



## Microplastics in Water: Occurrence, Human Health Impact, and Methods of Analysis

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### ABSTRACT

Microplastics are widely present in the environment, with some being deliberately produced while others are the result of plastic disintegration, degradation, or abrasion. The origins of their generation might be either terrestrial or aquatic, but they are present across the whole planet. Their existence leads to many ecological consequences, including risks to human health and economic losses. Recent studies focus on probable sources, sampling and analysis methods, and potential hazards to the environment and ecology. However, the absence of a standardized procedure for sampling and analysis makes it difficult to compare the existing results. The objective of this study is to investigate the complex processes involved in the formation of microplastics, occurrences in different environmental compartments, detrimental effects of microplastics on human health, and the methods used in the collection, laboratory analysis, identification, and characterization of samples.

**Keywords:** Microplastics, Degradation, Characteristics, Human health, Aquatic environment.

### INTRODUCTION

Plastics possess a diverse range of attributes, including their lightweight, chemical stability, effective insulating properties, low thermal conductivity, durability, notable impact resistance, resistance to rust, wear resistance, cost-effectiveness, and thus affordability in all countries<sup>1-3</sup>. These traits collectively contribute to the extensive utilization of plastics in several applications in the contemporary world. The phenomenon under consideration exerts a significant influence on the processes of modernization, industrialization, and economic development on a global scale. Hence, the development of plastic is widely regarded as a significant advancement of the 20<sup>th</sup> century<sup>4</sup>.

Thus, its production has increased from 1.7 million tonnes in 1950 to 390.7 million tonnes in 2021, globally<sup>5-6</sup>. However, its negative implications arise from the increasing production of plastic products, the introduction of new products, and extensive misuse coupled with inadequate waste management. Its notably slow-degrading characteristics endure its presence in the environment over extended periods ranging from 20 to 500 years, which leads to the accumulation of plastic waste of varying sizes in the various environmental compartments for a long duration<sup>7</sup>. The annual leakage of plastic waste of around 5 to 13 million metric tonnes into the oceans has been estimated<sup>8-9</sup>. Also, it is projected that a cumulative quantity of 250 million metric tonnes of plastic will be released into the environment by the year 2025<sup>8</sup>.



Plastic waste, when exposed to the environment, goes through several processes that cause breakdown and fragmentation of the polymer, resulting in the formation of debris. This debris accumulates in various parts of the environment and constitutes a threat to the ecology, environment, and human health.

Recent years have seen notable progress in microplastic research, focusing on terminology, categorization, methodology, distribution, and the adverse effects on marine life<sup>10</sup>. Microplastics can be defined as “Microplastics are any synthetic solid particle or polymeric matrix, with regular or irregular shape and with size ranging from 1 μm to 5 mm, of either primary or secondary manufacturing origin, that are insoluble in water”<sup>11</sup>.

The present study investigates the processes involved in the conversion of microplastics and factors affecting the regional and temporal distribution of microplastics in various environments, namely water, wastewater, sediment of water sources, etc., and their detrimental impact on humans as well as the ecological system. This paper will also review the methodology employed for sample collection, preparation, and laboratory analysis for the identification, characterization, and quantification of microplastics in various environments.

**Classification of Microplastics (MPs)**

The primary microplastics are characterized as particles of small size that are intentionally engineered to have microscopic dimensions that serve domestic, industrial, and biotechnological purposes<sup>12</sup>. The primary sources of microplastics encompass microbeads derived from personal hygiene, healthcare, and cosmetic products, such as shampoos, shower gels, lipsticks, sunscreens, masks, eye shadows, facial and body scrubs, toothpaste, medicine, etc.<sup>7,13</sup>. There is a chance to release primary microplastics during their production, transportation, and utilization for the manufacturing of various products and domestic purposes.

Secondary microplastics are formed when plastics or plastic waste are exposed to the environment for an extended period. This exposure leads to a decrease in the structural integrity of the plastics due to various physical, chemical, biological processes, and mechanical processes (Different processes involved in degradation are listed in Table 1). As a result, the plastics break down into smaller fragments<sup>2,14-15</sup>. Fig. 1 represents various sizes and common names of the fragmented debris of plastics.

