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# Microplastics: Detection methods-An update

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## Abstract

Microplastics are synthetic polymers with major dimension of ≤5 mm. The particles occur in a large variety of shapes, sizes, colors, and compositions. Microplastics enter the food chain, they may be biomagnified and bioaccumulated by larger organisms and ultimately reach humans. Apart from organisms, other food materials such as salt, honey, beer, tea bags, and drinking water have also been reported to have microplastic contamination. Organs reported to be contaminated by microplastics and nanoplastics include the gastrointestinal tract, respiratory system, skin, liver, kidneys, and even the brain. Effect of microplastic contamination on these organs can range from inflammatory responses to tissue damage and potential disruption of organ function and carcinogenesis. Microplastics have entered drinking water via various pathways, raising concerns about their potential health impacts. A number of fluorescent dyes, including Nile red, Rhodamine B, Safranin T, and fluorescein iso phosphate, can label plastic polymers and hence are used in the detection of microplastics. Among these, Nile red has been used widely as a rapid method for detecting microplastics. The three main methods for detecting and quantifying microplastic concentrations in water are FTIR Spectroscopy, py-GC/MS, and Raman Spectroscopy. FTIR and Raman Spectroscopy can determine the number of microplastic particles by plastic type and size range, whereas py-GC/MS can quantify concentrations of specific types of microplastics in mg/l. To overcome the challenges of time and labor-intensive, microplastic detection techniques, researchers are increasingly adopting machine learning and automation. These technologies can process large datasets with greater speed and accuracy, training algorithms to detect microplastic more efficiently.

**Keywords:** Fluorescent dyes; FTIR; Microplastic; Machine learning; Microplastic; Nile Red (NR); Raman Spectroscopy

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## 1. Introduction

Plastics were first developed in the early 1900s. After 1945 they became more widely used, with a resultant dramatic increase in plastic pollution and their breakdown to microplastics. Global plastic production has almost doubled compared to two decades ago and most of the plastics are ending up in landfill, incinerated, or leaking into the environment, with only 9% successfully recycled globally [1-141-172]. The term "microplastics" (MPs) was coined in 2004 by Professor Richard Thompson to describe small plastic particles found in various environments [7-9-141. 168, 169]. In developed countries, some plastics are recycled, whereas in low-income countries, no advanced equipment for recycling exists. Microplastics can be found in various environmental compartments such as oceans, rivers, lakes, soil, air, and even in organisms. According to recent research, microplastics were found in large concentrations in various food and beverage sources and were detected in human faeces. Microplastics are synthetic polymers with major dimension of ≤5 mm [1-141-166-172]. The particles occur in a large variety of shapes, sizes, colors, and compositions. Poly Vinyl Chloride (PVC), Polyethylene Terephthalate (PET), Polystyrene (PS), and Polyethylene (PE) are the most abundant microplastic polymers found in nature, and gastrointestinal ingestion has been identified as the primary pathway of exposure to microplastic [1-141-172]. Microplastics, pervasive environmental contaminants, pose a potential risk to human health, including the development of oral carcinoma [15, 17-141-172]. These minute plastic particles infiltrate various environmental niches and enter the human body through ingestion, inhalation, and dermal exposure [15, 17-141]. Detected in organs such as the gastrointestinal tract and respiratory system, microplastics may induce inflammation and organ dysfunction [15, 17-141-172]. Emerging research suggests their potential to harbor carcinogenic substances, leading to DNA damage and carcinogenesis [15, 17-141-169-172]. Overall, there are three routes of microplastic release into the environment – (i) direct discharge of plastic waste into water bodies; (ii) discharge from industrial and domestic wastewater treatment plants (WWTPs); (iii) plastic waste in fishing vessels, fisheries, and water operation industries.

