Discover Environment

Review

Current techniques for identifying, quantifying, and characterizing micro and nanoplastics with emphasis on strengths, limitations, and challenges

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Abstract
Microplastic and nanoplastic (MNP) contamination has infiltrated nearly every facet of life, including food, water, air, and soil. The persistence of MNPs within different matrices poses a significant environmental and human health risk, underscoring the necessity for standardised methods to detect, characterise, identify, and quantify these emerging pollutants of concern. Traditional detection methods, such as visual inspection and gas chromatography, have limitations and are thus unsuitable for rapid, repetitive analyses. As such, advanced techniques like Fourier-transform infrared (FTIR) spectroscopy, hyperspectral imaging, and artificial intelligence (AI) interfaces are becoming favoured. FTIR spectroscopy is the most popular method used today to identify and quantify MNPs. Furthermore, recent advancements in micro-FTIR (μ-FTIR)-AI image processing have enabled the semi-automation of MNP identification concentrated on different filter membranes without pre-sorting. Integrating AI tools in combination with μ-FTIR analysis can significantly boost its functionality as automated image processing facilitates quicker identification and quantification of MNPs. Overall, challenges remain in MNP detection due to plastic particle size constraints, contamination from organic matter, and a lack of standardised protocols, complicating the identification and quantification of these pollutants in environmental matrices and biological samples. Despite extensive research, there remains a pressing need for reliable, cost-effective analytical techniques. Moreover, establishing uniform definitions, characterisation methods, sampling techniques, risk assessment frameworks, and regulatory guidelines for MNPs is imperative for their effective management and regulation.

Keywords Microplastics · Nanoplastics · Micro-FTIR Spectroscopy Analysis · Artificial Intelligence Image Processing · Transmission Electron Microscopy

1 Introduction

The history of plastics dates back to 1869 when John Wesley Hyatt created the process of making celluloid. Leo Baekeland introduced the first synthetic plastic, Bakelite, in 1907. Its durability, moldability, water-chemical-heat resistance, and low conduction transformed manufacturing processes [1]. During World War II, plastics became essential for military applications, leading to the development of materials like Plexiglas and nylon. Post-WWII, plastics replaced conventional materials due to their cheapness, leading to new uses like polystyrene and polyethylene [2]. By 2023, plastic production reached 413.8

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million metric tons globally, leading to prevalent plastic pollution due to poor handling and improper disposal [3]. Efforts are ongoing to explore sustainable alternatives and improved recycling methods [4, 5].

Inappropriate disposal of plastics results in degradation through environmental factors and mechanical forces, leading to the fragmentation of larger plastics into smaller particles, known as microplastics (particles less than 5 mm) and nanoplastics (particles ranging from 1 to 1000 nm) [6], collectively referred to as MNPs. These MNPs can interact with biological systems and can be transported across environmental compartments like air, water, and sediment, posing risks to human and ecological health [7]. The propensity of plastics to transform in natural environments through fragmentation and aggregation, facilitated by weathering and ageing, necessitates the establishment of standardised experimental protocols for matrix sampling, detection, quantification, and characterisation. Protocols are crucial for uniform reporting, understanding degradation mechanisms, and identifying plastic types in different matrices, aiding environmental monitoring of pollutants [8]. However, the heterogeneity of environmental samples complicates the characterisation of MNPs, and the lack of standardised procedures hinders quality control and cross-study comparability. Key challenges include isolating MNPs from various matrices, reducing quantification errors, and developing universal protocols [9, 10].

Currently, methodologies are effective at the micrometre scale and for determining MNP clusters, but less efficient and more costly for nanoscale detection. MNP clusters are aggregates of tiny plastic particles formed through physical or chemical interactions, showing unique properties and behaviors relative to individual MNP particles [6, 11]. A thorough process of MNP sample cluster analysis, i.e., identification typing of individual MNPs in a cluster, remains elusive, further complicated by the physicochemical properties of individual plastics forming the MNP cluster [12, 13].

The diverse characteristics of MNPs, such as size, shape, and composition, further complicate the standardisation process and require varied assessment methods. Other developed quantification techniques like dynamic light scattering (DLS) and transmission electron microscopy (TEM) also have limitations [14]. Although DLS is quick, it struggles with polydisperse samples, cannot distinguish between plastic and non-plastic materials, and can be skewed by environmental or aggregation contaminants. TEM provides high-resolution images but necessitates complex sample preparation, examines only a small portion, can damage soft plastic particles, and does not provide chemical composition unless combined with other methods [15–17]. Additionally, the lack of universally accepted calibration standards impedes measurement accuracy [10]. Therefore, there is a need to align this standard with regulatory requirements across different jurisdictions.

High costs associated with complex sample preparation, including destructive analyses linked to Pyrolysis-Gas Chromatography/Mass Spectrometry (Py-GC/MS), are undesirable. Chromatography is considered destructive in MNP analysis due to its destruction of the original particles' structure, making it impossible to subsequently analyse their physical characteristics such as shape, size, or surface features [18]. FTIR is more desirable due to its high resolution, specificity, versatility, and nondestructive analysis [19]. Combining these techniques with artificial intelligence (AI)-machine and deep learning can minimize human errors, provide consistent results, automate data analysis, and be cost-effective [20]. Other advantages include morphological and spectral analysis and MNP source tracking. The combination of these techniques with AI-machine and deep learning can also result in improved data analysis and automation [19, 21].

This review explores the key aspects of MNP analysis, including sampling methods, detection techniques, sample preparation, and pretreatment procedures. It discusses various analytical methods, including chemometric and machine learning approaches, and highlights the need for standardisation. The review uses literature from 2015 to 2025 to analyse the processes, benefits, and drawbacks of current MNP analytical methods, including sampling, processing, detection, identification, quantification, and characterisation. It also explores AI-based classification algorithms and their interaction with FTIR, a topic not extensively addressed in published literature. The article also highlights significant knowledge gaps that need to be addressed.

2 Sampling methods for micro- and nanoplastics: environmental samples

Sampling MNPs in environmental samples necessitates specialised methods tailored to the distinct characteristics of rivers, sediments in riparian areas, open ocean, coastal, estuarine, and other aquatic systems. The selection of sampling instruments is influenced not only by the equipment at hand but also by the objectives of the research, the specific environmental context, and the type of matrices to be collected [22]. The choice of sampling devices significantly affects both the quantity and the representativeness of the collected samples. Consequently, there is no universally applicable instrument for MNP sampling in surface waters; each device presents its own set of advantages and limitations [23].