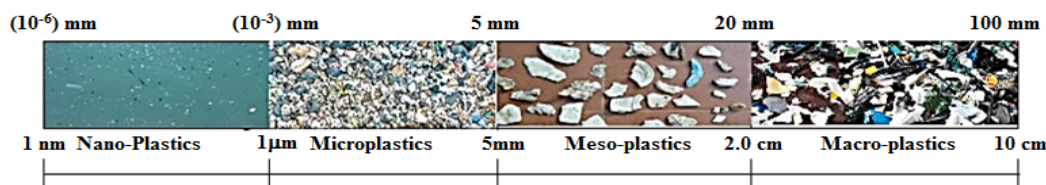


Fig. 1. Size range of plastic particle and their corresponding nomenclature

It is worth noting that previous research conducted by Eriksen *et al.*, (2013) has revealed that a significant portion of microplastics are secondary microplastics<sup>16</sup>. Municipal solid waste could act as a prospective reservoir of microplastics. Additionally, it has been observed

that the presence of these microplastics in water bodies tends to increase in proportion to the influx of plastic debris from various sources. The persistent accumulation of plastic waste results in the constant conversion of secondary microplastics<sup>13</sup>.

**Table 1: Degradation processes involved in the conversion of plastics to microplastics**

Degradation Process	Agent	Explanation	Reference
Biodegradation	<i>Bacillus cereus</i> , <i>Micrococcus</i> sp., or <i>Corynebacterium</i>	The process of organic material decomposition facilitated by microbes	[17], [18]
Photodegradation	UV light	The action of light or photons, usually sunlight in outdoor exposure (UV-A or greater, > 320 nm)	
Thermo-oxidative degradation	Temperature	The phenomenon of slow oxidative molecular degradation occurring at moderate temperatures	
Thermal degradation	Heat	High temperatures induce molecular degradation (not an environmental mechanism)	
Hydrolysis	Water	Reaction with water	
Mechanical Degradation	Wave action, Winds, surface currents, friction	Physical stress that may cause wear and tear	[19]

### Occurrence of Microplastics

Microplastics were initially documented in the early 1970s, since then significant advancements have been made in the field of microplastics research, particularly in the areas of sampling, quantification, and identification<sup>20</sup>. Microplastics are reported in various environmental components worldwide, encompassing the atmosphere<sup>21</sup>, oceans<sup>22</sup>, inland surface water<sup>23</sup>, Groundwater<sup>24</sup>, sediments<sup>25</sup>, soils<sup>26</sup>, and even in the remote location such as arctic ice<sup>27</sup>, alpine glacier<sup>28</sup>, arctic deep-sea<sup>10</sup>, polar ice regions<sup>29</sup>, Antarctic glacier<sup>30</sup> etc. As per concern for the surface water system, microplastics are reported in its water, sediment, fish, and other aquatic organism. Wastewater treatment facilities (WWTPs) are widely acknowledged for their substantial role in introducing microplastics (MPs) into the environment<sup>31</sup>. Additionally, the utilization of biosolids or sludge in agricultural practices serves as a substantial avenue for the dissemination of MPs into the surrounding ecosystems<sup>32</sup>.

### Detrimental Impact of Microplastics on Human health

The precise mechanisms by which microplastics and their related compounds are transmitted to humans, including potential routes such as ingestion, inhalation, dermal contact, or other unidentified techniques, and their exact health effects, currently lack comprehensive understanding. However, research has indicated that animals that ingest microplastics may encounter immunotoxicity and encounter numerous disruptive impacts on their digestive systems. Such effects encompass alterations in oxidative and inflammatory processes, dysbiosis, and perturbations in the epithelial permeability of the gut. Studies have also examined the mechanisms underlying the translocation and subsequent fate of microplastics in rats subsequent to inhalation or ingestion<sup>33</sup>.

