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Single-Pot Method for the Collection and Preparation of Natural Water for Microplastic Analyses: Microplastics in the Mississippi River System during and after Historic Flooding

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Abstract: We describe a simple single-pot method for collection and preparation of natural water for microplastic (MP) analyses. The method prepares samples in the same vessel (Mason jars) that they are collected in right up until the MPs are transferred onto filters or spectroscopic windows for analyses. The method minimized contamination, degradation, and losses, while increasing recoveries and throughput when compared to conventional sieving. We applied it to surface grab samples collected from the Mississippi River and its major tributaries during and after historic flooding in 2019. Microplastics (>~30 µm) were quantified using Nile Red fluorescence detection, and a small subset of samples were identified by micro-Fourier Transform Infrared Imaging spectroscopy (µFTIR-Imaging). Concentrations were lower during the flooding, likely due to dilution. Concentrations (MPs/L) ranged from ~14 in the Tennessee River during flooding to ~83 in the Ohio River during low-flow (summer) conditions. Loads of MPs tended to increase down river and ranged from ~87 to ~129 trillion MPs/day near New Orleans. Most of the MPs (>60%) were in the lower size fraction (~30–90 µm), consisted primarily of fragments (~85%), followed by fibers (~8%) and beads (~7%), with polyester, polyethylene, polypropylene, and polyacrylate as the primary MP type. Overall, we demonstrate that the single-pot method is effective and versatile, and, because it uses relatively inexpensive and easily assembled materials, it can be adapted for MP surveys worldwide, especially those involving volunteers from the community and schools.

Graphical Abstract
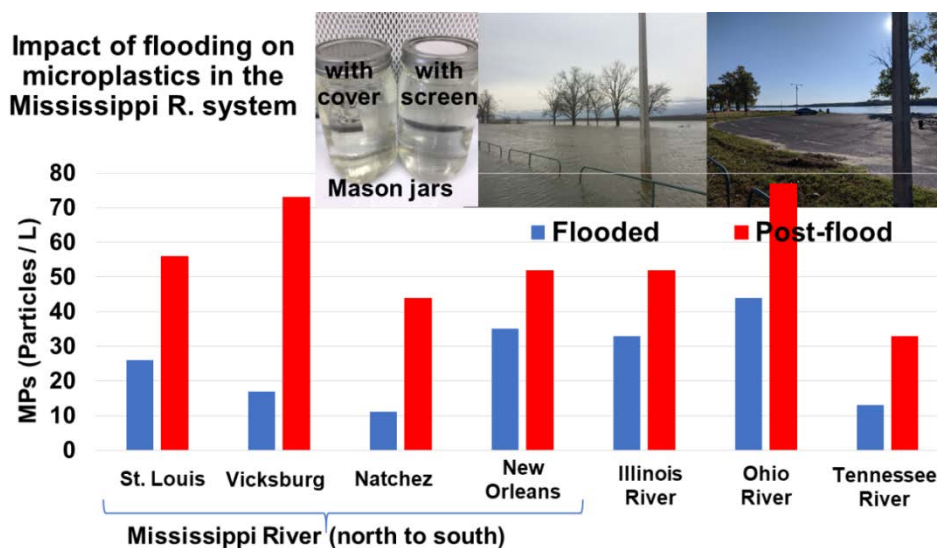
INTRODUCTION

Led by consumer products, the worldwide demand for plastic continues to

grow with global production at nearly 350 megatons in 2017 (Plastics Europe 2018).

Unfortunately, careless discarding of plastic and mishandling of the plastic waste

stream has resulted in widespread plastic pollution, including the infamous oceanic

garbage patches (Lebreton 2018). Further, plastics in the environment weather and

degrade as a result of ultraviolet radiation, microorganisms, temperature changes, and

mechanical forces (e.g. wave action), yielding smaller and smaller particles called

micro- and nano-plastics. Here, we focus on microplastics (MPs), which have been described as "any synthetic solid particle of polymeric matrix, with regular or irregular shape and with size ranging from 1 μm to 5 mm, of either primary or secondary manufacturing origin, which are insoluble in water" (Frias 2019).

The occurrence of MPs in the aquatic environment is well documented, with higher concentrations generally found near population centers (Li 2018). Microplastics have also been detected in remote areas, including the Arctic Ocean (Lusher 2015), deep-sea sediments (Free 2014), and mountain lakes (Cauwenberghe 2013). Given their small size and ubiquitous nature in lakes, rivers, and oceans, their ingestion and impact on aquatic life poses a serious threat, particularly for small suspension-feeding organisms (Auta 2017). Moreover, MPs have been shown to be substrates (vectors) for other contaminants, including persistent organic pollutants such as dichloro-diphenyl-trichloroethane (DDT), both in laboratory studies and in field studies (Teuten 2009; Costa 2017; Tourinho 2019).

Unfortunately, there are often wildly different estimates reported for MP abundances in natural water, even from the same waterbodies, making meaningful comparisons difficult and hindering the utility of real-world MP surveys (Lusher 2017; Lenz 2018; Jiang 2018). Some of these disparate results may be due to inherent variability at the sites, but part of the problem may be the different sampling, sample preparation, and analytical methods used. On one hand, the wide range of approaches to MP analyses is not surprising given that MPs (1) are a diverse class of contaminant encompassing a wide variety of sizes, morphologies, and chemical and physical properties (Rochman 2019), (2) partition into different environmental compartments depending on size, density, biofouling, and other factors (Hartmann 2019), and (3)

have only recently (in the last decade) caught the attention of the larger scientific community. On the other hand, microplastic analytical methods need to become more harmonized to increase the quality and comparability of experimental data.

