Microplastics and Nanoplastics

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Separation of microplastics from deep-sea sediment using an affordable, simple to use, and easily accessible density separation device

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Abstract

Microplastics accumulate in the environment but methods to extract particles from sediment for quantification and identification often lack accuracy and reproducibility. Existing methods vary greatly and many do not achieve adequate microplastic separation. During method development for extraction procedures, spike-recovery experiments (positive controls) are essential to ensure accurate and reproducible results from each sample matrix. Furthermore, the large variability in grain size and organic matter can affect the extraction of microplastics from the matrix. Scientists have used density separation to separate microplastics from matrices for decades, but apparatuses are often made of plastic, need to be custom made, and require multiple sample transfers from one apparatus to another. This study presents an affordable, easily accessible, and simple to use Density Separation Device (DSD) to remove plastics from deep-sea sediments. Eight polymers were spiked into replicates of environmental sediment, including six fragments: high density polyethylene (HDPE), polypropylene (PP), polyvinyl chloride (PVC), polystyrene (PS), nylon (PA6), and crumb rubber (CR) and two fibers: cellulose acetate (CA) and polyester (PEST). Two size classes of polymers were used: 100 µm to 300 µm and > 300 µm. Using a sodium polytungstate solution at a density of 1.9 g/mL and reflectance FTIR microscopy for particle identification, mean recoveries of all fragments exceeded 78% (CR: 92.7% ± 30.8%, PP: 78.4% ± 34.0%, HDPE: 93.8% ± 13.5%, PS: 86.9% ± 25.7%, PA6: 98.4% ± 63.2%, PVC: 100.0% ± 12.4%). Fiber recovery was much lower (PEST: 28.1% ± 28.1% and CA: 25.9% ± 17.3%) because they aggregated, passed through sieves vertically, or were obscured under other particles. The fragment recovery success, accessibility (available online, all parts under $200) and ease of use of this DSD should facilitate widespread use, thus helping to standardize sample preparation methods for microplastic metrology.

Keywords Microplastics, Method, Validation, Recovery rate, Sediment

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Introduction

Microplastics research has grown exponentially in the last decade [1]. As an emerging field, a focus of this research has been to quantify microplastics in a variety of media including sediment, water, and biota [2]. The methods used by laboratories, however, are quite different, from the way microplastics are isolated and extracted from the matrices to how the particles are polymer identified [3]. This has resulted in potential misrepresentation of microplastics concentrations [4] and the inability of researchers to compare their findings to other studies [5, 6]. In all, this stunts monitoring efforts and makes regulatory strategies challenging to enforce.

Microplastic extraction methods can and should be validated by spike-recovery experiments (positive controls) whereby known quantities of microplastics are added to the matrix of interest. The extraction and analytical methods are performed with known polymer spikes to determine a percentage recovery, providing a metric of sample preparation success. The majority of studies report recoveries less than 100%, indicating that methods underestimate microplastic concentrations in the environment (Table 1, [4]). Occasionally, recoveries are seen above 100%, which could be due to fragmentation of microplastic particles during sample handling and processing or background contamination, indicating methods that overestimate microplastic concentrations when measured as a particle count (Table 1).

Studies often consist of multiple techniques used in combination, such as a density separation, i.e. flotation, followed by oxidation [21] or an oil separation followed by cleanup with a surfactant [17]. In addition, the apparatuses used range from simple separatory funnels [11] (Fries et al. 2013) to more complex devices such as a sediment microplastics isolation unit [9] or the Munich Plastic Sediment Separator [12]. Methods developed by researchers often have a high recovery rate of spiked microplastics [9, 11, 18, 20]. Overall, no particular apparatus performs significantly better than another. Therefore, practical factors including the cost, access, and ease of use may determine preference for one apparatus over another (Table 2).

While a beaker or graduated cylinder are easily accessible and affordable, they lack the ability to separate the floating microplastics from the sinking sediments and agitate or mix the sediment at the same time. The Munich Plastic Separation System (MPSS) achieved almost perfect recoveries of polymers, but it was expensive (quoted at US $42,000) and custom made and is no longer being produced commercially. The literature has conflicting perspectives if matrix characteristics such as sediment particle size or organic matter affects microplastic extraction [24]. Because of this uncertainty, each method should consider grain size and organic content of the sediment and adjust if needed. Recoveries of microplastics are often greater from sandy sediments with larger grain sizes than silt with a much smaller grain size [6].

Table 1 Percent recovery of particle count or mass of multiple polymers of microplastics from sediment matrices determined by various methods in selected literature. Values are mean and standard deviation or standard error, depending on the study. Abbreviations are polypropylene (PP), polyethylene (PE), polystyrene (PS), nylon (PA6), polyethylene terephthalate (PET), and polyvinyl chloride (PVC). An expanded version of table 1 showing size and shape details of the spiked polymers is available in table S1. References included in this table: [7–20]

		Mean±standard deviation of percent recovery					
Reference	**Separation apparatus**	**PP**	**PE**	**PS**	**PA6**	**PET**	**PVC**
Duong et al. [10]	Beaker	90±7	93.3±2	96.7±3	-	71.7±3	36.7±3
Konechnaya et al. [13]	Beaker	100±0.5	100±1.3	101±0.4	97±0.6	102±2.9	98±1.5
Zobkov and Esiukov [20]	Beaker	-	-	-	-	92±7	-
Monteiro et al. [15]	Glass centrifuge tube, centrifuge	93±0	100±0	-	82±1	96±1	93±0
Maes et al. [14]	Plastic centrifuge tube, centrifuge	-	98	-	95	-	-
Fries et al. [11]	Separatory funnel	-	95±5.3	-	-	-	-
Cashman et al. [7]	Separatory funnel	100.07	100.69	86.18		45.13	126.64
Claessens et al. [8]	Elutriation Column	-	-	-	-	-	100
Vermeiren et al. [19]	Decanting column	80	70	-	-	100	100
Nuelle et al. [18]	air induced overflow (AIO) and volumetric flask	95.0 ± 7.6	100±0	90±11.5	-	93.3±7.5	100 ± 0
Vermeiren et al. [19]	overflow column with top inflow (OC-T)	100	100	-	-	100	90
Vermeiren et al. [19]	overflow column with mid-level inflow (OC-M)	20	10	-	-	30	50
Vermeiren et al. [19]	Sediment-Microplastic Isolation (SMI)	100	100	-	-	90	100
Coppock et al. [9]	Sediment-Microplastic Isolation (SMI)	-	97	-	92	-	94
Nel et al. [17]	Sediment-Microplastic Isolation (SMI)	83±2	83±2	-		87±3	-
Nakajima et al. [16]	JAMSTEC microplastic-sediment separator (JAMSS)	96.3±3.0	96.3±4.4	98.7±3.0	-	96.7±3.5	96.8±3.3
Imhof et al. [12]	Munich Plastic Sediment Separator (MPSS)	100	100	95	100	100	100
This study	Density Separation Device (DSD)	78.4±34.0	93.8±13.5	86.9±25.7	98.4.3±63.2	28.1±18.4	100.0±12.4

Table 2 Microplastic density separation apparatuses compared based on many factors. A green shaded box indicates a positive attribute, a red box a negative attribute, and a yellow box is in between the red and green. References included in this table: [8, 9, 11, 12, 15, 16, 18, 19, 22, 23]

