Article

Towards a Consensus Method for the Isolation of Microplastics from Freshwater Sediments

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Abstract: Environmental pollution caused by plastic waste is of global concern. There is growing interest in the study of microplastics in freshwater systems. However, the lack of harmonized analytical methodology makes it difficult to compare results obtained by different laboratories. This work compared methods for the recovery of microplastics from freshwater sediments based on density separation by flotation followed by digestion of organic matter. Simulated sediment was spiked with virgin polypropylene, polystyrene, polyamide (PA), polyethylene terephthalate (PET), and polyvinyl fluoride (PVF) pellets, and post-consumer polytetrafluoroethylene (PTFE) fragments. Density separation was carried out using distilled water and NaCl, $CaCl_2$, $ZnCl_2$, and NaI solutions, both for intact pellets/fragments and following grinding and sieving to three size fractions (<1 mm, 1–2 mm, and >2 mm). Digestions with HNO_3, NaOH, and Fenton's reagent were compared. Only NaI quantitatively recovered all types of polymers. However, $CaCl_2$ and $ZnCl_2$ recovered all but PVF and PTFE. Different flotation patterns were observed for different size fractions of the same polymer, highlighting the fact that density is not the only factor affecting recovery. Digestion efficiencies were 6–78% in HNO_3, 4–45% in NaOH, and 49–80% in Fenton's reagent. Overall, $CaCl_2$ is recommended for density separation and Fenton's reagent for organic matter removal.

Keywords: microplastics; freshwater sediments; flotation; organic matter digestion; Fenton's reagent

1. Introduction

Plastics are widely used in the modern world due to their flexibility and durability. Their manufacture and use have risen from about 2 million tonnes (Mt) in 1950 to 400 Mt in 2022 [1], with experts projecting that this number could reach 1.1 billion tons by 2050 [2]. As the demand for plastic products has increased, so has the volume of plastic waste generated. Only 8% of post-consumer plastics are currently recycled [3]. Much of the remainder (approximately 50% of all manufactured plastics) finds its way into the environment [4], affecting aquatic systems in particular [5]. Plastics in the environment can interfere with natural processes and biogeochemical cycles [6,7]. Aquatic organisms risk entanglement and ingestion of plastic materials, which can lead to injuries or death [8–11]. The accumulation of plastic debris in water bodies modifies habitats, while submerged ecosystems may degrade when macro and mega plastic debris are deposited [12].

Once in the environment, plastic wastes break down into smaller pieces through four main mechanisms: biodegradation, hydrolytic degradation, photodegradation, and thermo-oxidative degradation [13]. Of the fragments produced, microplastics—particles with diameters <5 mm [14]—are the most commonly studied. These comprise both secondary microplastics, generated by the degradation mechanisms noted above, and primary microplastics, deliberately manufactured in this size range, in the form of pellets, capsules, microbeads, or fibers found in consumer products such as detergents, toothpaste, facial cleansers, shower gels, and textiles [15]. Microplastics in aquatic systems are taken up by organisms and cause numerous adverse health effects [16,17]. For example, plastics taken

up by *Daphnia magna* bind to proteins and other molecules to form a corona, which remains in the gut and affects the organism's ability to feed [18]. On exposure to microplastics, corals preferentially ingest polypropylene, which leads to increased production of mucus, irregular feeding habits, and altered gene expression [11]. Controlled experiments have shown that exposure of *Danio rerio* and *Perca fluviatilis* to polyethylene (PE) particles induced oxidative stress, which then triggered changes in lipid peroxidation, DNA damage, and stimulated signal transduction pathways, which caused autophagy and apoptosis in fishes [10].

Microplastics can enter the human body through the consumption of fish and other seafood [19]. Since microplastics are also carriers of priority pollutants such as heavy metals, persistent organic compounds, and pharmaceutical products [20], their potential toxicity to humans and other organisms is of concern.

The majority of microplastics research to date has focussed on the marine environment, but there is growing interest in freshwater systems, in particular freshwater sediments. Studies on microplastics in freshwater sediments have majorly focused on rivers [21,22] and lakes [23,24], with rivers being of particular interest as they are major sinks for plastic wastes and facilitate their transport into Earth's oceans. Early studies of microplastics in freshwater systems have suggested that their occurrence and interactions are broadly comparable to those in marine systems. However, considerable further research is needed to fully understand their sources, abundance, distribution, fate, and impacts [25].

A challenge facing researchers is that there is as yet no standard method(s) for the isolation of microplastics from freshwater sediment samples [26]. Although many approaches share common features—a density-based separation of the microplastic particles from the majority of the sediment matrix by flotation in salt solution, preceded or followed by digestion of naturally occurring organic matter that otherwise makes visualization and physical isolation of microplastics difficult—they differ in detail.

A variety of salts have been employed for the density separation step. These include sodium chloride (NaCl) [21], calcium chloride ($CaCl_2$) [27], zinc chloride ($ZnCl_2$) [28], sodium iodide (NaI) [29], potassium formate [30], and sodium polytungstate [31]. The choice of salt has often been based on toxicity or cost [32] rather than a rigorous assessment of effectiveness, e.g., NaCl is widely used even though it may underestimate higher density microplastics, such as PET and PVC, which constitute a considerable volume of plastic wastes [33]. Another factor that has not been adequately investigated is the influence of microplastic particle size and shape on recovery by flotation in different salt solutions. Particle size is an important factor influencing both transport [34] and bioavailability of microplastics in aquatic ecosystems [35], and it is therefore important to understand how the choice of analytical protocol may influence the size distribution measured.

The removal of naturally occurring organic matter by digestion has used a variety of reagents, including hydrogen peroxide (H_2O_2) [28], Fenton's reagent [36], acids such as HCl [37], $HClO_4$ [38], HNO_3 [37,39], and H_2SO_4 [38], bases such as KOH [40,41] and NaOH [38,42,43], and enzymes such as amylase and proteinase K [44,45]. The exact conditions under which digestions were performed, including temperature, duration, and reagent concentration, have varied between studies (or sometimes have not been reported), which can lead to the adoption of arbitrary conditions by subsequent researchers [46]. This lack of standardization not only means that researchers may select analytical protocols based on the availability of apparatus and reagents rather than the efficiency of microplastics recovery [47] but also makes it difficult to compare results obtained by different laboratories [46].

