion, and uptake into the plant is thought to occur through the root epidermis and cortex. Once inside the apoplast, microplastics can move laterally along the root cell walls and through intercellular spaces to reach the xylem vessels (Roberts and Oparka, 2003). From xylem, they can be transported to the leaves and aerial parts of plant. The apoplast

route is thought to be the primary pathway for larger microplastics to traverse the plant.

The symplast is the interconnected network of plant cells via plasmodesmata, which are small channels that allow for direct communication and transport of molecules between cells. Microplastics can enter the plant cells through endocytosis or other mechanisms and move through the cytoplasmic continuum from cell to cell via plasmodesmata (Raliya et al., 2016). This route is thought to be the primary pathway for smaller microplastics or those with a hydrophilic surface. The probable pathway for microplastic transport within the plant is described in figure 3.20. Some general mechanisms that are mostly proposed include:

a) Adhesion and penetration: Microplastics may adhere to the root surface and penetrate into the root tissues through physical and chemical interactions (Nel et al., 2009).

b) Endocytosis: Microplastics can be taken up by plant cells through endocytosis, which involves the formation of vesicles around the particles (Etxeberria et al., 2006).

c) Translocation: Once inside the root, microplastics may be transported across the root cortex and into the xylem vessels, which carry water and nutrients up to the leaves. This transport can occur through diffusion or active transport mechanisms (Schwab et al., 2016).

d) Accumulation in leaves: Once in the xylem vessels, microplastics can be transported to the leaves, where they can accumulate in the leaf tissues. This accumulation can occur through transpiration, which is the loss of water through the leaves, and subsequent concentration of microplastics in the leaf tissues.

The uptake of microplastics by plants can have various effects, including alterations in plant growth, development, and metabolism. Furthermore, the presence of microplastics in edible plant tissues could pose potential risks to human health, and further research is needed to understand their impacts on plants. Hence, the migration of MPs inside plants is really important as it gives an indication on the accumulation and absorption of MPs on plant parts. Also, after accumulation and absorption of MPs, the phytoremediation potential can be determined to provide sustainable solution for environmental cleanup.

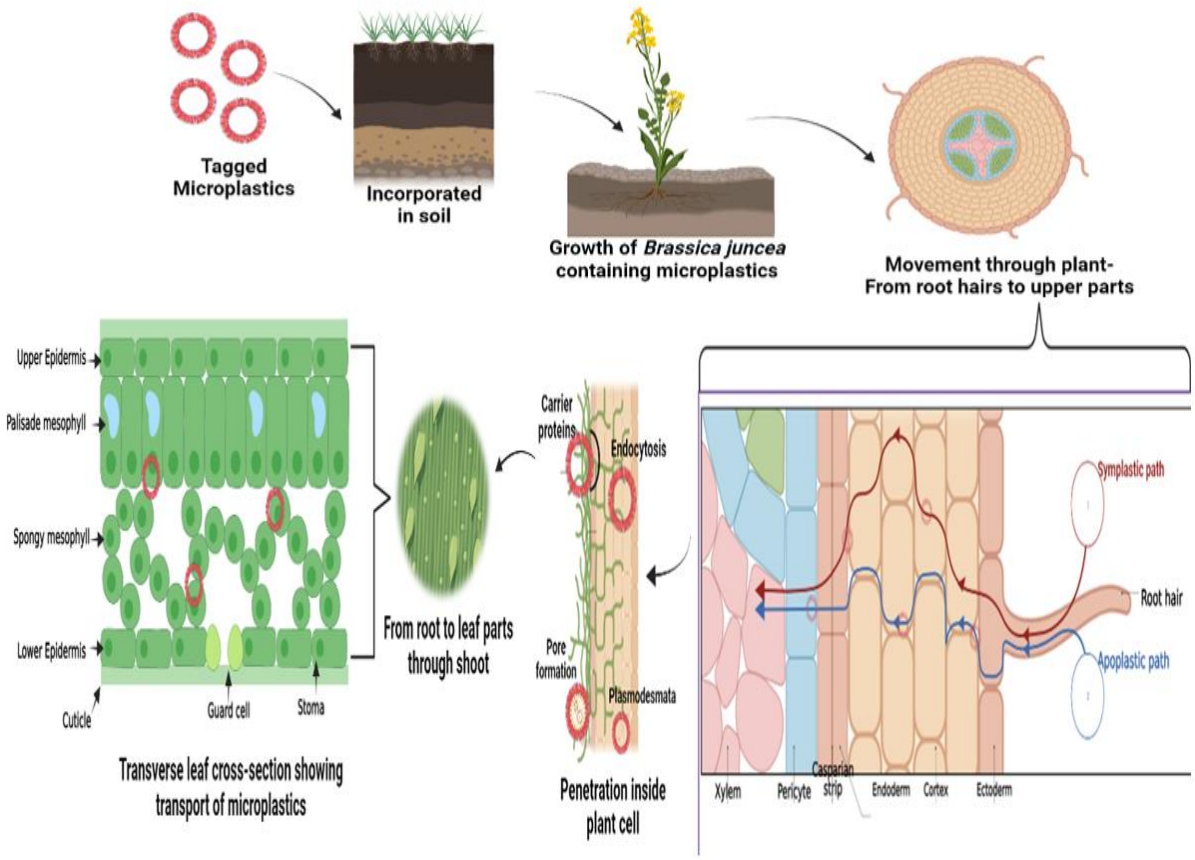


Figure 3.20: Mechanism of transport of microplastics from roots to different parts of plant

3.4. <u>OBJECTIVE 4</u>: Synergistic plant-microbe interaction and corresponding metabolic products in modulating micro/nano plastic degradation:

3.4.1. Isolation of bacterial strains capable of microplastic degradation

The soil used for the plantation of both the above wild plants was employed to test its efficacy for microplastic degradation. The comprehensive mechanism for isolation of bacterial isolates is depicted below (figure 3.34).

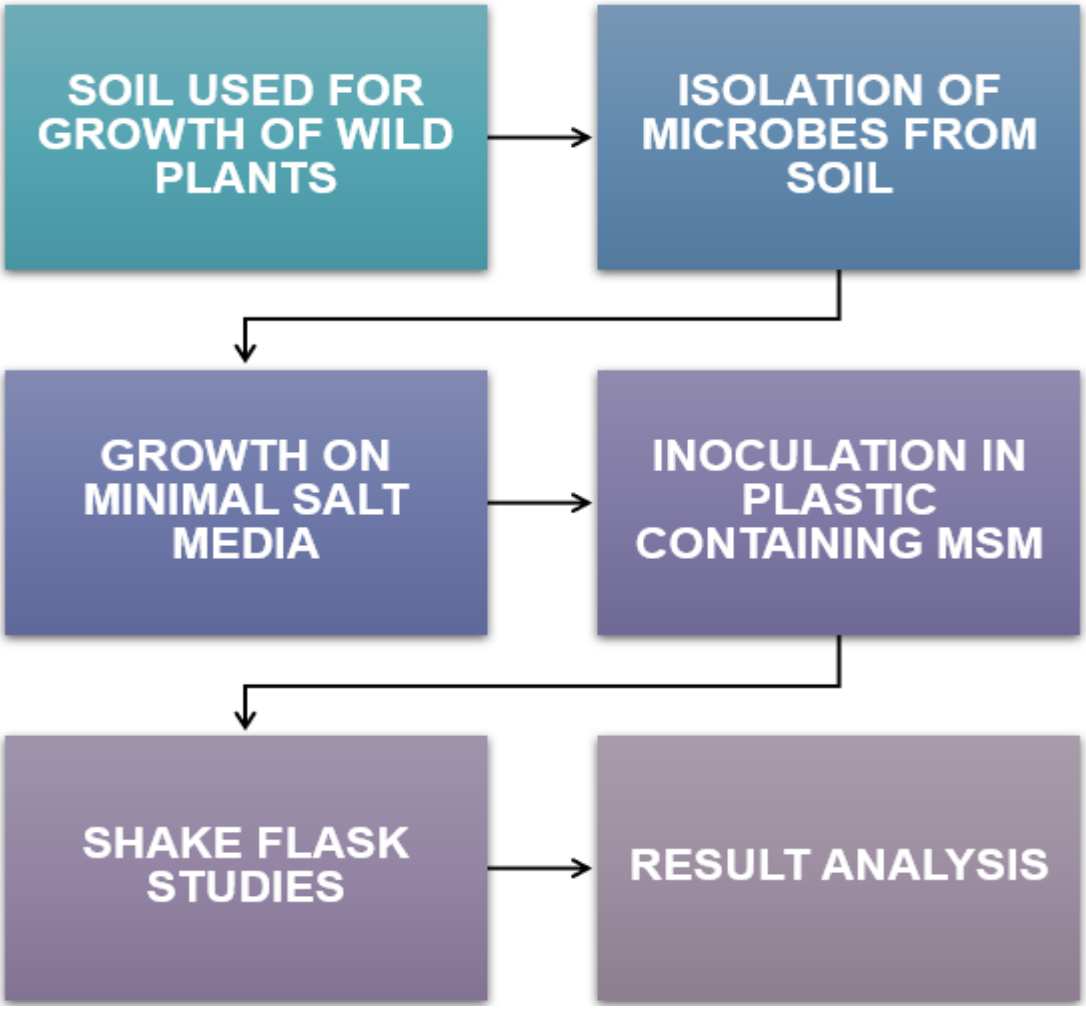


Figure 3.21: Mechanism of isolation of bacteria for microplastic degradation study

The process involved assembling libraries, creating clusters, sequencing, and then performing bioinformatic analysis once the DNA from the soil community was isolated. The results of this investigation showed that soil bacteria are distributed according to taxonomic phylum, as *Acinetobacter baumannii* (100%) dominated the soil sample when the phylum-level bacterial diversity was evaluated using a high-throughput 16S rRNA metagenomic sequencing technique. *Acinetobacter sp.* PMM5 (99.91%) was the second most numerous types of bacteria, after *Acinetobacter baumannii* strain AbCTX5 (99.81%). The soil was combined with an isotonic saline solution and allowed to settle for three hours.

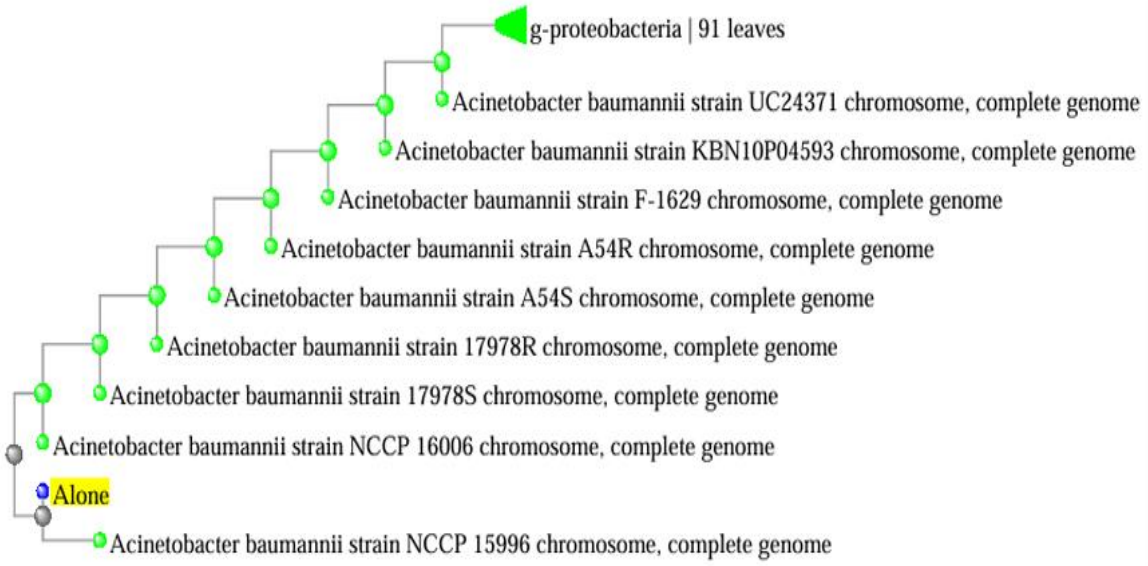
Following this, the supernatant was collected and added to LB broth for inoculation. The

ability of twelve bacterial strains specifically to degrade microplastics was assessed after they were isolated from a soil sample. Following their selective isolation from the LB agar plates, the bacteria were then injected into a basal medium containing 0.5% (w/v) microplastics, respectively. After that, every single colony was selected, and its ability to break down microplastics in the basal media while they were present was evaluated. From the twelve bacterial isolates, one bacterium had the maximum capacity for microplastic degradation. To find out how well this distinct culture degraded plastic, it was cultured under aerobic conditions for 15 days. Nucleotide homology analysis supported the identification of one of the bacterial cultures as *Acinetobacter baumannii* through investigation using 16S rDNA gene sequencing. The strain was labelled as Alone and tested for microplastics degradation potential for a span of 50 days. To prove the synergistic interaction of plant-microbe in micro/nano plastic degradation, bacterial isolates were screened and 16s RNA metagenomic sequencing analysis was performed. One microbial isolate showed maximum degradation efficiency as shown by the experimental study carried out for 50 days. The primers used for the sequencing of the bacterial isolate were 16S Forward - GGATGAGCCCGCGGCCTA and 16S Reverse- CGGTGTGTACAAGGCCCGG.

Sample: ALONE

- The Microbe was found to be *Acinetobacter baumannii* strain BCI1 16S ribosomal RNA gene
- Sequence ID: PP789708.1
- The next closest homologue was found to be *Acinetobacter baumannii* strain CEMTC 1538/1539 16S ribosomal RNA gene
- Sequence ID: OQ850120.1/

Phylogenetic Tree



BLAST Data:

Sl. No.	Organism Name	Accession No.	% Match
1	Acinetobacter baumannii strain BCI1 16S ribosomal RNA gene	PP789708.1	100.00%
2	Acinetobacter baumannii strain CEMTC_1538/1539 16S ribosomal RNA gene	OQ850120.1	99.91%
3	Acinetobacter sp. PMM5 16S ribosomal RNA gene	KF732993.1	99.91%
4	Acinetobacter baumannii strain AbCTX5 chromosome	CP060505.1	99.81%
5	Acinetobacter baumannii strain SIMBA089 chromosome	CP162145.1	99.81%
6	Acinetobacter baumannii strain SIMBA061 chromosome	CP161999.1	99.81%
7	Acinetobacter baumannii strain SIMBA034 chromosome	CP161992.1	99.81%
8	Acinetobacter baumannii strain SIMBA003 chromosome	CP161986.1	99.81%
9	Acinetobacter baumannii strain DETAB-P462 chromosome	CP161817.1	99.81%
10	Acinetobacter baumannii strain BCI 4 16S ribosomal RNA gene	PQ012606.1	99.81%

Figure 3.22: Phylogenetic Tree and BLAST Sequence of identified strain

3.4.2. FTIR analysis of microplastics

Microplastics' FTIR spectra before and after contact with an isolated microbe were shown in Figure 3.36 and figure 3.37. The primary distinctive absorption bands in PP were identified as follows: 2960 cm^{-1} (-CH3 stretching), 2856 cm^{-1} (-CHO stretching), 1500 cm^{-1} (C=C double bond stretching), and 690 cm^{-1} (C=C-H stretching). The FTIR analysis revealed that the PP_A Bacteria formed new functional groups, including hydroxyl groups (O-H stretch at 3329 cm^{-1}), methyl deformation (1493 cm-1), carbonyl groups (conjugated ketone or aldehyde R-C=O stretch at 1538 cm^{-1}), and 1267 cm^{-1} occurring in the end of methyl groups indicating the bio-oxidation and polymer chain breakage on the surface of the PP. Similar to the changes seen in polyethylene spectra analysis, the changes in polypropylene spectra primarily show a decrease in band intensity at specific wavenumbers (Wróbel et al., 2023).

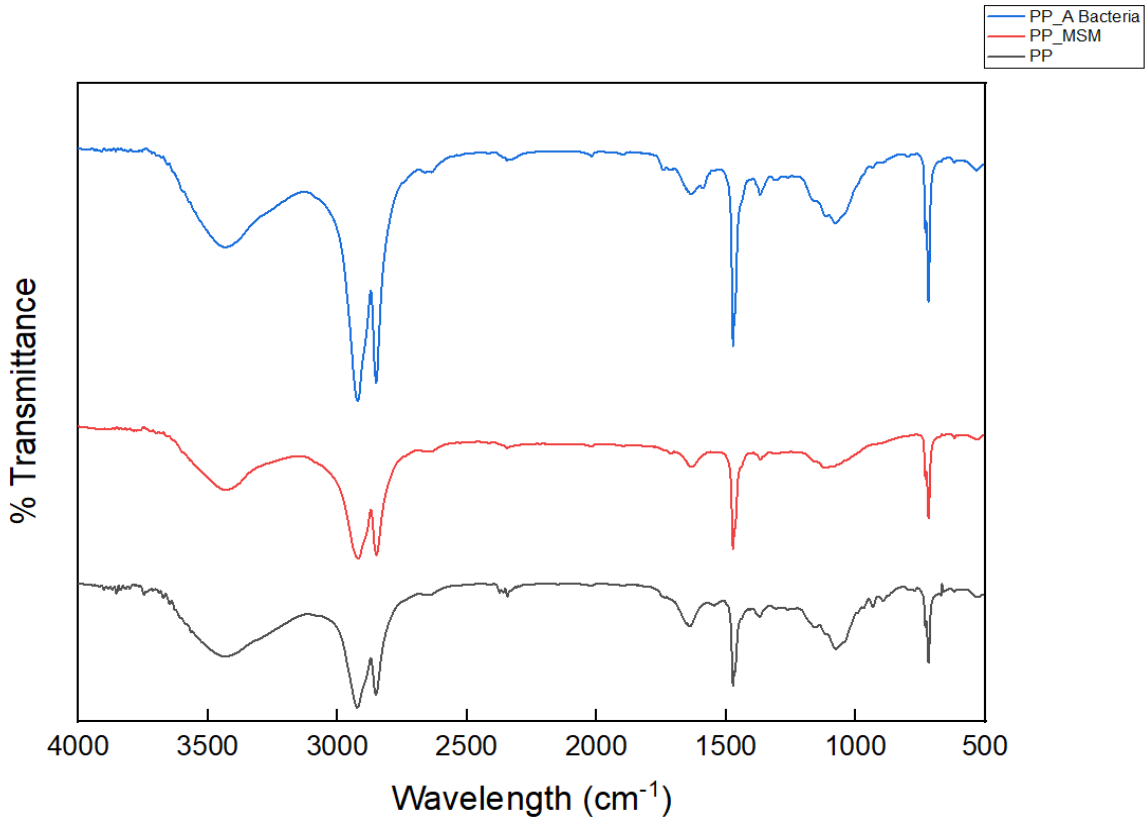


Figure 3.23: FTIR Spectra of Polypropylene Microplastics

The primary distinctive absorption bands in PVC were identified as follows: 3500 cm^{-1} (-CH_2 stretching), 1656 cm^{-1} (-CHO stretching), 1486 cm^{-1} (C=C double bond stretching), and 1230 cm^{-1} (C=C-H stretching). The FTIR analysis revealed that PVC_A Bacteria formed new functional groups, including hydroxyl groups (O-H stretch at 3270 cm^{-1} and 3340 cm^{-1}), chlorine groups (1613 cm^{-1}), carbonyl groups (conjugated ketone or aldehyde R-C=O stretch at 1500 cm^{-1} and 1107 cm^{-1}) occurring in the end of chlorine groups indicating the bio-oxidation and polymer chain breakage on the surface of PVC. When damaged PVC films were subjected to FTIR analysis, changes in bond formation and disappearance were observed along with variations in peak intensity. Analogously, the FTIR spectrum was utilized to enhance bacterial breakdown through the stretching and vibrating of functional groups and chemical bonds in the polymer structure. Oxidation of the polymer occurred as a result of dissolved oxygen, forming a carbonyl group that converts to carboxylic acid and travels via β-oxidation to join the citric acid cycle, ultimately producing CO_2 and H_2O (Khandare et al., 2021a).

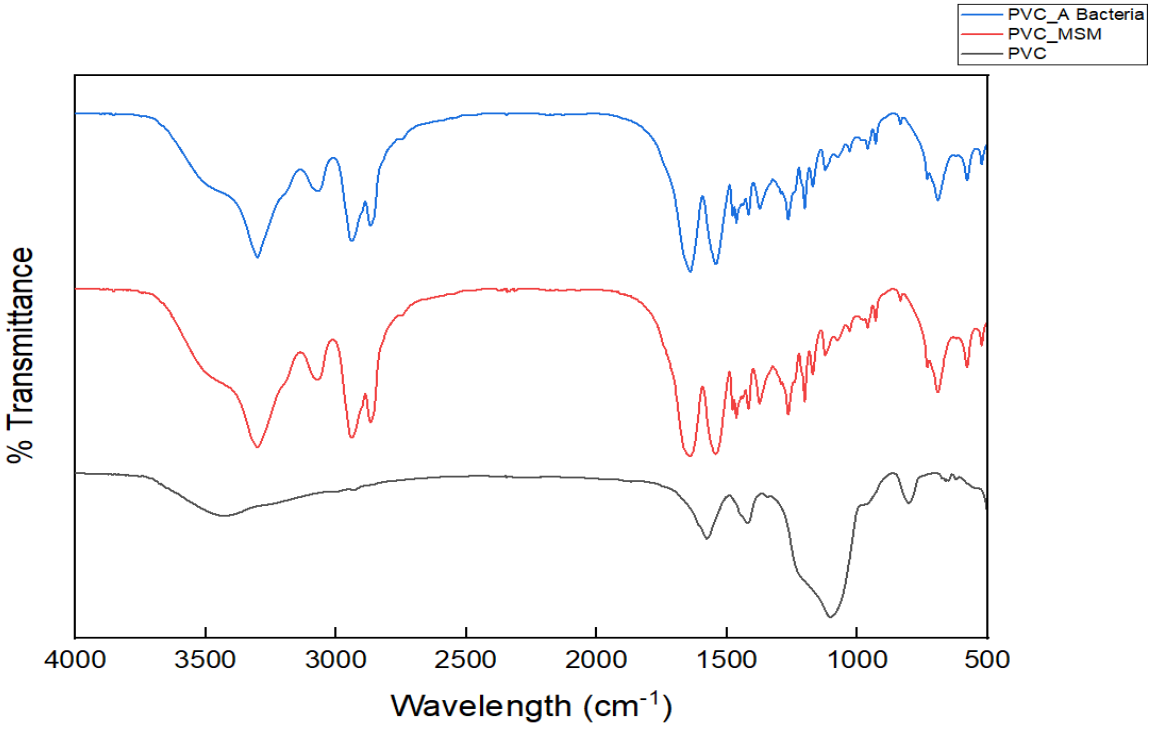


Figure 3.24: FTIR Spectra of Poly Vinyl Chloride Microplastics

3.4.3. Thermogravimetric analysis (TGA) of microplastics

Thermogravimetric analysis, or TGA, studies how polymers react to slow temperature rises. Mechanical properties including molecular weight and crystallinity, as well as chemical composition, are the main factors that determine heat stability. Changes in a polymer's mechanical and chemical properties can therefore be indicated by changes in its heat resistance. To investigate the composition and thermal stability of original microplastics and bacterially degraded microplastics, TGA was utilized. The degradation of PP microplastics begins at a temperature of around 280 °C with a weight loss of 80% after treatment with A Bacteria. On a contrary, the PP microplastic degrades at a temperature of 450 °C with a weight loss of 80% without any bacterial treatment. These results support the notion that some weight of the bacterially destroyed microplastics was maintained, indicating the possibility for the presence of small molecules that are byproducts of the degradation process. The findings suggested that the antioxidants might be in charge of a few variations in heat stability.

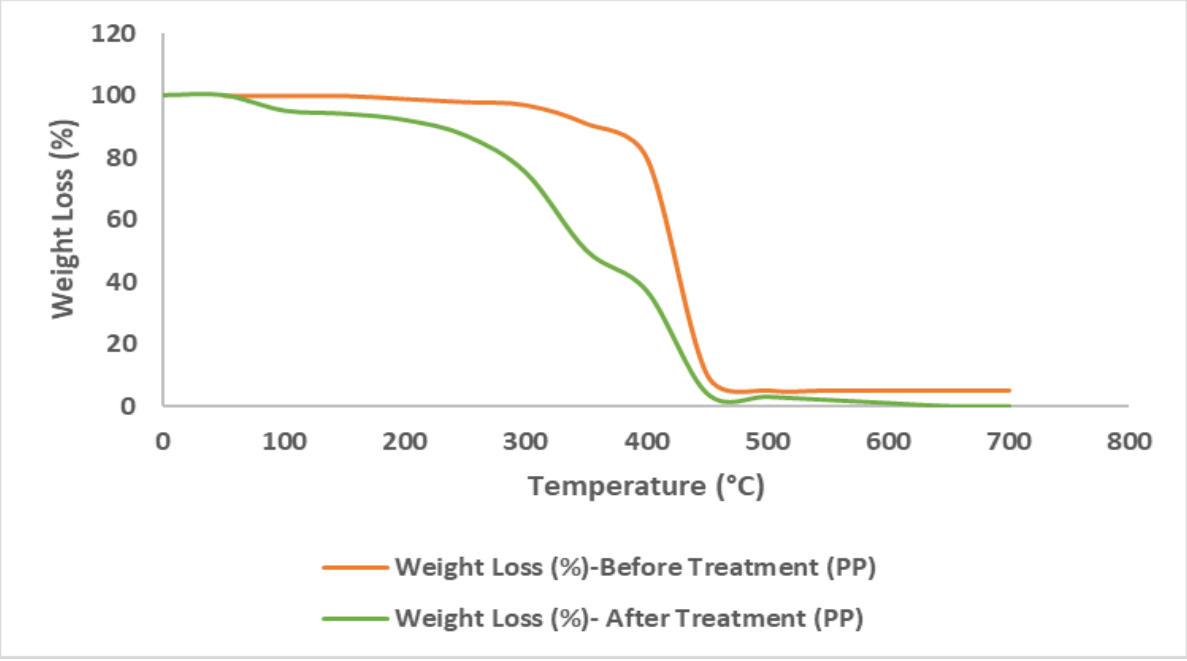


Figure 3.25: TGA of Polypropylene Microplastics

The degradation of PVC microplastics begins at a temperature of around 100 °C with a weight loss of 86 % after treatment with A_Bacteria. On a contrary, the PVC microplastic degrades at a temperature of 270 °C with a weight loss of 80 % without any bacterial treatment. This suggested that when a bacterial isolate is inoculated in microplastics, their thermal stability declines. The long chain structure may be broken down by bacteria into low molecular weight polymers, which are less resistant to temperature changes.

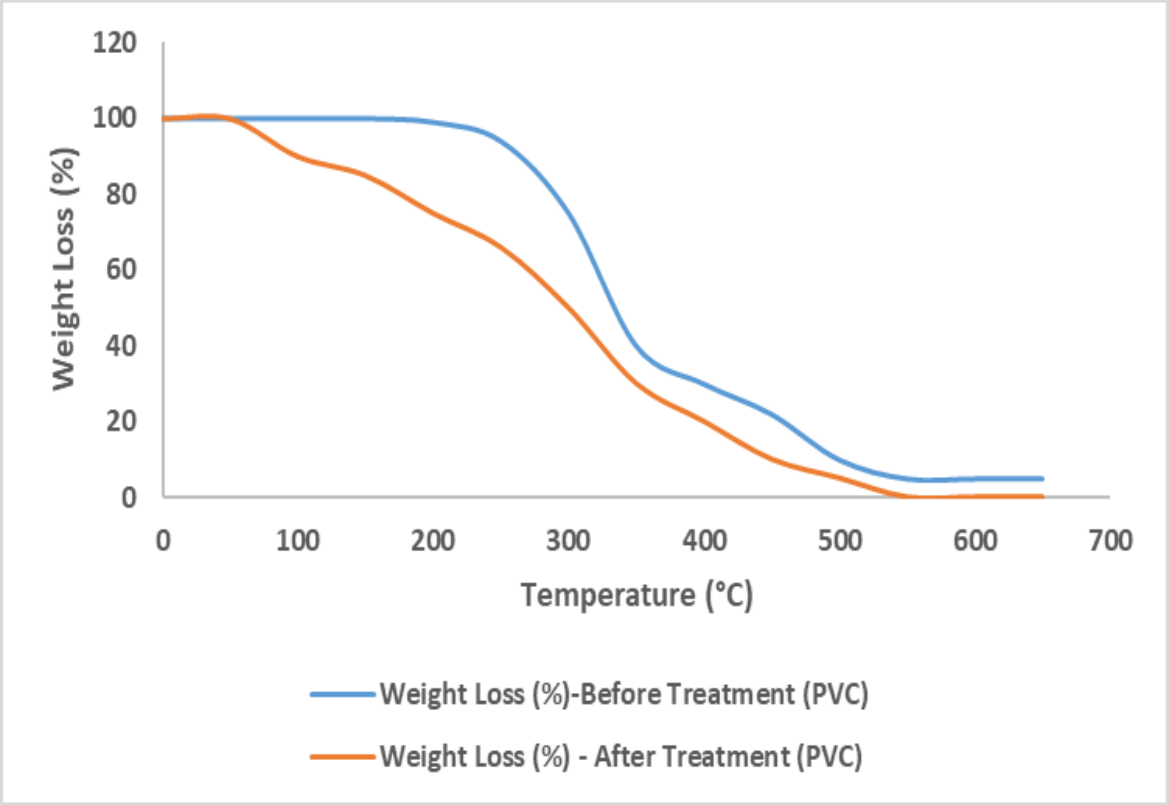


Figure 3.26: TGA of Poly Vinyl Chloride Microplastics

3.4.4. Microplastics dry weight determination post microbial degradation

Due to their ability to metabolize and break down microplastics, bacteria may be able to help reduce pollution through biodegradation. During the biodegradation study period, the negative control sample (MSM_PP) showed no weight loss; in contrast, the positive control sample (Bacteria_PP) showed a total weight loss of 33.33 %. Also, the positive control

sample (Bacteria_PVC) showed a total weight loss of 27.10 %. These results unequivocally demonstrate that variation in weight loss may be the result of microbial metabolism. The major reason observed for weight loss could be the bacterial enzymes. These enzymes have the potential for metabolizing and degrading microplastics through biochemical pathways (Feng et al., 2021). Polymer chains break down into smaller molecules during biodegradation, producing water, carbon dioxide, and other pollutants. The fragmentation process breaks down plastic particles into smaller components that microbes can use for growth and energy, which results in a reduction in the weight of the particles (Dave and Das, 2021). Because they may change their habitat according to what nutrients are available, microbes are able to adapt. Thus, it appears from the weight loss that microbial cells efficiently use the carbon in microplastics backbone by breaking down their chain structure (Auta et al., 2018).

Table 3.1: Weight Loss (%) of microplastics

Organism	Initial Weight (mg)	Final Weight (mg)	Weight Loss (%)
Control PP	5	5	0
PP _ A	5	3.33	33.3
Control PVC	5	5	0
PVC _ A	5	3.641	27.1

3.4.5. Morphological Analysis

The microbial interaction and attachment on microplastic surface were observed through Scanning Electron Microscopy (SEM). In the figure, it could be observed that a) and b) parts show *Acinetobacter baumannii* strain in healthy condition. The cells are intact with regular

morphology showing cocci and rod-shaped structure. However, after the inoculation of bacterium in MSM media as shown in parts c) and d), the cells shrink and show distortion in structure due to cell starvation and loss of nutrients leading to cell death. The attachment of bacteria to both the microplastics, PP and PVC, could be observed in parts e) and f). This morphology shows the entrapment, adherence and consumption of microplastics as a carbon source by the bacteria. This confirms that microbial adhesion is a sign of cellular desire for the carbon that is readily available on plastic surfaces. Analogous investigations have demonstrated the adherence of microorganisms onto the polymer surface in order to meet the carbon requirement (Huang et al., 2022).

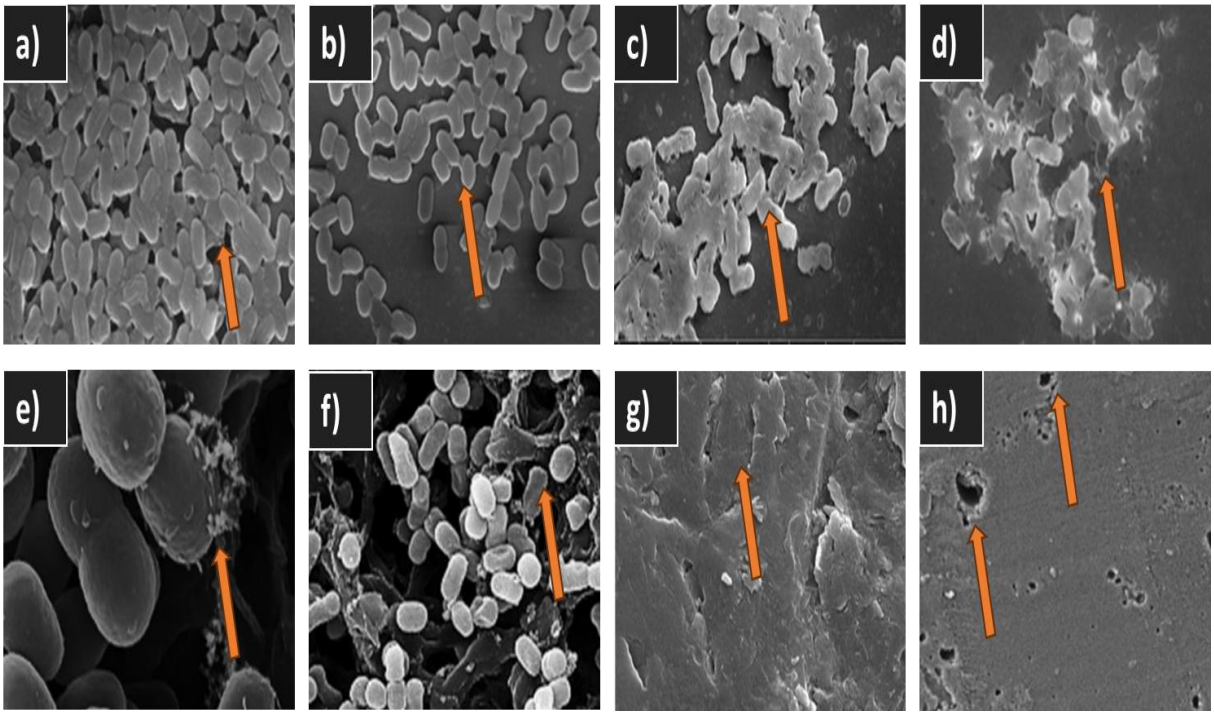


Figure 3.27: SEM images of bacterial isolate, adherence of bacteria to PP and PVC microplastics, and degradation of microplastics

Finally, parts g) and h) reveal the thinning, cracks and holes on PP and PVC microplastic surfaces after exposure to bacteria. The morphological analysis reveals the degradation of microplastics thereby proving the bacteria to have degradation potential.

3.4.6. Biometrical Parameters

3.4.6.1. Growth Response

Cyanodon dactylon (L.) (SG) and *Portulaca grandiflora* (PG) exposed to different concentrations of PE and nylon 6,6 showed a substantial change in shoot length and shoot biomass when compared to control plants after a span of 32 weeks.

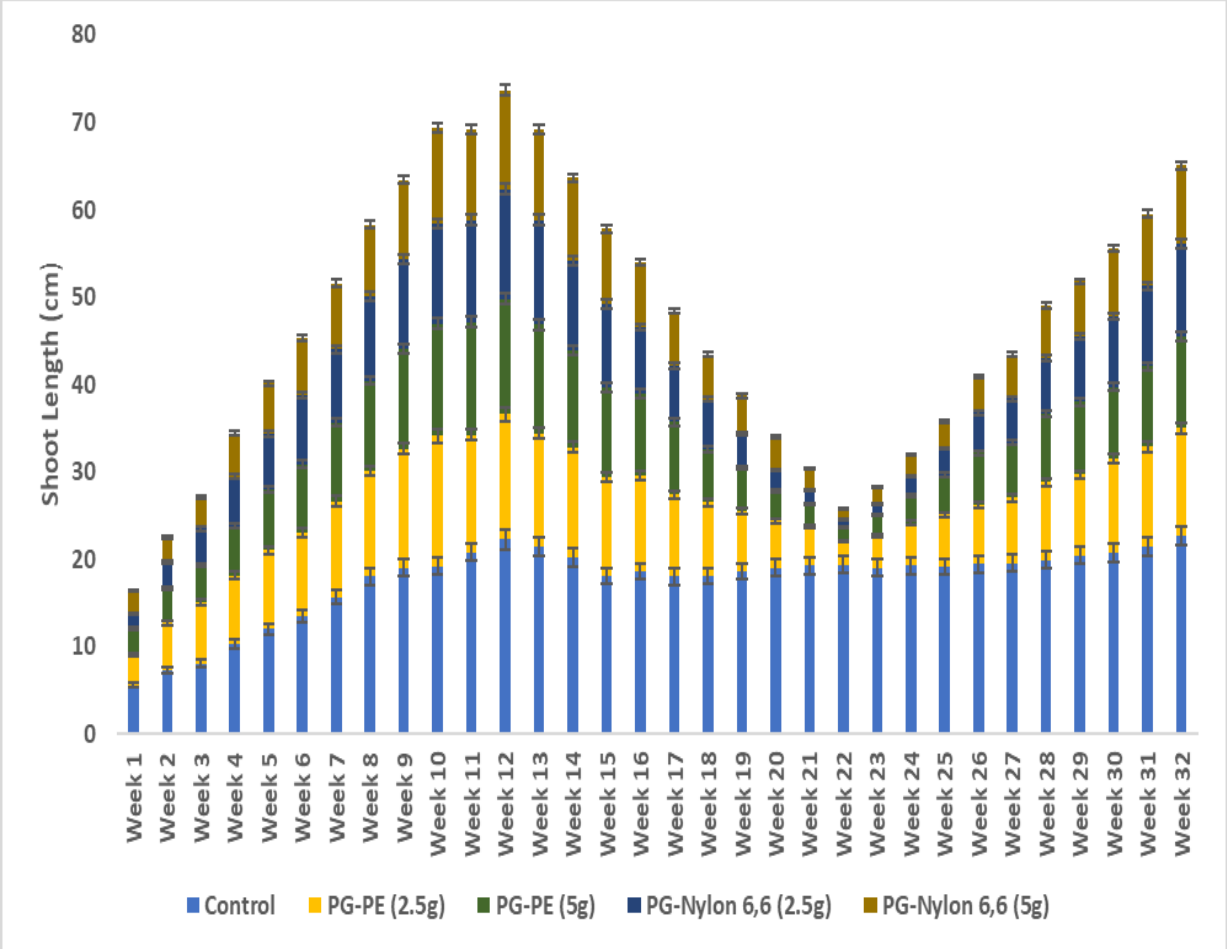


Figure 3.28: Influence of HDPE and Nylon 6,6 microplastics on *Portulaca grandiflora* (PG) plant samples by observing growth parameters: Shoot length for *Portulaca grandiflora* (PG) (The values are mean of three triplicates; Error bars denote 5% standard error)

The control shoot length for *Portulaca grandiflora* (PG) plant was 18.56 ± 0.27753 cm for 16th week and gradually increased to 22.64 ± 1.1115 cm for 32th week, while treated plants

showed a declining trend for 16th week with values for PG - PE (2.5g) = 10.98 ± 0.349 cm, PG - PE (5g) = 9.46 ± 0.2125 cm, PG - Nylon 6,6 (2.5g) = 7.58 ± 0.2105 cm, and PG - Nylon 6,6 (5g) = 7.48 ± 0.1835 cm. Similarly, after a span of 32 weeks, in contrast to control, the treated samples of *Portulaca grandiflora* (PG) showed high growth exhibiting values for PG - PE (2.5g) = 12.32 ± 0.7166 cm, PG - PE (5g) = 10.23 ± 0.6615 cm, PG - Nylon 6,6 (2.5g) = 10.56 ± 0.628 cm, and PG - Nylon 6,6 (5g) = 09.01 ± 0.567 cm. For microplastics treated to *Cyanodon dactylon* (L.) (SG) plants, shoot length for the control sample exhibited values of 6.32 ± 0.117 cm for 16th week with increasing trend by 32th week 7.109 ± 0.3445 cm. While the microplastics accumulated samples showed a lessening graphical analysis at 16th week with concentrations for SG - PE (2.5g) = 2.87 ± 0.099 cm, SG - PE (5g) = 1.982 ± 0.0725 cm, SG - Nylon 6,6 (2.5g) = 1.216 ± 0.089 cm, and SG - Nylon 6,6 (5g) = 1.531 ± 0.067 cm. Similarly, after a span of 32 weeks, in contrast, the treated samples of *Cyanodon dactylon* (L.) (SG) showed high growth exhibiting values for SG - PE (2.5g) = 4.83 ± 0.2005 cm, SG - PE (5g) = 3.33 ± 0.1605 cm, SG - Nylon 6,6 (2.5g) = 3.79 ± 0.1945 cm, and SG - Nylon 6,6 (5g) = 2.99 ± 0.1445 cm.

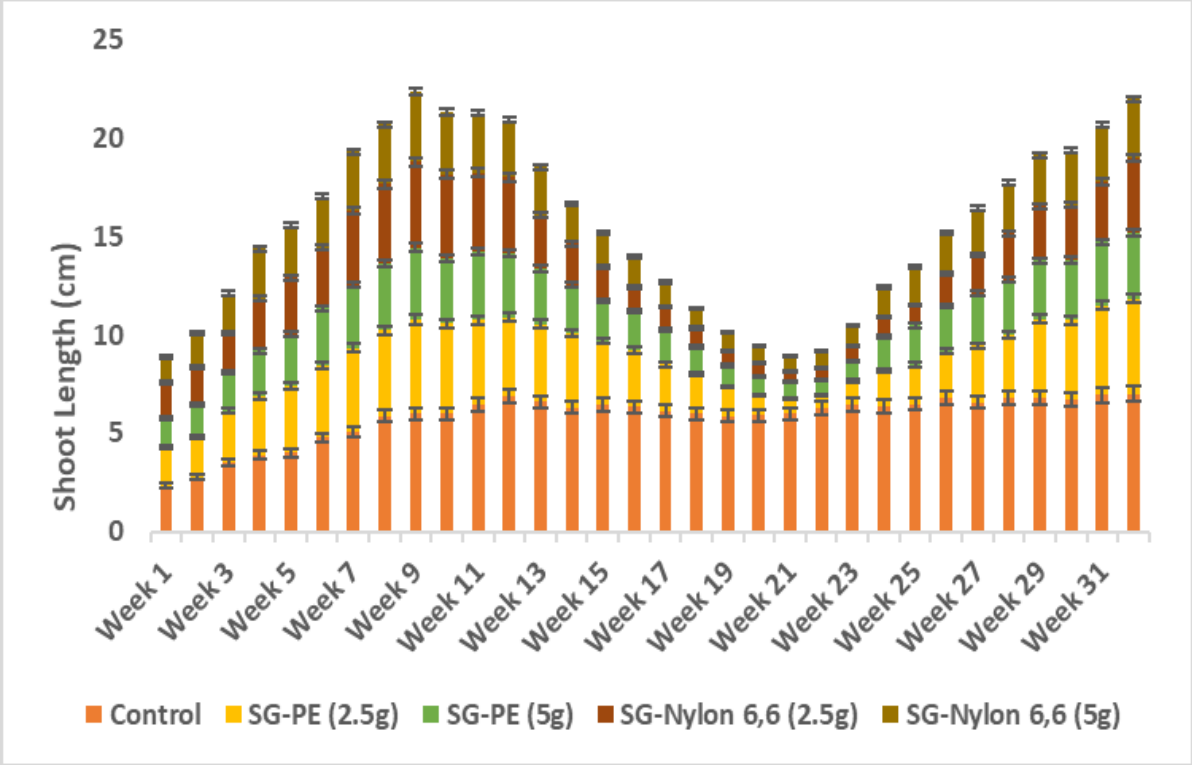


Figure 3.29: Influence of HDPE and Nylon 6,6 microplastics on *Cyanodon dactylon* (L.) (SG) plant samples by observing growth parameters: Shoot length for *Cyanodon dactylon* (L.) (SG); (The values are mean of three triplicates; Error bars denote 5% standard error)

With reference to shoot biomass, in contrast to the control sample, microplastics treated samples had the lowest shoot biomass, after a span of eight weeks, demonstrating that exposure to microplastics had a negative impact. However, in case of *Portulaca grandiflora* (PG) plants, control plant displayed values of 6.87 ± 0.0925 g in 16th week followed by 8.923 ± 0.3705 g by 32th week. For microplastics treated samples, in 16th week, *Portulaca grandiflora* (PG) plants showed values of PG - PE (2.5g) = 1.092 ± 0.05933 g, PG - PE (5g) = 0.724 ± 0.05933 g, PG - Nylon 6,6 (2.5g) = 0.738 ± 0.02967 g, and PG - Nylon 6,6 (5g) = 0.541 ± 0.04267 g. Similarly, after a span of 32 weeks, in contrast, the treated samples of *Portulaca grandiflora* (PG) showed high biomass exhibiting values for PG - PE (2.5g) = 4.333 ± 0.23867 g, PG - PE (5g) = 3.54 ± 0.2205 g, PG - Nylon 6,6 (2.5g) = 3.588 ± 0.20933 g, and PG - Nylon 6,6 (5g) = 3.02 ± 0.189 g.

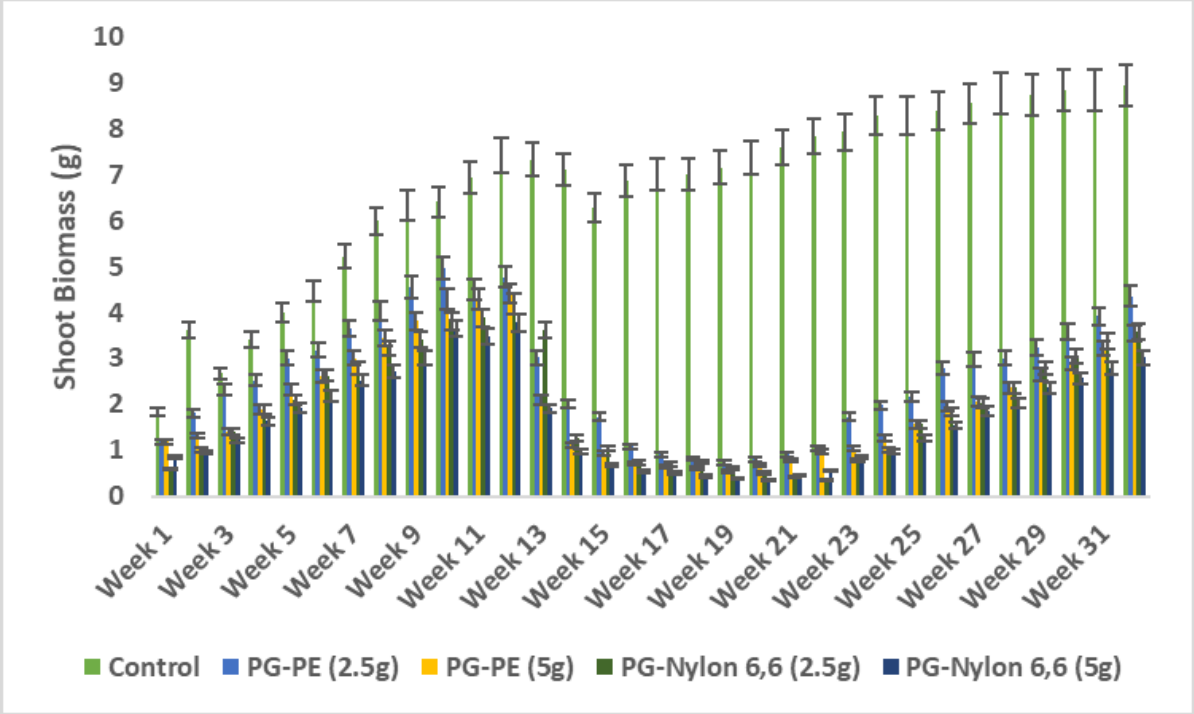


Figure 3.30: Influence of HDPE and Nylon 6,6 microplastics on *Portulaca grandiflora* (PG) plant samples by observing growth parameters: Shoot Biomass for *Portulaca grandiflora* (PG) (The values are mean of three triplicates; Error bars denote 5% standard error)

For *Cyanodon dactylon* (L.) (SG) plants, shoot biomass for control sample at 16th week was 2.078 ± 0.039 g and approximately 2.5667 ± 0.11483 g observed by 32th week. The plants treated with microplastics showed a declining trend in 16th week with respect to control containing shoot biomass of SG - PE (2.5g) = 0.653 ± 0.033 g, SG - PE (5g) = 0.4133 ± 0.02417 g, SG - Nylon 6,6 (2.5g) = 0.6533 ± 0.02967 g, and SG - Nylon 6,6 (5g) = 0.4667 ± 0.02233 g. However, after a span of 32 weeks, in contrast to control, the treated samples of *Cyanodon dactylon* (L.) (SG) showed high growth exhibiting values for SG - PE (2.5g) = 1.76 ± 0.06683 g, SG - PE (5g) = 0.97 ± 0.0535 g, SG - Nylon 6,6 (2.5g) = 0.89 ± 0.06483 g, and SG - Nylon 6,6 (5g) = 0.853 ± 0.04817 g.

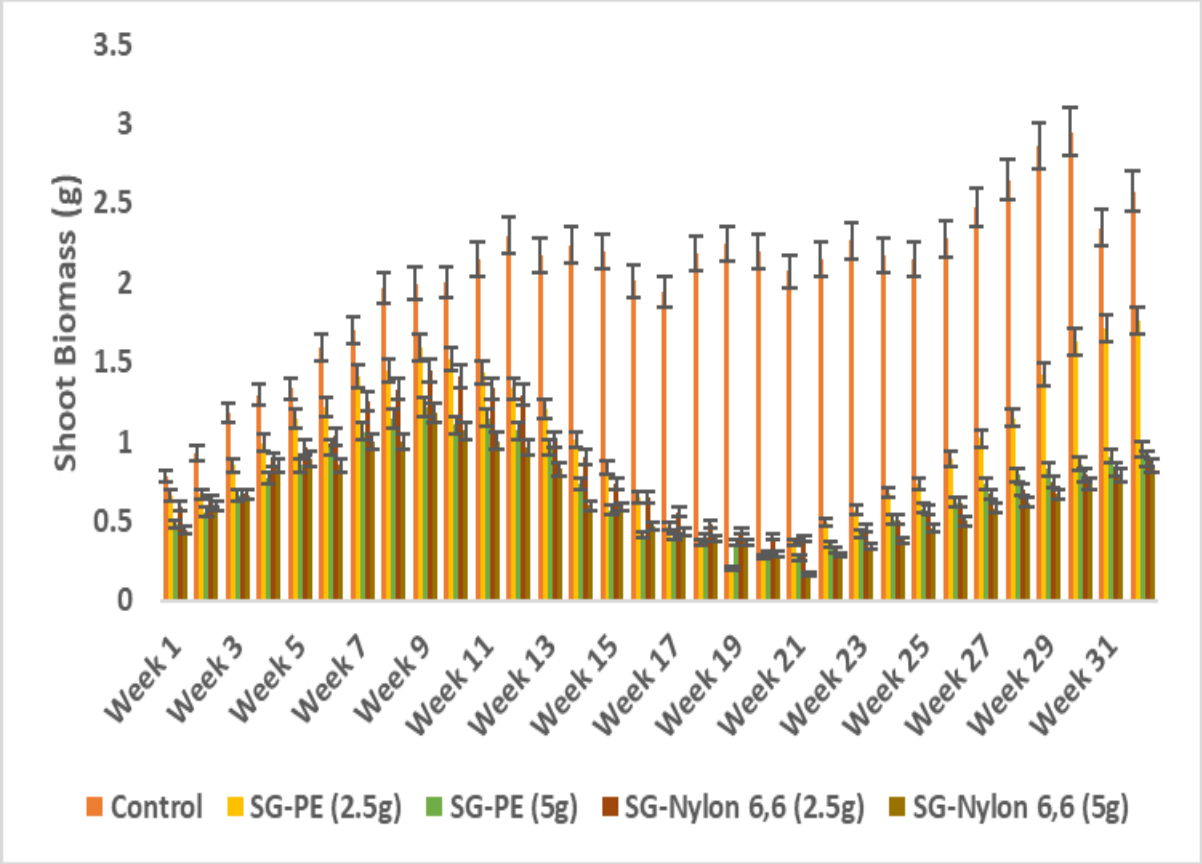


Figure 3.31: Influence of HDPE and Nylon 6,6 microplastics on *Cyanodon dactylon* (L.) (SG) plant samples by observing growth parameters: Shoot Biomass for *Cyanodon dactylon* (L.) (SG); (The values are mean of three triplicates; Error bars denote 5% standard error)

As the shoot length and biomass showed adverse impact on adding microplastics, a similar trend was observed for root length and biomass. *Cyanodon dactylon* (L.) (SG) and *Portulaca grandiflora* (PG) exposed to different concentrations of PE and nylon 6,6 showed a substantial change in root length and root biomass when compared to control plants.

The control root length for *Portulaca grandiflora* (PG) plant was 11.113 ± 0.13875 cm for 16th week and gradually increased to 10.115 ± 0.55575 cm for 32th week, while treated plants showed a declining trend for 16th week with values for PG - PE (2.5g) = 3.78 ± 0.089 cm, PG - PE (5g) = 3.0445 ± 0.07225 cm, PG - Nylon 6,6 (2.5g) = 3.89 ± 0.0445 cm, and PG - Nylon 6,6 (5g) = 3.728 ± 0.064 cm. However, after a span of 32 weeks, in contrast, the

treated samples of *Portulaca grandiflora* (PG) showed high growth exhibiting values for PG - PE (2.5g) = 6.782 ± 0.321 cm, PG - PE (5g) = 5.32 ± 0.316 cm, PG - Nylon 6,6 (2.5g) = 5.28 ± 0.314 cm, and PG - Nylon 6,6 (5g) = 4.67 ± 0.2835 cm.

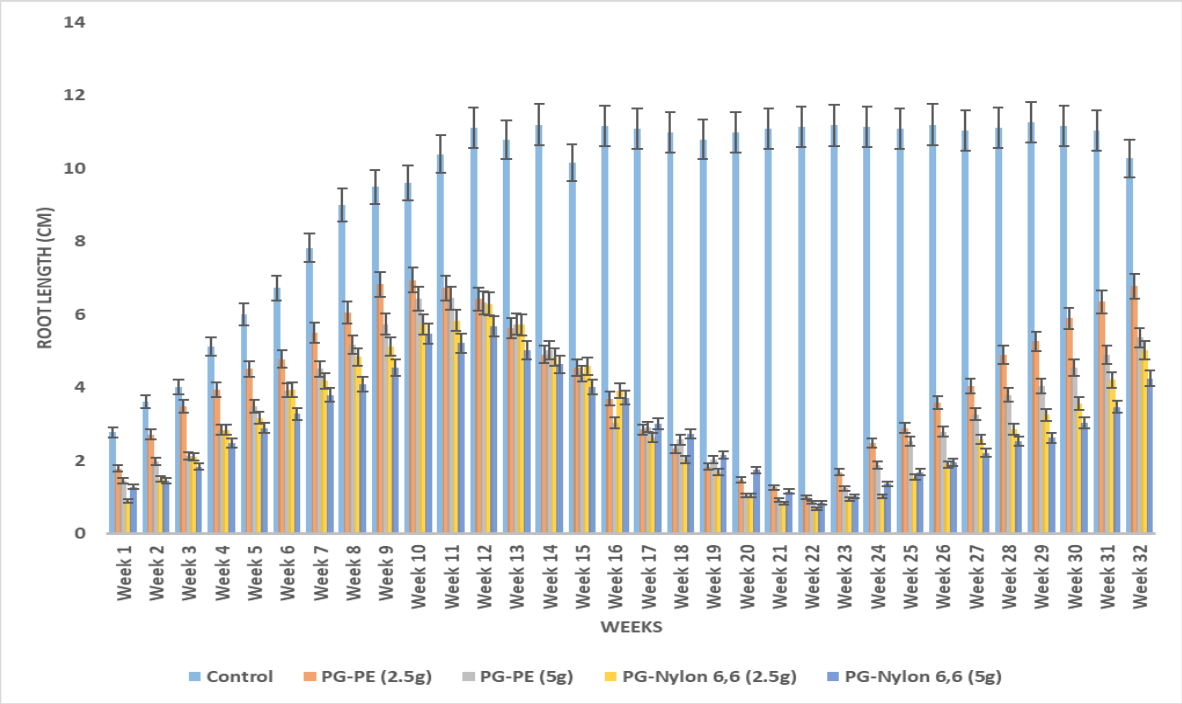


Figure 3.32: Influence of HDPE and Nylon 6,6 microplastics on *Portulaca grandiflora* (PG) plant samples by observing growth parameters: Root Length for *Portulaca grandiflora* (PG) (The values are mean of three triplicates; Error bars denote 5 % standard error)

For microplastics treated to *Cyanodon dactylon* (L.) (SG) plants, root length for the control sample exhibited values of 1.87 ± 0.0585 cm for 16th week with increasing trend by 32 week 3.145 ± 0.17225 cm. While the microplastics accumulated samples showed a lessening graphical analysis at 16th week with concentrations for SG - PE (2.5g) = 0.95 ± 0.0495 cm, SG - PE (5g) = 0.715 ± 0.03625 cm, SG - Nylon 6,6 (2.5g) = 0.85 ± 0.0445 cm, and SG - Nylon 6,6 (5g) = 0.74 ± 0.0335 cm. However, after a span of 32 weeks, in contrast, the treated samples of *Cyanodon dactylon* (L.) (SG) showed high growth exhibiting values for SG - PE (2.5g) = 2.005 ± 0.10025 cm, SG - PE (5g) = 1.705 ± 0.08025 cm, SG - Nylon 6,6 (2.5g) = 2.045 ± 0.09725 cm, and SG - Nylon 6,6 (5g) = 1.425 ± 0.07225 cm.

