

1 **Discovering Untapped Microbial Communities through Metagenomics for Microplastic** 2 **Remediation: Recent Advances, Challenges and Way Forward**

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28 29 **Abstract**

30 Microplastics (MPs) are ubiquitous pollutants persisting almost everywhere in the environment. With the
31 increase in anthropogenic activities, MP-accumulation is increasing enormously in aquatic, marine and
32 terrestrial ecosystems. Owing to the slow degradation of plastics, MPs show an increased biomagnification
33 probability of persistent, bioaccumulative, and toxic substances thereby creating a threat to environmental
34 biota. Thus, remediation of MP-pollutants requires efficient strategies to circumvent the mobilization of
35 contaminants leaching into the water, soil and ultimately to human beings. Over the years, several
36 microorganisms have been characterized with the potential to degrade different plastic polymers through
37 enzymatic actions. Metagenomics (MGs) is an effective way to discover novel microbial communities and
38 access their functional genetics for the exploration and characterization plastic degrading microbial
39 consortia and enzymes. MGs in combination with metatranscriptomics and metabolomics approaches are a
40 powerful tool to identify and select remediation-efficient microbes *in situ*. Advancement in bioinformatics
41 and sequencing tools allows rapid screening, mining and prediction of genes that are capable of polymer
42 degradation. This review comprehensively summarizes the growing threat of microplastics around the
43 world and highlights the role of MGs and computational biology in building effective response strategies
44 for MP-remediation.

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46 **Keywords:** Plastic; Pollution; Biodegradation; Microorganisms; Enzymes

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51 1. Introduction

52 The use of plastic has escalated tremendously over the last fifty years due to industrialization.
53 Plastic rise from 1.5 million metric tons (MMTs) in 1950 to 367 MMTs in 2020 is a testament to
54 the global plastic surge (Peng et al. 2021). Even though there has been a decrease of 0.3% in plastic
55 production, the shoot up in the usage of masks, gloves, sanitizer bottles, and medical equipment
56 during the ongoing COVID-19 pandemic has contributed to unforeseen environmental crisis
57 (Patrício Silva et al. 2021). MPs, the plastic fragments with less than 5 millimeters in size, are
58 insoluble, biodegradable, non-biodegradable waste particles having a half-life of about 100-1000
59 years. Based on the occurrence, MPs are classified into primary and secondary types. Primary MPs
60 exist in nature in standard MP-size such as microbeads and plastic pellets whereas, secondary MPs
61 arise from the breakdown of larger plastic materials like fishing nets, soda bottles, microwave
62 containers, and other plastic products. Chemically MPs are synthetic or semi-synthetic polymers
63 composed of carbon, nitrogen, oxygen, hydrogen, chloride, silicon, *etc.* Depending on the nature
64 of side chains, polymer backbone, physical properties, tensile strength, density, and thermal
65 resistant plastics are classified into seven types each numbered according to their recycling codes
66 as 1. Polyethylene terephthalate/PET (Beverage bottles, polyester clothing, rope), 2, High-density
67 polyethylene/HDPE (Detergent bottles, toys, buckets, rigid pipes), 3, Polyvinyl chloride/PVC
68 (Credit cards, medical tubing, rain gutters), 4, Low-density polyethylene/LDPE (Grocery bags,
69 beverage cups, bread bags), 5, Polypropylene/PP (Straws, packaging tape, disposable diapers), 6,
70 Polystyrene/Styrofoam/PS (Insulations, takeout food containers, cutlery), and 7, Others/O
71 (Bisphenol A, polyamimide, polycarbonate) (Verla et al. 2019; Henderson and Green 2020;
72 Veerasingam et al. 2020; Frias et al. 2021).

73 The top countries in the generation plastic waste per year in million tons in 2020 include the United
74 States (58.02) (Law et al. 2020), India (55.06) (Shams et al. 2021), the United Kingdom (39.7)
75 (Burgess et al. 2021), South Korea (38.1) (Shin et al. 2020), Germany (36) (Nelles et al. 2016),
76 Thailand (32.4) (Parashar and Hait 2021), Malaysia (29.8) (Fauziah et al. 2021), Argentina (29.7)
77 (Ronda et al. 2021), Russia (28) (Filiciotto and Rothenberg 2021), Italy (24.5) (Geyer et al. 2017),
78 and Brazil (23.2) (Almeida et al. 2021). Most ecosystems are under threat of plastic pollution
79 because of the properties like non-biodegradability, limited recovery, toxicity, higher ingestion,
80 accumulation, and incorporation associated with MPs (Campanale et al. 2020; Issac and
81 Kandasubramanian 2021). Since MP particles bear resemblance with the food of marine biota,
82 fishes, mammals, and plankton easily engulf it, accumulate in the body leading to blockage of the
83 digestive system (Walkinshaw et al. 2020). Wang et al. (2019a) studied the effect of ingested PS-
84 MPs on *Artemia parthenogenetica* (microcrustacean) and reported the occurrence of several
85 abnormal epithelial cells in the digestive tract. Exposure of zooplankton
86 crustacean *Daphnia magna* to PET textile microfibers resulted in increased mortality of daphnids
87 (Jemec et al. 2016). MPs not only affect the ecosystem directly, but they also act as carriers for
88 other environmental contaminants like heavy metals such as zinc and copper (Brennecke et al.
89 2016), polychlorinated biphenyl (Gerdes et al. 2019), polyaromatic hydrocarbons (Sørensen et al.
90 2020), and others (Ye et al. 2020a). Humans may suffer chronic effects by ingestion, inhalation
91 and dermal contact of MPs leading to cell damage, inflammation and hypersensitive reactions
92 (Visalli et al. 2021; Domenech and Marcos 2021; Blackburn and Green 2021). A 2016-17 UN
93 report documented about 800 animal species contaminated with plastic via entanglement and
94 ingestion, which is almost 70% greater than that of 1977 UN report. This makes humans prone to
95 harmful effects of plastic in the upcoming decades (Smith et al. 2018).

96 Hwang et al (2019) assessed the PP toxicity in human-derived cells and found that PP-MPs induce
97 pro-inflammatory cytokines in a size-dependent manner. Likewise, Wu et al (2019) studied the
98 size-dependent effects of PS-MPs on cytotoxicity and efflux pump inhibition in human colon
99 adenocarcinoma Caco-2 cells. They reported higher mitochondrial depolarization through 5 μm
100 PS-MPs while 0.1 μm PS-MPs induced higher inhibition of adenosine triphosphate-binding
101 cassette transporter. The traditional disposal methods like recycling, incineration, and landfill have
102 been reported to show negative effects by generating secondary pollutants that cause disastrous
103 effects on the environment (Rhodes 2018). Therefore, microbial degradation has emerged as a
104 method of choice for expunging plastic and other pollutants. Several studies have been carried out
105 in studying the biodegradation of MPs such as, PE (Restrepo-Flórez et al. 2014), PS (Kim et al.
106 2021), PP (Jeon et al. 2021), and PET (Farzi et al. 2019). Kim et al (2020) reported that the
107 *Pseudomonas aeruginosa* DSM 50071 strain, isolated from the gut of *Zophobas atratus* larvae
108 mediates the degradation of PS-MPs through enzymatic action. *Zalerion maritimum* (Paço et al.
109 2017), *Aspergillus versicolor* (Akhtar and Mannan 2020), *Vibrio parahaemolyticus* (Kesy et al.
110 2020), and *Psychrobacter* sp. (Chattopadhyay 2022) have been also reported to exhibit the MP-
111 remediation potential. A challenge in using microbial degradation on large scale is the slow rate
112 of plastic degradation. Moreover, most of the reports published on the biodegradation of MPs have
113 been performed in the laboratory set-ups.

114 Many microbes cannot be cultured in the laboratory conditions hence culture-based approaches
115 have proved to be insufficient for the exploration and characterization of microorganisms. Besides,
116 plastic biodegradation is also an outcome of the microbial consortia acting synergistically, which
117 is difficult to study through culture-based approach. Metagenomics offers a gateway to overcome
118 this problem (Handelsman 2004; Wani et al. 2022a). MGs in association with other meta-omics
119 approaches is proving to be standout approach for the identification of novel uncultivable
120 microorganisms capable of MP-remediation (Bharagava et al. 2018; Wani et al. 2022b). This
121 review offers a comprehensive outlook of the MP-threat around the globe besides highlighting the
122 fundamental MP-remediation studies mediated by microorganisms isolated through culture-
123 dependent and culture-independent approaches.

