



Comparison of microplastic isolation and extraction procedures from marine sediments^{☆, ☆ ☆}



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ABSTRACT

Microplastics (MPs) are small (< 5 mm) plastic particles which pose a threat to marine ecosystems. Identifying MPs is crucial for understanding their fate and effects. Many MP extraction methods exist, but procedural differences prevent meaningful comparisons across datasets. This method comparison examines the efficiency of five methods for extracting MPs (40–710 μm) from marine sediments. Known quantities of MPs were spiked into sediments. The MPs were extracted and enumerated to demonstrate percent recovery. Findings determined that sediment matrix, MP properties, and extraction method affect the percent recovery of MPs from sediments. Average recoveries of spiked microplastics were between 0 and 87.4% and varied greatly by sediment type, microplastic, and method of extraction. In general, larger particle and lower density MPs were more effectively recovered. Marine sediments low in organic matter and with larger grain size also had higher percent recoveries of MPs. These findings support the need for method optimization and unified procedures.

1. Introduction

Oceanic plastic pollution has garnered international attention as an example of waste mismanagement. Over 8.3 billion metric tons of plastic have been produced globally since the 1950s. Plastic consumption has surpassed the capacity of modern recycling infrastructure, leading to mismanaged disposal and environmental pollution. It is estimated that 8 million tons of plastic enter the oceans from land each year (Jambeck et al., 2015). However, floating plastics account for only 1% of the expected 8 million tons of plastic entering oceans annually (Van Sebille et al., 2015). Much of the 99% of the remaining plastics are expected to degrade into plastic fragments < 5 mm, known as microplastics (MPs) (Murphy, 2017) through a series of physical, chemical, and biological processes (Van Cauwenberghe et al., 2013), and ultimately accumulate in sediments (Fig. 1) (Andrady, 2011; Browne et al., 2011; Hidalgo-Ruz et al., 2012; Kowalski et al., 2016). MPs are quickly

rising to the forefront of emerging contaminant studies due to their unique quantification challenges and unknown toxic effects. Given this situation and their potential for environmental impacts, it is critical for researchers to have scientifically robust methods for extracting and isolating MPs from sediments.

There is an extensive list of published methods for isolating MPs from sediments (Prata et al., 2019; Mai et al., 2018). The variety of published procedures reflects the unique challenges associated with isolating MPs from marine sediments. Differences in extraction and isolation procedures ultimately determine the ability of various MPs to be accurately recovered and quantified, resulting in a wide range of recovery efficiencies. Therefore, it is difficult to compare MP recovery rates (number of plastic particles per sample) between environmental samples using different procedures. Developing methods to address a range of sediment and plastic matrices has resulted in a wide variety of extraction techniques. Procedural differences include the mass of

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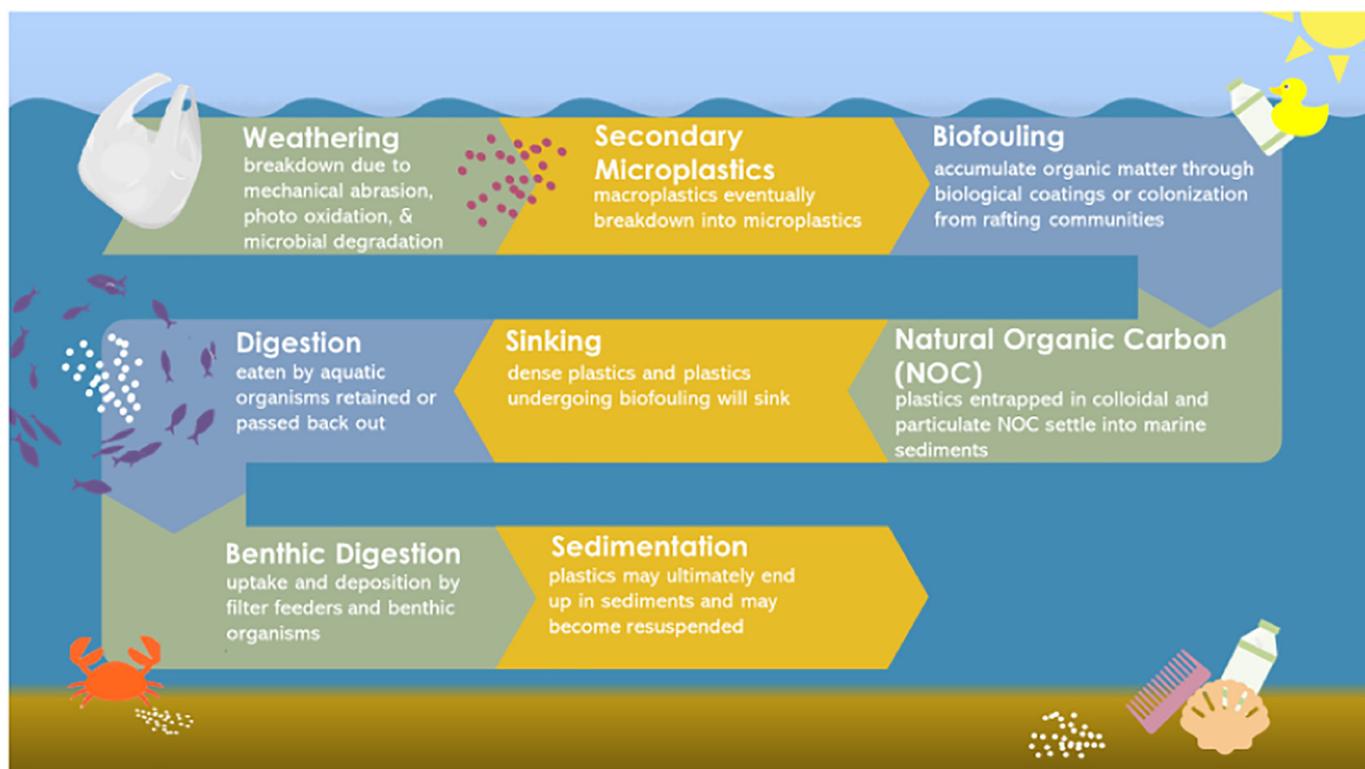


