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Analytical techniques and integrated microbial remediation of microplastic from aquatic system

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Abstract



Larger plastic pieces are disposed of in the environment, where they become weathered and degraded, resulting in the formation of microplastics (MPs), which are ubiquitous and have been increasing globally. MPs less than 5 mm in size are found in various sources, including oceans, sediments, surface water, groundwater, wastewater, tap water, bottled water, air, food products, aquatic organisms, and humans. They mostly originate from terrestrial sources, with rivers serving as essential transfer routes. MPs have a high ability to be absorbed into biological cells, where they are transported along the food chain, with humans as the final consumers of these products. This paper provides a comprehensive overview of the range of microplastic contamination in Aquatic system, focusing on the sources, types, distribution, and analytical methods used for their detection and quantification. This paper also explores the potential of plastic-degrading microorganisms for bioremediation by highlighting recent advances in the identification and characterization of plastic-degrading microorganisms, including bacteria and fungi, isolated from contaminated Aquatic system, and discusses the environmental factors that influence the efficiency of microbial degradation. It also discusses future research directions and the necessity of improving the comparability and efficacy of the fight against microplastic contamination. There is an urgent need for improved waste management practices, and microbial bioremediation is considered a sustainable approach for reducing microplastic contamination in Aquatic system.

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INTRODUCTION

Plastics, derived from petrochemicals, are used in various applications. The term "plastic" originates from the Greek word "Plasticos," which means something that can be molded into different shapes. Plastics are a significant innovation of the twenty-first century, serving various sectors such as agriculture, packaging, automotive, construction, and healthcare. They have become ubiquitous in daily life due to their light weight, strength, and low cost. Plastics are synthetic or semisynthetic materials primarily composed of polymers. Globally, the annual consumption of plastics in various industries exceeds 430 million tons. However, only 9% of plastic is recycled, while the

rest accumulates in landfills, rivers, and oceans. This large volume of waste, especially when not properly collected or managed, harms the environment and contributes to the growing problem of microplastic pollution.

Microplastics (MPs), with a diameter of less than 5 mm, originate from both primary and secondary sources. Primary microplastics are products less than 5 mm in size, used in various applications, including cosmetics, clothing, textiles, and fishing gear. Secondary microplastics result from the fragmentation of larger plastic items due to physical, chemical, and biological degradation under environmental conditions. These degraded plastic particles release significant amounts of microplastics into the environment. Microplastic particles can take various forms, including fragments, pellets, beads, and fibers [1].

Physical degradation involves wave action, sediment-induced erosion, and digestive segregation. Chemical degradation encompasses photodegradation, thermal/thermo-oxidative degradation, hydrolysis, corrosive chemical or solvent-mediated deterioration, and biodegradation. Microplastics accumulate in continental environments, particularly in regions with significant human impact, such as agricultural and urban areas. A major source of microplastic pollutants in aquatic systems is single-use plastics (SUP) from households and marketplaces [2]. The widespread use of SUP, such as bags, bottles, straws, packaging, and cups, which are used once and discarded, has caused significant environmental problems. Every year, the world produces over 300 million tons of plastic, nearly half of which is used in single-use products. In India, approximately 15,000 tons of plastic waste are generated daily, with 43% originating from single-use packaging [3].

Untreated sewage is often directly released into watercourses, and runoff from agricultural land or roads containing plastics is another significant source of microplastics in aquatic systems. Many microplastics remain undegraded through sewage treatment processes and are released into the environment through effluents or sludge applied to land. Recently, medical waste, such as protective masks and surgical gloves, has become a major contributor to microplastic pollution, particularly during the COVID-19 pandemic.

Microplastic pollution progressively increases along the course of a river, correlating with increasing human activity in both water and sediment environments [4]. In contrast, larger and denser microplastic particles tend to sink more easily and accumulate in sediment. Irregularly shaped microplastics are more likely to remain suspended underwater, while smooth spherical particles have a greater tendency to float on the surface. As rivers transport large quantities of plastic particles over long distances, microplastics often settle with sinking sediments, particularly in slow-moving riverbeds. MPs trapped in river sediments are remobilized during floods and transported downstream, eventually flowing into the oceans.

