

**Analyzing Select Hydrosphere Protocols
on Seasonal Samples of Microplastic
Concentrations Among Southeast's Rouge River**

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Abstract:

This research investigates the potential effects of seasonal changes on concentrations of microplastics and the influence of microplastic concentration on select hydrosphere and atmospheric parameters. Three samples were taken from the Parr Wayside site located in the Middle Branch of the Rouge River. This site was selected due to its proximity to various urban outlets, such as an elementary school, suburban communities, and public parks. Select parameters included conductivity, pH, turbidity, and water and air temperature. The Vernier series helped analyze conductivity, water temperature, and air temperature. In addition, the Hach 2100N turbidimeter and Hach Phenol Red kit were used to measure turbidity and pH, respectively. The researchers findings concluded that turbidity and conductivity are inversely proportional microplastic concentrations. Though pH levels did increase with every sample taken, the correlation was modest. In the future, the researcher hopes to expand the research to include additional hydrosphere parameters and multiple sites along the Lower Branch of the Rouge River to establish possible sources of microplastics in the Rouge River. Increasing the level of understanding in these areas is essential if to develop appropriate policy and management tools to address this emerging issue. Thus, to extend the scope of this research, the researchers hope for the implementation of regulations to monitor the impacts of microplastics as they are still poorly understood.

Key words: Hydrosphere protocols, Rouge River, microplastics, urban

Research Questions:

1. To what extent are microplastic concentrations impacted by seasonal change and accompanying precipitation?
2. How are pH, turbidity, conductivity, water and air temperatures affected by microplastic concentrations?
3. To what extent are microplastic concentrations impacted by urbanization?

Null Hypothesis:

1. Seasonal factors including snow melting and rainfall would not affect the concentrations of microplastics.
2. pH, turbidity, conductivity, water and air temperature will not be affected by the presence of microplastics.
3. Microplastic concentrations will not be affected in areas of urbanization.



Figure 1a. and Figure 1b. Site Visualization. The satellite image on the left shows the surrounding area of the sampling site. This site is located near many community outlets such as Parr Park, multiple trails and Hillcrest Elementary School. The image on the right is a close-up of the site in the lower branch of the Rouge River. This site was tested for all three samples.

Introduction and Review of Literature:

Plastics, due to their durability and affordability, have dominated modern industry, being utilized in almost every industrial sector, and finding their way into our daily lives. As plastic use continues to rise, a new wave of pollution has emerged: plastic pollution. Despite marine ecosystem pollution via microplastics becoming an increasing world concern, few studies have been conducted on the impact of microplastics in aquatic environments. Unlike large plastic debris that can be observed by the human eye, microplastics, defined as plastic particles <5 mm in size, are only discernible with use of microscopy. Through sunlight, wind, and waves, large plastic debris is broken down into smaller and smaller particles, posing a greater risk to living organisms if ingested (Bajt, 2021). Rivers play a crucial role in the transportation of microplastics due to their swift ability to capture and transport microplastics over vast locations.

Microplastic pollution can enter waterways from a variety of sources such as industrial effluent and urban runoff (Prata, 2018). Microplastic pollution can also originate from household waste as wastewater. Often originating from daily household tasks such as washing clothes or vacuuming carpets, countless microplastics are distributed every day. These plastics are often filled with additives to enhance their durability, flexibility, and other mechanical properties. These plastics are then either ingested by aquatic life or further weathered and leached into bodies of water (Cole et al., 2011). This leaching can further harm aquatic life as they continue to ingest even more plastics. Another growing concern is secondary microplastics. A notable example is synthetic fibers. Garments containing synthetic fibers can release >1,900 fibers per wash in a domestic household washer (Browne et al., 2011).

While preventing the degradation of microplastics is a hopeful solution, it is not currently viable with modern-day technology. Rather, decreasing the input of microplastics into waterways is a more productive process. The abundance of small plastic items within rivers such as bottle caps and plastic pellets pose a significant concern for marine life because of potential chemical leaching from the plastics after they have been ingested (Eerkes-Medrano et al., 2015). As microplastic research expands, furthering studies focused on chemical leaching from compounds is critical to the health of our aquatic ecosystems.

Additionally, developing solutions to decrease or stop the distribution of microplastics into waterways is crucial for efforts to protect waterways across the globe.

Monitoring microplastics is of utmost importance regarding the safety of the ecosystem as there are increasing concern in aquatic environments due to the ecotoxicological risks they pose, where

ingestion by a range of species can compromise energy reserves and can bioaccumulate and biomagnify through the food chain (Aktogan 2019). There is a lack of comprehensive research on microplastics and their common origins. Most of the research tends to favor the Middle Branch, including their distribution and impacts on ecosystems. The Lower Branch determines the source and potential pathways microplastics are distributed but isn't as thoroughly researched. If researchers were to further investigate the Lower Branch, the root of microplastics could be determined, allowing researchers to further develop an understanding and establish possible solutions. Therefore, analyzing concentrations of microplastics can help reduce the degradation of further plastic in the marine environment.