	Beaker, cylinder, flask, bottle, tube	Centrifugation in tube	Separatory Funnel	Elutration Column	Air Induced overflow (AIO) and volumetric flask	Overflow column with mid level inflow (OC-M)	Overflow column with top inflow (OC-T)	Sediment Microplastics Isolation (SMI)	JAMSTEC microplastic sediment separator	Munich Plastic Sediment Separator (MPSS)	Density Separation Device (DSD)
Reference	Rendell-Bhatti et al. 2023	Monteiro et al. 2022	Fries et al. 2013	Claessens et al. 2013	Nuelle et al. 2014	Vermeiren et al. 2020	Vermeiren et al. 2020	Coppock e tal. 2017	Nakajima et al. 2019	Imhoff et al. 2012; Zobkov and Esiukova 2017[b]	This study
Cost	$	$$$	$$	$$$	$$	$	$	$	$$$	$$$	$$
Accessibility	Common in labs	Need centrifuge	Common in labs	Need Centrifuge	Custom made	Custom Made	Hardware store	Hardware store	Custom made	Discontinued	Order online
Plastic materials?	No	No	Yes- Stopcock	Yes- PVC pipe	Yes- Pump and tubing	Yes- all PVC	Yes- all PVC	Yes- all PVC	No	Yes- o-ring	Yes- o-ring
Apparatus assembly complexity	Easy	Easy	Easy	Easy	Intermediate	Easy	Easy	Easy	Easy	Challenging	Easy
Sediment addition	Easy	Easy	Challenging	Easy	Easy	Easy	Easy	Easy	Easy	Easy	Easy
Dense solution restrictions	None	Corrosion	None	None	None	None	None	None	None	Corrosion	Corrosion
Mixing options	Stir bar, vortex, glass rod	Shake, vortex	Swirl	Water Flow	Air Flow	Air Flow	Addition of Dense Fluid	Shake, stir bar	Stir bar, gass rod	Internal blades	Shake, add balls
Can top and bottom chambers be partitioned?	No	No	No	No	No	No	No	Yes	No	Yes	Yes
Decanting	Pour, pipet	Pour, pipet	Top and bottom	Overflow	Overflow	Overflow	Overflow	Pour without sieve	Pour, Pipet	Pour without sieve	Pour with sieve
Additional cleaning steps within apparatus (vacuum, chemical digestion)	No	No	No	No	No	No	No	No	No	No	Yes
Rinsing ability during decantation to maximize particle recovery	No ability	No ability	Can rinse	Unnecessary	Unknown	Unnecessary	Unnecessary	Can rinse	Can rinse	Can rinse	Can rinse
Separation speed	Gravity	Fast	Gravity	Gravity	Gravity	Gravity	Gravity	Gravity	Gravity	Gravity	Gravity
Take down ease	Very simple	Very simple	Simple	Very simple	Very simple	Simple	Very simple	Simple-Moderate	Very Simple	Challenging	Simple-Moderate

All studies should include a spike-recovery experiment to ensure adequacy of the chosen method for that sediment sample type. No environmental reference materials for microplastics studies currently exist, so many studies will remove any microplastics already present from an environmentally collected sediment so it is microplastic free and ready to be spiked. For example, Claessens et al. "cleaned" their sediment by performing several elutriations to remove any microplastics before spiking [8]. If microplastics have not been removed from the sediment prior to spiking, it would be difficult to determine if the particles were already present in the sample or contamination. Some researchers have spiked sediments with plastics that are inherently distinguishable from those that would be found in the environmentlike fluorescently labeled microplastics. In addition to matrix effects, the density and shape of the plastic can also affect the extraction success of microplastics [21]. Smaller microplastics are more difficult to extract than larger microplastics [25] and more dense polymers like PVC require a denser solution than the commonly used sodium chloride [26].

The development of these methods is worthwhile as deep sea sediment samples hold vital information regarding the accumulation and abundance of microplastics in aquatic environments. Deep sea sediment cores offer an environmental sample that can be used for understanding both temporal and spatial trends of microplastic abundance, as well as plastic types in the ocean [27, 28]. The goal of this study was to develop an accessible and effective method for the separation of microplastics in deep sea sediments, informed by the successes and pitfalls of previously studied protocols. Every step in the workflow process was carefully considered to maximize recovery of microplastics from the sediment, minimize background contamination, and create a method that was user friendly for the research community. This included: stringent quality control techniques; experimental design that used an affordable, accessible and user-friendly separation device; novel methods to improve recovery, including ways to minimize transfer steps and maximize stirring; and improved separation of natural materials, including the application of a vacuum at the beginning of processing [29].

Methods

Quality control

Multiple steps were taken to minimize contamination of samples from background microplastics. Orange-dyed cotton lab coats were required for entry when working with microplastic samples because orange fibers are easy to identify. Any orange cotton fibers found in the spiked samples were assumed to be from the lab coats and not counted in final fiber counts. However, the research was performed during the Covid 19 pandemic and it was necessary to wear face masks. These masks were not dyed orange and may have shed fibers into the samples. No gloves were worn. All supplies were selected to avoid the use of plastic components. Metal and glass supplies were

pre-cleaned for 20 min with soap and water sonication, followed by three rinses with tap water, three rinses with high purity water, covered with aluminum foil, and baked at 450 ºC for 4 h. Components of the DSD were thoroughly cleaned in the same method as all other glassware, except the sight glasses were not baked (due to a silicone o-ring at the ends of the chambers), rather air dried in the laminar flow hood and the open ends covered with baked aluminum foil once dry. Prior to use the DSD components were rinsed with high purity water as a final assurance against microplastics from the lab. SPT was filtered through 8.0-µm filters (Polycarbonate membrane filters, 8.0 µm pore size, diameter of 25 mm, Sterlitech, Auburn WA) before use. Vinegar was filtered through a 0.45 µm syringe filter (PALL life sciences, Acrodisc 13 mm syringe filter, 0.45 µm, PA6 membrane, Lot 21723422). Exposure of samples or supplies to laboratory air was minimized by keeping items sealed, opening them only briefly, and performing as many steps as possible inside a HEPA-filtered laminar flow hood. Every surface inside the laminar flow hood, including the walls and front panel, was cleaned with high purity water and an orange dyed 100% cotton towel. One blank sample of the same deep-sea sediment was analyzed alongside each spiked sample to assess background lab contamination or plastic particles potentially already present in the deep sea sediment. Blank samples were open and adjacent to its corresponding sample during the spiking of the sediment samples. Particles in blank samples>100 µm (the smallest size spiked into the sample) were subtracted from the corresponding spiked sample. For example, if one PS particle was identified in blank #1, one PS particle was subtracted from the total PS count in Spike #1. In addition, balances were calibrated each day using NIST traceable weights (Troemner ISO/IEC 17025 ASTM Ultra) and recorded in a calibration record log.

Selected polymers

Seven polymers from the Hawaii Pacific University Center for Marine Debris Research Polymer Kit 1.0 were selected to assess the recovery of microplastics from sediment: crumb rubber (CR), polypropylene (PP), high density polyethylene (HDPE; HDPE.1 in Polymer Kit 1.0), polystyrene (PS), nylon (PA6 in Polymer Kit 1.0), polyvinyl chloride (PVC; PVC.1 in Polymer Kit 1.0), and polyester fibers (PEST). An additional polymer, cellulose acetate fibers (CA), were obtained from cigarette filters sold commercially (Top Premium King Size CA Cigarette Filters, Republic Tobacco, Glenview, IL). The PEST in Polymer Kit 1.0 has been determined to be polyethylene terephthalate (PET, data not shown). When referring to these spiked fibers, they will be called PEST for continuity with the kit material name.

