ABS ACTA BIOLOGICA SIBIRICA
A journal on the biodiversity of Siberia and the adjacent lands

Acta Biologica Sibirica 11: 1033–1053 (2025)
doi: 10.5281/zenodo.17332411
http://journal.asu.ru

RESEARCH ARTICLE

Methods of microplastic separation from soil and soil arthropods: a systematic review

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Academic editor: R. Yakovlev | Received 14 July 2025 | Accepted 2 August 2025 | Published 14 October 2025

http://zoobank.org/6169F7C3-3F57-44E5-874B-45A4D8C8DCC0

Citation: Al-Kinani B, Evsyukov AP (2025) Methods of microplastic separation from soil and soil arthropods: a systematic review. Acta Biologica Sibirica 11: 1033–1053. https://doi.org/10.5281/zenodo.17332411

Abstract

Microplastics – small plastic particles ≤ 5 mm – have quietly invaded soils, threatening the delicate balance of ecosystems and soil organisms. This review dives into the toolbox scientists use to detect and extract these hidden pollutants from soil and soil-dwelling organisms. We explore methods such as floating plastics in salty solutions, breaking down organic matter with hydrogen peroxide or enzymes, and using high-tech imaging like infrared spectroscopy. While some techniques, like sodium iodide separation, recover over 95% of microplastics, their high cost and complexity remain hurdles. Gentler approaches, such as enzyme-based digestion, protect fragile organisms but take longer. Despite advances, the field struggles with inconsistent methods and the near-invisible challenge of nanoplastics. Innovations like atomic force microscopy hint at future breakthroughs, but unifying global standards and blending old and new techniques will be key to turning the tide. This work calls for teamwork across disciplines to sharpen our tools, safeguard soils, and stem the silent spread of microplastic pollution.

Keywords

Microplastics, soil health, pollution detection, plastic identification, global standards

Introduction

Microplastics (MPs, plastic particles ≤ 5 mm) have emerged as a widespread environmental pollutant, with increasing evidence of their accumulation in terrestrial ecosystems, particularly soils (Rillig et al. 2017). While extensive research has focused on aquatic environments, terrestrial MPs – derived from agricultural plastics, sewage sludge, tire wear, and atmospheric deposition – pose unique ecological risks due to their persistence and potential interactions with soil organisms (Hurley and Nizzetto 2018).

Exposure to MPs has been shown to cause sublethal effects, such as reproductive disruption, reduced growth, alterations in metabolic activity, and induced immune responses (Kokalj 2023). Soil animals can accumulate MPs, enhance their fragmentation, alter their migration in the soil, and transfer them to higher trophic levels (Zhu et al. 2018; Wang et al. 2022; Helmberger et al. 2022; Möhrke et al. 2022; Zhang et al. 2022; etc.). Furthermore, some soil-dwelling arthropods have been shown to contribute to the biodegradation of microplastics via their gut microbiota and enzymes (Siddiqui et al. 2024). Together, these findings highlight the importance of investigating the interactions between soil-dwelling invertebrates, including arthropods, and microplastic particles.

The study of MPs in soil systems is critical for several reasons:

1. **Environmental consequences**: Organic matter can lead to soil structure, water retention, and microbial activity, which can destroy ecosystem and soil functions (de Souza Machado et al. 2018).
2. **The widespread presence of microplastics and their accumulation in soil.** Soil is the largest recipient of fine materials and microplastics, as it is contaminated by plant films, irrigation water, and treated water such as wastewater from treatment plants (Fig. 1). In a scientific review, it was confirmed that soil contains more microbes than what is found in seas and oceans (Horton et al. 2017).
3. **Negative impact on soil and its efficiency** MPs contribute to changing the physical and chemical properties of the soil, affecting its porosity, efficiency, density, and water-holding capacity, thus affecting root growth and microorganism activity The infiltration of microplastics into land systems is a cause for concern because of their potential impact on soil health and fertility. These particles can alter the physical properties of soil (Dissanayake et al. 2022).
4. **Dissemination of chemicals that pose a threat to the food chain.** Plant roots can absorb nanoplastic particles and accumulate them in their tissues, making them vulnerable to human and animal consumption. They are also ingested by soil organisms (such as earthworms), which form the basis of the food chain (Rillig 2012).
5. **Direct threat to environmental and biological diversity within the soil** MPs have become a threat to organisms that live in the soil and play a role in its fertility. For example the study of Huerta-Lwanga et al. (2016) revealed the dangers and effects of earthworms swallowing microplastic, which caused their death.

6. **It is considered a modern issue that requires more effective policies.** Treating wastewater before using it for irrigation and waste management is a first step to reducing plastic in the soil. As a report by the Food and Agriculture Organization (FAO) showed a major threat to soil due to plastic, which may lead to a decrease in its productivity and fertility (FAO 2021).