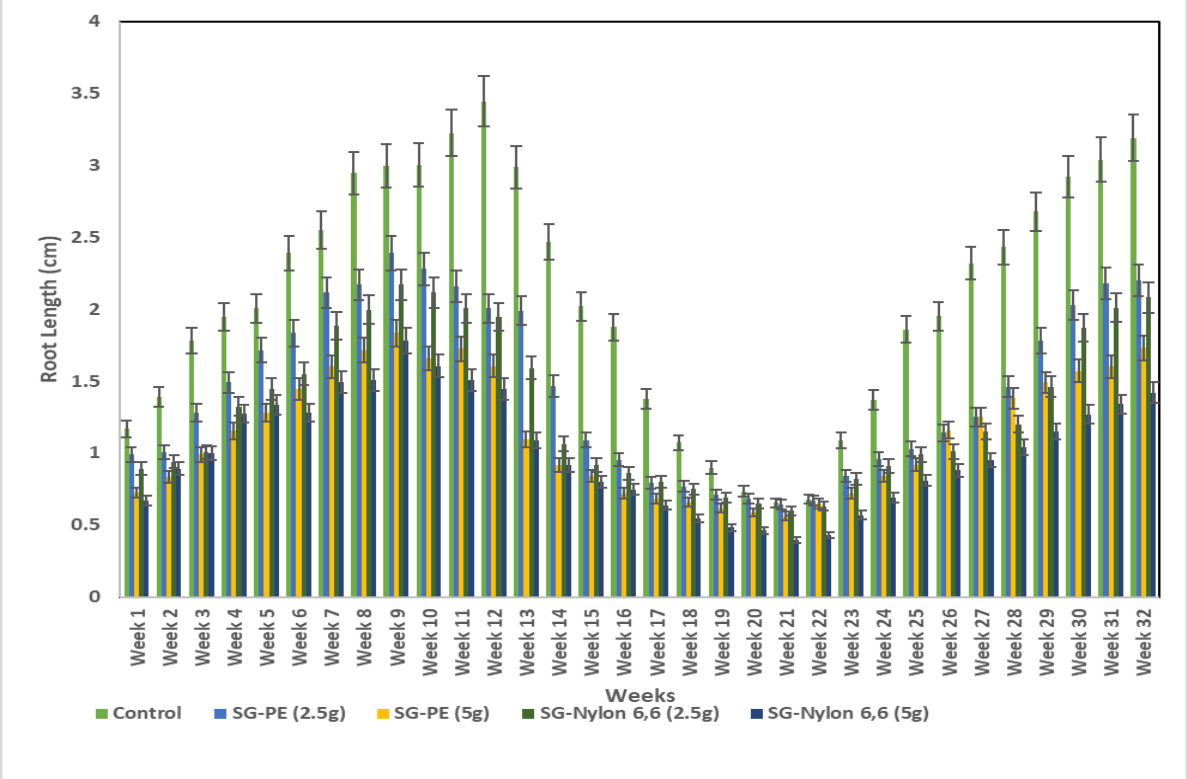


Figure 3.33: Influence of HDPE and Nylon 6,6 microplastics on *Cyanodon dactylon* (L.) (SG) plant samples by observing growth parameters: Root length for *Cyanodon dactylon* (L.) (SG); (The values are mean of three triplicates; Error bars denote 5 % standard error)

With reference to root biomass, in contrast to the control sample, microplastics treated samples had the lowest root biomass, demonstrating that exposure to microplastics had a negative impact. In case of *Portulaca grandiflora* (PG) plants, control plant displayed values of 3.625 ± 0.04625 g in 16th week followed by 3.695 ± 0.18525 g by 32 weeks. For microplastics treated samples, in 16th week, *Portulaca grandiflora* (PG) plants showed values of PG - PE (2.5g) = 1.24333 ± 0.02967 g, PG - PE (5g) = 1.26167 ± 0.02408 g, PG - Nylon 6,6 (2.5g) = 1.1067 ± 0.01483 g, and PG - Nylon 6,6 (5g) = 1.042667 ± 0.02133 g. However, after a span of 32 weeks, in contrast, the treated samples of *Portulaca grandiflora* (PG) showed high biomass exhibiting values for PG - PE (2.5g) = 2.27333 ± 0.23867 g, PG

- PE (5g) = 2.03 ± 0.2205 g, PG - Nylon 6,6 (2.5g) = 1.8667 ± 0.20933 g, and PG - Nylon 6,6 (5g) = 1.48 ± 0.189 g.

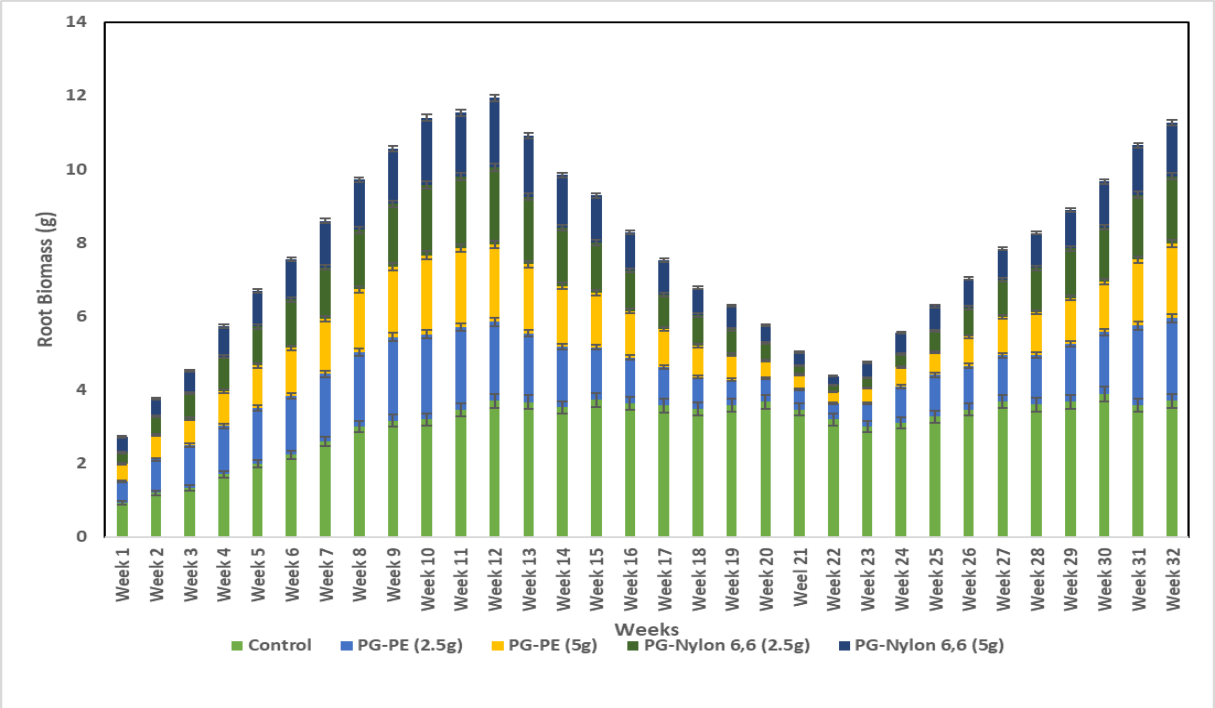


Figure 3.34: Influence of HDPE and Nylon 6,6 microplastics on *Portulaca grandiflora* (PG) plant samples by observing growth parameters: Root Biomass for *Portulaca grandiflora* (PG) (The values are mean of three triplicates; Error bars denote 5% standard error)

For *Cyanodon dactylon* (L.) (SG) plants, root biomass for control sample at 16th week was 1.039 ± 0.0195 g and approximately 1.0833 ± 0.05742 g observed by 32 weeks. The plants treated with microplastics showed a declining trend in 16th week with respect to control containing root biomass of SG - PE (2.5g) = 0.31 ± 0.0165 g, SG - PE (5g) = 0.25167 ± 0.01208 g, SG - Nylon 6,6 (2.5g) = 0.3667 ± 0.01483 g, and SG - Nylon 6,6 (5g) = 0.29333 ± 0.01117 g. However, after a span of 32 weeks, in contrast, the treated samples of *Cyanodon dactylon* (L.) (SG) showed high growth exhibiting values for SG - PE (2.5g) = 0.833 ± 0.42 g, SG - PE (5g) = 0.635 ± 0.02675 g, SG - Nylon 6,6 (2.5g) = 0.72833 ± 0.03242 g, and SG - Nylon 6,6 (5g) = 0.667 ± 0.02408.

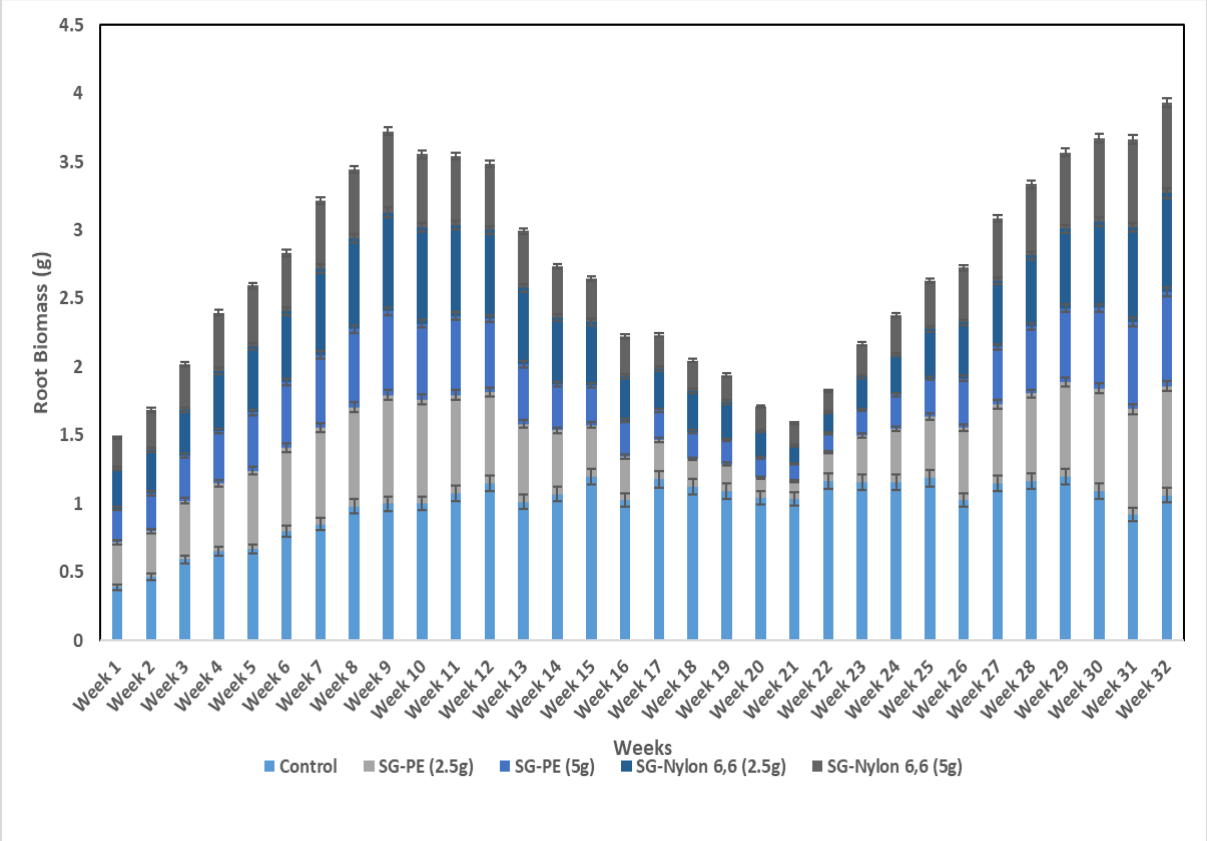
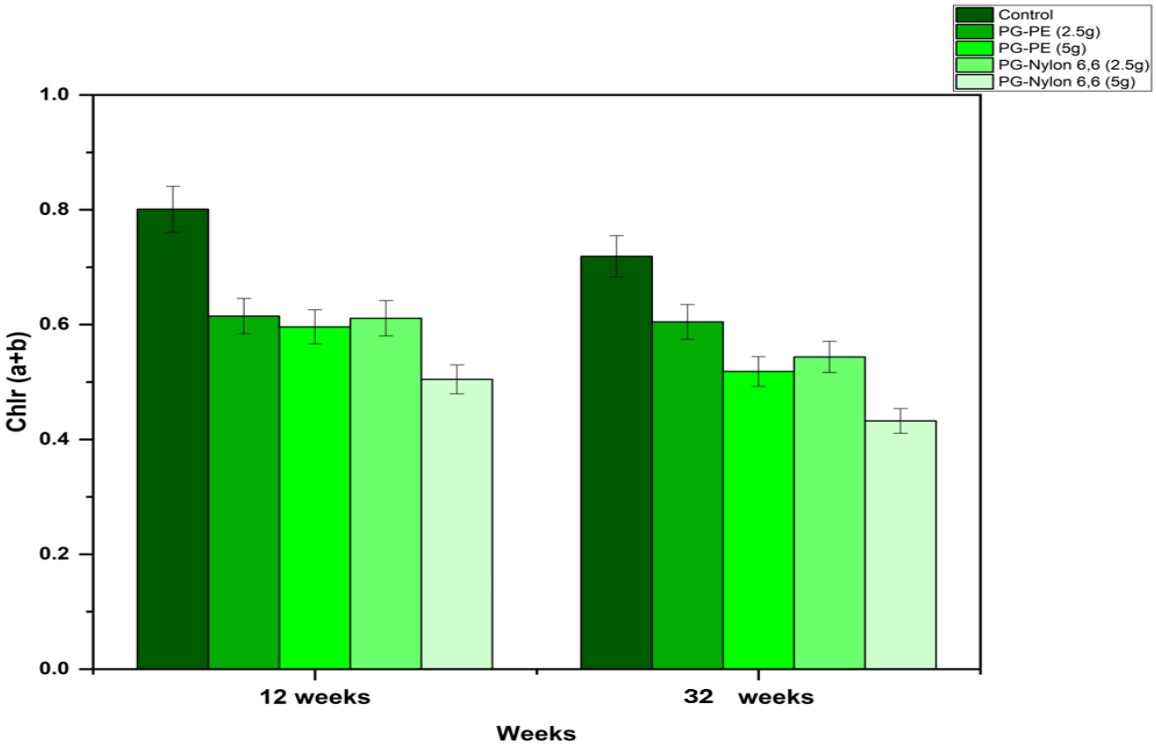


Figure 3.35: Influence of HDPE and Nylon 6,6 microplastics on *Cyanodon dactylon* (L.) (SG) plant samples by observing growth parameters: Root Biomass for *Cyanodon dactylon* (L.) (SG); (The values are mean of three triplicates; Error bars denote 5% standard error)

3.4.7. Chlorophyll content

Chlorophyll a is the major pigment engaged in photosynthesis, while chlorophyll b is an accessory pigment that delivers energy to chlorophyll a (Khaleghi et al., 2013). Total chlorophyll concentration (chl a + chl b) is an important indicator for photosynthetic activity, and fluctuations in this value indicate plant stress. Thus, chlorophyll a was found to be significantly lower in microplastics-treated *Portulaca grandiflora* (PG) and *Cyanodon dactylon* (L.) (SG) plant samples, showing that total chlorophyll concentration in leaves had a larger inhibitory effect on microplastics exposure after 16th week. The control plant observed the content of *chl a* as 3.783 ± 0.23915 in *Portulaca grandiflora* (PG) whereas

9.654 ± 0.5327 was observed in *Cyanodon dactylon* (L.) (SG). On a contrary, microplastics treated samples after 32 weeks exhibited *chl a* value of PG-PE (2.5g) = 4.267 ± 0.41335, PG-PE (5g) = 9.103 ± 0.40515, PG-Nylon 6,6 (2.5g) = 9.234 ± 0.4117, PG-Nylon 6,6 (5g) = 8.1654 ± 0.35827. Similarly, for *Cyanodon dactylon* (L.) (SG) treated plants, *chl a* concentrations were SG-PE (2.5g) = 9.842 ± 0.4821, SG-PE (5g) = 9.203 ± 0.41015, SG-Nylon 6,6 (2.5g) = 8.686 ± 0.3843, SG-Nylon 6,6 (5g) = 8.1654 ± 0.35827.



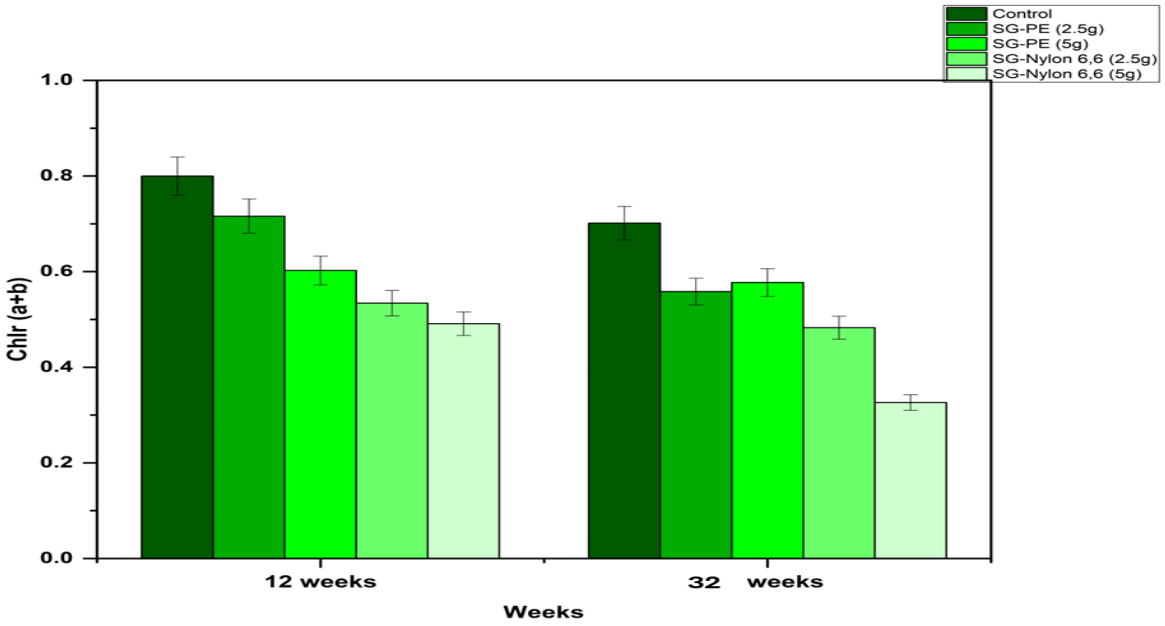


Figure 3.36: Chlorophyll content in leaves of *Portulaca grandiflora* (PG) and *Cyanodon dactylon* (L.) (SG) plant samples exposed to HDPE and Nylon 6,6 microplastics: (a) Chlorophyll content in *Portulaca grandiflora* (PG); (b) Chlorophyll content in *Cyanodon dactylon* (L.) (SG)

3.4.8. Antioxidant Activity

Portulaca grandiflora (PG) showed varied malondialdehyde content with control at 16th week (0.331 ± 0.0454 (nmole g^{-1}fw); PG - PE (2.5g) (0.498 ± 0.0412 nmole g^{-1}fw); PG - PE (5g) (0.567 ± 0.0342 nmole g^{-1}fw); PG - Nylon 6,6 (2.5g) (0.721 ± 0.02485 nmole g^{-1}fw); PG - Nylon 6,6 (5g) (0.791 ± 0.017810 nmole g^{-1}fw). *Cyanodon dactylon* (SG) also showed a similar trend with control (0.231 ± 0.04935 nmole g^{-1}fw); SG - PE (2.5g) (0.398 ± 0.03945 nmole g^{-1}fw); SG - PE (5g) (0.467 ± 0.03375 nmole g^{-1}fw); SG - Nylon 6,6 (2.5g) (0.621 ± 0.0248 nmole g^{-1}fw); SG - Nylon 6,6 (5g) (0.691 ± 0.0195 nmole g^{-1}fw). The findings suggest that incorporating microplastics increases MDA content in plants, causing stress.

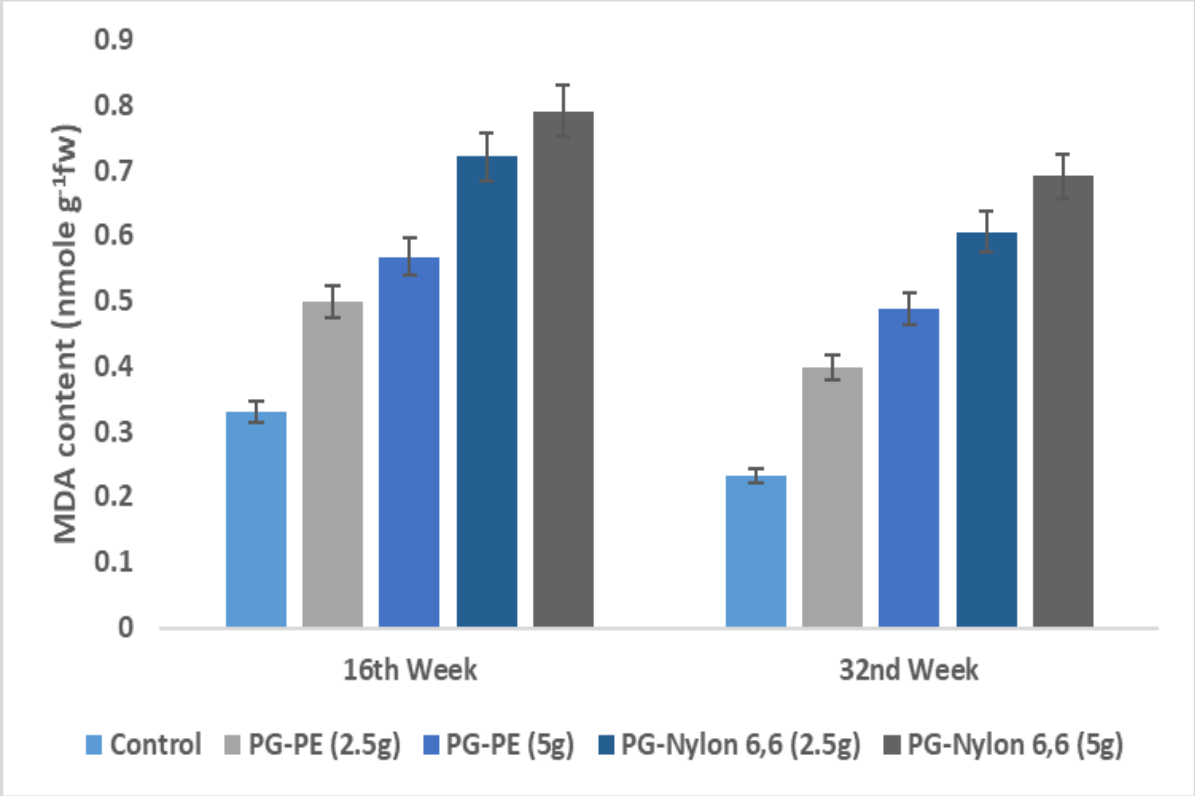


Figure 3.37: MDA content in leaves of *Portulaca grandiflora* (PG) plant samples exposed to HDPE and Nylon 6,6 microplastics: MDA content in *Portulaca grandiflora* (PG); (The values are mean of three triplicates; Error bars denote 5% standard error)

However, after a span of 32 weeks, *Portulaca grandiflora* (PG) showed varied malondialdehyde content with control (0.232 ± 0.0454 (nmole g^{-1}fw); PG - PE (2.5g) (0.398 ± 0.0412 nmole g^{-1}fw); PG - PE (5g) (0.487 ± 0.0342 nmole g^{-1}fw); PG - Nylon 6,6 (2.5g) (0.606 ± 0.02485 nmole g^{-1}fw); PG - Nylon 6,6 (5g) (0.691 ± 0.017810 nmole g^{-1}fw). *Cyanodon dactylon* (SG) also showed a similar trend with control (0.132 ± 0.04935 nmole g^{-1}fw); SG - PE (2.5g) (0.298 ± 0.03945 nmole g^{-1}fw); SG - PE (5g) (0.387 ± 0.03375 nmole g^{-1}fw); SG - Nylon 6,6 (2.5g) (0.496 ± 0.0248 nmole g^{-1}fw); SG - Nylon 6,6 (5g) (0.521 ± 0.0195 nmole g^{-1}fw). The findings suggest that impact of microplastics decreased gradually as shown by MDA content in plants, declining stress.

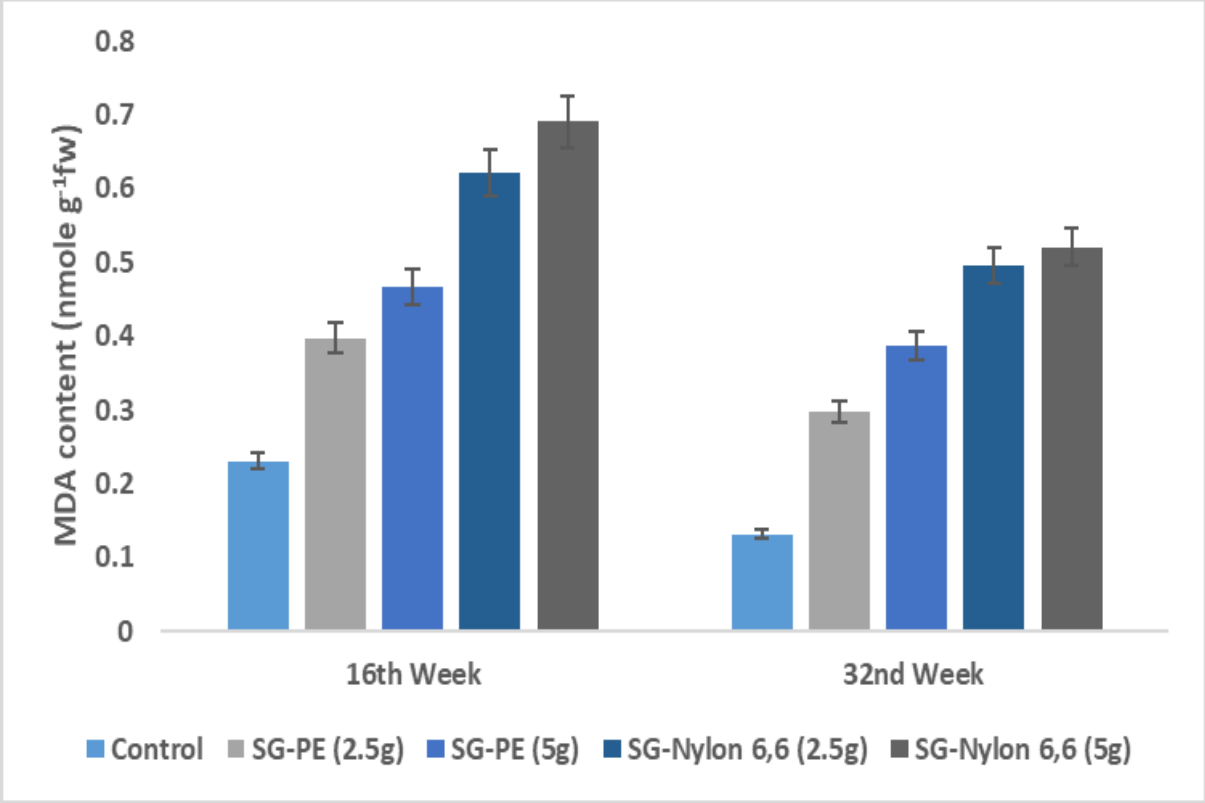


Figure 3.38: MDA content in leaves of *Cyanodon dactylon* (L.) (SG) plant samples exposed to HDPE and Nylon 6,6 microplastics: MDA content in *Cyanodon dactylon* (L.) (SG) (The values are mean of three triplicates; Error bars denote 5% standard error)

After exposure to MPs, the concentration of LOX gradually increased for 16th week, with PG - Nylon 6,6 (5g) showing the highest value of 0.990 ± 0.022 mg $^{-1}$ compared to the control with 0.491 ± 0.04915 mg $^{-1}$. Also, the control for SG treated plants was 0.576 ± 0.0488 mg $^{-1}$, and the highest content of LOX was observed at 0.932 ± 0.0216 mg $^{-1}$ for SG - Nylon 6,6 (5g). These data indicate that LOX is not able to decrease stress in *Portulaca grandiflora* (PG) and *Cyanodon dactylon* (SG) plants infected with microplastics. However, after a span of 32 weeks, PG - Nylon 6,6 (5g) showing the highest value of 0.930 ± 0.022 mg $^{-1}$ compared to the control with 0.481 ± 0.04915 mg $^{-1}$. Also, the control for SG treated plants was 0.436 ± 0.0488 mg $^{-1}$, and the highest content of LOX was observed at 0.902 ± 0.0216 mg $^{-1}$ for SG - Nylon 6,6 (5g).

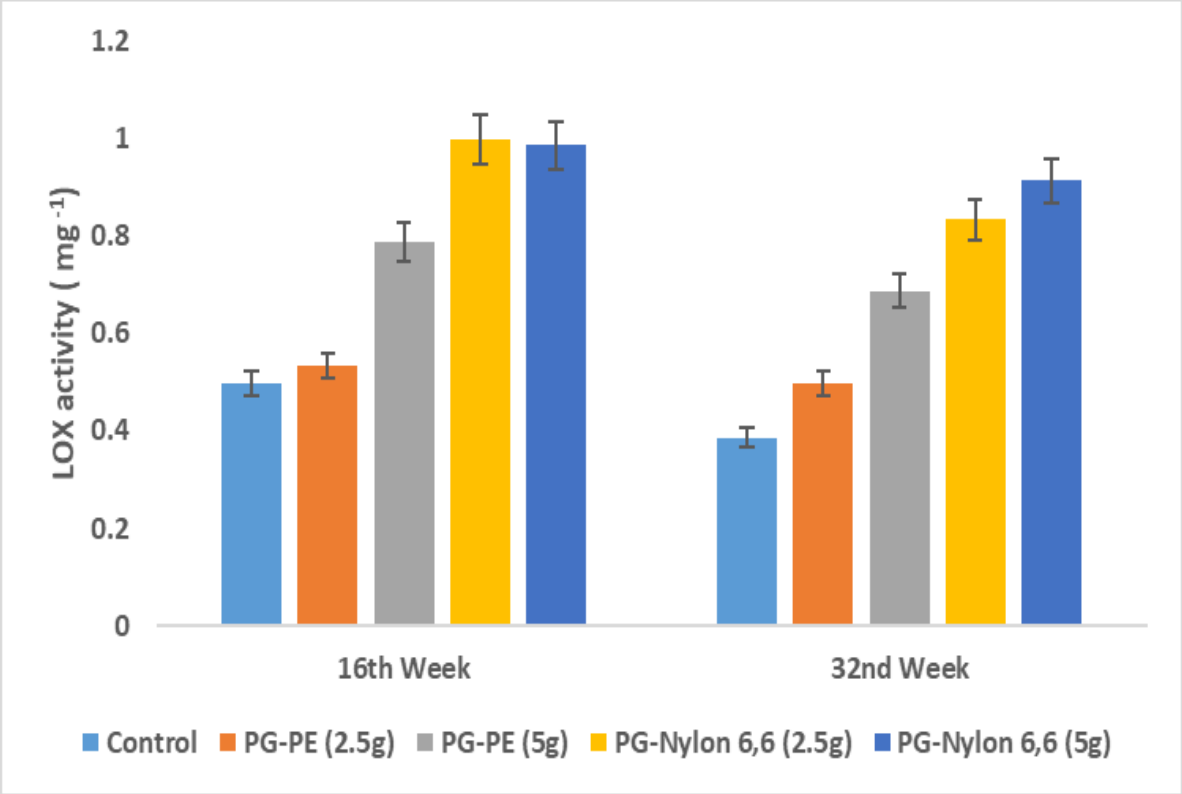


Figure 3.39: LOX content in leaves of *Portulaca grandiflora* (PG) plant samples exposed to HDPE and Nylon 6,6 microplastics: LOX content in *Portulaca grandiflora* (PG); (The values are mean of three triplicates; Error bars denote 5% standard error)

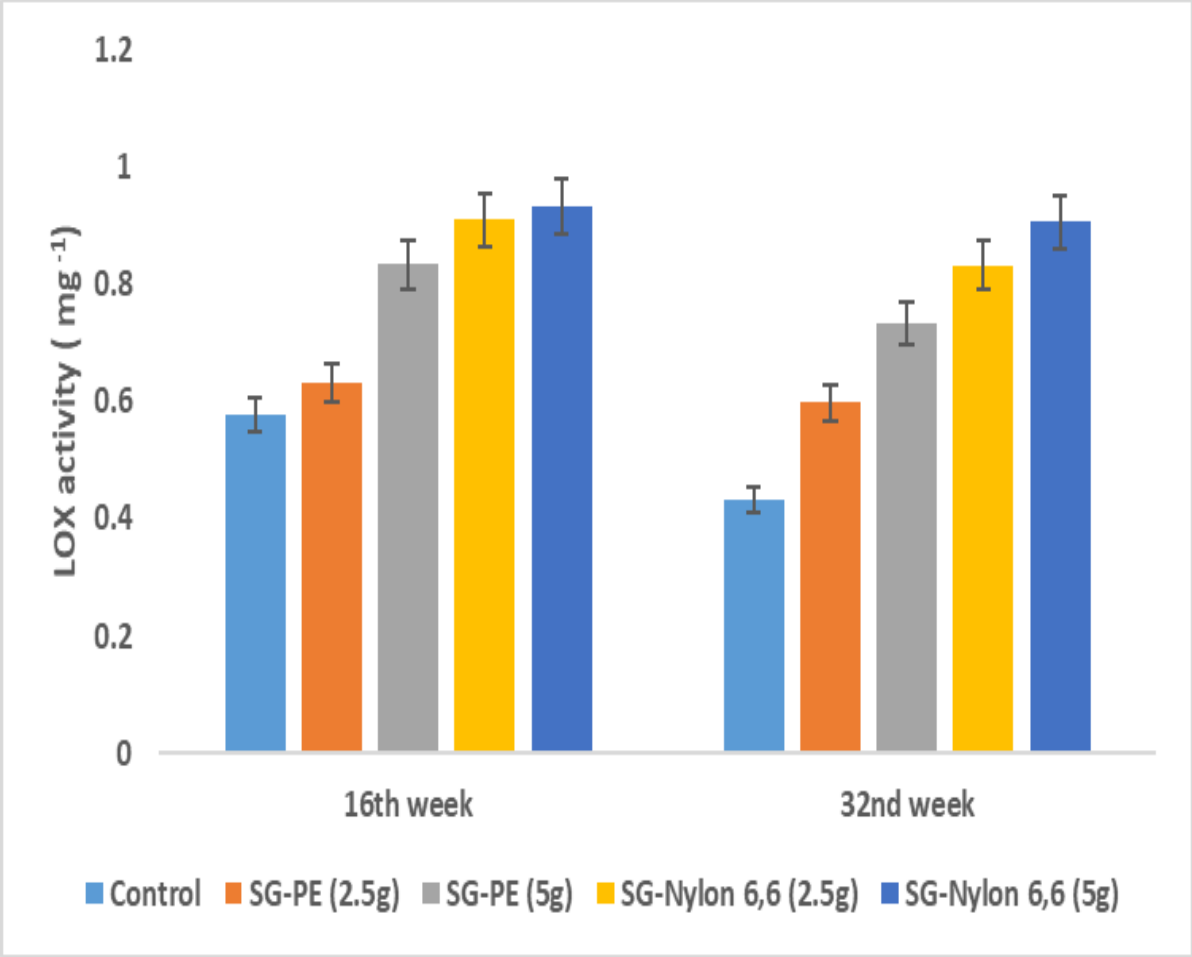


Figure 3.40: LOX content in leaves of *Cyanodon dactylon* (L.) (SG) plant samples exposed to HDPE and Nylon 6,6 microplastics: LOX content in *Cyanodon dactylon* (L.) (SG) (The values are mean of three triplicates; Error bars denote 5 % standard error)

Since the biometrical parameters, chlorophyll content, and antioxidant activity showed an increased content after 32 weeks, there might be a possibility of microplastic degradation during subsequent period of time. Therefore, to confirm the degradation, soil microorganisms were tested for their efficacy in microplastic degradation studies.

CHAPTER 4

SUMMARY, CONCLUSION & FUTURE SCOPE

Chapter-4: Summary, Conclusion & Future Scope

4.1. Summary

The persistent nature, extensive dispersion, and possible negative impacts on ecosystems of microplastics, make them significant environmental hazards. Microplastics' small size makes them readily ingested by a wide range of species, including larger marine creatures and plankton. This could lead to disturbances in the food chain and the build-up of toxic materials. To address the growing problem of plastic pollution, unique microorganisms must be identified and isolated for plastic cleanup. The synergistic action of plants and microbes can play a vital role in the degradation of microplastics and their elimination from the ecosystem. By means of enzymatic activity and metabolic processes, microbes exhibit a unique ability to degrade diverse types of plastics. Through the process of isolating and characterizing these specialized microbes, their applications for effective plastic remediation can be tailored to better understand the mechanisms underlying plastic degradation. This kind of isolation also makes it easier to assess how these bacteria interact with various types of plastic, how adaptable they are to different conditions, and whether or not they could have an impact on ecosystems.

A significant amount of leftover plastic from the extensive usage of plastic film mulch and effluents from surface runoff and industrial activities has accumulated and ultimately formed microplastics (MPs) in agricultural soils. However, it is uncertain how crops would be impacted by microplastics from plastic mulch film. In order to observe the effects of plastic fragments especially microplastics in plant and soil, the growth, physio-biochemical characteristics, and morphology of *Brassica juncea* (mustard plants) exposed to two types of HDPE microplastics – HDPE_MPs and HDPE_beads, were studied. Upon interaction with MPs and beads, the height, biomass, chlorophyll content, phenolic content and proline content of *Brassica juncea* plant were drastically lowered. The photosynthetic content of plants containing HDPE microplastics was significantly less compared to the control. Also, the microscopic images confirmed the translocation of microplastics within roots and leaves of plant thereby confirming its potential for harmful impact on plant. This work emphasizes

that MPs may have higher detrimental impacts for terrestrial ecosystems, which warrants additional investigation in future studies, and offers a fresh insight into the possible effects of MPs with varying biodegradability's on soil-plant systems.

Secondly, to observe the impacts of microplastics on wild plants, a simulated dump yard model was prepared studying impact of two different types of microplastics: high-density polyethylene (HDPE) and nylon-6,6, on tropical wild plants: *Cynodon dactylon* (L.) and *Portulaca grandiflora*. The effects of microplastics on the two plants were evaluated using confocal laser scanning microscopy for morphological inspection, antioxidant activity, chlorophyll content analysis, and biometrical parameters (root and shoot height, biomass output). This study fills in important knowledge gaps regarding the remediation of microplastic-contaminated soil and is the first to document the harmful effects of microplastics on tropical wild plants. The uptake of microplastics by plant parts could be observed through the symplastic and apoplastic pathways. The morphological studies could confirm the mechanism of uptake within plant parts. The accumulation of microplastics within the root and aerial parts of leaves could provide a phytoremediation strategy by phytoextraction of microplastics.

Finally, synergistic plant-microbe interaction was studied to determine the capability of soil microbes in degrading microplastics and also harnessing the plant nutrition and growth. For this, the isolation of soil microorganisms was carried out using metagenomics sequencing to identify the bacterial strain that showed the most degradation efficiency. Also, two other microplastics, PP and PVC, were taken for the research study to ascertain the importance of bacterial isolate for microplastic degradation. To confirm microplastic degradation by the isolated bacterial strain, *Acinetobacter baumannii*, both the microplastics were subjected to FTIR analysis, thermogravimetric study, weight loss % for a span of 50 days and morphological characterization to observe the changes in the structure of microplastics post bacterial inoculation. The results confirmed degradation efficiency in both the microplastics stating the efficacy of microbes for microplastics elimination for sustainable ecosystem.

To better comprehend the finding of this research, future insights on live imaging of microplastics within plant parts could provide substantial information on phytoaccumulation

of microplastics. The buildup of microplastics in soil is the last point that needs more attention. The remediation potential of soil could be determined by analyzing the amount of microplastics left over after accumulation in plants. Overall, it can help in the sustainable remediation of soil containing microplastics in nearby groundwater system for cleaner environment.

4.2. Conclusion

Micro-nano plastic pollution has increased dramatically in recent years, posing a threat to ecosystem diversity. Phytoremediation is a completely natural method for removing MNPs from agricultural ecosystems and restoring soil productivity and plant health. To determine the best use of phytoremediation technologies, it is required to investigate the fate of MNPs, their absorption and migration inside plant parts, trafficking along membranes, tolerance, and behavior in the rhizosphere under various environmental conditions. Plant species, root properties, MNPs size, and environmental variables, all influence MNPs uptake and phytoremediation. Furthermore, advanced phytoremediation tactics concentrating on the use of hyperaccumulator plant species, the use of plant-growth boosting bacteria, omics-based investigations, and genetic engineering using CRISPR-Cas9 technology are effective methodologies for MNPs ecosystem restoration. The use of microbial and enzymatic substances in the breakdown of MNPs has potential to solve this problem on a broad scale. As a result, a thorough understanding of these mechanisms is required for MNPs contaminated soil. Furthermore, the restrictions and future possibilities could be critical in creating cost-effective and environmentally acceptable ways for comprehensive MNPs degradation in terrestrial ecosystems. More research on the impact of MNPs on soil and developing an integrated approach to plant-based technologies for monitoring, assessment, and remediation of MNPs in terrestrial agroecosystems is needed.

4.3. Future Scope

Despite the cost-effective and eco-friendly approach to phytoremediation, challenges still continue in its implementation by the government and commercial sector (Saxena et al.,

2020). Application of phytoremediation towards biotic or climatic factors, food-chain adulteration, and utilization of MNPs pollutants are some constraints in utilizing phytoremediation technology (Gunarathne and Lee, 2019). Furthermore, low budgets by small-scale industries and short-term funds by government agencies limit the application of phytoremediation approaches on a wide scale. Furthermore, the molecular techniques of hyper accumulator species are not evidently described and may take a longer time span to degrade and remove MNPs pollutants. Hence, establishing effective management strategies and low-cost processing technology for decontamination of MNPs pollutants is essential for efficient phytoremediation (Anand et al., 2023).

4.3.1. Policies to eliminate MNPs from environment:

Soil inhabits various microorganisms that have proven to degrade MNPs. Bacterial strains are equipped for biodegrading plastics; however, bacterial consortia or biofilm offer less proficiency in the biodegradation processes, where a few strains are engaged with the disintegration and others are responsible for killing harmful metabolites discharged by the counterparts (Kumar et al., 2018). In spite of the fact that biodegradation by microorganisms appears to offer minimal expense and an eco-accommodating remediation approach, it remains a sluggish cycle for all intents and purposes profoundly reliant upon a few factors (biotic and abiotic):

a. One way to deal with advanced *in-situ* plastic bioremediation is through bio stimulation (with the use of development supplements, manures, normal surfactants, and nanoparticles, alongside the improvement of ecological prerequisites) or potentially bioaugmentation (Fomina and Gadd, 2014).

b. Another methodology incorporates applying current biotechnological procedures, for example, protein or chemical design. The advancement of enhanced microbial consortium, the use of hereditary design, systems science, and the use of hereditarily changed living beings are likely answers for further developing plastic biodegradation processes (Liu et al., 2020).

Notwithstanding, these creative remediation approaches do not tackle plastic contamination and should be joined by viable moderation methodologies that focus on source reduction. This could be accomplished by:

(i) fixing plastic decrease strategies underscoring a diminishing use furthermore;

(ii) streamlining waste executive frameworks;

(iii) looking for economical plastics to guarantee their environmental amicability;

(iv) expanding public awareness on plastic contamination alongside a social shift.

CHAPTER 5

REFERENCES

Chapter-5: References

Advantages And Disadvantages Of Bioremediation, 2018. URL https://agriculturistmusa.com/advantages-and-disadvantages-of-bioremediation/ (accessed 6.20.22).

Ainsworth, E.A., Gillespie, K.M., 2007. Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin–Ciocalteu reagent. Nat Protoc 2, 875–877. https://doi.org/10.1038/nprot.2007.102

Ali, H., Khan, E., Sajad, M.A., 2013. Phytoremediation of heavy metals—Concepts and applications. Chemosphere 91, 869–881. https://doi.org/10.1016/j.chemosphere.2013.01.075

Ali, S., Mehmood, A., Khan, N., 2021. Uptake, Translocation, and Consequences of Nanomaterials on Plant Growth and Stress Adaptation. Journal of Nanomaterials 2021, e6677616. https://doi.org/10.1155/2021/6677616

Amare, G., Desta, B., 2021. Coloured plastic mulches: impact on soil properties and crop productivity. Chemical and Biological Technologies in Agriculture 8, 4. https://doi.org/10.1186/s40538-020-00201-8

Anand, U., Dey, S., Bontempi, E., Ducoli, S., Vethaak, A.D., Dey, A., Federici, S., 2023. Biotechnological methods to remove microplastics: a review. Environ Chem Lett 21, 1787–1810. https://doi.org/10.1007/s10311-022-01552-4

Anyasi, R.O., Atagana, H.I., 2018. Profiling of plants at petroleum contaminated site for phytoremediation. International Journal of Phytoremediation 20, 352–361. https://doi.org/10.1080/15226514.2017.1393386

Arikan, B., Alp, F.N., Ozfidan-Konakci, C., Yildiztugay, E., Turan, M., Cavusoglu, H., 2022. The impacts of nanoplastic toxicity on the accumulation, hormonal regulation and tolerance mechanisms in a potential hyperaccumulator - Lemna minor L. Journal of Hazardous Materials 440, 129692. https://doi.org/10.1016/j.jhazmat.2022.129692

Ariza-Tarazona, M.C., Villarreal-Chiu, J.F., Barbieri, V., Siligardi, C., Cedillo-González, E.I., 2019. New strategy for microplastic degradation: Green photocatalysis using a

protein-based porous N-TiO2 semiconductor. Ceramics International, A selection of papers presented at CIMTEC 2018 45, 9618–9624. https://doi.org/10.1016/j.ceramint.2018.10.208

Auta, H.S., Emenike, C.U., Jayanthi, B., Fauziah, S.H., 2018. Growth kinetics and biodeterioration of polypropylene microplastics by Bacillus sp. and Rhodococcus sp. isolated from mangrove sediment. Marine Pollution Bulletin 127, 15–21. https://doi.org/10.1016/j.marpolbul.2017.11.036

Awasthi, S., Srivastava, P., Singh, P., Tiwary, D., Mishra, P.K., 2017. Biodegradation of thermally treated high-density polyethylene (HDPE) by Klebsiella pneumoniae CH001. 3 Biotech 7, 332. https://doi.org/10.1007/s13205-017-0959-3

Azubuike, C.C., Chikere, C.B., Okpokwasili, G.C., 2016. Bioremediation techniques–classification based on site of application: principles, advantages, limitations and prospects. World J Microbiol Biotechnol 32, 180. https://doi.org/10.1007/s11274-016-2137-x

Banerjee, K., Pramanik, P., Maity, A., Joshi, D.C., Wani, S. H., Krishnan, P., 2019. Chapter 4 - Methods of Using Nanomaterials to Plant Systems and Their Delivery to Plants (Mode of Entry, Uptake, Translocation, Accumulation, Biotransformation and Barriers), in: Ghorbanpour, M., Wani, Shabir Hussain (Eds.), Advances in Phytonanotechnology. Academic Press, pp. 123–152. https://doi.org/10.1016/B978-0-12-815322-2.00005-5

Bansal, M., Santhiya, D., Sharma, J.G., 2024. Mechanistic understanding on the uptake of micro-nano plastics by plants and its phytoremediation. Environ Sci Pollut Res 31, 8354–8368. https://doi.org/10.1007/s11356-023-31680-5

Bansal, M., Santhiya, D., Sharma, J.G., 2023. Exploring the Impacts of HDPE Microplastics on Growth and Physiological Behavior of Brassica juncea (Mustard Plant). Water Air Soil Pollut 234, 520. https://doi.org/10.1007/s11270-023-06548-6

Bansal, M., Santhiya, D., Sharma, J.G., 2021. Behavioural Mechanisms of Microplastic Pollutants in Marine Ecosystem: Challenges and Remediation Measurements. Water Air Soil Pollut 232, 372. https://doi.org/10.1007/s11270-021-05301-1

Baoune, H., Aparicio, J.D., Acuña, A., El Hadj-khelil, A.O., Sanchez, L., Polti, M.A., Alvarez, A., 2019. Effectiveness of the Zea mays-Streptomyces association for the phytoremediation of petroleum hydrocarbons impacted soils. Ecotoxicology and

Environmental Safety 184, 109591. https://doi.org/10.1016/j.ecoenv.2019.109591

Bhattacharya, P., Lin, S., Turner, J.P., Ke, P.C., 2010. Physical Adsorption of Charged Plastic Nanoparticles Affects Algal Photosynthesis. J. Phys. Chem. C 114, 16556–16561. https://doi.org/10.1021/jp1054759

Bian, F., Zhong, Z., Zhang, X., Yang, C., Gai, X., 2020. Bamboo – An untapped plant resource for the phytoremediation of heavy metal contaminated soils. Chemosphere 246, 125750. https://doi.org/10.1016/j.chemosphere.2019.125750

Bläsing, M., Amelung, W., 2018. Plastics in soil: Analytical methods and possible sources. Science of The Total Environment 612, 422–435. https://doi.org/10.1016/j.scitotenv.2017.08.086

Bol, R., Julich, D., Brödlin, D., Siemens, J., Kaiser, K., Dippold, M.A., Spielvogel, S., Zilla, T., Mewes, D., Blanckenburg, F. von, Puhlmann, H., Holzmann, S., Weiler, M., Amelung, W., Lang, F., Kuzyakov, Y., Feger, K.-H., Gottselig, N., Klumpp, E., Missong, A., Winkelmann, C., Uhlig, D., Sohrt, J., Wilpert, K. von, Wu, B., Hagedorn, F., 2016. Dissolved and colloidal phosphorus fluxes in forest ecosystems—an almost blind spot in ecosystem research. Journal of Plant Nutrition and Soil Science 179, 425–438. https://doi.org/10.1002/jpln.201600079

Bosker, T., Bouwman, L.J., Brun, N.R., Behrens, P., Vijver, M.G., 2019. Microplastics accumulate on pores in seed capsule and delay germination and root growth of the terrestrial vascular plant Lepidium sativum. Chemosphere 226, 774–781. https://doi.org/10.1016/j.chemosphere.2019.03.163

Boucher, J., Friot, D., 2017. Primary microplastics in the oceans: A global evaluation of sources. IUCN International Union for Conservation of Nature. https://doi.org/10.2305/IUCN.CH.2017.01.en

Briassoulis, D., Innocenti, F.D., 2017. Standards for Soil Biodegradable Plastics. Soil Degradable Bioplastics for a Sustainable Modern Agriculture 139–168. https://doi.org/10.1007/978-3-662-54130-2_6

Bussières, P., 2014. Estimating the number and size of phloem sieve plate pores using longitudinal views and geometric reconstruction. Sci Rep 4, 4929.

https://doi.org/10.1038/srep04929

Chaudhary, A.K., Chaitanya, K., Vijayakumar, R.P., 2021. Synergistic effect of UV and chemical treatment on biological degradation of Polystyrene by Cephalosporium strain NCIM 1251. Arch Microbiol 203, 2183–2191. https://doi.org/10.1007/s00203-021-02228-3

Cheng, L., Wang, Y., Cai, Z., Liu, J., Yu, B., Zhou, Q., 2017. Phytoremediation of petroleum hydrocarbon-contaminated saline-alkali soil by wild ornamental Iridaceae species. International Journal of Phytoremediation 19, 300–308. https://doi.org/10.1080/15226514.2016.1225282

Chirakkara, R.A., Cameselle, C., Reddy, K.R., 2016. Assessing the applicability of phytoremediation of soils with mixed organic and heavy metal contaminants. Rev Environ Sci Biotechnol 15, 299–326. https://doi.org/10.1007/s11157-016-9391-0

Christian, E., Beniah, I., 2019. Phytoremediation of Polluted Waterbodies with Aquatic plants: Recent Progress on Heavy Metal and Organic Pollutants. https://doi.org/10.20944/preprints201909.0020.v1

Colzi, I., Renna, L., Bianchi, E., Castellani, M.B., Coppi, A., Pignattelli, S., Loppi, S., Gonnelli, C., 2022. Impact of microplastics on growth, photosynthesis and essential elements in Cucurbita pepo L. Journal of Hazardous Materials 423, 127238. https://doi.org/10.1016/j.jhazmat.2021.127238

Corcoran, P.L., Norris, T., Ceccanese, T., Walzak, M.J., Helm, P.A., Marvin, C.H., 2015. Hidden plastics of Lake Ontario, Canada and their potential preservation in the sediment record. Environmental Pollution 204, 17–25. https://doi.org/10.1016/j.envpol.2015.04.009

Corradini, F., Meza, P., Eguiluz, R., Casado, F., Huerta-Lwanga, E., Geissen, V., 2019. Evidence of microplastic accumulation in agricultural soils from sewage sludge disposal. Science of The Total Environment 671, 411–420. https://doi.org/10.1016/j.scitotenv.2019.03.368

Crespy, D., Landfester, K., 2007. Preparation of Nylon 6 Nanoparticles and Nanocapsules by Two Novel Miniemulsion/Solvent Displacement Hybrid Techniques. Macromolecular Chemistry and Physics 208, 457–466. https://doi.org/10.1002/macp.200600487

cycles, T. text provides general information S. assumes no liability for the information

given being complete or correct D. to varying update, Text, S.C.D.M. up-to-D.D.T.R. in the, n.d. Topic: Global plastic waste [WWW Document]. Statista. URL https://www.statista.com/topics/5401/global-plastic-waste/ (accessed 5.17.21).

Dahl, M., Bergman, S., Björk, M., Diaz-Almela, E., Granberg, M., Gullström, M., Leiva-Dueñas, C., Magnusson, K., Marco-Méndez, C., Piñeiro-Juncal, N., Mateo, M.Á., 2021. A temporal record of microplastic pollution in Mediterranean seagrass soils. Environmental Pollution 273, 116451. https://doi.org/10.1016/j.envpol.2021.116451

DalCorso, G., Fasani, E., Manara, A., Visioli, G., Furini, A., 2019. Heavy Metal Pollutions: State of the Art and Innovation in Phytoremediation. International Journal of Molecular Sciences 20, 3412. https://doi.org/10.3390/ijms20143412

Dave, S., Das, J., 2021. Chapter 13 - Role of microbial enzymes for biodegradation and bioremediation of environmental pollutants: challenges and future prospects, in: Saxena, G., Kumar, V., Shah, M.P. (Eds.), Bioremediation for Environmental Sustainability. Elsevier, pp. 325–346. https://doi.org/10.1016/B978-0-12-820524-2.00013-4

De Gisi, S., Lofrano, G., Grassi, M., Notarnicola, M., 2016. Characteristics and adsorption capacities of low-cost sorbents for wastewater treatment: A review. Sustainable Materials and Technologies 9, 10–40. https://doi.org/10.1016/j.susmat.2016.06.002

de Souza Machado, A.A., Kloas, W., Zarfl, C., Hempel, S., Rillig, M.C., 2018. Microplastics as an emerging threat to terrestrial ecosystems. Global Change Biology 24, 1405–1416. https://doi.org/10.1111/gcb.14020

de Souza Machado, A.A., Lau, C.W., Kloas, W., Bergmann, J., Bachelier, J.B., Faltin, E., Becker, R., Görlich, A.S., Rillig, M.C., 2019. Microplastics Can Change Soil Properties and Affect Plant Performance. Environ. Sci. Technol. 53, 6044–6052. https://doi.org/10.1021/acs.est.9b01339

Ding, L., Zhang, S., Wang, X., Yang, X., Zhang, C., Qi, Y., Guo, X., 2020. The occurrence and distribution characteristics of microplastics in the agricultural soils of Shaanxi Province, in north-western China. Science of The Total Environment 720, 137525. https://doi.org/10.1016/j.scitotenv.2020.137525

Diwan, H., Ahmad, A., Iqbal, M., 2008. Genotypic Variation in the Phytoremediation

Potential of Indian Mustard for Chromium. Environmental Management 41, 734–741. https://doi.org/10.1007/s00267-007-9020-3

Doderer, A., Kokkelink, I., van der Veen, S., Valk, B.E., Schram, AndréW., Douma, A.C., 1992. Purification and characterization of two lipoxygenase isoenzymes from germinating barley. Biochimica et Biophysica Acta (BBA) - Protein Structure and Molecular Enzymology 1120, 97–104. https://doi.org/10.1016/0167-4838(92)90429-H

Dovidat, L.C., Brinkmann, B.W., Vijver, M.G., Bosker, T., 2020. Plastic particles adsorb to the roots of freshwater vascular plant Spirodela polyrhiza but do not impair growth. Limnology and Oceanography Letters 5, 37–45. https://doi.org/10.1002/lol2.10118

Dris, R., Gasperi, J., Rocher, V., Saad, M., Renault, N., Tassin, B., 2015. Microplastic contamination in an urban area: a case study in Greater Paris. Environ. Chem. 12, 592–599. https://doi.org/10.1071/EN14167

Ebere, E.C., Wirnkor, V.A., Ngozi, V.E., 2019. Uptake of Microplastics by Plant: a Reason to Worry or to be Happy? 12.

Environment, T., Commons, C., Environment, T., 2018. A simple method for the extraction and identification of light density microplastics from soil Zhang, S., Yang, X., Gertsen, H., Peters, P., Salánki, T., & Geissen, V. 1056–1065.

Enyoh, C.E., Verla, A.W., Verla, E.N., Ibe, F.C., Amaobi, C.E., 2019. Airborne microplastics: a review study on method for analysis, occurrence, movement and risks. Environ Monit Assess 191, 668. https://doi.org/10.1007/s10661-019-7842-0

Etxeberria, E., Gonzalez, P., Baroja-Fernández, E., Romero, J.P., 2006. Fluid Phase Endocytic Uptake of Artificial Nano-Spheres and Fluorescent Quantum Dots by Sycamore Cultured Cells. Plant Signaling & Behavior 1, 196–200. https://doi.org/10.4161/psb.1.4.3142

Fasani, E., Manara, A., Martini, F., Furini, A., DalCorso, G., 2018. The potential of genetic engineering of plants for the remediation of soils contaminated with heavy metals. Plant, Cell & Environment 41, 1201–1232. https://doi.org/10.1111/pce.12963

Feng, N.-X., Yu, J., Zhao, H.-M., Cheng, Y.-T., Mo, C.-H., Cai, Q.-Y., Li, Y.-W., Li, H., Wong, M.-H., 2017. Efficient phytoremediation of organic contaminants in soils using

plant–endophyte partnerships. Science of The Total Environment 583, 352–368. https://doi.org/10.1016/j.scitotenv.2017.01.075

Feng, S., Hao Ngo, H., Guo, W., Woong Chang, S., Duc Nguyen, D., Cheng, D., Varjani, S., Lei, Z., Liu, Y., 2021. Roles and applications of enzymes for resistant pollutants removal in wastewater treatment. Bioresource Technology 335, 125278. https://doi.org/10.1016/j.biortech.2021.125278

Fomina, M., Gadd, G.M., 2014. Biosorption: current perspectives on concept, definition and application. Bioresource Technology, Special Issue on Biosorption 160, 3–14. https://doi.org/10.1016/j.biortech.2013.12.102

Fred-Ahmadu, O.H., Bhagwat, G., Oluyoye, I., Benson, N.U., Ayejuyo, O.O., Palanisami, T., 2020. Interaction of chemical contaminants with microplastics: Principles and perspectives. Science of The Total Environment 706, 135978. https://doi.org/10.1016/j.scitotenv.2019.135978

Free, C.M., Jensen, O.P., Mason, S.A., Eriksen, M., Williamson, N.J., Boldgiv, B., 2014. High-levels of microplastic pollution in a large, remote, mountain lake. Marine Pollution Bulletin 85, 156–163. https://doi.org/10.1016/j.marpolbul.2014.06.001

Galloway, T.S., Cole, M., Lewis, C., 2017. Interactions of microplastic debris throughout the marine ecosystem. Nature Ecology & Evolution 1, 1–8. https://doi.org/10.1038/s41559-017-0116

Gao, M., Liu, Y., Dong, Y., Song, Z., 2021. Effect of polyethylene particles on dibutyl phthalate toxicity in lettuce (Lactuca sativa L.). Journal of Hazardous Materials 401, 123422. https://doi.org/10.1016/j.jhazmat.2020.123422

Gasperi, J., Wright, S.L., Dris, R., Collard, F., Mandin, C., Guerrouache, M., Langlois, V., Kelly, F.J., Tassin, B., 2018. Microplastics in air: Are we breathing it in? Current Opinion in Environmental Science & Health, Micro and Nanoplastics Edited by Dr. Teresa A.P. Rocha-Santos 1, 1–5. https://doi.org/10.1016/j.coesh.2017.10.002

Geisler-Lee, J., Wang, Q., Yao, Y., Zhang, W., Geisler, M., Li, K., Huang, Y., Chen, Y., Kolmakov, A., Ma, X., 2013. Phytotoxicity, accumulation and transport of silver nanoparticles by Arabidopsis thaliana. Nanotoxicology 7, 323–337.

https://doi.org/10.3109/17435390.2012.658094

Gong, X., Shi, G., Zou, D., Wu, Z., Qin, P., Yang, Y., Hu, X., Zhou, L., Zhou, Y., 2023. Micro- and nano-plastics pollution and its potential remediation pathway by phytoremediation. Planta 257, 35. https://doi.org/10.1007/s00425-023-04069-4

Goswami, S., Das, S., 2015. A Study on Cadmium Phytoremediation Potential of Indian Mustard, Brassica juncea. International Journal of Phytoremediation 17, 583–588. https://doi.org/10.1080/15226514.2014.935289

Gravouil, K., Ferru-Clément, R., Colas, S., Helye, R., Kadri, L., Bourdeau, L., Moumen, B., Mercier, A., Ferreira, T., 2017. Transcriptomics and Lipidomics of the Environmental Strain Rhodococcus ruber Point out Consumption Pathways and Potential Metabolic Bottlenecks for Polyethylene Degradation. Environ. Sci. Technol. 51, 5172–5181. https://doi.org/10.1021/acs.est.7b00846

Gunarathne, A.D.N., Lee, K.-H., 2019. Environmental and managerial information for cleaner production strategies: An environmental management development perspective. Journal of Cleaner Production 237, 117849. https://doi.org/10.1016/j.jclepro.2019.117849

Guo, J.-J., Huang, X.-P., Xiang, L., Wang, Y.-Z., Li, Y.-W., Li, H., Cai, Q.-Y., Mo, C.-H., Wong, M.-H., 2020a. Source, migration and toxicology of microplastics in soil. Environment International 137, 105263. https://doi.org/10.1016/j.envint.2019.105263

Guo, J.-J., Huang, X.-P., Xiang, L., Wang, Y.-Z., Li, Y.-W., Li, H., Cai, Q.-Y., Mo, C.-H., Wong, M.-H., 2020b. Source, migration and toxicology of microplastics in soil. Environment International 137, 105263. https://doi.org/10.1016/j.envint.2019.105263

Habib, S., Iruthayam, A., Abd Shukor, M.Y., Alias, S.A., Smykla, J., Yasid, N.A., 2020. Biodeterioration of Untreated Polypropylene Microplastic Particles by Antarctic Bacteria. Polymers 12, 2616. https://doi.org/10.3390/polym12112616

Haegerbaeumer, A., Mueller, M.-T., Fueser, H., Traunspurger, W., 2019. Impacts of Micro- and Nano-Sized Plastic Particles on Benthic Invertebrates: A Literature Review and Gap Analysis. Frontiers in Environmental Science 7.

