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Binary solvent extraction of microplastics from a complex environmental matrix

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Abstract

Separating microplastics (MPs) (smaller particle size, < 1 mm) from complex environmental samples such as particulate organic matter (POM) is challenging, particularly for polyethylene and polypropylene, which are buoyant like POM. It is often done using a time-consuming procedure, often with hazardous waste generation. We developed a simple, low-cost procedure using a binary solvent mixture (ethanol–water) followed by water solvation to separate MPs from estuarine POM and surface water. The isolated MPs were quantified and characterized using μFT-IR and scanning electron microscopy, with particle sizes ranging from 30 to 2500 μm and percentage mass from 2.62–21.3% wt/wt in POM and 0.04–0.42% wt/vol for surface water, respectively. Different polymer types, colors, and shapes were observed. Method recovery assessed using spiking yielded 89–93.1% and the method was validated by visual sorting with dye staining. This method is low-cost, simple, and aligns with Green Chemistry approaches while efficiently separating plastics of various particle sizes, shapes, and compositions. Furthermore, this low-cost approach and the near-universal availability of ethanol make this method more accessible in research and education throughout regions of the world where plastic debris is a major challenge but resources to study the problem are limited.

Microplastics (MPs) are found in various environmental matrices globally. They possess unique properties which render them mobile with the ability to sorb contaminants and permeate membranes (Fadare et al. 2019). MPs have been found in human stool (Schwabl et al. 2019), lungs (Amato-Lourenço et al. 2021; Jenner et al. 2022), colons (Ibrahim et al. 2021), placentae (Ragusa et al. 2021), and blood (Leslie et al. 2022). While little is known to date regarding its effects on humans, studies are beginning to enumerate MP impacts on organisms across species and ecosystems (Cole et al. 2013; Luo et al. 2022). Though innovative studies on MP occurrence and abundance are emerging daily, there are still limitations in the methods of detection, extraction, identification, quantification, and monitoring of MPs in the environment (Grbic et al. 2019). These issues coupled with limited access to analytical instrumentation may result in under or overestimation of MP abundance depending on study design, long sample processing times, hazardous waste generation, and cost (Nguyen et al. 2019; Okoffo et al. 2019; Alimi et al. 2021). In addition, these limitations are responsible for the lack of harmonized and universally accepted methods of sampling, extraction/separation, analysis, and reporting. Hence, improvements to existing methods or new methods are needed to extract MP from environmental matrices.

Unlike other environmental samples, the separation of MP from particulate organic matter (POM) is particularly challenging. Its low density and subsequent buoyancy result in its capture alongside a myriad of MP polymers with the most density separation methods. Hence, samples are often pretreated with

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Additional Supporting Information may be found in the online version of this article.

chemicals or enzymes to decompose the organic matter. The POM captured interferes with recoveries, identification, and quantification of MPs, particularly MPs ≤ 1 mm (Nguyen et al. 2019) which can be toxic to aquatic organisms (Gray and Weinstein 2017; Liu et al. 2020). For instance, MP combined with organic matter was found to enhance the accumulation and induce higher toxicity of heavy metals in fish tissue (Qiao et al. 2019). Furthermore, these smaller-sized items have relatively larger surface areas to sorb pollutants and are able to penetrate epithelial barriers due to their high mobility in various mediums (Fadare et al. 2020).

Methods to extract and quantify MPs from organic matter depend on environmental matrices, target particle sizes, and the proposed research questions. Some methods include manual removal via the naked eye or microscope, enzymatic digestion, chemical oxidation, and physical separation based on plastic density relative to the aqueous salt solution (NaCl, $ZnCl_2$, NaI, etc.; Nguyen et al. 2019; Okoffo et al. 2019). The density approach has also been used with only ethanol (96%; 0.8 g cm^3) by Herrera et al. (2018) to separate MP from vegetal-rich samples. Herrera et al. approach is notable as it deviates from most aqueous salt density separation methods. It follows a binary mixture separation method of ethanol to water (96 : 4), since 100% ethanol may not be obtainable because ethanol normally forms an azeotrope (a binary combination of two or more liquids exhibiting the same concentration and composition in both the liquid and vapor phases and vaporizes at certain temperature and pressure) with water. Binary mixture separation techniques have been described in the literature for over three decades (Hoffmann et al. 1993; Wakisaka and Ohki 2005; Dolai et al. 2018) but have not been explicitly optimized in MP research.

