Team MARINE: Microplastic Analysis and Removal in Industrial and Natural Ecosystems

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Thesis submitted in partial fulfillment of the requirements of the Gemstone Honors Program,

University of Maryland, 2024

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Abstract

Numerous methods have been developed for microplastics isolation and quantification in various environmental media, many of which require elaborate or expensive analytical equipment and decontaminated lab space. This study seeks to create a reproducible and economical method for isolating microplastics in surface water that rely on Nile Red staining. We use a Nile Red pre-staining step prior to sample digestion, density separation, and filtration to mitigate downstream in-lab contamination before quantification of microplastics via fluorescent microscopy. To test the method, we collected replicate surface water samples from several reaches of an urban stream in the Chesapeake Bay Watershed, USA seasonally for one year. The proposed sampling and quantification method found some success in these surface water samples with specific microplastics (≥20 µm) able to be enumerated.

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Introduction

Single-use plastics and plastic derivatives have become pervasive throughout the modern world, with plastic items serving as the de facto standard for cheap and quick-to-manufacture items with a limited purpose. Microplastics are an unavoidable byproduct of the widespread use of plastics and as a result, have been detected in nearly every location searched (Cole et al., 2011). Microplastics are defined as any piece of plastic that is 5mm or less in length, and nanoplastics form the subcategory of microplastics with sizes ranging between 1 nm and 1 µm (Materić et al., 2022). These plastics come from a variety of sources, which are generally broken down into two main categories: primary microplastics and secondary microplastics. Primary microplastics are those plastics that, due to their intended use, are manufactured as a microplastic (size less than 5mm) (Hwang et al., 2020). Primary microplastics include items such as microbeads found in cosmetic products and plastic fibers used in synthetic fabrics. Secondary microplastics are those plastics that begin their life cycle as macroplastics but, through their intended use or natural processes, break down and shed microplastics into the surrounding environment (Hwang et al., 2020). Frequent sources of microplastics include car tires, sneakers, and single-use plastic packaging. Certain items, such as car tires and sneakers, shed plastics as part of the normal wear and tear of the item. Car tires lose their tread as the tire makes contact with the road surface, producing microplastics, similar to sneakers as people walk from place to place. Car tires are made partially of synthetic rubbers, which are artificial elastomers made using a type of plastic that is formed from petroleum by-products (Kole et al., 2017). Other items, such as the vast majority of single-use plastic packaging, generate microplastics after they are discarded. These plastics make their way into the natural environment and gradually break down and produce microplastics as the plastic is subjected to winds, UV light, and the abrasion

that comes from being picked up in a river or stream and carried downstream (see Figure 1) (Song et al., 2017).

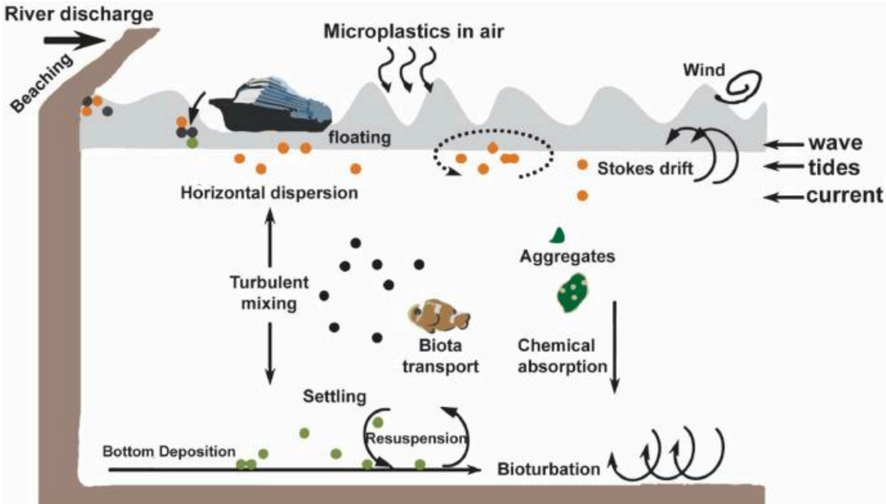


Figure 1: Diagram of Microplastics Transport Pathways in the Ocean (Ashrafy et al., 2023)

Microplastics can be broken into several size and shape categories, which ultimately affect where these plastics end up and how they impact the environment. The most common shapes for microplastics in water and sediment samples are fibers, fragments, beads, films, and foam (Kooi and Koelmans, 2019). Shape is an important factor in how microplastics interact with the environment. One study found that shape plays an important role in bioadhesion, adsorption, and biofouling; fragments adhered to plants greater than films, while films had the greatest adsorption capacity and development of biofilm (Rozman et al., 2023). Biofouling is the "accumulation of organisms on submerged surfaces and affects the hydrophobicity and buoyancy of plastic" (Kooi et al., 2017). As microplastics continue to break down once they enter the environment, their shapes and sizes are constantly changing. In freshwater, the density of the plastics plays an important role in transport and where they are concentrated; more dense microplastics tend to sink while less dense microplastics often float on the surface of the water

(Shamskhany et al., 2021). Density and size influence the rate of sedimentation of plastics, degradation, and biofouling (Kye et al., 2023). In seawater, a large fraction of microplastics are fragments and the collection of biofilm on these microplastics with larger surface areas may affect the sinking rate and thus distribution of the plastics in the water system (Kye et al., 2023). Transportation through marine environments is highly dependent on particle size, as they accumulate in different marine compartments based on hydrodynamic parameters (Shamskhany et al., 2021). In addition, depending on their size, microplastics interact differently with organisms (Kye et al., 2023). For example, larger microfibers led to a greater immune response in salmonid and a greater lethality of infectious hematopoietic necrosis virus than smaller microplastics (Seeley et al., 2023). Microplastics smaller than 20 µm can pass through cell membranes and those smaller than 1.5 µm can damage various types of cells, such as red blood cells, and lead to inflammation (Kye et al., 2023; Hwang et al., 2020). These microplastics pose a great risk to organismal health because of their ability to cross the cell membrane.

Once in the natural environment, microplastics can have a host of negative impacts on the surrounding ecosystem. While the impacts of microplastics are not yet fully understood, one concerning trait of microplastics is their ability to interact with harmful toxins in the water column and facilitate the processes of bioaccumulation and biomagnification (Peng, 2017; Turner and Holmes, 2015). Due to certain chemical properties of microplastics, chemicals and other contaminants can adhere to the surface of microplastics, allowing these chemicals to reach a higher concentration on the plastic than otherwise found in the ambient environment (Rochman et al., 2013). When the microplastic is consumed by an organism, these higher concentrations of contaminants are transferred into the organism. If this organism consumes high volumes of plastics, the contaminants found in the organism can reach levels far beyond what would be

normally found in the absence of plastics (Ribeiro et al., 2019; Ding et al., 2022). Notably, these contaminants can be transferred between trophic levels, potentially increasing risk of bioaccumulation (Carbery et al., 2018). The amount that microplastics actually adsorb is highly variable, as it is based on environmental factors ranging from salinity to hydrophobicity, making the risks of increased toxicity and bioaccumulation different for each environmental system (Rafa et al., 2024).

In addition to the effects of bioaccumulation, some studies have attributed the consumption of microplastics to a variety of negative health effects on the organisms that consume them. They may invade animals' tissues and organs, causing diminished feeding, growth inhibition, reduced mobility, and other conditions (Wang, 2019). Microplastic consumption has also been shown to impact the antioxidant abilities of fish, leading to neuromuscular damage (Elizalde-Velázquez, 2021). Animals ingesting microplastics may also be ingesting toxins that accumulate on the microplastics. For example, chemicals in microplastic fibers can act as endocrine disruptors, which skew fish and oyster sex ratios (Kim et al., 2015). In oysters, polyethylene terephthalate (PET) plastic may initially reduce growth for up to three months but do not seem to significantly affect overall growth (Sorini et al., 2021). However, the ratio of males to females was much lower than in control populations, being 54%-44% instead of 82%-18%, respectively, which could affect conclusions about oyster size (Sorini et al., 2021).

The effect of microplastics on human health is a particularly novel research focus, but the results of preliminary studies are an indication of the importance of continuing to expand this area of research. Microplastics have recently been found in multiple tissues in the human body, including human livers, kidneys, placentas (*From Pollution to Solution*, 2021). Plastics themselves may be harmful, and this is continuing to be understood, but microplastics are

particularly threatening because of the other chemicals that they contain or absorb. The International Union for the Conservation of Nature found that chemicals found in plastic products are carcinogenic and may cause developmental, neurological, reproductive, and immune disorders (*Issues Brief: Marine Plastic Pollution*, 2021). These new findings undermine the urgency with which new research about the presence of microplastics and their impact on our communities must be initiated.

As microplastic research moves to the forefront of environmental toxicology, it is important to make collection and processing methods consistent across studies. Currently, nets, pumps, sieves, bottles, and buckets all provide a potential source for variation leading to different counts of microplastics across studies (Prata et al., 2019). While the type of sample collected (surface water or sediment) plays a large part in the collection methodology, there are still factors that can be standardized to avoid counting biases in studies. Once samples are collected, researchers are tasked with the problem of turning a volume of water or sediment into a slide that can be quantified. Acidic, alkaline, and enzymatic solutions are used to digest samples and each affects the microplastics in the sample, such as through melting, yellowing, or degradation (Prata et al., 2019). Additionally, depending on the purity of the solution used and the lab environment, microplastics can be added to samples during processing.

Developing a consistent methodology based on previous research is crucial to make informed decisions about plastic regulation. Once a standardized methodology is in place, researchers can correlate population density and land use to microplastic concentrations (Yonkos et al., 2014). Additionally, variation exists between the size of particles in different bodies of water. Bikker et al. (2020) found that the mean particle concentration of the Chesapeake Bay was 0.160 particles per cubic meter. The size range for this study was 0.007 particles per cubic meter

and 1.245 particles per cubic meter. The Chesapeake watershed has also been analyzed for microplastics, namely in four tributaries of the bay which were found to have microplastic levels between 5 to 200 g/km^2 (Yonkos et al., 2014). Davey et al.'s (2023) microplastic research within the Anacostia watershed identified sites along Nash Run and found levels of 24-127 microplastic particles per liter (counted using a dissecting microscope). The presence of microplastics in local waterways are relevant issues for local agencies such as the Anacostia Riverkeeper who contribute to citizen science to define the nature of plastic pollution in the region (O'Donnell, 2019). With consistent processing methods, these concentrations could be compared more confidently.

One of the most pressing issues in the realm of microplastics research is the handling of contamination and error (Bogdanowicz et al., 2021). The three major error types we will discuss are false-positives, false-negatives, and exclusion bias. We will first examine a typical microplastic processing protocol intended to prepare slides for fluorescence microscopy. The major steps begin with sample digestion to remove organic matter from the sample which could clog future filtration steps. As discussed previously, this step can either be too weak, failing to sufficiently remove organic material, or too strong, potentially altering the integrity of the microplastic particle in a melting-like process (Prata et al., 2019). This step is followed by filtration through a glass-fiber filter, with other steps added to adjust to specific sample properties. For example, density separation may be added to samples with excesses of sediment present (Nava & Leoni, 2021). Next, a fluorescent stain is applied to the samples which allows for the dye to adhere to plastics and fluorescence under specific wavelengths (Sturm et al., 2023). Finally, the slide is examined using a fluorescence microscope and manual counting.

The utility of fluorescence microscopy is that a fluorescent stain can be added to a liquid sample which sorbs primarily to plastic particles within solution. In our case, Nile Red, a large hydrophobic organic molecule, attaches to plastic particles in the solution. The stain can be excited by various wavelengths of light and exhibits fluorescence which can then be distinguished from the background more easily than with light microscopy (Shruti et al., 2022).

Throughout this process there are several opportunities for exclusion, including sorption to the sides of a sampling bottle, never allowing the samples to be present on the filter. Another source of error is the overestimation of microplastic counts as a result of naturally fluorescing materials which resemble fluorescent plastics, such as undigested organic material (Sturm et al., 2023). However, the primary form of error that concerns us is the overcounting of plastics due to in-lab contamination before the application of the fluorescent dye onto the slides. Some traditional methods for avoiding this contamination include covering samples in aluminum foil when not being worked with directly, avoiding plastic-laden clothing, and air filtration systems (Bogdanowicz et al., 2021).

Many of these issues can be mitigated by using more analytical techniques such as Pyrolysis-Gas Chromatography-Mass Spectrometry which measures the vaporized plastics samples for mass (Seeley & Lynch, 2023). While this technique could improve accuracy and standardization, such instruments are extremely expensive and inaccessible for many laboratories, and especially for citizen scientists.

This study seeks to develop a replicable and cost-effective method of microplastic quantification in riverine water samples. Specifically, this study aims to minimize the risk of cross-contamination during the digestion and isolation phases while maintaining the integrity of the plastics contained within the sample. To accomplish this, collected riverine water samples

were pre-stained with Nile Red prior to any digestion or processing to minimize the risk of ambient microplastic contamination from being quantified in the final counts. This procedure was then applied to a study of the impacts of a large urban area on the concentration of microplastics in its surrounding waterways.

