JURNAL BIOEDUKASI
VOLUME 9, NOMOR 1, TAHUN 2026

P-ISSN : 2301-427X
E-ISSN : 2829-0844
E-mail : bioedukasi14@gmail.com
https://ejournal.unkhair.ac.id/index.php/bioedu

Spectroscopy-Based Approaches for Microplastic Detection across Environmental and Biological Matrices: A Mini Review

Pendekatan Berbasis Spektroskopi untuk Deteksi Mikroplastik di Berbagai Matriks Lingkungan dan Biologis: Kajian Singkat

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Abstract

Microplastic contamination is a global concern due to its widespread occurrence in environmental and biological matrices, including water, sediments, and marine organisms. Accurate detection and characterisation are essential to understand their distribution, sources, and potential risks to ecosystems and human health. This review summarises advances in spectroscopy-based and complementary analytical techniques for microplastic detection, focusing on Fourier-transform infrared (FTIR) spectroscopy, Raman spectroscopy, confocal laser scanning microscopy (CLSM), scanning electron microscopy (SEM), and pyrolysis–gas chromatography–mass spectrometry (Py-GC-MS). FTIR and Raman spectroscopy remain the primary tools for polymer identification through molecular fingerprinting, while CLSM enables rapid fluorescence-based screening and three-dimensional imaging. SEM provides detailed morphological information, revealing particle surface characteristics and weathering processes. In contrast, Py-GC-MS offers highly sensitive mass-based quantification and identification of polymer composition and associated additives. Each technique has distinct advantages and limitations related to sensitivity, resolution, throughput, and applicability to complex matrices. Therefore, integrated multi-method approaches are increasingly recommended to achieve comprehensive and reliable microplastic assessment. This review highlights current methodological developments and future directions to improve analytical accuracy and standardisation in microplastic research.

Article Information(

Keywords:
Biology; Detection; Environment; Microplastic; Spectroscopy techniques

Kontaminasi mikroplastik merupakan masalah global karena keberadaannya yang meluas di matriks lingkungan dan biologis, termasuk air, sedimen, dan organisme laut. Deteksi dan karakterisasi yang akurat sangat penting untuk memahami distribusi, sumber, dan potensi risikonya terhadap ekosistem dan kesehatan manusia. Tinjauan ini merangkum kemajuan dalam teknik analitik berbasis spektroskopi dan komplementer untuk deteksi mikroplastik, dengan fokus pada spektroskopi inframerah transformasi Fourier (FTIR), spektroskopi Raman, mikroskopi pemindaian laser konfokal (CLSM), mikroskopi elektron pemindaian (SEM), dan kromatografi gas-spektrometri massa pirolisis (Py-GC-MS). Spektroskopi FTIR dan Raman tetap menjadi alat utama untuk identifikasi polimer melalui sidik jari molekuler, sementara CLSM memungkinkan penyaringan berbasis fluoresensi yang cepat dan pencitraan tiga dimensi. SEM memberikan informasi morfologi terperinci, mengungkapkan karakteristik permukaan partikel dan proses pelapukan. Sebaliknya, Py-GC-MS menawarkan kuantifikasi dan identifikasi komposisi polimer dan aditif terkait yang sangat sensitif berdasarkan massa. Setiap teknik memiliki keunggulan dan keterbatasan yang berbeda terkait dengan sensitivitas, resolusi, kapasitas pemrosesan, dan penerapannya pada matriks kompleks. Oleh karena itu, pendekatan multi-metode terintegrasi semakin direkomendasikan untuk mencapai penilaian mikroplastik yang komprehensif dan andal. Tinjauan ini menyoroti perkembangan metodologis terkini dan arah masa depan untuk meningkatkan akurasi analitis dan standardisasi dalam penelitian mikroplastik.