Fig. 1. Suggested pathways for microplastics in marine environments.

sediment samples, sample preparation, and sample handling. With no sediment standard reference material for MPs, methods are developed with an array of sediment and plastic matrices. Sediments may go through pretreatment steps including oven drying (Su et al., 2018; Gilbreath et al., 2019; Nuelle et al., 2014), pre-sieving of coarse or fine materials (Gilbreath et al., 2019; Zobkov and Esiukova, 2017), or chemical oxidation (Zobkov and Esiukova, 2017; Masura et al., 2015; Maes et al., 2017; Hurley et al., 2018). The method efficacy is often dependent on sediment composition. Sediment properties such as grain size, organic matter content, and mineralogy largely affect results and method complexity. Differentiating between plastic and non-plastic particles in environmental samples is another major obstacle in isolating and visually identifying microplastics (Shaw and Day, 1994; Tamminga et al., 2017). Recent studies indicate that using selective fluorescent stains, such as Nile Red, may improve the detection of MPs in environmental samples (Maes et al., 2017; Shim et al., 2016). Nile Red is a fluorescent stain that adheres to hydrophobic substances including lipids and plastic. Hypothetically, staining environmental samples with Nile Red reduces the likelihood of false positive identification (Vianello et al., 2013).

This research assessed five current methods for the extraction and isolation of MPs from marine sediments. Two sediments (one sandy and one silty) were used as representative matrixes for amending known quantities of five common types of MPs. These matrixes are considered representative of the types of sediments found along the coasts of the United States including sand occurring at beaches and typical subtidal temperate silty muds. Microplastics are known to accumulate in these types of sediments. Consequently, methods for isolating and extracting MPs from sediments would need to be functional with these types of matrixes. The MPs were chosen to represent a range of MP polymer types, shapes, sizes, densities, and colors. Nile Red was also evaluated for improving MP visibility during extraction and isolation. We present a comparison of the efficiency of commonly used methods to characterize the number and types of MPs in marine sediments.

Most techniques used to isolate MPs from marine and estuarine

sediments involve density separation (i.e., floatation) by agitating sediment samples with aqueous salt solutions (Thompson et al., 2004). Methods that rely on floatation separations are restricted by the density of their respective salt solutions. Common plastics range in density from 0.8–2.35 g/cm³ (Hidalgo-Ruz et al., 2012). Low density salt solutions such as sodium chloride may be insufficient to separate higher density plastics from sediment (Hidalgo-Ruz et al., 2012; Coppock et al., 2017). However, high density salts (e.g., NaBr, NaI, ZnCl₂) may not allow differentiation among plastics and other sediment components making separation from sediment particles difficult. In addition, the various salts used in density separation methods vary greatly in price, toxicity, reactivity, and waste disposal. These considerations can be restrictive or prohibitive to laboratories seeking to use higher density salts.

Many extraction methods favor low density plastic particles, but environmental microplastics include a myriad of high-density plastic polymers, including polyester (Browne et al., 2011; Lusher et al., 2014; Lusher et al., 2013; Nor and Obbard, 2014), polyethylene terephthalate (PET) (Nor and Obbard, 2014; Peng et al., 2017), and polyethylene (PE) (Rios et al., 2007). Small variations in plastic chemical composition lead to large differences in polymer properties (Brydson, 1999). It is important to consider that many methods may inadvertently select for specific polymer fragments (i.e., microplastic spheres versus fibers) based on their physical properties (Nel et al., 2018). Another complication is microplastics stimulate biofilm formation. MPs with biofilm will increase their particle mass and complicate density separation (Rummel et al., 2017; Zettler et al., 2013). Surficial biofilms often mask the polymer type from spectrometers and can effectively camouflage plastic particles embedded within sediments. While this study does not address the complications of polymer spectrometry, many isolation methods use chemical oxidation as a means of further separating organic material and removing biofilms from the microplastic-sediment matrix (Gilbreath et al., 2019; Zobkov and Esiukova, 2017; Masura et al., 2015; Coppock et al., 2017). In summary, several factors greatly affect the overall method performance for recovering MPs from marine sediments. Many isolation and extraction methods exist, but there is no

information on their relative performance.

2. Materials and methods

2.1. Experimental set-up

Methods were chosen to represent a wide range of commonly used, literature documented procedures, and for the ability to be performed easily and inexpensively. Other considerations included minimal waste generation, low start-up costs, simple equipment and instrumentation set-up, and overall quick processing time. Each method was assessed using two model sediments, silty sediment from Long Island Sound, New York (USA) and beach sand from Narragansett Beach, Rhode Island (USA). Long Island Sound sediment (LIS) is a well characterized, fine-grained sediment, collected using a Smith MacIntyre grab sampler (0.1 m²) in September 2010 (Ho et al., 2000). Narragansett Beach sand (NAR) was collected by hand from the intertidal zone using a metal shovel in January of 2018. Sediments were press sieved through a 2 mm sieve prior to analysis to remove any coarse fragments, and the NAR was heated in a muffle oven at 550 °C for 6 h to remove organic material. Representative samples were analyzed for particle size distributions using a Restech CamSizer P4 (Haan, Germany) (Table 1).