On the basis of literature survey, it is likely that global contamination of microplastics will be eventually brought back to our dinner table through consumption of various food items. Although a few studies have quantitatively estimated the microplastic consumption of people from contaminated seafood , salt , and packaging materials , the extent of people's microplastic exposure via food consumption remains largely unknown [1-141-172] Once microplastics enter the food chain, they may be biomagnified and bioaccumulated by larger organisms and ultimately reach humans [4-15-141]. Apart from organisms, other food materials such as salt, honey, beer, tea bags, and drinking water have also been reported to have microplastic contamination [5-141]. These products are regularly used by humans and serve as sources for the entry of microplastics into the human body [1-141-172] Ingestion occurs primarily through contaminated food and water, inhalation through air contaminated with microplastic particles, and dermal exposure through direct contact with products containing microplastics or contaminated surfaces [1-15-141-172]. Organs reported to be contaminated by microplastics and nanoplastics include the gastrointestinal tract, respiratory system, liver, kidneys, and even brain [7-15]. Effects of microplastic contamination on these organs may include inflammation, oxidative stress, and disruption of cellular function [15, 17-141]. Organs reported to be contaminated by microplastics and nanoplastics include the gastrointestinal tract, respiratory system, skin, liver, kidneys, and even the brain [8-17-141]. Effects of microplastic contamination on these organs can range from inflammatory responses to tissue damage and potential disruption of organ function [15, 17-141]. Micro- and nanoplastics can serve as a source of carcinogenic or mutagenic substances, potentially causing DNA damage that can lead to carcinogenesis, the development of cancerous tumors [13-17-141]. Genotoxicity studies investigated the effects of microplastics and associated chemicals on DNA integrity, chromosomal structure, and genomic stability using sensitive assays [15, 17-141-172]. Due to the potential health risks and negative impacts on the environment, the use of microplastics in tea bags and other products is a major issue[1-13-17-141]. Li et al., (2023) [64] reported that microplastics are consumed by humans from an early age and in increasingly large quantities [12-17]. As microplastics pass through the gastrointestinal tract they interact with the normal physiological mechanism of the body, particularly in the colon and rectum, where they may interact with the protective colonic mucus layer[12-17]. Li et al., (2023) [64] reported several possible mechanisms of how microplastics may disrupt this mucus layer, thus reducing its protective effect and increasing the likelihood of colorectal cancer [12, 17-141-169]. Therefore, it is possible that the microplastics damage the barrier integrity of the colonic mucus layer, thus reducing its protective effect [64]. Li et al., (2023) [64] also indicated that further clarification needs to be sought regarding the interaction between microplastic, gut microbiota and the mucus layer [15]. This will need to be modeled in long-term animal studies to better understand how chronic consumption of environmentally-acquired microplastics may contribute to an increased risk of colorectal carcinogenesis [12-17-141-172].

## 2. Microplastics: Food

Now a days most of the food is contaminated with micro plastics. Some of the common food containing microplastics are table salt, sugar, herbal medicine, tea bags, honey, beer and milk [1-141-172]. Because table salt is most often produced by the distillation of seawater, it is difficult to avoid microplastics in final sea salt products without further purification steps because seawater contains microplastics[1-141-171]. In the last decade, researchers have identified the presence of microplastics in fish and shellfish captured in the wild and obtained from aquaculture farms or markets. Microplastics were also isolated from various processed foods[1-141-172]. They were investigated in liquids such as beer, honey and milk [1-141-168, 172]. The high concentrations of microplastics in beer samples required further confirmation because staining and visual counting may have overestimated the number of particles [1-141-172]. Although sugar contains nearly as much microplastic as sea salt, the only study on microplastics from sugars did not use spectroscopic identification methods, and it might include other particles rather than microplastics. Sugar might also be contaminated with microplastic during processing, requiring further investigations [1-141-172]. Dried food such as land animal-based, Chinese traditional medicine, processed seafood such as sardines and sprats, seaweed, dried fish, and teabags are also contaminated with microplastics [1-141, 168, 169-172]. The high microplastic concentrations in traditional medicine is due to high microplastic levels in the source materials [1-141-172]. Various studies have shown that microplastic causes adverse toxicity, including effects on behavioral patterns, oxidative stress, hyper immune response, genotoxicity, reproductive toxicity, developmental toxicity, neurotoxicity, and gastrointestinal toxicity [1-141-172].

