



REVIEW

Novel Techniques for Micro and Nanoplastics Analysis: A Review

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Microplastics contamination has received increased attention, due to its ubiquitous prevalence and possible hazards to the environment. It is critically necessary to have trustworthy techniques and tools for rapidly analyzing microplastics smaller than 5 mm. The analytical techniques for microplastics research such as sampling, identification and quantification are reviewed critically. Here both traditional and cutting-edge techniques for analyzing microplastics have been summarized. Furthermore, the present review focused on the current applications of microplastics analysis using, microscopy, Fourier transform infrared spectroscopy, scanning electron microscope, dynamic light scattering, thermal analysis, differential scanning calorimetry, pyrolysis-mass spectrometry and the highly advanced techniques includes automated image analysis, infrared hyperspectral imaging, microplastic sensors and electrochemical sensors.

Keywords: Environment, Microplastics, Nanoplastics, Techniques.

INTRODUCTION

Plastics have found extensive applications in a wide range of domains including aircraft, everyday necessities, agriculture, industry, medicine and the military. But as plastic items are used so widely, a lot of plastics waste that were never treated has found their way into the ecosystem [1]. Furthermore, the majority of these waste plastics are microplastics [2]. Plastic particles with a diameter smaller than 5 mm are known as microplastics [3]. The issue of microplastic pollution has become increasingly serious in recent years and it urgently has to be managed [4].

Microplastics can be categorized as main or secondary compounds based on their origins. Plastic particles that are released into the aquatic environment by rivers and wastewater treatment plants are known as primary microplastics. According to Guo & Wang [5], secondary microplastics are the plastic particles that are created when large plastic trash fragments and reduces in volume by chemical, physical and biological processes. Although microplastics can take many different forms,

the most common ones are granules, fibres, films and fragments [6]. Microplastics are extremely dangerous to both the environment as well as living things. Firstly, microplastics seriously affect the ecosystem and living things since they are difficult to dissolve and are exceptionally persistent in the surrounding environment [7,8]. Moreover, due to the huge specific surface area, microplastics are probably to be transporters of other contaminants in the surrounding environment, which might harm eco-systems more severely [9]. Finally, due to their tiny particle size, microplastics have a significant propensity to move throughout the environment.

The marine ecosystem faces significant contamination from microplastics, yet the extent to which this pollution has infiltrated the marine food chain remains uncertain [10,11]. According to reports, microplastics are consumed by plankton, which is found at the lowest tropical level of the marine environment. Later on in the food chain, predators consume plankton. Microplastics were shown to accumulate in plants, honey and water as well [12,13]. Thus, the apex predator, humans, eventually absorbs microplastics from lower tropical levels. Since both

plants and animals are consumed by humans, the quantity of microplastics that enter the body through food is rising. There has not been enough information released on the toxicity of microplastics that build up in the body yet [14,15]. Large trash is simple to collect and analyze, but microplastics, which are often described as particles less than 5 mm in diameter are more difficult to analyze [16]. Even for particles smaller in size than 500 μm , visual microplastic analysis is no longer an appropriate criteria; it is easy and affordable. In order to detect microplastic below 500 μm and to offer chemical proof that microplastic are synthetic polymers, chemical analytical approaches have become an essential component of microplastic research.

Due to their precision and spatial resolution, high-tech solutions utilizing imaging spectroscopy have established themselves as the “golden standard” but they are costly and tedious [17]. Moreover, it is a difficult process that requires the use of expensive and sophisticated equipment to identify and classify microplastics in the environment. Many methods for the analysis of microplastics have been introduced recently and their advantages and disadvantages of the techniques have been summarized in Table-1. However, the data on microplastics

gleaned from these detection techniques is typically disordered and unreliable. This review describes techniques for researchers to find new possibilities for the development of techniques to analyze micro and nanoplastics in environmental samples. Fig. 1 illustrated the various sophisticated analytical techniques for environmental micro and nanoplastics.

Microplastics sampling techniques: It is important to remember that the microplastics sample techniques have a significant impact on the analytical results [18]. As a result, collecting microplastic samples is crucial to the examination of microplastics. Consequently, we will initially explore several standard procedures for sampling microplastics before advancing to the analytical techniques. The techniques that are presently commonly employed to extract microplastics from the environment which include flotation, visual inspection, density separation, digestion method, sieving or filtration, chemical treatments, ingestion and biological removal and so forth [9,19].

When it comes to biological samples, they are mashed, dissected or subjected to enzymatic digestion in the presence of phosphate buffer saline to avoid sample rupture. Following the centrifugation of each under the appropriate circumstances

TABLE-1
THE PROS AND CONS OF THE CURRENTLY USED MICRO AND NANOPLASTICS DETECTION TECHNIQUES

Detection techniques	Pros	Cons
Visual examination	An established technique for determining and measuring microplastics. The low cost, ease of usage, and less chemical risk are the benefits of visual analysis.	Visual examination is a hard and time-consuming method. Furthermore, the physical testing method is no longer applicable when samples contain both organic and inorganic contaminants, or when microplastic particle sizes are too small. The accuracy and efficiency of the visual analysis technique are substandard. It is impossible to determine the chemical makeup of microplastics by visual inspection. Visual analysis is usually used as a supplemental method while looking at microplastics.
SEM-EDS	A potentially useful method for simultaneously evaluating the elemental composition and surface structure of microplastics.	The pretreatment procedure is intricate. Additionally, the work efficiency is low and the method is expensive. Moreover, the estimated amount of microplastics is not particularly accurate, and the color of microplastics cannot be effectively discriminated. It is important to note that this technique is primarily utilized to detect specific microplastics.
FTIR spectroscopy	Vibrational spectroscopy is a type of analysis that reveals details about chemical bonds and functional groups in materials. It is commonly used for qualitative analysis and evaluating microplastics.	This method is only effective for detecting microplastics that are larger than 20 μm . Additionally, various circumstances can easily affect the reliability of this approach.
Raman spectroscopy	An alternative approach to vibration spectroscopy is inelastic light scattering. Additionally, Raman spectroscopy can be used to detect microplastics that are smaller than 20 μm . Moreover, there is no requirement to dry or dehydrate materials before detection. This method is commonly employed for the qualitative detection and assessment of microplastics.	Raman spectroscopy has a longer detection time compared to other methods. Additionally, it needs to be improved for the study of microplastics.
Thermal analysis	A method for analyzing components is to examine their properties by considering time and temperature. This technique can be used to determine the chemical composition and concentration of microplastics as a whole.	The procedure for pretreating the samples is time-consuming, and it cannot be used to analyze the physical characteristics of microplastics due to the damage it causes to environmental materials.
Mass spectrometry	A critical approach for detecting polymers in microplastics. Polymer mass spectra can provide useful information on polymer structure, molecular weight, polymerization degree, main functional groups, and end group structure. This approach may be used to analyze chemical characteristics and quantify microplastics in environmental samples.	The applicability domain of this method is limited. Additionally, it is ineffective in quantifying all microplastics in the surrounding environment.

