Application Note

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Quantification of Microplastics in Soil and Sediment Using Dry-Ice-Assisted Fractionation

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Abstract

This application note presents a novel and efficient protocol for extracting and quantifying microplastics in soil and sediment using the Agilent 8700 LDIR chemical imaging system. The method follows conventional drying, density separation, and analysis workflows, but improves isolation efficiencies through dry-ice-assisted froth (foam) fractionation. Method performance was validated using spiked polypropylene fibers and polyethylene terephthalate (PET) and polytetrafluoroethylene (PTFE) fragments across multiple concentration levels, with recoveries generally within the accepted 60 to 140% range. An internal standard of polyethylene (PE) beads was incorporated into each sample to monitor method performance, yielding a mean recovery of 81 ± 12%. The protocol was successfully applied to environmental soil and sediment samples from Victoria, Australia, demonstrating robust, sample-specific quality control and reliable quantification of environmental microplastics.

Introduction

Microplastics are now widely reported in terrestrial environments, including soil and sediments, raising concerns about soil health and potential human exposure pathways (for example, in agricultural regions).[1,2] Microplastics can be classified by polymer type and particle characteristics (size, color, and morphology such as fragments and fibers) and may be primary (manufactured small) or secondary (formed from the breakdown of larger plastic items). In this workflow, microplastics were defined as particles ranging from 1 µm to < 1,000 µm.

Reliable and reproducible quantification remains challenging, limiting comparability between studies and complicating risk assessment and the development of defensible monitoring approaches. Challenges occur across the workflow, from sampling and extraction through to instrumental analysis and reporting, and are exacerbated by environmental heterogeneity, inconsistent reporting, and the limited availability of standard reference materials and routine quality control procedures.

Conventional soil microplastics workflows typically include drying, density separation and/or flotation, chemical digestion to remove organic matter, and filtration prior to spectroscopic identification.[3–5] In this application note, we combined a fast, practical extraction workflow with consistent, automated instrumental analysis by adapting foam fractionation to soil and sediment. Several bubbling strategies (soda water, compressed air, and nitrogen) were evaluated; dry ice was selected because it produced vigorous mixing and stable foam in the presence of a surfactant, enhancing exposure of particles to the foam layer and improving isolation from the matrix.

All method validation and environmental sample analyses were performed using an Agilent 8700 LDIR chemical imaging system, providing automated particle location, polymer identification, sizing, and counting. A particle-based internal standard was integrated into the workflow as a simple, sample-specific check on extraction and transfer performance.

Experimental

Sample preparation

Figure 1 outlines the extraction protocol. Using a special glass vial containing an overflow spout (Table 1), 5 g freeze-dried soil was added to the vial, along with ~ 3.5 g dry ice. Sublimation was initiated by addition of 0.1% Triton X-100, creating vigorous bubbling to mobilize microplastics. A mixture of saturated $CaCl_2$ solution and hexane was added to facilitate density separation across polymers with a wide range of densities. As sublimination progressed, microplastics concentrated in the foam and solvent layers, which were collected via an overflow spout.

As the surfactant layer also contains coextracted fine organic particles, these were removed by adding 30% H_2O_2 (60 mL) into the beaker and shaking it in an incubator at 120 rpm for 16 hours at 55 °C. The samples were then filtered, followed by a secondary $CaCl_2$-based density separation using shaking, sonication, and centrifugation. Final particles were collected on polycarbonate filters.

Table 1. Dimensions of the glass vial with overflow spout.