The objectives of this review are:

- To systematically evaluate methods for soil and soil arthropods.
- Compare efficiency, limitations, and applicability of density separation, digestion, and spectroscopic identification techniques.
- Identify knowledge gaps according to scientific methods and propose future directions for harmonized MP analysis in terrestrial environments.

Main challenges in current research:

- **Soil heterogeneity**: Organic matter and mineral particles interfere with MP extraction (Corradini et al. 2019).
- **Arthropod-specific issues**: These organisms require mild digestion to avoid MP degradation (Cole et al. 2014).
- **Detection limits**: Submicron-sized organic matter and micro- / nanoplastics elude traditional detection methods (Mitrano et al. 2021).

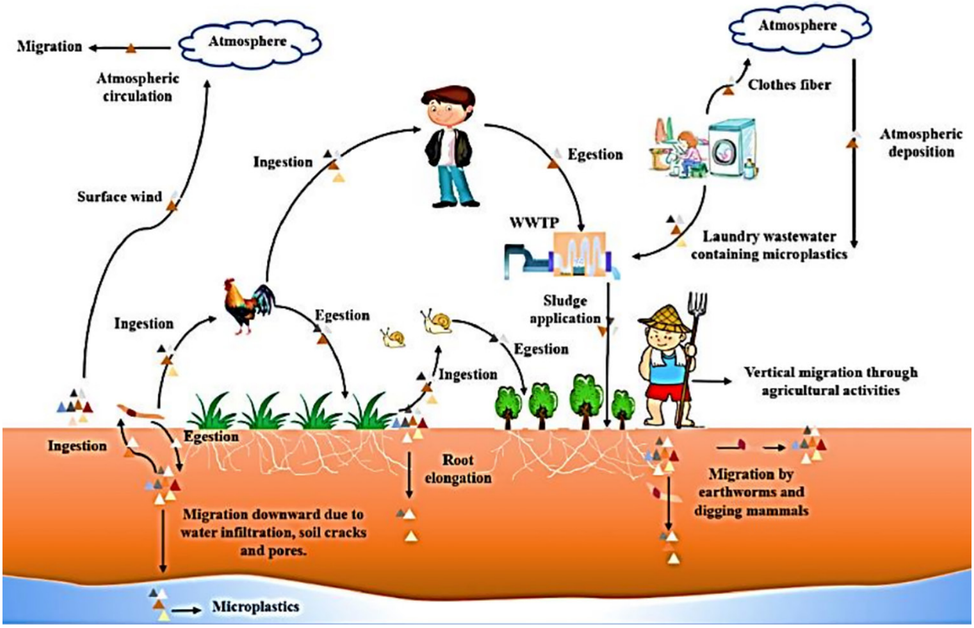

Figure 1. Distribution and migration of MPs in soil within the environment (Irhema 2022).

Results

Methods for microplastic separation and extraction from soil: a comparative analysis

Plastic particles in soil are generated from plastic films used in agriculture, wastewater irrigation, and recycling, in addition to the use of fertilizers, including organic fertilizers, and the leakage of plastic waste from landfills or disposal sites, in addition to vehicle tires. The challenge of extracting them is very difficult due to the complexity of the composition or mechanics of the soil matrix (Sajjad et al. 2022; Fan et al. 2023; etc.). In this review, we will focus on methods for separating and extracting microplastic particles, with an emphasis on cost and efficiency.

1. Density separation

Description: The density of a plastic depends largely on its chemical composition and structure. For example, the density of low-density polyethylene (LDPE) ranges from approximately 0.91 to 0.93 g/cm³, while the density of high-density polyethylene (HDPE) ranges from 0.94 to 0.97 g/cm³ (for other plastics density see Table 1). This variation in density is critical when choosing the concentration of solutions for the extraction of microplastic particles.

Pros: NaCl is cost-effective; NaI and $ZnCl_2$ offer higher recovery for smaller MPs (Nuelle et al. 2014).

Cons: NaI/$ZnCl_2$ are expensive; incomplete organic matter removal (Bläsing and Amelung 2018).

Principle: MPs float in high-density solutions while mineral/organic fractions sink.

Recommended solution: NaI (sodium iodide, 1.6–1.8 g/cm³) offers high recovery for most common polymers (PE, PP, PS). Saturated NaCl (1.2 g/cm³) is a low-cost, less toxic alternative but misses denser MPs (PET, PVC) (Liu et al. 2019; Corradini et al. 2019).

Protocol: Shake 10 g dry soil with 250 mL NaI solution for 1 hr. Settle for 4–24 hrs. Filter the floating portion onto a 5–10 μm pore size filter.

