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Developing a systematic method for extraction of microplastics in soils†

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Microplastics are an environmental issue of global concern. Although they have been found in a range of environments worldwide, their contamination in the terrestrial environment is poorly understood. The lack of standardised methods for their detection and quantification is a major obstacle for determining the risk they pose to soil environments. Here we present a systematic comparison of microplastic extraction methods from soils, taking into account the characteristics of the soil medium to determine the best methods for quantification. The efficiency of organic matter removal using hydrogen peroxide, potassium hydroxide and Fenton's reagent was measured. Soils with a range of particle size distribution and organic matter content were spiked with a variety of microplastic types. Density separation methods using sodium chloride, zinc chloride and canola oil were tested. Recovery efficiencies were calculated and the impact of the reagents on the microplastics was quantified using Attenuated Total Reflectance (ATR) Fourier Transform-Infrared (FTIR) spectroscopy. The optimal organic removal method was found to be hydrogen peroxide. The recovery efficiency of microplastics was variable across polymer types. Overall, canola oil was shown to be the optimal method for density separation, however, efficiency was dependent on the amount of organic matter in the soil. This outcome highlights the importance of including matrix-specific calibration in future studies considering a wide range of microplastic types, to avoid underestimation of microplastic contamination. We show here that methods for extracting microplastics from soils can be simple, cost-effective and widely applicable, which will enable the advancement of microplastic research in terrestrial environments.

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Introduction

Microplastics have been found globally in a wide variety of environments.¹ However only 3.8% of studies had, until recently, investigated microplastics in terrestrial soils.² This is despite the close proximity of terrestrial environments to many potential sources, as a large proportion of plastic waste is generated and disposed of on land.³ So far, high concentrations of microplastics have been found in soils.^{4–6} Scheurer and Bigalke⁷ found evidence for microplastics in 90% of tested soils, indicating there is a high likelihood of widespread contamination. It is necessary to measure and quantify the amount of microplastics in the terrestrial environment over a wide range of spatial and temporal scales to enable the risk of adverse effects to be determined. However, studies of this sort are currently

limited by the lack of suitable methods for quantifying microplastics in soils.

There is in general, an absence of standard operating procedures for quantifying microplastics in the environment and this is especially the case for soils, which can be extremely complex matrices.⁸ Soils are heterogeneous solid mixtures comprised of minerals with a range of particle size distributions and organic matter at varying stages of decomposition.⁹ The complexity of organo-mineral interactions and the variability of soil media makes the collection of soil microplastic data challenging, although it is recognised as an important emerging issue.¹⁰

Initial attempts have been made to quantify microplastics in soil. Density separation methods are some of the most commonly utilised techniques to isolate microplastics from environmental matrices. These methods isolate microplastics using high density saturated salt solutions, such as sodium chloride (NaCl, 1.2 g cm⁻³), sodium bromide (1.4 g cm⁻³) and zinc chloride (ZnCl₂, 1.7 g cm⁻³).^{7,11,12} Lower density solutions tend to be cheaper and less hazardous, but higher density solutions are required to extract more dense polymers such as polyvinyl chloride (PVC, 1.16–1.58 g cm⁻³) and polyethylene terephthalate (PET, 1.37–1.45 g cm⁻³).^{13–15} Alternatively, oil

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extraction methods have been developed, which use a combination of low density oil and the oleophilic property of plastic to accumulate microplastics in a layer of oil above an aqueous solution.^{16,17} Additional techniques such as ultrasonication^{18,19} and centrifugation¹⁵ may also be used to enhance these extractions.

While density separation techniques tend to target the inorganic fraction of a sample, organic matter, which has similar density to many types of plastic,⁹ can obscure the detection of microplastics and interfere with identification.²⁰ To overcome this problem, digestion methods to remove the organic matter have been used. Established soil organic matter digestion techniques involve strong acids;²¹ however they are not recommended for microplastic studies as they are damaging to some polymers.²² Instead, potassium hydroxide (KOH), hydrogen peroxide (H₂O₂), and enzymatic treatments may be used with minimal impact on microplastics.²³ Enzymatic treatments have been used successfully for less complex environmental samples (*e.g.* water and marine sediments).²⁴ However, the complex mixture of organic matter in soil is likely to require multiple types of enzymes to fully remove organic material which may become complex and costly.^{25,26} Chemical digestion methods have a broader specificity and are more likely to target a larger proportion of the soil organic matter.

Hydrogen peroxide is particularly effective at removing organic material in soil¹³ and can be used in combination with an iron catalyst (Fenton's reagent) to accelerate the reaction. Fenton's reagent has been shown to be particularly effective in sludge and soil samples.^{6,25,27} Increasing temperature may also enhance organic removal,²⁸ although this should be limited to 50 °C to remain within the heat deflection limits of most common microplastics.^{23,29}

An additional consideration of these methods is the sample matrix characteristics. In soils, this includes chemical and physical properties such as organic matter content, particle size distribution, pH, and bulk density. It is very likely that properties such as these impact the efficiency of extraction methods, similar to the matrix effect seen in analytical chemistry techniques, where the sample matrix characteristics influence

quantification and identification of contaminants.³⁰ Some studies have started to incorporate matrix characteristics into microplastic extraction method design, for example, testing the difference between sandy and clay soils.¹⁹ It has been suggested that different methods for extracting microplastics from soil should be applied depending on the proportions of clay and organic matter.² However, most studies lack a matrix characterisation, which is particularly important in soils due to their heterogeneous and variable nature. Method suitability must consider the impacts of reagents on microplastics³¹ and microplastics recovery efficiency. This is particularly important as some studies report recovery efficiencies of 85–100% (*ref.* 14) while others are much lower (5 to 75%).³²

In this study, the aim was to systematically explore and validate methods for extracting microplastics from soils, taking sample matrix characteristics into consideration. Organic removal efficiency and density separation techniques were tested in soils with a range of organic content and particle size. These techniques were combined to establish the most effective extraction methods for a range of microplastics types. Methods were further validated for use by assessing the physical impact on, and subsequent ease of identification of, microplastics. The outcomes of these trials can inform future studies looking to quantify microplastics in soil, enabling the most suitable method to be chosen based on the sample characteristics.

