

Binary Solvent Extraction of Microplastics from Complex Environmental Matrix.

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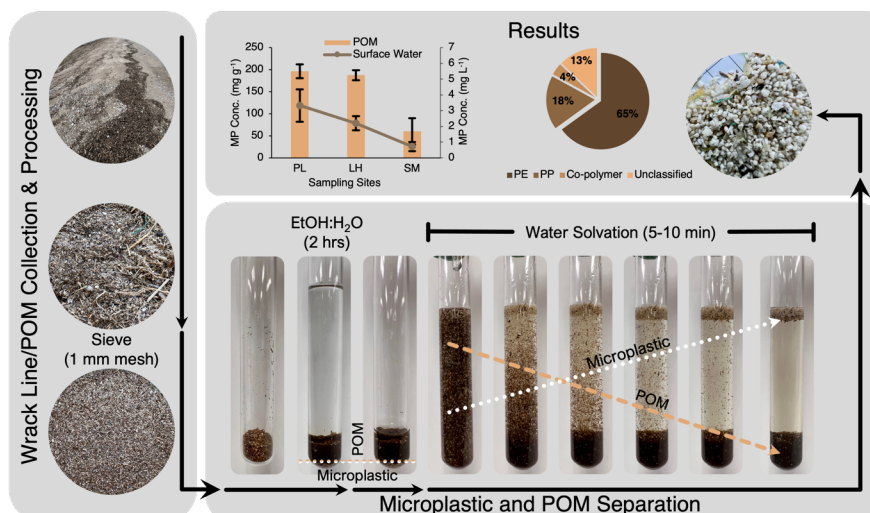
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Abstract

Separating microplastics from 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 method using a binary solvent mixture (ethanol-water) followed by water solvation to separate microplastics from estuarine POM and water. The isolated microplastics were quantified and characterized using μ FT-IR and SEM, with particle sizes ranging from 30-2500 μ m and percentage mass from 2.62-21.3% $^w/w$ in POM and 0.04-0.42% $^w/v$ 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 simple method aligns with Green Chemistry approaches by using ethanol, making it accessible to researchers throughout regions of the world where plastic debris is a major challenge but resources to study the problem are limited.



INTRODUCTION

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.¹ MPs have been found in human stool,² lungs,^{3,4} colons,⁵ placentae,⁶ and blood.⁷ While little is known to date regarding its effects on humans, studies are beginning to enumerate MP impacts on organisms across species and ecosystems.^{8,9} 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 microplastics in the environment.¹⁰ 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.^{11,12,13} Additionally, 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 results in its capture alongside a myriad of MP polymers with most density separation methods. The POM captured interferes with recoveries, identification, and quantification of MPs, particularly MPs > 1 mm¹² which are more toxic to aquatic organisms.^{14,15} For instance, MP combined with organic matter was found to enhance the accumulation and induce higher toxicity of heavy metals in fish tissue.¹⁶ 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.¹⁷

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 naked eye or microscope, enzymes digestion, chemical oxidation, and physical separation based on plastic density relative to aqueous salt solution (NaCl, ZnCl₂, NaI, etc.).^{12,13} The density approach has also been used with only ethanol (96%; 0.8 g cm³) by Herrera *et al.*¹⁸ 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 with water. Binary mixture separation techniques have been described in literature for over three decades,^{19–21} but has not been explicitly optimized in microplastic research.

Binary solvent mixtures overcome the hydrophobic interaction/effect of non-polar molecules, such as those on the surface of POM that prevent wetting. A solvent-water mixture acts as an "anti-hydrophobic agent"²²

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 microplastics from organic matter.²³ 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 mixture²⁴ 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 METHODS

Absolute ethanol (EtOH, [?]^{99.5%}) was obtained from EMD Millipore Cooperation, MA, USA (CAS No: 64-17-5). Methylene Blue (CAS:7220-79-3) and Nile Red (CAS: 7385-67-3) were obtained from ACROS Organics, NJ, USA. A stereo microscope (Meiji Techno, EMZ-8TR, Japan) 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 spectrometry (Thermo Scientific Nicolet iS10 FT-IR). Spectra were matched with polymers with an in-house library and verified using Open Specy.^{25,26} Scanning electron microscope (NeoScope, JCM-5000) was used to examine the surface morphology (Figure S8).

Method Development. Identification and isolation of smaller MPs (30 μ m - 1 mm) within complex environmental matrices like POM, which are similar in size and density to buoyant MP, is difficult and time-consuming. The proposed method that overcomes POM hydrophobicity using a binary solvent mixture to separate MP from environmental samples is shown in Figure S1.

Sample collection and preparation. Beach wrack line debris, which included POM and MP and surface water containing microplastics (quadruplicate, n=24) were collected from three shorelines of Lavaca Bay, Texas on December 15 and 16, 2021. These sites are named Peninsula Park (PL; 28 38'30"N 96 19'23"W), Lighthouse 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.