Five representative MPs were amended in known quantities into each sediment sample to evaluate the recovery efficiency of the selected microplastic extraction methods. The representative microplastics reflect a wide variety of polymer type, fragment shape, and particle size. For this study, we analyzed MPs ranging from 40 to 710 μm. The plastics used for spiking included polystyrene (PS), PE, polyvinyl chloride (PVC), PET, and polypropylene (PP) (Table 2). Fluorescent colored MPs were chosen for their ability to be easily enumerated as spiked reference materials. Both the PS and PE microbeads were purchased from Cospheric LLC (Santa Barbara, CA, USA). The other three microplastics purchased were PVC pipe (Home Depot, GA, USA), PET embroidery floss (J&P Coats, Middlesex, UK), and PP rope (SeaChoice, Pompano Beach, FL, USA). These three plastics were ground or cut into small pieces and sieved through a series of stacked mesh sieves to obtain desired size classes (Table 2). MPs were stored in a glass jar containing filtered seawater (20 μm) from Narragansett Bay (Narragansett, RI, USA) for a minimum of two weeks at 20 °C to develop a biofilm. Prior to sediment addition, each MP particle was individually inspected microscopically (Nikon SMZ745-T, Nikon, Minato, Tokyo, Japan) for shape abnormalities or fragmentation by two analysts. After inspection, a minimum of twenty plastic pieces per polymer type were carefully transferred to a sediment sample (20 pieces * 5 plastic types = 100 pieces of plastic/sample). The plastic-amended sediments were mixed on a roller mill (4 RPM) at 4 °C for a minimum of 48 h.

2.2. Quality control

Each method was evaluated with a total of 12 spiked sediment samples. An additional two sediment blanks (one sediment blank per sediment type) and a water blank were used per method to assess background and cross contamination during extraction. Airborne background contamination was assessed with one air blank per sample extraction. Air blanks were collected by wetting a 20 μm polycarbonate track-etched (PCTE) filter (Poretics, GVS North America, Sanford, ME, USA) with deionized water and placing the filter into a glass petri dish

covered in aluminum foil. The foil cover was removed whenever the working samples were exposed to air to assess possible air-born contamination. Each filter was inspected under the microscope with both normal light and UV excitation using a NightSea (Lexington, MA, USA) fluorescence filter (Excitation 360–380 nm, emission 415 nm long pass) to quantify the number of particles adhered to the filter. Further information on clean laboratory setup and quality control can be found in the Supplemental information section.

2.3. Methods compared

The following section outlines the general approach of each method. Each method was explicitly followed as described by the authors unless stated below. Detailed extraction steps can be found in published methods. Methods will be referred to by the last name of the first author for the remainder of this manuscript.

Fries et al. (2013): This method is a density separation approach using sodium chloride (NaCl) solution ($\rho = 1.2 \text{ g/cm}^3$). Wet sediment samples (175 g) underwent extraction in 2 L glass separatory funnels with the NaCl solution. Samples were vigorously shaken to float microplastics to the NaCl solution surface. After a settling period, sediment was removed through the bottom port of the separatory funnel. Suspended MPs in NaCl solution were filtered onto a 20 μm PCTE membrane filter and visually inspected using a Nikon SMZ745-T microscope.

Gilbreath et al. (2019): This manuscript extracts MPs from bio-retention ponds, but uses a methodology commonly used to extract MPs from sediments. This method is a modified version of **Stolte et al. (2015)** that extracts MPs > 45 μm using a calcium chloride (CaCl₂) solution ($\rho = 1.4 \text{ g/cm}^3$). Sieved (> 45 μm) sediment samples (150 g) were split into size fractions (45–500 μm, 501–1000 μm) and placed in 600 mL glass beakers with CaCl₂ solution. Samples were stirred vigorously and left to settle. All floating materials were transferred using a metal spoon to 1-L glass separatory funnels filled with CaCl₂. From there, separatory funnels were shaken, and the suspension allowed to settle. After settling, floating materials were filtered onto a 20 μm PCTE membrane filter and visually inspected using a Nikon SMZ745-T microscope.

Nuelle et al. (2014): This method is a density separation approach that uses both NaCl ($\rho = 1.2 \text{ g/cm}^3$) and sodium iodide (NaI) ($\rho = 1.8 \text{ g/cm}^3$) solutions. Sieved (< 1 mm) and dried (60 °C) sediment samples (1 kg) were initially separated with air induced overflow (AIO), which uses an aerated NaCl solution for density separation. Sediment samples were fluidized using the AIO method, which floats the MPs out of the fluidized sample causing them to overflow into secondary containment. All materials in secondary containment were transferred to 500 mL glass volumetric flasks filled with NaI solution. The volumetric flasks were shaken and decanted after a settling period. All decanted materials were filtered onto a 20 μm PCTE membrane filter and visually inspected using a Nikon SMZ745-T microscope.