The likely sources of microplastics (MPs) that might enter the human body include drinking water (from tap or bottled sources), diet (such as seafood and consumable items), air, medication, and personal care products. Packages and different types of plastic containers, such as cans, cups, wraps, water bottles, newborn feeders, and paper cups, are used to store water, food, and beverages to keep them fresh for a long time or to serve them for consumption. These consumable items are

supplemented with plastic flakes that are very small in size, either in the micron or nano range<sup>34</sup>. Although the ingestion of microplastics does not pose a direct risk to humans, but this could potentially lead to their entry into the circulatory system. There is speculation over the absorption of microplastics larger than 150  $\mu\text{m}$ , suggesting that they are unlikely to be absorbed. However, microplastics with a size less than 150  $\mu\text{m}$  have potential to migrate from the digestive system into the lymphatic and circulatory systems, resulting in systemic exposure. Nevertheless, it is anticipated that the assimilation of these microplastics will be restricted to a maximum of 0.3%. Microplastics of a size equal to or smaller than 20  $\mu\text{m}$  has the capability to infiltrate organs, but the percentage measuring between 0.1  $\mu\text{m}$  and 10  $\mu\text{m}$  can access all organs, traverse cell membranes, breach the blood-brain barrier, and cross the placenta<sup>35-36</sup>. Such translocation has been linked to a variety of negative outcomes, including inflammation, vascular blockages, pulmonary hypertension, damage to blood cells, and increased coagulation propensity<sup>36-37</sup>. The study conducted by Lu *et al.*, (2018), revealed the bioaccumulation of small polystyrene micro-particles in the liver, kidney, and gut following oral treatment in mice in an in vivo setting<sup>38</sup>.

Moreover, microplastics are associated with several heavy metals, chemicals used as additives, stabilizer in the process of plastic production, that may leach into the human body following consumption. The leaching of compounds such as Bisphenol A (BPA), Polybrominated Diphenyl Ether (PBDE), and Phthalates from plastic materials has been found to have the capacity to influence the endocrine system<sup>13</sup>. Direct interaction between human cells and heavy metals includes metal-estrogen interactions, congenital disabilities, the generation of reactive oxygen species (ROS), cytotoxicity, significant damage to cardiovascular, respiratory, haematological, gastrointestinal, renal, and hepatic systems, as well as the development of neurological, neurodegenerative, and mental disorders. Additionally, heavy metal exposure can lead to conditions such as anaemia, hypertension, miscarriages, infertility, and the potential for carcinogenic effects on organs such as the breast, liver, kidneys, and lungs<sup>13,39</sup>. The adverse effects on human health may not only be attributed to consumption; other variables such as the frequency, intensity, and duration of exposure, type

and abundance of associated heavy metals and chemicals, as well as the individual's health status, also play a crucial role. Nevertheless, the precise function and potential health consequences of these factors are still matter of research.

### Methods Employed to Measure Microplastics in Water

The determination of microplastics entails many distinct stages, such as sampling, sample processing or pretreatment, quality assurance and control, identification, and characterisation. The processes remain same across different samples, but differences in approach, procedure, and the use of chemicals and equipment provide distinct outcomes.

#### Sampling

The sampling devices used in microplastics analysis is sampling location dependent. As reported in literature, sampling devices can be categorized into four groups: (i) Container (such as a steel bucket, glass jar, telescopic sampling pole etc.), (ii) Autosampler, (iii) Pump (including pumping filtration and custom-made mobile pumps), and (iv) Surface filtration (such as plankton nets, neuston nets, and Taylor sieves).

Surface water sampling often involves the use of surface filtration, which is commonly done using a plankton net or neuston net with certain modifications. Most frequently mesh sizes used are 333  $\mu\text{m}$  or 335  $\mu\text{m}$ . Sometimes, the Hand-net method is used, whereby a handheld bucket is utilised to collect water and then filtered using a net<sup>40</sup>. Other sampling devices, such as the Ruttner sample, stacked sieves/filters, and Teflon pump, have also been mentioned in research studies<sup>41-43</sup>.

#### Pre-treatment of sample

This stage involves the purification of the sample and the isolation of microplastics from it. Typically, this stage is accomplished by the use of chemical forces. During the sampling procedure, other organic, inorganic, and miscellaneous substances may be inadvertently collected together with the sample. These additional components might potentially hinder the identification and characterization of microplastics. Hence, it is essential to exclude it from the sample for further examination. Sample purification may be accomplished by the utilization of chemicals, such as

strong acidic solutions, alkaline solutions, oxidizing agents, or a mixture of these agents, as well as enzymes. Various chemicals, including sodium hydroxide (NaOH), hydrochloric acid (HCl), nitric acid (HNO<sub>3</sub>), potassium hydroxide (KOH), alcohol, NaClO and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), are used for this process. However, a chemical is selected based on its susceptibility to existing microplastics. The organic sample is digested by using hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) with ferrous solution (Fe(II)) acting as a catalyst, which has been identified as the most frequently used chemical.