Sampling

Site Selection

The initial goal of this project was to quantify the University of Maryland's contribution to the prevalence of microplastics in the surrounding waterways, specifically the Paint Branch Creek watershed. As a result, we chose to sample from sites above, below, and on campus (see Figure 2). These sites were primarily chosen based on their relative location to campus and secondly based on sampling accessibility.

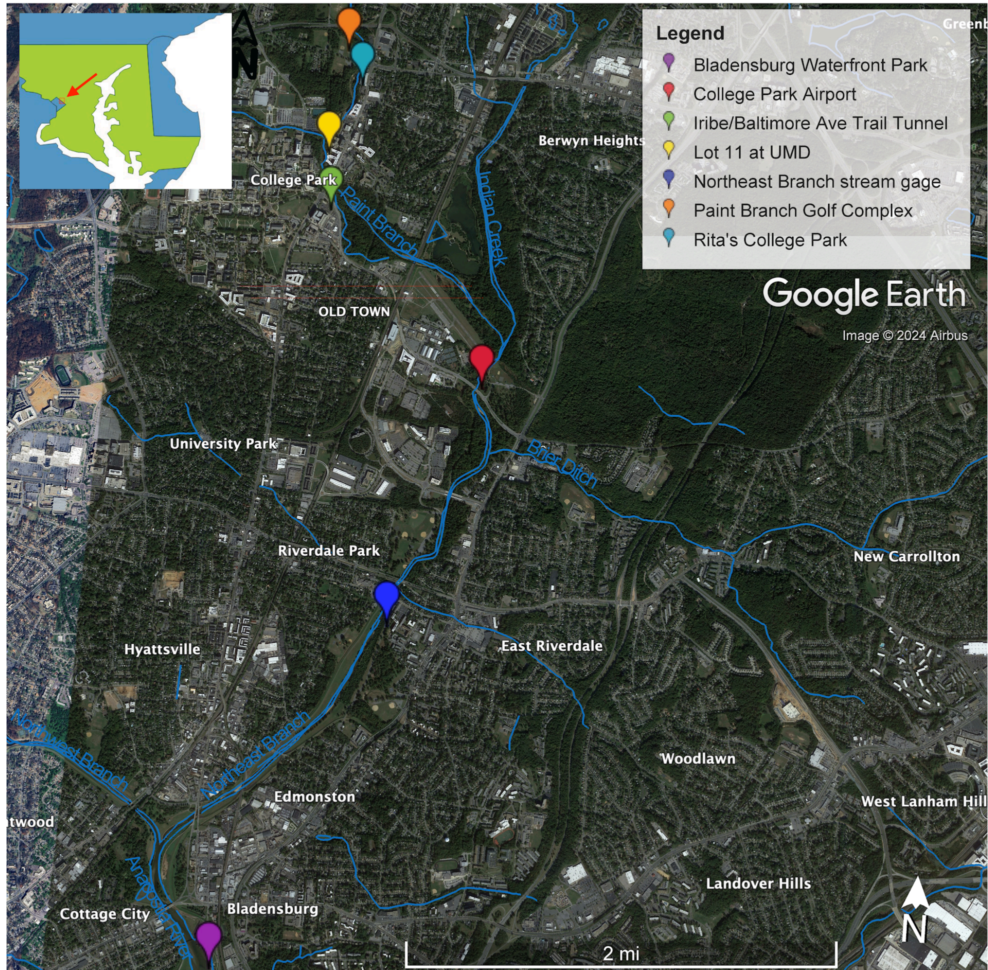


Figure 2: Anacostia watershed study site locations

The choice to focus on Paint Branch Creek was a practical choice so that we could consistently collect samples from the sample locations and compare them among sites. This is a more specific study region than the originally proposed larger Chesapeake and Anacostia watershed.

Sites for this study were chosen to capture both above and below the University of Maryland campus while staying on Paint Branch Creek. This sample range was a scaled down version of initial plans for study of the Anacostia watershed and greater Chesapeake Bay. Our final sample list was used to look more specifically at the microplastics impact of the University

of Maryland College Park campus impact. Our final sites were each nearby a landmark, one of the seven following: Bladensburg Waterfront Park, the Northeast Branch Anacostia River Stream Gauge, the College Park Airport, the Brendan Iribe Center for Computer Science and Engineering, Lot 11 at the University of Maryland, the Rita's Italian Ice of College Park, and Paint Branch Golf Complex. These landmarks were used to refer to the locations of our sites throughout the collection and processing of samples.

Table 1: Site Names, Numbers, and Coordinates

Site Identifier	Site Number	Coordinates
Bladensburg Waterfront Park	1	38°56'11.4"N 76°56'28.0"W
NE Gauge	2	38°57'36.4"N 76°55'32.9"W
Airport	3	38°58'38.5"N 76°55'06.5"W
Iribe	4	38°59'22.2"N 76°56'04.0"W
Lot 11	5	38°59'33.9"N 76°56'07.1"W
Rita’s College Park	6	38°59'33.9"N 76°56'07.1"W
Paint Branch Golf Complex	7	39°00'00.9"N 76°55'57.2"W

This initial site list additionally included a river bank directly west of the College Park Ikea for the purpose of capturing parking lot runoff. However, this site was not readily available for sampling due to fencing and forest brush, and was cut from our sample list. Furthermore, we chose to collect surface water samples from the shoreline due to ease of access. Surface water refers to water at the top of the water column.

An important consideration for sampling results from our ultimate site list is that rather than open waters, we chose to collect from creek surface waters, which presents issues with leaf litter and organic material in our samples.

Site Description

Bladensburg Waterfront Park is a public park in Prince George's County with a history of pollution and recent efforts toward remediation. Notably, Bladensburg Waterfront Park (see Figures 3-6) is our only tidally influenced site due to its size and proximity to more open water. Bladensburg is the site with the most visible debris upon selection for our study (see Figure 6).

Figure 3: Bladensburg Waterfront Park Sampling Site

Figure 4: Bladensburg Waterfront Park Sampling Site

Figure 5: Bladensburg Waterfront Park Sampling Site

Figure 6: Bladensburg Waterfront Park Sampling Site

The Northeast Branch Anacostia River Stream Gauge (see Figures 7-9) is located north of the Bladensburg sampling site across the street from an apartment complex and near an urban park. This location was chosen as measurements of water speed can be measured using data collected by the stream gauge (see Figure 8). Notably, this site involves two portions of the river re-mixing after separation at the bridge (see Figure 7); yet before a large rapid shown in Figure 9.

Figure 7: Northeast Branch Anacostia River Stream Gauge Sampling Site

Figure 8: Northeast Branch Anacostia River Stream Gauge Sampling Site

Figure 9: Northeast Branch Anacostia River Stream Gauge Sampling Site

The College Park Airport sampling site (see Figures 10-12) is slightly north of the Northeast Branch Anacostia River Stream Gauge, directly adjacent to the College Park Airport

runway. Similarly, there is a nearby urban park by this site as well as an outflow pipe (see Figure 13).

Figure 10: College Park Airport Sampling Site

Figure 11: College Park Airport Sampling Site

Figure 12: College Park Airport Sampling Site

The Iribe sampling site (see Figures 13-15) marks our first on-campus site, directly across from the Iribe Computer Science building on the University of Maryland campus. This site is in a forested area, surrounded on both sides with tree buffers. This site is directly below a fairly busy road in the city of College Park, US Route 1, as shown by the bridge in Figure 13.

Figure 13: Iribe Sampling Site

Figure 14: Iribe Sampling Site

Figure 15: Iribe Sampling Site

The Lot 11 sampling site (see Figures 16-18) is similar to the Iribe site with a walking bridge above the stream. There are a series of sandy beaches along with banks of the creek in this region as well as the merging of two sources, as shown by the adjoining stream shown in Figure 16.

Figure 16: Lot 11 Sampling Site

Figure 17: Lot 11 Sampling Site

Figure 18: Lot 11 Sampling Site

The Rita’s sampling site (see Figures 19-21) is below an urban bridge as shown in Figure 19. The banks of the river are surrounded by a rocky, sandy beach and forested area on one side and a human-built wooden fence on the other side (see Figure 20). This portion of the creek is directly adjacent to a parking lot and urban park. Over the course of this study, construction began on the road directly next to this site, leading to the creek.

Figure 19: Rita’s Sampling Site

Figure 20: Rita's Sampling Site

Figure 21: Rita's Sampling Site

The Golf Course sampling site (see Figures 22-24) is the most remote site we sampled at, with the nearest landmarks being the paved Paint Branch trails. There is a walking bridge above this site and a golf course within a quarter mile of our sampling site.

Figure 22: Golf Course Sampling Site

Figure 23: Golf Course Sampling Site

Figure 24: Golf Course Sampling Site

Collection

We collected two one liter samples from each site on a monthly basis along with one 1L blank sample using pre-collected distilled water from a laboratory setting. The purpose of taking duplicate samples is to be able to compare within replicates. However, only two samples were taken at each location due to material and transportation constraints.

Our intention for our collection process was to have 1 L duplicate samples as well as 1 L of a control for each sampling location and collection time. To minimize the time required to process each batch of samples, we opted for one control bottle per run filled with 1 L of filtered deionized water, opened each time a sample was collected. Originally, we attempted to collect our samples by placing a 1 L glass bottle directly into the running stream and allowing it to fill

with water. However, this created issues with air pocketing in which air leaving the bottle displaced sediment within the water column. This displacement biased samples due to increasing the collected sediment content within our samples. Due to the heavier microplastics which disproportionately exist in the sediment, this sampling method needed to be rethought. Furthermore, we wanted to collect within the middle of the water column, not the surface of the stream.

Our proposed solution was the equivalent of a two-sided jar, which would allow for stream water to flow through the apparatus before being closed on both ends simultaneously. This would allow for an effective “snapshot” of the stream at a given moment in time. When placed parallel to the flow of water, the apparatus can easily allow water to flow through and settle, before rapidly and simultaneously closing from both sides of the tube to collect 1 L of water. On site, we immediately funneled this into a 1 L glass bottle. Our apparatus used PVC piping fitted to the specific dimensions to prevent disturbance, as illustrated by Figure 25.

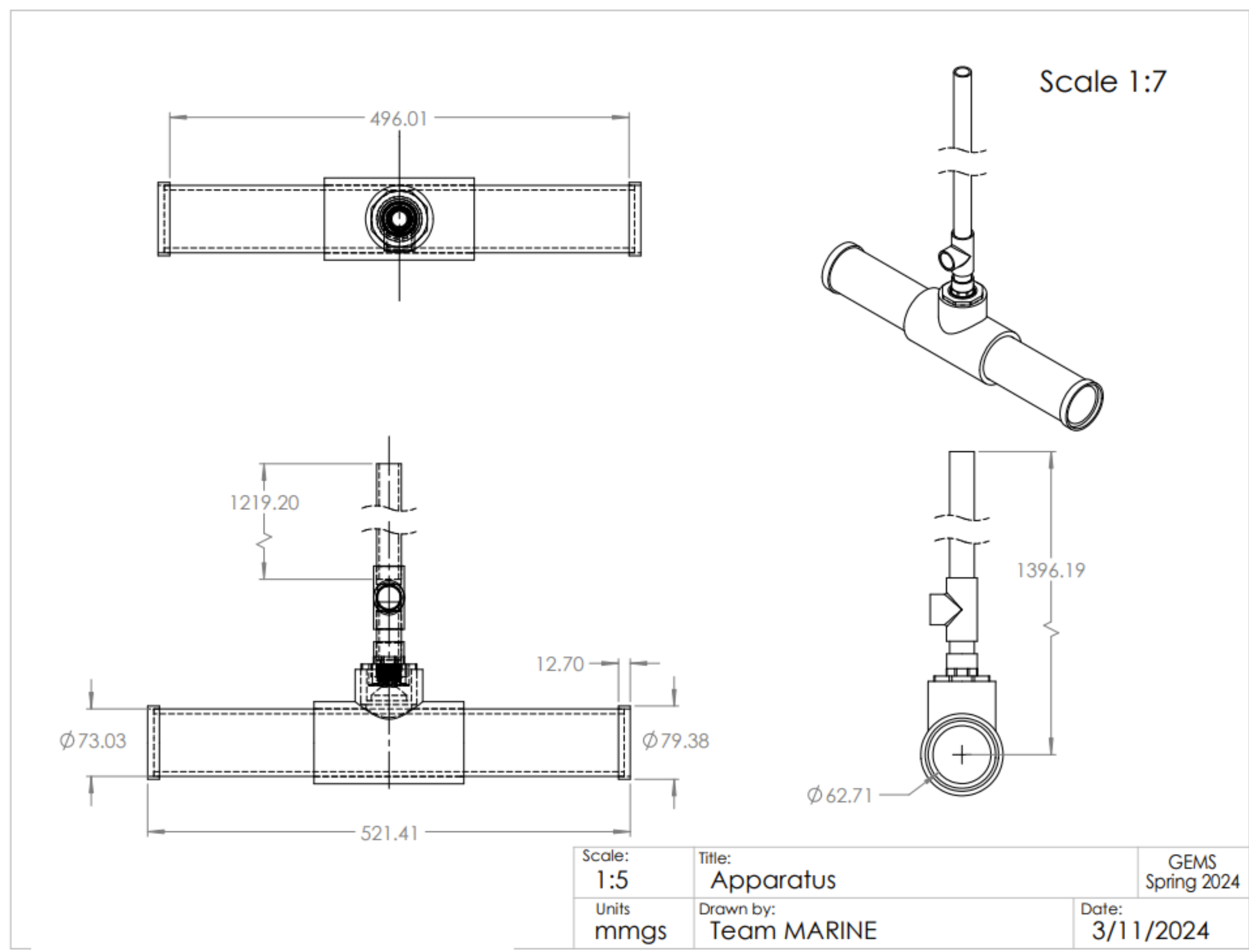


Figure 25: Technical Diagram of Sampling Apparatus Showing Dimensions

Table 2: Sampling Apparatus Parts List

Tag	Part Number
1	2.5" ASTM D1785 PVC Pipe
2	Inline Tee Reducing Adapter, Female Socket Connector (2.5" to 2")
3	Straight Bushing Reducing Adapters with Collar, Male Socket Connect × Female Threaded Pipe (2" to 0.75" with NPT Threads)
4	Straight Adapters, Female Socket Connect × Male Threaded Pipe (0.75" with NPT Threads)
5,7	0.75" ASTM D1785 PVC Pipe
6	0.75” Tee Female Socket Connect
8	2.5" ID Shaft Mounted Seals

