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REVIEW ARTICLE

Research on ‘Plastisphere’ in Environmental Health Perspectives-A Narrative Review

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Abstract

‘Plastisphere’ the term used to denote diverse microbial phyla inhabiting the outer surface of plastic debris, is recently emerged as hot research topic in environmental investigations. A growing body of research is actively involved in exploring holobiome of ‘Plastisphere’ and its impacts on three different components of ‘one health’. Further, a group of researchers are exploring the possibility of ‘Plastisphere’ as a niche for potential hydrocarbon degrading microbes and their functional genes to have possible applications in bioremediation research. A large number of microscopical and molecular tools are now being applied and developed for mining ‘Plastisphere’. Nevertheless, there are many gaps in the current knowledge of ‘Plastisphere’, demanding detailed further investigations focusing on three way interactions between plastics, microbes, and various consumers. The present review is intended to provide a comprehensive information on various aspects in ‘Plastisphere’ research.

Keywords: Microbes, Plastic, Pollution, Bioremediation, Environment.

1. Introduction

‘One Health’, a concept aiming the optimal health and well-being of all species, require an integrative effort of multiple disciplines (OHITF, 2008). Three major components requiring integral connections in ‘One Health approach’ are humans, animals and environment. An interdisciplinary collaboration between these three components are necessary to have a more holistic understanding and more effective actions against various public health threats (APHA, 2017). However, the third component (environment) remains as the most often neglected or less considered partner (Musoke *et al.*, 2016). The World Health Organization (WHO) recently made a rough estimation that thirteen million human deaths annually are being attributed by preventable environmental causes (WHO, 2019). The report also showed that 23% of total mortalities and 24% of global human disease burden are caused by environmental factors, and diseases due to environmental burden are 15 times greater in developing countries compared to developed countries. Recent studies have shown that, proper utilization of data, expertise and management approaches of various environmental professionals is necessary to have a deep understanding of the root cause of many diseases, and to have a better protection of natural resources to benefit the health (Eddy *et al.*, 2013; Musoke *et al.*, 2016). Pollution is recognized as the most serious global environmental threat causing severe health consequences in both humans and animals (Pandey *et al.*, 2019). Among different pollutants, plastic pollution is the most highly visible environmental issues in today’s world (Vince and Hardesty, 2016). It is estimated that almost 5 to 13 million tonnes of plastic has been passed into the ocean in 2010 alone (Jambeck *et al.*, 2015), contributing to 15 to 51 trillion floating plastic particles circulating in the marine environment (van Sebille *et al.*, 2015). This increasing stream of contamination and its ill effects on health has increased public awareness of plastic pollution in environment (World Economic Forum, 2016). Accordingly, various research efforts are being carried out worldwide, for exploring the influence of plastic litter on different components of ‘one health’ and on reducing the impacts of plastic pollution through effective remedial measures. The present paper is designed to provide an overview of an emerging and exciting research topic in plastic pollution namely, “Plastisphere’.

2. ‘Plastisphere’

Plastic pollutants are usually linked to the direct health hazards in larger animals following ingestion or entanglement damages (Watts *et al.*, 2015; Hermabessiere *et al.*, 2017; Jeong *et al.*, 2018). Microbial interactions with plastics are completely different and poorly studied (Zettler *et al.*, 2013; Prinz and Korez, 2020). Scientists have coined the term ‘Plastisphere’ to denote assemblage of different microbial taxa inhabiting outer surface of plastic debris

(Zettler *et al.*, 2013). Even though colonization on plastic materials have been reported in the early 1970s (Carpenter and Smith, 1972; Carpenter *et al.*, 1972), there was a long 25 year gap for getting close attention to the plastic associated microbes. It was Dang and Lovel (2000) who explored initial stages of biofilm formation on plastic plates in marine waters. Afterwards, comparison of biofilms formed on different substrates including plastics materials following short-term exposure experiments was done by Dang *et al.* (2008); Lee *et al.* (2008). These three early studies highlighted that plastic in the environment, as with any other available substrate will be colonized by diverse bacterial taxa. Followed to these findings, there has been an increasing interest in ‘Plastisphere’ evidenced by a gush of publications in this field.