Research Methods and Materials:

Throughout the Fall and Winter of 2023, a variety of water quality and air temperature data was collected in Dearborn Heights, MI. The site location chosen was the Parr Wayside, where the Rouge River passes through various urban area where families spend quality recreation time together. Near-by parks are also well-known centers where individuals can barbecue, ride bikes, and hike. The researchers selected Parr Wayside as the primary sampling location due to its proximity to a nearby district school, Hillcrest Elementary. Knowing the amount of microplastics present in local bodies of water within highly urbanized areas is essential to more fully understand the potential impact on the watershed. Using a Nasco Swing Sampler (12ft), Hach Phenol Red pH kit, Vernier LabQuest, Vernier conductivity probe, and Vernier temperature probe, we were able to measure the water pH, water and air temperature, turbidity, and conductivity during the months of November 2023 to February 2024. To minimize contamination and a skew in results, the researchers used 100% cotton-based materials when

sampling and testing the river water. Before conducting their research, they prepared a positive control sample. Loading multiple clothing pieces that were composed of either 100% polyester or 100% acrylic into a domestic washing machine, researchers sampled 500-mL of the water before the load was drained. This allowed a comparison of known fibers with those that could possibly be found in the river. The researchers took three of the samples at Parr Wayside site, located in the Middle Branch of the Rouge River. After collecting the water sample using a Nasco Swing sampler, they tested for the water temperature in degrees Celsius ($^{\circ}\text{C}$), using the Vernier LabQuest 2. Researchers then tested conductivity using a Vernier web conductivity probe and tested for pH using Hach Phenol Red test kit. The sample was taken back to Crestwood High School, Dearborn Heights, MI for further testing and filtration. Since samples may have settled between the time of collection and analysis, samples were shaken for 30 seconds before continuing with testing.



Figure 2a and Figure 2b. Collecting Samples. The image on the far left shows a student researcher collecting a 500ml (about 16.91 oz) sample of Rouge River water. In the picture on the right both student researchers are transferring 100ml (about 3.38 oz) from the 500ml (about 16.91 oz) sampling bottle.



Figure 3a and Figure 3b. pH Sampling: In the image on the left student researchers are dropping 4 drops of phenol red to for the pH of the water. The image on the right shows a student researcher analyzing the water sample and phenol red indicator to find the samples pH.

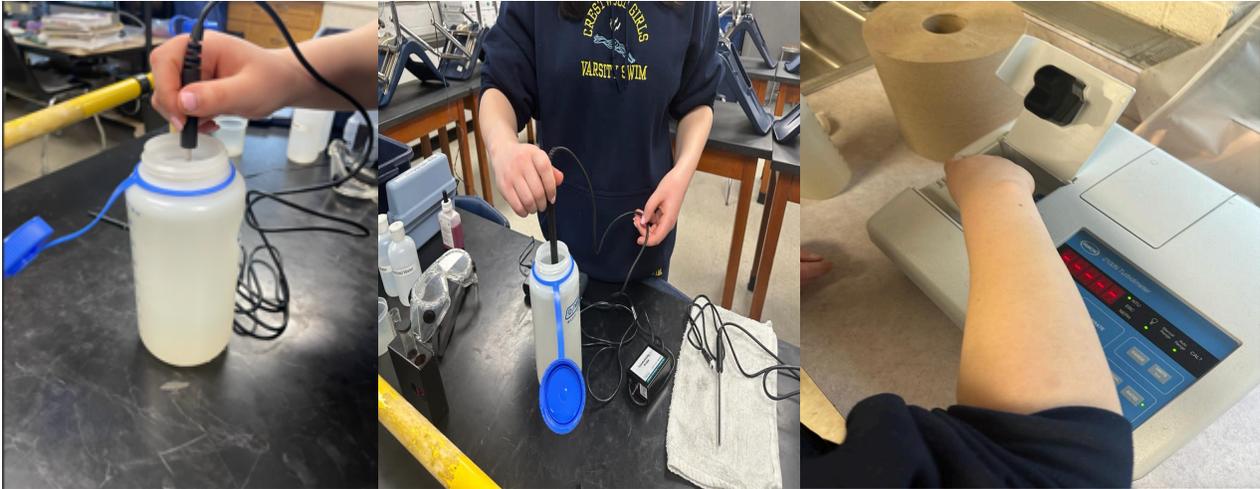


Figure 4a. Figure 4b. and Figure 4c. The image on the left shows a student researcher measuring the Rouge River water sample for water temperature. The image in the middle shows a student researcher measuring the sample for conductivity. The image on the right demonstrates a student researcher inputting the Rouge River water sample into the Hach 2100N Turbidimeter to analyze turbidity.

