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Microplastics and Nanoplastics

BRIEF REPORT **Open Access**

Positive controls with representative materials are essential for the advancement of microplastics research

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Abstract
Reporting accurate microplastics concentrations across environmental matrices is imperative for robust monitoring and regulation. However, recovering microplastics from complex matrices, such as soils and sediments, is hugely challenging. Numerous methods have been published to facilitate microplastics extraction from such matrices, but these protocols typically lack validation of microplastic recovery efficiency. We argue that environmentally realistic microplastic recovery rate experiments must be utilized consistently to increase the validity of microplastics pollution research, particularly for studies focused on complex matrices. Here, we outline the importance of harmonized recovery rate tests and demonstrate this experimentally using saltmarsh sediments as a case study. Building-upon established protocols, an iterative approach was used to test the recovery of four types of environmentally relevant microplastics: polypropylene (PP) fragments, polyethylene (PE) films, polyamide (PA) fibers and polyester (PET) fibers ranging in size from 180 to 1060 µm. For protocols attaining > 50% microplastic recovery, these methods were optimized to attain maximal recovery of all plastic types, and optimal methods replicated to determine precision. Most methods demonstrated efficient removal of organic and inorganic materials with reasonable recovery rates for fragments and films, but many methods failed to sufficiently recover fibers. This further underscores the need for environmentally representative reference microplastics for method validation. Owing to the differences and complexities across environmental matrices, the standardization of microplastic extraction methods is unlikely. Therefore, recovery rate experiments with representative reference microplastics should be a requirement to increase quality, harmonization, and comparability.

Keywords Method validation, Harmonization, Microplastic extraction, Environmental contamination, Case study, Salt marsh sediment

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Introduction

Microplastics pollution has garnered widespread attention in the public and governmental sectors, with their presence reported across all environmental compartments, food webs, and in human tissues [1–3]. This level of concern has resulted in a United Nations resolution to end plastic pollution (UNEA resolution 5/14), initiating intergovernmental negotiations to create the first legally binding instrument on plastic pollution [4]. As governments discuss and implement these policies, it will be necessary to have continual monitoring of microplastic contamination across environmental compartments. This will aid assessments of risk to ecosystems and evaluate the efficacy of management programs [5, 6]. Scientifically robust analytical methods are essential for ensuring quality of reporting and to maintain trust and reliability of the microplastics research field.

The harmonization of methods for microplastics analysis is an ongoing task. Interlaboratory comparison (ILC) studies comparing the performance and replicability of methods have found large variability in microplastic recovery across laboratories [7–13]. Notably, these tests almost exclusively focus upon laboratory methodology, however errors can be introduced at any point in the microplastics quantification procedure: environmental sampling, sample processing, enumeration, and identification [14–16]. Due to the variation and complexity of differing environmental matrices researchers may not be accurately reporting the number and types of microplastics present in the environment. This is particularly problematic for more complex matrices, such as sewage sludge, soil, fine estuarine sediment, or sediments with high organic content, that require multiple processing steps [15]. Increased processing steps and exposure time to equipment and to airborne microplastics in the laboratory and field may result in a higher risk of procedural contamination and of microplastic loss [11, 17, 18]. Weber & Kerpen (2022) highlight how data extrapolation from minor inaccuracies arising during processing can lead to inaccuracy by under- and over representation in reported results [16]. As such, it is imperative that studies are transparent in reporting their methodologies and consider quality control and assurance criteria [6, 14, 15, 19].

Many efforts have been made to limit the level of overestimation of microplastics contamination, through negative controls (i.e., procedural blanks), appropriate washing and handling procedures, and operating in clean laboratories [14, 18, 20]. However, positive controls (i.e., recovery rate or spike recovery experiments) used to evaluate method accuracy are not regularly reported across the literature, despite many proposing this as a necessary requirement (e.g [14, 19–25]). Historically, studies comparing laboratory methods have predominantly focused on evaluating mass reduction of samples via effective removal of organic and inorganic materials and on minimal adverse impacts on polymers rather than microplastic recovery [14, 15, 24, 25]. Without appropriate measures to evaluate microplastic recovery, such as positive controls, microplastic concentrations can be considerably underestimated [26, 27]. Moreover, biases in the recovery rates of different types of particles may result in an underestimation and misrepresentation of the particle shapes, polymers or sizes present in environmental samples. For example, environmental microplastic concentrations increase as microplastic size decreases, but small microplastics (<1 mm) have highly variable recovery rates in ILCs [7, 10, 11, 28]. The variation in microplastics processing and recovery makes across-study comparisons difficult and thus it is harder to evaluate the level of microplastic contamination in the environment.

