Abstract
This study investigates the presence, characteristics, and sources of microplastics in the Mississippi River, spanning the entire length of the river. Surface water grab samples were collected during a long-distance kayaking journey from May to July 2019 starting in the state of Minnesota and ending in the Gulf of Mexico. Microplastics were suspected at every sample site, with an average count of $7.1 \pm 3.1$ particles per liter (range: 2-14 particles per liter). The majority of particles were fibers (97%) and the remainder were fiber bundles, film, foam, or fragments. Raman spectroscopy was performed on a random subset of particles, which showed the majority were human made (67%). Particles were categorized as anthropogenic unknown (21%), anthropogenic cellulose (20%), natural (7%), and plastic (25%). Samples containing plastics were identified as fluoropolymers, polyethylene, polypropylene, and plastic-based dyes. This study estimated that median microplastic produced by someone living within the Mississippi River basin was 301,892 microplastics/day. A significant relationship between area-normalized load and discharge indicated that areas with greater runoff resulted in microplastic increases. Regression modeling found no significant relationships between microplastic concentration and spatial factors such as agriculture and forest cover. However, microplastic concentration was correlated with population density. These findings highlight the complexity of microplastic dynamics in river ecosystems. This study found widespread microplastic pollution in one of the largest river systems in the world. There is a need for further research to understand microplastic fate and transport in the Mississippi River as well as policy and regulatory efforts to reduce plastic loading and to mitigate its impact on aquatic ecosystems.
Thesis Readers
Thesis Advisor and Primary Reader: Dr. Dave Love

Thesis Reader: Dr. Ciaran Harman

Thesis Advisor: Dr. Kris Stepenuck
Acknowledgments

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Chapter 1: Introduction

The presence of plastics in the marine environment have been well documented since the 1970s (Carpenter & Smith, 1972). While plastics account for around 60-90% of floating marine debris, around 80% of those plastics come from terrestrial sources (Di Mauro et al., 2017). The durability of plastics, although useful, has resulted in significant consequences. Microplastics are defined as plastics smaller than 5 mm in one dimension (Moore, 2008). Microplastics can be a byproduct of macroplastics, plastics visible to the naked eye, that come from photodegradation or mechanical wear. Moreover, microplastics have been found throughout the water column, near shore, and in deep sea sediments in marine ecosystems (Cole et al., 2011; Vianello et al., 2013). As an emerging branch of pollution science, the ecological impacts of microplastics are not well understood. Nonetheless, microplastics pose a threat to biota due to displacement of food volume in the digestive system and the ability to serve as a transport medium for adsorbent toxins (Zhao et al., 2022). Because microplastics can break down as small as zooplankton, these plastics accumulate along the aquatic food chain (Di Mauro et al., 2017). This can result in the accumulation of microplastics in the digestive tracts of organisms that lead to lower energy intakes although the organism may feel satiated (Wright et al., 2013). Additionally, fat soluble, toxic pollutants can adsorb to microplastics. When these microplastics are consumed, toxic pollutants can accumulate in fat and become biomagnified (Rochman et al., 2013). Research on microplastics in humans requires long term observation and strong ethical consideration, thus results are slow to develop. However, toxicological studies in animals have demonstrated that there are deleterious effects at the cellular level (Hu & Palić, 2020). Cells that engulf nanoparticles elicit an inflammatory response making the entire organism vulnerable to disease (Rochman et al., 2013). Microplastics can be inhaled and ingested through the air, food, and
water (Koelmans et al., 2019; Lee et al., 2023; Smith et al., 2018). As toxicological research is developing, regulatory action could limit environmental and human exposure to microplastics.

Understanding the sources and transport of microplastics are key to developing actions that prevent or limit environmental contamination. Rivers are crucial ecosystems for studying microplastic dynamics. They serve as vital conduits between land based anthropogenic sources and marine ecosystems (Schmidt et al., 2017). It is estimated that rivers transport 0.47–2.75 million tons of plastic into marine ecosystems every year (Lebreton et al., 2017). Microplastics have been ubiquitously found in some of the most remote river ecosystems such as rivers in sparsely populated, mountainous environments (Lebreton et al., 2017). More microplastic studies are beginning to incorporate the examination of land-use on microplastics in river ecosystems. Land-based anthropogenic activities are highly correlated with microplastic concentrations in freshwater ecosystems (Li et al., 2023).

