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Matrix Matters: novel insights for the extraction, preparation, and quantitation of microplastics in a freshwater mesocosm study

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Abstract

The extraction and characterization of secondary microplastics, those formed through subjection to the environment, must continuously improve in accuracy and applicability in order to generate robust microplastics exposure and risk assessments. Currently, there is a dearth of reliable extraction and quantitation methods for solid microplastic particles in sediment with chemical specificity. Herein we present advances in methodologies to extract and quantify microplastic particles from sediment, utilizing an outdoor mesocosm to model a freshwater coastal environment. Model secondary microplastics of crosslinked polyurethane (PU) were studied in comparison to model secondary polypropylene (PP) microplastics. Techniques to characterize particles in sediment included pyrolysis gas chromatography mass spectrometry (py GC/MS), stereoscope microscopy, and scanning electron microscopy. To complement particle analysis, plastic-associated leachable molecules were extracted from sediment and analyzed semi-quantitatively by high performance liquid chromatography with high-resolution mass spectrometry (HPLC/HR-MS). After developing and optimizing extraction and analytical methods we quantitated PU microparticles by count and weight and discovered that model PU particles fell from the water column into the sediment over the course of a year, while no PP particles were located in sediment samples. In addition, target small molecules associated with the crosslinked PU were identified in sediment by HPLC/HR-MS but leachable molecules associated with polypropylene could not be identified in sediment samples. We share the new py GC/MS method to quantify highly crosslinked PUs in complex environmental matrices containing both inorganic and organic components. In the process of generating robust extraction methods for microplastics in sediment, we discovered important considerations for the quantitation of microplastics by py GC/MS and the impacts of sample matrix on the quantitation of PU and PP specifically. We provide guidance for the preparation of microplastics from complex environmental matrices (e.g., sediment and soil) for analysis by py GC/MS.

Keywords Polyurethane, Polypropylene, Microplastics, Sediment, Transport, Pyrolysis GC/MS, Microscopy, Extraction, Quantitation

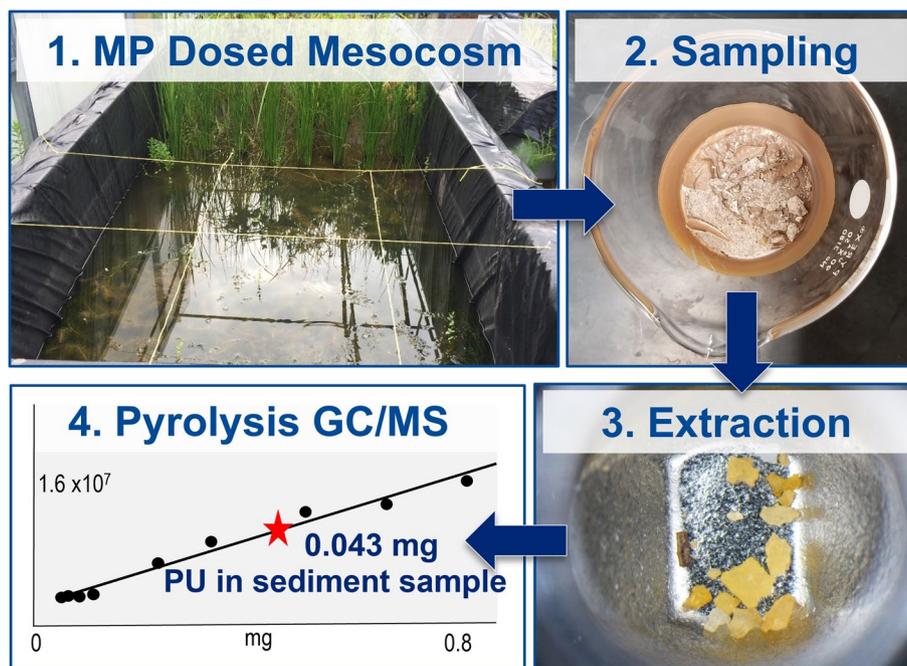
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Graphical Abstract



Introduction

To build an increasingly sustainable plastic and polymeric economy, we must investigate and understand the impact and fate of currently utilized solid polymeric materials. As such, the environmental exposure routes for microplastic particles and associated leachable compounds have become a societal issue of growing interest. The science of microplastics exposure assessment is rapidly evolving and continuously improving. A foundational picture for environmental exposure – particularly for marine ecosystems – is just beginning to take form [1–7]. Published studies demonstrating the presence or lack thereof for microplastics in environmental ecosystems continue to increase at an exponential rate [8–13]. Yet, there remains a critical need to develop high quality and robust analytical methods that will provide reliable data on environmental microplastic exposure including the fate and transport of *solid particles* and the fate and transport of *leachable compounds* [14–18]. Sampling and quantitation of microplastics in complex environmental matrices, especially those containing biofilms and/or solid materials like sediments or soils, presents major challenges to scientists eager to contribute to robust exposure assessments [19–28]. Advances that allow for higher quality data generation support better informed microplastics

exposure assessments and more appropriate hazard and risk assessments [29–31].

Little is understood about the effects of combined inorganic / organic environmental matrices on the chemical-specific quantitation of microplastics exposed to these complex matrices. A major focus of this research was to drive advancements in microplastic quantitation for particles located in environmental matrices containing both inorganic and organic components (i.e. sediment) and to advance the chemical quantitation of highly crosslinked microplastics that cannot be prepared through solvent extraction methodologies.

Common methods for environmental microplastic quantitation include mass-based analyses (e.g. py GC/MS [32–40] or thermal extraction-desorption (TED) GC/MS [41, 42] and count-based analyses (e.g. light microscopy, Fourier transform infrared microscopy, fluorescence microscopy, or Raman microscopy) [22, 24, 25]. Both analysis categories for quantitating microplastics are important and relevant to risk and hazard evaluations [3, 10, 43–46]. For this reason, we targeted developing improved methods for the quantitation of microplastics by both mass spectral and count techniques in this study. However, each quantitative analytical technique and each analysis category has specific sample conditioning requirements for microplastics. For example,

with mass-based technique py GC/MS it is important that organic and biological matrices be removed because the presence of organic matter can induce complex and unpredictable matrix effects during pyrolysis that impact the quantitation of micro- or nanoplastics. With microplastics located in sediments or soils, such an organic matrix could include biofilms, leaves, grass, and other plant matter. The size of individual microplastic particles may also impact quantitation results because larger microplastic particles (e.g., >250 μm) may not pyrolyze completely, despite adjustments in pyrolysis methodologies. Novel sample preparation methods developed to address this consideration include homogenization techniques (e.g., cryo-milling and thin film preparation), which have become increasingly popular [32, 33, 38, 40].

As the beginning of this introduction suggests, not all the issues with extraction, preparation, and mass-based analysis of microplastics in complex environmental matrices have been resolved. Appropriate removal of environmental matrices and physical preparation of samples allowing for complete pyrolysis of polyolefin particles remain major challenges [32, 35, 40, 41]. Previous py GC/MS studies have demonstrated repeated difficulties with consistent pyrolysis of microplastics for quantitation even with significant advancements in preparation methodologies [33, 35]. More complex polymers such as polyurethanes are often not included in pyrolysis microplastics studies and the applications of successful general methodologies to quantify polyurethanes are not well understood. As Py GC/MS analyses are analyte- and matrix-specific, new methods must be developed for these complex chemistries. While recent studies have advanced py GC/MS analyses of microplastic PUs [38, 47, 48] to the best of our knowledge, no py GC/MS method to quantify highly-crosslinked PU microplastics exists in academic literature, and no studies to test py GC/MS methods on PU microplastics located in environmental sediment exist.

Count-based analyses such as stereoscope microscopy have their own set of constraints associated with reproducibility that were accounted for in this study including preventing the physical fragmentation of environmental matrices while removing the surrounding environmental matrix to an appropriate degree. Inorganic matrix (sand, clay, minerals) and organic matrix should be sufficiently removed to properly visualize particles as necessary for the count-based method. Challenges for quantitating microplastics in sediment using count-based methods remain frequent as well and include appropriately removing environmental matrices and reducing sample loss during preparation steps. A robust comparison study published in 2020 by scientists at the US Environmental Protection Agency

and collaborators demonstrated wide variation (0 to 87.4%) in percent recoveries of microplastics across five common extraction methods for marine sediments [20].

