OPTIMIZING METHODS FOR QUANTIFICATION OF MICROPLASTICS IN AGRICULTURAL SOILS

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1. INTRODUCTION

In recent decades, the use of plastic materials in agriculture has become widespread due to their ability to enhance crop yields (Pereira et al., 2021). However, as these plastics degrade over time under environmental factors, they fragment into microplastics (MP) which may pose significant risks to soil health and plant growth in particular (Gao et al., 2019). While existing techniques have been successfully applied to sediments and ocean waters (Lee et al., 2023), the specific matrix of soil represents a significant challenge (Mushtak et al., 2024). This requires the adaptation of conventional methods for sediments to agricultural soils. To identify the most efficient method for isolating MP from soils, different methods for organic matter removal and colorimetric method were compared, also ensuring the morphological integrity of plastic particles (Lee et al., 2023; Mushtak et al., 2024).

2. OBJECTIVE

This study aims to optimize methods for quantifying MP in soils, particularly the agricultural soil covered with plastic mulch to protect against weeds, conserve soil moisture and regulate the soil temperature.

Fig. 1. Experimental field covered with plastic at the Innovation Hub of Fataca, Odemira, Southern Portugal.

3. METHODOLOGY

Soil samples (0-20 cm depth) were collected from an agricultural soil covered with a black geotextile (PP) film and planted with fertigated blueberries at the Innovation Hub of Fataca, Odemira, Southern Portugal (Fig. 1).

3.1 ORGANIC MATTER REMOVAL

Three distinct methods were applied to assess the organic matter removal in soil samples, without disturbing the morphology of plastic particles (MP). In Methods 1 and 2, different temperatures of incubation and two volumes of oxidizing solution were tested to evaluate effects on organic matter removal (Fig. 2). Physical and chemical changes in the MP were monitored.

3.2 DENSITY SEPARATION

After organic matter removal in three methods, soil samples underwent a density separation with a 40% sodium tungstate dehydrate solution (1.4 g cm^{-3}) to separate MP in filter paper (Fig. 2).

3.3 COLORIMETRIC METHOD: NILE RED

Nile Red dye was used to identify the MP in soil samples due to its affinity for hydrophobic polymers. Soil samples were incubated with a 2 µg mL^{-1} Nile Red solution at 70 °C for 3 hours, filtered and washed to remove excess dye. Fluorescent-stained MP were visualized using a Leica DM 6000B microscope.

3.4 POLYMER IDENTIFICATION: SPECTROSCOPY

The Fourier Transform Infrared Spectroscopy in Attenuated Total Reflectance (ATR-FTIR) was used to identify MP recovered in filter paper.

ATR-FTIR spectra were obtained at 25 °C using a Perkin Elmer® Spectrum Two spectrometer with a resolution of 4 cm^{-1} over the 4000–600 cm^{-1} range.