The extraction of microplastics from the original matrix is a critical process, as its effectiveness influences the amount of microplastics in the sample. Several techniques documented in the literature for isolating microplastics include simple filtering, density-based separation methods, centrifugation, flotation, oil extraction protocols, staining procedures, and various combinations thereof. The density separation method is the preferred technique for extracting microplastics. The selection of chemicals used in this approach is dependent on the likely kind of microplastics found in the sample. In the process of simple filtration, materials are either immediately filtered using membrane filters or passed through sieves and then subjected to vacuum filtration. The techniques of electrostatic separation, pressurized fluid extraction, ultrasonic dispersion, and density fractionation may be used to differentiate microplastics without the need to remove contaminants.

#### Identification and quantification

Microplastics exhibit a diverse range of composition and morphology that requires the use of several methods for their identification, quantification, and characterization. However, the identification techniques most commonly documented in the literature include visual examination using an optical microscope, Fourier-transform infrared spectroscopy (FTIR), Raman spectroscopy, and scanning electron microscopy (SEM).

#### Visual Identification

The process of analyzing microplastics will involve the utilization of an optical microscope for visual inspection<sup>22</sup>. However, the precision of estimating microplastics abundance through visual sorting may be undermined when other particles

like clay and algae are present. Hence, prior to doing a visual inspection, it is imperative to perform sample purification. Lenz *et al.*, (2015) reported that visual inspection correctly identified microplastics (MPs) in only 68% of cases, and as the size of the MP particles decreased, the accuracy diminished accordingly in order to improve the precision of identification, different technical tool including optical microscopes, Raman spectroscopy, and FTIR, can be employed for this purpose<sup>44</sup>. To identify and measure microplastics using a microscope, various magnifications are utilized, specifically 2.5X, 10X, 20X, 50X, and 100X<sup>45</sup>.

### Instrumental analysis

Microplastics collected through visual examination may potentially include additional substances, such as non-plastic materials, inorganic materials, or other types of matter, therefore may be chance of underestimation or overestimation. In order to classify the particles as microplastics, it is necessary to ascertain their chemical composition. There exist various methodologies for determining the chemical properties of microplastics. The methods discussed can be classified into two categories: destructive methods, such as Gas Chromatography-Mass Spectrometry (GCMS), Pyrolysis-GCMS (Py-GCMS), Thermal Desorption-GCMS (TDS-GCMS), and Liquid Chromatography; and non-destructive methods, such as Raman Spectroscopy, Fourier Transform Infrared Spectroscopy (FTIR), and Scanning Electron Microscopy (SEM)<sup>46</sup>.

FTIR is a commonly employed and reliable technique for the identification of the chemical composition of microplastics. This analytical method operates in two distinct modes, namely the Attenuated Total Reflectance (ATR) mode and the Focal Plane Array (FPA) mode. The ATR-FTIR technique is capable of measuring particles that are greater than 500  $\mu\text{m}$ , however it is not suitable for measuring smaller particles ( $< 20 \mu\text{m}$ )<sup>47</sup>. The FPA-FTIR technique has the capability to study individual particles that have been gathered on a filter by means of detection<sup>48</sup>. The system can operate in two distinct modes, namely reflectance and transmittance. The utilization of a technique known as micro-Fourier Transform Infrared Spectroscopy ( $\mu$ -FTIR) has been developed, which relies on the use of a focal plane array (FPA). The combination of

Fourier Transform Infrared Spectroscopy (FTIR) with an optical microscope is employed for this purpose<sup>49</sup>.

FTIR and Raman spectroscopy as frequently employed methods for microplastics identification<sup>50-51</sup>. Both of these techniques complement each other and involve sample identification through vibrational spectroscopy, producing a unique polymer spectrum that can be compared to a reference library of spectra. Raman spectroscopy is characterized by a laser beam that exhibits a reduced diameter when compared to FTIR spectroscopy. As a result, this technology allows for the identification of particles within the range of 1 to 20  $\mu\text{m}$ <sup>52</sup>, without being restricted by the size or thickness of the sample.