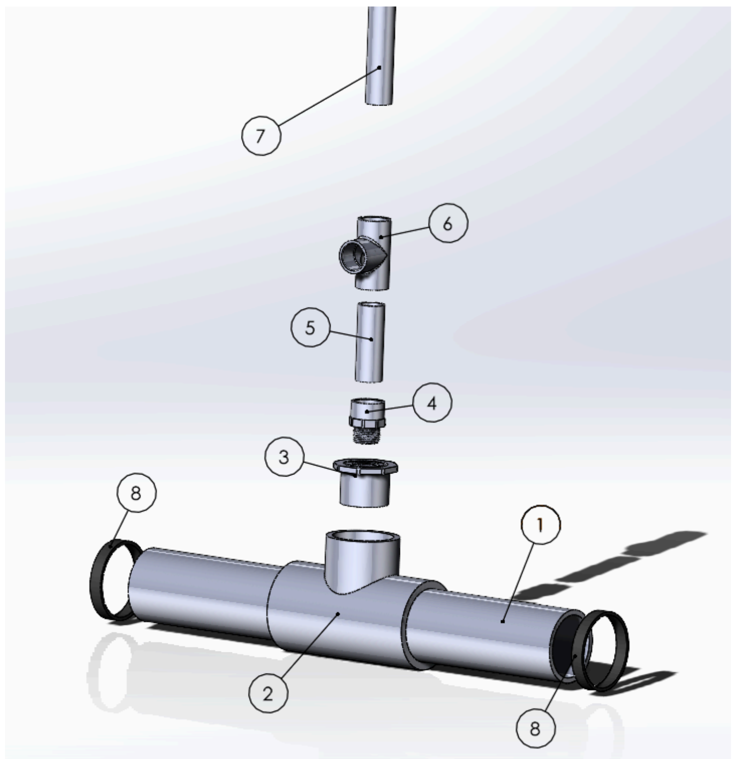


Figure 26: CAD Representation of Sampling Apparatus

Notably, filtered water samples were run through the apparatus and tested as a blank to ensure that the apparatus itself was not shedding microplastics into our samples. While a non-plastic apparatus would have been preferred to remove any concerns of contamination, budget constraints and the desire to develop a replicable and cost-effective methodology for use by citizen scientists prompted us to stick with the PVC sampling apparatus, given the confidence that it was not adding excessive contamination to our samples based on the blanks. To collect a sample, we lowered this apparatus below the surface of the water and allowed for the stream to flow through for 15 seconds before pulling a cord closing the apparatus, trapping 1.175 L of streamwater inside. Next, the apparatus was pulled out of the water and manually opened on one side allowing for water to pour into a 1 L glass bottle, assisted with a funnel to ensure sample

retention. Samples were shaken to ensure even distribution of the contents and leveled to exactly 1 L of water. Samples were then labeled based on the sampling run month, site, and replicate.

Figure 27: Apparatus Being Used to Collect a 1 L Sample

As samples were collected at each site, weather patterns as well as research observations were recorded. Importantly, recent rain events were noted along with the approximate height of the stream, turbidity, and qualitative flow rate (noted as fast or slow based on visual inspection).

We organized our samples with a three number code written on each bottle. The first number indicates which collection run the sample is from, the second the sampling location, and the third which replicate that sample was. Numbers ending in 00 indicate a control sample. This system was used to help more effectively organize and keep track of our samples.

Table 3: Collection Dates and their Numerical Code

Collection Run	Collection Date
0	Summer 2022 (May 4, July 6, and July 8 - depending on sample)
1	Oct. 16, 2022
2	Nov. 20, 2022
3	Jan. 7, 2023
4	Feb. 18, 2023
5	March 11, 2023
6	April 27, 2023
7	June 3, 2023
8	June 24, 2023
9	July 8, 2023
10	Aug. 26 and 27, 2023

Processing

Determining an optimal processing method for our samples was the most time-intensive part of the project. We used our collection method to collect extra samples as needed to test the effectiveness of each part of the process as we developed it. The full collection and processing protocol is available below (see Appendix A).

Acidification and Digestion

Our initial attempt was to directly pour each sample over a filter to then quantify the remaining microplastics; however, sediment and organic matter clogged the filters necessitating further filtration. These were glass fiber filters without binder resin and a pore size of 0.7 microns (MillporeSigma Glass Fiber Filters).

Figure 28: Filtration Set-Up

Figure 29: Sediment-Clogged Slides Which did not Receive Density Separation

For the first few sampling runs, we acidified each sample with 1 mL of nitric acid to prevent the growth of organic material during storage. We then digested each sample at a later date. Each sample was digested with 85% purity potassium hydroxide pellets sourced from Thermo Fisher Scientific Chemical, (added at 1M concentration) and then placed in a heated shaker for 48 hours. This process could lead to spilling and explosions so the bottles were required to be loosely capped to avoid pressure build. In later months, we were able to digest directly after collection and therefore skipped the acidification step.

In troubleshooting this digestion process, we found that loosely capping our bottles led to a pressure build-up within the media bottle, especially in samples with high amounts of sediment and possible organic matter. As a result, these pressurized bottles were leaking sample which had essentially spilled through the cap. For future tests, we ensured that the bottles remained tightly capped to prevent this problem.

Additionally, we considered a couple different digestion methods, including hydrogen peroxide (Tan et al., 2022). We limited our digesting reagent option to potassium hydroxide and hydrogen peroxide because we wanted to avoid overly harsh digestions which may degrade the

plastics within the sample. Hydrogen peroxide is more often used for plant and sediment-rich water samples and has been found to be less harsh on certain types of plastics including polyethylene terephthalate (PET) (Pfeiffer & Fischer, 2020). However, we opted for the potassium hydroxide digestion method due to the familiarity of this method in our lab and previous success with an existing methodology which we modified.

Staining

We chose to stain our samples with Nile Red stain to make the microplastics fluoresce when viewed through the microscope during the counting process. Originally, we tried the traditional method of adding Nile Red after the sample had been put onto a filter. However, as we added more steps into our processing procedure, we opted to add the stain to the sample before it was put onto the filter. This would prevent any microplastics that contaminated the filter through in-lab contamination from being stained and fluorescing later.

We found no noticeable difference in the effectiveness of Nile Red stain at different concentrations in the 1 L sample. We tried adding 0.5 mL, 1 mL, 2 mL, and 5 mL of Nile Red to samples, and the microplastics were just as fluorescent. Similarly, we added higher volumes of Nile Red dye to the samples by testing 10 mL and 15 mL additions. None of the tested samples had noticeably different levels of fluorescence. As a result, we chose the 5 mL volume in order to reasonably conserve materials while having a slight excess volume of Nile Red available in case a sample had a particularly high concentration of microplastics.

Allowing for Nile Red to sit for several hours without analyzing samples can result in Nile Red aggregates, similar to micelles, due to the hydrophobic nature of the dye (Mino et al., 2023). This could potentially cause concern given that we prepared Nile Red and allowed it to sit for up to a month before use as well as adding Nile Red to our digested samples and allowing for

these samples to sit for days or weeks. However, this methodology involved sending the samples through a sieve and only collecting the particles which are greater than 20 µm in diameter. These Nile Red aggregates are known to be smaller in size, estimated to be closer to 130 nm, and are therefore not a concern with the proposed method (Sutter et al., 2007).

We tried several different iterations of this technique including staining after digestion, before digestion, and simultaneous to digestion. We found no notable difference between these methods and therefore chose to stain simultaneously with digestion for the purpose of streamlining our laboratory processing and decreasing handling of our samples. As a result, immediately following the addition of 56 g of potassium hydroxide pellets, 5 mL of Nile Red was added to the sample media bottle. Each sample was left in the shaker for 48 hours after this step to ensure mixing and distribution of the stain throughout the sample.

Density Separation

Another issue we found - specifically from working with surface water samples - was that the samples quickly clogged our glass-fiber filters creating layers such that the samples could not be observed effectively under the microscope.

Our initial method for solving this involved decanting off the top portion of the digested sample, accounting for approximately 800 mL of the 1 L sample. Following this step, the remaining sediment-rich sample was divided into 250 mL round-bottom centrifuge tubes, which were then balanced with filtered water and centrifuged at 2500 rpm for 10 min. The top portion of water was then poured through a baked glass-fiber filter. This second decantation was done carefully to avoid clogging the filter. Next, the remaining sample was washed with additional water to dilute the potassium hydroxide present in the sample. This washing step is important to reduce the precipitate formed from future zinc chloride and potassium hydroxide interactions.

The washed sample was centrifuged again at 2500 rpm for 10 minutes and decanted into the filtration set up. Next, approximately 100 mL of 11 M (1.5 g/mL) zinc chloride (Zinc chloride, 98%, extra pure, Thermo Scientific Chemicals) was added to the centrifuge tube and the sample was shaken. This sample was centrifuged at 2500 rpm for 10 min and decanted into the filtration set up. This process retained a couple of the same issues that we were having before in that the filters continued to clog and therefore multiple filters had to be used per sample to get accurate counts. In addition, a huge amount of materials was being used to process each sample. For the purpose of making this method both accessible and not cost prohibitive, we decided to change this initial method by modifying the original sample. We additionally tried different separation methods including gravity separation using a separatory funnel but found that the separation layers were not sufficiently clear within a 48 hour period to warrant further investigation.

Figure 30: Centrifuge used for these analyses

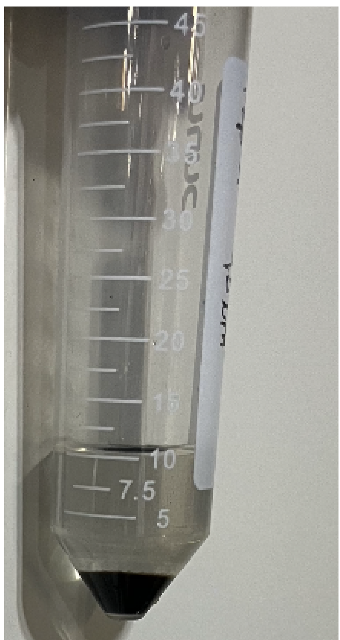


Figure 31: Sample Post-Density Separation

Our altered method begins by running the digested and stained 1 L sample through a 20 µm sieve and rinsing thoroughly with filtered deionized water. Following this rinse, a solution of filtered 11.0 M (1.5 g/L) zinc chloride was used to rinse the sieve directly into a 50mL centrifuge tube. This step reduces the need to move between glassware and reduces risk of both contamination and sample loss. Notably, such an alteration of the method reduced our study to only consider large microplastics over 20 µm in size. However, the amount of material used per sample is much smaller and interactions between potassium hydroxide and zinc chloride are minimized.

The zinc chloride collected sample is then centrifuged at 2500 rpm for 10 min to allow the sediments to pelletize. The top layer of zinc chloride is then decanted into the filtration set up to prevent excess sediment from clogging the filter. Then approximately 15 mL of zinc chloride is added to the remaining sediment compacted at the bottom of the sample. This sample is then shaken to resuspend the sediments and centrifuged again at 2500 rpm for 10 min to ensure

microplastics were not caught in the previous sediment pellet. The layer of zinc chloride is then decanted through the sample filter. Finally, the sample is washed with approximately 100 mL of filtered deionized water and then covered with aluminum foil and allowed to dry for approximately 10 min.

Some important precautions had to be taken for this method included a thorough wash of the sieve between samples as well as continuously covering the filtration set-up with aluminum foil when not directly decanting liquid through it in order to minimize in lab ambient contamination.

Quality Control

We conducted a rigorous stepwise check of our developed methods to ensure that the developed procedure was accurate and was not introducing unknown sources of error into our study. To do this, we created plastic fragments and shavings in three separate size categories (small, medium, large) that were prestained with Nile Red and added a set volume of 5ml to both control samples and actual unstained environmental samples to examine the impact of our procedure on these plastics throughout the process. These samples were then passed through our procedure, with each sample being stopped at a different step in the process and passed through a filter to examine the recovery rate of the spiked microplastics. We found through this process that our digestion process was not resulting in any overdigestion or degradation of the plastic particles nor was any specific step resulting in a large loss of the spiked plastics. This quality control stepwise process gave us the confidence that our developed method was fairly accurate and did not result in any undue contamination or microplastic loss, allowing us to begin collecting and processing field samples.

