

Article

Microplastics in the Mississippi River System during Flash Drought Conditions

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Abstract: The Mississippi River System is of great ecological and economic importance, making it crucial to monitor contaminants within it. While nutrient pollution is well studied, there are little data on microplastics (MPs) in the Mississippi River System (MSRS), especially during drought conditions. Herein, we characterize MP pollution from seven sites across the MSRS during both flash drought and non-drought periods using FTIR microspectroscopy (μ -FTIR). Additionally, we evaluate the impact of multiple water level conditions on MP polymer composition across five time points at a single sampling site. Of all MPs identified, polyethylene terephthalate (PET, 22%), resin (17%), and polyethylene (PE, 10%) were the most abundant polymers. Average concentrations ranged from 16 to 381 MPs/L across seven sites, with no significant difference in concentration between conditions. Irregular particles were the most common morphology, with most MPs falling in the lowest size range measured (30–100 μ m). Drought condition had a significant ($p < 0.001$) impact on polymer composition, and polymers most strongly correlated with flash drought were mostly fluoropolymers. For the single sampling site, concentrations differed, but not significantly, across the five timepoints. These results demonstrate the complex relationship between MP concentration and drought condition, and also highlight the importance of fully characterizing MPs in environmental studies.

Keywords: microplastics; Mississippi River System; flash drought; FTIR microscopy



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1. Introduction

The Mississippi River System is one of the largest in world, with a watershed area covering 32 states of the United States [1]. It is an extremely important river system as it provides habitats for around 780 different species of fish and wildlife [2], several of whom are listed as endangered, and the river system supports over 325 species of migratory birds as part of the Mississippi Flyway [3]. Additionally, the chemistry of the watershed has an outsized effect on the ecology of the northern Gulf of Mexico. Thus, chemical pollution in the Mississippi River has been the focus of many studies [4–6]. However, other types of pollution, such as microplastics (MPs), have received relatively little attention.

Defined as plastic particles less than 5 mm in size, MPs have been shown to have adverse effects on a variety of aquatic species [7–10]. While most MP toxicity studies have focused on the harm caused to the individual organism ingesting them, some studies have shown that MPs can cause increased juvenile mortality rates as well as decreased reproduction rates in both fish [9] and oyster [10] populations. These increased mortality rates coupled with declining reproduction rates indicate that MPs can have strong impacts on the viability of aquatic species populations. As such, it is crucial to determine the MP concentrations and polymer composition in areas of ecological importance, such as the Mississippi River System to both understand the magnitude of the problem and to target mitigation efforts.

Our previous research has shown that the Mississippi River System acts as a funnel for MPs, concentrating and transporting them into nearshore waters of the Gulf of

Mexico [11,12]. This prior research found lower concentrations of MPs during periods of high flooding (14–47 MP/L compared to 28–103 MP/L), likely due to the large influx of water into the system, diluting the concentration of MPs in samples. While flooding has been shown to affect MP concentrations in freshwater systems [13], there is far less research on the impact of drought conditions on MPs in similar systems, especially on the polymer types and other characteristics of MPs.

Previous studies have demonstrated that drought conditions can lead to a decrease in water quality due to contaminants being concentrated by the lack of water in the system [14,15]. However, it is also important to recognize that not all droughts are the same. In the past few years, the term “flash drought” has been used to describe intense droughts with a far more rapid onset than conventional droughts [16,17]. Although there is not yet a consensus that quantifies the rate of onset necessary for a flash drought, it seems to be generally accepted that an onset within a period of several weeks is categorized as a flash drought [16]. This contrasts with conventional droughts, whose onsets usually occur over several months to a year. This distinction is crucial as the rapid onset of a flash drought leads to different impacts than a conventional drought, though the environmental impact of flash versus conventional droughts is an area in need of more research [16].

In late 2022, the Mississippi River System experienced historically low water levels during a flash drought event (Figure 1). This could have large impacts on the water quality of this very important river system due to a potential increase in MP concentration. The goals of this research were therefore to (1) *characterize MP pollution in the Mississippi River System during flash drought periods for the first time* and (2) *determine how these rapidly occurring, low water conditions affect the abundance and characteristics of MPs within the river system*. While this study focuses on the Mississippi River System, it is not the only river system susceptible to drought. As changing climate conditions are expected to bring about drought conditions with increasing frequency and severity [18], it is crucial to understand how drought impacts MP pollution within aquatic ecosystems. MP toxicity is a complex topic, with research showing that it depends on a variety of factors such as polymer type, size, and morphology in addition to concentration [19]. As such, it is important to classify MPs in terms of all of these factors in order to provide the information needed by ecologists to predict toxicity to aquatic organisms as well as help target preservation and remediation efforts in drought-stricken river systems.

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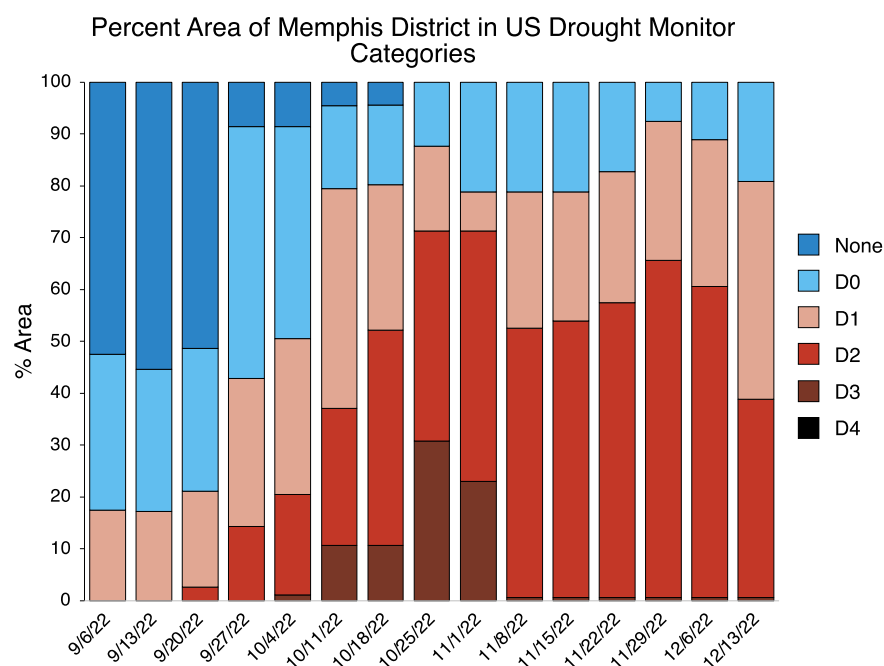


Figure 1. Percent area of the United States Army Corps of Engineers’ Memphis District in US Drought Monitor Categories [20]. Categories shown include normal or wet conditions (none, dark blue), abnormally dry conditions that have not yet met the threshold for a drought (D0, light blue), moderately dry conditions (D1, peach), severe drought (D2, light red), extreme drought (D3, dark red), and exceptional drought (D4, conditions not present).

