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Methodological Approach for Detecting Polypropylene Microplastics in Agricultural Soil in Southern Portugal

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Abstract

Plastics have been widely used in agriculture, particularly as mulching materials, due to their ability to improve soil conditions and enhance productivity. However, their degradation into microplastics (MPs) raises significant environmental and agronomic concerns, as these particles may change soil properties, affect microbial communities, and pose risks to surrounding ecosystems. While methodologies for MP detection in aquatic environments are well established, the analysis of MPs in soils remains challenging due to the complexity and heterogeneity of soil matrices. Currently, there is no standardized protocol for the determination of MPs in soils. This study critically evaluated and compared three different pre-treatment methods for removing organic matter from soil prior to MP analysis in an agricultural soil, and proposes a comprehensive methodology comprising two main phases: (i) organic matter removal, a crucial step of MP particles, and (ii) density separation of MP particles. Three distinct removal chemical methods were tested using samples from an agricultural soil in Southern Portugal. The most effective method was then applied to assess MP particles in an experimental field, using soil samples collected before mulching and 14 months later beneath a polyethylene-based soil cover. This was one of the first studies contributing to the establishment of a routine methodology for monitoring MPs in soils, particularly the agricultural soils, ensuring compliance with the future "Directive for Soil Monitoring".

Keywords: agricultural soil; soil organic matter removal; microplastic separation; polymer identification; ATR-FTIR spectroscopy

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1. Introduction

Plastics have become indispensable in human life due to their versatility, durability, and low-cost production. However, their persistence in the environment, due to their complex structures and large molecular weights has led to significant ecological concerns. One of the most significant issues is their gradual degradation into smaller particles known as "secondary microplastics (MPs)" by physical (mechanical degradation, temperature fluctuations, and agricultural practices), chemical, and biological processes [1–4]. Although there is no consensus on the definition of MPs, they can generally be defined as any synthetic solid particle or polymeric matrix, with a regular or irregular shape, and with size ranging from 1 μm to 5 mm. Microplastics exhibit unique properties, including their small size, high surface area and highly hydrophobic nature through covalent and electrostatic interactions, making them efficient vectors for environmental contaminants such as metals, organic pollutants, and pathogens [2,4–9]. But MPs can also be manufactured for commercial use (i.e., cosmetics or fertilizer coatings), in which case they are referred to as "primary MPs".

In agriculture, the use of plastics has increased significantly in recent decades, driven by their ability to enhance productivity [4,10,11]. A widely adopted agricultural practice involves the use of plastic mulching, especially those made of polyethylene (PE) or polypropylene (PP) to improve soil conditions and plant performance. This agricultural practice helps suppress weeds, conserves soil moisture, and regulates soil temperature in horticulture and fruit tree production systems [4,11,12]. Although these plastic soil covers effectively enhance productivity and reduce herbicide use, they can also cause significant environmental challenges. Over time, with prolonged exposure to the environmental conditions, plastic mulches may degrade and fragment into "secondary MP" particles, accumulating in the soil. This can negatively affect soil quality, microbial activity, and plant health, but also poses a risk of the contamination of nearby watercourses through leaching, runoff and soil erosion [13]. Thus, plastic mulch degradation not only poses environmental risks, but may also directly affect agricultural productivity [5,8,10,14]. As the mulch material degrades, its effectiveness also diminishes, compromising its agronomic benefits. Furthermore, the resulting MP may change the soil's physical, chemical, and biological properties. This may include changes in soil structure, porosity, pH, and nutrient availability, and disrupt the biological community, which is essential for soil health [4,6,15] and may interfere with plant growth by impairing photosynthesis, disrupting nutrient metabolism and inducing oxidative stress [3,16].

Although the environmental impact of plastic waste is well documented, the presence and effects of MPs in soils, particularly agricultural soils, have only recently gained attention [5,11,13,17]. The development of effective methodologies to quantify MPs in soils has become a critical area of research. Over the past, various techniques have been developed and refined for detecting MPs in different environmental compartments, such as water, sediments, and biota. And techniques like Fourier-transform infrared (FTIR) spectroscopy, Raman spectroscopy, and microscopy have proven to be effective in identifying and quantifying MPs in water bodies, sediments, and marine organisms [2]. Soils have a highly heterogeneous composition, with variations in bulk density, texture, pH, and organic matter concentration, which complicates the detection and quantification of MPs, for instance, by affecting MP distribution and sorption in soils [18].