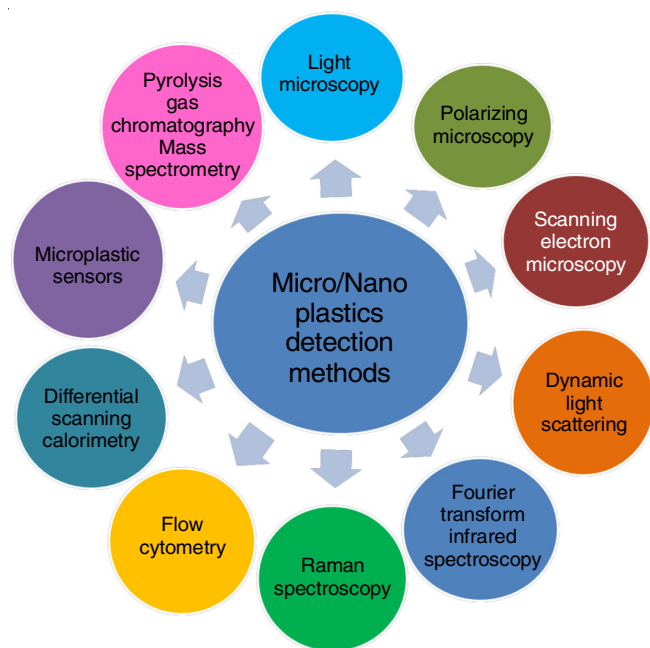


Fig. 1. List of various sophisticated analytical techniques for environmental micro and nanoplastics

to exclude any undesirable debris, an aliquot of the supernatant is examined utilizing detection methods [20]. In order to avoid losing any microplastics from the sample or underestimating the amount that are present, proper cleaning and filtration following the digestion process should be examined. Cross-contamination can be avoided by purifying samples to eliminate additional organic pollutants and preventing contamination and by employing advanced techniques for detection.

Chemical digestion: Some researchers use chemical digestion methods to break down organic matter in soil samples, leaving behind microplastics, which can then be separated and identified using microscopy or spectroscopy techniques. Microplastics can be extracted from the soil by using mild digestion to remove organic molecules. The ability of several digestion treatments to break down soil with varying amounts of organic matter was studied. About 10 g of soil samples were taken in triplicate and placed in the conical flask for digestion. A 200 mL of 30% H_2O_2 should be added gradually and incrementally to the conical flask holding the sample. In order to avoid an excessive rise in temperature, the conical flasks were immersed in a bath of cold water. To prevent H_2O_2 and dirt from clashing, the hot plate was used to heat the conical flasks to 70 °C, while being constantly swirled. To a flask, a few drops of butyl alcohol were added, when there was too much foam. The sample should be heated for about 12 h or until it becomes grey and becomes dry. The flasks were let to cool after digestion before the flotation procedure began [21].

In a conical flask, 30% H_2O_2 was used to wash the filter paper that contained microplastics after it had been filtered. Post-digestion is employed to separate microplastics from the agricultural soil, wherein the organic matter is eliminated prior to perform the qualitative analysis. The flask was brought to room temperature and put in an ice bath after the addition of 30% H_2O_2 and H_2SO_4 . Paper filters were used to filter the mixture

and then the filters were dried at 50 to 60 °C. By using Fourier transform infrared spectroscopy-attenuated total reflectance (FTIR-ATR), the extracted microplastics on filter paper was recognized after being seen using a stereo microscope [21].

Density separation with salt solutions: Density separation techniques involve suspending soil samples in a solution with a known density (e.g. salt solutions) to separate the microplastics, which often float, from denser soil particles. Owing to the lower density of microplastics, density floatation is frequently used to remove the minute plastic particles found in soil. Sodium chloride was used in this procedure as a density-separation solution. The soil sample was pre-digested and NaCl (200 mL) was added. The conical flask was then tightly capped and shaken for 1 h at 200 rpm in an orbital shaker. Shake well, let settle for 48 h and then pour off 150 mL of supernatant into a conical flask containing 100 mL of NaCl solution. The flask was again stirred for 30 min and finally, allow the mixture to stabilize and collect the residual liquid [21].

Filtration and sieving: Soil samples can be filtered through fine mesh or sieved to separate larger particles from microplastics. The retained particles can then be visually inspected or analyzed using other techniques. Numerous membrane filtration techniques, including dynamic membrane, reverse osmosis, microfiltration, ultrafiltration and membrane bioreactors, have been employed to manage the microplastics contamination. Furthermore, drinking water treatment plants (DWTPs) employed media filtration methods, including sand filtration and the application of activated carbon particles. Ma *et al.* [22] asserted that since the ultrafiltration membrane's pore size is smaller than the microplastics particle size, polyethylene (PE) microplastics may be entirely eliminated even in the absence of coagulation co-treatment [23]. However, adding coagulants would lead to filter membrane fouling in this procedure, particularly when small-sized microplastics are involved [22]. Comparably, another research demonstrated that polystyrene (PS) microbeads, ranging in size from 0.1 to 18 μm , were eliminated using filter membranes; however, large quantities of the smaller microplastics might worsen membrane fouling. Larger microplastics particles, on the other hand, often created a filter cake with greater porosity that reduced resistance to the clean the passage of water. Membrane filtering was often used in conjunction together with alternative technologies. As an instance, in DWTP, membrane filtration was frequently after which comes the coagulation treatment process. A prior study shown that membrane pollution can be considerably reduced following the action of coagulants based on aluminum such as aluminum sulfate (alum) or polyaluminum chloride (PACl). Microplastics might be effectively removed using ultrafiltration technology; however there are significant drawbacks, including membrane fouling, limited membrane flow and with the necessity of trans membrane pressure.

