TEAM MEMBERS

ADAM STROHM, ALLISON SMITH,
ANDREW PANNETON, SEBASTIAN TOMMASI

SPONSOR

LEIBNIZ INSTITUTE OF FRESHWATER ECOLOGY
AND INLAND FISHERIES (LEIBNIZ-IGB)

PROJECT ADVISORS

DANIEL DIMASSA AND FABIO CARRERA

A CITIZEN SCIENCE APPROACH TO MEASURING MICROPLASTICS IN BERLIN'S WATER

Abstract

Microplastic and nanoplastic pollution is a continuously growing threat to our environment. The goal of our project was to increase awareness of this risk in elementary students in the Treptow-Köpenick district of Berlin. Through our partnership with Leibniz-IGB and other district affiliates, we developed a lesson plan containing a student experiment, a presentation, infographics, and reflection materials to teach students about the problem. Increasing awareness in younger generations is an important step in hopes of attaining a more sustainable future.

Executive Summary

Plastic production has been growing annually since its popularization in the 1950's, and as of 2017, 8.3 billion total tons of plastic has been produced worldwide (Geyer et al., 2017). This rapid increase in production has caused an influx of pollutants in the environment. Microplastics and nanoplastics are small pieces of plastic that come from larger plastics breaking down by natural factors or deliberate manufacturing. These pollutants threaten water quality and affect aquatic species across the world. Although macroplastic pollution was first observed several decades ago, **microplastic and nanoplastic pollution** has recently emerged as a serious concern.

Current microplastic and nanoplastic testing is expensive and requires advanced equipment and analyses to obtain accurate results. Scientific reports regarding microplastic and nanoplastic testing have been carried out in oceans, rather than freshwater ecosystems. Furthermore, due to the difficulty in detecting microplastics and nanoplastics in water, people are unaware of the impact these particles have on the environment. For this reason, our project aims to raise awareness about microplastic and nanoplastic pollution through an educational program and student based experiments.

Community members of the **Treptow-Köpenick district of Berlin** utilize many major freshwater sources for travel, transportation of materials, and leisure. The recent partnership between the **municipality**, **Leibniz-IGB** (a research laboratory), and **TJP** (a STEM education facility) will increase community involvement and inspire students to take initiative on the growing challenge.

Our mission was to help Leibniz-IGB and TJP e.V./METEUM to improve awareness and knowledge about microplastics in Treptow-Köpenick, especially among school-aged children. To do this we created the following objectives:

1. To **compile** information on microplastic and nanoplastic and summarize the dangers they pose
2. To **design** a replicable experiment suitable for students to collect samples and detect microplastic, ensuring its effectiveness and feasibility
3. To **teach** 5th and 6th-grade students about the presence of microplastics and nanoplastics using informational material and engaging activities

In order to **compile** information, we used various credible sources that examine saltwater and freshwater, including studies done by Leibniz-IGB. We gathered information on potential dangers caused by microplastic and nanoplastic pollution from these sources and presented the material as a **literature review**, seen in Appendix C. We also organized an **annotated bibliography** of these sources, seen in Appendix D. The research conducted on microplastic and nanoplastic pollution enables us to begin informing the next generations of their presence and the threats they pose to the planet's health.

In order to **design** an experiment suitable for the students, we researched and performed several collection and detection methods from previously published studies. We performed two distinct experimental methods, one being a density separation method for sand samples and the other being a filtration method for water samples. We modified these methods to be suitable and cost-effective for a 5th and 6th-grade student experiment.

The *sand method* follows a previous WPI student experiment where the students used corn syrup to separate plastics from sand, rocks, and other particles of a sand sample (Schroeder, C., Alexander, T., Strauss, J., & Buzzell, A., 2018). We used this density separation technique because it is low-cost and effective for the scope of our experiment.

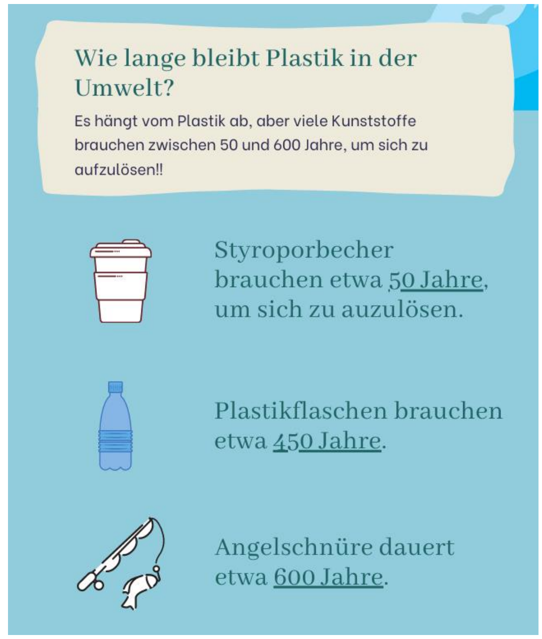
The second experimental protocol consisted of two *water filtration methods*, one for the lab and the other for on-site. Along with collection methods were two different identification techniques: magnification and fluorescence. These were compared and combined to give experimenters the best chance of detecting microplastics in their samples. We determined a **55-micron Bucket Filter** best suited our project's goals. It allowed for easy, fast on-site filtration of very large volumes of water. The **UV light** was the most effective at determining the presence of microplastics, but only truly effective when combined with the smartphone microscope or other magnification tool.

To enhance the performance of the student experiment, we have several suggestions for TJP. Using benchmarked concentrations from Leibniz-IGB will be useful references for the students to recognize the challenges microplastics pose. TJP should use nylon or glass filters instead of paper filters because they are much quicker, and are inexpensive. If the weather is nice, performing the experiment on-site with a bucket-filter will provide more accurate results and be more fun for students. We found it to be the most enjoyable and engaging experiment. To definitively detect microplastics, we suggest using both magnification and fluorescence techniques together. Our results showed that combining different methods gave us a higher chance at definitively determining microplastics in our sample. The detection tools we used were engaging and it would be beneficial for students to be introduced to rudimentary laboratory techniques.

