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# Separation and Identification of Conventional Microplastics from Farmland Soils

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## Abstract

Microplastics (MPs) pollution in the terrestrial environment has received increasing attention over the last decade, with increasing studies describing the numbers and types of MPs in different soil systems and their impacts on soil and crop health. However, different MPs extraction and analytical methods are used, limiting opportunities to compare results and generate reliable evidence for industry advice and policymakers. Here, we present a protocol that describes the methodology for sampling, separation, and chemical identification of conventional MPs from soil. The method is low-cost, and the materials are readily available. This enhances operational ease and may help with widespread adoption. The protocol provides detailed information on sample collection from the top 0-30 cm of soil using plastic-free utensils; simulation of different soil types through the use of various solid media (such as bentonite clay, silicon dioxide, and non-contaminated soil), with the addition of the same mass of polyethylene(PE)-MPs for subsequent quantification; density separation of plastic particles utilizing saturated sodium chloride (NaCl) solution and digestion of organic impurities in the supernatant using 4 M sodium hydroxide (NaOH) solution; quantification of particles using fluorescent microscopy after Nile Red staining; and polymer identification using micro Fourier-Transform Infrared Spectroscopy (μ-FTIR) or Laser-Direct Infrared (LDIR) spectroscopy. The MPs recovery rate ranged from 83% - 90% for the abovementioned media. This protocol presents an efficient method for soil MPs analysis that is optimized for

feasibility, applicability, and cost-effectiveness. Moreover, the video accompanied can guide the process of analyzing the soil MPs step-by-step virtually. This study is dedicated to standardizing the methods for soil MPs analysis, enhancing the connectivity and comparability of measurements, and establishing a foundation for more standardized and scientific research.

## Introduction

It is estimated that 4.8 to 12.7 million metric tons of plastic enter the ocean annually from terrestrial sources[1,2]. These plastic particles gradually degrade into smaller fragments in response to ultraviolet irradiation, mechanical abrasion, and biodegradation[3,4]. Microplastics (MPs) pollution, with plastic particles of diameter less than 5 mm, in the soil is becoming an increasing concern, particularly in terms of its potential effect on soil and crop health. It is primarily driven by the continual rise in plastic production and challenges surrounding the appropriate disposal of plastic waste[5,6].

The accumulation of MPs in soil can be attributed to various external factors. The potential sources of MPs in soils are complex, including the utilization of plasticulture practices (e.g., plastic mulch films, irrigation pipes, greenhouse films, and associated infrastructure)[7,8,9] and input of organic amendments (such as sewage sludge application, agricultural compost, and organic fertilizer)[10]. In addition, the inappropriate disposal of plastic litter[11], decomposition of digested food waste from food plastic packaging residue[12], utilization of coated fertilizers[13], wear and tear of rubber tires[14], and atmospheric deposition[15] are also known contributors to MPs in soils. China, the leading producer and user of agricultural plastics, particularly plastic mulch films, has been estimated to have an average abundance of MPs in heavily plastic-mulched agricultural farmland of ca. 4231 items $kg^{-1}$ (dry soil)[16]. In 2018, the quantities of MPs in Chinese farmland soils within the 0-10 cm depth ranged from $4.9 \times 10^6$ to $1.0 \times 10^7$ tons, with a significant contribution from agricultural mulch films[17]. Sludge applications to agricultural soils in Europe and North America may input over 63,000 and 44,000 tons of MPs per year, respectively[18]. A study in Germany showed that compost applications to arable fields also led to an annual input of plastic particles (>1 mm) into arable fields. The application of compost led to 35 billion to 2.2 trillion plastic particles[10].The contribution of atmospheric MPs to soils is still uncertain and requires further quantification[15]. For example, the annual average input of atmospheric MPs is estimated to be $7.9 \times 10^4$ items $m^{-2}$ $yr^{-1}$ in China[16]. The extremely wide range of sources of MPs in soil has attracted the attention of many researchers, but due to the diversity of sampling, extraction, and analytical detection methods, it is difficult to integrate and compare the results of various studies.

The accumulation of MPs from a wide range of sources poses a potential environmental threat to global soils[16], highlighting the clear need for studies of MPs in soil. Some studies have shown the effects of MPs on agricultural soil include altering soil properties, impeding the growth and development of plants and soil organisms, and impacting soil microbial activity[19,20]. Other studies have found that MPs can accumulate in organisms at higher trophic levels along the food chain[21], leading to a potential hazard to

human health[22]. To clarify the soil environmental effects of MPs, it is first necessary to understand the current status of their contamination, including their abundance, polymer identification, and distribution characteristics. Therefore, the accurate identification and detection of soil MPs are of paramount importance.