Coppock et al. (2017): This method is a density separation approach that uses zinc chloride (ZnCl₂) solution ($\rho = 1.5 \text{ g/cm}^3$) and a sediment microplastics isolation (SMI) unit. The SMI unit was constructed in our laboratory. Sediment samples (70 g) were placed in the SMI unit with ZnCl₂ and a stir bar. Plastics were separated through density separation driven by mixing with the stir bar. After settling, all floating materials were filtered onto 20 μm PCTE filters and underwent oxidation (30%

Table 1

Physical properties and sampling locations for representative sediments: Long Island Sound and Narragansett Beach. Sediment sizes classified using grainsize diameter 10, 50, and 90% cumulative percentile value.

	D ₁₀ (μm)	D ₅₀ (μm)	D ₉₀ (μm)	Water wt/wt%	Organic carbon %	GPS coordinates of collection location
Long Island Sound (LIS)	4.1	13.7	62.6	43	2	41° 7'N 72° 52'W
Narragansett Beach (NAR)	179.1	251.6	345.2	< 1	0	41° 26'N 72° 27'W

Table 2
Properties of microplastics used in this investigation.

	Size (μm)	Density (g/cm^3)	Shape	Color	Source
Polystyrene (PS)	40	0.96	Sphere	Transparent	Cospheric
Polyethylene (PE)	96–106	1.13	Sphere	Blue	Cospheric
Polyvinyl chloride (PVC)	500–710	1.35	Fragment	Orange	PVC pipe
Polyethylene terephthalate (PET)	250–500	1.38	Fiber	Pink	Embroidery floss
Polypropylene (PP)	500–710	0.91	Fiber	Yellow	Rope

H_2O_2) for 1 week. Oxidized samples were filtered onto new 20 μm PCTE filters and visually inspected using a Nikon SMZ745-T microscope.

The referenced ball valve used by Coppock et al. (2017) to construct the SMI was not commercially available in the United States. Therefore, our laboratory opted for a PVC ball valve constructed from a 6.4 cm slo-close valve made by Colonial Engineering Inc. (Portage, MI, USA). The unit was constructed using 63 mm outer diameter PVC piping with the ISO ball valve fixture adhered to a PVC plate (SI Fig. 1). Details for construction and operation can be found in Coppock et al. (2017).

Zobkov and Esiukova (2017): This method is an adaptation of the laboratory method published by the National Oceanic and Atmospheric Administration (NOAA) (Masura et al., 2015) that uses ZnCl_2 ($\rho = 1.6 \text{ g}/\text{cm}^3$) for density separation followed by oxidation catalyzed with a heated water bath. Sediment samples (400 g) were added to glass beakers containing aqueous ZnCl_2 solution. After stirring with stainless steel spoons and settling, floating debris and supernatant were filtered through a 170 μm stainless steel sieve. Debris retained on the sieve were rinsed into clean glass beakers by tilting the sieve and rinsing into a clean beaker with the addition of a 30% H_2O_2 and Fe (II) catalyst solution. Beakers were covered with aluminum foil and placed in a hot water bath (75 $^\circ\text{C}$) for 15 h. A solution containing 4.5% hydrochloric acid (4.5%) was then added to each beaker. Samples underwent another round of density separation with ZnCl_2 and then were filtered onto 20 μm PCTE filters and visually inspected using a Nikon SMZ745-T microscope.

2.4. Plastic characterization

Each sample was ultimately filtered onto a 20 μm PCTE membrane filter for visual inspection. Samples high in organic matter and sediment were often filtered onto several separate filters to more evenly distribute the debris including MPs. Filters were visually inspected under the microscope (Nikon SMZ745-T) using 2 \times magnification and identified as spiked MPs with white and fluorescent light. Physical properties of spiked MPs (color, fluorescence, shape and size) made them easily identifiable with microscopy. Two people verified each MP count using both normal light and cyan excitation with a NightSea (Lexington, MA, USA) fluorescence filter (excitation 490–515 nm, emission 550 nm long pass). Samples were recounted if there were discrepancies between the MP counts by both analysts. Spiked microplastics were counted on each filter and tallied by polymer type. Filters from sediment blank samples and water blank samples were visually inspected in the same manner. All filters were stored at 20 $^\circ\text{C}$ in glass petri dishes with foil lids after identification.

2.5. Nile Red addition

A secondary objective of this study was to determine the effects of Nile Red (NR) staining on the observational counting of microplastics from each sample. Lipophilic dyes such as Nile Red help differentiate microplastics from their environmental matrices during visual observation. Nile Red was purchased from Thomas Scientific (MP Biomedicals, Solon, OH, USA). A NR stock solution was prepared at 0.05 g/L in acetone according to methods developed by Maes et al. (2017). Prior to staining experimental samples, a laboratory trial was performed to determine an appropriate staining concentration and

temporal duration to effectively stain the five model plastics. The best results were obtained with a concentration of 0.025 g/L NR for a staining duration of 10 min.

As described previously, post-processed filters from each method were analyzed to determine percent recovery of MPs. After analyzing percent recovery, a subset of these filters were stained with NR and recounted to determine if NR staining affects percent recovery based on visual observation. Two samples (one sandy and one silty) from each method were randomly selected for staining. Samples were mounted onto a vacuum filter apparatus and stained with 10 mL of 0.025 g/L NR solution for 10 min, ensuring the entire filter was covered with stain. After 10 min, the samples were filtered and thoroughly rinsed with DI water to remove all NR stain. The filters were then inspected under on a Nikon SMZ745-T microscope equipped with NightSea fluorescence filter (excitation 490–515 nm, emission 550 nm long pass) to recount microplastics. MP counts were compared for each filter pre- and post-NR staining to determine whether NR affected percent recovery.