Parameter	Size
Tube Length	115 mm
Tube Diameter	27.7 mm
Spout Opening Height	102 mm from base of tube

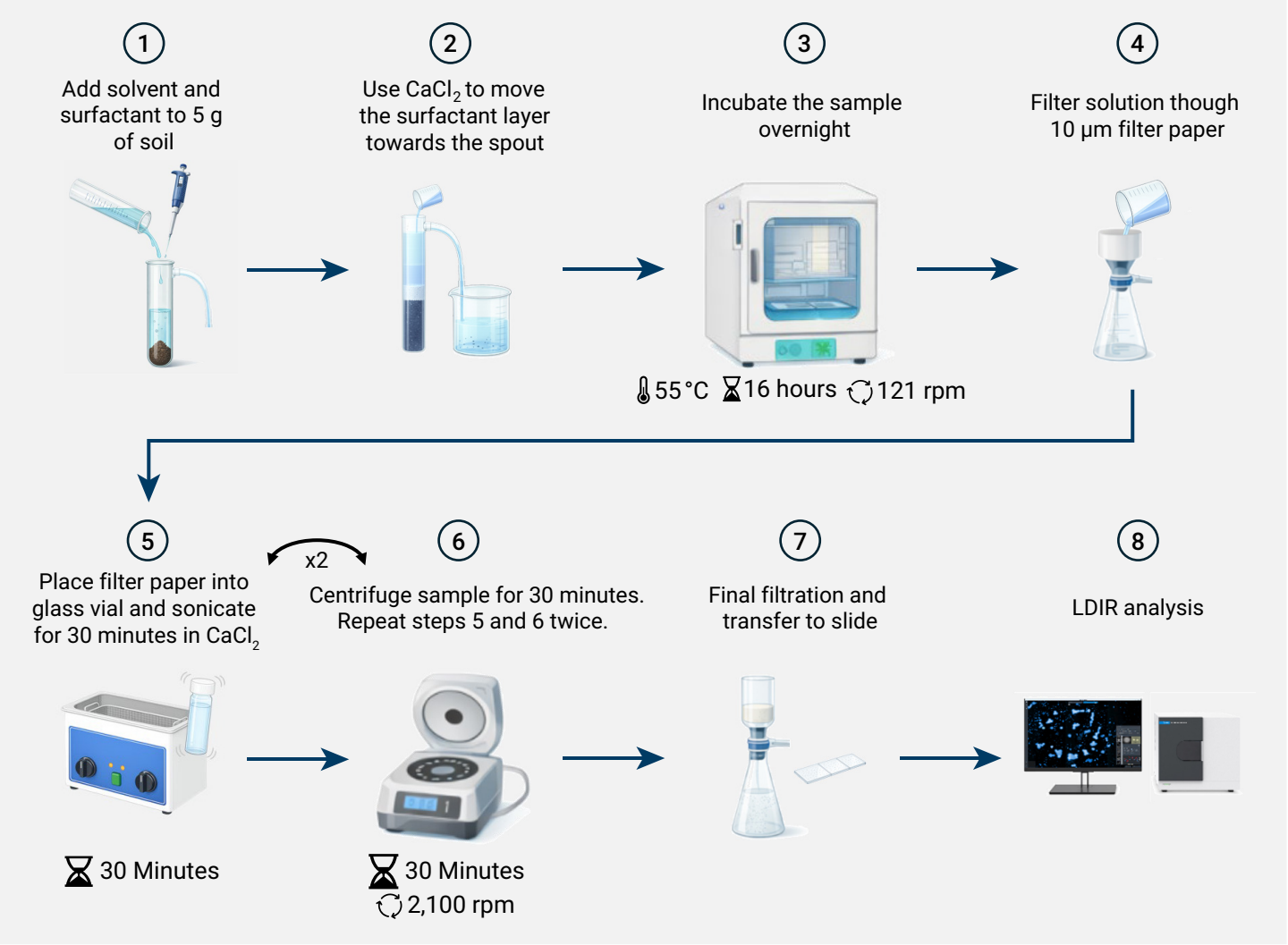

Figure 1. Dry-ice-assisted fractionation workflow for soil and sediment microplastics. Key steps include dry-ice foam generation and collection, digestion, filtration, $CaCl_2$ density separation/centrifugation, filter-to-slide transfer, and automated particle analysis using the Agilent 8700 LDIR chemical imaging system.