Binary solvent mixtures overcome the hydrophobic interaction/effect of nonpolar molecules, such as those on the surface of POM that prevent wetting. A solvent–water mixture acts as an "anti-hydrophobic agent" (Breslow et al. 1998) that solvates hydrophobic surfaces and allows water to penetrate the POM's internal matrix. The use of a binary solvent mixture can advance our understanding of MP presence, fate, behavior, and impact on the environment because it is an easy, fast, and cheap method of separating MPs from organic matter (Schrank et al. 2022). The method discussed below may also preserve aspects of MP chemistry allowing for the extraction of sorbed contaminants, particularly hydrophobic chemicals.

A new method of separating, quantifying, and verifying smaller MP (< 1 mm) from estuarine POM using a binary solvent mixture (Catalán et al. 2019) of ethanol and water is discussed below. This method can be used with various environmental matrices and combined with existing methods, particularly density separation using salt solutions, to target a variety of polymers. The binary solvent mixture used accelerates POM wetting, resulting in its loss of buoyancy. When the binary mixture is removed and only water is reintroduced to the sample, POM sinks while MP polymers float. The work focuses on extraction methods and their chemical mechanisms as well as the quantification and identification of the extracted MP polymers.

Materials and procedures

Materials

Absolute ethanol (EtOH; ≥ 99.5%) was obtained from EMD Millipore Cooperation (CAS No.: 64-17-5). Methylene blue (CAS:7220-79-3) and Nile red (CAS: 7385-67-3) were obtained from ACROS Organics. Methylene blue stock solution (10%) was prepared by dissolving methylene blue in water and subsequently, working solutions of 0.1% was used. Nile red stock solution of 100 mg L^{-1} was prepared in *n*-hexane with initial dissolution in a small amount of acetone and then made up to a known volume in hexane due to its low solubility in hexane. 0.5 mg L^{-1} working solution was used for staining (Michelaraki et al. 2020). A stereo microscope (EMZ-8TR; Meiji Techno) was used to select a subset of the isolated plastic particles. The selected subset of plastic debris was characterized after quantification using Fourier-transform infrared (FT-IR) spectrometry (Thermo Scientific Nicolet iS10 FT-IR). Spectra were matched with polymers with an in-house library and verified using Open Specy (Andrade et al. 2020; Cowger et al. 2021). A scanning electron microscope (SEM; JCM-5000; NeoScope) was used to examine the surface morphology (Supporting Information Fig. S8). Fluorescence microscope (Olympus CKX53 with Hamamatsu ORCA-Spark 431-3196) was used for the validation of the recovery method.

Method development

Binary solvent extraction is a liquid–liquid extraction or partitioning method used to separate compounds based on their relative solubilities in two different solvents. The proposed method employed the relative polarity of the binary solvent system to overcome POM hydrophobicity to separate MP from environmental samples (Supporting Information Fig. S1).

Sample collection and preparation.

Beach wrack line debris, which included POM and MP, and surface water containing MPs (quadruplicate, $n = 24$) were collected from three shorelines of Lavaca Bay, Texas on 15 and 16 December 2021. These sites are named Peninsula Park (PL; 28°38′30″N, 96°19′23″W), Light House Beach (LH; 28°38′21″N, 96°36′39″W), and Six Mile (SM; 28°41′37″N, 96°39′45″W). The wrack line samples were placed in a Ziplock bag, while water samples were stored in amber glass bottles.

Sample preparation and initial testing.