Quantification

Once the samples were processed, we were left with dozens of slides that could be counted at our leisure; it required that we manually counted what was on each slide. Due to the nature of what was on each slide, we needed to be strict on what we were classifying as microplastic, so that each person was observing the same counts.

In order to count the samples, each slide was analyzed with a Leica DM LA microscope at 40x magnification. We specifically use fluorescent microscopy with excitation at 510 nm. Starting from one end of the sample, the person counting would systematically scan through the entire slide, counting what they saw on each frame before moving on to the next. Most used a snake pattern to traverse the entire slide.

Each sample was counted by two to three people, one time per person, in an attempt to account for variability in counting. Each sample was counted twice initially, and, if the total particle count differed by greater than 20%, then it was counted a third time. Additionally, we separated the counts into categories for the types elements we saw on the slides to help with consistency. We named the categories the following: pinpoints, small particles, large particles, fibers, and nodes (see Tables 4 & 5). The categories were classified qualitatively with pinpoints being dull points of light with ill-defined edges which could not be focused on at the 40x magnification. Large and small particles were more traditional fragments which could be seen and easily identified as microplastics but were delineated based on size. The distinction was based on team consensus from our counting training to get everyone on the same page. Fibers are long, string-like pieces of plastic. Finally, nodes are nearly perfectly circular fluorescing objects which often were found in clusters. These nodes differ from other described plastics as typically plastics we found had more jagged edges whereas nodes were far smoother. The pinpoints

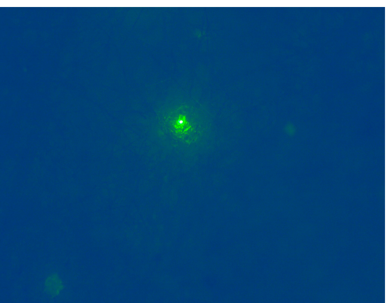
category was added after a few initial counts. Before, they were counted under small particles, but some people would count them while others would not, being unsure if that was a microplastic or something else. Interestingly, the pinpoints category mostly include fragments smaller than 20 µm, meaning that they should not have made it through our sieving process. As a result, pinpoints likely constitute some type of contamination from our processing method, potentially from a self-fluorescing species. As a result, the pinpoints were counted in all samples but were not considered as part of our environmental samples.

Table 4: Guidelines for Our Classification of Different Items that Fluoresce

	Pinpoint	Small	Large	Fiber	Node
Description	Small, dull light, not sharp/defined edges	Clearly fluorescing, sharp defined edges, small	Clearly fluorescing, sharp defined edges, large, easily visible at 10x magnification	Clearly fluorescing, long and thin	Clearly fluorescing, very circular, often occurring in groups
Example Photographs					

Table 5: Comparison of Fluorescent and Light Microscope View of Different Plastic Shapes and Types

Microplastic Shape	Type of Microscope View	
	Nile Red Fluorescent Microscope	Light Microscope
Fragment		
Fiber		
Node		
Comparison - small and large		

Pinpoint	

Because of time limitations, samples from only three sites were counted in addition to a control for each sample run. The sites were Iribe, the Golf Course, and Bladensburg. From each of the sites, a sample pair from each season was counted (see Appendix B).

Throughout this process, we looked into several other methods for quantification including use of Sedgwick rafter cells and full slide counting. Sedgwick Rafter cells are microscope slides with a precisely printed grid allowing for high resolution counting within a defined area, and by extension volume, of the slide. We considered incorporating this method into our counting by taking five representative 1 mL samples from our field-collected samples and pipetting this subsample onto the Sedwick Rafter cell (Pratesi et al., 2020). This method would allow for extrapolation from our subsample concentrations to find the total number of microplastics in the collected sample overall. One benefit of this method is the lack of required sample handling, which would then decrease risk of lab-introduced contamination. However, we opted against this method due to the size of our collection bottle. We are already collecting very small samples of large water bodies. As a result, taking even smaller subsamples contributes to potentially greater levels of sampling bias. We therefore chose to count all of the plastics on each processed sample filter, which was prepared as described above. Another issue found with this method was the presence of sediment in our field-collected samples which obstructed our view

of microplastics under the microscope. Such an issue can be circumvented with density separation, which then defeats the purpose of directly looking at our sample media under the microscope. Additionally, we found issues with identifying plastics on the Sedwick Rafter cells with the plastics grouping together as well as migrating to the sides of the glass cell. This is likely due to our samples being held within an aqueous solution, which repels the more hydrophobic plastics particles making the Sedgewick Rafter cell method infeasible in our environmental media.

After ruling out the Sedgewick Rafter method, we chose to pursue full filter counting by moving around the slide in standard search patterns described above. We trained all members of our team on the same three slides and counted in repetition until every member of the team was counting approximately the same number and distribution of microplastics of each type. Following analysis of these results, we found no counter bias and proceeded to count each of our selected samples in duplicate. Any samples which showed a discrepancy between counters were then subjected to a triplicate count and all three measurements were averaged.

Data and Results

Particle Classification Results

The composition of sample particles were categorized into large particles, small particles, fibers, and nodes (see Table 6). Small particles constituted the greatest percentage of total particles sampled at 75%, large particles were 18%, fibers were 3%, and node particles were 4% (see Figure 32).

All samples ($N = 28$, $n = 27$) were collected as duplicates and each duplicate was counted twice. The counts for each duplicate sample were then averaged yielding one average count for each duplicate. For any counts that were egregiously inconsistent between raters (variation by

greater than 20% between counters), we conducted a triplicate count. This triplicate count was then averaged with the original two counts. One sample was excluded from this study due to an inability to effectively quantify the sample particles with our procedures.

Table 6: Descriptive Statistics of Average Sample Particle Distribution per Liter across 27 Samples

Particle Size	*Mean*	*SD*	*Median*	*Min*	*Max*	*Range*
Small	842	446	773	269	1873	1604
Large	199	212	132	9	919	910
Fiber	32	21	30	6	81	76
Node	42	66	12	1	231	230
Total	1115	646	1067	346	2879	2533

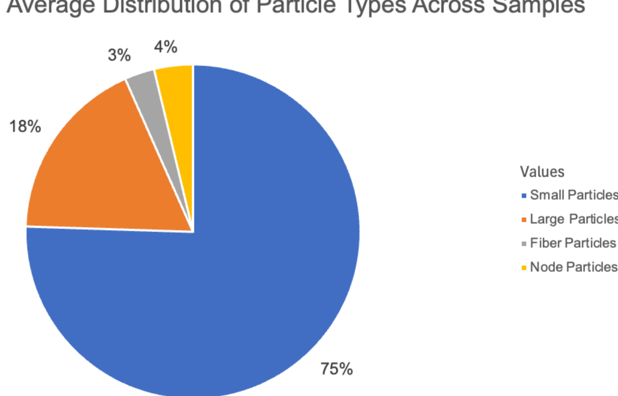


Figure 32: Average distribution of particle types across samples

Seasonality Results

We conducted a seasonality analysis to understand average sample particle quantities across each of the four seasons. We found that the average particles were highest in Spring at ($\bar{x}$ = 1603); followed by Fall ($\bar{x}$ = 1117); then Summer ($\bar{x}$ = 999); and lastly Winter ($\bar{x}$ = 724) (see Figure 33). Within each season the composition of particle types was largely consistent with the overall averages (see Figure 32). Nodes were an exception and were most prevalent in Spring and Summer; however, largely absent in the Fall and Winter. Spring had 71% small particles; 20% large particles; 3% fiber particles; and 6% node particles. Fall had 74% small particles; 22% large particles; 3% fiber particles; and 1% node particles. Summer had 81% small particles; 11% large particles; 4% fiber particles; and 4% node particles. Lastly, Winter had 81% small particles; 16% large particles; 2% fiber particles; and 1% node particles.

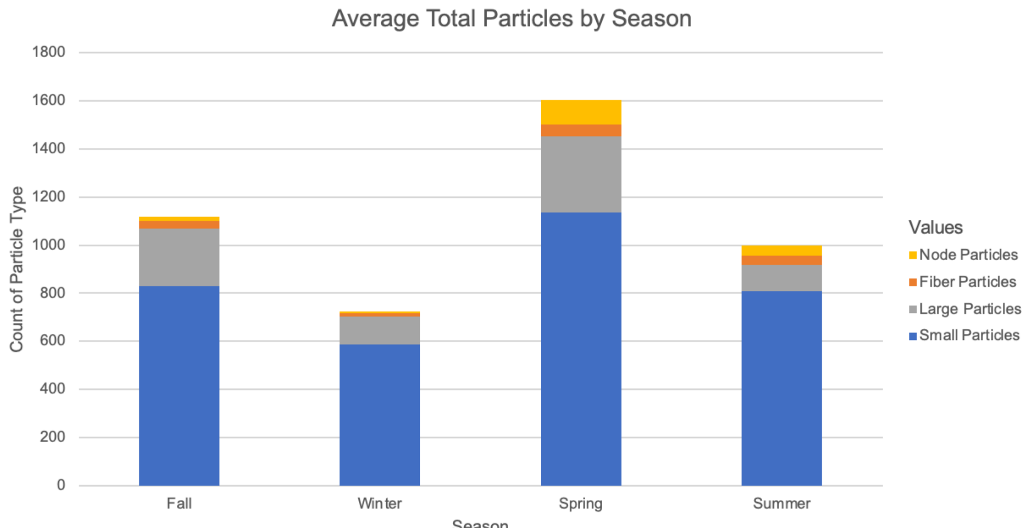


Figure 33*:* Average Total Particles by Season

Location Results

We conducted a location analysis to understand average sample particle quantities across each of the three sites and our control. We found that the average particles were highest in Bladensburg at ($\bar{x}$ = 1558); followed by Control ($\bar{x}$ = 1027); then by Golf Course ($\bar{x}$ = 926); and lastly Iribe ($\bar{x}$ = 875) (see Figure 34). Within each location the composition of particle types was largely consistent with the overall averages (see Figure 32). Bladensburg had 75% small particles; 17% large particles; 2% fiber particles; and 5% node particles. The Control had 67% small particles; 23% large particles; 4% fiber particles; and 6% node particles. Golf Course had 74% small particles; 20% large particles; 4% fiber particles; and 2% node particles. Lastly, Iribe had 83% small particles; 12% large particles; 3% fiber particles; and 2% node particles. We then compared the results of our seasonality and location analysis to understand how the two are related (see Figure 35).

