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A comprehensive toolkit for micro- to nanoplastic analysis

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Micro- and nanoplastic (MNP) particles have emerged as a novel class of anthropogenic contaminants, now recognized as pervasive across all environmental compartments and in food and drinking water. Their extreme heterogeneity in size, morphology, density, polymer type, surface chemistry, and degree of aging presents major analytical challenges, with reported abundances spanning up to ten orders of magnitude. Reliable assessment of their occurrence and impacts therefore requires advanced analytical approaches capable of identifying, quantifying, fractionating, and characterizing these particles across scales. This review systematically evaluates state-of-the-art analytical strategies for MNP detection, organized into four major categories: mass-based identification methods (*e.g.*, Py-GC/MS, TED-GC/MS, MALDI-ToF/MS), particle-based quantification techniques (*e.g.*, μ-FTIR, μ-Raman, ToF-SIMS), separation and fractionation methods (*e.g.*, FFF and HDC-SEC coupled with spectroscopy or mass spectrometry), and morphological and surface characterization tools (*e.g.*, SEM/EDX, AFM-IR, nano-FTIR, SP-ICP-MS). For each category, we critically assess detection limits, strengths, and limitations, highlighting their suitability for micro- *versus* nanoplastic detection. Special attention is devoted to emerging approaches that push detection toward the nanoscale, as well as the need for harmonization and standardization across methodologies. By comparing and integrating these techniques, we outline how complementary approaches can provide comprehensive characterization of MNPs and support reliable risk assessment. Finally, future perspectives are discussed for advancing analytical sensitivity, method automation, and cross-disciplinary standardization to address the global challenge of MNP pollution.

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Environmental significance

Micro- and nanoplastics (MNPs) are emerging contaminants of global concern, yet their detection, characterization, and quantification across environmental compartments remain methodologically challenging. The extreme diversity of particle sizes, morphologies, polymer chemistries, and weathering states demands a multi-method analytical approach. Our work advances this field by consolidating and critically assessing a micro-to-nano toolkit that integrates mass-based, particle-based, and imaging methods, highlighting their respective strengths, limitations, and complementarities. By identifying key methodological gaps—such as the lack of reference materials, harmonized QA/QC protocols, and validated nanoscale techniques—this study provides a roadmap for generating reliable, comparable, and environmentally realistic MNP data. These advances are crucial for understanding the environmental fate, transport, and impacts of MNPs, thereby informing risk assessment, policy, and mitigation strategies.

1. Introduction

Microplastics (MPs) and nanoplastics (NPLs) are small polymer fragments ubiquitously distributed across ecosystems, including marine and freshwater environments, soils, sediments, air, and even food and drinking water, and are now recognized as emerging pollutants of global concern.[1–9] The term microplastics was first introduced in 2004 by Thompson *et al.* in the context of marine pollution, with an upper size limit of 5 mm later formalized by Arthur *et al.* (2009).[10,11] Currently, particles smaller than ~1 μm are classified as nanoplastics,[12] while those between 1 μm and 1 mm are termed microplastics,[13–15] fragments in the 1–5 mm range are often referred to as "large microplastics."

Plastic materials possess a unique combination of properties —lightweight, versatile, durable, and resistant to corrosion, heat, and flames—that have profoundly enhanced the quality of life for billions of people worldwide. Plastics are increasingly accumulating in the environment and even entering the food chain, creating a growing global concern. While European production declined slightly between 2018 and 2019, global output has continued to climb, reaching approximately 413.8 million metric tons in 2023.[16] The most widely produced

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thermoplastics—HDPE, LDPE, PP, PS, PVC, and PET—also dominate microplastic pollution.[17] Recent studies, including Yang *et al.* (2024), report that polyethylene (PE) and polyethylene terephthalate (PET) are the dominant polymers detected in bottled drinking water, with polyvinyl chloride (PVC) occurring far less frequently.[18] This contrasts with broader freshwater surveys that typically show PE ≈ PP > PS > PVC > PET, reflecting differences in polymer sources and detection methodologies across studies.[19] Alongside conventional plastics (such as PMMA, PA, and PUR), the use and production of bioplastics—polymers that are bio-based and/or biodegradable—are steadily increasing. Notable examples are polylactide (PLA), often used in food packaging, and polybutylene adipate-*co*-terephthalate (PBAT), used in agricultural films. According to a recent formal definition,[20] the category of microplastics also encompasses degraded tyre particles (consist of about 40–60% synthetic rubber) and paint particles or surface-coating debris. Paints and coatings are multicomponent materials containing polymeric binders (film-forming resins such as polyesters, alkyds, epoxy or urethane resins, and acrylic or vinyl polymers in different formulations) along with pigments, fillers, and additives.[15,21] These too can generate microplastic-like particles in the environment.

MNPs arise from two main sources: primary particles, which are intentionally manufactured in microscopic form (*e.g.*, resin pellets, microbeads in cosmetics or abrasives), and secondary particles, which result from the breakdown or wear of larger plastic items in the environment or during use. This breakdown can be driven by mechanical abrasion, UV radiation, and microbial activity, among other processes.[15,22,23] For example, textile fibers (such as those made of nylon/PA or polyester) shed from clothing during washing are a source of secondary microplastics.

Microplastics (MPs) are now found worldwide—from the equator[20,24,25] to the poles,[26,27] deep-sea sediments[28,29] and even Mount Everest.[30] They pose risks through toxic additive release,[31–33] volatile organic compounds (VOCs) generation during photodegradation,[34] adsorption of persistent pollutants,[35,36] and by carrying pathogens or antibiotic-resistance factors.[37,38] However, the effects of MPs on living organisms reported to date are highly inconsistent – ranging from significant negative impacts (including lethal toxicity) to negligible or no observable effects, and even to apparent *detoxification* in certain cases (where an organism initially contained higher levels of pollutants than the ingested MPs, leading the plastics to sequester some of the pollutants). Many laboratory studies use MP concentrations far higher than those found in nature—sometimes by factors of 10^2–10^7 for particles <10 μm—highlighting the need for environmentally realistic exposure studies.[39–41] While the impacts of nanoplastics remain debated, evidence shows they can cross the blood–brain barrier in fish.[42,43] Progress in assessing realistic toxicity is limited by scarce environmental data on NPLs, while human exposure through air, water, and food is under active investigation.[44–46] MPs have been found in a variety of human diet items and beverages,[44,47,48] but humans are exposed to MNPs primarily through ingestion of contaminated food and drinking water, while inhalation of airborne particles (including plastic fragments and fibers) also represents an important secondary pathway, particularly in indoor and urban environments.[49–51] In general, smaller plastic particles are expected to pose greater hazards than larger ones. Information on the effects of NPLs on human health remains very limited so far,[52] but it has already been shown that nanoplastic particles can translocate across the human gastrointestinal barrier.[53] Accurate risk assessment of MNPs requires reliable data from environmental and food

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samples. Visual inspection, though inexpensive, becomes unreliable at smaller sizes, with one study confirming only 1.4% of visually identified particles as synthetic polymers.[54] This fact underscores the urgency of the proper selection of the compositional and chemical analysis of MNPs.

Vast sources, various uses, and emission pathways lead to MNPs with highly variable sizes, shapes, densities, polymer types, surface chemistries, and biofouling states.[3,4,19,55] This complexity makes MNPs one of the most challenging analytes in environmental and food research, requiring advanced analytical methods for accurate identification and quantification. Over the past decade, several reviews have discussed microplastic detection and analysis.[20,31,47,55–62] Early work emphasized the importance of chemical confirmation, with FTIR being the main tool in 2012 (ref. 58) while subsequent studies highlighted progress in spectroscopic and thermoanalytical techniques. More recent assessments have stressed the need for harmonized, cost-efficient methods[62] and growing attention has turned to the analysis of smaller MPs and nanoplastics.[6,7,63–65] However, significant challenges remain, including gaps in understanding the complementarity of mass spectroscopy *versus* particle-based methods, as well as issues of automation, validation, and standardization.

The purpose of this review is threefold: (i) to outline the main challenges in MNP research, (ii) to critically evaluate available methods for representative chemical analysis, and (iii) to discuss future directions. We compare mass- and particle-based approaches in terms of sensitivity, detection limits, and potential for automation, highlight promising emerging techniques, and emphasize the benefits of combining multiple methods for comprehensive characterization. Special attention is given to nanoplastic analysis, where extremely small particle size and mass pose unique challenges. Finally, we examine current efforts toward method validation and harmonization and provide an outlook on applying advanced analytical tools to MNPs and related particles. Unlike earlier reviews that primarily examined individual detection techniques or specific environmental compartments, this work takes an integrative and cross-compartmental approach. It unites mass-based, particle-based, and hybrid analytical strategies to construct a comprehensive toolkit for micro- and nanoplastic (MNP) quantification across scales. Rather than focusing on medium-specific case studies, this review systematically evaluates analytical workflows—from pretreatment and separation to detection and quantification—that can be adapted for diverse matrices including water, soil, air, and biological systems. By centring on methodological principles and workflow integration, the review establishes a comparative foundation for method transferability, harmonisation, and standardisation across different environmental contexts, thereby advancing toward a unified analytical framework for MNP analysis. Table 1 provides a comparative overview of analytical methods for micro- and nanoplastic detection, organised into four functional domains: identification, quantification, fractionation, and characterisation. For each method, typical detection limits, strengths, and weaknesses are summarised, enabling direct benchmarking of techniques. This structured framework highlights the complementary nature of different approaches and serves as a practical guide for selecting suitable methods depending on particle size, sample complexity, and research objectives.

2. Analysis of microplastics

2.1. Challenges in microplastic analysis

Microplastic pollution is highly complex and can be described across several key dimensions. These particles span a wide range of sizes from about 1 μm to 1 mm (and up to 5 mm for the largest), and they are composed of diverse polymers, including both conventional plastics and biopolymers with varying structures and densities (Fig. 1). Their shapes are equally varied, occurring as spheres, fragments, fibers, films, foams, and more. In addition, MPs often contain additives such as plasticizers, pigments, flame retardants, or UV stabilizers, and may carry weathering byproducts or adsorbed environmental contaminants like heavy metals, antibiotics, and persistent organic pollutants. Finally, their surfaces reflect different aging states, from pristine primary particles to weathered secondary ones, often with biofouling layers, altered charges, and shifts in hydrophobicity resulting from environmental exposure.

Given the vast diversity of microplastic characteristics and the extremely wide concentration ranges observed—spanning up to 10 orders of magnitude from about 10^{-2} to 10^{8} particles per m^3 in freshwater and drinking water[19] —their analysis presents significant challenges. A key issue is representative sampling: the appropriate sample size depends on the level of pollution in the medium (water, soil, air, *etc.*) and on the specific research focus, whether measuring particle counts, size distribution, or total polymer mass. Since smaller particles occur in much higher numbers, relatively small volumes may suffice for quantifying fine particles at the micron scale, whereas studies targeting larger particles or overall polymer mass require much larger, more representative samples.

Preconcentration and matrix removal. Identifying and quantifying MPs in complex environmental matrices can be like searching for a needle in a haystack. It typically requires concentrating the sample (for instance, by filtration or sedimentation) and efficiently removing organic and inorganic matrix components that could interfere with detection.

Analytical sensitivity and measurement metrics. Highly precision methods are necessary for the chemical identification and quantification of MPs. Many techniques can reliably identify polymer types (and sometimes additives), but quantification may be either mass-based or particle-based. Mass-based methods determine the total mass of each polymer present, without direct information on particle counts or sizes, whereas particle-based methods count individual particles and can measure their size distribution and shape. Furthermore, characterizing certain particle attributes (degree of degradation, surface chemistry, adsorbed chemicals, *etc.*) may require additional specialized techniques. Thus, depending on the

Table 1 Analytical methods for micro- and nanoplastics (MNPs) with detection limits, strengths, and weaknesses

Category	Approach	Techniques	Key applications	Typical detection limits	Strengths	Weaknesses
Identification	Mass-based	Py-GC/MS, TED-GC/MS, MALDI-ToF/MS, TD-PTR/MS, qNMR, HPLC, DSC	Identifies polymer types; bulk mass analysis; detects major plastic classes (PP, PE, PVC, PET, PS, PA, PMMA, PLA, PTFE)	~μg–mg polymer required	High accuracy in polymer identification; quantitative; applicable to complex matrices	Destructive; no particle-level info (size/-shape/counts); requires sample pretreatment
Quantification	Particle-based	ATR-FTIR, μ-FTIR, μ-Raman, ToF-SIMS, CARS, SRS, NIR	Determines particle counts, size distribution, and shapes; tracks aging and biofouling	FTIR: 10–20 μm; μ-FTIR: 5–10 μm; μ-Raman: ~1 μm (down to ~500 nm); ToF-SIMS/nano-IR: <1 μm–50 nm	Non-destructive; provides counts, size, morphology; chemical specificity	Limited NP detection (<100 nm for most methods); labor-intensive; slower throughput
Fractionation	Separation-based	HDC-SEC, FFF-Raman, FFF-Py-GC/MS	Separates MPs/MNPs by size, density, or type prior to further analysis	~20 nm to several μm (method-dependent)	Effective for size separation; enables pre-concentration of NPs; useful for complex samples	Needs coupling with other methods for identification; not stand-alone
Characterization	Morphology/surface	SEM/EDX, SEM-Raman, (SP)-ICP-MS, O-PT-IR, AFM-IR, nano-FTIR	Provides morphology, elemental composition, surface chemistry, additive/contaminant profiling, and nano-scale analysis	SEM/EDX: ~1 μm; SEM-Raman: ~500 nm; (SP)-ICP-MS: ~20–50 nm; AFM-IR/nano-FTIR: 20–50 nm; O-PT-IR: 50–100 nm	High-resolution imaging; detailed morphology; detects additives/contaminants; nano-scale capability	Expensive; time-consuming; limited throughput; complex sample preparation needed

information needed, one may have to deploy either a single method or a combination of multiple complementary methods.