Kata kunci:
Biologi; Deteksi; Lingkungan; Mikroplastik;Teknik spektroskopi

History
Manuscript received : 14/04/2026
Revised : 14/04/2026
Accepted : 29/04/2026
Published : 30/04/2026

A. INTRODUCTION

Microplastic pollution has become a global environmental issue due to the massive production of plastic (IUCN, 2024) and increasing presence of plastic debris across land, freshwater, and marine environments (Nayanathara Thathsarani Pilapitiya and Sandaruwan, 2024), as well as its increasing detection in food sources and human tissues (Smith et al., 2018; Forgione et al., 2026). Microplastics, generally defined as plastic particles ranging from 1 µm to 5 mm, originate from both primary sources and the degradation of larger plastic debris, leading to their widespread dispersal across environmental and biological matrices (Wang et al., 2021; Pereira et al., 2020; Jung et al., 2024). Recent research has verified the presence of microplastics in various compartments, including water, sediments, seafood, and even human biological samples, emphasising their potential impacts on ecosystem health and human exposure (Kreuze, 2024; Kissel et al., 2025). The complexity of these matrices, along with the diverse nature of microplastics in size, shape, and polymer type, presents notable analytical challenges for precise detection and characterization (Böke et al., 2022).

To address these challenges, a variety of analytical techniques have been developed, with spectroscopic approaches becoming the most widely used due to their ability to identify polymer types at the molecular level (Hidalgo-Ruz et al., 2012; Primpke et al., 2020; Käppler et al., 2018; Shim et al., 2016; Cowger et al., 2020). Vibrational spectroscopy methods, especially Fourier-transform infrared (FTIR) and Raman spectroscopy, produce unique spectral fingerprints of molecular vibrations, enabling both qualitative and quantitative analysis of microplastics (Böke et al., 2022). These techniques are highly valued because they are non-destructive, chemically specific, and capable of analysing particles across a broad size spectrum (Veerasingam et al., 2021). Additionally, Raman spectroscopy provides higher spatial resolution, enabling detection of smaller particles (down to the micrometre scale), whereas FTIR is ideal for rapid bulk analysis of polymer composition (Shiwani et al., 2025). Using these methods together has greatly enhanced the accuracy of microplastic identification in complex samples.

Despite these advancements, applying spectroscopy-based techniques to environmental and biological matrices remains difficult because of matrix interference, sample heterogeneity, and challenges in detecting very small particles or low concentrations. Biological samples, such as seafood or human tissues, often require complex preparation that may compromise particle integrity or introduce analytical bias (Dąbrowska et al., 2025). Moreover, environmental samples usually contain organic matter and mineral particles, complicating spectral interpretation (Khan and Zaidi, 2025). Recent advances, such as surface-enhanced Raman spectroscopy (SERS), hyperspectral imaging, and machine learning-assisted spectral classification, have shown promise in overcoming these issues and boosting detection sensitivity and throughput (Huang et al., 2024; Umurhan et al., 2025). While those advantages are mentioned, this approach remains largely complementary and has not replaced established benchmark methods. FTIR Spectroscopy continues to serve as a widely accepted reference for polymer identification, while Raman spectroscopy provides high-resolution chemical characterisation of smaller particles. Scanning Electron Microscopy (SEM) remains important for examining particle morphology and surface weathering, and Confocal Laser Scanning Microscopy (CLSM) provides fluorescence-based validation (Maes et al., 2017; Nalbone et al., 2021; Kalaronis et al., 2022; Stanton et al., 2019; Ribeiro et al., 2024). This review, therefore, aims to synthesise current progress in spectroscopy-based methods for microplastic detection across environmental and biological matrices, pointing out strengths, limitations, and potential directions for future research. Additionally, this review highlights the integrative framework as a unique contribution to the recent knowledge and basis for the next-generation analytical roadmap.

B. METHOD

Methodology Review: From Sample Extraction to Identification

The literature included in this review was systematically selected following the framework of the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses). A total of 200 records were initially identified through database searches using predefined keywords related to microplastic analytical techniques, including FTIR, Raman spectroscopy, SEM, CLSM, SERS, hyperspectral imaging, and machine learning-assisted classification. After duplicate removal and title and abstract screening, potentially relevant studies were assessed through full-text eligibility evaluation based on predefined inclusion criteria, including relevance to instrumental analysis, peer-reviewed status, methodological robustness, and contribution to current advances or limitations in microplastic characterisation. Studies lacking methodological detail, unrelated to analytical techniques, or not directly relevant to the review scope were excluded. Following this structured screening process, 39 studies were included in the final synthesis. The PRISMA-based selection process ensured transparency, reproducibility, and comprehensive coverage of the literature reviewed.