In this perspective, we argue that recovery rate experiments with harmonized reference materials are necessary for comparability and validation of microplastics studies, particularly when dealing with complex matrices. Using saltmarsh sediments as a case study, we highlight the difficulty in relying on protocols suitable for other types of sediments, and the importance of validating select methodologies by using a diverse array of representative microplastics in a spike-recovery experiment.

Principle of recovery rate experiments

Value of recovery rate experiments

A spike recovery experiment consists of dosing or ‘spiking’ a sample matrix with known amounts and types of analyte (e.g., microplastics of varying shapes, sizes, and polymers). The sample matrix is then processed using the protocol of interest and comparisons are made between the amount of analyte recovered and the original amount of analyte added. This test evaluates the efficiency of a protocol in recovering the analyte of interest. Use of spike recovery tests can help overcome four key challenges:

(1) **Methodological inconsistency**: Despite many studies using common methods to extract microplastics in the laboratory, not all methods can be used for every sample type due to differences in sample complexity [15]. Some sample types require more steps than others, different digestion solutions based on the type of organic matter (e.g., KOH for fatty tissues vs. H_2O_2 for vegetal matter), different density separation set-ups, or different sample sizes [15, 24]. Moreover, there will always be variations in equipment or materials used, with accessibility, cost and resources all being key factors. Researchers may slightly adjust methods to fit with what equipment or materials they have available. Even slight adjustments, such as vacuum filtration vs. sieving,

may affect recovery rates of microplastics [17]. Differences in personnel expertise may also affect recovery rates [29].

(2) **Sample inconsistency**: Individual sample properties can vary within a matrix. For example, sediments have large variability in grain size and organic content, and this can differ within one sampling area [24, 30]. These variations can affect microplastic recovery efficiency [16, 31–33]. Thus, even if the exact same extraction methods with the same materials, analyst and equipment were used for one sample type (e.g., sediments), recovery rates may still differ due to individual sample properties [26]. By including a recovery test of one method across sediment types, researchers can inform how efficient the method works across each one. With this information, results could be corrected based on that efficiency if needed, while maintaining comparability with harmonized extraction methods.

(3) **Mass reduction does not equate to microplastic recovery**: Studies quantifying microplastics in the environment often use an extraction method from a previously validated study, where the original research had high recoveries of microplastics. However, many studies rarely repeat the recovery rate test on their own samples, often finding that the validated method works well at reducing their own sample mass and is therefore appropriate for their work. As we highlight in our case study, mass reduction does not always correlate with recovery rates. One issue is that fibers and small particles have similar sizes and densities to fine sediment and organic matter so methods that reduce all fine sediment/organic matter may result in microplastic underestimation [34, 35]. There is a balance that must be met between sediment reduction– to be able to visualize the microplastics - and microplastic recovery within the sample.

(4) **Comparative studies**: A wide range of laboratories across the globe have contributed to microplastics research, developing an array of methods that are affordable and attainable to a given laboratory. Harmonized recovery rate experiments would ensure these diverse methodologies can all contribute comparable data for environmental microplastic monitoring and research. By including recovery rate tests as part of a harmonized protocol, we can maintain accessibility and inclusivity to microplastics research while allowing for comparability across method variations.

Challenges of recovery rate experiments

Despite their value, a meta-analysis of microplastic literature by Way et al. (2022) found only 8% of studies included recovery rate tests, and many failed to include representative microplastics or describe their methods in a replicable manner [26]. The challenges associated with conducting these validation steps largely relate to using reference microplastics that are representative of those found in the environment. Microplastics are a diverse contaminant suite varying in polymer type, shape, size, colour, and additive content [36]. These properties can affect microplastics recovery. For example, microplastics < 212 µm have been found to be difficult to extract from complex matrices, likely due to microplastic loss from multiple processing steps and difficulty visualizing particles in dirty samples [10]. Weathering of microplastics in the environment can also affect their identification in samples because of changes in colour and surface properties [37].