Transferring microplastics onto IR slides: For transfer of microplastic particles onto IR slides, a small drop of ethanol was first applied to both the Kevley IR slide and the polycarbonate (PC) filter paper. The PC filter, with the side containing the microplastics facing downward, was then placed onto the ethanol drop on the slide. While wearing clean nitrile gloves, gentle pressure was applied to the back of the PC filter using the thumb to ensure uniform distribution of the ethanol across the filter surface. Using tweezers, one corner of the filter was carefully lifted and peeled back diagonally, maintaining continuous downward pressure along the crease with the thumb to facilitate effective particle transfer. This process continued until only the final corner remained. Any residual ethanol droplet in the last corner was held against the glass Mirr IR slide to complete transfer of the remaining microplastics.

8700 LDIR chemical imaging system

Microplastic particles (10 to 1000 µm) were quantified using the 8700 LDIR chemical imaging system, operating in the infrared region between 975 to 1800 cm^{-1} and using Agilent Clarity software (version 1.7.17). Measurements were performed under continuous nitrogen purge (15 to 20 L/min) in a temperature-controlled laboratory (22 °C ± 0.5 °C) to minimize humidity and particle contamination. For analysis, the automated "particle analysis" method was used to locate, size, and identify particles based on infrared

spectral matching against a modified microplastics library.[6] Only identifications with medium (0.80 to 0.90) to high (0.90 to 0.99) confidence were accepted; polyamide identifications used a higher match threshold to minimize false positives (> 0.85).[7,8] The instrument parameters are shown in Table 2.

Table 2. Parameters used for the Agilent 8700 LDIR chemical imaging system automated method analysis of microplastics.

Parameter	Settings
Analysis Workflow	Particle Analysis
Attenuation %	Automatic (89.6%)
Size Range	10–1000 µm
Background Method	Automatic
Focus offset	Neutral
Focusing Method	Automatic
Library	Modified Microplastics Library
Scan Speed	Fast
Sweep Mode	Fast

Results and discussion

Quality control

To prevent microplastic contamination, sample extraction was done in a laminar flow cabinet with nitrile gloves. All equipment was washed with microplastic-free water before use. Each sample type had a blank method and a laboratory control sample (LCS). Field blanks were tested at random sites, with empty containers left open during sample collection.

Instrument repeatability

Instrument repeatability was assessed over five days by reanalyzing the same slide once per day, removing and reinserting the slide between runs to reflect typical real-world handling and repositioning during routine analysis.

Test 1 (low-count localization) used one polypropylene (PP) and one polytetrafluoroethylene (PTFE) particle. Both were detected in all five runs, with measured sizes of 100 ± 0.4 µm (PP) and 97 ± 0.3 µm (PTFE).

Test 2 (moderate particle loads) analyzed a defined area (15.0 × 17.9 mm) containing 75 PP and 37 PTFE particles five times. Counts were consistent at 74 ± 2 (PP) and 35 ± 1 (PTFE), with mean sizes of 148 ± 2 µm (PP) and 82 ± 4 µm (PTFE). Size-bin distributions were stable across runs (Figure 2).