Common approaches such as wet chemical digestion, density separation, and microscopy techniques have advantages and limitations for MP detection in soils [2], and their effectiveness depends on factors such as soil type, organic matter concentration, type of plastics, including thickness, and MP size [19]. Even though analytical techniques are well established for monitoring MPs in water and sediments, soil sample pre-treatment is essential to remove organic matter and other interfering compounds prior to MP determination [2,19]. Soil organic matter can adhere to MPs or form aggregates with them, hindering their separation and analysis. Furthermore, stable humic fractions may include polymeric macromolecules that may be read as false-positive MPs [19]. In addition, soil organic matter, organic fibers, and MPs have comparable densities, which interferes with the visual and spectroscopic identification of MPs [2,20]. Consequently, the removal of soil organic matter, preferably before, or if necessary after, density separation, is essential to improve the accuracy of MP recovery [20] should it be achieved, for instance, by digesting soil samples with hydrogen peroxide or strong acids or bases [2,20]. However, according to Lee et al. [2] and Pfeiffer and Fischer [21], acid and alkaline digestions can degrade certain polymers (e.g., polyamide, polyethylene terephthalate (PET), and polystyrene (PS)) compromising their identification. Density separation is effective for isolating lighter plastics like the PP and low-density polyethylene (LDPE), but may be less suitable for denser polymers, like PET, high-density polyethylene (HDPE), among others (Lee et al. [2] and Campanale et al. [22]). Similarly, filtration can be applied to soil samples, although does not fully separate MP from soil constituents [19]. Microscopy allows for the direct visualization of MPs, but it is time-consuming and often limited by the resolution of the equipment used [23]. Overall, methodological inconsistencies and the lack of standard protocols hinder comparisons between studies, and limit the accuracy to assessments of MP contamination in soils. Further developments are needed in methodologies for MP extraction, identification, and quantification as part of soil-based MP monitoring [19]. Based on an extensive literature review, we selected and tested the most relevant methods. Given these challenges, this study aimed at evaluating and comparing three pre-treatment methods for removing organic matter from soil prior to MP analysis. The most effective method was applied in a case study comparing MP levels in surface soil samples collected at the start of the experiment (before mulching) and 14 months after the application of a PP-based mulch. A density separation technique was used to isolate MPs from the soil matrix, and ATR-FTIR spectroscopy using an INVENIO® (Bruker, Germany) ATR-FTIR spectrometer, with a software OPUS 8.7 (Bruker, Germany), equipped with a DTGS detector, was employed to identify polymer types. By refining these analytical procedures, this study aimed to contributing to test different protocols for soil-based MP monitoring, an area of growing importance in light of forthcoming European soil monitoring regulations.

2. Material and Methods

2.1. Experimental Site

Soil samples were collected from the Innovation Hub of Fataca, in Odemira, Southern Portugal, lat.: 37.5903, long.: −8.7403. Over the past 30 years, temperatures have ranged from a minimum of 7 °C to a maximum of 31 °C (average of 19 °C), and average monthly rainfall ranged from 3 mm in summer to 75 mm in December, with a mean annual rainfall of 437 mm.

2.2. Soil Sampling

In June 2023, soil samples were collected in triplicate from unmanaged soil (Figure 1, site A). Additional soil samples were collected 14 months later, in August 2024, also in triplicate, from a soil covered with a black geotextile fabric material (polypropylene, PP) and cultivated with blueberries (Figure 1, site B). All soil samples were taken using a metallic soil corer from a depth of 0–20 cm. They were stored in glass containers, carefully handled to prevent airborne contamination and labeled for further analysis in the laboratory for MP particles using the adapted Method 2 (30% H_2O_2 digestion at 50 °C, for 48 h).

The soil had a sandy–loam texture, with a bulk density of 1.3 g cm^{-3}, pH of 5.8 ± 0.03, total N of 1.08 ± 0.05 g kg^{-1} dry soil, and organic C of 12.9 ± 0.16 g kg^{-1} dry soil [4].

2.3. Testing Methods for Microplastic Determination in Soil Samples

2.3.1. Soil Organic Matter Removal

In the present study, three different methods were compared to evaluate the efficiency in removing the soil organic matter (Table 1, Figure 2A). The soil samples collected from the field were air-dried until constant weight and sieved through a 2 mm mesh to remove larger particles. Each soil sample was prepared in triplicate in the laboratory to ensure reproducibility. According to [2,24,25], agricultural soil samples should be sieved at <2 mm, and weight less than 50 g, preferably 5–10 g for MP determination.