2.1 In riverines

Manta trawls are commonly used to collect microplastics from surface waters, but they have notable limitations. Their mesh sizes, normally between 300 and 500 µm, are too large to capture smaller microplastics and entirely ineffective for nanoplastics. This results in a significant underestimation of plastic pollution, particularly in the smaller size ranges. Furthermore, organic debris and biofilms can clog the mesh, thus reducing sampling efficiency even further. Therefore, manta trawls must be used with finer filtration or advanced analytical techniques to achieve a more precise assessment of MNP contamination [24]. A Manta net consists of a structural frame and a collecting net that leads to a collector, constructed from stainless steel or aluminium to maintain a consistent aperture size during sampling. The dimensions of the opening vary across studies, with mouth widths from 30 to 120 cm and heights from 10 to 75 cm [25], and the mesh size for manta nets can be varied depending on the research objectives.

Other sampling techniques, like pump filtration systems, can have filters with different pore sizes, as this is a critical factor in sampling results. Lower detected nanoplastic concentrations are a result of larger filters (> 300 µm) application, resulting in the capturing of larger microplastics while ignoring smaller fragments [26]. Although better information on the distribution of microplastics is provided by medium filters (100–300 µm), which gather a larger range of sizes, there are still challenges related to nanoplastic filters in filtration sample processing. The more prevalent smaller microplastic particles in freshwater are effectively captured by smaller filters (20–100 µm), underscoring the significance of filter selection for precise MNP research.

2.2 In sediments

Grab samplers allow for targeted microplastic collection at specific sediment depths, enabling stratification analysis. However, conventional sediment samplers, like grab samplers or corers, tend to disturb sediments, risking the loss of fine and low-density particles, including MNPs, although this loss is often unquantified [27]. The Van Veen grab sampler is effective for deep sediment collection in riparian environments, retrieving cores with minimal disturbance [28], but is preferably used for continuously changing sediment structure and is also useful for sampling sludge-type matrices containing MNPs [29]. The Van Veen grab sampler (stainless steel) comes in models with different capacities for different sediment types, with capacity ranges being 0–5 L (each measuring about 126 cm^2), 2 L (each measuring about 260 cm^2), 6 L (each measuring about 480 cm^2), and 12 L (each measuring about 880 cm^2). The grabber can sample disturbed loose materials and operate in depths greater than 30 m. They can be operated manually or with a davit, and the sets weigh between 2 and 41 kg [30].

Manual tools such as trowels, shovels, and handheld corers are useful for collecting shallow sediment samples, particularly in beach or riverbank locations. They are simple, cost-effective, and appropriate for fieldwork, although less precise than specialised equipment [24, 31]. Although less common, freeze-coring techniques help preserve sediments. Scoops and spatulas are good for surface sediments, which may more readily accumulate MNPs due to environmental factors.

2.3 In marine environments

Methods are similar to those used in rivers but adapted for ocean conditions. Manta trawls excel for large ocean areas, while benthic instruments gather ocean floor sediments, and plankton nets capture plankton and associated MNPs. Each method has distinct advantages, emphasizing the need for standardised protocols.

3 Method sensitivity in detecting, quantifying, and characterising micro- and nanoplastics

Sensitivity in the analysis of MNPs refers to the efficacy and accuracy of analytical techniques used to detect, quantify, and characterise MNPs of these minuscule particles [32]. The challenges associated with this sensitivity arise from the particles' diminutive size, varied structure, including shape, and thus indistinguishability in the presence of other particles, and the differentiated environmental matrices in which they are found [33]. Traditional methods, such as scanning electron microscopy (SEM), atomic force microscopy (AFM), vibrational spectroscopy, and others, are often insensitive to

detecting very small particles. However, while earlier or basic implementations have detection limits, advanced variants, and particularly workflows strengthened with AI-machine learning, can achieve considerably improved sensitivity [34].

This sensitivity challenge is further exacerbated by a multitude of additives in the source plastic waste, including plasticisers, pigments, antioxidants, flame retardants, and stabilisers, which have been found in MNPs and are commonly found in complex environmental matrices that contain a variety of pollutants [35]. This is largely attributed to the MNPs' large surface area, with the potential to be a repository of organic pollutants from the environment, drawing in pollutants, heavy metals, and microorganisms [36]. This can make detection more difficult and lead to false negative or positive results [37, 38]. Thus, the diverse chemical compositions of MNPs, stemming from different polymer types, require specialised analytical strategies to ensure accurate identification, given their distinct physical and chemical characteristics [39, 40].

4 Sample preparation and pretreatment for MNP samples

4.1 Physical separation methods

Sample preparation involves isolating MNPs from complex and differentiated matrices using density separation, floating, digestion, washing, filtration, and concentration. Unfortunately, there is no consensus on a standard or procedure for sample preparation, especially when it comes to nanoplastics in MNP research. The sample's background and the ensuing analytical technique play a major role in the method selection process [41]. Figure 1 is the schematic diagram illustrating the microplastic and nanoplastic analysis process. After collection, samples are separated using methods like density separation, filtration, and centrifugation. Matrix types are classified using specific analytical methods. Advanced methods such as Raman spectroscopy, TEM, FT-IR, SEM, micro-FTIR/Raman, pyrolysis-GC/MS, and fluorescence analysis are used for in-depth analysis and quantification.

For environmental samples, sample preparation can include sieving, magnetic separation, density gradient centrifugation, electrostatic filtration with specific pore sizes, field-flow fractionation that distinguishes based on size and hydrodynamic characteristics, and size-exclusion chromatography (SEC), with each method leveraging the distinct physical properties of MNPs.

