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# Examining How Tributaries Contribute to Anthropogenic Microfiber and Microplastic Pollution in an Urban Watershed in Nashville, TN, USA

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**ABSTRACT:** Recent research has shown that urban rivers often have large amounts of anthropogenic microfiber pollution (i.e. small thread-like pieces of litter that are most often microplastics and are <5mm in size). However, there is often a limited understanding of how tributaries and streams that flow into urban rivers contribute to these amounts. This study examined how the presence and abundance of anthropogenic microfiber pollution varied in six tributaries of the Cumberland River in Nashville, TN, USA which is a growing city with more than 2 million residents in the metropolitan area. To examine how anthropogenic microfiber pollution levels varied, surface water samples were collected over the course of two months in Spring 2022 from six tributaries in the Richland Creek Watershed ( $n=96$  samples total). Over the course of the study, anthropogenic microfibers were found in all tributaries and at all time points and at abundances that are similar to many other smaller tributaries and creeks that have been previously studied (mean of 96 samples = 17.4 microfibers/L or 17,400/m<sup>3</sup>). Interestingly, there were no significant differences between the individual tributaries or across the four sampling time periods in anthropogenic microfiber pollution abundance. However, the consistent levels of anthropogenic microfibers found supports recent research which suggests that this type of pollution represents an important threat in urban aquatic ecosystems.

**KEYWORDS:** Freshwater, microplastic, microfibers, pollution, urban river

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## Introduction

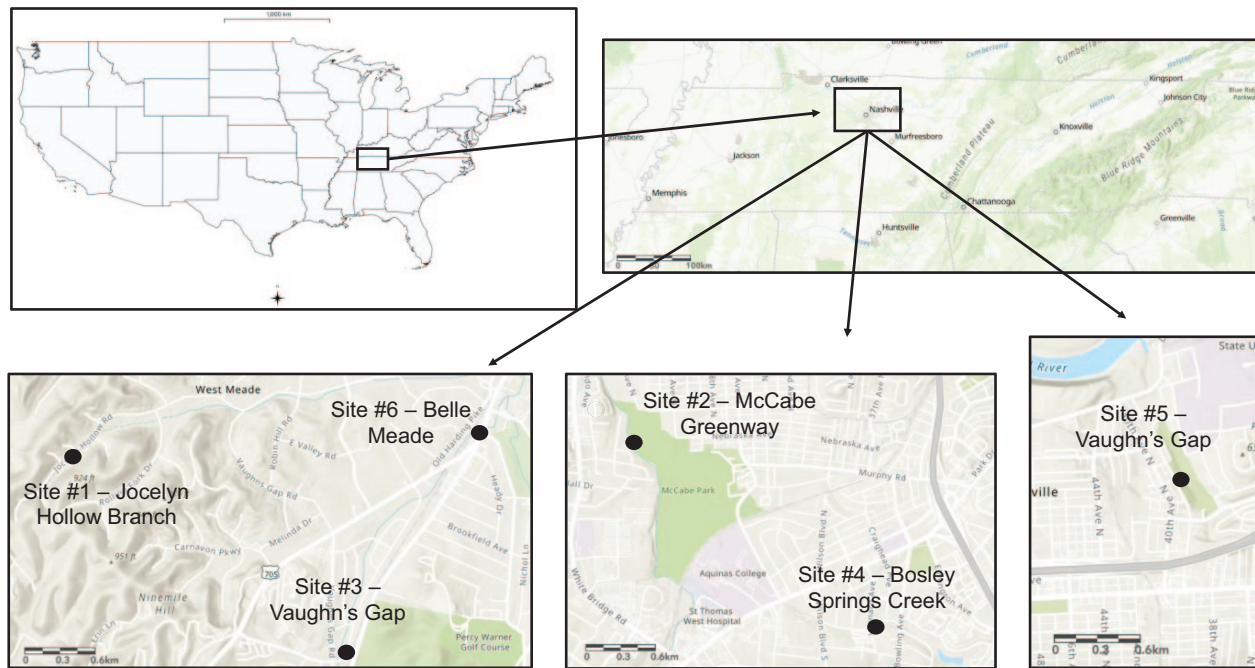
Urban waterways are facing increasing threats from plastic pollution and other anthropogenic litter (Hoellein & Rochman, 2021; Miller et al., 2017; Rochman, 2018; Wang et al., 2022; Xu et al., 2021). Of growing concern are anthropogenic microfibers—which include thread-like pieces of synthetic material (most commonly microplastics) and some human-supplied organic materials (e.g. cotton fibers from clothing) that are less than 5 mm in size (Athey & Erdle, 2022; Liu et al., 2019; Miller et al., 2024; Said & Heard, 2020; ). Anthropogenic microfibers are of particular concern because microplastics and other synthetic fibers can easily be consumed by organisms that live in freshwater ecosystems (Athey & Erdle 2022; Mateos-Cárdenas et al., 2021; Rochman, 2018) and because they are the most abundant form of microparticle pollution (<5 mm in size) in aquatic ecosystems (Carr, 2017; Miller et al., 2017). In addition, there is a growing body of evidence that anthropogenic microfibers and particularly microplastics may be able to move up the food chain and synthetic microfiber contamination could possibly lead to increases in human exposure over time (Athey & Erdle, 2022; Santonicola et al., 2021). For example, microplastic and anthropogenic microfiber consumption has been conclusively shown to impact some common organismal groups including plankton, mussels, fish, and turtles (Arat, 2024). Furthermore, microplastics can have high levels of toxins (Galloway & Lewis, 2016; Mishra et al., 2022) and potentially harbor pathogenic bacteria (Mishra et al., 2021; Pedrotti et al., 2022).