He, S., Jia, M., Xiang, Y., Song, B., Xiong, W., Cao, J., Peng, H., Yang, Y., Wang, W., Yang, Z., Zeng, G., 2022. Biofilm on microplastics in aqueous environment:

Physicochemical properties and environmental implications. Journal of Hazardous Materials 424, 127286. https://doi.org/10.1016/j.jhazmat.2021.127286

Heath, R.L., Packer, L., 2022. Reprint of: Photoperoxidation in Isolated Chloroplasts I. Kinetics and Stoichiometry of Fatty Acid Peroxidation. Archives of Biochemistry and Biophysics, Celebrating Eighty Years of Archives of Biochemistry and Biophysics 726, 109248. https://doi.org/10.1016/j.abb.2022.109248

Hermabessiere, L., Dehaut, A., Paul-Pont, I., Lacroix, C., Jezequel, R., Soudant, P., Duflos, G., 2017. Occurrence and effects of plastic additives on marine environments and organisms: A review. Chemosphere 182, 781–793. https://doi.org/10.1016/j.chemosphere.2017.05.096

Hodson, M.E., Duffus-Hodson, C.A., Clark, A., Prendergast-Miller, M.T., Thorpe, K.L., 2017. Plastic Bag Derived-Microplastics as a Vector for Metal Exposure in Terrestrial Invertebrates. Environ. Sci. Technol. 51, 4714–4721. https://doi.org/10.1021/acs.est.7b00635

Horton, Alice A., Walton, A., Spurgeon, D.J., Lahive, E., Svendsen, C., 2017. Microplastics in freshwater and terrestrial environments: Evaluating the current understanding to identify the knowledge gaps and future research priorities. Science of The Total Environment 586, 127–141. https://doi.org/10.1016/j.scitotenv.2017.01.190

Horton, Alice A, Walton, A., Spurgeon, D.J., Lahive, E., Svendsen, C., 2017. Microplastics in freshwater and terrestrial environments: Evaluating the current understanding to identify the knowledge gaps and future research priorities. Science of The Total Environment 586, 127–141. https://doi.org/10.1016/j.scitotenv.2017.01.190

Huang, D., Chen, H., Shen, M., Tao, J., Chen, S., Yin, L., Zhou, W., Wang, X., Xiao, R., Li, R., 2022. Recent advances on the transport of microplastics/nanoplastics in abiotic and biotic compartments. Journal of Hazardous Materials 438, 129515. https://doi.org/10.1016/j.jhazmat.2022.129515

Huerta Lwanga, E., Gertsen, H., Gooren, H., Peters, P., Salánki, T., van der Ploeg, M., Besseling, E., Koelmans, A.A., Geissen, V., 2017. Incorporation of microplastics from litter into burrows of Lumbricus terrestris. Environmental Pollution 220, 523–531.

https://doi.org/10.1016/j.envpol.2016.09.096

Hussain, F., Hussain, I., Khan, A.H.A., Muhammad, Y.S., Iqbal, M., Soja, G., Reichenauer, T.G., Zeshan, Yousaf, S., 2018. Combined application of biochar, compost, and bacterial consortia with Italian ryegrass enhanced phytoremediation of petroleum hydrocarbon contaminated soil. Environmental and Experimental Botany 153, 80–88. https://doi.org/10.1016/j.envexpbot.2018.05.012

Iqbal, A., Mukherjee, M., Rashid, J., Khan, S.A., Ali, M.A., Arshad, M., 2019. Development of plant-microbe phytoremediation system for petroleum hydrocarbon degradation: An insight from alkb gene expression and phytotoxicity analysis. Science of The Total Environment 671, 696–704. https://doi.org/10.1016/j.scitotenv.2019.03.331

Iqbal, S., Xu, J., Allen, S.D., Khan, S., Nadir, S., Arif, M.S., Yasmeen, T., 2020. Unraveling consequences of soil micro- and nano-plastic pollution on soil-plant system: Implications for nitrogen (N) cycling and soil microbial activity. Chemosphere 260, 127578. https://doi.org/10.1016/j.chemosphere.2020.127578

Jeevanantham, S., Saravanan, A., Hemavathy, R.V., Kumar, P.S., Yaashikaa, P.R., Yuvaraj, D., 2019. Removal of toxic pollutants from water environment by phytoremediation: A survey on application and future prospects. Environmental Technology & Innovation 13, 264–276. https://doi.org/10.1016/j.eti.2018.12.007

Jeon, J.-M., Park, S.-J., Choi, T.-R., Park, J.-H., Yang, Y.-H., Yoon, J.-J., 2021. Biodegradation of polyethylene and polypropylene by Lysinibacillus species JJY0216 isolated from soil grove. Polymer Degradation and Stability 191, 109662. https://doi.org/10.1016/j.polymdegradstab.2021.109662

Jeyavani, J., Sibiya, A., Shanthini, S., Ravi, C., Vijayakumar, S., Rajan, D.K., Vaseeharan, B., 2021. A Review on Aquatic Impacts of Microplastics and Its Bioremediation Aspects. Curr Pollution Rep 7, 286–299. https://doi.org/10.1007/s40726-021-00188-2

Jiang, X., Chen, H., Liao, Y., Ye, Z., Li, M., Klobučar, G., 2019. Ecotoxicity and genotoxicity of polystyrene microplastics on higher plant Vicia faba. Environmental Pollution 250, 831–838. https://doi.org/10.1016/j.envpol.2019.04.055

Junhao, C., Xining, Z., Xiaodong, G., Li, Z., Qi, H., Siddique, K.H.M., 2021. Extraction

and identification methods of microplastics and nanoplastics in agricultural soil: A review. Journal of Environmental Management 294, 112997. https://doi.org/10.1016/j.jenvman.2021.112997

Kader, M.A., Senge, M., Mojid, M.A., Ito, K., 2017. Recent advances in mulching materials and methods for modifying soil environment. Soil and Tillage Research 168, 155–166. https://doi.org/10.1016/j.still.2017.01.001

Kang, B.R., Kim, S.B., Song, H.A., Lee, T.K., 2019. Accelerating the Biodegradation of High-Density Polyethylene (HDPE) Using Bjerkandera adusta TBB-03 and Lignocellulose Substrates. Microorganisms 7, 304. https://doi.org/10.3390/microorganisms7090304

Karakolis, E.G., Nguyen, B., You, J.B., Rochman, C.M., Sinton, D., 2019. Fluorescent Dyes for Visualizing Microplastic Particles and Fibers in Laboratory-Based Studies. Environ. Sci. Technol. Lett. 6, 334–340. https://doi.org/10.1021/acs.estlett.9b00241

Kasirajan, S., Ngouajio, M., 2012. Polyethylene and biodegradable mulches for agricultural applications: a review. Agronomy for Sustainable Development 32, 501–529. https://doi.org/10.1007/s13593-011-0068-3

Kershaw, P.J., Rochman, C.M., 2015. Sources, fate and effects of microplastics in the marine environment: part 2 of a global assessment. Reports and studies - IMO/FAO/Unesco-IOC/WMO/IAEA/UN/UNEP Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection (GESAMP) eng no. 93.

Khaleghi, E., Arzani, K., Moallemi, N., Barzegar, M., 2013. Evaluation of Chlorophyll Content and Chlorophyll Fluorescence Parameters and Relationships between Chlorophyll a, b and Chlorophyll Content Index under Water Stress in Olea europaea cv. `Dezful'.

Khan, I., Awan, S.A., Rizwan, M., Hassan, Z.U., Akram, M.A., Tariq, R., Brestic, M., Xie, W., 2022. Nanoparticle's uptake and translocation mechanisms in plants via seed priming, foliar treatment, and root exposure: a review. Environ Sci Pollut Res 29, 89823–89833. https://doi.org/10.1007/s11356-022-23945-2

Khandare, S.D., Chaudhary, D.R., Jha, B., 2021a. Bioremediation of polyvinyl chloride (PVC) films by marine bacteria. Marine Pollution Bulletin 169, 112566. https://doi.org/10.1016/j.marpolbul.2021.112566

Khandare, S.D., Chaudhary, D.R., Jha, B., 2021b. Marine bacterial biodegradation of low-density polyethylene (LDPE) plastic. Biodegradation 32, 127–143. https://doi.org/10.1007/s10532-021-09927-0

Kibblewhite, M.G., 2018. Contamination of agricultural soil by urban and peri-urban highways: An overlooked priority? Environmental Pollution 242, 1331–1336. https://doi.org/10.1016/j.envpol.2018.08.008

Kowalczyk, A., Chyc, M., Ryszka, P., Latowski, D., 2016. Achromobacter xylosoxidans as a new microorganism strain colonizing high-density polyethylene as a key step to its biodegradation. Environ Sci Pollut Res 23, 11349–11356. https://doi.org/10.1007/s11356-016-6563-y

Kreider, M.L., Panko, J.M., McAtee, B.L., Sweet, L.I., Finley, B.L., 2010. Physical and chemical characterization of tire-related particles: Comparison of particles generated using different methodologies. Science of The Total Environment 408, 652–659. https://doi.org/10.1016/j.scitotenv.2009.10.016

Kumar, V., Shahi, S.K., Singh, S., 2018. Bioremediation: An Eco-sustainable Approach for Restoration of Contaminated Sites, in: Singh, J., Sharma, D., Kumar, G., Sharma, N.R. (Eds.), Microbial Bioprospecting for Sustainable Development. Springer, Singapore, pp. 115–136. https://doi.org/10.1007/978-981-13-0053-0_6

Kumar Yadav, K., Gupta, N., Kumar, A., Reece, L.M., Singh, N., Rezania, S., Ahmad Khan, S., 2018. Mechanistic understanding and holistic approach of phytoremediation: A review on application and future prospects. Ecological Engineering 120, 274–298. https://doi.org/10.1016/j.ecoleng.2018.05.039

Lambert, S., Wagner, M., 2017. Environmental performance of bio-based and biodegradable plastics: the road ahead. Chem. Soc. Rev. 46, 6855–6871. https://doi.org/10.1039/C7CS00149E

Li, B., Lan, Z., Wang, L., Sun, H., Yao, Y., Zhang, K., Zhu, L., 2019. The release and earthworm bioaccumulation of endogenous hexabromocyclododecanes (HBCDDs) from expanded polystyrene foam microparticles. Environmental Pollution 255, 113163. https://doi.org/10.1016/j.envpol.2019.113163

Li, J., Zhang, K., Zhang, H., 2018. Adsorption of antibiotics on microplastics. Environmental Pollution 237, 460–467. https://doi.org/10.1016/j.envpol.2018.02.050

Li, L., Luo, Y., Li, R., Zhou, Q., Peijnenburg, W.J.G.M., Yin, N., Yang, J., Tu, C., Zhang, Y., 2020a. Effective uptake of submicrometre plastics by crop plants via a crack-entry mode. Nat Sustain 3, 929–937. https://doi.org/10.1038/s41893-020-0567-9

Li, L., Luo, Y., Peijnenburg, W.J.G.M., Li, R., Yang, J., Zhou, Q., 2020b. Confocal measurement of microplastics uptake by plants. MethodsX 7, 100750. https://doi.org/10.1016/j.mex.2019.11.023

Li, L., Zhou, Q., Yin, N., Tu, C., Luo, Y., 2019. Uptake and accumulation of microplastics in an edible plant. Kexue Tongbao/Chinese Science Bulletin 64, 928–934. https://doi.org/10.1360/N972018-00845

Lian, J., Wu, J., Xiong, H., Zeb, A., Yang, T., Su, X., Su, L., Liu, W., 2020. Impact of polystyrene nanoplastics (PSNPs) on seed germination and seedling growth of wheat (Triticum aestivum L.). Journal of Hazardous Materials 385, 121620. https://doi.org/10.1016/j.jhazmat.2019.121620

Liu, J., Zhu, L., Luo, S., Bu, L., Chen, X., Yue, S., Li, S., 2014a. Response of nitrous oxide emission to soil mulching and nitrogen fertilization in semi-arid farmland. Agriculture, Ecosystems & Environment 188, 20–28. https://doi.org/10.1016/j.agee.2014.02.010

Liu, J., Zhu, L., Luo, S., Bu, L., Chen, X., Yue, S., Li, S., 2014b. Response of nitrous oxide emission to soil mulching and nitrogen fertilization in semi-arid farmland. Agriculture, Ecosystems & Environment 188, 20–28. https://doi.org/10.1016/j.agee.2014.02.010

Liu, L.-H., Yuan, T., Zhang, J.-Y., Tang, G.-X., Lü, H., Zhao, H.-M., Li, H., Li, Y.-W., Mo, C.-H., Tan, Z.-Y., Cai, Q.-Y., 2022. Diversity of endophytic bacteria in wild rice (Oryza meridionalis) and potential for promoting plant growth and degrading phthalates. Science of The Total Environment 806, 150310. https://doi.org/10.1016/j.scitotenv.2021.150310

Liu, M., Lu, S., Song, Y., Lei, L., Hu, J., Lv, W., Zhou, W., Cao, C., Shi, H., Yang, X., He, D., 2018. Microplastic and mesoplastic pollution in farmland soils in suburbs of Shanghai, China. Environmental Pollution 242, 855–862.

https://doi.org/10.1016/j.envpol.2018.07.051

Liu, T., Li, J., Qiu, L., Zhang, F., Linhardt, R.J., Zhong, W., 2020. Combined genomic and transcriptomic analysis of the dibutyl phthalate metabolic pathway in Arthrobacter sp. ZJUTW. Biotechnology and Bioengineering 117, 3712–3726. https://doi.org/10.1002/bit.27524

Liu, W., Zhang, J., Liu, H., Guo, X., Zhang, X., Yao, X., Cao, Z., Zhang, T., 2021. A review of the removal of microplastics in global wastewater treatment plants: Characteristics and mechanisms. Environment International 146, 106277. https://doi.org/10.1016/j.envint.2020.106277

Liu, Y., Guo, R., Zhang, S., Sun, Y., Wang, F., 2022. Uptake and translocation of nano/microplastics by rice seedlings: Evidence from a hydroponic experiment. Journal of Hazardous Materials 421, 126700. https://doi.org/10.1016/j.jhazmat.2021.126700

Lodeiro, C., Capelo, J.L., Oliveira, E., Lodeiro, J.F., 2019. New toxic emerging contaminants: beyond the toxicological effects. Environ Sci Pollut Res 26, 1–4. https://doi.org/10.1007/s11356-018-3003-1

Lourenço, J., Mendo, S., Pereira, R., 2019. Rehabilitation of Radioactively Contaminated Soil: Use of Bioremediation/Phytoremediation Techniques, in: Gupta, D.K., Voronina, A. (Eds.), Remediation Measures for Radioactively Contaminated Areas. Springer International Publishing, Cham, pp. 163–200. https://doi.org/10.1007/978-3-319-73398-2_8

Luo, Z., Zhou, X., Su, Y., Wang, H., Yu, R., Zhou, S., Xu, E.G., Xing, B., 2021. Environmental occurrence, fate, impact, and potential solution of tire microplastics: Similarities and differences with tire wear particles. Science of The Total Environment 795, 148902. https://doi.org/10.1016/j.scitotenv.2021.148902

Lv, Weiwei, Zhou, W., Lu, S., Huang, W., Yuan, Q., Tian, M., Lv, Weiguang, He, D., 2019. Microplastic pollution in rice-fish co-culture system: A report of three farmland stations in Shanghai, China. Science of The Total Environment 652, 1209–1218. https://doi.org/10.1016/j.scitotenv.2018.10.321

Ma, B., Xue, W., Ding, Y., Hu, C., Liu, H., Qu, J., 2019. Removal characteristics of

microplastics by Fe-based coagulants during drinking water treatment. Journal of Environmental Sciences 78, 267–275. https://doi.org/10.1016/j.jes.2018.10.006

Maes, T., Jessop, R., Wellner, N., Haupt, K., Mayes, A.G., 2017. A rapid-screening approach to detect and quantify microplastics based on fluorescent tagging with Nile Red. Sci Rep 7, 44501. https://doi.org/10.1038/srep44501

Magnin, A., Hoornaert, L., Pollet, E., Laurichesse, S., Phalip, V., Avérous, L., 2019. Isolation and characterization of different promising fungi for biological waste management of polyurethanes. Microbial Biotechnology 12, 544–555. https://doi.org/10.1111/1751-7915.13346

Mahon, A.M., O'Connell, B., Healy, M.G., O'Connor, I., Officer, R., Nash, R., Morrison, L., 2017. Microplastics in Sewage Sludge: Effects of Treatment. Environ. Sci. Technol. 51, 810–818. https://doi.org/10.1021/acs.est.6b04048

Mammo, F.K., Amoah, I.D., Gani, K.M., Pillay, L., Ratha, S.K., Bux, F., Kumari, S., 2020. Microplastics in the environment: Interactions with microbes and chemical contaminants. Science of The Total Environment 743, 140518. https://doi.org/10.1016/j.scitotenv.2020.140518

Managing Plastic Waste in India [WWW Document], n.d. URL https://www.cseindia.org/managing-plastic-waste-in-india-10352 (accessed 6.18.22).

Mason, S.A., Garneau, D., Sutton, R., Chu, Y., Ehmann, K., Barnes, J., Fink, P., Papazissimos, D., Rogers, D.L., 2016. Microplastic pollution is widely detected in US municipal wastewater treatment plant effluent. Environmental Pollution 218, 1045–1054. https://doi.org/10.1016/j.envpol.2016.08.056

Mateo-Sagasta, J., Medlicott, K., Qadir, M., Raschid-Sally, L., Drechsel, P., 2013. Proceedings of the UN-Water Project on the Safe Use of Wastewater in Agriculture.

Mateos-Cárdenas, A., van Pelt, F.N.A.M., O'Halloran, J., Jansen, M.A.K., 2021. Adsorption, uptake and toxicity of micro- and nanoplastics: Effects on terrestrial plants and aquatic macrophytes. Environmental Pollution 284, 117183. https://doi.org/10.1016/j.envpol.2021.117183

Medić, A., Stojanović, K., Izrael-Živković, L., Beškoski, V., Lončarević, B., Kazazić, S.,

Karadžić, I., 2019. A comprehensive study of conditions of the biodegradation of a plastic additive 2,6-di- tert -butylphenol and proteomic changes in the degrader Pseudomonas aeruginosa san ai. RSC Advances 9, 23696–23710. https://doi.org/10.1039/C9RA04298A

Montazer, Z., Habibi Najafi, M.B., Levin, D.B., 2020. Challenges with Verifying Microbial Degradation of Polyethylene. Polymers 12, 123. https://doi.org/10.3390/polym12010123

Moog, D., Schmitt, J., Senger, J., Zarzycki, J., Rexer, K.-H., Linne, U., Erb, T., Maier, U.G., 2019. Using a marine microalga as a chassis for polyethylene terephthalate (PET) degradation. Microbial Cell Factories 18, 171. https://doi.org/10.1186/s12934-019-1220-z

Nations), W.W.A.P. (United, 2009. Water in a Changing World. Earthscan.

Nel, A.E., Mädler, L., Velegol, D., Xia, T., Hoek, E.M.V., Somasundaran, P., Klaessig, F., Castranova, V., Thompson, M., 2009. Understanding biophysicochemical interactions at the nano–bio interface. Nature Mater 8, 543–557. https://doi.org/10.1038/nmat2442

Ng, E.-L., Huerta Lwanga, E., Eldridge, S.M., Johnston, P., Hu, H.-W., Geissen, V., Chen, D., 2018. An overview of microplastic and nanoplastic pollution in agroecosystems. Science of The Total Environment 627, 1377–1388. https://doi.org/10.1016/j.scitotenv.2018.01.341

Ohtaki, A., Nakasaki, K., 2000. Report: Ultimate degradability of various kinds of biodegradable plastics under controlled composting conditions. Waste Manag Res 18, 184–189. https://doi.org/10.1177/0734242X0001800210

Othman, A.R., Hasan, H.A., Muhamad, M.H., Ismail, N. 'Izzati, Abdullah, S.R.S., 2021. Microbial degradation of microplastics by enzymatic processes: a review. Environ Chem Lett 19, 3057–3073. https://doi.org/10.1007/s10311-021-01197-9

Palocci, C., Valletta, A., Chronopoulou, L., Donati, L., Bramosanti, M., Brasili, E., Baldan, B., Pasqua, G., 2017. Endocytic pathways involved in PLGA nanoparticle uptake by grapevine cells and role of cell wall and membrane in size selection. Plant Cell Rep 36, 1917–1928. https://doi.org/10.1007/s00299-017-2206-0

Pérez-de-Luque, A., 2017. Interaction of Nanomaterials with Plants: What Do We Need for Real Applications in Agriculture? Frontiers in Environmental Science 5.

Perren, W., Wojtasik, A., Cai, Q., 2018. Removal of Microbeads from Wastewater Using

Electrocoagulation. ACS Omega 3, 3357–3364. https://doi.org/10.1021/acsomega.7b02037

Pflugmacher, S., Sulek, A., Mader, H., Heo, J., Noh, J.H., Penttinen, O.-P., Kim, Y., Kim, S., Esterhuizen, M., 2020. The Influence of New and Artificial Aged Microplastic and Leachates on the Germination of Lepidium sativum L. Plants 9, 339. https://doi.org/10.3390/plants9030339

Piehl, S., Leibner, A., Löder, M.G.J., Dris, R., Bogner, C., Laforsch, C., 2018. Identification and quantification of macro- and microplastics on an agricultural farmland. Scientific Reports 8, 17950. https://doi.org/10.1038/s41598-018-36172-y

Prata, J.C., da Costa, J.P., Lopes, I., Duarte, A.C., Rocha-Santos, T., 2020. Environmental exposure to microplastics: An overview on possible human health effects. Science of The Total Environment 702, 134455. https://doi.org/10.1016/j.scitotenv.2019.134455

Pricinotto, L.F., Zucareli, C., Ferreira, A.S., Spolaor, L.T., Fonseca, I.C.D.B., 2019. YIELD AND BIOMETRIC CHARACTERISTICS OF MAIZE SUBMITTED TO PLANT POPULATION AND TRINEXAPAC-ETHYL DOSES. Rev. Caatinga 32, 667–678. https://doi.org/10.1590/1983-21252019v32n311rc

Prokin, A.A., Dubov, P.G., Bolotov, S.E., 2015. Formation of macroinvertebrates communities in duckweed (Lemnaceae) and artificial surface-floating substrate: Results of the experiment under natural conditions. Inland Water Biol 8, 373–383. https://doi.org/10.1134/S1995082915040136

Puiggené, Ò., Espinosa, M.J.C., Schlosser, D., Thies, S., Jehmlich, N., Kappelmeyer, U., Schreiber, S., Wibberg, D., Kalinowski, J., Harms, H., Heipieper, H.J., Eberlein, C., 2022. Extracellular degradation of a polyurethane oligomer involving outer membrane vesicles and further insights on the degradation of 2,4-diaminotoluene in Pseudomonas capeferrum TDA1. Sci Rep 12, 2666. https://doi.org/10.1038/s41598-022-06558-0

Qi, Y., Ossowicki, A., Yang, X., Huerta Lwanga, E., Dini-Andreote, F., Geissen, V., Garbeva, P., 2020. Effects of plastic mulch film residues on wheat rhizosphere and soil properties. Journal of Hazardous Materials 387, 121711. https://doi.org/10.1016/j.jhazmat.2019.121711

Qi, Y., Yang, X., Pelaez, A.M., Huerta Lwanga, E., Beriot, N., Gertsen, H., Garbeva, P.,

Geissen, V., 2018. Macro- and micro- plastics in soil-plant system: Effects of plastic mulch film residues on wheat (Triticum aestivum) growth. Science of The Total Environment 645, 1048–1056. https://doi.org/10.1016/j.scitotenv.2018.07.229

Qiu, J., Zhang, Y., Shi, Y., Jiang, J., Wu, S., Li, L., Shao, Y., Xin, Z., 2020. Identification and characterization of a novel phthalate-degrading hydrolase from a soil metagenomic library. Ecotoxicology and Environmental Safety 190, 110148. https://doi.org/10.1016/j.ecoenv.2019.110148

Rahbar, A., Farjadfard, S., Leili, M., Kafaei, R., Haghshenas, V., Ramavandi, B., 2016. Experimental data of biomaterial derived from Malva sylvestris and charcoal tablet powder for Hg2+ removal from aqueous solutions. Data in Brief 8, 132–135. https://doi.org/10.1016/j.dib.2016.05.032

Raliya, R., Franke, C., Chavalmane, S., Nair, R., Reed, N., Biswas, P., 2016. Quantitative Understanding of Nanoparticle Uptake in Watermelon Plants. Frontiers in Plant Science 7.

Ramos, L., Berenstein, G., Hughes, E.A., Zalts, A., Montserrat, J.M., 2015. Polyethylene film incorporation into the horticultural soil of small periurban production units in Argentina. Science of The Total Environment 523, 74–81. https://doi.org/10.1016/j.scitotenv.2015.03.142

Rathore, S.S., Shekhawat, K., Dass, A., Kandpal, B.K., Singh, V.K., 2019. Phytoremediation Mechanism in Indian Mustard (Brassica juncea) and Its Enhancement Through Agronomic Interventions. Proc. Natl. Acad. Sci., India, Sect. B Biol. Sci. 89, 419–427. https://doi.org/10.1007/s40011-017-0885-5

Raubenheimer, K., McIlgorm, A., 2018. Can a global fund help solve the global marine plastic debris problem? Journal of Ocean and Coastal Economics 5. https://doi.org/10.15351/2373-8456.1078

Raven, J.A., 2022. Commentary on the use of nutrient-coated quantum dots as a means of tracking nutrient uptake by and movement within plants. Plant Soil 476, 535–548. https://doi.org/10.1007/s11104-022-05507-0

Reineccius, J., Bresien, J., Waniek, J.J., 2021. Separation of microplastics from mass-limited samples by an effective adsorption technique. Science of The Total Environment

788, 147881. https://doi.org/10.1016/j.scitotenv.2021.147881

Ren Hong S.S. and T.A. for C.W.M., Luo Feng S.S. and T.A. for C.W.M., Xu Yan S.S. and T.A. for C.W.M., 2012. Comparison on methods of chlorophyll extraction in flowering chinese cabbage. Journal of Anhui Agricultural Sciences.

Rezania, S., Taib, S.M., Md Din, M.F., Dahalan, F.A., Kamyab, H., 2016. Comprehensive review on phytotechnology: Heavy metals removal by diverse aquatic plants species from wastewater. Journal of Hazardous Materials 318, 587–599. https://doi.org/10.1016/j.jhazmat.2016.07.053

Rillig, M.C., Ingraffia, R., de Souza Machado, A.A., 2017. Microplastic Incorporation into Soil in Agroecosystems . Frontiers in Plant Science .

Rilling, J.I., Acuña, J.J., Nannipieri, P., Cassan, F., Maruyama, F., Jorquera, M.A., 2019. Current opinion and perspectives on the methods for tracking and monitoring plant growth–promoting bacteria. Soil Biology and Biochemistry 130, 205–219. https://doi.org/10.1016/j.soilbio.2018.12.012

Roberts, A.G., Oparka, K.J., 2003. Plasmodesmata and the control of symplastic transport: Plasmodesmata and symplastic transport. Plant, Cell & Environment 26, 103–124. https://doi.org/10.1046/j.1365-3040.2003.00950.x

Rodrigo, O.-A., Daynet, S. del C., Jaime, N.-M., Nora, F., JosÃ©, Ã.-B., Alejandro, A., Jhonny, S.V., Milton, B.-A., 2021. Analysis of the degradation of polyethylene, polystyrene and polyurethane mediated by three filamentous fungi isolated from the Antarctica. Afr. J. Biotechnol. 20, 66–76. https://doi.org/10.5897/AJB2020.17200

Rodrigues, J.P., Duarte, A.C., Santos-Echeandía, J., Rocha-Santos, T., 2019. Significance of interactions between microplastics and POPs in the marine environment: A critical overview. TrAC Trends in Analytical Chemistry 111, 252–260. https://doi.org/10.1016/j.trac.2018.11.038

Rozman, U., Jemec Kokalj, A., Dolar, A., Drobne, D., Kalčíková, G., 2022. Long-term interactions between microplastics and floating macrophyte Lemna minor: The potential for phytoremediation of microplastics in the aquatic environment. Science of The Total Environment 831, 154866. https://doi.org/10.1016/j.scitotenv.2022.154866

Sarwar, N., Imran, M., Shaheen, M.R., Ishaque, W., Kamran, M.A., Matloob, A., Rehim, A., Hussain, S., 2017. Phytoremediation strategies for soils contaminated with heavy metals: Modifications and future perspectives. Chemosphere 171, 710–721. https://doi.org/10.1016/j.chemosphere.2016.12.116

Saxena, G., Purchase, D., Mulla, S.I., Saratale, G.D., Bharagava, R.N., 2020. Phytoremediation of Heavy Metal-Contaminated Sites: Eco-environmental Concerns, Field Studies, Sustainability Issues, and Future Prospects, in: de Voogt, P. (Ed.), Reviews of Environmental Contamination and Toxicology Volume 249, Reviews of Environmental Contamination and Toxicology. Springer International Publishing, Cham, pp. 71–131. https://doi.org/10.1007/398_2019_24

Scheurer, M., Bigalke, M., 2018. Microplastics in Swiss Floodplain Soils. Environmental Science & Technology 52, 3591–3598. https://doi.org/10.1021/acs.est.7b06003

Schwab, F., Zhai, G., Kern, M., Turner, A., Schnoor, J.L., Wiesner, M.R., 2016. Barriers, pathways and processes for uptake, translocation and accumulation of nanomaterials in plants – Critical review. Nanotoxicology 10, 257–278. https://doi.org/10.3109/17435390.2015.1048326

Scott Lambert, Chris Sinclair, and A.B., 2014. Occurrence, Degradation, and Effect of Polymer-Based Materials in the Environment, in: Reviews of Environmental Contamination and Toxicology. Springer International Publishing Switzerland, pp. vii–xi. https://doi.org/10.1007/978-3-319-01327-5

Shang, S., Liu, Youxing, Liu, M., Bai, Y., Wang, X., Wu, B., Chen, J., Dong, J., Liu, Yunqi, 2022. Studying the adsorption mechanisms of nanoplastics on covalent organic frameworks via molecular dynamics simulations. Journal of Hazardous Materials 421, 126796. https://doi.org/10.1016/j.jhazmat.2021.126796

Shao, H., Chen, M., Fei, X., Zhang, R., Zhong, Y., Ni, W., Tao, X., He, X., Zhang, E., Yong, B., Tan, X., 2019. Complete Genome Sequence and Characterization of a Polyethylene Biodegradation Strain, Streptomyces Albogriseolus LBX-2. Microorganisms 7, 379. https://doi.org/10.3390/microorganisms7100379

Sharma, S., Mallubhotla, S., 2019. Plastic Waste Management Practices.

Song, Z., Zhao, X., Dong, Y., Bai, L., Wang, S., Gao, M., 2023. Effects of polystyrene nanoplastics with different functional groups on the accumulation and toxicity of Pb on dandelion. Chemosphere 310, 136874. https://doi.org/10.1016/j.chemosphere.2022.136874

Staley, C., Sadowsky, M.J., 2016a. Application of metagenomics to assess microbial communities in water and other environmental matrices. Journal of the Marine Biological Association of the United Kingdom 96, 121–129. https://doi.org/10.1017/S0025315415001496

Staley, C., Sadowsky, M.J., 2016b. Application of metagenomics to assess microbial communities in water and other environmental matrices. Journal of the Marine Biological Association of the United Kingdom 96, 121–129. https://doi.org/10.1017/S0025315415001496

Steliga, T., Kluk, D., 2020. Application of Festuca arundinacea in phytoremediation of soils contaminated with Pb, Ni, Cd and petroleum hydrocarbons. Ecotoxicology and Environmental Safety 194, 110409. https://doi.org/10.1016/j.ecoenv.2020.110409

Stubenrauch, J., Ekardt, F., 2020. Plastic Pollution in Soils: Governance Approaches to Foster Soil Health and Closed Nutrient Cycles. Environments 7, 38. https://doi.org/10.3390/environments7050038

Su, Y., Ashworth, V., Kim, C., Adeleye, A.S., Rolshausen, P., Roper, C., White, J., Jassby, D., 2019. Delivery, uptake, fate, and transport of engineered nanoparticles in plants: a critical review and data analysis. Environ. Sci.: Nano 6, 2311–2331. https://doi.org/10.1039/C9EN00461K

Sun, H., Lei, C., Xu, J., Li, R., 2021. Foliar uptake and leaf-to-root translocation of nanoplastics with different coating charge in maize plants. Journal of Hazardous Materials 416, 125854. https://doi.org/10.1016/j.jhazmat.2021.125854

Sun, J., Pan, L., Li, Z., Zeng, Q., Wang, L., Zhu, L., 2018. Comparison of greenhouse and open field cultivations across China: Soil characteristics, contamination and microbial diversity. Environmental Pollution 243, 1509–1516. https://doi.org/10.1016/j.envpol.2018.09.112

Sun, L., Wang, X., Zhao, H., Wang, Z., Zhao, Y., Huang, H., Yang, R., Wang, S., Zhao,

W., Sun, L., Wang, X., Zhao, H., Wang, Z., Zhao, Y., Huang, H., Yang, R., Wang, S., Zhao, W., 2023. Micro/nanoplastics: a potential threat to crops. V 3. https://doi.org/10.48130/VR-2023-0018

Sun, W., Meng, Z., Li, R., Zhang, R., Jia, M., Yan, S., Tian, S., Zhou, Z., Zhu, W., 2021. Joint effects of microplastic and dufulin on bioaccumulation, oxidative stress and metabolic profile of the earthworm (Eisenia fetida). Chemosphere 263, 128171. https://doi.org/10.1016/j.chemosphere.2020.128171

Sundbæk, K.B., Koch, I.D.W., Villaro, C.G., Rasmussen, N.S., Holdt, S.L., Hartmann, N.B., 2018. Sorption of fluorescent polystyrene microplastic particles to edible seaweed Fucus vesiculosus. J Appl Phycol 30, 2923–2927. https://doi.org/10.1007/s10811-018-1472-8

Tagg, A.S., Labrenz, M., 2018. Closing Microplastic Pathways Before They Open: A Model Approach. Environ. Sci. Technol. 52, 3340–3341. https://doi.org/10.1021/acs.est.8b00961

Taheran, M., Naghdi, M., Brar, S.K., Verma, M., Surampalli, R.Y., 2018. Emerging contaminants: Here today, there tomorrow! Environmental Nanotechnology, Monitoring & Management 10, 122–126. https://doi.org/10.1016/j.enmm.2018.05.010

Talvitie, J., Mikola, A., Koistinen, A., Setälä, O., 2017. Solutions to microplastic pollution – Removal of microplastics from wastewater effluent with advanced wastewater treatment technologies. Water Research 123, 401–407. https://doi.org/10.1016/j.watres.2017.07.005

Tangahu, B.V., Sheikh Abdullah, S.R., Basri, H., Idris, M., Anuar, N., Mukhlisin, M., 2011. A Review on Heavy Metals (As, Pb, and Hg) Uptake by Plants through Phytoremediation. International Journal of Chemical Engineering 2011, e939161. https://doi.org/10.1155/2011/939161

Taylor, S.E., Pearce, C.I., Sanguinet, K.A., Hu, D., Chrisler, W.B., Kim, Y.-M., Wang, Z., Flury, M., 2020. Polystyrene nano- and microplastic accumulation at Arabidopsis and wheat root cap cells, but no evidence for uptake into roots. Environ. Sci.: Nano 7, 1942–1953. https://doi.org/10.1039/D0EN00309C

Tiwari, N., Santhiya, D., Sharma, J.G., 2020. Microbial remediation of micro-nano plastics:

Current knowledge and future trends. Environmental Pollution 265, 115044. https://doi.org/10.1016/j.envpol.2020.115044

Tun, T.Z., Kunisue, T., Tanabe, S., Prudente, M., Subramanian, A., Sudaryanto, A., Viet, P.H., Nakata, H., 2022. Microplastics in dumping site soils from six Asian countries as a source of plastic additives. Science of The Total Environment 806, 150912. https://doi.org/10.1016/j.scitotenv.2021.150912

Ullah, R., Tsui, M.T.-K., Chen, H., Chow, A., Williams, C., Ligaba-Osena, A., 2021a. Microplastics interaction with terrestrial plants and their impacts on agriculture. Journal of Environmental Quality 50, 1024–1041. https://doi.org/10.1002/jeq2.20264

Ullah, R., Tsui, M.T.-K., Chen, H., Chow, A., Williams, C., Ligaba-Osena, A., 2021b. Microplastics interaction with terrestrial plants and their impacts on agriculture. Journal of Environmental Quality 50, 1024–1041. https://doi.org/10.1002/jeq2.20264

van Weert, S., Redondo-Hasselerharm, P.E., Diepens, N.J., Koelmans, A.A., 2019. Effects of nanoplastics and microplastics on the growth of sediment-rooted macrophytes. Science of The Total Environment 654, 1040–1047. https://doi.org/10.1016/j.scitotenv.2018.11.183

Wallace, P.W., Haernvall, K., Ribitsch, D., Zitzenbacher, S., Schittmayer, M., Steinkellner, G., Gruber, K., Guebitz, G.M., Birner-Gruenberger, R., 2017. PpEst is a novel PBAT degrading polyesterase identified by proteomic screening of Pseudomonas pseudoalcaligenes. Appl Microbiol Biotechnol 101, 2291–2303. https://doi.org/10.1007/s00253-016-7992-8

Wang, F., Zhang, X., Zhang, Shuqi, Zhang, Shuwu, Sun, Y., 2020. Interactions of microplastics and cadmium on plant growth and arbuscular mycorrhizal fungal communities in an agricultural soil. Chemosphere 254, 126791. https://doi.org/10.1016/j.chemosphere.2020.126791

Wang, J., Coffin, S., Schlenk, D., Gan, J., 2020. Accumulation of HOCs via Precontaminated Microplastics by Earthworm Eisenia fetida in Soil. Environ. Sci. Technol. 54, 11220–11229. https://doi.org/10.1021/acs.est.0c02922

Wang, J., Liu, X., Li, Y., Powell, T., Wang, X., Wang, G., Zhang, P., 2019. Microplastics as contaminants in the soil environment: A mini-review. Science of The Total Environment

691, 848–857. https://doi.org/10.1016/j.scitotenv.2019.07.209

Wang, J., Luo, Y., Teng, Y., Ma, W., Christie, P., Li, Z., 2013. Soil contamination by phthalate esters in Chinese intensive vegetable production systems with different modes of use of plastic film. Environmental Pollution 180, 265–273. https://doi.org/10.1016/j.envpol.2013.05.036

Wang, Q., Adams, C.A., Wang, F., Sun, Y., Zhang, S., 2021. Interactions between microplastics and soil fauna: A critical review. Critical Reviews in Environmental Science and Technology 0, 1–33. https://doi.org/10.1080/10643389.2021.1915035

Wang, X., Li, Y., Zhao, J., Xia, X., Shi, X., Duan, J., Zhang, W., 2020. UV-induced aggregation of polystyrene nanoplastics: effects of radicals, surface functional groups and electrolyte. Environ. Sci.: Nano 7, 3914–3926. https://doi.org/10.1039/D0EN00518E

Wang, Z., Xie, X., Zhao, J., Liu, X., Feng, W., White, J.C., Xing, B., 2012. Xylem- and Phloem-Based Transport of CuO Nanoparticles in Maize (Zea mays L.). Environ. Sci. Technol. 46, 4434–4441. https://doi.org/10.1021/es204212z

Waśkiewicz, A., Muzolf-Panek, M., Goliński, P., 2013. Phenolic Content Changes in Plants Under Salt Stress, in: Ahmad, P., Azooz, M.M., Prasad, M.N.V. (Eds.), Ecophysiology and Responses of Plants under Salt Stress. Springer, New York, NY, pp. 283–314. https://doi.org/10.1007/978-1-4614-4747-4_11

Weldeslassie, T., Naz, H., Singh, B., Oves, M., 2018. Chemical Contaminants for Soil, Air and Aquatic Ecosystem, in: Oves, M., Zain Khan, M., M.I. Ismail, I. (Eds.), Modern Age Environmental Problems and Their Remediation. Springer International Publishing, Cham, pp. 1–22. https://doi.org/10.1007/978-3-319-64501-8_1

Wilkes, R.A., Aristilde, L., 2017. Degradation and metabolism of synthetic plastics and associated products by Pseudomonas sp.: capabilities and challenges. Journal of Applied Microbiology 123, 582–593. https://doi.org/10.1111/jam.13472

Windsor, F.M., Durance, I., Horton, A.A., Thompson, R.C., Tyler, C.R., Ormerod, S.J., 2019. A catchment-scale perspective of plastic pollution. Global Change Biology 25, 1207–1221. https://doi.org/10.1111/gcb.14572

Wróbel, M., Szymańska, S., Kowalkowski, T., Hrynkiewicz, K., 2023. Selection of

microorganisms capable of polyethylene (PE) and polypropylene (PP) degradation. Microbiological Research 267, 127251. https://doi.org/10.1016/j.micres.2022.127251

Wu, J., Liu, W., Zeb, A., Lian, J., Sun, Y., Sun, H., 2021. Polystyrene microplastic interaction with Oryza sativa: toxicity and metabolic mechanism. Environ. Sci.: Nano 8, 3699–3710. https://doi.org/10.1039/D1EN00636C

Wu, Y., Guo, P., Zhang, X., Zhang, Y., Xie, S., Deng, J., 2019. Effect of microplastics exposure on the photosynthesis system of freshwater algae. Journal of Hazardous Materials 374, 219–227. https://doi.org/10.1016/j.jhazmat.2019.04.039

Xia, B., Chen, B., Sun, X., Qu, K., Ma, F., Du, M., 2015. Interaction of TiO2 nanoparticles with the marine microalga Nitzschia closterium: Growth inhibition, oxidative stress and internalization. Science of The Total Environment 508, 525–533. https://doi.org/10.1016/j.scitotenv.2014.11.066

Xu, B., Liu, F., Cryder, Z., Huang, D., Lu, Zhijiang, He, Y., Wang, H., Lu, Zhenmei, Brookes, P.C., Tang, C., Gan, J., Xu, J., 2020. Microplastics in the soil environment: Occurrence, risks, interactions and fate – A review. Critical Reviews in Environmental Science and Technology 50, 2175–2222. https://doi.org/10.1080/10643389.2019.1694822

Xu, Q., Huang, Q.-S., Luo, T.-Y., Wu, R.-L., Wei, W., Ni, B.-J., 2021. Coagulation removal and photocatalytic degradation of microplastics in urban waters. Chemical Engineering Journal 416, 129123. https://doi.org/10.1016/j.cej.2021.129123

Xu, Z., Zhang, Y., Lin, L., Wang, L., Sun, W., Liu, C., Yu, G., Yu, J., Lv, Y., Chen, J., Chen, X., Fu, L., Wang, Y., 2022. Toxic effects of microplastics in plants depend more by their surface functional groups than just accumulation contents. Science of The Total Environment 833, 155097. https://doi.org/10.1016/j.scitotenv.2022.155097

Yang, X., Lwanga, E.H., Bemani, A., Gertsen, H., Salanki, T., Guo, X., Fu, H., Xue, S., Ritsema, C., Geissen, V., 2019. Biogenic transport of glyphosate in the presence of LDPE microplastics: A mesocosm experiment. Environmental Pollution 245, 829–835. https://doi.org/10.1016/j.envpol.2018.11.044

Yoshida, S., Hiraga, K., Takehana, T., Taniguchi, I., Yamaji, H., Maeda, Y., Toyohara, K., Miyamoto, K., Kimura, Y., Oda, K., 2016. A bacterium that degrades and assimilates

poly(ethylene terephthalate). Science 351, 1196–1199. https://doi.org/10.1126/science.aad6359

Yu, B., Peng, Y., Xu, J., Qin, D., Gao, T., Zhu, H., Zuo, S., Song, H., Dong, J., 2021. Phytoremediation potential of Youngia japonica (L.) DC: a newly discovered cadmium hyperaccumulator. Environ Sci Pollut Res 28, 6044–6057. https://doi.org/10.1007/s11356-020-10853-6

Zbyszewski, M., Corcoran, P.L., 2011. Distribution and Degradation of Fresh Water Plastic Particles Along the Beaches of Lake Huron, Canada. Water Air Soil Pollut 220, 365–372. https://doi.org/10.1007/s11270-011-0760-6

Zhang, G.S., Liu, Y.F., 2018. The distribution of microplastics in soil aggregate fractions in southwestern China. Science of The Total Environment 642, 12–20. https://doi.org/10.1016/j.scitotenv.2018.06.004

Zhang, H., Wang, J., Zhou, B., Zhou, Y., Dai, Z., Zhou, Q., Chriestie, P., Luo, Y., 2018. Enhanced adsorption of oxytetracycline to weathered microplastic polystyrene: Kinetics, isotherms and influencing factors. Environmental Pollution 243, 1550–1557. https://doi.org/10.1016/j.envpol.2018.09.122

Zhang, L., Cheng, Y., Qian, Y., Ding, T., Li, J., 2022. Phytotoxicity and accumulation of BPS to Pistia stratiotes under the influence of microplastics. Chemosphere 307, 135854. https://doi.org/10.1016/j.chemosphere.2022.135854

Zhang, X., Huang, G., Bian, X., Zhao, Q., 2013. Effects of root interaction and nitrogen fertilization on the chlorophyll content, root activity, photosynthetic characteristics of intercropped soybean and microbial quantity in the rhizosphere. Plant, Soil and Environment 59 (2013), 80–88. https://doi.org/10.17221/613/2012-PSE

Zhang, Z., Cui, Q., Chen, L., Zhu, X., Zhao, S., Duan, C., Zhang, X., Song, D., Fang, L., 2022. A critical review of microplastics in the soil-plant system: Distribution, uptake, phytotoxicity and prevention. Journal of Hazardous Materials 424, 127750. https://doi.org/10.1016/j.jhazmat.2021.127750

Zhou, G., Wang, Q., Li, J., Li, Q., Xu, H., Ye, Q., Wang, Y., Shu, S., Zhang, J., 2021. Removal of polystyrene and polyethylene microplastics using PAC and FeCl3 coagulation:

Performance and mechanism. Science of The Total Environment 752, 141837. https://doi.org/10.1016/j.scitotenv.2020.141837

Zhou, Q., Zhang, H., Fu, C., Zhou, Y., Dai, Z., Li, Y., Tu, C., Luo, Y., 2018. The distribution and morphology of microplastics in coastal soils adjacent to the Bohai Sea and the Yellow Sea. Geoderma 322, 201–208. https://doi.org/10.1016/j.geoderma.2018.02.015

Zhou, Y., Liu, X., Wang, J., 2019. Characterization of microplastics and the association of heavy metals with microplastics in suburban soil of central China. Science of The Total Environment 694, 133798. https://doi.org/10.1016/j.scitotenv.2019.133798

Zuo, L.-Z., Li, H.-X., Lin, L., Sun, Y.-X., Diao, Z.-H., Liu, S., Zhang, Z.-Y., Xu, X.-R., 2019. Sorption and desorption of phenanthrene on biodegradable poly(butylene adipate co-terephtalate) microplastics. Chemosphere 215, 25–32. https://doi.org/10.1016/j.chemosphere.2018.09.173

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f) Tiwari, N., **Bansal, M**., & Sharma, J. G. (2021). Metagenomics: a powerful lens viewing the microbial world. In Wastewater treatment reactors (pp. 309-339). Elsevier.

g) Tiwari, N., **Bansal, M.,** Santhiya, D., & Sharma, J. G. (2024). Microplastics contamination in agricultural ecosystems: Challenges and sustainable approaches for green environment (pp. 71-94). Elsevier.

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REVIEW ARTICLE

Mechanistic understanding on the uptake of micro-nano plastics by plants and its phytoremediation

Megha Bansal[1] · Deenan Santhiya[2] · Jai Gopal Sharma[1]

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Abstract

Contaminated soil is one of today's most difficult environmental issues, posing serious hazards to human health and the environment. Contaminants, particularly micro-nano plastics, have become more prevalent around the world, eventually ending up in the soil. Numerous studies have been conducted to investigate the interactions of micro-nano plastics in plants and agroecosystems. However, viable remediation of micro-nano plastics in soil remains limited. In this review, a powerful in situ soil remediation technology known as phytoremediation is emphasized for addressing micro-nano-plastic contamination in soil and plants. It is based on the synergistic effects of plants and the microorganisms that live in their rhizosphere. As a result, the purpose of this review is to investigate the mechanism of micro-nano plastic (MNP) uptake by plants as well as the limitations of existing MNP removal methods. Different phytoremediation options for removing micro-nano plastics from soil are also described. Phytoremediation improvements (endophytic-bacteria, hyperaccumulator species, omics investigations, and CRISPR-Cas9) have been proposed to enhance MNP degradation in agroecosystems. Finally, the limitations and future prospects of phytoremediation strategies have been highlighted in order to provide a better understanding for effective MNP decontamination from soil.

Keywords Soil remediation · Contaminants · Omics-based · Hyperaccumulator · CRISPR-Cas9

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Highlights
- Micro-nano plastics (MNPs) pose detrimental effects on agroecosystem.
- Mechanism of uptake of MNPs by plants is highlighted.
- Phytoremediation offers great insight to remediate MNPs from soil and plants.
- Advanced phytoremediation approaches (Omics and CRISPR-Cas9) are essential.
- Bioaugmentation and system science can enhance phytoremediation of MNPs.

✉ Deenan Santhiya
deenan.santhiya@dce.ac.in

Megha Bansal
megha_2k21phdbt01@dtu.ac.in

Jai Gopal Sharma
sharmajaigopal@dce.ac.in

[1] Department of Biotechnology, Delhi Technological University, Delhi, India

[2] Department of Applied Chemistry, Delhi Technological University, Main Bawana Road, Delhi 110042, India

Introduction

The most influential attribute of man-made activities is the discharge of plastic (Galloway et al. 2017), a polymer used in everyday life. According to reports from the Centre for Science and Environment (CSE) of India, 79% of the total plastic produced enters the environment in the form of waste, of which only around 9% is recycled ("Managing Plastic Waste in India," 2020). Various reports have claimed that most plastic materials are more integrated towards breakdown compared to degradation (Bansal et al. 2021). These large plastics generate smaller fragments of size less than 5 mm, referred to as microplastics (Mammo et al. 2020). Further deterioration of these microplastic fragments results in the formation of eventually smaller particles of size less than 0.1 µm, commonly called nanoplastics (Ng et al. 2018). Significant efforts have recently been made in the detection and analysis of micro-nano plastics (MNPs) (Tian et al. 2022; Pfohl et al. 2022), the current pollution study (Gong et al. 2023), and biological toxicity evaluation (Teng et al. 2022). Although many studies have focused on understanding the impacts of metal nanoparticles on plants

and soil, very little evidence exists on understanding the uptake and effect of MNPs on plants (Yadav et al. 2022; Kumar et al. 2023; Bansal et al. 2023). For example, the alleviation of lead toxicity in *Brassica juncea* by salicylic acid helped to minimize oxidative damage to the plant (Agnihotri et al. 2018). Another study showed antioxidant defense mechanisms in *Brassica juncea* L. by alleviating salinity stress through 24-Epibrassinolide (Gupta and Seth 2023). Although source control, such as cutting down on plastic consumption and developing alternative materials, is advocated, such processes may not be effective in a short time. Thus, it is imperative to seek efficient pathways to deal with the existing MNP pollution. A growing concern about the ingestion of microplastics and nanoplastics by aquatic species, some migration to microbial guts and trophic level transfers, has been reviewed since a long time (Galloway et al. 2017). However, the terrestrial landmasses are majorly empty for mapping global plastic distribution and its detrimental effects on agro-ecosystems. In the current context, the global expansion of a small fraction of plastics, namely, micro-nano plastics (MNPs), is gaining significant consideration from researchers around the globe because of their critical environmental effects (Wright et al. 2013).

MNPs and their sources

MNPs are divided into fiber, film, pellet, powder, and fragments based on their morphology. MNPs are composed of polypropylene (PP), polyethylene (PE), polystyrene (PS), polyvinyl chloride (PVC), and polyamide (PA) as chemical ingredients. MNPs are widely classified into primary and secondary classes based on their commercial applications. Primary MNPs are manufactured for specialized uses such as cosmetics, medicine delivery carriers, industrial use, and military aids. Furthermore, synthetic textiles account for roughly 35% of total primary MNPs emitted in bodies of water (Boucher and Friot 2017). Secondary MNPs are formed by the breakdown of large plastics through physical, chemical, and biological approaches. Mechanical forces, chemical breakdown, or microbial degradation of large plastic fragments result in the formation of secondary MNPs. Also, fishing, travelling, and catering contribute a significant proportion of secondary MNPs (Guo et al. 2020). Vehicular transport, including tyre wear, brakes, markings, is also a major source of microplastics in the ecosystem (Luo et al. 2021). Apart from major sources of MNPs in the environment, agricultural practices, recreational activities, sewage sludge application, and organic fertilizers can also contribute to MNP pollution (Guo et al. 2020). So, better knowledge of the potentially detrimental or deleterious consequences of these contaminants on agroecosystems is critical.

Impacts of MNPs to terrestrial and aquatic plants

Size, thickness, shape, and shade of MNPs control their bioavailability, and, subsequently, their toxicological impact (Haegerbaeumer et al. 2019). MNPs get adsorbed by plants, the soil rhizosphere, and soil organism thereby affecting soil physiochemical properties (Junhao et al. 2021). Likewise, the impacts of MNPs on aquatic plants are crucial to the well-being of flora and fauna (Fig. 1). Recently, airborne MNPs have also gained attention, suggesting that they are present both in indoor and outdoor air (Enyoh et al. 2019). Effluent discharge and surface runoff from rivers, lakes, and sediments have a direct impact on aquatic water bodies and related coastlines (Jeyavani et al. 2021). Also, MNPs aggregate and get ingested by marine species, thereby affecting the food chain (Prata et al. 2020). Apart from this, agricultural practices, road, and tyre wear discharge also emit MNPs on land and in the air (Luo et al. 2021). These sediments

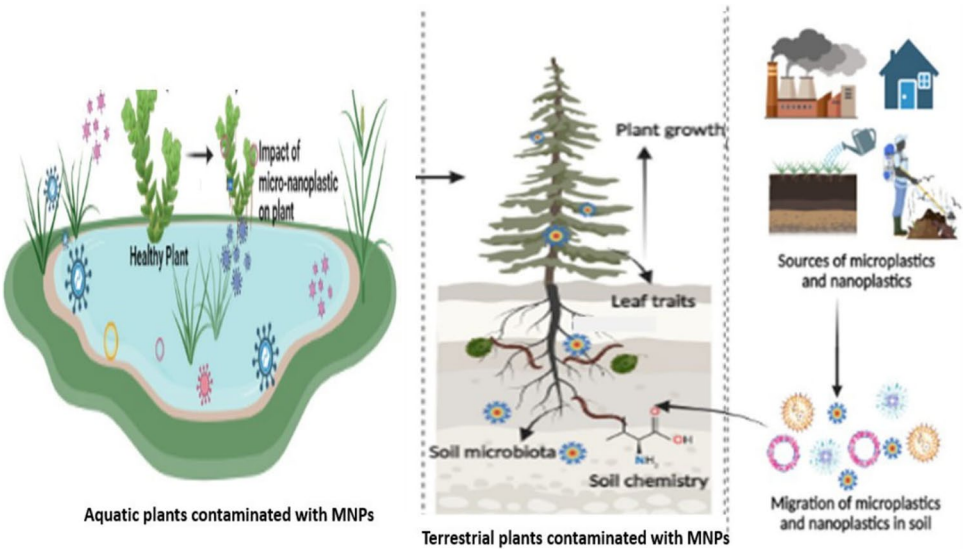

Fig. 1 Sources of MNPs, their migration, impact, and structural changes on terrestrial and aquatic plants

get infiltrated within the soil or discharge into water bodies, thereby affecting the ecosystem.

Toxicity to terrestrial plants and soil

MNPs can physically block or clog plant tissues, such as stomata or root hairs, limiting their ability to carry out essential functions like gas exchange and nutrient uptake (Banerjee et al. 2019). MNPs contain various additives, such as plasticizers and flame retardants, which can leach out and affect plants. These chemicals may have toxic effects on plants, disrupting their metabolism, photosynthesis, and hormonal balance (Tun et al. 2022). MNPs can adsorb nutrients such as nitrogen and phosphorus, reducing their bioavailability to plants. This can lead to nutrient deficiencies and affect plant growth and productivity (Dovidat et al. 2020). MNPs can provide a surface for microbial colonization, forming biofilms. These biofilms can alter the composition and activity of soil and water microbial communities, potentially affecting the plant–microbe interactions that are crucial for nutrient uptake and disease resistance (He et al. 2022). Despite having lower MNP emphasis than soil, air has a higher liquidity that allows for more accumulation of MNPs on leaf surfaces by means of fluid-elevated openings (de Souza Machado et al. 2019). MNPs appended to leaf surfaces might obstruct daylight and hamper photosynthesis, with comparable outcomes found in green growth (Wu et al. 2019). Due to the presence of MNPs in soil, a reduction in water-holding capacity occurs, which negatively causes a decrease in oxygen in soil aggregates (Liu et al. 2014). Similarly, micrometer-sized polystyrene (PS) microparticles may also penetrate inside the crop plants at the site of lateral root emergence, thereby contaminating the crops (Li et al. 2020a). Seeds may be harmed as a result of plastic leachate mixed with water, which seeds absorb during germination, or as a consequence of smaller MNPs altering soil structure (Pflugmacher et al. 2020).