Figure 1. Sampling sites at the Innovation Hub of Fataca, in Odemira, Southern Portugal.

Table 1. Summary of the tested methods for soil organic matter removal.

Method	Reagents	Concentration	Temperature (°C)	Contact Period (h)	Steps
Method 1	H_2O_2	30% (w/v)	25	24–96	- Mix the soil sample (10 g) with 30% H_2O_2 (5 and 12 mL g^{-1}). - Maintain at room temperature (25 °C), followed by stirring and filtration. - Allow for reaction to proceed until an overall complete organic matter oxidation [2].
Method 2	H_2O_2	30% (w/v)	50	24–96	- Mix the soil sample (10 g) with 30% H_2O_2 (5 and 12 mL g^{-1}). - Settle the mixture at 50 °C, with occasional stirring until an overall complete organic matter oxidation, and filtration [2].
Method 3	Dichloromethane + Hexane	100%/each reagent	25	24	- Mix the soil sample (10 g) with 10 mL 100% dichloromethane followed by stirring and 24 h settling. Filter with 1–3 µm pore size paper. - Add 10 mL 100% hexane, stirring and settling for 24 h, followed by a filtration [26].

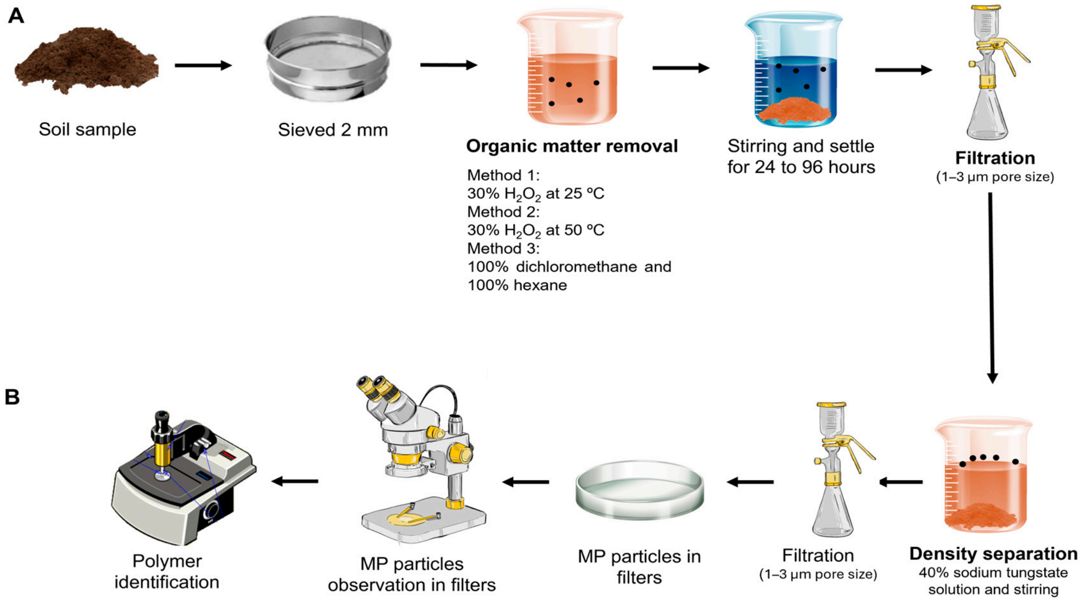

Figure 2. Steps for organic matter removal (**A**), and microplastic separation and polymer identification (**B**) in soil samples. Black round dots in glass containers (**A**,**B**) simulate the presence of MPs.

In Method 1, the soil samples (10 g) were treated with 30% H_2O_2 (w/v) solution, at room temperature (25 °C) [2]. In Method 2, the soil samples (10 g) were digested with 30% H_2O_2 (w/v) at 50 °C [2]. For both methods, two different volumes of H_2O_2 (5 and 12 mL g^{-1}) were tested. In Method 3, soil samples (10 g) were sequentially treated with 100% dichloromethane and 100% hexane, with a 24 h settling after the addition of each solvent [26]. Method 3 is a solvent extraction method widely used in chemistry and environmental science to extract (leach) specific compounds from matrices like soil based on their solubility, to isolate specific fractions of organic matter like lipids, humic fractions, and dissolved organic carbon [27–29]. Dichloromethane was selected as the extraction solvent due to its high dissolving efficiency for organic compounds. The three methods aimed to isolate the MPs from the soil matrix, improving their detectability and quantification. After the addition of reagents, all soil samples were magnetically stirred for 30–60 min to ensure proper homogenization. Thereafter, the mixtures were left to settle for 24–96 h to allow for complete soil organic matter removal.