Recommendation: Use $ZnCl_2$ (1.5–1.7 g/cm³) to balance cost. Always test the solution density using a hydrometer. Centrifugation (3000 rpm, 15 min) significantly reduces sedimentation.

2. Organic matter digestion

Quantifying microplastics in living organisms, excised tissues, or environmental samples is challenging because the plastic may be hidden by biological material, microbial biofilms, algae, and detritus. To isolate microplastics, organic matter can be digested, leaving only the resistant material. Traditionally, digestion is performed using strong oxidizing agents. However, synthetic polymers can be degraded or damaged by these chemical treatments, particularly at high temperatures. We combined chemical resistance data to highlight the sensitivity of polymers to a range of digestion agents and storage media. Environmentally exposed plastics, which may have been subjected to weathering, abrasion, and photodegradation, may have reduced structural integrity and chemical resistance compared to the virgin plastic used in these stress tests. As such, data verified using caustic digestion agents should be interpreted with caution, and the potential for plastic loss from digestion processing should be carefully considered.

Description: H_2O_2 or enzymes degrade organic material. Enzymatic methods (e.g., proteinase K) are gentler (Zhang et al. 2020).

Pros: H_2O_2 is rapid; enzymatic reduces MP degradation.

Cons: H_2O_2 may oxidize some polymers; enzyme digestion is slower and more expensive (Hurley et al. 2018).

Principle: Destroys biological/organic matter without degrading polymers.

Recommended Agents: Fenton's Reagent: 30% H_2O_2 + 0.05 M Fe(II) solution (e.g., $FeSO_4$). Very effective for cellulose-rich soils (Hurley et al. 2018).

Enzymatic Digestion: A combination of cellulase (≥ 2 U/mL) and protease. Gentle, preserves sensitive polymers (PET, PA) but is slower (48–72 hrs) (Cole et al. 2014).

Protocol: Pre-digest soil with density separation. Add the reagent (soil : solution ratio 1:5 w/v). Digest at 50°C (Fenton) or 37–40°C (enzymes) with stirring. Stop the reaction with $Na_2S_2O_3$ (Fenton) or inactivate with heat (enzymes). Filter the residue.

Recommendation: Avoid strong acids/alkalis (e.g., HNO_3, KOH) unless polymer-specific proof is available – they degrade nylon, PET, and cellulose acetate (Corradini et al. 2019). Fenton's reagent is optimal for most soils; enzymes are preferred for sensitive polymers or FTIR/Raman analysis.

Table 1. Summarizing the densities of some common plastics (Density of plastic ... 2025)

Plastic type	Density (g/cm³)
Low-Density Polyethylene (LDPE)	0.91–0.93
High-Density Polyethylene (HDPE)	0.94–0.97
Polypropylene (PP)	0.90–0.91
Polyvinyl Chloride (PVC)	1.30–1.45
Polystyrene (PS)	1.04
Acrylic (PMMA)	1.18
Polycarbonate (PC)	1.20–1.22
Nylon 6/6	1.14
Polybutylene Terephthalate (PBT)	1.34

3. Froth (Foam) flotation

Description: A foam made of the polymers cellulose and chitin removes up to 99.9% of microplastics from water and maintains its effectiveness after repeated use.

Pros: A specialized foam is generated that has unique properties that make it able to bind to microplastic particles Foam flotation technology is less expensive compared to other removal techniques that rely on electricity consumption.

Cons: Limited to hydrophobic MPs; requires specialized equipment (Liu et al. 2019).

Principle: Hydrophobic MPs attach to air bubbles with surfactants; soil sinks.

Recommended Setup: Use Sodium Lauryl Sulfate (SLS) or SDS (1–5 g/L) as surfactant in a customized flotation column (Wang et al. 2022). Adjust pH to 7–8.

Protocol: Mix pre-sieved soil (<2 mm) with the surfactant solution. Inject air at a controlled rate (e.g., 50 ml/min). Collect the foam for 10–30 mins. Spray the foam.

Recommendation: Very effective for small MPs (10–300 µm), especially polyethylene. Optimization of air flow, surfactant concentration, and pH is required. Suitable for low-grade clay or sandy soils. Removal of the surfactant after separation, such as by washing, is very important for spectroscopic analysis.

4. Electrostatic separation

Electrostatic separation machines rely on the principle of varying electrical conductivity of particles in a high-voltage electric field to achieve separation purposes. Plastic mixers separate materials based on the different paths resulting from varying electrical properties and stress conditions. The electrostatic separator is suitable for sorting various plastic materials which are difficult to separate by different separation methods on flotation according to specific gravity, such as ABS/ABS alloy/PC, PS/PET/PVC, PA/PE/PO, etc. The single-stage screening accuracy reaches over 98%, and the secondary-stage screening accuracy is approximately 100%.