## 3. Microplastics: Drinking water

Recently, it was announced that the presence of microplastics in drinking water bottles is a topic of significant concern [1-141-172]. The presence of microplastic particles in drinking water raises concerns about their potential impact on human health. Tap water and bottled water have been identified as significant pathways of human exposure to microplastics [9-141]. The primary sources of microplastic in the environment are textiles, medicine, and personal care items, while plastic containers, nets, fibers, and tiles constitute the secondary sources [1-9-141-172]. Polyethylene (PE), polystyrene (PS), polyethylene terephthalates (PET), and polypropylene (PP) constitute the most abundant polymers in water [1-141]. These polymers find their way into water bodies from various packaging materials such as plastic disposable bottles, bottle caps, food packaging materials, and plastic bags which are being discarded [1-141]. Even traditionally pure groundwater becomes vulnerable to microplastic infiltration through rainwater or plastic debris leaching [7-9-141-172]. The use stage (e.g., opening the bottles) also contributes to microplastics contamination because physical stress, heat, and abrasion can release microplastics into the water. Packaging materials, such as bottle caps and polyethylene foils, can also release microplastics [7-9-141-172].The toxicity of microplastics is known to increase with decreasing size of the particle, as smaller particles have higher chances of penetrating deeper into the organs [1-141-172]. Microplastics can enter the human body through ingestion, inhalation, and dermal absorption leading to their accumulation in various organs and tissues. These risks include the presence of toxic chemicals, accumulation within the body, promotion of microbial growth, and initiation of inflammation, due to unique characteristics such as hydrophobicity [1-141-172]. Microplastics in bottled water result from plastic degradation during production, transportation, and use [9-141-172]. Besides their own effects, microplastics in drinking water pose health risks by leaching additives/chemicals that can cause damage to the human digestive, neuroendocrine, reproduction, and other systems [9-141]. These chemicals can enter the human body through ingestion and dermal contact [9-141-172]. Improperly disposing of disposable plastic products, despite their convenience, raises significant concerns due to the adverse impacts on the environment and human health [7-9-172]. The accelerated degradation poses significant risks, as the released organics are identified as etiological, carcinogenic, and mutagenic, contributing to various harmful effects on the body [7-9-172]. These effects include oxidative stress, impairments in the gastrointestinal and reproductive systems, metabolic disturbances, and liver changes [7-9-172]. In India, the ecological risk posed by microplastics found in coastal sediments has been evaluated using a comprehensive analysis of metadata, employing three key indices: the Polymer Hazard Index (PHI), Pollution Load Index (PLI), and Potential Ecological Risk Index (PERI) [10]. These indices provide a quantitative assessment of sediment quality and the associated threat levels [10-141-172].

## 4. Microplastic : Detection Methods

A number of fluorescent dyes, including Nile red, Rhodamine B, Safranin T, and fluorescein iso phosphate, can label plastic polymers and hence are used in the detection of microplastics [1-141-172]. Among these, Nile red has been used widely as a rapid method for detecting microplastics [1-141-166-172]. Furthermore, Fourier transform infrared (FTIR) or Raman spectroscopy methods are commonly used for the detection of toxic polymer chemicals [1-141-172]. More

advanced methods, such as micro FTIR, pyrolysis-GC/MS, and thermo gravimetric analyser-GC/MS techniques, have also been used to identify microplastics with high precision. In addition to this, a number of animal models have been used to evaluate the toxicity of microplastic polymers [1-141-166-172]. The literature reports the use of invertebrates like *Caenorhabditis elegans*, *Daphnia magna*, and *Artemia*, and vertebrates such as mice, rats, rodents, and zebrafish [7-9-141-172]. These microplastics have the potential to alter marine ecosystems and pose health risks to aquatic species and, in turn, to humans [7-9-141-166-172].

## 5. Seperation of Microplastics from Sample of Interest

On the basis of literature survey, microplastics are usually collected from the samples by sieving or dissection from animal tissue of interest. Next, most samples will be treated with reagents to chemically digest or dissolve the matrix such as KOH, $HNO_3$, or $H_2O_2$ before trying to separate the microplastics by filtration, or gravimetrically by utilizing differences in density with or without centrifugation [1-141-166-172]. Fortunately, the chemical stability of the microplastic particles often allows analysts to dissolve and digest various matrices while leaving the polymeric particle intact [1-141-166-172]. However, care must be taken—especially when using nitric acid. One of the common methods used to digest samples is the use of a solution of concentrated (30–35%) hydrogen peroxide ($H_2O_2$)to remove organic matter [1-141-172]. In one of the study, digested residues of water samples were mixed with 20 mL of 30% hydrogen peroxide at $60^o$ Celsius for 72 h to digest organic material, then the polymer particles in the digested samples were separated by density overnight using saturated sodium chloride solution (D = 1.2 g/mL) [1-141-172]. In another study, microplastics present in air samples were collected and the sample filters were washed before digestion in 35 mL of 30% $H_2O_2$ at room temperature for 10 days to eliminate organic materials [1-141-166-172]. The remaining $H_2O_2$ solution was then vacuum-filtered through filter paper with a 2-micron pore size to remove any remaining particle matter [1-141-172]. Next, 50 mL of a saturated $ZnCl_2$ solution with a density of 1.6 to 1.8 g/cm$^3$ was added to each filter, and the mixture was agitated for five minutes at 350 rpm [1-141-172]. Then, the samples were allowed to remain still for one full hour before being centrifuged for 3 min at 4000 rpm to collect all microplastics [1-141-166]. The previous literature suggests that before the chemical analysis of microplastics, samples must be chemically digested, and the most common solution used for this purpose is the 30% $H_2O_2$ [1-141-172]. Separation by density (floating) for microplastic particles is a practical and common step to easily purify microplastics prior to chemical analysis [1-141-172]. In general, the protocol of sampling, filtration, chemical digestion, and density separation is routinely used for the sample preparation steps of microplastics analysis [1-141-172].