2.3.2. Microplastic Extraction by Density Separation

In the present study, after the removal of organic matter (Methods 1, 2, 3) (Figure 2A), each soil mixture was transferred to a decontaminated 1 L glass beaker, covered with a glass lid, and subjected to the density separation of MPs. This step was performed using 250 mL of sodium tungstate dihydrate ($Na_2WO_4 \cdot 2H_2O$, 40% concentration), with a density of 1.4 g cm^{-3} (Figure 2B), most suitable for the studied soil with a bulk density of about 1.3 g cm^{-3}, compared with the most usual NaCl solution (1.2 g cm^{-3}) [22]. The mixture was stirred mechanically for 30–60 min and left to settle for approximately 4 h. After settling, the supernatant was filtered under vacuum using a Millipore cellulose filter paper with a pore size of 1–3 μm. Filters were stored in covered Petri dishes and allowed to dry at room temperature (25 °C) until microscopic observation [2]. Filter selection can range from 0.2 to 20 μm, but should consider the MP particle size (1 μm–5 mm). Filters with pore sizes of 50 μm or larger may also be used depending on the target particle size [2]. Subsequently, the filters were examined with a stereomicroscope (in the present study, a Zeiss Axio Zoom.V16, magnification of 7×–112× was used) to directly observe the morphology and integrity of the plastic particles following exposure to pre-treatments under different conditions

(Figure 2B). Upon the identification of these particles, based on their color, shape and size, they were stored on a glass slide for further analysis using ATR-FTIR (Figure 2B).

2.3.3. Polymer Identification Using ATR-FTIR Spectroscopy

In the present study, all particles were manually picked from the filter paper after the density separation procedure (Section 2.3.2), and analyzed using an INVENIO® (Bruker, Germany) ATR-FTIR spectrometer, with a software OPUS 8.7 (Bruker, Germany), and equipped with a DTGS detector. Spectra were acquired in reflectance mode at 25 °C, with a resolution of 4 cm^{-1} across the range of 4000–600 cm^{-1}, and 32 scans were performed per sample. Polymer identification was based on the comparison of absorption bands with reference spectra. The MP particles determined by Methods 1, 2, and 3 (Section 2.3.1) and density separation (Section 2.3.2) were examined under a microscope to identify any modification in morphology, such as surface erosion, fragmentation, or deformation. Three critical variables were systematically analyzed during these experiments: the reagents used, the duration of the treatment, and the temperature at which the procedures were conducted. The choice of reagents, including their chemical composition and concentration, played a significant role in determining their potential to induce physical or chemical changes in the polymers. The treatment time was carefully controlled, as longer exposure could amplify any observed effect, such as degradation or structural weakening. Similarly, temperature was a crucial factor, as higher temperatures could accelerate chemical reactions and increase the likelihood of thermal or oxidative degradation in the polymers. By carefully monitoring and controlling these variables, it was possible to assess their individual and combined influence on the structural integrity of the MPs, providing valuable insights into the conditions under which synthetic polymers may undergo degradation or morphological changes (Figure 3).