Though anthropogenic microfiber pollution (most often microplastics) has been documented as a significant concern for urban rivers (Athey & Erdle 2022; Miller et al., 2017, 2024; Said & Heard, 2020), many geographic areas still lack a basic understanding of how pollution levels vary over space and time. In addition, there has been little work done examining how microplastic and anthropogenic microfiber waste varies in streams and tributaries of urban rivers. Furthermore, when there have been baseline studies conducted in urban streams and tributaries, there has been significant variation in the abundance of anthropogenic microfibers and microplastic pollution that has been found (e.g. from 0.0001 to 1,000 particles/L—Athey & Erdle, 2022). As a result, there is often lack an understanding of how pollution levels within local streams and tributaries may contribute to anthropogenic microfiber and microplastic pollution in urban rivers over time (Athey & Erdle, 2022; Doucet et al., 2021; Frank et al., 2021).

The Richland Creek Watershed comprises more than 17,000 acres in the Nashville, Tennessee, USA area and is the home of Richland Creek, which travels for approximately 28 miles through the western part of town before flowing into the Cumberland River—the main source of drinking water for the city of Nashville. Within the Richland Creek Watershed there are six tributaries that are accessible to the public that could be potentially important sources of anthropogenic microfiber pollution (Figure 1). These six tributaries also share similar soil types, geology, and elevational profiles (except site #1, which is ~150 m higher in elevation than other sites) and thus



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**Figure 1.** Map of six study tributaries in the Richland Creek Watershed in Nashville, TN, USA sampled in February and March of 2022.

are ideal for comparisons (Metropolitan Government of Nashville and Davidson County, 2016). Understanding and tracking the role of tributaries in driving pollution in the Richland Creek watershed is critical as this area is home to more than fifty thousand local residents (Metropolitan Government of Nashville and Davidson County, 2016). In addition, it is also important because Richland Creek flows into the Cumberland River, which serves as the source of drinking water for the city of Nashville, TN and has been documented to have high levels of microfiber pollution immediately downstream from Richland Creek (Said & Heard, 2020).