Membrane filtration: Dynamic membrane technology has several benefits over other filtering methods, such as cheap cost, low energy usage and ease of cleaning. Its primary method of operation depends on capturing impurities *via* a layer of filter cake. Contaminants captured by a large pore mesh serving as a support structure make up the filter cake layer [24]. This kind

of dynamic membrane is readily cleaned by backwashing and can operate in a vacuum-free environment under gravity [25].

However, one drawback of dynamic membrane is that it would cause cake layers due to captured impurities. The prior research suggested that the microplastic particles might cause a cake layer thickness to rise linearly with transmembrane pressure, which would have a major impact on the membrane performance [26]. A recent research found that dynamic membranes created a diatomite platform with a supporting mesh 90 μm in size. Following filtering treatment, the influent turbidity of water decreased from 195 nephelometric turbidity (NTU) to less than 1 NTU, demonstrating the successful removal of microplastics from the water [26]. Furthermore, it has been shown that rapid sand filtration (RSF), a DWTP technique that is frequently employed, has some removal effectiveness when it comes to large-scale microplastics from water [27,28]. According to a recent research, microplastic removal effectiveness ranged from 29.0 to 44.4% following sand filtration's interception action. However, the effluent of RSF did not include any small-sized microplastics, particularly those smaller than 10 μm . Furthermore, DWTP frequently employed activated carbon filtration as a cutting-edge treatment strategy. An activated carbon filtering treatment resulted in a removal rate of 56.8-60.9% for microplastics; notably, small-sized microplastic particles in the size range of 1-5 μm had a remarkably high removal efficiency of 73.7-98.5%.

Membrane bioreactors (MBRs), which combine membrane filtering technology with biological catalyst application, are commonly used [29]. For the elimination of microplastics, MBR has been shown to be more successful than either dynamic membrane technology or a single biodegradation treatment. Pretreatment, membrane filtration and bioreactor are only a few of the processes involved in MBR. Michielssen *et al.* [30] recently conducted a research at three WWTPs to assess the efficacy of several unit processes, such as MBR, tertiary treatment by granular sand filtration and secondary activated sludge treatment, in eliminating microplastics. Based on their findings, MBR had the maximum removal effectiveness of microplastics, which was 99.4%. Specifically, there were comparable amounts of microplastic fibres in the raw and effluent in the secondary treatment and the percentage of fibres removed was around 30% in the MBR process following sand filtering. More over half of the microplastics in the influent of the WWTP were fibres; nevertheless, removing them proved to be more challenging than removing other microplastic morphologies such as particle, bead and foam [31]. Talvitie *et al.* [32] found that in compared to other removal techniques as quick sand filtration and dissolved air flotation, MBR produced a comparatively high effectiveness for microplastics elimination, even exceeding 99.9%.

Flotation method: The flotation approach, one of several techniques for removing microplastics from the environment, will be crucial in doing so [33]. Microplastics can be segregated by the flotation method based on their wettability. Microplastics exhibiting hydrophobic surfaces are extracted from the flotation column by bubbles, whereas those with hydrophilic coatings descend to the column's base, facilitating the sorting of various microplastic kinds through flotation. For microplastics with com-

parable densities and charging characteristics, the flotation technique can be utilized for separation. The benefits of the flotation process are strong separation selectivity, cheap cost, simple equipment structure, superior purity and efficiency of separation [34]. For example, Grbic *et al.* [35] used silane to modify the surface of iron nanoparticles to make them hydrophobic. Microplastics were subsequently magnetized using these surface-hydrophobic iron magnetic nanoparticles. Eventually, the process of magnetic separation of 15 μm -sized microplastics involved adsorbing iron nanoparticles that had been adsorbed with the microplastics to the liquid surface using a magnet.

Magnetic separation: In some cases, magnetic properties of microplastics can be exploited to separate them from soil samples using magnets. Magnetic separation has been created recently to remove microplastics. Large specific surface magnetic nanoparticles were utilized as adsorbents in the removal procedure to mix with microplastics; magnetized microplastics can then be swiftly and readily removed from water by magnetic force. There are several different kinds of mechanisms involved in the magnetization activity of microplastics. The adsorption of magnetic carbon nanotubes (M-CNTs) by polyamide was primarily caused by the high hydrophobicity of microplastics and the combination of π - π electron interaction, electrostatic interaction complexation and hydrogen-bond interaction [36]. Over 90% of microplastics may be eliminated using magnetic biochar, M-CNTs and magnetic polyoxometalate-supported ionic liquid phase (magPOM-SILP) [37]. The following factors might affect the removal efficiency: the kind and size of microplastics, water pH, magnetic particle concentrations and other variables [38]. A recent study indicates that microplastics can be magnetized and subsequently extracted utilizing nano- Fe_3O_4 and the adsorption properties of magnets. According to Shi *et al.* [39], the elimination rates of four basic kinds of microplastics namely PS, PP PE and PET, ranged from 62.83 to 86.87% on average. The development of acidic oxygen-containing groups on the surface of carbon nanotubes reduced the hydrophobic impact of PE/PET and M-CNTs; yet, in a different research, it was demonstrated that the positively charged polyamide microplastics enhanced the electrostatic repulsion between M-CNTs and polyamide microplastics in an acidic environment [36]. An increase in the concentration of magnetic particles may lead to more microplastic collisions and the development of microplastic combinations. Furthermore, a high ion concentration may lessen the magnetic particles electrostatic repulsion effect, which would encourage their binding to microplastics [40]. Furthermore, super-paramagnetization, which induces desorption and regeneration of magnetic materials, often requires magnetic particles. Moreover, it is generally necessary to incorporate a substantial quantity of magnetic adsorbents to ensure that their concentration exceeds that of microplastics in water [39]. Therefore, the search for evidence of the total elimination of additional magnetic components post-treatment persists. Centrifugation was frequently utilized in the laboratory to accomplish this compound [36]. DWTP and WWTP practical implementation is challenging, nevertheless. Recycling and regenerating magnetic materials is a smart tactic. For instance, M-CNTs were shown to be recyclable by heat treatment at