Description: Charges particles to isolate MPs.

Pros: Non-destructive; minimal chemical use.

Cons: Low efficiency for heterogeneous soils (Fuller and Gautam 2016).

Principle: Differences in surface charge conductivitiy under high voltage.

Recommended Setup: Tribo-electrostatic separator or corona discharge unit. Pre-dry and sieve soil (<1 mm). Optimal voltage: 20–40 kV (Silva et al. 2018).

Protocol: Dry soil at 60°C. Feed into separator chamber with controlled humidity (<30%). Collect MPs from charged electrode.

Recommendation: Best for dry, mineral-rich soils. Low efficiency for wet/organic-rich soils or charged MPs. Primarily a lab-based technique requiring specialized equipment. Use after density separation and drying for best results.

5. Oil extraction

Description: Oil (e.g., canola) aggregates MPs, which are then isolated via density.

Pros: High recovery for small MPs (<1 mm).

Cons: Complex cleanup; oil residues may interfere (Liu et al. 2019).

Principle: MPs preferentially partition into oil; oil-MP mix is separated via density.

Recommended Oil: Ultrapure silicone oil (1.05 g/cm³) or canola oil.

Protocol: Mix soil with oil (1:2 w/v). Stir vigorously (30 min). Add water/propanol and centrifuge. MPs partition into oil phase. Recover oil layer, dilute with ethanol, and filter (Crichton et al. 2017).

Recommendation: Excellent for very small MPs (<100 μm) and complex matrices. Silicon oil is most effective but costly and requires thorough post-extraction cleaning (e.g., washes with ethanol/hexane) to remove residues before polymer identification. Avoid vegetable oils if downstream pyrolysis-GC/MS is planned.

6. Magnetic separation

Description: Iron-coated MPs are magnetically extracted.

Pros: Targets specific MP types.

Cons: Limited applicability; requires MP pretreatment (Möller et al. 2020).

Principle: MPs coated with magnetic nanoparticles (MNPs) are captured by magnets.

Recommended MNPs: Iron oxide (Fe_3O_4) nanoparticles. Functionalization (e.g., oleic acid) enhances selectivity (Sinha et al. 2025).

Protocol: Mix soil slurry with MNP suspension. Incubate (30–60 min). Apply strong neodymium magnet. Decant soil fraction. Wash captured MPs-MNP complex. Dissolve MNPs with mild acid (e.g., 0.5 M HCl) if needed.

Recommendation: Highly selective but requires MP functionalization step. Best as a targeted method for specific polymer types (e.g., after density separation) or for magnetic density separation (MDS). Not suitable for direct bulk soil extraction. Potential MNP contamination requires careful handling.

Brief information about the listed methods is summarized in Table 2.

Recommendations:

1. Standard workflow: Density separation (NaI or $ZnCl_2$) + organic digestion (Fenton's reagent) remains the most cost-effective and widely applicable approach for various soils and polymers.
2. Small MPs (<100 μm): Prioritize oil extraction (silicone oil) or foam flotation (using SDS/SLS).
3. Complex/organic-rich soils: Combine density separation with enzyme digestion to preserve sensitive polymers.

4. High-throughput needs: Magnetic density separation (MDS) is promising but requires specialized equipment.
5. Validation: Always include quality assurance and quality control procedures: blank procedural samples, positive controls (immobilized polymer materials), polymer recovery tests, and duplicate samples. Filter all solutions using <1 μm filters.

Table 2. Performance comparison of microplastic recovery techniques comparative analysis of microplastic (MP) recovery methods

Method	Recovery Rate	Cost	Time	Best For	Key Limitations
Density Separation	70–95%	Low	4–24 hrs	Bulk samples; PP, PE, PS	Misses dense MPs (PVC, PET); OM interference
Organic Digestion	80–98%	Medium	2–72 hrs	OM-rich soils; all polymers	Harsh chemicals degrade sensitive MPs
Froth Flotation	65–90%	Medium	1–2 hrs	Small MPs (<300 μm); PE, PP	Surfactant contamination; clay interference
Electrostatic Separation	50–75%	High	0.5–1 hr	Dry/mineral soils; charged MPs	Low efficiency in wet/organic soils
Oil Extraction	85–99%	High	1–3 hrs	Nanoparticles (<100 μm); all polymers	Oil residue complicates downstream analysis
Magnetic Separation	60–80%[1]	High	1–2 hrs	Fe-coated MPs; targeted extraction	Requires MNP coating; not for bulk samples

Notes: [1]After Fe-functionalization; OM = Organic Matter; MNP = Magnetic Nanoparticles.