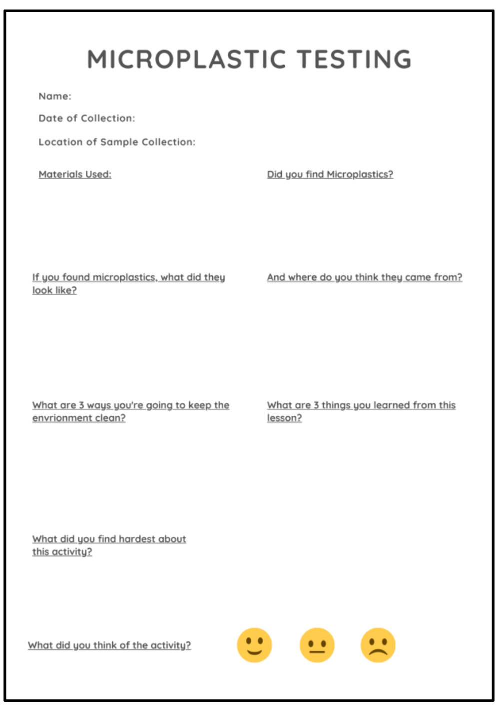
In order to **teach** 5th and 6th-grade students about microplastics, we researched the most effective way to present a condensed version of our researched material. We drew from our experiences and published lesson plans for 5th and 6th-grade students to structure our **overall lesson**, including educational materials. Using feedback from our sponsors, advisors, and outside references, we refined these materials to aid TJP staff in teaching the students. We also produced instructions that go through the activities and materials, from the student's and teacher's perspectives. The overall lesson contains a presentation, experimental activities, infographic booklet, reflection worksheet, and supplemental videos.

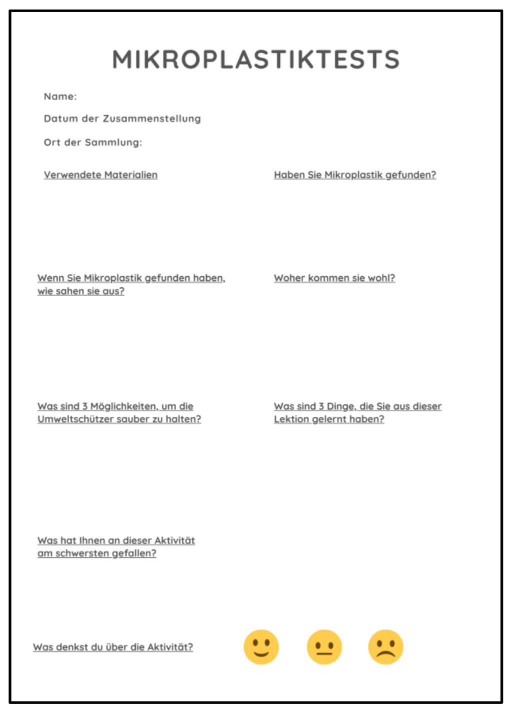
1. The **presentation** is a visual introduction for students to understand and get engaged with the material that will follow. It is the backbone of the lesson plan, containing background material to give context to students before the experiment as well as directions students can take to reduce plastic pollution after the experiment.

2. The lesson plan also contains finalized versions of the **experiments**. These are experiments to introduce students to the protocol using a pre-contaminated or contaminated sample, field experiments at local bodies of water, and beach clean-ups to emphasize preventing future pollution.
3. The **infographic booklet** is to be used as an aid to understanding the significant dangers that microplastics pose on the environment.



4. The **reflection worksheet** is focused on ensuring the students took away important information on the dangers of microplastics.





5. **Supplemental Videos** are added to the end of the lesson plan as an entertaining and informative option to conclude the lesson, if the instructor so chooses.

With the goal of ensuring educational outcomes, we have some **recommendations for our lesson plan** and providing additional takeaways. Firstly, we recommend that TJP prints out our infographics as posters and uses them as a group activity. This would be a fun way to introduce students to the topic before the presentation. Additionally, the district is home to many sustainability initiatives such as GreenKayak, Unverpackt, and Alles Im Fluss. Therefore, we recommend the municipality endorses and spreads awareness about the benefits of utilizing these opportunities.

As we continue to address the problem of plastic pollution, whether macroplastics, microplastics, or nanoplastics, we must consider that it is our responsibility to protect, mitigate, and manage the health of our planet and surrounding ecosystems.

Table of Contents

List of Figures

List of Tables

Glossary of Abbreviations and Acronyms

TJP- Technischer Jugendbildungsverein in Praxis

Leibniz-IGB- Leibniz-Institut für Gewässerökologie und Binnenfischerei

POP- Persistent Organic Pollutants

EPA- Environmental Protection Agency

ECHA- European Chemical Agency

RAC- Risk Assessment Committee

REACH- Registration, Evaluation, Authorization and Restriction of Chemicals

NaWi- Naturwissenschaften (Natural Sciences)

WPI- Worcester Polytechnic Institute

IQP- Interactive Qualifying Projects

STEM- Science, Technology, Engineering, and Mathematics

UV- Ultraviolet

Authorship

Contributors	
Adam J. Strohm: AJS	Allie E. Smith: AES
Andrew H. Panneton: AHP	Sebastian R. Tommasi: SRT

All team members participated in reading and editing each section.

Sections	Author(s)
Abstract	AES
Executive Summary	All
Chapter 1: Introduction	All
Chapter 2: Background	All
2.1 Emergence of Microplastic and Nanoplastic Pollution	AHP
2.1.1 Microplastic Regulations	AHP
2.2 The Hazards of Microplastic and Nanoplastics in Water	SRT
2.3 Microplastic Pollution in Treptow-Köpenick	AES
2.3.1 Major Waterways in Treptow-Köpenick	AES
2.4 Studying Pollution in Treptow-Köpenick	AJS
2.4.1 Leibniz-Institut für Gewässerökologie und Binnenfischerei (IGB)	AJS
2.4.2 Technischer Jugendbildungsverein in Praxis (TJP)	AJS
2.4.3 Engaging Citizens in Water Pollution Research	AES
2.4.4 Student Exposure to Natural Sciences	AES
Chapter 3: Methodology	All
3.1 Compiling Information on the Hazards of Microplastic and Nanoplastics	SRT
3.2 Designing an Experiment Suitable for Students	SRT
3.2.1 Preliminary Water Sampling Experiment	SRT

3.2.2 Testing the Sand Sampling Method	AES
3.2.3 Sponsor Trials and Feedback	AES
3.3 Teaching 5th and 6th Grade Students through Educational Material	SRT
Chapter 4: Results and Analysis	All
4.1 Compilation of Scientific Students on Microplastics and Nanoplastics	AHP
4.2 Student Experiment to Detect Microplastics	AJS
4.3 A Lesson Plan to Teach Students about Microplastics and Nanoplastics	AHP
Chapter 5: Conclusion & Recommendations	All
5.1 Conclusions and Recommendations for the Student Experiment	SRT
5.1.1 Perform Benchmark Testing to Obtain Accurate Concentrations	AHP
5.1.2 Use Nylon or Glass Filters and 10-15% Hydrogen Peroxide	AHP
5.1.3 Perform On-site Filtration when Weather Permits	SRT
5.1.4 Use Various Detection Techniques Together	AJS
5.2 Conclusions and Recommendations for the Lesson Plan	AJS
5.2.1 Turn the Infographics into a Group Activity	AJS
5.2.2 Work with Other Organizations in the Area	AJS
5.2.3 Endorse Sustainabilty Inititaives	AJS
References	All
Appendices	All

Acknowledgments

We would like to thank the following individuals and organizations for which our project would not have been possible.