Currently, a growing number of articles are exploring the global presence of MPs in soil, with considerable variation observed in the extraction and detection methods[23]. After the careful collection of samples (to minimize MPs contamination), the protocol for MPs analysis typically involves three key steps. First, density separation is widely adopted to isolate MPs particles from the soil matrix. This process commonly utilizes reagents such as distilled (DI) water (1.0 g $cm^{-3}$), sodium chloride (NaCl, 1.2 g $cm^{-3}$), or zinc chloride ($ZnCl_2$, 1.6 g $cm^{-3}$). Secondly, methods for removing organic impurities from the surface of MPs include cleaning with acidic and alkaline solutions or other oxidizing agents and enzymatic digestion[24]. The digestion of organic matter in the soil matrix or adhering to MPs particles is commonly carried out using 30% hydrogen peroxide ($H_2O_2$), 65% nitric acid ($HNO_3$), or 50% sodium hydroxide (NaOH)[25]. Following the density separation and organic matter digestion, the microscopic examination of MPs samples is required to determine the number of particles. This examination is supplemented with the analysis of the chemical composition of the polymers through techniques such as Fourier-Transform Infrared Spectroscopy (FTIR), Raman spectroscopy, or other near-infrared spectroscopy techniques[26].

However, each step in the MPs extraction and detection process carries the potential for either overestimation or underestimation of MPs occurrence. For instance, despite the widespread use of DI water as a reagent for density separation due to its cost-effectiveness and lack of hazardous properties, it may lead to the exclusion of MPs particles with higher density[27]. Conversely, the widespread application of high-density reagents may be limited by environmental hazards and increased costs[28]. Additionally, certain reagents used for organic digestion have the potential to cause damage to MPs particles[29]. Furthermore, visual classification using optical, stereoscopic, and anatomical microscopy is not without its challenges[26,30]. The determination of MPs particles heavily relies on the expertise and operation of the analysts, as well as the instrument settings. These findings emphasize the difficulty in achieving consistency and accuracy when employing various methodologies, thereby complicating the comparison of results across different studies.

To ensure the reliability and comparability of data across studies, it is imperative to establish a standardized protocol for MPs extraction and detection in soil. This standardization will not only enhance the accuracy of MPs occurrence assessments but also facilitate a more comprehensive and unified understanding of the environmental impact of MPs in soil ecosystems. To address the limitations of extraction and detection methods, the selected reagents for standardized methods should be readily available, should not affect the integrity or chemical composition of the MPs particles, and pose the lowest feasible environmental risk. Moreover, standardized methods should demonstrate high efficiency in both recovering MPs and removing organic matter from the soil matrix.

An easy-to-follow protocol is vital for widespread adoption across different research settings. Considering both MPs recovery rates and cost-effectiveness, saturated NaCl is the

optimal choice for large-scale soil sample density separation. For the digestion of organic matter, NaOH was used, as preliminary isolation experiments have shown that 4 M NaOH solution effectively decomposes soil sample impurities, such as plant residues, without causing significant damage to the MPs. In general, this experimental method utilizes readily available and cost-effective materials, has low operational complexity, and ensures a reliable extraction rate.

We recommend using the rapid and economical separation methodology proposed by Chinese Academy of Agricultural Sciences to determine MPs collected in agricultural fields[31]. For all following steps, ensure that all containers, instruments, and glassware are cleaned with DI water before use to minimize contamination. Also, ensure to run regular blanks alongside the samples to account for contamination introduced by the collection and extraction procedures.

## Protocol

NOTE: The following solutions need to be prepared at ambient temperature prior to the extraction process: 1) Saturated NaCl solution (5.7 M) - dissolve 1 kg of NaCl in 3 L DI $H_2O$; 2) 4 M NaOH - dissolve 480 g NaOH in 3 L DI $H_2O$; 3) Nile Red (100 µg $mL^{-1}$) - dissolve 10 mg of Nile Red in 100 mL of appropriate solvent (e.g., methanol, acetone).

### 1. Soil sampling and preparation

1. Collect a representative soil sample using a five-point sampling method in a "W" shape manner across the study area (**Figure 1**). Use a 30 cm stainless-steel soil auger for the collection procedure. Collect and store the samples in a non-plastic container, such as aluminum foil.