Two common ways to sample plastic debris suspended in water is through use of a surface or subsurface tow net or by collecting a known volume of water at a specific location (bulk water sampling). Nets are typically used in investigating large areas with results being reported in particles/m^3, whereas bulk water sampling is more accurate as a snapshot and is often reported in particles/L. A major drawback to sampling with a net is that it fails to capture particles smaller than the mesh opening (typically 333-µm), and these smaller particles tend to be the most abundant. In contrast, bulk water sampling captures all size fractions of particles in the water. Another advantage of bulk water sampling is the elimination of contamination from sampling equipment such as nylon nets and ropes. However, trawling with a net or bulk water sampling should be considered complementary techniques, covering different parts of the overall microplastic pollution (Tamminga 2019).

When using a net, the plastics caught in the cod end are typically rinsed out into a container for later processing in the laboratory. Determining the volume of water passing through the net or being pumped through collection sieves is important to accurately calculate MP concentrations. At some point the net and bulk sampling methods converge with the samples being filtered through a sieve or series of sieves to isolate particulates by size fraction(s). Larger particles can be removed by tweezers and analyzed by FTIR or other means. If the remaining solids collected on the sieves or filters are organic-rich they are typically subjected to either enzymatic digestion (Cole 2014) or wet peroxide oxidation, the latter sometimes in the presence of a Fe(II)

catalyst (Tagg 2017; Hurley 2018), to digest labile organic matter and "clean" the plastic surfaces. A final filtering step is used to concentrate the MPs which can then be examined directly on a filter by conventional light microscopy (Masura 2015), stained with Nile Red dye and examined by fluorescence microscopy (Erni-Cassola 2017), or transferred to a spectroscopic window/slide or a suitable filter for chemical imaging by Focal Plane Array (FPA)-µFTIR or Raman spectroscopy (Loder 2015; Tagg 2015; Olesen 2017; Wolff 2019). Note that prior to chemical imaging the MPs on the filter are transferred (dispersed) into a solvent such as ethanol (often by sonication) before an aliquot is applied on the spectroscopic window and dried (Liu 2019).

The above sample preparation scheme can be hampered by multiple sieving and transfer steps, which increase the likelihood of contamination and losses, while decreasing throughput. Regardless of the method used, it is imperative that it minimizes contamination, losses, and degradation of MPs to obtain meaningful (reliable and reproducible) data.

Here, we present a novel low-cost and efficient bulk water sampling method for the analysis of MPs in water. The single-pot method prepares samples in the same vessel (Mason canning jars) that they are collected in right up to the point where they are transferred onto filters for analysis (Figure 1). We compare the new method to conventional sieving, demonstrating that it lowers contamination, losses, and carryover between samples and improves recoveries. The method is particularly useful for the analyses of smaller MPs that can't be easily manipulated with tweezers or are too small to be seen with the naked eye. These smaller size fractions are also

more prone to contamination and require µ-spectroscopic imaging to identify the plastics.

Further, we applied the single-pot method to samples collected along a large transect of the main stem of the Mississippi River and in several of its major tributaries during historic flooding in the spring of 2019 and during more normal flow conditions later that summer. We quantified the MPs (down to ~30 µm in size) using Nile Red fluorescence detection and identified MPs in a small subset of samples using µFTIR-Imaging. Here, we report preliminary results for the concentrations, river loads, shapes, size distribution, and chemical composition of MPs in this important large-scale riverine network.

MATERIALS AND METHODS

Study site

To understand the occurrence, distribution, types, and sources of MP pollution in the Mississippi River system requires an extensive spatial and temporal study. The Mississippi River Basin, the largest drainage basin in the United States, consists of an intricate system of waterways, tributaries, and commercial routes. In this study, we collected surface water from 11 sites, 7 on the main stem of the Mississippi River extending from the northern-most site near St. Louis to the southern-most site near New Orleans, and 4 from its major tributaries, including the Illinois, Ohio, Tennessee and Yazoo Rivers (Figure 2). Specific sampling locations (GPS coordinates) and river flow information are provided in Table 1. Samples were collected during major (historic) flooding in May 2019 and during more normal flow conditions (post-flood) the following August. Thus, this work represents "snapshots" of the system during these two different seasons and flow regimes. The Missouri River and the Mississippi

River at Tunica and Greenville were inaccessible due to high waters and flood damage at sampling sites.

Sampling for the single-pot method

Two different sampling approaches were explored: high-volume (~360 L) field-filtered sampling and low-volume (~1 L) grab sampling (Figure 3). The high-volume method utilized a water transfer pump to sample ~360 liters of water over 5 minutes, which was passed through the 100 μm cod-end of a plankton net. The cod-end was then rinsed into a large stainless-steel bowl and the contents rinsed into a quart-sized (946 mL) Mason jar. Slightly larger 1 L volume Mason jars are available outside of the United States. Hereafter, we report abundances of MPs/L, adjusting for any differences in volume. The high-volume sampling approach was logistically challenging, time-consuming, and often yielded high amounts of particulate matter, particularly for river waters which are often turbid. High concentrations of particulates can lead to clogged filters and potential counting errors due to overlapping particles, necessitating the need for density separation. Thus, in this study we used the grab sample method which provided sufficient volumes for detecting MPs above blank levels. However, the high-volume method may be suitable for sampling in areas where MP abundances are especially low and the water has less particulate matter.