This research utilizes a model freshwater coastal system as an opportunity to develop advances in sample preparation, extraction, and quantification of microplastics originating in sediment by count and mass-based techniques. It also provides information on the potential transport of microplastic particles and associated leachable compounds. The main model secondary microplastic of interest is a crosslinked polyurethane (PU) adhesive used in contact with shorelines and aquatic regions. This oxygen-permeable polymer creates a strong, thin, and flexible film, allowing aquatic plants to anchor and rebuild the local ecosystem. Eventually, the permeable polymeric system may be physically fragmented, generating secondary microplastics with non-covalently bound compounds susceptible to leaching into the surrounding environment. The comparison model secondary microplastic tested was polypropylene (PP). Model PP microplastics represent a lower density, common plastic that may be present in the environment via waste leakage.

PU and PP microplastics originally dosed in water columns were isolated from the sediment fraction and analyzed by stereoscope microscopy. PU particles were further quantitated by pyrolysis gas chromatography mass spectrometry (py GC/MS). We developed a robust quantitative method to evaluate crosslinked PU from sediment by py GC/MS and a semi-robust sample extraction method for microplastics from sediment. Compounds that may have leached into the sediment from PU and PP model secondary microplastics were extracted using a novel sample preparation method and qualitatively analyzed by high-performance liquid chromatography with high resolution mass spectrometry detection (HPLC/HR-MS).

Throughout the research process, we found interesting impacts of environmental matrix and sample preparation on microplastics quantitation. This includes the finding that sample preparation reagents and byproducts can significantly impact the quantitation of microplastics by py GC/MS if samples are pyrolyzed with their filters. Inorganic compounds on filters also impacted microplastic particle counts as seen by light microscopy, leading to the conclusion that the developed sample extraction can be further refined. To solve these issues for quantitation, we conducted particle picking of microplastics from their respective filters and provide a mass- and count-based summary of PU microplastics quantitated in sediment over time versus PP microplastics. Lastly, we explored the impacts of various physical and chemical sample conditions on microplastics analysis, evaluated various sample

preparation protocols, and generate recommendations for future polyurethanes quantitative analysis.

Methods / experimental section

Model secondary microplastic description

The model secondary microplastics used in these experiments were previously analyzed for size distribution of PU particles using a Malvern Mastersizer Dynamic Light Scattering Instrument. Results for PU particles are as follows: $D_{[3,2]}$ 326 μm , $D_{[4,3]}$ 801 μm , $D_{v(10)}$ 187 μm , $D_{v(50)}$ 606 μm , $D_{v(90)}$ 1730 μm . Results for PP particles are: $D_{[3,2]}$ 194 μm , $D_{[4,3]}$ 747 μm , $D_{v(10)}$ 74.4 μm , $D_{v(50)}$ 550 μm , $D_{v(90)}$ 1720 μm with a bimodal size distribution. Further size distribution analysis of microplastics was conducted by stereoscope microscopy and is discussed in the main paper and the Supporting Information (SI) file.

Mesocosm setup & sample collection

The mesocosm setup has been previously described in a separate publication [49]. Briefly, freshwater mesocosm boxes ($3.7 \times 1.2 \times 0.8 \text{ m}^3$) were constructed outdoors in the Duke Forest Experimental Facility in Durham, North Carolina, consistent with designs used in previous studies of nanoparticle fate [49, 50]. Each box was filled with local sandy loam soil and groundwater that reached depths of 30 cm in the box. The mesocosm contained plant life (*Lobelia elongate*, *Carex lurida*, *Panicum virgatum*, *Juncus effusus*, *Egeria densa*, *Elodea canadensis* and *Lemna minor*), insects, freshwater snails (*Physella* sp. and *Lymnaea* sp.), and Eastern mosquito fish (*Gambusia holbrooki*). Conditions of the mesocosm water temperatures ranged 18°–25° C and pH varied between 7–10 throughout the year of the experiment.

PU and PP microplastics were dosed in the water column at time zero into separate mesocosm boxes to yield an initial concentration of 50 mg L⁻¹ of microplastics. An additional three mesocosms boxes containing no added microplastics were used as controls for other studies. Samples from one of the control boxes was used for this study. Sampling from each box took place 5 times during the year (5 days, 1.5 months, 3 months, 6 months, and 12 months) by hand. The first 2–4 cm of sediment were scooped directly using 40 mL glass vials. Surface sediment was sampled where it occurred at the deepest points below mesocosm water. At each sampling point, two sediment samples (10–20 g each) were taken from every box and placed in amber vials to be stored in a laboratory freezer (-20 °C) prior to analysis. Half of each sample set was utilized for particle isolation and quantitation and the other half was utilized for analysis of microplastic associated chemicals.

Sample extraction and preparation

Experimental materials and a discussion of sample preparation precautions can be found in the [SI File](#), Section II. Sample preparation efforts can be defined in two categories: (1) preparation of sediment samples for quantitation by stereoscope microscopy and/or py GC/MS, and (2) development of improved sample preparation for qualitative analysis by microscopy and py GC/MS. Sample extraction and preparation of samples for quantitation consisted of several major steps which are outlined here. QA/QC spike samples were run through the sample preparation process to determine percent recoveries of microplastic particles by weight. However, the number of sediment samples available for spike recovery determination was limited. Further details are found in the [SI File](#), Section II. The sample extraction and preparation steps were developed based on previous studies [20] and current needs in the field of microplastic quantitative analysis, including those discussed in the introduction section of this paper.

The following samples were prepared for analysis: one of each sediment sample from PU spiked mesocosm, sampled at the five time points: 0 months, 1.5 months, 3 months, 6 months, 12 months. One of each sediment sample from a mesocosm box spiked with PP at the preceding five time points, and one of each sediment sample from a “control” mesocosm box with no spiked microplastics. Researchers started with received sediment samples in amber glass vials and ended with microplastics, residual digested organics, and residual inorganic salts on Anodisc® filters. Field samples consisted of mesocosm sediment and several milliliters of incorporated water. The sediment included clay, pebbles, plant matter (e.g., seeds, grass, twigs/wood, other plant parts, organic matter, sand, and other unidentified material). No microplastics were visually observable in any field samples.

Sample isolation and preparation steps consisted of: (1) a quantitative transfer of sample from collection vial to clean glass drying dish and gentle drying of sediment sample at 51 °C overnight; (2) a quantitative transfer of dried sediment to a zinc chloride density gradient over a 5 mm metal sieve; (3) application of gentle vacuum to the density gradient to reduce amounts of buoyant organics and the removal of large pieces of organics by tweezers (e.g. wood, grass, seeds); (4) transfer of the top layer of the density gradient to a beaker; (5) a Fenton reaction on transferred material, addition of sodium thiosulfate, and periodic pH adjustment with sodium hydroxide; (6) addition of a second zinc chloride density gradient to same beaker; (7) a waiting period to allow inorganic crystals to settle in beaker; (8) a quantitative transfer of the top layer of the final density gradient to a 47 mm Anodisc® filter for analysis by stereoscope microscopy. Representative

images of the steps in the sample preparation process for field samples and spiked samples are shown in Fig. 1. Detailed information on each of the sample preparation steps are found in the [SI File](#), Section II.

Care was taken to minimize the amount of Fenton reagent used to digest the organics present in the sample, as the process generated a large amount of solid material, often referred to as “Fenton sludge” in literature. Through iterative tests, the total amount of Fenton reagent needed for a single sample containing approximately 4 to 10 g of dried sediment was approx. 40 mg of ferrous sulfate and 10 g of 30% hydrogen peroxide. Trace inorganic salts and organic material that remained following the Fenton reaction (e.g. partially digested plants, etc.) were analyzed by pyrolysis with and without virgin microplastics, and they were found to not significantly impact the relative levels of pyrolyzates used for quantitation of PU or PP materials.

In addition to preparing samples, several sediment microplastic spike samples were prepared using a different set of control mesocosm box samples: one PU QA/QC spike sample was prepared at 5.2 mg and two around

100 mg. A polypropylene spike sample was prepared at 8.1 mg, and two around 100 mg. An additional ~90 mg each of PU and PP model microplastics were run through the sample preparation process without spiking into sediment to determine the chemical effect, if any, the sample preparation process may have on the polymers.