Compatibility with laboratory chemicals

As part of quality assurance and method development, we recommend that researchers empirically test the compatibility of their selected polymers with the laboratory chemicals and conditions to which they will be subjected [30]. The Cr, PP, HDPE, PS, PA6, PVC, and PEST from the recovery experiment were used to assess compatibility. A cellulose acetate film (Goodfellow, Pittsburgh, PA) was also used for the compatibility trials. A pellet of each polymer was cut into 1 mm to 4 mm sized fragments (average mass 2.61 mg±1.82 mg) while PEST fibers were cut into 15 mm fibers (average mass 0.140 mg±0.146 mg) using a razor blade. Each "fiber" used in the compatibility trials consisted of multiple fibers twisted into a strand. Each individual fragment and fiber was weighed to 0.001 mg (Sartorius M3P Microanalytical Balance) and photographed under a dissecting microscope (Figure S1). An attenuated total reflection (ATR) Fourier Transform - Infrared (FTIR) spectrum (Thermo Scientific iS5) was taken on each material before cutting and chemical exposure. ATR-FTIR spectra of original polymers were added to an in-house library. The ATR-FTIR was used in the compatibility tests because the particle sizes were large enough to be handpicked by tweezers and the ATR-FTIR provides a high spectral resolution. Sodium polytungstate (SPT, Sodium metatungstate monohydrate, $Na_6O_{39}W_{1\,2}{\cdot}H_{20}$, CAS: 314075-43- 9, Thermo Fisher Scientific/Alfa Aesar, Chino, CA, USA) and household vinegar (White distilled vinegar, Good & Gather, Target Brands, Inc.) were tested for compatibility with the chosen polymers. Powdered SPT was dissolved in Millipore high-purity deionized water (resistivity=18 MΩ cm-1; 0.22 µm filter, hereinafter referred to as high purity water) until a density of 2.0 g/cm^3 was reached. Each fragment or fiber was placed in a clean glass test tube with 1 mL of the SPT or vinegar and covered with a cap. Polymer samples in sodium polytungstate were left at room temperature overnight while those in vinegar were placed overnight on a shaker table set to 130 RPM at 37.5 °C. Polymers in the water trials remained in the high purity water for 42 days, except for the PVC and CR that were left in high purity water overnight. The next day, polymers were removed from the chemical solution using tweezers, rinsed with high purity water, placed in individual aluminum dishes to dry at 40 °C for four days or until a constant mass was reached. Measurements were taken (mass and ATR-FTIR spectra) to compare to those measurements before chemical exposure. Mass change was calculated by Eq. 1, where $Mass_B$ and $Mass_A$ are the masses before and after the chemical exposure, respectively:

$$Mass\ Change\ =\ \frac{MassA - MassB}{MassB}\,\mathrm{X}\,100 \qquad (1)$$

The post-treatment spectra were searched through the in-house library of the "before" spectra using the OMNIC correlation algorithm from the OMNIC software (Thermo Fisher Scientific). The highest percent and polymer match was recorded. A polymer with a mass change of <10% and a polymer match of >90% was considered compatible with the chemical. A mass change of 10–50% or a spectra match of 70–89.9% is compatible with concern, and a mass change of >50% or a spectra match of <70% is considered incompatible with those chemicals. The paired before and after mass data was tested for normality, and the data was not normal. The non-parametric Wilcoxon signed rank test was performed to determine if the before masses and after masses differed significantly.

Sediment and polymer spiking

Sediment from the Bermuda Atlantic Time-series Study (BATS, 5.5; https://bats.bios.asu.edu/) was collected in October 2019 at 32° 18' 38.42" N, 64° 30' 45.95" W and at a depth of 2800 m. The top 15 cm of a box core was homogenized by mixing in a stainless-steel bowl and sieving through metal filter screens to remove remaining clumps. The sediment was subsequently dried at 50 °C. Subsamples of 150 g were placed in 16 oz wide mouth glass jars and shipped from Woods Hole Oceanographic Institution to the Hawaii Pacific University Center for Marine Debris Research (CMDR). All samples were again combined in a 2000 mL beaker and high purity water added and mixed until the sediment was wet and homogenous, resembling chocolate ice cream. Approximately 50 g of sediment (wet mass) were transferred while mixing to each clean 200 mL glass jars (six jars total). Three jars were to be spiked with polymers and three were prepared as blanks. The "blank" samples are the same environmental sediment as the spiked samples, and may already contain microplastics. The mass of the sediment was taken immediately after being placed in its jar.

The same eight polymers tested for compatibility were used to assess the recovery of microplastics from sediment, but were prepared differently. All polymers, excluding fibers, were ground using a stainless steel electric grain grinder mill (CGoldenwall, fineness: 70 µm to 300 µm mesh). Resulting particles captured on 53 µm, 100 µm, and 300 µm stacked sieves were stored separately. Microplastic particles from the 100 µm and 300 µm sieve size classes were used in this study (Figure S2). Particles from the 100 µm sieve (100 µm to 300 µm) will be referred to as the medium size class and particles >300 µm as the large size class. Particles >100 µm were chosen to facilitate the hand-picking of particles to spike into each sample. Fibers were prepared by separating a single fiber from the strand and then cut using a razor blade to approximately 2 mm in length.

Three jars were spiked with approximately 20 particles of each polymer (10 from the large size class and 10 from the medium size class) and 10 of each of the fibers. Particles and fibers were handpicked with tweezers from each specified polymer and size class and placed onto a gold slide (Figure S3). Particles were carefully counted using a dissecting microscope within a laminar flow hood. The number of particles differed slightly among replicates but were recorded. The slide was transferred to the Thermo Fisher Nicolet iN10 MX microscope FT-IR. The FTIR spectrum and size of each particle was taken using the automated Particles Wizard function included in the software. Spectra were collected in reflectance mode with a cooled detector and 64 scans and 8 cm^{-1} resolution from 675 cm^{-1} to 4000 cm^{-1}. All of the counted and sized particles were then rinsed into one replicate of the small jars containing 50 g of wet deep sea sediment using 1 mL of high purity water in a glass pipette. This process was repeated with each polymer and size class, until 20 particles per polymer and 10 of each fiber were added to each of the triplicate spiked-sediment samples. An equal amount of high purity water was added to each blank sediment sample ($n=3$) that was sitting adjacent to its corresponding spiked sample ($n=3$). The spiked samples and blank samples were processed in tandem to account for potential laboratory contamination on the processing day.

Density separation device (DSD)

A density separation device (DSD) was built using two glass sight chambers connected with a ball valve commonly used when brewing beer and commercially available through internet shopping sites (Fig. 1). A video demonstrating the assembly and use of the DSD is available (https://www.hpu.edu/cncs/cmdr/research/projects.html).

Each end of the chamber can be fitted with a metal cap (A) or a vacuum attachment (B). Each connection point consists of a silicone O-ring and a tri-clamp (D). Additional information on the purchase and construction of the DSD can be found in Figure S4 and Supporting Information text file. Polymer-spiked sediment samples were added to the lower chamber of the DSD using a spatula, rinsing with 50 mL to 60 mL of high purity water to ensure all sediment was transferred (Fig. 2, Step 1).

Three stainless steel balls were added, and the chamber was capped (Step 2) and mixed to suspend the sediment in high purity water (Step 3). An additional 10 mL to 20 mL of high purity water was used to rinse residue sediment on the cap into the chamber. The vacuum cap was attached and a vacuum was applied for 30 min (7.45×10^4 Pa is routinely used in our laboratory, but not recorded during this spike recovery experiment) (Step 4). Next the vacuum cap was removed and an open ball

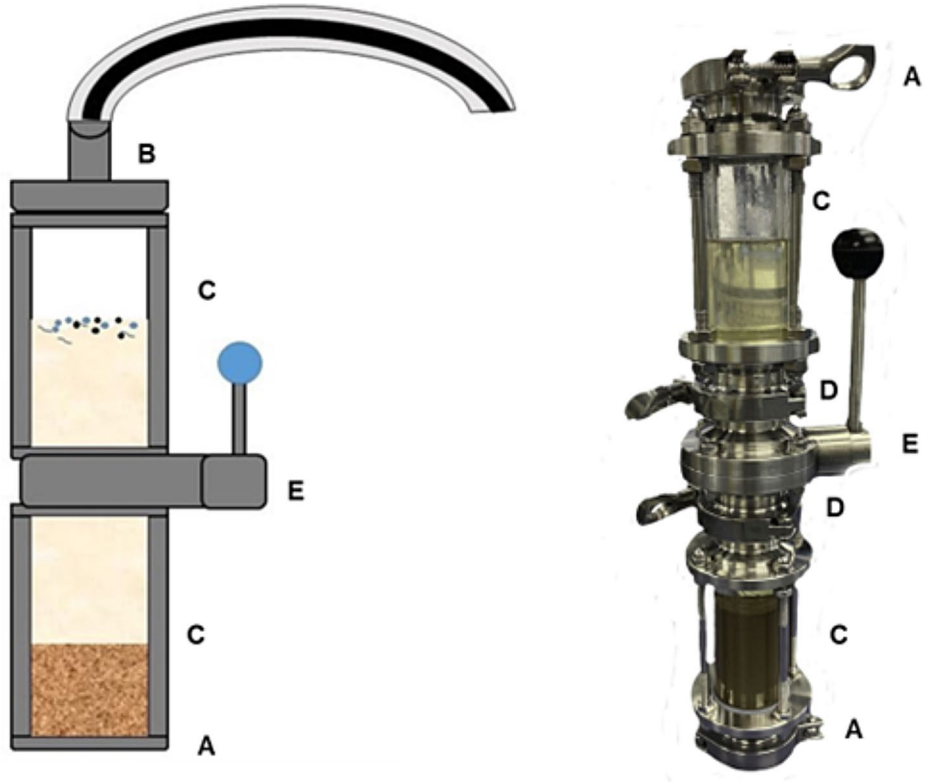

Fig. 1 A Density Separation Device (DSD) assembled from **(A)** End cap, silicone O-ring, and tri-clamp; **(B)** Vacuum attachment, silicone O-ring, and tri-clamp; **(C)** Sight glasses; **(D)** Silicone O-ring and tri-clamp; and **(E)** Ball valve