Figure 2 illustrates a flowchart reflecting the general principles of microplastic extraction from soil.

Comparison and challenges

Efficiency: Density separation with NaI achieves >95% recovery but is costly. Enzymatic digestion preserves MP integrity but is time-consuming.

Cost: NaCl is economical; enzymatic and NaI methods are expensive.

Soil Type: Clay-rich soils complicate density separation; soils rich in organic matter require vigorous digestion (Corradini et al. 2019).

Methods for separation and extraction of microplastics from soil arthropods

Soil arthropods, such as springtails, mites, woodlice, and beetles, are increasingly studied for their uptake of MPs in terrestrial ecosystems. Efficiently extracting and separating MPs from these organisms requires specially designed protocols to overcome challenges such as small body size, chitinous exoskeletons, and interference from organic matter. The main methods used to extract MPs from soil arthropods, supported by specific references, are listed below:

1. Sample collection and preparation

Arthropod extraction: Soil arthropods are typically collected using Berlese-Tullgren funnels, which drive organisms out of soil cores via temperature and light gradients (Southwood and Henderson 2000).

Surface cleaning: To remove externally attached organic matter, samples are rinsed with filtered water (e.g., 0.2 μm) or ethanol (Selonen et al. 2020). This reduces false positives resulting from environmental contamination.

Principle: Minimize contamination during arthropod collection and processing.

Protocol: Collect arthropods using stainless-steel forceps or glass straws; avoid plastic tools. Rinse samples with filtered ultrapure water to remove soil particles. Store in pre-incinerated (500°C, 4 hours) glass vials at −20°C.

Recommendations:

Field control procedures: Distribute empty containers during sampling to monitor airborne contamination.

Sizing to ensure sufficient material is available for analysis (e.g., DNA extraction and chemical analysis), arthropods are often pooled and stored together as a single composite sample. This pooling is necessary to meet the minimum biomass requirements for subsequent laboratory protocols (Selonen et al. 2020).

2. Organic matter digestion

Chemical digestion: Hydrogen Peroxide (H_2O_2): A 30% H_2O_2 solution is used to degrade soft tissues. This is effective for small arthropods but may require prolonged incubation (Cole et al. 2014).

Potassium Hydroxide (KOH): 10% KOH at 60°C digests proteins and lipids while preserving most plastics (Selonen et al. 2020).

Enzymatic digestion: Proteinase K or lipase solutions selectively break down proteins and fats without damaging MPs. Enzymes are preferred for sensitive polymers (e.g., polyethylene) (Dehaut et al. 2016).

Principle: Dissolve organic material without degrading MPs or chitin fragments.

Recommended Agents: A 10% KOH solution at 60°C effectively digests proteins and lipids while preserving most plastics (Selonen et al. 2020).

Enzymatic digestion: Proteinase K + chitinase (4 U/mL, 37°C, 72 hrs) for sensitive polymers (Cole et al. 2014).

Protocol: Incubate the samples with a strong oxidizing solution, most commonly hydrogen peroxide (H_2O_2) at elevated temperatures (e.g., 70°C).

Recommendations: Avoid using H_2O_2 or strong acids. H_2O_2 although commonly used, it is a strong oxidizing agent that requires high temperatures and prolonged incubation. This process can degrade and damage sensitive microplastic polymers, altering their properties and biasing your results (Cole et al. 2014).

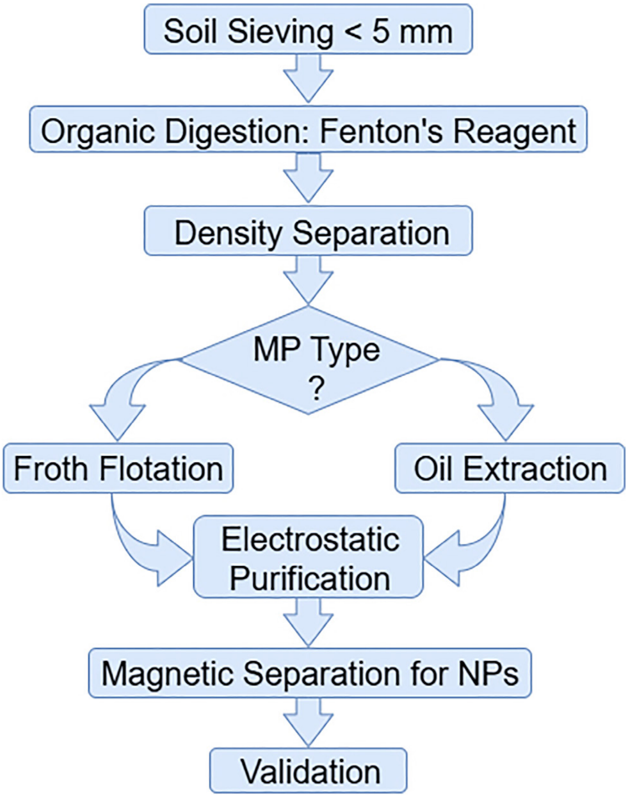

Figure 2. Integrated microplastic separation methods.