When filtering for microplastics, a 250-mL Nalgene filtration system and MF-Milipore 0.45 membrane filters were used. Considering our original Sample was taken in a 500-mL polyethylene bottle, the filtering process was repeated twice. To transmit the water through the filtering body, a vacuum was created in the lower compartment using a syringe. Researchers pulled the piston of the syringe and ejected the air. This process was repeated until all 500-ml of the water sample was passed through the filter paper. After filtration was completed, the MF-Milipore filter paper was removed and transferred to a petri dish. Researchers analyzed the 4 quadrants of the filter paper through a Parco LTM-400 series compound microscope and took images of each corresponding sector. After analyzing the microplastics, researchers distinguished the microplastics into 4 classifications: cellulose textile fibers, man-made fibers, plastic pieces, animal textile fibers, and unknown. Microplastics were classified under “unknown” if the filament but cannot be identified to fit one of the four categories. This analysis was done for all three water samples and the positive control sample. The data was then organized into a excel sheet where the data such as geometry, color, surface appearance, and assessment (cellulose textile fibers, man-made fibers, plastic pieces, animal textile fibers, and unknown) were monitored. Cellulose textile fibers are derived from the fibers of plants and are reported because many textile processing companies typically add chemical additives, such as plastic coatings, that make them a transporter of microplastics. These microplastics are classified under the category of cellulose textile if they had irregular twists and bends with irregular sections. Animal textile fibers are sourced from animals like silk, wool, cashmere, etc. Like cellulose textile fibers, animal textile fibers were reported due to the additives that could potentially transport microplastics. Animal textile fibers are identified by having a constant diameter over the length, soft bends, porous appearance, and sometimes with ends that are fibrillated. Man-made fibers are

classified as primary microplastics and though some are degradable in the long term, their plastic coating can contribute to microplastic pollution. Man-made fibers are highly dense; thus, they are not found on the surface, but rather mostly in sediment or agitated water bodies. To identify man-made fibers, researchers looked for flat section with non-frayed edges referred to as a “cut noodle”.

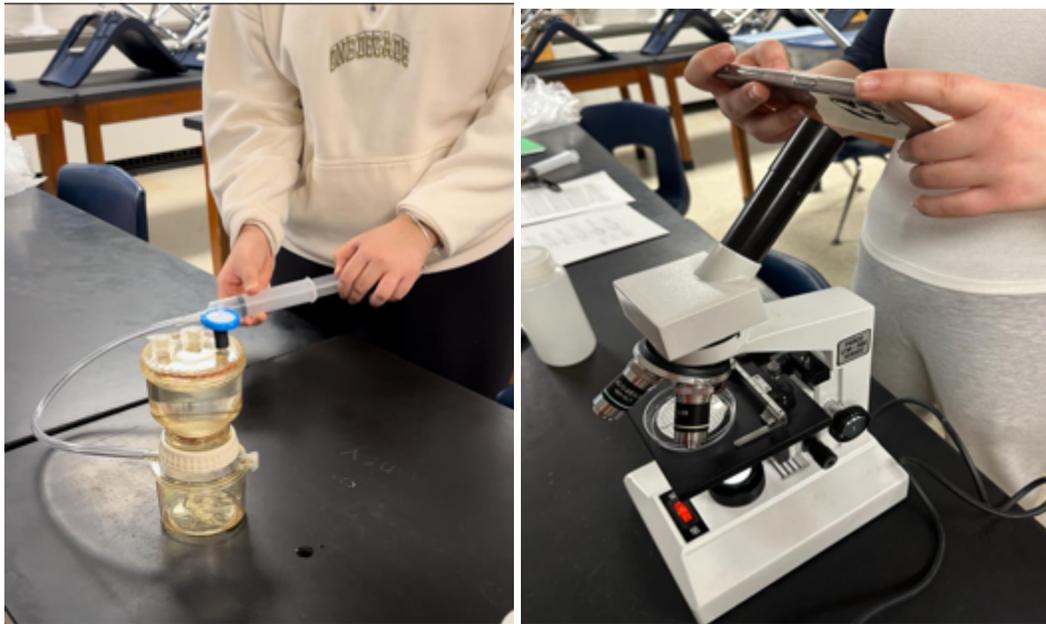


Figure 5A and Figure 5B. The image on the left shows a student researcher releasing air pressure from the Nalgene filtration vacuum and completing the filtration. The image on the right shows a student researcher taking images of the microplastic filtration system from a Parco LTM-400 series compound microscope.