The wrack line samples were air dried in the laboratory for 2 d before sieving with a 1 mm mesh (Supporting Information Fig. S2). Next, the sieved sample was briefly (< 10 min) soaked in deionized water to remove sand particles. The sand and miscellaneous materials that precipitated were examined for

MP under a stereo microscope. Within the materials that precipitated, no suspected plastic particles were observed. This was anticipated because most MP in the wrack line is deposited during tidal fluctuations due to their buoyancy. After sand removal, the remaining materials were sieved using 30 μm mesh and air dried for 2 d before 0.5 g aliquots were randomly selected and placed in test tubes. Next, 10 mL of a binary mixture of varying EtOH : water ratios (10 : 0, 9 : 1, 8 : 2, 7 : 3, 6 : 4, 5 : 5, 4 : 6, 3 : 7, 2 : 8, 1 : 9, 0 : 10) was added, gently shaken and allowed to soak for 2 h. The behavior of POM and MPs varied across the EtOH : water ratios (Supporting Information Fig. S4). From EtOH : water (0 : 10 to 2 : 8), nearly all material floated. For 3 : 7 and 4 : 6, some MP precipitated, while nearly all POM and most MP floated. From 5 : 5 to 9 : 1, increasing amounts of MP and POM precipitated into two distinct layers as the ratio approached 9 : 1 (Supporting Information Fig. S4). All ratios from EtOH : water (10 : 0 to 6 : 4) resulted in near complete POM buoyancy loss, which is the objective of this step. In addition, at ratio 5 : 5, POM also lost buoyancy, but the time required for soaking was greater than the 2 h needed with higher ratios of EtOH.

The ratio used to validate this method was EtOH : water (8 : 2), but future studies could use any ratio from 10 : 0 to 5 : 5 depending on the desire to minimize EtOH use and sample throughput. In addition, the chemical composition of POM may vary based on the vegetation source, influencing the EtOH : water ratio or soak time required. Therefore, this crucial binary solvent step must be optimized to meet the needs of each study.

After soaking the sample in the binary solvent mixture, the solution was removed by pipette, carefully avoiding the POM and MP (Supporting Information Fig. S4A). Next, 10 mL of DI water was added, and the tubes were gently shaken. Over 5 min, the MP previously at the bottom of the tube floated to the surface while the POM was initially resuspended before precipitating to the bottom (Supporting Information Fig. S6B–G). This step was repeated twice to maximize MPs separation from the POM. The isolated MPs were transferred via pipette from the test tube and placed on a filter membrane for further analysis.

Assessment

To assess the source of wrack line MP, water samples (100 mL) collected adjacent to each beach site were extracted for MPs using vacuum filtration using a membrane filter. The recovered MPs were cleaned up under the microscope and the subsample was characterized using FT-IR. To assess the thoroughness and extraction efficiency of this method, visual screening under a stereo microscope (×40 magnification) of both the MP isolated as well as the extracted POM was carried out. Negligible amounts of POM were found within the separated MP material (Fig. 1), while equally low amounts of MP were found in the extracted POM. The maximum MP particles found in any of the 12 samples of the POM extracts were 6. When these materials were analyzed using FT-IR, they matched for polyethylene (PE), indicating that an additional washing step would likely account for this minor amount of MP loss, if deemed necessary based on research objectives.

MPs characterization

FT-IR analysis (Supporting Information Fig. S7) was performed on 23–25 randomly selected items from each replicate of the isolated MPs (Supporting Information Fig. S5). Also, the morphological features of some of the MPs were captured (Supporting Information Fig. S8) using SEM (JCM-5000; NeoScope).