Toxicity to aquatic plants

Not only do MNPs have impacts on terrestrial plants, but recent research has found their potential to deteriorate aquatic flora. The formation of phytoplankton on the surface of water could encapsulate and entrap MNPs (Prokin et al. 2015). This sorption of MNPs may result in decreased length of principle outgrowths of established plants, repress root development, photosynthetic action, and suitability of freshwater phytoplankton. Considering past works, microplastics can influence the photosynthesis of green plants through attachment to the outer layer of xylem and phloem tissues and consequently assemble to repress their photosynthesis (Dovidat et al. 2020). PS nanoplastic adsorption also slows down green plant photosynthetic movement while speeding up the formation of responsive oxygen, which is dependent on the physiochemical qualities of plastics as well as the morphology and biochemical properties of green plants (Bhattacharya et al. 2010). Light is crucial for the photosynthetic process, and decreased light availability can impede plant growth and productivity (Mateos-Cárdenas et al. 2021). MNPs can induce oxidative stress in aquatic plants. These particles can generate reactive oxygen species (ROS) when interacting with plant tissues. ROS can cause cellular damage by oxidizing biomolecules, disrupting cellular processes, and impairing plant growth (van Weert et al. 2019). As higher trophic levels feed on these plants, the MNPs can be transferred up the food chain through a process called biomagnification, potentially affecting other organisms as well. The debilitating or even loss of porousness influences ordinary development and digestion cycle like the migration of compounds within and outside the cell walls of aquatic plants (Xia et al. 2015).

Keeping in view the above-stated facts and examples, most of the investigations have intensified on determining the effects of MNPs on terrestrial and aquatic plant species. The necessity of phytoremediation of micro-nano plastics results from the pressing need to address the negative environmental effects of plastic pollution, the shortcomings of current cleanup techniques, and the potential of plants to provide a viable and efficient response to this new environmental problem. The significance of phytoremediation stems from its capacity to offer environmentally sound, economically viable, and long-lasting solutions for waste management. Many advantages it provides include safeguarding human health, promote long-term environmental sustainability, and improve the general health of ecosystems. In light of this, this review is divided into five major objectives aimed at (i) exploring the importance of phytoremediation and highlighting the drawbacks associated with conventional methodologies to eliminate MNPs; (ii) examining the size of MNPs absorbed by plants, the mechanism of MNPs uptake, and the approaches involved in phytoremediation of MNPs; (iii) exploring the factors influencing phytoremediation of MNPs; (iv) proposing strategies for phytoremediation of MNPs in agroecosystems; and (v) policies and future directions to address the knowledge gaps and provide substantial inputs on combating MNPs in agroecosystems.

Objectives

Objective: importance of phytoremediation highlighting the drawbacks associated with conventional methodologies to eliminate MNPs

The aforementioned impact of MNPs on ecosystems necessitates the utilization of various methodologies to

tackle and reduce plastic contamination. Different conventional methods of eliminating plastic contaminants and their drawbacks are explained below:

1. Adsorption: In this technique, physical, chemical, or biological adsorbents such as carbon materials, zeolites, metal organic frameworks, and mesoporous materials are used to eliminate micro-nano plastic pollutants (Reineccius et al. 2021). MNPs can adhere to the surface of certain materials through physical interactions such as van der Waals forces, electrostatic interactions, and hydrophobic interactions. For example, a covalent organic framework like Tpa-H showed high adsorption energy for polyethylene, polyethylene terephthalate, and nylon-6 via molecular dynamics (Shang et al. 2022). MNPs can chemically bind to specific functional groups on the surface of adsorbent materials through covalent or coordinate bonding (Song et al. 2023). This mechanism is often employed using modified materials with functional groups such as amino groups, carboxyl groups, or sulfonic acid groups. For example, sulfate groups of polystyrene nanoplastics were degraded under UV radiation, thereby decreasing their electrostatic potential (Wang et al. 2020). The major disadvantage of using this technique is the generation of additional toxic wastes, and the cost of commercial adsorbents used for the treatment of MNPs is still high (De Gisi et al. 2016).
2. Coagulation: Different types of organic and inorganic coagulants are used for MNP removal, including ferric chloride, polyaluminum chloride, ferrous chloride, and polyamine (Zhou et al. 2021). These coagulants bind to microplastic particles by an uptake-complexation mechanism, thereby forming strong bonds with pollutants (Xu et al. 2021). For example, iron and aluminum coagulants were used for the removal of polyethylene microplastics under high polyacrylamide concentrations (Ariza-Tarazona et al. 2019). Also, metal hydroxide coagulants like iron and aluminum could help stabilize microplastics suspended in wastewater, thereby interacting via van der Waals forces to form sludge blankets (Perren et al. 2018). The major drawback of this method is its low selectivity; adsorbents are sensitive to pH, and competing ions tend to reduce the efficacy of adsorbents.
3. Membrane filtration: Dynamic membranes are utilized for influent flux and concentration of respective MNPs on the membrane during the process of filtration to enhance the removal of contaminants (Liu et al. 2021). For example, wastewater treatment plants were studied for their efficiency in removing microplastics in terms of shape, color, and dimensions using filtration processes. The microplastic removal efficiency reached approximately 90% (Ma et al. 2019). Another study showed that membrane bioreactors in combination with sand filters or disk filters, showed higher removal efficiency of microplastics when analyzed by Fourier transform infrared spectroscopy (FTIR) (Talvitie et al. 2017). The results revealed an abundance of polymers in the influent, with a high concentration of polyethylene terephthalate and polyester. The drawback of this technique is that the initial operational cost is high, and there is a need for post-treatment mineralization.
4. Microbial remediation: The technique of microbial remediation uses microorganisms to eliminate toxic MNPs from the environment (2018). Microbes produce enzymes, such as esterase, lipases, and proteases, which can break down the polymer chains of microplastics. These enzymes target specific chemical bonds present in plastics and initiate the process of degradation (Othman et al. 2021). Through enzymatic activity, microbes can gradually break down microplastics into smaller fragments. For example, *Phanerochaete chrysosporium* produces an enzyme, manganese peroxidase, which could help in the degradation of polyethylene (Kang et al. 2019). Also, *Ideonella sakaiensis 201-F6* was able to degrade polyethylene terephthalate by an enzyme called polyethylene terephthalate-ase (Yoshida et al. 2016). The method of microbial remediation is restricted to compounds that can easily biodegrade and is also time-consuming.

Importance of phytoremediation

The above-mentioned techniques are associated with drawbacks that facilitate the use of phytoremediation approaches which are considered eco-friendly and effective for elimination of pollutants from environment. Conventional methods used for exclusion of plastic pollutants are energy-dependent, time consuming, expensive, environmentally destructive and have adverse impact on ecosystem (Lourenço et al. 2019). Ability of various plants for phytoremediation is explored by numerous scientists (Rahbar et al. 2016; Rezania et al. 2016). Therefore, excessive interest is shown by researchers for improving the efficacy of conventionally used methods by an environmentally sound technique called phytoremediation. It refers to efficient green technology to dispose contaminants existing in air, water, and soil (Sarwar et al. 2017). Various plants act as efficient phytoremediators owing to their characteristic property of intake of pollutants and degradation by various bacteria and enzymes secreted by plant tissues (Chirakkara et al. 2016).

Objective: size of MNPs absorbed, mechanism of uptake and different approaches of phytoremediation for MNPs by plants

Size of MNPs absorbed by plants Plants can accumulate different sizes of MNP pollutants, thus acting as a potential source for remediation (Ebere et al. 2019). A study reported that nanoplastics ranging between 0 and 200 nm can be absorbed by plants internally and externally (Mateos-Cárdenas et al. 2021). Also, plants can uptake small microplastics (< 1000 µm) internally from xylem to phloem. Similarly, microplastics ranging in size from 1 to 5 mm can also be absorbed and transported to the aerial parts of plants from soil (Mateos-Cárdenas et al. 2021). Polystyrene microbeads (4.8 mm), for instance, were shown to be obstructing pores on the leaves, especially the root Garden cress (*Lepidium sativum* L.) hairs (Bosker et al. 2019). Additionally, there were microplastics of diameters 1–100 µm and 20 µm on the bladder wrack *Fucus vesiculosus* L., forming on its surface (Gutow et al. 2016), and the top layer of mucus (Sundbæk et al. 2018). Moreover, it has been observed that freshwater plants like *Lemna minor* L. may absorb < 600 µm polyethylene microbeads on the foliage of plants (Kalčíková et al. 2020). Wheat and lettuce are examples of agricultural plants that can internalize 200-nm polystyrene nanobeads and 2.0-µm polymethylmethacrylate microbeads (Li et al. 2020b). Plastic internalization appears to be influenced by particle charge. As demonstrated most recently by Wu et al. (2021a, b), *Arabidopsis thaliana* L. is capable of internalizing both positively and negatively charged 200-nm polystyrene beads in the stele of the root maturation zone. Micro-nanoplastics' (MNPs) absorption and internalization might have a significant impact on the environment.

Mechanisms of MNP uptake by plants The uptake of MNPs by plants is dependent on their physiological characteristics, demonstrating diverse absorption and accumulation inside plants and soil. Although very few studies have discussed the uptake mechanism of MNPs in plants, some evidence can be put forward through them. For example, one study observed the extracellular entrapment of polystyrene microbeads in the root caps of plant tissues. Furthermore, microbeads traversed from root to leaf parts through intercellular spaces via the vascular system following the transpiration stream (Li et al. 2019). Another study showed the absorption of microplastics within the endodermis of plant tissues through damaged root gaps and their transport to the aerial parts of the plant by transpiration (Sun et al. 2023). The apoplast and symplast are two pathways for MNPs to go through tissues once they have entered the plant. In contrast to symplastic transport, which involves movement of water and other substances between the cytoplasm of adjacent cells through specialized structures called plasmodesmata and sieve plates, apoplastic transport occurs outside the plasma membrane through extracellular spaces, the cell walls of adjacent cells, and xylem vessels (Pérez-de-Luque 2017). The apoplastic route is crucial for radial movement inside plant tissues and enables the passage of MNPs to vascular tissues and root central cylinder for further movement to the aerial portion (Li et al. 2020a). However, getting to the xylem through the root requires getting past a barrier to apoplastic pathway, the Casparian strip, which must be done by taking a symplastic route via endodermal cells. Using sieve tube components in the phloem, another significant symplastic transport is also conceivable, allowing distribution to non-photosynthetic tissues and organs (Liu et al. 2022b). Sieve tubes in phloem also provide a passage for MNP entry into the aerial parts of plants, as they have a thickness of approximately 0.77 to 1 µm, allowing small microplastics to traverse inside the plant tissues (Bussières 2014) (Fig. 2).

The uptake mechanisms of micro- and nanoplastics (MNPs) by plants can share similarities with the uptake mechanisms of other nanoparticles. While research on the specific uptake mechanisms of MNPs by plants is still emerging, studies on other nanoparticles provide insights into potential similarities. Here are a few examples of how the uptake mechanisms of MNPs may share similarities with the uptake mechanisms of other nanoparticles:

a. Size-dependent uptake: Plants can take up nanoparticles through various pathways, including root uptake, foliar uptake, and uptake through plant cell walls (Ali et al. 2021). This size-dependent uptake has been reported for a range of nanoparticles, including metal-based nanoparticles and carbon-based nanoparticles (Khan et al. 2022), and it may also apply to MNPs as postulated in different studies (Sun et al. 2021; Liu et al. 2022b; Huang et al. 2022).
b. Endocytosis: Some nanoparticles can be taken up by plants through endocytosis, which involves the internalization of particles by plasma membrane (Palocci et al. 2017). This mechanism has been reported for various nanoparticles, such as metal-based nanoparticles and quantum dots (Raven 2022). It is possible that MNPs can also be internalized by plants through endocytosis, for example, in rice seedlings exposed to polystyrene microplastics. The study observed accumulation of polystyrene microplastics in the roots of rice due to endocytosis (Wu et al. 2021a).
c. Passive diffusion: Nanoparticles can also passively diffuse across plant cell membranes. This process depends on the physicochemical properties of the particles, such as their size, surface charge, and hydrophobicity (Geisler-Lee et al. 2013). MNPs may exhibit similar passive diffusion mechanisms as other nanoparticles when entering plant cells (Xu et al. 2022).

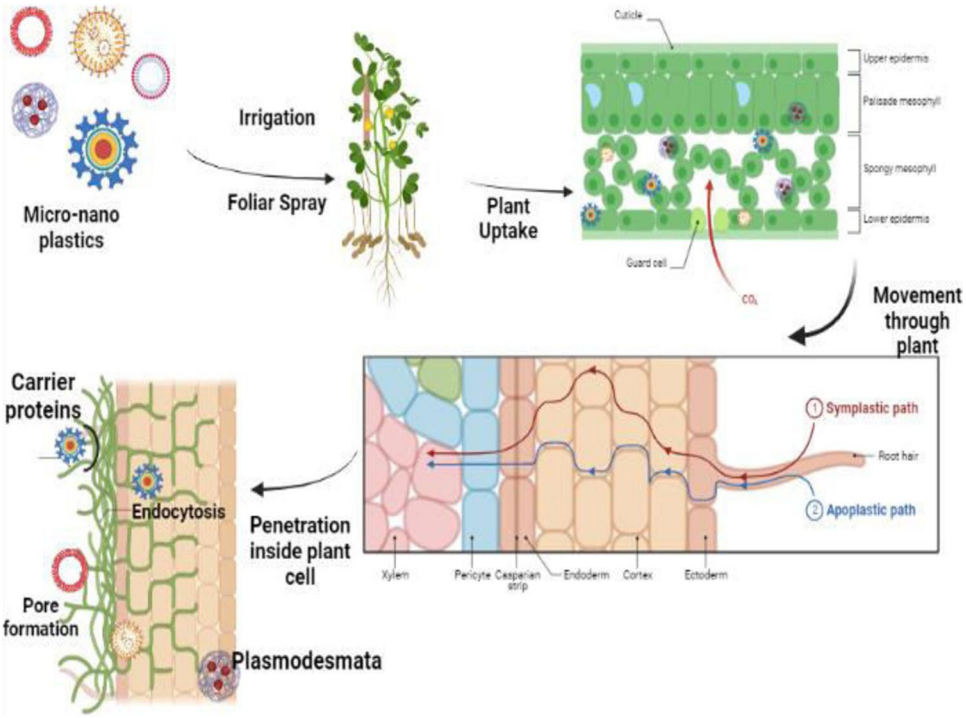

Fig. 2 Mechanism showing uptake of MNPs by plants from root hairs to plant cells and finally to inside parts of leaf through processes of endocytosis, intercellular spaces, plasmodesmata, carrier proteins, and pore formation

It is important to note that the uptake mechanisms of MNPs by plants are still an active area of research, and more studies are needed to understand the specific pathways and processes involved. Hence, the migration of MNPs inside plants is really important, as it gives an indication of the accumulation and absorption of MNPs on plant parts. Also, after the accumulation and absorption of MNPs, the phytoremediation potential can be determined to provide a sustainable solution for environmental cleanup.

Approaches of phytoremediation for MNPs Different approaches, including phytoextraction, phytostabilization, phytodegradation, phytovolatilization, and rhizosphere bioremediation, are employed by plants to facilitate the uptake of organic and inorganic pollutants from soil, thus forming the basis for phytoremediation technology. The major plants that are utilized for phytoremediation of MNPs are described in Table 1.

Phytoextraction Phytoextraction is a method to clean up contaminants from soil by absorption, accretion, and transfer of contaminants from soil to plant shoots, also referred to as phytomining (Tangahu et al. 2011). Plants act as hyper accumulators to absorb various pollutants in their shoots without any toxic effects on soil (Bian et al. 2020). An ideal hyper accumulator plant possesses the characteristic property of gathering large concentrations of MNPs within its shoots (Yu et al. 2021). For example, polystyrene microplastics and bisphenol-S showed no effect on *Pistis stratiotes* L. due to the accumulation of contaminants within the roots of plant and less translocation to other parts (Zhang et al. 2022). Another study showed accumulation of polystyrene

Table 1 Examples of different plants acting as potential sources for phytoremediation of various contaminants

Plant species	Accumulation part	Contaminant for remediation	Reference
Festuca arundinacea S	Shoots or roots	Petroleum hydrocarbon	Steliga and Kluk (2020)
Zea mays L	Roots	Phenanthrene	Baoune et al. (2019)
Chrysocoma Ciliate L	Roots	Petroleum aromatic hydrocarbons	Anyasi and Atagana (2018)
Lolium multiflorum L	Rhizosphere	Crude oil	Hussain et al. (2018)
Lolium perenne L	Rhizosphere microbes	Petroleum hydrocarbon	Iqbal et al. (2019)
Suaeda glauca L	Rhizosphere	Polycyclic aromatic hydrocarbons	Chaudhary et al. (2021a)
Iris dichotoma P	Roots	Petroleum hydrocarbon	Cheng et al. (2017)

microplastics within *Vicia faba* L. roots merely for around 48 h after being exposed to microplastics (Jiang et al. 2019).

Phytostabilization Phytostabilization, also referred to as phytoimmobilization, is a process of immobilizing contaminants in soil, roots, or shoots of plants thereby reducing their bioavailability in the environment (Tangahu et al. 2011). For example, polyethylene microplastics were able to adhere to aquatic macrophyte *L. minor*, due to surface stickiness and electrostatic interaction between MNPs and plant biomass (Rozman et al. 2022). Another study observed the immobilization of polystyrene microplastics in *F. vesiculosus* due to release of anionic polysaccharide on plant surface (Sundbæk et al. 2018). Also, microplastics captured within roots can reduce mobility and interaction with soil microorganisms and act as a potential source of phytostabilization. In a study by Gao et al. (2021), polyethylene microplastics adhered to root surface of *Lactuca sativa* L. without entering inside root hairs or other parts of the plant (Gao et al. 2021).

Phytovolatilization Phytovolatilization is a method that uses metabolic ability of plants and soil microorganism to change toxic plastic contaminants into volatile and less toxic forms, thereby releasing them into the atmosphere (Tangahu et al. 2011). While phytovolatilization is commonly associated with the uptake and release of organic compounds, such as volatile organic compounds (VOCs), there is limited research on its applicability to microplastics. Very few studies have investigated the potential for microplastic phytovolatilization by examining their uptake by plants as well as the subsequent release of volatile microplastic-associated compounds into the atmosphere. For example, laser confocal scanning microscopy and scanning electron microscopy provided evidence for the translocation of polystyrene nanoplastics from roots to shoots of *Triticum aestivum* L. without any effect on seed germination (Lian et al. 2020). Another study by Li et al. (2020a) observed transport of polystyrene and polymethylmethacrylate microplastics from roots to shoots of *T. aestivum* through crack-entry pathway and transpirational pull (Li et al. 2020a).

Phytodegradation Phytodegradation, also known as phytotransformation, is a method to decompose inorganic pollutants in soil by the application of enzymes such as oxygenases, nitroreductases, and dehydrogenases (Ali et al. 2013). The phytodegradation process occurs through the uptake of plastic contaminants within metabolic compartment of plants or microbes and their disintegration in soil. Degradation of pollutants occurs through two mechanisms: internal and external. In internal degradation mechanism, the MNPs are absorbed by plants that decompose through catalytic reactions by enzyme molecules, resulting in metabolic products utilized for plant growth and nutrition (Jeevanantham et al. 2019). In external degradation process, the plastic contaminants get absorbed by plant metabolic processes and hydrolyzed into smaller units (Jeevanantham et al. 2019). Formed monomer units are introduced into plant tissues for their growth and survival. For example, laccase and alkane hydrolase produced by *Staphylococcus epidermis* facilitated the depolymerization of polyethylene, forming monomer and oligomer units (Montazer et al. 2020). Also, the oxidase enzyme produced from *Pseudomonas vesicularis* PD could help in the degradation of polyvinyl chloride by oxidation of serine hydrolase active site present in polyvinyl chloride (Wilkes and Aristilde 2017).

Rhizosphere bioremediation Rhizosphere bioremediation is a process for eliminating MNPs from soil through their degradation and breakdown under the action of plant microorganisms (Jeevanantham et al. 2019). Besides, it is also referred to phytostimulation, rhizosphere degradation, and plant-assisted bioremediation (Kumar et al. 2018). The growth and proliferation of soil microorganisms occur due to the presence of carbohydrates in the soil. The different microorganisms employed for remediation of contaminants in soil are listed in Table 2. Various soil microorganisms can facilitate degradation of MNPs in soil. For example, *Bacillus cereus* and *Bacillus gottheilii* could change the structural properties of polyethylene microplastics (Auta et al. 2018). Also, *Pseudomonas capeferrum* TDA1 helped in the formation of a hydrolase enzyme that played an important role in the degradation of polyurethane (Puiggené et al. 2022). Root exudates also perform as excellent contributors to improving the degradation of pollutants by increasing their activity in rhizosphere. For example, MNPs induce stress with a negative influence on the growth of *T. aestivum* and genotoxic effects on *V. faba* (Jiang et al. 2019; Qi et al. 2020). Root exudates could alleviate the stress response in plants, thereby facilitating phytoremediation of MNPs.

Objective: factors influencing phytoremediation of MNPs

Phytoremediation, the use of plants to remediate pollutants from the environment, has gained attention as a potential method for addressing microplastic pollution. While research on phytoremediation of microplastics is still in its early stages, several factors are thought to influence the effectiveness of this approach like:

1. Plant species: Different plant species possess varying abilities to take up and accumulate microplastics (Colzi et al. 2022). Some plants may have higher affinity for microplastics due to their root structures or physiological characteristics. For example, certain aquatic plants

Table 2 Different microorganisms employed for remediation of various contaminants that are present in the soil

Microorganisms	Contaminant Remediation	Reference
Penicillium sp.	Low-density polyethylene (LDPE)	Rodrigo et al. (2021)
Klebsiella pneumoniae	High-density polyethylene (HDPE)	Awasthi et al. (2017)
Pseudomonas sp.	Polypropylene	Habib et al. (2020)
Penicillium sp.	Polyurethane	Magnin et al. (2019)
Vibrio sp.	Polyvinyl chloride	Khandare et al. (2021a)
Ideonella sakaiensis	Polyethylene terephthalate	Azubuike et al. (2016)
Lysinibacillus sp.	Polyethylene	Jeon et al. (2021)
Halomonas sp.	Low-density polyethylene (LDPE)	Khandare et al. (2021b)
Cephalosporium sp.	UV-treated polystyrene	Chaudhary et al. (2021b)

like water hyacinth (*Eichhornia crassipes* L.) and duckweed (*Lemna* spp.) have been found to effectively accumulate microplastics in water bodies (Christian and Beniah 2019; Rozman et al. 2022).

2. Root characteristics: The morphology and structure of plant roots can influence their ability to uptake microplastics. Plants with extensive root systems, such as those with fibrous or adventitious roots, have a larger surface area for interaction with microplastics. Plants with root exudates rich in enzymes and organic compounds may also enhance microbial activity around the roots, potentially facilitating microplastic degradation (Bosker et al. 2019). For instance, maize (*Zea mays* L.) and ryegrass (*Lolium perenne* L.) have shown promise in terms of their root characteristics for microplastic phytoremediation (Wang et al. 2012; Ullah et al. 2021).
3. Microplastic characteristics: The properties of microplastics, such as size, shape, and surface charge, can affect their interaction with plants. Smaller microplastics tend to have a larger surface area and may be more readily taken up by plants. Furthermore, the surface properties of microplastics can influence their adsorption to root surfaces and subsequent translocation within the plant (Wang et al. 2020; Xu et al. 2022).
4. Environmental conditions: Factors such as temperature, light intensity, and nutrient availability can impact the growth and metabolism of plants, which in turn may influence their ability to remediate microplastics (Gong et al. 2023). Certain environmental conditions may enhance plant-microplastic interactions or promote the activity of microorganisms involved in microplastic degradation (Ebere et al. 2019).

Hence, future research is necessitated to focus on phytoremediation techniques that are easy, inexpensive, and sustainable to environment. The merits and demerits of phytoremediation approaches are broadly listed in Table 3. Also, advanced strategies must be framed for effective phytoremediation of MNP contaminants.

Objective: proposed strategies for progressive phytoremediation of MNPs

Phytoremediation mechanisms offer great potential for the removal of MNPs. However, advanced strategies can

Table 3 Various approaches of phytoremediation highlighting their merits and demerits

Phytoremediation approach	Merits	Demerits
Phytoextraction	i. Cost-effective method compared to other strategies ii. Contaminant can be reused iii. Removal efficiency up to 95%	i. Enhanced uptake of plastic by roots ii. Leaches into groundwater iii. Phyto mass disposal is difficult
Phytovolatilization	i. Economically efficient method ii. Contaminant is less toxic	i. Redeposition of the contaminant back in the soil by precipitation
Phytostabilization	i. Low cost and efficient system ii. Reduction in soil erosion iii. Tolerates high concentration of pollutants	i. Soil not rendered suitable for plant growth ii. Obligatory checks necessary for effective remediation
Phytodegradation	i. Financially and economically stable system ii. Enzymatic breakdown of pollutants feasible	i. Dependent on soil abiotic conditions and plant species ii. Contaminants may re-emerge by soil microorganisms
Rhizosphere bioremediation	i. Microbial activity increases ii. Self-sustaining method for removal of pollutants iii. Environment friendly with low cost	i. Continuous monitoring of pH to uptake pollutants ii. Laboratory scale studies not stabilized for commercial and field purposes

deliver greater potential for phytoremediation to be effective (Fig. 3). Thus, various strategies have been proposed for efficient phytoremediation.

Selection of hyper accumulator plant species Exploration of various hyper accumulator plant species can revolutionize the technique of phytoremediation because of their ability to absorb contaminants 100 times more as compared to natural plants (Kumar Yadav et al. 2018). For example, *L. minor* is commonly used for phytoremediation of wastewater facilities, experimented with polystyrene nanoplastics to observe the impacts on accumulation and tolerance in plants. The results could provide direct evidence of no oxidative damage, unaltered chlorophyll contents, increased lipid peroxidation, and no growth suppression (Arikan et al. 2022). The polystyrene nanoplastics could only accumulate to some extent in the leaves of plants but not be translocated to other parts, thereby showing hyperaccumulation within specific plant parts (Arikan et al. 2022). Another study showed the accumulation of polystyrene nano- and microplastics within the root surface and cap cells of *A. thaliana* and *T. aestivum*. Laser confocal scanning microscopy and pyrolysis gas chromatography–mass spectrometry confirmed that polystyrene spheres accumulated only at root surface of each plant, without any evidence for internal uptake or accumulation (Taylor et al. 2020).

Utilization of plant growth–promoting bacteria for removal of MNPs Plants contain diverse microbial communities residing in the rhizosphere, phyllosphere, and endosphere (Feng et al. 2017). These microorganisms participate in essential roles for plant growth, nutrition, and degradation of contaminants (Kumar Yadav et al. 2018). For example, endophytic bacteria obtained from *Oryza meridionalis* L. were found to degrade phthalates, thereby reducing their accumulation in plants and increasing yield efficiency. A culture experiment containing various endophytic strains showed that the highest degradation of di-*n*-butyl-phthalate occurred using *Bacillus amyloliquefaciens*. The results confirmed the ability of endophytic bacterial strain to remove phthalates and promote plant growth and development (Liu et al. 2022a). Another study showed the isolation of *Achromobacter xylosoxidans* from soil that could degrade high-density polyethylene. Attenuated total reflectance Fourier transform infrared spectroscopy and scanning electron microscopy revealed degradation of microplastics by approximately 9% (Kowalczyk et al. 2016). Also, *Bacillus* spp. and *Rhodococcus* spp. strains isolated from mangrove sediments could help in degradation of polypropylene. Around 6% and 4% weight loss could be observed after 40 days of incubation in bacterial strains, which was confirmed by Fourier transform infrared spectroscopy and scanning electron microscopy

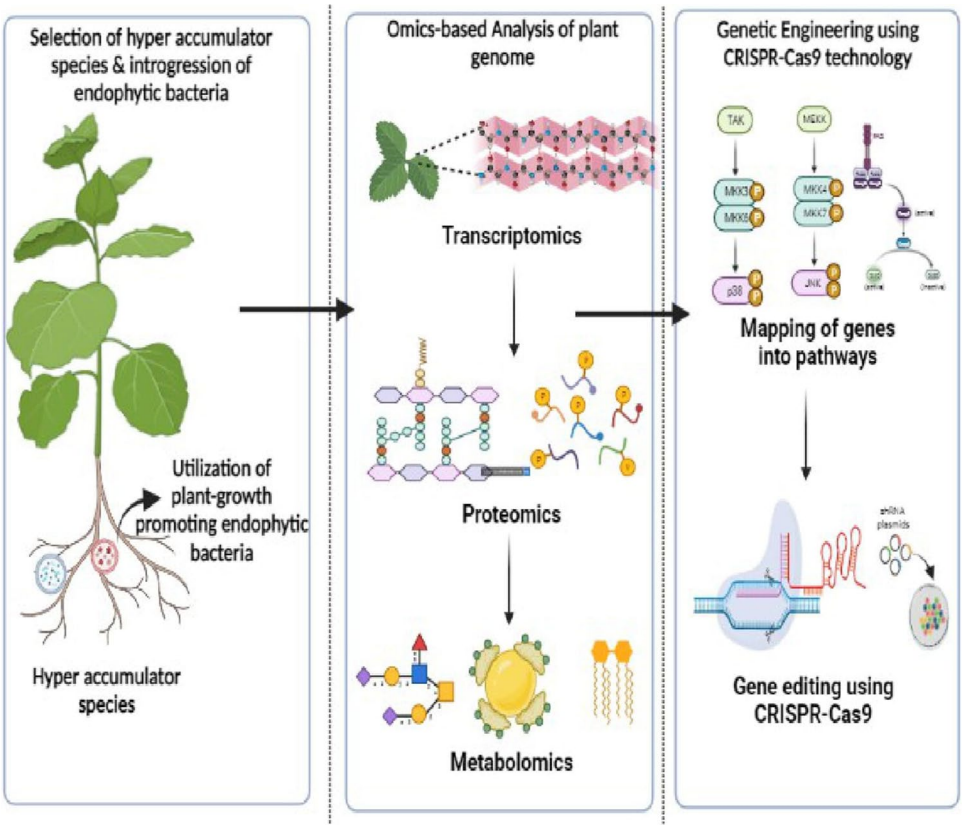

Fig. 3 Advanced strategies for phytoremediation of MNPs: selection of hyperaccumulator species, Omics-based analysis of plant genome, genetic engineering using Crispr-Cas9 technology

analysis (Auta et al. 2018). Thus, various bacterial strains could help in degradation of MNPs and increase plant growth yield.

Omics-based approaches to study MNPs degradation Plants respond differently to environmental conditions involving a range of routes, starting with changes in gene expression (transcriptomics), accumulation of protein products that help in degradation (proteomics), and formation of metabolites (metabolomics) (Forde and O'Toole, 2013). Metagenomics analysis could help in the identification of MNPs that degrade microbes and enzymes that could facilitate degradation (Staley and Sadowsky 2016). For example, cytochrome P450, esterase, and lipase enzymes were isolated from *Nocardioides* spp. and capable of degrading monoalkyl and dialkyl phthalate esters (Qiu et al. 2020). Apart from the identification of microbes for degradation, metabolic processes, gene identification, and expression are also essential for MNP degradation. For example, transcriptomics was applied to identify the mechanism of degradation of polyethylene by *Rhodococcus ruber* C20 strain (Gravouil et al. 2017). Another study showed the expression of pht and pca genes isolated from *Arthrobacter* sp. ZJUTW capable of degrading dibutyl phthalate. This study revealed a combination of metagenomic and metatranscriptomic studies to ascertain the degradation of phthalate (Liu et al. 2020). Besides transcriptomics and metagenomics, metaproteomics could also facilitate the mechanisms of protein synthesis that control metabolism and obtain metabolites (Medić et al. 2019). For example, proteomic profiling of *Pseudomonas pseudoalcaligenes* helped in the identification of a PpEst enzyme that could hydrolyze polybutylene adipate terephthalate (Wallace et al. 2017).

Gene Editing tools to increase MNP degradation Phytoremediation efficiency of plants can be increased by introducing plastic accumulating genetic determinants into the genomes of hyper accumulating species (DalCorso et al. 2019). Thus, genetic engineering tools can be explored to increase MNP accumulation by genes accountable for plastic uptake and their decontamination (Fasani et al. 2018). For example, *Ideonella sakaiensis* 201-F6 produces a polyethylene terephthalate-degrading enzyme. The genes of this bacterial strain can be genetically encoded in other bacterial strains to promote polyethylene terephthalate degradation (Anand et al. 2023). In a study by Moog et al. (2019), polyethylene terephthalate hydrolyzing enzymes were introduced into *Phaeodactylum tricornutum*, thereby showing efficient degradation (Moog et al. 2019). Apart from these genetic modifications, gene editing tools like clustered regularly interspaced palindromic repeats (CRISPR)/Cas9 could also promote manipulation of microbial species for faster degradation of MNPs. For example, *Streptomyces albogriseolus* LBX-2 could produce three different types of CRISPR sequences in which the main enzyme that helped in polyethylene degradation was oxygenase (Shao et al. 2019). Thus, genome editing could help in incorporating genes encoding MNP-degrading enzymes.

Objective: policies and future directions to address the knowledge gaps and provide substantial inputs on combating MNPs

Despite the cost-effective and eco-friendly approach to phytoremediation, challenges still continue in its implementation by the government and commercial sector (Saxena et al. 2020). Application of phytoremediation towards biotic or climatic factors, food-chain adulteration, and utilization of MNPs pollutants are some constraints in utilizing phytoremediation technology (Gunarathne and Lee 2019). Furthermore, low budgets by small-scale industries and short-term funds by government agencies limit the application of phytoremediation approaches on a wide scale. Furthermore, the molecular techniques of hyper accumulator species are not evidently described and may take a longer time span to degrade and remove MNP pollutants. Hence, establishing effective management strategies and low-cost processing technology for decontamination of MNPs pollutants is essential for efficient phytoremediation (Anand et al. 2023).

Policies to eliminate MNPs from the environment Soil inhabits various microorganisms that have proven to degrade MNPs. Bacterial strains are equipped for biodegrading plastics; however, bacterial consortia or biofilm offer less proficiency in the biodegradation processes, where a few strains are engaged with the disintegration and others are responsible for killing harmful metabolites discharged by the counterparts (Kumar et al. 2018). In spite of the fact that biodegradation by microorganisms appears to offer minimal expense and an eco-accommodating remediation approach, it remains a sluggish cycle for all intents and purposes profoundly reliant upon a few factors (biotic and abiotic).

1. One way to deal with advanced in situ plastic bioremediation is through bio stimulation (with the use of development supplements, manures, normal surfactants, and nanoparticles, alongside the improvement of ecological prerequisites) or potentially bioaugmentation (Fomina and Gadd 2014).
2. Another methodology incorporates applying current biotechnological procedures, for example, protein or chemical design. The advancement of enhanced microbial consortium, the use of hereditary design, systems science, and the use of hereditarily changed living beings are likely answers for further developing plastic biodegradation processes (Liu et al. 2020).

Notwithstanding, these creative remediation approaches do not tackle plastic contamination and should be joined by viable moderation methodologies that focus on source reduction. This could be accomplished by (I) fixing plastic decrease strategies underscoring a diminishing use furthermore; (ii) streamlining waste executive frameworks; (iii) looking for economical plastics to guarantee their environmental amicability; and (iv) expanding public awareness on plastic contamination alongside a social shift.

Conclusion

Micro-nano plastic pollution has increased dramatically in recent years, posing a threat to ecosystem diversity. Phytoremediation is a completely natural method for removing MNPs from agricultural ecosystems and restoring soil productivity and plant health. To determine the best use of phytoremediation technologies, it is required to investigate the fate of MNPs, their absorption and migration inside plant parts, trafficking along membranes, tolerance, and behavior in the rhizosphere under various environmental conditions. Plant species, root properties, MNP size, and environmental variables all influence MNP uptake and phytoremediation. Furthermore, advanced phytoremediation tactics concentrating on the use of hyperaccumulator plant species, the use of plant-growth boosting bacteria, omics-based investigations, and genetic engineering CRISPR-Cas9 technology are effective methodologies for MNP ecosystem restoration. The use of microbial and enzymatic substances in the breakdown of MNP has the potential to solve this problem on a broad scale. As a result, a thorough understanding of these mechanisms is required for MNP-contaminated soil. Furthermore, the review's restrictions and future possibilities could be critical in creating cost-effective and environmentally acceptable ways for comprehensive MNP degradation in terrestrial ecosystems. More research on the impact of MNPs on soil and developing an integrated approach to plant-based technologies for monitoring, assessment, and remediation of MNPs in terrestrial agroecosystems is needed.

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Ethics approval Not applicable.

Consent to participate Not applicable.

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Competing interests The authors declare no competing interests.

References

(2018) Advantages and disadvantages of bioremediation. https://agriculturistmusa.com/advantages-and-disadvantages-of-bioremediation/. Accessed 20 Jun 2022

Agnihotri A, Gupta P, Dwivedi A, Seth CS (2018) Counteractive mechanism (s) of salicylic acid in response to lead toxicity in Brassica juncea (L.) Czern. cv. Varuna Planta 248:49–68. https://doi.org/10.1007/s00425-018-2867-0

Ali H, Khan E, Sajad MA (2013) Phytoremediation of heavy metals—concepts and applications. Chemosphere 91:869–881. https://doi.org/10.1016/j.chemosphere.2013.01.075

Ali S, Mehmood A, Khan N (2021) Uptake, Translocation, and consequences of nanomaterials on plant growth and stress adaptation. J Nanomater 2021:e6677616. https://doi.org/10.1155/2021/6677616

Anand U, Dey S, Bontempi E et al (2023) Biotechnological methods to remove microplastics: a review. Environ Chem Lett 21:1787–1810. https://doi.org/10.1007/s10311-022-01552-4

Anyasi RO, Atagana HI (2018) Profiling of plants at petroleum contaminated site for phytoremediation. Int J Phytoremediation 20:352–361. https://doi.org/10.1080/15226514.2017.1393386

Arikan B, Alp FN, Ozfidan-Konakci C et al (2022) The impacts of nanoplastic toxicity on the accumulation, hormonal regulation and tolerance mechanisms in a potential hyperaccumulator - Lemna minor L. J Hazard Mater 440:129692. https://doi.org/10.1016/j.jhazmat.2022.129692

Ariza-Tarazona MC, Villarreal-Chiu JF, Barbieri V et al (2019) New strategy for microplastic degradation: green photocatalysis using a protein-based porous N-TiO2 semiconductor. Ceram Int 45:9618–9624. https://doi.org/10.1016/j.ceramint.2018.10.208

Auta HS, Emenike CU, Jayanthi B, Fauziah SH (2018) Growth kinetics and biodeterioration of polypropylene microplastics by Bacillus sp. and Rhodococcus sp. isolated from mangrove sediment. Mar Pollut Bull 127:15–21. https://doi.org/10.1016/j.marpolbul.2017.11.036

Awasthi S, Srivastava P, Singh P et al (2017) Biodegradation of thermally treated high-density polyethylene (HDPE) by Klebsiella pneumoniae CH001. 3 Biotech 7:332. https://doi.org/10.1007/s13205-017-0959-3

Azubuike CC, Chikere CB, Okpokwasili GC (2016) Bioremediation techniques–classification based on site of application: principles, advantages, limitations and prospects. World J Microbiol Biotechnol 32:180. https://doi.org/10.1007/s11274-016-2137-x

Banerjee K, Pramanik P, Maity A et al (2019) Chapter 4 - methods of using nanomaterials to plant systems and their delivery to plants (mode of entry, uptake, translocation, accumulation, biotransformation and barriers). In: Ghorbanpour M, Wani SH (eds) Advances in Phytonanotechnology. Academic Press, pp 123–152

Bansal M, Santhiya D, Sharma JG (2021) Behavioural mechanisms of microplastic pollutants in marine ecosystem: challenges and remediation measurements. Water Air Soil Pollut 232:372. https://doi.org/10.1007/s11270-021-05301-1

Bansal M, Santhiya D, Sharma JG (2023) Exploring the impacts of HDPE microplastics on growth and physiological behavior of Brassica juncea (Mustard Plant). Water Air Soil Pollut 234:520. https://doi.org/10.1007/s11270-023-06548-6

Baoune H, Aparicio JD, Acuña A et al (2019) Effectiveness of the Zea mays-Streptomyces association for the phytoremediation of petroleum hydrocarbons impacted soils. Ecotoxicol Environ Saf 184:109591. https://doi.org/10.1016/j.ecoenv.2019.109591

Bhattacharya P, Lin S, Turner JP, Ke PC (2010) Physical adsorption of charged plastic nanoparticles affects algal photosynthesis. J Phys Chem C 114:16556–16561. https://doi.org/10.1021/jp1054759

Bian F, Zhong Z, Zhang X et al (2020) Bamboo – an untapped plant resource for the phytoremediation of heavy metal contaminated soils. Chemosphere 246:125750. https://doi.org/10.1016/j.chemosphere.2019.125750

Bosker T, Bouwman LJ, Brun NR et al (2019) Microplastics accumulate on pores in seed capsule and delay germination and root growth of the terrestrial vascular plant Lepidium sativum. Chemosphere 226:774–781. https://doi.org/10.1016/j.chemosphere.2019.03.163

Boucher J, Friot D (2017) Primary microplastics in the oceans: a global evaluation of sources. IUCN International Union for Conservation of Nature

Burstein D, Harrington LB, Strutt SC et al (2017) New CRISPR–Cas systems from uncultivated microbes. Nature 542:237–241. https://doi.org/10.1038/nature21059

Bussières P (2014) Estimating the number and size of phloem sieve plate pores using longitudinal views and geometric reconstruction. Sci Rep 4:4929. https://doi.org/10.1038/srep04929

Chaudhary AK, Chaitanya K, Vijayakumar RP (2021a) Synergistic effect of UV and chemical treatment on biological degradation of Polystyrene by Cephalosporium strain NCIM 1251. Arch Microbiol 203:2183–2191. https://doi.org/10.1007/s00203-021-02228-3

Chaudhary AK, Chaitanya K, Vijayakumar RP (2021b) Synergistic effect of UV and chemical treatment on biological degradation of Polystyrene by Cephalosporium strain NCIM 1251. Arch Microbiol 203:2183–2191. https://doi.org/10.1007/s00203-021-02228-3

Cheng L, Wang Y, Cai Z et al (2017) Phytoremediation of petroleum hydrocarbon-contaminated saline-alkali soil by wild ornamental Iridaceae species. Int J Phytoremediation 19:300–308. https://doi.org/10.1080/15226514.2016.1225282

Chirakkara RA, Cameselle C, Reddy KR (2016) Assessing the applicability of phytoremediation of soils with mixed organic and heavy metal contaminants. Rev Environ Sci Biotechnol 15:299–326. https://doi.org/10.1007/s11157-016-9391-0

Christian E, Beniah I (2019) Phytoremediation of polluted waterbodies with aquatic plants: recent progress on heavy metal and organic pollutants. https://doi.org/10.20944/preprints201909.0020.v1

Colzi I, Renna L, Bianchi E et al (2022) Impact of microplastics on growth, photosynthesis and essential elements in Cucurbita pepo L. J Hazard Mater 423:127238. https://doi.org/10.1016/j.jhazmat.2021.127238

DalCorso G, Fasani E, Manara A et al (2019) Heavy metal pollutions: state of the art and innovation in phytoremediation. Int J Mol Sci 20:3412. https://doi.org/10.3390/ijms20143412

De Gisi S, Lofrano G, Grassi M, Notarnicola M (2016) Characteristics and adsorption capacities of low-cost sorbents for wastewater treatment: a review. Sustain Mater Technol 9:10–40. https://doi.org/10.1016/j.susmat.2016.06.002

de Souza Machado AA, Lau CW, Kloas W et al (2019) Microplastics can change soil properties and affect plant performance. Environ Sci Technol 53:6044–6052. https://doi.org/10.1021/acs.est.9b01339

Dovidat LC, Brinkmann BW, Vijver MG, Bosker T (2020) Plastic particles adsorb to the roots of freshwater vascular plant Spirodela polyrhiza but do not impair growth. Limnol Oceanogr Lett 5:37–45. https://doi.org/10.1002/lol2.10118

Ebere EC, Wirnkor VA, Ngozi VE (2019) Uptake of microplastics by plant: a reason to worry or to be happy? 12

Enyoh CE, Verla AW, Verla EN et al (2019) Airborne microplastics: a review study on method for analysis, occurrence, movement and risks. Environ Monit Assess 191:668. https://doi.org/10.1007/s10661-019-7842-0

Fasani E, Manara A, Martini F et al (2018) The potential of genetic engineering of plants for the remediation of soils contaminated with heavy metals. Plant Cell Environ 41:1201–1232. https://doi.org/10.1111/pce.12963

Feng N-X, Yu J, Zhao H-M et al (2017) Efficient phytoremediation of organic contaminants in soils using plant–endophyte partnerships. Sci Total Environ 583:352–368. https://doi.org/10.1016/j.scitotenv.2017.01.075

Fomina M, Gadd GM (2014) Biosorption: current perspectives on concept, definition and application. Biores Technol 160:3–14. https://doi.org/10.1016/j.biortech.2013.12.102

Forde BM, O'Toole PW (2013) Next-generation sequencing technologies and their impact on microbial genomics. Brief Funct Genomics 12(5):440–453. https://doi.org/10.1093/bfgp/els062

Galloway TS, Cole M, Lewis C (2017) Interactions of microplastic debris throughout the marine ecosystem. Nat Ecol Evol 1:1–8. https://doi.org/10.1038/s41559-017-0116

Gao M, Liu Y, Dong Y, Song Z (2021) Effect of polyethylene particles on dibutyl phthalate toxicity in lettuce (Lactuca sativa L.). J Hazard Mater 401:123422. https://doi.org/10.1016/j.jhazmat.2020.123422

Geisler-Lee J, Wang Q, Yao Y et al (2013) Phytotoxicity, accumulation and transport of silver nanoparticles by Arabidopsis thaliana. Nanotoxicology 7:323–337. https://doi.org/10.3109/17435390.2012.658094

Gong X, Shi G, Zou D et al (2023) Micro- and nano-plastics pollution and its potential remediation pathway by phytoremediation. Planta 257:35. https://doi.org/10.1007/s00425-023-04069-4

Gravouil K, Ferru-Clément R, Colas S et al (2017) Transcriptomics and lipidomics of the environmental strain rhodococcus ruber point out consumption pathways and potential metabolic bottlenecks for polyethylene degradation. Environ Sci Technol 51:5172–5181. https://doi.org/10.1021/acs.est.7b00846

Gunarathne ADN, Lee K-H (2019) Environmental and managerial information for cleaner production strategies: an environmental management development perspective. J Clean Prod 237:117849. https://doi.org/10.1016/j.jclepro.2019.117849

Guo J-J, Huang X-P, Xiang L et al (2020) Source, migration and toxicology of microplastics in soil. Environ Int 137:105263. https://doi.org/10.1016/j.envint.2019.105263

Gupta P, Seth CS (2023) 24-epibrassinolide regulates functional components of nitric oxide signalling and antioxidant defense pathways to alleviate salinity stress in Brassica juncea L. cv. Varuna J Plant Growth Regul 42:4207–4222. https://doi.org/10.1007/s00344-022-10884-y

Gutow L, Eckerlebe A, Giménez L, Saborowski R (2016) Experimental evaluation of seaweeds as a vector for microplastics into marine food webs. Environ Sci Technol 50:915–923. https://doi.org/10.1021/acs.est.5b02431

Habib S, Iruthayam A, Abd Shukor MY et al (2020) Biodeterioration of untreated polypropylene microplastic particles by antarctic bacteria. Polymers 12:2616. https://doi.org/10.3390/polym12112616

Haegerbaeumer A, Mueller M-T, Fueser H, Traunspurger W (2019) Impacts of micro- and nano-sized plastic particles on benthic invertebrates: a literature review and gap analysis. Front Environ Sci 7

He S, Jia M, Xiang Y et al (2022) Biofilm on microplastics in aqueous environment: physicochemical properties and environmental implications. J Hazard Mater 424:127286. https://doi.org/10.1016/j.jhazmat.2021.127286

Huang D, Chen H, Shen M et al (2022) Recent advances on the transport of microplastics/nanoplastics in abiotic and biotic compartments. J Hazard Mater 438:129515. https://doi.org/10.1016/j.jhazmat.2022.129515

Hussain F, Hussain I, Khan AHA et al (2018) Combined application of biochar, compost, and bacterial consortia with Italian ryegrass enhanced phytoremediation of petroleum hydrocarbon contaminated soil. Environ Exp Bot 153:80–88. https://doi.org/10.1016/j.envexpbot.2018.05.012

Iqbal A, Mukherjee M, Rashid J et al (2019) Development of plant-microbe phytoremediation system for petroleum hydrocarbon degradation: An insight from alkb gene expression and phytotoxicity analysis. Sci Total Environ 671:696–704. https://doi.org/10.1016/j.scitotenv.2019.03.331

Jeevanantham S, Saravanan A, Hemavathy RV et al (2019) Removal of toxic pollutants from water environment by phytoremediation: a survey on application and future prospects. Environ Technol Innov 13:264–276. https://doi.org/10.1016/j.eti.2018.12.007

Jeon J-M, Park S-J, Choi T-R et al (2021) Biodegradation of polyethylene and polypropylene by Lysinibacillus species JJY0216 isolated from soil grove. Polym Degrad Stab 191:109662. https://doi.org/10.1016/j.polymdegradstab.2021.109662

Jeyavani J, Sibiya A, Shanthini S et al (2021) A review on aquatic impacts of microplastics and its bioremediation aspects. Curr Pollution Rep 7:286–299. https://doi.org/10.1007/s40726-021-00188-2

Jiang X, Chen H, Liao Y et al (2019) Ecotoxicity and genotoxicity of polystyrene microplastics on higher plant Vicia faba. Environ Pollut 250:831–838. https://doi.org/10.1016/j.envpol.2019.04.055

Junhao C, Xining Z, Xiaodong G et al (2021) Extraction and identification methods of microplastics and nanoplastics in agricultural soil: a review. J Environ Manage 294:112997. https://doi.org/10.1016/j.jenvman.2021.112997

Kalčíková G, Skalar T, Marolt G, Jemec Kokalj A (2020) An environmental concentration of aged microplastics with adsorbed silver significantly affects aquatic organisms. Water Res 175:115644. https://doi.org/10.1016/j.watres.2020.115644

Kang BR, Kim SB, Song HA, Lee TK (2019) Accelerating the biodegradation of high-density polyethylene (HDPE) using Bjerkandera adusta TBB-03 and lignocellulose substrates. Microorganisms 7:304. https://doi.org/10.3390/microorganisms7090304

Khan I, Awan SA, Rizwan M et al (2022) Nanoparticle's uptake and translocation mechanisms in plants via seed priming, foliar treatment, and root exposure: a review. Environ Sci Pollut Res 29:89823–89833. https://doi.org/10.1007/s11356-022-23945-2

Khandare SD, Chaudhary DR, Jha B (2021a) Bioremediation of polyvinyl chloride (PVC) films by marine bacteria. Mar Pollut Bull 169:112566. https://doi.org/10.1016/j.marpolbul.2021.112566

Khandare SD, Chaudhary DR, Jha B (2021b) Marine bacterial biodegradation of low-density polyethylene (LDPE) plastic. Biodegradation 32:127–143. https://doi.org/10.1007/s10532-021-09927-0

Kowalczyk A, Chyc M, Ryszka P, Latowski D (2016) Achromobacter xylosoxidans as a new microorganism strain colonizing high-density polyethylene as a key step to its biodegradation. Environ Sci Pollut Res 23:11349–11356. https://doi.org/10.1007/s11356-016-6563-y

Kumar V, Shahi SK, Singh S (2018) Bioremediation: an eco-sustainable approach for restoration of contaminated sites. In: Singh J, Sharma D, Kumar G, Sharma NR (eds) Microbial Bioprospecting for Sustainable Development. Springer, Singapore, pp 115–136

Kumar D, Dhankher OP, Tripathi RD, Seth CS (2023) Titanium dioxide nanoparticles potentially regulate the mechanism(s) for photosynthetic attributes, genotoxicity, antioxidants defense machinery, and phytochelatins synthesis in relation to hexavalent chromium toxicity in Helianthus annuus L. J Hazard Mater 454:131418. https://doi.org/10.1016/j.jhazmat.2023.131418

Kumar Yadav K, Gupta N, Kumar A et al (2018) Mechanistic understanding and holistic approach of phytoremediation: a review on application and future prospects. Ecol Eng 120:274–298. https://doi.org/10.1016/j.ecoleng.2018.05.039

Li L, Zhou Q, Yin N et al (2019) Uptake and accumulation of microplastics in an edible plant. Kexue Tongbao/chinese Sci Bull 64:928–934. https://doi.org/10.1360/N972018-00845

Li L, Luo Y, Li R et al (2020) Effective uptake of submicrometre plastics by crop plants via a crack-entry mode. Nat Sustain 3:929–937. https://doi.org/10.1038/s41893-020-0567-9

Li Q, Feng Z, Zhang T et al (2020) Microplastics in the commercial seaweed nori. J Hazard Mater 388:122060. https://doi.org/10.1016/j.jhazmat.2020.122060

Lian J, Wu J, Xiong H et al (2020) Impact of polystyrene nanoplastics (PSNPs) on seed germination and seedling growth of wheat (Triticum aestivum L.). J Hazard Mater 385:121620. https://doi.org/10.1016/j.jhazmat.2019.121620

Liu J, Zhu L, Luo S et al (2014) Response of nitrous oxide emission to soil mulching and nitrogen fertilization in semi-arid farmland. Agr Ecosyst Environ 188:20–28. https://doi.org/10.1016/j.agee.2014.02.010

Liu T, Li J, Qiu L et al (2020) Combined genomic and transcriptomic analysis of the dibutyl phthalate metabolic pathway in Arthrobacter sp. ZJUTW Biotechnol Bioeng 117:3712–3726. https://doi.org/10.1002/bit.27524

Liu W, Zhang J, Liu H et al (2021) A review of the removal of microplastics in global wastewater treatment plants: characteristics and mechanisms. Environ Int 146:106277. https://doi.org/10.1016/j.envint.2020.106277

Liu L-H, Yuan T, Zhang J-Y et al (2022) Diversity of endophytic bacteria in wild rice (Oryza meridionalis) and potential for promoting plant growth and degrading phthalates. Sci Total Environ 806:150310. https://doi.org/10.1016/j.scitotenv.2021.150310

Liu Y, Guo R, Zhang S et al (2022) Uptake and translocation of nano/microplastics by rice seedlings: evidence from a hydroponic experiment. J Hazard Mater 421:126700. https://doi.org/10.1016/j.jhazmat.2021.126700

Lourenço J, Mendo S, Pereira R (2019) Rehabilitation of radioactively contaminated soil: use of bioremediation/phytoremediation techniques. In: Gupta DK, Voronina A (eds) Remediation Measures for Radioactively Contaminated Areas. Springer International Publishing, Cham, pp 163–200

Luo Z, Zhou X, Su Y et al (2021) Environmental occurrence, fate, impact, and potential solution of tire microplastics: similarities and differences with tire wear particles. Sci Total Environ 795:148902. https://doi.org/10.1016/j.scitotenv.2021.148902

Ma B, Xue W, Ding Y et al (2019) Removal characteristics of microplastics by Fe-based coagulants during drinking water treatment. J Environ Sci 78:267–275. https://doi.org/10.1016/j.jes.2018.10.006

Magnin A, Hoornaert L, Pollet E et al (2019) Isolation and characterization of different promising fungi for biological waste management of polyurethanes. Microb Biotechnol 12:544–555. https://doi.org/10.1111/1751-7915.13346

Mammo FK, Amoah ID, Gani KM et al (2020) Microplastics in the environment: interactions with microbes and chemical contaminants. Sci Total Environ 743:140518. https://doi.org/10.1016/j.scitotenv.2020.140518

Managing plastic waste in India. https://www.cseindia.org/managing-plastic-waste-in-india-10352. Accessed 8 Jul 2021

Mateos-Cárdenas A, van Pelt FNAM, O'Halloran J, Jansen MAK (2021) Adsorption, uptake and toxicity of micro- and nanoplastics: effects on terrestrial plants and aquatic macrophytes.