2. Materials and Methods

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2. Materials and Methods

2.1. Sampling Sites and Sample Collection

Samples were collected from seven sites across the Mississippi River System (Figure 2, Table 1). Sites were chosen to encompass both the Mississippi River and several major tributaries. Ease of river access and proximity to United States Geological Survey (USGS) surface water monitoring stations were considered, to allow for the monitoring of gage height and river flow rates at each site.



Figure 2. Sampling locations within the Mississippi River System. Sites include the Mississippi River (black circles) and tributary rivers (open circles). A description of the sampling sites is given in Table 1.

Table 1. Sampling locations within the Mississippi River System and sampling dates (D). Each site was sampled twice, once in November 2022 during the flash drought that occurred in the fall of that year and once in early June 2023, which was during a period of more normal flow. Furthermore, to investigate the differences in MPs across periods of flooding, normal conditions, flash drought, and conventional drought, additional samples

Site Number	Closest City	River	Latitude	Longitude	Sampling Date	
					Flash Drought	Normal Conditions
1	Grafton, IL	Illinois	38.968	−90.544	22 Nov 2022	8 Jun 2023
2	Florissant, MO	Missouri	38.861	−90.272	22 Nov 2022	8 Jun 2023

were collected from the Memphis site due to its proximity to our home institution (Table 2). One set of samples was collected in November of 2023, while additional samples from May and November of 2019 were analyzed from a previous research project [11].

Table 1. Sampling locations within the Mississippi River System and sampling dates (Day Month Year).

Site Number	Closest City	River	Latitude	Longitude	Sampling Date	
					Flash Drought	Normal Condition
1	Grafton, IL	Illinois	38.968	−90.544	22 Nov 2022	8 June 2023
2	Florissant, MO	Missouri	38.861	−90.272	22 Nov 2022	8 June 2023
3	St. Louis, MO	Mississippi	38.757	−90.171	22 Nov 2022	8 June 2023
4	Jackson, MO	Mississippi	37.455	−89.462	22 Nov 2022	9 June 2023
5	Metropolis, IL	Ohio	37.142	−88.711	22 Nov 2022	9 June 2023
6	Gilbertsville, KY	Tennessee	37.019	−88.279	21 Nov 2022	9 June 2023
7	Memphis, TN	Mississippi	35.180	−90.057	16 Nov 2022	8 June 2023

Table 2. Additional sampling dates and river conditions for the Memphis, Tennessee, USA, site (Day Month Year). Condition refers to the drought condition, which is based on the US Drought Monitor Categories. The US drought monitor gives a broad overview of physical conditions in an area and is based on a variety of factors such as “precipitations, streamflow, reservoir levels, temperature and evaporative demand, soil moisture, and vegetative health” [21]. Stage height and discharge are more directly focused on the river system itself. Stage height refers to the water level of the river with regard to a chosen reference point, while discharge refers to the amount of water flowing through the system. Both stage height and discharge may be used as input data when determining drought condition, but they are not the only factors considered. Stage height and discharge data obtained from the United State Geological Survey [22].

Condition	Sampling Date	Stage Height (m)	Discharge (m ³ /s)
Flooding	20 May 2019	10.2	37,095
Normal	2 Nov 2019	5.36	19,255
Flash Drought	16 Nov 2022	−0.677	6881
Normal	8 June 2023	−0.177	8410
Drought	28 Oct 2023	−2.68	4870

Samples were collected directly into quart-sized, wide mouthed Mason jars to avoid the use of plastic in the sampling apparatus. The quart-sized jars (946 mL) are widely availability and come in standardized sizes. Although the jars only have a capacity of 946 mL, all MP measurements in this study have been normalized to 1 L. Prior to sampling, all jars were rinsed with milliQ water and heat-cleaned at 450 °C for 3 h to remove any MPs. At each site, jars were screwed into a metal sampling arm and extended into the river to collect a grab sample. The jars were skimmed along the surface of the water approximately 1.83 m (6 ft) from the edge of the river during collection. After filling up the jar, the sampling arm was retracted and each jar was capped with a metal lid, which was screwed on using a metal ring. Triplicate samples were collected from each site. Prior to analysis, jars were stored at 4 °C to inhibit biological growth.

2.2. Sample Preparation

Samples were prepared using the single pot method as reported elsewhere, with a few modifications [11]. Briefly, the lid of each sample’s Mason jar was swapped with a Monel screen (200 × 600 mesh, ~30 µm) to allow the sampling jar to act not only as the reaction container but also as a filtration device. Samples were subjected to a wet peroxidation reaction using Fenton’s reagent (20 mL of 0.05 M iron (II) sulfate in 0.6% sulfuric acid and 25 mL of 30% hydrogen peroxide) to remove organic material as detailed in previous work [11], followed by a density separation to remove sediment. For the density separation, ~100 mL of zinc chloride (ZnCl₂) aqueous solution (1.63 g/mL) was added to each jar. The jars were swirled and then allowed to settle for a minimum of 12 h. Afterwards, the surface fraction of the solution was transferred to a 40 mL glass vial using a wide-tipped 5 mL

pipette. The process of stirring, allowing the solution to settle, and then collecting the surface fraction was repeated twice more for a total of 3 times. The collected surface fraction for each sample was then vacuum filtered onto its own 20 mm diameter, $\sim 13 \mu\text{m}$ pore sized silicon filter (SmartMembranes, Halle, Germany), which provide an IR transparent background for $\mu\text{-FTIR}$ analysis. Small amounts ($\sim 3 \text{ mL}$) of 2% hydrochloric acid were used to remove any zinc precipitate that formed on the filter. MilliQ water was then used to rinse the sides of the sample vials and filtration apparatus 3 times each to ensure that all MPs were rinsed onto the filter. Samples were then dried in a vacuum oven at 40°C for 12 h prior to $\mu\text{-FTIR}$ analysis.