Worcester Polytechnic Institute: **Professor Daniel DiMassa** and **Professor Fabio Carrera** for their guidance and support throughout this project. **Professor Katherine Foo** for her guidance and teaching of research designs, methods and analysis in our preparatory term.

Leibniz-Institut für Gewässerökologie und Binnenfischerei (IGB): **Ms. Angelina Tittmann** for her constant enthusiasm, feedback and support in refining our experiment and improving our educational material. **Prof. Dr. Hans-Peter Grossart** for his support, insight and recommendations to improve our experiment.

Technischer Jugendbildungsverein in Praxis (TJP): **Ms. Marie Dewender** and **Ms. Carmen Torres Raya** for the help and support refining the experiment throughout the course of this project.

The Treptow-Köpenick Municipality: **Mr. Dennis Lumme** for all of his support, feedback and suggestions in refining our project.

1 Introduction

Since 1950, yearly plastic production has increased exponentially from 2.3 million tons to 448 million tons in 2015, and researchers expect it to double by 2050 (Parker, 2019). This growth has the potential for irreversible environmental damage and biome disruption through pollution, which forces us to improve sustainability efforts worldwide. The United Nations (UN) proclaimed global water management goals in specified areas to manage and protect aquatic ecosystems by 2020 and significantly reduce aquatic pollutants by 2025. Additionally, the UN plans to improve most nations' water quality and sustainability by 2030 (*THE 17 GOALS | Sustainable Development*, n.d.). Although an admirable goal, the presence of plastic pollution and its many variances make this challenging to achieve.

In 2011, the Environmental Protection Agency (EPA) estimated that 6.4 million tons of waste accumulate annually in aquatic environments, up to 80% of which is plastic debris (Alimba & Faggio, 2019). This plastic pollution has penetrated waterways around the world, in part due to the microparticles that erode off larger plastic bodies. These microplastics (microplastic) and nanoplastics (microplastic) that enter the water are so minute that they can be challenging to detect with the naked eye. Even though they may be difficult to see, microplastics and nanoplastics pose harmful impacts to humans, animals, and the ecosystem. For example, the toxins present in microplastic and nanoplastic, as well as the biofilms that accumulate on them, can cause disturbances in aquatic organisms and the ecosystems they inhabit (Tu et al., 2020). Microplastic and nanoplastic pollution may be new to the public. However, scholars have acknowledged the diverse impacts they have on aquatic biota, some of which humans consume regularly.

Given the relatively recent attention to microplastic and nanoplastic, there is still a lack of conclusive evidence surrounding all aspects of microplastic and nanoplastic pollution. Current microplastic and nanoplastic testing is expensive and requires advanced equipment and analysis to obtain accurate results. Additionally, most testing and research are done in oceans; however, there has been a shift towards freshwater ecosystems in recent years. Due to the difficulty of seeing microplastic and nanoplastic in water, the largest problem is that many people are unaware of the impact these particles have on the environment and surrounding communities. For this reason, this project aims to initiate continual testing and help raise awareness through student experiments. By doing this, we can teach the next generation of students about the problems we will be facing for years to come.

Germany is highly susceptible to the impact of plastic pollutants in its waters, both from the ocean and numerous inland water bodies. The Treptow-Köpenick district of Berlin utilizes many major freshwater sources for travel, transportation of materials, and leisure. The district is working to improve sustainability through its local government and increase its community engagement to keep the surrounding environment clean. Our work aids the citizens of the Treptow-Köpenick district through our partnerships with the district's municipality, the IGB (a research laboratory), and TJP (a STEM education facility), in understanding how microplastics affect their local waterways. This project compiled scientific literature on the hazards of microplastic pollution and distilled this information in a comprehensible form for young students. Additionally, we introduced field experiments for students in the Treptow-Köpenick district to test their waters and detect the presence of microplastics in their community. Finally, we designed a lesson plan to ensure that students understood the severity of the issue and help their community stay clean, regardless of the experimental outcome. This project is a stepping stone in bridging the gap between the widespread issue of microplastic pollution and community engagement in local sustainability.

2 Background

Plastics are synthetic materials made from chains of organic polymers such as polyethylene, polyvinyl chloride (PVC), and nylon, among others. Different types of plastics are marked with a symbol that indicates which polymer chain it is and its properties. Below is a table of the seven types:

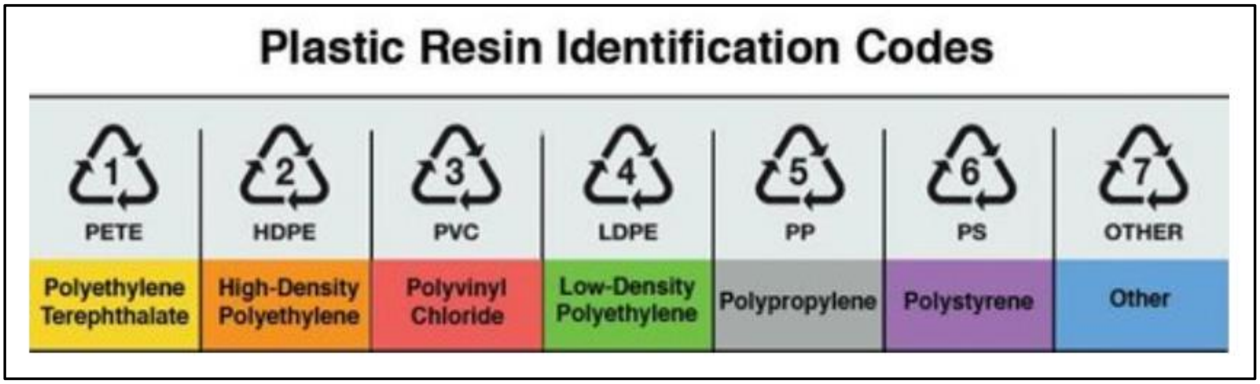


Figure 1. Different grades of plastics (*Plastics Facts — Different Types of Plastics*, n.d).