Over the past few years, citizen science projects and scientists have documented microplastics in only a few locations on the Mississippi River, yet no studies have systematically measured microplastics concentrations along the entire length of the river (Adventure Scientists Global Microplastics Project, n.d.; Bucci & Rochman, 2022; Scircle et al., 2020). To this date, few thru-river studies exist in documented literature especially of this magnitude (Kapp & Yeatman, 2018; Li et al., 2023). This study aims to 1.) study the concentration, distribution, and type of microplastics in the surface waters of the Mississippi River, and 2.) compare the effects of land-use on microplastic counts using both small-scale and large-scale spatial factors.
Chapter 2: Methods

Study Region
The Mississippi River is a 3,800 kilometer river that spans the length of the United States and flows through 10 states. It flows southbound from Minnesota into the Gulf of Mexico. The Mississippi River is a key economic, ecological, and cultural resource. The 3.2 million square kilometer watershed is home to approximately 18 million residents who receive drinking water from the Mississippi River and its contributing tributaries (National Park Service, 2022). Additionally, the river is a major flyway for 326 migratory bird species (National Park Service, 2022) with about 40% of migratory waterfowl in the United States using the river (National Park Service, 2022). The Mississippi River plays a significant role in the US and global economy. Agricultural products are the primary export from the Mississippi River basin. According to the National Park Service, “the [Mississippi River] basin produce[s] 92% of the nation's agricultural exports, 78% of the world's exports in feed grains and soybeans, and most of the livestock and hogs produced nationally” (National Park Service, 2022). Field sampling coincided with a historic flood. The spring and summer of 2019 was one of the longest lasting floods since the Great Flood of 1927 (Pal et al., 2020).

Sample Collection
Surface water grab samples were collected during a long-distance kayaking trip along the full length of the Mississippi River from May 25th 2019 to July 24th 2019. This sampling method was elected over neuston net tows due to field sampling logistics. Samples were collected every 50 miles for the first 600 miles of the river. Afterwards, samples were collected every 100 miles. Sampling distance was tracked by a Garmin Oregon 600 GPS. This resulted in 29 sample sites.
Sampling diverged from the main Mississippi River in Simmesport, Louisiana and continued down the Atchafalaya River. The Atchafalaya River diverts 30% of the Mississippi River’s volume into the Gulf of Mexico west of the main delta. Splitting from the Mississippi River avoided health and safety concerns for the kayak-based sampler due to the increase of industrial shipping and pollution exposure beyond Baton Rouge, Louisiana. The sampling strategy was influenced by designs in thru-river studies (Kapp & Yeatman, 2018). Samples were collected at a higher frequency for the first 600 miles of the river to capture large-scale rural to urban change surrounding the Mississippi River. The higher reaches of the Mississippi River are more forested and rural. After Minneapolis, Minnesota, land around the Mississippi River is more developed and populated. Additionally, the US Army Corps of Engineers lock system begins in Minneapolis, Minnesota. The higher sampling interval stretched beyond Minneapolis, Minnesota intentionally to ensure that this change was sufficiently documented. Surface water samples were collected from the Mississippi River using 1-liter high-density polyethylene (HDPE) bottles. At each sample location, bottles were triple rinsed in situ and then used to capture surface water. To minimize airborne contamination, samples were capped underwater and taken on the upwind side of the boat. At each sample site, duplicates were also collected. Triplicates were randomly collected at about one third of the sample sites. Within 48 hours of collection, samples were shipped at room temperature to be preserved in frozen storage. Freezing samples prevented biologic growth and has been a common method in other aquatic microplastic studies (Klasios et al., 2021). In a study on microplastics in tissue, freezing had no significant differences compared to other methods of preservation (Courtene-Jones et al., 2017). Samples were frozen from 2019 to the summer of 2023 when they were thawed for microplastic extraction and examination. Upon
processing, one sample (Sample Site 22) was removed due to a broken lid, and another (Sample Site 11) had a missing duplicate. This led to a total processing of 65 1-liter samples.

**Laboratory Contamination Control**
Contamination precautions were adapted from published methodology (Munno et al., 2023). Several methods were used to minimize plastic contamination into samples during microplastic extraction. Prior to sample collection, all equipment including glassware, tools, and laboratory surfaces were thoroughly cleaned with low foam dish soap to remove potential contaminants. Glassware was triple rinsed with tap water followed by three rinses with ultrapure water (water passed through a Milli-Q IQ 7000). Additionally, glassware and tools were triple rinsed with ultrapure water between samples. Non-plastic alternatives were used instead of plastic parts and equipment. For instance, natural sea sponges replaced plastic sponges, and glass Petri dishes were elected over plastic ones. Samples were covered with aluminum foil to reduce atmospheric deposition of contaminant microplastics. Additionally, samples were stored in a cabinet and lab use was minimized by other users. Cotton clothes and lab coats were donned by the primary laboratory personnel. Bright, unique colored cotton was worn to better identify contamination.