Methods

Methods of extraction were tested in four stages by measuring (1) the organic matter removal efficiency of selected digestion methods, (2) the extraction efficiency of spiked microplastics with and inorganic soils using density separation techniques, (3) the efficacy of these methods when combined on organic soils and (4) validation by assessing the impact of extraction reagents on microplastic identification.

Microplastics spikes

A mixed microplastic standard for spiked recovery was created. Consumer materials were used to create fragments and fibres

Table 1 Microplastic types used in spiking experiments: polymer type, shape, size, colour and original product. Density information relates to literature recording of polymer types^{59,60a}

Resin code	Abbreviation	Shape	Size (mm)	Colour	Original product	Density (g cm ⁻³)
1	PET	Fragment	0.5–1 mm	Blue	Drinks bottle	1.37–1.45
		Fibre	1–5 mm	Green	Craft ribbon	
2	HDPE	Fragment	0.25–0.5 mm	Pink	Cleaning product bottle	0.93–0.97
3	PVC	Fragment	0.5–1 mm	Red	Table cloth	1.16–1.58
4	LDPE	Fragment	0.25–0.5 mm	Purple	Carrier bag	0.91–0.92
			0.5–1 mm			
5	PP	Fragment	0.5–1 mm	White	Storage bottle	0.9–0.91
		Fibre	1–5 mm	Purple	Carpet	
6	PS	Fragment	0.25–0.5 mm	White	Packaging	0.015–0.03
			0.5–1 mm			

^a PET, polyethylene terephthalate; HDPE, high-density polyethylene; PVC, polyvinylchloride; LDPE, low-density polyethylene; PP polypropylene; PS, polystyrene.



under 5 mm in size³³ of polymers representing the six main resin codes (Table 1).³⁴ Fragments were created using a household coffee grinder and separated into small (0.25–0.5 mm) and large size fractions (0.5–1 mm), and fibres were cut to size (1–5 mm). Polymer type was identified by material labelling and confirmed using Attenuated Total Reflectance Fourier-Transform Infrared spectroscopy (ATR FT-IR) (Frontier, Perkin Elmer) with Spectrum infrared spectroscopy software (Perkin Elmer). Microplastic spikes were chosen with distinct characteristics making identification and separation from contamination sources possible. Each sample tested for recovery efficiency was spiked with five particles of each type of microplastic particle ($n = 60$) and shaken thoroughly prior to treatment to ensure microplastic distribution.

Soil materials

Soil materials for testing were specifically created for experimental procedures. The organic fraction of soils was represented by a commercial compost (John Innes Manufacturers Association approved, no. 1 compost, sieved to 2 mm to remove large debris) and the inorganic fraction was a fine sand. The two materials were mixed in varying ratios to form representative soils with specific levels of organic matter content. For soils of purely inorganic content the focus was particle size composition. Particle size was classified according to the Wentworth Scale,³⁵ where clay particles are $<4\ \mu\text{m}$ and sand particles are 0.125–2 mm. A clay material (Bentonite, Sibelco) was mixed with a fine sand (85.6% sand) in varying ratios to form six distinct soil types (see ESI, Table S1†). Organic matter content was measured in all samples using loss-on-ignition (LOI) at 550 °C and particle size distribution was analysed using the hydrometer method.³⁶ Unless otherwise stated, soils categorised for organic matter removal experiments as ‘high organic’ had an organic matter content of 73% (± 0.6 SE) and ‘low organic’ had 12% (± 0.9 SE). For each sample (both organic and inorganic), 10 g of soil was used.

Organic matter removal

The initial phase aimed to assess the amount of organic matter that could be removed from soil. Digestion treatments were tested on samples of low and high organic matter content to represent the extremes likely to be found in the environment,³⁷ carried out in glass jars (330 mL capacity) and repeated three times per treatment for each sample type.

Fenton's reagent (H_2O_2 , 30% w/v + Fe^{2+} catalyst, Fisher Scientific), H_2O_2 (30% w/v, Fisher Scientific) and KOH (10% w/v, Fisher Scientific) were selected for testing based on their reported organic removal efficiency and low impact on microplastics.^{23,25,38} For H_2O_2 and KOH treatments, a 50 mL aliquot was added to each sample. Fenton's reagent digestion was carried out using 25 mL of H_2O_2 with 25 mL of iron catalyst ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 1 g L^{-1} , Fisher Scientific) adjusted to pH 3 with concentrated sulphuric acid (H_2SO_4 , 95% v/v, Fisher Scientific), an ice bath was used to control the maximum temperature of the reaction to 50 °C until there was no longer a visible reaction. All samples were then placed in a shaking incubator at 100 rpm

at 50 °C for 24 hours or until all liquid had evaporated. The samples were then dried at 105 °C overnight, organic content was measured again in triplicate and the quantity of removed organic matter calculated. Each digestion was repeated on separate samples at 40 °C to assess the effect of temperature on digestion efficiency. Digestion treatments were additionally tested in combination with a dispersant, with the aim of dispersing the soil particles prior to digestion maximise organic removal efficiency. In separate samples, 50 mL of sodium hexametaphosphate ($\text{Na}_6\text{P}_6\text{O}_{18}$, 1% w/v, Fisher Scientific) was used to soak samples for 24 hours prior to digestion, and each digestion process was then completed on the samples as stated above at 50 °C.

Density separation

Microplastic recovery experiments were conducted using density separation methods to assess efficiency of microplastic separation from inorganic samples only. Each method was tested on six soils with distinct particle size composition in triplicate. Three different density separation media were tested: ZnCl_2 (1.7 g cm^{-3} , APC pure), NaCl solution (1.2 g cm^{-3} , food grade), and canola oil (food grade).

For ZnCl_2 and NaCl trials, 300 mL was added to the sample. The lid was tightly sealed and shaken vigorously by hand for 30 seconds to ensure full contact between the density separation medium and the sample, before leaving to settle overnight to allow dense particles to settle out. The top layer of the sample was then removed using an overflow method,³⁹ where excess ZnCl_2 or NaCl was gently added to the jar to spill the top layer of the sample into a surrounding glass crystallising dish and used to rinse the sides and inside of the lid of the jar.