3. Density separation

Density separation, where organic matter is floated in dense salt solutions (such as NaI and NaCl) to separate it from heavier soil minerals.

Principle: Isolate MPs from residual chitin/digestion debris via flotation.

Protocol: Resuspend digested pellet in NaI solution (1.6 g/cm^3).

Centrifuge (3000 ×g, 20 min).

Filter supernatant onto 1-µm polycarbonate membrane.

Recommendations: Pre-filter NaI (0.2-µm filter) to avoid false positives.

A low-cost, non-toxic, and environmentally friendly alternative to salt solutions for extracting lower density microplastics (e.g., PE, PP) from mineral-heavy samples (Claessens et al. 2013).

4. Filtration and identification

The floated solution is filtered and the remaining particles are analyzed visually under a microscope or using chemical methods like.

Principle: Extract polymer particles and distinguish them from chitin fragments.

Protocol: Transfer density-separated MPs to anodic aluminum oxide filters (0.2 μm pore size) for microscopic examination.

Stain with Nile Red (1 μg/ml) to fluorescently identify MPs.

Analysis using μFTIR or Raman spectroscopy (polymer identification).

Recommendations:

Chitin exclusion: Following density separation and filtration, subject the sample to enzymatic digestion using chitinase enzyme (Munno et al. 2018).

Automated imaging: Integrate fluorescence microscopy with software such as MP-VAT for particle counting.

Basic recommendations

1. **Workflow**: KOH digestion → NaI density separation → Raman/IR confirmation is the most reliable method for arthropods.
2. **Polymer caution**: Avoid enzymatic digestion if analyzing polymer (nylon) – chitinase degrades it.
3. **Contamination control**:
   Perform all steps in a laminar flow hood.
   Wear 100% cotton lab coats.
   Include blank samples with each batch.
4. **Validation**: Stabilize samples using reference MPs (e.g., 50-μm PE beads) to calculate recovery rates (target >85%).

Methods for identification of different microplastic types

Identifying the composition of MPs is critical for tracking sources, assessing environmental risks, and developing mitigation strategies. Below are the most important methods for distinguishing microplastic types, along with their advantages, limitations, and supporting references.

1. Visual identification

This method is the most basic and common, but it is the least accurate.

How it works: Samples are examined under a microscope (stereomicroscope), and particles are classified based on their physical properties, such as color, size and shape (fibers, fragments, granules, flakes), texture and transparency.

Advantages: Fast, inexpensive, and does not require complex equipment.

Disadvantages: Not very accurate, as microplastics can be confused with organic or inorganic particles.

2. Spectroscopic techniques

Fourier-Transform Infrared Spectroscopy (FTIR)

Principle: Detects infrared absorption by chemical bonds, generating polymer-specific spectra.

Applications: Effective for particles >20 μm; widely used in water, soil, and biota samples.

Strengths: Non-destructive, extensive spectral libraries (e.g., OpenSpecy, ATR-FTIR).

Limitations: Poor resolution for small/weathered particles; biofouling interference.

Reference: Käppler et al. (2016) optimized FTIR for identifying weathered MPs in environmental matrices.

Raman spectroscopy

Principle: Analyzes inelastic scattering of laser light to detect molecular vibrations.

Applications: Detects particles down to 1 μm; suitable for colored or pigmented MPs.

Strengths: High spatial resolution; minimal water interference.

Limitations: Fluorescence from additives or dyes can mask signals.

Reference: Renner et al. (2018) demonstrated Raman's utility for nanoplastics in drinking water.

Principle: Interaction of light with polymers generates unique spectral fingerprints.

FTIR (Fourier-Transform Infrared Spectroscopy):

Best for: Particles >20 μm; identification of common polymers (PE, PP, PS, PET).

Recommendations: Use μFTIR for single particles (10–500 μm); FPA-FTIR for imaging entire filters.

Prioritize reflection mode for dense/colored MPs, transmission mode for thin filters.

Library: HySpex FPI library or EuroChemo WG polymer database (Primpke et al. 2018).

Raman Spectroscopy:

Best for: Particles <20 μm (down to 1 μm); pigments/carbon-filled polymers.

Recommendations:

Apply 785 nm laser to reduce fluorescence.