124

125 **2. Microplastics (MPs): Generation and Escalation**

126 Millions of tons of plastics are released into the environment every year. As a result, the quantity,
127 and distribution of MPs have escalated in the atmosphere, aquatic, and terrestrial ecosystems
128 (Figure 1). It is estimated that by 2060 plastic accumulation can reach up to 155-265 million tons,
129 and it is believed that about 13.2 % of this weight could be MPs (Eriksen et al. 2014; Geyer et al.
130 2017; Bergmann et al. 2019). The presence of MPs in different environments was revealed during
131 the early 1970s (Buchanan 1971; Carpenter and Smith 1972). However, in recent times scientists
132 have started to investigate MPs spread, accumulation and ecological implications (Huang et al.
133 2021; Chen et al. 2021a; Vaid et al. 2021; Kallenbach et al. 2022). MP-pollution in terrestrial and
134 freshwater ecosystems has been less extensively studied in comparison to marine ecosystems
135 (Chen et al. 2021a). Afrin et al. (2020) investigated MP presence in landfill sites of Dhaka,
136 Bangladesh and reported the presence of LDPE, HDPE and cellulose acetate. Liu et al. (2018) also
137 reported PP (50.51 %) and PE (43.43 %) in the suburbs of Shanghai, China. 10 % of the plastic
138 ends up in the ocean and about 7-8 million plastic pieces escape into the oceans from land
139 terrestrial sources. At present most of the world's seas and oceans are MP-contaminated.
140 Mediterranean Sea, with a 1,500 m average depth, is recognized as a plastic contamination hotspot
141 because its MP-concentration is 4-fold greater than the North Pacific Ocean. This is attributed to

142 the distinguishing semi-enclosed morphology of the Mediterranean Sea, and surrounding plastic
 143 waste generating countries (Sharma et al. 2021). Table 1 gives insight about the growing MP
 144 contamination in different parts of the world. Lacerda et al. (2019) evaluated and characterized
 145 plastics in sea surface waters of the Antarctic Peninsula and did not find any statistical difference
 146 between the amount of MPs (54 %) and mesoplastics (46 %). They found smaller fragments
 147 composed of polyamide, PET, and Polyurethane (PU).

148
 149 **Table 1: Amount and type of microplastic contamination reported in different marine and**
 150 **terrestrial sites of the world**

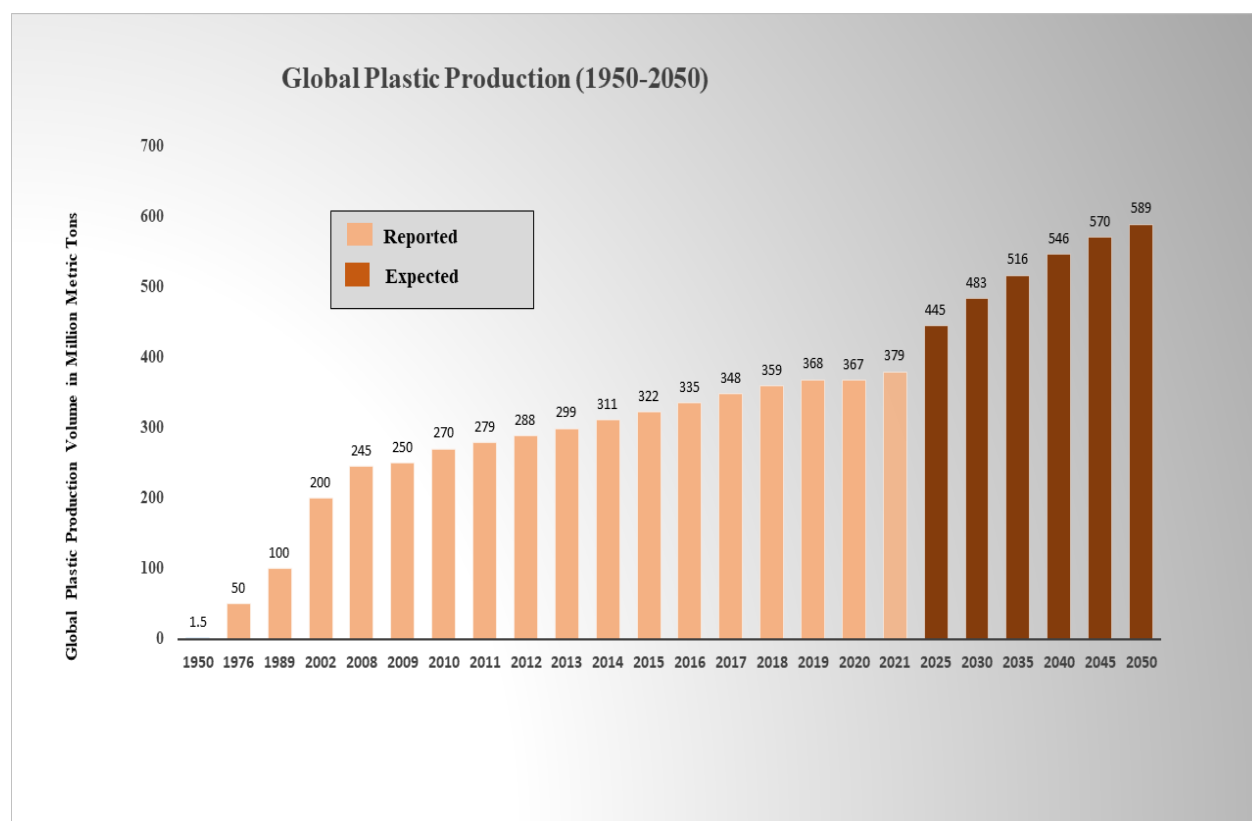
Country/Region	Sampling site	Sample type	Microplastic (MP) types/shapes	MP-Amount	References
Atlantic Ocean	South-North transect	Surface	PE and PP	1723 ± 1793 particles m ³ and 822 ± 1250 particles/ m ³	Pabortsava and Lampitt (2020)
Australia	Gardens	Soil	PE and PP	NA	Sobhani et al. (2021)
Brazil	Guanabara bay sublittoral sites	Sediment	Polyester	160-1000 items/kg	Alves and Figueiredo (2019)
	Patos Lagoon (Laranjal beach)	Water	LDPE, HDPE, and PFTE	0.00021g/L	Silva and de Sousa (2021)
China	Laizhou Bay	Water and Sediment	PET, Cellophane (CP), PE and PP	1.7 ± 1.5 particles/m ³ and 461.6 ± 167.0 particles/kg	Teng et al. (2020)
	Maowei Sea	Water	Polyester and Rayon	1.2-10.1 particles/L	Zhu et al. (2019)
	North Yellow Sea	Surface water and Sediment	PE and PP	545±282 items/m ³ and 37.1±42.7 items/kg	Zhu et al. (2018)
	Jiaozhou Bay	Water and Sediment	PET, PP, and PE	20-120 items/m ³ and 7-25 items/kg	Zheng et al. (2019)
French Polynesia	Tropical lagoons	Surface water and Pearl oyster	PE, and PP	0.2-8.4 items/m ³ and 2.1-125.0 items/g	Gardon et al. (2021)
	Coastal stretch of	Water, sediment,	PP, PE, polyesters, and	60-820 items/m ³ , 60-1620	Sunitha et al. (2021)

India	the Bay of Bengal	and dry sand	fluoro-polymers	items/kg, and 20-1540 items/kg	
	River shoreline Brahmaputra river	Sediment	PP, PE, and PVC	20-240 MP/Kg (particles larger than 150 μ m) and 531-3485 MP/kg (MP particles size range 20-150 μ m)	Tsering et al. (2021)
	Calicut beach, Kerela	Sediment	PE, PE+PP, PP, PS, PCU, PET, and PVC	80.56 items/Kg	Kumar and Varghese (2021)
Indonesia	Jakarta bay (Sunda Kelapa Port)	Sediment	PP, PE, PS, and PA	45066.67 \pm 2444.04 particles/kg	Azizi et al. (2021)
	Banten Bay	Sediment	Foam and PS	267 \pm 98 particles/kg	Falahudin et al. (2020)
Malaysia	Klang River, estuary	Surface water	PE, PA, fibres, and pellets	2.47 particles/L	Zaki et al. (2021)
Mediterranean Sea	Calabrian coasts	Surface water	PE	0.13 \pm 0.19 particles/ m ²	Marrone et al. (2021)
Nepal	Mount Everest	Stream water and Snow	Polyester fibers	1 item/L and 30 items/L	Napper et al. (2020)
Pacific Ocean	Western part	Sediment	PP, PE, and PET	240 items per kg dry weight	Zhang et al. (2020)
	Mid North	Surface water	PP and irregular fragments	0.51 \pm 0.36 items/m ³	Pan et al. (2022)
Portugal	Beaches of Portuguese coast	Sediment	Resin pellets, and PS	358-1679 items m ⁻² , and 63-169 items m ⁻²	Antunes et al. (2018)
South America	Two Tributaries of Cuiaba River	Water	Microfibers	9.6 \pm 8.3 \times 100/L	Faria et al. (2021)
Taiwan	Taiwan Strait	Sediment and surface seawater	Films, fragment, fibers, and granules	28-208 items/kg and 0.004-0.0058 items/m ³	Wu et al. (2021)