A key step towards the eventual harmonization of methods for the isolation of microplastics from freshwater sediments is to assess the relative performance of current approaches. In this context, Cashman et al. [48] observed wide variability and no consistent patterns in recoveries of post-consumer microplastics from (marine) sand and sediment using density flotation methods based on NaCl, $CaCl_2$, NaCl + NaI, and $ZnCl_2$. In contrast, Duong et al. [49] reported relatively little difference in the average recoveries of particles

from spiked river sediment by NaCl (80%), $ZnCl_2$ (84%), and NaI (82%) but obtained poorer performance using $CaCl_2$ (68%). Radford et al. [50] recommended canola oil over salt solutions (NaCl and $ZnCl_2$) for recovery of microplastics from simulated soils, whilst Han et al. [51] found a 1:1 (*v/v*) mixture of NaCl + NaI improved recovery of PET and PVC from sediment, relative to the use of NaCl alone.

For the organic matter removal step, Hurley et al. [41] found Fenton's reagent superior to H_2O_2, NaOH, or KOH for the determination of microplastics in soil and sludge. Prata et al. [52] applied a variety of substances (H_2O_2, Fenton's reagent, KOH, HNO_3, and even a surfactant) to a suite of natural organic materials (algae, driftwood, feathers, fish muscle, paraffin, and palm oil). Different reagents were found to be optimal for different substrates: Fenton's reagent was recommended for the digestion of plant-derived samples, but KOH was recommended for animal tissue. Radford et al. [50] concluded that H_2O_2 was suitable for removal of organic matter from most soils.

With no methodological consensus yet reached, the aim of the current study was to contribute to the development of optimized analytical approaches for the isolation of microplastics from freshwater sediment by carrying out a systematic investigation of both the density flotation and the organic matter removal steps. First, the ability of four salt solutions with different densities to isolate a suite of microplastics covering a wide density range from a simple surrogate sediment matrix was assessed. The microplastics were then ground, and the separation was repeated on various particle size fractions to, for the first time, assess the influence of microplastic size on recovery for virgin polymers. Next, a comparison of digestion methods was conducted featuring an acid (HNO_3), a base (NaOH), and a strong oxidant (Fenton's reagent). The parameters studied were reagent composition, digestion temperature, and digestion duration. The overall goal was to obtain a protocol that gave the highest microplastic recovery and most effective organic matter removal with minimal damage to the plastic particles. Finally, the procedure developed was applied to recover microplastics from two exemplar river sediments from West Central Scotland.

2. Materials and Methods

2.1. Samples

Five types of virgin plastic pellets (PA, PP, PS, PET, and polyvinyl fluoride (PVF)) and one post-product plastic, PTFE, were selected for study since these polymers are commonly used—hence found in the environment—and have a wide range of densities (Table 1).

Table 1. Microplastic samples.

Plastic	Origin	Reported Density (g/cm³) *	Particle Size (mm)	Shape
PP	Virgin (Merck)	0.85–0.93	4.4–4.6	Flat oval
PS	Virgin (Merck)	1.04–1.08	2.6–3.0	Cylindrical
PA	Virgin (Merck)	1.13–1.16	2.4–2.7	Cylindrical
PET	Virgin (Merck)	1.38–1.41	3.6–4.4	Flat squared
PVF	Virgin (Merck)	1.78	4.2–4.8	Spherical
PTFE	Post-consumer	2.2	Variable	Irregular

* as quoted in the information provided by the supplier.

Fine play-pit sand was used to simulate sediment in the investigation of flotation, and peat was used as a representative natural organic material for the comparison of digestion methods. Both were obtained from a commercial building/garden supplier (B&Q, Glasgow, UK). The procedure developed was applied to river sediment samples obtained from the Black Cart Water (sampling location 55°50′41″ N 4°29′42″ W) and White Cart Water (sampling location 55°48′42″ N 4°15′31″ W), which are tributaries of the River Clyde in West Central Scotland. A bulk composite sample of surficial sediment (sampling depth

5 cm) was collected from each site using a metal trowel, returned to the laboratory for analysis in a foil tray, and air-dried for seven days before analysis.

2.2. Reagents

The ability of four salts to separate microplastics from the surrogate sediment matrix was compared with that of distilled water: NaCl (Fisher Scientific, Loughborough, UK), $CaCl_2$ (Fisher Scientific), $ZnCl_2$ (Sigma Aldrich, Gillingham, UK), and NaI (Fisher Scientific). Salt solutions were prepared as described in Table 2. The density of each solution was determined using a hydrometer after preparation.

Table 2. Preparation and properties of solutions used for density flotation.

Salt	Mass of Salt Weighed (g)	Vol. of Solution Prepared (mL)	Density of Solution (g/cm^3)
NaCl	180	500	1.195
$CaCl_2$	300	500	1.350
$ZnCl_2$	450	500	1.450
NaI	500	500	1.800

Three digestion reagents were compared for the removal of organic matter: an acid (HNO_3, Fisher Scientific), a base (NaOH, Fisher Scientific), and a strong oxidizing agent (Fenton's reagent, which was a mixture of 30% H_2O_2 (Fisher Scientific) and 0.05 M $FeSO_4$ (Scientific Laboratory Supplies, Fairham, UK).

2.3. Procedures

2.3.1. Comparison of Salt Solutions for Recovery of Microplastics by Density Flotation

Surrogate sediment samples were prepared by mixing ten virgin PA, PP, PS, PET, or PVF pellets with 8 g of sand. For PTFE, 2 g of fragments (a similar weight to that used for the other plastics) was mixed with 8 g of sand. These were placed in narrow 250 mL beakers, and 100 mL aliquots of each salt solution were added. The mixtures were agitated vigorously, then covered and left to stand. After 24 h, the upper portion of each solution was collected by decantation, ensuring that all floating materials—including any microplastics—were recovered (but taking care not to disturb the settled sediment and non-buoyant microplastics). The decanted portion was then filtered through glass fiber membrane filters using a vacuum filtration apparatus. The filters were dried at 60 °C to constant weight. The recovery of virgin plastics was determined by counting the number of particles obtained. For PTFE, the extent of plastic recovery was determined by weight.

2.3.2. Effect of Microplastic Size on Recovery by Density Flotation

To assess the effect of particle size, microplastics that sank in one or more of the salt solutions were subjected to size reduction using a coffee grinder, then sieved into three particle size fractions using stainless steel sieves: <1 mm, 1–2 mm, and >2 mm. Polypropylene was excluded because it floated in all solutions, and PVF was excluded because it proved too hard to pulverize. Test portions (2 g) or each of the different size fractions of PA, PS, PET, and PTFE were mixed with 8 g sand, and then the flotation experiment described in Section 2.3.1 was repeated. Microplastic recovery was determined by weight.