Reference microplastics can be difficult to source and researchers often create their own microplastics, each choosing different polymers, shapes, and sizes. One proposed solution is to use pre-made soda tablets or capsules with known amounts and types of microplastics that researchers can use to spike their sample matrix [12, 38], but even these come with their own challenges. For instance, in an ILC study comparing candidate reference materials (RMs), gelatin capsules did not dissolve completely when added to different matrices, the creation of tablets containing fibers was time consuming, and the relative standard error of tablets often increased as the size of added particles decreased [12].

Another difficulty of recovery rate tests is discriminating between the spiked microplastics and environmentally present microplastics in the sample matrix. One option is to remove environmental microplastics from substrates, via density separation, combustion, or chemical digestion, before spiking with reference microplastics. However, these processes are highly labour intensive and might impact the results by changing sediment properties that influence microplastics recovery (e.g., organic matter content [32]). Another option is to cite a previous study that used a simplified but non-representative matrix of the main study (e.g., sand) or perform their own recovery tests on this simplified matrix and apply the recovery rate to a more complex sample type (e.g., mud). There is scope for this type of approach (it makes sense to use the same method as a previous study for comparability), however, without recovery validation in the 'new' or different matrix, it is likely that microplastics extraction efficacies will differ significantly among sample types.

A case study of saltmarsh sediments

To demonstrate the necessity of representative recovery rate experiments we conducted a case study of microplastics recovery from saltmarsh sediments. Saltmarsh sediments are complex matrices comprising a high proportion of clays and silts, with a high organic content and small particulate size. Extraction of microplastics from such complex sediments is challenging, typically requiring multiple processing steps that can lead to higher rates of contamination and microplastic loss [15, 17, 18]. Therefore, it is important that studies validate the laboratory methods used to ensure the results are representative of microplastics in the environment.

Microplastic extraction from saltmarsh sediments

From our analysis of relevant literature (see SI for methods), we established that only three of 15 studies that sampled microplastics from tidal marsh or saltmarsh sediments performed their own validation tests [39–41] (Table S2), while four papers referenced a previous study's recovery rates [42–45]. Only two studies included fibers in their recovery tests [41, 44], while most used fragments or beads [39, 40, 42, 43, 45]. Particles tested included polyethylene, polypropylene, polyamide, polyvinyl chloride, polystyrene, polyethylene terephthalate, and crumb rubber, ranging from 0.16 to >5 mm (Table S2); most studies tested between one and three different polymer types and one tested six different polymers. It is clear that recovery rate tests are not used consistently for complex matrices.

Test protocols

We tested 27 protocols to isolate microplastics from saltmarsh sediments (Table S1). Protocols included a combination of density-separation, using sediment-microplastic isolation units or centrifugation with $ZnCl_2$, NaBr or rapeseed oil, and chemical digestion, using NaClO, Fenton's and H_2O_2. Protocols were adjusted with the aim of achieving both sample mass-reduction, to facilitate visualization of microplastics, and high recovery rates for all microplastic types. Tests #1–24 were part of an iterative approach to find a method that recovered over 70% of a given microplastic type and were often only tested once, if recovery was low. Tests #25–27 were used to determine the efficacy and precision of optimized protocols and are highlighted in the 'Method Comparison' section. All tests were conducted in a positive-pressure laboratory under a laminar flow hood to minimize contamination.

Test sediment was collected from two sites: tests #1–8 used sediment from a saltmarsh located on the north Norfolk coast, United Kingdom (52°57'34.7"N 1°01'04.8"E) and the remaining tests used sediment from a saltmarsh in the Tamar estuary, United Kingdom (50°23'18.6"N 4°18'30.0"W). Properties of sediment from each marsh were similar, with mean grain size of 11.4 µm ± 0.29 SE and total organic matter content of 11.6% ± 0.19 SE for the north Norfolk coast site and mean grain size of 11.3 µm ± 0.46 SE and organic matter content of 13.1% ± 1.2 SE for the Tamar estuary site. Grain size was measured using a particle size analyzer (Beckman Coulter LS230) and organic matter content was determined using a loss-on-ignition protocol [46]. All sediment was frozen and stored at -20°C. Prior to testing, sediment was prepared by freeze drying in a ModulyoD freeze dryer (Thermo Electron Corporation) for a minimum of two days. Once dried, sediment was stored in sealed glass jars at room temperature. For all tests, sediment was sieved with a 1 mm stainless-steel sieve to remove microplastic > 1 mm and thereby focus testing on the smaller size fraction of microplastics.