Validation and comparability of methods. It is essential to validate analytical methods and to harmonize and standardize protocols so that results on MP contamination are reliable and comparable across studies. This in turn requires suitable reference materials. At present, reference materials that truly mimic the variety of microplastic particles found in real samples (in terms of polymer types, sizes, shapes, and aging states) are still lacking.

Contamination control. Because plastic debris is ubiquitous in the environment, strict measures are needed to prevent sample contamination throughout sampling, sample storage and processing, and even during instrumental analysis. Contamination control (*e.g.*, working in clean conditions, using filtered reagents and air, wearing natural-fiber lab clothing, procedural blanks, *etc.*) is indispensable for obtaining trustworthy results.

Selecting an analytical method for microplastic research must align with the study's objectives.[14,55,66] For monitoring or modeling, measuring total polymer mass may suffice, and destructive mass-based methods can be appropriate—though results may be skewed if a few large particles dominate the mass while smaller ones contribute little.[14] In contrast, studies on transport, fate, or biological impacts require particle-based, nondestructive methods that identify and quantify individual MPs, providing counts, size distributions, and shapes within the detection limits of the technique. Further analyses may be needed to assess properties such as degradation, surface chemistry, additives, or sorbed pollutants. Since no single method captures all aspects of diverse MP populations, combining multiple approaches is often necessary. Additionally, reliable outcomes depend on robust sampling and preparation protocols suited to the detection methods, sample complexity, and contamination levels.

2.2. Pre-treatment and fractionation of microplastic samples

Pre-treatment and fractionation are essential preparatory steps for the accurate analysis of microplastics (MPs) in environmental samples. The reliability of subsequent identification and quantification methods—whether spectroscopic, thermal, or microscopic—depends heavily on the quality of these early steps. Inadequate pre-treatment can lead to incomplete recovery, polymer degradation, or external contamination, all of which contribute to inconsistencies in reported data across studies.

Removal of organic matter. Microplastic samples from environmental or biological matrices often contain large amounts of organic material such as detritus, algae, or biofilm. These interfere with optical and spectroscopic detection and must be removed carefully without damaging the polymers. The most widely used oxidising agents include hydrogen peroxide (H_2O_2), Fenton's reagent (Fe^{2+}/H_2O_2), and potassium hydroxide (KOH). While H_2O_2 and Fenton's reagent are effective for organic matter degradation, they can partially oxidise certain polymers such as polyamide (PA) and

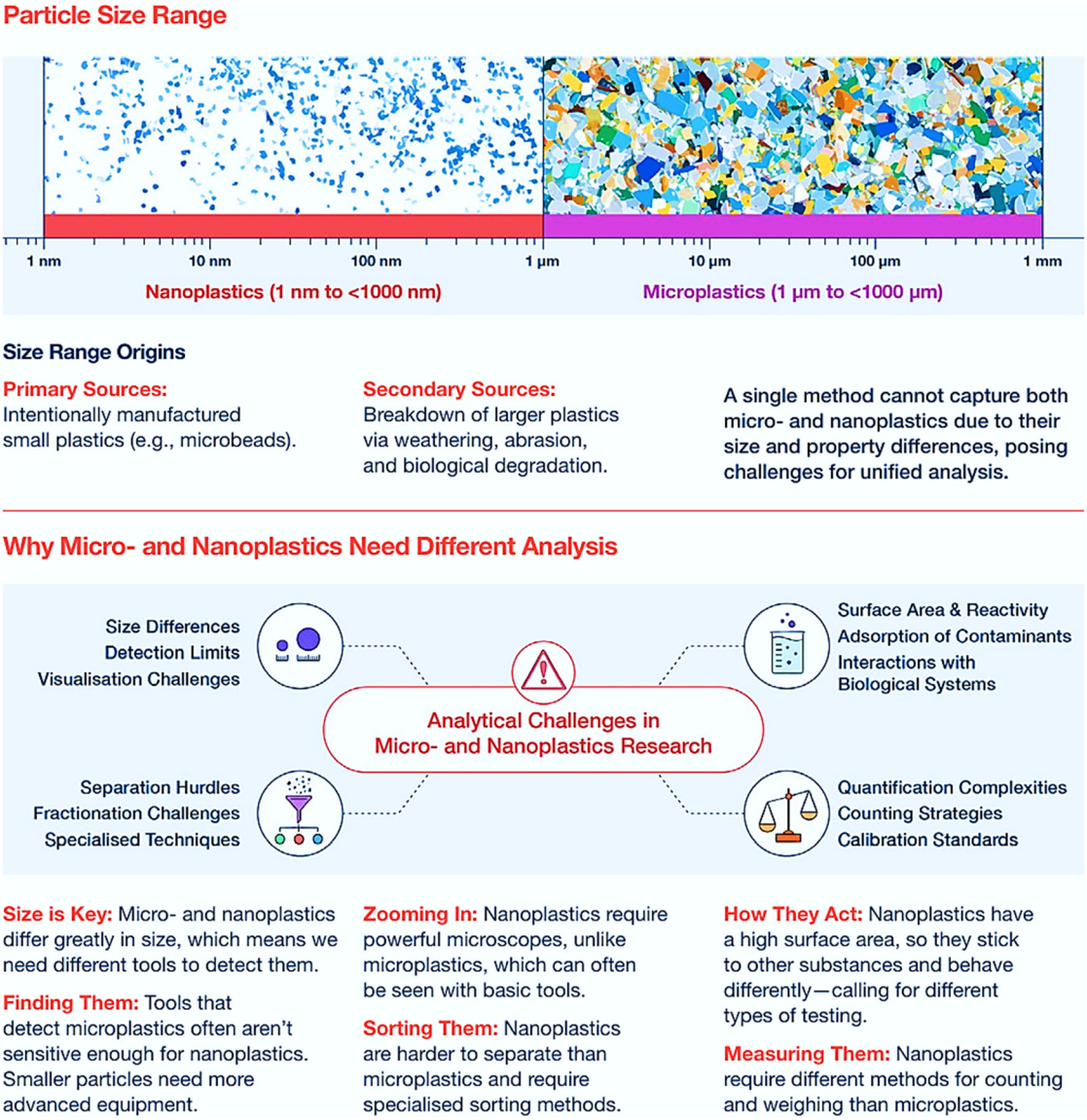


Fig. 1 Comparison of particle size ranges and analytical challenges in micro- and nanoplastics research. Nanoplastics (1–1000 nm) and microplastics (1–1000 µm) originate from both primary sources (*e.g.*, intentionally manufactured plastics) and secondary sources (*e.g.*, weathering and fragmentation of larger plastics). Due to significant differences in size, morphology, surface chemistry, and environmental behavior, a single method cannot reliably capture both fractions. Distinct analytical hurdles—such as detection limits, visualization challenges, separation and fractionation difficulties, surface reactivity, contaminant adsorption, biological interactions, and quantification complexities—necessitate the use of specialized and complementary techniques for robust characterization. Authors own the infographic.

polyethylene terephthalate (PET). In contrast, enzymatic digestion using lipase, protease, or cellulase provides a gentler option for biological samples but requires longer reaction times. The choice of reagent therefore depends on the matrix type, organic load, and polymer sensitivity.

Density separation. Following digestion, density separation is used to isolate microplastics from heavier inorganic particles. This technique relies on differences in density between plastics and sediments. Low-density solutions such as NaCl (1.2 g cm^{-3}) are suitable for recovering polyolefins (PE, PP), whereas denser media such as $ZnCl_2$ or NaI (1.6–1.8 g cm^{-3}) are necessary to extract higher-density polymers including PET, PVC, and PEEK. Despite high recovery efficiency, dense salt solutions are costly and require proper waste treatment. Recent approaches employ reusable density media or dual-density separation to capture a broader range of polymers while minimising chemical waste.

Filtration and sieving. After separation, microplastics are typically collected through filtration or sieving to obtain size-specific fractions. Filters or sieves made from non-plastic materials—such as glass fiber, stainless steel, or aluminum oxide (Anodisc)—are preferred to avoid contamination. A tiered system (*e.g.*, 5 mm, 300 µm, 50 µm) enables classification into large and small microplastic fractions. However, very fine particles (<20 µm) can adhere to surfaces or pass through filter pores, leading to underestimation. Closed filtration systems and gentle rinsing with pre-filtered

deionised water are recommended to maximise recovery and prevent airborne contamination.

Contamination prevention and recovery validation. One of the most significant challenges in microplastic analysis is contamination from laboratory environments. Airborne fibers, synthetic clothing, and plastic consumables can easily compromise samples. To mitigate this, all reagents should be pre-filtered, non-plastic tools (metal, glass) should be used, and procedural blanks included in every batch. Recovery efficiency should also be assessed using spiked reference materials to quantify potential losses during digestion or separation. Reporting recovery percentages and uncertainties enhances the reproducibility and comparability of microplastic data among laboratories.

Improving fractionation workflows. Although traditional density separation and filtration remain standard, they are not universally effective across all sample types. To improve representativeness, new matrix-specific workflows are being developed. These include the use of recyclable high-density solutions, microfluidic separation devices, and controlled ultrasonic dispersion to prevent aggregation of small microplastics. Standardisation of these procedures, along with the introduction of certified reference materials and inter-laboratory validation programs, will be critical for ensuring consistent and reproducible results across studies and laboratories.

2.3. Mass spectroscopy based identification for MP

Thermal transformation coupled with GC/MS involves polymer pyrolysis (heating without oxygen) followed by chromatographic separation and mass analysis of the resulting volatile fragments. The resulting pyrogram serves as a molecular fingerprint, enabling polymer identification and quantification in complex samples.[62,67]

Pyrolysis-GC/MS (Py-GC/MS) has long been used in polymer research—from detecting tire wear debris in 1966 to identifying polystyrene as an environmental pollutant in 1986.[65,66] It has since been applied to various matrices including sediments,[68–77] waters,[78–83] biota,[67,80,84,85] sewage sludge,[74,86] airborne particulates,[87] soil,[88,89] sea salt,[90] and drinking water[91] and more recently to nanoplastic analysis[92–95]

Two common configurations are (i) conventional Py-GC/MS and (ii) thermal extraction–desorption GC/MS (TED-GC/MS). Py-GC/MS can operate in several modes:

- Single-shot: rapid heating (>500 °C) fully decomposes polymers for direct GC/MS analysis.[62,76,96]
- Double-shot: sequential heating separates volatiles (additives, contaminants) from polymer fragments, allowing characterization of both.[97,98] Controlled desorption improves polymer identification in organic-rich matrices.[99,100]
- EGA-MS: continuous temperature ramp produces a thermogram of total ion current *vs.* temperature, rapidly indicating polymer types by decomposition profiles.[96]
- Thermochemolysis: pyrolysis with derivatization (*e.g.*, TMAH) enhances detection of polar polymers such as PET or polycarbonate through methylated monomers.[62,67,96]

Pyrolyzers may employ filament, Curie point, or microfurnace heating systems, differing mainly in temperature control and sample capacity.[72,75,77,101]

In TED-GC/MS, a thermogravimetric analyzer (TGA) heats samples under inert gas, and evolved volatiles are trapped and transferred to GC/MS. This allows larger sample masses (tens of mg) and improved detection limits. TED-GC/MS and TD-PTR/MS have detected polymers such as PS, PET, PVC, and PPC at ng levels in snow and aerosols.[102,103] Quantitative detection of PET in soil by TGA-MS achieved an LOD of 0.07 wt% and LOQ of 1.72 wt%.[104] Evolved gases can also be analyzed *via* FTIR (TGA-FTIR) for polymer identification.[105,106]

2.4. Other mass-based identification of MP

TGA–DSC quantifies MPs based on melting transitions, suitable for crystalline polymers (PE, PP, PA, PET).[107,108] Majewsky *et al.* identified distinct peaks for LDPE (101 °C), PP (164 °C), PA (216 °C), and overlapping peaks for PET/PES (250–261 °C).[109] Detection limits were 2.5 wt% for PE and 5 wt% for PP. DSC studies confirmed that particle size strongly affects melting profiles; pre-sieving samples improves consistency.[110] In industrial wastewater, extended DSC cycles detected PE, PP, PA, and PET at concentrations of 0.5–35.5 μg L^{-1}, with >99.99% MP removal efficiency in one WWTP.[107]

MALDI-ToF MS allows "soft" ionization of polymers with minimal fragmentation, yielding repeat-unit spectral patterns for polymer identification and molecular weight estimation.[111–113] It enables rapid, high-throughput analysis of extracts or particles mixed with a suitable matrix, identifying polymers such as PE, PP, PET, PS, and copolymers. However, ionization bias and mixture complexity can affect quantification.

ICP-based spectrometry (ICP-OES, ICP-MS) quantifies microplastics *via* tracer elements such as Ti (from TiO_2), Sb (in PET), or Br (in BFRs).[114–116] Single-particle ICP-MS (SP-ICP-MS) detects discrete particle ion bursts but is limited to plastics with metal or pigment markers. While ICP offers high sensitivity (sub-ppb), it does not provide molecular polymer identification and serves best as a complementary tool.