Canola oil separations were conducted based on the method developed by Crichton *et al.*¹⁶ 100 mL of distilled water and 5 mL of canola oil was added to each sample and again, shaken for 30 seconds. An additional 200 mL of distilled water was added into the jar to create a further separation between the top layer of canola oil and the bottom of the jar. This solution was then placed in an orbital shaker for 2 hours at room temperature, 100 rpm. The solution was then left to settle overnight, after which the canola oil layer was extracted with the same overflow method using distilled water. The overflowed layer of each treatment, containing microplastics, was then vacuum filtered onto a glass fibre filter (Whatman GF/A, 1.6 μm). The overflow process was completed twice per sample, from initial shaking to vacuum filtering, to achieve maximum extraction efficiency within a reasonable timeframe (see ESI, Fig. S2† for details). All filters were inspected under a low power microscope (Nikon SMZ1000, $\times 40$) and recovered microplastics were counted, distinguishable from contamination by their chosen colours (Table 1).

Each protocol with the density separation media was separately run and tested with ultrasound to break up the soils. After each time a sample was shaken it was subjected to 5 minutes of ultrasound in an ultrasonic bath (37 Hz, Fisher Scientific: FB15055). Samples were then overflowed, filtered and analysed as above.



Method combinations

To combine extraction methods, organic removal was included as an additional step prior to density separation in samples with organic matter. Informed by the results of the organic removal efficiencies (§results, organic matter removal), H₂O₂ at 50 °C was chosen as the optimum digestion method and was used in combination with each of the density separation methods. Each combined method was tested on samples with a range of organic matter content, ranging from 0.2–72% ($n = 18$ per method).

For the digestion, 50 mL of H₂O₂ was added to each sample and additional 50 mL once not visible reaction occurred to ensure maximum digestion. The samples were then heated and shaken (50 °C, 100 rpm) until all remaining liquid had evaporated. Using the same methods as stated previously (§methods, density separation), ZnCl₂, NaCl and canola oil extractions were carried out on samples. Recovery efficiencies were calculated for each sample.

Method reagent impact on the physical and spectroscopic properties of microplastics

To complete the validation, the impact of each method on the plastics was tested. Each type of microplastic particle used as a spike was exposed separately to each treatment involved in the methods in the absence of soil. Spikes subjected to H₂O₂, KOH and Fenton's reagent were tested at 40 and 50 °C to evaluate the effects of the digestion methods. Each of the microplastic types were added to glass vials containing 1 mL of each of the reagents, then heated to 40 °C or 50 °C for 24 hours and removed for analysis. Similarly, the digestion methods were evaluated by exposing each of the microplastic types to 1 mL of ZnCl₂, canola oil, and NaCl in glass vials. The effects of ultrasound were separately measured by adding 1 mL of distilled water to vials containing the microplastics and exposing them to the ultrasound treatment.

Virgin and exposed microplastics were analysed using ATR FT-IR with a wavenumber range of 4000–600 cm^{−1} with spectral resolution of 4 cm^{−1}. A library of virgin microplastics, which were not exposed to any reagents, was created including each of the 12 microplastic types used in the spiking experiments. The spectrum of microplastics exposed to each treatment ($n = 3$) was compared with the virgin microplastic library and assigned a hit quality index number (HQI, on a scale of 0–1)⁴⁰ to determine the effect of chosen reagents.

Statistical analysis

Organic matter removal rates were measured by calculating the amount of organic matter remaining in a sample after digestion (OM_a) as a percentage of the initial organic matter content (OM_i).

$$\text{Organic matter removed (\%)} = \frac{\text{OM}_a(\text{g})}{\text{OM}_i(\text{g})} \times 100$$

All statistical analyses were performed in RStudio (1.2.1335) software. Normal distribution of data was checked using Shapiro–Wilk tests and homogeneity of variance was checked using

Levene's test. Parametric tests were applied where assumptions of normality and equal variance had been met.

Statistical analysis in the form of Kruskal–Wallis tests (non-parametric), one and two-way analysis of variance (ANOVA, parametric) were used to compare differences between groupings, and pairwise comparisons were made using the post hoc analysis of Dunn's test for non-parametric and Tukey's tests for parametric data. This analysis applied to the amount of organic matter removed from samples with different digestion methods, recovery efficiencies in inorganic and organic samples using the different extraction methods, and differences in identification hit scores for microplastics treated with the different extraction reagents.

Differences between means were tested using a *T*-test and Wilcoxon rank sum test for the amount of organic matter removed at 40 °C and 50 °C and with initially high and low organic content, recovery efficiencies in inorganic samples with and without the use of ultrasound and H₂O₂. Correlations were tested using Spearman's Rank to assess relationships between particle size distribution and recovery of plastics in inorganic samples and the relationship between percentage organic matter content and microplastic recovery across all treatments.

Results

Organic matter removal

The three organic removal reagents worked at significantly different efficiencies across samples with low and high organic content ($p < 0.05$, Kruskal Wallis test). For samples with low organic content, removal of organic matter was similar for Fenton's and H₂O₂ and both these treatments were more effective than KOH ($p < 0.05$ for both, Dunn's test.). Samples with high organic content did not show a significant difference between the amount of organic matter removed by Fenton's and KOH (Fig. 1), but H₂O₂ removed more organic matter than both other treatments ($p < 0.05$ for both, Dunn's test). H₂O₂ at 50 °C removed 93% organic matter. This was significantly more than Fenton's at 40 and 50 °C, which removed 51% and 56% organic matter, respectively ($p < 0.05$ for both, Dunn's test). H₂O₂ at both 40 and 50 °C removed more organic matter than KOH at 40 °C which removed only 20% in samples with high initial organic content ($p < 0.01$, Dunn's test).

Temperature did not affect the efficiency of organic matter removal in samples with initial low or high organic content ($p > 0.05$ for both, Wilcoxon rank sum). The amount of organic matter removed by H₂O₂ was significantly reduced by the addition of dispersant ($p < 0.001$, one-way ANOVA) from 93 to −1.9%. There was no difference between the amounts of organic matter removed by KOH or Fenton's reagent with or without dispersant.