valve and top chamber were then attached to the bottom chamber, and 180 mL of sodium polytungstate (SPT) at a density of 2.3 g/mL were added to the chamber (Step 5). The density of the SPT was measured by dividing the mass of the SPT recorded to 0.00001 g by its volume of approximately 1 mL measured using a glass cylinder with 0.1 mL graduations. The addition of SPT at 2.3 g/mL gave a final density of 1.9 g/mL to 1.975 g/mL after mixing with the water already in the DSD. The top cap was then added and, with the ball valve open, the DSD shaken until all sediment was once again suspended (Step 6). The DSD was then left to settle overnight, approximately 18 h. When the sediment had settled to the point where the top of the sediment was visible in the lower chamber, the ball valve was closed and the DSD shaken again. The ball valve was then opened and closed three times to dislodge particles stuck to it, and then left open to allow the sediment to settle and the polymers to rise to the surface. After settling (approximately 3 h later), the ball valve was again closed and the DSD shaken for the last time. Again, the ball valve was opened and closed three times then left to settle. Once settled, the ball valve was opened and closed again to dislodge any particles and subsequently closed. Next, the bottom chamber of the DSD was disconnected below the ball valve, catching the sediment and SPT mixture in a clean, plastic beaker (Step 8). Then the top chamber of the DSD was flipped upside down and the ball valve was removed and rinsed with high purity water to ensure no particles are potentially lost on the inside of the ball valve. A 20 µm sieve was chosen to correspond to the lowest particle size we are confident can be identified by our FTIR microscope. Our empirical testing has shown we cannot reliably identify particles less than 20 µm in a real environmental sample with some matrix remaining (data not shown). However, smaller mesh attachments are available. The sieve was attached to the top chamber and the SPT was drained into a beaker for future recycling (Step 9). Information for recycling of SPT can be found in the Supporting Information text file. The top chamber was rinsed with high purity water and poured out through the sieve three times. The sieve top was carefully removed and any polymers were rinsed back into the top chamber with approximately 10 mL to 20 mL vinegar (Step 10). The chamber was then capped and placed on the shaker table at 37 °C and 122 RPM for 24 h. After 24 h, the top chamber was vacuum filtered through a 5-µm gold filter (polyester glycol gold-coated membrane filters with a pore size of 5.0 µm, 100 nm gold coating thickness, and a diameter of 25 mm, Sterlitech, Auburn WA) and allowed to dry in a drying oven at 40 °C for 4 days, or until dry (Step 11).

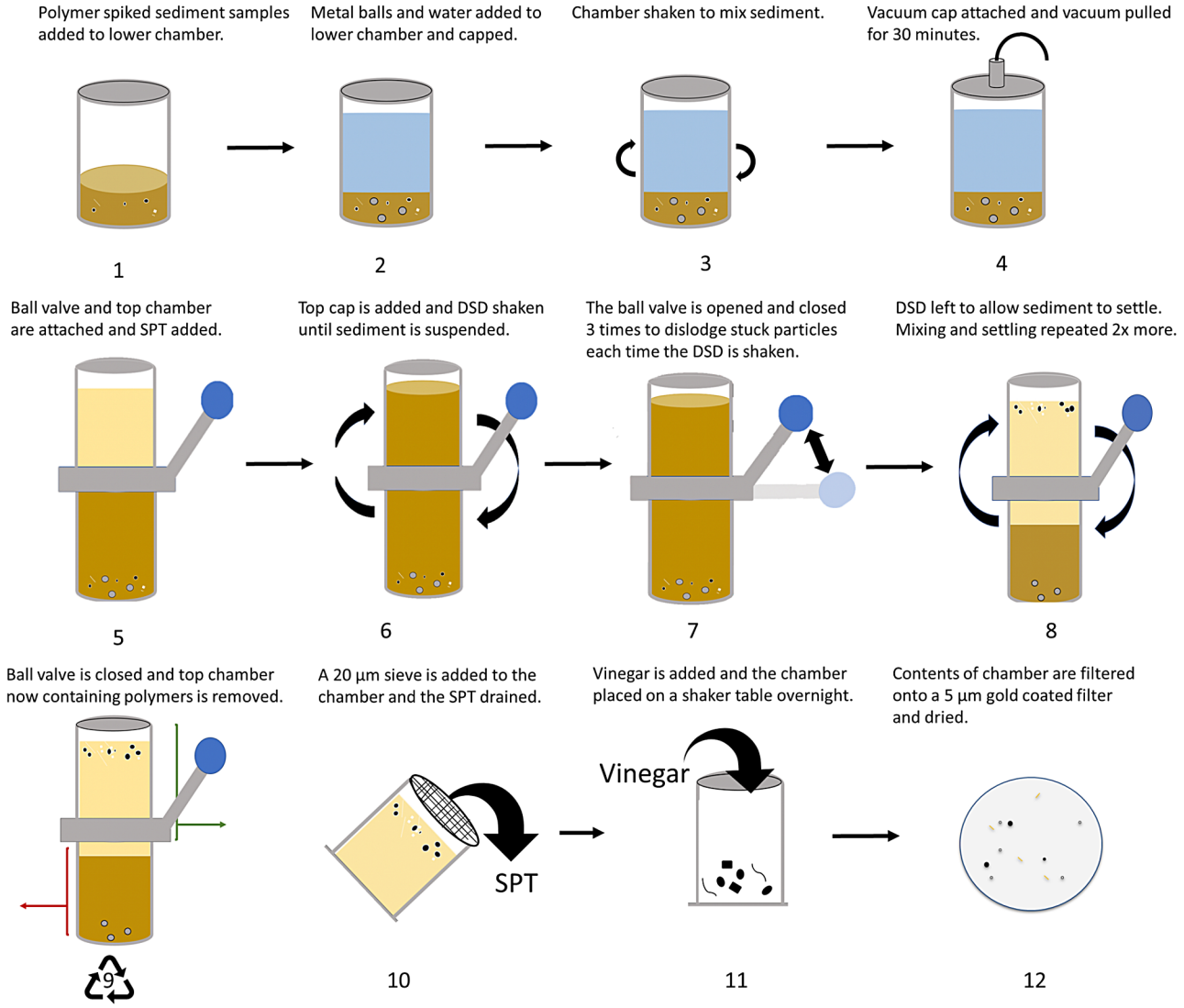

Fig. 2 Diagram of the steps to extract microplastics from sediment using the Density Separation Device. Brown = sediment; blue = water; yellow = 2 g/mL sodium polytungstate, gray circles = stainless steel balls, black/white circles/lines = microplastic particles. Recycle symbol indicates recycling of the sodium polytungstate solution for a future sample

Efficiency of vacuum

Six additional replicates of 50 g (wet mass) deep sea sediment (none spiked with polymers) were processed using the DSD to determine the efficiency of the vacuum step at removing natural material (compared to DSD without vacuum). Three or four stainless steel metal balls and 70 mL high purity water were added to the DSD chamber and the chamber shaken. A vacuum was applied for 30 min at 7.45×10^4 Pa for three replicates, while the three without vacuum were allowed to sit at room temperature for 30 min. All samples were treated the same from this point on following steps 5 to 8 above. Each replicate was then filtered onto a pre-weighed 20-µm polycarbonate filter (GVS North America, Polycarbonate Track Etched, 47 mm, 20 µm), dried in an oven at 40 °C for three days, and weighed. A non-parametric Kruskal-Wallis test was performed to compare floating particle masses with a vacuum applied vs. without.