## 6. Physical and Chemical Characeterization of Microplastics

After separating microplastics from the sample of interest, the next step is physical and chemical characterization [1-141-166-172]. Visual inspection is the quickest and the most popular way to identify suspected microplastic particles [142-172]. With the aid of optical microscopes, microplastic particles' size, shape, and color can be characterized [1-141-172]. This method has the advantage of being the simplest, lowest cost, and allowing for the largest diversity of microplastic to be detected in terms of size (primary diameter or length), color, and form (as fiber, film, fragment, and spherule). Dyes produce different colors which allow us to visually detect the type, shape, and size of microplastic [1-141-172].

Multiple dyes used include Oil Red EGN, Eosin B, Rose Bengal, Hostasol Yellow 3G, and NR (Nile Red) were used in the detection of microplastics [1-141-172]. Investigators soaked particles in the dyes for different durations between 5 min and 66 h. Nile Red was chosen as the optimal stain since it has the highest levels of adsorption and fluorescence intensity [1-141-172]. When exposed to blue light, the dye will fluoresce and simple photography with an orange filter is used to find fluorescence emission. Fluorescent particles can be recognized and counted using image analysis [1-141-172]. Particles that were as small as a few micrometers can be detected using magnified images that can be recorded and tiled/arranged to cover the entire filter area. Interestingly, Nile Red's solvatochromic properties provide the opportunity for plastic categorization based on the surface polarity traits of identified particles [1-141-172]. It was established that an incubation period of between 5 min and 66 h and a dye concentration of between 1 and 1000 g mL$^{-1}$ were ideal for visibility. A working concentration of 10 g m$^{-1}$ produced a nice balance between background signal, visibility, and speed [1-141-172]. An additional technique commonly used to characterize the size, shape, and chemical composition of microplastics is Scanning Electron Microscopy (SEM). This technique is used to image and measure objects with different diameters ranging from millimeters to nanometers in size [1-141-172]. The SEM technique is capable of nanometer spatial resolution. An effective and widely used method for identifying microplastics is FTIR microspectroscopy [1-141-172]. The signal is dependent on a change in molecular dipole moment occurring during a molecular vibration [1-141-166]. Another powerful method of analysis for microplastic particles is Raman spectroscopy. Raman spectroscopy is another form of vibrational spectroscopy; however, in contrast to FTIR, Raman

microscopy is more appropriate for small microplastics less than 20 μm [1-141-172]. Raman spectroscopy is based on the Raman effect, whereby the frequency of a small portion of dispersed or scattered radiation emanating from a sample differs from the frequency of monochromatic incident light. On the basis of literature survey, it is clear that no perfect technique exists for microplastic analysis which can provide both comprehensive chemical identification and high-resolution imaging capability [1-141-172].

## 7. Microplastic Detection Methods

Following are the few methods used for the detection of microplastics in plastic water bottles and food material.