Recovery rate tests used four types of microplastics: polypropylene (PP) fragments, polyethylene (PE) films, polyamide (PA) fibers, and polyester (PET) fibers (Figure S1). PP fragments were created by filing a yellow PP box with a flat steel file, PE films were cut from an orange plastic bag, and both types of fibers were shaved with a metal scalpel from two types of fleece-like fabrics (purple PA and red PET). All microplastics were separately vacuum filtered onto mesh sizes between 10 and 1000 µm. Microplastics were added to dried sediment (pre-sieved to < 1 mm) and mixed with a stainless-steel spoon prior to each tested protocol. The exception was tests #14–17, where microplastics were added after the digestion step and prior to density separation to elucidate in which step microplastic loss was occurring.

For tests #25–27, which compared optimized protocols, a random number generator was used to determine the number of each microplastic type per sample to ensure a blind count for the analyst. Numbers were generated between 5 and 10 for each polymer and shape of microplastic, and total microplastics added ranged between 29 and 38 (Table S3). The longest dimension for each plastic type ranged from 412 to 945 µm (mean ± standard deviation (s.d.): 612 ± 136 µm) for PP fragments, 265 to 959 µm (mean ± sd: 500 ± 142 µm) for PE films, 338 to 1063 µm (mean ± sd: 708 ± 176 µm) for PA fibers, and 187 to 981 µm (mean ± sd: 657 ± 207 µm) for PET fibers (Fig. 1).

Mass reduction of sediments was determined by subtracting the post-processing dry weight from the original dry weight and reporting as a percentage of weight removed. Samples were air dried on nylon filters until they maintained a constant weight (Mettler AE 200). Microplastic recovery rates were determined by visualizing filters under an Olympus SZX16 microscope and enumerating and measuring reference putative microplastics using CellSens imaging software. Putative microplastics were characterized using micro-FTIR to confirm polymer type (Perkin Elmer Spotlight 400; reflectance mode; 4000–600 cm^{-1}; 4 scans).