PU particle isolation method

PU particles located on the final filters were later isolated for py GC/MS analysis using a non-ionic solvent gradient. Crushed filters with sample were quantitatively transferred to glass vials, gently shaken, and plastic particles were manually isolated with an autopipette. Tests with virgin and chemically treated plastics revealed that chloroform was an appropriate solvent to separate the crushed filter, inorganic crystals, and microplastics by buoyancy. The separation of the microplastics from the acidic environment enabled accurate analysis by py GC/MS. Quality control studies were performed throughout the entire sample transfer process using spiked microplastics in pyrolysis cups with Anodisc® and inorganic crystals. Details on this sample preparation method and

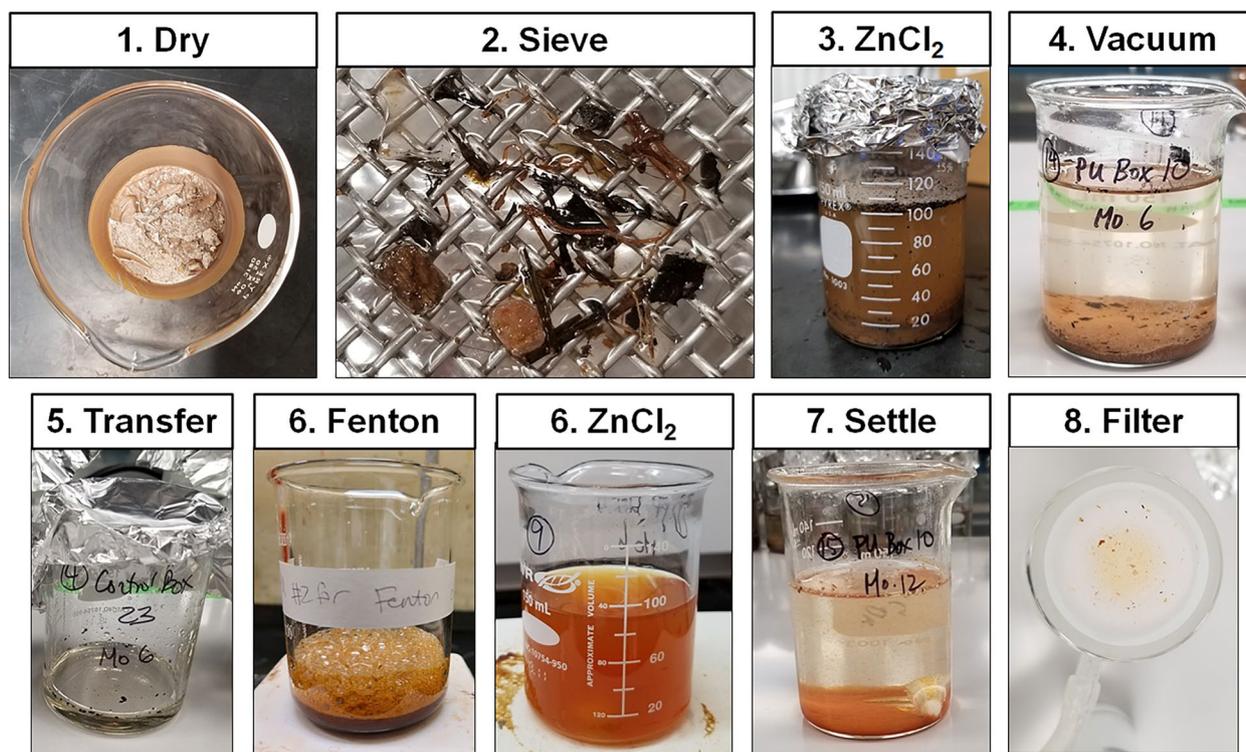


Fig. 1 Representative images of the eight steps in the sample extraction and preparation process: 1. The dried sediment sample is transferred to a Pyrex® dish ready to be weighed 2. The sample is quantitatively transferred over a no. 6 metal sieve into a density gradient using zinc chloride solution 3. The sample begins to settle in the density gradient 4. The sample in density gradient is exposed to light vacuum to reduce the content of floating organics in the supernatant 5. The upper layers of the density gradient are transferred to a clean beaker for Fenton reaction 6. The sample undergoes Fenton reaction, and more zinc chloride is added after the reaction is complete 7. The second density gradient settles overnight 8. The upper layers of density gradient are pipetted onto Anodisc® filter and filtered by vacuum. The filter with sample is rinsed and dried

QA/QC experiments can be found in the [SI File](#), Sections IV and V.

Separation and extraction improvement experiments

Novel sample preparation processes were investigated and developed to improve the final condition of samples as appropriate for microscopy and pyrolysis GC/MS in the future. The resulting procedure included the following steps: (1) a quantitative transfer and drying process to calculate the weight of dried sediment; (2) Fenton reaction on the whole transferred sample with adjusted reaction amounts (3) quenching of Fenton reaction using heat and adjustment of pH to neutral with sodium hydroxide; (4) quantitative transfer of dried sediment to a sodium iodide or sodium tungstate density gradient in a separatory funnel; (5) transfer of supernatant and microplastics to 13 mm Anodisc® filters. Further details can be found in the [SI File](#), Section VII.

Liquid extraction methodology (for HPLC/HR-MS analysis)

Sediment samples were prepared to conduct leachables semi-quantitative analysis for targeted analytes via HPLC/HR-MS. Sediment from each box and time point was freeze dried and ground into a fine powder using a ceramic mortar and pestle. A mass of 0.5 g sediment was spiked with 200 ng of bisphenol-A-d8 as a surrogate standard and mixed. Methanol was used to sequentially extract the sample using 3 mLs of solvent ultrasonicated (Branson Digital Sonifier, Danbury, CT, USA) for 5 min at 20% amplitude in triplicate. The combined 9 mLs of extract was diluted into 500 mLs of LC/MS grade water. This dilution was cleaned up on a glass Supelclean Envi-18 solid phase extraction tube (500 g, 3 mL, Millipore Sigma, St. Louis, MO, USA) to elute 8 mL of methanol. The eluent was then blown down to 0.1 mL of extract to be combined with 0.1 mL of LCMS grade acetonitrile and 0.8 mL water containing 100 ng/mL of caffeine-c13 as the internal standard. A complete table of recovery and internal standard performance can be found in the [SI File](#), Section X.

Stereoscope microscopy instrumental method information

A Nikon SMZ-1270 Stereozoom Microscope was used to photograph filters and microplastics. Prepared PU and PP mesocosm sediment samples were analyzed by stereoscope microscopy twice: once to visualize the intact sample filter and any microplastics located on the filter, and once on isolated microplastics that were separated from crushed filters, as described in the “[PU particle isolation method](#)”. Prepared samples from the control box mesocosm were analyzed on filter only. The original intention for sample analysis was to analyze microplastics by stereoscope microscopy on the filter, crush the filter

containing sample, and perform pyrolysis GC/MS on the resulting crushed material. In this way, any plastics that could not be visualized by stereoscope microscopy may be quantified by weight. However, issues stemming from matrix effects prevented this analysis, and a separate isolation step (outlined in the [SI File](#), Sections IV and V) was conducted to separate the microplastics from the filters and add them to pyrolysis sample cups.

Pyrolysis GC/MS instrumental method information

The instrument employed for pyrolysis GC/MS consisted of an Agilent 7890B gas chromatograph and an Agilent 5977A mass spectrometer equipped with a Frontier 3030D pyrolyzer. Conventional pyrolysis was performed at 600 °C for 1 min, while holding the interface temperature at 300 °C. The carrier gas was helium (99.999% purity) at a flow rate of 1.2 mL/min, and the injection was split 100:1. An Agilent HP-5MS® ultra-inert capillary column (30 m long, 250 µm ID, 0.25 µm film thickness) was employed for separation of sample pyrolyzates. The GC inlet temperature was 320 °C. The GC oven temperature was programmed from 40 °C and held for 2 min, subsequently ramped at 20 °C/min to 320 °C, and then held at the maximum temperature for 13 min. The mass spectrometer transfer line was kept at 320 °C. The ion source was held at 230 °C, and the electron multiplier voltage was set to 1290 V. Electron ionization (EI) mass spectra were recorded in the range of m/z 29–650 in SIM/Scan mode, selected ion monitoring for mass ions $m/z=106$, $m/z=182$, $m/z=197$, and $m/z=198$ for ions specific to PU and PP fragmentation.

SEM instrumental information

Particles and materials of interest were characterized using a Zeiss EVO MA-15 Scanning Electron Microscope operating in variable pressure backscattered electron imaging mode (VP-BSE). Dual Bruker Xflash 6/30 Energy Dispersive X-ray Spectrometers were used to perform elemental analysis (EDS).