- According to the method described by Mohan et al., (2023) [1], 20 different brands of plastic water bottles (500 mL capacity) were purchased in triplicates from local stores in Mangaluru (n = 60), Karnataka State, India[1]. A fluorescence staining method utilizing Nile Red (NR), a lipophilic dye, was used to visualize the microplastics [1-3-172]. Nile Red (NR), can detect microplastics quickly and identify the polymeric nature of a particle without the need for extra spectroscopic investigation [1-3-172]. The water samples from each bottle were poured into a stoppered glass bottle (1000 mL) under the laminar hood and were incubated with Nile red (NR) solution (10 μg/mL) for 30 min in the dark [1-3]. Nile Red solution (Sigma Aldrich) was prepared in acetone at a concentration of 1 mg/mL to yield a working solution of 10 μg/mL and was stored at 4 ° C in an amber bottle [1-4]. After incubation, the samples were vacuum filtered via a glass microfiber filter paper [1-4]. According to the method described by Mohan et al., (2023) [1], after filtration, the filter papers were kept in a sterile glass Petri plate and were observed under a fluorescence microscope [1-4]. The filter paper was marked and divided into quadrants for easy counting and sorting of the particles while imaging and characterizing the shapes [1-4]. Mohan et al., (2023) [1] reported that RO-treated water samples stored in glass containers were used as a negative control [1-4]. In addition, water samples obtained from the plastic bottles but not incubated with Nile Red were processed as described above and were imaged to determine the background fluorescence [1-4-130]. To characterize the type of polymer, 100 mL of the samples was filtered without Nile Red (NR) treatment [1-4]. The filtrate was then washed with 200 μl of deionized water, collected in a glass tube, and subjected to Fourier-transform infrared spectroscopy (FTIR) analysis [1-6]. This study by Mohan et al., (2023) [1], provides evidence for the concept that microplastics are abundant in the environment, they can enter the body through water, can accumulate in various organs, and can trigger oxidative stress [1-172]. It would be interesting to observe the changes that happen during long-term exposure to microplastics and the resulting changes in the physiological and biochemical processes in a cell [1-172]. According to the method described by Mohan et al., (2023) [1], Zebrafish embryos exposed to different concentrations of fluorescent-tagged polyethylene microplastics (PE-MPs) (10–150 μm) showed accumulation patterns at different time points in various organs [1]. This study by Mohan et al., (2023) [1], also confirmed that microplastics of various types were detected in different brands of packaged drinking water available in India [1]. Nile Red staining can be a simple and effective method for the detection of plastic polymers [1-172]. FTIR results indicated the abundance of polyethylene, polystyrene, and polyamide in the samples [1]. Polyethylene polymers altered the redox balance in zebrafish [1]. Correlations between oxidative stress and DNA damage were observed upon microplastic exposure to zebrafish [1].
- Although washing with deionized water and then visual inspection with or without staining is convenient for clean matrices, false-positive detection of microplastics is challenging to avoid [1-141-172].
- The simplest approach for physical characterization is visual identification, simply by naked eye observation using a dissecting or stereomicroscope [1-172]. This method is limited to microplastic sizes in the range of 2–5 mm and is often prone to error when identifying microplastics from complex environmental samples. can be used to count the number of microplastics in a sample and conduct a crude morphological study [1-172]. However, it is limited by a measurement error, that is the function of particle size, i.e., the smaller the size, the higher the percentage error [1-141-172]. With the use of a light microscope, larger particles between 1 and 5 mm can be identified. The light allows for the assessment of color, shape and helps distinguish plastic and non-plastic particles [1-166]. Electron microscopes can also be used to visualize the size, shape, colour, and texture of MPs within the size range of 0.5 to 5 mm [1-166-172]. Advanced equipment like cameras interfaced with the microscope and image refining software may be utilized to detect smaller microplastics and improved image resolution to clearly see details in the surface morphology [1-166-172]. Furthermore, to identify microplastics in the aquatic environment, fluorescent or lipophilic dyes prove useful as they can help easily visualize stained microplastics under a microscope [1-166-172].