The American Chemistry Council uses a much more in-depth chart of each type of plastic and the breakdown of the plastics' properties, applications, and potential hazards. Plastic types 2, 4, and 5 are considered relatively safe since they do not commonly spread into their surroundings, whereas types 1, 3, 6, and 7 cause various problems for humans and the environment. Some of these problems include the accumulation of bacteria, changing the balance of hormones in the human body, and infiltration into the digestive tract of animals. (*Plastic Packaging Resins*, n.d.). In the modern era, plastic pollution penetrates many environments across the globe. For example, in the Mediterranean Sea, a study found an average presence of one visible piece of floating plastic per four square meters (Cózar et al., 2015). This type of plastic presence is not unique to the Mediterranean, and it should be alarming to hear.

Plastic pollution may be a modern challenge, but it has a long history. John Wesley Hyatt created the first synthetic polymer in 1869 in response to a challenge to anyone who could provide a substitute material for ivory billiard balls (History and Future of Plastics, 2016). What made this invention so revolutionary is that it meant physical materials were not limited to what nature provides. His discovery allowed for Substitutions for things like ivory and tortoise shells. Plastic production surged during World War II due to the demand for materials with little natural resources. The boom in plastic production led to a similar increase in its presence as waste. Plastic waste in the open ocean was first observed in the 1960s and caused Americans to become more aware of environmental issues (History and Future of Plastics, 2016). However, it did not stop plastic production from growing and spreading into most facets of life.

Plastic pollution has become an extremely prominent environmental issue, primarily because of plastic's commercial convenience and physical persistence. The production of most plastics today comes from distilling crude oil using several different chemical processes (How Plastics Are Made, n.d.). As a cheap substitute for many materials, plastics are present in many industries, from automotive to beauty. These plastics can take hundreds or even thousands of years to fully degrade, meaning single-use plastic items that seem trivial, such as candy wrappers, leave a long-lasting impact on environmental quality. Another significant dimension of this issue is its scale. Around 8 million tons of plastic waste leaches into oceans from coastal nations each year. That is equivalent to spreading five full garbage bags of plastic on every foot of coastline in the world (Parker, 2019). The primary way plastics travel into the oceans is by rivers, but they can also travel by land. It is important to note that only about half of all plastic is buoyant enough to float, so more plastic pollution in waterways than just what is visible (US EPA, 2020). Once in the environment, plastics begin to break down into microplastics, which are less than 5mm in diameter, and nanoplastics, which are less than 100nm in diameter.

2.1 Emergence of Microplastic and Nanoplastic Pollution

Microplastic and nanoplastic are not just broken down pieces of larger plastic. Some microplastics are in production for consumer products like toothpaste or facial scrubs. These are called primary microplastics, as opposed to secondary microplastics, which fragment off of larger plastics. They drain down after use but are too small to be filtered out during most wastewater treatment processes (The Giant Issue of Microplastics, 2019). These microplastics are ever-present in our day-to-day lives. Not only are they prominent in tap water, but bottled water can contain even larger quantities, despite being seen as "safer" (Tyree & Morrison, n.d.). These concentrations are minimal, but their presence in our ecosystems is not. Since they have a wide variety of origins and take at least a few hundred years to degrade, the concern about microplastics is only going to worsen. Plastic pollution can even be introduced into the environment through a variety of avenues. For example, a study on synthetic clothes analyzed the microfibers breaking off in the washing cycle and found about 124 to 308 mg of microfibers per kilogram of clothes washed (De Falco et al., 2019). These microfibers drain with the wastewater but will not get filtered out during most of the treatment, leading them into the outlet water stream, affecting local rivers, lakes, and coastal waters. Microfibers, in turn, reach our agriculture, public water systems, and downstream aquatic ecosystems. Figure 1 illustrates the common ways that microplastics can leach into aquatic environments and the dangers they pose to the organisms situated in those environments.