Polymer identification

Filters from spiked and blank sediment replicates were analyzed using reflectance FTIR microscopy (Thermo Fisher Scientific Nicolet iN10 MX µFTIR). The µFTIR was used for the recovery experiment because the particle sizes used to spike the sediment were too small to be hand picked and analyzed on the ATR-FTIR. An in-house spectral library was created on the µFTIR with 125 known reference standards, which included 31 natural materials and 94 synthetic items representing 19 polymers. Gold filters were placed on the iN10 MX with a

15x objective. Spectra were collected in reflectance mode with a cooled detector and 64 scans and 8 cm^{-1} resolution from 675 cm^{-1} to 4000 cm^{-1}. Three data collection methods were used sequentially on each filter: the Particle Wizard, a manual point and shoot of individual particles, and an Area Map, in that order. Each filter was analyzed in quarters to not exceed the software data collection limitations. A Particle Wizard automatically detects particles on the filter within the mosaic (or the optical images stitched together), measures the length and width of each particle, collects a single spectrum in the center of each particle, and searches the selected library to identify the particle's material composition. The point and shoot function was then used to collect spectra of any particles which were missed by the Particle Wizard and manually searched against spectral libraries. Finally, an Area Map with a step size between 75 µm and 100 µm was collected to check the results of the first two functions. The acquired data from each quarter of the filter were inspected manually by comparing the data to the full optical mosaic to ensure every particle was counted and no particles were counted twice. Only particles with a match > 75% to a library spectrum were accepted. The spectra of all particles identified by the µFTIR as PA6 were examined manually to differentiate between PA6 and natural proteins basd upon peak percent transmittance and definition in the associated N-H regions 2800 cm^{-1} to 3400 cm^{-1} and 1400 cm^{-1} to 1700 cm^{-1} [31]. Furthermore, due to the tendency of crumb rubber spectra to match with carbon based organic material in the sample, all the spectra which matched to Crumb Rubber spectra in our libraries were also manually checked for black/dark coloration on the mosaic before being confirmed as a Crumb Rubber particle. Except for particles identified as CR, particles < 100 µm were excluded from counts before calculating the percent recovery, because only particles > 100 µm were spiked into the samples. Inclusion of smaller CR particles was justified, because < 100 µm particles of CR were spiked into the sample and CR easily fragments. All particles of CR regardless of size were included in final totals. The minimum size detection limit of this study was 20 µm. Any particles smaller than this were lost when sieving the DSD prior to adding vinegar.

Results and discussion

Polymer compatibility

Sodium polytungstate and vinegar are fully compatible with PP, PE, PS, and CA. Vinegar is also fully compatible with PA6 and PEST (Table 3). Both SPT and vinegar treatments resulted in poor ATR-FTIR matches for CR after the compatibility trials. Car tires are inherently heterogeneous, consisting of rubber, synthetic polymers, and fillers; and the crumb rubber was produced by the mechanical grinding of used tires. When untreated CR was searched through the in-house library, it only matched to a spectra of itself with an average match of 61.58% ± 2.48%. Itis likely neither the SPT nor vinegar that changed the spectrum of CR significantly, but rather the heterogeneous composition of the CR itself caused poor library matches. Crumb rubber is also black in color, making it difficult to obtain a clean spectrum using reflectance mode. The dark color causes lower amounts of radiation to reflect back to the detector, giving poor spectra [32]. In addition, PVC had a reduced ATR-FTIR match of 85.7% ± 3.77% and 76.5% ± 8.40% after treatment with SPT and vinegar, respectively. It is possible an additive such as calcium carbonate or phthalates leached from the PVC samples causing a lowered spectral match after treatment. The Wilcoxon signed rank test showed no significant differences in the before and after masses for any polymer in any treatment ($p > 0.05$).

Efficiency of vacuum

Natural, buoyant material present in environmental samples interferes with detection and identification of the synthetic polymers, so reducing it is a goal of effective sample preparation. Decaying plant matter contains intercellular space that is filled with gasses causing it to float in water. Bubbles trapped in diatom skeletons, which

Table 3 Compatibility of eight polymers with sodium polytungstate and household vinegar, shown as mean and one standard deviation in percent match to the polymer's original ATR FTIR spectrum and percent mass change of the polymer. Results highlighted in green indicate compatibility with the chemical (> 90% ATR FTIR match or < 10% mass change), orange indicates compatibility with caution (ATR match 70 – 89.9% or 10 – 50% change in mass) and red indicates incompatibility with the chemical (ATR match < 70% or mass change > 50%)

	Sodium Polytungstate		Vinegar		High Purity Water	
	ATR Percent Match	Percent Mass Change	ATR Percent Match	Percent Mass Change	ATR Percent Match	Percent Mass Change
Crumb Rubber	*51.3 ± 28.8*	0.293 ± 1.88	61.8 ± 19.8	-0.825 ± 0.846	94.4 ± 0.47	-1.14 ± 1.99
Polypropylene	98.0 ± 0.835	0.164 ± 0.835	98.3 ± 0.652	-1.025 ± 2.48	98 ± 0.879	-1.23 ± 1.55
High Density Polyethylene	98.6 ± 0.465	0.0780 ± 0.306	97.9 ± 1.03	0.0500 ± 0.173	99.2 ± 0.229	0.914 ± 1.13
Polystyrene	98.0 ± 0.308	0.0803 ± 0.299	95.8 ± 3.10	-0.175 ± 0.568	97.8 ± 0.817	0.626 ± 1.44
Nylon	93.5 ± 4.80	-11.6 ± 19.9	94.7 ± 3.80	-1.00 ± 0.245	97.6 ± 4.06	-1.31 ± 1.20
Polyvinyl Chloride	85.7 ± 3.77	0.0349 ± 0.293	76.5 ± 8.40	-0.0500 ± 0.252	86.9 ± 0.343	-0.127 ± 0.742
Polyester Fiber	91.1 ± 4.35	-12.7 ± 4.35	95.3 ± 4.13	-4.38 ± 3.50	96.8 ± 0.913	3.69 ± 12.2
Cellulose Acetate Film	93.8 ± 1.4	-3.75 ± 0.622	92.9 ± 1.03	-6.38 ± 0.806	95.0 ± 0.612	-9.29 ± 29.7

are abundant in deep-sea sediments, will also cause these natural materials to float. Applying a vacuum replaces the gas with water allowing cellulose or diatom skeletons, which are more dense than water, to sink [33]. Adding a vacuum step to the DSD procedure resulted in a visual decrease in the amount of natural material captured on the filter, though the difference was not statistically significant (t-value = -1.58, DF = 2, p value = 0.13; Fig. 3). In addition, the amount of material remaining was significantly less variable with the vacuum applied. The vacuum step can improve both accuracy and precision by reducing interfering particles and increasing reproducibility of material on the filter without the need of more harsh chemical cleanup techniques. The application of a strong vacuum is key to density separation, and this experiment suggests that 7.45×10^4 Pa is adequate. Further testing would be needed to determine the optimal vacuum for each sample type and more replicates would result in more power for statistical differences. Additional work to determine the ability of the vacuum to remove natural material is needed to fully determine the capability of the vacuum in reducing natural materials in a sample.

Microplastic recovery using the density separation device

Details for all particles recovered from the six sediment replicates (3 spiked and 3 blank), can be found in Table S3. Images of all six filters show few to no visible particles > 100 µm in the blank sediment and numerous particles from the spiked sediment as expected (Fig. 4).

The average recoveries of spiked fragments, regardless of original size at spiking, exceeded 78%, while the two fibers had recoveries lower than 26% (Table 4; Fig. 5). These recoveries indicate that the DSD is efficient at separating microplastic polymers of a range of densities (~0.9 g/mL PP to ~1.4 g/mL PVC) from fine sediment, but microfibers remain a challenge to extract or detect.

Recovered particle lengths were not significantly different from particle sizes before spiking for HDPE, PP, PVC, PA6, CR and CA (Wilcoxon p-values were > 0.05; Fig. 6). Polyester fibers were shorter upon recovery (z = -3.72, p = 0.0002), and PS fragments were longer (z = 2.11, p = 0.035) (Fig. 6).