- Another popular oxidation method is the use of Fenton's reagent. This method is suggested by the National Oceanic and Atmospheric Administration, USA, for marine organisms, although the method needs to be tested for a diversity of organic matrices [1-141-172].
- Digestion with alkaline solutions such as KOH and NaOH have predominantly been used for digesting fish and shellfish. It is advantageous for destroying proteins and other soft tissues. Suitable extraction recovery was found for polyethylene terephthalate (PET) and high-density polyethylene [1-141-172]. However, pH-sensitive polymers such as nylon and polyester can be disrupted at high pH [1-141-172]. Various strong acid solutions (e.g., $HNO_3$, $HCl/HNO_3$, and $HClO_4$) have been used to digest the soft tissues of fish, mussels, and other organisms [141,142]. Similar to strong basic solutions, the tissues were successfully decomposed, although low pH also led to the decomposition of pH-sensitive polymers [1-172].
- Microscopy, including optical and electron microscopy, is a standard method for identifying microplastic based on their size and shape due to its simplicity and low cost [142-166-172]. However, this approach is limited to microplastic, is time-intensive and relies heavily on the analyst's judgment, making it susceptible to errors influenced by environmental factors and sample impurities. Fluorescence staining is now widely applied as a supplementary technique to enhance microplastic identification by microscopy [1-142-166-172]. This method involves staining of microplastic with hydrophobic dyes and using specific wavelengths to trigger fluorescence, which aids in detection under specialized microscopes [1-142-172]. However, issues like false positives due to staining of organic materials or interference from natural fluorescence in samples remain challenges. The development of new fluorescent dyes that can recognize specific microplastic would greatly advance the field by improving the accuracy of microplastic identification in complex environmental samples. Advanced methods like scanning electron microscopy (SEM) and atomic force microscopy (AFM) are increasingly being used to study the smaller dimensions of nanoplastics [1-142-172]. SEM provides high-resolution imaging of morphological features, especially surface characteristics. When paired with energy-dispersive X-ray spectroscopy, it can also provide valuable insights into chemical composition [142-172]. However, SEM is slow and requires time-intensive sample preparation. In contrast, AFM is a promising tool for micro- and nanoscale analysis due to its ability to capture high-resolution images directly from the sample without pretreatment [1-142-171]. Furthermore, AFM is fast, simple and can distinguish material types within polymer blends, detecting compounds like heavy metals adsorbed onto microplastic surfaces [1-142-166].
- The two predominant methods used for microplastic identification in food were visual inspection under dissection microscope with or without staining and the absorption or reflection of IR with FT-IR or Raman spectroscopy [1-141-172].
- Two current approaches—counting microplastics with microscopy and destructive microplastic detection with thermal analysis—can be complementary [1-172]. In addition, contamination and decontamination of microplastics during food processing and cooking are important as microplastic exposure of people is primarily from the consumed final products, not on their ingredients [1-142-166].
- The three main methods for detecting and quantifying microplastic concentrations in water are FTIR Spectroscopy, py-GC/MS, and Raman Spectroscopy [1-141-172]. FTIR and Raman can determine the number of microplastic particles by plastic type and size range, whereas py-GC/MS can quantify concentrations of specific types of microplastics in mg/l [1-142-172]. These methods can also be used to detect microplastics in sludge and soil samples. Raman spectroscopy plays a key role in identifying the types and origins of microplastics. It is a part of the efforts to develop policies and procedures for controlling the amount of microplastics introduced into ecosystem [1-141-172].
- Micro- and nanoplastics are in our food, water and the air we breathe. They are showing up in our bodies, from testicles to brain matter [12, 13-141-172]. Nano and microplastics are byproducts of degrading plastic materials such as lunchboxes, cups and utensils. As very small particles with a large surface area, nanoplastics are particularly concerning to human health due to their increased ability to absorb toxins and penetrate biological barriers within the human body. Detecting these plastics typically requires skilled personnel and expensive equipment [12, 13-172]. Now, UBC, Vancouver, Canada researchers have developed a low-cost, portable tool to accurately measure plastic released from everyday sources like disposable cups and water bottles [13-172]. The device, paired with an app, uses fluorescent labeling to detect plastic particles ranging from 50 nanometres to 10 microns in size – too small to be detected by the naked eye – and delivers results in minutes. They created a small, biodegradable, 3D-printed box containing a wireless digital microscope, green LED light and an excitation filter [13]. To measure the plastics, they customized MATLAB software with machine-learning algorithms and combined it with image capture software [13]. The result is a portable tool that works with a smartphone or other mobile device to reveal the number of plastic particles in a sample. The tool only needs a tiny liquid sample – less than a drop of water – and makes the plastic particles glow under the green LED light in the microscope to visualize and measure them [13, 149]. The results are easy to understand, whether by a technician in a food processing lab or just someone curious about their morning cup of coffee [13]. The tool is currently calibrated to measure polystyrene, but the machine-learning algorithm could be tweaked

to measure different types of plastics like polyethylene or polypropylene [13]. Next, the researchers aimed to commercialize the device to analyze plastic particles for other real-world applications [13]. To reduce plastic ingestion, it is important to consider avoiding petroleum-based plastic products by opting for alternatives like glass or stainless steel for food containers [12, 13]. The development of biodegradable packaging materials is also important for replacing traditional plastics and moving towards a more sustainable world.