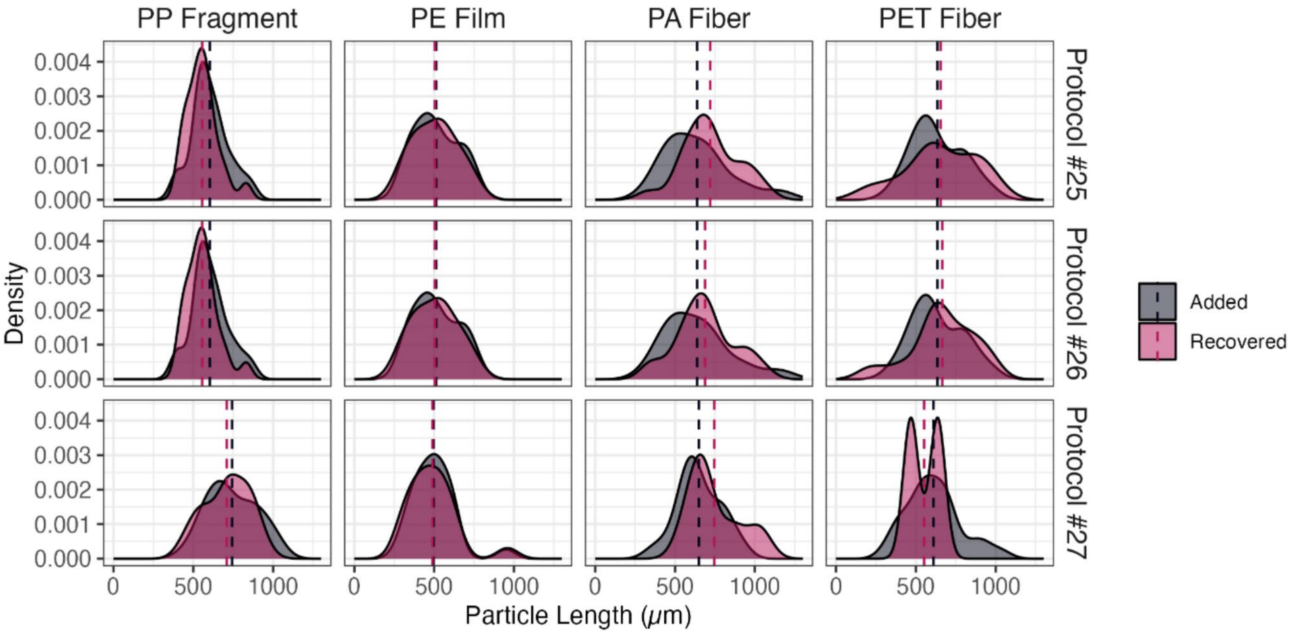

Fig. 1 Density plot showing the size distributions of microplastics added (grey) and recovered (pink) for tests #25–27. Dashed lines represent the mean

Microplastic recovery rates varied across tested protocols

The majority of tested protocols were successful at recovering fragments and films at acceptable levels, here defined as >70% recovery of a given microplastic type; however, recovery rates of microplastic fibers were typically < 50% (Table S1). Across all tested protocols, recoveries for PP fragments ranged from 40 to 100%, PE films ranged from 20 to 100%, PA fibers ranged from 0 to 99.5%, and PET fibers ranged from 0 to 120% (Table S1). The iterative approach shows how a method may need a lot of adjusting depending on the substrate of interest. Most methods tested in the literature have reported good recovery of microplastics but showed low recoveries when used here for saltmarsh sediments. For example, the oil extraction procedure (OEP), whereby oil and water are mixed with a sample and left to separate into solid, aqueous, and lipid phases, has been successfully used to recover microplastics from complex sediments across multiple studies [47–49]. However, we could not extract fibers consistently using the OEP (Table S1). Many studies that recommend the OEP do not include fibers in their validation tests. Only Lechthaler et al. (2020) used fibers to test recovery, but these were fiber agglomerates and were much larger than fibers typically found in the environment [49]. Differences in recovery rates might also stem from differences in the organic content of the substrate, the type of oil used, or the equipment set up [32, 47, 48]. Constant et al. (2021) also found lower recoveries using the OEP, especially for fibers and higher density microplastics [50]. However, the low recovery of fibers is not exclusive to the oil method. A review of extraction methods by Monteiro & Pinta da Costa (2022) indicated that fibers had lower recovery rates compared to fragments or granulates across multiple studies [33]. We also found this with a density separation using NaBr highlighted in the 'Method comparison' section below. Lower recoveries of fibers may be due to their sensitivity to chemical reagents and their unique morphology and flexibility may increase chances of passing through sieves [22, 33]. Overall, some methods may be appropriate for certain substrates but may not have the same recovery efficiency in others and using diverse and environmentally representative reference microplastics is imperative for increasing validation of analytical methods.

Regarding impacts on polymers, we found that some steps in the procedure did not affect particle colour for fragments or films but did affect fibers. We tested the effect of different concentrations of NaClO (0.5%, 1%, 2%, 4%, 7%) on the reference microplastics. Previous studies recommended 7.5% and 10% NaClO at 40–50 °C because it removed 88% and 92% of hard and soft organic materials, respectively, with minimal effects on polymer fragment weight [51]. However, we found a concentration of 7% NaClO removed the dye from the PA fibers (Figure S2). PA is often the most sensitive to digestion methods [51–54]. For our sediments, we found that 1% NaClO was most effective at reducing organic matter while still recovering all microplastics.

The centrifugation step also required some adjustments, as the number of rotations per minute affected recovery rates for fibers. We found that using a high RPM (i.e., 4000 RPM) during centrifugation did not recover fibers at high rates (< 50%) but was successful for fragments and films (Table S1, test #8). In comparing the efficacy of 500–2000 RPM, we found that between 1750 and 2000 RPM was the most ideal for extracting fibers while still allowing sediment to separate from the solution. A lower RPM is gentler on sample integrity and allows for a slower separation, potentially allowing more time for fibers to separate out from the sediment. Using a lower RPM still had some disadvantages. For instance, the sediment at the bottom of the tubes was more likely to come loose during vacuum filtration and rinsing, resulting in more sediment coated filters.