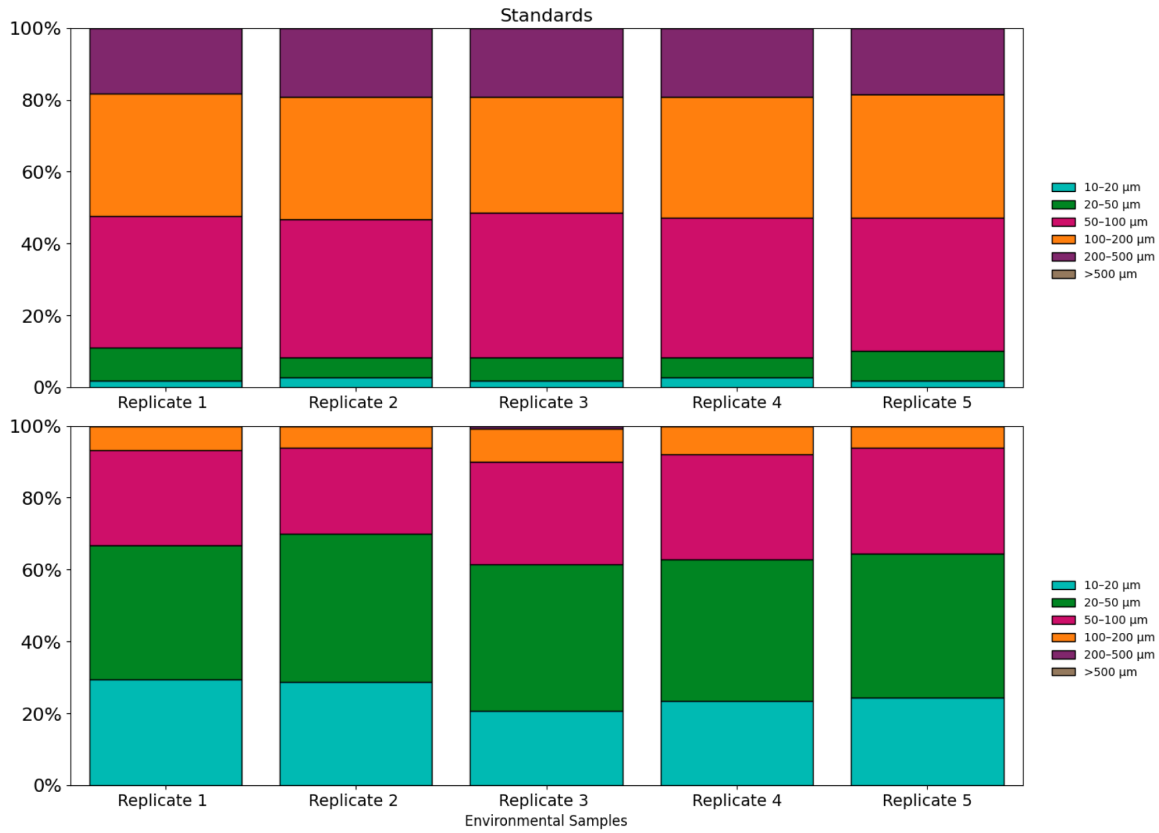

Figure 2. Size-bin composition (%) of microplastics across five repeatability tests. The upper panel shows standard materials with consistent size distributions across replicates. The lower panel shows environmental samples with minor variation near size-bin boundaries, while overall repeatability remained high.

Test 3 used an environmental soil sample (23.50 × 32.57 mm). Total particles (all matches) in a 23.5 × 22.6 mm region ranged from 9,403 to 10,376 (9,979 ± 427; CV 4%). Microplastics with match quality > 0.80 ranged from 133 to 174 (mean 153; CV 11%), with low run-to-run variability for individual polymers (SD 0 to 5) and size brackets (SD 0 to 10). Variability was greatest in the smallest size bins and for particles near bin thresholds, where minor differences in size can shift particles between adjacent brackets. Overall, the 8700 LDIR delivered consistent detection, sizing, and identification across repeated measurements, with small differences primarily attributable to slide handling.

Internal standard

Reliable microplastics reporting depends on verifying that particles are not lost during extraction, filtration, and transfer. To check performance, a particle-based internal standard (ISTD) was added to each sample.

Green PE beads (210 to 250 µm) were used because they are easily detected and visually distinct with the 8700 LDIR system. Since these beads do not occur naturally in environmental samples, spike-and-recovery checks are straightforward. A recovery window of 50 to 150% ensured routine quality control.

The ISTD was included in all sample types (n = 74), yielding recoveries between 48 and 100% (mean 81%). Only one sample was slightly below the lower limit, and no high carryover was observed.

Mean ISTD recoveries were 84% for soil, 82% for sediment, 58% for LCSs, and 73% for blanks. Lower and more variable recoveries in controls highlight the ISTD's value for confirming performance in each sample (Figure 3).

PE beads serve as a process check, not a correction factor. In low-matrix samples, bead retention can drop and fragmentation may occur, so all PE beads and fragments were excluded from microplastic counts. Overall, this approach allowed straightforward, sample-specific verification of extraction and transfer for LDIR-based microplastics analysis.