This study was the first survey of anthropogenic microfiber pollution in any tributaries found in the Richland Creek Watershed in Nashville, TN. It was hypothesized that microfiber pollution would be present in all six tributaries and that these waterways could be important sources of pollution that could eventually flow into the Cumberland River.

## Methods

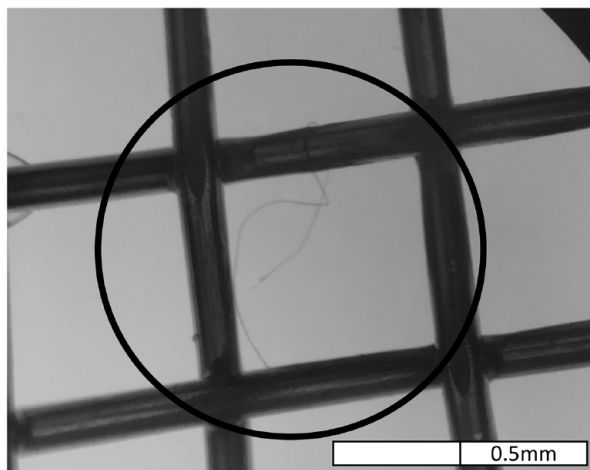
To assess anthropogenic microfiber abundance, grab samples were collected from the top 0.25 m of water from the six main publicly accessible tributaries of Richland Creek in Nashville, TN bi-weekly (Figure 1) during February and March 2022. At each of the six sites, four-250 mL water samples were collected at four sampling time points ( $n=96$  total). All water samples were collected in amber glass bottles that were sterilized to kill any bacteria which could consume microfibers and then rinsed with water from a reverse osmosis filter with a pore size of  $0.0001\ \mu\text{m}$ , which has no microfibers less than  $0.0001\ \mu\text{m}$  in size (Ziajahromi et al., 2017). For each water samples, the total number of anthropogenic microfibers (including microplastics and other human-supplied organic fibers like cotton threads)

was counted by pouring our 250 mL water samples through a steel sieve set with three different sized sieves (5.00, 1.02, and 0.38 mm) and conducting a grid search of each sieve. For the grid searches, both a dissecting scope and a Nightsea Microscope Adapter with royal blue excitation filter (Nightsea SFA, Lexington, MA, USA) were used identify microplastics and organic human-supplied microfibers, at  $45\times$  magnification (Payton, 2017; Said & Heard, 2020; Figure 2). The number of anthropogenic microfibers/L was determined by multiplying the number of microfibers found on the 1.02 and 0.38 mm mesh sieves multiplied by four. Following the processing of water samples, all of the metal sieves were cleaned with RO water to remove additional fibers.

## Limiting contamination of water samples

Two brief studies were conducted in the laboratory space where the samples were processed just before the completion of the field work to determine if there was any possible contamination risk. For the first study, 20.1 L sample bottles were rinsed with RO water and then filled with 1 L of RO water (which should not have fibers  $<0.0001\ \mu\text{m}$  in size present). These “blanks” were then poured through all three sieve sizes (discussed above) and then a grid search was conducted of each sieve. In this first study, there were 2 total pieces of microfibers in the 20 samples processed for a contamination rate of 0.1 microfibers/L. To account for this, the number of microfibers found in field samples was reduced by 0.1 microfibers/L (except in water samples that had zero).

The second study examined whether airborne contamination may have occurred in the lab space where field samples were processed. To do this, three sieves were placed on the open



**Figure 2.** Anthropogenic microfibers were identified using Nikon dissecting microscopes in combination with a Nightsea Adapter (with royal blue excitation filter/440–460nm). In this photo, there is an anthropogenic microfiber (likely a microplastic fiber) found in a sample from Richland Creek in Nashville, TN, USA.

lab benches where samples were processed for 15 min (the approximate time it took to conduct a grid search for a sample) and then a grid search was conducted to look for airborne microfiber particles. Following the grid search, sieves were rinsed with RO water (using the exact same methods as described above) and then this process was repeated four more times for a total of five airborne samples processed). This experiment was then repeated three more times on separate days to account for variation in the airborne contamination rate. In total across the five time points, 1 piece of microfiber was found on the 15 sieve examinations (equivalent to 0.27 microfibers/L). To account for this amount of potential contamination, microfiber numbers were reduced by 0.27 microfibers/L (except for samples that had zero found).

