Analyzing the Microbiome of Microplastics in the Hudson River

Desiree McGriff[1], Solveig Olson[2], Theodore Muth[3]

1. Department of Environmental Science, Oklahoma State University 2. Department of Biology, Grinnell College 3. Department of Biology, Brooklyn College

Introduction

Background

Although the existence of microplastics in waterways has been known for some time, the effects of microplastics on the surrounding environment have not been thoroughly examined. A compilation of data from the USGS found that water bodies near urban land use contain higher rates of microplastics. In addition, antibiotics have entered waterways due to heavy use in various fields and wastewater overflow. The microplastics in waterways can harbor biofilms, which are layers of bacteria adhered to surfaces, often encased in a thick 'extracellular matrix'. These biofilms promote conditions that enhance antibiotic resistance and are linked to many diseases caused by bacterial pathogens. Therefore, there is a potential for an increase of antibiotic resistance genes (ARGs) which would negatively impact human health systems.

Main Objectives

- **Bacterial Community Sequencing**: Analyze bacterial communities from the Hudson River to assess water quality indicators.
- **Microplastic-Antibiotic-Resistance Gene Connection**: Investigate the relationship between microplastics and the presence of antibiotic resistance genes (ARGs) within microbial communities.

End Goal

Understand the potential effects of the plastic's microbiome on the surrounding environment and human health, and to highlight the importance of preventing microplastic pollution to protect environmental and public health.

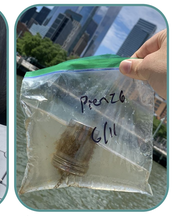
Methods: Collecting & Processing the Microplastic Biofilms

1. 16 g of microplastic beads into tea infusers

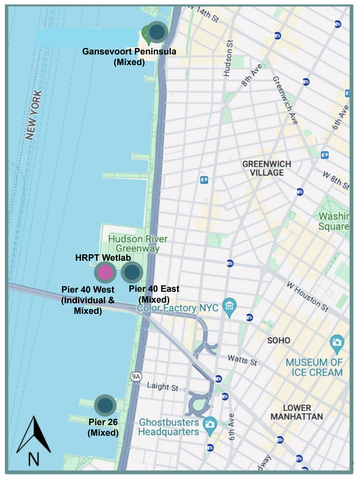
2. Zip tied infusers into minnow traps at HRPT Wet Lab and deploy on docks
3. Waited about 20 days for biofilm to accumulate on the microplastics

Step 2

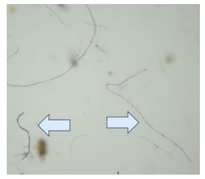
4. Collected traps and diffusers from the Hudson River
5. Rinsed the microplastics & washed off biofilm with diluted detergent and saline solution and vigorous shaking
6. Collected bacteria on a 0.22 micron pore filter
7. Extracted the DNA and prepared for DNA sequencing
8. Extracted DNA with Qiagen PowerWater kit
9. Used a Nanopore 16S barcoding kit
10. Used Qubit to quantify DNA concentrations
11. Ran Nanopore sequencing on a Flongle flow cell



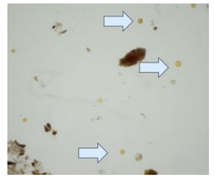
Step 4



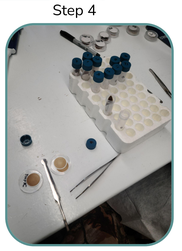
Non-experimental Research: Collecting and observing microplastics



Brightly colored microfibers collected from the Hudson River.



Presumed microplastic beads collected from the Hudson River.



Step 6

Discussion & Conclusion

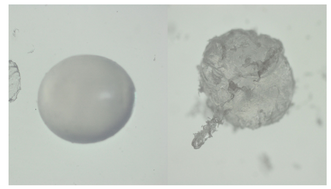
Results show similar bacteria at each site, and the most common bacteria are consistent with bacterial communities commonly found in aquatic ecosystems. This study confirmed the presence of bacteria that would be expected in saltwater environments. In addition, we also found evidence of antibiotic resistant genes in the biofilms present on the microplastics..

Our data further supports that:

1. The location within the Hudson River does not determine a significant difference in the types of bacteria found on microplastics.
2. The bacteria found in the biofilms are consistent with what we would expect to find.
3. Antibiotic resistant genes are present in the biofilms, but we are not sure if these are in a higher concentration than already found in the water.
4. We detected a few bacterial taxa that were surprising to find in this environment.

Ongoing and Future Research

1. Determining to what extent antibiotic resistant genes are encouraged by the growth of biofilm on microplastics compared to the river water.
2. Comparing the microbiomes of roughed up surfaces of microplastic beads to pristine beads to see if surface area is a significant factor.