Density separation

There was no significant correlation between soil particle size composition (*i.e.* amounts of clay in a sample) and total microplastics recovery efficiency across all methods ($r_s = 0.04$, $p > 0.05$, Spearman's Rank, Fig. 3). There was no significant difference in microplastic recovery efficiency when combining



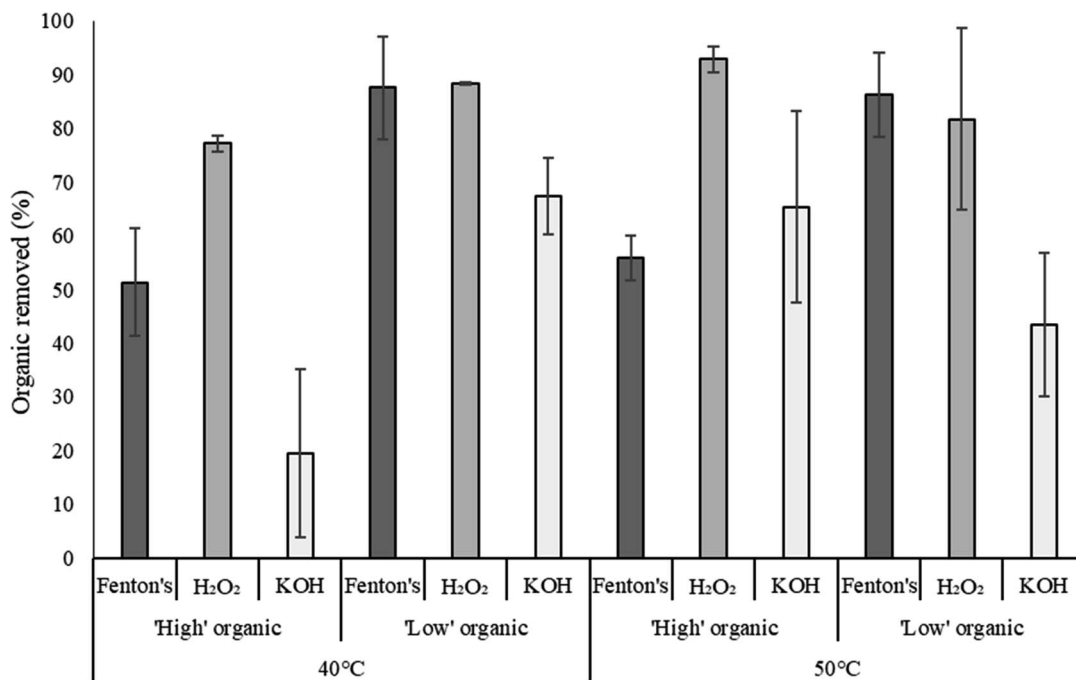


Fig. 1 Organic removed by tested chemicals (Fenton's reagent, potassium hydroxide (KOH)) and hydrogen peroxide (H₂O₂) at 40 and 50 °C in samples of initial high (73 ± 0.6% SE) and low (12 ± 0.9% SE). Organic matter content. Percent organic removed is reported as a mean ($n = 3$) with 95% CI error bars.

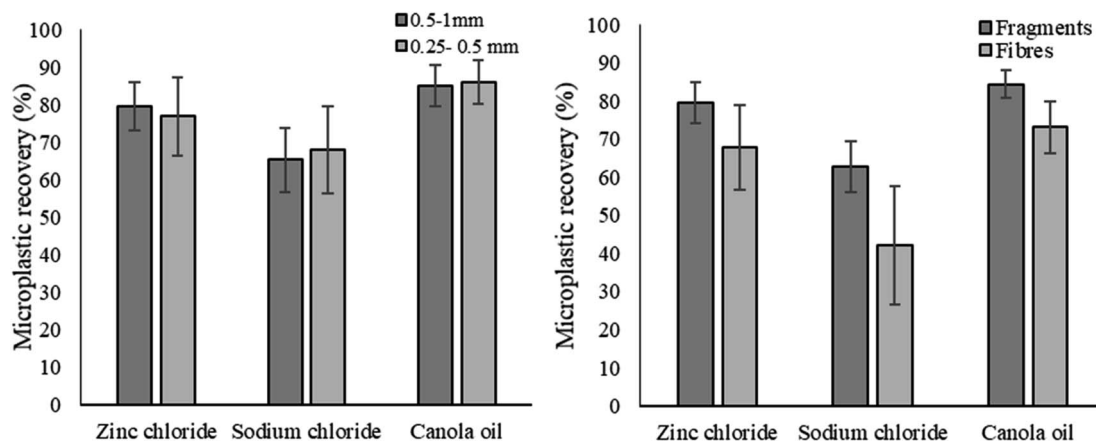


Fig. 2 Microplastic recovery for large (0.5–1 mm) and small (0.25–0.5 mm) microplastics (left) and fragments and fibres (right) across the three different density separation methods (zinc chloride, sodium chloride and canola oil). Percent recoveries are reported as a mean (fragments: $n = 3$, fibres: $n = 12$, large: $n = 24$, small: $n = 24$) with 95% CI error bars.

ultrasound with any of the three methods; ZnCl₂, NaCl or canola oil ($p > 0.05$ for all, t -test). Ultrasound samples were therefore not considered further. Total microplastic recovery efficiencies from inorganic samples were different between the extraction methods ($p < 0.01$, one-way ANOVA). Mean recovery efficiency was 59% (± 1.8 SE) for NaCl, 80% (± 1.7 SE) for ZnCl₂ and 84% (± 2.0) for canola oil extractions (Table 2). Canola oil and ZnCl₂ recovered significantly more microplastics than NaCl ($p < 0.01$ for both, Tukey's test) but there was no difference in total microplastic recovery between canola oil and ZnCl₂ extractions.

The methods recovered fragments and fibres with different efficiencies ($p < 0.05$, one-way ANOVA; $p < 0.05$, Kruskal Wallis) (Fig. 2). ZnCl₂ and canola oil recovered more fragments and fibres than NaCl ($p < 0.01$ for all), but there were no significant differences in the recovery of fragments or fibres between ZnCl₂ and canola oil methods. There were differences in recovery of large (0.5–1 mm) and small (0.25–0.5 mm) microplastics with the tested methods ($p < 0.05$, Kruskal Wallis test; $p < 0.05$ one way ANOVA). Small microplastics were better recovered with canola oil than NaCl ($p < 0.01$, Tukey's test) but showed no difference between canola oil and ZnCl₂ or ZnCl₂ and NaCl.