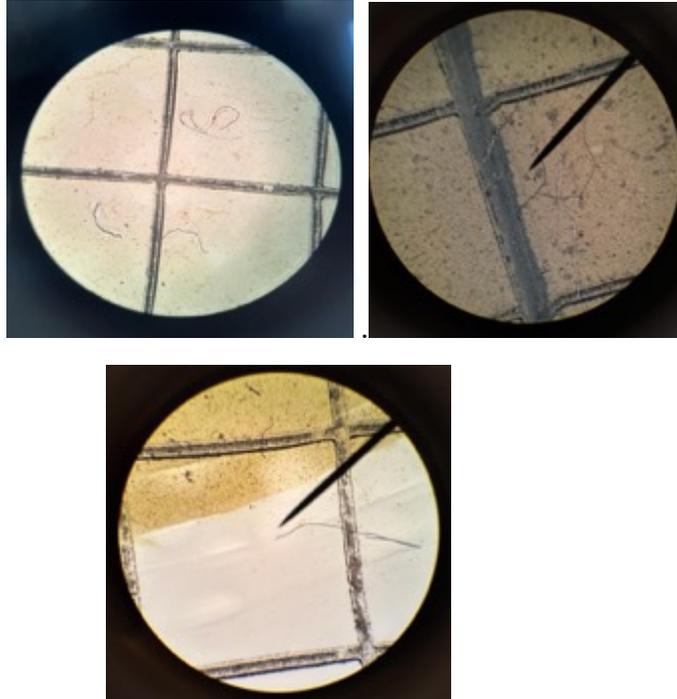


Figure 6a. Figure 6b. and Figure 6c. Microplastic Identification. The image on the left shows Sample 1, Quadrant 1 of the Rouge River sample, where visible concentrations of cellulose fiber, man-made fiber and animal fibers. The image on the right shows Sample 2, Quadrant 2 of the Rouge River water after flooding. Due to increased sediment that embedded possible microplastics, identifying microplastics required deep observation. However, cellulose fibers are still visible. The image on the bottom is from Sample 3, Quadrant 2. In this image, cellulose fiber, animal textile fiber, and man-made fibers are found.