HPLC/HR-MS instrumental information

Analysis of water-soluble organic contaminants was conducted by high performance liquid chromatography coupled to high-resolution mass spectrometry (HPLC/HRMS) using an Ultimate 3000 Liquid Chromatograph and Orbitrap Fusion Lumos MS (ThermoFisher Scientific, San Jose, CA). Chromatographic separation (10 µL injection) was performed on a Hypersil Gold aQ (100×2.1 mm; 1.9 µm) column (ThermoFisher Scientific, San Jose, CA) maintained at 30 °C. Each sample was analyzed using electrospray ionization (ESI) operating in positive and negative modes at 3300 and 2500 V electrospray voltage respectively. During positive mode

operation, solvent 'A' was water and 'B' was acetonitrile, both containing 0.1% formic acid. In negative mode, solvents were the same but without formic acid addition. The solvent flow rate was 300 $\mu\text{L min}^{-1}$. The mobile phase gradient was increased from 5 to 99% solvent 'B' within 30 min and was maintained for 5 min before returning to 5% solvent B. The mobile phase was maintained at 5% solvent 'B' for 2 min before and after the run. Total run time was 41 min with sample diverting to waste during the first 2 min. Source conditions included sheath gas 40 (arb), aux gas 12 (arb), ion transfer tube 360 °C, and vaporizer temperature 275 °C. The Orbitrap resolution was 240,000 FWHM and had a scan range of 120–1500 m/z . Thermo Scientific Tracefinder version 5.1 and FreeStyle version 1.5 software were used for qualitative and quantitative analysis of all analytes including bisphenol a- d_8 , caffeine- c_{13} , 3-cyano-4-methoxy-N-methyl-2-pyridone (ricinine), bis(2,2,6,6-tetramethyl-4-piperidyl) sebacate, and 2,2,6,6-tetramethyl piperidinol (TMPO).

Quantitation software

Agilent Mass Hunter Quantitative Analysis Software Version B.08.00 was used to quantify polyurethane by pyrolysis GC/MS. The quantitation ion used for PU level determinations was $m/z=198$, which is associated with 2,4'-methylenedianiline and 4,4'-methylenedianiline (2,4' and 4,4' MDA). Only the peak for 4,4' MDA was used for quantitation. The quantitation ion chosen for PP method development was $m/z=126$, which is associated with the PP trimer 2,4-dimethyl-1-heptene.

Results and discussion

Sample preparation experiments

The microplastic recovery from the sample preparation method was first determined gravimetrically using a microbalance by comparing filter weights before and after the supernatant from the final density gradient was transferred. Because the filtrate contained small amounts of residual plant matter and inorganic crystals, it was important to account for the weight of these "background" materials to determine microplastic percent recovery. The average weight gain for the filters was calculated for the prepared control box samples, which did not contain spiked microplastics. The absence of PU or PP microplastics in the optical size range was evaluated by stereoscope microscopy. The average weight of background material measured on the filters was $5.5 \text{ mg} \pm 1.1$ (the provided error was the standard deviation of weights from five of these samples). This 5.5 mg weight was used to adjust the calculated percent recoveries of spiked microplastic samples of 100 mg and 88 mg of PU in control box sediment.

After completing the sample preparation process and subtracting the averaged background mass from the control box samples, the average percent recovery was 97% ($\pm 8\%$). The same experiment was performed with 100 mg and 91 mg of PP, which resulted in an average microplastic percent recovery of 106% ($\pm 5.5\%$).

Low-level spike recovery percentages were calculated by weight for PU particles spiked in sediment vials at 5.2 mg and PP at 8.1 mg in sediment with the control box average weight subtracted as well. The PU spike sample had a calculated microplastic percent recovery of 716% and PP of 319%. This is not surprising given the control box background weight was similar to the weight of the spikes. We believe the high spike recoveries for these smaller spike samples are primarily due to the presence of inorganic crystals. In later studies, the number of spiked microplastic pieces were counted to determine percent recovery, and it was confirmed that ten visible spiked PP particles could be collected from sediment with 100% efficiency through the various steps.

The use of Anodisc[®] filters, which have small, misaligned pores throughout the alumina likely induced inorganics to crash out of solution and generated most of the undesired "background" weight of our final samples. Anodisc[®] filters were selected for our analysis because of their sub-micron pore sizes which are needed to capture microplastics and nanoplastics that may not be visible to the naked eye or by microscopic techniques. Additionally, Anodisc[®] filters can be crushed and homogenized along with the sample filtrate and analyzed quantitatively by py GC/MS [39], and this minimizes the chance of sample loss due to transference. Our original efforts to develop microscopy and py GC/MS-friendly preparation and quantitation methods for crosslinked PUs were based loosely on the guidance from the Fisher, et al. study, where they directly analyzed crushed (cryo-milled) filters with microplastics by py GC/MS [39]. However, our first experiments revealed that water rinses, acidic or basic rinses, and gravity filtration did not significantly reduce the amount of inorganic material captured on the Anodisc[®] filters. With time constraints, it was decided to proceed with the sample preparation method as described to prepare the mesocosm samples. Nevertheless, it was important to understand the origin and makeup of the inorganic material on the Anodisc[®] filters and investigate means to reduce crystal formation for future studies. Additional research was conducted, and information on the composition of the inorganic material is reported in the [SI File](#), Section VII. The results revealed possible research paths to improve microplastic preparation for quantitation by microscopy and pyrolysis GC/MS (described later in the [Results & discussion](#) section).

Microplastics quantitation by count

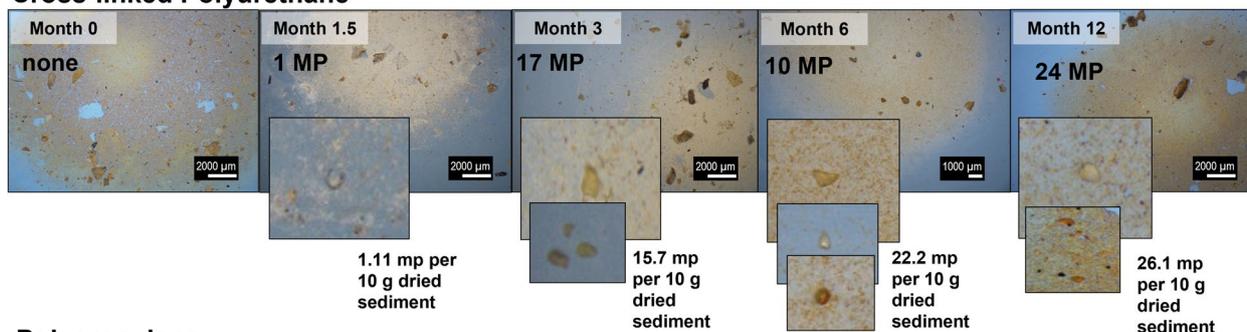
Representative images of the final prepared samples on Anodisc® filters are shown in Fig. 2. The number of microplastic particles (PU and PP) located in sediment over time was initially determined by count on filters using stereoscope microscopy. The results are shown in Table 1 both as total count and as normalized to the dry weight of sediment in each sample. The analysis of microplastic count on filters shows only PU microplastics were observed as extracted from mesocosm sediment samples.

The second count method, analyzing particles picked from crushed filters, is closely tied to requirements for pyrolysis GC/MS analysis. A discussion on the pyrolysis requirements and the count of microplastic particles from crushed filters will be explored in depth next.

Pyrolysis and challenges with matrix effects

In addition to count analyses on filters, extracted samples were to be analyzed by py GC/MS. During the development of the sample preparation methodology, qualitative

Cross-linked Polyurethane



Polypropylene



Fig. 2 Representative stereoscope microscope images of filters for particle count. Top Row: shows particle count of microplastics per field sample for a PU mesocosm box over five time points. Inlet boxes demonstrate examples of individual particles located on the filter. (MP refers to microplastic). Bottom row: shows the particle count for microplastics in a PP mesocosm box. No extracted microplastics were found for this PP spiked mesocosm box or for the non-spiked mesocosm control box. Fragments of partially-degraded plant matter remain

Table 1 Particle count results for extracted microplastics in sediment samples by stereoscope microscopy of Anodisc filters and by particle picking / manually isolating particles

Mesocosm	Count Type	Month 0	Month 1.5	Month 3	Month 6	Month 12
PU Box 10	On Filter	0	1	17	10	24
	Isolated	0	0	22	14	32
PP Box 4	On Filter	0	0	0	0	0
	Isolated	0	0	0	0	0
Control Box 23	On Filter	0	0	0	0	0
	Isolated	0	0	0	0	0

Mesocosm	Count / g Dry Sediment	Month 0	Month 1.5	Month 3	Month 6	Month 12
PU Box 10	On Filter	0	0.11	1.6	2.2	2.6
	Isolated	0	0	2.0	3.1	3.5

and semi-quantitative investigations were conducted on microplastic reference standards by py GC/MS to determine the effect of matrix on the nature and relative abundance of the pyrolyzates, especially those involved in the quantitation experiments. Virgin model microplastics were analyzed without treatment, after treatment with preparation reagents, and in environmental matrix to elucidate potential effects on mass ions $m/z=126$ and $m/z=198$ (quantitation ions for PP and PU, respectively). The results indicate that there were no major chemical changes to the PP and PU microplastics during the sample preparation process, nor from the limited exposure to the sediment.