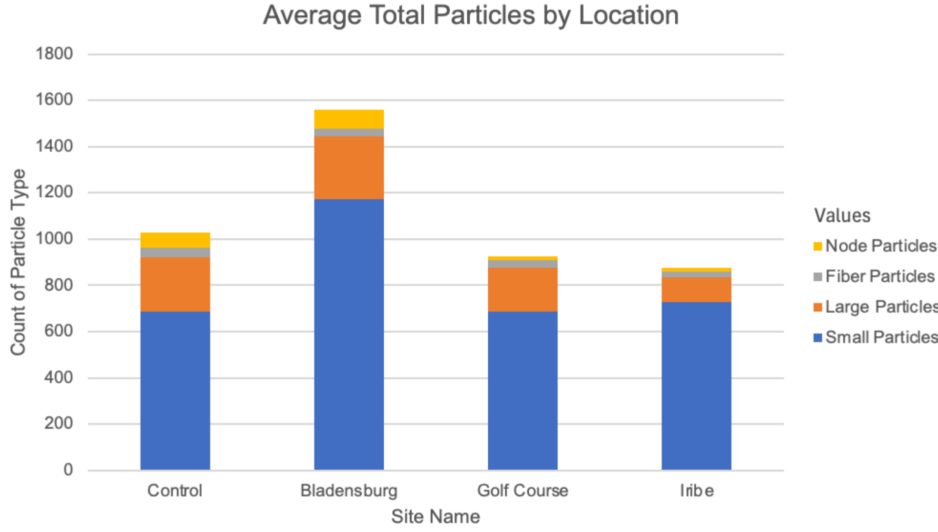


Figure 34: Average Total Particles by Location

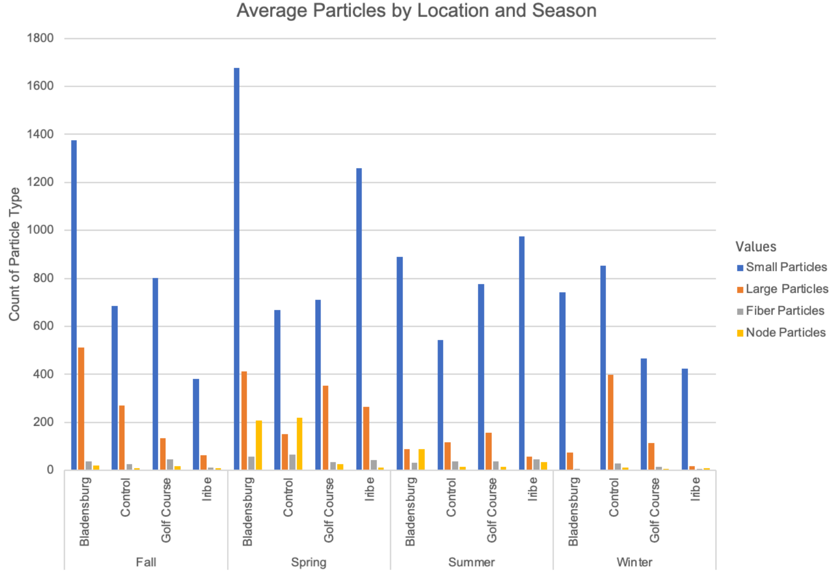


Figure 35: Average Particles by Location and Season

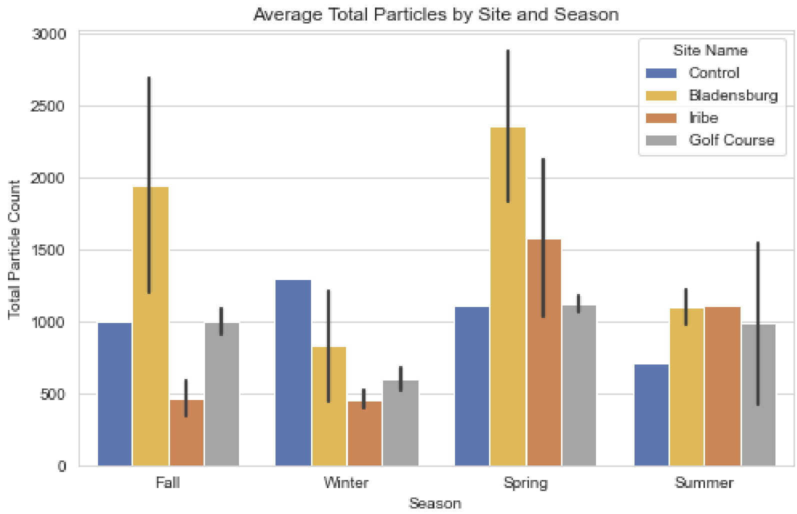


Figure 36: Total Microplastics Count Between Sites and Between Seasons. Error bars represent the range between the totals of two samples at each location (only one control sample was taken for each date, so there is insufficient data for error bars on control samples).

ANOVA Statistics

ANOVA Assumptions

We conducted a series of one-way ANOVAs to compare particle totals across all four seasons. We then conducted a second series of one-way ANOVAs comparing particle totals across our three sites and control. Before running these ANOVAs, we conducted the Shapiro-Wilks test of normality and found our data to be normally distributed following the removal of one outlier. This outlier was identified and removed from the dataset due to it falling outside of the normal distribution. There were excessive particles in this sample which could not be effectively quantified with our procedures. Next, we performed a Levene's test to assess homogeneity of variance where all tests were not statistically significant indicating the assumption was satisfied. All data were assumed independent.

ANOVA Statistics

All analyses were conducted in R Studio (Version 2023.09.1) and Microsoft Excel (Version 16.0.17324.42305). The ANOVA series addressed the comparison between particle totals across all four seasons and site locations, respectively. Each series of ANOVAs were run looking at small particles, large particles, fiber particles, node particles, and total particles. These categories were created to distinguish the different particle types that were visible on our samples. The first series compared these particles to seasons and the second to location. Tukey's t-tests were performed for any significant result to understand which variables significantly differed from one another.

The first series of one-way ANOVA tests were conducted to see if there were any significant differences in quantities of particle sizes across four seasons. There were no significant differences for small particles $F(3,23) = 1.997$, $p > 0.05$; large particles $F(3,23) = 1.625$, $p > 0.05$; or total particles $F(3,23) = 2.694$, $p > 0.05$. There were significant differences in fiber particles $F(3,23) = 5.812$, $p < 0.05$ and node particles $F(3,23) = 3.954$, $p < 0.05$. Tukey's post-hoc tests were run and found a significant difference in quantities of fiber particles between Spring and Winter ($M = -36.43$, $p < 0.05$). Another set of t-tests were run for nodes and found a significant difference in quantities of nodes between Spring and Winter ($M = -93.98$, $p < 0.05$); and Spring and Fall ($M = 86.26$, $p < 0.05$).

Table 7: One-Way Anova Results for Significant Differences in Quantities of Particle Sizes Across Seasons

Particle Size	*DFn*	*DFd*	*F*	*p*
Small	3	23	1.997	0.14
Large	3	23	1.625	0.21
Fiber	3	23	5.812	0.004*
Node	3	23	3.954	0.021*
Total	3	23	2.694	0.07

*$p < 0.05$.

A second series of one-way ANOVA tests were conducted to see if there were any significant differences in quantities of particle sizes across site locations. There were no significant differences for small particles $F(3,23) = 2.425$, $p > 0.05$; large particles $F(3,23) = 0.782$, $p > 0.05$; fiber particles $F(3,23) = 0.490$, $p > 0.05$; node particles $F(3,23) = 2.223$, $p >$

0.05; and total particles $F(3,23) = 2.694$, $p > 0.05$. Tukey's post-hoc tests were not run since there were no significant differences found.

Table 8: One-Way Anova Results for Significant Differences in Quantities of Particle Sizes Across Site Locations

Particle Size	*DFn*	*DFd*	*F*	*p*
Small	3	23	2.425	0.09
Large	3	23	0.782	0.52
Fiber	3	23	0.490	0.69
Node	3	23	2.223	0.11
Total	3	23	2.050	0.14

$^*p < 0.05$.

Interrater Agreeableness

To ensure different counters were not introducing over or undercounting biases into the results, a statistical analysis of individual counters was done. Each count from all counters was assigned a percentage differential compared to the other counting replicates of that sample. The percentage was calculated by finding the average count for the sample and dividing the difference of the individual count to the average by that same overall average. A percentage was chosen over a raw difference to avoid the true microplastic count influencing any potential bias. The mean and standard deviation of each counter's percentage differentials was calculated and used to administer a z-test (known population variance) with the null hypothesis that their mean differential was zero.

Table 9: Two-Way Z-Test for Counter Differentials Significantly Different from Zero

Counter Number	*n*	*μ*	*σ*	*z*	*p*
Counter 1	6	0.10800	0.251	0.430	0.834
Counter 2	6	0.00539	0.222	0.024	0.999
Counter 3	6	0.07380	0.125	0.590	0.778
Counter 4	7	-0.0354	0.170	0.208	0.918
Counter 5	8	-0.0601	0.220	0.273	0.893
Counter 6	7	-0.0669	0.105	0.637	0.762
Counter 7	8	-0.0211	0.121	0.174	0.931
Counter 8	6	0.05003	0.086	0.582	0.780

Based on this test, we cannot reject that the mean differential for each counter was equal to zero, $z_{max} = 0.637$, $p_{min} = 0.762$.

Discussion

Methods Utility and Sources of Contamination

This study aimed to develop a methodology that would be economical, easily accessible, and representative of popular techniques from the existing literature. Our collection protocol allowed for significant cost savings, as we used a plastic apparatus with a 1 L capacity that was able to be built by members of the research team without contracting an external makerspace.

One serious limitation of our study is the extensive contamination found in our control samples, ranging up to thousands of plastic particles in what should have effectively been distilled water. In order to address this issue, we ran a series of water samples through our staining, sieving, and density separation process which yielded very small levels of plastics contamination on our slides, which was more in-line with what we expected from our control samples. Similarly, we ran pre-filtered water (via Elga LabWater Purelab Water Purification System) through our sampling apparatus and also found minimal contamination. These values were nowhere near our actual control samples, suggesting that some element of our process was not represented within this lab test.

Table 10: Microplastics Counts from Lab Control Samples

Condition	Microplastics Count (MP/L)
Pre-filtered water with no ambient exposure	15
Pre-filtered water with 45 min ambient exposure	49
Unfiltered DI water with no ambient exposure	65 ± 22
Unfiltered DI water with 45 min ambient exposure	130.5 ± 30.5
Pre-filtered water exposed to interior of collection apparatus	83 ± 24

These results suggest that there is minimal contamination that results from our sample collection. However, because we stain at the beginning of our process, we made the assumption that contamination which occurred after this initial stain would not fluoresce unless it was introduced by some self-fluorescing substance (e.g., a mineral of some kind). In an effort to address these self-fluorescing substances, we added an additional density separation step with zinc chloride and a nitric acid wash to remove the self-fluorescing substances.

As a result we believe that this contamination of our controls occurred before or at the time of the initial staining. After generating a stained stock solution of potassium hydroxide (Potassium hydroxide, pellets, 85%, Thermo Fisher Scientific Chemical), we found extensive plastics present. These plastics were found extensively in all of our described size categories (including large fragments and fibers). Notably, we added our potassium hydroxide digesting reagent in pellet form at the same time as our Nile Red stain to our samples. This allows us to be reasonably confident that the potassium hydroxide pellets represent the majority of our contamination in both our control and collected samples (see Figure 37). This unfortunate finding came to light after all sample processing. As a result, we can best address this form of error by regarding the control level of plastics as background noise, which should be reasonably similar to those introduced to the collected samples. Therefore, we are still able to look at our samples comparatively but without a precise count of the plastics content of any individual sample. In addition, we would make the recommendation that any future microplastics work limit the use of reagent pellets as they may include plastics within the reagent. The zinc chloride reagent used (Zinc chloride, 98+%, extra pure, Thermo Scientific) was also analyzed and stained under the microscope, with no evidence of major contamination.

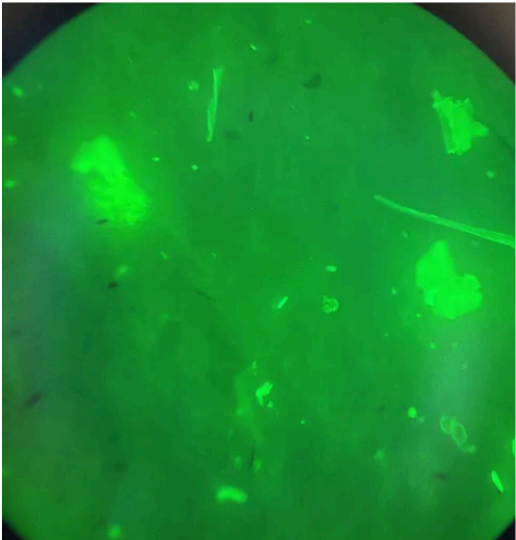


Figure 37: Microscope Photograph of Stained Potassium Hydroxide Solution

One unexplained type of contamination is the spherical node pieces which either self-fluoresce or are stained by Nile Red. Initially we believed them to be mold of some kind due to smooth exteriors which are not reminiscent of typical microplastics. Following the discovery of the potassium hydroxide contamination issue, we propose that the spherical pieces are potentially seed particles used in the potassium hydroxide manufacturing process. These particles allow for the salt to pelletize around the plastics which would explain the node category of plastics. Similarly, we found different levels of background contamination between sample processing batches. We propose that this batch effect could be attributed to using a different bottle of potassium hydroxide pellets. As a result, the switch between potassium hydroxide sources could account for the difference in background noise.

Another protocol that proved difficult was the definitions used during quantification of microplastics. The categories based on size lended themselves to subjectivity, which was seen mostly in the variance of counts for the Small category. Although the sieve stage was meant to remove particles below 20 µm, fluorescent particles smaller than this were visible. While these

smaller bits could be representative of larger plastics that broke down during processing, we counted them separately due to their unknown origin. These particles were counted as pinpoints to distinguish them from our other classifications which ultimately helped limit counter bias. We recommend for future fluorescent microscopy studies to use shape over size (fibers, fragments, beads/round, film), as these distinctions are more easily made given the limits of this methodology (Davey et al., 2023; Sturm et al., 2023).

While this study did not rely on polymer identification and chemical analysis methods to classify microplastics, peer studies using both these methods and fluorescent spectroscopy can shed light on possible origins of these particles. The pinpoints observed in the samples were likely related to the Nile Red stain. If excess Nile Red molecules were present on the wet filter following density separation, then they would likely form hydrophobic aggregates, similar to micelle formation, as a way to reduce interactions with ionic salt or polar water molecules. As a result, despite expecting Nile Red aggregate radii to be smaller than that of our sieve, the aggregates could form following density separation and remain on the filter if this formation took place later (Ray et al., 2019). These aggregates would be small enough that they could appear as highly fluorescent bits of light and may have been counted within our pinpoints category. As a result, the pinpoints category is considered as not reliably plastic and is excluded from our plastics analysis.

A similar analysis was applied to the sample identified and removed as an outlier for being many standard deviations outside of the population mean ($\bar{x}$ = 3980.5). Comparison of this imagery to that of Tong et al. 2021 suggests these many particles were of plastic origin. They were clearly fragments, resembling the image of polyurethane (PU) particles treated with potassium hydroxide and stained with Rhodamine B (Tong et al., 2021). This influx in the

sample from Summer 2022 at Iribe could potentially be due to a sealant used for nearby construction. The polymer images also suggest that polypropylene (PP) and polystyrene (PS) were prevalent in our samples, and that some particles identified as nodes share a resemblance with those identified as PVC (polyvinyl chloride) by Tong et al., 2021. Polypropylene (PP) is one polymer commonly associated with tire wear and tear (Kole et al., 2017), and PVC plastics are commonly used in the pelletization process (Zaman et al., 2016).