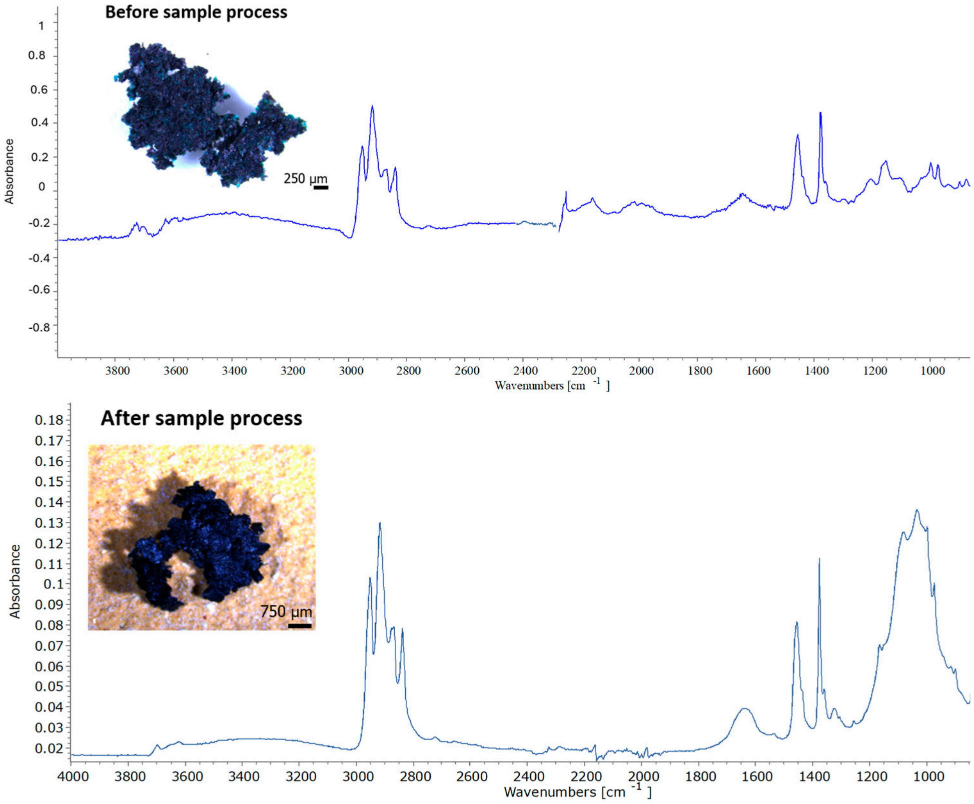

Figure 3. FTIR spectra showing: (**up**) the pristine polypropylene (PP) microplastic, and (**down**) the PP microplastic recovered after organic matter digestion, and the respective polymer (PP) identification by an ATR-FTIR spectrometer.

2.3.4. Statistical Analysis

Number of soil particles observed in filters after density separation method were compared using an ANOVA one-way analysis using Statistica 12, and MP particles were estimated and are presented in Table 2 as the means and standard deviation.

Table 2. Pre-treatment methods for the quantification of polypropylene (PP) particles in soil samples collected (0–20 cm) at the start of the experiment (June 2023) in an unmanaged agricultural soil, and 14 months later (August 2024) beneath a geotextile fabric (PP-derived material). The results are presented as the means ± standard deviation and expressed per 100 g dry soil.

Organic Matter Digestion				**Density Separation**			**Polymer**
Sampling Date	**Reagent**	**Temperature (°C)**	**Digestion Period (h)**	**Reagent**	**Total Particles/100 g Dry Soil**	**Total MP/100 g Dry Soil**	**Identification**
June 2023	30% H_2O_2	50	48	40% sodium tungstate	20 ± 17 (ns)	0	0
August 2024	30% H_2O_2	50	48	40% sodium tungstate	36 ± 19 (ns)	34 ± 18	34 PP

MP = microplastic; ns = non-significant, $p \geq 0.05$.

3. Results and Discussion

3.1. Evaluation of the Tested Methods

Soil organic matter and MPs have comparable densities and can interfere with the visual and spectral examination of MPs [20]. Moreover, the presence of organic matter on the surface of MP particles may affect polymer identification by spectroscopy [2,20]. Therefore, the removal of organic matter is a crucial pre-treatment step in soil sample analysis. The present results demonstrate that the reagent used in Methods 1 and 2 did not change the characteristic PP peaks at ~2950, 2915, 1455, and 1375 cm^{-1}, which remained visible in both spectra, indicating no significant chemical degradation (Figure 3). Minor spectral differences were attributed to residual soil matrix, particularly in the 1000–1100 cm^{-1} region, which is commonly associated with the Si-O stretching vibration of silicates and other mineral phases naturally present in soils (Figure 3). Additionally, no changes in the morphology of the MP particles were observed microscopically (Figure 3), confirming the suitability of Methods 1 and 2 as robust pretreatment approaches for soil samples providing a reliable foundation for further analytical protocols aimed at detecting and characterizing MPs in agricultural soils.