Tropical Eastern Pacific and Galapagos	Coast	Water and specimens	Fibers	NA	Alfaro-Núñez et al. (2021)
United States	Northwest Panhandle Florida and Central Florida	Water and snails	Microbeads, microfragments, and microfibers	8.375 items/L and 4.26 MPs/snail	Kleinschmidt and Janosik (2021)

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154 Figure 1: Escalation of plastic waste around the world from 1950 to 2050 (Ritchie and Roser 2018;
 155 Zhang et al. 2021; Jankowska et al. 2022; Luan et al. 2022)

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3. Bioaccumulation and Ecotoxicological repercussions of MPs

158 The resistance (Sharma and Chatterjee 2017), high durability (Lim 2021), high consumption (Chen
 159 et al. 2021b), and low recycling (Muncke et al. 2020) of plastic polymers contribute to the
 160 escalation of plastic in the environment. Oceans are the largest known sinks for MPs (Kvale et al.
 161 2020). The plastic debris from sewage treatment plants, transport and cosmetic industries,
 162 manufacturing, fishing, packaging, and shipping industries reaches the marine environment and is
 163 estimated to be 5-12 million metric tons per annum (Thushari and Senevirathna 2020; Vriend et
 164 al. 2021; Lim 2021; Peng et al. 2021). MP-accumulation in terrestrial and aquatic biota through
 165 absorption, ingestion, or respiration has been widely recognized (Duis and Coors 2016; Souza

166 Machado et al. 2018; Amobonye et al. 2021). *Arenicola marina*, an annelid species, has been
167 reported to have MPs embedded in its gastrointestinal tracts (Besseling et al. 2013). Some
168 crustaceans like *Carcinus maenas* have also been reported with the presence of MPs in digestive
169 and respiratory tracts (McGoran et al. 2020). These plastic particles are mistaken for food, leading
170 to the blockade of essential body tracts which results in the generation of incorrect signals (Smith
171 et al. 2018; Ugwu et al. 2021). Several studies have shown that MP-accumulation or continuous
172 exposure in aquatic organism leads to deterioration of inflammatory and oxidative intestinal
173 balance, and permeability disruption of gut epithelial cells besides promoting the growth of
174 pathogens on cell surfaces (Viršek et al. 2017; Limonta et al. 2019; Yang et al. 2020). Red tilapia
175 when exposed to 0.3, 5, and 70 μm PS fragments for 14 days induced oxidative stress,
176 neurotoxicity, and inhibition of cytochrome P450 enzyme activity (Ding et al. 2020). The
177 accumulation of PS in *Oryzias melastigmas* (Ye et al. 2021) and PE in *Dicentrarchus labrax*
178 (Barboza et al. 2020) have been reported to cause negative effects on histology, immunity, and
179 metabolism. Barboza et al. (2020), reported that PE and polyester in wild fish cause oxidative
180 damage in muscle and gills besides increasing acetylcholinesterase activity in the brain. Bisphenol
181 A and petroleum hydrocarbon aggravate immunotoxicity in blood clams and increase the toxicity
182 of cadmium in fishes (Prüst et al. 2020). Benthic sea cucumbers, a non-selective bottom feeder,
183 feed on the ocean floor debris and engulf a large amount of sediment (Sfriso et al. 2020). A study
184 reported that *Holothuria floridana*, *Thyonella gemmate*, and *Cucumaria frondose* ingested 2-20
185 times more filter feeders, have been reported to ingest MPs which decreases their filtration ability
186 leading to effects like neurotoxicity and immunotoxicity (Mohsen et al. 2019; Bulleri et al. 2021).
187 In 2019, marine biologists reported that seagrass beds in Makassar Strait, Indonesia contain MP-
188 contaminants in the form of beads, pellets, fragments, and fibres (Tahir et al. 2019). Zooplankton
189 also ingests MP-beads which upon excretion can stick to the exoskeleton and appendages
190 (Hasegawa and Nakaoka 2021).

191 The bioaccumulation of MPs in humans largely remains obscure, yet the MP-consumption by
192 crustaceans and fishes which are subsequently eaten by humans is still a matter of concern. There
193 has been no study that evaluates the direct effect of plastic polymers on humans. A major concern
194 in determining the negative effects of MPs on human is the lack of information on human exposure.
195 Thus, a better understanding of the MP-ability to cross epithelial barriers, skin, and gastrointestinal
196 tract is needed to alleviate the uncertainty in human risk assessment of MPs (Prata et al. 2020;
197 Vethaak and Legler 2021). However, several laboratory studies involving human cells and tissues,
198 and model organisms like rats and mice have shown negative implications of MPs. Researchers
199 have started to investigate the presence of MPs in human tissues to extrapolate the effects of MPs
200 that are directly human-oriented rather than in vitro. Ragusa et al, gave the first evidence of PPMP
201 presence in the human placenta (Ragusa et al. 2021). Even though presence and implications of
202 MP in human tissues is obscure, there is need to track and monitor MP-pollution continuously.
203 Exposure of mice to PE showed inflammation (Li et al. 2020) and smaller pups (Park et al. 2020),
204 and exposure to PS reduced sperm count in mice (Jin et al. 2021). In mice gut MPs increased
205 intestinal permeability, altered gut microbiota composition and enhanced intestinal inflammation
206 (Deng et al. 2020). One of the sub-chronic studies reported the accumulation of methacrylate
207 polymer beads only in the gastrointestinal tract of mice (Groborz et al. 2020). Rodriguez-Seijo et
208 al. (2017) reported the accumulation of PE-MPs in the earthworm gut causing damage to the
209 epithelium of the gut wall. Seabirds also feed on marine debris and several studies have reported
210 the presence of MPs in samples targeted for dietary studies, regurgitated cadavers and faeces. After
211 engulfing, seabirds likely get rid of MPs through excretion or regurgitation (Blight and Burger

1997; Gil-Delgado et al. 2017; Hamilton et al. 2021). However, there is a risk of exposing offspring to the MPs at the time of feeding. Kühn and van Franeker (2012), found plastic in the intestine of juveniles rather than adult birds.

Table 2 gives insight about the effect of different MPs on aquatic and terrestrial living systems of earth. Figure 2 illustrates the potential threat of MPs on the biotic components of earth.

Table 2: Effect of different MPs on the biota of aquatic and terrestrial ecosystems.

Microplastic type/shape	Organism	Effect	Reference
Aquatic Organisms			
HDPE	<i>Heliopora</i> , <i>Porites</i> , <i>Acropora</i> , and <i>Pocillopora</i> (Hermatypic corals)	Increase of coral susceptibility to stressors and increase in energy demand.	Reichert et al. (2019)
Microspheres	<i>Aiptasia</i> sp. and <i>Favites chinensis</i>	Disturbs anthozoan-algae symbiosis	Okubo et al. (2018)
PE	<i>Sparus aurata</i>	Intestinal distension, liquid accumulation, inflammation, epithelial desquamation.	Varó et al. (2021)
	<i>Pagurus bernhardus</i> (Hermit crabs)	Impairs shell selection and cognition that disrupts essential survival behavior	Crump et al. (2020)
	<i>Clarias gariepinus</i> (Catfish)	Reduction in swimming speed and increased opercular beat frequency	Tongo and Erhunmwunse (2022)
Polyester	Amphibians (Host) and Trematodes (parasite)	Reduces infection success when both are exposed to polyester contamination simultaneously.	Buss et al. (2021)
PP	<i>Dicentrarchus labrax</i> (Sea bass)	Upregulation of tumour necrosis factor- α and perturbations in gut microbiota	Montero et al. (2022)
	<i>Daphnia magna</i>	Acute toxicity	Jemec Kokalj et al. (2022)
PS/ PS- microbeads	<i>Pelteobagrus fulvidraco</i> (Yellow catfish)	Expression Inhibition of interleukin-8 and tumour necrosis factor- α	Li et al. (2021)
	<i>Mytilus coruscus</i> (Mussel)	Depletion of cellular energy stores like proteins, carbohydrates, and lipids.	Shang et al. (2021)
	<i>Danio rerio</i> (Zebrafish)	Inflammation, increased permeability, microbiota dysbiosis and mucosal damage	Qiao et al. (2019)