**Method Validation**
Particle recovery rates were determined using a spike and recovery experiment. Five 1-liter bottles were filled with Milli-Q water and five 1-liter bottles filled with 40μm sieved Mississippi River water were spiked with 50 plastic particles. The recovery rate of particles for control samples and river water was 102% ± 4.7 and 104% ± 9.4, respectively. There was no significant difference in recovery rates between Milli-Q and Mississippi River water (p = 0.61).
Microplastic Recovery from River Water

A density separation process was used to settle sediment from suspected microplastics. First, samples were thawed and shaken. Next, each sample was poured through metal sieves with mesh sizes of 5 mm, 100µm, and 40µm. Sample bottles were inspected for damage, rinsed at least three times with Milli-Q water, and poured through the sieve stack. Samples were fractioned into two size ranges: 40µm to 100µm and 100µm to 5mm. A 1.4 g/mL calcium chloride (CaCl2) solution was prepared following published protocols courtesy of the Rochman Lab at University of Toronto (“Resources for Microplastics Research,” 2021). Briefly, 1.2 L of Milli-Q water was added to a 2 L beaker containing a stir bar and placed on a stir plate. CaCl2 pellets were added gradually until a density of 1.4 g/mL was achieved. The solution was then filtered using a 20 µm filter and stored in a glass container. All samples with visible sediment underwent a density separation process with calcium chloride. Sieved contents were rinsed into a clean glass beaker with CaCl2 solution, stirred vigorously, and allowed to settle for at least 2 hours. The top floating layer was transferred to another clean beaker, and the process was repeated for the remaining sediment. After a settling period, the surface particles were sieved, triple rinsed with Milli-Q water, and placed into a clean beaker. To remove organic matter, the contents were sieved once more and rinsed into a clean glass beaker using H2O2 and allowed to digest at 45°C for 48 hours. The digested samples were then sieved, triple rinsed with Milli-Q water, and filtered through a 0.45 µm Whatman membrane filter using a vacuum filtration setup to capture particles. Vacuum filtration was performed using a cleaned glass filtration apparatus with Whatman filter paper. The filtered suspected microplastics were collected on gridded filter paper, which was then carefully removed and placed onto a glass petri dish for further analysis. One control was run for
roughly every nine field samples. One liter of ultrapure water was stored in an HDPE bottle, frozen, and thawed to simulate the treatment of field samples. Each control was also size fractioned and went through a density separation process, wet peroxide oxidation, and filtration. Amongst seven batch controls, 25 total particles were found. The mean was 3.29 ± 2.36 particles per liter. In controls, particle counts ranged from 0 to 6. Particles were predominantly fiber (96%).

Light Microscopy for Microplastic Identification
Particles collected on the filter paper were examined under a stereoscope (AmScope Trinocular Stereo Microscope, Irvine, California) for identification and enumeration. Superfine-tip forceps were used to handle suspected microplastics. The particles considered in this study were between 0.07mm - 5mm. Particles under 0.07mm were too difficult to assuredly handle with forceps. Digital images of microplastics were captured using a microscope digital camera attachment, AmScope 10 MP USB Camera Attachment, and analyzed using AmScope image analysis software for size and shape measurements. Microplastic identification was performed by two trained personnel to quantify length, morphology, and color. One primary individual counted all suspected plastics, another counter analyzed a subset of at least 20% of those samples for concordance. If there were deviances between sample counts, samples would be recounted. If samples counts were discordant after recounts, counts were then averaged. Discordance between sample counts was no greater than ±2 particles after this process. Size was measured for the first occurrence of a unique color morphology combination for each sample using AmScope camera software. Length was recorded in the largest dimension. This method was used to represent the general sizes for color-morphology combinations considering limited time. Morphology and
color were documented for every suspected microplastic. Morphology was classified into eight
categories: fragment, sphere, pellet, fiber, fiber bundle, foam, film, and tire/road wear.
Morphology and color definitions were guided by the Rochman Lab at the University of Toronto
(Munno et al., 2023).

Raman Microscopy for Microplastic Confirmation
A minimum 10% of particles from at least one replicate at each sample site was analyzed using
Raman spectroscopy to determine anthropogenic origins (Grbić et al., 2020; Huntington et al.,
2020). A Horiba LabRAM ARAMIS was utilized and equipped with a 532nm laser at 18 mW
and a 633 nm laser at 11.3 mW at the University of Maryland Surface Analysis Center. Spot size
for the 532 nm laser for a less than 10% laser power was 3.40 mW. For 50% laser power with the
532 nm wavelength, spot size was 7.18 mW. Hole and slit parameters were set to 400 and 100
respectively. Filter, exposure, and accumulations were adjusted to each particle. Dried and
extracted particles were individually placed on a glass slide and inserted into the Raman
spectrometer. This included at least 10% of particles from each control. A single operator
adjusted settings manually to capture the best spectra. The spectra from each particle was
matched to known chemical compositions using Wiley’s KnowItAll Software and Spectral
Libraries. Adapted from Klasios et al., 2021, identification outputs were classified into five
categories: natural, anthropogenic cellulose, anthropogenic unknown, plastic, and unidentifiable
(Klasios et al., 2021).
**Geospatial Data**