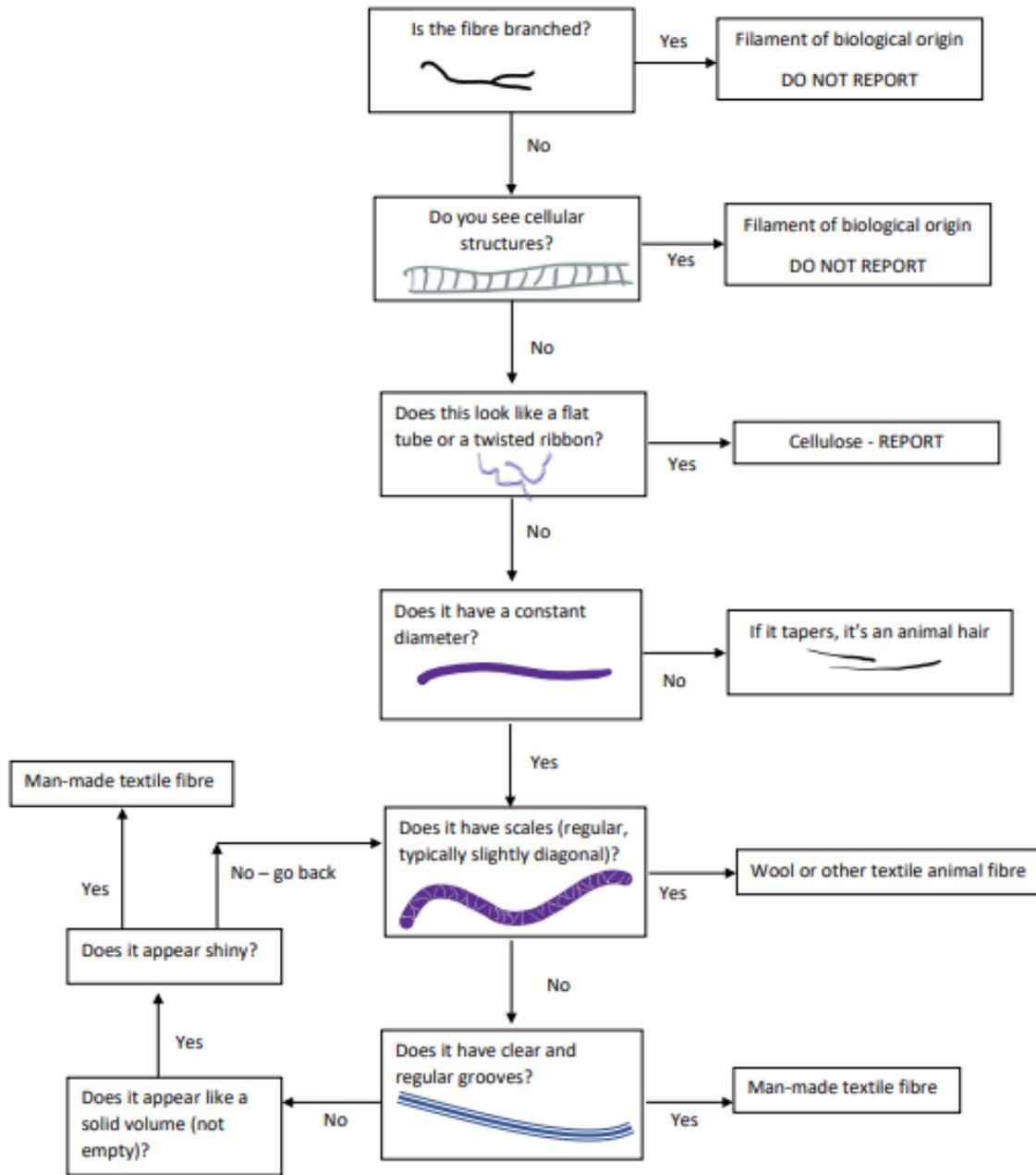


Figure 7. Microplastics identification guide. Shown above is a protocol included in a guide that Ms. Tracy Ostrom provided for identifying microplastics.

Results:

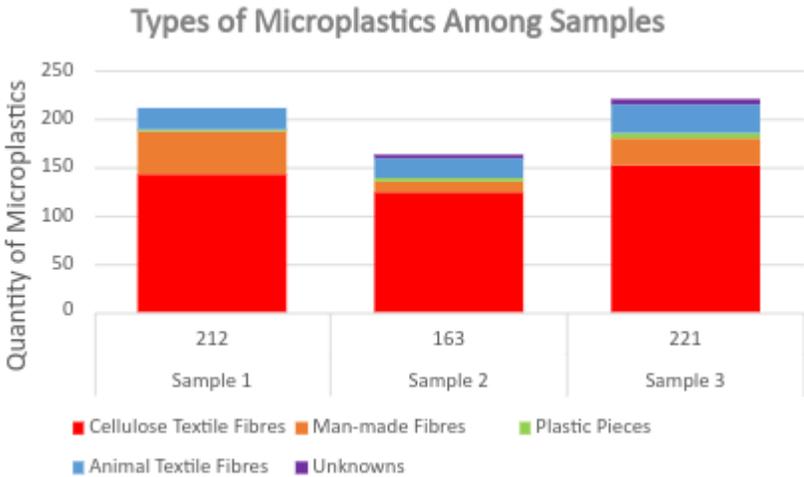


Figure 8. Types of Microplastics Among Samples. The bar graph above shows the comparison of different types of microplastics within each of the three samples.

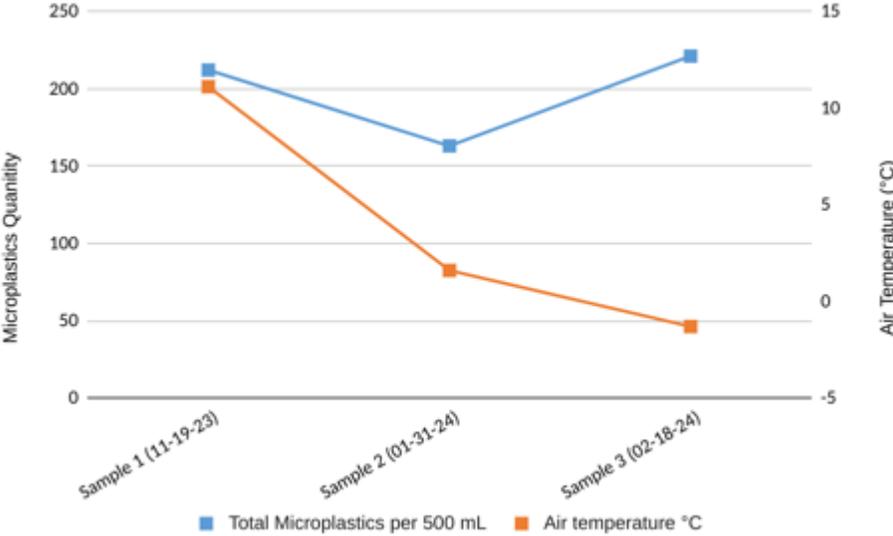


Figure 9. Air temperature °C Vs. Total Microplastics per 500-mL. The line graph above shows the comparison of air temperature and total microplastics identified between each sample of 500-mL.