All samples were analyzed using the LUMOS II FTIR Microscope System (Bruker Corp, Billerica, MA, USA). Prior to spectral collection, an optical image was taken of the silicon filter using the instrument's 8x objective and LED darkfield ring. The optical image was then used to identify and select particles on the silicon filter for spectral analysis using the software's "Find Particles" macro (Figure 3). All particles that were identified with the Find Particles macro were analyzed via FTIR. This decreases analysis time by excluding the blank areas of the filter. The instrument's mercury cadmium telluride (MCT) point detector was used to collect spectra in transmission mode. Both sample and background scans were collected with 4 cm^{-1} resolution, with a wavenumber range of $4000\text{--}650 \text{ cm}^{-1}$, and 16 co-added scans. The background scans were collected from a clean portion of the silicon filter. After spectral acquisition, the data was processed using the OPUS v8.7.4 software. All spectra were smoothed with the Savitzky–Golay method using 13 points prior to the program's Cluster ID function being used to compare each particle's spectrum to a series of polymer and additive libraries to identify them.

A hit quality index (HQI) score of 400 (out of 1000) was used as a cut off for identification. As shown in Figure 3, spectra of these HQI values have the characteristic peaks of the polymer they are identified as, providing a high degree of confidence in the identification, but may contain additional minor peaks due to additives or weathering. These libraries include the Bruker-KIMW ATR-IR Polymer Library, the Bruker-KIMW ATR-IR Additives Library, the BPAD-Bruker Optics ATR-Polymer Library, the Bruker General IR Library, the FTIR Library of Plastic Particles (FLOPP) [23], and the FTIR Library of Plastic Particles Sourced from the Environment (FLOPP-E) [23]. Following analysis, a custom R script was used to sort the particles into polymer categories (Table S1; Code S1) [24–26]. Particles identified as other materials were classified as non-plastic and excluded from further analysis, along with all unidentified particles.

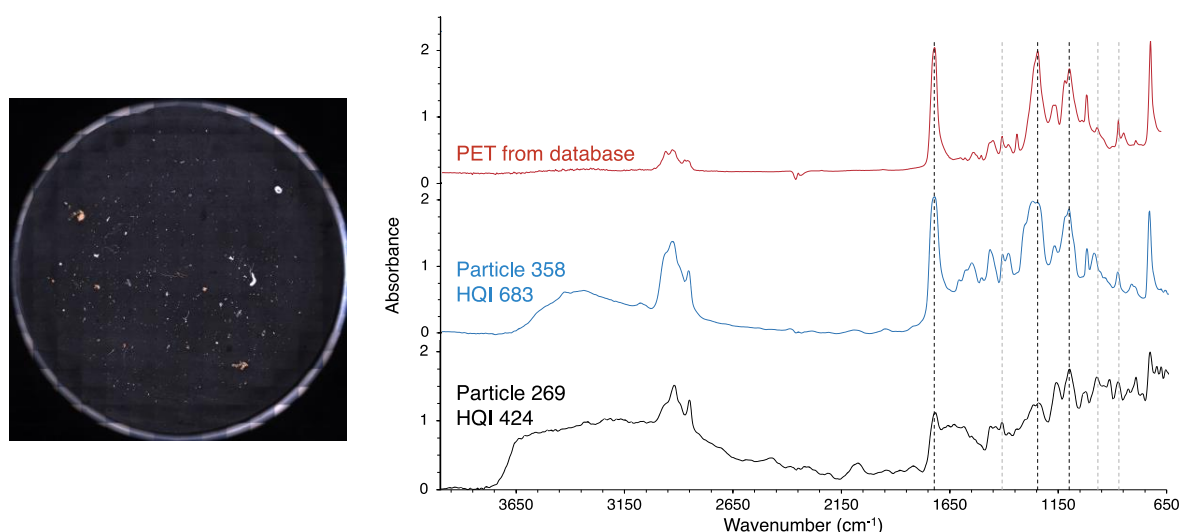


Figure 3. Microplastics tagged for FTIR analysis and representative spectra. Optical image of a silicon filter showing the distribution of particles to be tagged for analysis (left). Database spectrum of PET (top), along with two particles of HQI values 683 (middle) and 424 (bottom). Characteristic peaks of PET are depicted by black dotted lines at 1721 , 1245 , and 1100 cm^{-1} [27] with additional PET peaks shown in gray at 1409 , 967 , and 869 cm^{-1} [28].

(**top**), along with two particles of HQI values 683 (**middle**) and 424 (**bottom**). Characteristic peaks of PET are depicted by black dotted lines at 1721, 1245, and 110 cm^{-1} [27] with additional PET peaks shown in gray at 1409, 967, and 869 cm^{-1} [28].

2.4. Size and Morphology Analysis Using ImageJ v 1.54h

As OPUS v8.7.4 does not report the necessary parameters for morphology classification, ImageJ v 1.54h was used to determine the size and morphology of each MP [29]. To carry this out, a color threshold was set individually for the optical image of each sample. Manual selection of the threshold ensured that particle size and shape were being set accurately. Following this, the RGB image was binarized into a black and white image. The Particles8 plugin of the Morphology macro was then used to calculate various size and shape descriptors for each individual particle [30]. This approach is preferred over ImageJ's Analyze Particles feature as it returns more accurate results for certain shape descriptors [31]. Size is reported as the maximum Feret diameter for all microplastics and is given in microns. To determine the morphology, both the form factor and aspect ratio shape descriptors were used. Particles with an aspect ratio of 3 or greater were classified as fibers, particles with a form factor of greater than 0.6 were classified as spherical particles, and particles with a form factor of 0.6 or less were classified as irregular particles [31]. The terminology "spherical" and "irregular" particles is used instead of "bead" or "pellet" and "fragment", respectively, as suggested by Schnepf et al., as they are more inclusive and avoid assumptions about the particles' origin.

A second custom R script (code S2) was used to join the size/morphology data to the μ -FTIR data. During this process, it was discovered that occasionally the OPUS software had incorrectly identified a single particle as multiple smaller ones. When this occurred, the measurement position with the highest HQI value was joined to the size/morphology data and the other positions were removed from the data set. In cases where the polymer identification varied between multiple measurement positions on a single particle, the highest HQI value was also used to determine the particle's identity.