ArcGIS Pro 2.9 and the sf package in R were used to extract and process geographic data. We characterized land-use around a given sample site in a two-step process. First, the entire drainage basin for each sample site was delineated using a web-based watershed delineation tool (Heberger, n.d.). We specified USGS HUC-12 as the base map for watersheds in this tool. The resulting delineated basins were dubbed as “full basins” in this study. We adapted methods from several studies to define area sizes appropriate for the large scale of the Mississippi River (Grbić et al., 2020; Tran et al., 2010). In the second step of this approach, a 5km and 20km buffer was created around each sample location to accommodate the large width of the Mississippi River. To prevent overestimation of geospatial data, the circular buffer was clipped to intersecting HUC-12 subregions within the delineated basin (Figure 2). The clipped 5km and 20km buffers for each sample site basins are referred to as “5km and 20km basins”. The size of the contributing watershed basin to each sampling point was compared between the full basin, the 5km basin, and the 20 km basin. Geospatial features were estimated for each basin size including population and population density (US Census Bureau, 2020), area geometry, and land use percentages (U.S. Geological Survey, 2019a). Land-use types from the National Land Cover Database were collapsed into four categories: agriculture, forest, urban, and open water and transformed into percentages. Table S1 shows the NLCD land-use categories that were collapsed. Point data were also included in the geographic-count data regressions including wastewater treatment plants (WWTP) within 1km of the Mississippi River (U.S. EPA, 2019), rainfall (NOAA, 2019), flow rate in cubic foot per second (U.S. Geological Survey, 2019), locks and dams, and major rivers. An inverse distance weighted (IDW) calculation of discharge from WWTP was conducted using 2019 annual discharge rates from EPA’s ECHO database (U.S.
EPA, 2019). These IDW calculations compared powers 1-3 for all WWTP between the last sample point to the next.

**Data Analysis and Modeling**

Particle counts were averaged amongst replicates at each sample site. Due to a small sample size, low microplastic counts, and a proportionally high standard deviation, findings from controls and Raman spectroscopy were not subtracted out of averaged counts. Count data from environmental samples and controls were processed using tidyr and dplyr packages in R. Counts were grouped by sample site, color, and morphology. Bivariate regressions were conducted between count and geographic attributes grouped by sample site. The geographic data that were considered amongst these regressions as well as their sources are listed in Table S2. Comparisons between watershed traits of the full basin, the 20km basin, and the 5km basin were compared. We considered the model assumptions, which involved examining histograms, spatial dependence, and qq-plots to ensure the normality of residuals.

**Microplastic Load Calculations**

To estimate the quantity of microplastics generated per person per day in the Mississippi River, several factors were considered. We calculated microplastic concentrations by averaging counts from various sampling sites, considering interpolated discharge data, and referencing census information. In our calculations, we assumed that the average counts represented true microplastics and that the concentration remained consistent across the water column within the size range of 0.07mm to 5mm. Discharge data was sourced from the USGS National Water Information System server in R based on sampling date and time. To estimate discharge rates at
specific sample locations, we employed linear interpolation based on catchment areas and discharge data from river gauges. The concentration of microplastics at each sample site was then multiplied by its corresponding discharge rate to determine the load. This load was subsequently divided by the population residing within the contributing basin. Finally, the median value of these calculations was utilized to provide an estimate of the quantity of microplastics generated per person over a specified time-period in the Mississippi River.

Chapter 3: Results

Physical Characterization
Suspected microplastics were present in every sample site along the Mississippi River. A total of 424 particles were extracted from 65 samples over 29 sample sites. The mean particle count was 7.11 ± 3.09 particles per liter with a range of 3 to 14 particles per liter. Most particle types, around 97%, were fibers (Figure 3). Roughly 37% of particles were clear, followed by white at 19%, and blue at 14% (Figure 4.). Table S4 shows the distribution of particles by color and morphology. Length was only recorded for each unique color-morphology combination in every sample. This resulted in measurement of 79% of the recovered particles (n=333). The median length of particles was 0.11 mm.

Chemical Characterization
Approximately 27% of particles in the study were analyzed via Raman spectroscopy. Of the analyzed particles, 7% were classified as natural, 20% as anthropogenic cellulose, 21% as anthropogenic unknown, 25% as plastic, and 27% were unidentified (Figure 4). In this subset, 67% of the analyzed particles had human made origins. Within the plastic category, around 80%
of the microplastics were fluoropolymers. The minority 20% of plastics were split evenly between polyethylene, polypropylene, and an “other” category consisting of plastic-based dyes.

**Land-Use Regressions**

As a preliminary step, bivariate models were constructed to analyze the relationship between counts and each variable individually. The correlation coefficient for each spatial variable is listed in Table S3. We found that the correlations between count and the other spatial factors were insignificant. Next, we created a correlation heat map of all variables to limit collinearity (Figure S1). The results of the correlation heat maps informed variable selection in multilinear regressions. There were no significant trends for any given spatial variable or spatial relationship between or within 5km, 20km, or full basin models. This phenomenon was articulated in an anecdotal forest plot of coefficients for spatial factors compared between basin sizes (Figure S2). Overall, no significant consistent results were identified amongst any of the different models including comparisons between size boundaries and within size boundaries amongst different models.