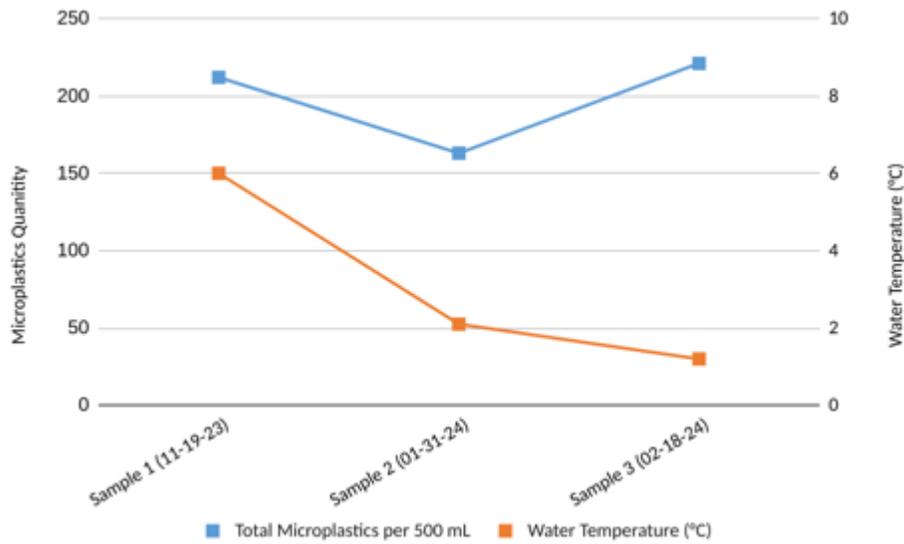


Figure 10. Water Temperature (°C) Vs. Total Microplastics per 500-mL. The line graph above shows the comparison of water temperature to the total quantity of microplastics.

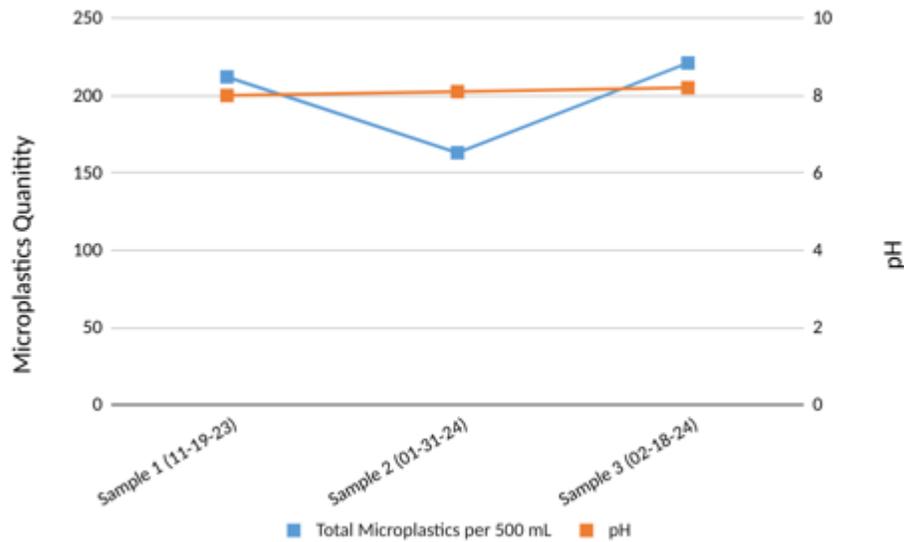


Figure 11. pH Vs. Total Microplastics per 500-mL. The line graph above shows the comparisons of pH to the total quantity of Microplastics for each of the three samples. Though microplastic quantities fluctuated between samples, pH values remained relatively constant with a pH of 8.0 for Sample 1, 8.1 for Sample 2, and 8.2 for Sample 3.