The detection of microplastics in environmental and biological samples depends on a combination of chemical identification and visual examination of their shape. The procedure begins with sample collection, sample preparation (including digestion, density separation, and filtration), screening and visual identification, and polymer characterisation (Figure 1). Among the most common methods, Fourier-transform infrared spectroscopy (FTIR) and Raman spectroscopy are the key techniques for identifying polymers because both produce unique vibrational fingerprints for different materials (Jung et al., 2024). In comparison, confocal laser scanning microscopy (CLSM) and scanning electron microscopy (SEM) are primarily imaging methods that provide spatial, topographic, and structural details and are often used as complementary techniques rather than primary tools for confirming polymers (Lin et al., 2025). Recent reviews emphasise that no single method is universally optimal; instead, method selection depends strongly on particle size, matrix complexity, throughput requirements, and whether the priority is chemical composition, abundance screening, or surface morphology (Xie et al., 2024; Huang et al., 2024; Olivatto et al., 2024; Lin et al., 2025).

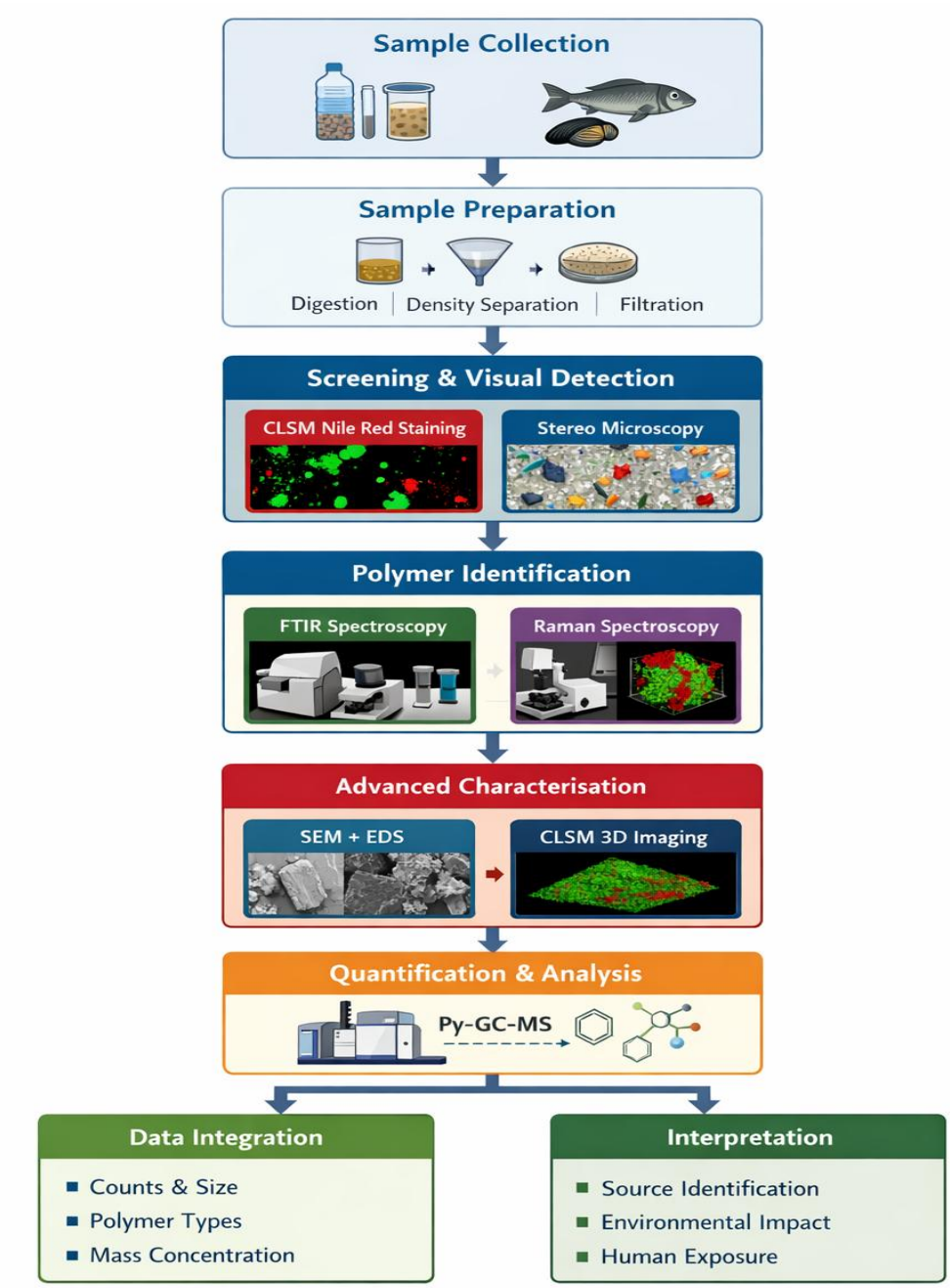

Figure 1. Microplastic detection workflow diagram

C. DISCUSSION

Comparative Perspective: FTIR vs Raman vs CLSM vs SEM

In comparative terms, FTIR is generally preferred for routine polymer identification and higher-throughput screening of larger microplastics, whereas Raman is favoured for smaller particles and detailed particle-level analysis. CLSM is advantageous for fluorescent imaging, three-dimensional visualisation, and rapid screening of stained particles, but it requires chemical confirmation because fluorescence alone is not polymer-specific. SEM offers the best morphological detail, yet it is mainly descriptive unless paired with complementary chemical methods. For complex environmental and biological matrices, the most robust strategy is often a multi-method workflow: digestion and filtration, followed by Nile Red-assisted CLSM or fluorescence screening, then FTIR or Raman confirmation, with SEM reserved for detailed surface characterisation. Current reviews consistently show that correlative and hybrid workflows are becoming the preferred approach in microplastic analytics because they balance throughput, specificity, and size resolution more effectively than any single technique. Comparison of each technique is presented in Table 1

1. Fourier-Transform Infrared Spectroscopy (FTIR)

FTIR remains the most established method for routine microplastic analysis because it is non-destructive, chemically specific, and relatively accessible for environmental laboratories (Primpke et al., 2020). The technique identifies polymers by measuring infrared absorption associated with molecular vibrations, and it is especially effective for common polymers such as polyethylene (PE), polypropylene (PP), polystyrene (PS), polyethylene terephthalate (PET), and