The shortening of PEST fibers may be an artifact of how the fibers were arranged on the filter after DSD. The majority of PEST fibers were tangled or folded on the filters. The automated sizing function can underestimate the length of folded fibers. While not significantly different, a similar trend is seen for the CA fibers (Fig. 6). Conversely, PS fragments significantly increased in size, and most other fragments also showed a lengthening trend. We can think of only two reasons to explain this finding. The smaller particles may be more challenging to recover, resulting in an increased size distribution. It was not possible to match individual particles after recovery to their original size. Alternatively and very likely, the increased size was an artifact of the automated sizing function measuring multiple adjacent small particles as one large particle. The recovered filters contained abundant smaller particles from the sediment sample that

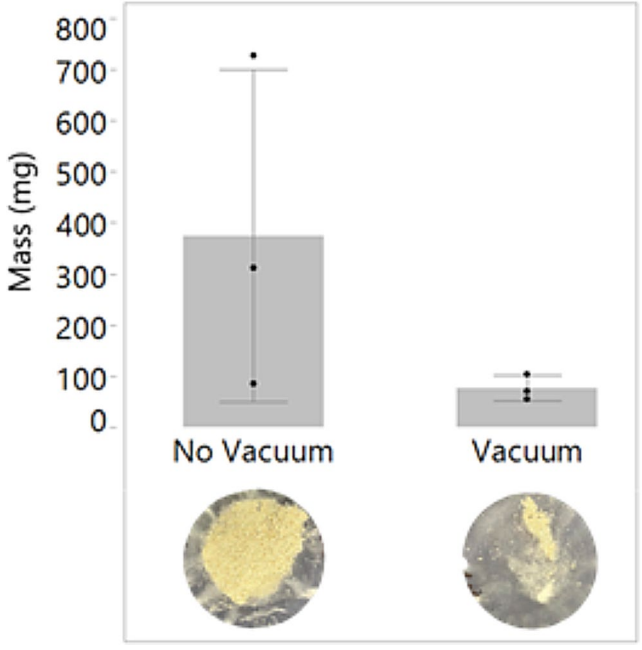

Fig. 3 The mass of deep-sea sediment material that floated in the DSD after being processed with and without the application of a vacuum. Though not statistically significant (p = 0.13), visually more natural material floated without vacuum

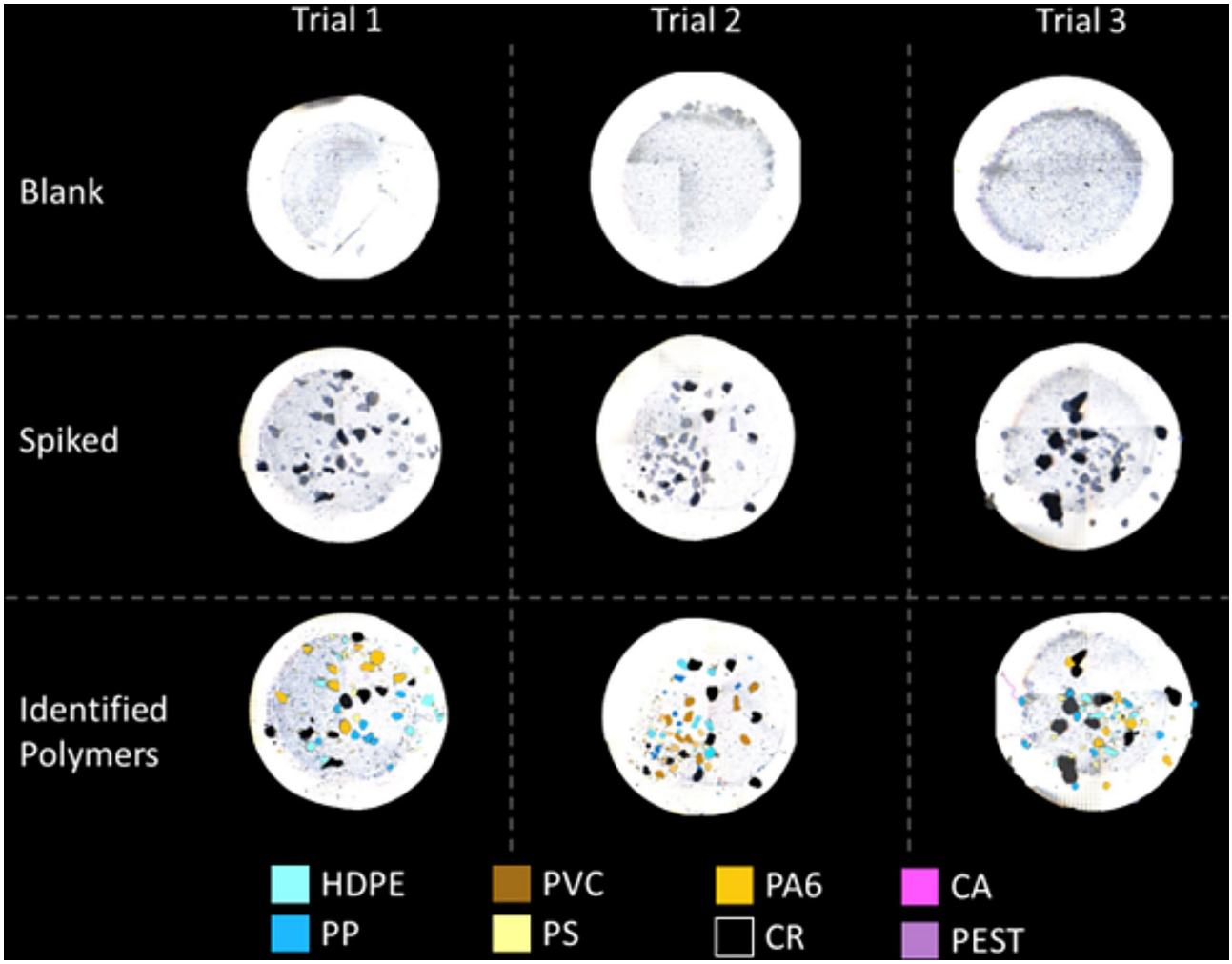

Fig. 4 Images of particles on the gold filters from three replicates of non-spiked (Blank) and microplastic-spiked deep-sea sediment (Spiked) processed with the Density Separation Device (DSD). The identity of the polymers in the spiked images were color coded using Photoshop in the bottom row

could have overestimated the length of recovered spiked particles (Fig. 4). Some particles were manually measured when the automated measurement was much longer than the other particles (these are noted in Table S3 column K).

The FTIR spectra of PA6 and natural polyamides are very similar and easy to confuse [34]. Before manual review of individual spectra, the percent recovery of "PA6" was much higher. Each spectrum that matched a library spectrum of PA6 or natural polyamide was therefore individually assessed by visually examining the peaks in the 1400 cm^{-1} to 1800 cm^{-1} and 2600 cm^{-1} to 3800 cm^{-1} ranges [31], Primpke personal communication 2021). The peaks in the spectra of natural proteins are less intense in the 2800 cm^{-1} to 3400 cm^{-1} region and the 1400 cm^{-1} to 1700 cm^{-1} region compared to PA6 (Fig. 7).

