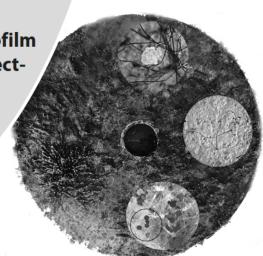
Inquiry & Investigation **Isolating Microplastics from Biofilm Communities: Connecting Project-**

Based Learning & Research

JESSE MEILLER, ANA SOSA, EVA MAY, J. ADAM



ABSTRACT

Plastic debris in aquatic and marine environments often breaks up into fragments that are smaller than 5 millimeters, which are then classified as microplastics. While there is not yet a standardized and validated methodology for characterizing microplastics, the protocol developed in this study uses methods for isolating and observing microplastics and for the investigation of how they interact with organisms present in biofilms from urban waterways. Project-based learning (PBL) has been proven to be a successful strategy in K-12 science education; the implementation of PBL provides opportunities for student-driven inquiry and provides teachers with a means to integrate curriculum with current research and to consider the effects of human impacts on the environment. This paper describes the protocol developed for high school teachers to educate students about microplastics and how to successfully isolate and observe them. Teachers and students in Maryland successfully isolated microplastics from biofilm samples from the Inner Harbor, Baltimore, Maryland, and shared their results. International teachers and students in Barcelona, Spain, involved in a related project, had similar results and shared experiences through images, video, and online meetings. These collaborations provide important opportunities for student-driven inquiry and for them to engage in methods of current scientific research.

Key Words: microplastics; biofilms; biodiversity; project-based learning; protocol; laboratory; field.

Introduction

The first synthetic plastic appeared in 1907 (Baekeland, 1909), and today hundreds of millions of tons of plastics are produced each year. The initial purpose of plastic was to produce goods that were easy to manipulate, inexpensive to manufacture, and durable. More than one hundred years later, most plastic goods are made to be used only once before disposal (Andrady et al., 2009). Globally, plastic production has outpaced that of any other bulk material over the past seven decades, with over 8 billion tons produced since 1950 (Geyer et al., 2017). Millions

of tons of plastic enter the marine ecosystem every year, with 80% originating from litter (Li et al., 2016). Sources for the other 20% include commercial fishing gear and waste products or effluent from wastewater treatment plants (Kalčíková et al., 2017; Ramírez-Álvarez et al., 2020). It's important to note that the majority of this plastic originates from land-based activities and is transported into waterways by stormwater runoff, wind, and illegal dumping. This study focused on microplastics, which "are any synthetic solid particle or polymeric matrix, with regular or irregular shape and with size ranging from 1 µm to 5 mm, of either primary or secondary manufacturing origin, which are insoluble in water" (Frias & Nash, 2019). While there are currently a number of definitions of microplastics in use, for the purpose of this paper, we will use this definition.

In order to better understand how aquatic and marine organisms are interacting with microplastics in the ambient environment, standardized and widely accepted methods are needed for the isolation and classification of microplastics. As of this writing, there is not yet an established and universally accepted methodology for this. With the understanding that microplastics are present in urban waterways, and that they are in fact interacting with the organisms there, we developed methods to isolate and observe microplastics from biofilm samples. The objectives of our study were threefold: to refine and improve methodology for the isolation and observation of microplastics from biofilm communities in urban aquatic and marine environments; to engage local, regional, and international teachers with their students as practitioners of the field and laboratory protocol developed to investigate microplastics in biofilm communities; and to enhance science curricula with project-based learning (PBL) that employs techniques in isolating microplastics from biofilm samples.

Activity Background

In the following activities, Maryland teachers and students in grades 10-12 were used as the target audience and were

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Table 1. Connections to high school NGSS organized by topic, performance expectation (PE), and the three dimensions of learning: science and engineering practices (SEP), disciplinary core ideas (DCI), and crosscutting concepts (CC).