Use SERS (Surface-Enhanced Raman Scattering) for nanoplastics (Lv et al. 2021).

Library: KnowItAll Raman Polymer Library (Bio-Rad).

Limitations: Both require particle isolation; humic acid/coatings cause interference.

3. Thermal analysis

Pyrolysis-Gas Chromatography-Mass Spectrometry (Py-GC-MS)

Principle: Thermal decomposition of polymers into diagnostic monomers/oligomers.

Applications: Quantitative analysis of complex matrices (e.g., sediments, biota).

Strengths: High sensitivity; identifies additives (e.g., phthalates).

Limitations: Destructive; requires expertise in fragment interpretation.

Reference: Dümichen et al. (2017) validated Py-GC-MS for polyethylene and polypropylene identification.

Thermal Extraction Desorption-GC-MS (TED-GC-MS)

Principle: Thermally extracts and analyzes polymer-specific volatile compounds.

Applications: High-throughput screening of bulk samples.

Strengths: Minimal sample preparation; semi-quantitative.

Limitations: Limited to thermally stable polymers.

Reference: David et al. (2018) applied TED-GC-MS for rapid MP detection in wastewater.

Pyrolysis-GC/MS (Pyr-GC/MS):

Best for: Mass quantification, additives identification (e.g., phthalates, BPA).

Protocol: Heat samples (600°C) in inert atmosphere; separate volatiles via GC; detect with MS.

Recommendations: Calibrate with polymer standards (e.g., 0.1–100 μg) for quantification (Dümichen et al. 2017). Combine with TED-GC/MS for bulk samples.

DSC (Differential Scanning Calorimetry):

Best for: Semi-crystalline polymers (PE, PP) via melting points.

Limitations: Cannot identify mixtures or additives.

Recommendation: Use Pyr-GC/MS for complex environmental samples requiring polymer mass and additive data.

4. Staining and microscopy

Nile Red Staining

Principle: Fluorescent dye binds to hydrophobic plastics under specific conditions.

Applications: Rapid screening and quantification of MPs in water samples.

Strengths: Cost-effective; works for particles <1 μm.

Limitations: False positives from organic matter; no polymer specificity.

Reference: Erni-Cassola et al. (2017) refined Nile Red protocols for marine MPs.

Scanning Electron Microscopy-Energy Dispersive X-ray Spectroscopy (SEM-EDS)

Principle: Combines surface imaging with elemental analysis.

Applications: Detects additives (e.g., Cl in PVC) and surface degradation.

Strengths: High-resolution morphology; semi-quantitative elemental data.

Limitations: Expensive; limited polymer identification.

Reference: Silva et al. (2018) used SEM-EDS to characterize MPs in urban dust.

Principle: Selective binding of dyes to plastics.

Nile Red Staining:

Best for: Rapid screening; distinguishes hydrophobic MPs from organics.

Protocol: Stain filters (1 μg/mL Nile Red in methanol); image with blue-light excitation (Erni-Cassola et al. 2017).

Recommendations:

False positives: Lipids may fluoresce. Confirm with FTIR/Raman.

Optimize for automated counting (e.g., with PlastiCounter software).

Electron Microscopy (SEM-EDS):

Best for: Morphology + elemental analysis (e.g., Cl in PVC, Ti in TiO_2-PET).

Limitations: Staining cannot identify polymer types; SEM requires gold-coating (destructive).

5. Advanced and emerging techniques

Atomic Force Microscopy-Infrared (AFM-IR)

Principle: Combines nanoscale imaging with infrared spectroscopy.

Applications: Identifies nanoplastics (<1 μm) in complex matrices.

Strengths: Sub-micron resolution; minimal sample preparation.

Limitations: High cost; technically demanding.

Reference: Lv et al. (2021) applied AFM-IR to detect nanoplastics in bottled water.

Laser Direct Infrared Imaging (LDIR)

Principle: Automated FTIR mapping using a quantum cascade laser.

Applications: High-throughput analysis of MPs in environmental samples.

Strengths: Rapid; combines chemical and spatial data.

Limitations: Limited to particles >10 μm.

Reference: Primpke et al. (2018) showcased LDIR for microplastic analysis in seawater.

LDIR (Laser-Direct Infrared Imaging):

Best for: High-throughput analysis (100–500 particles/hr); automated particle recognition.

Recommendation: Ideal for rapid screening of microplastics in environmental samples. Use for targeted analysis of particles >20 μm (Materić et al. 2022).

NMR (Nuclear Magnetic Resonance):

Best for: Nanoplastics in liquids; identifies polymer structure.

Limitation: Very low sensitivity (high mg/L range); requires high sample concentrations, making it unsuitable for complex environmental samples with low nanoplastic mass (Mohana et al. 2021).