The average recovery of the two fibers, CA and PEST, were 25.9% and 24.1%, respectively. Recovery of fibers relative to fragments was also lower in similar studies [6, 16, 45]. A common theory is that some fibers can pass through filters or sieves based on their diameter. PEST and CA fibers included in this study had a mean diameter of 22.3 µm ± 1.8 µm and 19.0 µm ± 2.1 µm, respectively. It is possible, but unlikely, that some fibers were lost when the SPT was poured out of the DSD through the 20 µm sieve. The fibers were unlikely lost at the final filtering through a gold 5 µm filter. From the literature review conducted, the majority of fibers used in spike-recovery studies were polyethylene terephthalate/PEST (Table S1) with recoveries ranging from 2.7 to 104.5%. The substantial difference in recoveries highlights the difficulties many researchers face when trying to remove or count fibers from natural matrices. The DSD may have helped the recovery of fibers in our study because the sample was processed in one apparatus, and did not require the transfer of the sample. The reduced recovery of fibers in the current study likely was a result of inaccurate

Table 4 Recovery of eight microplastic polymers spiked into deep sea sediment replicates. The * indicates all Crumb Rubber particles were counted regardless of size. For all other polymers, only particles > 100 µm in length were counted

Spiked polymer	1st Rep # spiked	1st Rep # recovered	# particles in paired blank sample > 100 µm in length	1st Rep % recovery	2nd Rep # spiked	2nd Rep # recovered	# particles in paired blank sample > 100 µm in length	2nd Rep % recovery	3rd Rep # spiked	3rd Rep # recovered	# particles in paired blank sample > 100 µm in length	3rd Rep % recovery	Mean % Recovery	SD of % Recovery
CR*	36	48	0	133.3	61	48	0	78.7	54	44	0	81.5	97.8	30.8
PP	23	25	0	108.7	24	11	1	41.7	27	23	0	85.2	78.5	34.0
HDPE	21	20	2	85.7	23	25	0	108.7	20	17	0	85.0	93.1	13.5
PS	21	21	0	100.0	18	10	0	55.6	22	22	0	100.0	85.2	25.7
PA6	22	37	0	168.2	21	10	0	47.6	20	15	0	75.0	96.9	63.2
PVC	22	23	0	104.5	20	22	0	110.0	22	19	0	86.4	100.3	12.4
PEST (Fiber)	9	5	0	55.6	10	5	3	20.0	10	0	0	0.0	25.2	28.1
CA (Fiber)	9	4	0	44.4	10	1	0	10.0	8	2	0	25.0	26.5	17.3

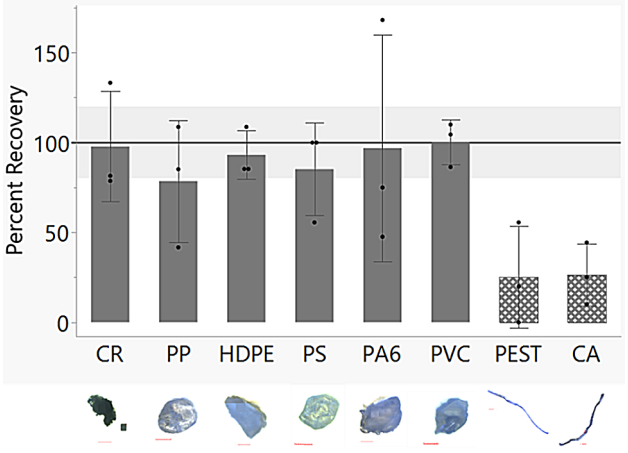

Fig. 5 The average percent recovery (one standard deviation) of eight polymers spiked into a deep-sea sediment material. The light gray shaded area represents 80–120% recovery. Under the polymer abbreviations is a photograph of each polymer before spiking into the sediment. The size bars are 100 µm

counting and the challenge of identifying fibers. The automated Particle Wizard allows for rapid polymer identification of particles on a sample filter, but it is challenged when large particles and tiny fibers are both found on the same filter because of focusing issues. Large fragments and fibers were not in the same plane of view, and the microscope must be focused on only one plane at the beginning of a Particle Wizard or Area Map. Another limitation is that some fibers were found tangled together or obscured under larger particles (Figure S5). Both of these observations would result in lower fiber counts. Untangling the fibers was extremely difficult because the fibers fragmented when pulled. Handling fibers that are successfully removed from the filter was challenging, and many fibers were lost during this process.

Microplastics were found in the blank sediment replicates as well. However, we could not determine if these microplastics were already present in the deep-sea sediment or a result of contamination from the ship or lab. A full description of microplastics found in the blank samples is available in Supporting Information text file and Fig. S6.

Apparatus comparison and benefits of the DSD

This study created and tested an affordable, simple to use, and easily accessible density separation device and method for extracting microplastics from deep sea sediment. Since acceptable recovery can be attained with any density separation apparatuses used previously in the literature (Table 1), the selection of an apparatus can be made based on accessibility, cost, ease of use, and ability to add cleanup steps in the same apparatus. All previously used apparatuses had at least one negative attribute, whereas the DSD tested here did not (Table 2). The DSD is a close derivative of the Sediment Microplastics Isolation Unit (SMI; [9].

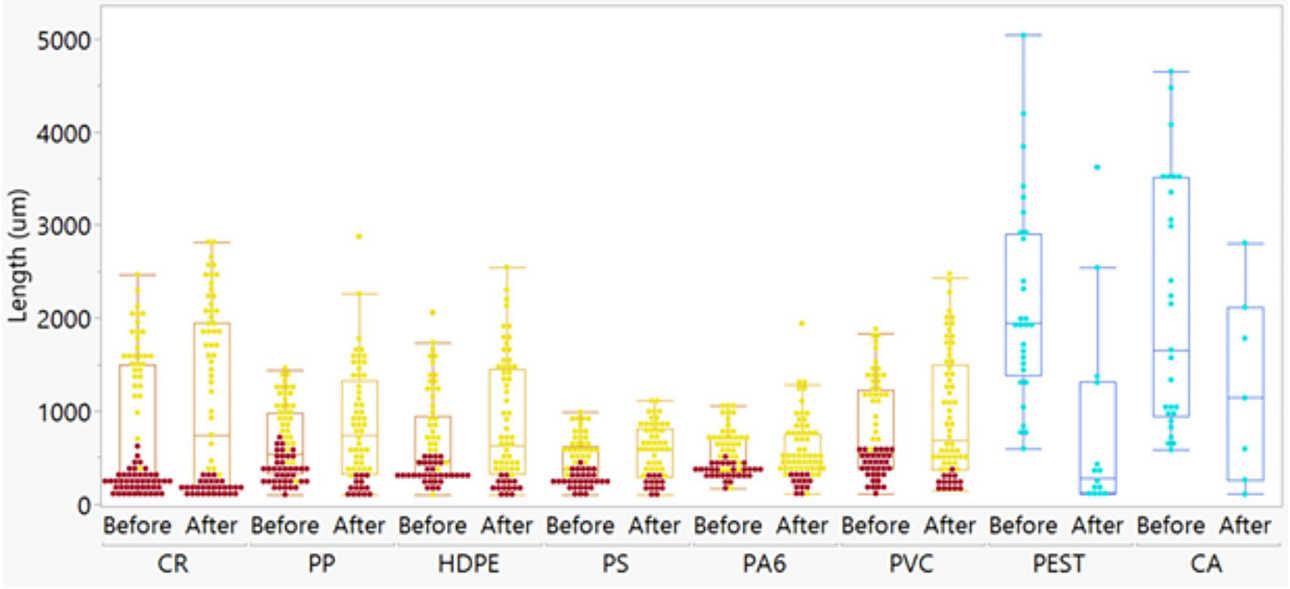

Fig. 6 The length (µm) of microplastic particles and fibers measured before being added to the sediment samples compared to after recovery using the DSD. Blue dots represent fibers, red dots represent medium particles (100–300 µm) and yellow dots represent large particles (> 300 µm). An * indicates a significant difference in length before and after recovery (Wilcoxon $p < 0.05$)