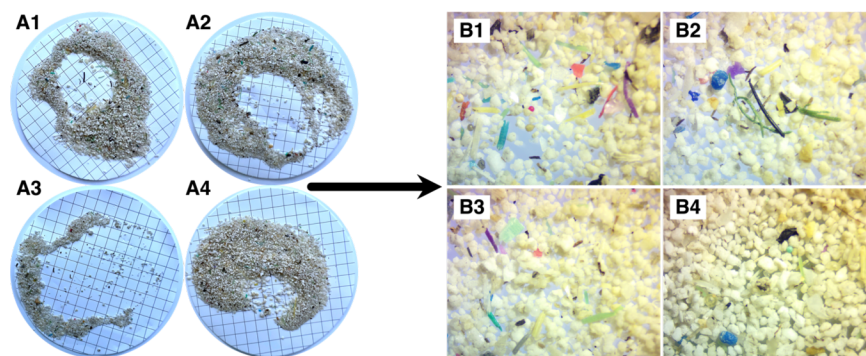
Sample Preparation and Initial Testing. The wrack line samples were air dried in the laboratory for two days before sieving with a 1 mm mesh (Figure S2). Next, the sieved sample was briefly (<10 min) soaked in deionized water to remove sand particles. Within the materials that precipitated, no suspected 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 μ m mesh and air-dried for two days before 0.5 g aliquots were randomly selected and placed in test tubes. The sand and miscellaneous materials that precipitated were examined for MP under a stereo microscope. 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 two hours. The behavior of POM and MPs varied across the EtOH:water ratios (Figure 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 (Figure 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. Additionally, at ratio 5:5, POM also lost buoyancy, but the time required for soaking was greater than the 2 hours 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. Additionally, 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 (Figure S4A). Next, 10 mL of DI water was added, and the tubes gently shaken. Over 5 minutes, the MP previously at the bottom of the tube floated to the surface while the POM was initially re-suspended before precipitating to the bottom (Figure S6B-G). This step was repeated twice to maximize MPs separation from the POM. The isolated microplastics were transferred via pipette from the test tube and placed on a filter membrane.

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 membrane filter. The recovered MPs were cleaned up under the microscope and sub-sample characterized using FT-IR. To assess the thoroughness and extraction efficiency of this method, visual screening under a stereo microscope (40x magnification) of both the MP isolated as well as the extracted POM were carried out. Negligible amounts of POM were found within the separated MP material (Figure 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.

Microplastics Characterization. FT-IR analysis (Figure S7) was performed on 23-25 randomly selected items from the isolated MP (Figure S5). Also, the morphological features of some of the microplastics were captured (Figure S8) using Scanning Electron Microscopy (SEM, NeoScope, JCM-5000).



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 hours and then storing covered to reduce airborne contamination.²⁷ 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.

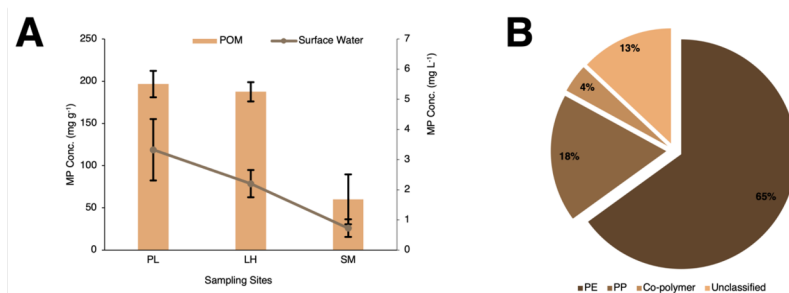
The percentage recoveries for the isolated MPs in POM were estimated (Table S1) by mixing a proportion of the isolated MPs and POM in 1:5 and repeated the whole extraction process using the binary solvent mixture of ethanol and water (7:3) to recover the MPs. We used two approaches to further evaluate 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 twelve 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, Japan). The fluorescent MPs were counted and estimated in particle per gram (particle.g⁻¹) of the POM (Figure S3).

RESULTS AND DISCUSSION

The method developed uses a binary solvent mixture of ethanol and water to separate POM from MP debris. 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 Figure 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 pre-production plastics. However, note that there are also small plastics that typically dominate estuarine systems without an irresponsible industrial polluter. When viewed at 40x magnification, these typical materials have multiple colors, shapes, and sizes (Figure 1B1-4).

Isolated microplastics 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% w/v (0.2 ± 9.5) for the surface water. Peninsula Park (PL) had the highest concentrations of microplastics 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 (Figure 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 polyethylene (PE; ~65%). Polypropylene (PP) and co-polymer (CP; mixed polymers) recorded 18% and 4% of the total sub-sampled MP respectively using FT-IR (Figure 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. (Figure 2B). **Chemistry of the separation technique.** POM is a complex environmental matrix that ensnares materials, including buoyant and non-buoyant macro and microplastics, as it moves across surface waters. The comparable density and size of POM to several microplastic polymers (PE, PP, foamed polystyrene, etc.) limit the effectiveness of the most used method, density separation, to partition microplastics 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.²⁸⁻³⁰ Depending on 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.²⁹ For instance, when cations (e.g., H⁺, Na⁺, NH⁴⁺, Fe³⁺, Ca²⁺, Mg²⁺, Hg²⁺, Al³⁺, and organic cations) are bound to the POM's exchange sites, intramolecular charge repulsion is minimized preventing POM wetting that results in hydrophobicity.^{31,32} 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 wetting of the organic matrix, resulting in hydrophobicity that drives buoyancy.³² There are also other mech-

anisms 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).³⁰ 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 get one material to float while the other sinks. This can be done by displacing insoluble cations present on the POM surface while disrupting intra- and inter-molecular 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.*¹⁸ used 96% ethanol for density separation of MP from organic matter which yielded improved and simplified method of MP extraction in organic matter. However, the study was limited to particle size of 1-5 mm, with no mention of MP <1 mm that are 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.

Limitations. Our observation in the current procedure revealed that this technique may not be suitable for microfiber extraction as we could not find any microfiber particle in all our isolated microplastics against our expectation. However, microfibrils were also not observed in the extracted organic matter when examined under the stereo microscope. It is also possible that some microfibrils 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 microplastic surfaces. This also requires additional investigation in our future studies.

Applications. This simple and cost-effective extraction method efficiently separates microplastics 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,³³ which should be a point of emphasis in microplastic 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.

Abbreviations

POM-Particulate Organic Matter; MPs-Microplastics; EtOH-Ethanol.

Supporting Information

The Supporting Information is attached

Author Information

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