Microplastics recovery studies

Overall recovery: To evaluate method recovery, soil and sediment samples were spiked with three representative polymers: PP fibers (10 to 1000 µm), PET fragments (10 to 100 µm), and PTFE fragments (10 to 100 µm). Each polymer was tested at low, medium, and high particle loads, with spike counts confirmed by LDIR before extraction and compared to postextraction counts.

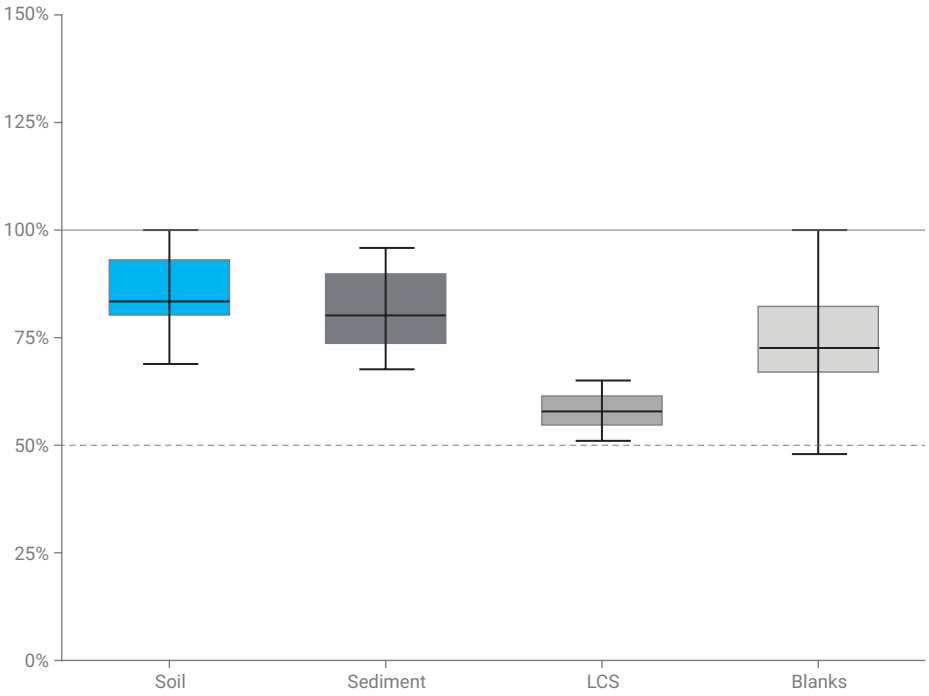

Figure 3. PE bead internal standard recovery. Top: Individual recoveries with 50–150% acceptance limits and a 100% target line. Bottom: Recovery distributions by sample type (soil, sediment, LCS, and method blanks).

For medium and high spike levels, recoveries in both soil and sediment fell within the accepted range of 60 to 140% (Figure 4). However, at low spike levels, PTFE recoveries dropped below 60% in both matrices. This highlights that particle-based recovery is increasingly sensitive to small absolute losses when only a few particles are present.

Overall, these results demonstrate that the dry-ice-assisted fractionation workflow reliably recovers common polymer types in soil and sediment across typical environmental particle loads.