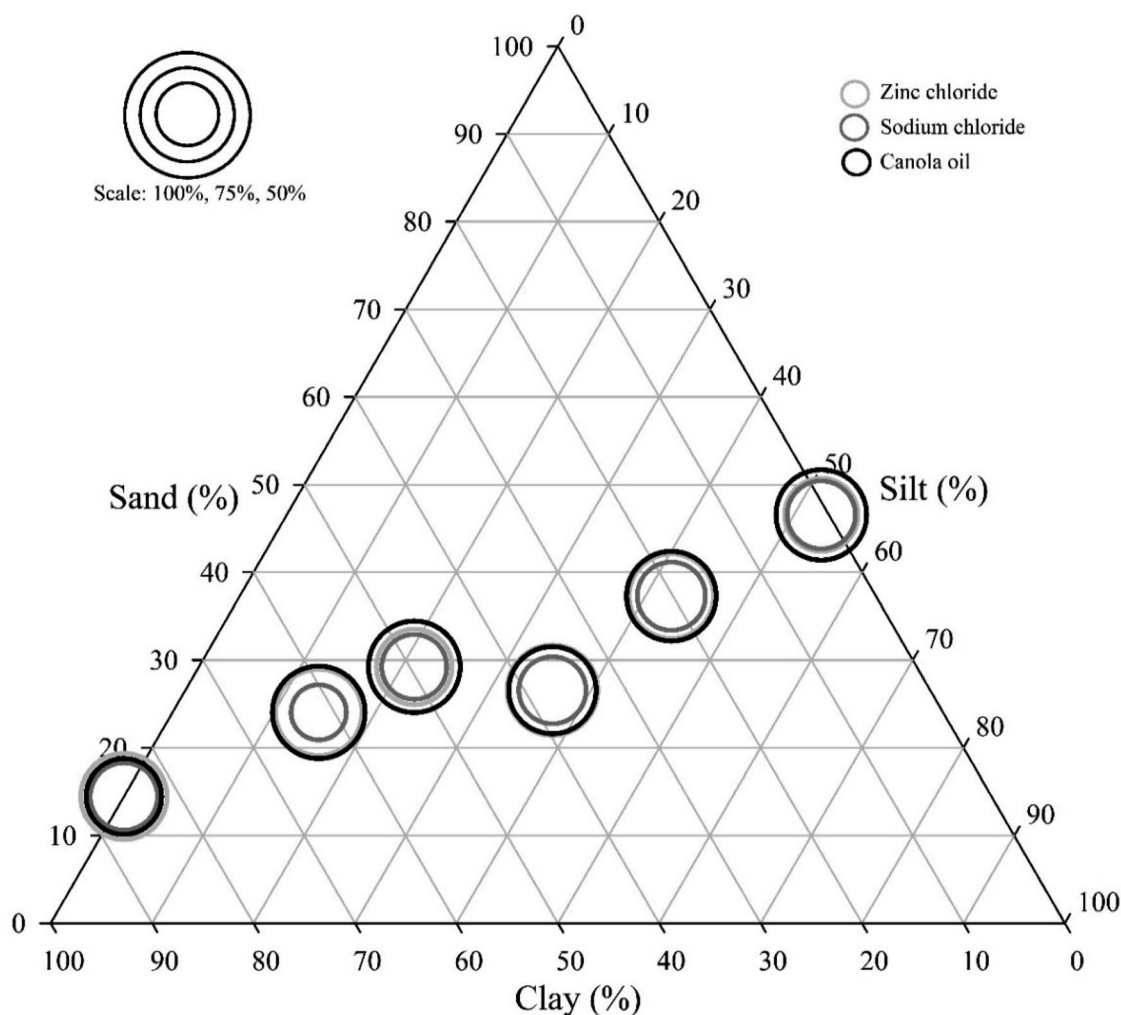


Fig. 3 Total microplastic recovery efficiencies from inorganic sediments with measuring particle size distributions using canola oil, sodium chloride and zinc chloride. Circle size represents microplastic recovery efficiency.

Table 2 Mean recovery efficiency (\pm SE) for individual microplastic types with the tested extraction methods. Inorganic only soils were treated with density separation alone and organic soils were treated with a hydrogen peroxide (H_2O_2) digestion prior to density separation^a

Microplastic type	Mean recovery efficiency (%) inorganic soils			Mean recovery efficiency (%) organic soils		
	Sodium chloride	Zinc chloride	Canola oil	Sodium chloride	Zinc chloride	Canola oil
PET fibre	10 (± 4.0)	62 (± 12)	68 (± 7.5)	27 (± 5.1)	59 (± 11)	51 (± 9.5)
PET fragment	27 (± 11)	93 (± 6.4)	77 (± 12)	10 (± 4.0)	57 (± 7.5)	21 (± 7.5)
Large HDPE	81 (± 8.9)	89 (± 7.6)	86 (± 11)	42 (± 6.8)	46 (± 7.6)	59 (± 6.8)
Small HDPE	78 (± 9.0)	90 (± 10)	96 (± 3.1)	34 (± 6.4)	32 (± 5.4)	28 (± 6.1)
Large PVC	38 (± 12)	83 (± 7.6)	96 (± 2.0)	11 (± 4.6)	74 (± 6.6)	20 (± 6.7)
Small PVC	34 (± 13)	88 (± 11)	99 (± 1.6)	12 (± 4.6)	72 (± 8.9)	54 (± 8.1)
Large LDPE	77 (± 5.3)	80 (± 14)	77 (± 6.3)	34 (± 7.4)	50 (± 7.6)	40 (± 10)
Small LDPE	97 (± 15)	108 (± 16)	74 (± 6.2)	57 (± 7.6)	54 (± 8.5)	39 (± 5.9)
PP fibre	56 (± 9.0)	51 (± 11)	78 (± 5.8)	47 (± 6.0)	57 (± 9.5)	66 (± 8.4)
PP fragment	71 (± 9.6)	76 (± 14)	87 (± 5.5)	44 (± 8.9)	51 (± 6.9)	60 (± 6.9)
Large PS	72 (± 10)	76 (± 8.0)	84 (± 8.0)	49 (± 5.9)	50 (± 8.1)	64 (± 8.3)
Small PS	73 (± 12)	62 (± 12)	84 (± 11)	22 (± 3.2)	33 (± 5.6)	56 (± 8.2)
Mean recovery	59 (± 1.8)	80 (± 1.7)	84 (± 2.0)	33 (± 2.9)	53 (± 4.4)	47 (± 3.7)

^a PET, polyethylene terephthalate; HDPE, high-density polyethylene; PVC, polyvinylchloride; LDPE, low-density polyethylene; PP polypropylene; PS, polystyrene.