Environ Pollut 284:117183. https://doi.org/10.1016/j.envpol.2021.117183
Medić A, Stojanović K, Izrael-Živković L et al (2019) A comprehensive study of conditions of the biodegradation of a plastic additive 2,6-di- tert -butylphenol and proteomic changes in the degrader Pseudomonas aeruginosa san ai. RSC Adv 9:23696–23710. https://doi.org/10.1039/C9RA04298A
Montazer Z, Habibi Najafi MB, Levin DB (2020) Challenges with verifying microbial degradation of polyethylene. Polymers 12:123. https://doi.org/10.3390/polym12010123
Moog D, Schmitt J, Senger J et al (2019) Using a marine microalga as a chassis for polyethylene terephthalate (PET) degradation. Microb Cell Fact 18:171. https://doi.org/10.1186/s12934-019-1220-z
Ng E-L, Huerta Lwanga E, Eldridge SM et al (2018) An overview of microplastic and nanoplastic pollution in agroecosystems. Sci Total Environ 627:1377–1388. https://doi.org/10.1016/j.scitotenv.2018.01.341
Othman AR, Hasan HA, Muhamad MH et al (2021) Microbial degradation of microplastics by enzymatic processes: a review. Environ Chem Lett 19:3057–3073. https://doi.org/10.1007/s10311-021-01197-9
Palocci C, Valletta A, Chronopoulou L et al (2017) Endocytic pathways involved in PLGA nanoparticle uptake by grapevine cells and role of cell wall and membrane in size selection. Plant Cell Rep 36:1917–1928. https://doi.org/10.1007/s00299-017-2206-0
Pérez-de-Luque A (2017) Interaction of nanomaterials with plants: what do we need for real applications in agriculture? Front Environ Sci 5:12. https://doi.org/10.3389/fenvs.2017.00012
Perren W, Wojtasik A, Cai Q (2018) Removal of microbeads from wastewater using electrocoagulation. ACS Omega 3:3357–3364. https://doi.org/10.1021/acsomega.7b02037
Pflugmacher S, Sulek A, Mader H et al (2020) The influence of new and artificial aged microplastic and leachates on the germination of Lepidium sativum L. Plants 9:339. https://doi.org/10.3390/plants9030339
Pfohl P, Wagner M, Meyer L et al (2022) Environmental degradation of microplastics: how to measure fragmentation rates to secondary micro- and nanoplastic fragments and dissociation into dissolved organics. Environ Sci Technol 56:11323–11334. https://doi.org/10.1021/acs.est.2c01228
Prata JC, da Costa JP, Lopes I et al (2020) Environmental exposure to microplastics: an overview on possible human health effects. Sci Total Environ 702:134455. https://doi.org/10.1016/j.scitotenv.2019.134455
Prokin AA, Dubov PG, Bolotov SE (2015) Formation of macroinvertebrates communities in duckweed (Lemnaceae) and artificial surface-floating substrate: results of the experiment under natural conditions. Inland Water Biol 8:373–383. https://doi.org/10.1134/S1995082915040136
Puiggené Ò, Espinosa MJC, Schlosser D et al (2022) Extracellular degradation of a polyurethane oligomer involving outer membrane vesicles and further insights on the degradation of 2,4-diaminotoluene in Pseudomonas capeferrum TDA1. Sci Rep 12:2666. https://doi.org/10.1038/s41598-022-06558-0
Qi Y, Ossowicki A, Yang X et al (2020) Effects of plastic mulch film residues on wheat rhizosphere and soil properties. J Hazard Mater 387:121711. https://doi.org/10.1016/j.jhazmat.2019.121711
Qiu J, Zhang Y, Shi Y et al (2020) Identification and characterization of a novel phthalate-degrading hydrolase from a soil metagenomic library. Ecotoxicol Environ Saf 190:110148. https://doi.org/10.1016/j.ecoenv.2019.110148
Rahbar A, Farjadfard S, Leili M et al (2016) Experimental data of biomaterial derived from Malva sylvestris and charcoal tablet powder for Hg2+ removal from aqueous solutions. Data Brief 8:132–135. https://doi.org/10.1016/j.dib.2016.05.032
Raven JA (2022) Commentary on the use of nutrient-coated quantum dots as a means of tracking nutrient uptake by and movement within plants. Plant Soil 476:535–548. https://doi.org/10.1007/s11104-022-05507-0
Reineccius J, Bresien J, Waniek JJ (2021) Separation of microplastics from mass-limited samples by an effective adsorption technique. Sci Total Environ 788:147881. https://doi.org/10.1016/j.scitotenv.2021.147881
Rezania S, Taib SM, Md Din MF et al (2016) Comprehensive review on phytotechnology: heavy metals removal by diverse aquatic plants species from wastewater. J Hazard Mater 318:587–599. https://doi.org/10.1016/j.jhazmat.2016.07.053
Rodrigo O-A, del Castillo Daynet S, Jaime N-M et al (2021) Analysis of the degradation of polyethylene, polystyrene and polyurethane mediated by three filamentous fungi isolated from the Antarctica. Afr J Biotechnol 20:66–76. https://doi.org/10.5897/AJB2020.17200
Rozman U, Jemec Kokalj A, Dolar A et al (2022) Long-term interactions between microplastics and floating macrophyte Lemna minor: the potential for phytoremediation of microplastics in the aquatic environment. Sci Total Environ 831:154866. https://doi.org/10.1016/j.scitotenv.2022.154866
Sarwar N, Imran M, Shaheen MR et al (2017) Phytoremediation strategies for soils contaminated with heavy metals: modifications and future perspectives. Chemosphere 171:710–721. https://doi.org/10.1016/j.chemosphere.2016.12.116
Saxena G, Purchase D, Mulla SI et al (2020) Phytoremediation of heavy metal-contaminated sites: eco-environmental concerns, field studies, sustainability issues, and future prospects. In: de Voogt P (ed) Reviews of Environmental Contamination and Toxicology, vol 249. Springer International Publishing, Cham, pp 71–131
Shang S, Liu Y, Liu M et al (2022) Studying the adsorption mechanisms of nanoplastics on covalent organic frameworks via molecular dynamics simulations. J Hazard Mater 421:126796. https://doi.org/10.1016/j.jhazmat.2021.126796
Shao H, Chen M, Fei X et al (2019) Complete genome sequence and characterization of a polyethylene biodegradation strain, Streptomyces Albogriseolus LBX-2. Microorganisms 7:379. https://doi.org/10.3390/microorganisms7100379
Song Z, Zhao X, Dong Y et al (2023) Effects of polystyrene nanoplastics with different functional groups on the accumulation and toxicity of Pb on dandelion. Chemosphere 310:136874. https://doi.org/10.1016/j.chemosphere.2022.136874
Staley C, Sadowsky MJ (2016) Application of metagenomics to assess microbial communities in water and other environmental matrices. J Mar Biol Assoc UK 96:121–129. https://doi.org/10.1017/S0025315415001496
Steliga T, Kluk D (2020) Application of Festuca arundinacea in phytoremediation of soils contaminated with Pb, Ni, Cd and petroleum hydrocarbons. Ecotoxicol Environ Saf 194:110409. https://doi.org/10.1016/j.ecoenv.2020.110409
Sun H, Lei C, Xu J, Li R (2021) Foliar uptake and leaf-to-root translocation of nanoplastics with different coating charge in maize plants. J Hazard Mater 416:125854. https://doi.org/10.1016/j.jhazmat.2021.125854
Sun L, Wang X, Zhao H et al (2023) Micro/nanoplastics: a potential threat to crops. V 3. https://doi.org/10.48130/VR-2023-0018
Sundbæk KB, Koch IDW, Villaro CG et al (2018) Sorption of fluorescent polystyrene microplastic particles to edible seaweed Fucus vesiculosus. J Appl Phycol 30:2923–2927. https://doi.org/10.1007/s10811-018-1472-8

Talvitie J, Mikola A, Koistinen A, Setälä O (2017) Solutions to microplastic pollution – removal of microplastics from wastewater effluent with advanced wastewater treatment technologies. Water Res 123:401–407. https://doi.org/10.1016/j.watres.2017.07.005

Tangahu BV, Sheikh Abdullah SR, Basri H et al (2011) A review on heavy metals (As, Pb, and Hg) uptake by plants through phytoremediation. Int J Chem Eng 2011:e939161. https://doi.org/10.1155/2011/939161

Taylor SE, Pearce CI, Sanguinet KA et al (2020) Polystyrene nano- and microplastic accumulation at Arabidopsis and wheat root cap cells, but no evidence for uptake into roots. Environ Sci Nano 7:1942–1953. https://doi.org/10.1039/D0EN00309C

Teng M, Zhao X, Wang C et al (2022) Polystyrene nanoplastics toxicity to zebrafish: dysregulation of the brain–intestine–microbiota axis. ACS Nano 16:8190–8204. https://doi.org/10.1021/acsnano.2c01872

Tian C, Lv J, Zhang W et al (2022) Accelerated degradation of microplastics at the liquid interface of ice crystals in frozen aqueous solutions. Angew Chem Int Ed 61:e202206947. https://doi.org/10.1002/anie.202206947

Tun TZ, Kunisue T, Tanabe S et al (2022) Microplastics in dumping site soils from six Asian countries as a source of plastic additives. Sci Total Environ 806:150912. https://doi.org/10.1016/j.scitotenv.2021.150912

Ullah R, Tsui MT-K, Chen H et al (2021) Microplastics interaction with terrestrial plants and their impacts on agriculture. J Environ Qual 50:1024–1041. https://doi.org/10.1002/jeq2.20264

van Weert S, Redondo-Hasselerharm PE, Diepens NJ, Koelmans AA (2019) Effects of nanoplastics and microplastics on the growth of sediment-rooted macrophytes. Sci Total Environ 654:1040–1047. https://doi.org/10.1016/j.scitotenv.2018.11.183

Wallace PW, Haernvall K, Ribitsch D et al (2017) PpEst is a novel PBAT degrading polyesterase identified by proteomic screening of Pseudomonas pseudoalcaligenes. Appl Microbiol Biotechnol 101:2291–2303. https://doi.org/10.1007/s00253-016-7992-8

Wang Z, Xie X, Zhao J et al (2012) Xylem- and phloem-based transport of CuO nanoparticles in maize (Zea mays L.). Environ Sci Technol 46:4434–4441. https://doi.org/10.1021/es204212z

Wang X, Li Y, Zhao J et al (2020) UV-induced aggregation of polystyrene nanoplastics: effects of radicals, surface functional groups and electrolyte. Environ Sci Nano 7:3914–3926. https://doi.org/10.1039/D0EN00518E

Wei Z, Van Le Q, Peng W et al (2021) A review on phytoremediation of contaminants in air, water and soil. J Hazard Mater 403:123658. https://doi.org/10.1016/j.jhazmat.2020.123658

Wilkes RA, Aristilde L (2017) Degradation and metabolism of synthetic plastics and associated products by Pseudomonas sp.: capabilities and challenges. J Appl Microbiol 123:582–593. https://doi.org/10.1111/jam.13472

Wright SL, Thompson RC, Galloway TS (2013) The physical impacts of microplastics on marine organisms: a review. Environ Pollut 178:483–492. https://doi.org/10.1016/j.envpol.2013.02.031

Wu Y, Guo P, Zhang X et al (2019) Effect of microplastics exposure on the photosynthesis system of freshwater algae. J Hazard Mater 374:219–227. https://doi.org/10.1016/j.jhazmat.2019.04.039

Wu J, Liu W, Zeb A et al (2021) Polystyrene microplastic interaction with Oryza sativa: toxicity and metabolic mechanism. Environ Sci: Nano 8:3699–3710. https://doi.org/10.1039/D1EN00636C

Wu X, Lu J, Du M et al (2021) Particulate plastics-plant interaction in soil and its implications: a review. Sci Total Environ 792:148337. https://doi.org/10.1016/j.scitotenv.2021.148337

Xia B, Chen B, Sun X et al (2015) Interaction of TiO2 nanoparticles with the marine microalga Nitzschia closterium: growth inhibition, oxidative stress and internalization. Sci Total Environ 508:525–533. https://doi.org/10.1016/j.scitotenv.2014.11.066

Xu Q, Huang Q-S, Luo T-Y et al (2021) Coagulation removal and photocatalytic degradation of microplastics in urban waters. Chem Eng J 416:129123. https://doi.org/10.1016/j.cej.2021.129123

Xu Z, Zhang Y, Lin L et al (2022) Toxic effects of microplastics in plants depend more by their surface functional groups than just accumulation contents. Sci Total Environ 833:155097. https://doi.org/10.1016/j.scitotenv.2022.155097

Yadav M, Gupta P, Seth CS (2022) Foliar application of α-lipoic acid attenuates cadmium toxicity on photosynthetic pigments and nitrogen metabolism in Solanum lycopersicum L. Acta Physiol Plant 44:112. https://doi.org/10.1007/s11738-022-03445-z

Yoshida S, Hiraga K, Takehana T et al (2016) A bacterium that degrades and assimilates poly(ethylene terephthalate). Science 351:1196–1199. https://doi.org/10.1126/science.aad6359

Yu B, Peng Y, Xu J et al (2021) Phytoremediation potential of Youngia japonica (L.) DC: a newly discovered cadmium hyperaccumulator. Environ Sci Pollut Res 28:6044–6057. https://doi.org/10.1007/s11356-020-10853-6

Zhang L, Cheng Y, Qian Y et al (2022) Phytotoxicity and accumulation of BPS to Pistia stratiotes under the influence of microplastics. Chemosphere 307:135854. https://doi.org/10.1016/j.chemosphere.2022.135854

Zhou G, Wang Q, Li J et al (2021) Removal of polystyrene and polyethylene microplastics using PAC and FeCl3 coagulation: performance and mechanism. Sci Total Environ 752:141837. https://doi.org/10.1016/j.scitotenv.2020.141837

Water Air Soil Pollut (2023) 234:520
https://doi.org/10.1007/s11270-023-06548-6

Exploring the Impacts of HDPE Microplastics on Growth and Physiological Behavior of *Brassica juncea* (Mustard Plant)

Megha Bansal · Deenan Santhiya · Jai Gopal Sharma

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Abstract In today's world, plastic pollution has become a big environmental concern. As a result, smaller plastic pieces, also known as microplastics (MPs), have received a lot of attention in recent years. The majority of study has focused on microplastic pollution in aquatic ecosystems and related shorelines. However, influence of microplastics (MPs) in the soil environment and on agroecosystem is minimal. To investigate the problem, this study is aimed to observe the effects of MPs in soil using *Brassica juncea* as a model plant. Because of its high biomass capacity, *Brassica juncea* can store considerable level of contaminants in its tissues. As a result, *B. juncea* was subjected to two microplastics, HDPE_MPs and HDPE_beads, followed by analysis of biometrical parameters, physio-biochemical features, and morphological analyses. Plants subjected to 20 g beads had the lowest concentrations of phenolic content, chlorophyll content, and proline content compared to other treated samples. Furthermore, fluorescence and confocal microscopy demonstrated the transfer of microplastics throughout plant roots and leaves, showing their potential for plant injury. To the best of our knowledge, this study focuses on the transfer of MPs from root to leaves of *Brassica juncea* for the first time. A method for microplastic uptake from plant roots to leaves has also been postulated. It merits further examination in future studies and provides new insight into the phytoaccumulation of MPs in soil.

Keywords Agroecosystem · Biometrical parameters · Microscopy · Physio-biochemical features · Phytoaccumulation

M. Bansal · J. G. Sharma
Department of Biotechnology, Delhi Technological University, Delhi, India
e-mail: megha_2k21phdbt01@dtu.ac.in

J. G. Sharma
e-mail: sharmajaigopal@dce.ac.in

D. Santhiya (✉)
Department of Applied Chemistry, Delhi Technological University, Shahbad Daulatpur, Main Bawana Road, Delhi 110042, India
e-mail: deenan.santhiya@dce.ac.in

1 Introduction

The accumulation of emerging contaminants (ECs) in agricultural ecosystems is one of the main concern in environment in today's scenario (Taheran et al., 2018). The behavior, fate and ecological impacts of ECs has led to inadequate management and loss of biodiversity (Lodeiro et al., 2019). Plastic materials with small particle sizes, such as microplastics (MPs) (plastics with size < 1000 μm) and nanoplastics (NPs) (with particle size less than 1 μm), are of particular interest because of ecotoxicity and health hazards (Bermúdez & Swarzenski, 2021). Despite occurrence of microplastics by degradation, they are

also incorporated as specific constituents in many products used in daily life. These microplastics bioaccumulate within the soil and have adverse effect on plant growth and development (Xu et al., 2020). For this reason, concern on impact of microplastics on plant performance, along with soil microbes and activity in soil has been the study for research (de Souza Machado et al., 2019; Ng et al., 2018).

Many recent studies have focused on understanding the impacts of various contaminants in soil and plants. For example, the leaves of *Prunus laurocerasus* L. obtained from diverse climatic habitats showed change in morphological characteristics due to different environmental conditions (YÏğit et al., 2018). Another study observed distinguished characteristics in various plant species exposed to magnesium, a major macronutrient for plants. Traffic density determined variations in magnesium concentrations in leaves of *Ficus bengalensis*, *Ziziphus mauritiana*, *Conocarpus erectus*, and *Azadirechta indica* species (Çetïn & Jawed, 2021). Accumulation of traffic-related heavy metals like aluminium, cadmium, iron, manganese, nickel, lead, and zinc in plants could be determined by using *Rosmarinus officinalis* L. (rosemary) as a model plant. This plant was mostly used in slopes and refuge along highway junctions and could act as a great tool for establishing traffic-related pollution in urban parts (Bozdogan Sert et al., 2019). Concentrations of heavy metals in soil can also increase on being accumulated with other contaminants like microplastics. For example, the uptake and inhibitory effects of cadmium on maize plants could be observed on being treated with HDPE microplastics. Different doses of microplastics induced phytotoxicity, change cadmium bioavailability and plant performance (Wang et al., 2020). Besides metal and other contaminant pollution in soil, microplastics could also have direct impacts on agroecosystem. For example, plastic mulch film significantly impact wheat growth, bacterial ecology and volatiles in the rhizosphere (Qi et al., 2020). Polylactic acid (PLA) microplastics severely decreased seedling height and prevented seed germination (Boots et al., 2019). In addition to plastic mulch film, scientists discovered that polystyrene-beads had effects on growth of plants on the basis of size, concentration, and that diameter may be a primary factor in determining whether micro/nanoplastics may permeate the tissues of plants. Regarding 100 nm polystyrene-beads, in addition to increasing wheat biomass (Lian et al., 2020), they might concentrate in the roots of *Vicia faba* and obstruct cell wall pores, which would impede delivery of plant nutrients (Jiang et al., 2019). According to a study, 10 mg kg^{-1} polystyrene-beads might shorten roots, whereas 100 nm enhanced seedling height (Ren et al., 2021). These findings demonstrated the buildup and movement of microplastics in the soil–plant system, highlighting the potential for microplastics to reach the food chain (Su et al., 2019). According to Dissanayake et al. (2022), applying agricultural sewage sludge alone leads to a large proportion of plastic residues into agricultural soils. Its input is predicted to be between 63,000 and 430,000 tonnes of microplastics per year in EU and North American farmlands, and between 2800 and 19,000 tonnes per year in Australian agroecosystems (Dissanayake et al., 2022). As per the study by Tun et al. (2022), 1411 pieces/kg of MPs were found in the Indian dumping soils which is just second to the highest pollution reported in Cambodia (Tun et al., 2022). Most of the microplastics that act as dominant polymers in soil include polyethylene, polyethylene terephthalate and polypropylene.

The major aim of present study is understanding uptake, accumulation, and translocation of high-density polyethylene (HDPE) microplastics along with their adverse effects on terrestrial plant. HDPE constitutes a large proportion of environmental pollution among all the microplastics (Awasthi et al., 2017). To facilitate this mechanism, *Brassica juncea*, commonly termed as Indian Mustard, was used as a model plant to assess sites of absorption, uptake, and accumulation within the plant. *Brassica juncea* is particularly useful for phytoremediation as it can accumulate high levels of heavy metals (lead, nickel, cadmium, mercury and selenium) in their tissues, a process called as phytoextraction (Rathore et al., 2019). *Brassica juncea* produce compounds called glucosinolates, which are broken down by enzymes to release toxic isothiocyanates. These isothiocyanates form complexes with heavy metals in the soil, where they are then absorbed by plant roots and stored in their tissues. This means they can quickly establish themselves in contaminated soil and start removing pollutants (Diwan et al., 2008). *Brassica juncea* has a high biomass, which means it can accumulate huge quantities of pollutants in tissues (Goswami & Das, 2015). Also, *Brassica juncea* has a deep root system that allows them to access pollutants that may

be located deep in soil. Overall, the combination of these characteristics makes *Brassica juncea* an effective plant for phytoremediation of soil. Therefore, it is considered as the most ideal plant to study potential for microplastics remediation in soil. To our knowledge, this study is the first to observe impacts of microplastics not only on roots, but also on leaves and shoots of plants. Biochemical analysis on roots, shoots and leaves has been demonstrated to provide further evidence of microplastics intake in plants. Finally, mechanism of uptake of microplastics by plants highlighting different pathways is briefed to observe phytoaccumulation and identify the possibilities for soil remediation of microplastics. Therefore, current study builds on examining the impacts of two different kinds of high-density polyethylene (HDPE) microplastics, namely HDPE_MPs and HDPE_beads, on *Brassica juncea*. To study the impact and accumulation of HDPE_MPs and HDPE_beads on the plant, biometrical parameters such as height and biomass was observed. Additionally, the amount of chlorophyll in leaves was used to determine the photosynthetic pigment. By analyzing the phenolic and proline concentrations in various plant sections, biochemical composition was estimated. An examination of the plant's morphology using fluorescence and confocal microscopy was done to see where microplastics were being absorbed. Finally, the mechanism of accumulation of MPs within plant roots and its translocation to aerial parts was highlighted to further our understanding of MPs impact in plants and soil.

2 Material And Methods

2.1 Experimental Design

2.1.1 Soil

To study impacts of different size microplastics on *Brassica juncea* (mustard seeds) in an open environment, a triplicate study was conducted. Pots containing equal concentrations of soil and mustard seeds with varying amounts of microplastics was used for the study. We harvested the mustard seeds after a week to determine the effects of our experiments on vegetative and reproductive growth. The sandy soil used in this study was obtained from an agricultural land in Ghaziabad, Uttar Pradesh, India, at 28° 39′ 14.1588" N and 77° 26′ 42.8784" E. The soil was composed of sand, silt, and clay with moderate amounts of organic matter. The air-dried soil was sieved with a 2 mm steel sieve before use.

2.1.2 Synthesis of Microplastics

In this experiment, two forms of microplastics were used: (1) high-density polyethylene (HDPE) microplastics and (2) high-density polyethylene (HDPE) beads. A method described by Crespy and Landfester, 2007, was used to prepare the HDPE microplastics (HDPE_MPs) and beads (HDPE_beads). A solution composed of 1 g of HDPE powder and 20 ml of xylene was mixed using a magnetic stirrer for 1 h until completely dissolved. The HDPE solution was then gently added to 100 ml of deionized water while maintaining the sonication at 70% amplitude (Branson W450 Digital sonicator, tip size 6.5 mm) for 30 s under ice cooling. The resulting solution was centrifuged, rinsed with water and ethanol, then air-dried before storage.

2.1.3 Mustard Seeds and Pots

Brassica juncea (mustard seeds) were obtained from Indian Institute of Agricultural Research (IARI), Pusa, New Delhi. The seeds were surface sterilized with 0.02% sodium hypochlorite (NaOCl) before immersion in 70% ethanol (Fig. 1: (A) *Brassica juncea* (mustard seeds) and (B) Germination done in complete dark). After sterilization, the seeds were rinsed several times with distilled water. The seeds were grown on tissue overnight before being planted in organic soil in November 2021. Pots were irrigated twice a week at first, then once every two days during seedling emergence in January and February of 2022. Because of the increase in temperature in February, the frequency of irrigation was increased. NPK was applied to the soil in each pot based on the mustard NPK requirement of 100, 20, and 60 kg/ha.

The pot used in the experiment was 20 cm long, 10 cm in diameter at the bottom, 13 cm in diameter at the top and had a volume of 2 l. We used a factorial experimental design. Furthermore, three control treatments with no microplastic residues were investigated. The experiment included 12 treatments performed in triplicate, as well as four independent pots containing tagged microplastics for imaging.

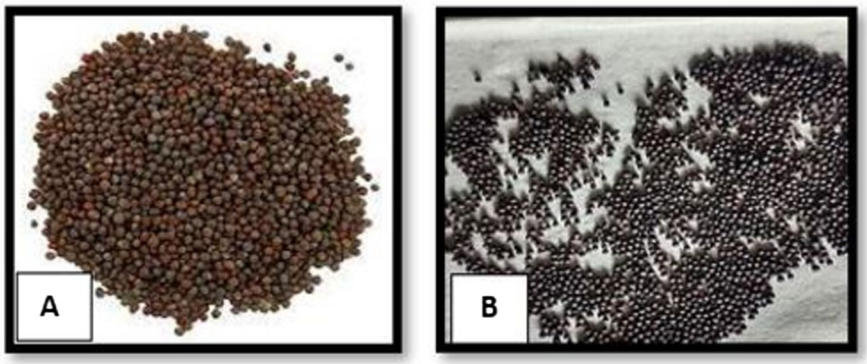

Fig. 1 (**A**) Brassica juncea (Mustard seeds) and (**B**) Germination done in complete dark

Each treatment was replicated three times, and total 36 pots of *B. juncea* seeds were grown. The mean of three potted plants was used to describe the study's findings.

2.2 Experimental Set-up and Climatic Conditions for Growth of Plant

2.2.1 Setting Up

Each pot constituted 2.5 kg of sieved soil and various concentrations of microplastics (except the three control treatments without microplastics) along with 150 ml of water. Prior to filling each pot with this mixture, a piece of geotextile was placed at the bottom of each pot to allow free circulation of air and water. After all the pots were filled, the soil moisture was uniformly set to 15%, which corresponds to the water capacity of the soil in the field. Before sowing *Brassica juncea* (mustard seeds), let sit for a week in each pot. Each container contained 12 g of litter (12.08 ± 0.06 g) and was sprayed with water to keep the litter moist.

2.2.2 Mustard Cultivation

Each pot contained 10–12 seeds, and post 2 weeks, 6–7 seedlings were selected from each pot for testing. The temperature was set at 15–16 °C during the day and 12 °C at night, with a photoperiod (14/10 h), a light intensity of 300 µmol m^{-2} s^{-1} and a relative humidity of 70% for day and night. The pots were watered weekly with tap water and soil moisture was adjusted to between 12 and 18% by weight. Pots were randomly placed in the climatic chamber and rotated each after two weeks.

2.2.3 Microplastic Tagging with Nile Red

For imaging, HDPE microplastic particles were labeled with Nile red as previously described (Karakolis et al., 2019). The dried microplastic particles were placed in an aqueous deionized (DI) solution of 100 µg/ml Nile red at a concentration of 50 mg of plastic particles per 10 ml of solution. This solution was prepared by dissolving 1 mg of Nile Red in 1 ml of acetone and the resulting solution was added to 10 ml of deionized water. The vials were left in the dark for two hours, rinsed three times with water, centrifuged and stored in deionized water for later use. Based on known staining procedures for microplastics, the Nile Red staining procedure was selected, including concentration of the Nile Red solution (Maes et al., 2017).

2.3 Measurements of Growth Parameters

2.3.1 Biometrical Analysis

Plant heights were measured using a steel tape measure on a regular basis from the 14th day after seeds were sown until the 90th day. Three months after planting, plants were divided into shoots and roots. Biometrical analysis provides the overall growth, biomass, and length of roots, shoots and leaves of plants after being exposed to different concentrations of a contaminant (Pricinotto et al., 2019). In this study, different concentrations of MPs were used as contaminant to observe the growth pattern in plants. At a span of three months from sowing of seeds, root and shoot height was measured and a mean of all three triplicates were used to determine the results of study. Similarly, root and shoot biomass was weighed

for each treated sample to determine mean difference with respect to control.

2.3.2 Chlorophyll Content

To determine photosynthetic pigment in leaves of plant, chlorophyll estimation was done. Relative chlorophyll content in plant leaves was measured and recorded as per the process defined by (Hong et al., 2012) using UV–Vis spectrophotometer (Biospectrophotometer, USA). Fresh leaves were obtained from each pot to determine the chlorophyll level. For each treated and control sample, 0.5 g of leaves were weighed from each pot. 10 ml of 80% acetone was added to chopped and homogenized leaves to make them transparent. The extract was centrifuged at 2500 rpm for five minutes. The resulting supernatant was diluted with 9 ml of 80% acetone before being measured with a UV–Vis Spectrophotometer at 663 nm and 644 nm. Total chlorophyll content in microplastic treated and control samples was evaluated using Mackinney's work and Arnon equations –

$$Chl_a = 12.7A_{663} - 2.69A_{645}; Chl_b = 22.9A_{645} - 4.68A_{663}$$
$$\text{Total chlorophyll} = Chl_a + Chl_b$$

2.4 Biochemical Analysis

2.4.1 Phenolic Content

A modified Folin–Ciocalteau test with gallic acid as the standard was used to assess total phenolic content (Ainsworth & Gillespie, 2007). 1 ml of plant extract was combined with 5 ml of Folin Ciocalteau's reagent after 1.5 ml of 20% Na_2CO_3 was added (diluted 1:10 with distilled water). Color development was accomplished by incubating the test tubes in dark for 30 min at room temperature, followed by measuring the absorbance at 765 nm. The total phenolic content of the sample was estimated as mg of dry mass equivalents of gallic acid. (GAE) mg^{-1}.

2.4.2 Proline Content

Modified ninhydrin chromogenic techniques were used to measure the proline content (Zhang et al., 2013). A glass tube containing freshly harvested roots (0.2 g) was filled with 5 ml of 3% sulfosalicylic acid. The glass tube was incubated for 10 min in a 100 °C water bath. 2 ml of the filtrate was digested in another glass tube after 4 ml of chromogenic solution (2 ml of 2.5% ninhydrin and 2 ml of glacial acetic acid) was added to the filter. The glass tube was then immersed for 30 min in a 100 °C water bath. Further, to stop the reaction, glass tube was submerged in an ice bath. 5 ml of toluene were placed in the glass tube, vortexed, and then allowed to stand. Using a spectrophotometer, the toluene layer's absorbance was observed at 520 nm.

2.5 Morphological Analysis

2.5.1 Fluorescence Microscopy

Fresh roots and leaves were removed from *Brassica juncea* plant and cleansed with deionized water. The roots were sectioned and placed on a glass slide with a few drops of clean water. The glass slide was then gently squeezed to flatten the pure water-covered root, ensuring that no air bubbles formed between the glass slide and cover slip. A fluorescent microscope was used to view each sample.

2.5.2 Confocal Microscopy

Fresh roots and leaves with tagged microplastics were picked out and cleansed with deionized water. On a glass slide with a few droplets of distilled water, cross-sections of roots were exhibited. Furthermore, to ensure no air bubbles between glass slide and cover slip, it was gently pressed to flatten the clean water-covered root. With the use of a confocal microscope (Nikon Laser Scanning Confocal Microscope), each sample was examined to observe the tagged microplastics. Similar observations were performed for leaf cross-sections of plant.

2.6 Statistical Analysis

All experiments were done in triplicate. Statistical analysis of experimental data was performed using Origin2023 software, and analysis of variance (ANOVA) was performed in SPSS 21.0 with a p-value of 0.05. For triplicate samples ($n=3$), all values were expressed as mean 5% standard error.

3 Results and Discussion

3.1 Biometrical Parameters

3.1.1 Growth Response

On exposure of *Brassica juncea* to HDPE_MPs and HDPE_beads, significant difference in shoot biomass and root biomass could be observed in contrast to control plants (Fig. 2 (a)). Shoot biomass for control was 3.155 ± 0.15 g compared to the treated plants showing a decreasing trend. The least shoot biomass was observed for 20 g MPs in contrast to control sample indicating an adverse effect when exposed to microplastics. Similarly, root biomass also showed a declining trend on being exposed to HDPE_MPs and HDPE_beads after a span of three months (Fig. 2 (b)). Root biomass for control was 0.85 ± 0.04 g compared to the treated plants having significant difference in values.

The shoot and root height also showed a declining trend on being treated with HDPE_MPs and HDPE_beads (Fig. 2 (c) & (d)). The shoot height for control plant was 33.23 ± 1.66 cm compared to microplastics treated samples showing declining trend. Also, the root height for control sample was 5.6 ± 0.28 cm compared to microplastics exposed samples showing a decreasing trend. Figure 2: Influence of HDPE_MPs and HDPE_beads on plant samples by observing growth parameters: (a) Shoot Biomass; (b) Root Biomass; (c) Shoot Height (The values are mean of three triplicates; Error bars denote 5% standard error).

3.1.2 Chlorophyll Content

The results showed that chlorophyll *a* (Chl *a*) was more easily impacted by varied microplastic concentrations than chlorophyll *b* (Chl *b*). Such a considerable variation in chl *a* and chl *b* content implies that total chlorophyll content in leaves of treated and control samples changed significantly (Fig. 3 (a) & (b)).

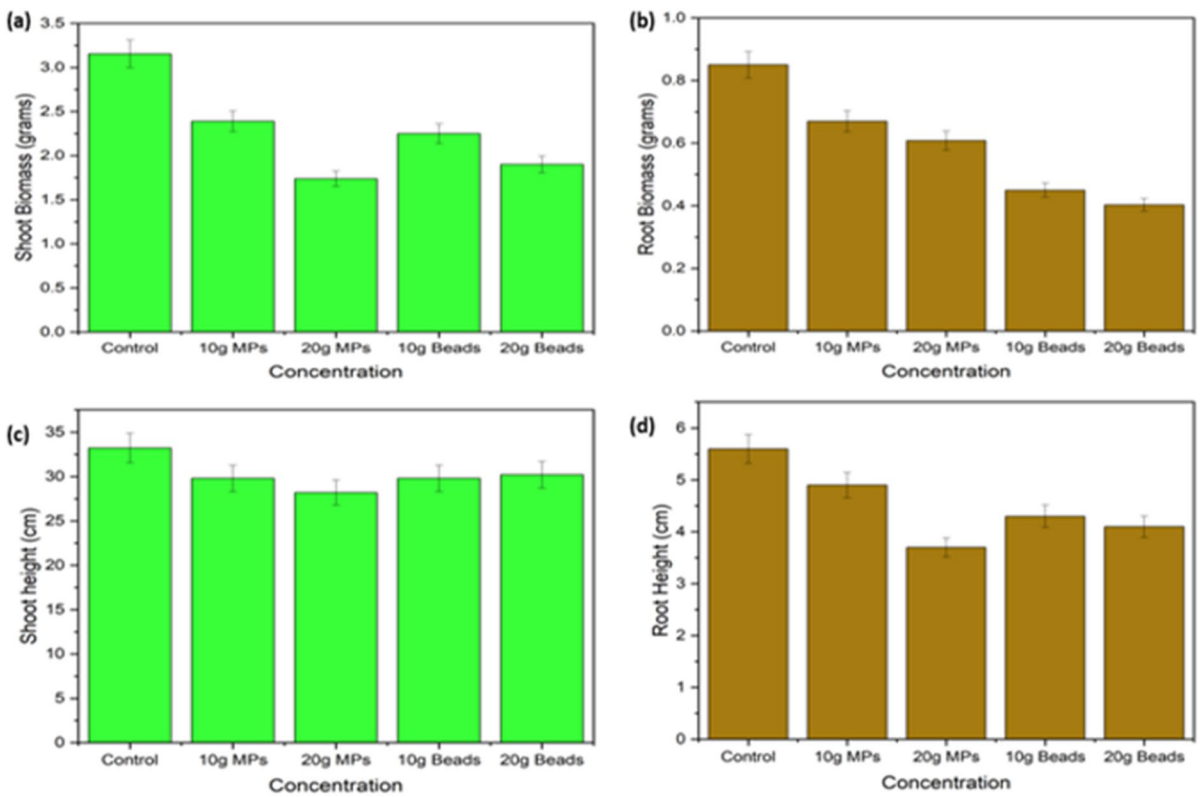

Fig. 2 Influence of HDPE_MPs and HDPE_beads on plant samples by observing growth parameters: (**a**) Shoot Biomass; (**b**) Root Biomass; (**c**) Shoot Height; (**d**) Root Height (The values are mean of three triplicates; Error bars denote 5% standard error)

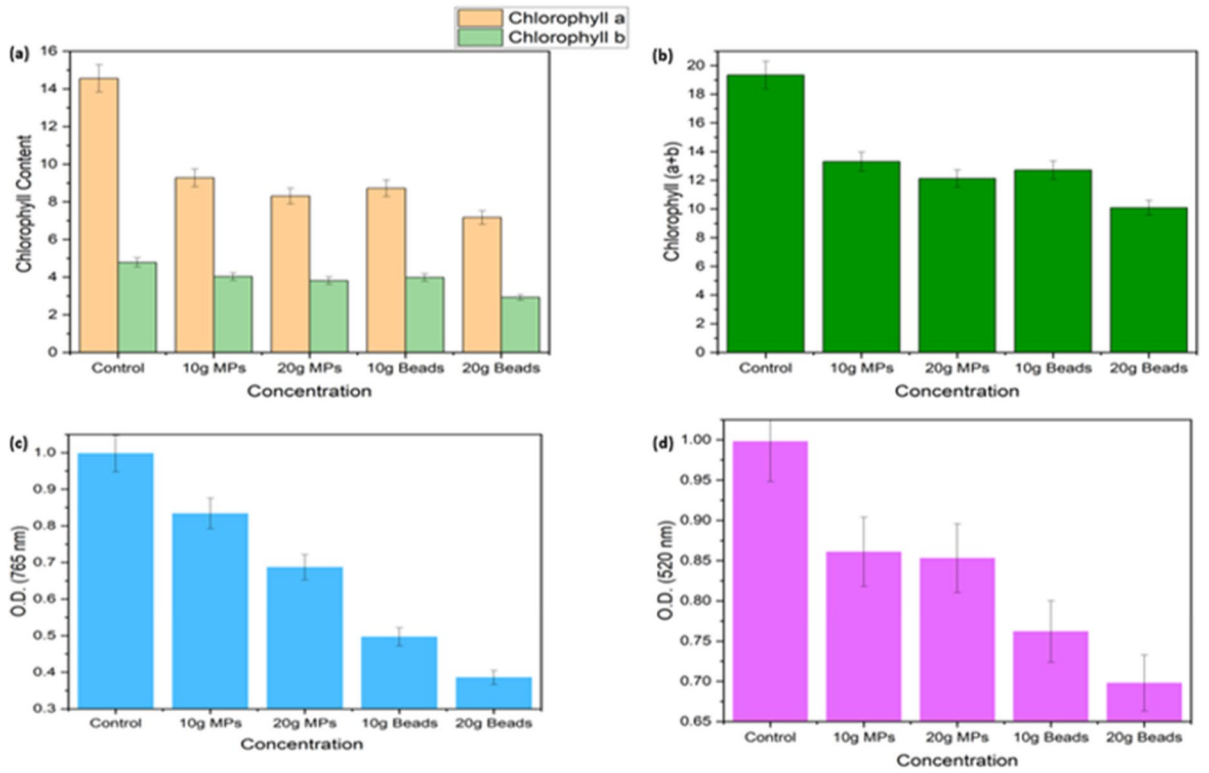

Fig. 3 Influence of HDPE_MPs and HDPE_beads on plant samples by observing growth parameters: (**a**) Chlorophyll a & b; (**b**) Total chlorophyll; (**c**) Phenolic content; (**d**) Proline content (The values are mean of three triplicates; Error bars denote 5% standard error)

Chlorophyll *a* is the primary pigment in the photosynthesis process, whereas chlorophyll *b* is an additional pigment that delivers energy to chlorophyll *a* (Khaleghi et al., 2013). An important metric for photosynthetic activity is the overall chlorophyll concentration (chl *a* + chl *b*), and variations in this value are a sign of stress in plants. Thus, it could be observed that chlorophyll *a* was comparatively less for microplastics treated plant samples indicating that total chlorophyll content in leaves showed greater inhibitory effect on exposure to microplastics.

3.2 Biochemical Analysis

3.2.1 Phenolic Content

The most common secondary metabolites found in plants are phenolics that serve as biological aid in plants, including defense against pathogens, protection from ultraviolet rays, pigmentation to draw pollinators, and defense against reactive oxygen species (Waśkiewicz et al., 2013). On being exposed to microplastics of different types, phenolic content in plants reduced showing a decreasing trend as observed in Fig. 3 (c). Compared to control having phenolic content of 0.998 ± 0.04 (GAE) mg^{-1}, phenolic content in plants treated with microplastics showed declining trend. The results indicate that incorporation of microplastics decreases phenolic content in plants thereby inducing stress. Figure 3: Influence of HDPE_MPs and HDPE_beads on plant samples by observing growth parameters: (a) Chlorophyll a & b; (b) Total chlorophyll; (c) Phenolic content; (d) Proline content (The values are mean of three triplicates; Error bars denote 5% standard error).

3.2.2 Proline Content

The current investigation demonstrated that *B. juncea* plants treated with HDPE_MPs and HDPE_beads had

a reduced proline content (Fig. 3 (d)). As proline is known to reduce oxidative stress, maintain osmotic balance, and regulate redox potential, increasing concentration of microplastics showed an opposite trend. On being exposed to MPs, content of proline gradually declined with 20 g beads showing lowest value of 0.698 ± 0.03 gg^{-1} compared to control with proline content of 0.998 ± 0.04 gg^{-1}. These values are indicative of the fact that proline is not able to reduce stress in *Brassica juncea* plants contaminated with microplastics.

The results presented in Fig. 2 (a) to (d) and Fig. 3 (a) to (d) are in good agreement and show the impact of microplastics on *Brassica juncea*. The decreasing trend observed in shoot biomass, root biomass, shoot height and root height ascertain the uptake of microplastics by plants and also provide evidence of plant contaminated with microplastics. Increasing concentration of microplastics in each of triplicate samples decreases the biometrical growth of *Brassica juncea*. Also, in contrast to chlorophyll *b*, chlorophyll *a* showed a major declining concentration on adding microplastics. Since, the major pigment involved in photosynthetic energy production is chlorophyll *a* (Khaleghi et al., 2013), its low content in microplastics treated samples indicates that total chlorophyll content gets significantly affected on addition of microplastics. Similarly, biochemical analysis conveys the impact of microplastics on *Brassica juncea* plants. As observed in Fig. 3 (c), phenolic content plays a major role as antioxidant agents that aid in free radical scavenging (Aryal et al., 2019). Decreasing concentration of phenolic agents on addition of microplastics justifies that plant have less redox potential thereby showing less antioxidant activity. Figure 3 (d) showing proline concentration signifies that microplastics addition induces stress in plants (Hayat et al., 2012).

3.3 Morphological Analysis

3.3.1 Fluorescence Microscopy

In this study, we show that *Brassica juncea* plant roots may absorb microplastics with sizes ranging between 5 to 10 µm from the surrounding soil (Fig. 4 (a) & (b)). We were able to find and see labelled microplastic particles embedded amid root cell structures using fluorescence microscopy. Fluorescing microplastic was found in exodermis, and vascular tissues, as well as in root hairs and outer epidermal layer, in addition to the inherent autofluorescence

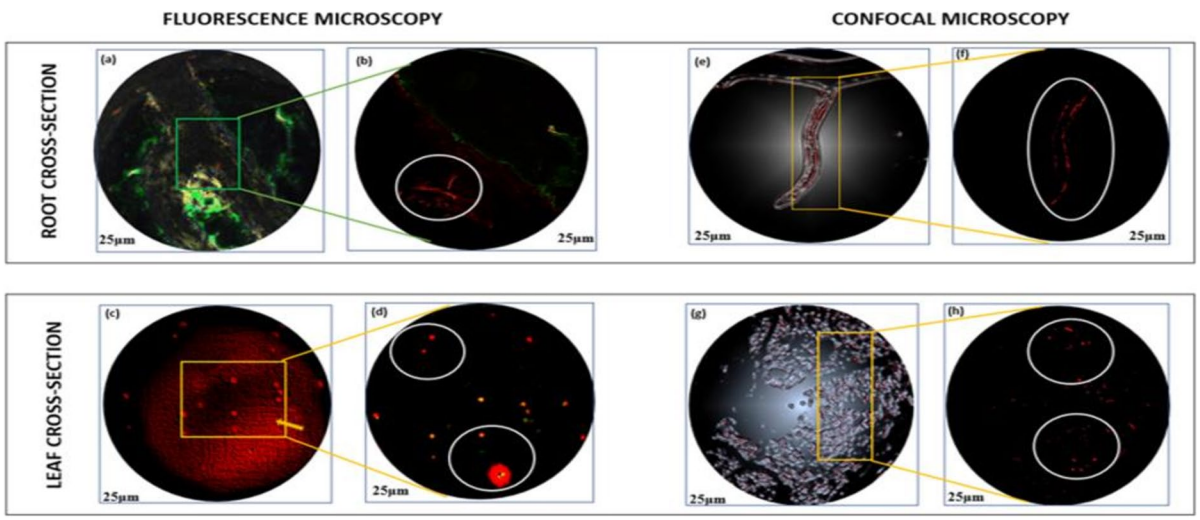

Fig. 4 Longitudinal cross-section of a Brassica juncea lateral root and leaf section in a three-month-old mustard plant after being exposed to a tagged microplastics. Clockwise from top left, flourescence microscopy shows root cross-sections (**a**) and (**b**), leaf sections (**c**) and (**d**), and confocal laser scanning microscopy shows root cross-sections (**e**) and (**f**), and leaf sections (**g**) and (**h**). Flourescing microplastic particles are indicated by white circles. All photographs were captured at magnifications of 40X and 100X and enhanced with contrast and brightness correction. Scales are displayed in the lower left and right corner of each image

of tree root. (Fig. 4(c) & (d)) provides evidence for microplastics incorporation in leaves of mustard plant as observed with red tagged MPs. Tagged microplastics could be observed in leaf sections and also induce structural changes in the morphology of leaf which is in line with our physiological and biochemical analysis (Sections 3.1. and 3.2.).

3.3.2 Confocal Microscopy

To decipher more significant findings of the study, confocal microscopy was performed to visualize tagged microplastics within the root lateral cross-sections and leaf parts. On visualization under red and green field at 40X and 100X magnification, it could be observed that microplastics were embedded in root hair segments and leaf internal veins at some points (Fig. 4 (e), (f), (g) & (h)). Because we could observe microplastics particles in inner root and leaf structures, our results suggest that micrometer sized microplastic can easily traverse from soil to root through crack-entry and apoplastic pathway, forming basis for entry to food chain. These results confirm microplastics uptake by *Brassica juncea* plants and also provide future insights for understanding the mechanism of action and impact on plants. Figure 4: Longitudinal cross-section of a *Brassica juncea* lateral root and leaf section in a three-month-old mustard plant after being exposed to tagged microplastics. Clockwise from top left, fluorescence microscopy shows root cross-sections (a) and (b), leaf sections (c) and (d), and confocal laser scanning microscopy shows root cross-sections (e) and (f), and leaf sections (g) and (h). Fluorescing microscopic particles are indicated by white circles. All photographs were captured at magnifications of 40X and 100X and enhanced with contrast and brightness correction. Scales are displayed in the lower left and right corner of each image.

To confirm the uptake of microplastics in root and leaf sections, morphological analysis using fluorescence and confocal microscopy is presented that is in line with the previous results. Biometrical parameters and biochemical analysis convey impact of microplastics on *Brassica juncea*. To provide further evidence for the same, fluorescence microscopy observed in Fig. 4 (a) to (d) signifies microplastics uptake in root and leaf cross-sections. Also, confocal microscopy shown in Fig. 4 (e) to (h), confirms the results and is in accordance with fluorescence microscopy observations.

3.4 Mechanism of Microplastics Uptake by Plants

Through this study, it could be observed that microplastics traverse from root hair sections to upper parts of plant like leaves and shoot. The results signify that microplastics have possible implications on *Brassica juncea* plant after being exposed. However, the mechanism of microplastic passage from soil to different plant parts is still in its infancy and needs more research. The apoplast and symplast routes are two pathways that microplastics can take to move through the plant from roots to the leaves (Su et al., 2019). Apoplast is the space outside plant cells, consisting of cell walls, intercellular spaces, and extracellular fluid. Microplastics can move through this space via diffusion, and uptake into the plant is thought to occur through the root epidermis and cortex as observed by morphological analysis in Fig. 4 (a), (b), (e) and (f). Once inside the apoplast, microplastics can move laterally along the root cell walls and through intercellular spaces to reach the xylem vessels (Roberts & Oparka, 2003). From xylem, microplastics are up taken by leaf parts and other tissues of plant as observed in Fig. 4 (c), (d), (g) and (h). The apoplast route is thought to be the primary pathway for larger microplastics to traverse the plant.

The symplast is the interconnected network of plant cells via plasmodesmata, which are small channels that allow for direct communication and transport of molecules between cells. Microplastics can enter the plant cells through endocytosis or other mechanisms and move through the cytoplasmic continuum from cell to cell via plasmodesmata (Raliya et al., 2016). This route is thought to be the primary pathway for smaller microplastics or those with a hydrophilic surface. The probable pathway for microplastic transport within the plant is described in Fig. 5: Mechanism of transport of microplastics from roots to different parts of plant. Some general mechanisms that are mostly proposed include:

a. Adhesion and penetration: Microplastics may adhere to the surface of roots and enter into root tissues by physical or chemical interactions (Nel et al., 2009). Above morphological analysis (Fig. 4) confirms the uptake of microplastics

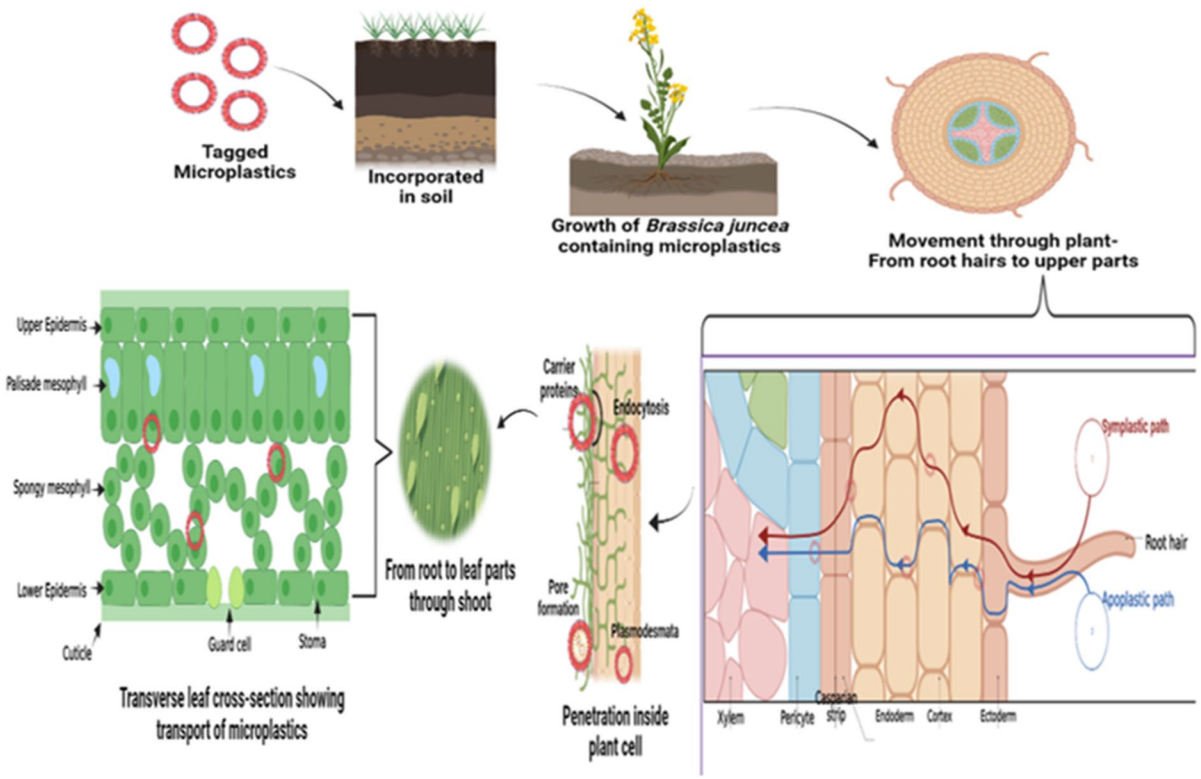

Fig. 5 Mechanism of transport of microplastics from roots to different parts of plants

from soil to root hairs and is in good agreement with the theoretical observations.

b. Endocytosis: Microplastics can traverse to plant cells through endocytosis, that encompasses formation of vesicles around the particles (Etxeberria et al., 2006). As observed in Fig. 4 (d), leaf sections show degradation of leaf surface due to accumulation of tagged microplastics. These results confirm the inflammation of leaf surface on being contaminated with microplastics.
c. Translocation: Once inside the root, microplastics may be transported across the root cortex and into the xylem vessels, which carry water and nutrients up to the leaves. This transport can cause microplastics uptake and occur through diffusion or active transport mechanisms (Schwab et al., 2016). The morphological analysis observed in Fig. 4 (a) to (h) shows that microplastics traverse from root hairs to leaf sections possibly through nutrients and water.
d. Accumulation in leaf parts: Once in xylem vessels, microplastics can be transported to the leaves, where they can accumulate in the leaf tissues. This accumulation can occur through transpiration, which is the loss of water through leaves, and subsequent concentration of microplastics in the leaf tissues. As observed in Fig. 4 (e) and (f), the leaf sections present accumulation of tagged microplastics at the surface and also degradation of a part due to accumulation.

The uptake of microplastics by plants can have various effects, including alterations in plant growth, development, and metabolism. This is well confirmed with the above findings stating impact of microplastics on biometry, biochemical analysis, and morphology. Furthermore, the presence of microplastics in edible plant tissues could pose potential risks to humans, and more research is required to understand their impacts on plants. Hence, the migration of MPs inside plants is really important as it gives an evidence on the accumulation and absorption of MPs on plant parts. Also, after accumulation and absorption of MPs, the phytoremediation potential can be

determined to provide sustainable solution for environmental cleanup.

4 Salient Findings of the Study

The results from overall study provide evidence for microplastics uptake by *Brassica juncea* and its impact on growth and development. The biometrical parameters and biochemical analysis signify that microplastics are transported from root to shoot parts of the plant as depicted by Figs. 2 and 3. Also, morphological analysis confirm uptake of microplastics from root to leaf sections of plant. The shoot biomass showed least concentration of 1.75 g on addition of 20 g MPs. Similarly, root biomass of 0.4 g was observed on addition of 20 g beads, that was least compared to all other concentrations. Also, the least shoot and root height was observed in plants treated with 20 g MPs showing shoot height at 27 cm and root height at 3.5 cm (Fig. 2). Similar to this, least chlorophyll concentration was observed in 20 g beads thereby showing least total chlorophyll content in microplastics exposed plants. Also, the antioxidant activity depicted by phenolic content and protection of plant against stress represented by proline content was least for 20 g beads exposed plants (Fig. 3). Morphological analysis confirmed the presence of microplastics uptake in root and leaf sections of plant. Confocal microscopy provides a rapid approach for visualization of MPs within plant parts (Li et al., 2020). The fluorescent dyes can generate stable emission signals that are easy to distinguish from the autofluorescence generated by plant tissues (Zhang et al., 2022). Thus, it is a rapid and efficient approach in detection of MPs within plant tissues (Ullah et al., 2021). The accumulation of tagged microplastics could also be observed in Fig. 4 (c) and (d) sections. These results portray uptake of microplastics not only by root but also by leaf sections of plant. Thus, *Brassica juncea* have been found to accumulate microplastics in their tissues, that could have significant impact on humans when consumed. This study also highlights uptake mechanism of microplastics from root to leaf sections as confirmed by morphological analysis. All the results present a significant finding providing evidence on impact on *Brassica juncea* plant after being exposed to different concentrations of microplastics. Further studies exploring phytoaccumulation of microplastics and remediation of soil need to be investigated for providing sustainability to agricultural ecosystem.

5 Conclusion

The inference from this study demonstrates absorption, uptake and phytotoxicity of microplastics to *Brassica juncea*, a vascular terrestrial plant for first time. Physiological and biochemical analysis reveals transport of MPs from root to leaf sections. Morphological analysis confirms the existence of MPs in various parts of plant as observed in vascular tissues of leaf sections and root hairs. Based on these findings, mechanism of transport of MPs from root to leaf sections through apoplast and symplast pathway is proposed. The accumulation of microplastics is evidenced in *Brassica juncea* plants providing a platform for future studies on remediation of soil in combination with other approaches to achieve optimal results. Nonetheless, it holds promise as a sustainable and environment friendly approach to addressing the issue of microplastic pollution.

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Data Availability All the data generated and analyzed during the current study is included in the manuscript.

Declarations

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References

Ainsworth, E. A., & Gillespie, K. M. (2007). Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin-Ciocalteu reagent. *Nature*

Protocols, 2(4), 875–877. https://doi.org/10.1038/nprot.2007.102

Aryal, S., Baniya, M. K., Danekhu, K., Kunwar, P., Gurung, R., & Koirala, N. (2019). Total phenolic content, flavonoid content and antioxidant potential of wild vegetables from Western Nepal. *Plants, 8*(4), 96. https://doi.org/10.3390/plants8040096

Awasthi, S., Srivastava, P., Singh, P., Tiwary, D., & Mishra, P. K. (2017). Biodegradation of thermally treated high-density polyethylene (HDPE) by Klebsiella pneumoniae CH001. *3 Biotech, 7*(5), 332. https://doi.org/10.1007/s13205-017-0959-3

Bermúdez, J. R., & Swarzenski, P. W. (2021). A microplastic size classification scheme aligned with universal plankton survey methods. *Methods X*, *8*, 101516. https://doi.org/10.1016/j.mex.2021.101516

Boots, B., Russell, C. W., & Green, D. S. (2019). Effects of microplastics in soil ecosystems: Above and below ground. *Environmental Science & Technology, 53*(19), 11496–11506. https://doi.org/10.1021/acs.est.9b03304

Bozdogan Sert, E., Turkmen, M., & Cetin, M. (2019). Heavy metal accumulation in rosemary leaves and stems exposed to traffic-related pollution near Adana-İskenderun Highway (Hatay, Turkey). *Environmental Monitoring and Assessment, 191*(9), 553. https://doi.org/10.1007/s10661-019-7714-7

Çetin, M., & Jawed, A. A. (2021). The chancing of Mg concentrations in some plants grown in Pakistan depends on plant species and the growing environment. *Kastamonu University Journal of Engineering and Sciences, 7*(2), 167–174.