Performance Expectation		Three Dimensions of Learning	
Topic: Interdependent Relationships in Ecosystems			
PE PE	HS-LS2-6. Evaluate claims, evidence, and reasoning that the complex interactions in ecosystems maintain relatively consistent numbers and types of organisms in stable conditions, but changing conditions may result in a new ecosystem. HS-LS2-7. Design, evaluate, and refine a solution for reducing the impacts of human activities on the environment and biodiversity.	SEP SEP SEP DCI DCI CC CC	Constructing Explanations and Designing Solutions Engaging in Argument from Evidence Connections to Nature of Science; scientific knowledge is open to revision in light of new evidence LS2.A: Interdependent Relationships in Ecosystems LS2.C: Ecosystem Dynamics, Functioning, and Resilience LS4.C: Adaptation Cause and Effect (HS-LS2-8) Stability and Change (HS-LS2-6)
Topic: Human Sustainability			
PE	HS-ESS3-4. Evaluate or refine a technological solution that reduces impacts of human activities on natural systems.	SEP SEP DCI DCI CC	Constructing Explanations and Designing Solutions Engaging in Argument from Evidence ESS3.A: Natural Resources ESS3.C: Human Impacts on Earth Systems Connections to Engineering, Technology, and Applications in Science / Influence of Science, Engineering, and Technology on Society and the Natural World (HS-ESS3-3) Connections to the Nature of Science / Science Addresses Questions About the Natural and Material World (HS-ESS3-2)

able to successfully participate in the field components of the project that includes deploying and retrieving discs and testing the laboratory protocol for the isolation and observation of microplastics in the classroom. The main science content areas integrated into these activities include biology, environmental science, physical science, and chemistry. Within these four disciplines there are overlapping concepts (described in Table 1) that can be expanded when examining a community of organisms, the influences of abiotic factors, and impacts of the built environment. The integrated nature of cross-curricular science content can bring together core ideas in a seamless manner.

The original work in the Baltimore Harbor used acrylic discs (Frederick et al., 2000), a substrate that results in rapid biofilm

Figure 1. A type of microfiber at 20× magnification.

colonization and further colonization by other aquatic organisms, which we have demonstrated with aluminum discs as well. For more advice see the websites of Biofilms and Biodiversity (https://www.mdseagrant.org/interactive_lessons/biofilm) and VIRTUE project (https://virtue.gmbl.se).

Samples are examined microscopically to look for the presence of microplastics and other related materials. Figures 1–3 depict various microplastics under microscopy.

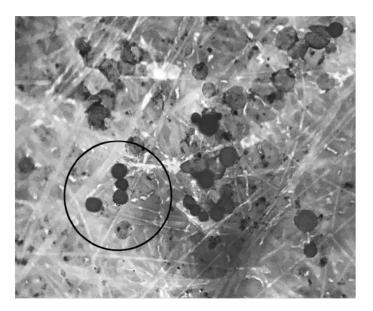


Figure 2. A group of microbeads at 40× magnification.



Figure 3. Miscellaneous microplastic and fiber.

General Tips for Activity Preparation

Avoiding Contamination

Contamination of samples by airborne microplastics is a very real concern and needs to be considered for all stages of the methods. Exposure of samples directly to air should be avoided.

- Use glass and metal supplies rather than plastic. Wear natural fiber clothing and gloves.
- Keep samples covered with foil or glass at all times (after collection, during transport, during processing, after separation). Limit exposure to air due to airborne microplastic contamination. Keep equipment covered when not in use.
- Use vinegar and distilled water for cleaning. Use only natural fibers to clean and dry materials.

Safety in the Laboratory

It is important for all students and teachers to follow site-specific safety guidelines as directed by their school system. In addition, we suggest that participants wear goggles and gloves for all work.

Activity 1: Biofilm Rack Design & Deployment

The overall design of the sampling rack and materials is simple and can be deployed in various aquatic or marine environments with proper planning (Figure 4). The following design is modified from Frederick and colleagues (2000, 2018) and the VIRTUE project.

Procedures

- 1. Drill one hole horizontally 2" from the top of dowel, 3/8" in diameter.
- Drill1/2" hole through middle of each aluminum disc (slightly larger than the diameter of the wooden dowel).
- Attach two hose clamps to the bottom of the dowel, approximately 2" from the bottom; tighten using the screwdriver.
- 4. Measure a 90° angle on each disc and notch the outer edges of the disc at this angle—this will allow you to stay consistent when sampling 25% of each disc later on.
- 5. Slide one disc onto the dowel above the hose clamps.



Figure 4. Sampling rack.

- 6. Attach one hose clamp about 4–5" above the first disc, and slide on another disc. Evenly space a total of 5–6 discs, approximately 4–5" apart, and attach hose clamps along the length of the dowel.
- 7. Tie the rope to the top of the dowel after feeding it through the hole at the top.
- 8. Lower the rack slowly into the water and tie securely off on a cleat or other fixture; ensure that the depth of the rack is below the low-tide mark (if applicable) and that the top of the rack is 1 m below the surface. If you prefer, a long rope can be used with a float attached so the rack will move with the tide. Racks will be oriented vertically in the water (for image see https://virtue.gmbl.se).