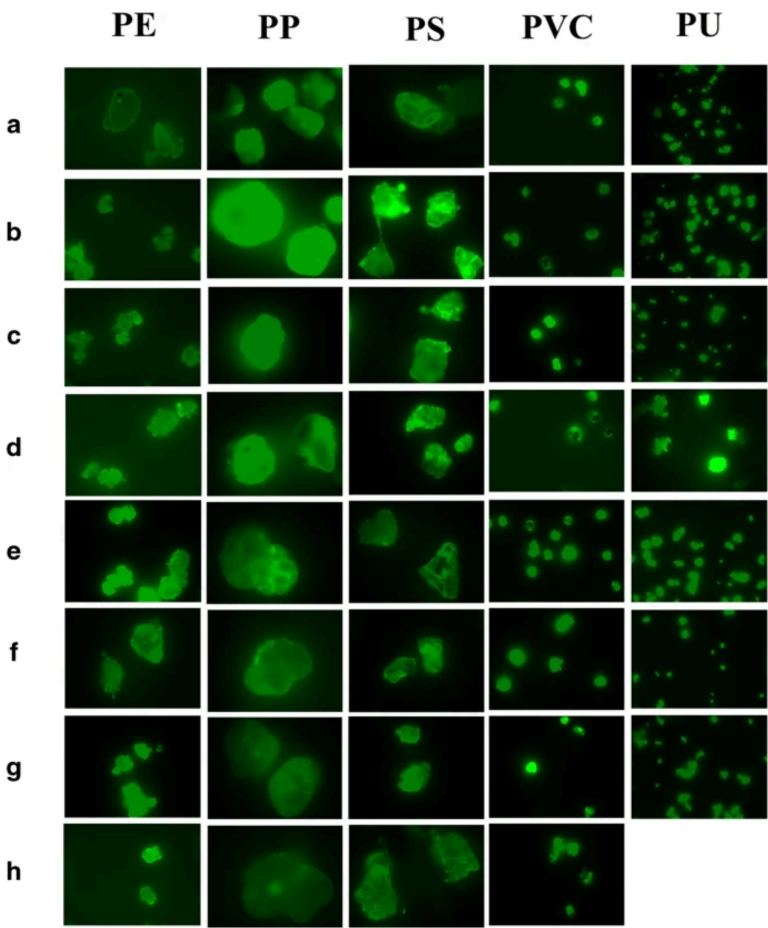


Figure 38: Collection of Microscope-view Photographs of Different Types of Plastics. Rows represent differing processing procedures, and (e) 24 hr treatment with 10% KOH is most comparable (Tong et al., 2021)

Comparable Local Study

Various studies of microplastic contamination have been completed across the Chesapeake region and its related watersheds with the most similar area evaluated being Nash Run: an Anacostia/Potomac River tributary about 3 km south of the Bladensburg sampling site (Davey et al., 2023). This study analyzed both water and sediment samples from Spring 2019 to Spring 2020. However, statistical significance was only found for their sediment samples. Davey et al. (2023) found water sample microplastic concentrations of around 10% of the average values found in this study. This is assuming classification size ranging from > 4000 micrometers to < 250 micrometers, including categorization by shape for fragments and filaments. These findings further provide evidence that sources of contamination existed in our samples. Davey et al. (2023) observed higher microplastic concentrations in Fall season samples; however, they did not find any significant correlations related to water velocity or sampling parameters (e.g., water velocity) which was in alignment with our findings. Additionally, chemical composition analysis and the use of dissection microscopy of samples showed the primary chemical composition of plastics were polypropylene and polyethylene (Davey et al., 2023).

Effect of Environmental Conditions

As with many waterborne pollutants, microplastic concentrations can be altered by countless environmental conditions obscuring anthropogenic trends and making them difficult to distinguish from background environmental variation. These environmental variables can take many forms, ranging from rain events and weather conditions to tidal fluctuations at sample locations, both of which can impact base concentration and dispersion throughout the water

column. As such, this variation must be considered prior to any conclusions being made about anthropogenic influences.

When examining water samples within freshwater rivers and streams, it is important to consider the flow characteristics of the water body at the time of sampling, particularly when discussing the transport of suspended particles such as microplastics from upstream sources. Additionally, runoff from rain events can result in increased transport of microplastics through storm drains, resulting in heightened concentrations in the timeframe immediately following the rain event. Three water quality variables were analyzed to examine their potential impact of variation within samples. These variables were turbidity, average discharge volume, and average water temperature, accessed from the USGS Northeast Regional Stream Gauge (see Figures 39-41). Precipitation and stream gauge height at Northeast Branch USGS Gauge and Bladensburg Waterfront Park were also analyzed (see Figure 42) (National Centers for Environmental Information [NCEI], 2024; United States Geological Survey [USGS], 2024; National Ocean Service [NOS], 2024). These factors differ greatly across seasons, suggesting that the correlations between these factors across seasons may add noise to the variation observed in this study. This then prompts caution when drawing conclusions about anthropogenic influence on microplastic concentration seen in this study. Given these potential sources of variation, future studies should exercise caution when selecting sampling dates and locations - accounting for environmental factors - to select dates that will best allow for uniform replication.

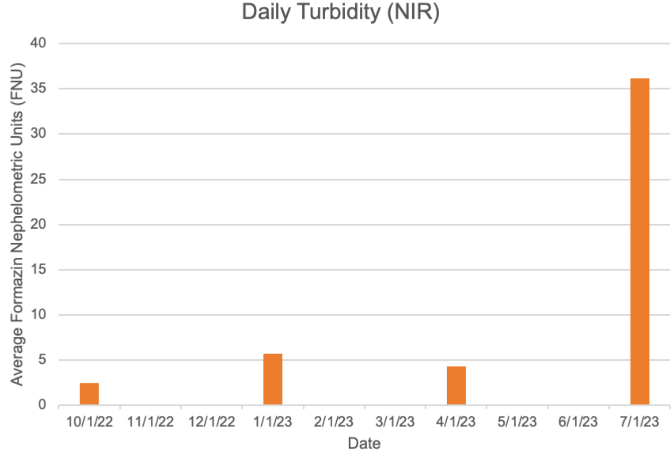


Figure 39: Turbidity on the Days that We Sampled as Reported by the Northeast Regional Stream Gauge (USGS, 2024)

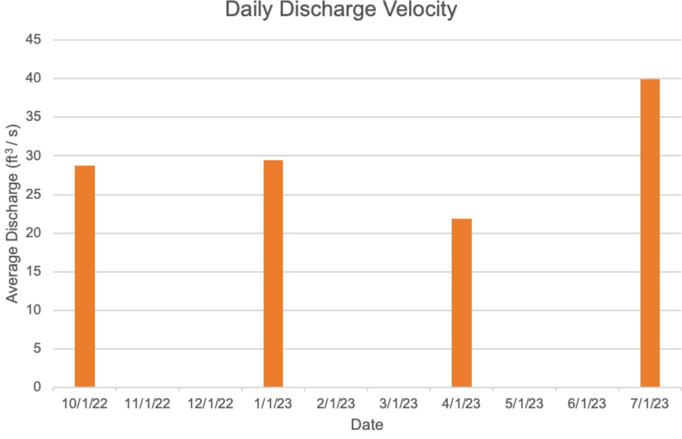


Figure 40: Discharge Volume on the Days that We Sampled as Reported by the Northeast Regional Stream Gauge (USGS, 2024)

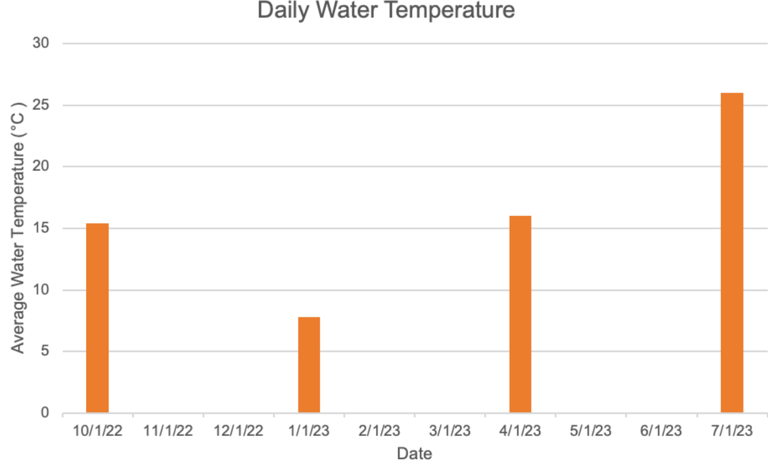


Figure 41: Water temperature on the days that we sampled as reported by the Northeast Regional Stream Gauge (USGS, 2024)

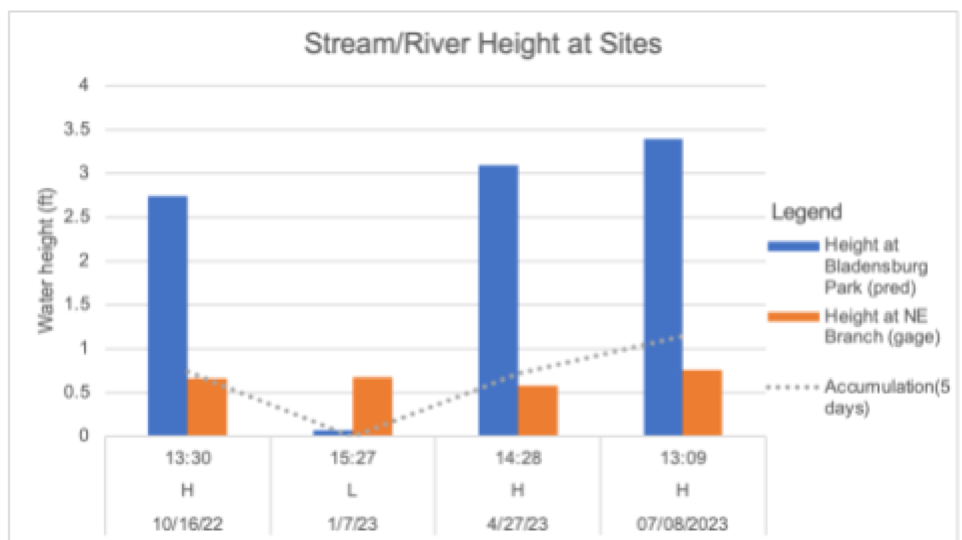


Figure 42: Water Height at both Bladensburg and Northeast Regional Stream Gauge Sites on Sample Days and Precipitation as Reported at NOAA HYATTSVILLE 0.7 N, MD US station (NCEI, 2024)

Our sample location at Bladensburg Waterfront Park is in a tidally influenced zone of the Anacostia River. As such, the daily tidal cycle can influence the concentration of microplastics at

this sample location, introducing a potential source of variation in our sample counts. At high tide, microplastics washed into the Anacostia by the upstream watershed meet at Bladensburg and likely stagnate at peak high tide, resulting in higher than average concentrations of microplastics (Ward, 1985). At the same time, microplastics from downstream can be carried upstream with the incoming tide, and the rising tide can also resuspend plastics that had been left on-shore by the previous tidal cycle, further increasing concentrations at Bladensburg (Stead et al., 2020). As such, collections and analysis conducted at a rising or high tide will likely experience higher levels of microplastics than those collected at a falling or low tide (Feng et al., 2023). Three out of our four samples collected at Bladensburg were collected during a rising or high tide, and therefore these samples likely represent an overestimate of the general microplastic levels. However, these tidal effects can also be confounded by other environmental variables, such as recent rain events and stream flow at the time of sampling, which could negate or reduce the effects of tidal trapping.

Table 11: Tidal Positions From Our Sampling Days as Reported by NOAA Anacostia Watershed Monitoring Site (NOS, 2024)

Date	Tidal Position
Fall (10/16/2022)	High
Winter (1/7/2023)	Low
Spring (4/27/2023)	High
Summer (07/08/2023)	High

Based on the statistical analysis and trends in the environmental factors, anthropogenic factors are likely responsible for variation in the results, as the differences in the data are not

fully explained by these trends (see Figure 42). Samples taken during the spring season have the highest amount of microplastics, however, almost all factors peak in summer (see Figures 39-41). Such trends are consistent with other studies' findings, such as in Australia where the abundance of microplastics increased 40 fold after storms (Kye et al., 2023; Hitchcock, 2020). Conversely, there was a strong correlation between water level and microplastics concentration in Netravati River, India (Kye et al., 2023; Amrutha and Warrier, 2020). As a result, it is important to consider both the normal environmental changes such as tidal shifts and precipitation, which may be biasing our microplastics counts, when addressing potential sources of anthropogenic introduction. Our Bladensburg site had by far the highest microplastics counts of our processed samples. This trend could be explained both by the historical pollution present in the Bladensburg watershed as well as ongoing construction occurring at the park with insufficient barriers to block plastic debris from entering the watershed.