	<i>Poecilia reticulata</i> (Juvenile guppy)	Impairs digestive performance, induces microbiota dysbiosis, and stimulates immune response	Huang et al. (2020)
	<i>Paracentrotus lividus</i> (sea urchin)	Increase in reactive oxygen and nitrogen species thus inducing stress on immune cells	Murano et al. (2020)
PVC	<i>Carassius auratus</i> (Goldfish)	Liver inflammation, oxidative damage in the brain, and histomorphological changes in the intestine	Romano et al. (2020)
	<i>Cyprinus carpio</i> var. larvae	Inhibition of weight gain and reduction in malondialdehyde level	Xia et al. (2020)
Terrestrial Organisms			
BPA	Sprague-Dawley rats	Perturbations in butanoate, alanine and aspartate metabolism	Mao et al. (2021)
PE	Mice	Increase in gut microbiota species and increase of interleukin-1 α in serum	Li et al. (2020)
	Mice	Increase in globulin and albumin levels	Sun et al. (2021)
PE and PVC	<i>Drosophila melanogaster</i>	Changes in fertility and sex ratio	Jimenez-Guri et al. (2021)
PET	<i>Achatina fulica</i> (Snail)	Villi damage in gastrointestinal walls and elevation in malondialdehyde levels	Song et al. (2019)
	Human	Alteration in colonic microbial community	Tamargo et al. (2022)
PP, PVC, PET, & PE	<i>Cucurbita pepo</i>	Root and shoot growth impairment, leaf size, and chlorophyll reduction	Colzi et al. (2022)
PS	<i>D. melanogaster</i>	Negative effect on locomotion and intestinal damage	Matthews et al. (2021)
	Rats	Apoptosis and pyroptosis of granulosa cells	Hou et al. (2021)
	<i>Triticum aestivum</i> (Wheat)	Inhibition of wheat root and stem elongation	Liao et al. (2019)

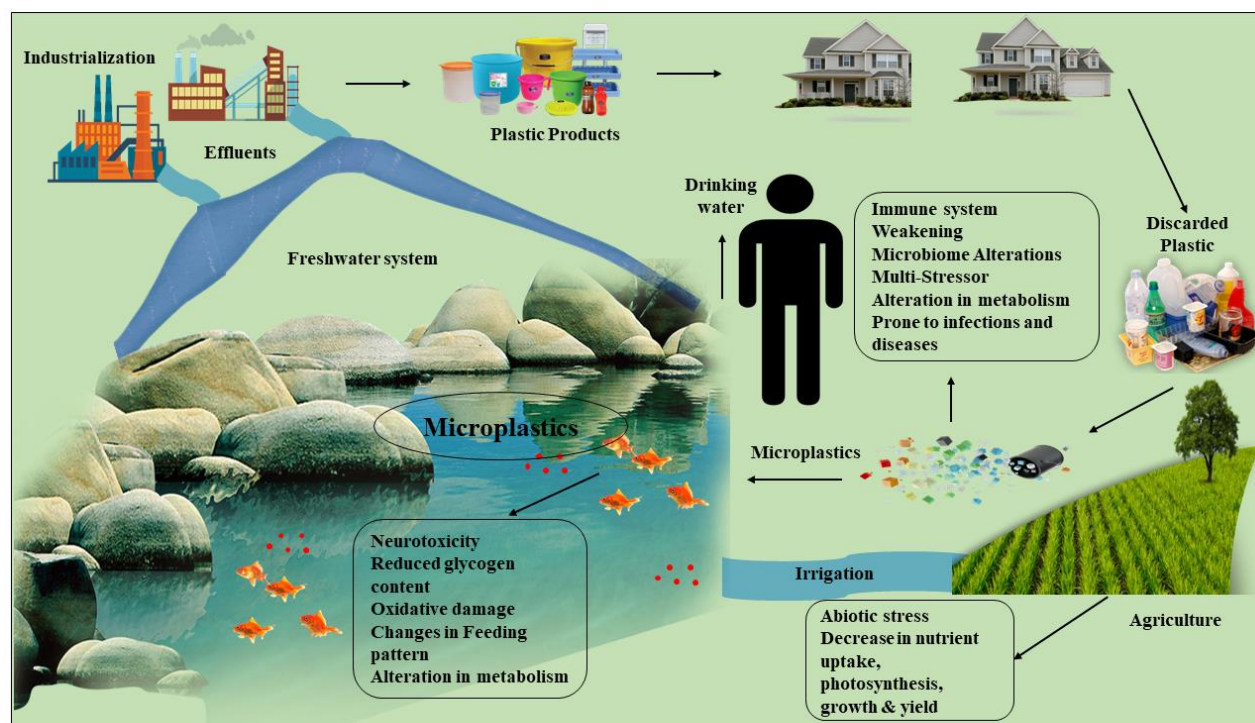


Figure 2: Impact of MPs on marine, terrestrial biota, and its potential threat to human beings

4. Microplastic remediation mediated by microorganisms

MPs degrade mechanically (Schyns and Shaver 2021), chemically (Zhou et al. 2021), and biologically (Arpia et al. 2021) in the environment. Degradation rates mainly depend on structure, chemical composition, temperature, humidity, and deposition environment (Soil, water, sand). Mechanical degradation of MPs occurs through particle contact with anthropogenic (littered trash, boats, vehicles, groynes) and natural items (sediment, woody debris, shells) (Strayer and Findlay 2010; Qiao et al. 2019). Mechanical abrasion of MPs produces items that are similar in morphology to sediment grains. Song et al. (2017), examined the effect of UV exposure on MPs in the replicated beach environment and reported that the degradation rate varies with plastic-type. PE and PP showed low degradation possibility through mechanical abrasion, but PS was found to fragment into more pieces. The exposure of floating plastic to UV light leads to the polymer degradation and generation of chain scission products (Gewert et al. 2018). Enfrin et al. (2020), investigated weathering of MPs when exposed to stress using pumping, ultrasonic irradiation, and stirring. They reported that MPs break down into nanoplastics (NPs) under low stress thus introducing more plastic debris to the environment. The weathering process of MPs is initiated or sometimes enhanced by chemical degradation through thermal oxidation, hydrolysis, and photooxidation. Plastics upon degradation produce different hydrocarbon gases such as methane, ethane, propylene and ethylene when exposed to the solar radiation. Thus, climate-relevant trace gases are expected to increase with the accumulation of more plastic in the environment (Royer et al. 2018). Besides, chemical degradation in seawater or replicated seawater has been reported to advance at a higher rate as compared to freshwater because of the variations in pH, and biotic community (Weinstein et al. 2016; Da Costa et al. 2018). Multiple chemical processes that are involved in the chemical degradation of MPs have been extensively reported and reviewed in great detail by different authors (Min et al. 2020; Ye et al. 2020b; Miao et al. 2020; Venkataramana et al. 2021;

250 Zhou et al. 2021; Akhtar et al. 2022). Both natural and synthetic plastics are degraded by microbial
 251 action (Zeenat et al. 2021). Microorganisms degrade MPs using oxygen as an electron acceptor in
 252 the case of aerobic biodegradation (Yoshikawa et al. 2016). MPs are not transported directly into
 253 microorganisms because of their large size and water-insoluble nature (Cavicchioli et al. 2019).
 254 The degradation of MPs occurs through a series of events including, microbial attachment forming
 255 biofilms (Oberbeckmann and Labrenz 2020), and utilization of MPs as a carbon source (Lear et
 256 al. 2021). The microbial attachment to the MPs leads to the secretion of enzymes changing large
 257 MPs into monomers and oligomers having a low molecular weight (Lin et al. 2022). The
 258 microorganisms can change the surface properties of MPs followed by their bio-fragmentation
 259 through enzymatic action (Pathak and Navneet 2017). Hou and Majumder (2021), identified
 260 cytochrome 4500s, monooxygenases, and hydrolases from microbial sources with PS-degrading
 261 potential. Several other microorganisms have been reported to have MP-degradation potential with
 262 varying biodegradation efficiency. *Pseudomonas fluorescens*, *Bacillus* sp. and *Paenibacillus* sp.
 263 degrade PE (Kathiresan 2003; Park and Kim 2019), *B. vallismortis*, *Aspergillus oryzae*, *B. cereus*,
 264 *Trichoderma viride*, *A. nomius* and *B. siamensis* degrade LDPE (Skariyachan et al. 2017; Montazer
 265 et al. 2018; Nourollahi et al. 2019), and *Klebsiella pneumoniae*, and *A. flavus* degrade HDPE
 266 (Awasthi et al. 2017; Taghavi et al. 2021). The bio-fragmented MPs enter microorganisms through
 267 cell membrane. The large monomers stay outside the microbial cells whereas small monomers
 268 move inside. Within the microbial cells the monomers undergo oxidation which leads to energy
 269 generation used for biomass production (Lucas et al. 2008; Ru et al. 2020). MP-biodegradability
 270 is largely affected by the factors like structural complexity, functional groups, morphology,
 271 polymer toughness, and bond strength (Klein et al. 2018). Biodegradability of MPs can be
 272 enhanced by combining MPs with several additives like nitric acid or pre-treating MPs with heat
 273 or UV (Montazer et al. 2018; Falkenstein et al. 2020). *B. amyloliquefaciens* degrades LDPE upon
 274 preliminary heat treatment by depolymerization reaction (Das and Kumar 2015). Similarly, *B.*
 275 *safensis* and *B. mycoides* degrades LDPE and HDPE upon pretreatment with 0.1% mercuric acid
 276 and sunlight respectively (Ibiene et al. 2013; Das and Kumar 2015). Microorganisms are known
 277 to adapt to varying environmental conditions including the pollution sites through a cascade of
 278 cellular and genetic pathways (Wani et al. 2022c). Microorganisms colonize surface of MPs which
 279 causes changes in mechanical properties like roughness, strength, and reduction in molecular
 280 weight (McGivney et al. 2020). The attachment changes hydrophobic MP surfaces into hydrophilic
 281 which makes them prone to degradation through the action of enzymes like tyrosinase, laccase,
 282 lipase, and peroxidase. For example, *K. pneumoniae* releases certain surfactants that mediate
 283 hydrophobic and hydrophilic phase exchange assisting in easy microbial penetration into PE for
 284 its degradation (Awasthi et al. 2017). Table 3 highlights the MP-degrading potential of
 285 microorganisms.