2.6. Statistical analysis

Mean percent recovery of microplastics achieved by each method was determined as a function of polymer and sediment type using Microsoft Excel (2016). All analyses of variance (ANOVA) were performed using the SAS statistical software (SAS Institute Inc., Cary, NC, USA; Version 9.4). Statistically significant differences ($p < 0.05$) among methods were determined for each polymer and sediment type using ANOVA. Significant differences identified by the ANOVA were further analyzed with a Bonferroni *f*-test to identify significant differences among recovery rates. A recovery threshold of 70% was determined before the start of experiments as a desirable recovery rate to evaluate the effectiveness of each method. A one-way *t*-test was used to compare each mean recovery to the 70% threshold between method, sediment type, and plastic. Samples with average percent recoveries of 0% were excluded from the *t*-test. Mean recoveries of MPs pre- and post-Nile Red staining were determined for each polymer type and then analyzed using a one-way *t*-test to determine significant ($p < 0.05$) differences.

3. Results and discussion

3.1. Overall trends

Recovered MPs were compared against known spiked MP quantities to determine percent recovery for each sample (SI Tables 1; 2). Overall, mean recoveries were slightly better in the sandy sediment with non-zero values ranging from 5% to 87% in the NAR sand compared to 2% to 77% in the silty LIS sediment (Fig. 2). In addition, 36% of the recoveries in the NAR exceeded 50% while only 20% of the recoveries from the silty sediment exceeded 50% (Fig. 2). Mean recoveries for PVC ranged from 33–86% for sand and 11–68% for silt. PE recoveries ranged from 32–61% for sand and 0–52% for silt. For both types of sediments, recoveries of PS were very low ranging from 0% to less than 20%. Mean recovery of PET ranged from 5–68% for sand and 2–58% for silt. Finally, for PP, recoveries ranged from 23–87% for sand and 0–77% for silt.

Across all methods, the hierarchical ranking of mean recovery of

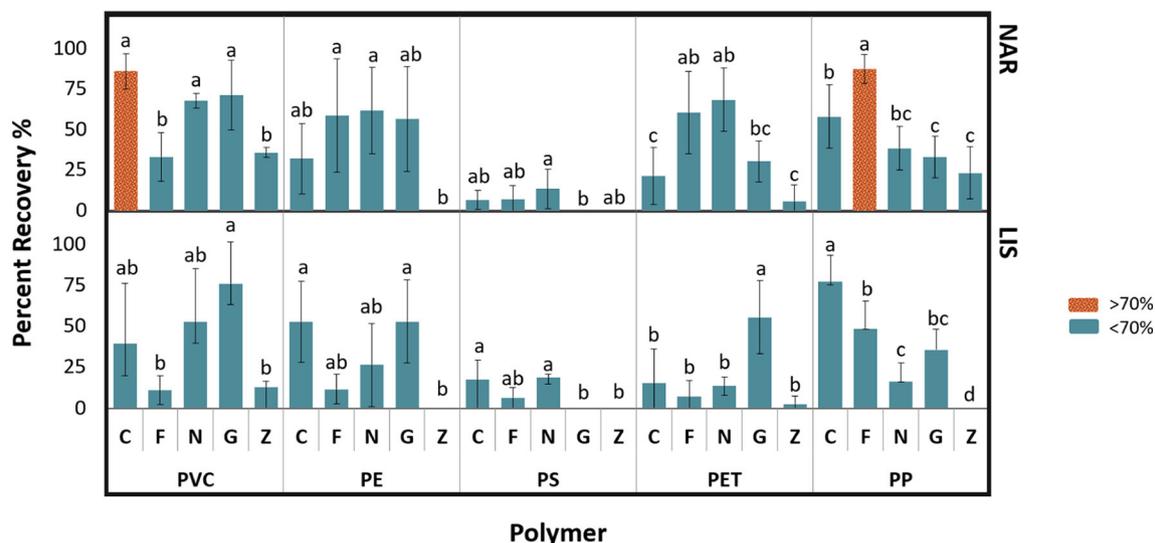


Fig. 2. Mean percent recoveries of microplastics (error bars are the standard deviations). Different letters represent statistical differences between polymer recovery ($p < 0.05$) per sediment type and method. Group “a” mean percent recovery is significantly greater than group “b”, which is significantly greater than group “c”. Bars with two letters are not significantly different from either group. Orange bar color signifies samples with significantly greater ($p < 0.05$) than 70% recovery. X-axis is organized first by plastic type (PVC = polyvinyl chloride, PE = polyethylene, PS = polystyrene, PET = polyethylene terephthalate, and PP = polypropylene), and further subdivided by extraction method (C = Coppock, F = Fries, N = Nuelle, G = Gilbreath, and Z = Zobkov).