Assessments of microplastic contamination in aquatic systems have consistently revealed high concentrations of microplastics. MPs have been reported in oceans, sediments, surface water, groundwater, wastewater, tap water, bottled water, air, food products, aquatic organisms, and humans. Largely originating from human activities on land, MPs have become increasingly common in aquatic environments, posing ecological and health risks. Every component of the environment soil, water, and air is now affected by the adverse effects of MP pollution. Alarming, humans are directly or indirectly affected by MPs. MPs exhibit multiple properties that can cause several issues in living organisms, raising ecological and health concerns. For example, MPs are highly stable molecules, allowing them to persist for long periods in nature once produced. They have a high potential to fragment, increasing their surface area and the likelihood of adsorbing harmful chemicals, such as poly organic polymers (POPs). These factors make microplastics a serious health risk to humans through inhalation, ingestion, or skin contact, leading to cell injury, hormonal disruption, and cardiovascular disease. Additionally, MPs significantly affect soil and plant growth, reducing yields and negatively impacting plant performance. Ultimately, MPs damage environmental matrices, harming the environment, economy, and human health.

Concerns over microplastic pollution, the assessment of microplastics in aquatic systems to identify the extent and sources of contamination, and the screening of plastic-degrading microorganisms have emerged as promising and

eco-friendly approaches for mitigating plastic waste pollution. Microorganisms offer potential solutions for biodegradation, aiding in the removal of microplastics from water and reducing their environmental impact. A wide range of microorganisms have been identified as capable of degrading plastics via various enzymes, including oxidases, amino acids, laccases, hydrolases, cutinases, and peroxidases. Degrading enzymes isolated from microorganisms can break down polymers into monomers, which microorganisms can utilize as a carbon source for growth [5][6].

This paper aims to provide an integrated overview of microplastic contamination in aquatic systems, focusing on their sources, types, distribution, and the analytical methods used for detection and quantification. Additionally, we highlight the potential of plastic-degrading microorganisms for bioremediation, showcasing recent advances in identifying and characterizing these microorganisms isolated from contaminated aquatic systems and the environmental factors that influence microbial degradation **Figure 1**.

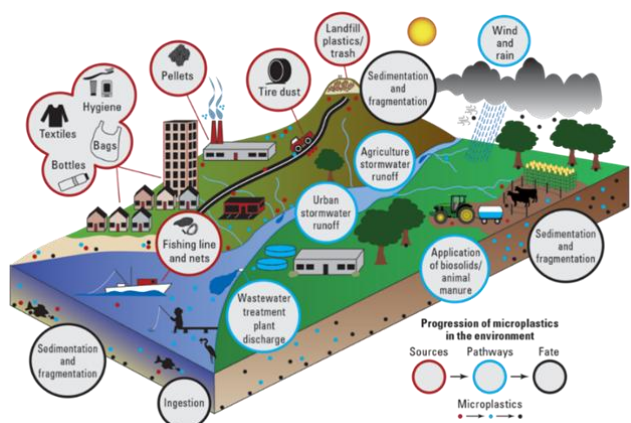


Figure 1 Microplastic Source, Pathways and Fate

(Credited to U.S. Geological Survey/photo by Jeffrey L. Corbett)

Methods for Assessment of Microplastic Contamination

The assessment of microplastics involves the identification, evaluation, and analysis of microplastic particles in diverse environmental samples. Therefore, it is crucial to study these small particles in various environmental samples. The methodology for assessing microplastics involves a multi-step approach to identify their type, size, shape, and potential sources [7][8]. The first and

most important stage is sampling, which entails gathering environmental samples from the intended locations. The selection of sampling location, depth, and frequency depended on the objective of the study and the specific environmental compartment being investigated (e.g., water and sediments). It is essential to acquire representative data that accurately depict the existence and quantity of microplastics in the environment. According to the Central Pollution Control Board [9] and the collected literature, the assessment of microplastics generally follows three main steps: a) sampling, b) sample extraction and isolation, and c) identification, characterization, and quantification of microplastics. These methodological approaches collectively contribute to understanding the distribution, types, and potential sources of microplastic contamination, thereby facilitating the development of targeted mitigation strategies for microplastic pollution.