2.5. Particle-based quantification methods for MP

Vibrational spectroscopy—mainly FTIR and Raman—is the cornerstone of nondestructive polymer identification. These methods detect characteristic molecular vibrations, providing polymer-specific fingerprints.

Micro-FTIR identifies particles down to 10–20 μm (and 5–10 μm with specialized optics) using transmission or reflection modes. Full-filter mapping or imaging with focal plane array (FPA) detectors allows automated screening of thousands of particles within hours.[117,118] Although diffraction limits mid-IR resolution (~10 μm), it remains highly effective for small MPs. Filters with minimal IR absorption (*e.g.*, aluminum oxide anodisc) are preferred. Micro-FTIR imaging provides polymer type, particle count, and size distributions efficiently, making it ideal for environmental MP analysis.

Raman microscopy uses laser light scattering and achieves higher spatial resolution (~1 μm or below) because it is limited by visible wavelengths (0.5–1 μm). Micro-Raman spectroscopy can therefore detect particles well below 10 μm, even approaching 1 μm, though it requires longer acquisition times and careful control of fluorescence interference. Similar to FTIR, Raman can operate in imaging mode by raster-scanning or using line-scan configurations to cover entire filters. Since water is a weak Raman scatterer, particles can be analyzed directly in aqueous suspensions, although filtration remains common. Automated Raman imaging has identified MPs as small as 1 μm in environmental samples—for example, ~338–628 MP particles per L in treated drinking water compared with only 0.022 ± 0.019 MP particles per L using FTIR imaging down to 6.6 μm.[91] This illustrates how smaller detection limits dramatically increase reported particle counts, emphasizing the need for highly sensitive methods for fine MPs.

Vibrational spectroscopy provides multidimensional information, including polymer type, particle count, size distribution, shape, and the ratio of plastic to non-plastic particles. For particles <20 μm, Raman is preferred due to its superior spatial resolution. In routine analyses, μ-FTIR remains dominant for 20–500 μm particles owing to faster throughput and reduced fluorescence interference, while Raman is used for smaller particles (~1 μm), providing complementary coverage.[91,119] Throughput continues to improve *via* quantum cascade laser (QCL)-based IR imaging and hyperspectral imaging combined with machine-learning algorithms (HIS + AI). Table 2 summarises representative analytical studies employing combined spectroscopic and thermal techniques for microplastic detection across various environmental matrices.

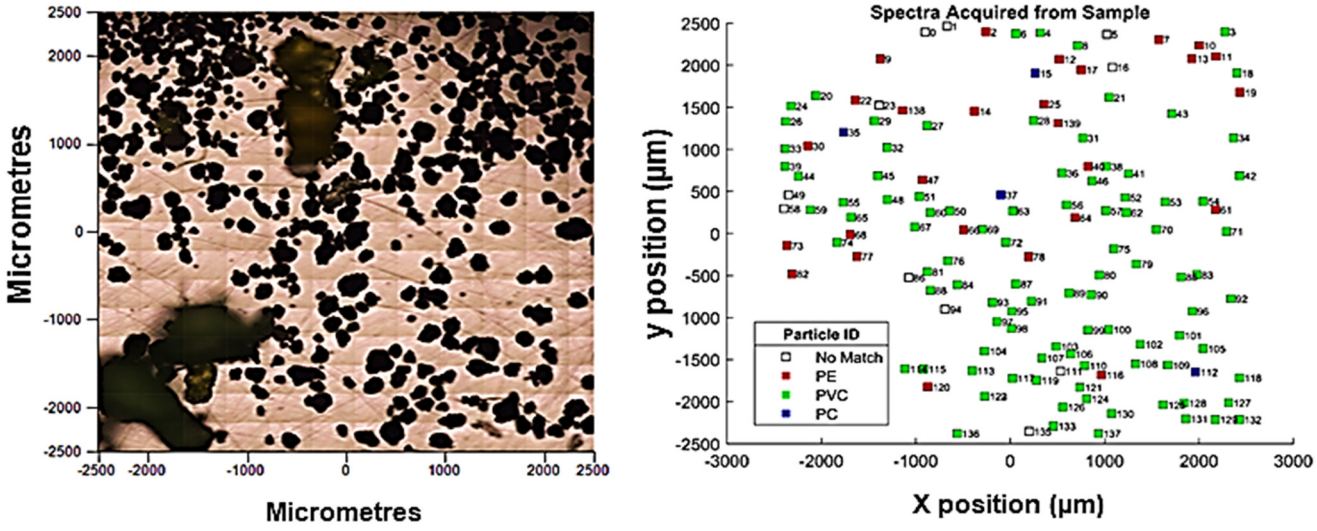
Beyond conventional FTIR and Raman, nonlinear optical methods such as coherent anti-Stokes Raman scattering (CARS) and stimulated Raman scattering (SRS) enhance sensitivity and suppress fluorescence. These techniques generate signals only from selected vibrational modes, allowing rapid *in situ* imaging without extensive matrix removal, although they require advanced laser systems and expertise.

Despite their power, vibrational techniques struggle to differentiate polymers with similar spectra (*e.g.*, PE *vs.* PP) or heavily weathered materials where oxidation obscures signals. Integrating spectroscopic data with thermal or elemental analysis improves reliability.

Micro-ATR-FTIR complements transmission FTIR by pressing particles against a crystal (diamond or germanium), enabling analysis of opaque or thick samples. It provides good spectra for individual particles ≥20 μm, though throughput is lower. ATR imaging using focal-plane-array detectors can extend this capability but with a smaller field of view. Overall, ATR-FTIR is valuable for small or non-transparent MPs despite reduced scanning speed (Fig. 2).

Table 2 Summary of analytical studies on microplastic detection across different matrices using combined spectroscopic and thermal techniques

Analytical focus	Types of MP	Size (μm)	Source	Techniques	Limitations	Ref.
Particle number, PSD	PP, HDPE, LDPE	400–5700	Seawater	FTIR, μ-FTIR, Raman	Polymer identity missing, only large particles	120
Mass fraction	PE, PET, PS, PP	145–174	Freshwater sediment	Py-GC/MS, TED-GC/MS, TGA-FTIR, TGA-MS, DSC	Pristine polymers, narrow particle size range	121
Particle number, polymer type, or particle mass	PE, PVC, PMMA, PS	8–140	Ultrapure water	Microscopy, μ-FTIR, μ-Raman spectroscopy, TED-GC/MS, SEM	Pristine MP, test material RSD 26–85%, ultra-pure water	122
Polymer type, particle number, polymer mass	PC, PS, PP, PET, LDPE, EPS	150–300 and 2000–4000	Soda tablets	Microscopy, gravimetric, ATR-FTIR, μ-FTIR, Py--GC/MS, Raman, μ-Raman spectroscopy	Only large particles	123
Polymer type, particle number	PVC, PET, PE, EPS, PS	1–500	Drinking water + gelatine	FTIR, Raman	—	124
Polymer type, particle number, particle size	PE, PS, PVC, PET	1–500	Drinking water	OM, μ-FTIR, μ-Raman spectroscopy	Clean water as matrix	125
Particle number, size, mass fraction	PET in water	30–200	Clean water	μ-FTIR, μ-Raman spectroscopy, Py-GC/MS, fluorescence microscopy, TGA, LDIR, NMR, HPLC	Clean water as matrix	126
Polymer type, polymer mass	PE, PS, PVC	125–355	Soda tablets	Microscopy, μ-Raman spectroscopy, LDIR	Narrow particle size range	127
Polymer type, particle number, particle mass, physical properties	PA66, PVC, PE (Py-GC/MS); PE dominant (LDIR, 53.6%)	Mean 35.6 μm (heterogeneous shapes)	Human thrombi (cerebral arteries, coronary arteries, deep veins)	Py-GC/MS, LDIR, SEM	Limited sample size (n = 30); potential confounding clinical factors; first study linking MPs with thrombi, requiring further validation	128
Polymer type, particle number, particle morphology	PET (47.8%), PP (34.7%), plus 5 other polymer types	LDIR: 20–500 μm; SEM: down to 2 μm	Human penile tissue (corpora)	LDIR, SEM	Small sample size (n = 6); limited to surgical patients; preliminary findings requiring validation	129



Row	Library Matches	Fraction of Matches
No Match	399	26.9%
PE	131	8.8%
PVC	914	61.6%
PC	39	2.6%
Total	1483	100.0%

Fig. 2 Micro FTIR of the samples collected from the local waste trap in the Sydney region of Australia. Authors own the analysis and characterisation.

Raman imaging and mapping. Modern Raman microscopes with automated stages and sensitive CCD detectors enable mapping of entire filters or membranes for microplastics. A typical workflow involves collecting particles on a filter, defining scan areas under an optical microscope, and acquiring Raman spectra at each pixel or selected particle sites using point or line mapping. Spectral libraries then classify polymers automatically. While this approach yields detailed data, high-resolution mapping can take hours to days, so targeted scanning of suspect particles (*e.g.*, fluorescence-marked regions) is often used. Combining Raman with optical trapping allows near-real-time analysis of particles in fluid streams.

Nonconventional Raman techniques. Advanced Raman-based methods such as coherent anti-Stokes Raman scattering (CARS) and stimulated Raman scattering (SRS) enhance sensitivity by generating signals only from specific vibrational modes. CARS uses two synchronized lasers to excite molecular vibrations, while SRS detects energy transfer between beams at resonance. Both provide high-contrast, fluorescence-free images and enable rapid, label-free imaging of microplastics in complex matrices. Though still confined to research applications, these nonlinear methods show promise for fast, *in situ* analysis.

Emerging Raman-based methods (optical tweezers *etc.*). Optical tweezers combined with micro-Raman spectroscopy ("Raman tweezers") use a tightly focused laser to both trap and excite microscopic particles. Originally developed for biological and nanomaterial analysis,[130–133] this approach has successfully identified micro- and nanoplastics down to ~50 nm.[132,134,135] Kniggendorf *et al.* demonstrated trapping of 100 µm plastic fragments in flowing water (1 L h^{-1}) and their identification *via* Raman spectra even in complex suspensions.[136] Although current systems are limited by throughput and sensitivity, they offer potential for continuous, *in situ* monitoring of plastics in liquids.

Fluorescence microscopy after staining. Fluorescent dyes like Nile Red selectively bind to hydrophobic polymer surfaces, allowing rapid visualization and counting of plastic particles.[114,137–140] While fast, this approach lacks polymer specificity and may yield false positives from natural organic debris. It is therefore often paired with spectroscopic confirmation for selected particles.

Electron microscopy with EDS. SEM and TEM provide high-resolution morphology but not polymer identity. When coupled with energy-dispersive X-ray spectroscopy (EDS), inorganic elements in or on plastics (*e.g.*, Ca in $CaCO_3$-filled PP) can be detected. These methods are valuable for nanoplastics (<1 µm), though confirming polymeric nature often requires EDS carbon mapping or correlative techniques such as AFM-IR for nanoscale chemical identification.

Asymmetric flow field-flow fractionation (AF4). AF4 separates particles (10 nm–100 μm) by size using laminar and cross-flow forces. When coupled with MALS, DLS, or on-line spectroscopic detectors (UV/IR), it characterizes nanoplastic size distributions and, after fractionation, can identify polymers using AF4-FTIR or AF4-MALS.

Chemical imaging and X-ray techniques. Advanced imaging techniques like μ-XRF (micro X-ray fluorescence) mapping or μ-PIXE (particle-induced X-ray emission) can scan filters for certain elemental signatures (*e.g.*, chlorine mapping might find PVC particles, titanium mapping finds TiO_2-loaded fragments). Likewise, STXM (scanning transmission X-ray microscopy) with NEXAFS (near-edge X-ray absorption fine structure) can differentiate polymer types by their carbon K-edge spectra at ~290 eV, even for nanoplastics – this has been used at synchrotrons to identify submicron plastics in environmental samples.[141,142] These X-ray methods require specialized facilities but add to the toolbox for challenging cases where traditional methods falter.

Holography and light scattering. Emerging optical tools such as digital holographic microscopy and multi-angle light scattering can detect and size microplastics in fluids *via* interference or scattering patterns. While they do not directly identify polymers, combining them with staining or spectral detection may enable high-throughput screening.

In summary, a range of complementary particle-level methods enhances detection sensitivity and size resolution for microplastics. While FTIR and Raman remain the primary techniques, these emerging approaches—optical, X-ray, and fractionation-based—offer valuable support for comprehensive micro- and nanoplastic characterization in complex samples.

2.6. Combined methods for MP analysis

Given the limitations of any single method, using multiple analytical techniques in tandem can provide a more holistic characterization of microplastic pollution (Fig. 3). There are several ways in which methods can be combined to overcome individual limitations:

Identification of individual MP particles. A common strategy is to pair mass-based and particle-based analyses. For example, Py-GC/MS can first determine polymer types and approximate mass fractions, followed by FTIR or Raman confirmation of individual particles. Conversely, imaging-based identification can precede destructive analysis for total mass quantification. Such cross-checking links polymer mass to particle counts and validates both datasets. Inter-comparison studies have shown method-dependent differences in detected polymers; for instance, μ-FTIR imaging and Py-GC/MS yielded slightly different polymer distributions in parallel water samples.[90] Using both ensures no major polymer type is overlooked.[91]

Integrated detection and quantification. Hybrid workflows often split a sample for complementary analysis—*e.g.*, a portion of a filter analyzed by Py-GC/MS for polymer mass, and the remainder by imaging spectroscopy for particle counts.[91,119] Comparing these results strengthens confidence: imaging reveals particle size and abundance, while pyrolysis gives absolute polymer mass. Discrepancies between methods can reveal biases (*e.g.*, many fine particles affecting counts but not mass, or large fragments skewing mass but not counts). Similarly, combining thermal and spectroscopic analyses enables additive tracking—linking brominated compounds from thermal analysis with PS fragments identified by Raman or SEM-EDS.