MPs by polymer was the same for both sediment types. PVC had the highest recovery (i.e., $59 \pm 25\%$ sand; $43 \pm 35\%$ silt), followed by PP ($53 \pm 27\%$ sand; $40 \pm 29\%$ silt), PE ($48 \pm 29\%$ sand; $34 \pm 29\%$ silt), PET ($43 \pm 29\%$ sand; $23 \pm 25\%$ silt), and PS ($6 \pm 9\%$ sand; $8 \pm 10\%$ silt). Mean recoveries across methods were higher in sandier sediment than silty sediments for each polymer, except for PS as the small size of PS ($40 \mu\text{m}$) prevented high recoveries for all methods. In general, the quantitative ranking of polymer recovery followed the size-ranking of each MP. That is, PVC and PP were the largest MPs ($500\text{--}710 \mu\text{m}$), and the most highly recovered from both sediments. PE ($96\text{--}106 \mu\text{m}$) was the next highest recovered, followed by PET ($250\text{--}500 \mu\text{m}$). Although PET was classed as a larger particle than PE, the fiber diameter ($20 \mu\text{m}$) in contrast to their long length made their recovery more difficult. PS was generally the most difficult MP to recover and was also the smallest plastic studied ($40 \mu\text{m}$). There was no recovery of PS from either the Zobkov ($170 \mu\text{m}$) or Gilbreath ($45 \mu\text{m}$) methods, as the initial sieve step for both methods removed smaller-sized particles.

There was no statistically significant trend of quantitative ranking of polymer recovery based on MP density. The ranking of density from greatest to least (Table 2) was PET, PVC, PE, PS, and PP, whereas the ranking of mean recovery from greatest to least was PVC, PP, PE, PET, and PS. However, it is challenging to draw comparisons among MPs based on properties without noting that MP color and shape may also affect recovery efficacy (i.e., colorful plastics are easier to see microscopically). There was no consistent pattern of quantitative ranking of polymer recovery based on method for either sediment type. More specifically, there was no recovery of PE from the Zobkov method, and no recovery of PS from the Zobkov or Gilbreath methods.

In a quantitative ranking of methods based on mean percent recovery, the Gilbreath method was the most successful at recovering dense plastics (PVC and PET) from silty sediments. The Coppock method was the most effective method for recovering light plastics (PP and PE) from silty sediments. The Nuelle method recovered the most PET, PS, and PE from sandy sediments, as well as PS from silty sediments. Overall, the Zobkov method was found to be the least effective for the isolation and extraction of our preselected microplastics. This is likely due to the higher size fraction cutoff of our samples ($45 \mu\text{m}$ Gilbreath and $175 \mu\text{m}$ Zobkov). It should be stressed that these quantitative rankings are not method recommendations. The difference in

ranking was often a vanishingly small margin, and this ranking does not consider the method's efficacy or recovery rate variability.

The Zobkov method consistently ranked the lowest in recovery per polymer and sediment type, but this is likely due to method constraints from size cutoffs. Many of the MPs tested for this study were smaller than the detection limit for this method (i.e. $< 175 \mu\text{m}$). Overall, the Fries method and Nuelle method had higher recoveries for most plastic polymers in sand. The Fries method yielded the highest mean recoveries for PET ($59 \pm 25\%$), PP ($87 \pm 9\%$) and PE ($59 \pm 35\%$) while the Nuelle method ranked the highest for mean recoveries of PE ($62 \pm 27\%$), PET ($68 \pm 20\%$), and PS ($13 \pm 12\%$). However, the Coppock method achieved a mean 86% recovery of PVC in sand, the second highest recovery of any polymer by any method. The Coppock method and Gilbreath method generally have the highest mean recoveries of plastic polymers in silty sediments. In addition, the Coppock method had the highest mean recoveries for PE ($53 \pm 25\%$), PP ($77 \pm 16\%$), and PS ($17 \pm 12\%$). The Gilbreath method had highest mean recovery for PE (tied with Coppock, $53 \pm 25\%$), PET ($55 \pm 22\%$), and PVC ($76 \pm 25\%$).

3.2. Comparison of recovery to a standard

When performing relative comparisons, we established a target goal of $\geq 70\%$ recovery as achievable and desirable. No singular method effectively or consistently recovered $> 70\%$ of each polymer in either sediment. The mean recovery was significantly greater than 70% in only two extractions from NAR sand. PP plastic was extracted with a mean efficiency of $87\% (\pm 9\%)$ using the Fries method and PVC was extracted with a mean efficiency of $86\% (\pm 11\%)$ using the Coppock method. This analysis indicates that less than 10% of the isolation and extraction procedures meet the sandy sediment 70% standard and none of the procedures met the silty sediment standard. Had the standard been set at the low value of $\geq 50\%$ recovery, 40% of the isolation and extraction procedures have met or exceeded the standard for sandy sediment. For the silty sediment, the procedures meeting the standard were approximately 30%.

3.3. Variability associated with the methods

Coefficients of variance ($\text{CV} = (\text{standard deviation}/\text{mean}) * 100$)

were calculated to measure the relative variability of the recoveries (Supplemental information Table 1). CVs ranged from 6 to 141%, indicating large variation in recovery of MPs. Mean recovery values of 0 were excluded from this analysis. For PET extracted using the Zobkov method, the CVs of 141% for both sand and silt indicated higher variability of percent recoveries compared with other polymers and methods. CVs for PVC, PET and PP were consistently lower in sand. CVs for PS were consistently lower in silty sediment, and CVs for PE extractions were method dependent. In general, CV values for PP and PVC were lower than PE, PET, and PS. This suggests that the recoveries of PP and PVC were more consistent with variability. Consequently, PVC and PP were on average, the most highly recovered MPs independent of sediment and method.