Sampling

The selection of a sampling method for collecting microplastic samples depends on the environment to be sampled and the size limitations of the targeted microplastics. Three sampling methodologies were employed: selected sampling, bulk sampling, and volume-reduction sampling. Selective sampling is used when plastic items are large enough for identification by the naked eye, but it has a high size limitation for detectable microplastics, making less obvious items easy to overlook. Bulk sampling collects the entire sample without reducing its volume; however, it only allows a small amount of sample, which may affect its representativeness. Volume-reduced sampling reduces the total volume of the bulk sample through rapid filtration, retaining only a minor portion for further analysis. This method is advantageous for covering large quantities or areas of samples but can result in a substantial loss of microplastics, particularly those smaller than the mesh size of the sampling tools. The selective method is typically used for beach sampling. Bulk sampling is used for sediment and water samples, and volume-reduced sampling is the most popular approach for water samples. The selection of sampling points plays an important role in the sampling process. Site selection was based on the study's objectives. The samples should be representative of the entire system, and a well-

mixed zone is essential. If possible, obtain a composite sample from the surface to the bottom at the center of the lake or river, or from side to side at middepth. If only one sample can be collected, it is collected in the middle of the water body at mid-depth, avoiding areas of turbulence and weirs. Generally, samples are collected beneath the surface with the mouth directed towards the current [9].

To detect and determine microplastics in Aquatic system, suitable sampling points must be identified. Sampling points, whether random or systematic, are often chosen to ensure the representativeness of an entire waterbody. Surface water collection is essential for collecting floating microplastics and obtaining the requisite number of samples. Periodic sampling at the same locations can provide insights into the temporal trends and seasonal variations of microplastic contamination [10]. Large volumes of water were collected using nets, sieves, and pumps. Towing neuston nets or manta nets (333 mm) allows for sampling near-surface or surface water. Bongo nets are paired nets used to gather replicate samples from the water column [11]. Manta nets allow the sampling of large volumes of water and are widely used for standardization. Plankton nets have smaller mesh sizes, allowing sampling for less than a minute and recovering concentrations 30 times higher than those of the manta nets. The mesh size had a substantial influence on the reported concentration. An 80 mm mesh filter can filter 250 times more fibres than a 330 mm net.

Sediment sampling for microplastics requires careful planning and execution to obtain accurate and representative results. First, it is necessary to evaluate the sampling design, location, number of sampling points, and sampling frequency. Therefore, random and systematic sampling methods are often used to ensure representativeness. The depth of sediment samples is generally dictated by the individual research purpose and the vertical distribution of microplastics, which can range from a few millimeters to several centimeters. At each sampling location, sediment samples were obtained using a core or grab sampler to prevent disruption of the surface layer or loss of suspended microplastics. Sediment analysis revealed the concentration and type of microplastics accumulated over time, providing a historical

record of the pollution. Using sampling methods and tools, samples of the environment, water, and sediment will be gathered to learn more about the existence of microscopic plastic particles.

Sample extraction and isolation

Sample collection for the Microplastics were separated from both water and sediment samples. To ensure the accuracy and reliability of microplastic analysis, various pretreatment techniques have been applied to remove debris from water and sediment samples. After pretreatment, the samples were subjected to several separation techniques. Water samples were filtered using a mesh sieve to retain suspended microplastics and eliminate larger debris and organisms. Density separation was conducted to extract MPs from both water and sediment samples using a saturated NaCl solution to float the microplastics owing to their low density [8][12]. The floating materials were separated and collected using custom filters. For the sediment samples, an additional wet peroxide oxidation (WPO) step was used to digest labile organic matter. The WPO-treated mixture was then subjected to density separation, and the floating plastic debris was air-dried and weighed to determine its microplastic concentration. Finally, the isolated particles are characterized using microscopy, spectroscopy, or chemical analysis to identify their types, sizes, and concentrations [13].

Identification, quantification and Characterization

MPs have been separated from samples, the next critical step is identification, quantification, and characterization. This includes determining the types of microplastics present, quantifying them, and examining their physical and chemical properties. Optical microscopy is commonly used for visual identification, allowing researchers to assess the shape, color, and size of particles. Following chemical identification, the particle size was analyzed using image analysis software to determine the size and size distribution from microscopic observations [14]. However, this technique has limitations, particularly in the detection of microplastics smaller than the resolution of the microscope. In such cases, advanced analytical methods such as Fourier-transform infrared (FTIR) spectroscopy, and Raman spectroscopy are employed to determine

the polymeric composition of the particles [15] **Table 1.** The results of these analyses were used to quantify the concentration and abundance of microplastics in a given ecosystem. Depending on the sample size and microplastic count, the particles can be counted manually or using automated image analysis software. Additionally, weighing microplastics after isolation aids in determining their mass concentrations in the sample. Among the available techniques, FTIR and Raman microspectroscopy are the most widely applied methods for analyzing microplastics in environmental samples. Understanding the characteristics of microplastics is essential for developing strategies to mitigate their environmental impacts.