**Microplastic Load Estimate**

Based on the assumptions established in the methods, the estimated median daily load of microplastics (MP) was $2 \times 10^{12}$ MP/day in the Mississippi River (range $5.7 \times 10^{11}$ to $1.2 \times 10^{16}$ MP/day). Using American Community Survey 5-year Data from the US Census Bureau, it was estimated that around 86 million people live within the Mississippi River Basin (US Census Bureau, 2020). From this, a rate of 301,893 MP/person/day was derived. First, non-area normalized estimates were compared. In Figure 6, the logarithm of discharge was plotted against
the logarithm of load, revealing a slope of 1.07. Figure S3 and Figure S4 display the logarithm of discharge plotted against the logarithm of concentration, and the logarithm of population versus the logarithm of load, respectively. Area-normalized load was compared with population density and area-normalized discharge, as illustrated in Figure 7 and 8. In Figure 7, there was a relationship between area-normalized load and population density (p-value<0.01). There was also a relationship between a regression of area-normalized discharge and area-normalized load (p-value<0.01).

Chapter 4: Discussion

In this study, we conducted an exploratory investigation into the presence, characteristics, sources, and distribution of microplastics in the Mississippi River. Our research aligns with prior studies, which consistently highlight the prevalence of microfibers as the primary constituent in surface waters (Balla et al., 2022; Grbić et al., 2020; Kapp & Yeatman, 2018; Zhu et al., 2021). Furthermore, other research has found the dominance of microfibers amongst other morphologies in river systems (Koelmans et al., 2019). Some studies suggest that spherical particles may settle out faster, leaving fibers remaining in the surface region due a lower settling velocity (Dittmar et al., 2024; Goral et al., 2023).

We did not find relationships between microplastic concentration and spatial factors. In the literature, land-use analyses have resulted in relatively mixed results. Only vegetative cover has been consistently negatively correlated with microplastic concentration (Bian et al., 2022; Tanentzap et al., 2021). For instance, the influence of agriculture on microplastic is not well understood as studies have found insignificant or negative correlations (Lin et al., 2022, Talbot & Chang, 2022, Wang et al., 2022).
Raman spectroscopy analysis provided further insights into the composition of microplastics in the river. Within the subset of Raman-analyzed samples, most particles were human made and roughly one third were confirmed as plastic. Compared to an oceanic surface water study that found predominantly microfibers, the fraction of confirmed plastics were relatively higher (Suaria et al., 2020). Conversely, the fraction of confirmed plastics compared to a surface water river study was two times lower (Kapp & Yeatman, 2018).

Based on our methodological assumptions, we estimated the median microplastic load per day in the Mississippi River. In a review of microplastics research, Koelmans et al. plotted microplastic concentrations from various river studies. Our findings, ranging from 3.3 to 4.1 log(MP/m^3), fall within the upper quartile of their reported range for microplastic concentration (Koelmans et al., 2019). There was a significant relationship between population density and area-normalized load. This was consistent with current observations in the literature (Kapp & Yeatman, 2018; Kunz et al., 2023; Talbot & Chang, 2022; Yang et al., 2021). Microplastic load was also correlated with area-normalized discharge which is a function of runoff. This finding suggests that surface water microplastics may be a transport limited pollutant.

**Limitations**

Inadequate power was a major limitation of this study. This is the likeliest reason no significant findings appeared amongst land-use regressions. Additionally, the study was limited by the relatively small number of sample sites, with only 29 sites included in the analysis. This limited spatial coverage may have restricted the ability to detect significant relationships between microplastic presence and land-use variables, particularly at finer spatial scales. Furthermore, this study did not consider distance from shore.
**Strengths**

This study examines microplastics continuously from source to sea on a river greater than 3,200km, a methodology previously explored in only a few instances in microplastic literature (Kapp & Yeatman, 2018; Yang et al., 2021). Of these studies, the study region may be the longest prompting more research on this body of water and other long rivers. This study has demonstrated enhanced capabilities in defining relevant watershed boundaries for river ecosystems. By conforming buffers to delineated basins, this allows for a more comprehensive and accurate representation of the complex interactions between land use patterns and their impacts on the microplastics, thereby advancing an understanding of environmental dynamics in these ecosystems.