Alumina, the material in Anodisc[®] filters, was also added to the PP and PU microplastics and pyrolyzed to determine its impact on pyrolysis. Figure 3 show stacked pyrograms of PP and PU with and without alumina, revealing the effect that alumina has on the pyrolysis of PP and PU microplastic particles. These results indicate that the PP trimer chosen for quantitation undergoes isomerization in the presence of alumina during pyrolysis, resulting in the appearance of several peaks containing mass ions with a $m/z=126$ near its retention time in

the chromatogram (Fig. 3, left bottom panel). Alumina exhibited significant reactivity towards 4,4'-methylene diphenyl diisocyanate (MDI) in the PU sample, and a quantitative conversion to 4,4'-methylene diamine (MDA) was observed. An ion extraction for mass ion $m/z=250$ revealed no evidence for MDI, which was detected in trace amounts when PU was pyrolyzed without alumina.

Samples for pyrolysis were prepared in a similar fashion to previously reported methods by crushing filters together with microplastics and adding the whole mixture to pyrolysis sample cups [39]. While the inorganic crystals from the sample preparation and the virgin Anodisc[®] had limited impact on the pyrolysis of PU, the presence of large amounts of prepared Anodisc[®] filter (filter run through the sample preparation process) in the samples had unexpected effects during pyrolysis of PU microplastics in test samples. There was a near-complete disappearance of the MDA quantitation ion $m/z=198$ and MDI quantitation ion $m/z=250$ when a small amount of PU was pyrolyzed with Anodisc[®] that had been exposed to the sample preparation reagents. Mass spectral evidence was found for hydrochloric acid

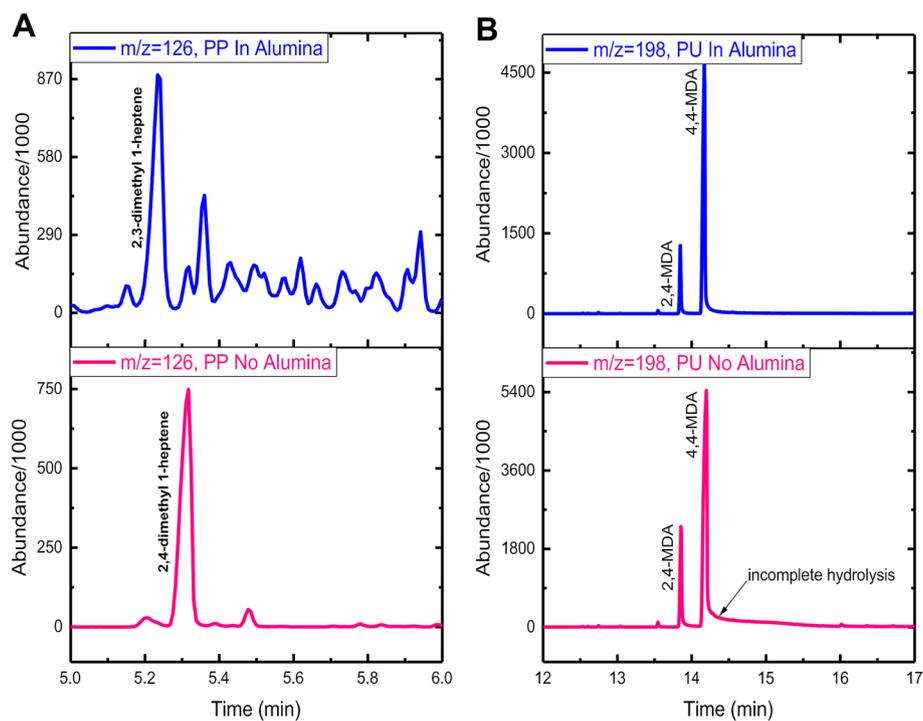


Fig. 3 Left Panel: Top: Pyrogram of PP standard without Alumina (conventional pyrolysis at 600 °C for 1 min). A single peak assigned to 2,3-dimethyl 1-heptene is observed at ~5.32 min. Bottom: Pyrogram of PP standard with Alumina (conventional pyrolysis at 600 °C for 1 min). Several peaks containing mass ions $m/z=126$ are observed indicating isomerization of 2,4-dimethyl 1-heptene. Right Panel: Top: Pyrogram of PU standard with Alumina (conventional pyrolysis at 600 °C for 1 min). Evidence for MDI was not observed indicating hydrolysis of MDI to MDA proceeds in the presence of alumina. Bottom: Pyrogram of PU standard without Alumina (conventional pyrolysis at 600 °C for 1 min). Evidence for MDI is seen between 14 and 16 min

in the pyrogram, which may have reacted with the MDA and MDI generated during pyrolysis and confounded our analysis. All Anodisc® filters were repeatedly rinsed with deionized water prior to analysis (see [SI File](#), Section II), so it was not expected that a high concentration of acidic components would remain embedded in filters. Regardless, it was clear that the approach towards pyrolyzing the entire filter with the PU microplastics needed to change.

To circumvent the challenges related to sample preparation discussed above, we developed an isolation method using chloroform as a non-ionic density gradient (see [Methods / experimental section](#) section and [SI File](#), Section V). In the future, such a density separation step using solvent may be utilized for whole filters with samples in cases where isolation of microplastics is desired. Previous work from BASF SE colleagues has demonstrated the utility of solvent density separation for PET particles on intact filters using methanol, per their work in the Joint Research Center and German Federal Institute for Materials (JRC/BAM) interlaboratory study to quantify microplastics in 2020 [51]. A similar process was performed for the isolation of any potential PP particles

from crushed sample filters. These materials were transferred into clean petri dishes to image using stereoscope microscopy and SEM analysis rather than pyrolysis cups. (Given that the focus of this study was on the extraction and quantitation of PU microplastic particles & pyrolysis method development, the analysis of any PP particles in sediment was performed using microscopy only, not pyrolysis GC/MS.)

Microscopic evaluation of particles isolated to address chemical matrix effects

Once the PU particles were manually isolated from the chloroform, the particle counts on-filter versus the isolation method were compared (Table 1). Representative images of the appearance of the particle-picked microplastics are found in Fig. 4.

Table 1 shows slightly higher PU particle counts by the manual isolation method than imaging on the filter. This may be due to some small particles being hidden from view by inorganic film/crust on the Anodisc® filter. Regardless of the counting method, a clear trend in concentration of PU microplastics in sediment was observed

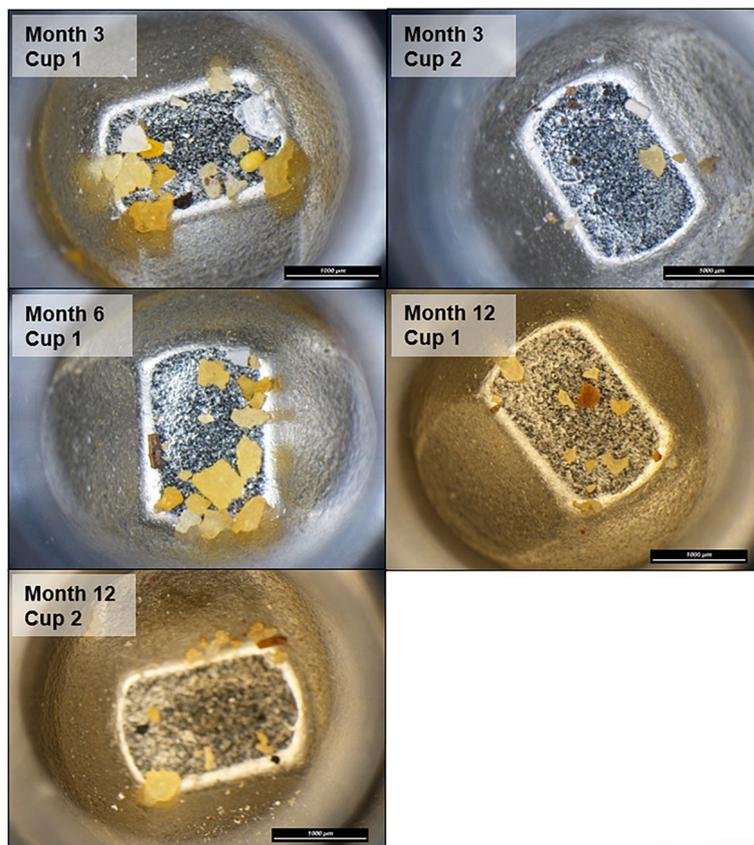


Fig. 4 Representative images of microplastics manually isolated for particle counting and analysis by pyrolysis GC/MS. Note a small amount of digested plant matter remains with particle-picking. Scale bars are for 1000 µm

for these samples. While no microplastics were observed immediately after dosing the particles into the water column, over time an increase in microplastic concentration (count per dried weight of sediment) is observed until the last collection point at 12 months. This indicates that the PU microplastics were settling in the sediment (although no conclusions can be made for the rate of settling, due to the small sampling size and a single sample analyzed per box at each time interval). In contrast, PP particles were not observed on any filters or after buoyance extraction using light microscopy and SEM–EDS analyses (see the [SI File](#), Section VIII for details).