2.3.3. Comparison of Digestion Procedures for Organic Matter Removal

A 3-factor (time, temperature, and reagent concentration/composition) 2-level full factorial design was performed on Minitab to generate the experimental parameters. The digestion temperature was set at either 30 °C or 60 °C for HNO_3 and NaOH but at either ambient or 30 °C for Fenton's reagent since the reaction proved too vigorous to control at

60 °C (resulting in sample loss). The duration of the digestion was either 60 min or 360 min. An extended digestion time (24 h) was also studied for Fenton's reagent only in an attempt to compensate for the lower temperatures used. Two different concentrations of HNO_3 and NaOH were investigated (1 M and 10 M), and two different ratios of H_2O_2:$FeSO_4$ (1:1 and 2:1 *v/v*) in the Fenton's reagent.

Approximately 0.5 g of peat was accurately weighed into a DigiPREP® borosilicate digestion tube, and 10 virgin PA, PP, PS, or PET pellets were added. Polytetrafluoroethylene was not studied since it was not available as pure pellets (suitable for FTIR analysis) or PVF, as it is less common in the environment [53]. A 20 mL aliquot of each digestion reagent was added and stirred for 1 min; then, the tubes were transferred into a block digester (DigiPREP® Jr, Qmx Laboratories, Thaxted, UK) set at the appropriate temperature.

When the required digestion time had elapsed, the tubes were removed from the block digester, and the contents were immediately filtered through pre-weighed glass fiber membrane filters. The digestion tubes were rinsed multiple times with distilled water, and the washings passed through the same filter. The microplastic pellets were removed from the filter using tweezers and rinsed with ethanol and distilled water, taking care to ensure that any adhering undigested organic material was rinsed back onto the filter. The filter papers were then removed from the vacuum apparatus and dried at 60 °C for 2 h before their final weight was recorded.

Digestion efficiency (DE) was calculated based on the weight (*wt*) of residual organic matter remaining at the end of the digestion procedure using the following expression:

$$DE \ (\%) = \frac{Initial \ wt \ of \ org \ matter - final \ wt \ of \ org \ matter}{Initial \ wt \ of \ org \ matter} \times 100$$

The recovered microplastic pellets were taken for physical observation and imaging using a digital microscope and chemical characterization using ATR-FTIR.

ATR-FTIR spectra were obtained on a Nicolet IS5 spectrometer (Thermo Scientific, Loughborough, UK) equipped with a Specac Golden Gate diamond ATR accessory (Specac Ltd., Orpington, UK). Sixty-four scans per sample were acquired at a resolution of 4 cm^{-1} over the range of 4000–600 cm^{-1}. Boxcar apodization was employed, and spectra were baseline and background-corrected, with a new background collected every ninety minutes. Spectra were processed using the instrument's in-built OMNIC software.

2.3.4. Analysis of Freshwater Sediment Sample

The air-dried Black Cart Water and White Cart Water sediments were extracted (10 g test portions, *n* = 3) using the procedure developed. Further test portions were 'spiked' with microplastics (a mixture containing approximately 1 g each of PP, PS, PA, PET, and PTFE) before being extracted. The organic matter content of the sediments was estimated by loss-on-ignition [54].

3. Results and Discussion

3.1. Comparison of Salt Solutions for Recovery of Microplastics by Density Flotation

The recoveries of PP, PS, PA, PET, PVF, and PTFE from the simulated sediment when separation by density flotation was carried out using different salt solutions are shown in Figure 1.

The only microplastic quantitatively recovered in distilled water was PP, which is as expected since it was the only polymer studied with a density (0.93 g/cm^3) less than that of H_2O. Zhang et al. [55] were also able quantitatively to isolate PP (and LDPE) from sand using distilled water. All of the PP, PS, and PA pellets were recovered using NaCl solution, which is consistent with expectations from density values. This salt has been widely used for the isolation of PP and PS from environmental samples [27,56,57]. One study [49] reported that NaCl could also float PET and other denser microplastics such as PVC. However, these were naturally weathered samples whose densities may have differed from virgin polymers.
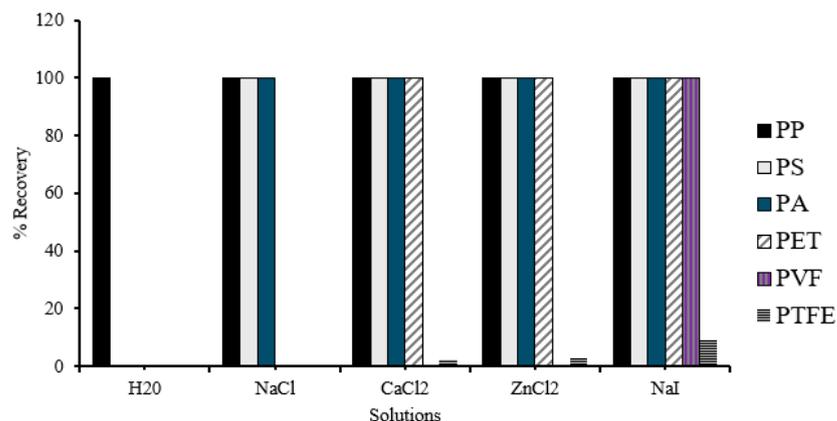
Figure 1. Recoveries of different types of microplastic by density flotation in various salt solutions (error bars represent one standard deviation, *n* = 3). PP = polypropylene; PS = polystyrene; PA = polyamide; PET = polyethylene terephthalate; PVF = polyvinyl fluoride; PTFE = polytetrafluoroethylene.

All of the PP, PS, PA, and PET pellets floated in $CaCl_2$ and $ZnCl_2$ and were recovered quantitatively by these solutions. This was expected for PP, PS, and PA, all of which have lower densities than both salts, but not for the PET sample used, which (according to the supplier) had a density range of 1.38–1.41 g/cm^3, slightly greater than that of $CaCl_2$ (1.35 g/cm^3), and so would be expected to sink in solutions of this salt. Perhaps surprisingly, a small amount of PTFE was recovered in both $CaCl_2$ and $ZnCl_2$ even though its density (2.2 g/cm^3) far exceeds that of either solution. As expected, all PP, PS, PET, PA, and PVF were recovered in NaI (density 1.78 g/cm^3). A small proportion of the PTFE (around 9%) also floated. The flotation observed for PTFE in $CaCl_2$, $ZnCl_2$, and NaI may be attributed to irregularity in particle shape and size since—in contrast to the other microplastics—the PTFE sample was created by scrapping material from a post-consumer product, together with the hydrophobicity of the polymer, which allows microparticles to float even in pure water [58].

Although density appeared to be the primary factor affecting flotation, and hence recovery, of different types of microplastic from different types of salt solution, PET floated in $CaCl_2$ (where it should theoretically have sunk), and small quantities of PTFE were obtained with $CaCl_2$, $ZnCl_2$, and NaI even though the polymer has a much higher density than any of these solutions. It was further noted that some microplastics initially floated in particular solutions but sank after agitation. This suggests the extent of recovery may be partially operationally defined, further highlighting the need for the development of standardized laboratory protocols.