**Chapter 5: Conclusion**

The pervasive presence of microplastics throughout the Mississippi River emphasizes the urgent need for concerted efforts to address this environmental challenge. The complexity of microplastic dynamics remains apparent, as no significant relationships were identified between microplastic concentration and various spatial factors. However, area-normalized microplastic load was correlated with population density and area-normalized discharge. These findings highlight the intricate interplay of factors influencing microplastic distribution in river ecosystems. Given the complexity of microplastic dynamics, future studies would benefit from increased sampling power and a comprehensive assessment of microplastic distribution throughout the water column, enabling a more nuanced understanding of microplastic concentration in the Mississippi River. As the prevalence of microplastics continues to pose risks to aquatic ecosystems and human health, further research and monitoring efforts are imperative.
By enhancing our understanding of microplastic sources, transport pathways, and ecological impacts, we can develop targeted strategies to mitigate the adverse effects of microplastic pollution.
Tables

Table 1. Descriptive table of sample sites.

Descriptive information for each sample site. Includes the day sample was acquired in kayak journey, river mile at which the sample was collected, the replicates taken at each sample site, and the confirmation of microplastics by Raman spectroscopy. In the “Raman Confirmed Microplastic” column, “Y” represents yes, “N” represents no, and “NA” means that replicates were not analyzed by Raman. Particle counts are a replicate-averaged particle count per liter. Sampling began 5/25/2019 and ended 7/24/2019.

<table>
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<th>Particle Count</th>
<th>Raman Confirmed Microplastic</th>
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<td>2</td>
<td>7</td>
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<td>26</td>
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<td>2</td>
<td>8</td>
<td>N</td>
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<td>28</td>
<td>900</td>
<td>2</td>
<td>5.5</td>
<td>NA</td>
</tr>
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<td>17</td>
<td>31</td>
<td>1000</td>
<td>2</td>
<td>5.5</td>
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</tr>
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<td>33</td>
<td>1100</td>
<td>3</td>
<td>3</td>
<td>N</td>
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<td>8.5</td>
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<td>1300</td>
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<td>5.5</td>
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</tr>
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<td>42</td>
<td>1400</td>
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<td>12</td>
<td>Y</td>
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**Supplementary Tables**

**Table S1. Collapsed 2019 NLCD land-use categories.**

<table>
<thead>
<tr>
<th>Land Use Code</th>
<th>Definitions</th>
<th>Collapsed Category</th>
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<tr>
<td>11</td>
<td>Open water</td>
<td>Open Water</td>
</tr>
<tr>
<td>21</td>
<td>Developed open space</td>
<td>Urban</td>
</tr>
<tr>
<td>22</td>
<td>Developed low intensity</td>
<td></td>
</tr>
<tr>
<td>23</td>
<td>Developed medium intensity</td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>Developed high intensity</td>
<td></td>
</tr>
<tr>
<td>41</td>
<td>Deciduous forest</td>
<td>Forested</td>
</tr>
<tr>
<td>42</td>
<td>Evergreen forest</td>
<td></td>
</tr>
<tr>
<td>43</td>
<td>Mixed forest</td>
<td></td>
</tr>
<tr>
<td>52</td>
<td>Shrub or scrub</td>
<td></td>
</tr>
<tr>
<td>71</td>
<td>Grassland or herbaceous</td>
<td></td>
</tr>
<tr>
<td>90</td>
<td>Woody wetlands</td>
<td></td>
</tr>
<tr>
<td>95</td>
<td>Emergent herbaceous wetlands</td>
<td></td>
</tr>
<tr>
<td>81</td>
<td>Pasture or hay</td>
<td>Agriculture</td>
</tr>
<tr>
<td>82</td>
<td>Cultivated crops</td>
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**Table S2. Geographic data dictionary and sources.**