   NOTE: The soil samples can be split into different desired depths (e.g., 0-10, 10-20, and 20-30 cm). To minimize plastic contamination, avoid using plastic sampling equipment and storage containers. Instead, non-plastic containers such as aluminum foil are used to collect and store the samples. These samples can either be bulked to provide one composite sample or kept as independent replicates.

2. Dry the soil at room temperature away from direct sunlight.

   1. If a soil drier is available, use it to process multiple soil samples simultaneously, as the filter within the individual chambers minimizes the risk of cross-contamination.

   2. Otherwise, use an oven set to 40 °C and dry the soil for a minimum of 24 h, until completely dry.

   3. Grind and sieve the dry soil using a 2-5 mm metal sieve. Remove visible plant material, stones, and other inert materials.

      NOTE: Loosely cover the soil with aluminum foil to minimize airborne contamination within the oven/drier. Collect visible plastic debris (> 5 mm) from the soil with metal tweezers and place them in a plastic-free storage container, if later analysis of macroplastic is to be carried out.

3. Using a 2 decimal-place scale, weigh out 5.0 g ± 0.05 g of the soil sample onto a plastic-free weighing paper or aluminum foil. Use a new weighing paper for different samples to minimize cross-contamination. Samples can be stored in plastic-free containers (e.g., glass vials).

## 2. Density flotation

1. Transfer the 5.0 g dried soil sample into a clean 600 mL glass beaker (Beaker A). Ensure accurate labeling of all storage containers and beakers.
2. Add 230 mL of saturated NaCl solution to beaker A. Place beaker A on a magnetic stirring plate and add a glass magnetic stirrer. Stir the solution for 30 min at 260 rpm, until fully homogenized.
3. Once fully homogenized, remove the magnetic stirrer from the solution and rinse with saturated NaCl solution to prevent plastic particles from being carried out of the solution. Place the beaker on a flat surface without direct sunlight and leave it standing overnight, until full density separation has occurred.
   NOTE: When performing the entire procedure, it is necessary to fully cover with aluminum foil to avoid plastic contaminants from the air.

## 3. Impurity digestion

1. Once the contents of beaker A have completely separated, carefully transfer the supernatant to a new, clean 600 mL glass beaker (beaker B). Carefully rinse the inner walls of beaker A with saturated NaCl solution. Transfer the supernatant to beaker B. Perform this procedure 2-3 times.
   NOTE: A total of 200 mL of supernatant is recommended. Ensure that the total supernatant volume is consistent while maintaining the same concentration of digestion solution in the next step.
2. Add 4 M NaOH solution to the sample in beaker B to reach a fixed volume of 500 mL. Place beaker B on a magnetic stirring plate, add a glass magnetic stirrer, and stir the solution for 30 min at 260 rpm until fully homogenized. Keep the beaker covered with aluminum foil during this process to minimize airborne contamination.
3. Once fully homogenized, remove the magnetic stirrer from the solution and rinse with saturated NaCl solution to remove any attached particles. Place beaker B on a flat surface without direct sunlight and leave it standing overnight until full density separation and organic matter digestion have occurred.
   NOTE: When performing the entire procedure, it is necessary to fully cover with aluminum foil to avoid plastic contaminants entering the solution from the air. The digestion duration depends on the quantity and the type of organic material. Extend the digestion time if required to ensure complete organic matter digestion. After successful digestion, the supernatant should look clear with no visible organic matter floating in the beaker.

## 4. Coloration with Nile Red solution

1. Once the contents of beaker B have completely separated, carefully transfer the supernatant to a new, clean 600 mL glass beaker (beaker C). Rinse the inner walls of beaker B with DI water to ensure maximum particle transfer.
   1. If the volume in beaker C is less than 500 mL, make up the value to 500 mL with DI water to unify the solution volume.
2. Add the Nile Red solution to beaker C to achieve a final maximum concentration of 0.5 M. Stir the solution with a glass rod until completely homogenized, then let the solution incubate for 30 min in the dark by covering the beaker with aluminum foil.