600 °C and were even employed to eliminate around 80% of microplastics following four cycles of use [36]. To confirm the efficacy of this method, it must be employed extensively. Consequently, it is essential to meticulously consider the elimination of magnetic adsorbent residue to mitigate any environmental risks.

Microplastics identification and quantification methods

Physical characterization: Hidalgo-Ruz *et al.* [41] stated that physical evaluation study of microplastics may be used to identify the particle shape, size, colour, morphology and corrosion degree, preliminary typing and ageing degree of microplastics. The physical features analysis approaches of microplastics are briefly introduced here. These techniques include laser diffraction particle size analysis, dynamic light scattering analysis and visual evaluation [42,43]. Fig. 2 shows the analytical procedure framework for the analysis of microplastics.

Laser diffraction particle size analysis: A wide range of advanced devices has been developed due to the rapid advancement of material science. The examination of soil and sediment particle size distribution may be done with laser diffraction particle size analysis, which is a quick, dependable and computerized technique [44]. The accuracy, resolution and detail of the experimental data are excellent. Essential samples can be retrieved using this largely non-destructive procedure [45]. Particles in the size range of 0.04–2000 µm may be examined using this approach [45]. However, certain contaminants in ambient samples might skew the outcomes of the investigation. Furthermore, the purpose of employing this method is to eliminate and separate the microplastics that are found in the surrounding environment. Though this approach hasn't been applied extensively in the detection of microplastic granular distribution, as technology advances, it will ultimately become a crucial tool for doing so.

Dynamic light scattering: Plastic particles that are smaller than 1 µm are referred to as nanoplastics [46]. The examination of nanoplastics can be greatly enhanced by the use of dynamic light scattering (DLS) analysis. Sorasan *et al.* [43] conducted dynamic light scattering study and found that solar photochemical ageing could lead additional microplastics to transform into nanoplastics. Still, some contaminants in ambient samples might skew the findings of the experiment, similar to laser diffraction particle size analysis.

The samples were diluted in phosphate buffer saline (PBS) to a concentration of 50 µg/mL prior to testing. Samples were subjected to multi-angle dynamic light scattering (MADLS) using the Zetasizer Ultra (Malvern Panalytical, UK). Typically, 1 mL of sample was placed into a DTS0012 cuvette. To account for light scattering from the front (13°), side (90°) and rear (173°) of each sample, detectors were positioned at three distinct angles to quantify the particle size mode, size distribution and particle concentration. Every sample was examined at every angle in triplicate, utilizing adaptive correlation on every measurement, in an effort to improve the overall quality of the data [47]. The Zetasizer Ultra research includes total concentration in addition to an intensity- and volume-weighted particle size distribution. The samples D-F underwent DLS studies using the Malvern Zetasizer ZS at two different wavelengths, 633 nm and 532 nm, in compliance with the SOP developed by the European Nanomedicine Characterization Lab (EUNCL) [47]. Visual inspection may only be utilized for the preliminary detection of microplastics with a particle size of 1–4 mm [48].

Microscopy techniques

Light microscopy: Microplastics which are less than a few hundred micrometres are commonly identified using light microscopy. The physical response qualities of microplastics,