286
287

Table 3: Microorganisms with MP-degrading potential isolated from different sites

Microorganisms	Sample	MP-type	MP- Initial concentration (Grams)	Weight loss (%)	Incubation period in days
<i>Massilia</i> sp. FS1903	<i>Galleria mellonella</i> gut	PS	0.15	12.97 ± 1.05	30 (Jiang et al. 2021)
<i>B. siamensis</i>	Waste disposal	LDPE	100	8.46 ± 0.3	90 (Maroof et al. 2021)

<i>B. cereus</i>	Landfill area	LDPE	0.13	1.53	120 (Zerhouni et al. 2018)
<i>Pseudomonas sp.</i>	Soil	Bisphenol -A	0.0001	54.6±3.7	60 (Matsumura et al. 2009)
<i>Lysinibacillus sp.</i>	Soil grove	PE and PP	0.3 and 0.39	7.5 and 3	28 (Jeon et al. 2021)
<i>Microbacterium paraoxydans</i> and <i>P. aeruginosa</i>	Pure cultures used	LDPE	0.25	61 and 50.5	60 (Rajandas et al. 2012)
<i>Pseudomonas sp.</i> and <i>Rhodococcus sp.</i>	Antarctic soil	PP	0.100	17.3 and 7.3	40 (Habib et al. 2020)
<i>Rhodococcus sp.</i>	Mangrove sediment	PP	0.500	6.4	40 (Auta et al. 2018)
<i>Aspergillus tubingensis</i> and <i>A. flavus</i>	Coastal area soil	HDPE	0.200	6.02 ± 0.1 and 9.34 ±0.2	30(Sangeetha Devi et al. 2015)
<i>Paenibacillus sp.</i>	Landfill	PE	0.0147	11.6	90 (Bardají et al. 2019)
<i>Lysinibacillus xylanilyticus</i> and <i>Aspergillus niger</i>	Landfill	LDPE	0.300	8.9 and 17.4	63 and 126(Esmaeil i et al. 2013)
<i>Stenotrophomonas sp.</i> and <i>Fusarium sp.</i>	Compost soil	Nylon	0.03	16 and 14	28 (Tachibana et al. 2010)
<i>P. aeruginosa</i>	Surface water	PE	0.80	6.25	30 (Mouafo Tamnou et al. 2021)
<i>Dethiosulfovibrio sp.</i> ; <i>Sporobacter sp.</i> , and <i>Cupriavidus sp.</i>	Marine litter and water	PVC	10	3.51±0.81,3.71±0.28, and 3.91±0.2,	90 (Giacomucci et al. 2020)
<i>Mycobacterium neoaurum</i>	Soil	Dimethyl phenol	0.5	6.7	60 (Ji et al. 2020)

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5. Enzymatic degradation of MPs

290 Owing to the presence of the homoatomic and heteroatomic backbone in plastics, MP-degradation

291 by microorganisms is an arduous process (Edmondson and Gilbert 2017). There is considerable

292 weight loss in the plastic polymer with the action microorganisms but the process is significantly

293 slower than chemically mediated biodegradation processes (Jaiswal et al. 2020). The polymer

294 chains of MPs are broken by enzymes secreted by microbes (Mohanani et al. 2020; Lv et al. 2022;
 295 Kaur et al. 2022; Gaur et al. 2022). ATP-binding cassette transporters couple hydrolysis process
 296 to mediate the uptake and efflux of small fragments across the cell membrane in prokaryotic and
 297 eukaryotic cells. These transporters also play role in the secretion of toxins (Giuliani et al. 2011).
 298 Enzymatic actions like oxidation, hydrolysis and hydroxylation cleave the MPs into monomers
 299 (Rana et al. 2022). The high molecular weight MPs are degraded first by extracellular enzymes
 300 and then incorporate into microbial cells (Urbanek et al. 2018). Within the microorganisms, the
 301 degraded MPs are catabolically channeled to yield energy for intracellular polymerization and
 302 integration into cellular structures (Müller et al. 2019; Rogers et al. 2020). Cutinase, an esterase
 303 sub-class, isolated from *F. solani*, *Thermobifida fusca*, *T. alba*, and *T. cellulositytica* is effective
 304 in hydrolyzing polyester MPs (Ribitsch et al. 2012; Dong et al. 2020). Several studies have
 305 reported that PET degradation is mediated by PET-hydrolases belonging to cutinases (Kawai et al.
 306 2019; Furukawa et al. 2019; Carr et al. 2020). The enzymatic degradation of PET occurs either by
 307 surface modification of polyester fibres or polymer hydrolysis (Bååth et al. 2020). Several
 308 hydrolases have been reported to cause PET surface hydrophilization, such as lipases from
 309 *Thermomyces* sp., *Candida antarctica* (Carniel et al. 2017), cutinases from *Penicillium citrinum*,
 310 *Humicola insolens*, and *Saccharomonospora viridis* (Liebminger et al. 2007), and
 311 carboxylesterases from *T. halotolerans* (Samak et al. 2020). PU degradation by membrane-
 312 associated (*PudA*) and extracellular (*PueA*, *PueB*) esterases isolated from *Comamonas*
 313 *acidovorans*, *P. fluorescens*, and *P. chlororaphis* have been characterized (Stern and Howard
 314 2000). The blending of certain natural polymers like starch with synthetic MPs has been shown to
 315 increase the rate of MP-biodegradation (Vroman and Tighzert 2009). This is attributed to the rapid
 316 hydrolysis of starch making the MPs susceptible to microbial degradations. Karimi and Biria have
 317 reported LDPE degradation by the action of amylase when blended with starch (Karimi and Biria
 318 2019). Currently, the least information on the enzymes acting on MPs with high molecular weight
 319 like PVC, PP, PS and Polyamide is available. Even though mixed microbial communities have
 320 been reported to cause the weight loss of these MPs, the effectiveness of gene products is yet to be
 321 ascertained completely. Extreme environments are rich reservoirs of hydrolytic enzymes stable at
 322 fluctuating environmental conditions like temperature, pH, salinity, and pressure. The search for
 323 MP-degrading microorganisms and enzymes is already gaining research attention through
 324 metagenomic strategies. Table 4 gives an overview of the enzymes isolated and characterized
 325 from microbial sources with MP-degrading potential.

326

327

328 **Table 4: Enzymes derived from different microorganisms and their MP-degrading potential**

MP-type	Enzyme	Microorganism	References
Biodegradable plastic	Esterase	<i>Pseudozyma antartica</i>	Sameshima-Yamashita et al. (2019)
HDPE	Peroxidase	<i>Citrobacter</i> sp.	Ojha et al. (2017)
LDPE	Laccase	<i>Lysinibacillus</i> sp.	Ghatge et al. (2020)
	Laccase	<i>Rhodococcus ruber</i>	Santo et al. (2013)

PE	Alkane hydroxylase	<i>Pseudomonas</i> sp.	Jeon and Kim (2015)
PET	PETase	<i>Ideonella sakaiensis</i>	Webb et al. (2013)
	Cutinase	<i>Thermobifida fusca</i>	Müller et al. (2005)
	Cutinase	<i>Fusarium</i> sp., & <i>Humicola</i> sp.	O'Neill et al. (2007); Ronkvist et al. (2009)
	MHETase	<i>Ideonella sakaiensis</i>	Yoshida et al. (2016)
	Oxidoreducase	<i>Klebsiella pneumoniae</i>	Peter Guengerich and Yoshimoto (2018); Kawai et al. (2019)
Polycaprolactone	Lipase	<i>Alcaligenes faecalis</i>	Oda et al. (1997)
Polycaprolactone and Polyhydroxybutyrate	Manganese peroxidase	<i>Amycolaptosis</i> sp. and <i>Tremetes versicolor</i>	Deguchi et al. (1998); Fujisawa et al. (2001)
Polyester	Polyesterase	<i>Cyanobacteria</i> sp.	Hajighasemi et al. (2018); Wani et al. (2021)
	Protease	<i>P. fluorescens</i>	Howard and Blake (1998)
	Serine hydrolase	<i>Pestalotiopsis microspore</i>	Russell et al. (2011)
Polylactic acid	Cutinase like enzyme	<i>Cryptococcus</i> sp.	Masaki et al. (2005)
PP	Monoxygenase	<i>Rhodococcus</i> sp.	Toda et al. (2012)
	Hydrolases	<i>Rhodococcus</i> sp. and <i>Bacillus</i> sp.	Auta et al. (2018)
	Hydrolases	<i>Rhodococcus ruber</i>	Mor and Sivan (2008)