3.2. Effect of Microplastic Size on Recovery by Density Flotation

Since the comparison of salt solutions suggested that microplastic shape and size may have influenced recovery, four of the polymers were ground, and the density separation procedure was repeated on different particle size fractions (<1 mm, 1–2 mm, and >2 mm). The results are shown in Table 3.

Appreciable but different amounts of all three particle size fractions of the PS were recovered by flotation in distilled water (67%, 59%, and 54% for <1 mm, 1–2 mm, and >2 mm particles, respectively). All the PA particles <1 mm in diameter floated, as did 93% of the 1–2 mm particles, but particles >2 mm sank. For PET, 41% of the <1 mm size fraction and 46% of the 1–2 mm size fraction were recovered in water, as were 28% of the <1 mm and 38% of the 1–2 mm PTFE particles. Some of the PET particles of all sizes floated in NaCl, with the highest recoveries (90%) obtained for the smallest size fraction (<1 mm). Between 9% and 36% of PTFE particles were also recovered in NaCl solution. All of the other salt solutions recovered some PTFE. In general, the highest recoveries were obtained for the smallest particle size fraction (<1 mm) and the lowest for the largest size fraction

(>2 mm). The exception was $ZnCl_2$, where the highest recovery (66%) was obtained for the 1–2 mm fraction. However, the recovery of the <1 mm fraction was only slightly lower at 61%, and the value had a high uncertainty.

Table 3. Recoveries of various size fractions of microplastics by density flotation.

		PS (%)	PA (%)	PET (%)	PTFE (%)
H_2O	<1 mm	67.0 ± 1.4	100 ± 0.0	40.6 ± 0.9	28.2 ± 11.4
	1–2 mm	59.0 ± 1.4	92.8 ± 3.7	46.2 ± 2.3	38.4 ± 8.4
	>2 mm	54.5 ± 2.1	0	0	4.00 ± 1.4
	Unground	0	0	0	0
NaCl	<1 mm	-	-	90.2 ± 4.5	11.2 ± 2.5
	1–2 mm	-	-	64.6 ± 0.7	35.6 ± 14.8
	>2 mm	-	-	13.5 ± 2.8	8.60 ± 5.10
	Unground	100	100	0	0
$CaCl_2$	<1 mm	-	-	-	80.2 ± 2.9
	1–2 mm	-	-	-	30.2 ± 27.3
	>2 mm	-	-	-	12.1 ± 5.7
	Unground	100	100	100	2.50 ± 2.10
$ZnCl_2$	<1 mm	-	-	-	60.7 ± 17.4
	1–2 mm	-	-	-	65.7 ± 3.3
	>2 mm	-	-	-	11.0 ± 1.5
	Unground	100	100	100	3.0 ± 2.0
NaI	<1 mm	-	-	-	60.2 ± 12.9
	1–2 mm	-	-	-	10.9 ± 3.0
	>2 mm	-	-	-	7.65 ± 5.20
	Unground	100	100	100	9.00 ± 1.40

All values are mean $\pm$ SD (n = 3). The density flotation was repeated only in solutions where the unground samples had not been quantitatively recovered in the previous experiment. PS = polystyrene; PA = polyamide; PET = polyethylene terephthalate; PTFE = polytetrafluoroethylene.

This highlights the fact that polymer density (relative to that of the flotation solution) alone does not control microplastic recovery, even for virgin plastics whose properties are well-defined. Particle size is also important. Real environmental samples are likely to contain post-consumer plastics of widely varying size and shape, the densities of which may have been altered by the presence of additives or due to environmental processes such as the formation of inorganic deposits or biofilms [58]. Ensuring quantitative recoveries of such materials for analysis remains challenging.

3.3. Comparison of Digestion Procedures for Organic Matter Removal

3.3.1. Digestion of Organic Matter with HNO_3

The efficiency of organic matter removal in HNO_3 under different digestion conditions is shown in Figure 2.

Similar trends were obtained for all of the polymers studied. The 10 M HNO_3 gave significantly higher digestion efficiencies than the 1 M acid under all conditions (as compared using the Student's *t*-test at a 95% confidence interval). Increasing the digestion time from 1 to 6 h had relatively little effect at 30 °C but markedly increased digestion efficiency at 60 °C, in particular for the more dilute acid. The best performance (70–78% digestion efficiency) was obtained using 10 M HNO_3 at 60 °C for 6 h.

Nitric acid is widely used for the digestion of environmental samples for trace element analysis. Studies on its use specifically as part of a protocol for the removal of naturally occurring organic matter in the isolation of microplastics from sediment are limited. However, Prata et al. [52] similarly reported increased digestion efficiency for 35% *v/v* HNO_3 (at 50 °C) when reaction time was increased from 1 to 6 h.
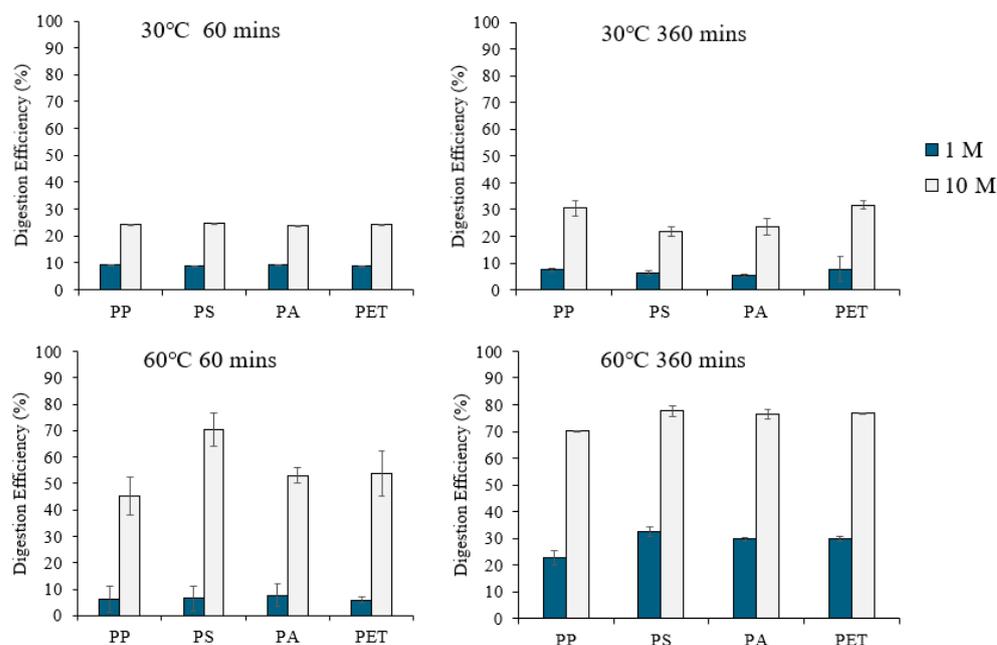
Figure 2. Digestion of natural organic matter under different conditions using HNO_3 (error bars represent one standard deviation ($n = 3$)).