Crespy, D., & Landfester, K. (2007). Preparation of nylon 6 nanoparticles and nanocapsules by two novel miniemulsion/solvent displacement hybrid techniques. *Macromolecular Chemistry and Physics, 208*(5), 457–466. https://doi.org/10.1002/macp.200600487

de Souza Machado, A. A., Lau, C. W., Kloas, W., Bergmann, J., Bachelier, J. B., Faltin, E., et al. (2019). Microplastics can change soil properties and affect plant performance. *Environmental Science & Technology, 53*(10), 6044–6052. https://doi.org/10.1021/acs.est.9b01339

Dissanayake, P. D., Kim, S., Sarkar, B., Oleszczuk, P., Sang, M. K., Haque, M. N., et al. (2022). Effects of microplastics on the terrestrial environment: A critical review. *Environmental Research, 209*, 112734. https://doi.org/10.1016/j.envres.2022.112734

Diwan, H., Ahmad, A., & Iqbal, M. (2008). Genotypic variation in the phytoremediation potential of Indian mustard for chromium. *Environmental Management, 41*(5), 734–741. https://doi.org/10.1007/s00267-007-9020-3

Etxeberria, E., Gonzalez, P., Baroja-Fernández, E., & Romero, J. P. (2006). Fluid phase endocytic uptake of artificial nano-spheres and fluorescent quantum dots by sycamore cultured cells. *Plant Signaling & Behavior, 1*(4), 196–200. https://doi.org/10.4161/psb.1.4.3142

Goswami, S., & Das, S. (2015). A study on cadmium phytoremediation potential of indian mustard, Brassica juncea. *International Journal of Phytoremediation, 17*(6), 583–588. https://doi.org/10.1080/15226514.2014.935289

Hayat, S., Hayat, Q., Alyemeni, M. N., Wani, A. S., Pichtel, J., & Ahmad, A. (2012). Role of proline under changing environments. *Plant Signaling & Behavior, 7*(11), 1456–1466. https://doi.org/10.4161/psb.21949

Hong, R., Feng, L., & Yan, X. (2012). Comparison on methods of chlorophyll extraction in flowering chinese cabbage. *Journal of Anhui Agricultural Sciences*. https://scholar.google.com/scholar_lookup?title=Comparison+on+methods+of+chlorophyll+extraction+in+flowering+chinese+cabbage&author=Ren+Hong%2C+Sanya+Science+and+Technology+Academy+for+Crop+Winter+Multiplication%2CSanya%28China%29&publication_year=2012. Accessed 5 February 2023

Jiang, X., Chen, H., Liao, Y., Ye, Z., Li, M., & Klobučar, G. (2019). Ecotoxicity and genotoxicity of polystyrene microplastics on higher plant Vicia faba. *Environmental Pollution, 250*, 831–838. https://doi.org/10.1016/j.envpol.2019.04.055

Karakolis, E. G., Nguyen, B., You, J. B., Rochman, C. M., & Sinton, D. (2019). Fluorescent dyes for visualizing microplastic particles and fibers in laboratory-based studies. *Environmental Science & Technology Letters, 6*(6), 334–340. https://doi.org/10.1021/acs.estlett.9b00241

Khaleghi, E., Arzani, K., Moallemi, N., & Barzegar, M. (2013). Evaluation of chlorophyll content and chlorophyll fluorescence parameters and relationships between chlorophyll a, b and chlorophyll content index under water stress in Olea europaea cv. Dezful. International Journal of Agricultural and Biosystems Engineering 6(8), 636–639. https://doi.org/10.1016/j.mex.2021.101516

Li, L., Luo, Y., Peijnenburg, W. J. G. M., Li, R., Yang, J., & Zhou, Q. (2020). Confocal measurement of microplastics uptake by plants. *MethodsX, 7*, 100750. https://doi.org/10.1016/j.mex.2019.11.023

Lian, J., Wu, J., Xiong, H., Zeb, A., Yang, T., Su, X., et al. (2020). Impact of polystyrene nanoplastics (PSNPs) on seed germination and seedling growth of wheat (Triticum aestivum L.). *Journal of Hazardous Materials, 385*, 121620. https://doi.org/10.1016/j.jhazmat.2019.121620

Lodeiro, C., Capelo, J. L., Oliveira, E., & Lodeiro, J. F. (2019). New toxic emerging contaminants: Beyond the toxicological effects. *Environmental Science and Pollution Research, 26*(1), 1–4. https://doi.org/10.1007/s11356-018-3003-1

Maes, T., Jessop, R., Wellner, N., Haupt, K., & Mayes, A. G. (2017). A rapid-screening approach to detect and quantify microplastics based on fluorescent tagging with Nile Red. *Scientific Reports, 7*(1), 44501. https://doi.org/10.1038/srep44501

Nel, A. E., Mädler, L., Velegol, D., Xia, T., Hoek, E. M. V., Somasundaran, P., et al. (2009). Understanding biophysicochemical interactions at the nano–bio interface. *Nature Materials, 8*(7), 543–557. https://doi.org/10.1038/nmat2442

Ng, E.-L., Huerta Lwanga, E., Eldridge, S. M., Johnston, P., Hu, H.-W., Geissen, V., & Chen, D. (2018). An overview of microplastic and nanoplastic pollution in agroecosystems. *Science of the Total Environment, 627*, 1377–1388. https://doi.org/10.1016/j.scitotenv.2018.01.341

Pricinotto, L. F., Zucareli, C., Ferreira, A. S., Spolaor, L. T., & Fonseca, I. C. D. B. (2019). Yield and biometric characteristics of maize submitted to plant population and

trinexapac-ethyl doses. *Revista Caatinga, 32*, 667–678. https://doi.org/10.1590/1983-21252019v32n311rc

Qi, Y., Ossowicki, A., Yang, X., Huerta Lwanga, E., Dini-Andreote, F., Geissen, V., & Garbeva, P. (2020). Effects of plastic mulch film residues on wheat rhizosphere and soil properties. *Journal of Hazardous Materials, 387*, 121711. https://doi.org/10.1016/j.jhazmat.2019.121711

Raliya, R., Franke, C., Chavalmane, S., Nair, R., Reed, N., & Biswas, P. (2016). Quantitative Understanding of Nanoparticle Uptake in Watermelon Plants. *Frontiers in Plant Science*, *7*. https://www.frontiersin.org/articles/10.3389/fpls.2016.01288. Accessed 18 October 2022

Rathore, S. S., Shekhawat, K., Dass, A., Kandpal, B. K., & Singh, V. K. (2019). Phytoremediation mechanism in Indian mustard (Brassica juncea) and its enhancement through agronomic interventions. *Proceedings of the National Academy of Sciences, India Section B: Biological Sciences, 89*(2), 419–427. https://doi.org/10.1007/s40011-017-0885-5

Ren, X., Tang, J., Wang, L., & Liu, Q. (2021). Microplastics in soil-plant system: Effects of nano/microplastics on plant photosynthesis, rhizosphere microbes and soil properties in soil with different residues. *Plant and Soil, 462*(1), 561–576. https://doi.org/10.1007/s11104-021-04869-1

Roberts, A. G., & Oparka, K. J. (2003). Plasmodesmata and the control of symplastic transport: Plasmodesmata and symplastic transport. *Plant, Cell & Environment, 26*(1), 103–124. https://doi.org/10.1046/j.1365-3040.2003.00950.x

Schwab, F., Zhai, G., Kern, M., Turner, A., Schnoor, J. L., & Wiesner, M. R. (2016). Barriers, pathways and processes for uptake, translocation and accumulation of nanomaterials in plants – Critical review. *Nanotoxicology, 10*(3), 257–278. https://doi.org/10.3109/17435390.2015.1048326

Su, Y., Ashworth, V., Kim, C., Adeleye, A. S., Rolshausen, P., Roper, C., et al. (2019). Delivery, uptake, fate, and transport of engineered nanoparticles in plants: A critical review and data analysis. *Environmental Science: Nano, 6*(8), 2311–2331. https://doi.org/10.1039/C9EN00461K

Taheran, M., Naghdi, M., Brar, S. K., Verma, M., & Surampalli, R. Y. (2018). Emerging contaminants: Here today, there tomorrow! *Environmental Nanotechnology, Monitoring & Management, 10*, 122–126. https://doi.org/10.1016/j.enmm.2018.05.010

Tun, T. Z., Kunisue, T., Tanabe, S., Prudente, M., Subramanian, A., Sudaryanto, A., et al. (2022). Microplastics in dumping site soils from six Asian countries as a source of plastic additives. *Science of The Total Environment, 806*, 150912. https://doi.org/10.1016/j.scitotenv.2021.150912

Ullah, R., Tsui, M.T.-K., Chen, H., Chow, A., Williams, C., & Ligaba-Osena, A. (2021). Microplastics interaction with terrestrial plants and their impacts on agriculture. *Journal of Environmental Quality, 50*(5), 1024–1041. https://doi.org/10.1002/jeq2.20264

Wang, F., Zhang, X., Zhang, S., Zhang, S., Adams, C. A., & Sun, Y. (2020). Effects of co-contamination of microplastics and Cd on plant growth and Cd accumulation. *Toxics, 8*(2), 36. https://doi.org/10.3390/toxics8020036

Waśkiewicz, A., Muzolf-Panek, M., & Goliński, P. (2013). Phenolic content changes in plants under salt stress. In P. Ahmad, M. M. Azooz, & M. N. V. Prasad (Eds.), *Ecophysiology and responses of plants under salt stress* (pp. 283–314). Springer. https://doi.org/10.1007/978-1-4614-4747-4_11

Xu, B., Liu, F., Cryder, Z., Huang, D., Lu, Z., He, Y., et al. (2020). Microplastics in the soil environment: Occurrence, risks, interactions and fate – A review. *Critical Reviews in Environmental Science and Technology, 50*(21), 2175–2222. https://doi.org/10.1080/10643389.2019.1694822

Yİğit, N., ÇetİN, M., & ŞevİK, H. (2018). The change in some leaf micromorphological characters of Prunus laurocerasus L. species by their habitat. *Turkish Journal of Agriculture - Food Science and Technology, 6*(11), 1517–1521.

Zhang, X., Huang, G., Bian, X., & Zhao, Q. (2013). Effects of root interaction and nitrogen fertilization on the chlorophyll content, root activity, photosynthetic characteristics of intercropped soybean and microbial quantity in the rhizosphere. *Plant, Soil and Environment, 59*(No. 2), 80–88. https://doi.org/10.17221/613/2012-PSE

Zhang, Z., Cui, Q., Chen, L., Zhu, X., Zhao, S., Duan, C., et al. (2022). A critical review of microplastics in the soil-plant system: Distribution, uptake, phytotoxicity and prevention. *Journal of Hazardous Materials, 424*, 127750. https://doi.org/10.1016/j.jhazmat.2021.127750

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Behavioural Mechanisms of Microplastic Pollutants in Marine Ecosystem: Challenges and Remediation Measurements

Megha Bansal · Deenan Santhiya · Jai Gopal Sharma

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Abstract Plastic pollution is the biggest threat to marine ecosystem owing to its high rates of disposal and low recovery from the environment. Due to inefficiency in degradation, most of plastic is fragmented into microplastics that are reported as ubiquitous toxicants in marine environment. The abundance of toxic microplastics in marine ecosystem causes adverse impacts on aquatic flora and fauna including oceans, lakes, rivers, coastal areas, and seas. This aggravates its toxicity and induces genomic instability, oxidative stress and disruption of marine organisms. Hence, it is necessary to understand the potential sources, types and behaviour of microplastic in marine environment. In this review, considering the pollution of aquatic ecosystem, major contributors of microplastics in marine environment along with their classification are brought out. Also, behaviour mechanisms of microplastics including physical, chemical and biological behaviours together with their ecological and toxicological impacts on marine ecosystem are illustrated. Finally, the remediation measures to combat against toxic microplastic pollution in aquatic ecosystem are highlighted to bring out an instant remedy for the environment.

Keywords Microplastics · Marine ecosystem · Toxicants · Behaviour · Remediation

Highlights
- Microplastics alter the characteristics of marine environment.
- The types and sources of microplastics in marine ecosystem are highlighted.
- Behavioural mechanisms of microplastics on marine ecosystem are illustrated.
- Potential remediation measures are proposed owing to impact of microplastics.

M. Bansal · J. G. Sharma
Department of Biotechnology, Delhi Technological University, Delhi, India
e-mail: megha_mt2k19@dtu.ac.in

J. G. Sharma
e-mail: sharmajaigopal@dce.ac.in

D. Santhiya (✉)
Department of Applied Chemistry, Delhi Technological University, Main Bawana Road Delhi, Shahbad Daulatpur 110042, India
e-mail: deenan.santhiya@dce.ac.in

1 Introduction

Plastic pollution is considered as major threat to marine environment affecting large number of aquatic organisms. The marine ecosystem has ubiquitous presence of plastic contaminants, prevailing on surface of oceans, on seabed and in water columns (Ritchie & Roser, 2018). Globally, the production of plastic has reached around 360 million tonnes of which a significant proportion is directly thrown into oceans without proper waste management (Jambeck et al., 2015). The major contributors to plastic waste in oceans originate from land-based sources including

landfill operations, agricultural activities, construction and waste released from industries (Geyer et al., 2017). Moreover, various bio solids leached from wastewater treatment plants also contribute substantial proportion of plastic fragments. The microbeads in cosmetic products and fibres also cause pollution of plastics in aquatic ecosystem (Mason et al., 2016). The pollution due to plastic accumulation in marine ecosystem is identified as the most prominent contaminant owing to its properties of flexibility, durability, low cost, corrosion resistance and easy handling (Botterell et al., 2019). In spite of wide range of applications of plastic worldwide, the issues related to its contamination and adverse impact on marine environment cannot be overlooked. Among the major plastic particles present in aquatic ecosystem, smaller fragments have garnered greater concern because of their ability to get ingested by marine organisms. The large plastic particles in marine environment are broken down into smaller pieces by ultraviolet degradation, physical abrasion and wave action, eventually forming microplastics (Dolatabadi & Ahmadzadeh, 2020). Approximately, 90% of plastic waste in oceans is microplastics owing to their very small size of less than 5 mm (Auta et al., 2017). The abundance of microplastics in marine environment poses a potential threat to aquatic flora and fauna with significant adverse impacts on oceans, lakes, rivers, coastal areas and seas.

1.1 Importance to Explore the Behaviour of Microplastics in Marine Environment

Anthropogenic activities and waste from rivers, winds, sewage sludge and water runoff cause accumulation of microplastics in marine environment. Also, tourism and recreational activities at shoreline create large amounts of waste litter that paves its pathway into aquatic water bodies (Avio et al., 2017). Military and research fleets, offshore installations and commercial vessels also cause detrimental effects on seashore (Galgani et al., 2015). Not only they cause damage to water, but they also get dispersed in soil and air causing threat to biodiversity (Wang et al., 2021). Plastic waste reach coasts by influence of water currents and meteorological conditions that cause spatial distribution of litter at coastline which further enter in oceans (Ourmieres et al., 2018). On entering the water column, microplastics having higher density like polyvinyl chloride tend to sink, whereas lower density microplastics like polyethylene and polypropylene tend to float in water. Change in density of particles along water column occurs due to colonization and biofouling of organisms on microplastic surface causing them to collapse at sediments (Avio et al., 2017). Water quality indices including pH, biological oxygen demand, dissolved oxygen and total nitrogen content change when exposed to microplastics (Kataoka et al., 2019). These characteristics of water significantly alter the biological matrix and cause destruction in environment.

Microplastic pollution in marine ecosystem causes potential threats and disorders in aquatic species because of their ingestion and entrapment (Bellasi et al., 2020; Galloway et al., 2017). Microplastics offer more surface area-to-volume ratio for accumulation of various contaminants including toxic metals and polychlorinated biphenyls (PCB) (Ozcan et al., 2013). These chemicals can bio accumulate in biological tissue and cause adverse effects in aquatic food chains. Moreover, added chemicals and additives in plastic manufacturing and organic pollutants are real threats to marine organisms (Hong et al., 2018). Microplastics bioaccumulation in marine environment increases with decreasing size and serve as a surface for proliferation of bacterial pathogens (Michielssen et al., 2016). Hence, in order to minimize the risks linked with ingestion of microplastic particles, it becomes essential to identify potential response and behaviour of microplastics in aquatic ecosystem.

In this review, major sources for various types of microplastic fragments in marine ecosystem are highlighted. Behavioural characteristics of microplastics focusing on physical, chemical and biological mechanisms are discussed to identify their fate in marine environment. In addition, potential impacts of microplastics on marine environment along with plausible remediation measures to overcome microplastic pollution have been provided.

2 Sources of Microplastics in Marine Ecosystem

Prevalence and abundance of microplastic particles in marine environment can be attributed to various sources. The major sources of microplastic can be attributed as primary microplastic that results from

direct release of pellets or powders and microbeads from cosmetic formulations, household products and raw materials from industries, whereas secondary microplastics arise from fragmentation of large plastic particles by ultraviolet radiation, oxidative stress and microbial degradation (Thompson, 2015). Also, evidences suggest the littering of microplastics in marine environments from plastic product manufacturing and waste management industries, from household activities and other commercial sectors. Thus, different source sectors provide substantial amount of microplastics in aquatic ecosystems. A brief summary of all the sources of microplastics from different usage sectors polluting the marine environment is depicted (Fig. 1).

3 Types of Microplastics in Marine Environment

Microplastics are classified into various types because of their different size and shapes (Guzzetti et al., 2018). Additionally, the toxicity and adverse effects of microplastics in marine ecosystem make it necessary to identify them. Hence, microplastics are categorized on the basis of origin, their source and on basis of properties.

3.1 Primary and Secondary Microplastics

This is the most common method to classify microplastics depending on their initial sizes when they enter the aquatic ecosystem. Microplastics fragments

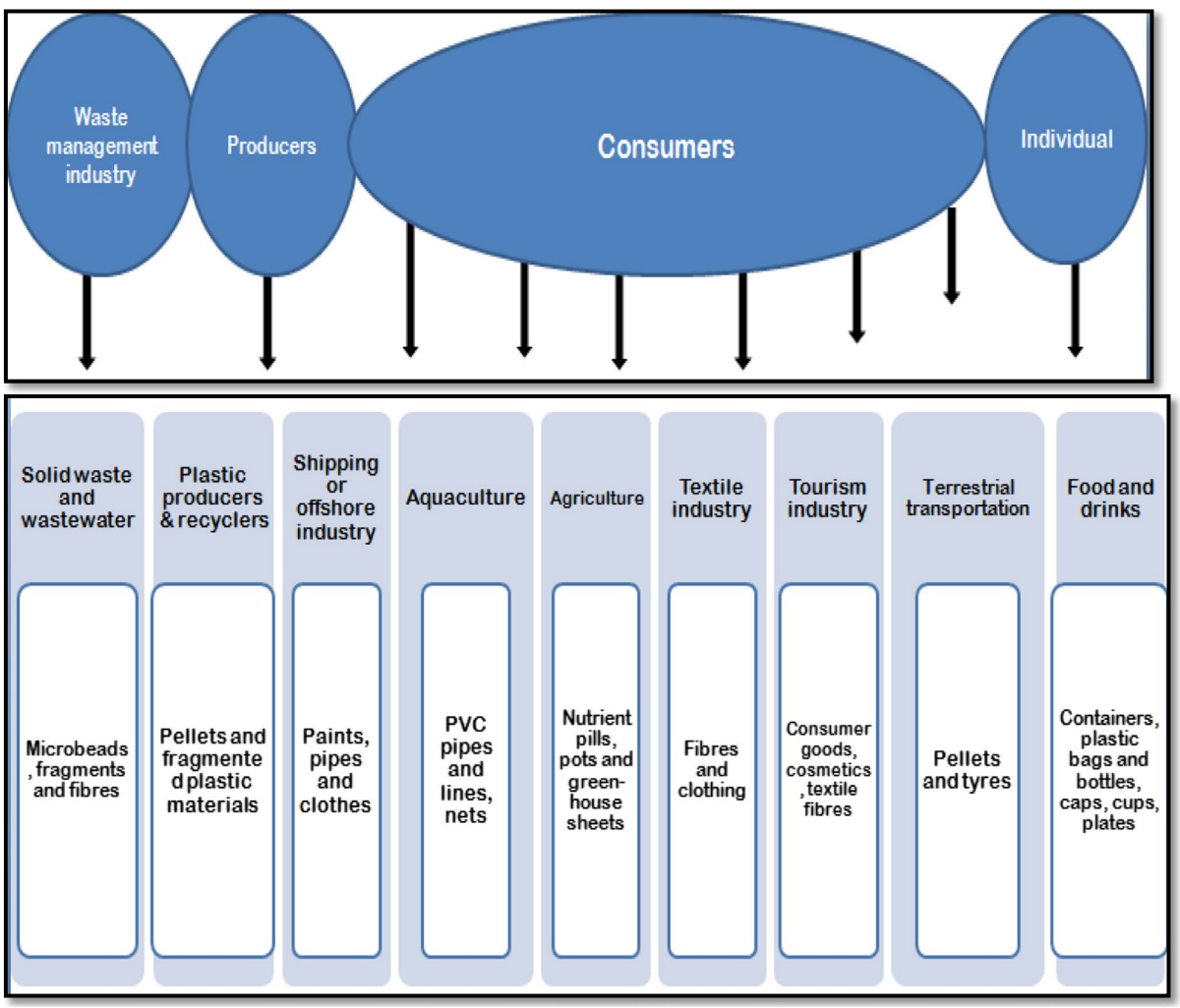


Fig. 1 Classification of microplastics in rivers and coastline

are categorized as primary or secondary microplastics on the basis of their origin. Primary microplastics include the plastic fragments synthesized by small size and used in various commercial applications. For example, microplastics are present in microbeads used for personal care products, abrasives in toothpaste, tiny beads used in exfoliation and pellets used for grinding and polishing (Fu & Wang, 2019).

Secondary microplastics are formed by fragmentation and degradation of bigger microplastic fragments under various atmospheric conditions (Yuan et al., 2020). Different processes including solar radiation, thermal oxidation and hydraulic power in rivers and oceans cause cracking and breakdown of large plastic particles (Liu et al., 2020). Therefore, small fragments with size less than 5 μm are formed commonly called as secondary microplastics. Different sources of secondary microplastics are industrial and plastic goods including plastic bottles, packaged bags, boxes, agricultural plastic films, marine paints, synthetic turfs, clothing, instruments and production wastes (Ammala et al., 2011).

3.2 Industry and Domestic Microplastics

Different industries generate primary or secondary microplastic in marine environment and human activities also add substantial load of microplastic contaminants in water (Kelly et al., 2019). Textile industry generates fibres including natural, regenerated and synthetic fibres that contain large amounts of microplastics released into water columns (Prata, 2018). Various researches have reported presence of microfibres in textile sewage containing microplastic particles approximately accounting for 35% of pollution in marine environment. The domestic washing also contributes to around 700,000 microfibres from wash load of 6 kg of acrylic fabric resulting in huge pollution (Napper & Thompson, 2016). Plastic microbeads occur in various products including shampoos, soaps and lotions used widely for domestic purposes thereby causing contamination when released from domestic sewage and wastewater treatment plants (Cheung & Fok, 2017). Different types of monomer microplastics are produced from automotive tyres. Most emission of microplastics is from car tyres with the road runoff (Kole et al., 2017).

Agricultural soils contain various types of microplastics because of utilization of agricultural plastic films and compost in soils. Farmers utilize various compost and plastic mulches for sustaining crops and ensuring food security (Ding et al., 2020). This indirectly paves the path for proliferation of microplastics that are released from soils to aquatic systems. Different construction sites and landfill operations along with domestic sewage from households also generate microplastics that are considered to be primary in origin (Galafassi et al., 2019).

3.3 Classification on Molecular Basis

Compared to the above two methods, the classification of microplastics at molecular level is achieved based on qualitative analysis of polymeric plastics. This classification helps to study the degradation potential of microplastics as well as recognize their chemical composition at time of degradation mechanisms (Yuan et al., 2020). The classification of microplastics on molecular basis and their properties are summarized in Table 1.

4 Behaviour of Microplastics in Marine Environment

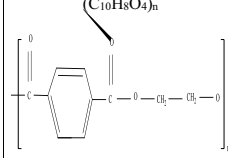
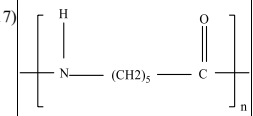
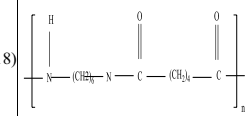
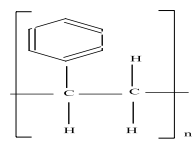
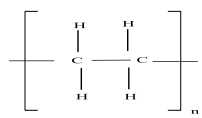
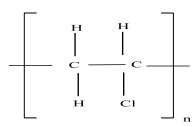
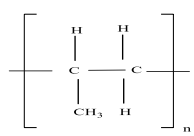
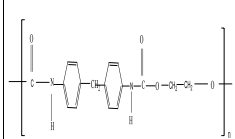
Microplastic bioaccumulation and biomagnifications can cause potential toxicity in humans and marine organisms. Thus, it is essential to determine behaviour of microplastics fragments and their mechanisms to understand harmful impacts in marine environment. The behaviour of microplastics in marine ecosystem can be classified as physical behaviour, chemical behaviour and biological behaviours (Wang et al., 2016). The various mechanisms underlying these behaviours are discussed further to analyse their impacts on aquatic system.

4.1 Physical Behaviour

4.1.1 Sedimentation

The residence of floating plastic debris in aquatic ecosystem causes an increase in the density of plastic debris (Barboza et al., 2019). The different microplastics interact with biotic and abiotic environment to reach high densities in sediment water (Rodrigues et al., 2018). Microplastics that reside on surface of water

Table 1 Microplastics polymers and their properties

Microplastic	Chemical Property	Physical Property			
	Chemical & Structural Formula	Crystallinity	Size (µm)	Glass Transition Temperature (Tg)	Melting Point
Polyethylene terephthalate (PET) (Zhao et al. 2018)	$(C_{10}H_8O_4)_n$ 	Semi-crystalline	100-150	80 ºC	260 ºC
Polyamide 6 (PA6) (Parodi et al. 2017)	$(C_6H_{11}NO)_n$ 	Crystalline	100-150	60 ºC	223 ºC
Polyamide 66 (PA66) (Pellini et al. 2018)	$(C_{12}H_{22}N_2O_2)_n$ 	Crystalline	100-150	58 ºC	260 ºC
Polystyrene (PS) (Sundbæk et al. 2018)	$(C_8H_8)_n$ 	Amorphous	100	100 ºC	240 ºC
Polyethylene (PE) (Peez et al. 2019)	$(C_2H_4)_n$ 	Semi-crystalline	100-150	-125 ºC	130 ºC
Polyvinyl Chloride (PVC) (Wu et al. 2019)	$(C_2H_3Cl)_n$ 	Amorphous	100-150	87 ºC	100-200 ºC
Polypropylene (PP) (Khoironi et al. 2020)	$(C_3H_6)_n$ 	Amorphous and semi-crystalline	100-150	0 ºC	160 ºC
Polyurethane (PU) (Zhang et al. 2018b)	$C_{17}H_{16}N_2O_4$ 	Amorphous	100-200	-20 ºC	90 C

can be drawn down into deep water column and deposited in sediments. The vertical and horizontal forces allow sediment particles to act as carriers of microplastics within rivers (Horton & Dixon, 2018). Sinking or floating behaviour of different sediment particles influence the occurrence of microplastics within the rivers and tend to have similar dynamics as clay sediment particles (Chubarenko et al., 2020). Also, abiotic factors weaken the molecular structure of plastic fragments and enhance their degradation into small microplastics that reach sediment zone due to change in sediment velocities and settling methods (van der Hal et al., 2017). Subsequently, adsorption and interaction of microplastics with sediment particles allows their sedimentation into large water systems.

4.1.2 Migration

Plastic pollution can quickly transfer from one site to another within the seawater by drifter buoys and physical oceanographic models (Law et al., 2010). Various types of microplastic monomers including polyethylene and polypropylene are buoyant and easily transferred within seawater (Ritchie & Roser, 2018). Underlying oceanic currents help in transfer of microplastics that are denser than seawater, for example, polyvinyl chloride (PVC) and polyethylene terephthalate (PET). These microplastics potentially sink in water due to high density and float on the surface only when they have entrapped air (Engler, 2012). Ocean currents are commonly referred as migration of water by different forces acting upon it including wind, temperature and salinity (Mahanty et al., 2016). Moreover, plastic particles could also get transferred by wind, and tsunami occurring in oceans (Lebreton et al., 2019). The strong wind can help to accelerate vortexing and redistribute plastic fragments at surface layers of water (Collignon et al., 2012). Additionally, tides and tsunamis could also offer transfer of plastic debris from one site to another within the seawater (Sadri & Thompson, 2014).

4.1.3 Accumulation

The migration of microplastics from one place to another by littering, landfill, dumping, wastewater treatment plants and accidental inputs causes their accumulation in coastal areas and aquatic system (Phelan et al., 2020). Since microplastics are non-degradable in nature, this allows them to accumulate within sediments and seabed and reside for prolonged period of time (Mason et al., 2016). The major cause of microplastics accumulation in ocean surface is due to their less density as compared with seawater (Gago et al., 2018). Seabed is also likely a sink for microplastics as large amounts of microfibres are also present in deep sea sediments (Sanchez-Vidal et al., 2018) that cause their accumulation. Fragmentation of plastic particles using different processes including physical abrasion, UV radiation and photo degradation could also result in accumulation of microplastics within the seawater (Zettler et al., 2013). The accumulation of plastic debris within the marine environment also results in formation of plastisphere (Zettler et al., 2013). This indirectly helps various microbial species to grow and proliferate on plastic surface and contribute to contamination (Lobelle & Cunliffe, 2011). Plastisphere acts as a habitat for microorganisms to survive and contains various microplastic fragments that are obtained from various sources. Accumulation of plastic debris can create new environment for organisms which indirectly increases their abundance and diffusivity (Diepens & Koelmans, 2018).

4.2 Chemical Behaviour

4.2.1 Degradation

A change in various properties of polymer including tensile strength, colour and shape of polymer under influence of several environmental factors could result in degradation (Bazli et al., 2020). The process of degradation could be categorized as thermal degradation, catalytic degradation, mechano-chemi-

cal degradation, photo-oxidative degradation, ozone-induced degradation and biodegradation (Singh & Sharma, 2008). Different types of microplastics such as polyethylene (PE), polypropylene (PP) and polystyrene (PS) would undergo degradation after exposure to solar radiation or physical abrasion thereby polluting marine environment (Andrady, 2011). Degradation plays an important role in recycling and reusing of microplastics fragments to reduce environmental pollution (Karbalaei et al., 2018). However, full conversion of microplastic particles in to carbon dioxide, water and inorganic compounds is extremely slow especially in marine environment where primary source of degradation is solar-UV radiation (Lithner et al., 2011). Various studies have reported slow degradation of microplastics by other processes as compared to solar radiation (Booth & Sørensen, 2020). Besides this, presence of additives in plastic particles can enhance the tendency for photo-oxidative degradation in marine environment (Zou et al., 2020). Also, oxygen concentrations and temperature changes would lead to degradation of various microplastic monomers (Andrady, 2011; Lithner et al., 2011).

Researchers are focused on determining the chemical reactions that result in degradation of polymers along with potential hazards from chemicals released by degradation. Degradation of plastic polymers usually follows abiotic or biotic pathways. Generally abiotic degradation involves the chemical breakdown process followed by biotic degradation that is initiated by biodegradation pathways (Fazey & Ryan, 2016). The various examples of polymers undergoing chemical degradation pathways are explained further in detail (Fig. 2).

Polyethylene (PE) degradation is initiated by exposing the polymer to ultraviolet radiation for breaking the main polymer chain to produce a free radical (Costa et al., 2018). After the initiation step, propagation phase occurs in which auto-oxidation process helps to form complex oxygenated low molecular weight compounds by reacting with free radicals (Gravouil et al., 2017). These low molecule weight fragments include aliphatic carboxylic acids, aldehydes, alcohols and ketones (Lapointe et al., 2020). In the auto-oxidation step, oxygen is incorporated in polymers thus its presence is necessary to initiate the whole process (Matthews, 2018). After the propagation phase, termination occurs that involves random cross-linking of molecules to generate degradation products including ethane, ethene, propane, propene, butane and hexane (Vasile, 2018). The polymer is degraded and becomes brittle with low molecular compounds formed as degradation products (Bäckström et al., 2017).

Polyvinyl chloride (PVC) is the most sensitive polymer towards UV radiation (Arnaud et al., 2017) and hence photo degradation is necessary (Feldman, 2016). When PVC is exposed to sunlight, it is dechlorinated with formation of conjugated double bonds in polymer and hydrochloric acid along with certain traces of other products (Prociak et al., 2018). The degradation of PVC is enhanced by photo-induced dechlorination in aerobic conditions, presence of HCl, mechanical stress, humidity, presence of other chemicals and high temperatures (Prociak et al., 2018; Seleem et al., 2017). The resulting unsaturated double bonds formed are less stable towards photo-degradation and are prone towards further degradation to smaller fragments (Hatakeyama-Sato et al., 2019).

Polyethylene terephthalate (PET) degradation in marine environment occurs through UV radiation, photo-oxidation as well as hydrolytic degradation (Fotopoulou & Karapanagioti, 2019). As discussed earlier in case of PE, UV radiation results in formation of free radicals that produce carboxylic acid end group in case of PVC (Morgan et al., 2017). The photo-degradation is followed by photo-oxidation process resulting in formation of hydrogen peroxide by various radical reactions (Jia et al., 2020). PET is also susceptible to hydrolytic degradation in marine environment. The low temperature degradation of PET under hydrolysis is the most important procedure and considered as reverse reaction of esterification of PET (Qin et al., 2020; Silva et al., 2018).

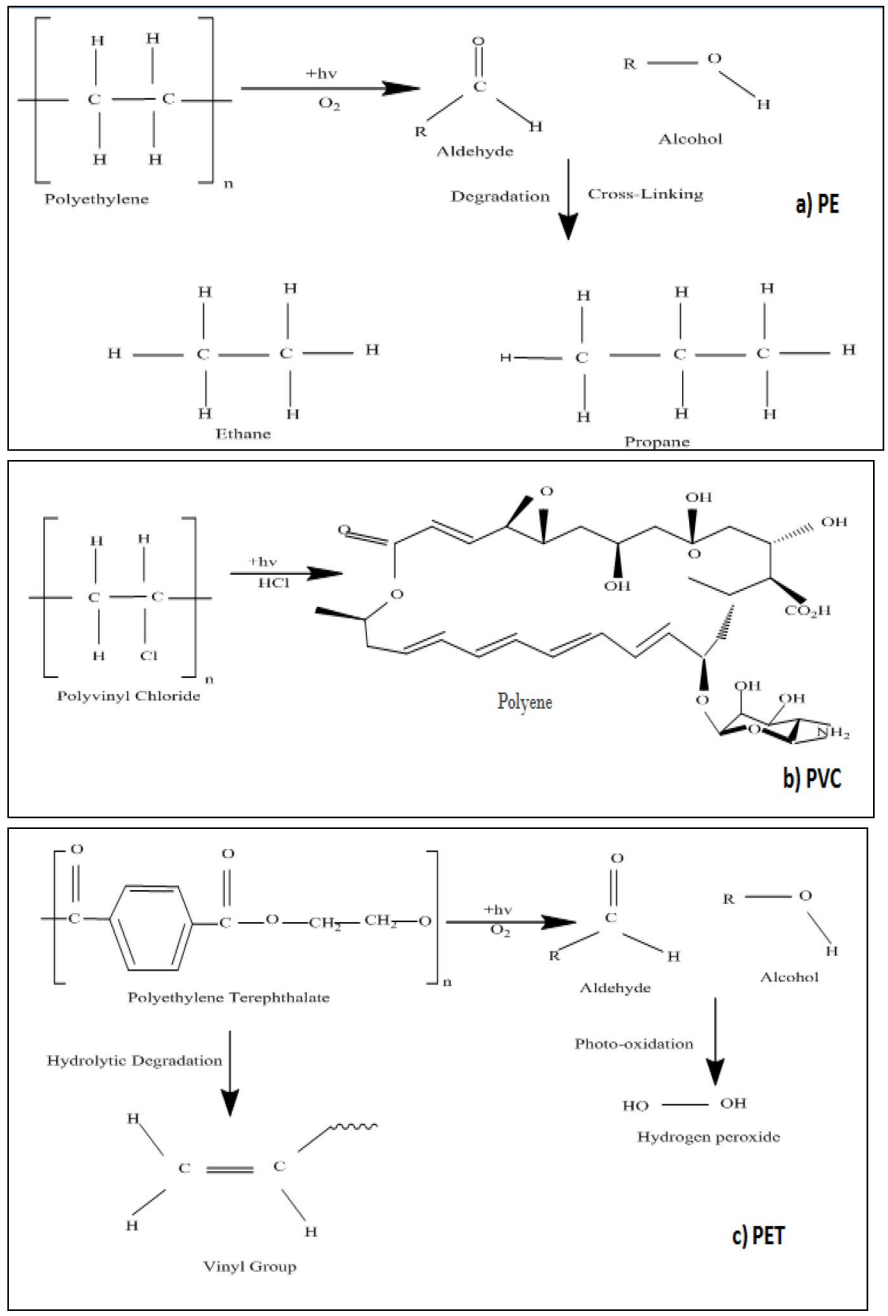


Fig. 2 Chemical reactions of different polymers

4.2.2 Adsorption

Adsorption was considered as a physical behaviour but it also shows significant chemical effects. The adsorption by physical means was relying on surface area as well as forces that increased adsorption of pollutants (Zhang et al., 2018a). On a contrary, chemical adsorption allows higher affinity for various pollutants on hydrophobic microplastic surface, and their sorption is influenced by crystallinity, diffusivity and surface area (Karapanagioti & Klontza, 2008). The environment is endowed with various organic polymers that constitute crystalline and amorphous regions (Sun et al., 2019). Crystalline region allows for lattice arrangement of molecules whereas molecules are randomly arranged

in amorphous region (Balzano et al., 2019). Thus, amorphous region exhibits a loose structure that allows greater sorption of organic pollutants on microplastic surfaces (Tien et al., 2009).

Also, properties of sorbates, weathering and residence time conditions, have an influence on adsorption in marine environment (Ogata et al., 2009). Moreover, polymer weathering could effectively increase surface area thereby enhancing the diffusivity (Losaria & Yim, 2020). Even photo-oxidation process could alter the plastic surface by developing cross-links, chain scissions, groups in polymers such as carbon moieties, resulting in cracks on the surface (Grause et al., 2020). Besides, weathering of plastic debris is considered to have long residence time in marine environments thereby resulting in greater sorption of organic pollutants (Lohmann, 2012). Different foulants attached to plastic debris also allow for increased sorption of contaminants including organic pollutants and metals (Hu et al., 2020). Persistent organic pollutants including polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs) and dichlorodiphenyltrichloroethane (DDTs) are major pollutants adsorbed on microplastics (Hirai et al., 2011). Charged or neutral regions of plastic surfaces could also direct the adsorption of cations or complexes of metals (Holmes et al., 2012). Study by Turner & Holmes, 2015 reported greater adsorption of various metals including Ag, Cd, Pb, Zn, Ni and Co to plastics surfaces as pH of the water environment was increased. Studies have reported that microplastics make significant contributions to exposure of various chemical contaminants (Avio et al., 2017; Murray & Cowie, 2011). These substances are directly released from gut of marine species or may leach out in marine environment on weathering (Ribeiro et al., 2019).

4.3 Biological Behaviour

4.3.1 Ingestion

Plastic debris has polluted the whole marine environment with significant harmful effects on marine ecosystem. Uptake and behaviour of microplastics has been demonstrated in above sections that shows various microplastics might accumulate in marine species, from zooplankton to crabs, including whales, fishes, mussels, sea reptiles and sea birds (Gallo et al., 2018). Plastic debris is known to be present in gut content of fishes globally including estuaries (Dantas et al., 2012), and demersal habitats (Lusher et al., 2013). Also, wild seafood bivalves have been a source for ingestion of plastic debris (Van Cauwenberghe & Janssen, 2014). Microplastics also allow attachment of microalgae on their surfaces acting as food source for filter feeders (Kershaw & Rochman, 2015).

The most common microplastic debris prevailing in marine environment is polystyrene (PS) (Blettler et al., 2017). The ingestion of microplastic by fish majorly occurred through gills and intestine. Gills provide high surface area for accumulation of most microplastic debris and ingestion could also occur through digestive tract (Ribeiro et al., 2019). These microplastic contaminants could remain in tissue and organs of fish thereby inducing toxic effects (Booth & Sørensen, 2020). The foraging time indicating the activity of searching food also reduced after the accumulation of polymer (Roch et al., 2020). Polystyrene microplastics exposure also reduced the swimming speed of fishes suggesting a weak constitution (Foulon et al., 2016).

Different chemicals disrupt the marine ecosystem including endocrine disrupting chemicals (EDCs) that alter the synthesis of endocrine hormones (Munn & Goumenou, 2019). Various consequences of disrupted endocrine systems are low birth rates, thyroid functioning, metabolism and increased hormone-sensitive cancers (Gore et al., 2015). Chemicals including tetrabromobisphenol A present in *Danio rerio* cause increased oxidation in fish (Yu et al., 2020) whereas silver in *Lemna minor* and *Daphnia magna* causes eco-toxicity in digestive system of these species (Kalčíková et al., 2020). Some precursors employed in textile and paper industry including perfluorooctanesulfonic acid (PFOS) and perfluorooctanoic acid (PFOA) can also cause microplastic ingestion in marine environment (Schaider et al., 2017).

The chronic exposure of these harmful chemicals on marine species has been directly linked to reduced growth and survival of offspring thereby having negative effect on marine biodiversity (Desforges et al., 2015).

4.3.2 Translocation

After ingestion of various microplastics by marine organisms, they may be retained in digestive tract, exit in form of feces, absorbed into epithelial lining of gut or translocated to other tissues within an organism (Hale et al., 2020). Various studies have reported the presence of microplastics in gills and digestive tract of mussels using polarized light microscopy (von Moos et al., 2012). Also, microplastics have been found in the ovary, gills and hepato pancreas of crabs (Farrell & Nelson, 2013). These microplastics can be easily translocated from gut to circulatory system (Jovanović, 2017). Depending on the organs of digestive system and difference in dimensional characteristics of ingested microplastics, it is easier to translocate them to other organs (Brennecke et al., 2015). Microplastics may be broken down to smaller fragments and enter the lipid-rich organs of fishes (Anderson et al., 2016). Oxidative stress and transient changes in regulatory activities could also occur in aquatic organisms (Cassia et al., 2018). Microplastics could also be transported from lower trophic level organisms to their predators, for example, from *mytilus edulis* to *carcinus maenas* (Farrell & Nelson, 2013) and from zooplankton to mysid shrimp (Setälä et al., 2014).

4.3.3 Biodegradation

Different properties of microplastics including their hydrophobicity and lack of metabolic activity to polymerize the plastics make it difficult to undergo biodegradation (Chowdhary et al., 2020). However, biodegradation is possible by formation of microbial biofilms on surface of microplastic fragments (Rummel et al., 2017). These biofilms allow for growth of bacteria and other organisms that could potentially help in degradation of plastic (Lobelle & Cunliffe, 2011). Also, the weight of various plastic polymers can be reduced by incubating different microbial strains (Harshvardhan & Jha, 2013). Additionally, surface of microplastics containing pits could also be an indicator for bacterial species to degrade the polymers (Zettler et al., 2013). Enzymes play an essential role in degradation of plastic polymers after they are subjected to chemical degradation (Adrio & Demain, 2014). The plastic fragments formed after the chemical (abiotic) degradation are buried deep in marine environment and take years for degradation (Fotopoulou & Karapanagioti, 2019). The microbial biofilms attached to the surface of polymer allow the formation of various enzymes that induce breakdown of plastic by hydrolysis (Ho et al., 2018). Various microorganisms have potential to produce enzymes that result in degradation of polymers, for example, *Thermobifida fusca* produces an enzyme, hydrolase, capable of degrading PET (Barth et al., 2016; Jabloune et al., 2020).

Strains of *Bacillus cereus* and *Bacillus spheri-cus* produce peroxidase that helps to degrade PE (Yuan et al., 2020) (Figure 3). The primary procedure for biodegradation of plastic is initiated by sticking of microbes on polymer surface and their proliferation (Kawai et al., 2019). These microbes help in excretion of extracellular enzymes that result in breakdown of plastics (Alshehrei, 2017). The enzymatic hydrolysis occurs in two ways: first is oligomers, dimers and monomers release degradation products that are converted to carbon-dioxide and water when enzyme attaches to polymer and hydrolytic division occurs (Roohi et al., 2017). Secondly, polymers are degraded by microbes in absence of air and new enzymes are needed to degrade the plastic in anaerobic conditions (Pathak and Navneet, 2017). Thus, biodegradation of polymers results in production of microbial biomass, carbon-dioxide and water that can be used by aquatic flora and fauna.

The enzymatic degradation of PET using hydrolases, esterases, proteases and cutinases has shown to hydrolyze PET surfaces. Modification in enzymes can improve the specificity and effi-

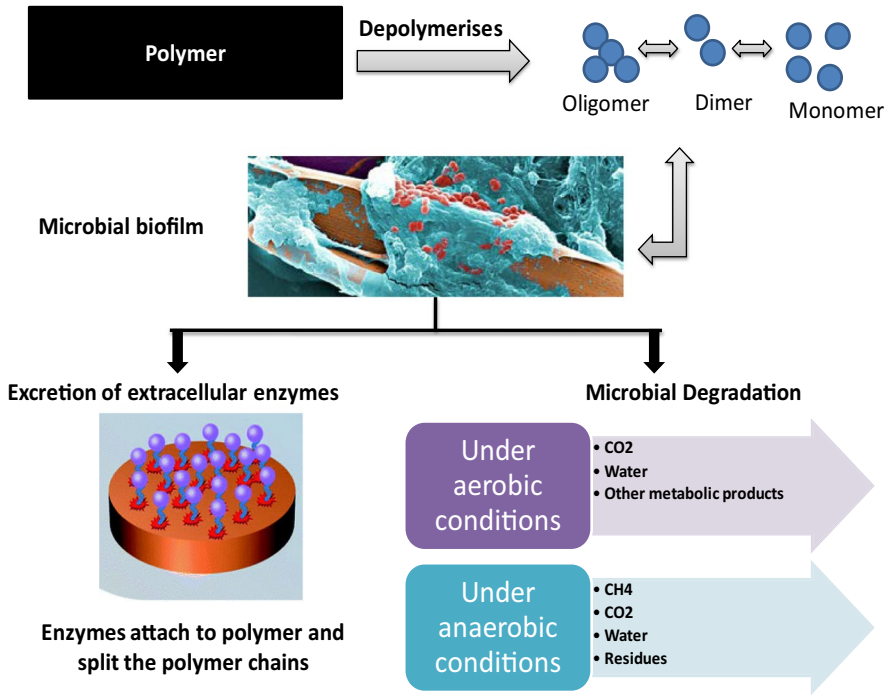


Fig. 3 Polymer degradation mechanism

ciency of PET degradation. For example, recent studies have shown that microbial species, *Ideonella sakaiensis*, has the capability to degrade PET by action of enzyme PETase. A hydrolytic reaction of PET under the enzyme, PETase, has efficiency to produce ethylene glycol and terephthalate that are required for microbial growth (Vandermaesen et al., 2016). Similarly, other enzymes are employed for degradation of microplastics PU, and 6-aminohexanoate oligomers (PA) (Wei & Zimmermann, 2017) (Fig. 4).

5 Challenges and Future Prospects

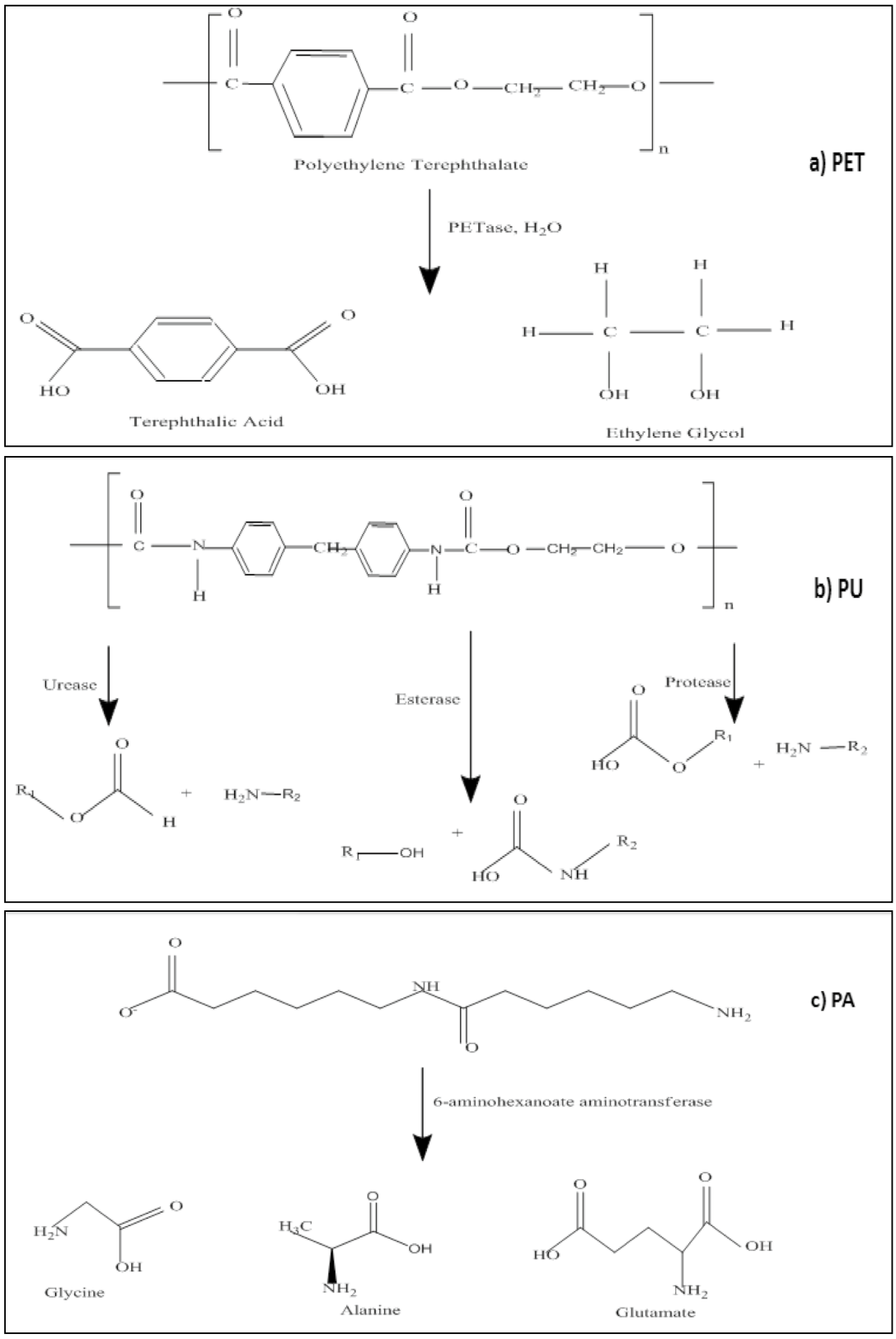
Extensive research studies have focused on the presence and effects of microplastics in marine environment. However, from the above discussions, knowledge gaps and major research questions in understanding the dynamics of microplastics in marine environment need utmost attention. Some broad issues with possible measures to safeguard aquatic environment are briefly summarized below.

Strategies to control different sources of microplastic pollutants in marine environment

- Source control of microplastic pollution in marine environment is commonly achieved by awareness programs and proper legislation (Picó & Barceló, 2019). Decreasing microplastic release can be achieved most appropriately by banning utilization of microplastic beads in cosmetic products. Some countries like the USA, Australia and Canada are on the forefront of implementing measures to combat microplastic in cosmetics. Also, secondary microplastics obtained from single-use plastics should be restricted for sale and consumption (Steensgaard et al., 2017). The combinatorial approach of restricting plastic usage and levy may attempt to educate public of environmental hazards from microplastics (Picó & Barceló, 2019).

Quantify microplastic pollutants in marine ecosystem and assess water quality parameters at field scale post microplastic contamination.

- Remediation of aquatic environment to eliminate organic matter is performed since a long time but quantifying microplastics is essential since



◂ **Fig. 4** Enzymatic degradation of PET, PU and PA

they are mistaken for various anthropogenic particles (Picó & Barceló, 2019). Visual analysis of microplastics is not adequate that makes physical detection a more reliable approach to quantify microplastics (Sol et al., 2020). FT-IR, Raman spectroscopy, pyrolysis–gas chromatography/mass spectrometry and thermogravimetry coupled to differential scanning calorimetry can help to speculate new insights into microplastics (Gong & Xie, 2020).

- To conquer the problem of water quality analysis following microplastic contamination, new instrumental tools that cater to real-time analysis should be utilized for effective monitoring of pollutants (Varotsos et al., 2020). Application of optical device-making system (ODMS) allows for efficient monitoring of water quality based on detection of emissions from photometry and ellipsometry (Varotsos et al., 2019).

Possible approaches to reduce the physical–chemical behavioural mechanisms of microplastics in marine environment

- The characteristic behaviour of microplastics in marine environment is a major problem which raises questions on the interactions between microplastics and chemicals that cause implications in organisms. Therefore, understanding the surface properties and ecocorona of microplastics is necessitated to address their environmental impact (Galloway et al., 2017). The application of atomic force microscopy, pyrolysis gas-chromatography mass-spectrometry and optical nanosensors can facilitate the characterisation of microplastic particles and explore composition of ecocorona to provide greater insights into surface methodology of microplastics (Burrows et al., 2020). Other techniques like scanning electron microscopy and optical profilometry can help to determine the roughness of microplastic surfaces and enable the measurement of interactions between chemicals that have harmful impact on environment (Connors et al., 2017). Also, action towards chemical management and disposal of polymers from chemical industries could provide beneficial effect in reducing microplastic load in aquatic ecosystem (Hamidian et al., 2021). This can be achieved by adopting green synthetic methodologies like use of green solvents in membrane fabrication process (Xie et al., 2021) and bio-based reagents for manufacturing polymers (Sternberg et al., 2021). Also, bioplastic as alternative to synthetic polymers could provide positive environmental benefits and sustainability to the economy (Pellis et al., 2021).

Control the shortcomings arising from biological behaviour of microplastics in marine environment

- Microbial remediation is necessary to reduce the potential biological impact of microplastics in ecosystem (Tiwari et al., 2020). As discussed above, biodegradation of microplastics is facilitated by microorganisms like *Rhodococcus* sp., *Bacillus* sp., *Clostridium* sp., *Staphylococcus* sp. and *Cladosporium* sp. (Shen et al., 2019). Also, addition of biodegradable additives like starch and stimulants (Satti et al., 2018) to microplastic polymer makes the polymer hydrophilic allowing greater efficiency of enzyme to act on microplastic (Zadjelovic et al., 2020). The formation of biofilms on microplastic surface allows for growth and proliferation of various microorganisms which can facilitate degradation of polymer within aquatic system (Tu et al., 2020).
- Sea animals including annelids (sandworms) and echinoderms (sea cucumber) could be efficient source of microplastic bioremediation (Masiá et al., 2020). Sea grasses like *Halophila ovalis*, *Halophila beccarii* and *Zostera japonica* can help in trapping microplastics and mangrove rhizospheres including *Avicennia marina* and *Aegiceras corniculatum* could act as a sink for microplastic particles (Huang et al., 2021).
- Different algae like *Anabaena spiroides*, *Cocconis, Navicula*, *Chlorella fusca* and *Chlamydomonas reinhardtii* can colonize on microplastic surfaces and result in their biodegradation (Chia et al., 2020). Not only does algae help in biodegradation of microplastics, but it also acts as a potential source for bioplastic production (Rahman & Miller, 2017). Microalgae biomass, biorefinery processing, genetic engineering tools like CRISPR technology and intermixing with other blended materials could help in the production

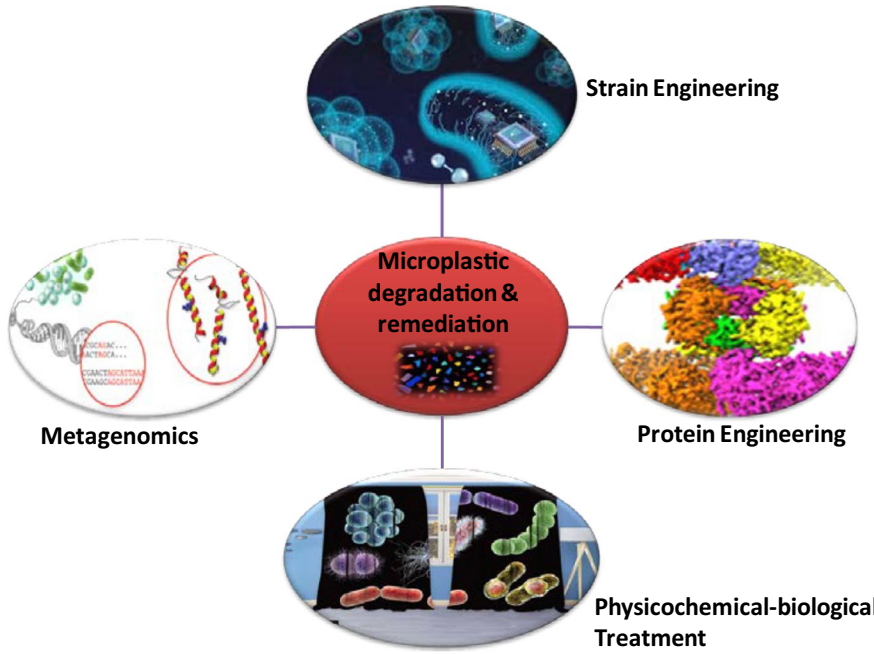


Fig. 5 Methods to remediate and degrade microplastics in marine environment

of algal-based bioplastic that promise non-toxic effect on environment (Karan et al., 2019).

Futuristic strategies to control microplastic pollution in marine environment

- To mitigate the effects of microplastic pollution, futuristic objectives aiming at different bioengineering-based solutions need to be considered (Coyle et al., 2020). Hence, the following tools can provide plausible solutions to microplastic contamination (Fig. 5):
- Strain engineering: This technique relies on using genetic engineering and recombinant DNA technology to manipulate microbial cells for increasing their efficiency of microplastic degradation (Ferreira et al., 2018; Jaiswal et al., 2020). For example, *Bacillus subtilis* strain could efficiently produce PETase enzyme capable of degrading PET microplastic (Huang et al., 2018). Also, microalgae, *Phaeodactylum tricornutum*, generated a microbial cell factory that produced PETase from *Ideonella sakaiensis* for biological decomposition of PET in seawater (Moog and Blank 2019).
- Protein engineering: This approach helps to enhance the activity and efficiency of enzymes for microplastic degradation (Wei et al., 2016). The protein engineered enzymes help to degrade the building blocks of microplastic. For example, the engineered hydrolase were able to degrade and hydrolyze PET films faster as compared to normal enzymes (Han et al., 2017; Ma et al., 2018). Another study by Islam et al., 2019 demonstrated that cutinases derived from *Thermomonospora curvata* could establish faster degradation of polyurethane.
- Metagenomics: This novel approach provides detailed understanding on classification of microorganisms and characterises enzymes capable of degrading microplastic polymers (Bhatt et al., 2021; Ufarté et al., 2015). The microbial biofilms formed on microplastic surfaces can be analysed using targeted or shotgun metagenomic technique to reveal distinct microbial profile (Tiwari et al., 2021) which can be found in the National Centre for Biotechnology Information (NCBI) portal (Bryant et al., 2016). Enzymes responsible for microplastic degradation can be found in biofilms through shotgun metagenomic sequencing process (Pinnell & Turner, 2019). Microplastic bioremediation could also be accelerated using in silico genome mining integrated with metagenomic datasets (Rai et al., 2021; Ziemert et al., 2016).

- Combined physicochemical-biological treatment: Recent studies have reported the use of chemical and biological methods simultaneously to improve degradation of microplastics and provide possible remediation measure in marine environment (Sánchez, 2020). For example, combination of chemical and biological methods was assessed by Tsiota et al., 2018 to degrade secondary microplastics in marine environment. In this study, polyethylene was exposed to ultraviolet radiation resulting in microplastics fragments which were incubated with pelagic microbiomes for increasing their degradation efficiency (Tsiota et al., 2018). Similarly, the microbial strain of *Penicillium variabile* could greatly enhance mineralization of polystyrene under ozone pre-treatment (Sharma et al., 2021).

6 Conclusion

Microplastic particles are emerging pollutants in aquatic ecosystem and also draw attention to other emerging contaminants. Due to the size and feasibility of entering the marine environment, microplastics bioaccumulation is high. The concentration of microplastics from different sources endangers the food security, biodiversity and marine ecosystem. Plastic polymers have different additives and stabilizers that adsorb various toxic pollutants and contaminants from surrounding environment. Their toxic effects are closely related with physical, chemical and biological properties that alter the functioning and characteristics of aquatic flora and fauna. The behavioural mechanisms not only demonstrate their fate but also the response of microplastics to certain environmental conditions. Based on this review, better understanding on types and behaviour of microplastics in marine environment along with their potential impacts could be identified. Development of biodegradable plastic products could help in combating against plastic pollution in marine ecosystem. The utilization of various microorganisms for bioremediation of plastic contaminants can provide sustainable solutions to plastic free marine environment. Further research on engineered enzymes and microbial strains could provide significant insights on degradation and remediation of microplastics in aquatic ecosystem.

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References

Adrio, J. L., & Demain, A. L. (2014). Microbial enzymes: Tools for biotechnological processes. *Biomolecules, 4*, 117–139. https://doi.org/10.3390/biom4010117

Alshehrei, F. (2017). Biodegradation of synthetic and natural plastic by microorganisms. Environ Microbiol 12

Ammala, A., Bateman, S., Dean, K., et al. (2011). An overview of degradable and biodegradable polyolefins. *Progress in Polymer Science, 36*, 1015–1049. https://doi.org/10.1016/j.progpolymsci.2010.12.002

Anderson, J. C., Park, B. J., & Palace, V. P. (2016). Microplastics in aquatic environments: Implications for Canadian ecosystems. *Environmental Pollution, 218*, 269–280. https://doi.org/10.1016/j.envpol.2016.06.074

Andrady, A. L. (2011). Microplastics in the marine environment. *Marine Pollution Bulletin, 62*, 1596–1605. https://doi.org/10.1016/j.marpolbul.2011.05.030

Arnaud, S. P., Wu, L., Chang, M.-A.W., et al. (2017). New bio-based monomers: Tuneable polyester properties using branched diols from biomass. *Faraday Discussions, 202*, 61–77. https://doi.org/10.1039/C7FD00057J

Auta, H. S., Emenike, C. U., & Fauziah, S. H. (2017). Distribution and importance of microplastics in the marine environment: A review of the sources, fate, effects, and potential solutions. *Environment International, 102*, 165–176. https://doi.org/10.1016/j.envint.2017.02.013

Avio, C. G., Gorbi, S., & Regoli, F. (2017). Plastics and microplastics in the oceans: From emerging pollutants to emerged threat. *Marine Environment Research, 128*, 2–11. https://doi.org/10.1016/j.marenvres.2016.05.012

Bäckström, E., Odelius, K., & Hakkarainen, M. (2017). Trash to treasure: Microwave-assisted conversion of polyethylene to functional chemicals. *Industrial and Engineering Chemistry Research, 56*, 14814–14821. https://doi.org/10.1021/acs.iecr.7b04091

Balzano, L., Coussens, B., Engels, T., et al. (2019). Multiscale structure and microscopic deformation mechanisms of gel-spun ultrahigh-molecular-weight polyethylene fibers. *Macromolecules, 52*, 5207–5216. https://doi.org/10.1021/acs.macromol.9b00247

Barboza, L. G. A., Cózar, A., Gimenez, B. C. G., et al. (2019). Chapter 17 — macroplastics pollution in the marine environment. In: Sheppard C (ed) *World Seas: an Environmental Evaluation* (Second Edition). Academic Press, pp 305–328

Barth, M., Honak, A., Oeser, T., et al. (2016). A dual enzyme system composed of a polyester hydrolase and a carboxylesterase enhances the biocatalytic degradation of polyethylene terephthalate films. *Biotechnology Journal, 11*, 1082–1087. https://doi.org/10.1002/biot.201600008

Bazli, M., Zhao, X.-L., Jafari, A., et al. (2020). Mechanical properties of pultruded GFRP profiles under seawater sea sand concrete environment coupled with UV radiation and moisture. *Construction and Building Materials, 258*, 120369. https://doi.org/10.1016/j.conbuildmat.2020.120369

Bellasi, A., Binda, G., Pozzi, A., et al. (2020). Microplastic contamination in freshwater environments: A review, focusing on interactions with sediments and benthic organisms. *Environments, 7*, 30. https://doi.org/10.3390/environments7040030

Bhatt, P., Pathak, V. M., Bagheri, A. R., & Bilal, M. (2021). Microplastic contaminants in the aqueous environment, fate, toxicity consequences, and remediation strategies. *Environmental Research, 200*, 111762. https://doi.org/10.1016/j.envres.2021.111762

Blettler, M. C. M., Ulla, M. A., Rabuffetti, A. P., & Garello, N. (2017). Plastic pollution in freshwater ecosystems: Macro-, meso-, and microplastic debris in a floodplain lake. *Environmental Monitoring and Assessment, 189*, 581. https://doi.org/10.1007/s10661-017-6305-8

Booth, A. M., & Sørensen, L. (2020). Microplastic fate and impacts in the environment. In T. Rocha-Santos, M. Costa, & C. Mouneyrac (Eds.), *Handbook of Microplastics in the Environment* (pp. 1–24). Springer International Publishing.