A pre-treatment may be done in sediment samples, which includes utilising larger sieves. This is followed by density separation, filtration, and centrifugation. Variations in density can facilitate the separation of plastics from sediment, typically attained by mixing the sediment thoroughly with salt-saturated solutions and gathering the supernatant containing microplastics for additional filtration [42]. It is widely acknowledged that solutions greater than 1.4 g cm^{-3} are necessary for separating microplastics from sediments, as their density depends on additive concentration, polymer type, and adsorbed substances and organisms. A simple density separation using water can recover some types of plastics,

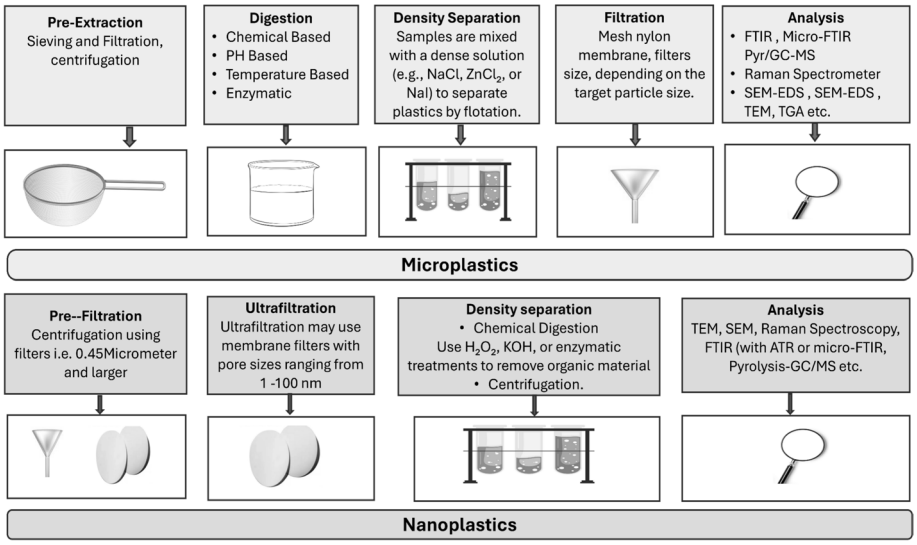

Fig. 1 Schematic diagram of micro- and nanoplastics from separation to analysis

such as PE (polyethylene) and PP (polypropylene), from soil samples or fibres from sediments, due to their shape and large surface [42, 43].

Analysing nanoplastics in environmental matrices is still in its initial stages. A key challenge in destructive methods, especially Pyr-GC/MS, is separating less than 1 µm before the analysis to avoid reporting a combined concentration of plastics (MNPs). Prefiltering the digested samples through a 1 µm filter can achieve this [44]. Nonetheless, with complex organic matter-rich matrices, such as marine sediments and soil, this is hard to achieve without a highly effective digestion pretreatment. This presents a methodological challenge [45] as most current methods, like Raman spectroscopy and FTIR, grapple with resolution and sensitivity at the nanoscale, particularly in complex sediment matrices [17].

Sieving serves to separate MNPs from sediments by granulometric fractions post-drying and is generally employed as a step to remove particles exceeding a defined size. Microplastics (MPs) are extracted by using sieves with varying mesh sizes; retained material is subsequently sorted. This method allows the classification of microplastics into multiple-size fractions, typically employing one to six sieves with mesh sizes from (38 to 4750) µm [46]. However, nanoplastics are not retained by conventional sieves or filters commonly used in microplastic sampling protocols due to their extremely small size. Consequently, they often pass through standard mesh sizes, leading to their underrepresentation or complete omission in environmental samples. This has significant repercussions for downstream analyses, including quantification, risk assessment, and ecological impact studies, as the absence of nanoplastics in collected samples may result in the underestimation of total plastic pollution and its potential effects [47]. On the other hand, chromatography is particularly useful for concentrating and purifying environmental MNPs from larger debris and other sample constituents [48] and, therefore, can be harnessed specifically for nanoplastic retrieval for analysis. Regarding performance, chromatography offers high selectivity and recovery rates, particularly when combined with advanced detectors like multi-angle light scattering (MALS) or UV–Vis spectroscopy. This allows for detailed characterisation of nanoplastics, including polymer type and size distribution. The technique, however, is sensitive to interferences such as salts, surfactants, and natural organic matter, which can co-elute or alter retention times. Furthermore, matrix effects from environmental samples may require extensive pre-treatment to avoid clogging [49].

Filtration in MNP research can be applied at various stages of sample processing, including post-density separation, following chemical digestion. Conventional filtration methods, such as vacuum filtration and membrane filtration, are commonly utilised in laboratory analyses [46]. Membrane filters are made from materials like alumina, ceramics, and polycarbonate, with commonly used types in MNP analysis being glass fibre, cellulose acetate, cellulose nitrate, polycarbonate, nylon, and alumina. After filtration, filters and retained MNPs are dried at ambient temperature or in desiccators, with drying temperatures varying between 60 °C and 90 °C. However, standards recommend a maximum temperature of (50 ± 2) °C for MNP samples, drying for 24 h, and a gradual return to ambient conditions to prevent changes in MNP properties.

Density separation proves to be particularly advantageous for large quantities of water and sediment samples. It involves the separation of gravel, sand, silt, and clay. MNPs are separated from their matrix by exploiting the differences in density between the extraction solution and the MNPs. Observingly, clay, due to its adhesive properties, can entrap a larger quantity of MNPs. Density separation can be followed by a second phase that entails the oxidation of organic matter, which tends to rise to the surface alongside the separated MNPs, often complicating the extraction and detection of the MNPs. This density-based extraction method has been the primary technique in research over the last two decades, utilising differing densities of polymers and flotation mediums [46]. It separates materials by immersing them in an intermediate-density liquid, allowing lighter materials to float. Sediments and soils averaging between (1.70 to 2.65) g/cm^3 can be treated with sodium chloride (NaCl), a process deemed cost-effective and environmentally friendly. The concentration of NaCl that can be used is roughly between 20 and 30% (w/v) for the effective separation of sediments and soils with an average density between 1.70 and 2.65 g/cm^3 [50].

Overall, for different separation techniques, high selectivity, cost-effectiveness, and scalability must be considered in MNP research. Disadvantages, such as sample characteristics, the possibility of MNP loss during processing, interference from other materials in samples, restrictions on the effectiveness of particle size range, and the requirement for technical expertise, must all be considered [51, 52].

4.2 Chemical digestion and biodegradation

Chemical digestion plays an essential role in the pretreatment of environmental samples that include MNPs. The main purpose of chemical digestion is to remove or reduce organic matter, which may interfere with subsequent analysis,

particularly infrared spectroscopy. This aspect is especially significant when assessing the presence and concentration of MNPs, as these pollutants may be masked by other substances within the sample.