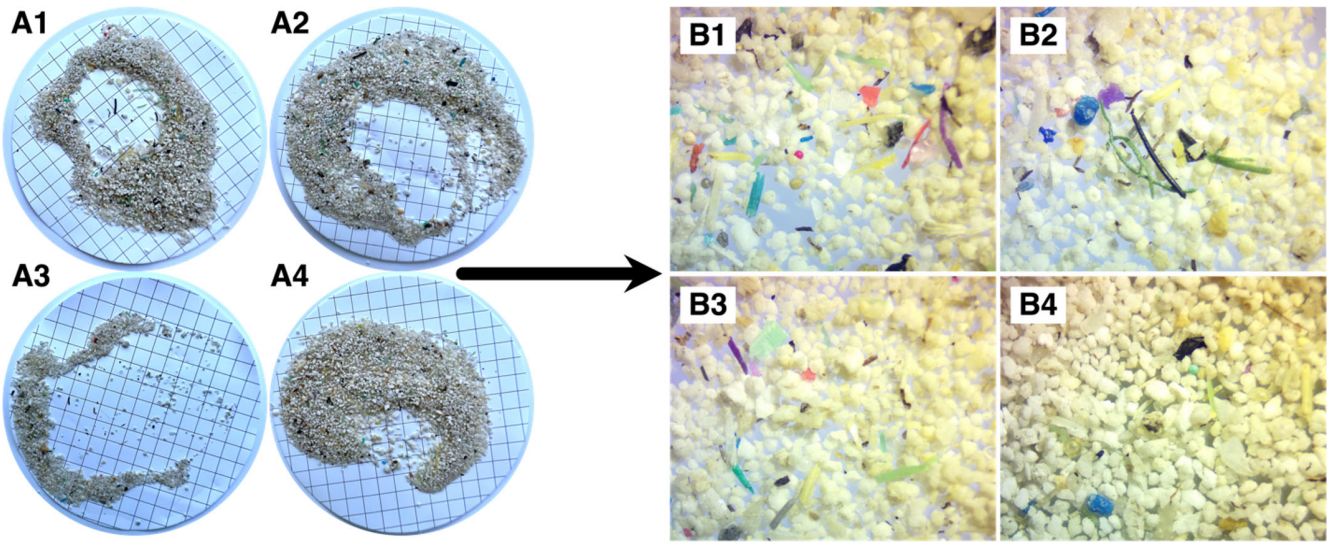

Fig. 1. (**A1–4**) Representative microplastic samples isolated from POM in sampling locations within Lavaca Bays, Texas. (**B1–4**) ×40 Magnification of the representative samples.

Quality assurance and quality control

The objective of this research was to extract MP debris (30 μm to 1 mm) from wrack line samples. Therefore, the quality control used reflects the need to limit contamination within that size range, which is mostly particulate in shape, rather than smaller MP, including fibers, and nanoplastics. Briefly, quality control procedures included washing and rinsing all glassware and utensils with DI water before being muffled at 500°C for 4 h and then storing covered to reduce airborne contamination (Hermsen et al. 2018). Laboratory contamination of samples was assessed using four blank samples. The materials from the blank samples consisted of an average of 12.8 ± 3.0 microfibers. Similar fibers were not found in the samples and therefore, no corrections were done to the final results. Dyed Cotton laboratory coats and gloves were worn at each step of the analysis. Analysis was done in a laboratory in an air filtration system and all surfaces were cleaned with DI water each time before use.

The percentage recoveries for the isolated MPs in POM were estimated (Supporting Information Table S1) by mixing a proportion of the isolated MPs and POM in 1 : 5 and repeating the whole extraction process using the binary solvent mixture of ethanol and water (7 : 3) to recover the MPs. We used two approaches to further evaluate our recovery method. First, we visually observed the POM under the stereo microscope where 6 MP particles was the maximum number found in only 1 of the 12 wrack line samples assessed (PL). Second, 1 g each of the recovered POM was stained with methylene blue to mask the fluorescence of organic matter and reduce interference. After that, the POM was stained with Nile red and viewed under the fluorescence microscope (Olympus CKX53 with Hamamatsu ORCA-Spark 431-3196). The fluorescent MPs were counted and estimated in particle per gram (particle g^{-1}) of the POM (Supporting Information Fig. S3).

Discussion

The method developed uses a binary solvent mixture of ethanol and water to separate POM from MP debris. The results and the underlying chemistry of this method are discussed below. A representative selection of the MPs isolated from POM in wrack line debris at sites in Lavaca Bay is shown in Fig. 1. White blocky particles overwhelmingly dominated the samples collected. These atypical materials are released at this size and shape from a nearby industrial facility that produces preproduction plastics. However, note that there are also small plastics that typically dominate estuarine systems without an irresponsible industrial polluter. When viewed at $\times 40$ magnification, these typical materials have multiple colors, shapes, and sizes (Fig. 1B1–4).

Isolated MPs were quantified by mass rather than count due to their small size. MP constituted 2.62–21.3% (14.8 ± 7.1) of the POM sample mass and 0.04–0.42% wt/vol (0.2 ± 9.5) for the surface water. Peninsula Park (PL) had the highest concentrations of MPs in both the beach POM and surface water at 196.6 ± 15.7 mg g^{-1} and 3.3 ± 1.0 mg L^{-1}, respectively (Fig. 2A). Six Mile (SM) beach had the lowest MP concentrations at 60.1 ± 29.6 mg g^{-1} and 0.7 ± 0.3 mg L^{-1} in the POM and surface water, respectively.