3.3.2. Digestion of Organic Matter with NaOH

The efficiency of organic matter removal in NaOH under different digestion conditions is shown in Figure 3. As with acid digestion, 10 M NaOH gave higher digestion efficiencies than 1 M NaOH. Increasing the digestion time increased the digestion efficiency at 60 °C but not at 30 °C. The best performance was only 38–45% digestion efficiency, obtained using 10 M NaOH at 60 °C for 6 h. After treatment with NaOH, the residue on the filter paper appeared blackened, which made visualization of the microplastics difficult.
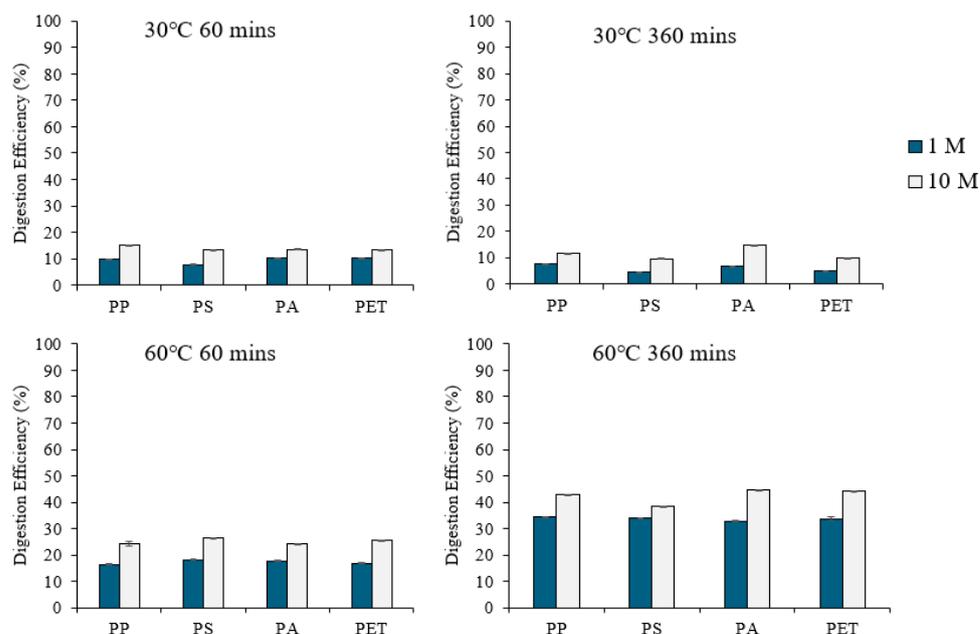
Figure 3. Digestion of natural organic matter under different conditions using NaOH (error bars represent one standard deviation ($n = 3$)).

Some previous workers have favored alkaline digestion for organic matter removal in microplastics studies. For example, a 10% w/v KOH solution at 50 °C [52] was found to

provide an average digestion efficiency of 58.3% over a range of test substrates. However, performance varied markedly, from complete digestion of fish muscle tissue to ca. 15% digestion of driftwood. Low digestion efficiencies (3–14%) were also obtained in another study [42] when 10 M NaOH was applied to samples rich in vegetal matter (leaves, seeds, seaweed, etc.), and the reagent was observed to damage polyester (PET) fibers.

3.3.3. Digestion of Organic Matter with Fenton's Reagent

The efficiency of organic matter removal in Fenton's reagent under different digestion conditions is shown in Figure 4.
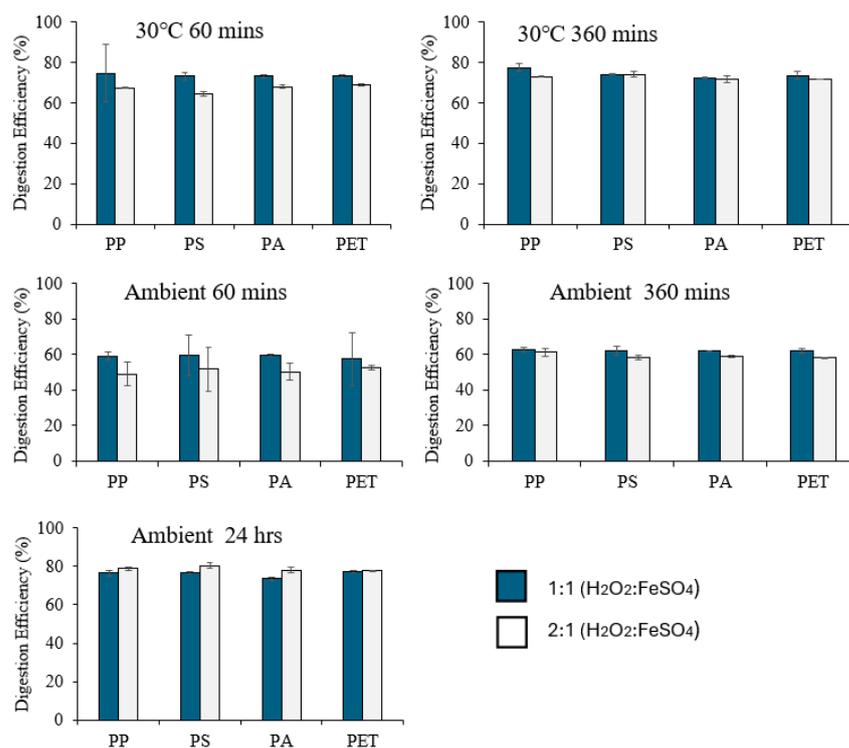
Figure 4. Digestion of natural organic matter under different conditions using Fenton's reagent (error bars represent one standard deviation (*n* = 3)).

In terms of composition, Fenton's reagent, comprising a 1:1 ratio of H_2O_2 to $FeSO_4$, sometimes gave higher digestion efficiencies than the 2:1 ratio. However, differences were not significant ($p > 0.05$) when compared using the Student's *t*-test. Decreasing digestion temperature from 30 °C to ambient (around 21 °C) decreased digestion efficiency by at least 10%, but this could be compensated by increasing digestion time. The best performance (78–80% digestion) was obtained by digesting samples for 24 h at room temperature in the 2:1 Fenton's reagent. Treatment for 6 h at 30 °C in the 1:1 reagent was almost as effective (73–75% digestion).