All methods reduced initial sediment mass by >99%. Past this level of mass reduction, filter clarity and number of filters were used as a proxy to compare treatment efficacy. The oil extraction procedure resulted in the clearest filters but had low and inconsistent recovery rates for all microplastic types. The method with the highest recovery rates (1% NaClO with NaBr centrifuge extraction; #23–26) maintained a layer of sediment on the filters and often resulted in 1–7 filters per sample, depending on original sample mass (10 g vs. 40 g). More filters required more time to count the microplastics and the layer of sediment would have inhibited micro-FTIR focal plane array (FPA) imaging as a feasible alternative. Using a smaller sample mass (10 g) allows for fewer filters for analysis but could limit the representativeness of the sample [24]. The trade-off is a higher sample mass that would require more analytical effort. Ultimately, there is a balance that must be met between sediment mass reduction and microplastic recovery.

Here, we took protocols from the literature that were commonly used for sediment microplastic analysis and applied them to saltmarsh sediments– a difficult matrix to extract from owing to its high organic content and small grain size. These tests show that complex substrates will need adjustments to microplastic extraction methods depending on access to materials and matrix properties. Differences in equipment, chemical concentrations, sample mass, and procedural order will all affect microplastics recovery rates. There is no method that will work for every sample type or laboratory, but these differences can be controlled if microplastic recovery rates are reported and potentially used as a correction factor.

Method comparison

Optimized protocols were repeated and compared to test efficacy and precision (tests #25–27). A procedural blank was run for each method ($N = 3$) following the same procedure as spiked samples but without sediment or reference microplastics. No contamination was observed on the filters. These tests used the same sample mass (40 g), digestion solution (1% NaClO), and density separation solution (NaBr, d = 1.48 g/mL) but differed in the density separation set up:

Protocol #25 and #26

Each 40 g sample was split into four 10 g subsamples and digested overnight with 50 mL each of 1% NaClO (50 °C, 120 RPM) in an orbital incubator (Sanyo). Each subsample was sieved onto a 20 µm stainless-steel sieve (Fisherbrand, ISO 3310/1), triple rinsed with ultrapure water, rinsed once with NaBr, then rinsed into 50 mL falcon tubes and topped with NaBr to the 50 mL mark. Contents were mixed for 5 min by shaking falcon tubes manually and with a vortex. Samples were centrifuged at 1750 RPM for 5 min with 5 brake speed. The supernatant was vacuum filtered onto 20 µm nylon mesh and triple rinsed with ultrapure water. For protocol #26, samples were centrifuged a second time, by adding 35 mL NaBr to the remaining sediment in the falcon tubes after the first supernatant was decanted, homogenizing the sample by hand and with a vortex, centrifuging, and filtering as previously described.

Protocol #27

Spiked sediment was incubated overnight with 200 mL of 1% NaClO at 50 °C and 120 RPM in an orbital incubator (Sanyo). Digested sediment was sieved onto a 20 µm stainless steel sieve (Fisherbrand, ISO 3310/1), triple rinsed with ultrapure water, followed by one rinse with NaBr, and then rinsed with NaBr into a 250 mL glass beaker. Sediment-Microplastic Isolation (SMI) units were prepared by purging the ball valve with NaBr and left to settle for 5 min before filtering off any potential contamination in the unit, as per Coppock et al. (2017) [55]. The sample, a magnetic stir bar, and additional NaBr was then added to the SMI unit. The glass beaker was rinsed with NaBr into the SMI to ensure all sample contents transferred. Each sample was mixed for 5 min on a stir plate, then left to settle in the SMI unit for 24 h. The next day, the supernatant above the headspace was vacuum filtered onto a 20 µm nylon mesh filter.