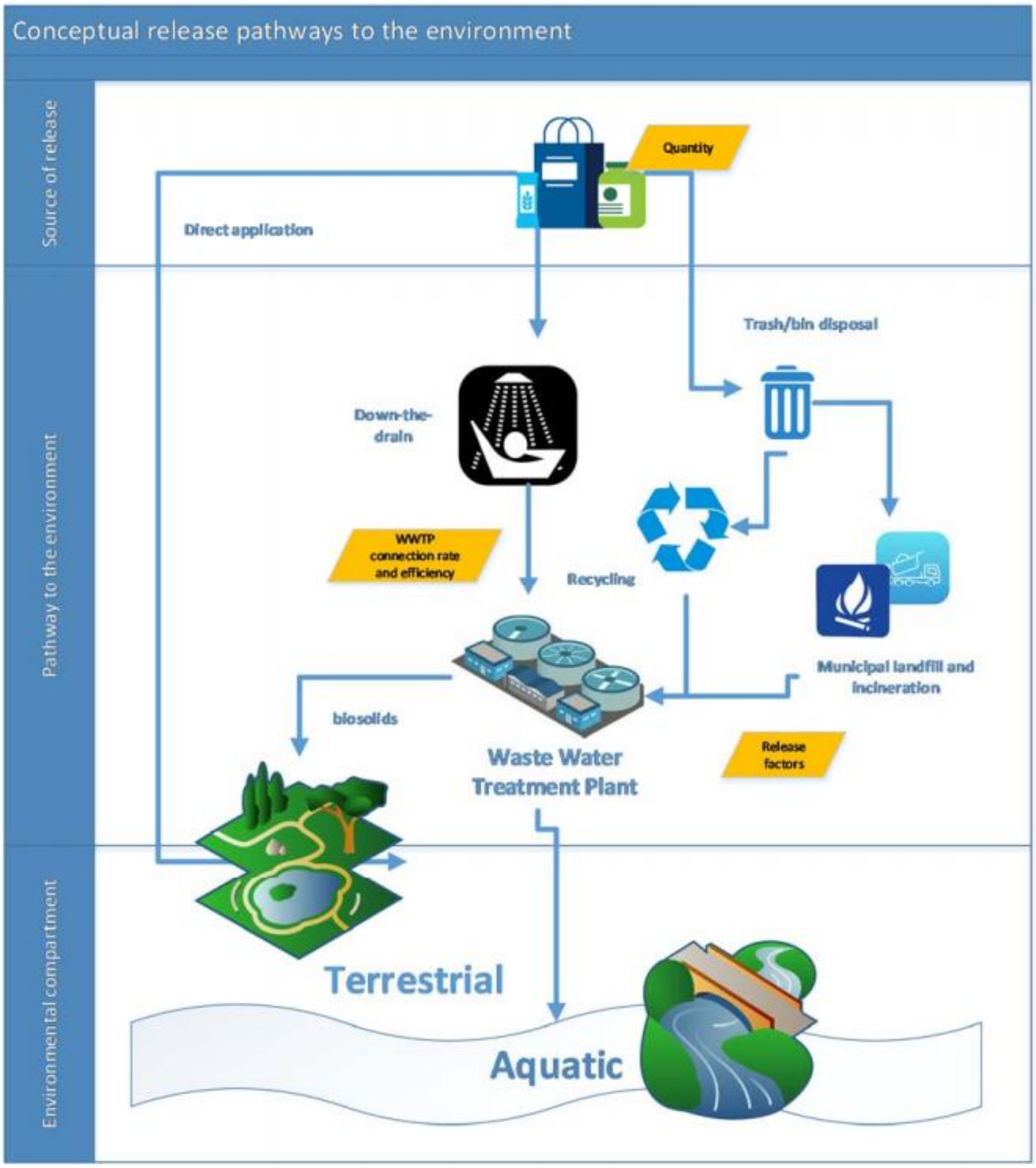


Figure 2. Potential pathways of primary microplastics (Annex XV Restriction Report:, 2019).

Although the threats that microplastic and nanoplastic pose are recognized, the extent of the issue is still being discovered. A study performed in Paris found the presence of microplastics in wastewater, treated water, and surface water, as well as in the atmosphere, and soil (Dris et al., 2015). With three different sampling methods, the scientists found high levels of microfibers in wastewater, lower levels in treated water, and the lowest levels in total atmospheric fallout. This air quality contamination, even with its low levels, is alarming, and a separate study, also performed in France, found the presence of microplastics in both the urban and suburban atmospheres (Dris et al., 2016).

Researchers at German and Swiss institutions analyzed snow near urban sites in Germany and the Alps and found an average microplastic concentration of 24,600 per 34 ounces of snow, arguing that aerial transport allows microplastics to be present everywhere, even in the Arctic (Bergmann et al., 2019). Additionally, Food Federation Germany claims that multiple individual research projects have reported microplastics in food, but these were at incredibly low levels (Microplastics and Food, 2018). While there is not much evidence on the health risks related to microplastics, they can leach into these foods in a variety of ways, be it through the air, seawater, freshwater, groundwater, soil, or even degrading from the food packaging itself.

2.1.1 Microplastic Regulations

Microplastics are a new topic of legislation. In 2018, the European Union formed a plan around plastic pollution, intending to have all plastic packaging in Europe be recyclable by 2030. Additionally, they want to reduce the presence of single-use plastics, and restrict the intentional use of microplastics (Plastic Waste, 2018). In its 2019 Annex XV Restriction Report of its Registration, Evaluation, Authorization and Restriction of Chemicals (REACH) program, the ECHA focused on limiting the use of primary microplastics, released into the environment. In 2020, the European Chemical Agency's (ECHA) Risk Assessment Committee (RAC) announced that they supported potential restrictions. The report also enforced more strict criteria for biodegradable plastic (RAC Backs Restricting Intentional Uses of Microplastics, 2020). Secondary microplastics were outside the scope of this report (*Annex XV Restriction Report:*, 2019). These primary microplastics can be from various industries, including agriculture, cosmetics, paints, construction, and many more.

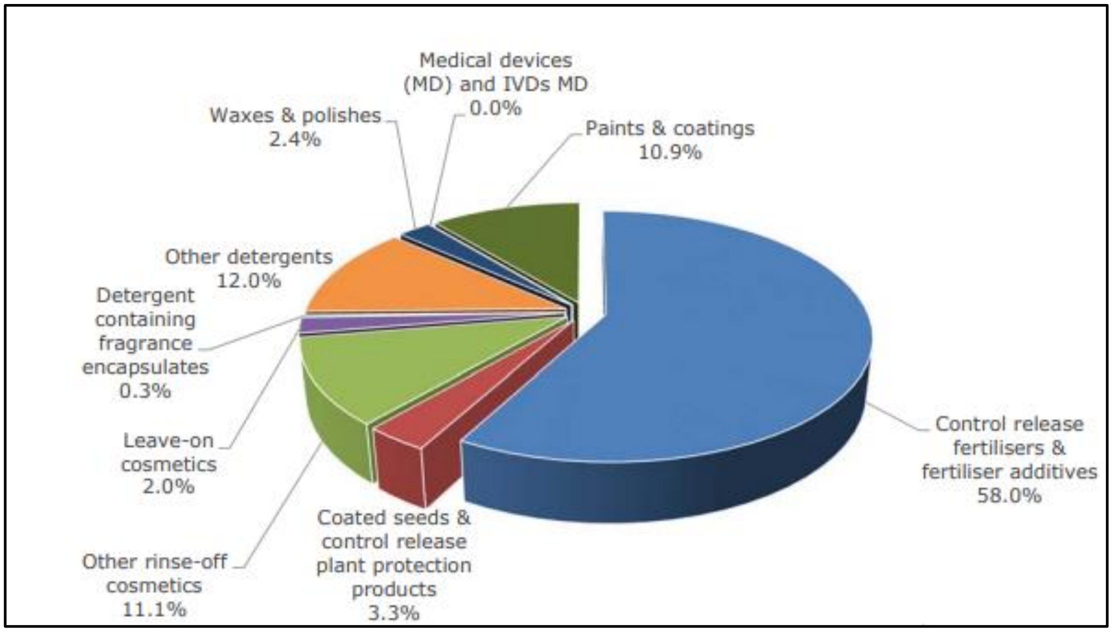


Figure 3. Percentage of microplastics emitted by use (*Annex XV Restriction Report:*, 2019).