polyvinyl chloride (PVC). Recent reviews note that FTIR, including ATR-FTIR, micro-FTIR, and focal plane array (FPA)-FTIR imaging, is currently the most widely used infrared platform for microplastic analysis, with FPA-FTIR representing a major advance for automated mapping and high-throughput particle screening (Circelli et al., 2024). However, FTIR is constrained by the optical diffraction limit and is generally less effective at resolving very small particles below roughly the low-micrometre range, particularly in complex biological matrices. ATR-FTIR is also typically better suited to larger particles or manually isolated items because it usually requires direct contact between the sample and crystal (Veerasingam et al., 2021).

In practical terms, FTIR is highly suitable for sediments, filters, shoreline debris, and larger particles recovered from digested biota, especially when the goal is to obtain reliable polymer confirmation with moderate-to-high throughput (Xu et al., 2020). Its main advantages are reproducibility, compatibility with spectral libraries, and strong performance for weathered environmental plastics. Its main limitations are reduced sensitivity for very small particles, spectral interference from water and organic residues, and the need for careful background correction and sample purification (Alfonso et al., 2021). For this reason, FTIR is often preferred when the study focuses on polymer-level identification of microplastics larger than about 10–20 µm, whereas other techniques may be needed for smaller fractions.

2. Raman Spectroscopy

Raman spectroscopy is widely regarded as complementary to FTIR and is often superior for smaller particles, as it can achieve higher spatial resolution and identify particles down to the micrometre scale under optimised microscopy-based configurations. Raman analysis is based on inelastic light scattering and provides polymer-specific spectra that are often highly discriminative, making it valuable for identifying fine particles in environmental and biological samples (Shiwani et al., 2025). Recent work and reviews highlight Raman microscopy, including

confocal Raman approaches, as especially useful for particles too small for conventional FTIR workflows and for cases requiring detailed particle-by-particle confirmation.

Despite these strengths, Raman spectroscopy has important practical constraints. Fluorescence from dyes, pigments, biofilms, and residual organic matter can obscure spectra, and dark or heavily weathered particles may be difficult to analyse. Throughput is also typically lower than imaging FTIR because Raman often involves slower point-by-point acquisition, particularly in particle-rich samples. Nevertheless, Raman is highly valuable for microplastics in seafood tissues, digested biological samples, and very small environmental particles, especially when researchers require greater spatial resolution than FTIR can provide. In many workflows, FTIR is used for bulk screening, and Raman is then applied to ambiguous particles or the smallest size classes.