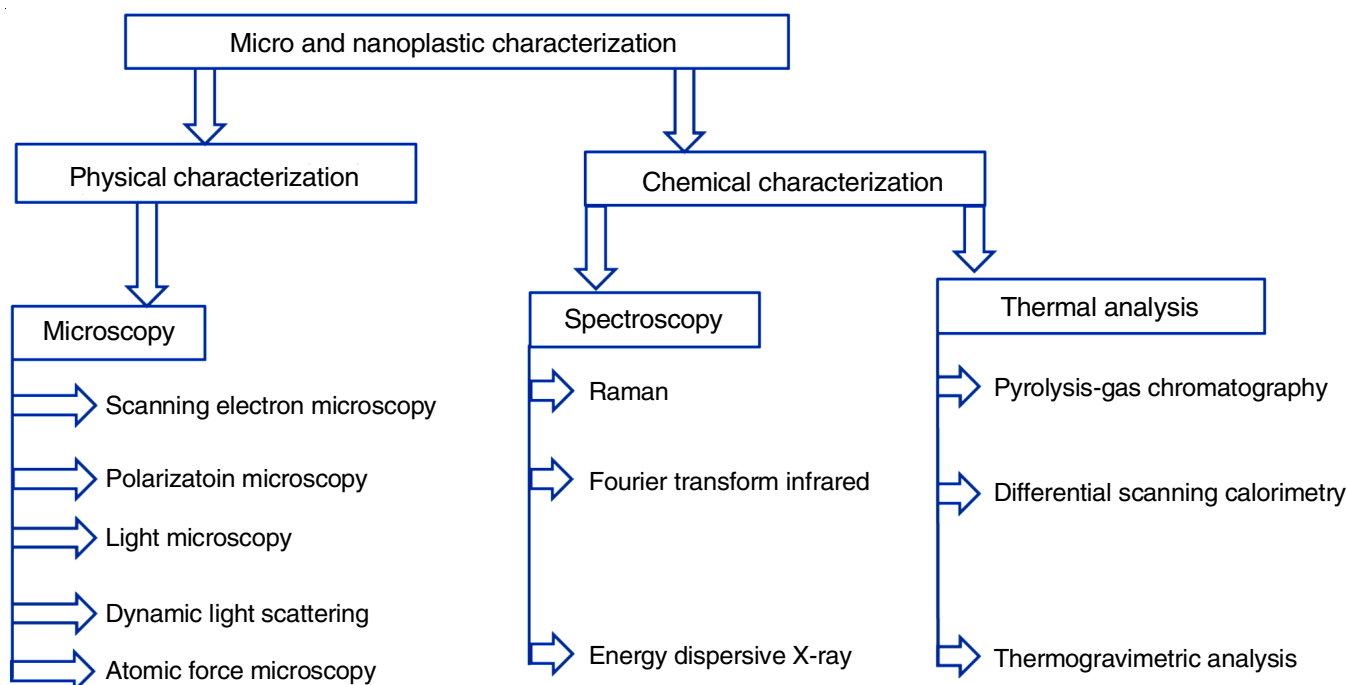


Fig. 2. Physical and chemical characterization techniques for micro and nanoplastic analysis

for particular elasticity or hardness, are used to identify them since they often lack lustre [49]. Microplastic samples can be obtained with this predominantly non-invasive method [50]. An optical microscope can be used to identify the coloured polymer compounds produced with the addition of dye [51,52]. Determining the characteristics of colourless or non-obviously shaped plastic particles in the sub-micrometer size range is a challenging task. It can also be difficult to identify the microplastics under the microscope if sample particles are not separated well enough. Furthermore, sediments and living elements that are not completely removed by chemical breakdown make it difficult to evaluate microplastics under a microscope. According to earlier research, the percentage of microscopic misidentification for plastic-like particles is more than 20%, while for transparent particles; it is higher than 70%. Natural and synthetic fibres are comparable particles with interfering characteristics that make it challenging to tell them apart using a microscope alone.

In an attempt to detect particles, destructive tests have been carried out; nevertheless, cotton fibres are frequently mistaken for plastic. To make up for this, studies have employed a technique to detect such particles: the plastic particles melt when they come into contact with a heated needle [48,53]. Bleaching compounds are the most often utilized plastic additives in the textile and plastic synthesis industries. A fluorescence microscope may be used to identify plastics since whitening agents typically exhibit fluorescence. Due to some compounds ability to self-fluoresce among minerals, detection mistakes may still happen [52].

Optical microscopy: Traditional optical microscopy can be employed to visually detect larger microplastics in soil. This technique is effective for particles larger than 100 micrometers. Several hundred micrometer-sized microplastics may be identified using light microscopy. The physical response qualities of microplastics, such as their particular hardness as well as elasticity, are used to examine them since they often lack lustre [49]. Furthermore, microplastics often appear in the environment as fibres, pieces and beads [50]. An optical microscope may be used to recognize quickly coloured polymers to which dye has been added during production [52]. However, it is difficult to characterize the colourless or asymmetrical plastic particles in the size range below 100 μm . Furthermore, the microscopic detection of microplastics may be hampered by the insufficient separation of sample particles. Moreover, the sediments and biological materials that are not completely removed by chemical degradation make it difficult to discern microplastics under a microscope. According to earlier research, transparent particles are misidentified at a rate of more than 70% and plastic-like particles at a rate of more than 20%. Under a microscope alone, it might be challenging to discern between synthetic and natural fibres since they are identical particles with interfering elements. There have been “destructive tests” to identify the particles, although cotton fibres are frequently mistaken for plastic. To make up for this, studies have developed a technique to detect these particles that involves putting the particles in contact with a heated needle, which causes the plastic particles to melt [53]. Whitening agents are the most widely used plastic additives, used in both the textile and plastic

synthesis sectors. A fluorescence microscope can be employed to detect plastics, as bleaching agents generally display fluorescence. However, detection errors may still occur due to certain minerals containing chemicals that demonstrate self-fluorescence [52].

Electron microscopy increased magnification and crisper imaging allow it to differentiate microplastics from particulate contaminants more easily than optical microscopy [48]. As per Wagner *et al.* [54], the resolution of a SEM, which is widely employed, may attain 0.1 μm . When identifying microplastics with particle sizes as small as 1 nm, SEM can be utilized [48]. Consequently, to the lower identification error rate of microplastics, electron and optical microscopy are frequently coupled.

Scanning electron microscopy (SEM): A promising method for analyzing the surface morphology and elemental composition of microplastics concurrently is SEM-EDX [55,56]. SEM can provide high-resolution images of soil particles, allowing researchers to visually identify and characterize the microplastics based on their surface features. Even for extremely small particles, such as nanoplastics, SEM may provide pictures that are crisp and magnified several times [57]. It is simple to judge the organic particles from microplastics by the texture of the particle surface, which may be verified by high-resolution images [58]. Microplastics are also identified using transmission electron microscopy (TEM) or transmission electron microscopy. Although the visual identification of microplastics by TEM could change based on user, the data may be cross-validated to reduce errors from several users using a tried-and-true technique. Al, Mg, Na, Ca, and Si serve as examples of antioxidant or additive components that are collected as markers for the purpose of microplastic identification through EDX analysis [54]. Carbon-rich polymers may be distinguished from inorganic particles by analyzing a particle surface elemental composition [50]. However, there is a limit to the number of samples that can be processed by TEM and SEM due to the costly equipment and the time- and labour-intensive sample preparation and inspection operations.

Nevertheless, SEM-EDX still has certain drawbacks. First and foremost, conductive specimens are a necessary need for the analysis of samples utilizing SEM-EDX technology. However, the majority of microplastics fail to carry. Consequently, in order to analyze microplastics utilizing an SEM-EDX, intricate processes for pretreatment like gold plating are needed [59]. Furthermore, the colour of microplastics cannot be determined by SEM-EDX [54] and also due to the technological constraints, SEM-EDX can sometimes be insufficient to detect microplastics [54].

Fluorescence microscopy: Fluorescence microscopy, combined with fluorescent dyes that bind to plastics, enhances the visibility of microplastics, making them easier to detect. Since bleaching chemicals usually display fluorescence, plastics may be detected with a fluorescence microscope. Nevertheless, whitening chemicals can also be utilized on paper. However, identification mistakes may happen because certain minerals encompass chemicals that display the self-fluorescence feature [52].