After an initial period of a few days to one week, a biofilm will develop on the discs.

Formative assessment prompt. (It is suggested that this prompt be performed over a series of days as students learn about the project.) Students will work in small groups (three or four) to develop strategies that will propose to improve or alter methods for deploying racks and will account for local conditions

(water flow, light penetration, overall water quality, etc.). Students should write a plan for deployment that includes the length of time on the water and how often the discs will be checked.

Activity 2: Disc Retrieval, Sampling, Separation & Processing

Small groups of students (three or four) can be organized to perform tasks related to disc retrieval, transport, and processing in the classroom and can provide a check for ensuring that proper procedures are followed.

Procedures

- Retrieve the rack from the water slowly. Carefully remove the number of discs needed and place in a metal tray for transport. Cover tray with foil or a metal lid. Keep discs separate from each other. (Note: Placing the discs in water during transport will cause the biofilm to slough off the disc. Water from the site can be transported in a separate container and used to completely submerge the disc in a glass dish for observation under the microscope.)
- At this point, visually observe disc biofilm assemblages for biodiversity under a dissecting microscope.
- 3. Make a 70 ppt saltwater solution in a 1L glass graduated cylinder. Verify salinity with a refractometer.
- 4. Select a biomass sample up to 25% of the total disc mass and scrape it off using a metal razor blade. Place in a glass petri dish and cover with a lid.
- 5. Any larger organisms or inorganic material, such as rocks and shells, should be carefully removed from the sample with metal forceps and rinsed with distilled water over the separation funnel to remove any possible microplastics. The items can then be removed from the sample completely as they could impede the separation and clog the funnel. The sample is now ready for separation with the glassware specified.
- 6. Use 200 mL to 400 mL of 70 ppt saltwater to suspend the sample in the 500 mL separation funnel. Base the amount of saltwater added to the funnel on how much organic material looks to be on your biofilm sample—more organic material will require more saltwater for suspension and separation. Be sure not to add more water than the total volume of the separation funnel (Figure 5).
- 7. Use DI water to spray the entirety of the inside of the petri dish in order to ensure that all of the sample makes its way into the funnel or use a regular glass funnel when pouring both your saltwater and your biofilm sample into the separation funnel. Shake well to mix.
- 8. Allow the sample to settle for at least 30–45 min (longer is better). Check on the sample every few minutes in the separation funnel—if you notice the funnel getting clogged or organic material sticking to the sides, give the sample an occasional shake and allow the material to settle again (Figure 6).
- 9. Drain any settled material into a 500 mL beaker and discard. This portion of the sample may contain dense microplastics, but for the purposes of these methods, students will focus on isolating less dense, more buoyant microplastics. The remaining liquid in the separation



Figure 5. Sample added to separation funnel.



Figure 6. Sample settling in separation funnel.

- funnel will be used for the filtration onto the filter paper with the manual vacuum pump system.
- Drain 150–200 mL of remaining liquid into a clean 200 mL beaker and cover with foil. This will be used in the filtration process to look for microplastics.
- Connect the 150 mL Buchner funnel to the 500 mL Erlenmeyer flask at the ground joint and connect the vacuum pump.
- 12. Place a piece of gridded 60 mm filter paper into the bottom of the Buchner funnel. From the 200 mL beaker, pour the liquid into the Buchner funnel along the inside of the glass. Cover the funnel with foil.
- Use the vacuum pump system to pull the sample through the paper; the waste liquid will collect in the Erlenmeyer flask below.
- 14. Use metal forceps to remove the filter paper from the Buchner funnel and place it face up on top of a 75 mm x 50 mm glass slide, centered as best as possible.
- 15. Place a second glass slide on top of the paper, centered as best as possible. The 2 glass slides form a "sandwich" around the filter paper. Some of the filter paper will be exposed.
- 16. Repeat steps 9–15 for the remaining liquid in the separation funnel.

Formative assessment prompt. (It is suggested that this prompt be performed after students have completed at least one attempt at isolating microplastics from a biofilm sample.) Students will work in small groups (3–4) to propose methods for altering or improving the isolation of microplastics from the biofilm sample. Students should keep in mind the safety guidelines and tips for avoiding contamination in their proposed methods.

Activity 3: Observing & Counting Microplastics

There are many options for capturing images using microscopy with a digital camera and/or smartphone adapter. These methods culminate in highly engaging activities for students to examine samples for microplastics and hone their microscopy skills (Figure 7 and Figure 8).