Policy Implications

The location of this research is particularly fixated at relevant areas for environmental policy intiatives. From 1996 to present day, the Anacostia River has been listed by the Maryland Department of the Environment as an impaired water for many reasons: trash, polychlorinated biphenyls, total phosphorus, total nitrogen, biochemical oxygen demand, total suspended solids, enterococcus, heptachlor epoxide, chloride, sulfate, habitat alterations, and a lack of riparian buffer (*Maryland's Searchable Integrated Report Database [Combined 303(d)/305(b) List]*, 2022). Because of this, the Anacostia River requires a Total Maximum Daily Load (TMDL). Since the Anacostia River watershed covers land in both Maryland and the District of Columbia, a trash TMDL was created in cooperation between the Environmental Protection Agency Region 3, the District Department of the Environment, and Maryland Department of the Environment

(Total Maximum Daily Loads of Trash for the Anacostia River Watershed, Montgomery and Prince George's Counties, Maryland and the District of Columbia, 2010). The TMDL identifies impaired waterways, sets targets to limit pollution, establishes guidelines for monitoring, and summarizes trash reduction plans. This is an essential component of creating a collaborative, interjurisdictional plan for preventing plastic entry into the Anacostia River watershed, but there is an ongoing need to address the issue.

Creating a uniform methodology for quantifying or categorizing microplastics in waterways is an essential foundation for making policy changes that mitigate plastic pollution. The most impactful and cost-effective way of reducing environmental plastic pollution is through source reduction and intervention before plastics enter the environment (Environmental Protection Agency, 1990). This method is preferred over waste management and removal, as plastic breaks down once it enters the environment and collecting and separating plastic from other waste is a complicated process. Small countries with underdeveloped infrastructure for waste management tend to burn plastics to reduce their volume, emitting a significant amount of greenhouse gas and other poisonous gas in the process (Kibria et al., 2023). Therefore, plastics not only pose their own challenges to environmental and human health, but their disposal can also worsen the climate crisis.

Since secondary microplastics originate from weathered macroplastics, identifying where microplastics exist in high concentrations and predicting their source material can inform policy decisions to regulate plastic production. Data accessibility, quality, and sharing are three of the top four challenges faced by state governments when using data in the policymaking process (Bergh et al., 2018). However, having specific data and evidence to apply to an issue can make it easy to pass effective legislation regarding the issue (Bergh et al., 2018). In addition, anti-litter

campaigns and litter-management solutions can be effectively targeted to specific communities based on data about the types of litter that accumulate as well as the area in which it accumulates. Carpenter & Wolverton (2017) utilized a behavioral archaeology approach to understand the littering patterns in four sites along a stream in Texas and provide solutions. For instance, they posit that in an area with a great amount of plastic that is high access, a locally targeted campaign with signage both encouraging environmental stewardship and highlighting the consequences of littering in conjunction with media coverage and greater educational outreach would be the best strategy for reducing litter (Carpenter and Wolverton, 2017). In contrast, to reduce litter at a site with fewer direct sources of litter and more lightweight plastic, the study suggests plastic bag bans would yield the greatest effect. The site-specificity of plastic introduction into local environments requires specific strategies to yield the greatest effects. Thus, tailoring campaigns to reduce plastic waste towards local communities utilizing data from analysis of local sites would be an effective strategy for diminishing the amount of plastic found in local waterways.

One example of policymakers using local data to inform legislation has already occurred on the local College Park scale. In early 2023, the College Park City Council unanimously passed the Better Bag Bill, banning single-use plastic bags for College Park businesses (Wilson, 2023). Our preliminary research was cited during the hearing for the bill in College Park City Hall (Artero, 2023), and council member Maria Mackie was motivated to vote for the bill to keep plastic pollution out of the Anacostia watershed (Wilson, 2023). Similar initiatives have taken place in other major cities and states that have passed policies banning or restricting various forms of single-use plastics (Herberz et al., 2020). Plastic bag bans are generally supported by local residents, but their effectiveness is contingent upon enforcement, as a survey conducted in

Ohio found that consumers are not self-motivated to stop using plastic bags (Bartolotta & Hardy, 2021). With store policies and legislation, however, consumers used reusable bags and still supported businesses that enforced plastic bag taxes or did not offer plastic bags (Bartolotta & Hardy, 2021). In the long-term, disposable plastic bag taxes consistently incentivize consumers to use reusable bags and disincentivize plastic bag use, according to a study conducted in Montgomery County, Maryland, where a plastic bag tax was passed in 2011 (Simon, 2020).

On the national level, the Organization for Economic Co-operation and Development is directing policy efforts toward the regulation of microfibers shed from garments and tire and road wear particles (Organization for Economic Co-operation and Development [OECD], 2021). Emissions of these particles occur during several stages of their life cycles, allowing regulation at various stages of production and use. Although preventative measures early in the production processes are likely to be the most cost-effective of the solutions, improving waste management for these products and containing them before they reach the environment are also areas of possible regulation to diminish their presence in the environment. Regulating these particles is made more complicated by a lack of consensus in the community that studies microplastics, as well as a wide variation in sampling and characterization methods. This study seeks to rectify the gap in methods by proposing a streamlined method for collecting, processing, and analyzing freshwater microplastics samples. Standardizing the methods for collecting and processing microplastics will allow easier comparison of results across areas, which will increase the impact of data on policy making (OECD, 2021).

Much of the literature on plastics policy is international and focuses on marine macroplastics, specifically revolving around those related to the Pacific Garbage Patch. Policymakers are often prompted to pass legislation by seeing graphic photos of megafauna that

are negatively impacted by macroplastics (GESAMP, 2016). As a result, the lower visibility plastics and impacts of plastic are not targeted as often because they do not provide the same visual motivation. Regulating the production of larger plastics is an effective mechanism for reducing the amount of microplastics in the environment, as many of these microplastics are secondary microplastics. India targeted macroplastics through a single-use plastic ban and adopted extended producer responsibility to manage waste from plastic production (Singh & Biswas, 2023). To avoid economic losses and malpractice, lawmakers focused on investment in finding compostable plastic alternatives to plastic items. Enforcement teams and border checkpoints were also created to monitor the illegal manufacture, transport, and use of banned single-use plastics (Singh & Biswas, 2023). A holistic literature review of the effectiveness of plastic bag bans internationally found that plastic bag bans have been rendered ineffective by lapses in monitoring and enforcement (Muposhi et al., 2022). They are similarly undermined by corporations deflecting accountability to the consumer and national governments. The study also found that a global treaty that standardizes the ban could aid in the enforcement of the bans and prevent smuggling. Furthermore, another key to the effective bans is to encourage the voluntary participation of consumers through education and the promotion of reusable shopping bags (Muposhi et al., 2022).

The United Nations has various working groups dedicated to minimizing impacts from anthropogenic plastic production and consumption, primarily Working Group 40 of the Joint Group on the Scientific Aspects of Marine Environmental Protection (GESAMP). According to this report, much of microplastic contribution can be traced back to consumer use; however, upstream solutions enacted by producers are generally more cost-effective than downstream solutions (GESAMP, 2016). In the same report, GESAMP highlighted the importance of

collaborating with national governments, which should focus on investing in infrastructure, creating incentives for following government regulations, supporting research and development, and encouraging enhanced producer and consumer responsibility. Governments should promote private sector investment in green chemistry and other aspects of product design aimed at the reduction of microplastic generation and waste. Local governments can focus on proper wastewater treatment and port reception infrastructure to prevent microplastics from making their way into the marine environment (GESAMP, 2016).

On the local scale in College Park, Bladensburg offers fishing and boating activities, putting people in direct contact with the water that is downstream from construction sites. Microplastic concentrations seem to peak during springtime and remain higher in the summer-times when people are outside and more likely to come into contact with the water, especially children. Thus, creating local campaigns to prevent littering would directly benefit highly traversed sites, such as Bladensburg, where a great deal of litter comes from direct littering. Government regulations to discourage the use of plastic and promote the development and use of compostable products will diminish the damage caused by litter when these products inevitably make their way into the environment.

Across freshwater microplastics, levels are highest in urban and estuarine streams (Luo et al., 2019; Kye et al., 2023). A variety of land use covers our study sites, including designated park areas, parking lots, and roadside areas buffered by trees. Of the three sites included, the Iribe site was the closest to a major road, US Route 1 (see Figure 43). This factor is a way to track the potential prevalence of tire-related microplastics in the region (Kole et al. 2017; Davey et al., 2023). Collecting local data such as this will enable policymakers to create targeted

campaigns to reduce microplastics, such as by allowing them to determine if targeting consumers or producers will yield the greatest change.

Figure 43: Distance from Major Roads between Bladensburg, Golf Course, and Iribe Sampling Sites

Future Research Directions

This research contributes to the larger literature primarily in its focus on freshwater and economical methods in microplastic detection. For further development, we suggest integration of more computer vision assisted methods of quantifying microplastic concentration in samples, and sampling in freshwater areas that are more impacted by tidal cycles. Additionally future work should exclude the use of unfiltered potassium hydroxide pellets due to their plastics contamination. We believe future sampling should occur at standard tidal influx times and seasonality should be considered more robustly. We also recommend taking an optical density measurement at each site to have a quantified measurement of turbidity rather than one based on visual observations of the sample site, which could lead to better analysis of any relationship between turbidity and microplastics. We believe that the use of a pre-staining procedure is useful for limiting in-lab contamination from ambient sources. These ambient sources are important to

consider, especially when processing samples outside of a “clean room” setting as they have been found to contribute particles with diameter < 50 µm (Zobkov et al., 2019; Kye et al., 2023). We maintain an abundance of processed and filtered environmental samples which could be counted using fluorescence microscopy. However, these filtered samples have the same contamination issues as those counted in this report. As a result, future steps should involve sample collection and processing, using our same methodology with the exception of digestion with a pre-filtered concentrated potassium hydroxide solution.

A variety of questions remain in regard to microplastics which should be answered. Related to our work, we believe that more extensive quantification is important in local streams and waterways, especially in highly populated areas. We believe that our work helps to troubleshoot microplastics methodology for these samples and lays the groundwork for more effective quantification in the future.

Equity Impact Analysis

Establishing an equity impact statement for this research is crucial for ensuring that the project has the intended social impacts and avoids undue harm to impacted communities. In establishing this report, the first step is to identify stakeholders and currently or potentially impacted communities. As members of an affluent campus community upstream from communities threatened by environmental injustice, our project aims to quantify our community’s contribution to microplastic quantities in the watershed in order to create a foundation for policies addressing the issue.

Environmental Justice

Environmental pollutants such as microplastics pose a widespread threat once they enter the environment. Preliminary research indicates that microplastics are prolific in the human

body. Microplastics have been identified in human blood, lung tissue, breastmilk, and placenta (Goodman et al., 2021; Ragusa et al., 2022). Researchers have begun to report adverse effects of microplastics on human health, such as respiratory inflammation and lesions, inhibition of cell proliferation, oxidative stress, and an accumulation in cells (Blackburn & Green, 2022; Goodman et al., 2021). While research defining the full extent of microplastic impacts on human health is ongoing, the pervasiveness of these pollutants is enough to require expanded research into microplastic quantities and concentrations in the environment. In order to figure out where to target remediation efforts, we need to know how microplastics are distributed to focus our efforts on the hardest hit areas. Additionally, we need to be able to compare results across studies in order to understand the relative severity of the areas. By looking to create an accessible and uniform collection and quantification procedure, we hope to enable cross comparison between studies and help policymakers learn where to target remediation policies.

Our watershed of study covers communities with a variety of resources to support health access, environmental safety, and climate change resilience. The two southernmost sampling sites, NE Gauge and Bladensburg, are located in areas that face higher levels of environmental injustice than the rest of the sites. According to the EPA's EJScreen, NE Gauge and Bladensburg face the highest levels of diesel particulate matter, traffic proximity, and superfund proximity, among other environmental justice indices (US EPA, 2014). Additionally, the EJScreen identifies that the NE Gauge sampling site is within the 95 - 100 percentile for lack of health insurance, and the Bladensburg site is within the 90 - 95 percentile for the same. These are not the only disparities these communities face, and the EJScreen does not quantify microplastics, but they contribute to the larger, important narrative that communities downstream from College Park are subject to higher levels of environmental injustice.

Microplastics within our watershed of study, the Anacostia watershed, affect the community downstream in Bladensburg, the wider Washington, DC area, and all residents who rely on food harvested or grown in the Chesapeake Bay region. Bladensburg, MD is an environmental justice hotspot within Prince George's County, which is 67% Black, faces some of the highest levels of environmental injustice in the state (U.S. Census Bureau, 2021; Dosu et al., 2019; Ieronimo, 2021). Marine litter and microplastics can be much more highly concentrated in vulnerable communities, especially those that depend on wild seafood, so through quantifying microplastics in our local watershed, we are providing the baseline information required to create policies that will prevent environmental pollutants from entering the community (UN News, 2021).

We place particular emphasis on the potential health impacts of Washington, DC residents consuming water collected from the Potomac River leading into the Chesapeake Bay. While socioeconomically advantaged populations may have the opportunity to drink water or eat food from outside sources that are free from microplastics, less affluent populations have little autonomy over their exposure through ingestion. This only adds to the existing discrepancy between urban versus non-urban environments, given urban environments consistently have much higher concentrations of microplastics in water bodies and sediments. As a result, this disparity of health opportunities creates inequities that may have far-reaching effects on overall health and well-being. From an economic perspective, the majority of seafood harvesters living in the Delmarva region are reliant on Chesapeake Bay organisms. Given how microplastics are known to move up food webs and bioaccumulate in higher-level predators, like larger fish and crabs, the influx of microplastics can have negative financial implications for an already economically struggling population of seafood harvesters (Blackburn & Green, 2022).