Polarizing microscopy: In toxicity investigations, polyethylene (PE) particle identification has been accomplished with

success using polarized light microscopy [60]. The measurement of the transmission of polarized light can be influenced by the crystal structure present in the plastic [61]. The extent of crystallinity varies based on the kind of polymer and the production method, even within the same polymer. To enable enough polarized light to go through, microplastic samples must be thin. Specimen of translucent microplastics cannot be processed using this procedure.

Chemical characterization: Microplastics often consist of complex and diverse combinations of heterogeneous plastic particles. The process of identifying the chemical composition of microplastics involves employing specific techniques to ascertain the polymerization degree, structure, molecular weight and functional groups of the polymers present in the microplastics. Determining the chemical makeup of microplastics is crucial for both the provenance analysis and the choice of treatment techniques [62]. Today, methods such as FTIR [62], SEM-EDX [56], thermal analysis [63], Raman spectroscopy [64], mass spectrometry [65], *etc.* are frequently used for the chemical composition analysis of microplastics. In this part, several common techniques as well as a few useful tools like novel advancements will be presented.

Raman spectroscopy: The Raman spectroscopy technique also provides a specific spectrum for every polymer subjected to laser radiation, as the molecular structure and atom count of the target determine the backscattered light's frequency. Moreover, Raman spectroscopy, like FTIR, distinguishes between polymers and gives a polymer composition profile for every sample [66]. With the use of data libraries and algorithms, it is possible to deduce the outcomes that are acquired [67]. When it comes to the integration of non-destructive chemical analysis with microscopy, Raman spectroscopy and the FTIR approach are comparable [68]. The shift in the polarizability of molecular chemical bonds results in the production of Raman spectra, a type of signature that carries the distinctive characteristics of the materials to be examined [51].

Raman spectroscopy allows for the identification of microplastics as tiny as a few micrometres in size due to the smaller diameter of the laser beam. The benefit of using Raman spectroscopy for non-invasive evaluation is that the microplastic specimen may be preserved prior to as well as following the evaluation for potential future examination. Raman spectroscopy presents a challenge in identifying the target polymer type since the resultant signal is susceptible to compounds used in microplastics pigments and preservatives [69]. Furthermore, the surface curvature of worn microplastics may give rise to inaccuracies. However, there is still room for improvement when it comes to the ability of Raman spectroscopy to identify microplastics. Raman spectroscopy has seen a number of advancements recently. Araujo *et al.* [64] achieved automated identification of microplastics with particle sizes ranging from 1 μm to 500 μm by combining optimized algorithms with Raman spectroscopic technology. This greatly enhanced the capability of Raman spectroscopy to determine microplastics. In future, the characterization of microplastics in actual specimens will be heavily dependent on Raman spectroscopy [64].

Fourier transform infrared spectroscopy: Using FTIR spectroscopy, one may ascertain the precise chemical bonds between individual particles. With this technique, carbon based polymer compounds are rapidly recognized. Various binding combinations in polymers chemical compositions give rise to a distinct spectrum that sets them apart from other organic and inorganic particles [70,71]. Moreover, firmly rooted polymeric spectrum libraries may be used to identify plastics [72,73]. The quantification of non-plastic particles may be prevented by using infrared (IR) spectra to arrange information for each plastic. This also lowers the possibility of finding plastic particles that lack a distinct texture or colour. Furthermore, the substance's makeup is revealed by FTIR authentication that might offer hints about the specimen's provenance and mode of manifestation [74].

Micro-FTIR, which encompasses both characteristics and enables the microscopic viewing of microscopically, sized plastic-like fragments before spectroscopic validation on just one system, makes microplastic identification simpler [49]. Furthermore, there are a lot of benefits to using FTIR spectroscopy for analyzing microplastics. Firstly, specimens from the environment are less adversely affected by the non-invasive FTIR technique [75,76]. Secondly, the autofluorescence from the additional substances in samples is less likely to disrupt with FTIR spectroscopy. Moreover, sustainability and rapid identification are two benefits of FTIR method [64]. Currently, quantitative monitoring and chemical ingredient evaluation of microplastics are often conducted using FTIR spectroscopy [10,77]. However, specimens must have completely dried before being analyzed using FTIR spectroscopy, as moisture in the specimens might tamper with the diagnosis.

Nevertheless, the FTIR spectroscopy investigation of microplastics continues to have certain limitations. Initially, the FTIR technique can only detect microplastics with particle dimensions greater than 20 μm , as the spectral resolution of this technique is limited to 10-20 μm [78]. Furthermore, the diverse composition of microplastics, their degree of ageing and other organic materials in the environment can all affect FTIR spectroscopy. For instance, using FTIR spectroscopy to analyze transparent or black microplastics is challenging [79]. In order to identify microplastics in a vast area with high accuracy, Ojeda *et al.* [80] established a technique called focal plane array-FTIR spectroscopy. In order to sum up, more advancement in FTIR spectroscopy is required for a more accurate determination of microplastics in actual environmental specimens.

Thermal analysis: Thermal analysis, a method that assesses alterations in the chemical and physical properties of polymers with heat resistance, has been used in recent years to assess approaches for microplastic detection. These techniques rely on finding polymers in the degradation products of the material.

Differential scanning calorimetry: In order to validate the physical characteristics of polymers, it displays variations in crystallization, transition temperatures, dissolution and the associated enthalpy and entropy [81]. It is possible to distinguish between different polymer kinds using DSC since every plastic product has unique features [82]. When DSC detects

microplastics with comparable melting points, there are restrictions because of overlapping peaks [83]. Only a few basic microplastics, such polyethylene (PE) and polypropylene (PP), can be identified using DSC [84].