Microscopy for morphological validation. After chemical identification, SEM provides detailed surface morphology, revealing weathering, biofilm presence, or filler content (*via* EDS). For nanoplastics, TEM offers higher magnification and elemental mapping, confirming particle composition and structure. Although low-throughput, electron microscopy provides crucial ground-truth visualization of particle shape, roughness, and origin (Fig. 4).

Assessing weathering and additive migration. Complementary techniques such as XPS and FTIR can quantify surface oxidation or chemical aging of identified plastics (*e.g.*, carbonyl or hydroxyl indices). GC/MS of leachates detects oxidative breakdown products, while micro-FTIR or Raman mapping can visualize additive loss or migration. Correlating these chemical and morphological changes helps assess degradation history and environmental exposure.

In summary, integrating bulk and particle-scale methods—combining thermal, spectroscopic, and microscopic analyses—enables accurate identification, quantification, and aging assessment of microplastics. Such multi-method workflows maximize reliability and provide a holistic understanding of polymer composition, morphology, and environmental transformation.

3. Analysis of nanoplastics

3.1. Challenges in nanoplastics analysis

Nanoplastics (typically defined as plastic particles ≲1 μm in size) present a suite of additional challenges compared to microplastics. Their extremely small size means that detection limits of traditional methods are pushed to the brink, and new sources of background noise and contamination emerge. Nanoplastics often exist as colloidal dispersions or agglomerates, making them difficult to isolate and count. They can also exhibit different behavior (*e.g.*, staying suspended in water, passing through filtration steps that capture larger MPs, or interacting with organisms at the cellular or subcellular level) and thus demand specialized analytical strategies. Key challenges include:

Ultratrace concentrations. Environmental concentrations of nanoplastics are largely unknown but are expected to be very low (potentially orders of magnitude lower in mass than microplastics), requiring methods with exquisite sensitivity.

Separation from matrices. Effectively separating and concentrating nanoplastics from complex matrices (water with natural colloids, soil leachate, tissue digests, *etc.*) is nontrivial. Nanoplastics can pass through filters that retain microplastics and may adhere to container walls or other surfaces.

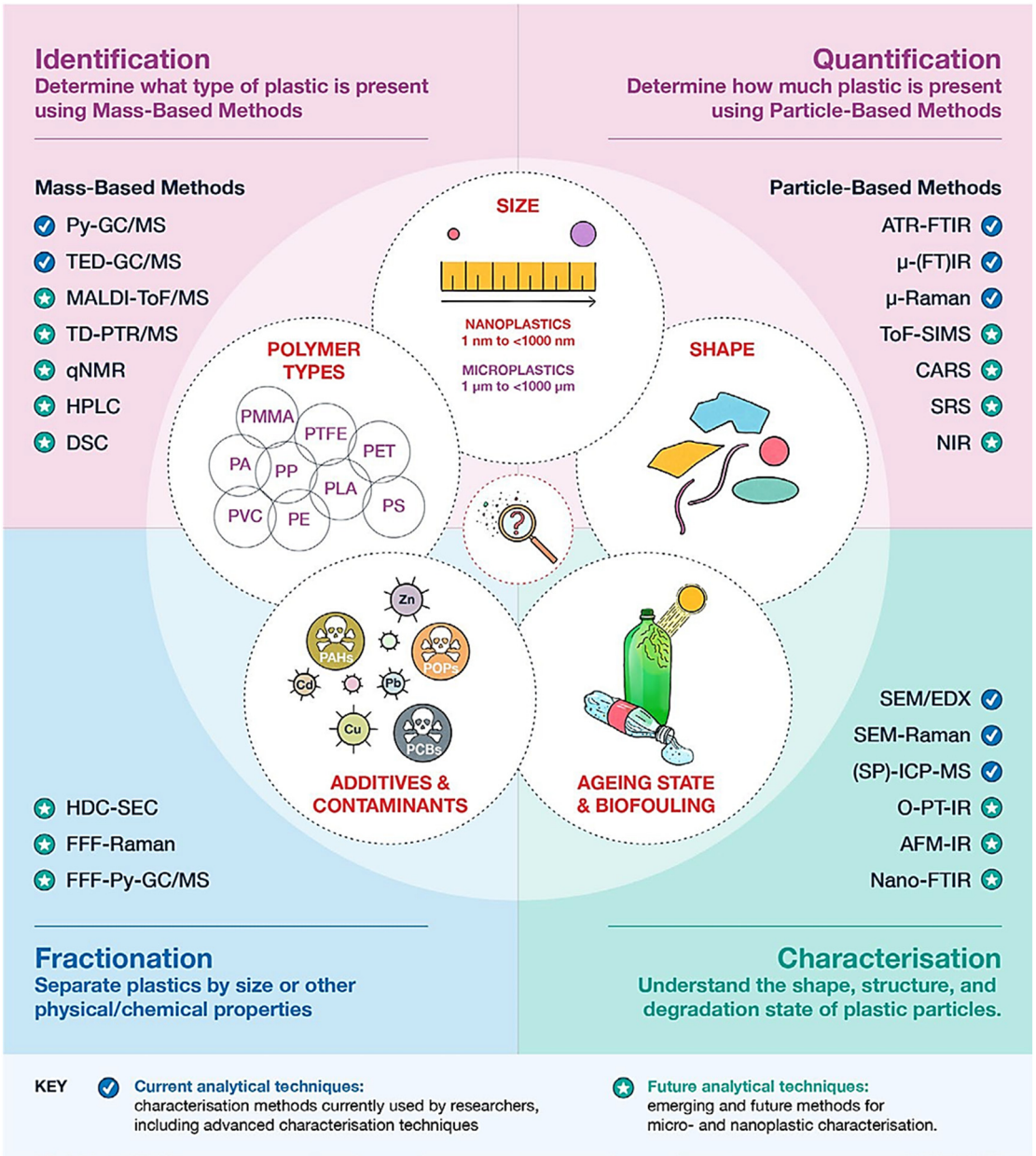


Fig. 3 Microplastic analysis demands evolving from basic identification methods to comprehensive, standardised analytical frameworks that can track sources, assess environmental impacts, and inform effective mitigation policies across all environmental compartments. Authors own the infographic.

Distinguishing nanoplastics from natural nanoparticles. Environmental samples contain myriad natural nanoparticles (clays, organic detritus, combustion soot, *etc.*). Discriminating engineered or weathered plastic nanoparticles from these background particles calls for highly specific chemical identification.

Lack of reference materials and validated methods. Standardized protocols for sampling, recovering, and measuring nanoplastics are not yet established. Few reference nanoplastic materials are available to test method performance (*e.g.*, monodisperse fluorescent nanospheres can be proxies, but they may not represent irregular, weathered nanoplastics found in reality).

Aggregation and surface effects. Nanoplastics tend to aggregate due to van der Waals forces and surface charges, especially in the presence of natural organic matter or salts. They may also acquire coronas of organic molecules (like proteins or humic substances), altering their behavior and complicating analysis (*e.g.*, by changing their spectral signatures or making them stick to labware).

The objectives for nanoplastic analysis are similar to those for microplastics – reliable identification, quantification, and characterization – but at a far smaller scale. This often means that methods need to be borrowed or adapted from fields like nanotechnology, colloid chemistry, and bioanalytical chemistry. The goal is to develop techniques that can detect

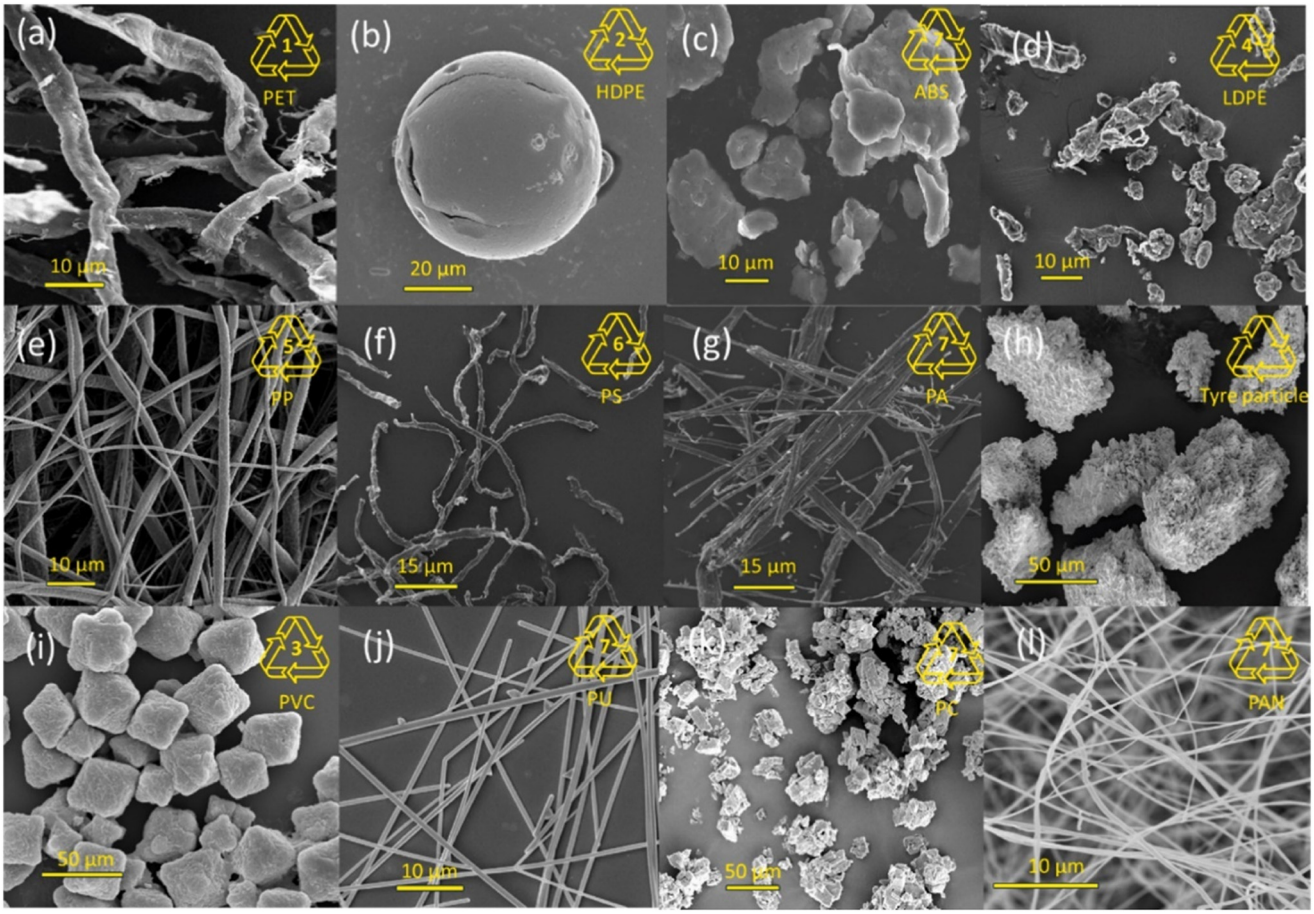


Fig. 4 (a) PET microfibers from the kitchen towel, b) HDPE microbeads from the facial scrub cosmetics, c) ABS microplastic debris from children toys, d) LDPE microplastic debris from waste soft plastics due to aging, e) PP microplastic fibers debris from kitchen scrubber, f) PS microfibers from textiles, g) PA microfibers from fabric, h) rubber Tyre debris, i) laboratory generated PVC microplastics, j) PU microfibers used to produce synthetic leather, k) PC debris from the fractured headlamp of old cars, and l) PAN precursor nanofiber. Authors own the analysis and characterisation.

nanoplastics in environmental and biological media, determine their particle size distribution and concentration, identify their polymer composition (and any additives or coatings), and ideally assess their surface chemistry and any adsorbed pollutants or biomolecules.

3.2. Pre-treatment and fractionation of nanoplastic samples

Before nanoplastics can be analyzed, they usually must be isolated from large volumes of sample and separated from other components. Several approaches are used for preconcentration and fractionation.

Ultrafiltration. Passing water samples through membranes with very small pore sizes (*e.g.*, 0.2 µm, 0.05 µm, or using molecular weight cut-off filters) can retain nanoplastic colloids while letting truly dissolved substances through. Tangential flow filtration systems, where the sample flows parallel to the filter to minimize clogging, are helpful for processing larger volumes.

Centrifugation and ultracentrifugation. High-speed centrifugation can pellet nanoparticles, especially if they aggregate or if density gradient media are used. By choosing appropriate centrifugal force and time, particles above a certain size can be separated. Density gradient ultracentrifugation has been explored to separate nanoplastics from natural colloids by layering solutions of different densities (*e.g.*, sucrose or silica gradients) and spinning at very high G-forces.

Flocculation and cloud point extraction. In some cases, chemicals can be added to induce aggregation of nanoplastics, which then either settle or can be filtered more easily. For instance, adding salts or organic polymers might flocculate nanoplastics. Cloud point extraction involves using a surfactant solution that, when warmed, phase-separates and drags hydrophobic particles into a small coacervate phase. This method has been used to concentrate nanoparticles including plastics.