Grab samples were collected from shore at the water surface directly into 946 mL Mason (canning) jars using a pole (Figure 3). The Mason jars are available from most major grocery or home hardware stores and can be readily be attached to a variety of sampling equipment for collection of water samples from the shore of spillways, rivers or lakes, or off boats or docks. Larger size mason jars can also be

used but are more difficult to handle with the added weight and volume. For other sampling scenarios, such as deep-water samples collected using a Rosette sampler, the water can simply be transferred (poured) into the Mason jar. Once full the Mason jar is tightly capped and placed in a crate or cooler for transport, although the samples do not need to be kept cold. However, if the samples are to be stored for extended periods (>1 month) before processing, they can be preserved with addition of isopropanol (1:1 v/v). In this study, we did not preserve samples as they were processed within 3 weeks of collection, instead keeping them in their original container (full) until sample preparation.

Sample preparation for natural water using the single-pot method

Mason jars containing water from the Mississippi River and its tributaries were placed in a clean laminar flow hood located in a HEPA-filtered clean room. The lids were removed and replaced with lids that were cut with a round 57 mm diameter opening and outfitted with a round 84 mm diameter 200 x 600 mesh (~30 µm) screen made of Monel, a nickel copper alloy (Unique Wire Weaving Co. Inc. Hillside, NJ, USA) (Figure 4). Unlike stainless steel, the Monel screen (filter cloth) is flexible enough to mold into the lid's seal when tightened to prevent leaks, yet strong enough to be cleaned and reused. Next, the jar was turned upside down and a jet of air was used to break the surface tension and allow the water out of the jar through the screen. Any source of clean compressed gas will work, including canned compressed air. This process can be done at a sink or into a bucket in the clean hood. Once the water was removed the lid was opened and the screen carefully rinsed back into the Mason jar using a Teflon squeeze bottle with ultrapure water (purified, deionized, and 0.2 µm-filtered; Milli-Q, Millipore, Burlington, MA, USA). While this washing step should

be thorough to quantitatively transfer MPs off the filter back into the container/solution, the volume of water should be kept to $< \sim 100$ mL to avoid dilution of reactants during the subsequent digestion step.

Next, a wet peroxide oxidation was used to remove the natural organic material which can interfere in the analyses. Using a peroxide oxidation avoids both strong acids and heat which can damage the MPs (Claessens 2013; Erni-Cassola 2017; Munno 2017). Specifically, 20 mL of 30% H_2O_2 and 20 mL of 0.05 M Fe(II) solution (Fenton's reagent) was added directly to each sample in the Mason jars. The lid was placed on but not screwed down to avoid pressurization. Note: the Monel screen cover can also be used as it will allow CO_2 to escape. Typically, the mixture bubbled and became amber colored, but as the reaction proceeds to completion the color shifted to a pale yellow. Depending on the amount of organic material remaining, additional aliquots of H_2O_2 were added until the digestion was complete. Note: larger debris that is clearly not plastic such as pieces of wood can be removed with tweezers. Following digestion, the 30 μm mesh Monel screen/lid combination was secured back on the Mason jar and the solution was forced out with pressured air as before. Then the screen was carefully removed and rinsed 3 times back into the Mason jar.

Finally, the samples were vacuum filtered using an all glass filtration apparatus to concentrate the MPs for analysis. Here, we used two different filters, a 25 mm diameter, 10 μm pore size, polycarbonate track-etched filter (PC filter) (Sterlitech Corporation Kent, WA, USA) for fluorescence microscopy, and a 25 mm diameter, ~30 μm pore size, 200 x 600 mesh Monel wire screen for μFTIR-Imaging.

Enumeration of microplastics using fluorescence microscopy

To detect MPs and assess their size distribution and morphology, fluorescence microscopy was used after staining the plastic particles with Nile Red dye fluorochrome (Erni-Cassola 2017). Briefly, Nile Red dye solution (10 µg/mL in methanol) was added directly (dropwise) to the PC filter containing putative MPs until the filter was covered with the dye solution and left to dry in the laminar flow hood for ~15 min. Note: the dye should be applied gently to the filter to avoid displacing particles off the filter. Then the 25 mm PC filters were carefully placed between a microscope slide and coverslip and taped together to ensure that objects on the filter were secure. The slides were stored in a glass petri dish in the clean room until analysis.

Putative MPs were detected and counted using a Nikon Ti2 Eclipse Fluorescence Microscope. The specific microscope, camera, and counting settings used for this work are provided in Table 2. The procedure was a four step process that took ~20 minutes/sample: (1) images were collected across the entire filter area and stitched together into one larger image, (2) automatic image thresholding was performed to convert the image into a binary image, (3) the built in object count software was run, (4) the MPs data were sorted based on size or morphology. We counted and categorized uniformly fluorescing objects in the shape of fibers, fragments (particles with sharp edges), or beads (circular objects); objects that were clearly not plastic or that had biological features such as spines or striations were not counted. If in doubt we did not count the object as plastic, making our estimates conservative.