2.5. Statistics

Permutational multivariate analysis of variance (PERMANOVA) is frequently used to identify differences in community populations amongst groups [32]. PERMANOVA is well suited for analyzing MP data as it does not require the data to fit the assumption of multivariate normality and is useful for zero-inflated data [32]. For the main set of samples, PERMANOVA (permutations = 999) was used to assess the significance of the factors of drought condition and sampling site and the interactions thereof, using polymer population counts as input and a Bray–Curtis dissimilarity matrix as the method, using the *adonis2* function in R [33]. Results of this analysis were considered significant when $p < 0.05$. Following PERMANOVA, the data's dispersion heterogeneity was assessed using the *betadis* function in R, as PERMANOVA's power can decrease significantly when the groups' sample dispersions differ [32]. Non-significant results ($p > 0.05$) indicated a lack of differences in sample dispersion, which in turn indicated that the PERMANOVA results were truly significant. Additionally, since PERMANOVA can only determine which groups are significantly different from each other, a distance-based redundancy analysis (dbRDA) was used to determine which polymers contribute most strongly to the differences between groups. A similar process was used to assess differences in the Memphis samples. Here, the only factor assessed was drought condition as all samples were from a single site.

2.6. Contamination Control and Blank Subtraction

All sample preparation steps were carried out in laminar flow hood (AirClean 6000 Workstation) located within a HEPA filtered clean room environment. Where possible, glass and metal tools were used in place of plastic. Glassware was heat-cleaned at 450 °C

for three hours and rinsed three times with milliQ filtered (0.22 μm) water prior to use. Metal tools such as the tweezers used for manipulating the filters were rinsed three times with milliQ water both before use and between samples. Silicon filters were first sonicated in acetone for three minutes, then in methanol for five minutes to remove any particles. Following this, the filters were optically inspected under the microscope to ensure cleanliness prior to use. During sample preparation, analysts wore 100% cotton lab coats dyed bright orange to help identify contamination. Nitrile gloves were used as PPE, and a spectrum of the gloves was collected via ATR-FTIR and added to the spectral libraries used for identification. During sample preparation, all samples were kept covered unless being actively worked on.

Blanks consisted of reagents carried through the sample preparation process to monitor any contamination that occurred. All blanks were prepared and analyzed using the exact same methods as the samples. The MP concentrations reported herein for all samples have been blank-subtracted. Blank subtraction is a complex topic in MP analysis and there is no current standard used within the field. While it is possible to blank subtract based on overall count alone, subtracting based on individual polymer count may more accurately account for the contamination present. As such, the average numbers of MPs present in the blanks for each individual polymer were calculated. These were then subtracted from the sample data. In those few cases where there were fewer MP of a specific polymer in a sample than the average number of that polymer in the blanks, the number of MPs of that specific polymer for that sample was reported as zero.

3. Results

3.1. Characteristics of MPs in the MS River System

3.1.1. Chemical Composition of MPs

There were 38 unique polymers identified in the MS River System samples, with a further 11 mixed polymers also present. Of these, polyethylene terephthalate (PET), resin, and polyethylene (PE) were the most abundant making up 22%, 17%, and 10% of all MPs, respectively (Figure 4). PET and PE are some of the most commonly produced plastics, so it makes sense that they represent a large portion of the MPs present. It should be noted that the polymers identified as resin in this study are made up of several different types of resin. Of these, epoxy resin was the most prevalent (83.6% of all resins), followed by phenol formaldehyde resin (14.5%), and vinyl ester resin (1.9%). Epoxy resin has a wide variety of applications, including paints, coatings, and fiber-embedded plastic, which makes it difficult to determine a source for these MPs [34]. Phenol formaldehyde resin is typical used as a binder in plywood and also in injection molded products and fiber-embedded plastics [35]. Vinyl ester resins are frequently used in consumer paints, marine coatings, and fiberglass applications, which could be a potential source for the MPs identified in this study given the large number of marine vessels on the Mississippi River System. However, several types of marine resin-based paints, such as antifouling, lead-based, and epoxy primer, have significantly higher densities than the ZnCl_2 solution used for MP separation in this study [36]. As such, there may be an even larger number of resin MPs present in the river system than shown here.

3.1.2. Physical Characteristics of MPs

MP sizes are reported using the max Feret diameter, as is common in MP studies [31]. Results showed that over 90% of all MPs identified fell within 30–300 μm , while over half (55%) were within the 30–100 μm range (Figure 5). This is consistent with numerous other studies that have shown an increasing number of MPs as their size decreases [19,37]. The most common morphology was irregular particles (70%), which is also consistent with literature. Interestingly, spherical particles were the next most common morphology (26%), followed by fibers (4%). This contrasts with most studies, where spherical particles are often the least common morphology.

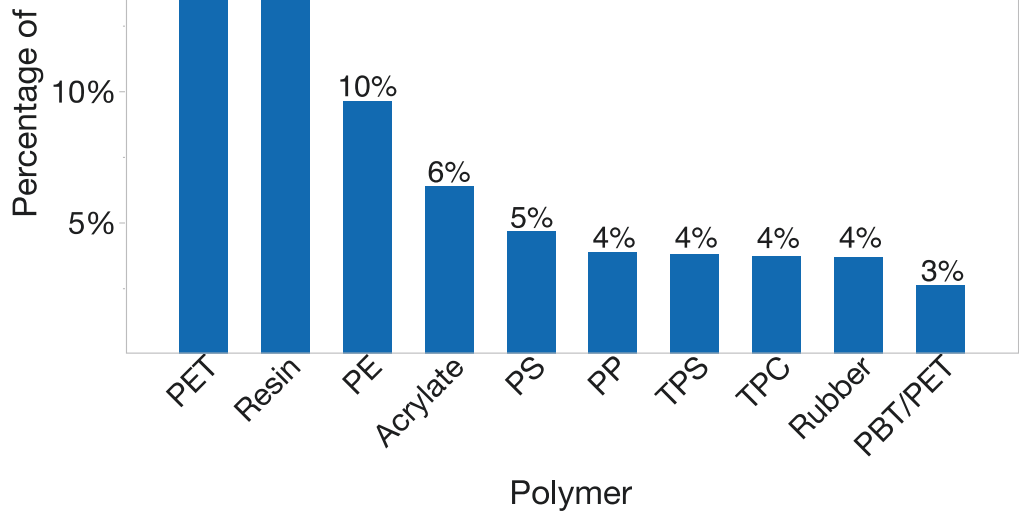


Figure 4. Polymer composition of total microplastics identified in this study ($n = 6209$). For readability, only the top 10 polymers have been shown. See supplemental information for a list of the other MP identified (Figure S1).