Lastly, the relationship between PU particle physics, chemistry, and particle location was evaluated. With a small number of PU particles picked from sediment samples, the particle sizes found in sediment are roughly representative of the sizes distributed throughout the stock material (see [SI File](#), Section I for further analysis). No conclusions on size of particles and correlation to likelihood of settling could be made. The most obvious trend for observation in settling was chemical type. No PP particles were observed on sample filters or found after particle picking and advanced microscopy investigations. One simple hypothesis supporting this finding is that the crosslinked PU is of slightly higher density and polarity than PP. The small difference in density and potential to incorporate water could theoretically impact settling rate. PU particles may also have an increased likelihood to develop biofilms than PP particles due to the inherent chemistry and physics of the particle surfaces. Note again, the number and size of sediment samples were too small to make definitive conclusions on the settling rates for PU versus PP particles overall.

Quantitation of polyurethanes by mass spectrometry

Now, we return to the goal to develop a novel, robust pyrolysis method to quantify crosslinked PU in sediment. Having pivoted from the whole-filter analysis approach, the qualitative experiments provided us a path forward to quantify particle-picked PU microplastics. Instrument manufacturer recommendations for pyrolysis of polymer samples for quantitation often include solvent casting a thin film in a sample cup. This improves homogeneity of the sample for pyrolysis compared with analyzing irregularly shaped solid fragments, and it allows for reproducible sub-sampling of amounts in the linear range of the detector. Highly crosslinked polymers, however, cannot always be readily dissolved into solvents. Thus, the approach for quantitative pyrolysis of these polymer types is mechanical homogenization of a sample or standard when feasible (e.g., cryo-milling) and distribution of particles in appropriate physical diluents as needed.

Several physical diluents were evaluated for use with the quantitation of cross-linked PU, including alumina, calcined sand, and calcium carbonate. In all cases, we found that placing quartz reaction disks on top of the samples in the pyrolysis cups helped to prevent the release of the diluent into the autosampler, pyrolysis furnace, and inlet liner. However, the alumina was found to travel outside of the pyrolysis cup despite this measure, causing the need for frequent instrument maintenance. Calcined sand and calcium carbonate both worked well as a physical diluent, but the calcium carbonate was preferred because it also functioned as a hydrolysis reagent, facilitating quantitative conversion of MDI to MDA during pyrolysis.

To prepare calibration standards, PU was cryo-milled with powdered calcium carbonate (details in the [SI File](#), Section VI). Given the potential errors arising from ultramicrobalance drift and static charge, this approach ensured accurate and reproducible low-level standards in the linear range of the detector. Two stock standards were generated – the first at a 1:100 ratio of PU to calcium carbonate by weight, and the second an aliquot of the first stock standard was used to generate a 1 in 10,000 concentration of PU in calcium carbonate. The stock standards were then used to prepare calibration standards in the weight range from 0.0005 mg to 0.0799 mg of PU. Resulting calibration curves had a coefficient of determination ranging from 0.987 to 0.993. A calibration for 2,4' MDI could be performed if analyzing larger sample weights is desired and a lower detection limit is not necessary.

To determine the reproducibility of the instrument method, seven replicate weigh-ups of a stock standard were analyzed. Results are shown in [Table 2](#). Variation in results can be attributed to both the instrumental method and the analytical balance because samples are prepared gravimetrically, and results show reproducibility when analyzing PU that is cryo-milled and homogenized with calcium carbonate. Using this data, the method detection limit (MDL) of the method was calculated by Agilent MassHunter[®] software from this data to be 0.0052 mg, the limit of detection (LOD) was calculated to be 0.0050 mg, and the limit of quantitation (LOQ) was calculated to be 0.0165 mg as shown in [Table 3](#). However, the signal-to-noise ratio of our lowest calibration standard (0.0005 mg) was 50, and the associated peak for 4,4' MDA was found to have mass ion qualifier ratios consistent with PU, which indicates that the LOD and LOQ of the instrumental method were much lower than that calculated using the replicate weigh-ups of stock standard. This also suggests that the analytical balance was a significant source of variation in this method.

It was deemed that the manually isolated samples should not be cryo-milled in calcium carbonate as were the standards for this project due to the small amounts of sample and the potential for loss during the cryomilling process. The samples were instead divided between pyrolysis cups, ensuring that the mass added would fall within the linear calibration range of the detector. Approximately 5 mg of calcium carbonate was added to each sample cup before analysis by py GC/MS to ensure full conversion of MDI to MDA and keep matrix effects consistent.

It was important to establish if residual matrix from the mesocosm and/or the sample preparation process would impact PU recovery as measured by the quantitative py GC/MS method. PU particles of a similar size range to our particle-picked environmental samples were spiked into sediment and run through the entire sample preparation process. Particles were then weighed using an ultramicrobalance and analyzed using the pyrolysis GC/MS method. Percent mass accuracy was determined by dividing the calculated amount of PU from py GC/MS by

the measured weight of PU. Table 4 shows the results of the analysis. The average mass accuracy was 96.7% with 8.3% relative standard deviation (RSD). The high RSD is likely attributable to variation in measurements on the ultramicrobalance, as determined in a subsequent experiment evaluating method reproducibility when microplastic are weighed on an ultramicrobalance (see SI for additional discussion). Our quality assurance measurements demonstrate a robust method with stability. We believe the stability and QA/QC data demonstrate this method may be directly applicable to additional PU systems. This method utilized calcium carbonate to transform MDI to MDA via hydrolysis, helping to prevent the underestimation of the PU in the environment. We caution readers to test for full hydrolysis of any PU of interest with calcium carbonate compared to other hydrolysis agents.

As discussed in the introduction section, we aimed to develop quantification methods for PU by both mass and count analytics as both types of analytical measurements are important for downstream exposure and hazard

Table 2 Instrument reproducibility demonstration. Individual measurements for reproducibility test

Name	Area	PU in Standard (mg)	Measured PU (mg)	%Accuracy
PU Standard 5 - Reproducibility Test 1	13188994	0.046142	0.043013	93.2
PU Standard 5 - Reproducibility Test 2	13638298	0.046987	0.044458	94.6
PU Standard 5 - Reproducibility Test 3	13701439	0.046987	0.044661	95.0
PU Standard 5 - Reproducibility Test 4	13230540	0.045403	0.043146	95.0
PU standard 5 - Reproducibility Test 5	12524662	0.045720	0.040876	89.4
PU Standard 5 - Reproducibility Test 6	13366813	0.047092	0.043584	92.6
PU Standard 5 - Reproducibility Test 7	12376800	0.045403	0.040400	89.0

Table 3 Resulting average measured PU calculations from the set of 7 data points including standard deviation, MDL, LOQ, LOD, and noise

Avg Accuracy	Avg Measured PU (mg)	Std Dev. (mg)	Avg Measured PU RSD	MDL	LOQ	LOD	Noise
92.70%	0.0429	0.0017	3.9%	0.0052	0.0165	0.0050	9600.38

Table 4 Mass accuracy analysis

Name	Weighed PU (mg)	Measured PU (mg)	Mass accuracy (%)	CaCO ₃ (g)
QC spike (CHCl ₃) #1	0.0461	0.0507	109.9	0.00535
QC spike (CHCl ₃) #2	0.0543	0.0468	86.2	0.00522
QC spike (CHCl ₃) #3	0.0426	0.0423	99.4	0.00534
QC spike (CHCl ₃) #4	0.0460	0.0445	96.8	0.00520
QC spike (CHCl ₃) #5	0.0488	0.0445	91.1	0.00517

assessments. To compare our count and mass analyses, quantitation results by pyrolysis GC/MS reveal an increase in PU microplastic weight in the sediment over time but there is not a direct correlation with observed particle count (Table 5). For example, in months six and twelve, we see a close weight match in sampled sediment while the number of particles has increased. This is an important consideration for future studies.