As previously mentioned, this is just the tip of the iceberg and microplastics can impact all populations. However, with these populations in mind, we define the primary way that a positive impact for equity on this issue could be made is to (1) create an informative map about microplastic exposure that is made available to the public and (2) suggest policy to the Maryland Department of the Environment based on the findings from microplastic research (Maryland Department of the Environment, 2023). Completing these goals will provide impacted communities with easily accessible information on microplastics so that they can better influence policy decisions. Finally, the policy shift must be written with equity and anti-discrimination principles in mind to protect low-income communities and other vulnerable populations from environmental hazards. Often, the burden of environmental laws can fall on low-income populations, because they don't have the resources to completely transform their consumption in order to abide by the new policies. Instead of creating taxes and fees for the consumer, solutions can focus on the producers of plastics via quotas and pollution control.

Accessible Protocols

Our project also creates the potential for the protocols that we developed to be adopted by other labs facing similar resource constraints. While the costs of some materials we used were high, and we did not have to pay costs upfront to access our microscope, our collection apparatus was made with inexpensive materials that are available to the average consumer. This method is effective in creek surface waters and could be adapted for local citizen science initiatives (Barrows et al., 2018). This could address the information gap of microplastic quantities and contribute to a more comprehensive understanding of microplastic concentrations in waterways in different locations.

Budget Addendum

Our team received funding from various sources including: Gemstone Honors Program, Sustainability Mini-Grant, Sea Grant, The Do Good Institute, and from Giving Day donors. This budget funded our lab operations throughout the duration of the project from Spring 2021 through Spring 2024. Throughout this research, we purchased various materials including: glass bottles, sieves, dyes, microscope repairs, and other lab equipment. This budget also funded members of our team to attend the SETAC Conference in Seville, Spain.

We are grateful for the contributions of our research lab, the Gemstone Honors Program, the University of Maryland, and all other generous donors.

Acknowledgments

Team MARINE would like to extend our deepest gratitude to all who helped make this research possible. We would like to first and foremost thank our incredible mentor Dr. Lance Yonkos for his unwavering support and encouragement throughout this process and for the expertise he brought to our research, as well as Sabine Malik and Rosie Vichi for their assistance in developing our methodology. We would like to thank the Maryland Sea Grant and our donors for their financial support. Thank you to Wenyu Chen, Matt Chaisson, Ryan Halli, Nicholas Portwood, and Andrew Zhong for their contributions to the team. Finally, we would like to extend our gratitude to our librarian Ben Shaw, Dr. David Lovell, Dr. Allison Lansverk, and the entire Gemstone Program and its many staff members, both present and past, for helping make this incredible research program possible and for providing us with this incredible research opportunity.

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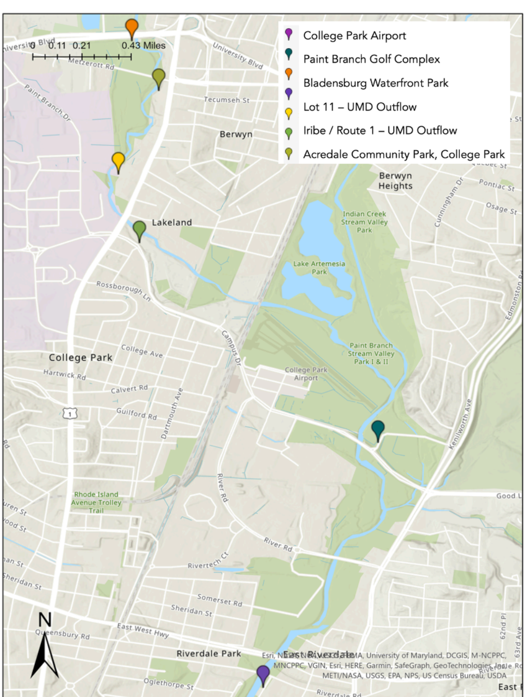
Appendix A

Methodology Protocols

A. Collect.
    a. Ensure all materials are thoroughly washed with double filtered DI water (deionized water that has been passed through a glass fiber filter twice) prior to leaving the laboratory and fill up the control sample with double filtered DI water.
        i. On each bottle, rip off a piece of tape and put it on the side of the bottle.
        ii. Fill one of the cleaned bottles with 1 L of DI water to act as the control.
        iii. Label this bottle with the first number based on the sampling run followed by two zeros (ex. The third sampling run's control is 300).
    b. Carefully pack all of the materials into a car and travel to each of the seven sites
        i. All of the sites are labeled (bit.ly/samplingsites)

ii. 

iii. All sites have parking either directly at the site or adjacent to the site.

c. After arriving at the site, walk to the edge of the water and lay out all materials where they are easily accessible.

   i. Appropriately label the bottles with the (Sample Run #, Site #, Replicate #).

d. Position the control bottle on a flat surface and remove the cap from the bottle so that it is exposed to the air for the duration of the collection.

e. Next, ensure that the apparatus is correctly hooked and wade into the water with the open apparatus.

   i. Warning: Wear Rain Boots when wading into the river (At All Times).

   ii. Warning: Do not enter the water if flooding or rushing white water.

f. Then, allow for the sediment to settle, and then dip the apparatus approximately 4 inches beneath the surface for 30 seconds.

i. It is important to allow the dust to settle from wading into the water.

g. After 15 seconds, pull the string on the apparatus while it is still underwater.

h. Lift the apparatus out of the water and pour the contents of the apparatus into an already open, and appropriately labeled, 1 L Glass Jar.

  i. If the sample is less than 1 L, redo and collect again.

  ii. If the sample is over 1 L, shake and immediately pour out the excess.

i. Cap the Glass Jar, rehook the apparatus, and collect a second replicate.

j. After both replicates are collected, cap the control sample, and carefully return all of the materials to the car.

k. Repeat Steps #3-10 for all seven sample sites.

l. After all samples are collected, return to the lab and clean any unused bottles, the apparatus, and funnel with DI water.

m. After returning to the lab, shake each sample and dump out any excess until the sample is exactly 1 L (marked by the line near the neck of the bottle).

B. Stain and Digest.

a. Put the beaker on the scale and tare the scale (Hit the big red “T” button).

b. Measure out 56g of KOH tablets on the scale.

c. Add 5mL of Nile Red to each sample bottle.

d. Pour the KOH tablets into the 1 L sample bottle, tightly close the lid and shake the sample until dissolved and the bottle is warm.

  i. The solution should be cloudy and turbid.

e. Put sample bottle in the shaker (Big white machine near fish tank).

f. After all of the samples are loaded up, shut the lid of the shaker. Select the bottom right blue button to turn the light on. Then, flip the switch so that the shaker is on.

g. The shaker should run for approximately 48 hours (err on the longer side if necessary).

h. Clean up all materials and wash any necessary materials. Make sure that the excess sample is disposed of appropriately into the Nitric Acid Waste Container.

C. Sieve.

a. Rinse the sieve and equipment with regular DI water to clean it.

b. Put the sieve in an open wide glass container that can hold at a minimum 1 L of water.

i. If the glass container fills up, you can discard the excess water in a KOH waste container.

c. Slowly pour the stained and digested sample into the 20µm sieve (do NOT shake the sample) to avoid overflowing the sieve (where you would need to restart).

i. When you get to the last 200ml, shake the sample to loosen any particulate matter off of the bottom of the container.

ii. Then, pour the rest of the sample through. If residue is stuck to the bottom of the sampling bottle, take a 25ml (or as much as necessary) 50% nitric acid and shake until residue is suspended (only if really necessary - not needed for every sample).

d. Afterwards, rinse the sample container with Double Filtered DI water.

e. Next, remove the sieve from the waste water container and tap and rinse the sieve to get as much sediment through as possible. Do this by rinsing it all to the bottom of the sieve using a DI water squirt bottle.

f. Dispose of waste water in KOH container and rinse out the open glass container.

g. Using a funnel and a conical centrifuge tube, turn the sieve over and rinse the sediment into the centrifuge tube using a zinc chloride squirt bottle. (Try not to fill the centrifuge tube past 27.5 mL).

h. Weigh the centrifuge tube and fill another tube with an equal weight of water (margin of error 1g).

i. Centrifuge sediment for 10 minutes.

j. Move on to filtering.

k. Clean-up: we tried submerging in water and that seemed to work but technically we are supposed to by an ultrasonic shaker.

D. Filter.

a. Put the hourglass filter on top of the 1000mL erlenmeyer flask.

b. Using tweezers, put an oven baked filter with the rough side facing up centered on top of the glass filter.

c. Take a 250mL ridged glass beaker on top of the filter.

d. Attach a clamp that holds the 250mL ridged beaker and the 1000mL erlenmeyer flask together.

e. Next, take one of the gray tubes and attach it to the vacuum adapter.

f. Then, turn on the vacuum (Yellow Knob) by turning it counterclockwise.

g. Carefully decant the sample out of the centrifuge tube onto the filter.

h. Immediately after, turn the red knob counterclockwise until vertical and parallel with the gray tube.

i. Pour the remaining sample (a little bit at a time) into the glass filter until all of the remaining sample flows through.

j. Throughout the process at any time when not adding water, cover the top of the 250mL ridged beaker with tin foil.

k. After pouring the sample onto the filter, add 10-15 mL zinc chloride to the bottle and re-centrifuge for 10 minutes and pour back over the filter.

l. Once all of the sample has passed through, add 100mL of filtered DI and pass that through the filter.

m. Cover and wait 10 minutes to allow the filter to dry.

Appendix B

Results Supplementary Figures

Sample ID	Season	Site Name	Small	Large	Fibers	Nodes	Total
100	Fall	Control	686.6666667	272	26.33333333	10.33333333	995.3333333
111	Fall	Bladensburg	1672.333333	919	63.66666667	37.33333333	2692.333333
112	Fall	Bladensburg	1077	104	13.5	6	1200.5
141	Fall	Iribe	269	58.5	10	8.5	346
142	Fall	Iribe	494.6666667	65.66666667	13	10.33333333	583.6666667
171	Fall	Golf Course	860	146.3333333	55	23	1084.333333
172	Fall	Golf Course	741	122.3333333	40.33333333	11.33333333	915
300	Winter	Control	853.6666667	399	29.66666667	11.66666667	1294
311	Winter	Bladensburg	1104	98	6.5	6	1214.5
312	Winter	Bladensburg	383	51.5	5.5	3.5	443.5
341	Winter	Iribe	477	26	8	12	523
342	Winter	Iribe	370.5	9	7	7	393.5
371	Winter	Golf Course	437	73.33333333	11.66666667	0.666666667	522.6666667
372	Winter	Golf Course	494	153	16.66666667	12	675.6666667
600	Spring	Control	667	152.5	66	220.5	1106

Sample ID	Season	Site Name	Small	Large	Fibers	Nodes	Total
611	Spring	Bladensburg	1873	693.5	81	231	2878.5
612	Spring	Bladensburg	1479.5	131.5	35	186	1832
641	Spring	Iribe	1746.5	314.5	60.5	5	2126.5
642	Spring	Iribe	773	217.6666667	27	17.66666667	1035.333333
671	Spring	Golf Course	601	518	32	26.5	1177.5
672	Spring	Golf Course	819	185.5	38.5	24	1067
900	Summer	Control	544	116	38	15	713
911	Summer	Bladensburg	821	45.5	21	92.5	980
912	Summer	Bladensburg	959.3333333	132.6666667	43	85	1220
941	Summer	Iribe	975	56.33333333	47	36.33333333	1114.666667
971	Summer	Golf Course	273.5	98	47.5	2	421
972	Summer	Golf Course	1277.5	214	27	26.5	1545

Note. This table includes averaged results from individual counters for each sample at each site and during each season by particle type.

Appendix C

Budget Addendum

Provided below is an itemized receipt of what was purchased for our project. The largest expense was the Glass Bottles which were purchased for our collection procedure.

Order Date	Item Name	Price	Description
5/2/2022	Glass Bottle	254.58	FISHER SCIENTIFIC Reusable Glass Media Bottles with Cap
9/7/2022	Sedgewick Rafter	131.78	FORESTRY SUPPLIERS Sedgewick-Rafter Counting Cell (#77227)
9/7/2022	Glass Bottle	274.89	FISHER SCIENTIFIC 3 PYREX Reusable Media Storage Bottles (#06-414-1D)
12/20/2022	Sieves	261.33	Hogentogler-2 µm Sieves (#1325)
1/20/2023	Glass Bottle	176.04	FISHER SCIENTIFIC 2 1L Glass Bottles (#FB8001000)
4/27/2023	Glass Filters	576.21	FISHER SCIENTIFIC: 3 Glass Fiber Filters, Plastic Petri Slides & Pot Hydrox
5/2/2023	Microscope Repair	290	Microscope Repair from Alpha & Omega Microscopes
5/10/2023	Sieve	156.40	Hogentogler-1325 3" Test Sieve, No 635 Mesh, Full Height, Stainless Frame, Stainless Cloth
8//28/2023	Potassium Hydroxide	201.65	Potassium hydroxide, ca. 85%, for analysis, pellets, Thermo Scientific Chemicals (2500g)