Chemical digestion is crucial for isolating MNPs in environmental samples by decomposing organic matter and preserving synthetic polymers. Organic residues can hinder polymer separation. Key reagents include: 1) organic acids like nitric acid (HNO_3), which digests organics but may partially oxidise MNPs, and hydrochloric acid (HCl), which is less damaging to many polymers; 2) Bases like potassium hydroxide (KOH) and sodium hydroxide (NaOH) can digest organic materials but may oxidise MNPs. Overall, alkaline hydrolysis effectively breaks down proteins and removes MNPs, especially with 10% KOH at 60 °C. However, some cellulosic and chitinous materials can be recalcitrant to such hydrolysis, although it can be used for sludge and soil samples. Since alkali-insoluble humins often dominate soil organic content, resulting in lower removal rates [53], such an approach might also not be suitable; 3) Oxidising agents, especially hydrogen peroxide (H_2O_2), are considered effective for digesting various materials, including grease and chitin. H_2O_2 can also be used for MNP sample preparation, but its overall effectiveness for MNPs in biota has been questioned [54]. One study showed that 25% of the biotic organisms were destroyed after utilising a 35% H_2O_2 solution within seven days [55].

Enzymes enable selective digestion but can be expensive and may need oxidisers for complete residue removal, requiring a careful choice of agents for effective MNP recovery. They prevent the loss of MNPs, their degradation, and surface chemistry changes, and are eco-friendly. They are mainly used alone but can be combined with other methods, depending on the sample's specifics [46]. Among these agents, enzymes are considered the best key agents due to their specificity, efficiency, reusability, and adaptability. They target specific substrates to break down organic polymeric substances [53].

5 Methods for identifying, quantifying, and characterising micro- and nanoplastics

To effectively identify and characterise MNPs, a range of techniques must be used following appropriate pre-treatment procedures. These include microscopy techniques, scanning electron microscopy combined with energy-dispersive X-ray spectroscopy (SEM–EDS), transmission electron microscopy (TEM), fluorescence, vibrational spectroscopy (Raman and FTIR), mass-based thermal methods coupled with mass spectrometry, e.g., Py-GC/MS, and flow cytometry [56], including Near-infrared (NIR) spectroscopy for nanoplastic detection. Each of these methods possesses unique applications, along with distinct advantages and limitations. Vibrational spectroscopy (Raman, FTIR) provides non-destructive analysis and broad applicability, but has interference issues. Mass-based thermal methods with mass spectrometry offer strong analysis capabilities but face sample destruction and data complexity challenges [57]. Whereas MPs can be easily seen using microscopy, flow cytometry is a highly sensitive tool to study plastic nanoparticles. It offers advantages over other techniques, such as Raman spectroscopy, which can generally resolve (1–2) µm particle sizes. Cytometry is an analytical technique that measures particle characteristics in fluid via laser, offering high sensitivity, rapid quantitative analysis, multiparametric data collection, and high throughput, making it ideal for studying plastic nanoparticles [56]. See Table 1, which depicts some of the methods and their advantages and disadvantages, and Fig. 2, which illustrates a combination of methods used. Although some of these are commonly at the first phase of analysis, their integration with AI tools is largely unknown.

SEM is a powerful technique for examining the morphological surface structure of MNPs, producing high-resolution images, and offering chemical composition data through detectors like EDS. While SEM–EDS is generally viewed as costly, characterising MNPs can be conducted efficiently and economically using the environmental SEM mode/route, which eliminates the need for nitrogen gas in the chamber and avoids sputtering gold or carbon, although such processes can improve image quality [22]. Transmission electron microscopy (TEM) is frequently utilised for nanomaterial characterisation, delivering chemical insights and images at atomic resolution levels. The method involves an electron beam interacting with a thin specimen, resulting in scattered electrons [22].

Fluorescence microscopy is an effective analytical tool for visualising MNPs in diverse environments, including aquatic systems, soils, and biological samples. It exploits the ability of certain materials to absorb light and then emit it at longer wavelengths, allowing researchers to label plastic particles with fluorescent dyes. When exposed to specific light wavelengths, these labelled plastics fluoresce, enabling detection through appropriate microscopy filters, thus providing insights into the size, shape, distribution, and concentration of the particles [72].

Although TEM offers high-resolution structural analysis, it also presents preparation and financial difficulties, while SEM lacks insights into the internal structures of analytes and is useful for the surface analysis of larger particles, striking a balance between detail and usability. Although it is useful for detecting types or additives at low concentrations,

Table 1 Methods and their advantages and disadvantages

Method	Advantages	Disadvantages	References
Scanning Electron Microscopy (SEM)	High-resolution imaging morphology, shape, and size characterization of micro and nano plastics 3D imaging capability Elemental analysis with EDS Wide size range analysis	Requires conductive coating, may damage delicate particles under vacuum, potential electron beam damage, limited particle size distribution data, and entails high cost and complexity	[58, 59]
Transmission Electron Microscopy (TEM)	High-Resolution Imaging Coupled with EDS, TEM enables elemental analysis to identify different polymer types It provides insights into the shape, size, and distribution of particles	Complex sample preparation, potential sample damage, limited sample size, high costs, and vacuum requirements	[60]
Fluorescence microscope	Visual imaging enables detailed observation of microplastic morphology, including shape and size Multiplexing with fluorescent dyes aids in distinguishing plastic types	- False positives from organic matter- limited polymer identification-diffraction issues for nanoplastics, and potential signal interference from pigments	[61, 62]
Spectromicroscopic AFM-Roman	Spectroscopy on a single platform provides powerful tools for characterizing both the physical morphology and chemical composition of materials at high resolutions while maintaining non-destructive analysis capabilities	Expensive, setup complexity, environmental sensitivity, depth profiling limitations, sample preparation requirements, and data interpretation difficulties	[63]
Spectromicroscopic AFM-IR	A combination of morphological imaging and chemical identification in a single experiment. Identification of specific functional groups and chemical bonds present within particles	Costly, imitations related to sample types, risks of damage, contamination issues, and lengthy data collection times	[64]
Microscope AFM	Advanced in analysing morphological surfaces with exceptional resolution adaptability for nanoscale restricted scan size	Costly and prolonged scan durations because of high-resolution demands, and sensitivity to environmental conditions	[65]
Micro-FTIR	Non-destructive analysis, high spatial resolution, chemical identification, and high sensitivity for identifying MNP particles	Atmospheric interference and complex spectral interpretation; require expertise in plastic polymer knowledge and spectroscopy for accurate analysis	[66–69]
Micro-Raman	Minimal sample preparation, allowing for the identification of specific plastic polymers Chemical identification of MNP particles, high spatial resolution, non-destructive analysis	Sample degradation, instrument sensitivity, potential fluorescence interference, and complex spectral interpretation	[41]
FTIR	Chemical identification, high sensitivity, non-destructive analysis, and flexibility for detecting small amounts of plastic polymers in environmental samples	Sample size limitations, time-consuming sample preparation, and interference from other materials	[68, 70]
Chromatography	Separation and identification of plastic polymers and additives, quantification of MNPs, and their detection, making it suitable for environmental sample analysis	Requires complex sample preparation and specialized instrumentation, a destructive technique for analysis, breaking down plastic particles through thermal processes like pyrolysis into their chemical components	[92, 106]
Fluorescence	High-resolution imaging capabilities, high sensitivity, specificity, and non-destructive analysis for identifying MNPs	Limited applicability for nanoplastics, interference, time-consuming sample preparation, and quantification challenges due to variability in fluorescence properties	[61, 62]