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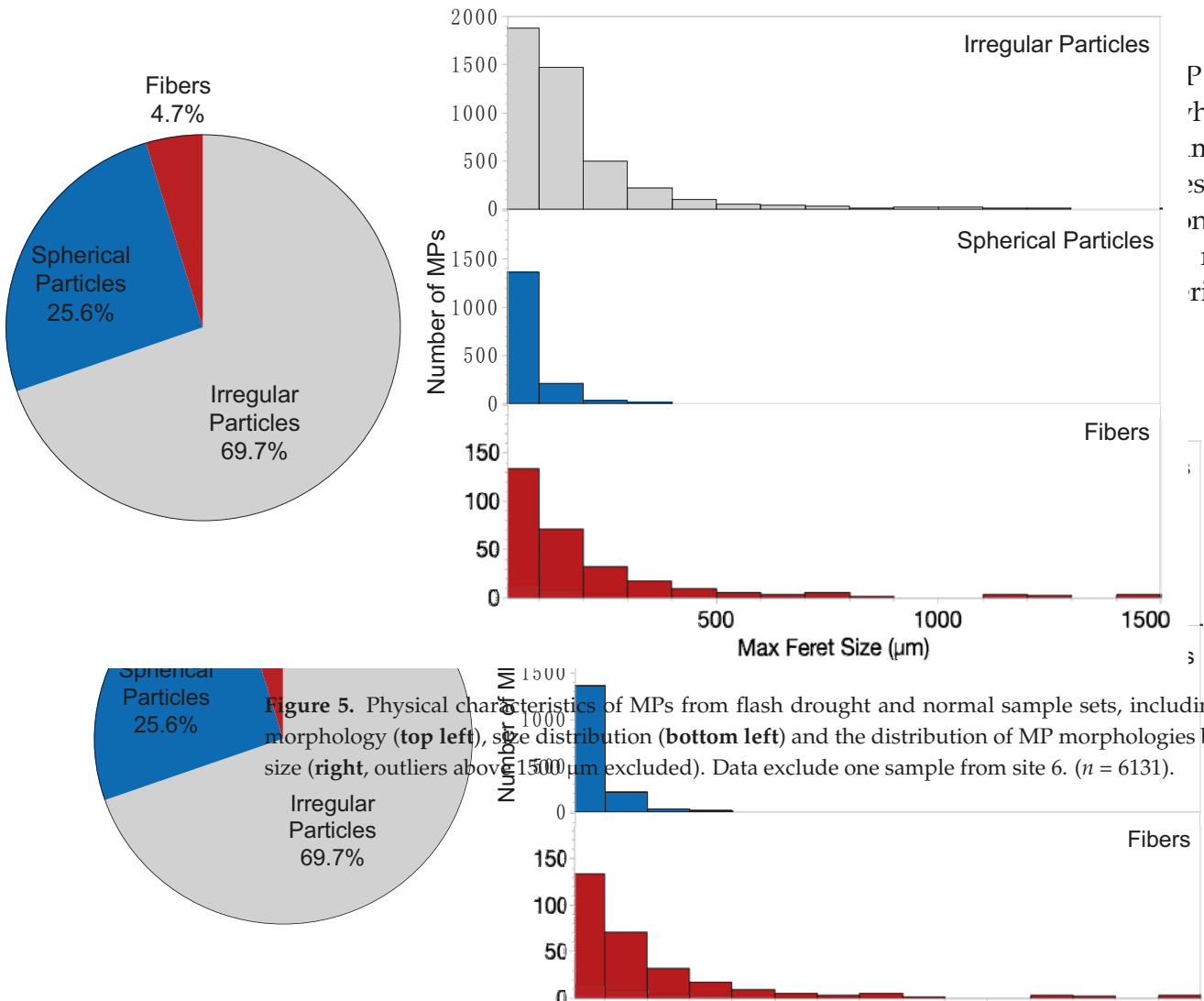


Figure 5. Physical characteristics of MPs from flash drought and normal sample sets, including morphology (top left), size distribution (bottom left) and the distribution of MP morphologies by size (right, outliers above 1500 μm excluded). Data exclude one sample from site 6. ($n = 6131$).

3.2. MPs in Flash Drought vs. Normal Conditions

MP concentrations ranged from 16 to 381 MPs/L across the seven sites and two drought conditions studied (Figure 6). A two-way analysis of variance (ANOVA) was performed to assess the effect of site, drought condition, and the interactions thereof on the concentration of MPs in the Mississippi River System. Results showed that neither site ($p = 0.70$) nor condition ($p = 0.22$) had a statistically significant effect on the concentration of MPs. The interaction ($p = 0.83$) of these effects was also found not to be significant. However, these results may be due to limitations of the statistical method used (see Section 4.4). Despite the fact that no statistical differences could be found, some trends can be seen when observing