3. ‘Plastisphere’ Community

The first study exploring ‘Plastisphere’ community composition using NGS approaches was done by Zettler *et al.* (2013). Later, more studies characterizing ‘Plastisphere’ community were carried out from different environments. A summary of major studies characterizing ‘Plastisphere’ community is represented in Table 1. Altogether, these studies have shown that ‘Plastisphere’ represent a new distinct niche for microbes. Plastisphere’ community is unique in its composition from the surrounding environment (Amaral-Zettler *et al.*, 2015; De Tender *et al.*, 2015; Frere *et al.,* 2018). Photoautotrophic bacteria like cyanobacteria are reported to dominate the surface ‘Plastisphere’ (Zettler *et al.*, 2013; De Tender *et al.*, 2017; Dussud *et al.*, 2018a), while the seafloor and subsurface ‘Plastisphere’ are dominated by Bacteroidetes and Proteobacteria (Zettler *et al.*, 2013; Bryant *et al.*, 2016; Dussud *et al.*, 2018a). Further, taxonomic composition of ‘Plastisphere’ vary with various factors *viz.*, season, geographical location, polymer type, surface roughness (rugosity), hydrophobic surface and size of plastic material (De Tender *et al.*, 2015; Oberbeckmann *et al.*, 2016; Hoellein *et al.*, 2017; Frere *et al.*, 2018). Studies focusing on the successive colonization stages of plastics showed that, initial colonization was mainly by γ-Proteobacteria and α-Proteobacteria (Oberbeckmann *et al.*, 2015), while members of Bacteroidetes become increasingly abundant as time progresses (Lee *et al.*, 2008). The carrying capacity of ‘Plastisphere’ community remains unknown with conflicting results from various studies (Frere *et al.*, 2018; Amaral-Zettler *et al.*, 2020). However, most of the studies indicated a lower richness and greater evenness among ‘Plastisphere’ (McCormick *et al.*, 2014; Amaral-Zettler *et al.*, 2015). In brief, there are many gaps in current knowledge of ‘Plastisphere’ holobiome which needs to be evaluated in future studies, to have a clear knowledge on the ecological impacts of ‘Plastisphere’ (Amaral-Zettler *et al.*, 2020).

4. Tools in ‘Plastisphere’ Research

Early studies of ‘Plastisphere’ relied primarily on bright field microscopy, which identified morphologically distinct organisms (Sieburth, 1975). Now, scanning electron microscopy (SEM) is used to have a useful firsthand and a detailed look at microbes on plastic surfaces (De Tender *et al.*, 2017). However, taxonomic resolution of microbial communities is very limited with this approach (De Tender *et al.*, 2017). Among microscopical methods, epifluorescence microscopy using phylogenetic probes *via* Fluorescence *In Situ* Hybridization (FISH) has the potential to 1) provide taxonomic information about community 2) to give spatial structure of community and 3) to provide actual abundance data rather than relative abundance (De Tender *et al.*, 2017). However, this method will not be accurate for the communities that are not growing actively as it relies on phylogenetic probes against ribosomal RNA. Further, there is a limitation in number of different fluorophores (up to 3) that could be used simultaneously. Therefore, various tools are being developed now to overcome these limitations in epifluorescence microscopy *viz.*, (1) enhancing the signal through nested FISH technique using multiple probes, catalyzed reporter deposition FISH (CARD-FISH) or next-generation *in situ* hybridization chain reaction (HCR) (Choi *et al.*, 2014; Harrison *et al.*, 2014; De Tender *et al.*, 2017), (2) use of different fluorophores (6 or more) through Combinatorial Labelling and Spectral Imaging FISH (CLASI-FISH) *etc* (Mark Welch *et al.*, 2016; De Tender *et al.*, 2017; Schlundt *et al.*, 2020). Conventional microbiological methods using cultivation dependent and enrichment methods has been applied in “Plastisphere’ research. However, major limitation of this method is uncultivable nature of >99% microbes in environment by standard cultivation techniques. Application of culture independent methods using advanced molecular tools, like use of clone libraries, Denaturing Gradient Gel Electrophoresis (DGGE), Terminal restriction fragment length polymorphism (T-RFLP) and high-throughput sequencing have increased our understanding of ‘Plastisphere’ (De Tender *et al.*, 2017). Among high-throughput sequencing technologies, amplicon sequencing *via* Illumina MiSeq is the most widely used method. However, shotgun metagenomics is the most comprehensive method for mining both functional and phylogenetic diversity, with additional benefit of discovering metabolic functions of microbes (Bryant *et al.*, 2016; Bouchez *et al.*, 2016). A detailed review on merits and demerits of different tools in ‘Plastisphere’ research was given by De Tender *et al.* (2017). Presently, SEM is the most commonly used technique to visualize ‘Plastisphere’ communities. However, high throughput next-generation sequencing (NGS) methods are now gaining momentum as the preferred method for profiling ‘Plastisphere’.