The most abundant MP polymer-type across all the samples was PE ($\sim$ 65%). Polypropylene (PP) and copolymer (mixed polymers) recorded 18% and 4% of the total subsampled MP, respectively, using FT-IR (Supporting Information Fig. S7). Although about 13% of the particles give a convincing feature of polymers when visually observed under the stereo microscope, however, no confident material match could be determined hence they were named as unclassified (Fig. 2B).

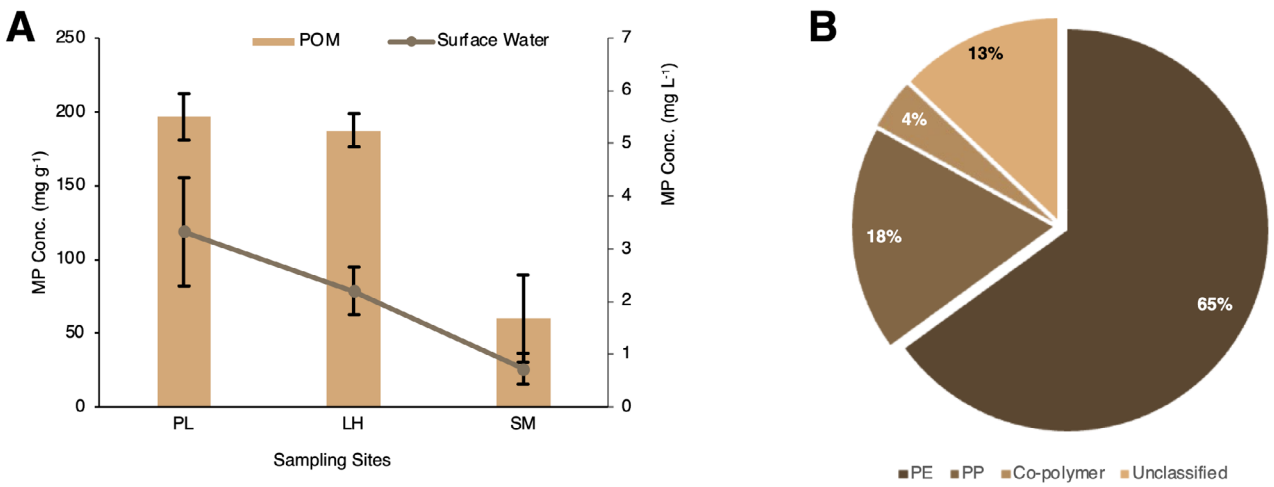

Fig. 2. (**A**) The total average concentration of MP from the beach wrack line POM and surface water from three sampling locations in Lavaca Bay, Texas. PL (Peninsula Park), LH (Light House Beach), and SM (Six Miles Beach). Error bar—SD, $n = 4$. (**B**) represents the overall percentage distribution of the polymer types of the characterized microplastics from the three sampling locations.

Chemistry of the separation technique

POM is a complex environmental matrix that ensnares materials, including buoyant and non-buoyant macroplastics and MPs, as it moves across surface waters. The comparable density and size of POM to several MP polymers (PE, PP, foamed polystyrene, etc.) limit the effectiveness of the most used method, density separation, to partition MPs from POM. The use of a binary solvent mixture enables the separation of these similar-density materials by altering POM surface chemistry to induce wetting and decrease buoyancy.