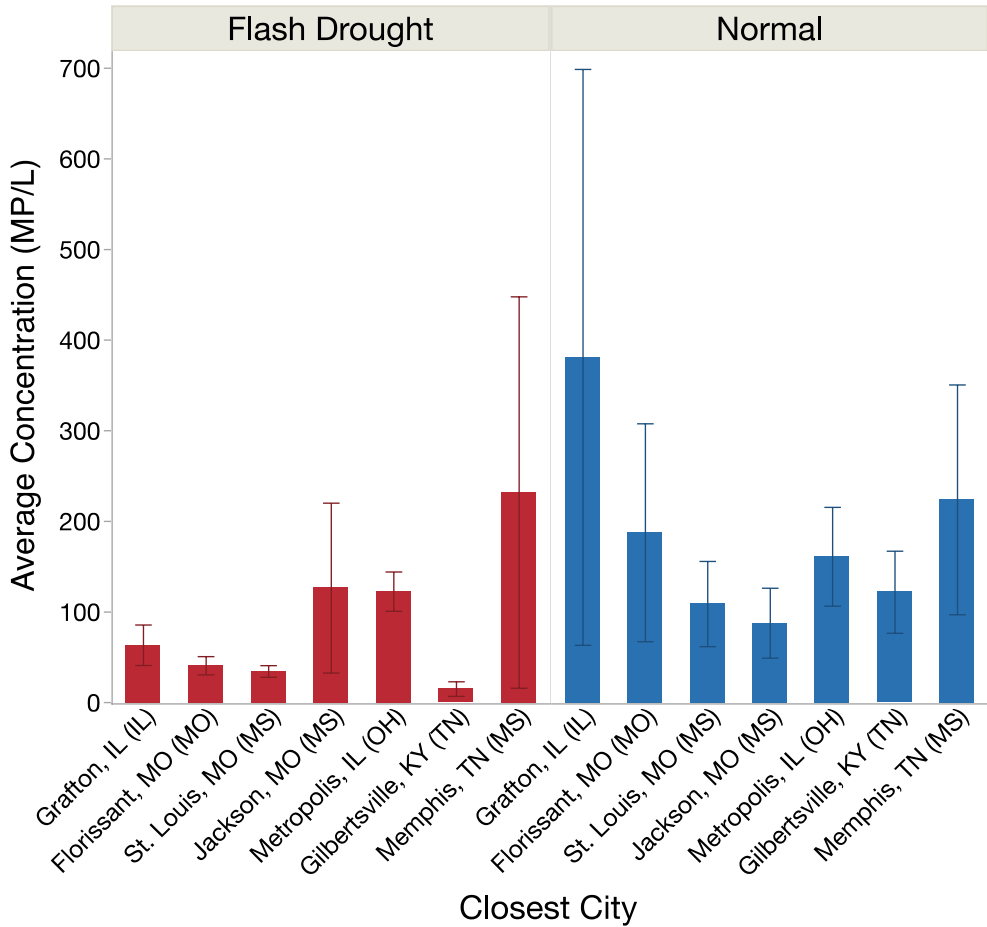


Figure 6. Average MP concentrations by sampling site across both flash drought and normal flow conditions. Abbreviation of river name listed in parenthesis after site name. Error bars represent one standard error.

There is a general trend of higher MP concentrations during normal conditions as compared to the flash drought conditions. While there was no significant effect of site on MP concentration, PERMANOVA results showed that the flash drought conditions had a significant impact ($p < 0.001$) on polymer composition. These results were further investigated using PERMANOVA, which found no significant differences ($p = 0.39$) in group dispersions. The effects of the sampling site ($p = 0.75$) and the interaction of flash drought conditions and the site ($p = 0.91$) were also studied but were ultimately found not to be significant. The highest concentration of MPs was found at the Grafton, IL, site during normal conditions in June 2023. This site is located within Pere Marquette State Park on the Illinois River, which is a tributary of the Mississippi River. The sampling site is located downstream from the outlet of several lakes and a wastewater treatment plant. The increased amount of ethylene propylene copolymer (EPP), polyethylene terephthalate (PET), polypropylene (PP), and polyethylene glycol (PEG) were indicative of the flash drought conditions while acrylic, PE/EVA, SAN, SMA, PS, and ABS were indicative of the normal conditions.

Table 3. Distance-based Redundancy Analysis (dbRDA) results for top 4 polymers correlated with each condition. For all polymer scores, see Table S2.

the marina during the summer months may explain the much larger MP concentration compared to during the flash drought sampling when boats were up on the hard.

While there was no significant effect on MP concentration, PERMANOVA results showed that flash drought conditions had a significant impact ($p < 0.001$) on polymer composition. These results were further investigated using PERMDISP, which found no significant differences ($p = 0.39$) in group dispersions. The effects of the sampling site ($p = 0.75$) and the interaction of flash drought conditions and the site ($p = 0.91$) were also studied but were ultimately found not to be significant.

Following this, a dbRDA was used to determine the polymers that were most strongly correlated with each group of drought conditions (Tables 3 and S2). We found that PC, ETFE (ethylene tetrafluoroethylene), EFEP (fluorinated ethylene propylene copolymer), and MFA (tetrafluoroethylene perfluoromethylvinylether) were indicative of the flash drought conditions while acrylic, PE/EVA, SAN, SMA, PS, and ABS were indicative of the normal conditions.

Table 3. Distance-based Redundancy Analysis (dbRDA) results for top 4 polymers correlated with each condition. For all polymer scores, see Table S2.

	Polymer	dbRDA Score
Flash Drought		0.7051 ¹
	Ethylene Tetrafluoroethylene (ETFE)	0.700
	Polycarbonate (PC)	0.712
	Fluorinated Ethylene Propylene Copolymer (EFEP)	0.712
	Tetrafluoroethylene Perfluoromethylvinylether (MFA)	0.726
Normal Flow		−0.7051 ¹
	Polymethylmethacrylate (acrylic)	−0.738
	Polyethylene/Ethylene Vinyl Acetate Blend (PE/EVA)	−0.659
	Styrene Acrylonitrile (SAN)	−0.779
	Styrene Maleic Anhydride Copolymer (SMA)	−0.781

¹ Group centroid score.

3.3. Temporal Variation in MPs at the Memphis Site

The proximity of the Memphis, TN, site allowed samples to be collected at multiple times points and across varying conditions. Of these, the 2022 flash drought and summer 2023 normal conditions showed much higher average concentrations of MPs than the 2019 flooding and normal condition samples as well as the October 2023 drought samples. Average concentrations ranged from 6 to 241 MPs/L (Figure 7). A one-way ANOVA showed that there were no statistical differences in MP concentration among the five time points ($p = 0.39$). However, PERMANOVA results showed that drought conditions did have a significant impact ($p = 0.002$) on polymer composition. PERMDISP showed a significant difference ($p < 0.001$) in group dispersions among these samples; however, graphically plotting the data did show differences in location in addition to dispersion. Similarly to the multi-site comparison above, a dbRDA was used to determine which polymers were most closely correlated to each condition. The results of this analysis are displayed in Table 4 below. Notably, there were no polymer species that showed strong correlation to the normal flow period during November of 2019, which may be due to the low number of MPs identified in those samples.

the multi-site comparison above, a dbRDA was used to determine which polymers were most closely correlated to each condition. The results of this analysis are displayed in Table 4 below. Notably, there were no polymer species that showed strong correlation to the normal flow period during November of 2019, which may be due to the low number of MPs identified in those samples.