Table 1: Microbial communities enlisted on "Plastisphere

Sl. No	Broad category	Microbial phyla	Reference
1	Bacteria	Bacteroidetes (*Flavobacteriaceae*), Planctomycetes (*Planctomycetaceae*), α Proteobacteria (*Phyllobacteriaceae, Rhodobacteraceae*), γ Proteobacteria (*Alteromonadaceae*), β Proteobacteria	Dang *et al*., 2008; Dang and Lowel, 2016; De Tender *et al*., 2017; Pinto *et al*., 2019
		γ Proteobacteria (*Acinetobacter* sp., *Alteromonas* sp., *Vibrio* sp., *Pseudoalteromonas* sp., *Psuedomonas* sp., *Idiomarina* sp., *Oceaniserpentilla* sp., *Psychrobacter* sp.), α Proteobacteria (*Albidovulum* sp., *Devosia* sp., *Erythrobacter* sp., *Filomicrobium* sp., *Henriciella* sp., *Hyphomonas* sp., *Labrenzia* sp., *Nitrotireductor* sp., *Parvularcula* sp., *Pelagibacter* sp., *Rhodovulum* sp., *Roseovarius* sp., *Rubrimonas* sp., *Thalassobius* sp., *Thalassobius* sp.) Bacteroidetes (*Amoebophilus* sp., *Fulvivirga* sp., *Haliscomenobacter* sp., *Hellea* sp., *Lewinella* sp., *Marinoscillum* sp., *Microscilla* sp., *Muricauda* sp., *Sediminibacterium* sp., *Tenacibaculum* sp.), *δ* Proteobacteria (*Bacteriovorax* sp., *Bdellovibrio* sp.), Planctomycetes (*Blastopirellula* sp., *Phycisphaera* sp.)	Zettler *et al*., 2013
		γ Proteobacteria (*Thiotrichales* and *Alteromondales*), Bacteroidetes *(Flavobacteria*)	Oberbeckmann *et al*., 2014
		Bacteroidetes (*Lewinella* sp.), *δ* Proteobacteria (Nannocystaceae), Verrucomicrobia	Oberbeckmann *et al*., 2016
		α Proteobacteria, β Proteobacteria, γ Proteobacteria, Actinobacteria, Acidobacteria, Bacteroidetes, *δ* Proteobacteria	Debroas *et al*., 2017
		γ Proteobacteria (*Alcanivorax* sp., *Aestuariicella hydrocarbonica*, *Alteromonas* sp., and *Thalassolituus* sp., *Marinobacter* sp., Neptiniibacter sp. and *Maricurvus* sp.), α Proteobacteria (*Phycisphaerae, Planctomycetia* and Sphingobacterii*a*), Bacteroidetes (*Tenacibaculum* sp.)	Dussud *et al*., 2018a
		Bacteroidetes, γ Proteobacteria (*Vibrionaceae)*, α Proteobacteria (*Rhodobacteraceae*),	Schlundt *et al*., 2020
		Bacteroidetes (*Flavobacteriaceae, Winogradskyella* sp,.), γ Proteobacteria (*Vibrionacea, Pseudoalteromonadaceae, Oceanospirillales, Glaciecola* sp.), α Proteobacteria, ε Proteobacteria, Verrucomicrobia	Delacuvellerie *et al*., 2019
		α Proteobacteria (*Erythrobacter* sp., *Roseobacter* sp.)	Pinto *et al*., 2019
2	Blue algae/ Cyanobacteria	Cyanophyta (*Phormidium* sp., *Pleurocapsa* sp., *Prochlorococcus* sp., *Rivularia* sp., *Synechococcus* sp.)	Zettler *et al*., 2013;
		Cyanophyta (*Stanieria* sp. and *Pseudophormidium* sp.)	Oberbeckmann *et al*., 2014
		Cyanophyta (*Phormidium* sp., *Rivularia* sp., *Leptolyngbya* sp.)	Bryant *et al*., 2016; Oberbeckmann *et al*., 2016
		Cyanophyta (*Microcystis* sp.)	Muthukrishnan *et al*., 2019
3	Fungi	Ascomycota, Basidiomycota, Zygomycota	De Tender *et al*., 2017
		Chytridiomycota, Cryptomycota, Ascomycota	Kettner *et al*., 2017
		Basidiomycota (*Malassezia* sp.)	Amend *et al*., 2019
4	Protozoa	Ciliophora (*Ephelota* sp.)	Kobayashi *et al*., 2011; Zettler *et al*., 2013
		Radiolaria (Circorrhegma sp.), Ciliophora (Thiobios sp.)	Zettler *et al*., 2013; Carson *et al*., 2013
5	Diatoms/Microalgae	Ochrophyta (*Licmophora gracilis, Cylindrotheca closterium*)	Briand *et al*., 2012
		Bacillariophyta (*Sellaphora* sp., *Mastogloia* sp.) Ochrophyta (*Amphora* sp., *Nitzschia* sp.)	Muthukrishnan, 2013
		Bacillariophyta (*Mastogloia* sp., *Pleurosigma* sp.), Ochrophyta (*Cyclotella* sp.)	Zetter *et al*., 2013
		Bacillariophyta (*Bacillariophyceae)*	Oberbeckmann *et al*., 2014
		Ochrophyta (*Amphora* sp., *Nitzschia* sp. and 6 other genera), Haptophyta (7 genera), Dinoflagellata (*Ceratium* sp.)	Reisser *et al*., 2014