## 5. Vacuum filtration

1. Set up the vacuum filtration devices in the following order: glass funnel, metal clamp, vacuum filtration base, collection beaker, connection hose, moisture trap, and vacuum pump. Carefully remove a new membrane (0.2 µm pore size, 47 mm diameter) from its storage container using metal tweezers. Place the filter membrane centrally and flat on the top of the vacuum filtration base.
   NOTE: Ensure a secure connection by aligning the vacuum filtration base with the glass funnel and fastening it with a metal clamp.
2. Activate the vacuum filtration and slowly pour the liquid from beaker C into the glass funnel. Rinse beaker C several times with DI water to maximize particle recovery. Rinse the sides of the glass funnel with DI water after sample filtration to ensure minimal particle loss.
   NOTE: Cover the glass funnel with aluminum foil to minimize airborne contamination during the filtration process. If the sample has a high particle number and the filtration speed decreases, multiple membranes can be used for the same sample. This will ensure an even distribution of particles across the membrane and minimize the risk of particles aggregating and overlapping for later quantification.
3. Once filtration is completed, carefully retrieve the filter membrane from the porous plate using tweezers and place each membrane in an individual glass Petri dish. Let the membrane fully dry before closing the Petri dish and wrapping it in aluminum foil. Store it in a dry and dark place until further analysis.

## 6. MPs particle quantification by fluorescence microscopy

1. If the exact location of fluorescent particles on the membrane is required for later polymer identification (e.g., by using FTIR), please refer to the steps below:
   1. Use a black gel pen to gently mark the beginning position and 10 marks on the filter membrane, following the shape of the "Z" (**Figure 2**). Place the membrane carefully on the glass slides on the microscope stage using tweezers, ensuring a flat sample surface.
   2. Activate the fluorescence instrument in the following order: the host, fluorescent sources, monitor, and fluorescence microscope. Turn on the instrument and set the source's light knob to maximum brightness. Utilize the **bright field (BF)** and **fluorescent light (FL)** switch buttons to take BF and FL images, respectively.
   3. Using the software for sample observation and recording (e.g., DP2-BSW), take bright field pictures under the BF position. Turn the knob into FL position and take pictures with the fluorescence filter in the dark. Ensure the field of view observation sequence runs from 1 to 10. Make sure the FL and BL pictures are taken in the same position.
      NOTE: Fluorescent microscopic analysis should be carried out within 24-48 h after filtration to ensure optimal fluorescence of particles. Ensure the BF and FL images are taken in the same position (**Figure 3**). Ensure all membranes are analyzed using the same magnification and instrument settings.
2. For polymer identification by using LDIR, perform the microscope steps as below:

1. Set up the microscopy system as follows: the camera, filters, magnifications and microscope stage, and computer. Select the appropriate filter mode best suited for the desired excitation and emission wavelengths (e.g., 470 nm and 495 nm, respectively) depending on sample and background fluorescence.
2. Wipe the filter membrane holders with dust-free tissues, then secure the membrane in the holder and slide onto the microscope stage. Scan the entire membrane visually to ensure an even distribution of particles.
3. Ensure the camera is connected, and the microscope magnification is appropriate for all sample types and consistent across all samples from the same set. Determine the size of the area in mm captured with the camera before starting the analysis by using a measuring tape.
   NOTE: If particles are evenly distributed, analyze a minimum of 10% of the total membrane area by selecting the required number of sampling points on the membrane. Take a picture of each sampling point using the camera.

## 7. MPs polymer identification using FTIR or LDIR spectroscopy

1. If FTIR is used to identify polymer particles, please refer to the steps below.
   1. Turn on the FTIR spectrometer and corresponding software for sample observation and recording. Clean the probe before measuring each sample.
   2. Identify the particles for monitoring through real-time screen recording. Adjust position and sharpness by manipulating the rocker. Bring the operating platform to the center and capture the current air background spectrum.
      NOTE: Target particles that correspond to both BF and FL images.
   3. Measure 3-5 fixed points on each particle to attain spectra within the infrared wavenumber range of 400-4,000 $cm^{-1}$. On the results page, save the original data, obtain the spectrum, and compare it with the plastic spectrum in the standard library to confirm the sample's hit quality index (**Figure 4**).
      NOTE: In this study, due to the small size of MPs and the challenges in quantifying them across the entire filter, 5-7 representative particles were selected from each sample for FTIR analysis. To ensure an even distribution, particles following a "Z-shaped" pattern across the filter were selected. However, it should be noted that some uncertainty may arise, as the particles were selected randomly rather than by scanning the entire filter.
   4. A match is accepted if the hit quality index (HQI) ≥ 0.7. **Figure 4** shows an example of particles identified by FTIR spectroscopy in a soil sample spiked with 0.04% PE-MPs.
2. If LDIR is used for polymer particle identification, follow the steps below:
   1. Once the fluorescent microscope steps are completed, resuspend the particles for analysis on the LDIR. Place the filter membrane into a new glass vial and add 20 mL of pure ethanol. Close the vials tightly and wrap the lids with paraffin film to prevent leakage.