POM consists of humic substances having hydrophilic and hydrophobic groups, charged sites, and counter ions (Santschi 2018; Buruga et al. 2019; Piccolo et al. 2019). Depending on the parent material, POM has varying portions of polysaccharides and lignin. POM is difficult for water to penetrate (wetting) due to its chemical characteristics, surface cations, and interactions with other organic moieties and minerals in the marine environment (Buruga et al. 2019). For instance, when cations (e.g., H^+, Na^+, NH^{4+}, Fe^{3+}, Ca^{2+}, Mg^{2+}, Hg^{2+}, Al^{3+}, and organic cations) are bound to the POM's exchange sites, intramolecular charge repulsion is minimized preventing POM wetting that results in hydrophobicity (Swift 1996; Zhao et al. 2021). Furthermore, multivalent cations may also form crosslinked intra and intermolecular bonds with multiple charged sites on the POM. This crosslinking further compacts the POM molecular structure, restricting the wetting of the organic matrix, and resulting in hydrophobicity that drives buoyancy (Swift 1996). There are also other mechanisms that influence POM wetting including dispersion or van der Waal's forces, polarizability, polarity or dipole interactions, permanent dipole–dipole interactions, or hydrogen bonding interactions (electron exchange) (Piccolo et al. 2019). The overall effect of these mechanisms produces strong bonding on the POM surface which contributes to hydrophobicity.

Therefore, understanding the physicochemical behaviors of the POM composition helps when selecting the optimal method for MP separation. For example, one way to easily separate MPs, particularly buoyant polymers, from POM, is to get one material to float while the other sinks. This can be done by displacing insoluble cations present on the POM surface while disrupting intramolecular and intermolecular associations (bonding) and surface interactions. This was achieved through a binary solvent medium that penetrated the POM and exposed the bonding within its moiety.

Herrera et al. (2018) used 96% ethanol for the density separation of MP from organic matter which yielded an improved and simplified method of MP extraction in organic matter. However, the study was limited to a particle size of 1–5 mm, with no mention of MP < 1 mm which is more toxicologically problematic. Also, water–ethanol–water interactions were not employed in their method as only ethanol (96%) was used. The method described here can be optimized to use 50% ethanol, reducing costs and solvent use. Therefore, relative to Herrera et al. (2018), this method can be optimized to use as little as 50–60% ethanol to reduce costs and solvent use, while also separating smaller MP sizes (< 1 mm) from POM.

Second, Herrera et al. reported that their method does not capture polystyrene, and polyurethane foams because they are buoyant in 96% ethanol used. These two polymers were not identified during our FT-IR analysis. However, because of the additional water solvation step in this method, foamed polystyrene and polyurethane, which are buoyant in water, will be captured with this method.

Comments and recommendations

Limitations

Our observation of the current procedure revealed that this technique may not be suitable for microfiber extraction as we could not find any microfiber particles in all our isolated MPs against our expectations. However, microfibers were also not observed in the extracted organic matter when examined under the stereo microscope. It is also possible that some microfibers are lost during sieving.

Also, improved methods for separating MP from complex environmental matrixes with little or no alteration to the chemistry of the isolated MP are needed, especially for studies that quantify contaminants sorbed to environmentally weathered plastic. This method, because it uses diluted ethanol, may not be sufficient for hydrophilic chemicals sorbed to plastic. However, because of ethanol's higher polarity, it may not interfere with the stronger bonds of hydrophobic chemicals on MP surfaces. This also requires additional investigation in our future studies.

Applications

This simple and cost-effective extraction method efficiently separates MPs from 30 μm to 1 mm. It can be combined with existing density separation methods to capture higher-density MP by using an aqueous salt solution instead of DI water for the water solvation step. Furthermore, it can be adapted and optimized to extract MP from POM in other environmental settings that have not been heavily studied, particularly riparian zone POM as well as soils and sediment that contain organic matter. Binary solvent mixtures could also be used to extract MP from wastewater with its high organic matter content. In addition to versatility, POM separation from MP uses only water and EtOH (which can be easily recovered and reused), aligning with the principles of Green Chemistry (Anastas and Warner 1998), which should be a point of emphasis in MP research. Last, this simplistic and low-cost approach with broad applicability can expand research potential for areas where plastic pollution is significant, but resources to study and address the problem are limited.

Environmental safety

Complex environmental samples need extra steps like digestion along with density treatment to separate MPs. The chemicals used in organic matter digestion including strong acids, bases, and oxidants (hydrochloric acid, sodium hydroxide,

hydrogen peroxide, zinc chloride, and sodium iodide) are mostly harmful and corrosive. This novel method does not employ any of these chemicals, which is an advancement in MP research.

Data availability statement

All information on this study is given in Supporting Information.

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Conflict of interest

None declared.

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