Large microplastics had higher recovery efficiencies with canola oil and ZnCl_2 than NaCl ($p < 0.01$ for both, Dunn's test) but there was no difference between canola oil and ZnCl_2 methods.

Different types of microplastics had different recovery efficiencies with each of the methods of extraction (Table 2). PET fibres had the lowest recovery efficiency in both NaCl and oil extractions with a mean of 10% (± 4.0 SE) and 68% (± 7.5 SE), respectively. PP fibres had the lowest recovery efficiency using ZnCl_2 with a mean of 51% (± 11 SE). The highest recovery efficiency in both ZnCl_2 and NaCl extractions was with small LDPE which had mean recoveries of 108% (± 16 SE) and 97% (± 14 SE). Recovery efficiencies were highest for small PVC in oil extractions ($99\% \pm 1.6$ SE).

Recovery efficiencies of the densest polymers (PET fragments and fibres, and large and small PVC) were higher with ZnCl_2 and canola oil than NaCl ($p < 0.05$, Dunn's test). Large and small HDPE and PS, large LDPE or PP fragments showed similar recovery efficiencies for all three extraction methods.

Method combinations

There was a difference in the total recovery efficiency of microplastics in organic samples when using the three density separation methods ($p < 0.01$, one-way ANOVA; Fig. 4); 95% confidence intervals for each treatment ranged from 8.6 to 9.0%. In combination with H_2O_2 as a digestion method, ZnCl_2 and canola oil showed similar microplastic recovery efficiencies from organic samples and both recovered more microplastics than NaCl ($p < 0.05$ for both, Tukey's test). There was a significant correlation between percent organic content and total microplastic recovery efficiency using the canola oil method ($r = -0.50$, $p < 0.05$, Spearman's Rank); the higher the organic

content, the lower the microplastics recovery. However, this was not the case for NaCl or ZnCl_2 , which both showed no correlation between percent organic content and total microplastic recovery.

Microplastic fragment recovery efficiencies were different across treatments ($p < 0.05$, one-way ANOVA). Higher recovery efficiencies were seen for fragments using ZnCl_2 compared with NaCl ($p < 0.01$, Tukey's test). Similarly for large microplastics, the recovery efficiencies were significantly different across treatments ($p < 0.01$, one-way ANOVA). ZnCl_2 recovered more large microplastics than NaCl ($p < 0.05$, Tukey's test) but there was no difference between these treatments and canola oil. Recovery efficiencies for both fibres and small microplastics did not vary between methods across all these samples ($p > 0.05$ for both, Kruskal Wallis). There was no correlation between fibre recovery efficiency and organic content using any of the treatments or for small microplastics using ZnCl_2 or NaCl but there was a significant but weak negative correlation for small microplastics using canola oil ($r = -0.48$, $p < 0.05$, Spearman's Rank).

Recovery efficiencies varied for each microplastic type with the different methods of extraction (Table 2). PET fragments had the lowest recovery efficiency in NaCl with a mean of 10% (± 4.0). Small HDPE had the lowest recovery efficiencies in ZnCl_2 (32%, ± 5.4 SE) and large PVC has the lowest recoveries in canola oil (20% ± 6.7 SE). Small LDPE showed the highest recovery efficiencies with NaCl (57%, ± 7.6 SE), large PVC showed the highest recovered microplastic in ZnCl_2 (74%, ± 6.6) and PP fibres were the highest in canola oil (66%, ± 8.4). There was a strong negative correlation between recovery efficiencies of PET and small LDPE fragments and organic content using the canola oil method; the higher the organic content, the fewer

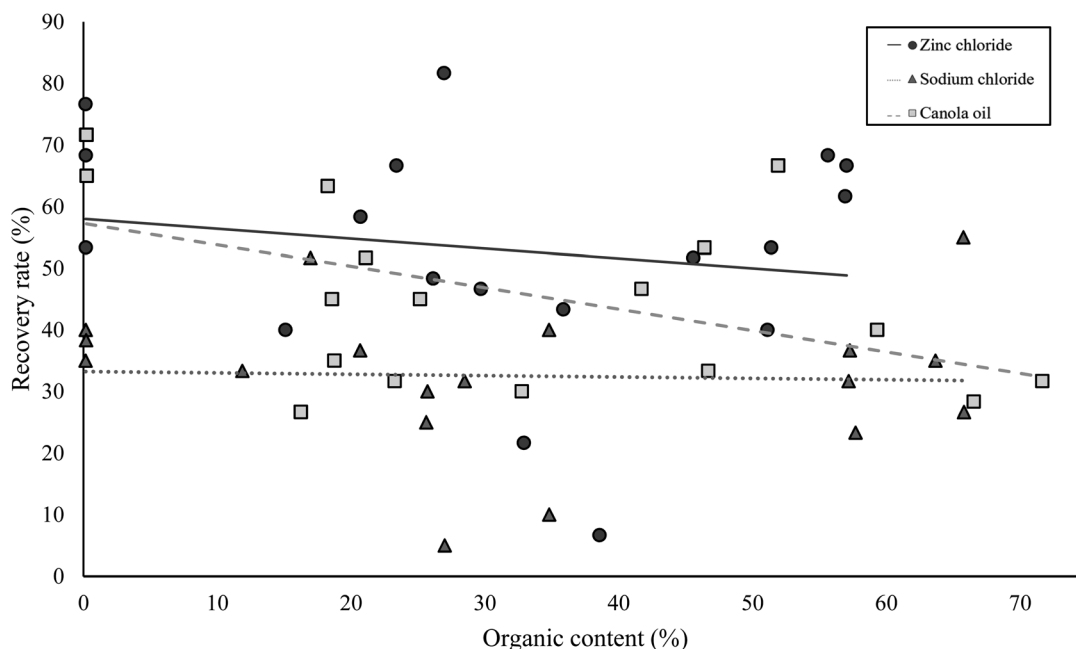


Fig. 4 Recovery efficiency of total microplastics compared to organic content of samples using hydrogen peroxide to digest and three different density separation techniques: zinc chloride, sodium chloride and canola oil.



fragments were recovered ($r_s = -0.69$, $p < 0.01$; $r_s = -0.55$, $p < 0.05$, Spearman's Rank). There were no correlations between recovery efficiencies of any of the other types of microplastics with organic content or density separation technique. In combination with H_2O_2 digestion, multiple density separations significantly increased the number of microplastics recovered using canola oil ($p < 0.05$, two-way ANOVA), but not with ZnCl_2 or NaCl .