3. Confocal Laser Scanning Microscopy (CLSM)

CLSM is not, strictly speaking, a vibrational spectroscopy method like FTIR or Raman, but it has become increasingly important in microplastic research as an advanced fluorescence-based imaging technique, especially when used with dyes such as Nile Red (Meyers et al., 2022; Shruti et al., 2022). The main advantage of CLSM is its ability for optical sectioning and three-dimensional visualisation, which enhances particle detection in heterogeneous samples and allows for volumetric assessment of small particles embedded within filters, tissues, or complex residues. Recent analytical reviews describe CLSM as particularly promising for fine-particle imaging because it can improve contrast, minimise out-of-focus signals, and support three-dimensional reconstruction of stained particles (Lin et al., 2025).

However, CLSM has a significant limitation: it usually cannot provide definite polymer identification on its own. Instead, it is mainly used for localising particles, counting them, estimating their size, and analysing their spatial distribution after fluorescent staining. Nile Red-based workflows are quick and sensitive, but they can produce false positives because the dye may also stain some non-plastic organic materials unless additional counterstaining or confirmation steps are taken. Therefore, CLSM is best viewed as a high-resolution screening and imaging tool that should ideally be combined with FTIR, Raman, or thermal techniques for chemical confirmation. This makes CLSM particularly useful for studies of small fluorescently stained particles in water, dust, sediments, or digested food and tissue matrices (Mohamed et al., 2025).

4. Scanning Electron Microscopy (SEM)

SEM is an imaging method that provides high-resolution surface details, making it valuable for describing the physical features of microplastics, including cracks, pits, abrasions, weathering traits, fibre texture, and particle shape. It is especially useful when researchers want to distinguish pristine from aged particles or assess surface roughness that may affect contaminant adsorption and biological interactions. Recent comparative studies show that SEM is effective for morphological analysis and can reveal details far beyond optical microscopy.

On its own, however, SEM cannot reliably identify polymer type. When coupled with energy-dispersive X-ray spectroscopy (SEM-EDS), it can provide elemental composition information, which is useful for detecting inorganic fillers, pigments, or adhered minerals, and for differentiating plastics from certain non-plastic particles (Soliz et al., 2024). Even so, SEM-EDS remains a supporting technique rather than a definitive method for polymer identification, because elemental composition alone is usually insufficient to distinguish among many organic polymers. SEM therefore works best as a complementary tool alongside FTIR or Raman,

particularly in studies of weathered particles, fibres, and complex environmental residues where surface microstructure is analytically important.

Additional Common Technique Used: Pyrolysis–Gas Chromatography–Mass Spectrometry (PY-GC-MS)

Pyrolysis–gas chromatography–mass spectrometry (Py-GC-MS) has become a powerful analytical tool for quantifying and characterising microplastics, especially in complex environmental and biological samples where spectroscopic methods may be limited. Unlike FTIR and Raman spectroscopy, which rely on vibrational fingerprints, Py-GC-MS thermally breaks down polymeric materials at high temperatures in an inert atmosphere, generating distinctive pyrolytic fragments that are then separated by gas chromatography and identified by mass spectrometry. This method enables highly specific identification of polymer types, such as polyethylene (PE), polypropylene (PP), polystyrene (PS), polyethylene terephthalate (PET), and polyvinyl chloride (PVC), as well as related additives, such as phthalates and stabilisers. One major benefit of Py-GC-MS is its capacity to provide mass-based quantification, enabling the measurement of total polymer concentrations rather than particle counts, which is especially useful for risk assessments and exposure studies (Fischer & Scholz-Böttcher, 2017; Duemichen et al., 2019).

However, Py-GC-MS is inherently a destructive technique and does not provide information on particle size, shape, or morphology, which limits its application when particle-level characterisation is required. Sample preparation typically involves homogenisation and may include pre-treatment steps such as organic matter digestion and density separation to reduce matrix interference. Despite these limitations, Py-GC-MS is especially advantageous for analysing small microplastics and nanoplastics that are difficult to detect using FTIR or Raman spectroscopy due to size constraints or spectral interference. Moreover, the technique is highly sensitive and can detect low concentrations of polymers in complex matrices such as sediments, sludge, and biota. Consequently, Py-GC-MS is increasingly used as a complementary method alongside spectroscopic techniques, providing robust chemical confirmation and quantitative data that enhance the reliability of microplastic assessments in environmental and food-related studies (Hermabessiere et al., 2017; Primpke et al., 2020).