6	Microalgae	Dinoflagellata (*Ostreopsis* sp., *Coolia* sp., *Alexandrium* sp.)	Maso *et al*., 2003
		Dinoflagellata (*Alexandrium* sp., *Ceratium* sp.)	Zetter *et al*., 2013; Reisser *et al*., 2014
		Dinoflagellata (*Pfiesteria* sp.)	Kettner *et al*., 2017
7	Brown algae	*Phaeophyceae*	Oberbeckmann *et al*., 2014
8	Green algae	Chlorophyta (*Micromonas* sp.) Chlorophyta (*Ulva* sp.)	Zettler *et al*., 2013 Kettner *et al*., 2017

5. Impact of 'Plastisphere' in Environment and Animals

'Plastisphere' research is mainly focused to have a better understanding on the impact of plastic pollutants on different ecosystem functions. Among various areas, impact of 'Plastisphere' on biogeochemical cycling of different ecosystems is an active area of interest (Roager and Sonnenschein, 2019; Rogers *et al*., 2020). Incorporation of plastics and its degradation intermediates into carbon cycle through microbial interactions is known to affect biogeochemical carbon cycling (Rogers *et al*., 2020). Impact of 'Plastisphere' on health of aquatic and marine animals is another burning issue.

It was found that metabolic/derivative products of plastic degradation by 'Plastisphere' will decrease size of plastic litters, thus can be better transported through different components of food webs (Jorgensen, 1995; Zettler *et al*., 2013). This will increase the chance of carrying over of these polymers and their leachates (derivatives/microplastics) into various food source and a broader range of consumers (Botterell *et al*., 2019). It was reported that derivatives/microplastics ingested by aquatic animals will become further enriched with gut microbiota that have biodegradation properties for these pollutants, contributing further decreasing of size and more dissemination into different food web components (Lu *et al*., 2019).

'Plastisphere' communities can further affect the health of aquatic animals by disrupting intestinal homeostasis, proper functioning of host gut microflora, and through production of key biogeochemical compounds like methane (Rogers *et al*., 2020). Nevertheless, the current knowledge is insufficient to draw a clear picture of this kinds of impacts (Jacquin *et al*., 2019). To have a better understanding on the environmental impacts of 'Plastisphere', detailed investigations on three dimensional interactions between plastics, microorganisms, and consumers need to be targeted in future (Rogers *et al*., 2020).

6. Plastisphere': A Vector for Potential Pathogens and Diseases

It has been now increasingly recognized that plastics can serve as a vector for the transport of numerous pathogens over long distances than biodegradable materials (Quilliam *et al*., 2014). This long distance transportation can potentially introduce many invasive and pathogenic microbes into different ecosystems. This kind of a 'vector potential' was initially suspected after the observation of high abundance of *Vibrio* spp. on plastic fragments recovered from the North Atlantic and report of *Escherichia coli* on plastic in beach waters (Oberbeckmann *et al*., 2014).