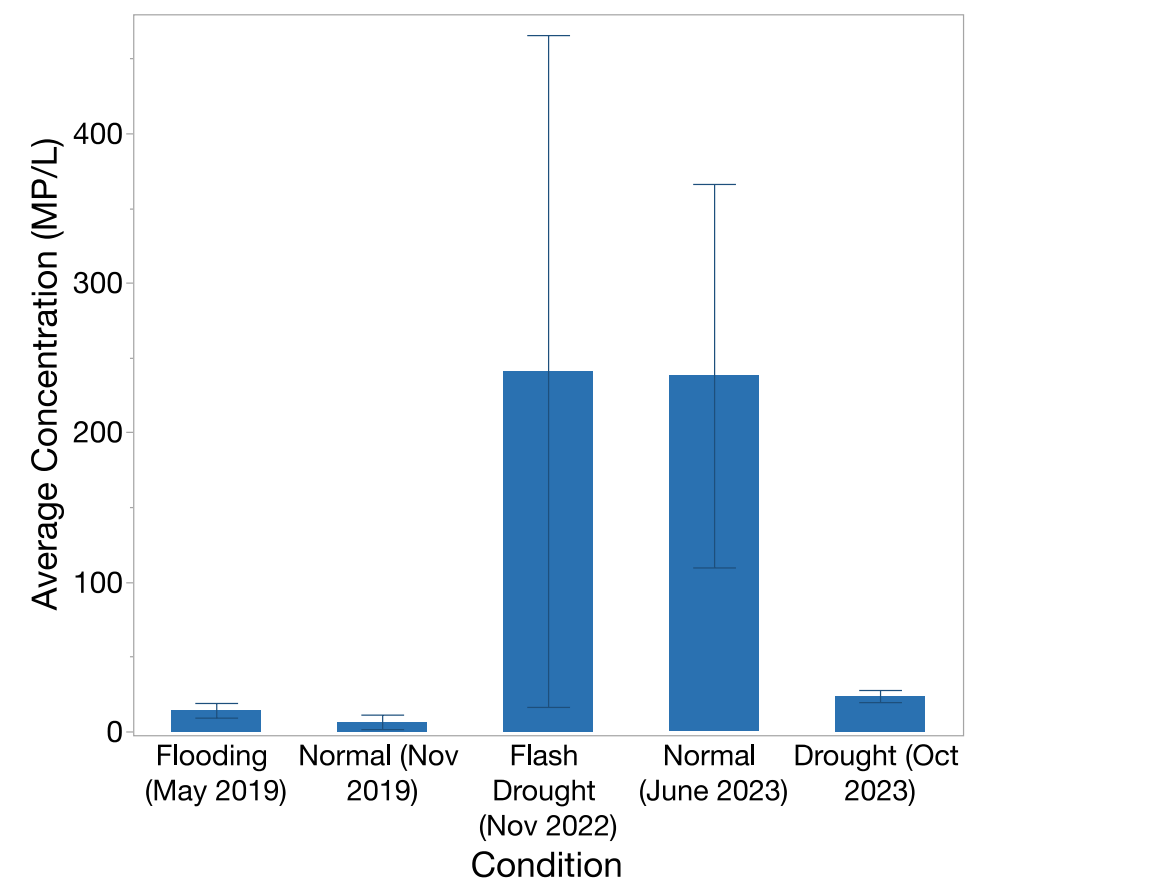


Figure 7. Average microplastics concentration (MP/L) by condition at Memphis, TN (Site 7). Error bars represent one standard error.

Table 4. Distance-based Redundancy Analysis (dbRDA) results for the top 2 polymers correlated with each condition at the Memphis site. For all polymer scores, see Table S3.

Polymer		dbRDA Score
Flooding		−0.986 ¹
	Polycarbonate (PC)	−0.911
	Polycarbonate/ acrylonitrile butadiene styrene (PC/ABS)	−0.929
Normal Flow 2019		−0.679 ¹
	None	
Flash Drought		0.555 ¹
	Thermoplastic copolyester (TPC)	0.574
	Polybutylene terephthalate (PBT)	0.550
Normal Flow 2023		1.003 ¹
	Polyurethane (PU)	1.021
	Polyethylene/ethylene vinyl acetate (PE/EVA)	0.986
Drought		0.108 ¹
	Modified polytetrafluoroethylene (TFM)	0.143
	Tetrafluoroethylene perfluoromethylvinylether (MFA)	−0.028

¹ Group centroid score.

4. Discussion

4.1. Characteristics of MPs in the MS River System

One possible explanation for the abundance of spherical particles in this study appears when analyzing the size distribution of the MPs by their shape (Figure 5). Here, it can

be seen that the average size of the spherical particles is much lower than that of the irregular particles and the fibers, and that their spread is narrower as well. Given the fixed 8x objective on the LUMOS, it is likely that smaller particles may not be resolved well enough to accurately determine their morphology [31]. This highlights one of the many challenges associated with MP analysis. Research has shown that both morphology and size have an impact on MP toxicity [19,38,39]. As such, it is crucial to be able to accurately determine these metrics when studying environmental MPs. Our results suggest that optical resolution may be just as important as spectral resolution when working with FTIR microscopy.

4.2. MPs in Flash Drought vs. Normal Conditions

The fact that MP concentration did not change during times of decreased rainfall may at first seem counterintuitive. After all, one might expect that less water in the river system would increase the concentration of MPs. However, this assumes that the overall number of MPs in the system remains constant. Studies have shown that stormwater runoff is a significant contributor to MPs in aquatic systems [37,40]. As such, the reduction in stormwater runoff may lead to a reduction in the number of MPs entering the river system. The two factors of less water and fewer MPs may compete and ultimately lead to similar MP concentrations in the flash drought samples compared to the normal flow ones.

While there are multiple factors that influence MP toxicity, polymer composition is one of the most well studied and understood [19]. The fact that polymer composition did vary with drought condition, even though the overall concentration did not, therefore has significant impacts for understanding the risks to aquatic species associated with MP pollution. These differences in polymer composition are likely driven by differences in the MPs' mobilities. Several factors can impact the ability of MPs to move throughout a river system. Some of these, such as size and morphology, are independent of polymer identity, while others such as the density of the particle are inherent to the polymer [41]. During higher flow periods, river waters are more turbulent, which can help move higher density MPs further laterally. In contrast, lower flow rate periods, such as those that occur during drought conditions, have less turbid waters which may result in decreased transportation of high-density MPs. This may explain why fluoropolymers were correlated with the flash drought samples, given their relatively high densities.