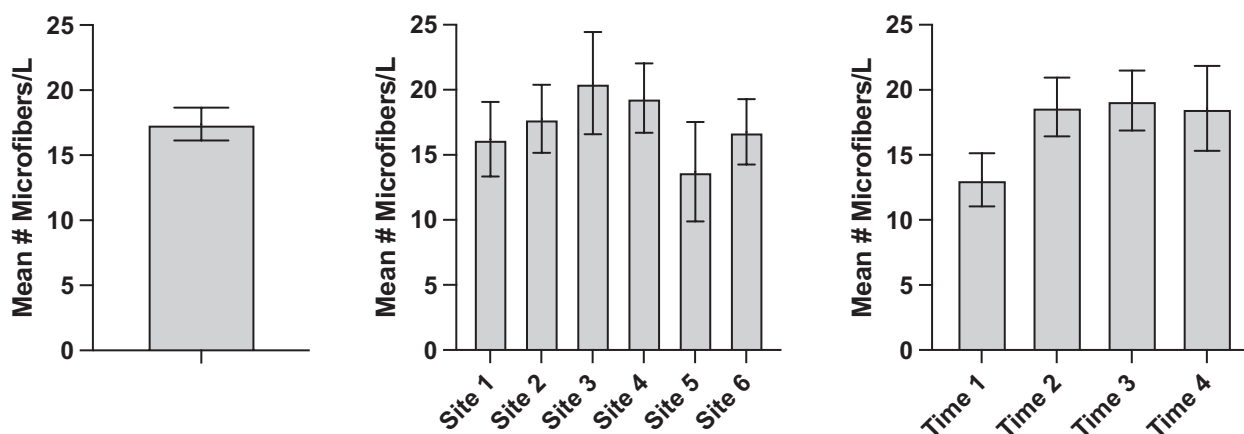
### Statistical analyses

This study looked for differences in the abundance of anthropogenic microfibers/L at the six tributaries using a two-way ANOVA test with site, time, and their interactions as potential effects. A Box-Cox transformation was used and a constant of 5 was added to initial sample numbers to transform data to meet the assumptions of normality. Normality was assessed using a Kolmogorov-Smirnov Test of Normality because the sample size was greater than 50. Microfiber numbers were also corrected to account for the 0.27 microfibers/L contamination values found in the two control experiments. All statistical tests and graphs were conducted using Prism version 10.00 for Mac, Graphpad Software, La Jolla California USA, www.graphpad.com. Maps were produced using ArcGIS and topography basemap (<https://www.arcgis.com/home/item.html?id=7dc6cea0b1764a1f9af2e679f642f0f5>).

### Results and Discussion

There was an average of 17.4/L anthropogenic microfibers per sample (after corrections) in the tributaries, which is equivalent to 17,4000 anthropogenic microfibers/m<sup>3</sup> (Figure 3a). This level of anthropogenic microfiber pollution levels was significantly similar to a review paper by Athey and Erdle (2022) that showed potential ranges in samples from 0.0001 to 1,000 particles/L. In addition, other studies that were not included in that review paper have also shown similar levels of anthropogenic microfiber abundance in freshwater samples (e.g. Balestra et al., 2024; Treilles et al., 2022 which ranged from 2 to 654 particles/L).