The report suggested market restrictions based on the industry and application, with some recommendations for transitional periods for industries like cosmetics, detergents, and medical devices. These restrictions are estimated to reduce microplastic emission by about 400,000 tons over the two decades following their enactment (*Annex XV Restriction Report:*, 2019). The report also suggests labeling requirements for consumers to properly use/dispose of the product, limiting potential release into the environment. Industry requirements for reporting manufactured microplastics also allows for more transparent and accessible information. These restrictions are still heavily discussed and as of March 2021, no legislative action has been taken as a result. However, the potential impacts of these primary microplastics is concerning for the European Union.

2.2 The Hazards of Microplastic and Nanoplastics in Water

The single-use plastics we see every day often end up in bodies of water across the world. Through several factors such as sunlight and weathering, the plastics break apart into microplastic and nanoplastic (Yang et al., 2020). The macroplastics in our oceans can be easily seen; however, microplastics and nanoplastics cannot. According to *Woods Hole Oceanographic Institute*, a 2015 study showed that of the 8 million tons of plastic that enter the ocean yearly, we can only observe around one percent of these plastics. This fact leads them to believe that the other 99% of plastics are lurking beneath the water's surface or are in the form of microplastic and nanoplastic.

Microplastic and nanoplastic has a significant impact on the aquatic environment through two main methods. Firstly, animals can consume microplastic and nanoplastic through accidental ingestion, clogging their digestive tract and transmitting toxic materials. Common toxins are heavy metals and persistent organic pollutants (POPs) that adhere to their surface. Nanoplastics also carry the risk of being passed into the bloodstream, impacting the aquatic food chain, including humans as significant seafood consumers (Gopinath et al., 2019). Secondly, when microplastic and nanoplastic enter the water, they form a biofilm, a layer of bacteria. The organic layer on their surface forms almost instantly, allowing bacteria to colonize or live and feed on them. The surface of microplastic and nanoplastic also affects how bacteria can colonize them. Examples of this are the texture, chemical make-up, and the charge of the plastic. The bacteria on biofilms can disturb the flow of nutrients in bodies of water, posing a threat to the overall health of the source (Tu et al. 2020).

Bacteria are also heavily involved in the nitrogen and phosphorus cycles. Some bacteria are responsible for taking nitrogen from the air and making it useful for plants and animals, while other bacteria do the opposite. Similarly, bacteria can affect the phosphorus cycle through sorption, or the process by which one substance attaches to another. The concentration of nitrogen and phosphorus facilitates the growth of algae and other beneficial microorganisms in the ecosystem, impacting the overall health of the oceans and other water bodies (Rummel et al. 2017). The processes of the nitrogen and phosphorus cycles can be seen in Appendix A.

Along with the effects microplastic and nanoplastic have on the balance of an ecosystem, they also cause problems for aquatic organisms. In a recent study in southern Germany, 1,167 fish ranging across 22 species in 17 different inland bodies of water were found to contain microplastics in their digestive tracts. One-fifth of the total fish sampled tested positive for microplastic and nanoplastic presence. In each contaminated fish, the average number of particles was between one and four. Despite this fact, this study estimates that roughly 95% of microplastics and nanoplastics are not detectable with current technologies. Difficulties in identification can call the accuracy of several studies around microplastic and nanoplastic and how widespread the problem is into question (Roch et al. 2019). Without accurate measurement techniques or the actual concentration of microplastic and nanoplastic, the problem could be more widespread than previously believed.

2.3 Microplastic Pollution in Treptow-Köpenick

The Treptow-Köpenick district sits at the confluence of two major waterways. The district contains highly traveled rivers and could potentially have exposure to microplastic pollution. It is one of the twelve boroughs of Berlin, in the south-east, bordering NeuKölln, Friedrichshian-Kreuberg, Lichtenberg and Marzahn-Hellersdorf. Compared to any other borough, Treptow-Köpenick has the largest area of forests, waterways, and lakes, stretching 168.42 km^2, making up approximately 70% of the district's total surface area (Municipality of Berlin-Mitte, n.d. & Districts of Berlin, n.d.). Recently, an innovative partnership between the municipality, Leibniz-IGB, and TJP (see sections 2.4.1 and 2.4.2 for more information) pushed the district to improve its overall water management initiative. This partnership aims to foster ways to educate their communities on the topic of microplastic and nanoplastic to prevent further water pollution.

Figure 4. The Treptow-Köpenick District of Berlin that depicts major bodies of water (Courtesy of TJP).

2.3.1 Major Waterways in Treptow-Koepnick

As shown in Figure 4, the Treptow-Köpenick district includes many water sources where students could collect data. The largest of these is the Müggelsee, with an area of 2.857 mi^2, and it serves as the main drinking water reservoir of East Berlin. In the past five years, the sulfate values in the lake water have increased and exceeded the sulfate value limit of drinking water (Sulphate in River Spree and Lake Müggelsee (Leibniz-IGB), 2020). Typically sulfate has a minimal impact on the human body. However, when ingested, it can cause several health issues, such as dehydration and bowel problems.

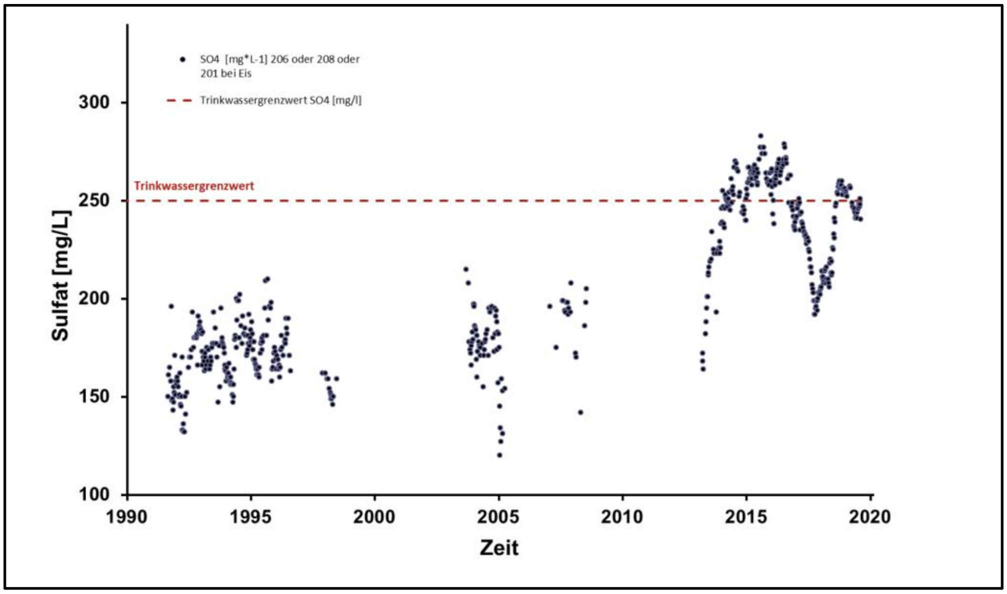


Figure 5. The Müggelsee Lake sulfate concentration over the past 30 years. The red reference line indicates the sulfate value limit of drinking water (Sulphate in River Spree and Lake Müggelsee (Leibniz-IGB), 2020).