Field-flow fractionation (FFF). As mentioned earlier, asymmetric flow field-flow fractionation is a powerful technique to size-separate nanoparticles. In AF4, a gentle cross-flow field causes smaller particles to diffuse further from the accumulation wall than larger particles, resulting in different elution times. AF4 can be coupled online with

detectors (light scattering, RI, UV, or even ICP-MS if particles contain metal) to obtain size distributions. It has been applied to nanoplastic mixtures to achieve some level of size sorting prior to analysis.

Magnetic separation. If nanoplastics can be made to associate with magnetic nanoparticles (*e.g.*, through adsorption or by grafting functional groups that bind), a magnetic field could then pull them out of suspension. This is not a broadly used method yet, but conceptually could be interesting for certain sample cleanup steps (for example, using magnetic sorbents that preferentially capture hydrophobic particles).

Selective solvent extraction. While not exactly a physical fractionation, selectively dissolving one component of a sample (*e.g.*, removing natural organic matter *via* oxidation, or dissolving carbonates with acid) can enrich the relative concentration of nanoplastics. However, extreme caution is needed to avoid dissolving or altering the nanoplastics themselves (most common solvents will dissolve or swell plastics if attempted).

Each of these methods must be optimized to maximize nanoplastic recovery while removing as much background matrix as possible. Often, a combination is used (*e.g.*, filter a large volume, then subject the retentate to FFF for analysis). It's also crucial to perform procedural blanks, since contamination with airborne microfibers or dust can easily introduce nanoplastic-like particles at this scale.

3.3. Nanoplastic characterisation using mass-based methods

Many of the mass-based techniques described for microplastics can, in principle, detect nanoplastics as well – the main limitation is sensitivity. Because nanoplastics contribute very little mass, one often has to accumulate or concentrate a sufficient amount of them to reach the detection threshold of instruments like Py-GC/MS or TGA.

Pyrolysis-GC/MS has been successfully applied to detect nanoplastics, but typically this is demonstrated in controlled settings (*e.g.*, spiking known nanopolymers into a matrix). As noted earlier, researchers have shown that Py-GC/MS can identify polymers in size ranges approaching the nanometer scale.[93–95,118] For example, one study might grind or otherwise produce nano-sized polymer particles, mix them into an environmental matrix, and then retrieve a signal by Py-GC/MS for a characteristic fragment (like a specific styrene dimer for PS). Achieving this requires either analyzing a large mass of sample (to get enough nanoplastic mass) or using methods like TED-GC/MS with highly sensitive MS detection. Advances such as thermal extraction directly in a TGA linked to MS (as described in section 2.2.2) could be particularly useful: by slowly heating a relatively large sample, even trace amounts of polymer might be detected by their unique volatile products.

Another promising mass-based approach for nanoplastics is mass spectrometry of characteristic polymer fragments or additives without full chromatographic separation. For instance, some researchers are exploring direct MS techniques like thermal desorption MS or pyrolysis-photoionization MS for rapid screening of plastics. These could be tuned for nanoplastics, especially if the goal is just to confirm presence of a certain polymer type by a unique mass fragment.

It's worth mentioning that traditional solvent extraction followed by liquid chromatography (LC) is generally not feasible for intact polymers (they're insoluble or too large), but for nanoplastics that have aged, there might be soluble oligomers or depolymerization products that can be extracted and analyzed by LC-MS. For example, if one suspects PET nanoplastics, detecting trace amounts of terephthalic acid or other PET monomers in water could indicate their presence. Such approaches lean more into the realm of indirect inference, however, rather than directly measuring the nanoplastic particles.

Radiolabeling techniques have also been used in laboratory studies of nanoplastics, *e.g.*, using 14C-labeled polymer nanoparticles and then measuring 14C in environmental compartments to track them.[143] While not an environmental monitoring tool (because environmental plastics aren't pre-labeled), such techniques help validate recovery and detection methods by providing a clear signal of where the nanoplastic goes.

In summary, mass-based detection of nanoplastics is extremely challenging and often requires *enriching the sample* in plastics first. When that can be done, pyrolysis-based methods (Py-GC/MS, TED-GC/MS) remain among the most powerful tools for confirming the polymer identity and quantifying total mass. The development of more sensitive MS detectors and preconcentration techniques will improve the prospects of mass-based nanoplastic analysis.

In Table 3, the reviewed studies each have important limitations that must be considered when interpreting their findings. Many investigations were restricted to laboratory-controlled conditions, often using engineered polystyrene nanoplastics that may not fully represent the complexity of environmental particles. In several cases, experiments involved short-term or acute exposures with only one or two particle sizes, limiting extrapolation to chronic or real-world scenarios. Plant uptake studies were generally conducted under hydroponic conditions, which do not account for the variability of field soils, while trophic transfer experiments were simplified to single food chains, reducing ecological realism. Degradation and remediation studies were often confined to model waters or controlled furnace systems, focusing on a narrow set of polymers and overlooking the chemical diversity of plastics encountered in the environment. Furthermore, emerging analytical techniques, while highly sensitive, have so far been validated on limited polymers and matrices, and require further standardization and application to diverse environmental samples. Collectively, these drawbacks underscore the need for more comprehensive, long-term, and environmentally realistic approaches to studying micro- and nanoplastics.

Table 3 Example of recent studies on nanoplastic detection, uptake, transport, degradation, and characterization across different environmental and biological matrices. The table highlights polymers studied, particle sizes, matrices investigated, analytical methods employed, and the key limitations of each approach, providing an overview of experimental designs ranging from controlled laboratory exposures to novel field-based detection techniques

Measured properties	Polymers	Particle size ($\mu m\ nm^{-1}$)	Matrix	Methods	Limitations	Ref.
Uptake, distribution, depuration kinetics; polymer type; tissue localization	Radiolabeled polystyrene nanoparticles (nPS24, nPS250)	~24 ± 13 nm and ~248 ± 21 nm	*Pecten maximus* (scallop soft tissues)	Radiolabeled synthesis (^{14}C-PS); TEM; FTIR; liquid scintillation counting (LSC); quantitative whole-body autoradiography (QWBA)	Only acute (pulse) exposure tested; limited to two particle sizes; requires extrapolation for chronic exposures; radiolabeling may alter behavior. Laboratory controlled study	144
Uptake, accumulation, localization (roots *vs.* shoots)	Polystyrene nanoparticles doped with europium chelate (PS–Eu)	200 nm	Edible plants (wheat *Triticum aestivum*; lettuce *Lactuca sativa*)	ICP-MS (quantification *via* Eu signal); time-gated luminescence; SEM (confirmation/visualization)	Limited shoot transport (<3% at 5000 $\mu g\ L^{-1}$); laboratory exposure only; engineered NPs (PS–Eu) may not fully represent environmental particles	145
Uptake, transport (root → petiole → leaf), oxidative stress, hormone disruption, gene expression changes, transcription factor regulation	Polystyrene nanoplastics (PS-NPs, fluorescently labeled)	~50 nm	Pakchoi (*Brassica rapa* subsp. chinensis)	CLSM (fluorescence localization); SEM (distribution in xylem); RNA-seq (transcriptome); qRT-PCR; ROS assays (DAB, NBT); enzyme activity (SOD, POD, CAT, GPX, GST); HPLC (hormones IAA, ZT, ABA)	Conducted under hydroponic lab conditions (not field soils); only PS-NPs tested; short-term exposure; extrapolation to real field requires validation	146
Uptake, accumulation in roots/shoots; trophic transfer to snails; effects on biomass and growth	Polystyrene nanoplastics (PS-NPs)	Not specified (NP scale)	Lettuce (*Lactuca sativa*) roots/shoots; snails (herbivores, soft tissue, feces)	Pyrolysis-GC/MS (mass quantification)	Laboratory exposure only; single NP type; bio-dilution may underestimate long-term effects; limited trophic complexity	147
NP quantification (TOC, COD, turbidity); particle size and morphology; chemical surface modifications; degradation under AOPs	Polystyrene nanoplastics (PS-NPs)	140, 252, 460, 909 nm	Aqueous suspension (model water)	TOC, COD, turbidity (bulk measures); TEM, DLS, NTA, AFM (size & morphology); FTIR (surface chemistry); photo-Fenton oxidation test	Laboratory-based model study; only PS tested; AOP tested under controlled conditions (ambient water, single treatment type); monitoring methods still need validation in complex waters	148
Controlled generation of aerosolized NPs; particle size distribution; activation efficiency; thermal/oxidative degradation behavior	Polyethylene (PE/LDPE), polypropylene (PP), polyethylene terephthalate (PET, PETp)	2.8–40 nm (up to ~80 nm)	Aerosol nanoplastics (tube furnace output)	Tube furnace (110–220 °C) for NP generation; differential mobility particle sizer (DMPS); condensation particle counters (CPC, 50% cutoff); TEM; thermal degradation analysis	Limited to lab-controlled furnace setup; only PE, PP, PET tested; material additives influenced particle output; chemical composition of generated particles not fully resolved	149
Chemical fingerprinting; polymer identification in complex organic matrices; sensitivity and detection limits	Polystyrene (PS), polyethylene terephthalate (PET), polyvinyl chloride (PVC), polypropylene carbonate (PPC)	Micro- to nanoplastics; PET detected in nanometer range	Snow samples (surface snow and snowpit, Austrian Alps)	Thermal desorption–proton transfer reaction-mass spectrometry (TD-PTR-MS)	Novel method, but currently validated on limited polymers; field application restricted to snow; further validation needed for diverse environmental matrices	150

Table 3 *(continued)*

Measured properties	Polymers	Particle size (μm nm^{-1})	Matrix	Methods	Limitations	Ref.
Occurrence, recovery, particle distribution, polymer identification in STP influent and effluent	Polystyrene (PS), polyvinyl chloride (PVC), polyethylene (PE), polytetrafluoroethylene (PTFE), polyamide (PA), polypropylene (PP)	50–2500 nm (with 44% in 50–<100 nm range)	Sewage (raw and treated effluent, full-scale STP, Saudi Arabia)	Nano-flow cytometry (quantification); Micro-Raman spectroscopy; SEM-EDX (composition and morphology)	First application of nano-flow cytometry to wastewater; polymer identification limited to representative subsets; single STP studied; conventional treatment inefficiency highlighted but not fully resolved mechanistically	151
Detection/identification in complex matrices; polymer-specific pyrolysis markers; NOM interference; validation on environmental nanoplastics (EnvNPs)	Polypropylene (PP), polystyrene (PS)	<1 μm (NP suspensions; EnvNPs 200–500 nm)	Suspensions with natural organic matter (algae, humic acid, NOM); environmental debris-derived NPs	Pyrolysis-GC/MS (600 °C) with marker analysis (C9, C12, C15i, C15s for PP; styrene dimer/trimer for PS); DLS (size); TEM-EDX (morphology, additives); H_2O_2 + UV purification to remove NOM	Quantification not yet possible (only qualitative detection); PS markers obscured by NOM without purification; NOM interference requires pre-treatment; lab validation limited to PP/PS only	152
Size distribution, particle number, polymer identification, mass quantification, recovery optimization	Polystyrene (PS), poly(methyl methacrylate) (PMMA)	60–350 nm	Water samples (laboratory spiked suspensions)	AF4-MALS (size fractionation, particle count & distribution); Py-GC/MS (polymer ID & mass quantification); pre-concentration by optimized filtration with SDS (0.05%)	Aggregation during filtration; not yet effective for larger particles (350 nm^{-1} μm); sensitivity still limited; validated only on PS and PMMA	153
Release of nanoplastics, microplastic fibrils, and microplastic fibers during washing and abrasion; particle number, size, morphology, chemical confirmation	Polyester (synthetic textiles, fleece)	Nanoplastics: 173–188 nm; fibrils: 3 ± 1 μm (20–160 μm length); MPFs: 16 ± 7 μm (up to 5 mm length)	Textile washing effluent and abrasion residues	STXM (chemical ID, NEXAFS spectra); SEM & TEM (morphology, size); NTA (quantification)	First study to confirm nanoplastic release from textiles; only polyester tested; real-world washing conditions not fully replicated; particles <100 nm not confirmed	154

3.4. Spectroscopy-based non-destructive methods for nanoplastics

For nanoplastics, conventional FTIR and Raman microscopy approach their practical limits. The diffraction limit for FTIR (~10 μm) is far above the nanoscale, and although one can detect ensembles of nanoparticles (for example, many nanoparticles together on a filter might produce a weak IR absorbance band), identifying single nanoplastic particles *via* far-field FTIR is not possible. Raman microscopy, with a laser spot of perhaps ~1 μm, can sometimes detect particles down to a few hundred nanometers if they are strongly scattering and if one is lucky to target them, but the sensitivity drops with volume, and fluorescence or photodegradation become severe for tiny particles. To overcome these issues, researchers are turning to specialized techniques that combine high spatial resolution with chemical specificity.

Near-field techniques (scanning probe-coupled spectroscopies). These include methods like AFM-IR (also called nano-IR) and tip-enhanced Raman spectroscopy (TERS), which were briefly mentioned earlier.

In AFM-IR, an AFM tip is in contact with the sample surface and an IR laser is used to irradiate the sample. When the sample absorbs IR light, it heats and thermally expands slightly, which the AFM tip detects as a deflection or oscillation. By tuning the laser wavelength and scanning, one obtains an IR absorption spectrum at spatial resolutions on the order of tens of nanometers (essentially defined by the AFM tip radius rather than the light wavelength). AFM-IR has been successfully used to identify nanoplastic particles down to ~50–100 nm, distinguishing different polymers by their IR spectra.[141,142,155,156] It has, for example, been applied to nanoplastic residues on filters or to nano-scale microplastic fragments extracted from samples, providing clear identification of polymer type. The limitation is that AFM-IR typically can analyze only one particle at a time (it's a point-by-point technique) and requires an AFM setup with an IR laser tunable to key wavelengths.