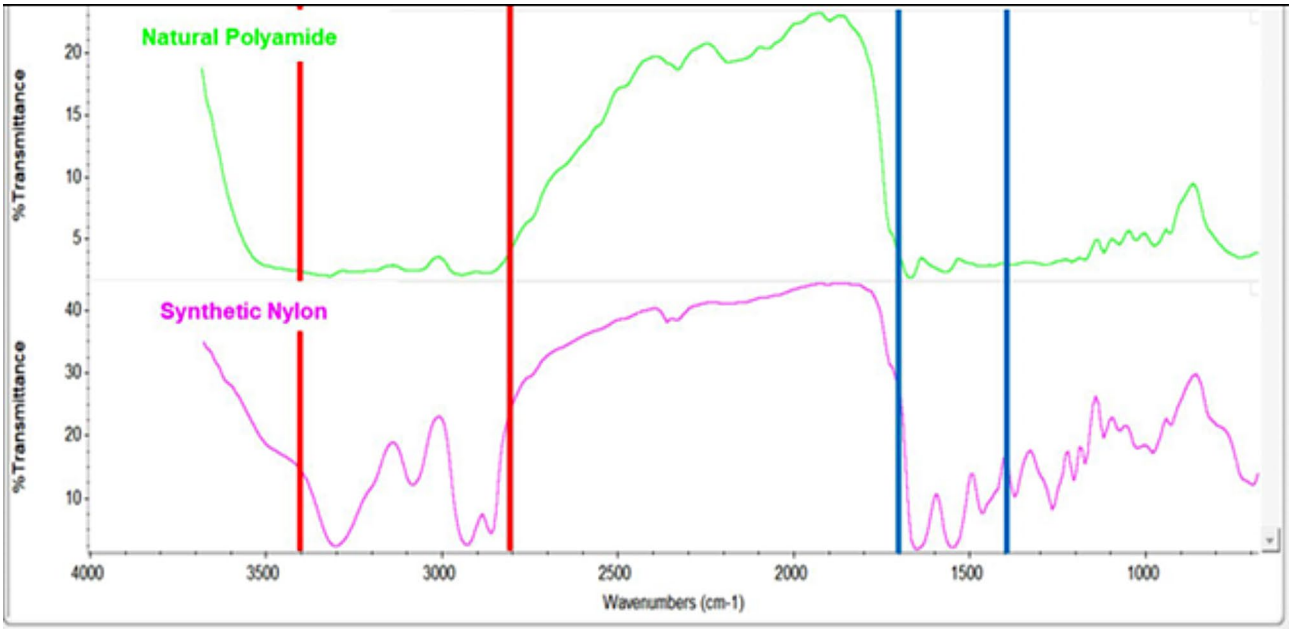

Fig. 7 Comparison of PA6 (bottom) and natural protein (top) FTIR reflectance spectra. The 2800 cm^{-1} to 3400 cm^{-1} region (red) and 1400 cm^{-1} to 1700 cm^{-1} region (blue) have larger relative peaks in the PA6 spectra than the natural polyamide spectra

Both of these apparatuses consist of two chambers with a ball valve in between. The DSD is made almost entirely of glass and metal parts, reducing potential plastic contamination. Only a small portion of silicone o-rings touch the sample. Conversely, the SMI is made entirely of PVC. This greatly increases the chance of contaminating the sample with PVC. Though only three polymers were tested with the SMI, the recovery rates of these polymers, PE, PA6, and PVC are very similar between the DSD and SMI. The Munich Plastic Sediment Separator (MPSS) had 95% to 100 recoveres for all polymers tested: PP, PE, PS, PA6, PET, and PVC [12]. However, since the MPSS was discontinued, custom fabrication is quoted above $42,000. The price of the MPSS greatly limits its accessibility to many researchers.

The overflow column with mid-level inflow (OC-M) and top inflow (OC-T) only differ in the depth at which

additional liquid is added but have vastly different outcomes. This illustrates how one small modification can reduce microplastic recovery from 90 to 100% (OC-T) to 10 to 50% (OC-M; [19]. We rated an aditional overflow device, the Air Inducted Overflow (AIO) apparatus, as having an intermediate complexity apparatus setup due to multiple beakers, tubing, and a pump required to ensure correct flow of the dense liquid. This apparatus has multiple plastic components including the pump and tubing, but also achieved over 90% recovery of PP, PE, PS, PET, and PVC 18]. The overflow method was also used with beakers in a similar fashion, achieving high recoveries for most polymers [10, 13]. Even though these simpler methods can achieve high recoveries of polymers, one of the best and most unique features of the DSD is the ability to process a sample through the entire separation and digestion workflow without changing glassware. Decanting the separation solution without disturbing the bottom sediment layer is simply performed after isolating the two chambers with the ball value. This separation can also be achieved with the SMI or MPSS. However, the DSD allows the solution to be poured out through a sieve attachment that retains the floated particles inside the top chamber. This functionality makes it easy to switch between dense separation liquids and digestion reagents without transferring the extracted particles. While this greatly simplifies the workflow, it also should minimize particle loss.

Parts for the DSD are available from online retailers for less than US$200. It is easy to assemble and the wide mouth makes adding sediment easy. Sodium polytungstate was used in this experiment because of its superior density, non-corrosive properties, and low toxicity, but it is expensive. Other dense liquids could be used in place of the SPT, making it more affordable, but salt solutions, especially sodium chloride, may cause metal parts of the DSD to rust. Mixing was done with manual shaking of the DSD, which can be tiring, but this could be automated with a shaker or rotator. Stainless steel balls can be added if desired to improve mixing of the sample, though the metal balls could cause microplastic particle fragmentation. Furthermore, the DSD allows for mixing of the sediment in the bottom chamber separated from the already-floated microplastics in the top chamber.

Another benefit of the DSD is a diversity of apparatus sizes. The DSD used here had a diameter of 1.5 in., but the parts of the DSD are available in diameters of 0.5 in. and 3 in., respectively. The sample mass used in this experiment was approximately 50 g, which works well in the 1.5 in. diameter DSD. The 3 in. DSD can process a larger amount of sediment without subsampling. The pore size of the sieve can also be adjusted. Both 5 µm and 20 µm sieves are available online.

Use of the DSD is not limited to sediment samples. Any samples requiring density separation and/or digestion can be processed in the DSD including fish gastrointestinal (GI) tract, water, and roadway runoff samples. Each sample matrix should be tested in the DSD so method adjustments can be made. For example, samples with a high amount of organic matter, such as GI tracts or wastewater sludge, may benefit from undergoing a digestion before the density separation. The digestion step can be done first in the DSD, the chemicals and digested matter drained out through the sieve attachment, and then the dense liquid added for density separation. In these ways the DSD can be modified for a wide range of matrices.

Conclusions

An efficient and reproducible extraction method is critical for microplastic studies. Researchers should perform chemical compatibility and spike-recovery experiments to ensure adequate recoveries from each particular matrix. Though one specific workflow will probably not work across all sediment or sample types, the DSD can be adapted for a wide variety of matrices by changing the type of dense liquid, digestion chemicals, sieve size, or size of the DSD itself. Its adaptability can transform the microplastics research field by offering a standardized apparatus that many laboratories can easily access.

Abbreviations

DSD	Density Separation Device
HDPE	High density polyethylene
PE	Polyethylene
PP	Polypropylene
PVC	Polyvinylchloride
PS	Polystyrene
PA6	Nylon
CR	Crumb rubber
CA	Cellulose acetate
PEST	Polyester
SMI	Sediment Microplastics Isolation
JAMSS	JAMSTEC microplastic sediment separator
MPSS	Munich Plastic Sediment Separator
ATR-FTIR	attenuated total reflection- fourier transform infrared spectroscopy
SPT	Sodium polytungstate
CMDR	Center for Marine Debris Research
µFTIR	Microscope FT-IR

Supplementary Information

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Supplementary Material 1

Supplementary Material 2

Supplementary Material 3

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Author contributions

KRS: Methodology, Formal analysis, Investigation, Data Curation, Writing-Original Draft, Visualization. RS: Methodology, Formal analysis, Investigation, Data Curation, Writing- Review & Editing, Visualization. CF: Methodology,

Investigation, Writing- Review & Editing. JB: Methodology, Investigation, Writing- Review & Editing. AF: Methodology, Investigation, Writing- Review & Editing. JTS: Investigation, Supervision. SG: Conceptualization, Methodology, Investigation, Resources, Writing- Review & Editing, Supervision, Project administration. JML: Conceptualization, Methodology, Validation, Formal Analysis, Resources, Writing- Review & Editing, Visualization, Supervision, Project Administration, Funding acquisition.

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Data availability
Full literature review (Table S1), raw data (Table S2); Supporting Information text file including: 1) Supplementary Fig. 2) Sodium polytungstate use and recycling 3) Microplastic quantification in non-spiked sediment and 4) Links to purchase DSD.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

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The authors declare no competing financial interest. Certain commercial equipment, instruments, or materials are identified in this paper to specify adequately the experimental procedure. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology, nor does it imply that the materials or equipment identified are necessarily the best available for the purpose.

Competing interests
The authors declare no competing interests.

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