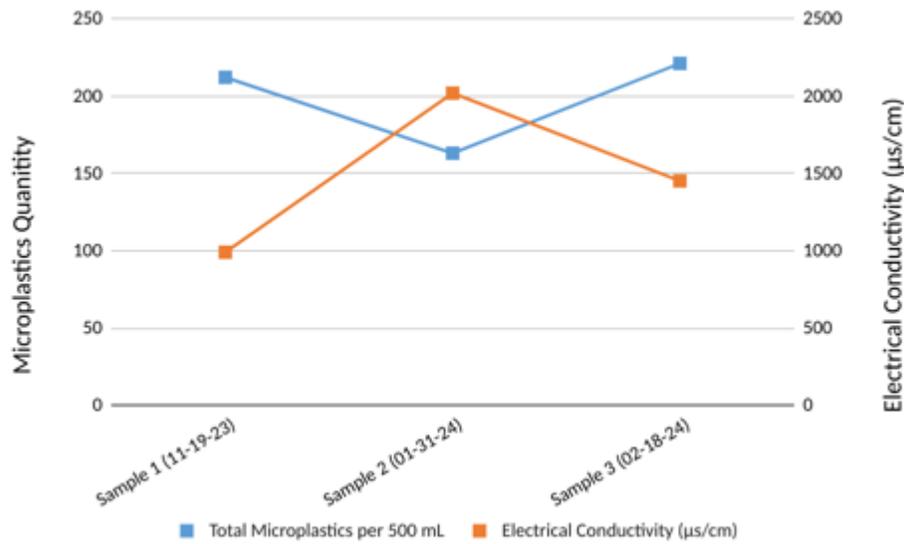


Figure 12. Electrical Conductivity (µs/cm) Vs. Total Microplastics per 500-mL. The line graph above shows the comparison of conductivity and the total amount of microplastics between the three samples. The data shows electrical conductivity to be inversely related to the amount of microplastics identified.

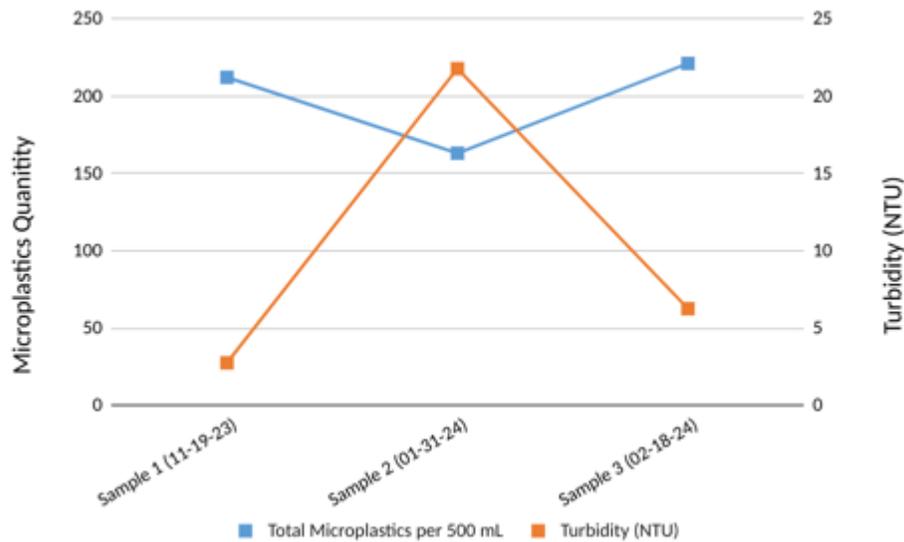


Figure 13. Turbidity (NTU) Vs. Total Microplastics per 500-mL. The line graph above shows the correlation between turbidity in NTU to the total abundance of microplastics. The correlation between microplastics and turbidity is inversely related.

Discussion:

The results of these experiments varied in supporting the hypotheses made. Through the hypothesis, parameters like water temperature, air temperature and pH levels were accepted due to not displaying correlation with microplastic concentrations. Turbidity and conductivity presented clear relations based on microplastic concentrations. As microplastic concentrations increased, turbidity and conductivity decreased. This could be as result of the increased sediment in Sample 2 which hindered the visibility of microplastics, landing to a decreased quantity documented. The researchers' third hypothesis of potential urban effects on microplastic concentrations could not be concluded due to the limited number of samples.

Considering the insufficient number of samples, implementing a more frequent testing schedule is a critical goal of establishing concrete correlation between all four seasons on microplastic concentration. A longitudinal study would be preferable as it would account for the potential correlation between seasonal changes and microplastic densities which in turn can affect concentrations. A larger data sample would allow a more comprehensive and statistically significant conclusion as it will reduce standard error and outliers. In addition, the researchers would also like to expand testing locations, having made plans to test at two additional locations located in the Lower Branch and Main Branch. Expanding testing locations to include other branches of the Rouge River allows us to conclusively analyze the distribution of microplastics and the possible source of these microplastics. Testing in the Lower Branch where the river flows through the Detroit River Wastewater Treatment Plant can help identify potential sources of microplastic input. The researchers could also confirm the causes of the increased microplastic

types due to the seasonal factors surrounding the testing location. With all these modifications, the researchers know that the project can be monumental for their community.