TERS uses a metallized AFM or STM tip to enormously enhance the local Raman signal (through plasmon resonance at the tip) in the vicinity of the tip apex. When a nanoplastic particle is positioned under the tip and a laser is focused, the Raman scattering from the region just a few nanometers around the tip is amplified, yielding a Raman spectrum of a single nanoparticle that would otherwise be undetectable. TERS has achieved ~10 nm resolution in some cases. Applying TERS to nanoplastics is cutting-edge, but researchers have demonstrated its ability to get Raman spectra of nanobeads or small plastic fragments that are beyond the reach of normal Raman microscopy. It remains a technically demanding method requiring very stable setups and often under ultra-high vacuum or controlled conditions, so it's not routine yet for environmental samples.

Advanced light scattering and spectroscopy in solution. Another approach is to analyze nanoplastics while still suspended in liquid, to avoid losses and artifacts from drying on filters. Techniques like dynamic light scattering (DLS) or nanoparticle tracking analysis (NTA) can measure the hydrodynamic size distribution of particles in a suspension. While these can't chemically identify the particles, they give an idea of the presence and size of nano-scale particles. If combined with a staining method (for instance, fluorescently tagging the nanoplastics), NTA can specifically track those particles.

There are also specialized flow cytometry methods being developed for nanoparticles. For example, some studies have used flow cytometry with side-scatter detection and fluorescence triggering (using Nile Red staining of nanoplastics) to count and size nanoplastic particles in water.[157] This can statistically assess large numbers of nanoparticles, though distinguishing plastic from other colloids might rely on the dye's selectivity.

Spectral imaging on filters with high-power objectives. Pushing traditional methods to their limits, one can use Raman with longer integration times or specialized substrates. One trick is to use surface-enhanced Raman scattering (SERS) by depositing samples on SERS-active substrates (like gold nanoparticle-coated filters). If nanoplastics happen to interact with the SERS substrate, their Raman signals might be amplified sufficiently for detection. This approach is not fully reliable for quantification, but it's an area of research.

Electron energy-loss spectroscopy (EELS) in TEM. For nanoplastics that have been located in a TEM, one can use EELS to glean chemical information. EELS can detect bonding features (*e.g.*, the carbon K-edge fine structure) somewhat analogous to NEXAFS mentioned earlier. It requires an advanced TEM with EELS capability, but it could differentiate, say, polyethylene *vs.* PET by the different shape of their carbon edge spectra.

In practice, analyzing nanoplastics often involves a combination of approaches to concentrate them, separate them by size (to remove larger MPs that could interfere), then use a high-resolution microscope (AFM-IR, TEM, *etc.*) to identify a few representative particles. Meanwhile, use a bulk method (like Py-GC/MS) on the concentrate to confirm the presence of the polymer in the sample as a whole. The field of nanoplastic analysis is rapidly evolving, and each method has major hurdles: for example, distinguishing a 100 nm plastic particle from a 100 nm biogenic organic particle is extremely hard by visual means, so chemical fingerprinting is essential.

Scanning probe microscopy coupled to spectroscopy. As described, techniques like AFM-IR offer nanoscale IR spectroscopy, and TERS offers nanoscale Raman spectroscopy. Another related technique is photothermal induced resonance (PTIR) which is a type of AFM-IR that can even be done with quantum cascade lasers to cover a broad spectral range quickly. These scanning probe methods are some of the most promising for directly identifying nanoplastics. They do, however, require the nanoplastics to be deposited on a very clean, flat substrate (like a ZnSe prism or Au-coated slide for AFM-IR), and they analyze one particle at a time. So, they might be used to verify the identity of nanoplastics after other methods suggest their presence.

Optical tweezers integrated Raman approach for nanoplastic detection. In section 2.3, we discussed how optical tweezers combined with Raman can trap and analyze individual microplastics. For nanoplastics, the same principle can be applied, but trapping nanometer-sized particles is more challenging because the trapping force is weaker for smaller particles. Nonetheless, with powerful focused laser beams and a very stable setup, even ~100 nm particles can be optically trapped in solution. Recently, researchers have shown that *Raman tweezers* can detect polystyrene nanospheres down to tens of nanometers.[134] The Raman spectra of such tiny particles are faint, but by prolonging acquisition or using resonant Raman enhancement (choosing a laser wavelength that resonates with a particular polymer chromophore), it is feasible.

One of the advantages of Raman tweezers is that it works in aqueous environments, so nanoplastics can be studied *in situ* in water samples without extensive preparation. A limitation is throughput: typically one particle is trapped at a time. However, one could envision scanning through many individual particles sequentially (automation could trap one particle, take a spectrum, then release it and trap another from the flow). This could eventually allow some statistical survey of nanoplastics in a sample.

In summary, the analysis of nanoplastics often pushes techniques to their sensitivity and resolution limits. It requires combining enrichment steps with cutting-edge spectroscopy or microscopy. Over the coming years, improvements in these methods (and possibly entirely new techniques) will likely close the current gap in our ability to detect and characterize nanoplastics in the environment.

3.5. Particle-based quantification of nanoplastics

While most nanoplastic (NP) studies have relied on mass-based analytical techniques such as Py-GC/MS or TED-GC/MS for quantification, these approaches do not reveal the number, size

distribution, or morphology of individual nanoparticles — parameters that are often more relevant to environmental exposure and toxicological risk. Particle-based quantification methods are therefore gaining increasing importance for nanoscale analysis.

Optical and light-scattering techniques such as dynamic light scattering (DLS) and nanoparticle tracking analysis (NTA) can determine particle number concentrations and hydrodynamic diameters in the 30–1000 nm range, although they struggle with heterogeneous or polydisperse samples. Flow cytometry offers higher throughput for fluorescently labelled nanoplastics, allowing differentiation from natural colloids when combined with dye staining (*e.g.*, Nile Red or SYBR-Green) or refractive-index gating.

Advanced optical methods such as optical tweezers Raman spectroscopy ("Raman tweezers") can trap and identify single nanoplastic particles in suspension, providing both size and chemical information simultaneously. Likewise, near-field spectroscopies like AFM-IR and TERS achieve nanoscale spatial resolution (≤50 nm) and enable semi-quantitative mapping of particle number and distribution on surfaces.

Importantly, particle number concentration rather than polymer mass may better correlate with biological responses, since surface area and particle count govern interactions with cells and biomolecules. Developing standard protocols for number-based calibration, such as using nanoparticle reference suspensions with certified particle counts, will therefore be crucial for future nanoplastic risk assessment and regulatory monitoring. Fig. 5 presents the Py-GC/MS analytical conditions for tyre dust characterization and HRTEM images showing agglomerated, amorphous tyre dust particles with embedded crystalline metallic contaminants.

In summary, integrating mass- and particle-based quantification approaches provides a fuller picture of nanoplastic pollution — combining total polymer load with particle count

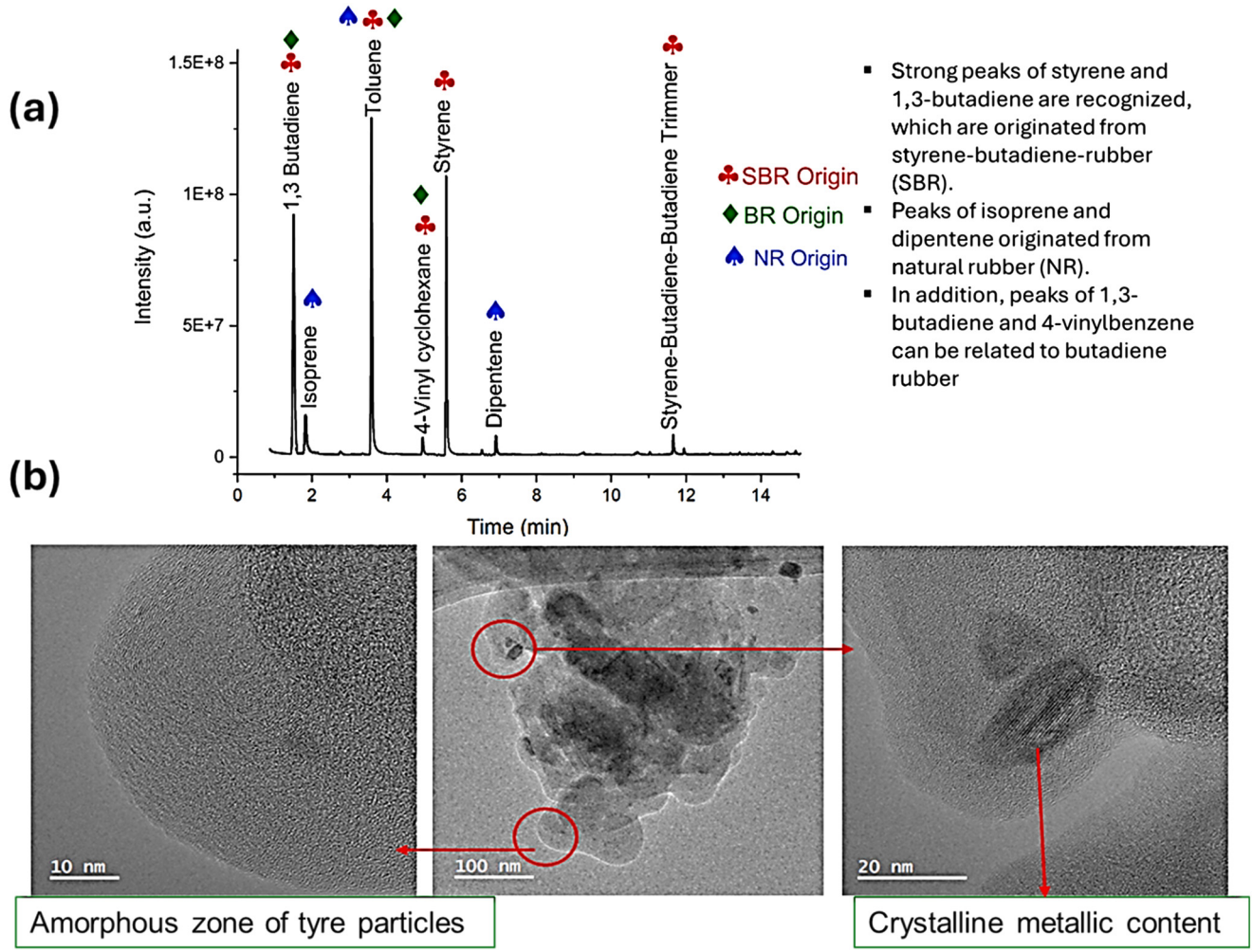


Fig. 5 (a) Pyro-GCMS furnace temperature is 600 °C; sample weight 0.175 gm; MS scan rate: *m/z* 29 to 600; flow rate: 1 mL min^{-1}, split ratio: 1/100; GC oven temp: 40 °C (5 min hold)-320 °C (20 °C min^{-1}, 15 min hold); (b) the HRTEM image in figure a depicts that the Tyre dusts are agglomerated. the matrix of the Tyre dust is amorphous. The metallic content (crystalline contamination) is embedded in the amorphous matrix of the Tyre dust (figure c). Authors own the analysis and images of the Tyre dust.

and morphology to link analytical metrics with environmental and health effects.

Each analytical approach offers distinct advantages and limitations that complement one another. Mass-based methods such as Py-GC/MS, TED-GC/MS, and MALDI-ToF MS provide highly accurate polymer identification and quantification, including additives and degradation products, but are destructive and require complex calibration. Particle-based methods like μ-FTIR and μ-Raman enable non-destructive identification and mapping of individual particles, delivering valuable size, shape, and compositional information, though they are time-intensive and limited by diffraction or fluorescence interference. Fractionation techniques including HDC-SEC and field-flow fractionation (FFF) serve as preparatory tools for isolating size-specific fractions, particularly nanoplastics, yet remain slow and technically demanding. Characterisation methods such as SEM/EDX, AFM-IR, and nano-FTIR reveal morphology, elemental composition, and ageing state at micro- to nanoscale resolution, but require costly instrumentation and skilled operation. Collectively, these complementary tools form an integrated analytical toolkit: mass-based approaches ensure accurate quantification, spectroscopic and microscopic methods provide morphological and compositional context, and fractionation enhances resolution across particle size ranges. Their combined use is therefore essential to obtain a comprehensive and reliable assessment of micro- and nanoplastic contamination in complex environmental matrices. Table 4 underscores that no single technique can comprehensively characterise MNPs across all size and compositional ranges. Each method offers complementary strengths: mass-based approaches ensure robust polymer quantification, spectroscopic and microscopic

Table 4 Advantages and limitations of analytical and characterisation techniques for micro- and nanoplastic (MNP) detection