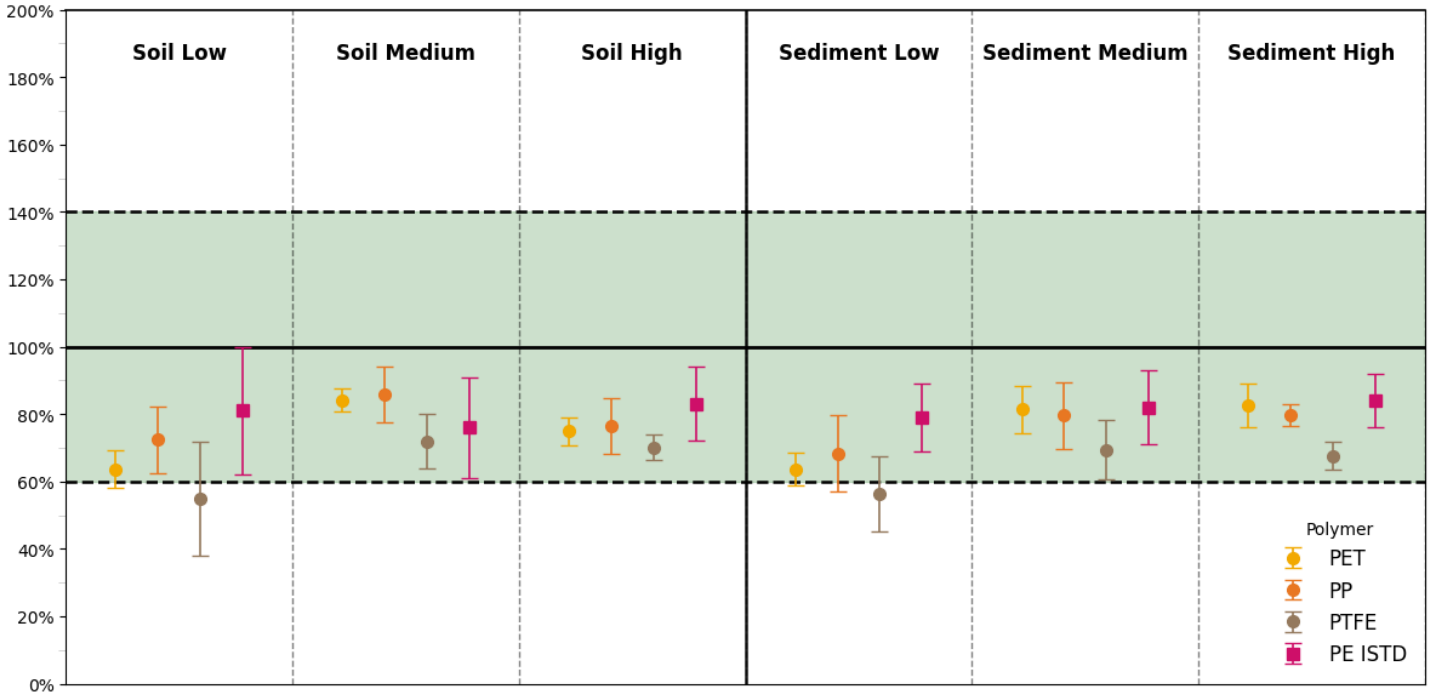

Figure 4. Spike recovery for PET, PP, and PTFE in soil and sediment. Results are shown at low, medium, and high levels; error bars indicate replicate standard deviation. The shaded band (60 to 140%) shows the acceptance range and the solid line marks 100% recovery.

Small, dense fragments like PET and PTFE are more prone to loss during transfer steps compared to lower-density fibers, especially at low particle counts. To mitigate this, saturated $CaCl_2$ was used for density separation, supporting recovery of a wide range of polymer densities while remaining compatible with the overall workflow. Validation relied on both LDIR identification and visual confirmation of particle color, but PTFE particles became discolored during extraction (Figure 5), reducing their visual traceability. This makes PTFE less suitable as a particle standard in workflows requiring color confirmation.