PS	Styrene monooxygenase	<i>Nocordia sp.</i>	Jacquin et al. (2019)
	Isomerase, dehydrogenase, & monooxygenase	<i>Micrococcus, Nocordia, & Bacillus</i>	Jacquin et al. (2019); Danso et al. (2019)
	Cytochrome P450s	<i>Enterococcus sp.</i>	Hou and Majumder (2021)
	Peroxidase, esterase, dioxygenase, and monooxygenase	<i>B. paralicheniformis</i>	Ganesh Kumar et al. (2021)
	Oxygenase	<i>Exiguobacterium sp. RIT 594</i>	Parthasarathy et al. (2022)
PU	Esterase	<i>Alicycliphilus sp.</i>	Oceguera-Cervantes et al. (2007)
	Lipase	<i>Candida rugosa</i>	Gautam et al. (2007)

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331

6. Metagenomics (MGs): Gateway to microbial and enzyme mining

332 Even though microorganisms are present everywhere in the environment, limitations in traditional

333 culture techniques have crippled the exploration of vast microbial flora (Lewis et al. 2021).

334 Microbiologists estimate that only 1-2% of the total microbial flora is culturable, which leaves

335 majority of the microorganisms unexplored. MGs offers an efficient lens to reveal the hidden

336 microbial diversity in a culture-independent manner (Handelsman 2004; Wani et al. 2022d). Figure

337 3 highlights the fundamental methodology of the sequence- and function-based metagenomic

338 approach for the exploration of microorganisms and gene products. The taxonomic profiling and

339 functional gene annotation of microbial communities of river Ganga (sediment) using whole-

340 genome MGs has also been done (Rout et al. 2022). Several other research groups have identified

341 novel bacteria from different sites including extreme environments like hot springs, deserts, and

342 deep-sea sediments for bioprospecting using a MG approach (Tang et al. 2018; Najjar et al. 2020;

343 Alotaibi et al. 2020; Zhu et al. 2022; Wani et al. 2022b). Global ocean sampling revealed about 40

344 million non-redundant novel genes from more than 30,000 species, whereas over 97% of the 150

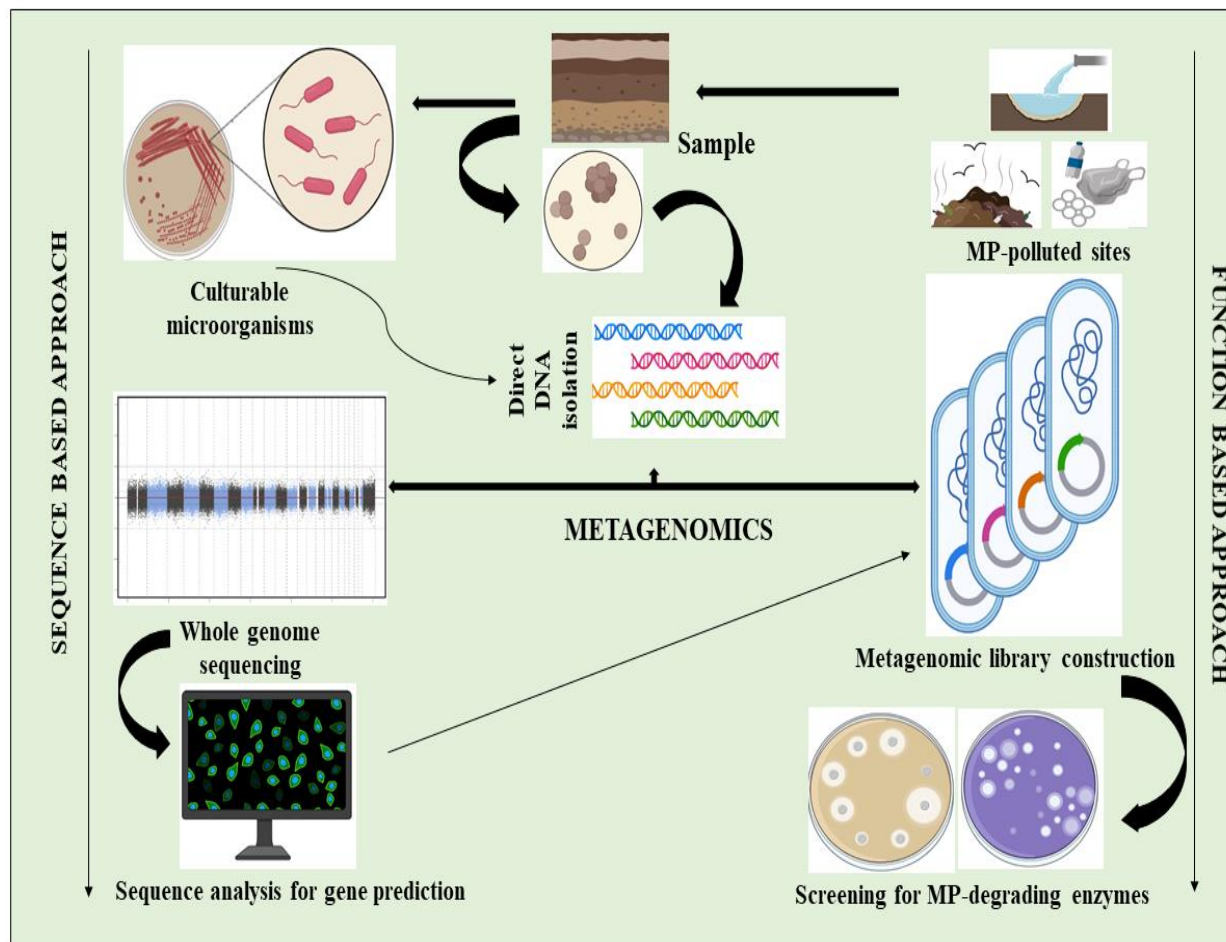
345 million genes reported in topsoil globally cannot be found in the existing gene catalogue. This is a

346 strong indicator that microbiomes carry huge functional potential, with unculturable

347 microorganisms as acting enzyme reservoir (Sunagawa et al. 2015; Bahram et al. 2018). In a study,
348 hidden Markov models were constructed from experimentally verified enzymes and mined soil
349 and ocean metagenomes to assess the ability of microorganisms in degrading plastics. They
350 compiled almost 30,000 non-redundant enzymes that were homologues with known enzymes
351 having plastic degrading potential (Zrimec et al. 2021). Chow et al (2023) presents a sequence-
352 based in silico strategy for screening and characterization of PETases from MG datasets. The MG
353 screening of a novel PET esterase through in vitro expression system has also been developed
354 using next generation sequencing (Han et al. 2023). In a recent study, distinct microbial
355 communities have been unveiled through MGs that degrade hydrocarbon chains, which are units
356 of plastic polymers (Hauptfeld et al. 2022). Using 16S rRNA datasets obtained through MGs, the
357 taxonomic and functional characteristics of PE-degrading microorganisms have been analyzed
358 from one of the waste recycling sites in Tehran, Iran (Hesami Zokaei et al. 2021).
359 Integrated Microbial Genome (IMG) helps to identify candidate genes from different
360 metagenomes (Zaidi et al. 2021). In a MG study, two heat stable enzymes with application in
361 plastic degradation were partially characterized (Danso et al. 2018). Shotgun MGs has revealed
362 the microbial community response to plastic contamination in coastal environments (Pinnell and
363 Turner 2019). Shotgun MGs generated 3,314,688 contigs (DNA sequences that overlap providing
364 contiguous representation of a genomic region) and 120 microbial genomes. This was followed by
365 the functional gene annotation to identify microbiomes that harbor genes encoding esterases,
366 lipases, and monooxygenases that are known to degrade different types of plastics (Radwan et al.
367 2020). Hu et al (2021) reported hydrolysis of PET by a metalloprotease and a serine protease. The
368 study provided intrinsic insight into PET degradation and opened a gateway for hunting more
369 plastic-degrading enzymes. Bollinger et al. (2020), also characterized a novel polyester hydrolase
370 from *P. aestusingri* for the degradation of synthetic PET. Table 5 highlights some of the abundant
371 microbes and enzymes isolated and characterized from microorganisms through culture-based and
372 sequence- and function-based MG approaches having MP-degrading potential. Even though the
373 MP-degradation by microorganisms and their gene products is effective, the rate of degradation
374 has always been a matter of concern. MG investigation allows upscaling the degradation rate by
375 modifying the microbial composition and genome engineering.