Microplastic Contamination in Indian Aquatic Systems

Studies on microplastic pollution across various aquatic environments in India have highlighted the concentration ranges, polymer types, and analytical methods used to investigate this issue. These studies emphasize the growing concern over microplastic contamination in river and lake ecosystems, utilizing advanced techniques such as Raman spectroscopy, FTIR, and microscopic analysis to identify and quantify microplastic particles. The data reveal the widespread presence of common polymers such as polyethylene (PE), polypropylene (PP), and polystyrene (PS) in both water and sediment samples, highlighting the

Table 1 presents a comparative analysis of the primary assessment techniques used for detecting (MPs). It presents a list of commonly used techniques that are in practice for MPs detection and compares them based on their advantages and limitations

Analytical technique	Advantages	Limitations	References
Optical Microscope	Facilitates the classification of MPs according to their shape, size, color, and origin. Simple to perform. Low-cost equipment, Fast initial screening.	There is a high error rate, especially for small or transparent MPs. The chemical composition cannot be identified. Non-plastics can be counted as plastics based on their size range.	[14] [15]
FTIR	Non-destructive, preserving samples for further testing or archiving. The most promising method for characterizing MP polymer types. Better lateral resolution with a larger spectral coverage. Can detect MPs of size even 1 μm . This method is non-contact (unlike Microscope).	MPs below a size range of 10 μm are difficult to detect. Sample preparation is required before performing the experiment (as FTIR is strongly active for water molecules). FTIR spectra obtained from different modes for MPs not identical. MPs with irregular shapes provide non-interpretable FTIR data (refractive error).	
Raman spectroscopy	Along with MPs detection, it helps in determining size distribution, particle number, and other morphological features. Provides a highly specific fingerprint spectrum (lesser interference from water).	Raman spectra of weathered MPs prone to change (due to lack of a specific database for them). Raman microscopy for MPs (< 20 μm) produces weak signals. To obtain a proper signal to noise ratio, longer acquisition time required, for weak intensity Raman scattering.	

Table 2 offers a Microplastic studies in India in terms of concentration, types, and assessment methods

S.NO	Location	Sample type	Size	Polymer type	Detection method	Refer ences
1	Karamana River, Killiyar, and Akkulam-Veli Lake (Kerala)	Sediment	300 mm	LDPE	Binocular Microscope and LabRAM HR Evolution Raman Microscope	[3]
2	Mahanadi River estuary (Odisha)	Water Sediment	<1mm	Polyesters, PE, PVC, PP, PA, PS and PC	ATR FTIR	[4]
3	Chennai's Red Hills Lake	Water Sediment	0.3 mm, 1 mm, and 2 mm	HDPE, LDPE, PP, PS	FTIR and X-ray EDX	[16]
4	Ganga river	Sediment	<5mm	PET, PE, PP, PS	Microscope and ATR FTIR	[17]
5	Puducherry	Sediment	300mm to 5mm	PP, HDPE, LDPE, PS, PU	Microscope and Raman Spectroscopy	[18]

urgent need for standardized monitoring and mitigation efforts **Table 2**.

Microplastic-Degrading Microorganisms

Recent studies have explored the use of microplastic-degrading microbes as a sustainable remediation technique, alongside efforts to determine the frequency and properties of microplastics in aquatic systems. Various bacterial and fungal strains have demonstrated the ability to colonize and biodegrade synthetic polymers under both laboratory and environmental conditions. Understanding the diversity, enzymatic pathways, and ecological roles of these microorganisms is essential for developing effective bioremediation strategies to mitigate microplastic pollution.

In the presence of pollutants, the adaptive properties of microorganisms significantly enhance their metabolism, sometimes promoting degradation. During polymer degradation, microorganisms adhere to the polymer surface, and extracellular enzymes bind to the polymer, causing hydrolytic cleavage. Enzymes, which are biological catalysts, participate in reactions by acting on specific substrates and accelerating their conversion into valuable products. Intracellular enzymes, present within the microorganism's cells, break down microplastic particles once they pass through the cellular membrane. The use of microorganisms for biodegradation enhances

microplastic breakdown without causing harm to the environment. Consequently, identifying microorganisms capable of degrading microplastics presents a viable, ecologically sound technique for enhancing natural bioremediation and positively impacting ecosystem restoration without detrimental effects.