Fluorescence microscopy provides a cost-effective, high-throughput way to detect MPs with images that can be processed for size and morphology. However, it is worth pointing out some of its limitations. One limitation is that sample preparation (digestion) may leave some non-plastic particles intact that may also fluoresce (e.g. chitin), although adjusting fluorescence parameters and visual inspection can be used to minimize false positives (Erni-Cassola 2017). Plastics can also be negatively affected by certain digestion techniques, especially if done at elevated temperature or under acidic or basic conditions, potentially impacting their size and causing discoloration (Nuelle 2014). Another limitation is that MPs made of tire rubber don't readily fluoresce (Erni-Cassola 2017). Despite these limitations, Nile Red combined with fluorescence microscopy is a powerful technique that is increasingly being used to assess MP contamination in a variety of matrices (Maes 2017; Fischer 2019; Scircle 2019).

Identification of microplastics in select samples by FPA-µFTIR-Imaging

To assess suitability of the single-pot method for FPA-µFTIR-Imaging and to identify the major type of MPs in a subset of water samples, we used an Agilent Cary 620 FTIR microscope coupled to an Agilent Cary 670 FTIR spectrometer at Aalborg University, Denmark. µFTIR-Imaging is currently considered the most suitable technique to analyze small MP ($< 500\ \mu$m) without pre-sorting and providing unbiased results (Loder 2015; Primpke 2017; Vianello 2019). Water samples were prepared as before but filtered onto 25 mm Monel screens. These screens were individually placed into 25 mL glass scintillation vials and submerged in 2.5 mL of 50% ethanol. The vials were sealed and placed in an ultrasonic bath for 5 minutes, after which the Monel screens were removed and rinsed with 2.5 mL of 50% ethanol.

The 5 mL sample was split and a 2.5 mL portion was shipped to Denmark for FPA-µFTIR-Imaging. There, aliquots of known volume were deposited onto a zinc selenide (ZnSe) windows held in compression cells (PIKE Technologies, Fitchburg, WI, USA) using a capillary glass pipette (micro-classic, Brand GmbH, Germany), and dried at 55˚C on a heating plate overnight. The windows were then placed onto the FTIR-microscope stage. The system uses a 128 x 128 pixel Focal Plane Array (FPA) detector capable of simultaneously acquiring 16,384 spatially resolved spectra over an area of 704 x 704 microns/tile using 15x IR Cassegrain, which provides a pixel size of 5.5 µm. The analysis was performed by scanning the entire active area of the windows, which is approximatively 78.5 mm^2. The instrument operated in transmission mode with an active spectral range from 850 to 3750 cm^{-1}, collecting 120 co-scans for the background (also collected on a ZnSe window) and 30 co-scans for the samples at a spectral resolution of 8 cm^{-1} and a beam attenuation of 50%. The scan time was ~4 hours.

Data analysis was carried out using siMPle, a software developed by Aalborg University (AAU) and Alfred Wegener Institute (AWI) (siMPle, 2019); a paper detailing the software was under review at the time of submission. Much like its predecessor software MPhunter (Primpke et al. 2017), SiMPLe allows for automatically detecting the particles on the scanned surface, correlating the raw spectra, the 1st and 2nd derivative of all sample spectra to a custom-built database containing more than 100 reference spectra (polymers, paints and resins, and non-synthetic materials). Moreover, siMPle automatically measures the size of the particles and can also provide a mass estimation using the area, the density and an estimated thickness of the identified particles (Liu 2019).

Blanks, replicates, and spike recovery tests

Many of the early papers on MP pollution do not include sufficient quality assurance measurements to demonstrate the reliability of the data. Such measurements are especially important when developing and validating a new method. Here, we evaluate our single-pot method with multiple blanks, replicates, and spike recovery measurements. To assess contamination, we prepared blanks to assess various aspects of the sample processing including total procedural blanks. To assess precision, we split samples and compared each half both to each other and to other un-split samples from the same site. To assess accuracy, we spiked samples with known amounts of MPs, processed the samples, and determined recoveries.

For the recovery experiments, 50 bright red acrylonitrile butadiene styrene (ABS) particles were added to DI water (filtered through 10 µm filters) in Mason jars. Twelve samples for each of two size categories (250-500 µm and 500-1000 µm) were spiked. Within each of these size fractions, 6 samples were processed with the single-pot method, as described earlier, and 6 using the conventional sieving method. In the conventional method, samples from the jar were transferred into the standard test sieve (20.5 cm diameter; Advantech, New Berlin, WI, USA) and washed with 0.45 µm filtered water. The particles on the sieve were then rinsed back into Mason jar. All samples were then processed the same as real samples, except Nile Red dye was not added to the final filters. The filters were sandwiched between microscope slides and securely taped. Recoveries were determined by visual counting using a magnifying glass and tweezers.

Recovery tests for smaller particles that are difficult to manipulate or that are invisible to the naked eye is challenging. Here, we used MPs in the 125-250 µm size

range that were generated from weathered plastic (high density polyethylene) found in the environment (Sardis Lake, Mississippi, USA). The weathered fragments were cut to smaller pieces and ground into MPs using a cryomill (SPEX Certiprep, Metuchen, NJ, USA). The particles were then sorted into different size fractions using standard sieves (Gilson Company, Inc.). A few mg of the 125-250 µm size fraction were dispersed in 0.05% (v/v) sodium dodecyl sulfate (SDS) in ultrapure water and a 0.5 ml aliquot of the solution was pipetted into ~100 mL of ultrapure water which was then filtered and stained with Nile Red dye as described earlier. These filters were examined by microscopy and the number of particles in the 125-250 µm size fraction ranged from 34 to 54, which overlaps the amount we measured for this size category in 1 L of river water. The filters were carefully placed into small glass covered petri dishes, brought back to the clean MPs laboratory, and thoroughly rinsed into Mason jars in the clean hood. These samples, along with blanks, were then processed as before and re-examined by fluorescence microscopy to determine recoveries.