2. Sonicate samples in an ultrasonic bath for a minimum of 1 h until all particles have been resuspended. Remove and discard the membrane from the glass vial.
   NOTE: Ensure the membrane side with particles faces inwards, i.e., away from the vial wall. Sonication time depends on the membrane type used; the membrane may leach color, but this will not interfere with the polymer identification.
3. Confirm the successful particle resuspension by reanalyzing membranes under the fluorescent microscope and ensuring particle removal is > 95%.
4. Place the glass vials with the ethanol solution on a magnetic stirring plate and add a small magnetic glass stirrer to the vial. Let the ethanol evaporate to less than 5 mL by setting the plate temperature to 100 °C and stirring at a low speed to keep particles suspended.
   NOTE: Cover the vials lightly with aluminum foil to minimize airborne contamination of the samples. Optionally dry the solution under a gentle stream of nitrogen for ethanol evaporation.
5. Transfer the sample into a small 8 mL glass vial, using new ethanol to rinse the 20 mL glass vial to maximize particle recovery.
   NOTE: If required, add new ethanol to the small glass vial to reach exactly 5 mL.
6. To prepare the sample for analysis on the LDIR, shake the sample thoroughly until all particles are homogenously suspended in the solution and quickly pipette 10 µL of the sample onto the slide and let the ethanol evaporate. Repeat this step two more times to analyze 3 replicates per sample on each slide.
7. Use the particle analysis tool in the associated software to measure the infrared spectra of each particle within the set size range and match them against the internal library for polymer identification.
   NOTE: One point on each particle is automatically measured in the infrared wavenumber range of 800-1800 $cm^{-1}$, and particles are automatically matched against known library spectra of polymers and other organic and inorganic materials.
8. If the hit quality index (HQI) ≥ 0.8, the match is accepted. **Figure 5** shows an example of particles identified by LDIR spectroscopy in a soil sample spiked with 0.04% PE-MPs.
   NOTE: Sample volume can be increased for samples with low particle numbers.

## 8. Particle quantification of fluorescent membrane images using ImageJ

1. Open ImageJ[32] (version 1.54f) and load images into the software. Adjust the scale of the image to the correct measurements corresponding to the real image size. Use the functions **Analyze > Set scale** to input image pixel and size (mm) values, then select **Global** to apply these settings to all images during the session.
2. Transform the image into a binary image and convert it to 8-bit, using the functions **Process > Binary** and **Image > Type > 8-bit**, respectively. To select the parameters for particle analysis, use the functions **Analyze > Set measurements** and select the desired parameters, e.g., **Area, Shape descriptors**, and **Feret's diameter**. Select **Add to overlay** and determine the number of decimal places for data output.

3. To analyze particles, select **Analyze > Analyze particles** and determine the particle size range of interest. Set **Circularity** to 0-1, unselect **Pixel units**, enable **Overlay** to be shown, and select the following boxes: **Display results, Exclude edges, Clear results, Include holes, Summarize,** and **Overlay**. Export results as .csv files from the results window.

   NOTE: The analysis of fluorescent images will only provide information on particle number, not polymer number; therefore, an additional identification procedure is required, for example, using IR spectroscopy techniques.

## Representative Results

To validate the recovery rates of this methodology, samples from three different solid matrices (silicon dioxide (SD), bentonite clay (BT), and soil) were analyzed in sets of three replicates. Samples were analyzed with and without the addition of 0.04% *w/w* white polyethylene (PE) microplastic (particle size range 40-48 µm). Soil samples were collected from Haidian District, Beijing, China (China Agricultural University West Campus), and soil was classified as umber soil. Additionally, three replicates of blanks were included to account for any potential contamination introduced throughout the extraction and analysis process.

The average recovery rates were 84%, 83%, and 90% of BT, SD and soil, respectively. On average, 86% of the PE particulates were successfully recovered within a set size range of 20-500 µm. The interference of results from the blank sample and chemical identification were eliminated. The particle loss could be accounted for by supernatant transfer, filtration, or incorrect identification. The variation in recovery rates can be influenced by factors such as the type of salt solutions, digestion solutions, and reaction times[33,34]. Furthermore, a global meta-analysis reported that recovery rates in soil MPs extraction experiments typically range from 71% to 93%[35]. Therefore, the experimental results in this study are valid and meet the standards required for microplastic analysis in soils.