Anthropogenic microfibers were also found in every tributary and in every sampling period. However, there were no significant differences between tributaries in anthropogenic microfiber abundance ( $F_{5,72}=0.93$ ;  $p=.46$ ; Figure 3b). There



**Figure 3.** (a) Mean abundance of anthropogenic microfibers for all sites and all sampling time points. Error bars represent standard error. (b) There were anthropogenic microfibers in all six tributaries, but there were no significant differences between tributaries in mean abundance. Error bars represent standard error. (c) There were anthropogenic microfibers at all timepoints and in all tributaries, but there were no significant differences in mean abundance over time in microfiber abundance. Error bars represent standard error. \*Though actual sampling numbers are depicted, all statistical analyses were conducted using Box-Cox transformed data to meet the assumptions of normality.



were also no significant differences in anthropogenic microfiber abundance due to sampling time ( $F_{3,72}=1.52$ ;  $p=.22$ ; Figure 3c) and there were no interactive effects between site and sampling time ( $F_{15,72}=1.20$ ;  $p=.29$ ). This finding was somewhat surprising given that the study sites vary from residential areas, to parks, to near commercial centers. However, this shows that despite the heterogeneity of the study sites, pollution from anthropogenic microfibers is common and possibly stable across this urban watershed. Prior research supports these data as well and suggests that anthropogenic microfibers represent a dominant form of pollution in many different types of urban freshwater ecosystems (Athey & Erdle, 2022; Liu et al., 2019).

The presence of anthropogenic microfibers, which includes microplastics and other synthetic materials in small creeks and streams in an urban watershed raises important questions about potential sources of pollution and impacts to wildlife and public health. The numbers of microfibers found in these small creeks and streams is similar to what is often found in larger-scale urban rivers (Barrows et al., 2018; Miller et al., 2017; Said & Heard, 2020) and indicates that tributaries represent an important source of microplastic and microfiber pollution and that it is not all the result of wastewater treatment plants in large urban rivers.

#### *Limitations of the study*

One of the key limitations for this study is that it is snapshot from a single watershed and from a single year. Increased temporal and spatial coverage can help to create a more accurate baseline for anthropogenic microfiber pollution since levels can significantly vary both seasonally and spatially (Athey & Erdle, 2022; Liu et al., 2022; Said & Heard, 2020; Treilles et al., 2022). In addition, it is important to note that anthropogenic microfiber abundance can be impacted by the methods that were used for assessment and counting. For example, in some studies researchers focus solely on synthetic fibers and often miss organic fibers, which can decrease their count (Athey & Erdle, 2021).

#### **Conclusion**

This research represents the first study that has examined how anthropogenic microfiber pollution levels varied across space and time in an urban watershed in the southeastern United States. The collection of this baseline data is an important first step for understanding how urban streams and tributaries contribute to the pollution of urban rivers over time. In addition, it allows for comparison to other geographic areas that are now being studied using similar methods.

The results of this study indicate that anthropogenic microfibers and microplastics do ultimately represent an important threat to urban streams and tributaries and can contribute significant levels of pollution to urban rivers over time. In addition, these findings indicate that anthropogenic microfiber

pollution is likely to be a consistent threat over time and regardless of the land-use type in adjacent ecosystems. This finding is critical because it indicates the widespread nature of the threat posed by anthropogenic microfiber pollution and because it suggests that there is still a limited understanding of the causal mechanisms that drive pollution abundance.

Moving forward, it will be critical for future studies to investigate how these anthropogenic microfibers and microplastics are impacting urban creeks and streams and the organisms that persist within them. There has been research which has shown to date that many of the organisms that persist in these ecosystems have the potential to be significantly impacted over time through consumption or exposure. And there is also evidence that these have the potential to move into the food chain and potentially impact humans over time.

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#### **Author Contributions**

A.A., C.S., and M.H. contributed to the development of this project, data collection, data analysis, and wrote the manuscript. All authors have read and approved this submission.

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#### **Ethical Approval**

Not Applicable

#### **Consent to Participate**

Not Applicable

#### **Data Accessibility Statement**

Data used in this paper will be submitted to Dryad upon acceptance of the manuscript.

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