RESULTS AND DISCUSSION

Laboratory and reagent blanks

Using fluorescence microscopy, we analyzed a variety of blanks to assess contamination and carryover between samples. Reagent blanks (n=3) consisting of Nile Red dye solution deposited directly on the filters yielded 2 or fewer MPs. Similarly, air blanks prepared by leaving a filter exposed in the laminar flow while preparing other samples (~1 day) were also negligible. Full procedural (method) blanks using ultrapure water (purified, deionized, and filtered through a 0.22 µm membrane with a Milli-Q A10 system) as the sample and for rinsing yielded blanks that averaged 35 ± 4 total putative MPs (n=9; ±1 standard error), with most being in

the lowest size fraction (30-90 µm) (Figure 5). Method blanks were always less than samples from the Mississippi River and its tributaries. We emphasize that it is important to include multiple blanks in each batch of samples because contamination can vary from batch to batch. Steps to lower blanks should include avoiding plastic (e.g. pipette tips), filtering reagents, avoiding clothing that can readily shed fibers, and working in a clean laboratory environment.

Recovery and replicate/split sample experiments

Average recoveries for MPs spiked into water ranged from 97% – 116% (Table 3). For the larger particles (250-500 µm and 500-1000 µm), mean recoveries for the single-pot method were 97% for both size fractions, compared to 90% and 93% for the conventional method, demonstrating a modest improvement. For smaller MPs (125-250 µm), the mean recovery was 116% (range 105-130%). These results are assuring, particularly for the smallest size fraction, considering the experimental challenges in detecting, spiking, and quantifying them.

Given the inhomogeneity of natural water samples and the challenges of isolating and quantifying smaller MPs, there will always be some variability between samples. Reproducibility for real-world samples is presented in the *Microplastics in the Mississippi River and several of its major tributaries* section of this paper. Here we highlight a comparison between two river samples collected moments apart, with one analyzed "whole" (~1 L) and the other split into two equal halves (~0.5 L each). Results showed that the split samples had a similar number of MP particles (145 and 160) compared to 242 particles in the 1 L (whole) sample. On the one hand, this shows that that the numbers of MPs in the split samples decreased nearly in half as expected. On the other hand, because the split samples had 60% and 66% of the total

number of MPs detected in the un-split sample, it emphasizes the importance of thoroughly cleaning equipment and screens that are to be reused to minimize contamination and carryover between samples.

Variations and remarks on the single-pot method

The single-pot method could be a useful tool in the growing practice of citizen science owing to the relative ease of sample collection and availability of materials used. The use of citizen science in assessing MP pollution is an exciting opportunity to get communities involved in science and increase environmental stewardship in local waterways. There are a few published examples of citizen science MP studies and this single-pot method is a suitable starting point for such studies, particularly since these studies almost always rely on grab samples (Bosker 2017; Barrows 2018). Whereas fluorescence microscopy and µFTIR-Imaging is likely beyond the scope of most citizen science-based MP surveys, the single-pot method's screens/filters can be photographed and/or assessed using magnifying glasses or optical microscopy, with larger MPs removed with tweezers for further study.

Indeed, the single-pot method is versatile and can be adapted as needed by researchers. For example, besides the high-volume sampling method described earlier, other variations include adding pre-filtering or density separation for turbid water with higher silt and sand content. A 1-mm (or other mesh size) screen can be added to the Mason jar (as before) and the water simply poured into a second clean mason jar. This allows rapid removal of larger debris that can be present in samples collected from turbulent waters. The filter can be inspected for larger MPs and the water (containing MPs less than the screen opening size) can be processed in the new

(second) Mason jar as before. In the current study, however, we found this unnecessary and kept all analyses to a single pot.

Another variation allows sampling larger volumes of water in the field by adding the screen top (instead of the closed cover), inverting the setup, and displacing the water with a stream of air such as from a bicycle tire pump. Then temporarily removing the cover/screen, refilling the jar without over-spilling, and placing the screen/cover back on. This process can be repeated multiple times as desired accumulating MPs from the water if done carefully and if the total volume is kept track of.

We also found that Mason jar metal covers tended to rust over time and while the Monel screen doesn't readily corrode, the rust from the covers can transfer or stain the Monel screen. Thus, it is best to replace the covers periodically before rust becomes a problem.

Microplastics in the Mississippi River and several of its major tributaries

While several studies have examined MP pollution in the northern Gulf of Mexico (e.g., Wessel 2016; Di Mauro 2017), studies on MPs in the Mississippi River system are scarce. Martin used micro-attenuated total reflectance FTIR to quantify and characterize the Mississippi River's contribution of MP debris to the Gulf of Mexico (Martin 2018). The authors report a total of 7,600 suspect MPs were quantified from 24 samples (an average of $\sim 11.6 \pm 3.8$ MPs/L) by removing them from filters using tweezers and using micro-attenuated total reflectance-FTIR for confirmation.