Additionally, apart from PE, the phenolic resins, polyamide (PA), polypropylene (PP), and polyvinyl chloride (PVC), were also identified (**Table 1**). These contaminations may have originated from the filtration device, laboratory equipment, atmospheric deposition, or DI water. Although saturated NaCl may result in relatively lower recovery rates for high-density microplastics (MPs), both our results and previous studies have reported recovery rates of 92%-100% for high-density MPs, such as polyamide (PA, 1.12-1.69 g/cm$^3$) and polyvinyl chloride, (PVC, 1.35-1.45 g/cm$^3$)[33,34].

The main result of MPs sampling and sample analysis is the quantification and identification of MPs particles per sample. The formula used to determine abundance (items kg$^{-1}$) is:

$$N_1 = \frac{S_t \times \frac{n}{S_f}}{w}$$

where $N_1$ and $n$ represent the particle abundance and number of each sample under ImageJ software, respectively. $S_t$ and $S_f$ denote the effective filtration area of a single filter membrane and each vision field area, respectively. The $w$ stands for the weight of each sample, which is 5 g in this example.

$$N_2 = recovery\ rate \times N_1$$

$$recovery\ rate = \frac{plastic\ particle}{all\ particles\ detected} \times 100\%$$

where $N_2$ represents the abundance of plastic particles after identification, and the recovery rate is calculated as the

ratio of plastic fragments to the total number of detected particles. The hit quality index (HQI) indicates the level of similarity between measured sample spectra and the best match found in the library. In this study, the FTIR and LDIR HQI standards for polymer matches are set at 70% and 80% or above, respectively. That means the sample spectra are 70% and 80% or more, similar to the closest match in the library[36].The recovery rate and polymer identification of the BT, SD, soil samples, and blanks after extraction and subsequent FTIR analysis are shown in **Table 1**.

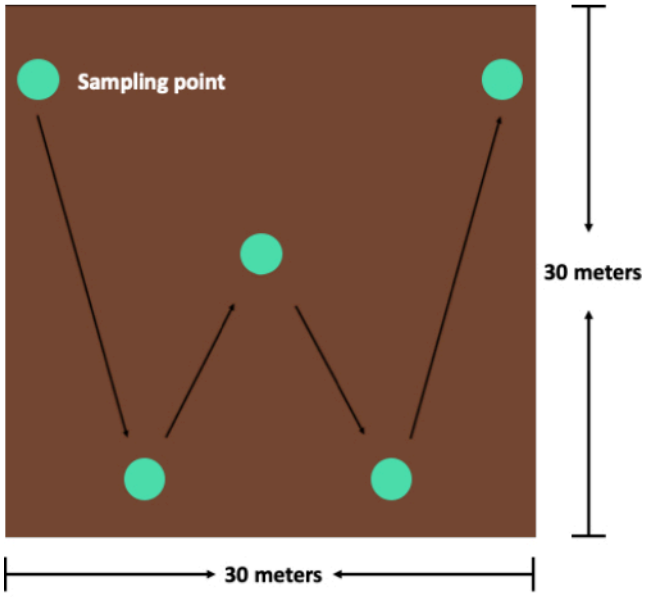


**Figure 1: A schematic representation of a soil sampling strategy using the 'W' approach.** Please click here to view a larger version of this figure.

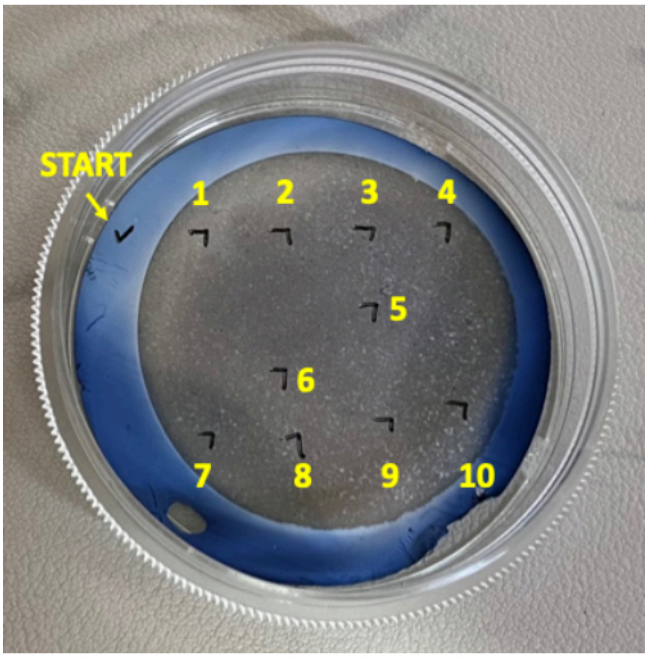


**Figure 2: The filter membrane for FTIR detection is marked with a black gel pen in a Z-shaped sequence of 10 positions.** Please click here to view a larger version of this figure.