It is notable that three of the four plastics most correlated to flash drought conditions are fluoropolymers. Fluoropolymers are a distinct class of per- and polyfluoroalkyl substances (PFAS) which are generally regarded as environmentally safe due to their chemical stability, limited solubility, and lack of bioaccumulation [42,43]. However, while these polymers may be chemically stable, they are still prone to physical degradation. Mechanical breakdown decreases the size of these particles, which in turn increases their bioavailability [44]. Lohmann et al. argue that end-of-life issues such as this and their ability to release PFAS leachates into the environment are one reason why fluoropolymers should not be classified as being of low environmental concern [44]. As such, while it is always important to monitor changing polymer compositions in MP pollution, the fact that fluoropolymers are so closely linked to flash drought conditions may be a cause for special concern.

4.3. Temporal Variation in MPs at the Memphis Site

Interestingly, the two normal conditions (November 2019 and June 2023) showed very different concentrations of MPs. While both these time points are defined by not meeting the thresholds for any of the D1-D4 drought monitor categories reported jointly by the National Drought Mitigation Center at the University of Nebraska-Lincoln, the United States Department of Agriculture, and the National Oceanic and Atmospheric Administration, this does not mean that the same amount of water was present in the system at these times. The normal conditions during 2019 had much higher water levels than the 2023 normal conditions, as seen when comparing stage height (Figure S2). It is likely that the larger amount of water is responsible for the much lower concentration

of MPs during the 2019 sampling. These results also suggest that concentration of MPs is more closely related to the amount of water in the system rather than to the specific drought category.

This could also explain why higher concentrations were seen in the flash drought and June 2023 normal conditions as compared to the October 2023 drought samples. The flash drought of late 2022 was characterized by rapidly increasing drought conditions over a period of a few weeks (Figure 1). This resulted in a large amount of water leaving the river system in a very short period of time, leading to high concentrations of MPs at this site. By early 2023, the drought monitor conditions had returned to normal, but water levels were still lower than in previous years. The June 2023 samples were collected during a normal condition period, but drought conditions set in within the following weeks. As such, water levels were still lower than usual, meaning that there were still relatively high concentrations of MPs. By October 2023, drought conditions had been present for several months, leading to even lower water levels. However, the long-term lack of stormwater runoff may have constituted a significant decrease in the number of MPs entering the river system, thus leading to lower MPs concentrations. It should also be noted that while the long-term drought had lower MP concentrations than the flash drought period, it did have higher concentrations than the flooding and 2019 normal periods. These results suggest that both flash and long-term droughts do impact the concentration of MPs. However, given that not all sampling sites experienced the same degree and duration of drought, this trend was not apparent in our multi-site study. The Memphis site results also highlight the importance of long-term sampling for MP monitoring. While there was no significant difference in the MP concentration between the flash drought and the 2023 normal samples, it is possible that a significant difference might have been found if the multi-site study had included samples from the 2019 normal period.

4.4. Limitations

One of the main limitations of this study is the large variation in samples collected from the same site and timepoint. ANOVA is sensitive to large differences in within-group variances, which could be the reason that no statistical difference was seen in MP concentration between the flash drought and normal samples or between sites despite the fact that the group means alone do appear to be different. This is best illustrated by Figure 6, where the average concentration at the Grafton, Illinois, site was 381 MPs/L during normal conditions and 64 MPs/L during the flash drought. Given that the average concentration during normal conditions was over five times larger than during flash drought, one would expect these values to be statistically distinct. However, the large variance between samples results in overlapping error bars, making it impossible for ANOVA to find statistical differences between them.

Large variations were also present across the multiple timepoints sampled at the Memphis, Tennessee site. This leads to a similar statistical issue where despite the much higher concentrations of MPs in the flash drought and 2023 normal flow samples compared to the other conditions, no statistically significant differences were found between timepoints. The underlying variance issue is an inherent limitation of grab sampling, particularly when dealing with small sample volumes. In this study, each sample collected consisted of slightly less than 1 L of water. Multiple replicates were taken at each site and timepoint, but while averaging the results of the replicates yields a more accurate depiction of the MP present in the river, it does not address the large differences between samples. However, other sampling techniques that collect larger volumes of water come with their own set of limitations. Net trawling is a common sampling method, but limits the lowest size fraction of MPs that can be collected due to the holes in the mesh [11]. Bulk sampling in jars avoids this issue, making it possible to characterize the smaller MP size fractions that are both more prevalent and of greater toxicological relevance. As such, grab sampling was the chosen method for this study, despite its other limitations.

5. Conclusions

MPs can affect the reproduction and mortality rates of several aquatic species [7–10], making it crucial to characterize them within river systems, especially during times that may result in increased hardship for these species, such as during droughts. To our knowledge, this work represents the first characterization of MP pollution in the Mississippi River System during a flash drought period. Our results showed that MP concentrations varied widely across sites and drought conditions, ranging from 16 to 381 MPs/L. There was no statistical difference in MP concentration among sites or drought condition in these samples, though there was a significance in polymer composition between the flash drought and normal conditions. The additional timepoints at the Memphis site provide additional insight into these results. Here, both normal condition timepoints had MP concentrations that were more in line with timepoints of similar river stage heights and water discharge rates than with each other. This suggests that MP concentration in river systems may be more dependent on the amount of water flowing through the system than on the drought condition itself. However, drought condition clearly also plays a role, given that the flash and long-term drought samples showed very different MP concentrations despite being collected at times of similar water levels. Ultimately, the relationship between MP concentration and drought condition is complex, and further research is needed to fully characterize it.

However, while these findings suggest that MP concentrations only change over longer time scales, polymer composition of the MP present did statistically differ between the flash drought and normal condition samples. Given that polymer identity can greatly impact the toxicity of MPs [19], knowing that the composition of MPs in a river system can change over the relatively short time span of roughly six months is vital to those designing similar studies. It also underlines the importance of fully characterizing all MPs in a study. Although less common now, many studies in the past have focused on quantifying MPs using techniques such as optical or fluorescence microscopy and only characterizing a small portion of their samples using FTIR microspectroscopy. While these methods do serve to confirm that analysts are properly identifying MPs, failing to characterize the polymers present in all samples reduces the utility of the results for those seeking to use them for risk assessment and remediation efforts. As such, the benefits of fully characterizing all MPs within a study justify the time and resources required to do so.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/environments11070141/s1>, Document S1: Figures S1–S3 and Tables S1 and S2; Code S1: Polymer Classification; Code S2: Joining Spectral and Optical Data.

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