Here, we used the single-pot method with fluorescence microscopy and µFTIR-Imaging to quantify and identify MPs in water samples collected from the Mississippi River and its' tributaries during flood and post-flood conditions. Concentrations and loads of MPs in the Mississippi River system are given in Table 4. Concentrations (MPs/L) ranged from 14 in the Tennessee River during flooding to 83 in the Ohio River during low-flow (summer) conditions. Loads of MPs tended to increase down the Mississippi River, ranging from ~87 to ~129 trillion MPs/day near New Orleans close to the river's mouth. These levels are higher than those reported by Martin, however our analysis includes smaller MPs that can't be removed with tweezers for quantification (Martin 2018). We note that concentrations of smaller MPs (~5-333 µm) in the open ocean were recently reported on the order of 10^2-10^3 particles/L, with even higher concentrations (and variability) near shore (Brandon 2019).

Whereas flooding decreased concentrations of MPs, likely due to dilution, its impact on overall loads varied between sites. It should be noted that sampling during the flood typically required collection from low-lying areas adjacent to the river channel. Also, flooded samples were more likely to include invertebrate exoskeletons that can, to some extent, adsorb the Nile Red dye. However, their bio-structure is readily identified and they were excluded from the final MP count. The prevalence of this type of debris was atypical and wasn't nearly as prevalent in samples collected during non-flooded river conditions. The size distribution of MPs was relatively uniform between flood and post-flood samples (Figure 5). However, differences and trends in size distribution may be masked by the uncertainty in the data. Overall, the MPs consisted primarily of fragments (~85%), followed by fibers (~8%) and beads

(~7%). This distribution of morphology was remarkably consistent between sites and during the two flow regimes.

Other trends observed in the data were (1) the Mississippi River generally had higher concentrations of MPs/L than samples from its tributaries (except for the Ohio River), and (2) the loading of MPs tended to increase downriver with highest loads in the lower Mississippi River near its mouth. How these trends are influenced by seasons and certain other factors such proximity to wastewater treatment plant outflows are under investigation.

Select water samples were also processed and analyzed by µFTIR-Imaging to show that the single-pot method is amenable to the technique. A detailed comparison of MPs abundances determined by fluorescence microscopy and FPA-µFTIR-Imaging is beyond the scope of this pilot project. Here, we focused on using µFTIR-Imaging to identify the major type of MPs in select samples from the Mississippi River. Again, we found that river samples yielded higher MPs counts than blanks, with the most common polymer types being polyester, polyethylene, polypropylene, polyacrylate, and polyurethane. For example, we detected 47 particles in a sample from the upper Mississippi River near St. Louis, with 34 particles identified as polyester and 6 as polypropylene, and 62 particles in a sample from the lower Mississippi River near New Orleans, with 38 identified as polyester and 17 as polypropylene. Additional µFTIR-Imaging of samples is needed to fully characterize spatial and temporal trends of MPs in the system.

CONCLUSIONS

We developed and validated a versatile single-pot method for collection and preparation of natural water for MP analyses. The method reduces sample preparation

time, while minimizing outside contamination and carry-over between samples, and is amenable to multiple analytical techniques for detection and characterization, including optical and fluorescence microscopy and imaging techniques that center on smaller MPs (<333 µm), including µFTIR and µRaman. The method was applied to assess MP contamination in the Mississippi River system, the largest drainage basin in the USA, during both flooded and low-flow conditions. Concentrations of MPs were lower during flooding. Loading of MPs generally increased as the Mississippi River approached its terminus at the Gulf of Mexico. Given the availability of the materials and ease of use, the single-pot approach can potentially harmonize sample collection and preparation for many MP surveys worldwide, especially those involving volunteers from the community and schools.

Data Availability statement-- Data and associated metadata are available from the corresponding author through supplemental files (czidziel@olemiss.edu).

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Figures

Figure 1. Flow-chart showing the general scheme for characterizing microplastic pollution <1-mm in size, including the portion covered by the single-pot method. The single-pot method prepares samples in the same vessel (Mason jars) that they are collected in right up until they are transferred onto filters or windows for final spectroscopic or microscopic analysis.
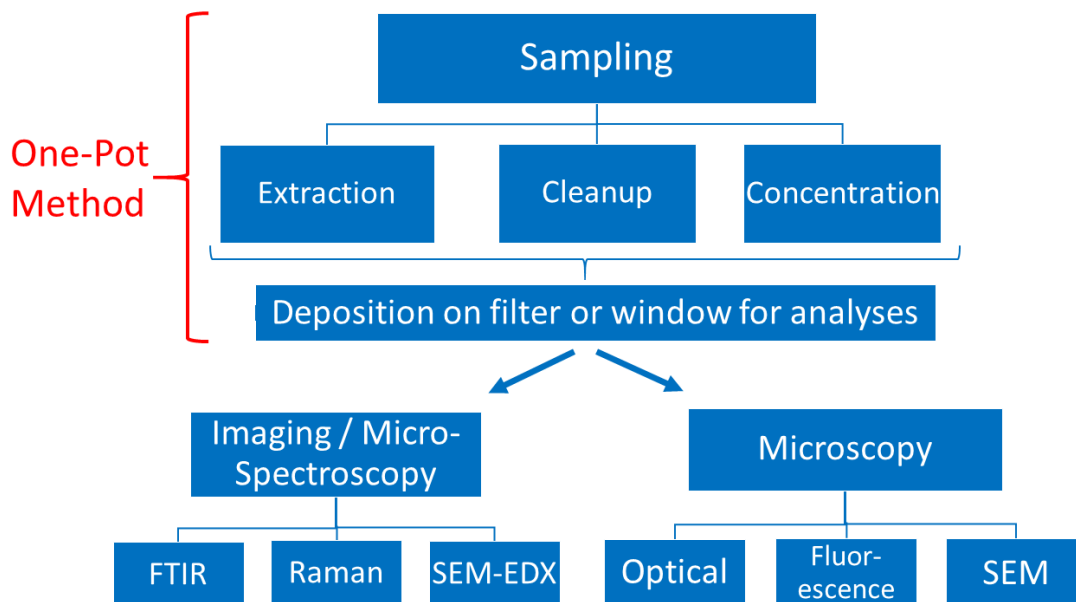
Figure 2. Map showing the locations in the Mississippi River (dark circles) and in major tributaries (open circles) that were sampled during the spring and summer of 2019. Numbers are Major cities and state boundaries are shown for reference. Site numbers are listed north to south, with corresponding GPS locations and river flow data in Table 1.