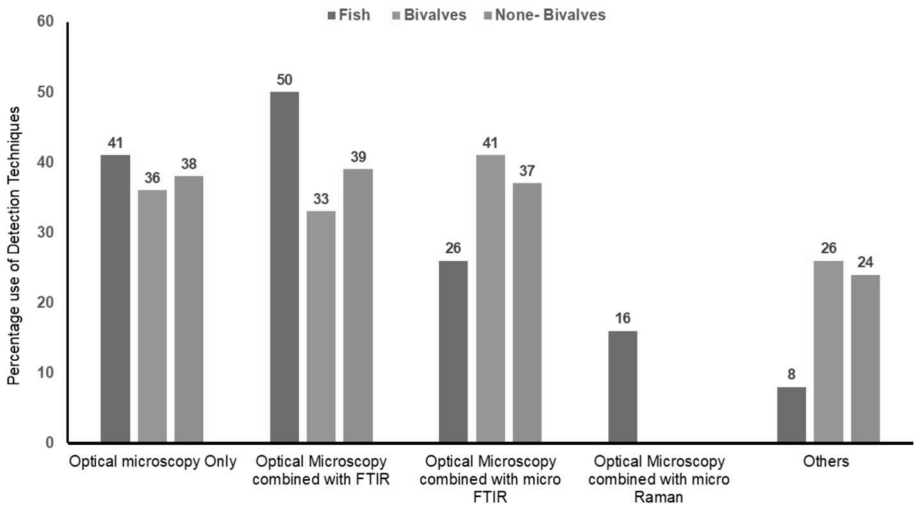

Fig. 2 Techniques for detecting and quantifying MNPs with few occurrences grouped as others from the literature reviewed (2015 to 2025) [66]

fluorescence microscopy lacks structural resolution and is best suited for focused investigations in biologics. The technique selected is determined by the goals of the MNP research [23, 58]. Table 2 illustrates some of the different identification and characterisation methods of MNPs and some integrated methods.

With atomic force microscopy (AFM), a higher resolution for images that provides three-dimensional topographical data at the nanoscale can be achieved. It operates by scanning a sharp tip across the surface of a sample and measuring forces between the tip and the sample to create detailed images. AFM is especially relevant in the investigation of MNPs due to its ability to precisely characterise surface morphology, mechanical properties, and interactions at the nanoscale [12].

AFM-Infrared (IR) merges atomic force microscopy's high spatial resolution with infrared spectroscopy's chemical analysis capabilities. It works by using an AFM probe to detect thermal expansion in a sample when it absorbs infrared radiation, exciting specific molecular vibrations. Recent advancements focus on improving sensitivity and reducing measurement noise, with innovative detection methods using piezo components rather than traditional cantilever deflection, enhancing precision for imaging smaller volumes and diverse samples [73]. AFM-Raman and AFM-IR are effective for analysing MNPs. AFM-Raman offers high spatial resolution and non-destructive molecular identification, albeit AFM-IR provides better chemical sensitivity and depth profiling. Using both techniques can yield complementary insights into the complexities of MNPs in research.

When compared to microscopy and vibrational spectroscopy techniques, there is still wide use of Py-GC/MS, which uses thermal decomposition to break down MNPs into mass spectrometer measurable constituents, i.e., chemical identification of macromolecules, and performing a match to pre-existing spectral libraries to identify them. Significant growth in its use for the identification of MNPs found in environmental samples is mentioned elsewhere [74, 75]. Accurate MNP identification and quantification in a variety of samples is made possible by this method [76]. Despite its large sample capacity, the sensitivity of this method remains comparatively low—a major disadvantage—albeit it is not constrained by the dimensions of plastic particles. Another disadvantage is that it requires homogeneous representative portion microgram-scale sample sizes, something difficult to achieve in MNP analysis and pyrolysis optimization, which increases sample processing times, including high energy requirements and inert carrier gas usage.

The combination of optical microscopy with polymer identification methods like FTIR, μ-FTIR, and μ-Raman is widely used, appearing in 57%, 59%, and 58% of studies on fish, bivalves, and non-bivalve invertebrates, respectively. μ-FTIR is known to have limitations in terms of spatial resolution, especially when analysing materials that are on the nanoscale. μ-Raman can detect nanoplastics (NPs) but faces challenges like fluorescence interference. μ-FTIR remains important for detecting NPs, while μ-Raman provides superior resolution and less interference from water. Combining both techniques can yield comprehensive insights into NPs' characteristics; however, using μ-FTIR alongside fluorescence may address its limitations and enhance MNP detection [66, 77].

Two sampling campaigns were conducted at 11 Amsterdam canal sites during summer and winter. They compared μ-Fourier-transform infrared imaging and pyrolysis–gas chromatography-mass spectrometry for the identification and quantification of MPs in urban water samples. The results showed that the MP concentrations ranged from about

Table 2 Different identification and characterisation methods of MNPs and some integrated methods