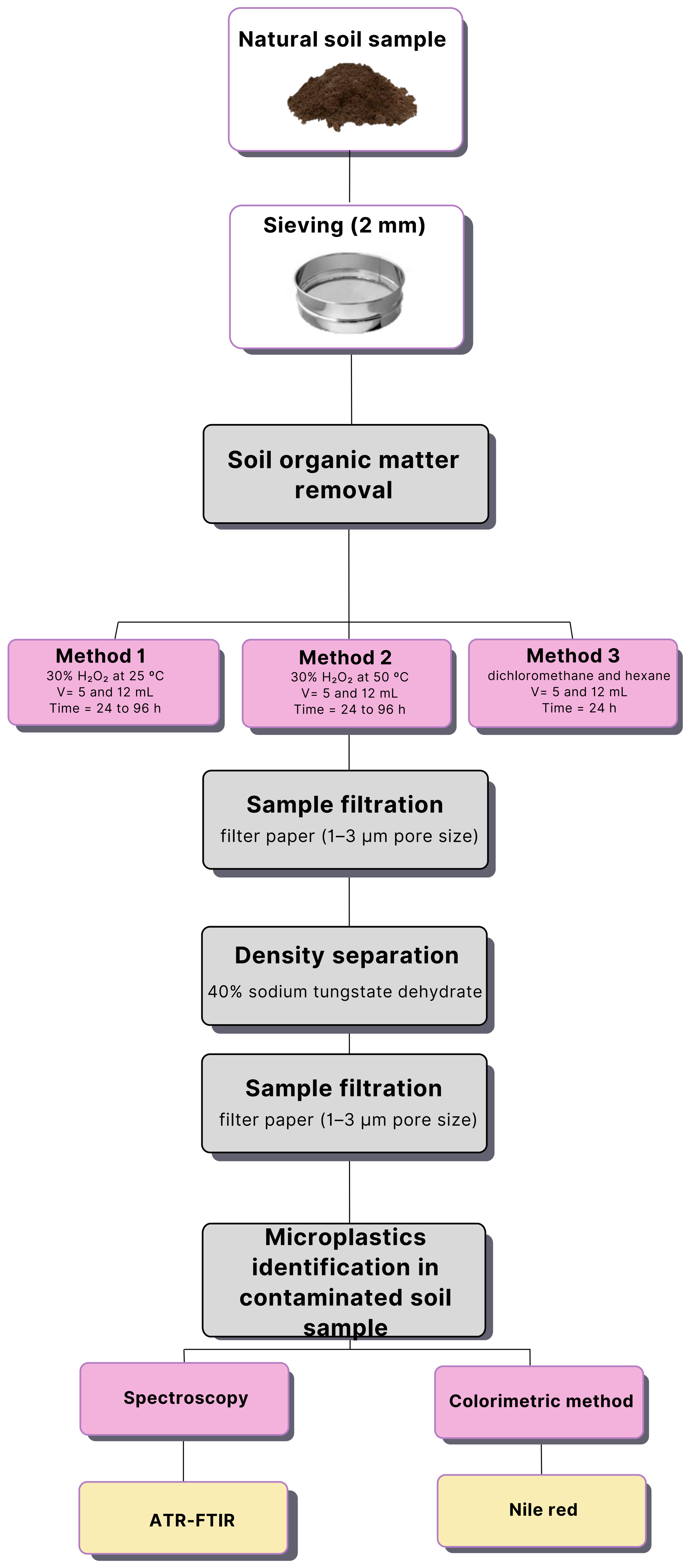

Fig. 2. An overview of the methodologies used for microplastics detection in soil samples.

4. RESULTS AND DISCUSSION

4.1 ORGANIC MATTER REMOVAL

- Method 1 (30% H_2O_2, 25 °C): after 48 h incubation, organic matter (OM) digestion was incomplete, but the MP morphology was preserved. A PE polymer was found.
- Method 2 (30% H_2O_2, 50 °C): after 48 h incubation, a more complete OM removal was observed, preserving the MP integrity. Three PP and two PE polymers were recovered.
- Method 3 (dichloromethane and hexane): after 24 h incubation at 25 °C, this method yielded the highest particle counts (six particles). But MP identification was inconclusive, likely due to solvent-induced morphological changes and analytical interferences.

4.2 POLYMER IDENTIFICATION: SPECTROSCOPY

- The spectra pf polymers (PE and PP) were clearly identified by ATR-FTIR in particles extracted by Methods 1 and 2 (Fig. 3A, B).
- The spectra by ATR-FTIR of particles from Method 3 had much noise thus compromising polymers identification. This suggests that reagents used in Method 3 would have altered the MP morphology, affecting their spectral signatures.

4.3 POLYMER IDENTIFICATION

ATR-FTIR was used to assess the efficacy of each organic matter removal method and particles separation (Fig. 3A,B,C).