<table>
<thead>
<tr>
<th>Column Name</th>
<th>Unit</th>
<th>Explanation</th>
<th>Source</th>
<th>Calculation</th>
<th>Citation</th>
</tr>
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<tbody>
<tr>
<td>sample_site</td>
<td>1 through 29</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rivermile</td>
<td>River distance tracked by GPS.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-------------------</td>
<td>-------------------------------</td>
<td>----------</td>
<td>----------</td>
<td>----------</td>
<td>----------</td>
</tr>
<tr>
<td>num_replicates</td>
<td>Replicates in each sample</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>count</td>
<td>Averaged microplastic count amongst replicates.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CFS</td>
<td>Cubic feet per second of river.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>CFS monitored at USGS gauge nearest to sample point</td>
<td>USGS NWIS server</td>
<td>USGS, 2024</td>
<td></td>
<td></td>
</tr>
<tr>
<td>rainfall</td>
<td>Greater than 1 cm of precipitation within 24 hours of sampling at SS location.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Climate Data Online</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>NOAA, 2023</td>
<td></td>
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<td>Feature</td>
<td>Type</td>
<td>Description</td>
<td>Source</td>
<td>2024</td>
<td></td>
</tr>
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<td>-------------------------------</td>
<td>--------</td>
<td>-----------------------------------------------------------------------------</td>
<td>---------------------------------------------</td>
<td>---------------------------</td>
<td></td>
</tr>
<tr>
<td>dams_above</td>
<td>continuous</td>
<td>Quantity of dams above between last point to next.</td>
<td>Army Corps of Engineers</td>
<td></td>
<td></td>
</tr>
<tr>
<td>dams_distance</td>
<td>km</td>
<td>River distance to nearest dam.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mjriver_distance</td>
<td>km</td>
<td>Distance to nearest major river.</td>
<td>major river is defined as river with greater than 2000 CFS per year discharge</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WWTP_distance</td>
<td>km</td>
<td>Distance to nearest WWTP.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5km_WWTP_above</td>
<td>binary</td>
<td>Is there a WWTP within 5km?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20km_WWTP_above</td>
<td>binary</td>
<td>Is there a WWTP within 20km?</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>WWTP IDW</td>
<td>Million gallons per day</td>
<td>WWTP IDW calculated from 2019 discharge rates and river distance.</td>
<td>EPA ECHO Database</td>
<td>p = 1</td>
<td>US EPA, 2019</td>
</tr>
<tr>
<td>------------</td>
<td>-------------------------</td>
<td>-----------------------------------------------------------------</td>
<td>-------------------</td>
<td>------</td>
<td>---------------</td>
</tr>
<tr>
<td>WWTP IDW_1</td>
<td>million</td>
<td></td>
<td></td>
<td></td>
<td>p = 1</td>
</tr>
<tr>
<td>WWTP IDW_2</td>
<td>million</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WWTP IDW_3</td>
<td>million</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>population</td>
<td></td>
<td>Population of each sample site's basin</td>
<td>2019 5 Year Estimate</td>
<td></td>
<td>US Census Bureau, 2020</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Population by Census Tract</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Euclidean geometry of watershed.</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table S3. Correlation coefficient of all variables against count.

<table>
<thead>
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<th>Parameter</th>
<th>Correlation Coefficient</th>
<th>p-value</th>
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<tr>
<td>dams-above</td>
<td>-0.01888</td>
<td>0.96</td>
</tr>
<tr>
<td>rainfall</td>
<td>-1.005</td>
<td>0.392</td>
</tr>
<tr>
<td>rivermile</td>
<td>0.0010199</td>
<td>0.237</td>
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<tr>
<td>mjriver_above</td>
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<td>0.813</td>
</tr>
<tr>
<td>mjriver_distance</td>
<td>-0.02157</td>
<td>0.41812</td>
</tr>
<tr>
<td>mjriver_cfs</td>
<td>1.99E-05</td>
<td>*</td>
</tr>
<tr>
<td>WWTP_above</td>
<td>0.1016</td>
<td>0.939</td>
</tr>
<tr>
<td>WWTP_distance</td>
<td>0.01967</td>
<td>0.321</td>
</tr>
<tr>
<td>WWTP_quant</td>
<td>-0.08631</td>
<td>0.678</td>
</tr>
<tr>
<td>WWTP_IDW_3</td>
<td>-0.014074</td>
<td>0.0821</td>
</tr>
<tr>
<td>WWTP_above_20km</td>
<td>0.6284</td>
<td>0.595</td>
</tr>
<tr>
<td>WWTP_above_5km</td>
<td>0.3806</td>
<td>0.794</td>
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</tbody>
</table>

Full

| agriculture                   | 0.005017                 | 0.884   |
| forested                      | -0.008408                | 0.83034 |
| urban                         | 0.2033                   | 0.652   |
| open water                    | -0.01171                 | 0.927   |

5km

| agriculture                   | -0.01517                 | 0.601   |
| forested                      | 0.002511                 | 0.927   |
| urban                         | 0.01503                  | 0.692   |
open water 0.01526 0.769

20km
agriculture -0.01645 0.565
forested -0.001411 0.956
urban 0.01218 0.83
open water 0.05819 0.45

Population
full 2.42E-08 0.148
20km 1.40E-06 0.743
5km 6.27E-06 0.656

Size
full 6.01E-07 0.21
20km 0.001105 0.572742
5km -0.001697 0.675

There was a significant association between particle count and the flow of the nearest major river to a given sample site (p-value = 0.02) (Table X). Despite the statistical significance, the correlation coefficient was close to zero, rendering this finding not meaningful. All correlations between count and the other spatial factors were insignificant.

Table S4. Distribution of color-morphology combinations.

<table>
<thead>
<tr>
<th></th>
<th>fiber</th>
<th>fiber bundle</th>
<th>film</th>
<th>foam</th>
<th>fragment</th>
<th>Total</th>
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<td>0</td>
<td>0</td>
<td>0</td>
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<td>0</td>
<td>0</td>
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<td>58</td>
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<td>1</td>
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<td>0</td>
<td>0</td>
<td>0</td>
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<td>0</td>
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<td>1</td>
<td>0</td>
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<td>yellow</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>411</td>
<td>2</td>
<td>4</td>
<td>2</td>
<td>5</td>
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</table>
Figures

Figure 1. Mississippi River study region.