Method 1 was effective in preserving the MP morphology; however, it did not achieve the complete oxidation of organic matter as foaming persisted after 96 h of reaction, regardless of oxidant volume (5 or 12 mL g^{-1}). Method 2 proved to be the most effective, successfully degrading the soil organic matter (foam ceased after 48 h of digestion), while maintaining the MP morphology, including their size, shape and surface characteristics. In soils rich in organic matter, a 30% H_2O_2 solution generally produces a dense foaming [20] which may be mitigated by using a lower H_2O_2 concentration in combination with a high temperature [20]. Couceiro [30] reported similar results using a 48 h digestion period at 60 °C for MP recovery in a sandy soil. The highest temperature employed in Method 2 (50 °C) enhanced organic matter digestion, compared to Method 1, as evidenced by the cessation of foaming after 48 h digestion. Seo et al. [19] noted that temperatures above 50 °C may induce thermal degradation of MPs, changing their chemical signatures and potentially compromising the accuracy of identification and quantification techniques such as ATR-FTIR.

Method 3, which employed 100% dichloromethane effectively removed soil organic matter after 24 h, but compromised the MP structural integrity. Certain solvents (e.g., dichloromethane) are known to dissolve or chemically degrade specific plastic polymers, especially under elevated temperatures (50 °C) [19]. This effect is more pronounced in sensitive polymers such as polystyrene (PS), polyvinyl chloride (PVC), and polylactic acid (PLA), but not the polypropylene (PP). Such degradation can result in an underestimation of microplastic concentrations. The ATR-FTIR spectra of the MPs isolated using Method 3 were inconclusive, likely due to chemical alterations in the polymer structure or surface characteristics and increased spectral noise that masked MP signals, hindering accurate polymer identification. Therefore, this chemical method is not recommended for MP detection and characterization in soil samples.

Due to their hydrophobic nature, most plastics do not absorb water and tend to float in aqueous solutions of suitable density. This property was exploited to separate the MPs from soil. Using a high density solution not only enhances the overall extraction efficiency of MPs, but also helps remove impurities adhering their surfaces [19]. The density of common MPs typically ranges from 0.80 to 1.40 g cm^{-3}, which is generally lower than that of most soils ($\geq$1.30 g cm^{-3}). When the density of MPs is unknown, a high-density salt solution is recommended to ensure effective separation by flotation. Several reagents can be used for this purpose, including water, NaCl, $CaCl_2$; $ZnCl_2$, among others. However, not all MPs can float in water alone. While most MPs float due to their lower density compared to water, some small MPs, especially after the surface adsorption of microorganisms, metals, or organic contaminants, can become denser and sink.

Although a NaCl solution (1.20 g cm^{-3}) is the most commonly used for the density separation of MPs, its lower density relative to many soils such as the present one makes it less effective for recovering high-density polymers. For this reason, 40% sodium tungstate dihydrate (1.4 g cm^{-3}) was adopted in the present study.

The optimized protocol adapted here for organic matter removal using Method 2, at 50 °C for 48 h, with a 1:4 (w:v) soil to oxidant ratio, followed by density separation with 250 mL of 40% sodium tungstate dihydrate proved to be the most suitable approach for eliminating organic matter and isolating MPs from soil extracts.

3.2. Quantification of Microplastics and Polymer Identification in Agricultural Soil: A Case Study Using Method 2

In the present study, organic matter removal was conducted before density separation using a 40% sodium tungstate dihydrate solution (Figure 4). The determined number of MP particles was expressed in 100 g dry soil.

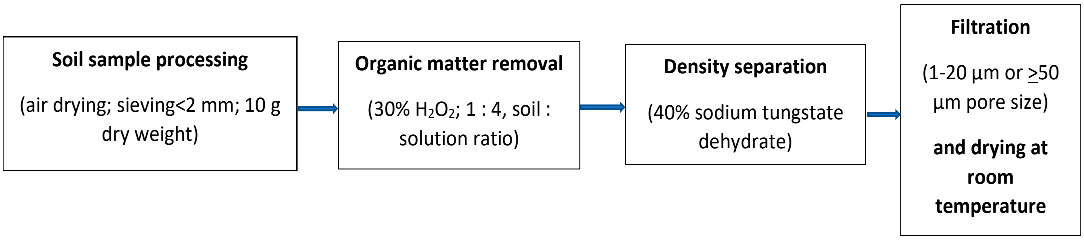

Figure 4. Flowchart of the proposed pre-treatment soil procedures.