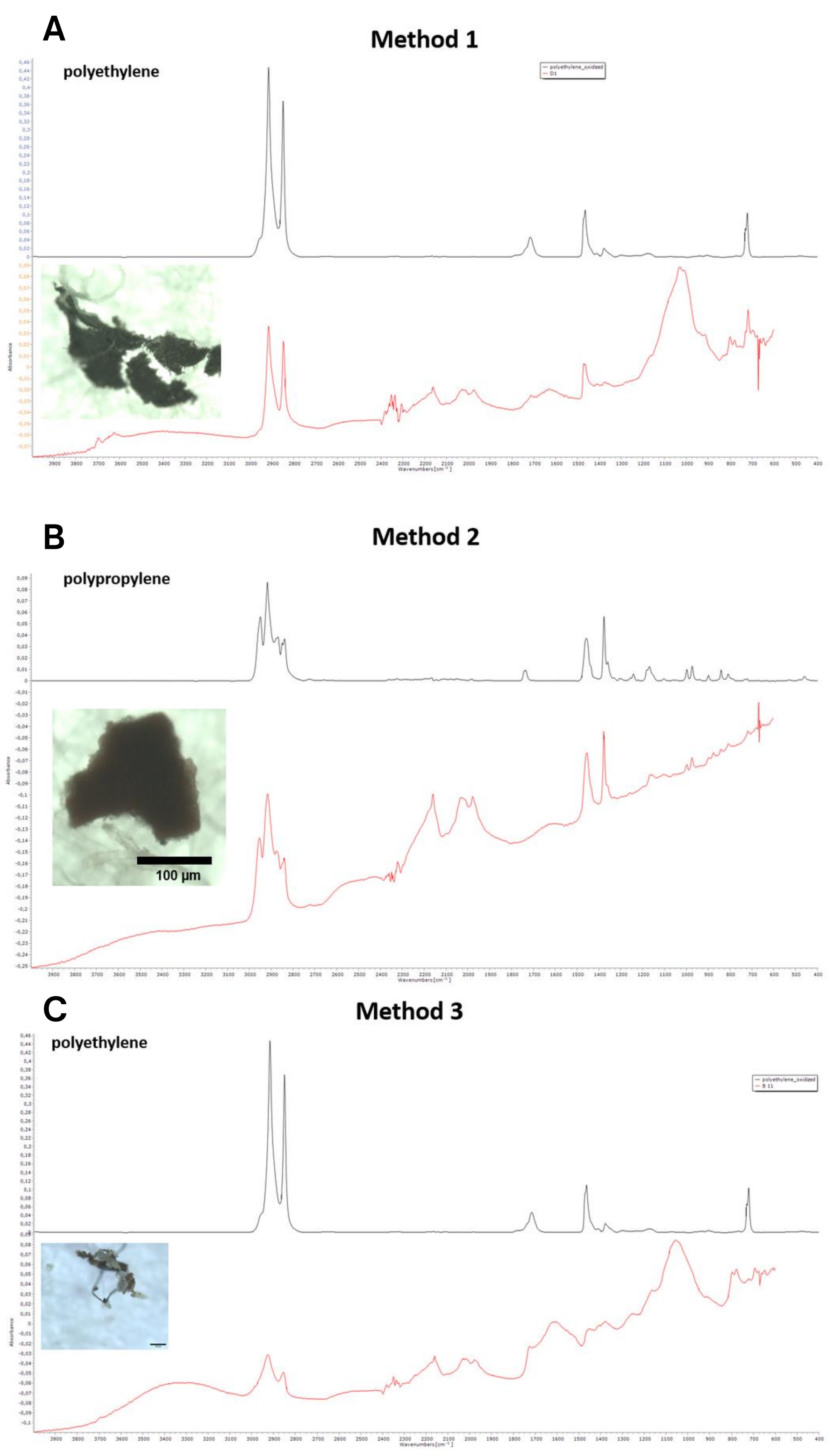

Fig. 3A,B,C Polymer identification with ATR-FTIR of microplastics collected in soil samples pre-treated with: A) hydrogen peroxide (H_2O_2 30%, w/v) for organic matter digestion at 25 °C (Method 1), B) 50 °C (Method 2), C) solvent-based extraction of organic matter with dichloromethane and hexane (Method 3). In all methods, density separation with tungstate dehydrate ($Na_2WO_4·2H_2O$; density: 1.4 g cm^{-3}) was used.
The bleu line represents the spectrum of a pure particle; the red line represents the spectrum of particle in soil sample.

4.3 COLORIMETRIC METHOD: NILE RED

- The Nile Red staining method failed to specifically detect MP due to organic matter interference.
- It is recommended to remove the organic matter prior to staining to improve method efficiency.

5. RECOMMENDATIONS

- The study highlights the importance of selecting an appropriate organic matter removal method to ensure an appropriate MP recovery.
- Method 2 (12 mL 30%H_2O_2/g soil, 48 h incubation, 50 °C) was the most effective method, with an apparent complete organic matter oxidation, also preserving the MP morphology.

6. REFERENCES

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