Aquatic systems have traditionally been favored as dumping sites for solid waste disposal. This review aims to evaluate the presence of microplastics in water bodies and offer a remediation solution for environments contaminated with microplastics using microbial isolates.

Researchers have investigated and isolated microorganisms capable of degrading plastics in natural environments. Microorganisms such as algae, fungi, bacteria, and actinomycetes have been shown to degrade plastics due to their unique enzymes. Polymer-degrading microbial species associated with degradation include *Streptococcus*, *Klebsiella*, *Micrococcus*, *Staphylococcus*, and *Pseudomonas*. Microorganisms involved in plastic biodegradation are discussed below:

Algae

Research has shown that microalgae, such as blue-green algae and freshwater non-toxic cyanobacteria, can effectively degrade polymers in sewage. These algae, which are less hazardous and non-toxic, can colonize polyethylene surfaces,

thereby reducing the energy required for its degradation. The most effective species are *Phormidium lucidum*, *Oscillatoria subbrevis*, *Chlorella vulgaris*, and *Chlamydomonas reinhardtii*, which exhibit the highest biodegradation percentages [19].

Fungi

Research has found that fungi play a pivotal role in plastic biodegradation within a few days by secreting degrading enzymes. The species that showed the highest biodegradation percentages include *Aspergillus nidulans*, *Aspergillus glaucus*, *Aspergillus flavus*, *Aspergillus oryzae*, *Aspergillus nomius*, *Penicillium griseofulvum*, *Bjerkandera adusta*, *Phanerochaete chrysosporium*, *Cladosporium cladosporioides*, and other saprotrophic fungi such as *Pleurotus abalones*, *Pleurotus ostreatus*, *Agaricus bisporus*, and *Pleurotus eryngii* [20][21].

Bacteria

Research has found that bacteria involved in plastic degradation include *Azotobacter sp.*, *Bacillus megaterium*, *Ralstonia eutpha*, *Pseudomonas sp.*, *Halomonas sp.*, *B. brevis*, *A. delafieldii*, *P. amylocticus*, *B. pumilus*, *B. petrii*, *P. aeruginosa*, and *Shewanella sp.* Studies on polyethylene degradation have shown the potential of *Arthrobacter sp.*, *Acinetobacter baumannii*, *A. viscosus*, *Bacillus amyloliquefaciens*, *Pseudomonas sp.*, *Bacillus thuringiensis*, *Cereus*, *Pumilus*, *Mycoides*, *Staphylococcus cohnii*, *Pseudomonas fluorescens*, *Xylosus sp.*, *Micrococcus luteus*, *M. lylae*, *Rahnella aquatilis*, *Flavobacterium sp.*, *Paenibacillus macerans*, *Ralstonia sp.*, *Delftia acidovorans*, *R. erythropolis*, *P. aeruginosa*, and *B. brevis* [21].

Actinomycetes

Actinomycetes, a group of filamentous bacteria found in soil and marine environments, are known for their metabolic versatility and biotechnological applications, including bioremediation. These include *Streptomyces*, *Rhodococcus ruber*, *Actinomadura spp.*, and *Thermoactinomyces* species, all of which have shown significant plastic biodegradation potential [22].

Screening and Isolation of Microorganisms

Microbe isolation involves collecting environmental samples, such as water or sediment,

from locations with high plastic contamination. The collected samples are placed in sterile plastic bags and transported to the laboratory for further analysis. These samples are incubated in a specialized culture medium, where plastic serves as the sole carbon source, promoting the growth of microorganisms that can use plastic as an energy source. Plastic-degrading bacteria are identified from samples obtained from diverse sources using a selective enrichment technique on low-salt agar. To formulate the minimal salt medium (MSM), 2.27 g KH₂PO₄, 5.97 g Na₂HPO₄, 0.5 g NH₄Cl, 0.25 g MgSO₄, 0.0025 g CaCl₂, 0.001 g FeSO₄, 0.0005 g MnSO₄, and 0.001 g ZnSO₄ are dissolved in 500 ml of distilled water in an autoclaved flask. Microorganisms utilize the MSM, with plastic as their sole carbon source. One milliliter of each sample is spread on Petri plates and incubated at 37 °C for optimal growth. Following growth, 1 ml of 0.9% saline solution is added to each Petri plate and homogenized with a spreader to collect the culture. Subsequently, 1 ml of the culture is extracted with a micropipette and transferred into a flask containing 50 ml of minimal salt solution. After incubation, cultures on the surface of each plate are observed and streaked onto the same medium to isolate pure culture colonies for further analysis. Further identification of the isolated organisms is carried out through various biochemical tests, following the guidelines outlined by the Clinical and Laboratory Standards Institute. Promising microbes that exhibit degradation activity are identified and further analyzed using molecular techniques, such as 16S rRNA sequencing, to determine their species and potentially uncover novel strains with enhanced degradation properties. This systematic approach allows researchers to discover, characterize, and harness microbes with the potential to biologically break down plastics [23][24].