Table 1. Comparison of Analytical Techniques for Microplastic Detection

Technique	Principle	Information Obtained	Detection Size Range	Strengths	Limitations	Typical Applications
FTIR (ATR / µ-FTIR / FPA-FTIR)	Infrared absorption of molecular vibrations	Polymer identification (chemical fingerprint)	~10–20 µm to mm	Non-destructive; reliable polymer ID; high throughput (FPA imaging); extensive spectral libraries	Limited for very small particles; interference from water/organic matter	Sediments, filters, shoreline debris, and large MPs in biota
Raman Spectroscopy	Inelastic scattering of laser light	High-resolution polymer identification	~1 µm and below (nano-scale possible)	High spatial resolution; detects small particles; minimal water interference	Fluorescence interference; slower analysis; sensitive to pigments	Small MPs in water, seafood tissues, fine particles
CLSM (Nile Red staining)	Fluorescence imaging with optical sectioning	Particle visualization, size, distribution (3D)	~1–10 µm (depending on optics)	Rapid screening; 3D imaging; high sensitivity; good for complex matrices	Not polymer-specific; false positives from organic matter	Water, sediments, digested biota (screening stage)
SEM (±EDS)	Electron beam	Surface	<1 µm (high	Ultra-high	No polymer ID	Morphological

Technique	Principle	Information Obtained	Detection Size Range	Strengths	Limitations	Typical Applications
	imaging	morphology; elemental composition (EDS)	resolution)	resolution; reveals weathering, texture, fibres	(without coupling); destructive coating; expensive	characterization, fibre analysis
Py-GC-MS	Thermal decomposition → GC-MS analysis	Polymer type + **mass-based quantification**; additives	No size limitation (bulk analysis)	Highly sensitive; detects nano-MPs; quantifies polymers; identifies additives	Destructive; no particle size/shape info; complex prep	Complex matrices (sediment, sludge, tissue); exposure studies

This review provides a specific contribution beyond conventional instrument comparisons by critically integrating both established and emerging analytical tools into a unified framework for microplastic characterisation. Unlike previous reviews that often evaluate techniques individually, this article synthesises the complementary roles of FTIR Spectroscopy, Raman spectroscopy, SEM, CLSM, Py-GC-MS, and emerging approaches such as SERS, hyperspectral imaging, and machine learning-assisted classification, highlighting how these methods address different analytical gaps in sensitivity, throughput, morphology, and chemical identification. A key contribution of this review is the proposal of a multi-method, tiered analytical perspective in which conventional instruments are positioned as benchmark reference methods for validation and comparability, while newer technologies are assessed as complementary enhancements rather than direct replacements. The review also contributes by identifying persistent gaps in standardisation, spectral interpretation, and resource-sensitive application, while offering a conceptual pathway for harmonised workflows that can support more robust monitoring, cross-study comparability, and future methodological development in microplastic research.

D. CONCLUSION

The rapid progress of analytical techniques has greatly enhanced the detection and characterisation of microplastics across various environmental and biological matrices. Spectroscopy-based methods such as FTIR and Raman remain essential for accurate polymer identification, while additional tools, including CLSM, SEM, and Py-GC-MS, offer vital insights into particle distribution, shape, and mass-based composition. A major advantage of FTIR, Raman, SEM, and CLSM is that these techniques are already widely adopted, relatively mature, supported by established protocols, and increasingly used as reference methods for validation and cross-verification, making them indispensable for maintaining continuity and comparability with the existing microplastic literature. However, no single technique can fully address the complexity of microplastic contamination, especially in heterogeneous matrices like sediments and biological tissues. Variations in sample preparation, analytical procedures, and reporting metrics continue to hinder comparability between studies, emphasising the urgent need for harmonised methodologies and standardised workflows.

Future research should prioritise integrating multi-method approaches that combine rapid screening, high-resolution identification, and quantitative analysis to achieve more comprehensive assessments. Emerging technologies, including automated spectral imaging, machine-learning-assisted classification, and enhanced-sensitivity techniques, offer promising pathways to improve detection accuracy, particularly for smaller microplastics and nanoplastics. In addition, greater emphasis should be placed on linking analytical data with exposure assessment and ecological risk frameworks, especially in regions with high seafood consumption. Advancing standardisation, improving analytical sensitivity, and strengthening interdisciplinary

collaboration will be essential to support robust monitoring and informed policy development in addressing global microplastic pollution.

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