Thermogravimetric analysis: The thermogravimetric analysis (TGA) method, which verifies the qualitative and quantitative information of the sample by analyzing the dependency on temperature and time, measures the weight reduction in the sample as it is heated at a specific rate under predetermined atmospheric and temperature conditions [85]. TGA and DSC were used in conjunction in earlier research to analyze microplastics in wastewater. But in these investigations, only PE and PP were easily distinguished. There was no identification of polyester (PES), polyamide (PA) or polyvinyl chloride (PVC). It is challenging to differentiate phase transition signals that overlap like those of polyurethane (PU) and polyethylene terephthalate (PET) [63,86].

Pyrolysis-gas chromatography mass spectrometry: Py-GC-MS is an alternate method for assessing pyrolyzed gases in polymers [87]. An established polymer standard can be used to compare a sample pyrogram to determine whether or not it is plastic. GC-MS analysis is performed on a very small number of plastic samples that have been pyrolyzed at a temperature greater than TGA [48]. In bulk examinations of suspended solid particles, sediment polystyrene (PS), styrene butadiene rubber (SBR), poly(acrylonitrile butadiene styrene) (ABS), polyvinyl acetate (PVA) and PVC are all present. Only derivatives of PS may be identified as microplastics using Py-GC-MS. The search for molecular markers for different kinds of plastics requires more research [88]. Fig. 3 illustrates the analytical techniques commonly used for microplastics analysis based on particle size.

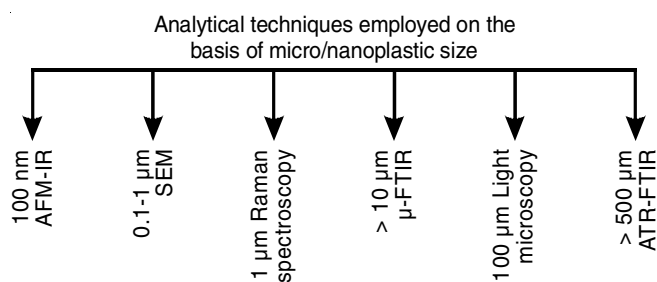


Fig. 3. Analytical techniques frequently used for micro and nanoplastics analysis based on particle size

Novel advancements: The current difficulties in identifying microplastics should be overcome by developing new analytical tools and combining cutting-edge detection technologies with already-in-use instruments. The maximum detectable size is one of the problems in microplastic analysis that has to be resolved. Contemporary analytical techniques have a minimum observable dimension limit of several micrometres. It is of greater significance nowadays to understand the existence, distribution, toxicity and destiny of nanoplastics since smaller plastic particles can have greater harmful consequences. As a result, protocols for nanoplastics sampling, extraction, purification and concentration should be established with novel identification methods.

Automated image analysis: Advanced image processing software and machine learning algorithms are increasingly being used to automate the identification and quantification of microplastics in soil samples based on visual characteristics. Micro-Fourier transform infrared (mFTIR) microscopy imaging data processing is a potent technology that makes it possible to analyze microplastics that are enriched on membrane filters. An automated method to lessen the amount of time currently required for data analysis. A novel analytical pipeline was developed utilizing Python and Simple ITK image processing modules for image analysis, based on Bruker's OPUS® Software. The analysis of datasets from focal plane array (FPA) mFTIR mapping of samples with up to 1.8 million single spectra was made possible by this recently created workflow. Considering a range of methods and quality and accuracy-focused benchmark tests, all spectra were compared to a database of various natural and synthetic polymers. Image analysis was made possible by the optimization of the spectral correlation for the development of high-quality data. These findings led to the development of an image analysis method that gave details on the sizes and quantities of particles for each polymer found. Even for complicated sample matrices, gathering all the data was rather simple. This method significantly improves the quality of the data while also reducing the amount of time needed to evaluate complicated FTIR imaging data [89].

Recently, hyperspectral imaging (HSI) has been used to analyze microplastic data, with promising outcomes. HSI has been used for material identification in a number of disciplines since it was first created for remote sensing of the earth's surface [90-92]. Most significantly, the recycling sector frequently uses HSI to classify plastics based on the kind of polymer [93]. This proof of concept shows promise and has direct connections to microplastic analysis. The foundation of a hyperspectral image is the same as that of a conventional RGB image. I rows and J columns of a matrix can be used to represent a picture, giving it dimensions of $I \times J$. The image's size is represented by these two spatial dimensions. Every item in the matrix corresponds to a single pixel. Pixels are actually point measurements, even if they are represented by squares [94]. The term "squares" is more accurately defined as the spatial resolution, in which each pixel (point measurement) denotes a separate area. Every pixel in the electromagnetic spectrum represents a location in actual space that is both absorbing and reflecting light. The intensity of reflected light is recorded as a number. A greyscale picture with high intensity shown as white and low intensity as black is produced when just one wavelength is captured. Three wavelength bands are used in colour images to indicate how much light is reflected by an item in a particular area of the electromagnetic spectrum. These bands of colour represent visible light in the wavelength range of 400-800 nm, which includes red, green and blue light. The sensitivity of the human eye to these photopigments is the reason these areas were selected. Red, green and blue (RGB) intensity values are combined to represent each pixel in a picture, giving the matrix three dimensions [95]. As previously, I and J are spatial, while K is the new, spectral third dimension. One way to see the three K components is as stacked layers of dimensions $I \times J$. The

resultant datacube is hyperspectral when the number of spectral bands exceeds 100. According to Goetz *et al.* [90], foundational study high-speed imaging (HSI) is defined as the acquisition of images in hundreds of contiguous, registered, spectral bands such that a radiance spectrum can be derived for each pixel. Multispectral imaging is not included in this definition as the bands aren't always contiguous. It is preferable to think of the outcome of this continuous sampling as a spectrum. Because this spectrum provides an incredibly accurate depiction of the many light wavelengths that an item reflects, pixels may be categorized based on their chemical makeup rather than merely colour.