Figure 3. River water passing through the 100 µm cod-end net using the high-volume sampling method (left); intake chamber and hose for the high-volume sampling method (middle); and low-volume grab sampling using a ~1 L Mason jar (right).

Figure 4. Single-pot sample preparation and analytical scheme used in this study. Sample collected in the field in a Mason jar (A). Replacement of the solid lid with a 30 µm mesh Monel screen cover (B). Injecting a stream of clean air to pass the water through the screen (C). Digestion of organic matter using H_2O_2 in the presence of an iron catalyst after rinsing the screen back into the Mason jar (D). The digested solution is filtered through the screen cover and rinsed back into the jar as before. This final rinse solution then filtered through either a 25-mm diameter 10 µm pore size polycarbonate (PC) filters for fluorescence microscopy or a 30 µm wire (Monel) screen for micro-spectroscopic imaging (E). The PC filters are stained with Nile Red dye and placed on microscope slides (F) and analyzed by fluorescence microscopy (G). False color image obtained using fluorescence microscopy showing putative microplastics (stained with Nile Red dye) from a typical sample collected from the Mississippi River (H).
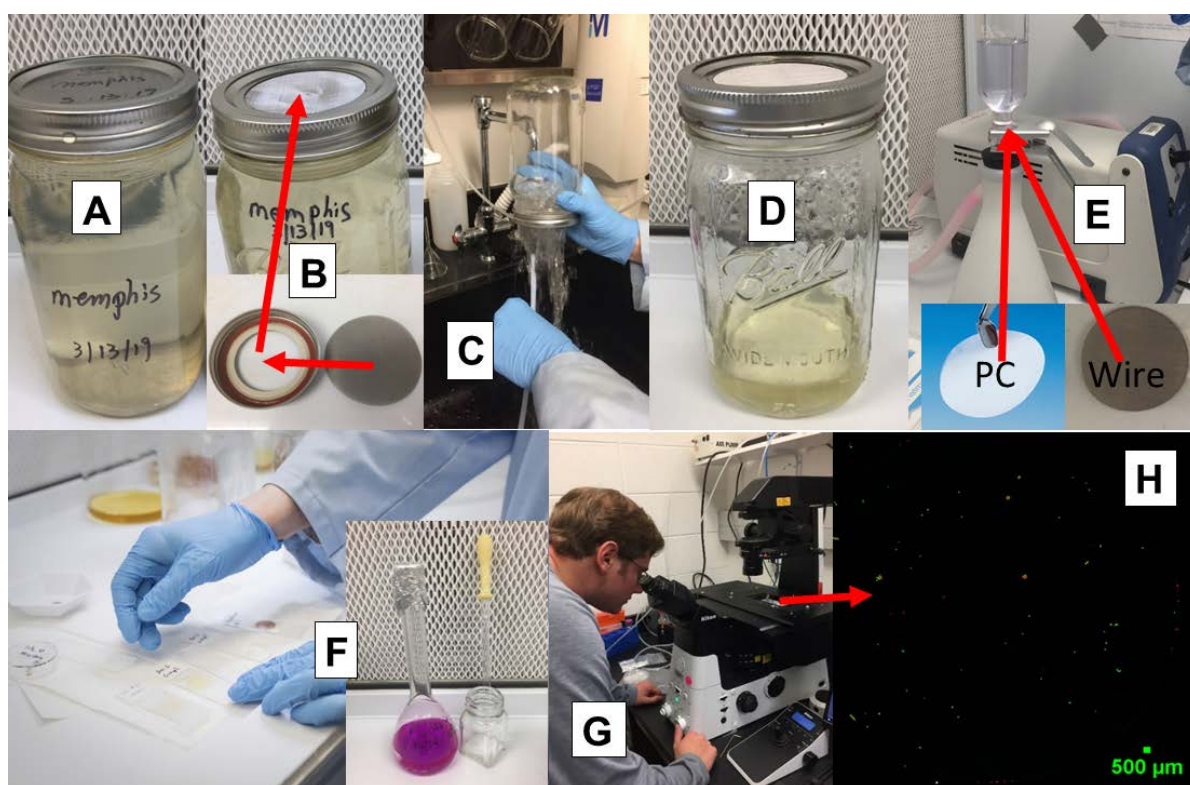
Figure 5. Microplastics as a function of particle size in method blanks and samples from the Mississippi River near New Orleans, LA and the Ohio River near Fort Massac, IL determined using fluorescence microscopy. The same y-scale is used to aid comparison. Error bars are ±1 standard deviation.
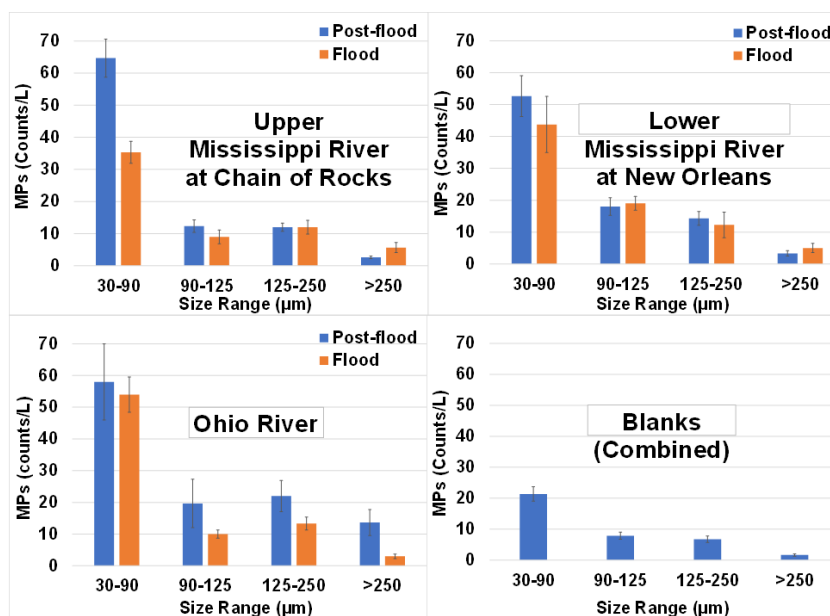