Several of the individual recovery replicates were greater than 100%. This highlights the important issue of MP fragmentation during isolation and extraction. Both the ground PVC and manufactured PE beads were noted as highly friable. Methods that used abrasive measures such as dry sieving likely caused these plastics to break down further and resulted in artificially high recoveries (i.e., > 100%). Several methods had consistent recoveries of 0%, especially for smaller sized microplastics. As previously noted, Gilbreath and Zobkov methods had higher size cut-off ranges (45 μm and 175 μm , respectively) that caused the loss of small MPs from sediments. None of the plastics tested in this evaluation were greater than the 1 mm upper size threshold used by several methods.

The variability in mean recovery is much larger in this evaluation than the variability reported by each author's individual methodology validation in the scientific literature. For the four published methods, reported MP mean recoveries ranged from 70 to 100% (Nuelle (91–99%), Fries (80–100%), Zobkov (85–99%), and Coppock (70–100%)). The discrepancies between published recoveries and our laboratory trials clearly highlight the influence of sediment matrix, and MP properties of size, shape and density when reporting microplastic abundance in environmental samples. Standardization of isolation and extraction techniques needs to be paired with explicit limitations of recovery. Based on this comparison, it is unreasonable to assume that one method will extract all MPs from all matrices with the same level of efficiency. As discussed above, statistical analyses indicated PP extracted from sandy sediment by the Fries method and PVC extracted from silt by the Coppock method were the only two mean recoveries significantly greater than 70%.

3.4. Effectiveness of Nile Red

There was no statistically significant benefit to using NR to identify MPs on filters (Fig. 3). Initial investigations from Maes et al. used NR to recover an average of 96.6% spiked MPs from various sediments (Maes et al., 2017). In our study, mean recovery was higher before NR staining for PE (50.6 vs. 44.9%), PET (42.6 vs. 38.7%), and PP (52.9 vs. 44.9%). Mean recovery of PVC (60.7 vs. 67.9%) and PS (14.8 vs. 15.5%) was higher after staining with NR (SI Tables 3; 4). NR did not uniformly stain the spiked plastics on each filter. This suggests the potential to miss certain MPs due to low stain uptake. Another major difficulty in using NR to stain MPs came from the incidental false-positive staining of organic debris such as benthic organisms and diatoms also present in the final filter samples. The silty sediment's high organic carbon content made identifying stained plastics particularly difficult, especially when differentiating smaller plastics such as PS and PE. These results suggest that the use of NR may confuse MP identification in high organic carbon sediments rather than providing improved identification.

3.5. Factors affecting method efficacy

Based on this investigation, we suspect physical properties (i.e., grain size and distribution, mineralogy and % carbon) play a significant role in microplastic extraction efficacy. For example, the beach sand

with its large grain size and lack of organic matter consistently generated better mean recoveries than the silty sediment. Silty sediment samples consistently took longer to extract and had lower percent recoveries. High sediment cohesion complicated procedural steps involving bulk sediment transfer, sediment suspension, and/or oven drying. As noted regarding the NR stain, moderate levels of organic matter add complexity to the plastic identification in the silty sediment. It is important to note the challenges of working with fine grained sediments because they represent a large fraction of global sediment inventories (Thorpe, 1937), particularly in low energy depositional environments such as estuaries and protected bays where MPs and other anthropogenic contaminants will likely settle (Hume and Herdendorf, 1992; Reineck and Wunderlich, 1968; Pettijohn and Ridge, 1932). In addition, sediment property variation can inadvertently influence microplastic visual identification. Plastics that mimic or are masked by sediment composition may be under-reported depending on the isolation and extraction method. Sandy beaches are distributed globally and represent an important aesthetic, recreational, economic and ecological resource. Some of the methods compared here demonstrated considerable promise with sandy sediments.

3.6. Recommendations

The diversity of MPs and range of sediment matrixes may be too broad to standardize recoveries for isolation and extraction (e.g., 70%) with a single extraction procedure. While we saw positive aspects of each extraction method, we cannot make a recommendation for a single method that functions best for all sediment and microplastic types. Therefore, it is imperative that researchers first define what types (particularly size) of plastics they would like to quantify and how their environmental samples may affect the extraction process. In terms of method efficacy, the Gilbreath method was the easiest to use with sandier sediments, whereas the Coppock method and Fries method were the easiest to apply with silty sediments. However, efficacy does not reflect best percent recovery, nor does it describe the total number of nonpolymer particles (e.g., sediment, natural organic carbon) that remain on the final filters for polymer analysis. In these instances, laboratories conducting polymer spectral analysis (e.g., Raman, FT-IR) after MP extraction need to perform organic matter oxidation to reduce the number of particles on each filter. Oxidation steps do lengthen the processing time, but they were crucial for sediment high in organic matter. The biofilms were often removed in oxidation, and some MP particles with surficial dyes lost some of their coloration. These points may be notable if researchers are looking to identify MP surface characteristics. The Zobkov method is an adaptation of the NOAA sediment method and would work well on larger MPs (Masura et al., 2015). Given our laboratory setup, we found the Nuelle method most challenging to replicate; however, the Nuelle method is the only one that examined sediment sample masses in the 1 kg range. The other four methods were easier to perform partly due to their smaller sediment mass. This method is advantageous for larger sediment samples.