The municipality showed a growing interest in sustainable water management practices due to the impending loss of a major reservoir. The increased use of the Müggelsee for recreational activities caused substantial deterioration of water quality, and algae development is becoming a limiting factor in current and future utilization. Research is still being conducted on climate changes and nutrient input related to the increased algae development. Additionally, phosphorus is influential in algae development during the spring and fall seasons. Hence, measures to reduce phosphorus inputs into the Müggelsee and Spree are in motion by modifying how detergents and chemicals are used (Behrendt et al., 1999).

The concept of water conservation in Treptow-Köpenick is not solely concerning the Muggelsee. The district is an industrial center situated at the confluence of the Spree and Dahme Rivers, which emphasizes the importance of the municipality’s conservation efforts. The Spree, one of the largest rivers in Berlin, serves as the Lusatia lignite mining region (Pusch, Martin, & Andreas Hoffmann, 2000). This draining causes many consequential effects on the ecosystems that surround the Spree, which is home to a multitude of aquatic species, including fishes, invertebrates, and plankton. The fauna of the river, especially in nearby Lusatia, is impoverished for freshwater ecosystems because pollutants are in their drinking water. Due to the input of pumped groundwater, river discharge increased and resulted in a deterioration of water quality (Pusch et al., 2000). This ultimately affected the aquatic inhabitants that resided there.

2.4 Studying Pollution in Treptow-Köpenick

The local government officials of the Treptow-Köpenick district have taken the initiative to study microplastic and nanoplastic pollution in their local waterways. In order to gain a better understanding of the current situation, they asked several organizations in the area to help in their efforts to measure microplastic and spread awareness to communities in the area. The Leibniz Institute of Freshwater Ecology and Inland Fisheries (Leibniz-IGB) studies freshwater bodies' water quality and sustainability worldwide. In the past decade, several researchers at the institution have taken an interest in the impacts of microplastic and nanoplastic on aquatic biota and the surrounding environment. Meanwhile, the Technischer Jugendbildungsverein in Praxis (TJP) (Technical Youth Education Association in Practice) educates younger students and adults in the STEM fields through experimental methods. In school, students learn about Naturwissenschaften, or NaWi, an elementary science curriculum. Alongside NaWi, teachers at TJP educate students on fun and exciting experiments to give participants an understanding of science. Experiments can be just for fun or to address larger underlying challenges. The municipality of Treptow-Köpenick is interested in utilizing organizations, such as Leibniz-IGB and TJP, to research microplastic and nanoplastic pollution presence in their waterways and inform communities of microplastic and nanoplastic presence.

2.4.1 Leibniz-Institut für Gewässerökologie und Binnenfischerei (IGB)

The IGB Leibniz Institute of Freshwater Ecology and Inland Fisheries is considered one of the leading research centers globally for freshwater. Leibniz-IGB's research is published on their website, which acts to inform the public. They want to “combine scientific freedom and excellence with social responsibility and effectiveness” (*Leibniz Institute of Freshwater Ecology and Inland Fisheries (Leibniz-IGB),* n.d.). The Leibniz-IGB facility is located on the north side of the Müggelsee and can offer assistance for scientific research that is conducted on Treptow-

Köpenick's water systems. Their work helps protect freshwaters, which are deemed some of the most species-rich habitats on Earth and need to be protected.

Leibniz-IGB has produced hundreds of research papers and projects on biodiversity, environmental change, pollutants, and water management. Throughout many of Leibniz-IGB's projects, they have raised awareness on how humans continually impact freshwater systems and the potential impacts of their actions. Additionally, they are spreading information about how vital freshwaters are to warning scientists about global ecological changes. Although they have started researching the impacts of microplastic and nanoplastic, they are working to gain more information on the microbial films which manifest on microplastic and nanoplastic debris. Understanding how contaminants, such as microplastic and nanoplastic, affect the interactions between flora and fauna will better inform Berlin and the rest of the scientific community of the long-term dangers.

2.4.2 Technischer Jugendbildungsverein in Praxis (TJP)

TJP is a science-focused organization that promotes scientific experiments with people of all ages. In exchange for participating in their experiments and research, students and adults pay five euros per participant (or less if they bring their materials). The TJP facility is located in the Treptow-Köpenick district of Berlin. It is within walking distance of the Dahme and Müggel-Spree rivers, as well as the Müggelsee.

The TJP's goal is "to bring teachers, students, initiatives, providers and interested parties together" (*Technischer Jugendbildungsverein in Praxis (TJP)*, n.d.). They use experiments to enlighten and awaken an understanding of various scientific topics. METEUM, a sub-group of TJP, focuses on biological projects and involves students with biochemistry and renewable energy. They aim to arouse interest in STEM and help students develop skills they can use in the future. Umweltbildungszentrum is a relatively new group in TJP and focuses on environmental aspects of their community. This group emphasizes the importance of keeping our environment clean. Essentially, TJP is an activity center that teaches youth about current societal challenges by using experiments as a way to engage them (*Technischer Jugendbildungsverein in Praxis (TJP)*, n.d.). Through METEUM and Umweltbildungszentrum, participants can learn about how this type of pollution impacts their environment and why it is crucial to improve sustainability by reducing the number of plastics discarded.