- The global microplastic detection market is experiencing significant growth due to rising environmental concerns, regulatory pressures, and advancements in detection technology [1-143-172]. With microplastic contamination found in water, soil, air, and food products, industries are investing in high-precision spectroscopy, chromatography, and microscopy-based solutions to ensure accurate detection and compliance [1-143-172]. Key players like Thermo Fisher Scientific, Agilent Technologies, Bruker, and PerkinElmer are leading the innovation in real-time monitoring and high-sensitivity testing methods [1-143].
- To overcome the challenges of time and labor-intensive, microplastic detection techniques, researchers are increasingly adopting machine learning and automation. These technologies can process large datasets with greater speed and accuracy, training algorithms to detect microplastic more efficiently [142-166-172]. Machine learning models can analyze spectral data or images, identifying patterns that distinguish microplastic from other particles [130-142-172]. Several studies have now proven the potential of machine learning for microplastics identification using SEM, fluorescence, Ramen spectroscopy and FTIR. One study introduced PlasticNet, a deep learning model specifically trained to recognize microplastics from images generated by FPA-based micro-FTIR spectroscopy [130-142-166-172]. PlasticNet, trained on spectra from 11 types of virgin plastic particles, achieved over 95% classification accuracy, demonstrating the huge potential for deep learning in microplastic detection [120-142-172]. However, while the model shows high accuracy for virgin plastics, this model's efficacy on environmentally sourced microplastic remains untested [142-166]. In another study, a dataset of over 64,000 Raman spectra from 47 environmental or wastewater samples was used to develop a human-computer hybrid approach. This method achieved high recall (≥99.4%) and precision (≥97.1%) for identifying microplastic and reduced the annotation time from hours to under one hour per sample compared to human-only analysis [120-142-172].
- Currently, in situ detection and quantification of microplastic is difficult or even impossible, because of a lack of applicable methods. Certain environments, like wastewater treatment plants and water-intensive industries, contain high levels of organic and inorganic solids, complicating the detection of low-abundance microplastic without sample pretreatment [142-172]. Environmental factors, such as temperature and pressure fluctuations in natural water bodies, add further complexity by altering conditions at different depths, affecting the properties of microplastic locally. Recent advancements in compact light sources, detectors and optical components have led to the availability of portable and handheld photometers and spectrometers for environmental monitoring [142-172]. Among these, commercial portable devices based on fluorescence, FTIR and Raman have shown promise. For example, a cost-effective portable Raman sensor has been designed to detect micrometer-sized magnetic plastic particles in water using a quartz cuvette [142-172]. Additionally, a recent study utilized a portable photometer and fluorescent staining to measure the presence of microplastic in water samples, representing significant progress toward accessible, field-ready detection methods [142-172].
- Microwave technology is a novel method for monitoring microplastics. It is a very promising approach for quickly determining the size and concentration of selective microplastic sensing [169]. Although there are some limitations, and are working on ways to overcome the weaknesses by enhancing the microwave part of the device and improving the sensitivity of the sensor [169]. This enhancement might allow us to detect microplastic particles as accurately as optical spectroscopic methods but would be much faster and cheaper [140-169]. Microwaves are highly sensitive to the properties of the materials with which they interact. Microwave sensors are based on the difference in permittivity of the host medium (e.g., water) and the microplastic contaminant [169]. This contrast is high, enabling researchers to accurately count the number of particles present, even at low concentration levels [169]. Microwave sensors also can be combined with planar technology to obtain compact, light, robust, and low-price fabrication[169]. There are strengths and weaknesses associated with any detection methodology, including microwave sensing. One downside is that microwave sensors cannot detect particles smaller than 20 micrometers (in samples with concentrations less than 1000k particles/L or those with concentrations less than 50k particles/L) [140-169]. Another challenge with this method is that it cannot differentiate between two different plastics in a sample, but this could be addressed in the future as the technology improves [169].