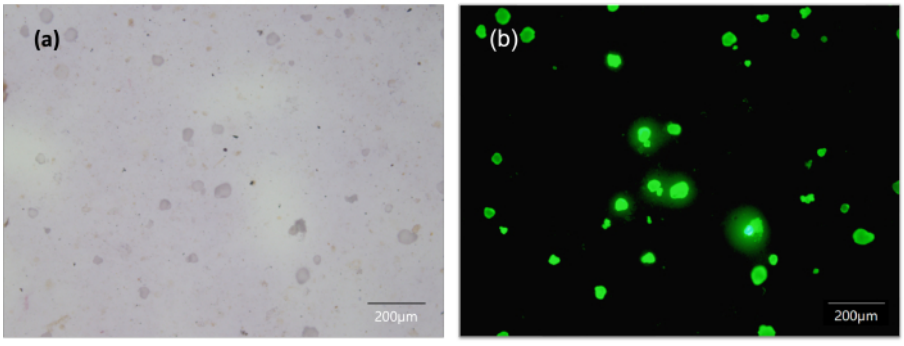


**Figure 3: The membrane image of suspected MPs particles (in green) from soil taken using the fluorescence microscope.** A) Example of particles under bright field (BF) and B) fluorescent light (FL) microscopy. Please click here to view a larger version of this figure.

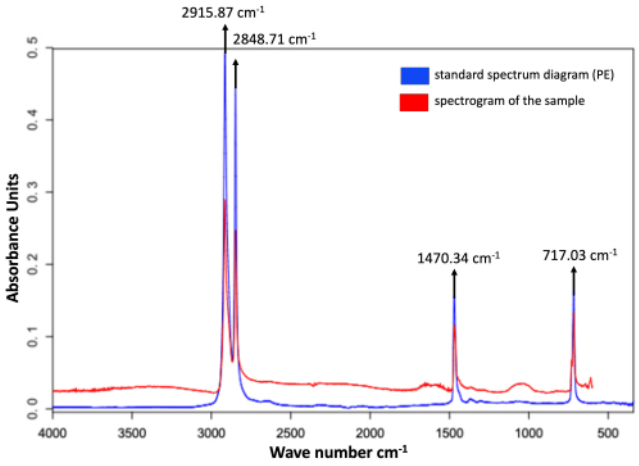


**Figure 4: A schematic diagram of the results based on the FTIR method.** Comparison of the spectrogram of the sample and the PE standard, and PE particle spectra (red line) matched against the closest library spectra (blue line) with a match quality of 0.98. Please click here to view a larger version of this figure.

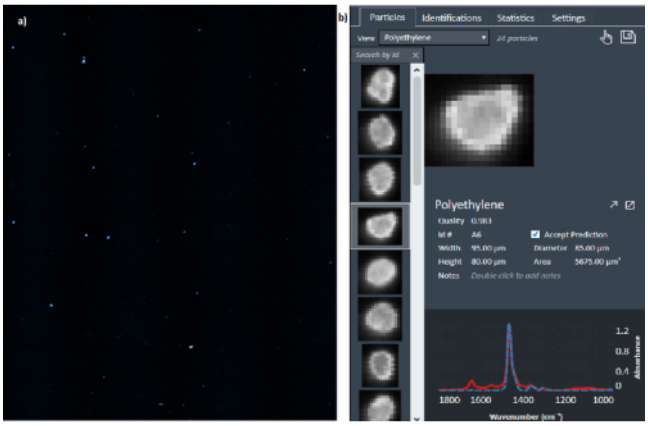


**Figure 5: A schematic diagram of the results based on the LDIR method.** A) Particles identified using LDIR chemical imaging in 10 µl of a 5 ml ethanol solution, after extraction of soil spiked with 0.04% *w/w* PE; B) PE particle spectra (red line) matched against the closest library spectra (blue dotted line) with a match quality of 0.98. Please click here to view a larger version of this figure.

| Samples | Bentonite clay (BT) | Silicon dioxide (SD) | Soil |
|---|---|---|---|
| 1 recovery rate | 89.30% | 81.50% | 95.20% |
| 2 recovery rate | 81.70% | 87.60% | 86.70% |
| 3 recovery rate | 82.40% | 79.60% | 88.30% |
| Total recovery rate (%) | 84.50% | 82.90% | 90.10% |
| Polymer type | PE (93.3%)<br>Phenolic resin (6.7%) | PE (86.4%)<br>Polyvinyl chloride (13.6%) | PE (89.6%)<br>Polyamide (6.8%)<br>Polypropylene (3.6%) |