377

378



379
 380 **Figure 3:** Metagenomic (MG) driven search operation for MP-degrading microorganisms through
 381 function and sequence-based metagenomic approaches. The function-based approach is followed
 382 by random screening for different enzymes while the sequence-based approach ensures the
 383 prediction of several genes that are effective in producing MP-degrading enzymes.

384
 385 **Table 5: Sequence based (SB) and Function-based (FB) metagenomic approaches for the**
 386 **identification of abundant microbes and /or enzymes useful in targeting different plastic**
 387 **substrates**

388

Microbes/Enzymes	Metagenome source	Metagenome Sequencing approach	Metagenome strategy	Target plastic substrate	References
<i>Bryozoa, Cyanobacteria, Alphaproteobacteria, and Bacteroidetes</i>	Sea water	Shotgun metagenomics	SB	Mixed plastic debris	Bryant et al. (2016)
<i>Flavobacteriaceae,</i>	Surface sea water	16S metagenom	SB	PS	Sekiguchi et al.

<i>Methylophilaceae</i> , <i>Rhodobacteraceae</i> , <i>Planctomycetaceae</i> , <i>Nocardiaceae</i> , and <i>Verrucomicrobiae</i>		ics (V4-V6 and V9)			(2009); Kirstein et al. (2019)
<i>Rhodococcus sp.</i> (YC-SY1, YC-BJ1, and YC-GZ1)	Soil	Illumina HiSeq 16S metagenomics (V3+V4)	SB	Triphenyl phosphate (Plasticizer)	Wang et al. (2019b)
PET hydrolase	Marine water	Next-generation metagenome sequencing	FB	PET	Danso et al. (2018)
<i>Thalassospiraceae</i> , <i>Alteromonadaceae</i> , <i>Alcanivoraceae</i> , and <i>Vibrionaceae</i>	Beach sediment	Meta-omics (16S metagenomic approach)	SB and FB	PET	Wright et al. (2021)
<i>Proteobacteria</i> , <i>Firmicutes</i> , <i>Actinobacteria</i> , and <i>Firmicutes</i>	Landfill soil	High throughput metagenomics	SB	PE and PS	Kumar et al. (2021)
Polyurethane esterase	Landfill	Shotgun metagenomics	FB	PU	Gaytán et al. (2019)
Cutinase	Compost	Shotgun metagenomics	FB	PET	Sulaiman et al. (2012)
Esterase	Seawater	Illumina HiSeq	FB	Polyhydroxybutyrate, and polylactic acid	Tchigvintsev et al. (2015)
Esterase	Compost	Shotgun metagenomics	SB and FB	PU	Kang et al. (2011)
Protease	Marine sediment	Bidirectional end sequencing	FB	Polyester	Lim et al. (2005); Sun et al. (2020)

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391

6.1. Microbial manipulation

392 The manipulation of human, animal, soil, plant and water microbiome is the contemporary strategy
393 followed for increasing the benefits offered by them (Huynh et al. 2016; Hussain et al. 2018; Jochum
394 et al. 2019). It includes several cellular, molecular, and chemical methods for extensive
395 manipulation with higher specificity and magnitude. The prebiotic (chemical) approach enables
396 modification in microbial communities to increase their adaptability and functionality in a particular
397 environment (Gianoulis et al. 2009; Raes et al. 2021). Polysaccharides and oligosaccharides affect
398 microbiome composition and support the growth of MP-degrading microorganisms (Grondin et al.
399 2017). Chitin, starch, lipopeptides, glycolipids, etc. help in biofilm formation by acting as
400 surfactants on MP-surfaces (Shilpa et al. 2022). Similarly, probiotic cultures are applied for the
401 better performance of MP-degraders through bioaugmentation (Kamilya and Devi 2022). The
402 microorganisms like *Pseudomonas*, *Micrococcus*, *Moraxella*, *Streptomyces*, *Thermoactinomyces*,
403 *Penicillium*, and *Aspergillus* are preferred over the native microorganism (Spini et al. 2018).
404 Microbiome transplantation and probiotic bioaugmentation remain unsuccessful owing to the slow
405 microbial growth, low cell viability, limited distribution, and reduced functionality. These issues are
406 likely to be solved by metagenome engineering followed by bioaugmentation.

407 Microorganisms are genetically modified to produce novel strains that express unique and well-
408 defined genetic determinants or to introduce genetic variants that cause phenotypic changes. The
409 process is used to investigate the biotechnological potential linked to environmentally useful
410 microorganisms and to make use of functional genes when put into the right host (Zeaiter et al.
411 2018). There have also been attempts to chemically alter marine microbes. Besides natural
412 competence, wild-type and DNase-negative *Vibrio cholerae* strains are effectively electroporated
413 and transformed by the researchers for biotechnological applications (Marcus et al. 1990; Jaskólska
414 et al. 2018). Although the outcome of the electroporation can also be influenced by other parameters,
415 including growth conditions, the pulse used, and the type of exogenous DNA, the electroporation
416 efficiency is strain dependent. Several marine strains from various genera, including *Roseobacter*,
417 *Vibrio*, *Pseudoalteromonas*, *Caulobacter*, *Cyanobacteria*, and *Halomona*, have been successfully
418 modified for expression of environment-useful genes (Kivelä et al. 2008; Borg et al. 2016;
419 Laurenceau et al. 2020).

420

421 **6.2. Genetic engineering**

422 With the progress in molecular biology and genetic engineering, the development of genetically
423 modified microorganisms as potent MP-degraders has advanced significantly. The construction of
424 metagenomic libraries makes it likely to create genetic circuits with novel and precise functionalities
425 (Bacha et al. 2021). The synthetic microbial cells created through genome editing, protein
426 engineering, or genetic engineering can be employed for metagenome engineering in the
427 plastisphere (Austin et al. 2018; Jaiswal et al. 2019). Since biodegradation of MPs involves a
428 cascade of oxidation processes which is difficult and slow by the action of single species (Klein et
429 al. 2018). Metagenome engineering can be applied for complementing multiple genes involved in
430 MP-degrading metabolic pathways. This will ensure the production of multiple enzymes that
431 regulate biofilm formation and quorum sensing. Genome modification of *B. subtilis* and *E. coli* for
432 expression of *PETase* enzyme for the degradation of PET is a common example. *PETase* and
433 *MHETase* have been identified in *Ideonella sakaiensis* 201-F6 and cloned in a suitable PUCIDT
434 vector for the creation of recombinants with higher PET-degrading potential (Janatunaim and
435 Fibriani 2020). Puspitasari et al. (2021), showed that the rate of *PETase* hydrolysis increases
436 significantly in the presence of hydrophobin. Since the core metagenome of any site is constant,
437 therefore rather than modifying a single genome it is possible to engineer the entire metagenome.

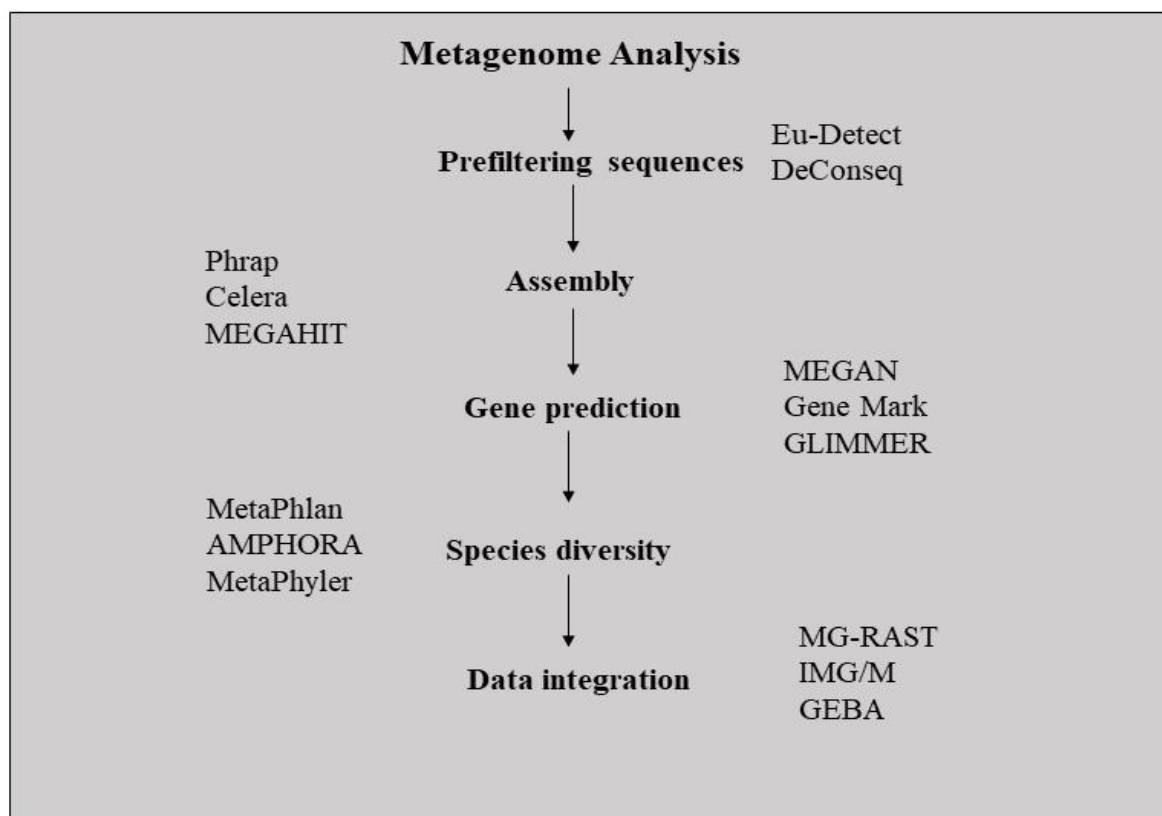
438 The direct in situ metagenome engineering of microbial population is achievable through horizontal
439 gene transfer of plasmid construct through genetic augmentation. The applicability of
440 bacteriophages as gene delivery agents is advancing. The strategy can very well be applied to the
441 gene delivery with having MP-degrading potential. However, there is a growing concern about the
442 release of genetically engineered microorganisms into the environment owing to their adverse
443 effects. There are chances that engineered microorganisms may affect the biodiversity by creating
444 more infectious pathogens, harm non-target species, and disrupt ecological balance (Lenski 1993;
445 Clark 2006).