HPLC/MS studies on leachables in sediment

To complement information related to the microplastic particles located in sediment, an extraction was performed on a second set of mesocosm sediment samples for analysis of microplastic associated chemicals from PU or PP by HPLC/HR-MS. Additional sediment samples were spiked with PU and PP microplastics at 10 mg, 50 mg, and 100 mg into 0.5 g of sediment prior to extraction. 3-cyano-4-methoxy-N-methyl-2-pyridone, a naturally occurring compound associated with castor oil, and otherwise known as ricinine, was the PU associated chemical identified in the extract of the PU. The PU was partly biosourced from castor oil and this compound was highlighted as a potential marker to qualitatively assess the presence of PU microplastics in sediment. Results, found in Fig. 5, demonstrate that 3-cyano-4-methoxy-N-methyl-2-pyridone could be recovered from sediment that had PU microplastics spiked at concentrations ranging 10-100 mg PU and that very low area responses of 3-cyano-4-methoxy-N-methyl-2-pyridone were periodically detected in the PU mesocosm sediments. In contrast, this compound was not found in any control box samples. This evidence suggests that exposure of the mesocosm to PU model microplastics led to the presence of 3-cyano-4-methoxy-N-methyl-2-pyridone in the sediment.

However, a direct correlation between the presence of 3-cyano-4-methoxy-N-methyl-2-pyridone and the presence of particles in the sediment could not be determined. Fitting a 7-point calibration curve (0.5 – 200

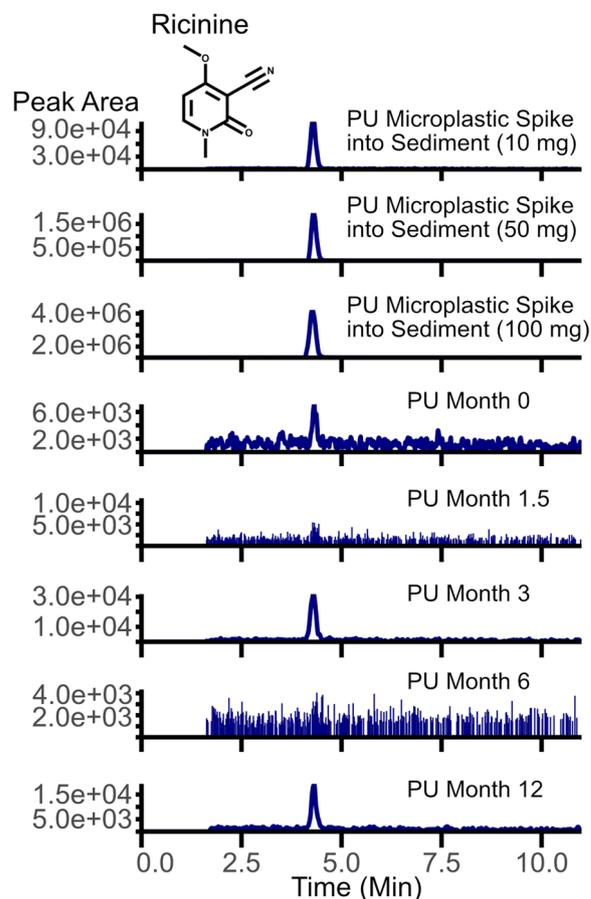


Fig. 5 HPLC/HR-MS chromatograms of 3-cyano-4-methoxy-N-methyl-2-pyridone (m/z 164.16) detected in sediment sample extractions and extractions of sediment spiked with PU microplastics (10, 50, 100 mg PU)

ng/mL, $r^2 = 0.9971$) of 3-cyano-4-methoxy-N-methyl-2-pyridone to this data shows that only extracts from 50 and 100 mg of spiked sediment were above the limit of quantitation (3 ng/mL). A spike of 50 mg PU and 100 mg PU microplastics in 0.5 g of sediment resulted in

Table 5 Concentrations of PU microplastics in sediment comparing pyrolysis to count

Month	PU Weight (mg)	Dry Weight Sediment (g)	ppm (µg/g)	Particle # per g on filter	Particle # per g, picked
0	0	12.47665	0	0	0
1.5	0.00069	8.93744	0 ^a	0.112	0
3	0.01188	10.78546	1.10	1.58	2.04
6	0.03847	4.4967	8.56	2.22	3.11
12	0.04003	9.19179	4.36	2.61	3.48

^a Calculated weight of PU is similar to the average amount measured in blank samples and therefore below the limit of quantitation

0.21 ng 3-cyano-4-methoxy-N-methyl-2-pyridone mg^{-1} PU microplastics and 1.0 ng 3-cyano-4-methoxy-N-methyl-2-pyridone mg^{-1} PU microplastics, respectively. The particle quantitation data above determined that PU microplastics were not detected at quantities ranging 100–200 mg PU g^{-1} sediment and indicated that relating polymer associated chemicals to microplastic quantities in sediment may not be appropriate at the current polymer loadings of this study.

For PP, the analytes of interest were bis(2,2,6,6-tetramethyl-4-piperidyl) sebacate and TMPO as both were detected in direct PP microplastic solvent extractions. However, ions associated with both molecules were found in every field sediment sample analyzed including those from control boxes. Due to the complexity of the composition of the sediment, which serves as the environmental matrix for these analyses, we cannot rule out a matrix interference contribution to the m/z of interest for our target molecules. Therefore, no conclusions could be made for the presence of these compounds in mesocosm sediments to supplement our findings on lack of PP particles (see [SI File](#), Section X).

Sediment sample preparation: solutions for the future

Finally, it can be noted that while the quantitative analysis method for pyrolysis was demonstrated robust, the sample preparation method for pyrolysis was not ideal. There were two areas of interest to improve the sample preparation methodology: the first was to reduce the amount of crystal formation that is generated if samples need to be filtered onto small pore-size filters directly. The second is to ensure that the pH of the sample and any remaining matrix would not impact pyrolysis of polyurethanes. Tests were conducted on model native sediment spiked with PU and/or PP microplastics. Native sediment consisted of Southeast Michigan clay, dirt, pine needles, oak and other deciduous tree leaf fragments, dried grass, commercial sand, and construction gravel. To first address the unwanted crystallization on Anodisc[®] filters, we considered the use of elemental alternatives to zinc, a reactive transition metal, with less reactive elements in the density gradients.

Next, the order of steps was streamlined so the protocol time could be reduced. In this case, a Fenton reaction would be performed first on the whole sediment sample with enough Fenton reagent to digest the unwanted organic matrix fully. In the original method, the amount of Fenton reagent used was an issue due to the formation of orange crystals that were easily re-dispersed in density gradients. The use of a separatory funnel and lack of transition metal salt solution were expected to improve the amount of Fenton sludge generated and transferred to the final filter. These methodological adjustments allowed

for the use of a single density gradient which could be performed in a separation funnel after the Fenton reaction was performed on the sample. The use of vacuum to allow for easier removal of large organic pieces manually prior to Fenton reaction should be evaluated per sample needs in the future.

Next the Fenton reaction was improved, and the sample pH issue was addressed. As opposed to using a chemical means of accelerating hydrogen peroxide degradation by adding sodium thiosulfate, which further required increasing the pH once again using sodium hydroxide, gentle heat was used. 50 °C for 1 h reliably quenched our Fenton reactions to completion. Following quenching, the pH of the solution was adjusted to neutral using sodium hydroxide and then a density gradient was added. The goal for this neutralization step was to prevent the destruction of the PU functional groups as observed under acidic pyrolysis conditions.

Of the tested density gradients (experimental details found in the [SI File](#), Section VII): sodium iodide, sodium tungstate, sodium bromide, and calcium chloride, the former two density gradients were most promising. SEM analysis was conducted on microplastics that were run through the Fenton reaction and density gradients. Results for light microscopy and SEM-EDS can be observed in [Fig. 6](#). Light microscopy revealed crystal formation on Anodisc[®] filters could not be avoided but sodium iodide and sodium tungstate systems appeared to have vastly reduced crystal formation compared to zinc chloride. SEM analysis revealed that crystals do not have the same rose-like structure as observed before, indicating a complexation including iron does not occur. Crystallization on polyurethane appeared to occur to a greater degree with the use of sodium bromide than sodium iodide or tungstate for the tested samples.