Debraj and Mulky (2025) [168] are of the opinion that currently, the most commonly used method for microplastic identification is the use of a stereomicroscope [168]. It has a high error rate and often results in false positives. Spectroscopy methods are more efficient. However, they require expensive and bulky equipment and trained personnel for operation [168]. Moreover, depending on the environmental sample, they require long pre-treatment steps. Multiple

datasets generated could be sorted and classified with the help of machine learning tools [168]. However, associated issues include high dimensionality, limited accuracy, low performance, and redundancy [166-169]. Conventional methods of microplastic sorting rely greatly on the physical state of the sample, the protocol for sample preparation, and the possibility of contaminants. Furthermore, new techniques continue to develop, from mechanisms that exploit the electrostatic properties of MPs, to microfluidics and even the use of the Internet of Things [168].

While no single technique provides a one-size-fits-all solution for detecting microplastic advancements in spectroscopy, mass spectrometry, imaging and AI-driven approaches re rapidly improving our ability to track these pollutants [142-166-169]. The challenge now is to make these technologies faster, more cost-effective and scalable for widespread environmental monitoring. By developing portable tools and integrating machine learning, we may soon have the capability to map the global distribution of microplastic, as well as distinguish between different types of plastics, trace their origins and monitor their movement through ecosystems and food chains [142-166-169].By enhancing our detection capabilities, the scientific community can work toward informed, evidence-based policies and interventions aimed at reducing plastic pollution and protecting both environmental and human health [142-166-169].

Currently, the detection of microplastics primarily relies on visual analysis using stereoscopes or a combination of spectroscopic techniques, including Fourier transform infrared spectroscopy (FT-IR) and Raman spectroscopy, fluorescent staining methods (e.g. Nile Red), thermal analysis, and chromatographic analysis [1-96–98-172]. However, these methods have limitations and drawbacks. For instance, the use of Nile Red fluorescent staining may complicate the identification of specific polymeric materials [1-99-172]. Some methods are subjective, time-consuming, and prone to errors due to the varying appearances and properties of microplstics. Therefore, more objective criteria and the assistance of AI or data analysis are needed to help scientists accurately identify the morphological features of microplastics. There is an urgent need for scientists to develop more time-efficient and precise methods for detecting MPs in the environment and human bodies, to address the challenges faced in microplastics detection. With advancements in microplastics detection technology, especially in the exploration of methods for detecting microplastics within the human body, it is anticipated that more direct evidence of the correlation between microplastics and human health will be uncovered in the future.

## 8. Conclusion

Microplastics have been found in various environmental media, including soil, water, air, and have been shown to have negative impacts on wildlife. Microplastics have entered drinking water via various pathways, raising concerns about their potential health impacts. Multiple dyes used include Oil Red EGN, Eosin B, Rose Bengal, Hostasol Yellow 3G, and NR (Nile Red) were used in the detection of microplastics. When exposed to blue light, the dye will fluoresce and simple photography with an orange filter is used to find fluorescence emission. Fluorescent particles can be recognized and counted using image analysis. The three main methods for detecting and quantifying microplastic concentrations in water are FTIR Spectroscopy, py-GC/MS, and Raman Spectroscopy. FTIR and Raman can determine the number of microplastic particles by plastic type and size range, whereas py-GC/MS can quantify concentrations of specific types of microplastics in mg/l. These methods can also be used to detect microplastics in sludge and soil samples. Raman spectroscopy plays a key role in identifying the types and origins of microplastics. It is a part of the efforts to develop policies and procedures for controlling the amount of microplastics introduced into ecosystem. There are several, well-established methods for detecting and monitoring microplastic particles – the most common being Fourier-transform infrared (FTIR) and Raman spectroscopy. Although these techniques are very accurate and can detect the types of plastic in the sample, they require intense training of the technician and bulky and expensive measurement equipment. They also use offline methods and require samples direct from water facilities for lab experiments. Overall, these approaches end up being time-consuming, labor-intensive, and expensive for researchers, especially when they have a large number of samples to process, The most significant challenge in microplastic detection, much like microplastic extraction and sampling, is the lack of a standardized method of identification. Due to the plethora of polymer compositions and numerous complex environmental matrices, it is almost impossible to have a singular identification protocol. Moreover, it is also dependent on the availability and accessibility of the instruments, technicians, and related materials. Different instruments provide different physico-chemical property information, and each has its own set of advantages and limitations.

## Compliance with ethical standards

### *Disclosure of conflict of interest*

No conflict of interest to be disclosed.

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