Average recovery rates for all reference microplastics were 84% (Protocol #25), 95% (Protocol #26) and 49% (Protocol #27). All methods were successful at recovering >70% of the fragments and films, however protocol #27 recovered <22% of fibers (Fig. 2). The added centrifuge step in protocol #26 increased the recovery rates of fibers but not fragments or films. While this added step increased recovery rates, it required seven filters (compared with three filters for protocol #25) and increased overall processing time by an hour. There were two replicates in protocol #25 and #26 where recovery of PP fragments was higher than the number of added fragments. While this could be from contamination, such fragments were not observed in the procedural blank; visual observations and FTIR analysis suggest that the spiked PP plastics broke apart during sample processing. Moreover, the size distributions between the added and recovered

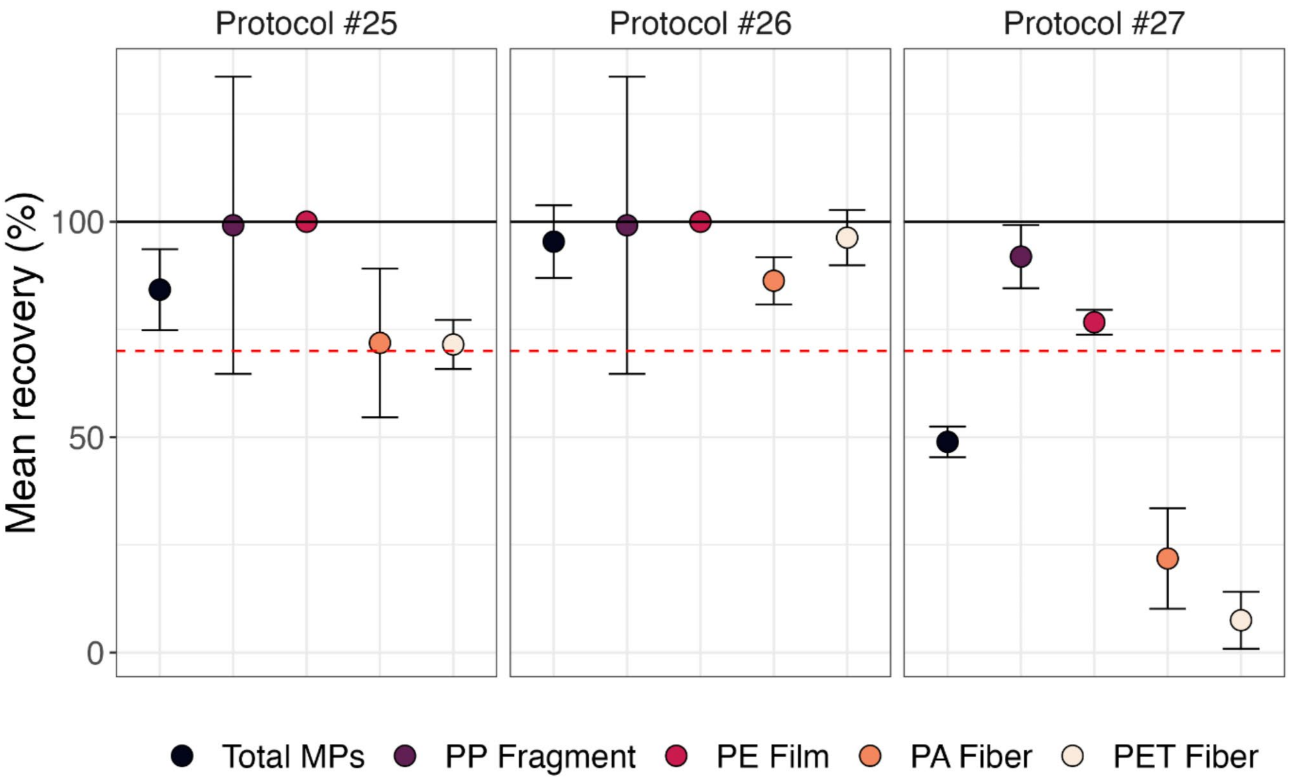

Fig. 2 Mean ± standard deviation of microplastic recovery rates for three protocols (*n* = 3; Protocol #25: Centrifuge, Protocol #26: repeated centrifuge step, Protocol #27: SMI unit). Red dashed line marks 70% recovery rate. Black solid line marks 100% recovery rate. Black circles = total microplastic recovery, purple circles = polypropylene (PP) fragments, pink circles = polyethylene (PE) film, orange circles = polyamide (PA) fibers, white circles = polyethylene terephthalate (PET) fibers

PP fragments were similar, with a slight skew toward the lower size range in the recovered fragments (Fig. 1). Radford et al. (2021) also observed increased fragmentation of spiked microplastics in a $ZnCl_2$ separation [32]. Using a mass-based approach to quantify microplastics could help account for potential fragmentation during laboratory processing. However, many studies report environmental microplastic concentrations as a count-based value and researchers may be overestimating some polymers due to fragmentation in the extraction process. Both mass and number-based techniques are important for capturing the impacts of extraction protocols on microplastic recovery, though not every laboratory will be equipped to measure both units and this will be dependent on the research objectives.