Botterell, Z. L. R., Beaumont, N., Dorrington, T., et al. (2019). Bioavailability and effects of microplastics on marine zooplankton: A review. *Environmental Pollution, 245*, 98–110. https://doi.org/10.1016/j.envpol.2018.10.065

Brennecke, D., Ferreira, E. C., Costa, T. M. M., et al. (2015). Ingested microplastics (>100μm) are translocated to organs of the tropical fiddler crab Uca rapax. *Mar Pollut Bull* 96:491–495. https://doi.org/10.1016/j.marpolbul.2015.05.001

Bryant, J. A., Clemente, T. M., Viviani, D. A., et al. (2016). Diversity and activity of communities inhabiting plastic debris in the North Pacific Gyre. *mSystems* 1:. https://doi.org/10.1128/mSystems.00024-16

Burrows, S. D., Frustaci, S., Thomas, K. V., & Galloway, T. (2020). Expanding exploration of dynamic microplastic surface characteristics and interactions. *TrAC, Trends in Analytical Chemistry, 130*, 115993. https://doi.org/10.1016/j.trac.2020.115993

Cassia, R., Nocioni, M., Correa-Aragunde, N., & Lamattina, L. (2018) Climate change and the impact of greenhouse gasses: CO2 and NO, friends and foes of plant oxidative stress. *Front Plant Sci* 9.https://doi.org/10.3389/fpls.2018.00273

Cheung, P. K., & Fok, L. (2017). Characterisation of plastic microbeads in facial scrubs and their estimated emissions in Mainland China. *Water Research, 122*, 53–61. https://doi.org/10.1016/j.watres.2017.05.053

Chia, W. Y., Ying Tang, D. Y., Khoo, K. S., et al. (2020). Nature's fight against plastic pollution: Algae for plastic biodegradation and bioplastics production. *Environ Sci Ecotechnology, 4*, 100065. https://doi.org/10.1016/j.ese.2020.100065

Chowdhary, P., Raj, A., Verma, D., & Akhter, Y. (2020). *Microorganisms for sustainable environment and health*. Elsevier.

Chubarenko, I., Efimova, I., Bagaeva, M., et al. (2020). On mechanical fragmentation of single-use plastics in the sea swash zone with different types of bottom sediments: Insights from laboratory experiments. *Marine Pollution Bulletin, 150*, 110726. https://doi.org/10.1016/j.marpolbul.2019.110726

Collignon, A., Hecq, J.-H., Glagani, F., et al. (2012). Neustonic microplastic and zooplankton in the North Western Mediterranean Sea. *Marine Pollution Bulletin, 64*, 861–864. https://doi.org/10.1016/j.marpolbul.2012.01.011

Connors, K. A., Dyer, S. D., & Belanger, S. E. (2017). Advancing the quality of environmental microplastic research. *Environmental Toxicology and Chemistry, 36*, 1697–1703. https://doi.org/10.1002/etc.3829

Costa, J. P. D., Nunes, A. R., Santos, P. S. M., et al. (2018). Degradation of polyethylene microplastics in seawater: Insights into the environmental degradation of polymers. *Journal of Environmental Science and Health, Part A Environmental Science, 53*, 866–875. https://doi.org/10.1080/10934529.2018.1455381

Coyle, R., Hardiman, G., & Driscoll, K. O. (2020). Microplastics in the marine environment: a review of their sources, distribution processes and uptake into ecosystems. *Case Stud Chem Environ Eng* 100010.https://doi.org/10.1016/j.cscee.2020.100010

Dantas, D. V., Barletta, M., & da Costa, M. F. (2012). The seasonal and spatial patterns of ingestion of polyfilament nylon fragments by estuarine drums (Sciaenidae). *Environmental Science and Pollution Research, 19*, 600–606. https://doi.org/10.1007/s11356-011-0579-0

de Silva, J., AA e, Rodrigues JKG, Carvalho MW de, , et al. (2018). Mechanical performance of asphalt mixtures using polymer-micronized PET-modified binder. *Road Mater Pavement Des, 19*, 1001–1009. https://doi.org/10.1080/14680629.2017.1283353

Desforges, J.-P.W., Galbraith, M., & Ross, P. S. (2015). Ingestion of microplastics by zooplankton in the Northeast Pacific Ocean. *Archives of Environmental Contamination and Toxicology, 69*, 320–330. https://doi.org/10.1007/s00244-015-0172-5

Diepens, N. J., & Koelmans, A. A. (2018). Accumulation of plastic debris and associated contaminants in aquatic food webs. *Environmental Science and Technology, 52*, 8510–8520. https://doi.org/10.1021/acs.est.8b02515

Ding, L., Zhang, S., Wang, X., et al. (2020). The occurrence and distribution characteristics of microplastics in the agricultural soils of Shaanxi Province, in north-western China. *Science of the Total Environment, 720*, 137525. https://doi.org/10.1016/j.scitotenv.2020.137525

Dolatabadi M, Ahmadzadeh S (2020) Microplastics pollution in the aquatic environment: problems and challenges. *J Environ Health Sustain Dev*. https://doi.org/10.18502/jehsd.v5i2.3383

Engler, R. E. (2012). The complex interaction between marine debris and toxic chemicals in the ocean. *Environmental Science and Technology, 46*, 12302–12315. https://doi.org/10.1021/es3027105

Farrell, P., & Nelson, K. (2013). Trophic level transfer of microplastic: Mytilus edulis (L.) to Carcinus maenas (L.). *Environmental Pollution, 177*, 1–3. https://doi.org/10.1016/j.envpol.2013.01.046

Fazey, F. M. C., & Ryan, P. G. (2016). Biofouling on buoyant marine plastics: An experimental study into the effect of size on surface longevity. *Environmental Pollution, 210*, 354–360. https://doi.org/10.1016/j.envpol.2016.01.026

Feldman, D. (2016). Polyolefin, olefin copolymers and polyolefin polyblend nanocomposites. *J Macromol Sci Part A, 53*, 651–658. https://doi.org/10.1080/10601325.2016.1212313

Ferreira. R. da G., Azzoni, A. R., Freitas, S. (2018). Techno-economic analysis of the industrial production of a low-cost enzyme using E. coli: the case of recombinant β-glucosidase. *Biotechnol Biofuels 11*:81. https://doi.org/10.1186/s13068-018-1077-0

Fotopoulou, K. N., & Karapanagioti, H. K. (2019). Degradation of various plastics in the environment. In H. Takada & H. K. Karapanagioti (Eds.), *Hazardous Chemicals Associated with Plastics in the Marine Environment* (pp. 71–92). Springer International Publishing.

Foulon, V., Le Roux, F., Lambert, C., et al. (2016). Colonization of polystyrene microparticles by vibrio crassostreae: Light and electron microscopic investigation. *Environmental Science and Technology, 50*, 10988–10996. https://doi.org/10.1021/acs.est.6b02720

Fu, Z., & Wang, J. (2019). Current practices and future perspectives of microplastic pollution in freshwater ecosystems in China. *Science of the Total Environment, 691*, 697–712. https://doi.org/10.1016/j.scitotenv.2019.07.167

Gago, J., Carretero, O., Filgueiras, A. V., & Viñas, L. (2018). Synthetic microfibers in the marine environment: A review on their occurrence in seawater and sediments. *Marine Pollution Bulletin, 127*, 365–376. https://doi.org/10.1016/j.marpolbul.2017.11.070

Galafassi, S., Nizzetto, L., & Volta, P. (2019). Plastic sources: A survey across scientific and grey literature for their inventory and relative contribution to microplastics pollution in natural environments, with an emphasis on surface water. *Science of the Total Environment, 693*, 133499. https://doi.org/10.1016/j.scitotenv.2019.07.305

Galgani, F., Hanke, G., & Maes, T. (2015). Global distribution, composition and abundance of marine litter. In M. Bergmann, L. Gutow, & M. Klages (Eds.), *Marine Anthropogenic Litter* (pp. 29–56). Springer International Publishing.

Gallo, F., Fossi, C., Weber, R., et al. (2018). Marine litter plastics and microplastics and their toxic chemicals components: The need for urgent preventive measures. *Environmental Sciences Europe, 30*, 13. https://doi.org/10.1186/s12302-018-0139-z

Galloway, T. S., Cole, M., & Lewis, C. (2017). Interactions of microplastic debris throughout the marine ecosystem. *Nat Ecol Evol, 1*, 1–8. https://doi.org/10.1038/s41559-017-0116

Geyer, R., Jambeck, J. R., & Law, K. L. (2017). Production, use, and fate of all plastics ever made. *Science Advances, 3*, e1700782. https://doi.org/10.1126/sciadv.1700782

Gong, J., & Xie, P. (2020). Research progress in sources, analytical methods, eco-environmental effects, and control measures of microplastics. *Chemosphere, 254*, 126790. https://doi.org/10.1016/j.chemosphere.2020.126790

Gore, A. C., Chappell, V. A., Fenton, S. E., et al. (2015). Executive Summary to EDC-2: The Endocrine Society's Second Scientific Statement on Endocrine-Disrupting Chemicals. *Endocrine Reviews, 36*, 593–602. https://doi.org/10.1210/er.2015-1093

Grause, G., Chien, M.-F., & Inoue, C. (2020). Changes during the weathering of polyolefins. *Polymer Degradation and Stability, 181*, 109364. https://doi.org/10.1016/j.polymdegradstab.2020.109364

Gravouil, K., Ferru-Clément, R., Colas, S., et al. (2017). Transcriptomics and lipidomics of the environmental strain Rhodococcus ruber point out consumption pathways and potential metabolic bottlenecks for polyethylene degradation. *Environmental Science and Technology, 51*, 5172–5181. https://doi.org/10.1021/acs.est.7b00846

Guzzetti, E., Sureda, A., Tejada, S., & Faggio, C. (2018). Microplastic in marine organism: Environmental and toxicological effects. *Environmental Toxicology and Pharmacology, 64*, 164–171. https://doi.org/10.1016/j.etap.2018.10.009

Hale, R. C., Seeley, M. E., Guardia, M. J. L., et al. (2020). A global perspective on microplastics. *J Geophys Res Oceans* 125:e2018JC014719. https://doi.org/10.1029/2018JC014719

Hamidian, A. H., Ozumchelouei, E. J., Feizi, F., et al. (2021). A review on the characteristics of microplastics in wastewater treatment plants: A source for toxic chemicals. *Journal of Cleaner Production, 295*, 126480. https://doi.org/10.1016/j.jclepro.2021.126480

Han, X., Liu, W., Huang, J.-W., et al. (2017). Structural insight into catalytic mechanism of PET hydrolase. *Nature Communications, 8*, 2106. https://doi.org/10.1038/s41467-017-02255-z

Harshvardhan, K., & Jha, B. (2013). Biodegradation of low-density polyethylene by marine bacteria from pelagic waters, Arabian Sea, India. *Marine Pollution Bulletin, 77*, 100–106. https://doi.org/10.1016/j.marpolbul.2013.10.025

Hatakeyama-Sato, K., Masui, T., Serikawa, T., et al. (2019). Nonconjugated redox-active polymer mediators for rapid electrocatalytic charging of lithium metal oxides. *ACS Appl Energy Mater, 2*, 6375–6382. https://doi.org/10.1021/acsaem.9b01007

Hirai, H., Takada, H., Ogata, Y., et al. (2011). Organic micropollutants in marine plastics debris from the open ocean

and remote and urban beaches. *Marine Pollution Bulletin, 62*, 1683–1692. https://doi.org/10.1016/j.marpolbul.2011.06.004

Ho, B. T., Roberts, T. K., & Lucas, S. (2018). An overview on biodegradation of polystyrene and modified polystyrene: The microbial approach. *Critical Reviews in Biotechnology, 38*, 308–320. https://doi.org/10.1080/07388551.2017.1355293

Holmes, L. A., Turner, A., & Thompson, R. C. (2012). Adsorption of trace metals to plastic resin pellets in the marine environment. *Environmental Pollution, 160*, 42–48. https://doi.org/10.1016/j.envpol.2011.08.052

Hong, S. H., Shim, W. J., Jang, M. (2018). Chapter 9 - Chemicals associated with marine plastic debris and microplastics: analyses and contaminant levels. In: Zeng EY (ed) Microplastic Contamination in Aquatic Environments. Elsevier, pp 271–315

Horton, A. A., & Dixon, S. J. (2018). Microplastics: an introduction to environmental transport processes. *WIREs Water 5*.https://doi.org/10.1002/wat2.1268

Hu, B., Ai, Y., Jin, J., et al. (2020). Efficient elimination of organic and inorganic pollutants by biochar and biochar-based materials. *Biochar, 2*, 47–64. https://doi.org/10.1007/s42773-020-00044-4

Huang, X., Cao, L., Qin, Z., et al. (2018). Tat-independent secretion of polyethylene terephthalate hydrolase PETase in Bacillus subtilis 168 mediated by its native signal peptide. *Journal of Agriculture and Food Chemistry, 66*, 13217–13227. https://doi.org/10.1021/acs.jafc.8b05038

Huang, Y., Xiao, X., Effiong, K., et al. (2021). New insights into the microplastic enrichment in the blue carbon ecosystem: Evidence from seagrass meadows and mangrove forests in Coastal South China Sea. *Environmental Science and Technology, 55*, 4804–4812. https://doi.org/10.1021/acs.est.0c07289

Islam, S., Apitius, L., Jakob, F., & Schwaneberg, U. (2019). Targeting microplastic particles in the void of diluted suspensions. *Environment International, 123*, 428–435. https://doi.org/10.1016/j.envint.2018.12.029

Jabloune, R., Khalil, M., Moussa, I. E.B., et al. (2020). Enzymatic degradation of *p*-nitrophenyl esters, polyethylene terephthalate, cutin, and suberin by sub1, a suberinase encoded by the plant pathogen *Streptomyces scabies*. *Microbes Environ 35*.https://doi.org/10.1264/jsme2.ME19086

Jaiswal, S., Sharma, B., & Shukla, P. (2020). Integrated approaches in microbial degradation of plastics. *Environmental Technology and Innovation, 17*, 100567. https://doi.org/10.1016/j.eti.2019.100567

Jambeck, J. R., Geyer, R., Wilcox, C., et al. (2015). Plastic waste inputs from land into the ocean. *Science, 347*, 768–771. https://doi.org/10.1126/science.1260352

Jia, H., Ben, H., Luo, Y., & Wang, R. (2020). Catalytic fast pyrolysis of poly (ethylene terephthalate) (PET) with zeolite and nickel chloride. *Polymers, 12*, 705. https://doi.org/10.3390/polym12030705

Jovanović, B. (2017). Ingestion of microplastics by fish and its potential consequences from a physical perspective. *Integrated Environmental Assessment and Management, 13*, 510–515. https://doi.org/10.1002/ieam.1913

Kalčíková, G., Skalar, T., Marolt, G., & Jemec Kokalj, A. (2020). An environmental concentration of aged microplastics with adsorbed silver significantly affects aquatic organisms. *Water Research, 175*, 115644. https://doi.org/10.1016/j.watres.2020.115644

Karan, H., Funk, C., Grabert, M., et al. (2019). Green bioplastics as part of a circular bioeconomy. *Trends in Plant Science, 24*, 237–249. https://doi.org/10.1016/j.tplants.2018.11.010

Karapanagioti, H. K., & Klontza, I. (2008). Testing phenanthrene distribution properties of virgin plastic pellets and plastic eroded pellets found on Lesvos island beaches (Greece). *Marine Environment Research, 65*, 283–290. https://doi.org/10.1016/j.marenvres.2007.11.005

Karbalaei, S., Hanachi, P., Walker, T. R., & Cole, M. (2018). Occurrence, sources, human health impacts and mitigation of microplastic pollution. *Environmental Science and Pollution Research, 25*, 36046–36063. https://doi.org/10.1007/s11356-018-3508-7

Kataoka, T., Nihei, Y., Kudou, K., & Hinata, H. (2019). Assessment of the sources and inflow processes of microplastics in the river environments of Japan. *Environmental Pollution, 244*, 958–965. https://doi.org/10.1016/j.envpol.2018.10.111

Kawai, F., Kawabata, T., & Oda, M. (2019). Current knowledge on enzymatic PET degradation and its possible application to waste stream management and other fields. *Applied Microbiology and Biotechnology, 103*, 4253–4268. https://doi.org/10.1007/s00253-019-09717-y

Kelly, M. R., Lant, N. J., Kurr, M., & Burgess, J. G. (2019). Importance of water-volume on the release of microplastic fibers from laundry. *Environmental Science and Technology, 53*, 11735–11744. https://doi.org/10.1021/acs.est.9b03022

Kershaw, P. J., Rochman, C. M. (2015). Sources, fate and effects of microplastics in the marine environment: part 2 of a global assessment. *Rep Stud - IMOFAOUnesco-IOCWMOIAEAUNUNEP Jt Group Experts Sci Asp Mar Environ Prot GESAMP Eng No 93*

Khoironi, A., Hadiyanto, H., Anggoro, S., & Sudarno, S. (2020). Evaluation of polypropylene plastic degradation and microplastic identification in sediments at Tambak Lorok coastal area, Semarang. *Indonesia. Mar Pollut Bull, 151*, 110868. https://doi.org/10.1016/j.marpolbul.2019.110868

Kole, P. J., Löhr, A. J., Van Belleghem, F. G. A. J., & Ragas, A. M. J. (2017). Wear and tear of tyres: A stealthy source of microplastics in the environment. *International Journal of Environmental Research and Public Health, 14*, 1265. https://doi.org/10.3390/ijerph14101265

Lapointe, M., Farner, J. M., Hernandez, L. M., & Tufenkji, N. (2020). Understanding and improving microplastic removal during water treatment: Impact of coagulation and flocculation. *Environmental Science and Technology, 54*, 8719–8727. https://doi.org/10.1021/acs.est.0c00712

Law, K. L., Morét-Ferguson, S., Maximenko, N. A., et al. (2010). Plastic accumulation in the North Atlantic Subtropical Gyre. *Science, 329*, 1185–1188. https://doi.org/10.1126/science.1192321

Lebreton, L., Egger, M., & Slat, B. (2019). A global mass budget for positively buoyant macroplastic debris in the

ocean. *Science and Reports, 9*, 12922. https://doi.org/10.1038/s41598-019-49413-5

Lithner, D., Larsson, Å., & Dave, G. (2011). Environmental and health hazard ranking and assessment of plastic polymers based on chemical composition. *Science of the Total Environment, 409*, 3309–3324. https://doi.org/10.1016/j.scitotenv.2011.04.038

Liu, Y., Huang, J., Jin, J., et al. (2020). The classification of micro-plastics and biodegradation of plastics/micro-plastics. 10

Lobelle, D., & Cunliffe, M. (2011). Early microbial biofilm formation on marine plastic debris. *Marine Pollution Bulletin, 62*, 197–200. https://doi.org/10.1016/j.marpolbul.2010.10.013

Lohmann, R. (2012). Critical review of low-density polyethylene's partitioning and diffusion coefficients for trace organic contaminants and implications for its use as a passive sampler. *Environmental Science and Technology, 46*, 606–618. https://doi.org/10.1021/es202702y

Losaria, P. M., & Yim, J.-H. (2020). Enhancement of strain-sensing performance through gas phase incorporation of siloxane into thermoplastic polyurethane-conducting polymer composite. *Macromolecular Chemistry and Physics, 221*, 2000155. https://doi.org/10.1002/macp.202000155

Lusher, A. L., McHugh, M., & Thompson, R. C. (2013). Occurrence of microplastics in the gastrointestinal tract of pelagic and demersal fish from the English Channel. *Marine Pollution Bulletin, 67*, 94–99. https://doi.org/10.1016/j.marpolbul.2012.11.028

Ma, Y., Yao, M., Li, B., et al. (2018). Enhanced poly(ethylene terephthalate) hydrolase activity by protein engineering. *Engineering, 4*, 888–893. https://doi.org/10.1016/j.eng.2018.09.007

Mahanty, M. M., Mohanty, P. K., Pattnaik, A. K., et al. (2016). Hydrodynamics, temperature/salinity variability and residence time in the Chilika lagoon during dry and wet period: Measurement and modeling. *Continental Shelf Research, 125*, 28–43. https://doi.org/10.1016/j.csr.2016.06.017

Masiá, P., Sol, D., Ardura, A., et al. (2020). Bioremediation as a promising strategy for microplastics removal in wastewater treatment plants. *Marine Pollution Bulletin, 156*, 111252. https://doi.org/10.1016/j.marpolbul.2020.111252

Mason, S. A., Garneau, D., Sutton, R., et al. (2016). Microplastic pollution is widely detected in US municipal wastewater treatment plant effluent. *Environmental Pollution, 218*, 1045–1054. https://doi.org/10.1016/j.envpol.2016.08.056

Matthews, M. E. (2018). *Crystallisation behaviour of commercial polyethylenes: A fundamental study*. Stellenbosch University.

Michielssen, M. R., Michielssen, E. R., Ni, J., & Duhaime, M. B. (2016). Fate of microplastics and other small anthropogenic litter (SAL) in wastewater treatment plants depends on unit processes employed. *Environmental Science: Water Research & Technology, 2*, 1064–1073. https://doi.org/10.1039/C6EW00207B

Moog, U., Blank, R., et al. (2019). Entwicklungsstörungen und Behinderungen. In G. F. Hoffmann, M. J. Lentze, & J. Spranger (Eds.), *Pädiatrie: Grundlagen und Praxis* (pp. 1–6). Springer.

Morgan, A., Cocca, M., Vega, K., et al. (2017). Vacuum UV photo-oxidation of poly(ethylene terephthalate). *Journal of Adhesion Science and Technology, 31*, 2542–2554. https://doi.org/10.1080/01694243.2017.1308994

Munn, S., & Goumenou, M. (2019). Thresholds for endocrine disrupters and related uncertainties. *Report of the Endocrine Disrupters Expert Advisory Group*

Murray, F., & Cowie, P. R. (2011). Plastic contamination in the decapod crustacean Nephrops norvegicus (Linnaeus, 1758). *Marine Pollution Bulletin, 62*, 1207–1217. https://doi.org/10.1016/j.marpolbul.2011.03.032

Napper, I. E., & Thompson, R. C. (2016). Release of synthetic microplastic plastic fibres from domestic washing machines: Effects of fabric type and washing conditions. *Marine Pollution Bulletin, 112*, 39–45. https://doi.org/10.1016/j.marpolbul.2016.09.025

Ogata, Y., Takada, H., Mizukawa, K., et al. (2009). International Pellet Watch: Global monitoring of persistent organic pollutants (POPs) in coastal waters. 1. Initial phase data on PCBs, DDTs, and HCHs. *Marine Pollution Bulletin, 58*, 1437–1446. https://doi.org/10.1016/j.marpolbul.2009.06.014

Ourmieres, Y., Mansui, J., Molcard, A., et al. (2018). The boundary current role on the transport and stranding of floating marine litter: The French Riviera case. *Continental Shelf Research, 155*, 11–20. https://doi.org/10.1016/j.csr.2018.01.010

Ozcan, S., Tor, A., & Aydin, M. E. (2013). Investigation on the levels of heavy metals, polycyclic aromatic hydrocarbons, and polychlorinated biphenyls in sewage sludge samples and ecotoxicological testing. *CLEAN – Soil Air Water 41*:411–418. https://doi.org/10.1002/clen.201100187

Parodi, E., Govaert, L. E., & Peters, G. W. M. (2017). Glass transition temperature versus structure of polyamide 6: A flash-DSC study. *Thermochimica Acta, 657*, 110–122. https://doi.org/10.1016/j.tca.2017.09.021

Peez, N., Janiska, M.-C., & Imhof, W. (2019). The first application of quantitative 1H NMR spectroscopy as a simple and fast method of identification and quantification of microplastic particles (PE, PET, and PS). *Analytical and Bioanalytical Chemistry, 411*, 823–833. https://doi.org/10.1007/s00216-018-1510-z

Pellini, G., Gomiero, A., Fortibuoni, T., et al. (2018). Characterization of microplastic litter in the gastrointestinal tract of Solea solea from the Adriatic Sea. *Environmental Pollution, 234*, 943–952. https://doi.org/10.1016/j.envpol.2017.12.038

Pellis, A., Malinconico, M., Guarneri, A., & Gardossi, L. (2021). Renewable polymers and plastics: Performance beyond the green. *New Biotechnology, 60*, 146–158. https://doi.org/10.1016/j.nbt.2020.10.003

Phelan, A., (Anya), Ross H, Setianto NA, , et al. (2020). Ocean plastic crisis—mental models of plastic pollution from remote Indonesian coastal communities. *PLoS ONE, 15*, e0236149. https://doi.org/10.1371/journal.pone.0236149

Picó, Y., & Barceló, D. (2019). Analysis and prevention of microplastics pollution in water: Current perspectives

and future directions. *ACS Omega, 4*, 6709–6719. https://doi.org/10.1021/acsomega.9b00222

Pinnell, L. J., & Turner, J. W. (2019). Shotgun metagenomics reveals the benthic microbial community response to plastic and bioplastic in a coastal marine environment. Front Microbiol 10.https://doi.org/10.3389/fmicb.2019.01252

Prata, J. C. (2018). Microplastics in wastewater: State of the knowledge on sources, fate and solutions. *Marine Pollution Bulletin, 129*, 262–265. https://doi.org/10.1016/j.marpolbul.2018.02.046

Prociak, A., Kurańska, M., Cabulis, U., et al. (2018). Effect of bio-polyols with different chemical structures on foaming of polyurethane systems and foam properties. *Industrial Crops and Products, 120*, 262–270. https://doi.org/10.1016/j.indcrop.2018.04.046

Qin, Y., Qu, M., Kaschta, J., et al. (2020). Studies on recycled polyester. In S. S. Muthu (Ed.), *Recycled polyester: Manufacturing, properties, test methods, and identification* (pp. 29–67). Springer.

Rahman, A., & Miller, C. D. (2017). Chapter 6 - Microalgae as a source of bioplastics. In R. P. Rastogi, D. Madamwar, & A. Pandey (Eds.), *Algal Green Chemistry* (pp. 121–138). Elsevier.

Rai, P. K., Lee, J., Brown, R. J. C., & Kim, K.-H. (2021). Micro- and nano-plastic pollution: Behavior, microbial ecology, and remediation technologies. *Journal of Cleaner Production, 291*, 125240. https://doi.org/10.1016/j.jclepro.2020.125240

Ribeiro, F., O'Brien, J. W., Galloway, T., & Thomas, K. V. (2019). Accumulation and fate of nano- and micro-plastics and associated contaminants in organisms. *TrAC, Trends in Analytical Chemistry, 111*, 139–147. https://doi.org/10.1016/j.trac.2018.12.010

Ritchie, H., & Roser, M. (2018). Plastic pollution. Our World Data

Roch, S., Friedrich, C., & Brinker, A. (2020). Uptake routes of microplastics in fishes: Practical and theoretical approaches to test existing theories. *Science and Reports, 10*, 3896. https://doi.org/10.1038/s41598-020-60630-1

Rodrigues, M. O., Abrantes, N., Gonçalves, F. J. M., et al. (2018). Spatial and temporal distribution of microplastics in water and sediments of a freshwater system (Antuã River, Portugal). *Science of the Total Environment, 633*, 1549–1559. https://doi.org/10.1016/j.scitotenv.2018.03.233

Roohi, B. K., Kuddus, M., et al. (2017). Microbial enzymatic degradation of biodegradable plastics. *Current Pharmaceutical Biotechnology, 18*, 429–440. https://doi.org/10.2174/1389201018666170523165742

Rummel, C. D., Jahnke, A., Gorokhova, E., et al. (2017). Impacts of biofilm formation on the fate and potential effects of microplastic in the aquatic environment. *Environmental Science & Technology Letters, 4*, 258–267. https://doi.org/10.1021/acs.estlett.7b00164

Sadri, S. S., & Thompson, R. C. (2014). On the quantity and composition of floating plastic debris entering and leaving the Tamar Estuary, Southwest England. *Marine Pollution Bulletin, 81*, 55–60. https://doi.org/10.1016/j.marpolbul.2014.02.020

Sánchez, C. (2020). Fungal potential for the degradation of petroleum-based polymers: An overview of macro- and microplastics biodegradation. *Biotechnology Advances, 40*, 107501. https://doi.org/10.1016/j.biotechadv.2019.107501

Sanchez-Vidal, A., Thompson, R. C., Canals, M., & de Haan, W. P. (2018). The imprint of microfibres in southern European deep seas. *PLoS ONE, 13*, e0207033. https://doi.org/10.1371/journal.pone.0207033

Satti, S. M., Shah, A. A., Marsh, T. L., & Auras, R. (2018). Biodegradation of poly(lactic acid) in soil microcosms at ambient temperature: Evaluation of natural attenuation, bio-augmentation and bio-stimulation. *Journal of Polymers and the Environment, 26*, 3848–3857. https://doi.org/10.1007/s10924-018-1264-x

Schaider, L. A., Balan, S. A., Blum, A., et al. (2017). Fluorinated compounds in U.S. Fast Food packaging. *Environmental Science & Technology Letters, 4*, 105–111. https://doi.org/10.1021/acs.estlett.6b00435

Seleem, S., Hopkins, M., Olivio, J., & Schiraldi, D. A. (2017). Comparison of thermal decomposition of polystyrene products vs. bio-based polymer aerogels. Ohio J Sci 117:50–60. https://doi.org/10.18061/ojs.v117i2.5828

Setälä, O., Fleming-Lehtinen, V., & Lehtiniemi, M. (2014). Ingestion and transfer of microplastics in the planktonic food web. *Environmental Pollution, 185*, 77–83. https://doi.org/10.1016/j.envpol.2013.10.013

Sharma, S., Basu, S., Shetti, N. P., et al. (2021). Microplastics in the environment: Occurrence, perils, and eradication. *Chemical Engineering Journal, 408*, 127317. https://doi.org/10.1016/j.cej.2020.127317

Shen, M., Zeng, G., Zhang, Y., et al. (2019). Can biotechnology strategies effectively manage environmental (micro) plastics? *Science of the Total Environment, 697*, 134200. https://doi.org/10.1016/j.scitotenv.2019.134200

Singh, B., & Sharma, N. (2008). Mechanistic implications of plastic degradation. *Polymer Degradation and Stability, 93*, 561–584. https://doi.org/10.1016/j.polymdegradstab.2007.11.008

Sol, D., Laca, A., Laca, A., & Díaz, M. (2020). Approaching the environmental problem of microplastics: Importance of WWTP treatments. *Science of the Total Environment, 740*, 140016. https://doi.org/10.1016/j.scitotenv.2020.140016

Steensgaard, I. M., Syberg, K., Rist, S., et al. (2017). From macro- to microplastics — analysis of EU regulation along the life cycle of plastic bags. *Environmental Pollution, 224*, 289–299. https://doi.org/10.1016/j.envpol.2017.02.007

Sternberg, J., Sequerth, O., & Pilla, S. (2021). Green chemistry design in polymers derived from lignin: Review and perspective. *Progress in Polymer Science, 113*, 101344. https://doi.org/10.1016/j.progpolymsci.2020.101344

Sun, Q., Aguila, B., & Ma, S. (2019). Opportunities of porous organic polymers for radionuclide sequestration. *Trends Chem, 1*, 292–303. https://doi.org/10.1016/j.trechm.2019.02.010

Sundbæk, K. B., Koch, I. D. W., Villaro, C. G., et al. (2018). Sorption of fluorescent polystyrene microplastic particles to edible seaweed Fucus vesiculosus. *Journal of Applied*

Phycology, 30, 2923–2927. https://doi.org/10.1007/s10811-018-1472-8

Thompson, R. C. (2015). Microplastics in the marine environment: Sources, consequences and solutions. In M. Bergmann, L. Gutow, & M. Klages (Eds.), *Marine Anthropogenic Litter* (pp. 185–200). Springer International Publishing.

Tien, C.-J., Wu, W.-H., Chuang, T.-L., & Chen, C. S. (2009). Development of river biofilms on artificial substrates and their potential for biomonitoring water quality. *Chemosphere, 76*, 1288–1295. https://doi.org/10.1016/j.chemosphere.2009.06.013

Tiwari, N., Santhiya, D., & Sharma, J. G. (2020). Microbial remediation of micro-nano plastics: Current knowledge and future trends. *Environmental Pollution, 265*, 115044. https://doi.org/10.1016/j.envpol.2020.115044

Tiwari, N., Bansal, M., & Sharma, J. G. (2021). 14 - Metagenomics: a powerful lens viewing the microbial world. In: Shah MP, Rodriguez-Couto S (eds) Wastewater Treatment Reactors. Elsevier, pp 309–339

Tsiota, P., Karkanorachaki, K., Syranidou, E., et al. (2018). Microbial degradation of HDPE secondary microplastics: preliminary results. In: Cocca M, Di Pace E, Errico ME, et al. (eds) *Proceedings of the International Conference on Microplastic Pollution in the Mediterranean Sea*. Springer International Publishing, Cham, pp 181–188

Tu, C., Zhou, Q., Zhang, C., et al. (2020). Biofilms of microplastics. In D. He & Y. Luo (Eds.), *Microplastics in terrestrial environments: Emerging contaminants and major challenges* (pp. 299–317). Springer International Publishing.

Turner, A., & Holmes, L. A. (2015). Adsorption of trace metals by microplastic pellets in fresh water. *Environmental Chemistry, 12*, 600–610. https://doi.org/10.1071/EN14143

Ufarté, L., Laville, É., Duquesne, S., & Potocki-Veronese, G. (2015). Metagenomics for the discovery of pollutant degrading enzymes. *Biotechnology Advances, 33*, 1845–1854. https://doi.org/10.1016/j.biotechadv.2015.10.009

Van Cauwenberghe, L., & Janssen, C. R. (2014). Microplastics in bivalves cultured for human consumption. *Environmental Pollution, 193*, 65–70. https://doi.org/10.1016/j.envpol.2014.06.010

van der Hal, N., Ariel, A., & Angel, D. L. (2017). Exceptionally high abundances of microplastics in the oligotrophic Israeli Mediterranean coastal waters. *Marine Pollution Bulletin, 116*, 151–155. https://doi.org/10.1016/j.marpolbul.2016.12.052

Vandermaesen, J., Horemans, B., Bers, K., et al. (2016). Application of biodegradation in mitigating and remediating pesticide contamination of freshwater resources: State of the art and challenges for optimization. *Applied Microbiology and Biotechnology, 100*, 7361–7376. https://doi.org/10.1007/s00253-016-7709-z

Varotsos, C. A., Krapivin, V. F., & Mkrtchyan, F. A. (2019). New optical tools for water quality diagnostics. *Water, Air, and Soil Pollution, 230*, 177. https://doi.org/10.1007/s11270-019-4228-4

Varotsos, C. A., Krapivin, V. F., Mkrtchyan, F. A., et al. (2020). A novel approach to monitoring the quality of lakes water by optical and modeling tools: Lake Sevan as a case study. *Water, Air, and Soil Pollution, 231*, 435. https://doi.org/10.1007/s11270-020-04792-8

Vasile, C. (2018). Polymeric nanocomposites and nanocoatings for food packaging: A review. *Materials, 11*, 1834. https://doi.org/10.3390/ma11101834

von Moos, N., Burkhardt-Holm, P., & Köhler, A. (2012). Uptake and effects of microplastics on cells and tissue of the blue mussel Mytilus edulis L. after an experimental exposure. *Environmental Science and Technology, 46*, 11327–11335. https://doi.org/10.1021/es302332w

Wang, J., Tan, Z., Peng, J., et al. (2016). The behaviors of microplastics in the marine environment. *Marine Environment Research, 113*, 7–17. https://doi.org/10.1016/j.marenvres.2015.10.014

Wang, Q., Adams, C. A., Wang, F., et al. (2021). Interactions between microplastics and soil fauna: A critical review. *Critical Reviews in Environment Science and Technology, 0*, 1–33. https://doi.org/10.1080/10643389.2021.1915035

Wei, R., & Zimmermann, W. (2017). Microbial enzymes for the recycling of recalcitrant petroleum-based plastics: How far are we? *Microbial Biotechnology, 10*, 1308–1322. https://doi.org/10.1111/1751-7915.12710

Wei, R., Oeser, T., Schmidt, J., et al. (2016). Engineered bacterial polyester hydrolases efficiently degrade polyethylene terephthalate due to relieved product inhibition. *Biotechnology and Bioengineering, 113*, 1658–1665. https://doi.org/10.1002/bit.25941

Wu, P., Cai, Z., Jin, H., & Tang, Y. (2019). Adsorption mechanisms of five bisphenol analogues on PVC microplastics. *Science of the Total Environment, 650*, 671–678. https://doi.org/10.1016/j.scitotenv.2018.09.049

Xie, W., Li, T., Tiraferri, A., et al. (2021). Toward the next generation of sustainable membranes from green chemistry principles. *ACS Sustain Chem Eng, 9*, 50–75. https://doi.org/10.1021/acssuschemeng.0c07119

Yu, F., Yang, C., Huang, G., et al. (2020). Interfacial interaction between diverse microplastics and tetracycline by adsorption in an aqueous solution. *Science of the Total Environment, 721*, 137729. https://doi.org/10.1016/j.scitotenv.2020.137729

Yuan, J., Ma, J., Sun, Y., et al. (2020). Microbial degradation and other environmental aspects of microplastics/plastics. *Science of the Total Environment, 715*, 136968. https://doi.org/10.1016/j.scitotenv.2020.136968

Zadjelovic, V., Chhun, A., Quareshy, M., et al. (2020). Beyond oil degradation: Enzymatic potential of Alcanivorax to degrade natural and synthetic polyesters. *Environmental Microbiology, 22*, 1356–1369. https://doi.org/10.1111/1462-2920.14947

Zettler, E. R., Mincer, T. J., & Amaral-Zettler, L. A. (2013). Life in the "Plastisphere": Microbial communities on plastic marine debris. *Environmental Science and Technology, 47*, 7137–7146. https://doi.org/10.1021/es401288x

Zhang, H., Wang, J., Zhou, B., et al. (2018a). Enhanced adsorption of oxytetracycline to weathered microplastic polystyrene: Kinetics, isotherms and influencing factors. *Environmental Pollution, 243*, 1550–1557. https://doi.org/10.1016/j.envpol.2018.09.122

Zhang, Q., Hu, X.-M., Wu, M.-Y., et al. (2018b). Effects of different catalysts on the structure and properties of polyurethane/water glass grouting materials. *Journal of*

Applied Polymer Science, 135, 46460. https://doi.org/10.1002/app.46460

Zhao, J., Ran, W., Teng, J., et al. (2018). Microplastic pollution in sediments from the Bohai Sea and the Yellow Sea, China. *Science of the Total Environment, 640–641*, 637–645. https://doi.org/10.1016/j.scitotenv.2018.05.346

Ziemert, N., Alanjary, M., & Weber, T. (2016). The evolution of genome mining in microbes – a review. *Natural Products Reports, 33*, 988–1005. https://doi.org/10.1039/C6NP00025H

Zou, W., Xia, M., Jiang, K., et al. (2020). Photo-oxidative degradation mitigated the developmental toxicity of polyamide microplastics to zebrafish larvae by modulating macrophage-triggered proinflammatory responses and apoptosis. *Environmental Science and Technology, 54*, 13888–13898. https://doi.org/10.1021/acs.est.0c05399

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MINI-REVIEW

Insights into microbial diversity on plastisphere by multi-omics

Neha Tiwari[1] · Megha Bansal[1] · Deenan Santhiya[2] · Jai Gopal Sharma[1]

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Abstract

Plastic pollution is a major concern in marine environment as it takes many years to degrade and is one of the greatest threats to marine life. Plastic surface, referred to as plastisphere, provides habitat for growth and proliferation of various microorganisms. The discovery of these microbes is necessary to identify significant genes, enzymes and bioactive compounds that could help in bioremediation and other commercial applications. Conventional culture techniques have been successful in identifying few microbes from these habitats, leaving majority of them yet to be explored. As such, to recognize the vivid genetic diversity of microbes residing in plastisphere, their structure and corresponding ecological roles within the ecosystem, an emerging technique, called metagenomics has been explored. The technique is expected to provide hitherto unknown information on microbes from the plastisphere. Metagenomics along with next generation sequencing provides comprehensive knowledge on microbes residing in plastisphere that identifies novel microbes for plastic bioremediation, bioactive compounds and other potential benefits. The following review summarizes the efficiency of metagenomics and next generation sequencing technology over conventionally used methods for culturing microbes. It attempts to illustrate the workflow mechanism of metagenomics to elucidate diverse microbial profiles. Further, importance of integrated multi-omics techniques has been highlighted in discovering microbial ecology residing on plastisphere for wider applications.

Keywords Plastisphere · Microbes · Metagenomics · Next generation sequencing · Bioremediation · Multi-omics

Introduction

Ocean plastics

Since 2014, usage of plastic materials has been increasing worldwide with over 300 million tons in production every year (Eriksen et al. 2014). As a result, plastic waste has become a serious environmental concern in seas and oceans, posing immense threat to marine life and human health (Bansal et al. 2021). Despite widespread recognition of these threats, plastics are still abundantly released into oceans through various pathways like rivers, atmospheric transmission, beach littering, shipping, aquaculture and oil spills (Kershaw and Rochman 2015). These plastic wastes accumulate in marine ecosystems because of various characteristics such as prolonged durability, lower rate of recycling, improper waste management and prolonged use (Barnes et al. 2009). Figure 1 portrays an estimate of global plastic wastes across various ocean surfaces (adapted from Eriksen et al. 2014).

Plastic wastes disposed of in oceans can be classified into three categories based on size: macroplastics (having

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Neha Tiwari and Megha Bansal contributed equally.

✉ Deenan Santhiya
deenan.santhiya@de.ac.in

Neha Tiwari
nehatiwari_phd2k18@dtu.ac.in

Megha Bansal
megha_mt2k19@dtu.ac.in

Jai Gopal Sharma
sharmajaigopal@dce.ac.in

[1] Department of Biotechnology, Delhi Technological University, Delhi, India

[2] Department of Applied Chemistry, Delhi Technological University, Shahbad Daulatpur, Main Bawana Road, Delhi 110042, India

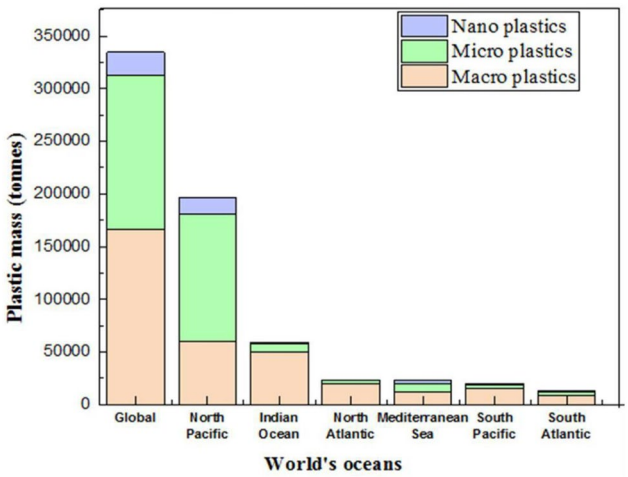

Fig. 1 Global plastic wastes across various ocean surfaces (adapted from Eriksen et al. 2014)

size greater than 5 mm), micro plastics (size less than 5 mm) and nano plastics (size lower than 1 µm) (Galloway et al. 2017). Rivers and lakes also get contaminated by plastics of various size ranges resulting in transfer of these wastes to other freshwater ecosystems, ground water sources like tube wells and ultimately the ocean (Koelmans et al. 2019). The headwaters of major Asian rivers, longest rivers in different countries and prominent lakes that get directed towards seas are heavily contaminated with plastic pollutants. Examples of contaminated river sites at various locations around the globe are listed below:

1. Lake Geneva (Switzerland) was reported to contain microplastics in the range of 1–7 particles/L predominated by polystyrene (Faure et al. 2012).
2. Saint Lawrence River (Canada) was shown to contain large accumulation of plastic microbeads of 0.5–2 mm size range (Castañeda et al. 2014). San Gabriel River (USA) was also found to contain nanoplastic fragments of size ranging from 0.5 to 0.8 mm (Moore et al. 2011).
3. Australian shorelines were characterized by various sizes and shape with fragments > 58% and pellets > 30% of plastic particles. Further investigation revealed that the shoreline microplastics were mostly polyethylene, polypropylene and polystyrene (Carbery et al. 2020).
4. Various river surfaces such as Deep Bay, Tolo Harbor, Tsing Yi and Victoria Harbor in Hong Kong were found to contain microplastics ranging between 51 and 27,909 particles/100 m^3 (Tsang et al. 2017).
5. Microplastics and macroplastics were calculated to be around 11–43 wt% in Saigon river of Vietnam (Lahens et al. 2018).
6. Many great river surfaces of Tibet Plateau were assessed for presence of microplastics ranging from 483 to 967 items/m^3 (Jiang et al. 2016).
7. Lake Victoria in East Africa was recently reported to contain 0.3–4.9 mm sized microplastics (Egessa et al. 2020).

Oil spills occurring worldwide also contribute heavily towards marine pollution. Petroleum hydrocarbons are mostly composed of plastics such as polystyrene, poly(ethylene terephthalate) (PET), and low-density polyethylene (LDPE) (Chen et al. 2020). Toxic chemical composition and weathering reactions associated with these oil spills show adverse effects on aquatic environment (Das and Chandran 2011; Zhang et al. 2019). Micro-nanoplastics that accumulate in the environment have been known to be remediated by employing microorganisms. Recently, a general review on present knowledge and future trends in microbial remediation of micro-nanoplastics has been published by Tiwari et al. (2020, 2021).The current review article specifically explains the efficiency of modern metagenomics and its work flow mechanisms compared to traditional techniques in disclosing oceanic plastic environment. Finally, need of multi-omics techniques to identify a wide range of potential benefits emerging out of unveiling plastisphere are highlighted to direct future research.

Oceanic plastic environment

It has been evaluated that 5.25 trillion micro-nanoplastic fragments are ingested by marine organisms that aggregate within their bodies, thereby deteriorating the aquatic ecosystem (Eriksen et al. 2014). Plastic litters aggregate and get deposited at the centre of the ocean by air and wind. Thus plastic wastes converge to form floating debris called "garbage patches" on encountering water currents (Lebreton et al. 2018). Similarly, this plastic debris also gets accumulated and aggregate at the bottom of the ocean resulting in the formation of the benthic zone (Eerkes-Medrano et al. 2015). Micro-nanoplastics have large surface area which provides habitat for growth of diverse microbial communities (Zalasiewicz et al. 2016; Keswani et al. 2016; Viršek et al. 2017). Such an ecological niche that supports the growth of microbial diversity on plastic surfaces is termed as "plastisphere" (Zettler et al. 2013). Biogeographic origin, freshwater to marine environmental conditions and polymer type along with their surface properties and size (Amaral-Zettler et al. 2015) are essential factors that contribute to the formation of plastisphere. Microorganisms colonizing the plastisphere play an important role in bioremediation of ocean plastics (Chowdhary et al. 2020).

A careful analysis of microbial colonies of plastisphere is important to resolve various environmental issues related

to plastic pollutants (Bryant et al. 2016; Jacquin et al. 2019). Until now, a handful of microbes and their secreted enzymes have been isolated and characterized from plastisphere for plastic degradation, but with lower efficiency. Interestingly, current developments in sequencing technologies and in silico analysis could enable the rapid screening of metagenome at contaminated sites such as detection of hydrocarbon degradation genes from black yeast (Radwan and Ruiz 2021a), identification of multiple bacterial species from biofouled plastic fabric for hydrocarbon degradation (Radwan and Ruiz 2021b) and rapid detection of structural and molecular basis of enzymes (Skariyachan et al. 2022). This should lead to the discovery of the whole microbial community by providing detailed information on novel genes as well as enzymes (esterase, hydrolase, laccase, etc.) that are responsible for plastic degradation.

Microbes in plastisphere

The microorganisms inhabiting the plastisphere depend on their ecosystems for survival. The microbes residing in plastisphere are not only helpful in degradation of plastics but also act as major source of nutrients (Amaral-Zettler et al. 2015), enzymes and catalysts, and other beneficial compounds formed by a combination of microbes (Caruso 2020). Numerous techniques have been employed for isolation and characterization of the diverse microbial species colonizing the plastisphere for various applications. For example, photo heterotrophic bacteria, including *Erythrobacter* and *Roseobacter* were identified through bacterial cell morphology analysis (Luo and Moran 2014). Visual analysis of microbial population structures (VAMPS) revealed presence of fungal groups, including *Malassezzia* on the plastisphere (Amend et al. 2019). Using scanning electron microscopy (SEM), diverse filamentous cyanobacteria attached to plastic surface in marine environment has been recognized (Foulon et al. 2016). The exposure of plastic films to UV light or other thermal treatment methods help in isolation of mesophilic *Pseudomonas* strain that can degrade polyethylene (MoonGyung et al. 2012). Arctic microorganisms, including bacterial strains, *Rhodococcus* species and *Pseudomonas* species, were isolated from the Martin and LB agar plates medium and showed significant potential for plastic degradation (Urbanek et al. 2018). Processing of plastisphere and sea foam containing various plastic fragments by advent culturing methods helped in identification of *Sensustricto*, a marine fungi used for the development of natural products (Overy et al. 2019).

A detailed study was conducted by Pinnell and Turner (2019), to characterize microbial communities from biofilms on plastic (polyethylene terephthalate; PET) and bioplastic (polyhydroxyalkanoate; PHA) in coastal benthic habitat using ceramic pellets as a control sample. Initially, isolation of DNA from plastic samples was carried out by sodium dodecyl sulphate (SDS) method and was amplified using Nextera XT DNA Library Preparation Kit. Then DNA sequencing was carried out by employing Illumina HiSeq 2500 platform followed by screening of sequencing reads using SeqPrep. Herein, bioinformatics tools including QIIME and SILVA helped in assigning operational taxonomic units. Interestingly, such advent analysis of microbial community residing on plastic surfaces revealed distinct characteristics of microbes with PHA-associated assemblage facilitating its degradation and sulphate reduction by enrichment of enzymes such as esterases, depolymerases, adenylyl sulphate reductases (aprBA), and dissimilatory sulphite reductases (dsrAB) (Pinnell and Turner 2019). 16S rRNA microbiome profiles of surface and sediment plastic-associated microbial biofilms from the Mediterranean Sea revealed presence of bacteria (*Bacteroidetes* and *gammaproteobacteria*) in abundance and their plastic degrading potential (Delacuvellerie et al. 2019). A comparative study on composition of prokaryotes in polystyrene (PS) biofilms incubated in seawater and industrial water released from petrochemical plant was demonstrated by Tourova et al. (2020). Using 16S rRNA gene sequencing, it was observed that microbial communities resulting from PS biofilms in industrial water differed with seawater in PS degradation potential and also carbohydrate and amino acid metabolism (Tourova et al. 2020). Microbes functioning as styrene-degraders in seawater includes bacteria of genera *Erythrobacter*, *Maribacter*, and *Mycobacterium*, whereas *Pseudomonas* and *Arenimonas* were potential degraders in biofilms in industrial water (Tourova et al. 2020). Recently unidentified plastisphere containing microbes has been found to be a major source for generation of antibiotic resistance genes that help in providing antibiotic resistance (Yang et al. 2020a). Ultimately, identification of novel microbes from plastisphere is expected to serve potential benefits for ecosystem (Malla et al. 2018). Hence it becomes essential to identify such novel microbes of oceanic plastic environment. Most importantly, identification and understanding the function of these plastisphere microbes for a variety of potential applications will require more efficient modern techniques.

Traditional culture methods and drawbacks

Traditional culture methods have been considered as standard procedures for identification and characterization of environmental samples including plastisphere-related microbial communities till date. In these methods, recognition of microbial heterogeneity (Vannini et al. 2020), anatomy and combination of genes present in DNA of a specific microbe (Kirstein et al. 2019) require culture techniques. Further,

identification of microbes at species level requires various molecular level biochemical analyses. A few classic culture-dependent methods being employed for exploring microbial diversity on plastics are briefed below:

1. Plating: this is the most common technique employed till today to culture novel microorganisms from marine environment (Overy et al. 2019). Plating involves different methods such as pour plate technique in which molten agar is solidified on plate and colony forming units help in generating growth curves of microbes (Jackson et al. 2013). Pour plate technique is usually employed to count the number of microorganisms in a mixed sample culture (Kathiresan 2003). Another technique is spread plate method used for screening and selection of microorganisms spread uniformly over the agar plate (Delgado-Viscogliosi et al. 2009). For example, isolation of a single bacterial colony can be done by streaking (Odusanya et al. 2013), which is used to obtain a pure culture from a mixed culture (Molitor et al. 2020) provided colony morphology is known (Hamood Altowayti et al. 2020). Besides easy culture, preparation of pour plate is time consuming, and spread plate technique might lead to loss of viability of organisms when sample comes in contact with hot agar. These plating techniques can also cause reduction in growth rate of obligate aerobes and embedded colonies of microbes that are much smaller as compared to colonies on surface (Tankeshwar 2016).
2. Micromanipulation: micromanipulation helps to obtain accurate information on single bacterial cell by isolating it from mixed environmental samples (Köpke et al. 2005; Zhang et al. 2018a, b). Herein, target bacteria from enrichment cultures are isolated by fluorescent in situ hybridization (FISH) with fluorescently labelled probes (Thomsen et al. 2004) that help in visualizing the bacteria (Ferrari and Gillings 2009) with proper magnification using capillary tube with a bevelled tip (Franco-Duarte et al. 2019). This method is advantageous over plating technique in determining the growth pattern of specific microbes and also risk associated with death of heat-sensitive cells by hot molten agar is negligible (Paul et al. 2017). The main limitation of micromanipulation method is low recovery rate for the growth of single bacterial cell in culture medium (Hohnadel et al. 2018). Also, it might cause cell death while picking up by a tip (Ashida et al. 2010), time consuming process and not quite economical (Kennady et al. 2019).
3. Differential centrifugation: this technique is useful in isolation of pure microbial cultures from a sample of mixed population by varying the centrifugation speed (Eckert et al. 2018). Crude cell fractions obtained after differential centrifugation are layered over sucrose gradients to obtain target microbial species (Rossmanith and Wagner 2011; Radhika and Murugesan 2012). Further, cultures are extracted from the centrifuged medium by observing the layers at which they are expected to reside (Suzuki et al. 2018). This method is advantageous in identifying and subsequent culturing of samples from environment since it can differentiate the microbial cells within layers after centrifugation (Puglisi et al. 2019). The drawback of this technique is longer time required for culturing microbes and concentration of end-product is very less (Yu et al. 2018). Moreover, centrifugation for short duration will sediment fastest particles, leaving slower particles in suspension mixture and therefore optimum speed to isolate target species remains a major drawback (Tan et al. 2012).

Since only a small fraction of microorganisms can be successfully cultured from a specimen of multiple microbes; these traditional culture techniques are suitable solely for fingerprinting microbial communities to elucidate taxonomy, morphology and diversity. This is due to various factors such as relatively faster growth requirements for some, non-viable organisms altering the growth pattern or inhibition of pathogenic organisms due to production of various inhibitors by other microbes such as bacteriocin (Lewis et al. 2020). Despite various molecular techniques employed currently, culture methods fail to provide collective information on DNA sequences of whole microbial communities, insights into microbial diversity, metabolic pathways in microbial systems and gene expression associated with metabolic processes of microbes (Purohit et al. 2020). Hence, it is difficult to understand the composition of microbial communities on the plastisphere and their impact on degradation of plastics by traditional methods (De Tender et al. 2015; Krueger et al. 2015). To summarize a detailed picture of plastisphere for potential biotechnological applications, modern techniques are very much in need. Such approaches offer new platforms to study the entire nucleic content of the microbial diversity from plastic environment without any loss or culturing.

Importance of modern techniques

Current estimates demonstrate that around 90% of microorganisms existing in various natural environments are not readily culturable and therefore not available for biotechnology research and applications (Bøifot et al. 2020). This assessment suggests the requirement of alternative biotechnological techniques which could provide insights into particularly novel microbes from various environments with their potential genes and genomes (Kirubakaran et al. 2020). Plastisphere-specific communities are useful for exploring beneficial enzymes and bioactive compounds for various applications (Gacesa et al.

2018). Thus, disclosing microbial communities inhabiting plastisphere require high throughput sequencing technology for better understanding of their profile (Tender et al. 2017). Moreover, to overcome the limitations posed by conventional molecular techniques, modern approaches using metagenomics and next generation sequencing are being developed that should advance our knowledge regarding full-scale characterization of microbial diversity, composition, structure and activity.

Metagenomics

Metagenomics enables genome-wide analysis of all microbial species inhabiting an environment that are otherwise difficult to culture using conventional methods (Nazir 2016). This technique allows microbiome isolation from a specific sample followed by DNA sequencing to generate sequence reads (Abia et al. 2018). These sequence reads are further analyzed to identify the phylogenetic relationship among microbes and to compare their genetic makeup with other species present in the environmental niche (Vijayvargiya et al. 2019). The data obtained by the metagenomics library can be compared with bioinformatics tools that provide relevant information on the species composition, profile and characteristics (Gilbert and Dupont 2011). Recent identification and analysis of various microorganisms present on plastic particles in marine environment using metagenomics data revealed the existence and importance of antibiotic and metal resistance genes in influencing the resistome of the microbiota (Yang et al. 2019). Metagenomics analysis of *Oceanospirillales* and *Alteromonadales* helped in degradation of plastics in water (Wright et al. 2020c). Another metagenomics based study by Pinnell and Turner (2019) identified novel species including *Desulfovibrio*, *Desulfobacteraceae* and *Desulfobulbaceae* capable of bioplastic degradation and reducing sulphur from various plastic surfaces of coastal marine environment. In addition, this technique was also explored to recognize various microbes that could produce enzymes such as six putative PETase-like enzymes and four putative MHETase-like enzymes. Such enzymes promote deterioration of aliphatic-aromatic polymers from plastics (Meyer et al. 2020). Thus, a complete range of interesting gene products of plastisphere and their functions can be elucidated by metagenomics that may unlock valuable information and provide developments in key areas of ecology and environment.