Table 1. Sampling location and river flows for the Mississippi River and major tributaries. Sites are listed north to south, with site numbers depicted in Figure 1

Site #	River	Nearest City	GPS Coordinates		River Flow (m³/s)	
			Lat.	Lon.	Spring	Summer
1	Illinois	Grafton, IL	38.968	-90.544	2379	708
2	Mississippi	St Louis, MO	38.757	-90.171	19539	11893
3	Ohio	Metropolis, OH	37.142	-88.711	1814	1699
4	Tennessee	Paducah, KY	37.019	-88.279	2775	1235
5	Mississippi	Memphis, TN	35.180	-90.057	37661	18972
6	Mississippi	Tunica, MS	34.738	-90.446	NA	NA
7	Mississippi	Greenville, MS	33.356	-91.136	41617	29601

8	Yazoo	Vicksburg, MS	32.351	-90.884	NA	NA
9	Mississippi	Vicksburg, MS	32.325	-90.896	41150	34263
10	Mississippi	Natchez, MS	31.588	-91.429	43019	36812
11	Mississippi	New Orleans, LA	29.884	-89.970	31149	26901

NA = Not Available

Table 2. Fluorescence microscopy settings and parameters

Components	Instrument Settings
Microscope	Nikon Ti2 Eclipse
Optics	Plan Apo λ 4x
Filter Set	FITC (470 nm excitation)
Dichroic Mirror	505 nm lp
Emission Filter	535 nm bp
Excitation Source	Lumencor Spectra, 470 nm
Modality	Wide-field fluorescence
Camera	pco.edge
Camera Specs	Aperture: 0.2; Exposure: 100 ms; Readout: 110 MHz; 16 bit
Image Processing	Nikon Software
Counting Range	$100~\mu m^2$ and larger

Table 3. Recoveries for water spiked with microplastics using conventional (open) sieving and one-pot (closed) sieving

Counting Method	n	Size Fraction (µm)	% Recovery: Mean (Range)	
			Conventional Method	One-Pot Method
Visual	6	500-1000	93 (90 - 98)	97 (94-100)
Visual	6	250-500	88 (82 - 98)	97 (92-100)
Fluorescence	3	125-250	NA	116 (105-130)

Table 4. Concentrations and loads of microplastics (>30 μm) in the Mississippi River and its tributaries

during the spring and summer of 2019 determined using the one-pot method and fluorescence microscopy[a]

Site #	River	Site	Spring (flooding)			Summer (post-flood)		
			n	Mean (Range) (Particles/L)	Loads (Counts x 10^{12}/day)	n	Mean (Range) (Particles/L)	Loads (Counts x 10^{12}/day)
1	Illinois	Grafton, IL	3	35 (0-62)	7.21	3	56 (34-67)	3.38
2	Mississippi	St. Louis, MO	3	28 (1-43)	46.69	3	60 (34-85)	61.22
3	Ohio	Metropolis, OH	3	47 (40-51)	7.34	3	83 (30-172)	12.02
4	Tennessee	Paducah, KY	2	14 (12-15)	3.32	3	35 (32-38)	3.74
5	Mississippi	Memphis, TN	3	33 (9-60)	107.80	3	28 (0-77)	17.44
6	Mississippi	Tunica, MS		NA	NA		NA	NA
7	Mississippi	Greenville, MS	4	15 (10-27)	53.55		NA	NA
8	Yazoo	Vicksburg, MS	4	45 (17-78)	NA	2	73 (28-118)	NA
9	Mississippi	Vicksburg, MS	3	18 (4-30)	64.30	3	103 (71-	306.86
10	Mississippi	Natchez, MS	3	24 (19-29)	90.31	3	52 (27-87)	148.87
11	Mississippi	New Orleans, LA	4	38 (0-109)	86.54	3	56 (23-85)	128.57

[a] Data is blank-subtracted. NA = Not Available (site inaccessible or flow data unavailable).