The final methodology was tested with sodium iodide and sodium tungstate. Percent recovery was tested by count; 100% of the particles could be recovered on filters if particles were spiked by hand in model sediment. After running PU plastic and a 13 mm Anodisc[®] filter through the entire improved sample preparation process using the sodium iodide or sodium tungstate density gradient, the plastic and the filter were crushed together and pyrolyzed. An extracted ion pyrogram for $m/z=198$ is found in the [SI File](#), Section IX for the sample using sodium iodide gradient ([Fig. S19](#)). This data reveals that the MDA functional group remains intact during pyrolysis of samples with Anodisc[®] and quantitation can be performed using py GC/MS following the sodium iodide gradient. However, a low signal-to-noise ratio was observed for the peak associated with 4,4'-MDA following the sodium tungstate sample preparation, so we were unable to confirm that sodium tungstate is an appropriate

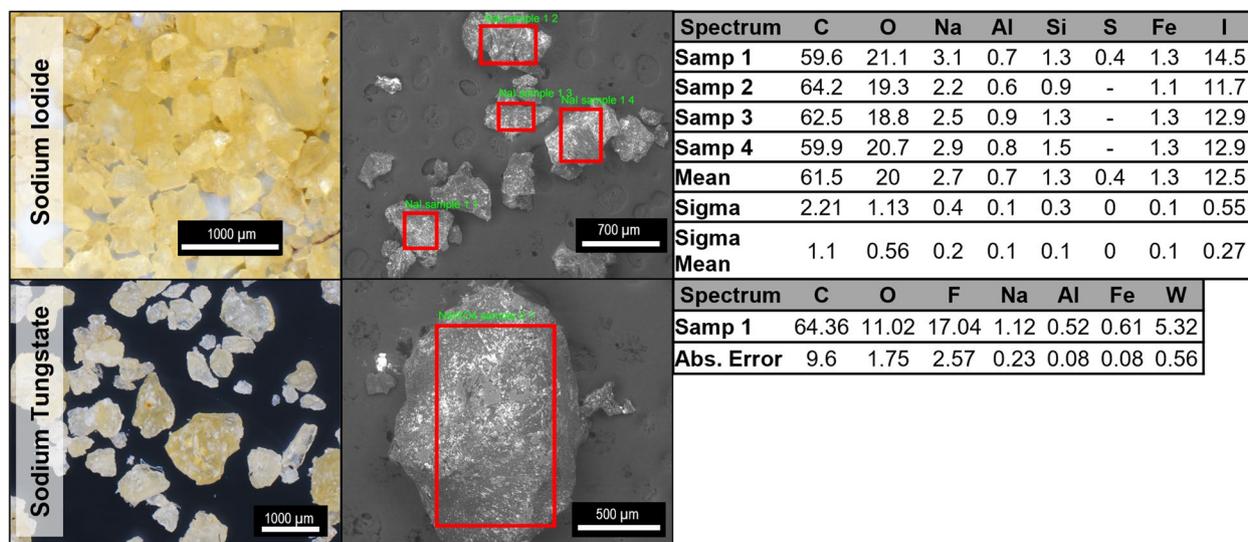


Fig. 6 Light microscopy and SEM with EDS of inorganic residuals after sample preparation

density gradient to use for the quantitation of PU with a processed Anodisc® filter. From these limited studies, the sodium iodide gradient appeared to be the best sample preparation procedure for PU and Anodisc® filter to be analyzed by py GC/MS. We recommend further exploration of pH-neutralized methods to remove organic matrices in sediment and/or soil, and as needed, combined with density gradients of sodium iodide in the future. Additional density gradients should be further explored for their applicability to quantitatively pyrolyze PU microplastics extracted from complex environmental matrices.

Conclusions

These studies allowed us the unique opportunity to develop sample preparation and analytical methodologies to more appropriately probe PU microplastics in freshwater systems and evaluate leachable compounds in correlation to the solid plastic particles. We have demonstrated a robust method to quantify crosslinked polyurethanes by py GC/MS, based loosely on guidance from instrument manufacturers and industry consortia. We have also developed an improved sample preparation of PU microplastics in sediment for quantitation by py GC/MS and provide recommendations to consider for environmental sample extraction. Our count- and mass-based analyses indicate that PU microplastics in our samples migrated from the water column to the sediment compartment of these freshwater mesocosm systems over 1 year. In contrast, PP microplastics were not detected in the sediment at any sampling point up to 1 year. HPLC/

HR-MS analyses reveal the presence of the PU-associated compound 3-cyano-4-methoxy-N-methyl-2-pyridone in extracted sediment during the year. No conclusions could be made for the presence of any PP-related leachable compounds in sediment.

Several complications for samples to be analyzed by py GC/MS have been uncovered and addressed: the final pH of the sample must be neutral, the amount of inorganic solids generated from sample preparation should be minimized in order to reduce risk of undesired reactions under pyrolytic conditions, and attention should be paid to the presence of chemicals that may induce hydrolysis and/or isomerization during pyrolysis, such as calcium carbonate, and alumina, respectively. We recommend that the following sample preparation be explored in the future to extract microplastics from sediment in combination with robust QA/QC measures: Fenton reaction followed by quenching with heat, pH neutralization, and density gradient in separatory funnels using sodium iodide or another acceptable density gradient that does not induce side reactions during pyrolysis.

This work does not address additional sample preparation improvements associated with the reduction of salt formation on Anodisc® filters. But we do provide a recommended route forward if one desires to collect microplastics on sub-micron filters for analysis of micro- and nanoplastics extracted from sediment and complex environmental matrices by py GC/MS. In all cases, density gradients, sample filters, and sample preparation methods should be tested for their impacts on microplastics and analyses prior to quantitating specific polymers.

Abbreviations

SI File	Supplemental information file
PU	Polyurethane
PP	Polypropylene
$D_{(3,2)}$	Surface area moment, the surface-weighted average particle size
$D_{(4,3)}$	Volume moment mean, the volume-based average particle size
$D_{v(10, 50, 90)}$	Diameters of sample particles below which falls 10, 50, and 90% of particle population from volume-based data
$D_{s(10, 50, 90)}$	Diameters of sample particles below which falls 10, 50, and 90% of particle population from surface-based data
MDI	4,4'-Methylene diphenyl diisocyanate
MDA	4,4'-Methylene diamine
GC/MS	Gas chromatography mass spectrometry
SIM	Single ion monitoring
EI	Electron ionization
EM	Electron multiplier (voltage)
QA/QC	Quality assurance / quality control
QC sample	Quality control sample
SEM	Scanning electron microscopy
EDS	Energy dispersive X-ray spectroscopy
VP-BSE	Variable pressure backscattered electron imaging mode
HPLC/HR-MS	High performance liquid chromatography with high resolution mass spectrometry
mg	Milligrams
μ g	Microgram
g	Gram
μ p	Microplastic
ppm	Parts per million
ICV	Independent calibration verification standard
RSD	Relative standard deviation
MDL	Method detection limit
LOQ	Limit of quantitation
LOD	Limit of detection
TMPO	2,2,6,6-Tetramethyl piperidinol
BPA	Bisphenol A

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s43591-023-00062-6>.

Additional file 1.

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Authors' contributions

JMH managed the overall laboratory research at BASF Corporation, led the microplastics extraction research with hands-on efforts, contributed data to and wrote major portions of the paper. JLH managed the pyrolysis gas chromatography mass spectrometry research with hands-on efforts, designed methodology, contributed data to, and wrote major portions of the paper. IW sampled and prepared the sediment from the mesocosm boxes, conducted and led leachables research at Duke University, led liquid chromatography mass spectrometry research, contributed data and wrote portions of the paper. KS conducted research and methodology for portions of the paper including particle picking method development, contributed data and

wording to portions of the paper. JG conducted research for portions of the paper including studying impacts of different density gradients, contributed data. JJ led all microscopy efforts / research for the paper, contributed major portions of data and some writing. CK conducted research in microplastics extraction portion, contributed sample preparation data to paper. DB conducted research in pyrolysis GC/MS portion of paper, contributed data, contributed to writing and editing of paper. WW was the principal investigator for BASF portion of project and provided feedback and scientific guidance. LF was the principal investigator for Duke University portion of project and provided feedback and scientific guidance. All authors read and approved the final manuscript.

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Availability of data and materials

Additional information can be found in the supplemental file of this paper. Additional details and data may be provided upon request by contacting the corresponding author.

Declarations

Ethics approval and consent to participate

Not Applicable.

Consent for publication

All authors provide consent for publication.

Competing interests

The authors declare the following competing financial interest(s): JMH, JLH, WW, CK, JJ, JG, and DB are employees of a company producing and marketing polymeric and plastic materials.

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