In Coppock et al. (2017), the SMI unit worked well for recovering fragments and filaments from sand and silty sediments [55]. However, these sediments were collected from sites without vegetation and likely had a lower organic matter content compared to the sediments used here. In contrast, only one method (protocol #26) in our study showed recovery of fibers > 80% with the salt-marsh sediments. These comparisons show that there are limitations to any method and the composition of a sample substrate alters the effectiveness of a method. We therefore cannot rely on previous studies recovery rates when applying a method to a new matrix. However, many methods are useable and can be applied in different contexts.

Limitations and further considerations

Moving forward, the challenge will be harmonizing reference materials and the procedure of validation tests. Matrix-matched certified reference materials (CRMs) could be used to validate analytical methods, provide metrological traceability of the results, and generate uncertainty budgets [56–59]. This involves creating a matrix resembling the sample matrix of interest (e.g., sediment) containing a known and verified amount of analyte (e.g., microplastics) and is accompanied by a certificate containing the value of the specified property, its associated uncertainty, and a statement of metrological traceability [57, 58, 60, 61]. Matrix-matched CRMs have been used for the analysis of organic and inorganic pollutants such as airborne dust, heavy metals, pesticides, and PAHs [57, 59, 60]. However, there are many difficulties in creating certified reference materials. The creation of CRMs is often dependent on the creation of standardized analytical methods, generating a causality dilemma [60, 62].

Additionally, different environments (marine vs. freshwater; water vs. sediment) and local economic activities (industrial facilities, fishing ports, tourist areas, etc.) may have different compositions of microplastic pollution. Method validation tests for these different systems might require varying combinations of microplastic polymers, shapes, and sizes as reference microplastics to ensure validation tests are representative of what might be expected in a local area. Other considerations for reference microplastics include using aged microplastics and natural particles (e.g., cotton fibers) [7, 10, 33]. Finding reference microplastics that are representative of environmental microplastics while still being identifiable from ambient environmental contamination will be difficult, unless matrix-matched CRMs are produced. Alternatively, reference microplastics could be fluorescently tagged for easy identification. Some studies have used laboratory made sample matrices and controlled the amount of organic matter and soil properties to avoid ambient environmental contamination [32]. Though not yet standardized, this technique could be used to allow adjustments based on the specific sample composition of interest. This, in combination with the reference materials being developed by many laboratories, could be a path forward for the harmonization of microplastics research [12, 38, 61, 62].

Analytical methods to extract microplastics will always differ across laboratories based on availability of funding and resources, the research question, type of substrate, and experimental design. A one-size-fits-all approach would limit access to microplastics research and hinder scientific creativity. Standardized protocols with measurement standards should be reserved for accredited laboratories focused on monitoring. Where the research question investigates beyond monitoring, it may be more valuable to apply harmonized recovery rate tests with verified and diverse reference materials. Results can then be compared via extraction efficiencies with known uncertainties rather than applying a single protocol across one matrix with varying properties. Ultimately, the microplastics research field can only advance when recovery rate tests become a requirement and reference materials are harmonized to facilitate the comparability and reliability of field data.

Supplementary Information

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Supplementary Material 1

Supplementary Material 2

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Author contributions

HKM: Conceptualization, Methodology, Formal Analysis, Investigation, Visualization, Writing– Original Draft. PKL: Conceptualization, Methodology, Supervision, Writing– Review & Editing. TJT: Conceptualization, Methodology, Supervision, Writing– Review & Editing. MC: Conceptualization, Funding acquisition, Methodology, Supervision, Writing– Review & Editing.

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Data availability

Data is provided within the manuscript or supplementary information files.

Declarations

Competing interests

The authors declare no competing interests.

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