## Discussion

The soil sampling strategy in the field, including approaches such as simple random sampling or systematic grid sampling, as well as the sampling area and depth, must be tailored to the specific research questions and clearly defined prior to the sample collection. Some studies have focused on the topsoil layer of 0-10 cm[34,37], whereas other collected soil samples with a depth of 0-40 cm[38]. Since the size and abundance of MPs vary in different soil depths, it is necessary to unify the depth for soil sampling. We suggest considering a depth of 0-30 cm to be appropriate and practical for agricultural fields, as this is the typical plow depth[39,40]. Moreover, the research objective, sampling approaches, and sampling area must be designed to address the predefined research question. Additionally, during the collection and transportation of the samples, every effort should be made to avoid contamination from plastic products by replacing them with suitable sampling containers like aluminum boxes, paper bags, or cardboard containers.

Selecting the most appropriate solutions for density separation and organic digestion is the critical step in the protocol, as they affect MPs extraction efficiency and minimize the potential damage of MPs, thereby affecting the final MPs extracted from samples. Density separation protocols are commonly employed for the separation of MPs from the soil, with high-density salt solutions being the most frequently used extraction media. The recommended salt solutions include NaCl (1.2 g $cm^{-3}$), NaI (1.8 g $cm^{-3}$), $Na_6[H_2W_{12}O_{46}]$ (1.4 g $cm^{-3}$), $ZnCl_2$ (1.6-1.7 g $cm^{-3}$), and NaBr (1.55 g $cm^{-3}$). Considering both MPs recovery rates and cost-effectiveness, saturated NaCl is the optimal choice for large-scale soil sample density separation, though it may lead to relatively lower recovery for high-density MPs (such as PVC). However, sample preprocessing in previous studies has indicated that NaCl can also extract high-density polymers[34,33]. In addition, $ZnCl_2$ is a more suitable extraction solvent for biodegradable plastics. A recently published $H_2O$-$ZnCl_2$ extraction method specifically for biodegradable polymers by Li et al., (2023) can maximize the integrity of biodegradable plastics themselves while ensuring high recovery efficiency (91.7% ± 7.5%)[41]. Regarding the extraction time, we have chosen a significant period of settling to ensure high extraction efficiency for MPs. This extended duration facilitates thorough contact between the solution and the soil sample. However, some studies suggest that settling for 8-12 hours is also acceptable[42,31]. The minimum extraction time required is typically between 8 to 12 hours, although this duration may increase depending on the soil

texture. Additionally, if the extracted soil sample is larger than the 5.0 g used in this protocol, two flotation processes can be conducted[34].

The majority of current research suggests utilizing acidic and alkaline solutions to digest the organic fraction from the sample[43,44]. Preliminary isolation experiments of MPs from soil have indicated that the remaining impurities after the density separation can be mainly attributed to dry plant residues. Therefore, low concentrations of strong acidic or alkaline solution are recommended to decompose the impurities without causing noticeable damage to the MPs. Some studies use 30% $H_2O_2$ solution[45], however, since soil typically has a higher organic matter content compared to air, water, and sediment samples, a stronger solution (i.e., NaOH) is recommended in this protocol. It is worth noting that controlling the concentration and reaction time of the digestion solution is essential, as excessively high concentrations or prolonged reaction times may damage MPs, leading to a change in the physical properties of MPs[31].

The initial method of MPs analysis involved visual identification under a light microscope. However, this technique by itself is highly error-prone due to the potential to introduce bias[46]. Currently, there are various identification methods for polymers, with FTIR spectroscopy and Raman spectroscopy being the most commonly used[47]. FTIR spectroscopy can further be categorized into attenuated total reflectance (ATR)-FTIR spectroscopy and μFTIR spectroscopy, which are suitable for chemical structure identification based on particle sizes (particles > 0.5 mm and particles < 0.5 mm, respectively). These methods are simple, convenient, and can provide fast and accurate results, making infrared spectroscopy the most popular technology for identifying MPs[48]. Additionally, a more recent technique known as laser direct infrared (LDIR) chemical imaging has gained attention[49]. This method can reliably detect MPs particles within a size range of 20-500 μm with a faster detection speed, but its higher cost has limited widespread adoption for now.

## Disclosures

The authors have nothing to disclose.

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