Category	Technique	Advantages	Limitations
Mass-based methods	Py-GC/MS	Accurate polymer identification; quantitative for mixed matrices; detects additives and degradation products	Destructive; no particle morphology; costly instrumentation; matrix-dependent calibration
	TED-GC/MS	Quantitative; handles larger sample masses; improves detection limits for low polymer content	Slower heating alters breakdown pattern; requires individual polymer calibration
	MALDI-ToF MS	Soft ionisation preserves molecular weight info; rapid and minimal fragmentation	Limited for complex mixtures; matrix optimisation needed; biased toward ionisable polymers
	TD-PTR/MS	Ultra-sensitive (ng-level); suitable for airborne or aqueous nanoplastics	Expensive; sensitive to trace impurity interference
	qNMR	Quantitative without external calibration; structural fingerprinting of polymers	Requires pure, concentrated samples; limited for polymer mixtures
	HPLC	Separates additives and oligomers; useful for degradation and additive studies	Cannot directly identify polymer backbones; solvent compatibility critical
	DSC	Fast, low-cost thermal identification; distinct melting profiles for crystalline polymers	Overlapping peaks; not effective for amorphous polymers or complex samples
Particle-based methods	ATR-FTIR	Non-destructive; suitable for opaque samples; simple sample prep	Low spatial resolution (~20 μm); low throughput
	μ-(FT)IR	Automated mapping/imaging of thousands of particles; strong spectral libraries	Diffraction limit (~10 μm); filter and water interference
	μ-Raman	High spatial resolution (<1 μm); detects smaller MPs/NPs; minimal water interference	Fluorescence interference; long acquisition time
	ToF-SIMS	High surface sensitivity; detects additives, oxidation states, coatings	Destructive; complex spectra interpretation; small area coverage
	CARS/SRS	Fluorescence-free nonlinear Raman; enables *in situ*, rapid imaging	Requires advanced laser setup; limited spectral range; expensive
	NIR	Portable, fast, low-cost; suited for bulk plastic sorting	Low sensitivity; unsuitable for nanoplastics or complex mixtures
Fractionation methods	HDC-SEC	Size-based separation under mild conditions; reproducible	Requires calibration; not suitable for >10 μm particles
	FFF-Raman/FFF-Py-GC/MS	Combines size fractionation with chemical identification; ideal for nanoplastics	Technically demanding; long runtime; low throughput; costly
Characterisation techniques	SEM/EDX	High-resolution morphology; elemental detection (fillers, coatings)	Conductive coating needed; vacuum compatibility required
	SEM-Raman	Correlates morphology with chemical composition	Complex alignment; time-consuming; high cost
	(SP)-ICP-MS	Detects element-tagged polymers; high sensitivity for metals/additives	Only works for polymers with elemental markers; background interferences
	O-PT-IR	Photothermal IR with nanoscale resolution; combines optical and chemical contrast	Expensive; limited commercial availability; small field of view
	AFM-IR	Nanoscale chemical mapping (10–100 nm); ideal for NPs	Small scan area; slow analysis; requires expert operation
	Nano-FTIR	Ultra-high spatial resolution (<50 nm); direct nano-chemical imaging	Requires synchrotron or specialized IR source; extremely costly

tools provide morphological and compositional context, and fractionation techniques enhance resolution and recovery across particle sizes. Therefore, combining these approaches within multi-method workflows remains essential for obtaining accurate, reproducible, and environmentally meaningful MNP assessments.

Mass-based methods are generally expensive but well-established in analytical laboratories; particle-based spectroscopic tools are more accessible but limited by throughput and resolution. Fractionation and advanced nanoscale characterisation instruments remain cost-intensive and geographically concentrated, restricting global adoption. Developing cost-efficient, portable, and standardised systems —for instance, compact Raman or simplified pyrolytic detectors—will be key to extending MNP monitoring beyond specialised facilities (Table S1).

4. Pretreatment and sample preparation: current challenges and future needs

Pretreatment remains one of the most critical and error-prone stages in micro- and nanoplastic (MNP) analysis. The accuracy of any subsequent mass- or particle-based quantification relies heavily on how effectively the sample is processed prior to measurement. Inadequate pretreatment can lead to polymer degradation, contamination, or the loss of fine particles, resulting in significant discrepancies across laboratories.

Typically, pretreatment involves three key steps: (i) removal of organic matter through chemical oxidation (*e.g.*, hydrogen peroxide, Fenton's reagent, or enzymatic digestion); (ii) density separation to isolate plastics from sediments, biota, or organic debris; and (iii) filtration or sieving to obtain size-defined fractions. While these methods are well established for microplastics, their efficiency declines sharply for nanoplastics, which tend to agglomerate, pass through filters, or adhere to container surfaces. Furthermore, harsh oxidising treatments can partially depolymerise or oxidise sensitive polymers such as polyamide and PET, altering their spectroscopic or thermal signatures.

Pretreatment introduces three major sources of uncertainty:

1. Incomplete recovery, particularly for particles <20 μm, due to adherence to glassware or filter clogging.

2. Chemical alteration, where aggressive digestion affects polymer chemistry or surface oxidation state.

3. External contamination, arising from airborne fibres, plastic labware, or impure reagents.

Despite awareness of these issues, there is still no universally accepted digestion or separation protocol applicable to all sample types (water, soil, sludge, air, biota). As a result, reported concentrations often vary by orders of magnitude between studies analysing the same matrix. To overcome these limitations, new approaches should focus on matrix-specific and polymer-preserving workflows. Promising directions include:

- Enzymatic or mild oxidative digestion tailored to biological matrices to avoid polymer damage.
- Closed-system filtration and microfluidic concentration to minimise airborne contamination and loss of nanoparticles.
- Magnetic or density-tunable separation media to enhance recovery across wide particle size ranges.
- Pretreatment validation using reference materials of known composition and recovery tracking *via* spiked samples.
- Standardised quality assurance protocols, such as laboratory blanks, replicate analyses, and reporting of recovery efficiency.

Developing validated, matrix-specific pretreatment workflows will be vital for ensuring that data generated by different laboratories are directly comparable and traceable. Harmonised pretreatment, coupled with certified reference materials and inter-laboratory studies, represents the necessary foundation for the standardisation of MNP analysis.

5. Method validation and QA/QC protocols

5.1. Calibration and reference materials for micro- and nanoplastics

One of the pressing needs in microplastic research is the development of standardized reference materials and intercomparison exercises to validate analytical methods. Reference materials are well-characterized samples with known quantities and types of microplastics (or nanoplastics) that can be used to test and calibrate analytical procedures. To date, creating such materials has proven difficult, because real microplastic pollution is so heterogeneous.

Nonetheless, efforts are underway to produce and utilize reference samples. For microplastics, some researchers have used industrial resin pellets or ground plastic powders of known polymer type and size distribution as proxy reference materials. Others have created spiked samples – for example, adding a known number of microplastic particles to an environmental matrix (like clean sediment or water) to serve as a test sample for recovery studies. Initiatives by standards organizations and research consortia (*e.g.*, the European Commission's Joint Research Centre (JRC) and Germany's BAM, as well as ISO) are focusing on preparing reference materials, such as monodisperse spherical microplastics of polystyrene or polyethylene, or custom polymer mixtures, that laboratories can use to benchmark their methods.[158]

For nanoplastics, providing reference materials is even more challenging. Some approaches include synthesizing radiolabeled nanoplastics (*e.g.*, polymers labeled with ref. 61) which can be spiked into samples and later quantified *via* radioanalytics or isotope-ratio MS to evaluate method recovery.[143,159] Other researchers have prepared metal-doped nanoplastics, where metal nanoparticles or ions (like Ag or

Au) are embedded in or attached to nanoplastic particles, making them detectable by ICP-MS as a surrogate for the plastic.[160] While these exotic labels are not present in real pollution, they allow for controlled lab studies to develop and validate nanoplastic analysis techniques by providing an unmistakable marker.

Another strategy is to generate standardized weathered plastics. For example, well-defined plastic films or fragments can be subjected to accelerated aging (UV exposure, heat, mechanical abrasion) and then characterized in detail (surface oxidation level, brittleness, *etc.*). These weathered materials can serve as references to mimic environmental microplastics during method development – ensuring that methods work not just on pristine polymer, but on aged polymer that might have biofilms or oxidized layers.

Interlaboratory comparison studies (round-robin tests) have started to be organized to assess how different labs measure microplastics. In a recent exercise, multiple laboratories were given identical samples (*e.g.*, water spiked with a known amount of microplastics) and asked to analyze them with their methods.[161,162] The results often showed a wide variance between labs, underlining the necessity of standardized protocols. Some labs might undercount or overcount, or identify different polymer types for the same sample. These studies highlight issues like losses during sample handling, contamination, and differing detection limits as major sources of variability.

Quality assurance and quality control (QA/QC) protocols are being established to accompany any microplastic analysis. These include the use of procedural blanks (to check for background contamination), positive controls (analysis of samples with a known added quantity of microplastic to verify recovery), and, where possible, the use of internal standards. An internal standard in microplastic analysis might be, for example, adding a few known plastic particles of an uncommon polymer (one that is not expected in the environment, like a particular fluoropolymer) to each sample to see if they are recovered.

Standard organizations have begun issuing guidelines: for instance, ASTM and ISO have working groups on microplastic measurement. The goal is to eventually have validated methods that can be used for regulatory monitoring, much as there are standard methods for, say, measuring heavy metals or pesticides. Germany's DIN has released a technical report on methods for microplastics, and ISO is working on terminology and sampling guidelines.[13,15]

To support QA/QC, labs also use flow tracers or markers to understand their process efficiency. For example, a known number of fluorescent microspheres can be added to a sample before any processing as a tracer. After analysis, the number of those fluorescent spheres recovered can be checked; if significantly lost, the sample prep might be adjusted.

In summary, establishing reliable reference materials and QA/QC practices is an active and crucial area of microplastic research. Without these, data from different studies may not be comparable and could even be misleading. The community is moving towards greater standardization: for microplastics, a core set of methods (like FTIR imaging and Py-GC/MS) are likely to be standardized first. For nanoplastics, reference material development is still at an early stage, but ongoing research into labeled nanoparticles and highly characterized synthetic nanoplastics will pave the way for future validation studies.

5.2. Interlaboratory comparison studies and harmonization efforts

Ensuring that different laboratories obtain comparable results when analyzing microplastics is a major concern. Interlaboratory comparison exercises (sometimes called round-robin tests or proficiency tests) have been carried out to evaluate the consistency of microplastic analysis. For instance, a recent large interlaboratory study under the auspices of the European Union involved laboratories across different countries analyzing identical water samples spiked with a mix of microplastics.[161–163] The findings of such studies often reveal significant discrepancies – not all labs could detect all polymers present, and the counts and masses reported varied widely. These discrepancies arise from factors like differences in sample handling (some labs might inadvertently lose the smallest particles), differences in instrumentation (*e.g.*, some used FTIR *vs.* Raman, or different pyrolysis setups), and differences in the criteria for identifying a "plastic" (spectral matching libraries, human *vs.* software interpretation, *etc.*).

Recognizing these issues, efforts have been intensified to harmonize methods. Harmonization doesn't necessarily mean everyone uses exactly the same technique, but that the approaches are standardized enough that results are compatible. One example of harmonization is agreeing on units and reporting formats – whether to report microplastic concentration in particles per liter and in mass per liter, how to bin size fractions, *etc.*, so that two studies can be directly compared. Another is developing standard operating procedures (SOPs) for common tasks like density separation of microplastics from sediments or digestion of organic material in biota samples (with many labs converging on using wet peroxide oxidation or enzyme digestion in a similar manner).

Quality control measures such as using spiked recovery tests and blank contamination checks are becoming routine. A laboratory should report its blank levels (how many fibers or fragments were found in procedural blanks) so readers can judge the signal-to-noise in their data. They should also report recovery percentages for any surrogates spiked in, to provide transparency about how much of the sample might have been lost.

International bodies are working on formal standards. For instance, ISO (International Organization for Standardization) had a technical committee (ISO/TC 147/SC 2) looking at microplastics in water, and one output was a technical report

(ISO TR 21960:2020) summarizing the state of knowledge and methods.[15] The report recommends definitions and outlines various analytical approaches without endorsing a single one, reflecting that the field is still developing. It emphasizes the need for careful QA/QC and suggests validation using reference materials once available.

Another dimension of QA is the competence of analysts: identifying microplastics by microscopy or spectroscopy can require a trained eye or good software. Training and certification of analysts may be considered in the future (similar to how labs might get certified for analyzing certain pollutants). Collaborative networks and workshops help with this, where analysts gather to compare techniques and learn from each other.

It's also worth noting the move toward automated data analysis to reduce subjective bias. For example, using software for FTIR/Raman spectral matching rather than relying on an individual's judgment can improve consistency between labs (provided they use the same spectral libraries and quality indices for matches). Some initiatives have created shared spectral libraries of common polymers and are encouraging all labs to use those libraries so that polymer identification is uniform.

In summary, the community is converging on best practices: multiple complementary methods are encouraged (to cross-verify results), and thorough QA/QC protocols are mandated (including blanks, recoveries, replicates). Interlaboratory studies so far underline how far apart measurements can be, but each round of such studies tends to improve methodologies. The ultimate goal is that, in the near future, regulatory bodies could set environmental microplastic monitoring requirements with confidence that data collected by different agencies or labs will be comparable and reliable.

6. Standardisation, data harmonisation, and environmental relevance

Despite the rapid growth of analytical capabilities for micro- and nanoplastic (MNP) detection, the field remains fragmented by inconsistent data reporting, variable detection limits, and non-standardised workflows. This lack of harmonisation impedes the comparability of datasets across laboratories and undermines confidence in environmental risk assessments. Establishing shared standards, reference materials, and harmonised metrics is therefore essential to translate laboratory-scale detection into regulatory and ecological contexts.

One major challenge lies in defining comparable detection thresholds. Studies using μ-FTIR, Raman, or Py-GC/MS often report results in incompatible units — particles per litre, micrograms per gram, or polymer mass per surface area — which prevents direct cross-study synthesis. Creating standard conversion protocols between particle counts and polymer mass (using known size–density relationships) could enable the first global MNP databases that integrate both abundance and compositional data. Such harmonised quantification would allow correlation between environmental loads and biological uptake rates, which is currently limited by incompatible reporting conventions.