Previous workers achieved 65.9% average digestion efficiency across a variety of organic substrates using Fenton's reagent at 50 °C [52] (a temperature that could not be achieved in the current study without significant sample loss). Another study [50] found efficiency to vary from around 50% for an organic-rich soil (loss-on-ignition = 73%) digested at 40 °C to 85% for a soil containing 12% organic matter digested at 50 °C. Similarly, complete destruction of organic matter was reported for a soil with low organic content (5.8%) at temperatures not exceeding 40 °C, but only 87% digestion efficiency was achieved for a sludge containing 52% organic matter [41].

3.4. Effect of Digestion Procedures on Microplastics

3.4.1. Effect of Digestion with HNO_3 on Microplastics

Digital microscope images of the microplastics before and after digestion of the simulated sediment using HNO_3 are shown in Figure 5. Changes in physical appearance were apparent for all four types of polymers. The PP was discolored. The PS developed cracks and dulling of its surface. The PA became strongly colored in 1 M acid, especially at 60 °C, and dissolved entirely in 10 M acid. The PET, which was initially relatively transparent, became translucent. As might be expected, the alterations were generally more marked at higher temperatures, acid concentration, and following longer digestion periods.

Figure 5. Digital microscope images of microplastics before and after digestion in HNO_3.

The FTIR spectra of the plastics were, however, largely unaffected by these changes in physical appearance (see Supplementary Material). A study [59] on microplastics in biota that used an enhanced nitric acid solution (4:1 HNO_3: H_2O_2) at 50 °C similarly reported

that spectra for most polymers remained unchanged after sample digestion. The exception was PET. The difference between the literature work and the current study may be due to their use of higher acid concentration and the addition of a supplementary oxidizing agent.

3.4.2. Effect of Digestion with HNO_3 on Microplastics

Less marked changes in physical appearance were observed for microplastics recovered from samples digested in NaOH, except for PET, where roughening, cracks, and, at 60 °C in 10 M solution, coloration were observed (Figure 6).

	PP	PS	PA	PET
Untreated				
30°C 60 mins 1 M				
30°C 60 mins 10 M				
30°C 360 mins 1 M				
30°C 360 mins 10 M				
60°C 60 mins 1 M				
60°C 60 mins 10 M				
60°C 360 mins 1 M				
60°C 360 mins 10 M				
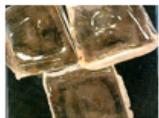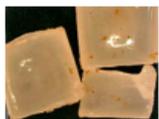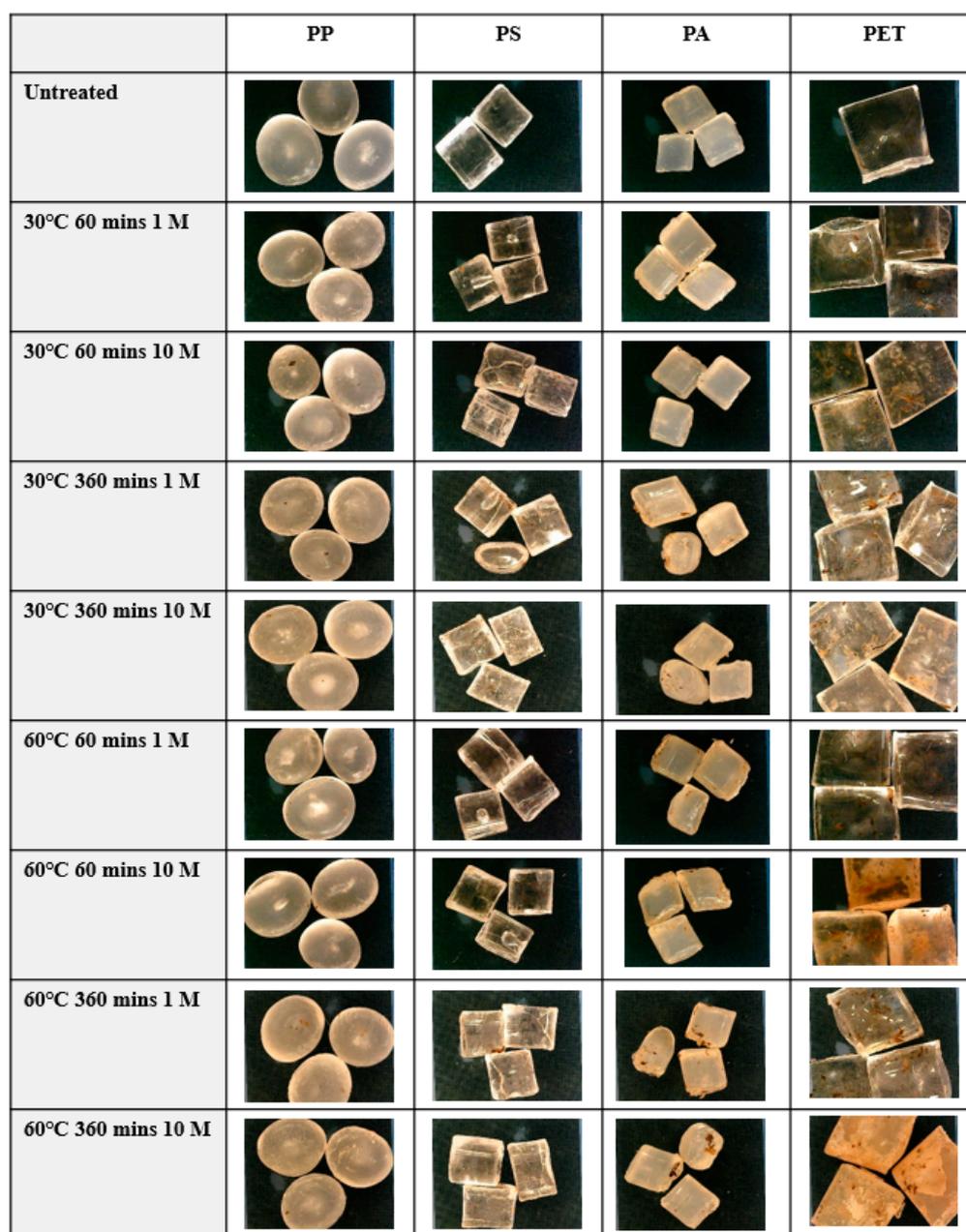
Figure 6. Digital microscope images of microplastics before and after digestion in NaOH.