Methods	Source of sample	Range	Pre-treatment	Outcomes	References
Scanning Electron Microscopy (SEM)	Microplastics in sediments	500 µm	Density separation with NaCl	Identification of fragments of PE, PP, PS, PA, chlorinated polyethylene, and chlorosulfonated polyethylene with blue, white, and transparent colouring	[22]
Transmission Electron Microscopy (TEM)	Nanoplastics in soil	80 to 100 nm	Preservation with osmium tetroxide and dehydration with acetone	Identification of fragments, fibres, and membranes with smooth surfaces and irregular shapes	[22]
SEM–EDS	Microplastics in river sediments	2.8 mm to 11 µm	Extraction and separation by density	Identification of pellet granules	[79]
Stereomicroscopy and micro-Fourier transform infrared spectroscopy (µ-FTIR)	Microplastics in sand and Water Microplastics in water	1 to 3 mm Sand and water 90 µm to 1 mm	Air-floating in saturated NaCl and density separation in saturated NaI	Identification of polyethylene, polypropylene, polyester, rayon, acrylic, and polyamide	[80]
ATR-FTIR	Microplastics Waters from aquatic ecosystems	Particles < 400 µm and > 500 µm	Samples were extracted, purified, and filtered	identification of PE, PP, PVC, polycarbonate, PS, PET, and PES	[81]
Raman spectroscopy	Nanoplastics and Microplastics \ Aquatic environments include pure water and seawater	Particles included 100 nm, 500 nm, and 10 µm	SERS detects MNPS via synthesis, coating, measurement, and analysis	Identified polystyrene (PS), polyethylene (PE), and polypropylene (PP)	[82]
Pyrolysis gas chromatography-mass spectrometry (Py-GC–MS)	Nanoplastics and Microplastics in Wastewater Treatment Plants	Concentrations between 0.04 and 7.3 µg/L. PP (0.1–4.5 µg/L), Nylon 66 (0.2–7.3 µg/L), PET (0.1–2.2 µg/L), PE (0.1–6.6 µg/L), and Nylon 6 (0.1–3.6 µg/L)	Nanoplastics were extracted by pre-treatment with H2O2 and concentration via Amicon® Stirred Cell, then vacuum-filtered	(PMMA, nylon 6, PC, PET, PS, PP, nylon 66, PE) identified and quantified	[44]

2.0 to 789 µg/m3 for µ-FTIR imaging and 8.5 to 754 µg/m3 for Py-GC–MS. µ-FTIR imaging appears to have slightly better sensitivity and a higher upper detection range, making it more versatile for detecting. However, the results also showed a significant correlation among the trends in MP abundance; differences in polymer compositions were observed because of the intrinsic differences in the methodologies used [78]. Another study used ML models based on Random Decision Forests to analyse large µ-FTIR datasets to automate microplastic identification in environmental samples. This method allowed for semi-automated analysis of thousands of particles, thus lessening the amount of manual labour [20].

In comparison with vibrational spectroscopy techniques, Py-GC/MS disassembles the sample and elucidates its polymeric makeup, including any additives and contaminants [83]. FTIR assesses the functional groups present on the surface of a sample by analysing infrared absorption patterns, facilitating the identification of polymer types with minimal sample preparation. Furthermore, the advent of µ-FTIR with ATR, which is a surface technique, including localised analysis of microscopic regions and heterogeneous sample processing capabilities, further imparts in-situ non-destructive analysis, thereby negating the necessity for sample purification and concentration [84, 85]. With µ-FTIR, sample characterisation can be performed using either transmitted or reflected light modes, with transmitted light yielding high-quality spectral data. Nevertheless, the successful application of transmission µ-FTIR necessitates meticulous sample preparation to ensure that samples are sufficiently thin to allow for effective light transmission, which is critical for obtaining precise results [86]. Although FTIR serves as a valuable instrument for identifying functional groups in polymers and characterising materials, Py-GC/MS demonstrates superior capabilities in both identification and quantification through thermal degradation analysis, albeit it encounters challenges in analysing complex mixtures and exhibits limitations in large sample quantitative assessments, whereas µ-FTIR excels in identifying polymer types and examining surface chemistry and distribution in small sample sizes [87].

Key differences between FTIR and µ-FTIR can be summarily explained as follows: 1) the spatial resolution of µ-FTIR is at a microscale, with averaged FTIR data being at a larger scale. These are averages in bulk sample information compared to those observed at a microscopic scale for µ-FTIR, 2) µ-FTIR utilises ATR for non-specific surface-sensitive analysis, with FTIR relying on a thin slice for sampling transmission, and most importantly, 3) µ-FTIR is suited for heterogeneous sample handling, while normal FTIR sample uniformity is assumed, particularly when environmental samples are analysed. Additionally, optical capabilities in µ-FTIR further demonstrate its superiority to normal FTIR, which lacks this capability. While FTIR collects spectral data by scanning wavelengths, µ-FTIR may use imaging detectors to capture multiple spectra at once, allowing for quick chemical imaging [19, 88–90]. It can also integrate an infrared light source, microscope optics, and a Michelson interferometer to focus on tiny particles. Table 3 clearly illustrates the distinctions between FTIR and µ-FTIR regarding sample size and preparation, resolution, and their respective strengths and limitations.

Overall, both techniques exhibit commendable chemical specificity; nonetheless, µ-FTIR presents a distinct advantage when examining heterogeneous samples that comprise various plastics or contaminants at a micro-scale. The superior resolution of µ-FTIR enables researchers to differentiate between diverse polymer types more efficiently than conventional FTIR. While both techniques are essential analytical instruments in the field of spectroscopy, they fulfil different roles depending on the scale and characteristics of the samples under investigation. Standard FTIR may fall short of providing adequate detail for minute particles or intricate mixtures typically encountered in environmental samples [91]. In contrast, although µ-FTIR is particularly adept at analysing small samples, it may necessitate more advanced instrumentation and extended analysis durations [91]. For rapid and large sample processing to mitigate human error minimization, a vibrational spectroscopy-chemometrics, AI/machine learning nexus approach is necessary.

6 Chemometrics machine learning approach for micro- and nanoplastic identification and characterisation

Artificial intelligence (AI) is an evolving discipline that holds the promise of transforming various dimensions of our daily lives. The incorporation of an AI approach to developing machine learning (ML) algorithms based on chemometrics into MNP research has greatly advanced our insights into MNP pollution across multiple environmental matrices [92, 93]. The utilisation of AI for MNP analysis involves the application of ML techniques to accurately identify and classify MNPs found in water and sediment samples, thereby improving both detection accuracy and operational efficiency. This analytical framework consists of several key phases: data collection, which entails the gathering of images from environmental samples, and data preprocessing, which involves the cleaning and annotation of images obtained. AI-driven approaches have considerably enhanced the detection, quantification, and characterisation of

Table 3 Strengths and limitations of FTIR and μ-FTIR

Methods	Sample size and preparation	Spatial resolution	Strengths	Weaknesses	References
FTIR	Generally, requires larger samples and extensive preparation	Lower spatial resolution limiting its ability to differentiate closely spaced features	Comprehensive spectral data across a wide range and suitability for bulk analysis	Limited spatial resolution, necessitates careful sample prep May not provide sufficient detail for very small particles (nanoplastics)	[68]
Micro-FTIR	Accommodates minute samples with minimal preparation, making it ideal for delicate specimens	Provides high spatial resolution for detailed mapping of organic matter and minerals in samples	Excels in high spatial resolution and non-destructive analysis, FTIR excels at analysing small samples	faces spectral range limitations and complexity in data interpretation due to overlapping peaks	[68, 69]

MNPs by processing large data volumes, improving precision, and uncovering hidden distribution patterns, thus making them an important tool in MNP analysis [93]. Chemometrics, defined as the application of statistical and mathematical techniques to chemical data analysis, can significantly enhance the understanding of intricate datasets generated by vibrational spectroscopy, i.e., μ-FTIR spectroscopy, in the study of MNPs.