The entire study region is overviewed by the leftmost panel, which shows three segments of the river (A, B and C). Panels A, B, and C show granular details of sampling locations (sample sites 1–29), wastewater treatment plants (WWTP) and lock and dam sites. In panel C, the diversion of
the Atchafalaya River (West) from the Mississippi River (East) is depicted at the delta. Sample site 28 is located on the Atchafalaya River and sample site 29 is located on the Louisiana coastline near the Atchafalaya River mouth.

Figure 2. Defining a “5km” basin at sample site 2.

Panel A depicts the delineated basin for sample site 2 divided by HUC 12-digit subwatersheds. The NLCD layer represents land use within the basins. Panel B highlights a 5km buffer surrounding sample site 2. The subwatersheds that intersect the buffer are highlighted in red. Panel C articulates the final 5km basin where the two intersecting subwatersheds are clipped from the polygon in Panel A, with borders dissolved. This process was applied to all sample sites with 5km and 20km buffers.
Figure 3. Distribution of microplastic morphology.

Microplastic morphology amongst the entire sample is depicted in the bar above with fibers dominating type. The remaining morphologies (3% of the sample) are described in the bar below.

Figure 4. Distribution of microplastic color.

Microplastic color was ranked from highest to lowest (n=424).
Microplastic color was ranked from highest to lowest (n=424).

**Figure 5. Chemical composition of sample subset.**

Categorization of chemical identification of subsample (n=117) via Raman spectroscopy. Most of the subsample was human made. Plastics were the largest category of human-made particles (n=30).
Figure 6. Scatterplot of log-log of discharge (m³/s) and microplastic load (microplastics/m³/s).

The log of discharge (x-axis) is regressed with the log of load (y-axis). Discharge is represented in m³/s and load is represented by microplastics per m³/s. Two clusters of data are noted due to the confluence of the Missouri River.

Figure 7. Scatterplot of area-normalized load (microplastics/ km²/day) and population density (population/km²).
This figure shows a regression of population density with microplastic load as a function of basin area. This figure excludes one outlier, sample site 1, due to a small population.

**Figure 8.** Scatterplot of area-normalized discharge (m$^3$/km$^2$/day) and area-normalized load (microplastic/km$^2$/day).

The regression analysis of area-normalized discharge and area-normalized load demonstrates a significant relationship with a p-value equal to $1.03 \cdot 10^6$. 

\[ y = 28616x^{0.7633} \]

\[ R^2 = 0.6315 \]
Supplementary Figures

Figure S1. Correlation heat map.

We focused on comparing correlations grouped within the 5km, 20km, and full basin attributes. In the full basin attributes, collinearity was observed, particularly among urban areas and other human-made point data such as wastewater treatment plants (WWTPs) and dams. To mitigate this, population was used as a proxy for urban areas or vice versa in any full basin models. In the 20 km basin models, moderate correlations were found between basins area, population, urban areas, and anthropogenic structures. For the 5 km basin models, strong correlations were noted between open water and basin size, potentially explained by geographical factors.

Figure S2. Multi-linear regression output.

Model 1 Comparison:
$Y(\text{microplastic per liter}) = B_0 + B_1(\% \text{ Agriculture}) + B_2(\% \text{ Urban}) + B_3(\% \text{ Forest}) + B_4(\% \text{Open Water})$

Model 2 Comparison:

$Y(\text{microplastic per liter}) = B_0 + B_1(\text{population}) + B_2(\text{WWTP above}) + B_3(\% \text{ Forest})$

Model 3 Comparison:

$Y(\text{microplastic per liter}) = B_0 + B_1(\text{Rainfall}) + B_2(\text{Major River Above}) + B_3(\% \text{ Urban})$
These models represent three combinations of variables compared between basin sizes. In the Model 1 comparison, the “urban” variable had both positive and negative coefficients compared between the 5km, 20km, or full basin. In Model 3, the “urban” variable also had mixed insignificant results that are very different from Model 1.

**Figure S3.** Scatterplot of log-log discharge (m³/s) and microplastic concentration (microplastics/m³/s).
The log of discharge (x-axis) is regressed with the log of concentration (y-axis). Discharge is represented in m$^3$/s and load is represented by microplastics per m$^3$.

**Figure S4.** Scatterplot of log of population and log of load.
References


https://doi.org/10.1016/j.redox.2020.101620


https://doi.org/10.1016/j.envpol.2020.116260

https://doi.org/10.1016/j.watres.2019.02.054

https://doi.org/10.1016/j.envpol.2023.121096


NOAA. (2019). *Climate Data Online (CDO)—The National Climatic Data Center’s (NCDC)*. 

*Climate Data Online (CDO) provides free access to NCDC’s archive of historical*
weather and climate data in addition to station history information. | National Climatic Data Center (NCDC). https://www.ncdc.noaa.gov/cdo-web/