Changes in the FTIR spectra of PET particles also occurred, though only when the digestion was carried out in 10 M NaOH for 6 h (Figure 7). The decrease in the intensity of the C=O peak (~1720 cm^{-1}) at 30 °C and its disappearance at 60 °C, along with the diminishing of the C-O peak of the ester (~1000 to 1100 cm^{-1}), are consistent with the hydrolysis of PET. Indeed, the spectrum obtained following digestion for 6 h in 10 M NaOH

at 60 °C strongly resembles that of disodium terephthalate. The 1 M NaOH reagent was likely too mild, even when applied at 60 °C for 6 h, to hydrolyze the polymer in the solid state below its glass transition temperature.
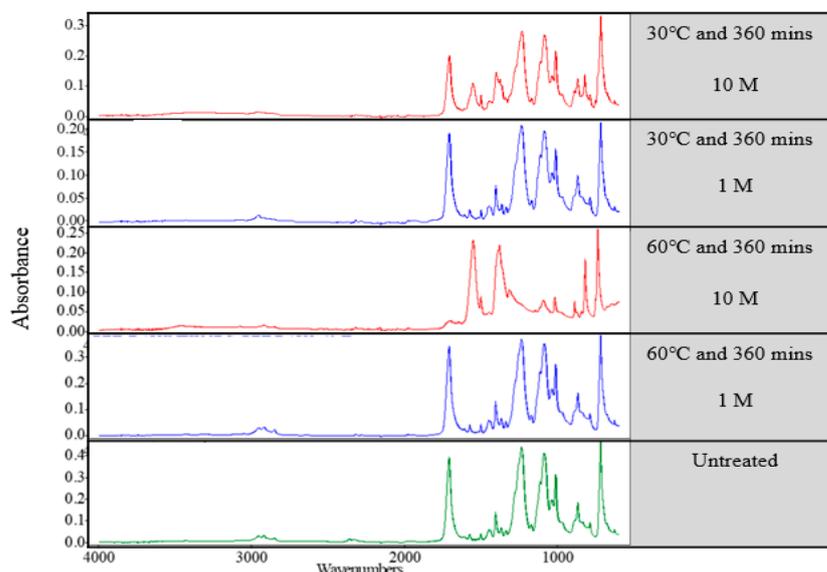
Figure 7. FTIR spectra of PET before and after digestion in NaOH. Green indicates the untreated sample, blue indicates samples treated with 1 M NaOH and red indicates samples treated with 10 M NaOH.

3.4.3. Effect of Digestion with Fenton's Reagent on Microplastics

There were no marked changes to the appearance of microplastics treated with Fenton's reagent, even under the two sets of digestion conditions that gave the most effective organic matter removal (Figure 8). Only a slight surface discoloration of the PA was observed.

**Figure 8.** Digital microscope images of microplastics before and after digestion in Fenton's reagent.

The FTIR spectra were similarly unaffected, i.e., spectra obtained for samples that had experienced treatment with either 1:1 H_2O_2:$FeSO_4$ at 30 °C for 6 h or 2:1 H_2O_2:$FeSO_4$ at ambient temperature for 24 h were virtually identical to those of untreated samples (see Supplementary Material) meaning that there was minimal risk of polymer misidentification. This corroborates other studies [41,60,61] that concluded that treatment with Fenton's reagent did not visibly change the spectra of microplastics such as PP, PS, and PA.

3.5. Analysis of Freshwater Sediment Samples

The best compromise analytical procedure—density separation in $CaCl_2$ followed by removal of organic matter by digestion in 1:1 H_2O_2:$FeSO_4$ at 30 °C for 6 h—was applied to two locally sourced freshwater sediments.

3.5.1. Recovery of Microplastics Added to the Sediment Samples

As shown in Figure 9, the proposed method recovered all of the PP, PA, PS, and PET microplastic particles added, as well as a proportion of the PTFE (40% from the Black Cart Water sediment and 47% from the White Cart Water sediment). This is consistent with the results obtained for the simulated sediment (Section 3.1) and confirms the effectiveness of the $CaCl_2$ salt solution for the isolation of the majority of common microplastics. The two sediments were found to have similar organic matter contents (5.7% for the Black Cart and 4.8% for the White Cart), and no difficulties were encountered with sample digestion or subsequent polymer identification.
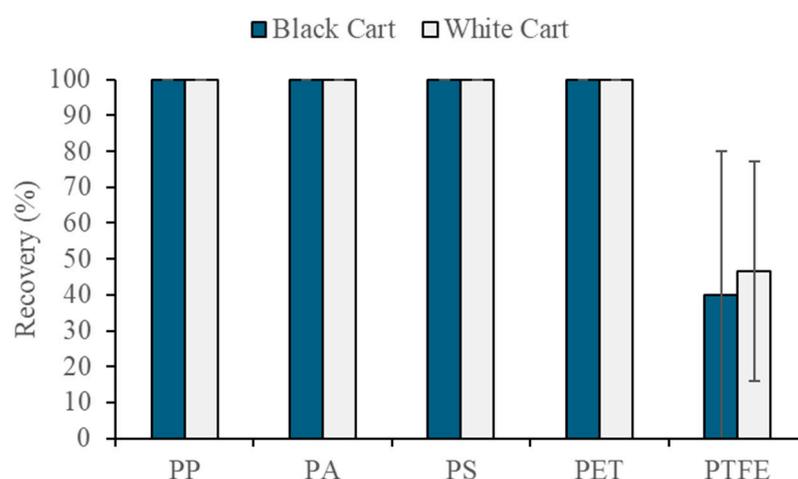

Figure 9. Recovery of spiked microplastics from Black Cart and White Cart Water sediments.

3.5.2. Study of Additional Microplastics Recovered from the Sediment Samples

Microplastics were recovered from the two sediment samples in addition to those deliberately added to assess the performance of the analytical method. On average, 4.3 ± 1.0 particle/10 g sediment (equivalent to 430 particles/kg) was found in the Black Cart, and 2.0 ± 1.9 particle/10 g sediment (200 particles/kg) in the White Cart. This represents material already present at the time of sampling. The higher microplastic abundance in the Black Cart sediment sample is likely due to the fact that it was collected in a built-up area, whereas the sampling location for the White Cart was in a rural location. Results obtained were much lower than the 50 particles/g (50,000 particles/kg) previously reported for sediments from the River Clyde [62] but similar to the values of 50–244 particles/kg [22] and 16–432 particles/kg [63] reported for the River Kelvin, which is also a tributary of the Clyde passing through both rural and urban areas.