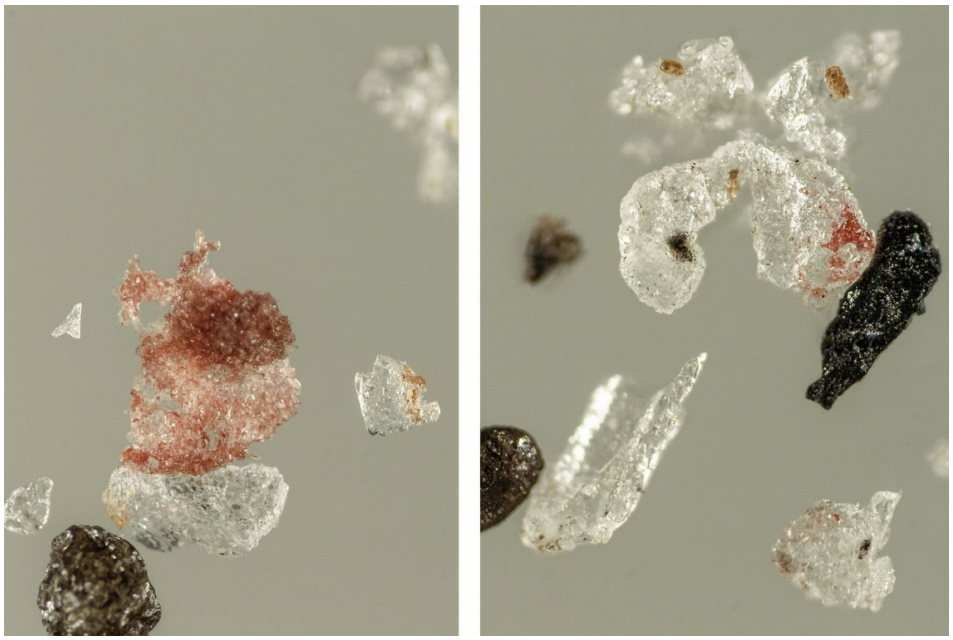

Figure 5. Visual images acquired using the LDIR of PTFE particles after extraction show discoloration, which can reduce visual confirmation during spike-and-recovery checks.

Soil recovery tests: In soil samples, mean recoveries (± SD) were: low level, 64 ± 10% (PET), 72 ± 10% (PP), and 55 ± 17% (PTFE); medium level, 84 ± 8% (PET), 86 ± 8% (PP), and 72 ± 8% (PTFE); and high level, 75 ± 4% (PET), 76 ± 8% (PP), and 70 ± 4% (PTFE). All medium- and high-level recoveries met acceptance criteria (Figure 3).

Sediment recovery tests: Similarly, in sediment samples, mean recoveries (± SD) at low levels were 64 ± 5% (PET), 68 ± 11% (PP), and 56 ± 11% (PTFE); medium level: 81 ± 7% (PET), 80 ± 10% (PP), and 69 ± 9% (PTFE); high level: 83 ± 7% (PET), 80% (PP), and 69 ± 9% (PTFE). As observed in soil, PTFE showed greater variability at low particle counts, but medium and high levels met acceptance criteria.

Comparison of internal standard and spiked polymer recoveries: Within individual samples, PP recoveries closely matched the PE bead ISTD, while recoveries for PET and PTFE were generally lower. This pattern aligns with the increasing density of PET and PTFE relative to PE and PP, reinforcing the advantage of pairing a particle-based ISTD with polymer spikes for thorough workflow performance verification.

Environmental sample overview

The workflow was applied to environmental samples from Victoria, Australia, including three soil types (chromosol, dermosol, and hydrosol) and freshwater sediment from three locations. In all samples, the 8700 LDIR provided automated particle identification, sizing, and counting of microplastics following dry-ice-assisted fractionation and density separation.

Soil concentrations ranged from 4,360 to 102,000 microplastics kg^{-1}, with acrylonitrile butadiene styrene and polyamide the most frequently detected polymers. Sediment concentrations ranged from 41,400 to 127,000 microplastics kg^{-1} and were dominated by fragments in the 10 to 50 µm size range.[8]

Conclusion

This study demonstrates the suitability of the Agilent 8700 LDIR chemical imaging system for automated, particle-based quantification of microplastics in complex soil and sediment matrices. When combined with dry-ice-assisted foam fractionation, the 8700 LDIR provided reliable identification, sizing, and counting of microplastics across a broad size and density range with high repeatability.

The integration of a particle-based ISTD within the LDIR workflow enabled sample-specific performance assessment, addressing a key limitation in current microplastic methodologies. Application to environmental samples confirmed the robustness of the approach and highlighted the capability of the 8700 LDIR to support high-confidence, reproducible microplastics analysis for research and routine monitoring applications.

References

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Further information

- Agilent 8700 LDIR Chemical Imaging System
- Agilent Clarity Software
- Microplastics Technologies FAQs
- Microplastics Analysis in Water
- Agilent MicroLab Software
- ATR-FTIR Spectroscopy Overview

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