In Table 2, the initial soil samples collected in June 2023 contained few particles, none of which were identified as MPs. This indicates that native soil at Fataca was free from MP contamination. In August 2024, the black MP-PP particles identified in covered soil with a geotextile fabric accounted for 34 ± 18 MP-PP per 100 g soil, according to the polymer spectral library in Frias et al. [31] (Figure 5). This finding reflects the geotextile fabric degradation in the field after 14 months, and corresponds to approximately 94%

of the total MP particles detected in soil beneath the black geotextile fabric. Overall, these MP particles were fragments exhibiting a black color, consistent with the original mulch material, with sizes varying substantially, from 45 to 763 µm. The efficiency of polymer identifying analyzed through ATR-FTIR is typically assessed by the similarity index (match score—70/80%) between the sample spectrum and the reference spectrum in the polymer library. The sample spectra were of good quality, being free of noise (Figure 5). Other particles detected in covered soil after 14 months were likely due to occasional contamination or irrigation tubes.

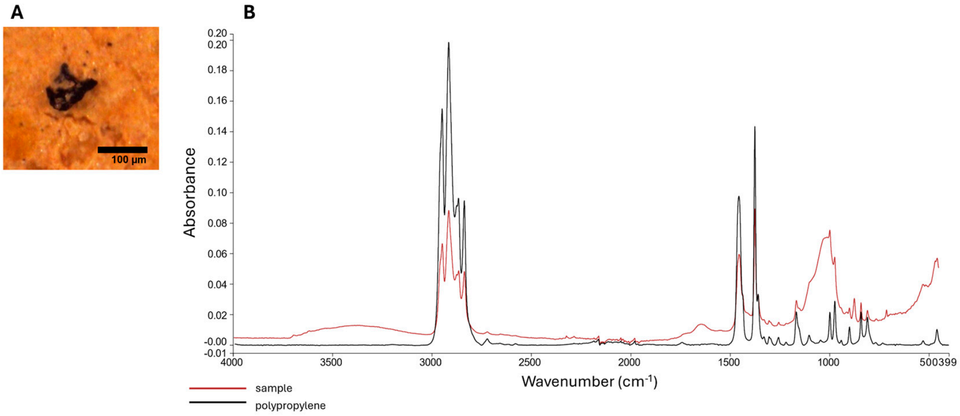

Figure 5. (**A**) Particle in soil after 14 months covered with a geotextile; (**B**) polymer identification (PP) in comparison with a polymer spectral library [31].

4. Conclusions

This study evaluated various methods for isolating microplastics from soil and polymer characterization, identifying their respective strengths, limitations, and potential for routine analysis. Our findings contributed to developing of an effective and accessible methodology for monitoring microplastics in soil.

Among the tested methods for organic matter removal, Method 2, using 30% hydrogen peroxide at 50 °C for 48 h, with a 1:4 (w:v) soil to oxidant ratio, proved to be the most effective method for organic matter removal, also providing an accurate polymer identification by ATR-FTIR. The elevated temperature accelerated organic matter breakdown while preserving microplastic morphology integrity, which is essential for accurately identifying sources and understanding particle behavior in soil ATR-FTIR, confirming it to be a reliable non-destructive and rapid technique for routine microplastic analysis. However, there are limitations regarding particle size and the detection of a single particle at a time, making this procedure time-consuming.

Nevertheless, the overall cost effectiveness and accessibility of chemical Method 2, combined with density separation using 40% sodium tungstate and ATR-FTIR make this workflow a highly feasible option for routine microplastic quantification in soils with a bulk density of approximately 1.3 g cm^{-3}, or higher. Its straightforward implementation in laboratories opens important opportunities for the regular monitoring of microplastics in agricultural soils, especially in light of the forthcoming European "Monitoring Law for Soils".

Author Contributions: All authors contributed to this study by field experiment, laboratory tests, and the manuscript preparation and revision. Conceptualization of this study was mainly performed by C.C.; the methodology was planned by C.C., P.A., J.A., F.P. and R.V.; the laboratory tests and measurements were carried out by P.A. and J.A.; supervision was undertaken by C.C. and R.V.; writing the original draft preparation was performed by P.A. and J.A.; reviewing and editing were undertaken by C.C., F.P. and R.V. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: The original contributions presented in this study are included in the article; further inquiries can be directed to the corresponding author.

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Conflicts of Interest: The authors declare no conflicts of interest.

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