Figure 10 shows some of the microplastics collected as observed using a light microscope. The most abundant shapes were films (86%), followed by fragments (8%) and fibers (6%). Most particles were white or translucent, but some black and brightly colored material was also present. Fibers, fragments, and films are all common shapes of microplastics found in sediments, although others—including foams, pellets, and spheres—have also been reported [64,65]. Similarly, although various colors have been observed, white microplastics are generally the most common [66]. Particle sizes ranged from 0.5 to 3 mm, with the majority <1 mm. This is consistent with other studies [22,67], which reported that microplastics <1 mm in size were more abundant in freshwater sediments.
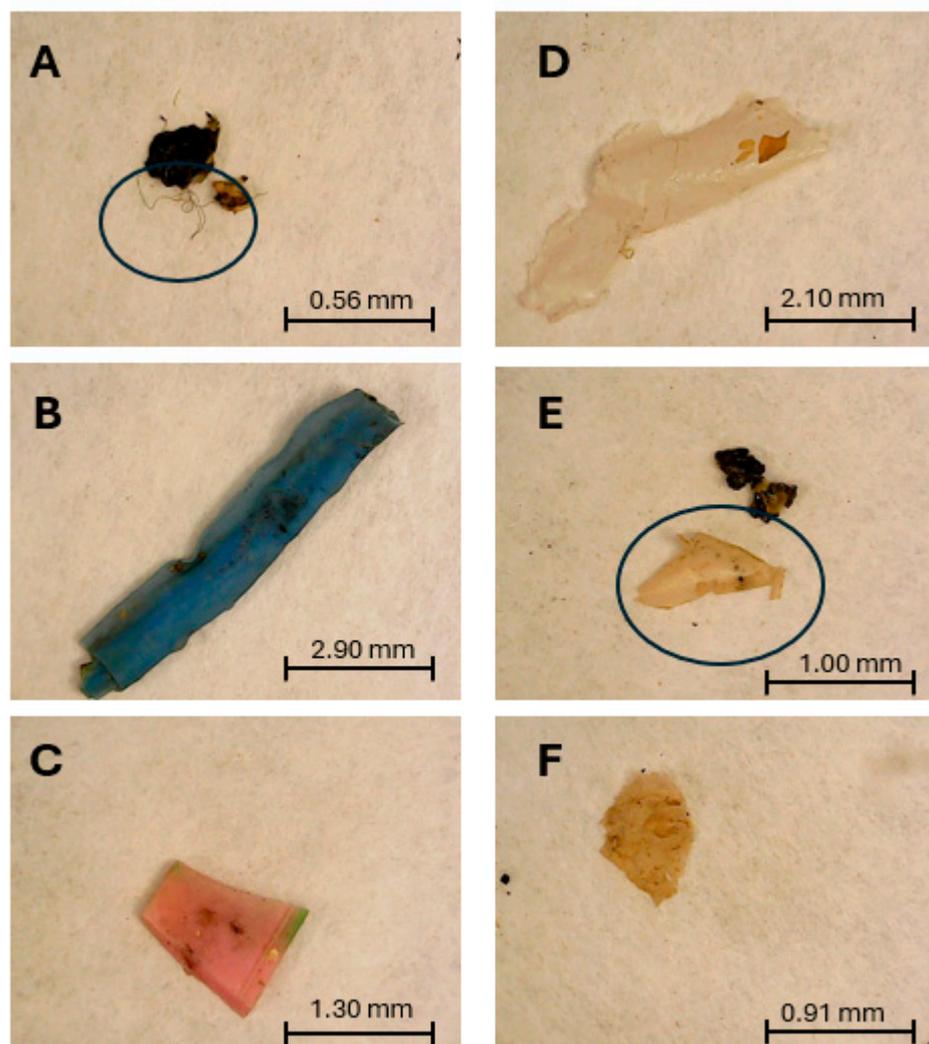
Figure 10. Images of some microplastic fibers (**A**), fragments (**B**,**C**), and films (**D**–**F**) recovered from the sediments of the Black Cart and White Cart Waters.

Analysis by ATR-FTIR indicated that the isolated microplastics included examples of the following polymers (theoretical densities given in parenthesis): butadiene rubber (~0.91 g/cm^3); LDPE (~0.93 g/cm^3); PE (~0.96 g/cm^3); polydimethylsiloxane (~0.97 g/cm^3); polyurethane (~1.26 g/cm^3); cellophane (~1.42 g/cm^3); PVA (~1.27 g/cm^3); and PVC (~1.44–1.48 g/cm^3). This demonstrates that the flotation protocol using CaCl$_2$ was effective in recovering common polymers, including types not included in method development, with densities up to almost 1.50 g/cm^3, and that the digestion procedure using Fenton's reagent did not degrade the polymers to an extent that compromised their identification by FTIR spectroscopy.

4. Conclusions

This study compared five different flotation solutions and three different digestion reagents for the isolation of microplastics from simulated sediment matrices. Sodium iodide (density 1.8 g/cm^3) gave quantitative recovery of virgin PP, PS, PA, PET, and PVF pellets, whilst CaCl$_2$ (density 1.35 g/cm^3) and ZnCl$_2$ (density 1.45 g/cm^3) recovered only PP, PS, PA, and PET. However, since NaI and ZnCl$_2$ are expensive and/or toxic, and the majority of plastics present in the environment have densities below that of PET, the use of CaCl$_2$ as a general-purpose reagent is recommended (whilst investigations of sites specifically known to be contaminated with higher density polymers may require use of higher density solutions). Despite having a higher theoretical density (2.2 g/cm^3), fragments of post-

consumer product PTFE partially floated in CaCl$_2$, ZnCl$_2$, and NaI, indicating that particle shape and size, as well as polymer density, affected recovery. This was confirmed when samples of PS, PA, PET, and PTFE were ground and sieved into different-sized fractions, and the flotation experiments were repeated.

Fenton's reagent was preferred to HNO$_3$ and NaOH for the removal of natural organic matter. It offered the highest digestion efficiency and had the least effect on the microplastics. The recommended operating conditions are either ambient temperature for 24 h or 30 °C for 360 min of digestion. These conditions did not cause marked changes in the physical appearance of the microplastics, nor alterations to the ATR-FTIR spectra that could lead to misidentification of the polymer present.

When applied to spiked and unspiked freshwater sediment samples, the developed protocol performed well, recovering all of the added microplastics (except PTFE, which was partially recovered) and allowing the identification of a variety of additional microplastics that had accumulated in the sediments whilst in the environment on the riverbed. These included examples of polymers not included in the method development phase of the study.

Whilst the method developed is suitable for general application in studies of microplastics in freshwater sediments, more research is still needed on factors affecting particle recoveries. Since size critically affects microplastic mobility and bioavailability, further study and perhaps method development is required to ensure that analytical methods accurately capture particle size distributions. Extraction efficiencies for post-consumer microplastics from real sediment samples of different compositions warrant detailed evaluation. Many polymers in actual use are filled with inert minerals, which alters their density and may make identification via spectral libraries more difficult, whilst complex interactions between microplastics and the environment, such as weathering, occlusion, and biofilm formation, will affect their properties and hence the ability to be recovered even by standardized methods.

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