Microplastic sensors: Some researchers are developing portable sensors and devices that can detect and quantify microplastics in real-time or in the field. These techniques can be used individually or in combination, depending on research goals and available resources. Advances in microplastic detection methods continue to improve the ability to investigate the existence and characteristics of microplastics in agricultural soil, aiding in better understanding their ecological and agricultural implications. It has recently been shown that it is possible to identify rough, transparent tiny plastics from their measured near-infrared (NIR) transmission spectra and from the dispersion of their refractive index. The study also suggested other optical techniques to deal with the difficult issue of microplastic detection in the aquatic environment [96]. In continuation, the current work examines the use of two distinct optical phenomena to identify transparent and translucent microplastics in fresh water that vary in size and form. The two polymers that are the subject are translucent low-density polyethylene (LDPE) and transparent polyethylene terephthalate (PET), which is frequently used for water bottles. Utilizing the interaction of a diverging laser beam with the microplastics to identify the various materials is made possible by the fact that coherent laser radiation may find minute optical imperfections along its path length.

A microplastic inside the cone formed by this diverging beam will have an impact on the optical characteristics of the microplastics, including their ability to reflect, refract, scatter, diffraction and transmit light. A portable apparatus that was previously proposed for liquid wetting investigations [97] is used for this purpose; however, it is modified to add a charge-coupled device (CCD) camera. The idea is to take advantage of the ability to detect the intensity of the specular component of the re-emitted light in the case of diffuse light reflection from translucent microplastic or light reflection from transparent smooth microplastic in the context of geometrical optics. In contrast, in the transmission mode, one detects the pattern formation of light interference from the microplastics. This novel prototype is suggested to have uses in the field of measuring microplastics in samples from rivers, lakes and seas as well as in filtration water samples from wastewater treatment facilities. Though currently intended for onshore measurements, this gadget may theoretically be adapted to function inside aquatic bodies to provide real-time data [98].

Asamoah *et al.* [98] presented a portable prototype optical detector for detecting the presence of transparent and opaque

microplastics in water. The forward interference pattern from the interactions between light and microplastics in water and the specular light reflection signal recorded simultaneously serve as the basis for the detection. For the various sizes, the clear PET produced a stronger specular signal than the translucent LDPE. In contrast, a speckle pattern was formed by the interference pattern being significantly deformed by the transparent LDPE. The size of the floating transparent LDPE contributed more to the creation of the speckle pattern and the amount of the specular reflection signal in the complicated scenario of two microplastics in the volume of water than it did in the case of a single microplastic. By combining these two optical detection techniques, one may determine the type of microplastic and estimate its size. Although the statistics from the training set are encouraging, more work has to be done on the prototype and analytic techniques. In theory, this prototype optical sensor might be utilized in fixed position sedimentation of PET to identify items at various sea, lake, or river depths [98]. Additionally, it may be utilized in wastewater treatment facilities to find floating and submerged microplastics of various kinds in water filter samples.

Electrochemical sensors: The detection of environmental contaminants, including leachates from microplastic, has been a common application for electrochemical sensors [99]. However, the use of these sensors for microplastic detection is very uncommon and is just now receiving attention. The creation of electrochemical sensors for microplastic is highly intriguing because to the devices' quick reaction times, ease of use, portability and affordability. Unlike other conventional procedures, the electrochemical methods may easily be extended to infield testing of various sample types and do not require prior microplastic separation or purification. Microplastic is now detected and monitored using voltammetry, amperometry and label-free electrochemical impedance spectroscopy (EIS). PE microplastics were found by combining flow cytometry with an EIS-based sensor [100]. The sensor was made up of a particle detector flow cytometer and an EIS detection flow cell with Au-plated circuit boards that held all Au-plated electrodes. The underlying principle of this method was that, at low frequencies, the real impedance should vary in accordance with the volume of particles. Consequently, the microplastic particles flowing across electrodes cause the change in impedance at low frequencies, which is proportional to the volume of the microplastic particles. Using this technique, the plastic and biological particles may be distinguished at any measured frequency.

It is significant to observe that biological particles resulted in a negative alteration in impedance, but microplastics caused a positive alteration. This suggests that this platform might be utilized to detect microplastics in complicated media. The pure water flow was monitored in real-time and compared with water that had been tainted with known microplastics in order to determine the sensitivity and ability of sensor to identify and discriminate between various microplastic sizes. If microplastic was present in the solution being tested, the impedance would change, resulting in peaks that would allow the particle size to be measured. The relationship between the impedance change and the particle size was established using the linear

relationship between the particle diameter and the cube root of the actual impedance change. Finally, the PE microplastics (212-1000 μm) and microplastic beads (210-1200 μm size range, comprising biological components) could be measured and quantified using this sensor. The sensor showed a recovery rate of more than 90% for microplastic in the 100-300 μm size range and a 1% false positive rate when it came to classifying biological materials as microplastics [100]. Furthermore, using particle impact electrochemistry, spherical PE microplastics (1-22 μm) have been detected [101]. The particle-electrode impact method is a widely used method for examining particles suspended in solution. A rapid current response was seen during chronoamperometry testing when the particle collided with the carbon fibre microwire electrode. To find the appropriate response, an undivided three-electrode setup was kept at a certain voltage in the electrochemical analysis setup. The observed change in signal was caused by a collision between particles and electrodes.

Conclusion

Various analytical techniques are required for the identification of micro and nanoplastics in complex environmental samples. The smaller the particles, the longer it takes to identify them. Understanding the risks and impacts of microplastics on the environment and human health increasingly relies on sub-micron studies. To meet the growing need for monitoring microplastic contamination, it is crucial to develop new techniques and improve existing ones in order to reduce identification time and effort. Additionally, precise and practical identification methods are essential for detecting and quantifying nanoplastics in environmental samples. Future research should focus on developing a fully or partially automated analytical method that combines chemical analysis to identify the individual plastic components with image analysis-based approaches to determine the physical characteristics (such as shape and size) of microplastics. Moreover, synthetic cellulose fibers, referred to as polyester in Europe and rayon in the United States, contaminate the environment in a manner that is similar to microplastics. Therefore, when studying microplastics, it is important to consider this aspect as well. To avoid confusion, it is necessary to investigate the characteristics that distinguish microplastics from similar materials. Furthermore, ongoing research should continue to develop new dyeing methods, nanotechnology and analytical tools to detect and remove microplastics from environmental samples.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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