Factors Affecting Microbial Degradation

The major factors affecting microbial biodegradation depend on the polymer characteristics and environmental conditions. Morphology, functional groups, flexibility, additives, molecular weight, crosslinking, copolymers, crystalline structure, and blends are all characteristics of polymers [25]. Several environmental factors significantly affect the microbial degradation of plastics, including temperature, pH, humidity, oxygen levels, and UV

radiation, which not only influence polymer degradation but also have a crucial impact on microbial populations and enzyme activity. Higher temperatures can enhance enzymatic activity; however, extreme temperatures can inhibit microbial activity. Most microorganisms thrive within a specific pH range, and deviations from this range can impact their activity and plastic degradation rates. Plastic degradation can be significantly reduced in environments with low humidity and water availability.

Challenges and Future Perspectives

A comparison of the main evaluation techniques revealed that no single method currently offers a completely thorough and reliable way to identify microplastics in aquatic environments. An inadequate understanding of the temporal and geographical distribution of microplastics is also due to inconsistent and complex sampling techniques. Methodological inconsistencies and inadequate reporting of procedural details undermine the representativeness and reproducibility of the results of several studies. Future research should prioritize the development of efficient alternatives that ensure reliable detection not only in aquatic systems but also in more complex environmental matrices, such as soil and food.

Adopting standardized and innovative techniques for sampling, separation, and characterization is the way forward in quantifying microplastics. Although previous studies on MPs have been conducted worldwide, further research is needed to understand the specific mechanisms by which microorganisms degrade microplastics, including the enzymes involved and the degradation products. This knowledge can be used to design effective biodegradation strategies for such pollutants. The removal of MPs from the environment has become a research hotspot, contributing to a better understanding of the problem and supporting the development of sustainable solutions for mitigating plastic pollution.

Conclusion

Microplastic pollution is becoming an increasingly significant global issue. The rapid expansion of urban areas, agricultural runoff, and improper disposal of plastic waste, including the widespread use of disposable medical items, have exacerbated

these problems. MPs originate from various primary and secondary sources that enter aquatic systems. A major source of these microplastic fragments in aquatic systems comes from land, with rivers acting as critical transfer media for these particles. Mismanagement or improper disposal of plastics, along with prolonged UV radiation, can break down plastics into tiny particles, making their entry into aquatic systems more challenging to prevent. MPs, largely originating from human activities on land, have become more common in aquatic environments, posing both ecological and health risks. The persistent nature of plastic materials and their accumulation in living organisms raises serious ecological and health concerns regarding the presence of microplastics in aquatic systems. In this context, a thorough assessment of microplastic contamination in aquatic systems is essential for understanding the scope of the pollution. One sustainable approach to addressing this issue is to investigate microbes capable of breaking down plastics. Together, these efforts contribute to a better understanding of the problem and support the development of sustainable solutions to mitigate plastic pollution. This study provides detailed information on the collection, preparation, and analysis of solid and liquid microplastic samples. Furthermore, the individual tools available for the characterization and quantification of microplastic concentration and type in environmental samples are presented, with recommendations for future studies.

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Ethical Approval

No ethical approval was necessary for this study.

Author Contribution

All authors made substantial contributions to the conception, design, acquisition, analysis, or interpretation of data for the work. They were involved in drafting the manuscript or revising it critically for important intellectual content. All authors gave final approval of the version to be published and agreed to be accountable for all aspects of the work, ensuring its accuracy and integrity.

Conflict of Interest

The authors declare no conflict of interest, financial or otherwise.

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