Certified reference materials (CRMs) for microplastics are another critical need. Although several initiatives (*e.g.*, NIST, BAM, JRC) have begun developing polymer mixtures and size-calibrated particles, few are validated below 10 μm or for weathered polymers. Reference materials spanning both pristine and aged states are crucial for calibration, as environmental plastics often undergo oxidation, fragmentation, and sorption of organic compounds that alter spectral signatures. Without CRMs covering these variations, analytical bias persists, particularly in automated classification using AI-based spectral libraries.

Equally important is inter-laboratory validation and round-robin testing. Comparative studies have shown that even using similar instruments, variability can exceed an order of magnitude when different pretreatment or data-processing protocols are applied. Establishing community-endorsed best practices—such as standardized pretreatment, polymer libraries, and reporting formats—would significantly enhance reproducibility. Harmonisation efforts should also include metadata standards: sample matrix, digestion procedure, particle size range, and detection limits should be mandatory in all MNP publications to enable meta-analyses.

Another emerging priority is linking analytical data to environmental and toxicological relevance. Many studies quantify MNPs with great precision but fail to interpret what these concentrations mean for ecosystem or human health. Integrating analytical results with exposure modelling, ecotoxicity testing, and chemical leachate analysis can provide a more meaningful assessment of risk. For instance, coupling MNP characterization with assays of oxidative stress or endocrine disruption in model organisms can help determine which polymer fractions pose the greatest hazard. Such correlation between analytical precision and ecological interpretation will elevate MNP analysis from descriptive to predictive science.

Lastly, digital standardisation and data interoperability must evolve in parallel. Establishing open-access repositories —similar to those in genomics or materials databases— would allow harmonised spectral libraries, retention times, and calibration datasets to be shared globally. Integration with AI-based spectral matching and cloud computing could automate identification pipelines, reduce redundancy, and democratise access to advanced data interpretation tools.

7. Breakthrough insights and emerging perspectives

Looking ahead, the next breakthrough in micro- and nanoplastic (MNP) research will not come from incremental improvements in detection limits alone but from a paradigm shift toward integrative, automated, and predictive analysis.

Analytical chemistry, materials science, data science, and environmental modelling must converge to build an end-to-end analytical ecosystem—one that links sampling, pretreatment, detection, and interpretation within a single, interoperable framework. Artificial intelligence and machine learning will play a central role by enabling automated spectral classification, pattern recognition of polymer mixtures, and data-driven correction of measurement biases. Future progress will also hinge on sensor miniaturisation and field deployability, allowing real-time MNP monitoring through portable spectroscopic or electrochemical devices. These tools could eventually support continuous surveillance in drinking water, wastewater, and atmospheric monitoring networks.

Another transformative direction lies in coupling analytical precision with environmental relevance. This means moving beyond particle counts to define exposure thresholds and toxicity-relevant metrics that can inform risk-based regulation. Integrating MNP analytics with omics-based biological assays and computational toxicology models will enable predictive understanding of how particle properties—size, surface chemistry, and aging state—govern bioavailability and effects. Equally, cross-disciplinary efforts should aim to design benign-by-design polymers whose environmental signatures can be rapidly identified by standardised analytical fingerprints, reducing future uncertainty.

Ultimately, the breakthrough insight for the field is to treat MNP analysis not merely as measurement science but as a dynamic, systems-level discipline that unites advanced instrumentation, data integration, and policy translation. Achieving this synthesis will transform MNP detection from an analytical challenge into a cornerstone of sustainable materials management and environmental protection.

8. Summary and outlook

Building upon the transformative perspectives outlined above, the following section consolidates current analytical achievements and outlines the practical directions for continued advancement. Microplastics – and more recently nanoplastics – have been recognized as emergent pollutants of global concern, spurring a high level of scientific and public interest. Significant progress has been made in our ability to detect, identify, and quantify these particles in various matrices over the past decade. Advanced analytical methods now allow researchers to measure microplastics in environmental samples at ever lower size ranges and concentrations. However, many critical knowledge gaps remain, and the field faces ongoing challenges that will shape research in the coming years.

Analytical advancements

The development of both mass-based and particle-based methods has vastly improved the detection of microplastics. Thermal analytical techniques (like Py-GC/MS and TED-GC/MS) provide quantitative polymer mass data and have proven effective for complex samples, though they inherently lose information on particle size and count. Vibrational spectroscopic imaging (FTIR and Raman) has enabled the enumeration and characterization of microplastic particles down to the low micrometer scale in water, sediments, and even air. Emerging techniques such as AFM-IR, TERS, and Raman tweezers are pushing the detectable size limit into the nanoscale, heralding a new era of nanoplastic research. At the same time, simpler screening tools (*e.g.*, dye staining and fluorescence detection) are being refined for rapid monitoring purposes, though they require careful validation. The synergy of multiple methods – for example, combining spectroscopic identification with pyrolysis-based quantification – has been shown to yield a more complete picture of contamination and will likely become standard practice.

Current limitations

Despite these advances, detecting smaller particles (<1 μm) at trace levels is still extraordinarily difficult. Every step of the process, from sampling to analysis, risks contamination or loss of these tiny particles. There is a clear need for further innovation in sample processing (*e.g.*, concentrating nanoplastics from large volumes without losses) and in detection (perhaps leveraging novel sensors or spectroscopy techniques). Furthermore, while polymer identification is usually straightforward for larger microplastics, heavily weathered or biofouled particles can produce ambiguous spectra. Developing methods to assess particle aging – for instance, measuring oxidation level or surface cracks – in tandem with identifying the polymer is an area of active research, since the environmental and health impacts of plastics likely depend on their weathering state.

Inclusion of complex and functionalised polymers

While the majority of analytical studies have focused on common commodity polymers such as polyethylene (PE), polypropylene (PP), and polystyrene (PS), there is a growing need to characterise more complex and functionalised plastics that increasingly dominate modern waste streams. These include engineering polymers (*e.g.*, polycarbonate, polyamide, polyetheretherketone), elastomers, and multi-layer composites, as well as biodegradable and biobased plastics such as PLA and PBAT. Detecting and differentiating these materials poses greater analytical challenges due to overlapping spectral features, copolymer structures, and additives that modify degradation behaviour. Advanced methods such as thermochemolysis-GC/MS (using derivatising agents like TMAH), 2D correlation spectroscopy (2D-FTIR/Raman), and high-resolution mass spectrometry are beginning to address these gaps by providing distinctive molecular or fragment fingerprints. Integrating these complex polymers into reference databases and calibration libraries will be crucial to ensure that emerging detection frameworks remain representative of real-world plastic mixtures rather than limited to a few conventional types.

Harmonization and standardization

One of the most important perspectives for the field is the drive toward standardized methods and inter-comparable data. As discussed, efforts by international organizations are laying the groundwork for reference methods. In the near future, we can expect official standard methods to be published for certain common sample types (*e.g.*, microplastics in drinking water or wastewater effluent). These will likely stipulate everything from how samples should be taken and stored (to avoid contamination), to how they are to be treated (digestion, density separation protocols), and which analytical techniques are acceptable. The establishment of certified reference materials – say a sediment with a certified microplastic content, or a water with known microplastic count of specific polymers – will greatly aid method development and quality control.

Despite major analytical progress, pretreatment and matrix removal remain rate-limiting steps for reliable MNP quantification. Variations in digestion efficiency, density media, and nanoparticle recovery continue to drive inter-laboratory variability. Future harmonisation efforts should prioritise matrix-specific, polymer-preserving workflows and validated recovery benchmarks to ensure that subsequent analytical advances yield reproducible and globally comparable results.

Beyond analytical chemistry – sources, fate, and effects

Improved analytical capabilities are not an end in themselves, but a means to answer pressing environmental questions. With better tools, researchers can more accurately determine sources and sinks of microplastics in the environment, track their transport pathways, and study their interactions with organisms. For example, knowing that a certain fraction of airborne microfibers is actually semi-synthetic (like rayon with additives) *vs.* truly plastic can inform source attribution (textile sources *vs.* others). As methods become routine, large-scale monitoring programs may be implemented (similar to how air quality or water quality is routinely monitored for other pollutants). This will generate data to model the flow of plastics through ecosystems and to identify hotspots that need mitigation.

On the nano-sized end, once detection challenges are met, we will need to understand nanoplastics' behavior – for instance, their propensity to cross biological barriers as initial studies suggest they can cross cell membranes or even the blood–brain barrier in fish.[43,53] This raises questions about human exposure: early studies indicate we inhale and ingest microplastics regularly, but it is still unclear what fraction of those might be nano-sized and potentially more bioavailable. The field of nanoplastics toxicology will significantly benefit from the analytical developments described; being able to dose realistic nanoplastic materials and measure their uptake and effects at low concentrations will shed light on potential risks.

Interdisciplinary approaches

Addressing the microplastic pollution issue will require input not only from analytical chemists, but also from materials scientists, polymer engineers, toxicologists, and policy makers. On the analytical side, one can foresee greater collaboration with the broader nanomaterial community – many techniques used for nanoparticles of other types (*e.g.*, metal or mineral nanoparticles) can be adapted to plastics, and *vice versa*. Additionally, data science and machine learning are starting to play a role in dealing with the complex datasets generated by imaging techniques (for example, automated image analysis to differentiate plastic *vs.* organic debris particles based on morphology and spectral signals). Interdisciplinary innovations may lead to field-deployable sensors for microplastics (imagine a portable device that could scan water in real-time for microplastic content *via* some optical or acoustic signature).

Policy and mitigation perspectives

As analytical methods become more robust, they will undoubtedly inform regulatory measures. Already, some jurisdictions (like California) are moving to mandate monitoring of microplastics in drinking water and coastal waters.[7] The establishment of a standard analytical toolkit is a prerequisite for implementing such regulations and for measuring the effectiveness of any mitigation strategies (*e.g.*, improved wastewater filtration or stormwater treatment aimed at capturing microplastics). In the coming years, we will likely see guidelines or limits set for microplastic contamination in various environments, analogous to those for conventional pollutants, and those will rely on the methods reviewed here.

Future directions

To build on current achievements, future work must prioritise three interconnected goals: (1) integration and validation of analytical workflows, (2) scalability and accessibility of techniques, and (3) linkage of analytical data to environmental and biological outcomes. Inter-laboratory comparisons and certified reference materials are urgently needed to harmonise detection limits, recovery efficiencies, and reporting units across studies. Advancing hybrid workflows that combine mass-based quantification (*e.g.*, Py-GC/MS or TED-GC/MS) with spectroscopic and microscopic imaging will ensure both compositional accuracy and morphological insight. At the same time, developing cost-effective and field-deployable systems—such as portable Raman or miniaturised pyrolytic detectors—will democratise monitoring capacity beyond research laboratories. Finally, analytical progress must be tightly coupled with toxicological, ecological, and modelling research to define thresholds of concern and translate analytical precision into meaningful environmental indicators. By moving from methodological innovation to validated, standardised, and applied frameworks, the field will be equipped to generate globally comparable data and guide evidence-based mitigation strategies.

By moving from methodological innovation to validated, standardised, and applied frameworks, the field will be equipped to generate globally comparable data and guide evidence-based mitigation strategies. Despite these advances, several critical knowledge gaps remain that must be addressed to achieve consistent and meaningful micro- and nanoplastic (MNP) analysis. Reliable detection of nanoscale particles (<100 nm) remains a major challenge, as no universally validated or inter-laboratory-tested protocols currently exist for techniques such as AFM-IR, TERS, or Raman tweezers. Pretreatment variability continues to cause significant uncertainty in recovery efficiency and polymer alteration, particularly for samples with high organic or mineral loads. A clear cost–performance gap also persists—high-end instruments (*e.g.*, TED-GC/MS, TERS) provide exceptional sensitivity but are rarely accessible for routine monitoring, whereas low-cost field methods remain underdeveloped. Moreover, the absence of certified reference materials and harmonised calibration datasets limits quantitative comparability between studies. Complex and functionalised polymers, including engineering, elastomeric, and biodegradable plastics, are still underrepresented in spectral and thermal libraries, restricting analytical inclusivity. Finally, the lack of integration between mass-based and number-based data hampers translation of analytical results into risk-relevant metrics such as surface reactivity, aging state, and additive release. Bridging these gaps through coordinated inter-laboratory validation, the development of reference materials, and cross-disciplinary data integration will be crucial for advancing MNP analytics from descriptive measurement to predictive environmental assessment.

In conclusion, the field of microplastic and nanoplastic analysis has made remarkable strides in a short time, evolving from simple visual examination to a suite of sophisticated chemical analyses. While significant challenges remain – particularly at the nanoscale and in ensuring data comparability – the trajectory is clear. Continued technological innovation, coupled with concerted standardization efforts, will enable the scientific community to reliably monitor these contaminants. This, in turn, will support risk assessments and the development of strategies to reduce plastic pollution. Ultimately, the progress in analytical capabilities fuels our capacity to understand and address the implications of microplastics and nanoplastics in the environment and in public health, a critical endeavor as plastic production and use show no immediate signs of waning.

Author contributions

Rumana Hossain: conceptualization, data curation, formal analysis, funding acquisition, investigation, methodology, validation, visualization, writing – original draft, writing – review & editing. Veena Sahajwalla: conceptualization, project administration, resources, funding acquisition, supervision.

Conflicts of interest

There are no conflicts to declare.

Data availability

Data will be made available on request from the corresponding author.

Supplementary information (SI) is available. See DOI: https://doi.org/10.1039/d5en00856e.

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