Machine learning/AI:

Tools: ML-FTIR (train models on spectral libraries); Raman + CNN (convolutional neural networks).

Recommendation: Use open-source platforms like MPAI for spectral matching (Primpke et al. 2020).

Critical recommendations

1. Workflow synergy:

Screening: Nile Red → μFTIR/Raman confirmation.

Quantification: Pyr-GC/MS for mass, FTIR/Raman for particle counts.

Nanoplastics: SERS + AFM-IR.

2. Technique selection guide:

Sample Type	Priority Method
Single particles >20 μm	μFTIR
Particles <20 μm	Raman
Bulk environmental	Pyr-GC/MS
High-throughput needs	LDIR

3. QA/QC Essentials:

Blanks: Include air, reagent, and procedural blanks.

Validation: Spike reference MPs (e.g., PE, PET, PS) to calculate recovery.

Spectral Threshold: Match >70% with reference libraries.

Challenges and future directions

Size and Complexity: Smaller MPs (<10 μm) require advanced tools like AFM-IR or Raman.

Standardization: Lack of unified protocols complicates cross-study comparisons.

Hybrid Methods: Coupling spectroscopy with chromatography (e.g., μFTIR + Py-GC-MS) enhances accuracy.

The methods for extracting and determining MPs from soil arthropods are summarized in Fig. 3.

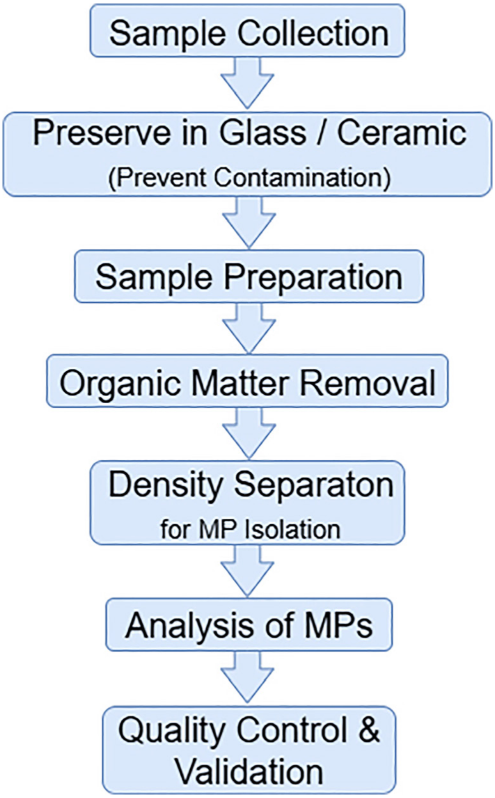

Figure 3. Extraction and determination of microplastics from soil arthropods.

Key considerations

Contamination control: Critical in all steps (e.g., air/water filtration, non-plastic instruments).

Method choice: Enzymatic digestion preserves sensitive polymers (e.g., PET), while H_2O_2/KOH suits robust polymers (e.g., PE/PP).

Detection limits: Smaller pore sizes (0.45 μm) capture nanoplastics but increase analysis complexity.

Reporting: Follow the International Technology Research Committee (ITRC) guidelines for MP data transparency.This workflow balances efficiency, sensitivity, and ecological relevance for MP studies in soil invertebrates.

This workflow balances efficiency, sensitivity, and ecological relevance for MP studies in soil invertebrates.

Conclusions

Microplastics in soil represent a widespread, albeit under-researched, threat to ecosystems and human well-being, requiring urgent attention. Meanwhile, the impact of MP on many groups of soil invertebrates remains poorly studied. The methods described in the article can be used to study several aspects of soil arthropod interactions with MP debris.

This review highlights the complexity of extracting and identifying these invisible contaminants, given the variability in soil composition and the balance required to separate microplastics without destroying them and without compromising data accuracy. Techniques including density separation, enzyme digestion, and advanced spectroscopic methods have their advantages, but are also limited by cost, efficiency, and suitability for different soil types.

Recovering microplastics from soil arthropods adds another dimension of complexity, requiring robust and efficient protocols to maintain the integrity of the plastic during dissolution of biological tissue. Emerging AFM-IR and LDIR techniques hold promise for identifying plastic particles, but standardization and widespread application remain challenging. The way forward requires unifying research efforts through universal protocols, interdisciplinary collaboration, and the integration of complementary technologies. Translating laboratory research into practice relies on collaboration between researchers, policymakers, and local communities. By prioritizing innovation, standardization, and global knowledge transfer, we can mitigate the invisible microplastic crisis in soil and protect the valuable organisms that underpin terrestrial ecosystems.

Acknowledgements

The present study was supported by RSF, project number 25-24-00295.

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