Method reagent impact on the spectroscopic properties of microplastics

Organic removal treatments had minimal effects on the ease of identification with FT-IR. All microplastics subjected to organic removal treatments had good mean HQI (>0.7 , as defined by Renner *et al.*, 2019). The lowest scores were for large PVC treated with KOH at 40 °C and 50 °C, which had HQIs of 0.71, 0.77, respectively. However, there was no overall difference in HQIs for polymers with different chemical treatments or temperatures. It was not possible to measure the HQIs for PET fibres treated with H_2O_2 at 50 °C or KOH at 40 °C.

There were some differences in microplastic identification with the different density separation treatments ($p < 0.01$, Kruskal Wallis). NaCl and ultrasound treatments both had significantly better identification HQI scores than canola oil ($p < 0.01$, for both, Dunn's test) and ZnCl_2 ($p < 0.01$, for both, Dunn's test) for overall identification. There were significant differences between the different types of microplastic and the identification across density separation treatments ($p < 0.01$, Kruskal Wallis). Despite differences between treatments, all polymers subjected to density separation treatments good mean HQI (>0.7 , Renner *et al.*, 2019) with the exception of large PVC treated with canola oil, which had a HQI score of 0.49.

Discussion

Standardised methods for quantifying microplastics in the environment, and in particular effective methods for soils, are urgently required. Here we have tested and validated several methods and found differences in the efficiency based on the reagents used and soil characteristics. The first step of testing organic matter digestions indicated that KOH is an unsuitable method for removing organic matter from soils, despite its reported success when used on biological samples.⁴¹ Pre-treatment with the dispersant sodium hexametaphosphate was deemed unsuitable as it decreased the efficiency of H_2O_2 and had no impact on the efficiency of KOH or Fenton's reagent; therefore, it is an unnecessary additional step.

H_2O_2 and Fenton's reagent both resulted in considerable digestion of organic matter ($>70\%$) indicating their suitability for removing organic matter from soils (Fig. 1). This is in line with previous studies²⁰ which also showed that there was minimal difference between organic removal with both treatments in intertidal sediments, although this was also dependant on original organic content. We recommend here that H_2O_2 is the preferred method as, although both reagents removed similar amounts of organic matter overall, H_2O_2

removed more from soils with initially high organic content than did Fenton's reagent. Additionally, it is a simpler method to perform, requiring fewer reagents and reduced costs. The reaction can be more easily controlled, as the exothermic reaction of Fenton's reagent requires additional monitoring and control which, if not properly regulated, may result in temperatures >90 °C (ref. 31) leading to the likelihood of polymer damage.²⁹ As there was no difference in the efficiency of reagents at the two temperatures tested (40 °C and 50 °C), we suggest that processing samples at 50 °C may be optimal to speed up processing times, allowing for larger number of samples to be processed without altering the chemical structure of polymers by remaining within the heat deflection limits of most common polymers.²⁹ Additionally, prolonged exposure to 30% H_2O_2 may cause degradation to some polymer types and therefore should be shortened where possible.⁴²

In general, density separation has been developed for aquatic sediments and different methods have been tested with good recovery rates.^{43–45} Here we found that density separation methods had differences in extraction efficiencies in both organic and inorganic soils (Table 2). The composition of inorganic soils had no impact on recovery rate for any of the methods tested, similar to previous studies that have shown no difference in recovery efficiency between fine, medium and coarse sediments.¹⁶ This suggests that particle size does not need to be adjusted for when applying a density separation method when within these ranges, however it may affect to the time required to effectively process a sample, as it relies on particles settling out in a solution, which according to Stoke's law denotes that the smaller the particle size, the longer it will take to settle.³² This should be considered when calculating density separation processing time as soils with higher clay content may take longer to separate fully.⁴⁶ We suggest that ultrasound is not required in the density separation step of extraction. Despite its use in previous studies,^{13,18,19} it did not increase microplastic recovery efficiency in the present study and can therefore be excluded to simplify methods.

Of the tested density separation methods, NaCl had the lowest extraction rates from both organic and inorganic soils. The recoveries of the higher density polymers PET and PVC, which together make up over 17% of the global plastic demand,⁴⁷ were particularly low. Despite this clear bias towards low-density polymers, it is a method that has been used extensively since it was first tested in 2004, predominantly due to its low cost and limited potential for harm.^{11,44,48} In soils with purely inorganic content, both oil and ZnCl_2 had much higher recovery rates than NaCl and which extended across the different polymer types. Extraction with canola oil recovered the most small microplastics so is preferential for extractions from soils with high inorganic content, as environmental samples tend to be dominated by smaller microplastics.^{32,49,50} Additionally, ZnCl_2 can be more expensive, more hazardous to work with, and more toxic to aquatic biota, whereas oil offers a cheap and relatively safe method.¹⁶ It is important to note that in some cases recovery rates exceeded 100%, this may be a result of fragmentation of the microplastic particles during the extraction process, or possibly due to contamination of the initial soil



mixtures. Blank media are not usually analysed in spiking studies of this kind, and while the occurrence in the soils of identical microparticles to those we introduced is unlikely, this highlights the importance of including contamination measures and controls when processing environmental samples to minimise the risk of microplastic contamination.⁵¹

In soils containing organic matter, extraction efficiencies were generally much lower (Table 2). The organic fraction of soils increases the difficulty of microplastic extraction, even with the addition of a digestion step.⁹ In this case, canola oil and ZnCl₂ showed similar overall recoveries, however the canola oil method was more obviously impacted by the presence of organic matter as extraction efficiency decreased as organic content increased, particularly for PET fragments and small LDPE. This highlights the importance of including a digestion step to reduce this effect, particularly in environmental samples where microplastics are likely to be coated or aggregated with biological material, which may further reduce efficiencies.¹⁷ Additionally, it is important to note that, even with high efficiency, there was high variability in microplastic recovery within treatments for organic soils (Table 2), this is likely due to the collection of non-plastic material which may obscure the identification and analysis of microplastic particles,²⁴ again, suggesting the high importance of the digestion step.