Even though these five methods are a small fraction of the existing methods in use, they represent distinct processes common to many methods. Readers may also find these results helpful in developing their own extraction methods, but we emphasize that these are only opinions of the laboratory researchers. MPs were frequently lost from the samples in extraction steps that involved transferring the sample from one container to another. In addition, methods that limit the amount of sediment transfer are easier to perform. Many MPs were also observed as sticking to the walls of containers during density separation. Therefore, extraction methods need multiple rinsing steps to ensure complete transfer. The fine grained sediment was consistently more difficult to work with. Methods that included sediment drying are not recommended for silty sediments, as this resulted in sediment "bricks" that were difficult to sieve and further process. Wet sediment matrixes with high amounts of fine silts and clay are often difficult to sieve and

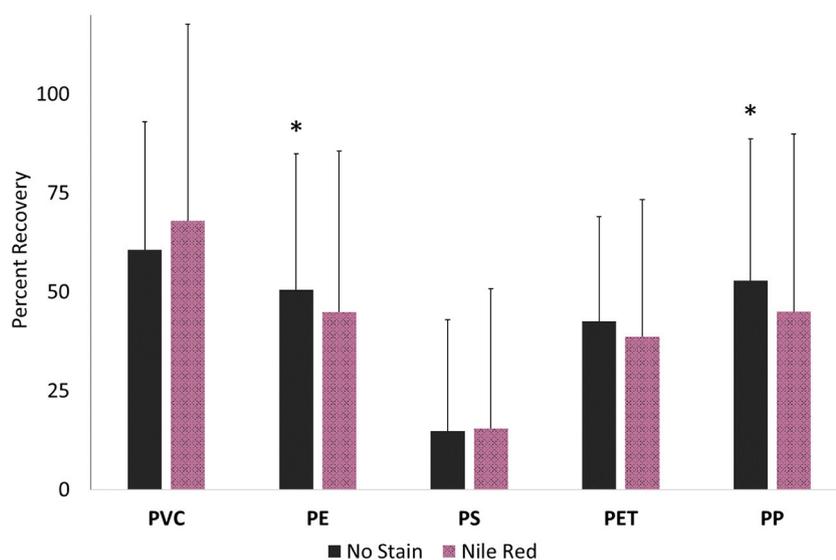


Fig. 3. Mean recovery of microplastics using visual spectroscopy prior to staining (“No Stain”) vs. after staining (“Nile Red”). “*” = $p < 0.05$ *t*-test. Error bars are the standard deviations.

filter. We recommend removing the fine fraction of MPs, sediments, and organic matter from samples ($< 45 \mu\text{m}$) prior to analysis to greatly improve method efficacy. MPs smaller than $45 \mu\text{m}$ in size cannot be easily seen under current stereomicroscopes. While this investigation did not look at polymer spectral analysis, many methods implore researchers to transfer suspected MPs with tweezers off of filters and onto clean surfaces prior to spectral analysis. Researchers interested in MPs $< 45 \mu\text{m}$ might consider further delineating size fractions to help with visual observations. A positive aspect of all five methods is that salt solutions for density separation can be reused. For these studies, used salt solutions were filtered, reconstituted up to appropriate density, and re-filtered to cutdown on the purchasing of salts and generation of waste.

Potential observation bias must be documented when reporting data on MP abundance in sediments. It may be ultimately necessary to move towards developing a MP internal standard for sediments to help identify bias in MP isolation. Our recommendation is to develop a suite of MPs that are representative of, and span the types of MP particles (size, shape) of interest. This suite could be amended into environmental matrixes and extracted along with environmental MPs to estimate efficiency. This suite of MPs should be determined by the research project objectives. Recovery rates for the internal standard MPs should extrapolate the estimated recovery of environmental MPs isolated from environmental samples. An internal standard would allow for better standardization of data across environmental sampling and a better understanding of challenges posed by sediment matrixes.

4. Conclusions

The comparison of five methods to extract varying MPs from two sediment types indicate that method, sediment matrix, and plastic properties play substantial roles in the isolation of MPs from environmental sediment matrixes. Sediments high in organic matter and with smaller grain sizes were generally more difficult to extract MPs from and had lower mean recoveries when compared to MP recoveries from sand. In addition, most methods reviewed had higher mean recoveries for larger and low-density plastics. These findings highlight potential biases in the current approximations of MP distribution in sediments worldwide. Further, the variability associated with each method was elevated with CVs ranging from 8% to 140% and 6% to 110% for the silt and sand, respectively. These CVs suggest that larger MPs ($> 500 \mu\text{m}$) are easier and more consistently recovered than smaller

MPs. The isolation and extraction of MPs from sediments is a crucial first step in the identification of MPs by polymer. Differences in MP extraction procedures prevent meaningful comparisons across field analyses. Further, differences in sediment matrix and MP properties can substantially affect extraction efficacy of MPs from sediments. The development of an internal standard composed of multiple types of MPs is urgently needed to allow standardization of MP extractions in marine sediments.

CRediT authorship contribution statement

Michaela Cashman: Conceptualization, Methodology, Investigation, Visualization, Validation, Writing - original draft. **Kay T. Ho:** Conceptualization, Investigation, Validation, Resources, Writing - review & editing, Visualization, Project administration, Funding acquisition. **Thomas B. Boving:** Validation, Formal analysis, Resources, Data curation, Writing - review & editing. **Stephen Russo:** Validation, Investigation, Writing - review & editing. **Sandra Robinson:** Validation, Investigation, Data curation, Writing - review & editing. **Robert M. Burgess:** Conceptualization, Validation, Formal analysis, Data curation, Writing - review & editing, Visualization, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.marpolbul.2020.111507>.

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