Since then, presence of different potential pathogens such as members of *Vibrio* spp. *Aeromonas* spp., *Arcobacter* spp. and *Campylobacteraceae* has been reported from different environmental plastic litters worldwide, in both temperate and tropics including both marine and fresh water environments (McCormick *et al*., 2014; De Tender *et al*., 2015; Kirstein *et al*., 2016; Oberbeckmann *et al*., 2016; Harrison *et al*., 2018; Jiang *et al*., 2018; Amaral-Zettler *et al*., 2020). In addition to pathogens posing risk to humans from marine activities, plastic has been shown to transport potential protistan coral pathogens (Goldstein *et al*., 2014) and many other known fish and shellfish pathogens (Virsek *et al*., 2017). Roughly one third of the plastic-associated bacterial or archaeal sequences identified by Oberbeckmann *et al*. (2016) were assigned to the genus *Tenacibaculum* sp. (*Flavobacteriaceae*) that harbors several fish pathogens. Dussud *et al*. (2018b) found that half of the putative pathogens in plastic samples belonged to *Vibrio* spp., and 20% and 11% were identified as *Tenacibaculum* sp. and *Staphylococcus aureus* respectively.

Recent study by Sun *et al*. (2020) showed a higher relative abundance of bacteria belonging to *Vibrio* sp., *Pseudoalteromonas* sp. and *Alteromonas* sp. on microplastics than those in their surrounding seawater and sediments, indicating that microplastics might act as vectors for enrichment of potential pathogens, enhancing the ecological risk of microplastics to mariculture industry.

Phototrophic species responsible for harmful algal blooms were also reported in 'Plastisphere' (Maso *et al*., 2003; Maso *et al*., 2016; Casabianca *et al*., 2019). Further, plastic pollutants are recognized to play an important role in the emergence and dispersal of antimicrobial resistant (AMR) pathogens and AMR genes into different ecosystems (Moore *et al*., 2020). As plastics and microplastics can harbor many chemicals, organic pollutants, heavy metals and pharmaceuticals (Katole *et al*., 2013), it can act as a perfect platform for the co-selection of AMR genes and AMR phenotypes (Li *et al*., 2018). The hypothesis was then confirmed by Arias-Andres *et al*. (2018), who showed that horizontal gene transfer between phylogenetically distinct microbes in 'Plastipshere' -

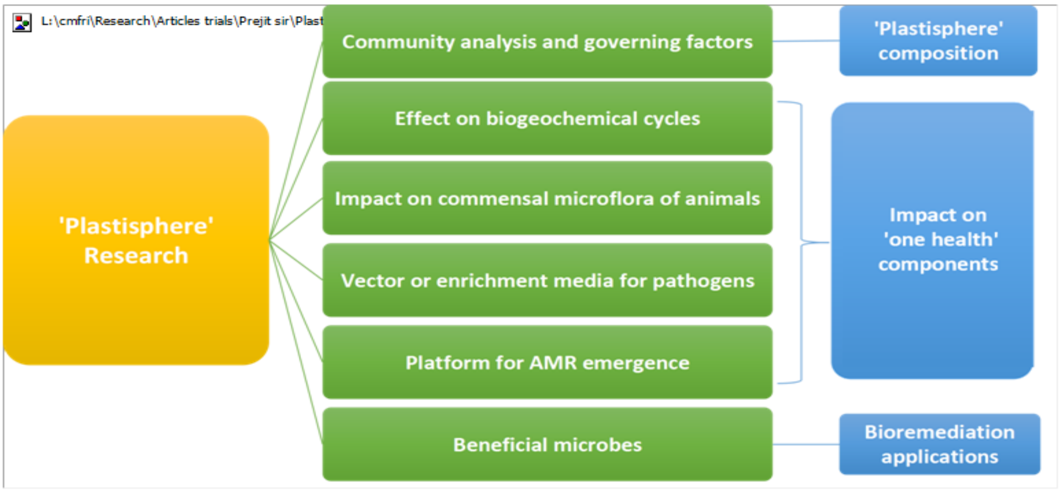

Fig 1: Schematic representation of an overview of current ‘Plastisphere’ research: Major research questions and possible answers/uses in “Plastisphere’ research projects are indicated in second column and third column respectively.

occur much faster than free living microbes. Yang *et al.* (2019) found that ‘Plastisphere’ communities harbor higher abundance of multi-drug resistance genes, aminoglycoside resistance genes and unclassified AMR genes. However, most of evidences for role of plastics in dissemination of diseases and AMR pathogens stems from molecular sequence data. Pathogenicity or virulence of these pathogens is not confirmed (Oberbeckmann *et al.*, 2016). Therefore, exact role of plastic debris as a fomite for pathogenic microbes warrants further research (Amaral-Zettler *et al.*, 2020).