446

447 **7. Metagenome analysis through computational tools**

448 Development in computational tools and advancement of computational power has enormously
449 aided in metagenome refinement and analysis. The sequencing of metagenome samples with the
450 potential to degrade contaminants is a method of choice for identifying novel microorganisms and
451 predicting genes. Shotgun MGs gives insight into the microbial community members and the
452 possible metabolic pathways mediated by them. Since metagenome collection from environments
453 is largely uncontrolled, the organisms present in abundance are highly represented in sequence data.
454 To achieve equal coverage of all the microbial members, the random shotgun sequencing resolves
455 genomes uniformly and ensures the identification of lesser presented organisms. The metagenome
456 data is often enormous containing fragmented and raw data (Wooley et al. 2010). The metagenome
457 sequencing of cow rumen generated more than 250 gigabases, while the gut microbiome of human-
458 generated more than 550 gigabases of sequence data (Qin et al. 2010; Hess et al. 2011). Thus, the
459 identification, collection, and curation of useful data from huge metagenome datasets are
460 challenging for many researchers. Almeida et al, employed *in silico* screening method for the
461 identification of potential *PETase-like* enzymes. They identified the *PETase-like* gene SM14est in
462 *Streptomyces* after analyzing more than 50 genomes (Almeida et al. 2019). Figure 4 represents the
463 basic methodology of metagenome data analysis useful for understanding microbial diversity and
464 predicting useful genes. One of the standalone metagenome analyzing tools is Meta Genome
465 Analyzer (MEGAN). It was initially used for studying metagenomes obtained from mammoth bone
466 (Poinar et al. 2006). The tool is used to perform functional and taxonomic binning using the lowest
467 common ancestor algorithm. More efficient, accurate, and faster computational tools are being
468 developed to keep up the pace with high-throughput sequencing. Metagenomic Rapid Annotations
469 using Subsystems Technology (MG-RAST) is one of the biggest metagenome repositories
470 developed for automatic phylogenetic and functional analysis of metagenomes. Wani et al. (2022e)
471 has comprehensively reviewed the maximum number of computational tools used in the analysis of
472 metagenome data-sets.

473



474
 475 **Figure 4:** Basic methodology of the metagenome analysis through computational tools.
 476 The generated metagenome sequences are prefiltered for the removal of low-quality, and
 477 redundant sequences using Eu-detect, & DeConseq. To increase the analytical efficiency
 478 of computational tools, the metagenome assemblies are developed using Phrap or Celera
 479 or MEGAHIT assembler. This is followed by the prediction of genes using the MEGAN
 480 or Gene Mark or Gene Locator and Interpolated Markov Modeler (GLIMMER) program.
 481 Function-based annotation and taxonomic profiling are carried out MetaPhlan or
 482 Automatic phylogenomic inference application (AMPHORA) or Metaphyler followed by
 483 integration into MG-RAST, Integrated Microbial Genomes and Metagenomes (IMG/M)
 484 and Genomic Encyclopedia of Bacteria and Archaea (GEBA) like tools.

485
 486

487 **8. Limitations and way forward**

488 MGs based studies allows exploration of microbial diversity, genetic evolution, species
 489 composition, and bioprospecting. However, bottlenecks in MGs right from sample collection until
 490 the analysis have always been challenging (Scholz et al. 2012). Sample collection is one of the
 491 confounding factors that affect the sequencing outcomes owing to concerns like contamination,
 492 transportation, storage, and safety. The developments in sequencing technology have significantly
 493 advanced computational tools for functional annotations and analysis (Bharti and Grimm 2021).
 494 However, multiple challenges still exist owing to the complexity of metagenomic data. While
 495 analyzing the complex metagenome data sets challenges like multiple genomes, and inter- and
 496 intra-genomic repeats lead to uneven sequencing with a higher degree of sequencing errors.
 497 Although the gene prediction tools have an efficiency of about 90%, the small number of genes
 498 escaping detection can be novel and more useful (Coleman and Korem 2021). Downstream

499 processing of MG data is also much crucial for understanding microbiome structures and metabolic
500 pathways, but due to multivariate metagenomic data, the downstream analysis is difficult
501 (Lindgreen et al. 2016). The discovery of enzymes is prevented by other limitations like limited
502 thermostability, low stereoselectivity, and insufficient expression. Ribosome engineering can be
503 useful in retrieving all possible candidate genes for synthesis and testing the activities (Uchiyama
504 and Miyazaki 2009). Fungi despite their affinity for plastics have been largely neglected. MG
505 findings provide evidence that the plastisphere is a suitable niche for various fungal organisms,
506 including pathogenic species (Gkoutselis et al. 2021).

507 The technical glitches and problems in data evaluation and interpretation confronted during
508 metagenome studies can be overcome by the combination of MGs and machine learning tools like
509 artificial intelligence (Rhoads 2020; Wani et al. 2022f). This will help in accurate, and timely
510 characterization of microorganisms and microbial products useful in remediation processes.
511 Artificial intelligence can be utilized in developing new models to design effective bioremediation
512 tools and evaluate the performance and functionality of microorganisms. The development of
513 smart biomarkers as indicators of pollution is an efficient way to track environmental fluctuations
514 (Krishna Kumar et al. 2011). Moreover, gene engineering within genomes and metagenomes using
515 gene-editing tools like Clustered regular interspaced short palindromic repeats-associated protein
516 (CRISPR-Cas) system can revolutionize the microbe-mediated degradation processes owing to its
517 specific nature (Jaiswal et al. 2019; Wani et al. 2022g; Mir et al. 2022). This will help to upregulate
518 contaminant-degrading genes and pave way for understanding the molecular pathway involved in
519 it. The applicability of artificial intelligence environmental and genome editing for microbial
520 simulation will continue to be the method of choice in combatting plastic and other pollution.

521

522 **9. Conclusion**

523 The emergence of MP-contamination has become a serious concern for the biota owing to
524 the small size and their ability to reach into the human body through secondary sources
525 like food. Moreover, research investigations and evidence based on the ecological toxicity
526 of microplastics to aquatic biota revealed numerous toxic effects on organisms, posing
527 serious ecological risks. The hazardous effect of microplastic is outlined as
528 single and combined toxicity of various pollutants, which has reportedly impacted
529 mortality rates, development, food intake capacity, reproductive capability, and gene
530 expression in aquatic organisms. Considering the degradation potential of microbes and
531 enzymes, it is possible to detoxify and degrade MPs into non-toxic end products. Thus, it
532 is necessary to explore microorganisms that can mediate the bioremediation process of
533 these MPs. MGs is a powerful genome centric culture-independent technique to identify
534 novel microorganisms and their products for bioprospecting including the degradation of
535 environmental contaminants. MGs with other meta-omics strategies can be useful in
536 building a timely response strategy for combatting the growing plastic threat and its
537 associated concerns. Overall, MGs has enabled scientific studies of complex microbiomes,
538 which have assisted to explain certain metabolic processes of polymer degradation. As a
539 result, extensive research in this area is required, which may significantly reduce global
540 plastic pollution while also ensuring the health of future generations.

541

542

543 **Author contributions**

544

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555 Writing – original draft, Visualization, Writing – review & editing, Supervision, Project
556 administration.

557

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