The scanning electron microscope (SEM) is a highly useful tool that offers comprehensive structural insights into particle surfaces<sup>53</sup>. This is achieved by the generation of high-resolution images by scanning the sample's surface with a focused electron beam<sup>54</sup>. By means of its analysis, scanning electron microscopy (SEM) has successfully produced visual representations of microplastics (MPs) exhibiting diverse morphologies and dimensions, spanning from a few microns to 5 mm. The chemical composition of the polymer is not identified. In addition, previous studies have utilized SEM-energy dispersive X-ray spectroscopy (SEM-EDS) and environmental scanning electron microscopy-EDS (ESEM-EDS) techniques to analyse the diffraction and radiation impression of MPs surfaces. These methods have been employed for the purpose of categorizing the surface morphology of MPs and identifying the fundamental polymeric structures present<sup>16</sup>.

Thermoanalytical techniques, including pyrolysis-GC/MS and Thermogravimetric analysis-Mass spectrometry (TGA-MS), have been employed in the analysis of microplastics. Firstly, the samples undergo thermal degradation, after which the resulting compounds are subsequently analysed using mass spectrometry. However, the thermal analysis approach used is destructive in nature and does not provide information on the size distribution<sup>55</sup>. Pyrolysis-gas chromatography mass spectrometry (Py-GCMS) is a non-visual method that offers simple sample preparation and rapid identification of microplastics (MPs) in a given sample. This technique involves

the application of thermochemical procedures to facilitate the study of MPs using mass spectrometry<sup>56</sup>. Nevertheless, the methodology lacks the capability to accurately determine the precise quantity of MPs, their specific morphology, and presents challenges in managing sample materials<sup>57</sup>. A notable progress has been made in integrating pyrolysis with various spectroscopic techniques, which can be effectively employed for the quantification of microplastics (MPs). The utilization of thermal desorption gas chromatography mass spectrometer (TDS-GCMS) in conjunction with thermogravimetric analysis (TGA) and solid phase extraction (SPE) improves the analytical procedure, enabling accurate identification of microplastics (MPs) even when present in small sizes and amounts<sup>56-57</sup>.

However, the lack of established characterization methodologies for microplastics (MPs) in natural samples increases the likelihood of incorrect identification and counting, hence impeding reliable assessment. The establishment of standardized protocols for the collection, processing, and analysis of natural samples in the future will enable researchers to directly compare findings across studies. This would greatly facilitate the assessment of the origins, transit pathways, and possible risks associated with microplastic pollutants.

## CONCLUSION

The study reveals that microplastics are ubiquitously present in various environmental compartments, spanning from groundwater to ocean, indoors to remote Arctic regions and great Himalayan regions, lower invertebrates to higher trophic levels of food. These microplastics have been detected across a wide range of organisms, including fish, bivalves, crustaceans, seabirds, cetaceans etc. along with different body parts of human, and have even been identified in human breast milk. Nevertheless, empirical studies have revealed considerable variation in the concentration of microplastics across different samples, with quantities ranging from a few numbers of particles to hundreds of particles per unit volume. In addition,

discrepancies in microplastic levels might be attributed to the range of samples analyzed, as well as alterations in sampling methods, sample preparation, extraction and purification procedures, and analytical approaches. Despite significant advancements and refinements in the sampling and analysis techniques employed for microplastics, the lack of universally standardized methods of analysis has hindered the attainment of comparable results. Therefore, there is a need for further meticulous investigation in the realm of microplastics, with a specific emphasis on the development of a comprehensive approach encompassing sampling, pretreatment, extraction, analysis, and the interpretation of findings. Furthermore, it is imperative to conduct a thorough analysis of different dietary components in order to determine the concentration of microplastics and the extent to which additives are leaching into the food. Additionally, it is crucial to assess the variability in the quantity of these components. The existence of microplastics has significant implications for both the natural environment and human well-being, leading to adverse economic consequences. The health consequences of microplastics for people, including their exposure levels and the potential variations in health effects based on different concentrations, are currently not well understood. Future research endeavors should prioritize the investigation of the potential amplification of microplastics in diverse aquatic ecosystems, as well as the identification of their possible origins. Additionally, in light of the detrimental impacts associated with microplastics, it is imperative to establish a standardized protocol aimed at mitigating their production, managing their degradation, and ultimately reducing their prevalence in the natural environment.

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## Conflict of interest

The author declare that we have no conflict of interest.

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