7. Plastisphere’: A New Niche for Bioremediation Research

There has been increasing interest in exploration of microbes and microbial enzymes for plastic and other hydrocarbon degradation purpose, to provide solutions to the increasing pollution problem. In this aspect, much attention has recently turned to the members of ‘Plastisphere’. Exposure experiments for checking the microbial colonization of plastic showed a decrease in plastic buoyancy with concurrent increase in bacterial counts (Webb *et al.*, 2009; Lobelle and Cunliffe, 2011; Cozar *et al.*, 2014). These findings arose interest in researchers to know whether ‘Plastisphere’ may degrade either the plastics and/or plastic-adsorbed co-pollutants in natural environments. The first study exploring ‘Plastisphere’ microbes by Zettler *et al.* (2013) showed presence of ‘pits and grooves’ in plastic, using electron microscopy suggestive of microbial degradation of plastics. Two other studies characterizing microbial community on plastic surfaces (Carson *et al.*, 2013; Reisser *et al.*, 2014) also identified the presence of ‘pits’ and ‘grooves’ in plastic surfaces. Oberbeckmann *et al.* (2016); Kirstein *et al.* (2018) hypothesized that uncovering the rare species on ‘Plastisphere’ will be the first necessary step to identify efficient microbes having potential for plastic degradation. Recently, Syranidou *et al.* (2017) developed microbial consortia capable of degrading weathered polystyrene and polyethylene fragments from ‘Plastisphere’. Similarly, Bryant *et al.* (2016) reported for the first time that many candidate genes involving in hydrocarbon degradation were abundant in ‘Plastisphere’, suggesting the possibility of these microbes to serve as the source of potential hydrocarbon degrading microbes/functional genes. Further, the possibility of finding such potential microbes is increased in ‘Plastisphere’ near oil reserves/mangroves (Lustosa *et al.*, 2018; Streit, 2018; Urbanek *et al.*, 2018). Delacuvellerie *et al.* (2019) showed that ‘Plastisphere’ in marine ecosystem hosts potential specific microbial degraders of low-density polyethylene (*Alcanivorax borkumensis*). Dussud *et al.* (2018b) identified that 34.4% of the total microbial sequences on ‘Plastisphere’ belonged to putative hydrocarbonoclastic bacteria (HCB), compared to 4.1% in control seawater. In short, many researchers have now pointed out the potential of ‘Plastisphere’ in hydrocarbon biodegradation (Amaral-Zettler *et al.*, 2015; De Tender *et al.*, 2017; Dussud *et al.*, 2018b; Oberbeckmann *et al.*, 2018; Delacuvellerie *et al.*, 2019; Kirstein *et al.*, 2019), and further findings in this arena is awaited.

8. Conclusion

‘Plastisphere’ is the term used to denote various microbial taxa inhabiting outer surface of plastic debris. An overview of current ‘Plastisphere’ research is represented schematically in Fig 1. Research have shown that ‘Plastisphere’ represents a new distinct niche for microbes, with unique microbial communities from that of its surrounding environment. Further, ‘Plastisphere’ composition is driven by different spatial factors, seasonal effects and substrate type. Presently, SEM is the most commonly used technique to visualize

'Plastisphere' communities. However, high throughput next-generation sequencing methods are now becoming more preferred method for profiling 'Plastisphere'. In parallel to the research exploring taxonomic composition of 'Plastisphere', impacts of 'Plastisphere' on different components of 'one health' i.e. human, animals and environment remains as an active area of research. A different line of research exploring 'Plastisphere' for plastic degrading microbes and genes are also getting attention. Still, gaps existing in the current knowledge of 'Plastisphere' and its critical role in different ecosystem components make it a burning research topic and warrants future attention.

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