Conclusions:

The researchers conducted experiments to determine whether environmental factors were influenced by microplastic concentrations. The team also researched whether human interactions with the environment influence microplastic concentrations within the Rouge River. Since the samples are taken from the Middle Branch of the Rouge River, where there are no wastewater treatment plants, the team cannot determine a possible source of microplastics besides normal urban runoff. To determine the effect of treated sewage effluent on microplastic levels, samples would need to be taken from the Lower Branch of the Rouge River which drains the water coming from the Ypsilanti Sewage Treatment Plant found upstream. It will also be important to conduct a period of extended testing to determine the potential effect of environmental factors, such as air and water temperature, electrical conductivity, turbidity, and pH on microplastics. However, with the research gathered, the team determined that pH is independent of microplastic concentrations. The pH of the water samples was relatively constant, but microplastic concentrations fluctuated. These findings allow the team to conclude that pH is an environmental factor that does not influence the concentration of microplastics, which supports the null hypothesis rather than rejecting it. The data appeared to indicate that the higher the turbidity of a sample, the less microplastics were present. It is important to note that in turbid Sample 2, some microplastics may have been embedded in the sediment and subsequently not counted. All other microplastic counts are assumed to be highly accurate since careful protocols were implemented.

The results also indicated influence the concentration of microplastics. Due to limited sampling data, the researchers cannot derive conclusions on the impact of water temperature and air temperature on microplastic concentrations. While it was expected microplastic concentrations would decrease with colder air and water temperatures, the opposite effect occurred within the data gathered, which supported the null hypothesis. While the findings provide mixed results on environmental factors impacting microplastic concentrations, it was concluded that some environmental factors are influenced by microplastic concentrations while others are not.

Submitting GLOBE Data Verification:

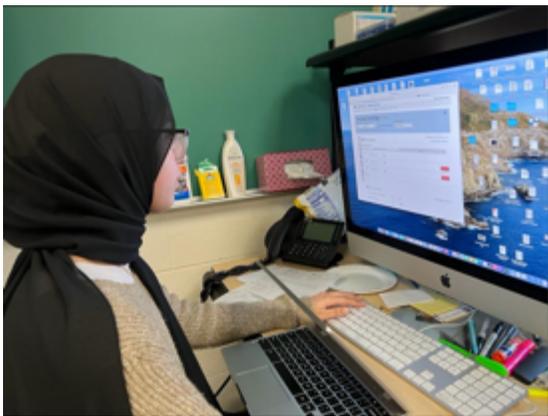


Figure 14. Data Entry.

Student researcher submits data to a GLOBE database for GLOBE verification.

Citations:

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Acknowledgments:

The researchers would like to thank Ms. Tracy Ostrom, Co-Program coordinator and trainer at the University of California Berkely, for guiding them through their research. She provided them with the materials needed (water quality monitoring) to complete their research and guided them through online zoom lectures. She also trained them in the identification of microplastics and the filtration processes. With her support researchers were able to clarify questions and continue with their research. They would also like to thank the Friends of the Rouge for their financial support, which helped them with equipment needed for their research. Finally, the researchers would like to thank Mrs. Diana Johns, for her guidance and support as they continued to pursue their research.

Badges:**I Am A STEM Professional:**

The researchers hope to receive the “I Am A STEM Professional” badge. We received assistance from STEM professional, Tracy Ostrom, through an online meeting that included training for identifying microplastics and understanding the significant impacts of the growing research field. Ostrom provided the researchers with expensive material that could not have been obtained otherwise, such as filtering units, syringes, filter paper, and tubing that totaled around \$2,500. Beyond that, Ms. Ostrom provided the researchers with a thorough recognition guide to identify microplastics accurately and improve precision of analysis. Ostrom guided the team along their journey of analyzing microplastic concentration within the Rouge River, answering their questions along the way.

I Am A Data Scientist:

The researchers hope to receive the “I Am A Data Scientist” badge for their data collection, Over a 4-month period we tested for 3 samples individually inputting data to a Google document. This document was used to organize the data we gathered to create graphs. The team then created and analyzed these graphs to draw conclusions on the research they did.

I Make an Impact:

The researchers hope to receive the “I Make an Impact” badge as the implementation of this research can make both a local and global difference. Directing the finding of our research to the Rouge River City Council, being the first to announce standard methods for quantifying microplastic concentrations in drinking water, with the aim of monitoring water over the next

four years and publicly reporting the results. Providing these findings to the team's city can allow for the innovation of newer and more efficient methods of microplastic identification and quantification of microplastics within the Rouge River.

I Am A Stem Story Teller:

The researchers hope to receive the "I Am A Stem Story Teller" badge as they have documented their journey on an Instagram page with the username, "microplastic.counters." The team has utilized this social media page to expose members of their community to their research and educate them on the impacts of microplastics within waterways. The team posts periodic updates of their research every time they sample and gather data. With a focus on the scientific methods utilized by the team, they have been able to craft a compelling story detailing their progress on their GLOBE project, hoping to inspire others to craft their own stories through the GLOBE program.