Metagenomics process: elucidation of microbial profile

In this section, two methods namely targeted and shotgun metagenomics that are widely being used for screening a metagenomic library from plastisphere (Amaral-Zettler et al. 2020) has been elucidated. Steps involved in these methods have been discussed below:

1. *DNA isolation from the plastisphere sample* though DNA extraction by cell lyses is a simple procedure, isolation of DNA from plastic fragments faces certain hurdles that need to be taken into consideration. To identify original species, DNA should be extracted from a wide variety of microorganisms present within the plastisphere (Kirstein et al. 2019). During extraction, DNA should not result in the formation of chimeric products and should be free of any contaminants that might interfere in the sequencing procedure (Yang et al. 2020a, b). Chimeric products are hybrids, formed between multiple parent sequences that can be misinterpreted as new organisms, thus showing novel diversity. To remove any 16s chimeric contaminants, several computational methods have been utilized such as are Pintail (Ashelford et al. 2005), Chimera Slayer (CS) (Haas et al. 2011) and Bellerophon (DeSantis et al. 2006). The sequencing and cloning of DNA requires DNA to be present in microgram quantities for generating information on plastic samples. Sequencing and cloning insufficient DNA quantities may alter the genomic profile of the specific plastisphere. This is due to the fact that minimal amount of DNA may not sufficiently provide all relevant information needed from microbes and their genetic diversity (Davidov et al. 2020).
2. *Collection of the non-sequencing dataset from the plastisphere* the DNA extract from plastic samples may also contain certain non-sequencing datasets called "metadata" (Oberbeckmann and Labrenz 2020). These datasets help in interpreting the information obtained from sequence profiles of microbial species within plastisphere (Kirstein et al. 2019). For example, freshwater lake sediments containing plastisphere-specific community have the potential to mineralize carbon into greenhouse gases. Thus, identification of microbial species having functionality to mineralize carbon can be obtained by comparing the plastisphere in marine ecosystem to control obtained from any surrounding environment (Yakimovich 2017).
3. *Cloning methods for constructing metagenomics library* the extracted DNA sample from plastisphere needs to be cloned to construct a metagenomics library. Cloning of DNA depends on objective of the investigation being carried out. Hence, it is essential to amplify DNA clones so that they can be sequenced to attain necessary information (Meyer-Dombard et al. 2020).
4. *Techniques for screening DNA library: targeted metagenomics*

Targeted metagenomics

Focuses on identifying phylogenetic relationship among species while shotgun metagenomics helps to elucidate gene function of microbes identified from plastisphere and their role in the marine environment. Thus, targeted sequencing technique helps to recognize complete microbial structure along with distinct species present in a plastic particle and contamination caused by pollutant. However, targeted metagenomics has limited applications in various fields of research as it can demonstrate only the structure, relationship among species, constituents of microbial diversity and phylogenetic composition of microorganisms present in plastisphere (Trindade et al. 2015). For example, targeted metagenomics was employed to distinguish between bacterial community in water and diversity of microbes residing in free-living amoebae (Delafont et al. 2019). However, this approach could only analyze the microbiome for characterizing bacteria associated with amoebae and could not provide functional and metabolic potential of microbes. Hence, the most used method for identifying microbial profiles in plastisphere relies on using shotgun metagenomics. Figure 2 provides a schematic representation of targeted and shotgun metagenomics processes to screen microbes on plastisphere.

Shotgun metagenomics

Steps involved in Shotgun metagenomics for elucidating microbial profiles of a given sample are as follows.

1. *Sequence reads screening* DNA sequences obtained after microbial isolation from plastisphere are pre-processed to eliminate low-quality bases that are repeated within a sequence dataset. For example, metagenomics sample analysis of plastic debris in Laguna Madre was done using Nextera XT DNA Library Preparation Kit fol-

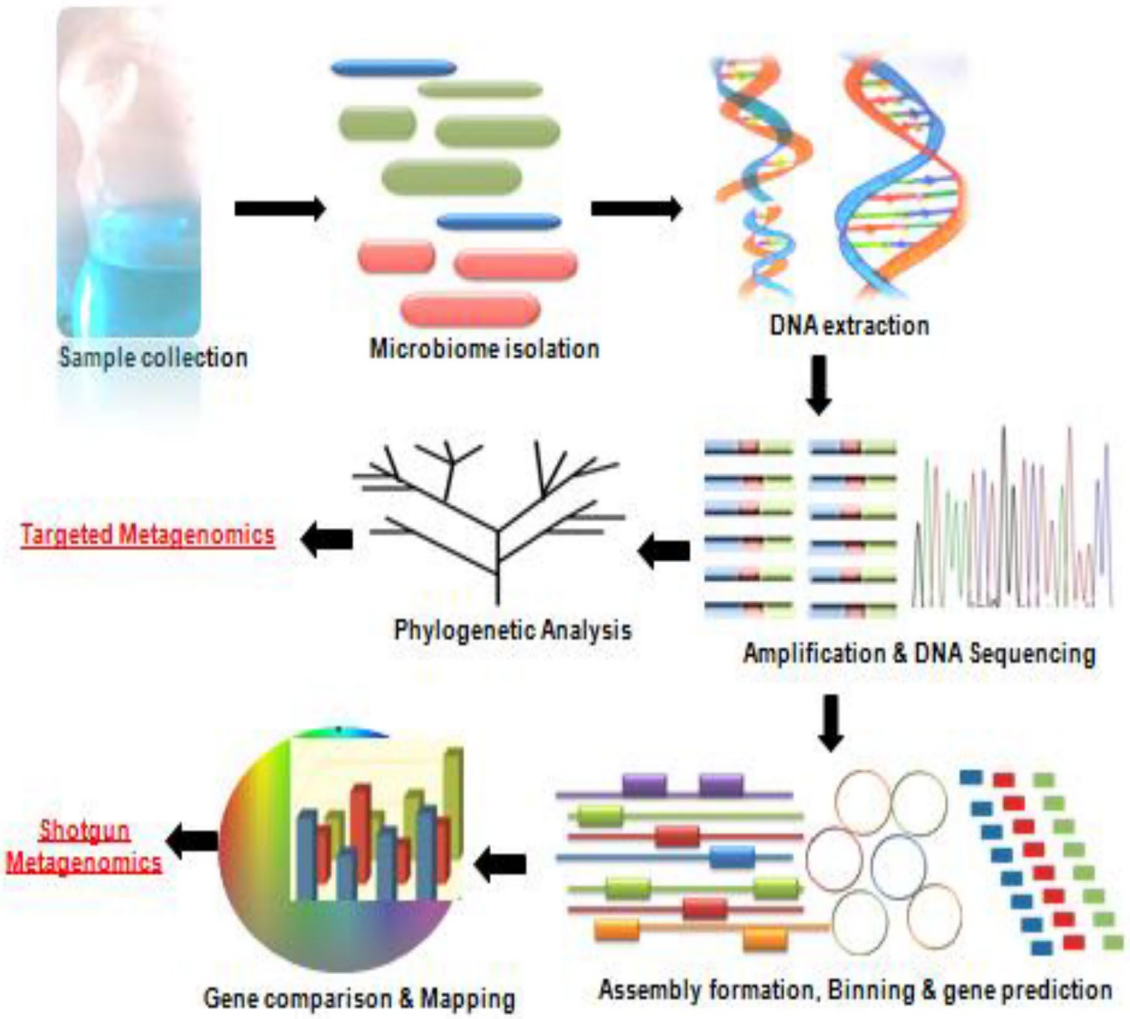

Fig. 2 Schematic representation of targeted and shotgun metagenomics processes employed to screen microbes on plastisphere

lowed by screening of sequence reads using SeqPrep (Pinnell and Turner 2019).

2. The sequence reads obtained from DNA sequences of microbial plastisphere combine to form large stretches of DNA resulting in formation of *Contigs* (Delacuvellerie et al. 2019). An example demonstrating the mineralization of PBAT-based blend film by marine culture was observed using metagenomics approach. Metagenomics library was assembled, binned and sequence reads were mapped with bins to identify species abundant within plastic debris (Meyer-Cifuentes et al. 2020).
3. *Metagenomics data analysis* there are various tools and software that help in elucidation of information from metagenomics datasets obtained from plastisphere communities. The software tools used can determine multifunctional aspects of genes, DNA, proteins and metabolites within a microbial community specific to plastic (Purohit et al. 2020). Some of the computational tools and bioinformatics software for metagenomics analysis include QIIME (Tender et al. 2017), MG-RAST (Fadiji et al. 2020), SRA (Yang et al. 2019).

   The DNA sequencing of microbes in plastisphere traditionally relied on using conventional sequencing methods to characterize the whole plastisphere. For example, Sanger sequencing used for generating DNA profile of plastisphere communities had limitations of generating faster sequence datasets (Krüger et al. 2020). These problems can be overcome by use of NGS technologies that have provided great potential for faster analysis of DNA sequences.

Techniques associated with next generation sequencing (NGS)

NGS technologies have major advantages including short duration for analyzing data, low cost of operation and high accuracy of sequence reads. Due to the capacity for big-data acquisition, NGS technologies are beneficial for various research fields including exploration of microorganisms residing in plastisphere of marine ecosystem (Stitzlein 2018). Profiling of diverse microbial community for identifying important enzymes traditionally relied on using large computer algorithms and useful computers to generate data (Ravi et al. 2018). One of the majorly utilized sequencing techniques is Illumina Next Generation Sequencing. Illumina Next Generation Sequencing stages are fit for paired-end sequencing. The sequencing that happens from the two closures of a DNA section, producing excellent sequence information with inside and out inclusion and big quantities of reads. Illumina's reversible eliminator innovation, alongside matched end sequencing, makes it the most reliable base-by-base sequencing with a mistake pace of 0.1% (essentially replacement blunders, seldom inclusions/cancellations) (Hu et al. 2021). This technique also ease the investigation on the interaction between plastic and biofilm forming microorganisms and a detailed characterization of microbial gene pool on plastics, which provides an insight on the degraders of plastics (Bhagwat et al. 2021; Luo et al. 2022). Also, the emergence of other NGS technologies has the potential to explore genetic diversity of plastisphere-specific communities (Forde and O'Toole 2013).

A few NGS methods employed on various plastisphere samples along with the corresponding outcome are listed in Table 1.

Multi-omics techniques

Metagenomics helps in analyzing microbial profile and identifying genes present in a microorganism (Zhang et al., 2019). Complete profiling of microbial community is achieved using DNA sequencing. Since presence of genes does not necessitate protein expression, metaproteomics can provide precise functional information on expressed proteins (Verberkmoes et al. 2009). Direct measurement of transcripts or proteins is performed using metatranscriptomics or metaproteomics approaches. Their combination enhances knowledge of microbial genome assembly and gene identification along with detection of induced/repressed genes (Heintz-Buschart et al. 2016). Additionally, active transcription allows for identifying genes from metabolically active microbes compared to inert or dead microorganisms (Rastogi and Sani 2011). Metaproteomics can also facilitate identification of host-associated microbiomes *in-vivo* and quantify host proteins irrespective of their phylogenetic origin (Zhang et al. 2018a). Profiling metabolic outcomes of microbes using metabolomics helps to directly identify metabolites acting as markers for host and microbiome interactions as well as in identifying other bioactive compounds (Gavin et al. 2018).

Metatranscriptomics

Metatranscriptomics is a study on gene expression of various microbes from natural environment (Shi et al. 2018). It is a powerful tool for analyzing the metabolic functions (Gosalbes et al. 2011), activities, regulations, taxonomic composition, predicted open-reading frames and novel sites of transcription and translation of microbial genomes from plastics. Metatranscriptomics helps in assessing the genes that are expressed at different environmental conditions at time of sampling plastics while encoding specific physiological functions (Moran et al. 2013). Several bioinformatics tools such as BOWTIE (Langmead and Salzberg 2012) along with high-throughput analysis of datasets (MG-RAST)

Table 1 A list of NGS methods employed on various plastisphere samples to disclose microbial profiles

Sample location	NGS method	Description	Outcome	References
"BiologischeAnstalt Helgoland", 60 km off German coastline	Illumina Sequencing	DNA fragments obtained from microbial biofilms were amplified and attached to primers containing Illumina adaptor sequences. Resulting amplicons were sequenced on Illumina MiSeq platform	Helps in analyzing distinct microbes and identify plastic specific microorganisms	Kirstein et al. (2019)
Mediterranean Coast of Israel	Nano-pore sequencing	DNA obtained from plastisphere was amplified and MinION adaptors were attached to ligate DNA. These ligated sequences were passed through nanopore, to provide DNA bases	Observed high diversity of microorganisms with algae domination on plastisphere. Many plastic-specific species were found to have potential to degrade plastics	Davidov et al. (2020)
Cruise on board between areas of Bremerhaven, Germany and Cape Town, East Atlantic Ocean	Pyrosequencing	DNA isolated from plastic samples was amplified using PCR followed by binding of dinucleotide (dNTPs) to pyrophosphate releasing ATP	Diversity of microbial biofilms on anthropogenic and natural particles were distinguished from free-living bacterial species	Papadatou (2013)
Beaches east of Gijon port, Asturias, Spain	Ion torrent sequencing	DNA extracted from plastic samples were amplified using PCR and analyzed using Ion Torrent PGM (ThermoFisher Scientific, USA)	Biofouling communities present on different materials including textile and plastic surfaces were compared. Plastic litter was observed as a vector for dispersal of various species	Ibabe et al. (2020)
The Laguna Madre, coastal lagoon in Northern Gulf of Mexico	Whole genome shotgun sequencing	DNA isolated from plastic samples was sequenced into contigs and assemble to classify them and identify genes and chromosomal maps	Gene identified after metagenomic analysis of datasets revealed various bacterial species having potential for plastic degradation and sulphate reduction	Pinnell and Turner (2019)
South Pacific Ocean of Chile	Real-time PCR sequencing	Isolated DNA strands were amplified using PCR and bacterial species abundance was analyzed using real-time PCR system by removal of primers, repeats and low-quality bases	Plastisphere communities were dominated in consumption and production of N_2O and CO_2. These results showed direct impact of plastic pollution on marine ecosystem	Cornejo-D'Ottone et al. (2020)

(Glass et al. 2010) are subjected to provide the overall gene expression profile of mRNA reads within a plastisphere sample. The transcriptomics study approach enabled the path to identify genes encoding enzymes involved in the consumption of polymers such as Polyethylene (PE) in *R. ruber* (C208) (Gravouil et al. 2017).

Metaproteomics

Metaproteomics provides a qualitative recognition and quantification of proteins from various microbial communities thereby providing direct insights into the phenotypic characteristics of microorganisms (Schneider et al. 2012). The study of protein expression within the microbial diversity (metaproteome) becomes necessary to understand the functional profile of microbial components of the various environmental samples within ecosystems (Li et al. 2019). Recent literature highlights the significance of metaproteomics and its role in identification of PETase-like enzymes from sponge microbiome for their potential application in various biotechnological industries (de Oliveira et al. 2020). Metaproteomics could also help in analyzing proteins essential for degradation of poly aromatic hydrocarbons within groundwater (Herbst et al. 2013). The degradation of terephthalate (TA), a monomer of known plastic (PET) was revealed through metaproteomics analysis of sludge sample, in which a total of 482 proteins were identified and showed a distinct distribution pattern of microbial functions expressed in situ by *Pelotomaculum* spp. (Wu et al. 2013).

Metabolomics

Metabolomics enables the study of various endogenous and exogenous small molecules and intermediates, commonly termed as metabolites, that are leached out by the biological system of an organism during interaction with its surrounding environment (Liu and Locasale 2017). Thus metabolomics allows detection of intermediates and end products of cellular metabolism from environmental pollutants (Deng et al. 2019). Metabolomics approach helped in identification of two novel marine isolates (*Thioclava* sp. BHET1 and *Bacillus* sp. BHET2) including PET hydrolytic intermediates from a plastisphere that could degrade PET (Wright et al. 2020a). Apart from plastics, plastic additives which are highly toxic to marine life also contribute towards pollution of marine environment. The degradation of plastic additives through various enzymes finds potential in remediation. These degraded intermediates and related metabolites can be identified by mass spectrometry through metabolomics approach (Kumari and Chaudhary 2020). The study by Wright et al. (2020a, b, c) highlight the potential of plastisphere-specific communities in degradation of plasticizers present in marine environment along with intermediate products formed during degradation process.

All of the above techniques demonstrate significant potential to decipher information on microbial diversity of plastisphere (Schlundt et al. 2020). Various environmental samples when tested using metagenomics, metatranscriptomics, and metaproteomics techniques could help in identification of PET-like enzymes that can promote degradation of an aromatic-aliphatic copolyester blend (Meyer-Cifuentes et al. 2020). The integrated data obtained from metaproteomics along with metagenomics and meta-transcriptomics information promises to provide enhanced knowledge on microbial functional profiles of plastisphere and their effective contribution for the functioning of ecosystem (Yang et al. 2020b) (Fig. 3). However, studies of microbial community using these techniques are still in its infancy owing to difficulty in processing environmental samples and their recovery for novel products. Advancements in metagenomics bioinformatics tools for large-scale analysis of genomic DNA and understanding microbial ecology using advent methods including metatranscriptomics, metabolomics and metaproteomics is necessary to provide genome-wide analysis of plastisphere-specific communities.

Advantages of multi-omics

The emergence of metagenomics technique has enabled large scale study of diverse microbial diversity utilizing NGS technology (Abia et al. 2018). Enhancements in DNA profiling of microbes along with low cost of operation has allowed metagenomics to emerge at rapid rate. Targeted metagenomics has provided novel insights into structure and function of microorganisms (Islam et al. 2019). Many novel genes, proteins and metabolites utilized for various applications from microbes have been identified using shotgun metagenomics (Pinnell and Turner 2019). Microbes and their interactions with various substrates can alter the geological composition, dissolution and deterioration of plastic present in marine ecosystem (Gadd 2010). This helps to remediate and lessen the toxic effects of pollutants on aquatic environment. Moreover, plastisphere acts as a novel substrate in marine ecosystem facilitating microbial dispersal and enabling research in microbial ecology through metagenomics (Amaral-Zettler et al. 2015). Metagenomics finds novel application in bioremediation of pollutants in environment by development of enzymes that help in degradation of plastic (Table 2). For example, PETase enzyme obtained from *Ideonella sakiensis* is useful for degradation of PET within marine ecosystem (Huang et al. 2018). Use of NGS technology could help in detection of pathogenic species residing on plastisphere. For example, pathogenic species of *Tenacibaculum* and *Phormidium* were observed

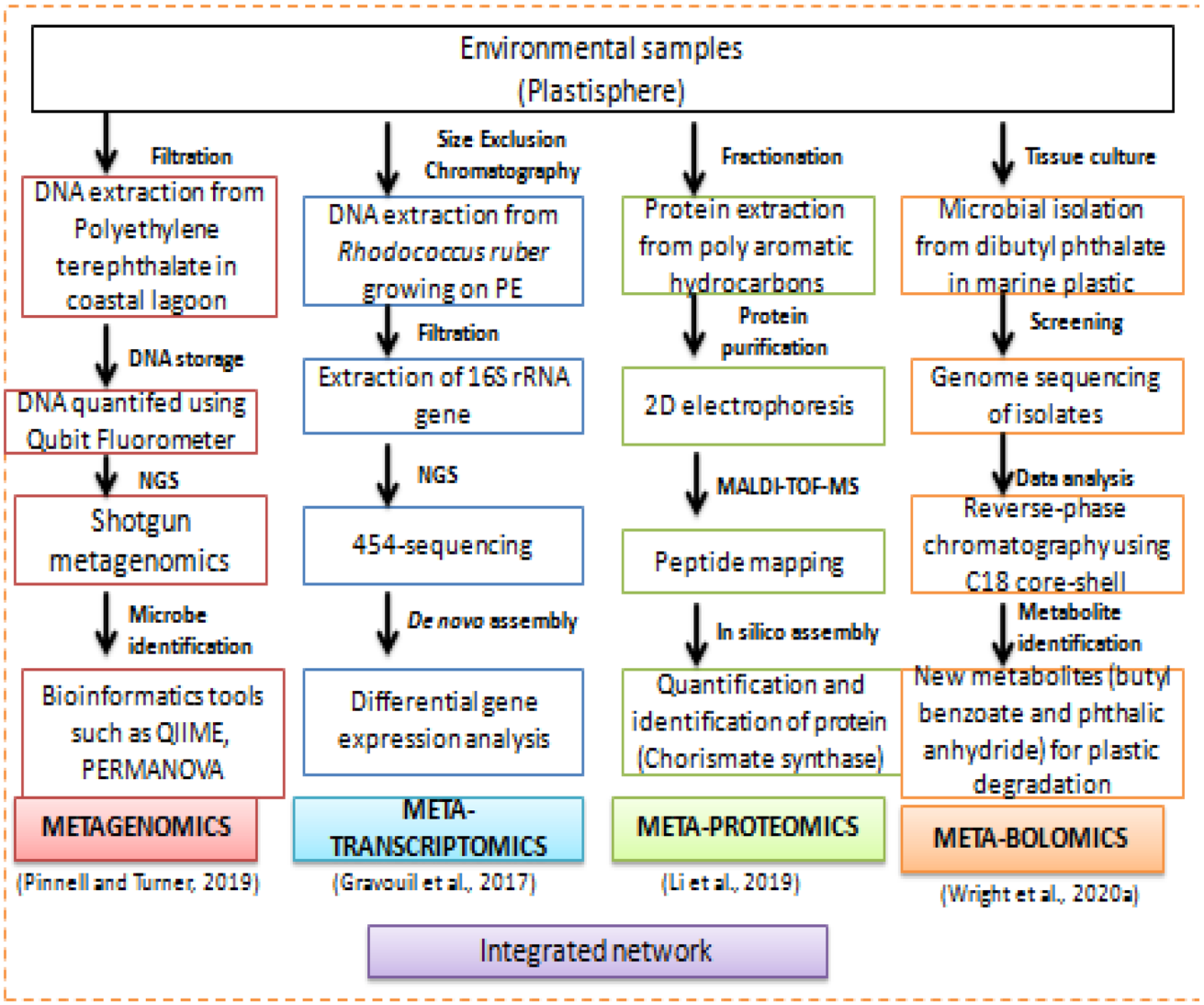

Fig. 3 Integrated network of multi-omics techniques

more on plastic surfaces as compared to surrounding water after 16S rRNA metabarcoding method (Jacquin et al. 2019). These pathogenic species act as a source of food for various aquatic animals and indirectly by humans that consume the sea food containing pathogenic microbes (Novoslavskij et al. 2016). Metagenomics also helped in identifying novel enzymes from microbial species, for example, isolation of laccase from *Rhodococcus ruber*, could facilitate the degradation of polyethylene (Santo et al. 2013). Processing of plastisphere and sea foam containing various plastic fragments by advent methods helped in the production of *Sensustricto*, marine fungi and used for the development of natural products (Overy et al. 2019). Isolation of fungal communities from plastisphere including *Fusarium* species and *Humicola insolens* can also help in degradation of PET by enzyme cutinase (Ronkvist et al. 2009).

Metagenomics can also help in identification of various species prevailing in plastic debris for years. For example, plastic debris isolated from Mediterranean coast of Israel were analyzed using Nanopore sequencing, generating diverse species and also determining plastic-specific communities (Davidov et al. 2020). Plastisphere possess distinct microbial communities that have also been found in sewage generated from wastewater treatment plants, such as, *Arcobacter* species found in landfill microbiome in USA (Stamps et al. 2016). Microbial communities could also be identified in plastisphere of soil that facilitate plant protection and provide various metabolic functions. For example, endophytic microorganisms were observed in rhizosphere soil that could demonstrate various activities including anti diabetic, production of phytohormones and secretion of lytic enzymes (Fadiji and Babalola 2020).

Table 2 List of enzymes isolated from plastisphere-specific communities using multi-omics for plastic degradation

Microbial strain from plastisphere	Location	Enzymes obtained	Technique	Use	References
Rhodococcus ruber	Bacterial strain grown in mineral salt media under laboratory conditions	Laccase	RNA sequencing coupled with mass-spectrometry-based transcriptomics	Degradation of polyethylene	Gravouil et al. (2017)
Ideonella sakaiensis	2–3 cm below water surface at yard of PET-bottle recycling factory, Sakai city, Japan	PETase and MHETase	RNA and Illumina sequencing	Degradation of polyethylene terephthalate	Yoshida et al. (2016)
Thermobifida cellulosilytica	Artificial marine medium containing copolysterplastic	Hydrolase	Metagenomics, metatranscriptomics and metaproteomics	Poly(butylene adipate-co-terephthalate) degradation	Meyer-Cifuentes et al. (2020)
Comamonas sp. 51F	Leachate collected from Municipal Solid Waste landfill site at Ghazipur, Delhi, India	Terephthalate 1,2-dioxygenase reductase	16S rRNA sequencing and mass spectrometric analysis	Degradation of phthalic acid esters used for plastic manufacturing	Kumar et al. (2017)
Geldibacter sp.	Artificial marine medium containing copolyster plastic	Esterase	Metagenomics, metatranscriptomics and metaproteomics	Degradation of phenol formaldehyde resins	Meyer-Cifuentes et al. (2020)
Pelotomaculum sp.	Anaerobic fixed film bioreactor inoculated with terephthalate degrading sludge derived from laboratory	Cyclohexa-1,5-dienecarbonyl-CoA hydratase, 6-hydroxy-cyclohex-1-ene-1-carboxyl-CoA dehydrogenase and 6-oxo-cyclohex-1-enecarbonyl-CoA hydrolase	Shotgun metagenomics and metaproteomic analysis	Terephthalate degradation	Wu et al. (2013)
Burkholderiales	Activated sludge from industrial wastewater treatment plant in Tianjin Lingang, China	PAH-ring hydroxylating dioxygenase	454 pyrosequencing and metaproteomic analysis	Biodegradation of polycyclic aromatic hydrocarbons	Li et al. (2019)
Halomonas sp. and *Mycobacterium vanbaalenii*	Marine plastic debris collected from Plymouth Sound, Devon and Portaferry, Northern Ireland, UK	Decarboxylase	Metabolomic and proteogenomic analysis	Degradation of dibutyl phthalate, bis(2-ethyl hexyl) phthalate and acetyl tribunyl citrate	Wright et al. (2020a)

Therefore, multi-omics are expected to provide significant insights into the inaccessible and undiscovered microorganisms for research in genomics, evolution and ecology.

Conclusion

Microorganisms are abundant throughout environment with majority of them being difficult to cultivate by classical microbiological techniques. The advent of metagenomics and next generation sequencing has revolutionized field of microbial biotechnology. Metagenomics approach bypasses the requirement for isolation or culturing microbes by way of direct profiling complex environmental samples such as plastisphere. With advances in next generation sequencing technologies, millions of complex metagenomic datasets can be generated from plastisphere, which in turn would lead to formulation of various bioinformatics tools for efficient analysis of data with respect to phylogeny and metabolic diversity. Genetic diversity and metabolic activities of uncultivable microbes are currently linked to large-scale gene expression studies and proteome studies of microbial diversity.

Metatranscriptomics, metaproteomics and metabolomics offer significant potential to elucidate activities, functional characteristics and production capabilities of microbial consortia. Together with accessibility of these novel approaches, a prompt metamorphosis is obtained in understanding the profile of microbial diversity. Applications of genetic DNA obtained from plastisphere samples can help in bioremediation, conservation biology, environmental assessment, trophic and community ecology. Integration of multi-omics techniques can help in identification of genes, proteins and pathways that can be distinguished from healthy subjects. Taken as a whole, metagenomics along with NGS technologies can help in characterization of the plastisphere-specific communities and ultimately discover hidden microbes with novel genes and enzymes for plastic degradation and other potential applications.

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Declarations

Conflict of interest The authors have no conflicts of interest to declare that are relevant to the content of this article.

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References

Abia ALK, Alisoltani A, Keshri J, Ubomba-Jaswa E (2018) Metagenomic analysis of the bacterial communities and their functional profiles in water and sediments of the Apies River, South Africa, as a function of land use. Sci Total Environ 616–617:326–334. https://doi.org/10.1016/j.scitotenv.2017.10.322

Amaral-Zettler LA, Zettler ER, Slikas B et al (2015) The biogeography of the plastisphere: implications for policy. Front Ecol Environ 13:541–546. https://doi.org/10.1890/150017

Amaral-Zettler LA, Zettler ER, Mincer TJ (2020) Ecology of the plastisphere. Nat Rev Microbiol 18:139–151. https://doi.org/10.1038/s41579-019-0308-0

Amend A, Burgaud G, Cunliffe M et al (2019) Fungi in the marine environment: open questions and unsolved problems. Mbio. https://doi.org/10.1128/mBio.01189-18

Ashelford KE, Chuzhanova NA, Fry JC et al (2005) At least 1 in 20 16S rRNA sequence records currently held in public repositories is estimated to contain substantial anomalies. Appl Environ Microbiol 71:7724–7736. https://doi.org/10.1128/AEM.71.12.7724-7736.2005

Ashida N, Ishii S, Hayano S et al (2010) Isolation of functional single cells from environments using a micromanipulator: application to study denitrifying bacteria. Appl Microbiol Biotechnol 85:1211–1217. https://doi.org/10.1007/s00253-009-2330-z

Bansal M, Santhiya D, Sharma JG (2021) Behavioural mechanisms of microplastic pollutants in marine ecosystem: challenges and remediation measurements. Water Air Soil Pollut 232:372. https://doi.org/10.1007/s11270-021-05301-1

Barnes DKA, Galgani F, Thompson RC, Barlaz M (2009) Accumulation and fragmentation of plastic debris in global environments. Philos Trans R Soc B Biol Sci 364:1985–1998. https://doi.org/10.1098/rstb.2008.0205

Bhagwat G, Zhu Q, O'Connor W et al (2021) Exploring the composition and functions of plastic microbiome using whole-genome sequencing. Environ Sci Technol 55:4899–4913. https://doi.org/10.1021/acs.est.0c07952

Bøifot KO, Gohli J, Moen LV, Dybwad M (2020) Performance evaluation of a new custom, multi-component DNA isolation method optimized for use in shotgun metagenomic sequencing-based aerosol microbiome research. Environ Microbiome 15:1. https://doi.org/10.1186/s40793-019-0349-z

Bryant JA, Clemente TM, Viviani DA et al (2016) Diversity and activity of communities inhabiting plastic debris in the North Pacific Gyre. mSystems. https://doi.org/10.1128/mSystems.00024-16

Carbery M, MacFarlane GR, O'Connor W et al (2020) Baseline analysis of metal(loid)s on microplastics collected from the Australian shoreline using citizen science. Mar Pollut Bull 152:110914. https://doi.org/10.1016/j.marpolbul.2020.110914

Caruso G (2020) Microbial colonization in marine environments: overview of current knowledge and emerging research topics. J Mar Sci Eng 8:78. https://doi.org/10.3390/jmse8020078

Castañeda RA, Avlijas S, Simard MA, Ricciardi A (2014) Microplastic pollution in St. Lawrence River sediments. Can J Fish Aquat Sci 71:1767–1771. https://doi.org/10.1139/cjfas-2014-0281

Chen C, Ng DYW, Weil T (2020) Polymer bioconjugates: modern design concepts toward precision hybrid materials. Prog Polym Sci 105:101241. https://doi.org/10.1016/j.progpolymsci.2020.101241

Chowdhary P, Raj A, Verma D, Akhter Y (2020) Microorganisms for sustainable environment and health. Elsevier, Amsterdam

Cornejo-D'Ottone M, Molina V, Pavez J, Silva N (2020) Greenhouse gas cycling by the plastisphere: the sleeper issue of plastic pollution. Chemosphere 246:125709. https://doi.org/10.1016/j.chemosphere.2019.125709

Das N, Chandran P (2011) Microbial degradation of petroleum hydrocarbon contaminants: an overview. Biotechnol Res Int 2011:1–13. https://doi.org/10.4061/2011/941810

Davidov K, Iankelevich-Kounio E, Yakovenko I et al (2020) Identification of plastic-associated species in the Mediterranean Sea using DNA metabarcoding with Nanopore MinION. Sci Rep 10:17533. https://doi.org/10.1038/s41598-020-74180-z

de Oliveira BFR, Carr CM, Dobson ADW, Laport MS (2020) Harnessing the sponge microbiome for industrial biocatalysts. Appl Microbiol Biotechnol 104:8131–8154. https://doi.org/10.1007/s00253-020-10817-3

De Tender CA, Devriese LI, Haegeman A et al (2015) Bacterial community profiling of plastic litter in the Belgian part of the North Sea. Environ Sci Technol 49:9629–9638. https://doi.org/10.1021/acs.est.5b01093

Delacuvellerie A, Cyriaque V, Gobert S et al (2019) The plastisphere in marine ecosystem hosts potential specific microbial degraders including Alcanivorax borkumensis as a key player for the low-density polyethylene degradation. J Hazard Mater 380:120899. https://doi.org/10.1016/j.jhazmat.2019.120899

Delafont V, Perrin Y, Bouchon D et al (2019) Targeted metagenomics of microbial diversity in free-living amoebae and water samples. In: Buchrieser C, Hilbi H (eds) Legionella: methods and protocols. Springer, New York, pp 421–428

Delgado-Viscogliosi P, Solignac L, Delattre J-M (2009) Viability PCR, a culture-independent method for rapid and selective quantification of viable *legionella pneumophila* cells in environmental water samples. Appl Environ Microbiol 75:3502–3512. https://doi.org/10.1128/AEM.02878-08

Deng P, Li X, Petriello MC et al (2019) Application of metabolomics to characterize environmental pollutant toxicity and disease risks. Rev Environ Health 34:251–259. https://doi.org/10.1515/reveh-2019-0030

DeSantis TZ, Hugenholtz P, Larsen N et al (2006) Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. Appl Environ Microbiol 72:5069–5072. https://doi.org/10.1128/AEM.03006-05

Eckert EM, Di Cesare A, Kettner MT et al (2018) Microplastics increase impact of treated wastewater on freshwater microbial community. Environ Pollut 234:495–502. https://doi.org/10.1016/j.envpol.2017.11.070

Eerkes-Medrano D, Thompson RC, Aldridge DC (2015) Microplastics in freshwater systems: a review of the emerging threats, identification of knowledge gaps and prioritisation of research needs. Water Res 75:63–82. https://doi.org/10.1016/j.watres.2015.02.012

Egessa R, Nankabirwa A, Ocaya H, Pabire WG (2020) Microplastic pollution in surface water of Lake Victoria. Sci Total Environ 741:140201. https://doi.org/10.1016/j.scitotenv.2020.140201

Eriksen M, Lebreton LCM, Carson HS et al (2014) Plastic pollution in the World's oceans: more than 5 trillion plastic pieces weighing over 250,000 tons afloat at sea. PLoS ONE 9:e111913. https://doi.org/10.1371/journal.pone.0111913

Fadiji AE, Babalola OO (2020) Elucidating mechanisms of Endophytes used in plant protection and other bioactivities with multifunctional prospects. Front Bioeng Biotechnol. https://doi.org/10.3389/fbioe.2020.00467

Fadiji AE, Ayangbenro AS, Babalola OO (2020) Metagenomic profiling of the community structure, diversity, and nutrient pathways of bacterial endophytes in maize plant. Antonie Van Leeuwenhoek 113:1559–1571. https://doi.org/10.1007/s10482-020-01463-w

Faure F, Corbaz M, Baecher H, Felippe L (2012) Pollution due to plastics and microplastics in Lake Geneva and in the Mediterranean Sea. Arch Sci 6:No. POST_TALK

Ferrari BC, Gillings MR (2009) Cultivation of fastidious bacteria by viability staining and micromanipulation in a soil substrate membrane system. Appl Environ Microbiol 75:3352–3354. https://doi.org/10.1128/AEM.02407-08

Forde BM, O'Toole PW (2013) Next-generation sequencing technologies and their impact on microbial genomics. Brief Funct Genom 12:440–453. https://doi.org/10.1093/bfgp/els062

Foulon V, Le Roux F, Lambert C et al (2016) Colonization of polystyrene microparticles by *Vibrio crassostreae*: Light and electron microscopic investigation. Environ Sci Technol 50:10988–10996. https://doi.org/10.1021/acs.est.6b02720

Franco-Duarte R, Černáková L, Kadam S et al (2019) Advances in chemical and biological methods to identify microorganisms—from past to present. Microorganisms 7:130. https://doi.org/10.3390/microorganisms7050130

Gacesa R, Baranasic D, Starcevic A et al (2018) Bioprospecting for genes encoding hydrocarbon-degrading enzymes from metagenomic samples isolated from Northern Adriatic Sea Sediments. Food Technol Biotechnol 56:270–277. https://doi.org/10.17113/ftb.56.02.18.5393

Gadd GM (2010) Metals, minerals and microbes: geomicrobiology and bioremediation. Microbiology 156:609–643. https://doi.org/10.1099/mic.0.037143-0

Galloway TS, Cole M, Lewis C (2017) Interactions of microplastic debris throughout the marine ecosystem. Nat Ecol Evol 1:1–8. https://doi.org/10.1038/s41559-017-0116

Gavin PG, Mullaney JA, Loo D et al (2018) Intestinal metaproteomics reveals host-microbiota interactions in subjects at risk for type 1 diabetes. Diabetes Care 41:2178–2186. https://doi.org/10.2337/dc18-0777

Gilbert JA, Dupont CL (2011) Microbial metagenomics: beyond the genome. Annu Rev Mar Sci 3:347–371. https://doi.org/10.1146/annurev-marine-120709-142811

Glass EM, Wilkening J, Wilke A et al (2010) Using the metagenomics RAST server (MG-RAST) for analyzing shotgun metagenomes. Cold Spring Harb Protoc. https://doi.org/10.1101/pdb.prot5368

Gosalbes MJ, Durbán A, Pignatelli M et al (2011) Metatranscriptomic approach to analyze the functional human gut microbiota. PLoS ONE 6:e17447. https://doi.org/10.1371/journal.pone.0017447

Gravouil K, Ferru-Clément R, Colas S et al (2017) Transcriptomics and lipidomics of the environmental strain *Rhodococcus ruber* point out consumption pathways and potential metabolic bottlenecks for polyethylene degradation. Environ Sci Technol 51:5172–5181. https://doi.org/10.1021/acs.est.7b00846

Haas BJ, Gevers D, Earl AM et al (2011) Chimeric 16S rRNA sequence formation and detection in Sanger and 454-pyrosequenced PCR amplicons. Genome Res 21:494–504. https://doi.org/10.1101/gr.112730.110

Hamood Altowayti WA, Almoalemi H, Shahir S, Othman N (2020) Comparison of culture-independent and dependent approaches for identification of native arsenic-resistant bacteria and their potential use for arsenic bioremediation. Ecotoxicol Environ Saf 205:111267. https://doi.org/10.1016/j.ecoenv.2020.111267

Heintz-Buschart A, May P, Laczny CC et al (2016) Integrated multi-omics of the human gut microbiome in a case study of familial type 1 diabetes. Nat Microbiol 2:1–13. https://doi.org/10.1038/nmicrobiol.2016.180

Herbst F-A, Bahr A, Duarte M et al (2013) Elucidation of in situ polycyclic aromatic hydrocarbon degradation by functional metaproteomics (protein-SIP). Proteomics 13:2910–2920. https://doi.org/10.1002/pmic.201200569

Hohnadel M, Maumy M, Chollet R (2018) Development of a micromanipulation method for single cell isolation of prokaryotes and its application in food safety. PLoS ONE 13:e0198208. https://doi.org/10.1371/journal.pone.0198208

Hu T, Chitnis N, Monos D, Dinh A (2021) Next-generation sequencing technologies: an overview. Hum Immunol 82:801–811. https://doi.org/10.1016/j.humimm.2021.02.012

Huang X, Cao L, Qin Z et al (2018) Tat-independent secretion of polyethylene terephthalate hydrolase PETase in *Bacillus subtilis* 168 mediated by its native signal peptide. J Agric Food Chem 66:13217–13227. https://doi.org/10.1021/acs.jafc.8b05038

Ibabe A, Rayón F, Martinez JL, Garcia-Vazquez E (2020) Environmental DNA from plastic and textile marine litter detects exotic and nuisance species nearby ports. PLoS ONE 15:e0228811. https://doi.org/10.1371/journal.pone.0228811

Islam S, Apitius L, Jakob F, Schwaneberg U (2019) Targeting microplastic particles in the void of diluted suspensions. Environ Int 123:428–435. https://doi.org/10.1016/j.envint.2018.12.029

Jackson CR, Randolph KC, Osborn SL, Tyler HL (2013) Culture dependent and independent analysis of bacterial communities associated with commercial salad leaf vegetables. BMC Microbiol 13:274. https://doi.org/10.1186/1471-2180-13-274

Jacquin J, Cheng J, Odobel C et al (2019) Microbial ecotoxicology of marine plastic debris: a review on colonization and biodegradation by the "plastisphere." Front Microbiol. https://doi.org/10.3389/fmicb.2019.00865

Jiang Y, Xiong X, Danska J, Parkinson J (2016) Metatranscriptomic analysis of diverse microbial communities reveals core metabolic pathways and microbiome-specific functionality. Microbiome 4:2. https://doi.org/10.1186/s40168-015-0146-x

Kathiresan K (2003) Polythene and Plastics-degrading microbes from the mangrove soil. Rev Biol Trop 51:629–633

Kennady V, Virmani M, Verma R et al (2019) Micromanipulation of embryos: an overview. J Entomol Zool Stud 7:439–441

Kershaw PJ, Rochman CM (2015) Sources, fate and effects of microplastics in the marine environment: part 2 of a global assessment. Rep Stud - IMOFAOUnesco-IOCWMOIAEAUNUNEP Jt Group Experts Sci Asp Mar Environ Prot GESAMP Eng No 93

Keswani A, Oliver DM, Gutierrez T, Quilliam RS (2016) Microbial hitchhikers on marine plastic debris: human exposure risks at bathing waters and beach environments. Mar Environ Res 118:10–19. https://doi.org/10.1016/j.marenvres.2016.04.006

Kirstein IV, Wichels A, Gullans E et al (2019) The plastisphere—uncovering tightly attached plastic "specific" microorganisms. PLoS ONE 14:e0215859. https://doi.org/10.1371/journal.pone.0215859

Kirubakaran R, ArulJothi KN, Revathi S et al (2020) Emerging priorities for microbial metagenome research. Bioresour Technol Rep 11:100485. https://doi.org/10.1016/j.biteb.2020.100485

Koelmans AA, Mohamed Nor NH, Hermsen E et al (2019) Microplastics in freshwaters and drinking water: critical review and assessment of data quality. Water Res 155:410–422. https://doi.org/10.1016/j.watres.2019.02.054

Köpke B, Wilms R, Engelen B et al (2005) Microbial diversity in coastal subsurface sediments: a cultivation approach using various electron acceptors and substrate gradients. Appl Environ Microbiol 71:7819–7830. https://doi.org/10.1128/AEM.71.12.7819-7830.2005

Krueger MC, Harms H, Schlosser D (2015) Prospects for microbiological solutions to environmental pollution with plastics. Appl Microbiol Biotechnol 99:8857–8874. https://doi.org/10.1007/s00253-015-6879-4

Krüger A, Schäfers C, Busch P, Antranikian G (2020) Digitalization in microbiology—paving the path to sustainable circular bioeconomy. New Biotechnol 59:88–96. https://doi.org/10.1016/j.nbt.2020.06.004

Kumar V, Sharma N, Maitra SS (2017) Comparative study on the degradation of dibutyl phthalate by two newly isolated *Pseudomonas* sp. V21b and *Comamonas* sp. 51F. Biotechnol Rep 15:1–10. https://doi.org/10.1016/j.btre.2017.04.002

Kumari A, Chaudhary DR (2020) 21—Engineered microbes and evolving plastic bioremediation technology. In: Pandey VC, Singh V (eds) Bioremediation of pollutants. Elsevier, Amsterdam, pp 417–443

Lahens L, Strady E, Kieu-Le T-C et al (2018) Macroplastic and microplastic contamination assessment of a tropical river (Saigon River, Vietnam) transversed by a developing megacity. Environ Pollut 236:661–671. https://doi.org/10.1016/j.envpol.2018.02.005

Langmead B, Salzberg SL (2012) Fast gapped-read alignment with Bowtie 2. Nat Methods 9:357–359. https://doi.org/10.1038/nmeth.1923

Lebreton L, Slat B, Ferrari F et al (2018) Evidence that the Great Pacific Garbage Patch is rapidly accumulating plastic. Sci Rep 8:4666. https://doi.org/10.1038/s41598-018-22939-w

Lewis WH, Tahon G, Geesink P et al (2020) Innovations to culturing the uncultured microbial majority. Nat Rev Microbiol. https://doi.org/10.1038/s41579-020-00458-8

Li S, Hu S, Shi S et al (2019) Microbial diversity and metaproteomic analysis of activated sludge responses to naphthalene and anthracene exposure. RSC Adv 9:22841–22852. https://doi.org/10.1039/C9RA04674G

Liu X, Locasale JW (2017) Metabolomics: a primer. Trends Biochem Sci 42:274–284. https://doi.org/10.1016/j.tibs.2017.01.004

Luo H, Moran MA (2014) Evolutionary ecology of the marine *Roseobacter clade*. Microbiol Mol Biol Rev 78:573–587. https://doi.org/10.1128/MMBR.00020-14

Luo G, Jin T, Zhang H et al (2022) Deciphering the diversity and functions of plastisphere bacterial communities in plastic-mulching croplands of subtropical China. J Hazard Mater 422:126865. https://doi.org/10.1016/j.jhazmat.2021.126865

Malla MA, Dubey A, Yadav S et al (2018) Understanding and designing the strategies for the microbe-mediated remediation of environmental contaminants using omics approaches. Front Microbiol. https://doi.org/10.3389/fmicb.2018.01132

Meyer I, Werner J, Jehmlich N et al (2020) Synergistic biodegradation of aromatic-aliphatic copolyester plastic by a marine microbial consortium. Nat Commun 11:1234567890. https://doi.org/10.1038/s41467-020-19583-2

Meyer-Cifuentes IE, Werner J, Jehmlich N et al (2020) Synergistic biodegradation of aromatic-aliphatic copolyester plastic by a marine microbial consortium. Nat Commun 11:5790. https://doi.org/10.1038/s41467-020-19583-2

Meyer-Dombard DAR, Bogner JE, Malas J (2020). A review of landfill microbiology and ecology: A call formodernization with 'next generation'technology. Frontiers in Microbiology, 11:1127. https://doi.org/10.3389/fmicb.2020.01127

Molitor R, Bollinger A, Kubicki S et al (2020) Agar plate-based screening methods for the identification of polyester hydrolysis by *Pseudomonas* species. Microb Biotechnol 13:274–284. https://doi.org/10.1111/1751-7915.13418

MoonGyung Y, HyunJeong J, MalNam K (2012) Biodegradation of polyethylene by a soil bacterium and alkB cloned recombinant cell. J Bioremediation Biodegrad 3:4

Moore CJ, Lattin GL, Zellers AF (2011) Quantity and type of plastic debris flowing from two urban rivers to coastal waters and beaches of Southern California. Rev Gest Costeira Integrada 11:65–73. https://doi.org/10.5894/rgci194

Moran MA, Satinsky B, Gifford SM et al (2013) Sizing up metatranscriptomics. ISME J 7:237–243. https://doi.org/10.1038/ismej.2012.94

Nazir A (2016) Review on metagenomics and its applications. Imp J Interdiscip Res 2:10

Novoslavskij A, Terentjeva M, Eizenberga I et al (2016) Major foodborne pathogens in fish and fish products: a review. Ann Microbiol 66:1–15. https://doi.org/10.1007/s13213-015-1102-5

Oberbeckmann S, Labrenz M (2020) Marine microbial assemblages on microplastics: diversity, adaptation, and role in degradation. Annu Rev Mar Sci 12:209–232. https://doi.org/10.1146/annurev-marine-010419-010633

Odusanya SA, Nkwogu JV, Alu N et al (2013) Preliminary studies on microbial degradation of plastics used in packaging potable water in Nigeria. Niger Food J 31:63–72. https://doi.org/10.1016/S0189-7241(15)30078-3

Overy DP, Rämä T, Oosterhuis R et al (2019) The neglected marine fungi, *Sensu stricto*, and their isolation for natural products' discovery. Mar Drugs 17:42. https://doi.org/10.3390/md17010042

Papadatou M (2013) Bacterial diversity associated with anthropogenic particles and neuston in the surface layer of the East Atlantic Ocean. University Gent, Gent

Paul T, Akomolafe G, Okogbaa J, Omojola J (2017) Responses of different tomato varieties to disease stress under different conditions of gravity. Fulafia J Sci Technol 3:69–75

Pinnell LJ, Turner JW (2019) Shotgun metagenomics reveals the benthic microbial community response to plastic and bioplastic in a coastal marine environment. Front Microbiol. https://doi.org/10.3389/fmicb.2019.01252

Puglisi E, Romaniello F, Galletti S et al (2019) Selective bacterial colonization processes on polyethylene waste samples in an abandoned landfill site. Sci Rep 9:14138. https://doi.org/10.1038/s41598-019-50740-w

Purohit J, Chattopadhyay A, Teli B (2020) Metagenomic exploration of plastic degrading microbes for biotechnological application. Curr Genom 21:253–270. https://doi.org/10.2174/1389202921999200525155711

Radhika D, Murugesan AG (2012) Bioproduction, statistical optimization and characterization of microbial plastic (poly 3-hydroxy butyrate) employing various hydrolysates of water hyacinth (*Eichhornia crassipes*) as sole carbon source. Bioresour Technol 121:83–92. https://doi.org/10.1016/j.biortech.2012.06.107

Radwan O, Ruiz ON (2021a) Black yeast genomes assembled from plastic fabric metagenomes reveal an abundance of hydrocarbon degradation genes. Microbiol Resour Announc. https://doi.org/10.1128/MRA.01459-20

Radwan O, Ruiz ON (2021b) Metagenome-assembled genomes of 12 bacterial species from biofouled plastic fabrics harbor multiple genes for degradation of hydrocarbons. Microbiol Resour Announc. https://doi.org/10.1128/MRA.01458-20

Rastogi G, Sani RK (2011) Molecular techniques to assess microbial community structure, function, and dynamics in the environment. In: Ahmad I, Ahmad F, Pichtel J (eds) Microbes and microbial technology: agricultural and environmental applications. Springer, New York, pp 29–57

Ravi RK, Walton K, Khosroheidari M (2018) MiSeq: a next generation sequencing platform for genomic analysis. In: DiStefano JK (ed) Disease gene identification: methods and protocols. Springer, New York, pp 223–232

Ronkvist ÅM, Xie W, Lu W, Gross RA (2009) Cutinase-catalyzed hydrolysis of poly(ethylene terephthalate). Macromolecules 42:5128–5138. https://doi.org/10.1021/ma9005318

Rossmanith P, Wagner M (2011) 11—Sample preparation for the detection of foodborne pathogens by molecular biological methods. In: Brul S, Fratamico PM, McMeekin TA (eds) Tracing pathogens in the food chain. Woodhead Publishing, Sawston, pp 237–262

Santo M, Weitsman R, Sivan A (2013) The role of the copper-binding enzyme—laccase—in the biodegradation of polyethylene by the actinomycete *Rhodococcus ruber*. Int Biodeterior Biodegrad 84:204–210. https://doi.org/10.1016/j.ibiod.2012.03.001

Schlundt C, Welch JLM, Knochel AM et al (2020) Spatial structure in the "plastisphere": molecular resources for imaging microscopic communities on plastic marine debris. Mol Ecol Resour 20:620–634. https://doi.org/10.1111/1755-0998.13119

Schneider T, Keiblinger KM, Schmid E et al (2012) Who is who in litter decomposition? Metaproteomics reveals major microbial players and their biogeochemical functions. ISME J 6:1749–1762. https://doi.org/10.1038/ismej.2012.11

Shi M, Zhang Y-Z, Holmes EC (2018) Meta-transcriptomics and the evolutionary biology of RNA viruses. Virus Res 243:83–90. https://doi.org/10.1016/j.virusres.2017.10.016

Skariyachan S, Taskeen N, Kishore AP, Krishna BV (2022) Recent advances in plastic degradation—from microbial consortia-based methods to data sciences and computational biology driven approaches. J Hazard Mater 426:128086. https://doi.org/10.1016/j.jhazmat.2021.128086

Stamps BW, Lyles CN, Suflita JM et al (2016) Municipal solid waste landfills harbor distinct microbiomes. Front Microbiol. https://doi.org/10.3389/fmicb.2016.00534

Stitzlein TM (2018) The marine plastic microbiome: microbial colonization of polymer surfaces in the arctic marine environment. https://hdl.handle.net/10037/15348

Suzuki M, Tachibana Y, Oba K et al (2018) Microbial degradation of poly(ε-caprolactone) in a coastal environment. Polym Degrad Stab 149:1–8. https://doi.org/10.1016/j.polymdegradstab.2018.01.017

Tan J, Lee B-D, Polo-Parada L, Sengupta S (2012) Kinetically limited differential centrifugation as an inexpensive and readily available alternative to centrifugal elutriation. Biotechniques 53:104–108. https://doi.org/10.2144/0000113853

Tankeshwar A (2016) Pour plate method: Principle, procedure, uses, and (dis) advantages. In: Learn Microbiol. Online. https://microbeonline.com/pour-plate-method-principle-procedure-uses-dis-advantages/. Accessed 30 Jan 2021

Tender CD, Schlundt C, Devriese LI et al (2017) A review of microscopy and comparative molecular-based methods to characterize "plastisphere" communities. Anal Methods 9:2132–2143. https://doi.org/10.1039/C7AY00260B

Thomsen TR, Nielsen JL, Ramsing NB, Nielsen PH (2004) Micromanipulation and further identification of FISH-labelled microcolonies of a dominant denitrifying bacterium in activated sludge. Environ Microbiol 6:470–479. https://doi.org/10.1111/j.1462-2920.2004.00580.x

Tiwari N, Santhiya D, Sharma JG (2020) Microbial remediation of micro-nano plastics: current knowledge and future trends. Environ Pollut 265:115044. https://doi.org/10.1016/j.envpol.2020.115044

Tiwari N, Bansal M, Sharma JG (2021) 14—Metagenomics: a powerful lens viewing the microbial world. In: Shah MP, Rodriguez-Couto S (eds) Wastewater treatment reactors. Elsevier, Amsterdam, pp 309–339

Tourova T, Sokolova D, Nazina T et al (2020) Biodiversity of microorganisms colonizing the surface of polystyrene samples exposed to different aqueous environments. Sustainability 12:3624. https://doi.org/10.3390/su12093624

Trindade M, van Zyl LJ, Navarro-Fernández J, Abd Elrazak A (2015) Targeted metagenomics as a tool to tap into marine natural product diversity for the discovery and production of drug candidates. Front Microbiol. https://doi.org/10.3389/fmicb.2015.00890

Tsang YY, Mak CW, Liebich C et al (2017) Microplastic pollution in the marine waters and sediments of Hong Kong. Mar Pollut Bull 115:20–28. https://doi.org/10.1016/j.marpolbul.2016.11.003

Urbanek AK, Rymowicz W, Mirończuk AM (2018) Degradation of plastics and plastic-degrading bacteria in cold marine habitats.

Appl Microbiol Biotechnol 102:7669–7678. https://doi.org/10.1007/s00253-018-9195-y

Vannini C, Rossi A, Vallerini F et al (2020) Microbial communities of polyhydroxyalkanoate (PHA)-based biodegradable composites plastisphere and of surrounding environmental matrix: a comparison between marine (seabed) and coastal sediments (dune sand) over a long-time scale. Sci Total Environ. https://doi.org/10.1016/j.scitotenv.2020.142814

Verberkmoes NC, Russell AL, Shah M et al (2009) Shotgun metaproteomics of the human distal gut microbiota. ISME J 3:179–189. https://doi.org/10.1038/ismej.2008.108

Vijayvargiya P, Jeraldo PR, Thoendel MJ et al (2019) Application of metagenomic shotgun sequencing to detect vector-borne pathogens in clinical blood samples. PLoS ONE 14:e0222915. https://doi.org/10.1371/journal.pone.0222915

Viršek MK, Lovšin MN, Koren Š et al (2017) Microplastics as a vector for the transport of the bacterial fish pathogen species *Aeromonas salmonicida*. Mar Pollut Bull 125:301–309. https://doi.org/10.1016/j.marpolbul.2017.08.024

Wright RJ, Bosch R, Gibson MI, Christie-Oleza JA (2020a) Plasticizer degradation by marine bacterial isolates: a proteogenomic and metabolomic characterization. Environ Sci Technol 54:2244–2256. https://doi.org/10.1021/acs.est.9b05228

Wright RJ, Bosch R, Langille MG, et al (2020b) A multi-OMIC characterisation of biodegradation and microbial community succession within the PET plastisphere (**in review**)

Wright RJ, Langille MGI, Walker TR (2020c) Food or just a free ride? A meta-analysis reveals the global diversity of the plastisphere. ISME J. https://doi.org/10.1038/s41396-020-00814-9

Wu J-H, Wu F-Y, Chuang H-P et al (2013) Community and proteomic analysis of methanogenic consortia degrading terephthalate. Appl Environ Microbiol 79:105–112. https://doi.org/10.1128/AEM.02327-12

Yakimovich K (2017) Examining anaerobic microbial communities that direct the fate of terrestrial carbon in lake sediments. Thesis, Laurentian University of Sudbury

Yang Y, Liu G, Song W et al (2019) Plastics in the marine environment are reservoirs for antibiotic and metal resistance genes. Environ Int 123:79–86. https://doi.org/10.1016/j.envint.2018.11.061

Yang K, Chen Q-L, Chen M-L et al (2020a) Temporal dynamics of antibiotic resistome in the plastisphere during microbial colonization. Environ Sci Technol 54:11322–11332. https://doi.org/10.1021/acs.est.0c04292

Yang Y, Liu W, Zhang Z et al (2020b) Microplastics provide new microbial niches in aquatic environments. Appl Microbiol Biotechnol 104:6501–6511. https://doi.org/10.1007/s00253-020-10704-x

Yoshida S, Hiraga K, Takehana T et al (2016) A bacterium that degrades and assimilates poly(ethylene terephthalate). Science 351:1196–1199. https://doi.org/10.1126/science.aad6359

Yu L-L, Zhu J, Liu J-X et al (2018) A comparison of traditional and novel methods for the separation of exosomes from human samples. BioMed Res Int. https://doi.org/10.1155/2018/3634563

Zalasiewicz J, WatersIvar do Sul CNJA et al (2016) The geological cycle of plastics and their use as a stratigraphic indicator of the Anthropocene. Anthropocene 13:4–17. https://doi.org/10.1016/j.ancene.2016.01.002

Zettler ER, Mincer TJ, Amaral-Zettler LA (2013) Life in the "Plastisphere": microbial communities on plastic marine debris. Environ Sci Technol 47:7137–7146. https://doi.org/10.1021/es401288x

Zhang X, Deeke SA, Ning Z et al (2018a) Metaproteomics reveals associations between microbiome and intestinal extracellular vesicle proteins in pediatric inflammatory bowel disease. Nat Commun 9:2873. https://doi.org/10.1038/s41467-018-05357-4

Zhang Z, Wu Y, Zhang X-H (2018b) Cultivation of microbes from the deep-sea environments. Deep Sea Res Part II Top Stud Oceanogr 155:34–43. https://doi.org/10.1016/j.dsr2.2017.07.008

Zhang B, Matchinski EJ, Chen B et al (2019) Chapter 21—Marine oil spills—oil pollution, sources and effects. In: Sheppard C (ed) World seas: an environmental evaluation, 2nd edn. Academic Press, pp 391–406

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