By analysing the spectral properties of microplastics, researchers can significantly improve the accuracy of their classification using ML techniques [94]. This technique presents numerous advantages, including enhanced precision in identifying different polymer types, reduced processing times relative to conventional methods, the ability to process extensive datasets, and increased reliability and reproducibility of findings [20, 95]. This can lead to the standardisation of methods that will be acceptable at an international level. Additionally, the implementation of ML techniques not only enhances detection abilities but also contributes to a better understanding of the ecological ramifications of microplastic pollution [96].

While improvements in AI detection mechanisms for MNPs show promise, combining ML with other methods like μ-FTIR improves detection and quantification accuracy, simplifies data analysis, and yields actionable insights that researchers can use. However, there are still challenges because of the complexity of particle shapes, compositional variability, and the requirement for standardised analysis protocols to guarantee consistent results. Training datasets must be updated continuously [41, 97, 98].

Observingly, the framework of integrating μ-FTIR with ML based on chemometrics is presumed advantageous. The incorporation of ML into μ-FTIR analysis markedly enhances its capabilities. ML facilitates automated image processing, which accelerates the identification and quantification of microplastics [92], imparting a "just-in-time" (JIT) approach. This automated approach to identification permits swift evaluations of MNP contamination in unidentified samples, thereby ensuring both reliability and precision in the detection of MNPs [99, 100]. Furthermore, it integrates data from multiple analytical methodologies, yielding a more thorough comprehension of MP contamination. ML also supports real-time monitoring, allowing for prompt interventions regarding pollution concerns for source riverine for drinking production, and employs predictive analytics to anticipate future pollution patterns in the advent of seasonality variation due to climate change. The combined advantages of this integration not only improve detection rates but also enhance our understanding of the interactions between MPs, NPs, and the environment [101, 102].

Moses et al. [103] compared two well-known data analysis algorithms, i.e., siMPle alongside MPAPP (MicroPlastic Automated Particle/fibre analysis Pipeline) and BPF (Bayreuth Particle Finder), in relation to microplastic (MP) outcomes concerning abundance, size distributions, and polymer composition taken from environmental water samples (11–500) μm. Although the results indicate a generally strong alignment, inconsistencies occur for certain polymer types and the smallest MP size categories. Findings also highlight the necessity of detailed comparisons of MP algorithms for enhanced data comparability [103]. Another study by Fan et al. [104] developed a dual principal component analysis (PCA) method combined with ML to analyse hyperspectral Raman imaging data for MNP identification. The approach addressed challenges in signal noise and high-dimensional data, achieving automated classification of particles as small as 100 nm in environmental samples [104].

Table 4 shows a comparative overview of six well-known ML-based tools designed for microplastic identification and analysis, each tool varying in its underlying ML approach, strengths, limitations, and source of reference. BPF is a model-based ML approach and has been used in various studies focusing on microplastic contamination in freshwater environments [103]. Microplastic Finder (MPF) and Microplastic AI Finder contrast significantly in their approach, capabilities, and intended use. Microplastic AI Finder uses AI-enhanced Raman spectroscopy with plasmonic amplification, allowing it to detect both microplastics and nanoplastics in under 20 min without sample pre-treatment. It is especially appropriate for biological applications but necessitates specialised hardware and is still in early development, restricting its scalability and accessibility. In contrast, MPF relies on model-based machine learning using Random Forests applied to high-quality FTIR or Raman spectral data, making it effective for identifying more than 20 polymer types in complex environmental samples. Nevertheless, it is proprietary, dependent on input quality, and is less transparent [105, 106]. The application of machine learning/AI in μ-FTIR spectroscopy or Raman signifies a crucial development in addressing significant environmental issues associated with MNP pollution.

Table 4 Strengths, limitations, and references for various automated tools and AI-based systems used for microplastic analysis

Tool	Model	Strengths	Limitations	Reference
Purency Microplastic Finder (MPF)	Model-based ML (Random Forests)	Identifies polymer types; Fast automated analysis; Low human bias; Scalable for complex matrices	Proprietary licensing; Requires high-quality FTIR/Raman input; Limited transparency	[105]
siMPle	Instance-based ML (spectral similarity)	Open source; Transparent and interpretable; Good for routine lab use	Poor performance on small/degraded particles; Dependent on reference libraries; Limited additive detection	[103]
MPAPP	Instance-based ML (pipeline extension of siMPle)	Useful for environmental monitoring; Integrates with siMPle; Automates particle/fibre analysis	Lower accuracy for small particles; Requires manual tuning; Shares siMPle's limitations	[103]
BPF (Bayreuth Particle Finder)	Model-based ML (Random Decision Forests)	Retrainable for new polymers; Improved accuracy for small particles; Validated in comparative studies	Less transparent; Training data-dependent; Requires expert setup	[103]
Automated Particle Tracking	Image-based ML (object tracking)	Integrates with video microscopy; Enables real-time motion analysis; Useful for transport studies	Sensitive to noise and lighting; No chemical identification; High computational demand	[34]
Microplastic AI Finder	AI-enhanced Raman with plasmonic amplification	No pre-treatment needed; Detects microplastics; Works in biological samples	Limited scalability for large datasets; Requires specialized hardware; Early-stage technology	[106]

7 Standardisation of methods for identifying, quantifying, and characterising micro- and nanoplastics

International Standards Organisation's (ISO) ISO/TR 80004-12:2017 and ISO/TR 80004-1:2015 are crucial standards in the field of nanomaterials and nanoplastics, providing a framework for terminology and communication among researchers, manufacturers, and regulatory authorities. ISO/TR 80004-1:2015 is particularly significant as it provides foundational definitions and terminology for all nanomaterials, including nanoplastics [107, 108]. Adherence to ISO/TR 80004-1:2015 ensures reliable and comparable research on nanomaterials, particularly their environmental effects. ISO 5667 is a series of international standards that stipulate guidelines for water sampling. It includes guidance on the design of sampling programs, sampling techniques, and preservation and handling of water samples. Specifically, ISO 5667-27:2025 outlines methods for sampling suspended microplastics in water, including domestic, freshwater, seawater, and treated and untreated wastewater [109]. Suspended particles may include synthetic or semi-synthetic polymeric materials. The standard does not include chemical analysis, biological methods, physical methods, or pre-treatment or digestion methods. It elucidates general methodologies for grab sampling using cascade filtration for water samples with low, medium, and high suspended solid content and net sampling using manta, plankton, or neuston nets.