We recommend here that canola oil can be used for soils with low organic content, but ZnCl₂ is required to obtain sufficient extraction efficiencies in soils with higher organic matter content (>30% (ref. 52)). We anticipate that the canola oil method will be suitable for the large majority of soil types as organic matter rarely exceeds 30%.⁵³ Only soils with high organic content, for example peats,⁵⁴ will exceed this and require the use of ZnCl₂. When ZnCl₂ is used it must be carefully considered in terms of hazards to operators and environmental concern, and precautions must be taken to reduce its impact.^{12,55} Alternatively, salt solutions with densities higher than NaCl but less hazardous than ZnCl₂, such as calcium chloride (1.46 g cm⁻³) may be explored as an intermediate between the two.¹⁶ Additionally, the oil extraction method may be further optimised by using alternative types of oil, *e.g.* castor oil, which may be more efficient at extracting microplastics due to their higher viscosity,¹⁷ although this may further reduce suitability for soils with high organic matter content. Furthermore, we recommend that, as simulated soils were used here, these methods may require further adjustment to account for the natural variability in environmental samples as recovery may vary depending on specific soil conditions including the type of organic and minerogenic matter present. We also suggest that the methods recommended here will require tailoring to the required sample size to ensure maximum efficiency, as particularly the canola oil method may require different ratios of reagents for larger sample volumes.⁵⁶

Little impact was seen on the identification of microplastics treated with the method reagents, with the majority of HQIs above 0.7. This was expected as reagents were chosen for their previously reported low impact on plastic particles.^{25,31,38} PVC proved to be most susceptible to the tested reagents as it returned the lowest hit scores. This may be due to the

characteristically broad C–Cl peak of PVC seen at 690 cm⁻¹ which is at the edge of the spectral range measured (4000–650 cm⁻¹).⁵⁷ This suggests that consideration of visually matching to reference spectra when identifying PVC with FT-IR is required, and that a more conservative assessment of hit scores may be required to avoid false identification. Additionally, a decrease in HQI scores was seen for some polymers treated with canola oil and ZnCl₂. This may be due to the high viscosity of both liquids and hydrophobicity of canola oil, which results in residues remaining on the particles that reduced ease of identification.⁴⁰ It is therefore important that a cleaning step (*e.g.* alcohol rinse) to remove these residues should be further considered.¹⁶

This study is the first to compare systematically different methods for extraction of microplastics from soils, and highlights the importance of considering sample characteristics when selecting a method for extracting microplastics. Sample-dependent efficiencies should be considered and applied when quantifying microplastics in environmental samples (Table 3), similar to the principle of matrix-matched calibrations used in other areas of analytical chemistry.⁵⁸

Microplastic recovery efficiency is dependent on the polymer type, shape and size, therefore we suggest that study-specific calibrations using a range of different polymers with different shapes and sizes similar to those used here are employed to account for this variation. It should also be considered that weathered microplastics and those smaller than the size ranges used in this study remain a challenge despite being highly likely to be found in the environment,³² therefore should be considered in future studies, particularly as their respective fragility and large surface area to volume ratios may increase susceptibility to chemical degradation. Additionally, the type of recovery microplastics should be tailored to the type of microplastics

Table 3 Overall recommendations of method suitability for extracting microplastics from soils of low (<30%) and high (>30%) organic content

	Reagent	Suitable for use on low organic soils? (<30% organic matter)	Suitable for use on high organic soils? (>30% organic matter)
Organic removal	Hydrogen peroxide	Yes	Yes
	Fenton's reagent	Yes ^c	Yes ^c
	KOH	n/a	No
Density separation	Oil	Yes	Yes ^{a,b}
	Zinc chloride	Yes	Yes ^a
	Sodium chloride	No	No

^a Rinsing with ethanol is required to minimise impacts on identification of microplastics. ^b To be used only on soils of lower organic content. ^c To be used with caution to avoid exceeding polymer heat deflection limits.



considered within a study as, if a study aims to consider smaller plastics, it should use spiking plastics within that size range. Environmental samples tend to show large compositional differences in types of polymer found,⁷ including different shapes and sizes^{5,15} making it especially important to establish methods that will account for this and avoid an underestimation of environmental microplastic concentrations.

Conclusions

We recommend that for the majority of common soils, which are likely to have low organic matter content, the preferred method for extracting microplastics from soils involves a digestion step using H₂O₂ at 50 °C to remove organic matter followed by a canola oil density separation. These methods proposed do not require specialized equipment, are relatively cheap and have reduced complexity to extract microplastics from soils, while minimising environmental impact and hazard to operators. This approach meets the need of the microplastics research community to allow for method harmonisation, however it is clear that method efficiency must be accounted for to prevent underestimation of microplastic concentrations and study-specific calibrations must be employed to enable high accuracy within studies. This will allow for the expansion of future research and a greater understanding of microplastic concentrations in soils.

Data availability

Data supporting this study are openly available from the University of Southampton repository at: <https://doi.org/10.5258/SOTON/D1455>.

Author contributions

Freya Radford: Conceptualization, Methodology, Formal analysis, Investigation, Writing – Original draft. Lina M. Zapata-Restrepo: Writing – Review & Editing, Methodology, Investigation. Alice A. Horton: Writing – Review & Editing, Supervision. Malcolm D. Hudson: Conceptualization, Writing – Review & Editing, Supervision, Peter J. Shaw: Conceptualization, Writing – Review & Editing, Supervision. Ian D. Williams: Conceptualization, Writing – Review & Editing, Supervision.

Conflicts of interest

There are no conflicts to declare.

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