Procedures

- Allow the filter paper/slides sandwich to dry overnight wrapped loosely in foil. Excess filter paper extending outside of coverslips can be trimmed with a razor blade or scissors.
- Leave the filter paper sample between the glass slides for purposes of counting and general microscopy. This will also assist in limiting contamination from microfibers in the air.
- 3. Count each gridded filter paper sample at least three times for consistency using a random sampling method to select the grids on the filter paper to be counted. The number of grids counted will also be determined by the amount of time that can be dedicated to the project and the density of the sample. If not too dense, ideally the entire filter should be counted.
- 4. Take an in-depth look at the whole surface of the filter paper with a good quality stereoscope or compound microscope at low power. Another alternative is to use a stereoscope with a 2.0x Barlow lens threaded onto the bottom of the body tube. This will allow for a macro view of the sample, and reflective lighting makes easy viewing.



Figure 7. Students examining samples for microplastics.



Figure 8. Students examining samples for microplastics.

- 5. If necessary, take a picture of an area of the sample for viewing and count at a later time. It is helpful to use a copy of the blank gridded filter paper as a way to mark the location of microplastic that is found on the sample without disturbing the sample.
- Microfibers, fragments, and other types of microplastics should all be included in the counts.

Summative assessment prompt. (It is suggested that this prompt be performed after students have completed activities 1–3 and have familiarized themselves with background related to microplastics.) Students will work in small groups (3–4) to propose ideas about the frequency and potential impacts of microplastics contamination in and on the school campus. Students should think creatively about sources of microplastics and how they might be managed so that there is a reduction in air, water and soil microplastics. It is suggested that students perform this activity in a research poster session format.

Results

We were able to successfully isolate and observe the occurrence of microplastics through the methods we developed in a collaborative manner with teachers and their students over a three year period. During this time, we refined our protocol and conducted five teacher professional development workshops. In addition, we also leveraged funding from an existing NSF project to develop loaner kits for teachers and students that included microscopes, glassware, and all other supplies. The majority of the microplastics observed in biofilm communities by teachers and students involved in this project were microfibers, consistent with other studies in urban freshwater systems (Ramírez-Álvarez et al., 2020; Wilkens et al., 2020). Hernandez and colleagues (2017) investigated the factors affecting microfiber release from textiles, such as those in laundry effluent released in urban areas, contributing to these high microfiber concentrations. Microfibers tend to be less dense than other types of microplastics and, as described in the methods, the density separation steps isolate less dense microplastics.

Once a protocol was established, teacher professional development workshops followed that included demonstration of field and laboratory techniques and hands-on practical experience. Engaging the education community in this process leads to a greater likelihood of success in the classroom coupled with increased confidence demonstrated by the classroom teacher, as it allows them to gain critical content knowledge and applied laboratory skills. Teachers in five local Maryland schools and one international school in Barcelona, Spain, used the microplastic isolation and observation methods with students and found success in isolating microplastics from biofilm samples provided from the Baltimore Inner Harbor and waterways near Barcelona, Spain.

Conclusion

This project has brought together scientists, teachers, and students around an emerging area of research in a way that moves the science forward and provides meaningful learning opportunities about a real-world problem. Students and teachers provided data and feedback to scientists about the interactions of microplastics in biofilm

communities. Understanding microplastics in terms of these types of interactions as well as their sources, transport mechanisms, fate, and their subsequent impacts on ecosystems is crucial for the success of prevention and management. Geyer and colleagues (2017) and Pipkin (2020) conducted comprehensive scientific and literature reviews on plastic pollution that focused on research priorities and potential solutions to the plastics problem. Our work contributes to the collective knowledge and study of microplastics in the field and the lab through the development of a reliable and effective protocol for isolation and characterization of microplastic particles in an aquatic urban environment.

Project-based learning allows students to improve their problem-solving and critical thinking skills. It also provides teachers with a model to follow based on the PBL gold standard. This project in particular provides the experience of using a density separation protocol to reveal a complex issue that will get participants thinking of their own contributions to plastic pollution, as well as that of their community. By integrating this activity into curriculum in a variety of schools, the hope is that a growing number of students will increase their awareness and understanding of plastic pollution in their local watersheds, while also gaining crucial hands-on scientific classroom skills. Future collaborative work will continue to include an iterative process with teachers and scientists that allows for the revision of methods, capturing qualitative and quantitative data, and the development of best practices in the classroom and the research laboratory.

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