Results

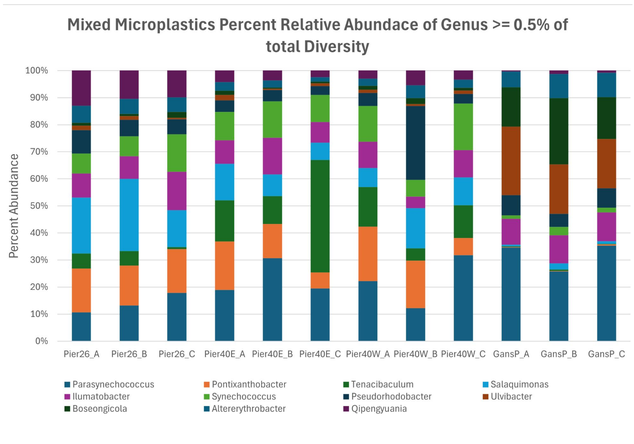


Figure 1: Percent abundance of bacteria genera that make up at least 0.5% of the total diversity from mixed plastic samples at the locations Pier 26 (26 A-C), Pier 40 East (40E A-C), Pier 40 West (40EW A-C), and the Gansevoort Peninsula (G A-C). Note: the unclassified taxa are excluded from this dataset.

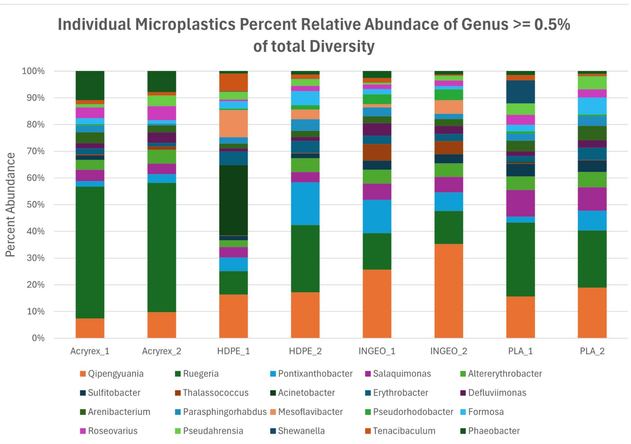


Figure 2: Percent abundance of bacteria genera that make up at least 0.5% of the total diversity from individual types of plastics all from Pier 40 West. Note: the unclassified taxa are excluded from this dataset.

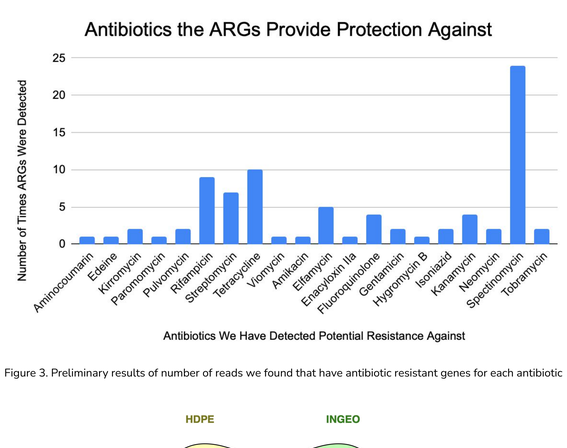


Figure 3. Preliminary results of number of reads we found that have antibiotic resistant genes for each antibiotic

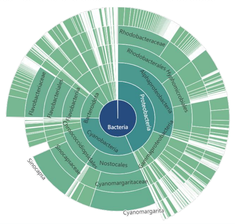


Figure 4: (left) Sunburst plot of the microbiome sample from pier 26 rep A showing hierarchical data: each layer of the circle represents a taxonomic rank. The color indicates the abundance of that taxon

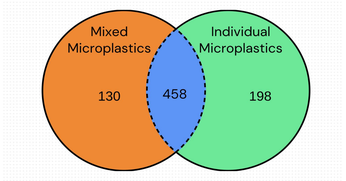


Figure 5. Venn Diagram comparing the number of genera shared by the Mixed vs Unmixed Microplastics

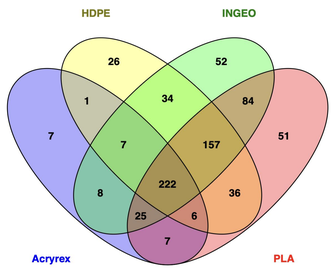


Figure 6. Venn Diagram comparing the genera for each individual type of plastic

References

1. Marathe, N.P., Bank, M.S. (2022). The Microplastic-Antibiotic Resistance Connection. In: Bank, M.S. (eds) Microplastic in the Environment: Pattern and Process. Environmental Contamination Remediation and Management. Springer, Cham. https://doi.org/10.1007/978-3-030-78627-4_9
2. *Microplastics in our Nation's Waterways.* (n.d.). https://labs.waterdata.usgs.gov/visualizations/microplastics/index.html
3. Oluwole, O. M. (2022). BIOFILM: FORMATION AND NATURAL PRODUCTS' APPROACH TO CONTROL – A REVIEW. *African Journal of Infectious Diseases*, *16*(2 Suppl), 59-71. https://doi.org/10.21010/Ajid.v16i2S.7
4. Vestby, L. K., Grønseth, T., Simm, R., & Nesse, L. L. (2020). *Bacterial Biofilm and its Role in the Pathogenesis of Disease.* Antibiotics, 9(2). https://doi.org/10.3390/antibiotics9020059

Acknowledgements

Thank you to Dr. Muth and the Muth lab for their support and assistance. We also thank the BUEE program and the National Science Foundation for the opportunity to conduct this research. Lastly, our thanks to the Hudson River Park Trust Fund for sponsoring this project. Without their support, this research would not be possible.