ISO/TR 80004-12:2016 and ISO/TR 80004-1:2023 entail standardised terminology for nanotechnologies. They define terms on nanoobjects and nanostructured materials, but do not directly address nanoplastic or provide guidance on environmental sampling or analytical methods. This creates a gap for regulators and researchers focusing on MNP pollution, who need more targeted standards and methodologies [110, 111].

ISO 24187:2023 is recognised as a standard for analysing microplastics in various environmental matrices, such as soil, water, sediments, biota, air, food, and consumer products. It aims to harmonise methodologies and ensure comparability of results across studies and laboratories. In contrast, ISO 24187: 2024 does not prescribe a single method but outlines several techniques such as Raman spectroscopy, Py-GC/MS, FTIR (ATR/FPA), TED-GC-MS, DSC, NIR, and QCL-IR. It helps laboratories choose the most appropriate approach based on their needs. ISO/DIS 16094-3 provides standardised procedures for analysing microplastics in clean water samples using thermo-analytical techniques, designed for samples with low levels of suspended solids [112, 113].

The challenges of ISO/DIS 16094-3 and ISO 24187:2023 in MNP analysis result from limitations in scope, methodology, and standardisation. ISO 24187:2023 offers broad principles but lacks detailed protocols, introduces non-standard definitions, faces sampling and contamination issues, especially for nanoplastics, and requires complex sample preparation. ISO/DIS 16094-3 focuses on thermo-analytical methods and presents polymer mass data, but not particle count or size. This makes it less suitable for the comprehensive characterisation of MNPs. Both standards emphasise the need for more refinement to tackle the unique challenges presented by MNPs in environmental monitoring [114].

The ISO has made progress in addressing challenges related to MNP pollution through various guidelines focusing on sampling methods, analytical techniques, and risk assessment frameworks. This includes standardised sampling from environments and protocols for analysing microplastics using FTIR or Raman spectroscopy. These guidelines are relevant to sectors such as environmental monitoring, food safety, consumer product evaluation, and so on (ISO 2023). Despite ISO's progress, the measurement of MNP levels faces challenges such as standardisation, analytical sensitivity, characterisation, risk management, interdisciplinary collaboration, public education, and universally accepted standards. Variations in methodologies among research groups and rapid advancements in analytical technologies necessitate ongoing updates to existing standards. These limitations highlight the need for further development of international standardised methods for regulatory compliance and, thus, adoption.

8 Research gaps and future developments

The environmental persistence of MNPs poses a considerable threat to both ecosystems and human health, highlighting the critical need for improved detection and mitigation approaches. One of the primary challenges in current detection techniques is the absence of standardised protocols; this complicates the identification and analysis of these particles. Additionally, the small size of MNPs further complicates their detection and characterisation. Existing sampling methods are inadequate for capturing the full diversity of particle sizes and types, which limits comprehensive impact assessments.

Future innovations in methodologies for MNP research are anticipated to greatly enhance their utility across various sectors. As research advances, several important areas are expected to see significant improvements:

- Advancements in detection technologies for enhanced sensitivity, allowing for the identification of smaller particles at the nanoscale and lower concentrations.
- Integrated vibrational spectroscopy techniques, i.e., combining methods like Raman spectroscopy and FTIR for detailed particle characterisation with machine learning in AI.
- Further improvements in AI-driven classification algorithms and machine learning that can analyse environmental datasets, improving MNP identification accuracy.
- Standardisation initiatives by developing universally accepted sampling and analysis protocols are crucial for research consistency.
- Public participation in monitoring to expand research and to raise awareness about the consequences of plastic pollution.
- Further ISO standards development is essential for managing MNP pollution, with key focus areas being refining definitions, enhancing testing methods, and evaluating ecological impacts.

9 Conclusion

MNPs pose significant health and ecosystem risks, necessitating accurate identification and quantification for effective mitigation strategies. Both traditional and modern analytical methods are used, highlighting the need for standardised detection methods. While conventional methods like visual inspection and gas chromatography-mass spectroscopy methods offer some flexibility, advanced methods such as FTIR and hyperspectral imaging are preferred, despite challenges related to size limitations and sample contamination. Overall, there is a need for reliable, cost-effective analytical methods and standardised definitions and protocols for MNP identification, quantification, and characterisation. Improving identification and quantification techniques is crucial for effective management and regulation of MNP contamination, albeit this requires innovative approaches. The integration of μ-FTIR-AI-machine learning analysis can enhance our understanding of MNP composition, size, and morphology, as well as their potential environmental and human health impacts. It streamlines automated image processing and can enable rapid identification and quantification of MNPs as AI machine learning synthesises data from various analytical methods, providing a comprehensive understanding of MNP contamination, and providing real-time monitoring and predictive analytics enabling swift responses to pollution challenges. This integration cannot only improve detection rates but also deepen our understanding of interactions between microplastics, nanoplastics, and environmental sinks. Overall, the application of AI in μ-FTIR spectroscopy indicates a significant advancement in addressing pressing environmental challenges posed by MNP pollution.

10 Recommendations

Below are some recommendations on addressing the limitations in MNP analysis methods and the lack of standardisation:

- Use combined methods that are both count-based (e.g., microscopy, FTIR) and mass-based (e.g., Py-GC/MS) also integrate Machine learning methods for robust analysis on MNPs.
- Extraction and quantification methods must be tailored to the different environmental matrices (e.g., water, sediment, biota, etc.)
- To ensure the applicability of methods, test them within realistic environmental conditions.
- To facilitate comparability of data across regions, encourage the adoption of ISO standards.

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