2.4.3 Engaging Citizens in Water Pollution Research

Citizen science is an underutilized procedure that has been around for the last century but has become increasingly helpful in recent years. It engages the general public to collect large amounts of data, typically taking months or years for a few scientists alone. It can also be used as an educational tool, as demonstrated by a Chilean experiment that successfully informed and engaged the participants about microplastics (Hidalgo-Ruz & Thiel, 2013). A systematic literature review of 56 peer-reviewed research articles identified factors that contributed to successful citizen science projects. Three sets of design principles for citizen science projects in regards to water quality were identified, as shown in Figure 6 below:

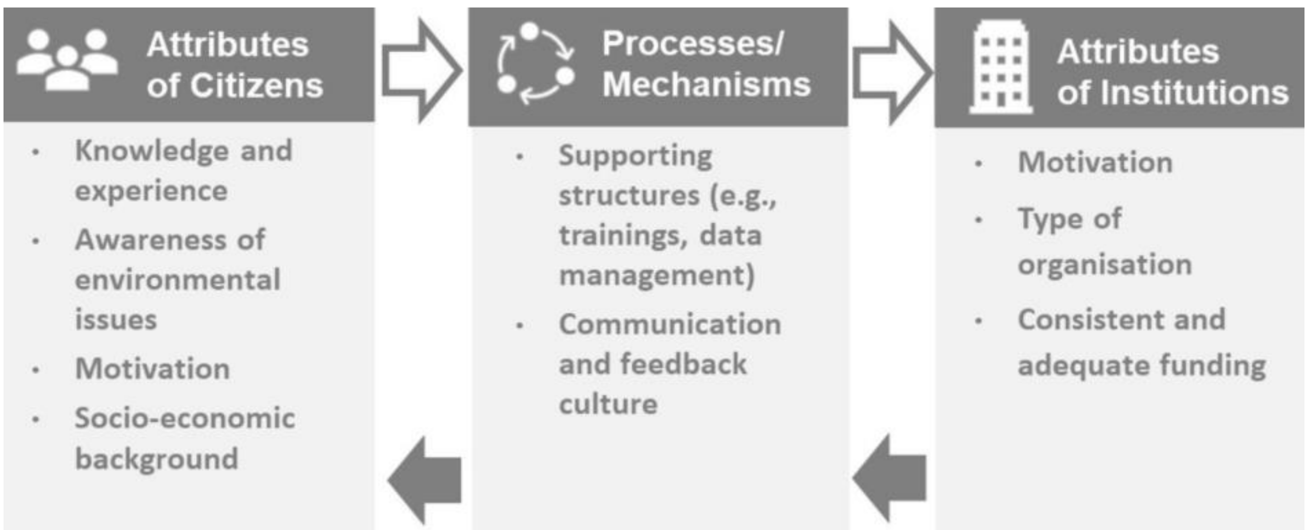


Figure 6. How to successfully design a citizen science experiment (Capdevila, 2020).

Due to the lack of sufficient data on water quality, citizen science projects show strong potential to address microplastic pollution. Since its emergence, many understand the importance of this technique and the symbiotic relationship it fosters between communities and science. By engaging the public, scientists can benefit by receiving large amounts of data to study, and focus on the scientific scope of the research. On the other hand, citizens can dive into a topic and become aware of certain aspects of science, biology, and chemistry. This allows them to gain perspective on societal challenges. To engage people, there needs to be a reason, and in this case, microplastic and nanoplastic pollution is growing rapidly and cannot be left unaddressed. Utilizing a citizen science approach could encourage communal desire to aid in local efforts towards monitoring plastic pollution.

2.4.4 Student Exposure to Natural Sciences

Currently, students of the district have a limited understanding of scientific topics and how they correlate to global challenges. Naturwissenschaften (NaWi) is the elementary science curriculum taught to 5th and 6th-grade German students. The objective of NaWi is to educate students on natural sciences through lessons and experiments (NaWi, 2015). Each series of lessons covers three topics of choice decided upon by teachers, including physics, chemistry, biology, and geology. The key components of NaWi experiments are the implementation of experiments alongside lesson plans (NaWi, 2015). Teachers have training sessions on conducting experiments, and NaWi provides experimental kits based on the lessons to participating schools (NaWi, 2015). Our sponsors informed us of some current scientific methods that the students will understand through the NaWi program, some of which include: indicators, detection reactions, sedimentation, filtration, separation of mixtures in substances, and the use of microscopes. Students are also introduced to procedural steps of experiment conduction, including hypothesis, observation, measuring, analyzing, and discussing. NaWi is an "action" based program that focuses on science education exposure in young students. Similar to many other programs and schools in Berlin, STEM education is growing in demand. Additional environmental education programs focused on STEM fields provide students with opportunities outside of the standard school curriculum, similar to an afterschool program, like TJP or a science camp.

Elementary school students receive a broad understanding of scientific topics through NaWi. Unfortunately, this does not mean students fully comprehend the role science plays in the world. With the rapidly growing concerns around microplastic and nanoplastic pollution, helping the district maintain its prosperity will rely on the education of the district's future generations.

3 Methodology

Our mission was to help Leibniz-IGB and TJP e.V./METEUM to improve awareness and knowledge about microplastics in Treptow-Köpenick, especially among school-aged children. To do this we decided on the following objectives:

1. To compile information on microplastic and nanoplastic and summarize the dangers they pose
2. To design a replicable experiment suitable for students to collect samples and

detect microplastic, ensuring its effectiveness and feasibility

3. To teach 5th and 6th grade students about the presence of microplastics and nanoplastics using informational material and engaging activities

This project only considered plastics that are less than 5mm in diameter in waterways in the Treptow-Köpenick District of Berlin. The compilation of research information focuses on the hazards of these microplastic and nanoplastic. This experiment is for those who have an introductory understanding of the scientific method. The research on microplastic is relatively new; therefore, the team only considered papers from the last 20 years. The experimental design of this project focuses on microplastics, as the feasibility of an experiment to collect nanoplastics was beyond the scope of consideration. The focuses of the experimental designs are collection and quantification. The team omitted the identification of specific plastic types due to the large variety of polymers. The coronavirus pandemic lockdown in Germany did not allow students to use this experiment during our project, so pilot trials were conducted by our team and by our sponsors.

The roadmap shown below outlines the rough methodology which the group followed throughout the process. Steps in blue represent our objectives, which are discussed in more detail later in the chapter. Steps in gray represent the methods used to complete each objective and our results. We researched the dangers of microplastic and nanoplastic as toxins and the threats posed by biofilms. We found additional methods to collect and test both sand and water samples using low-cost materials and focusing on a citizen based approach. We used the scientific process to complete and refine the experiment. The team and the sponsors conducted experimental trials, which allowed us to complete an efficacy and feasibility study. Our final step was to produce a lesson plan consisting of several pieces of educational material for 5th and 6th grade students in the Treptow-Köpenick district. The lesson plan contained distilled information from the literature review in the form of an infographic and slideshow presentation for TJP. Additional experiments were added to enhance the students' learning and involvement.

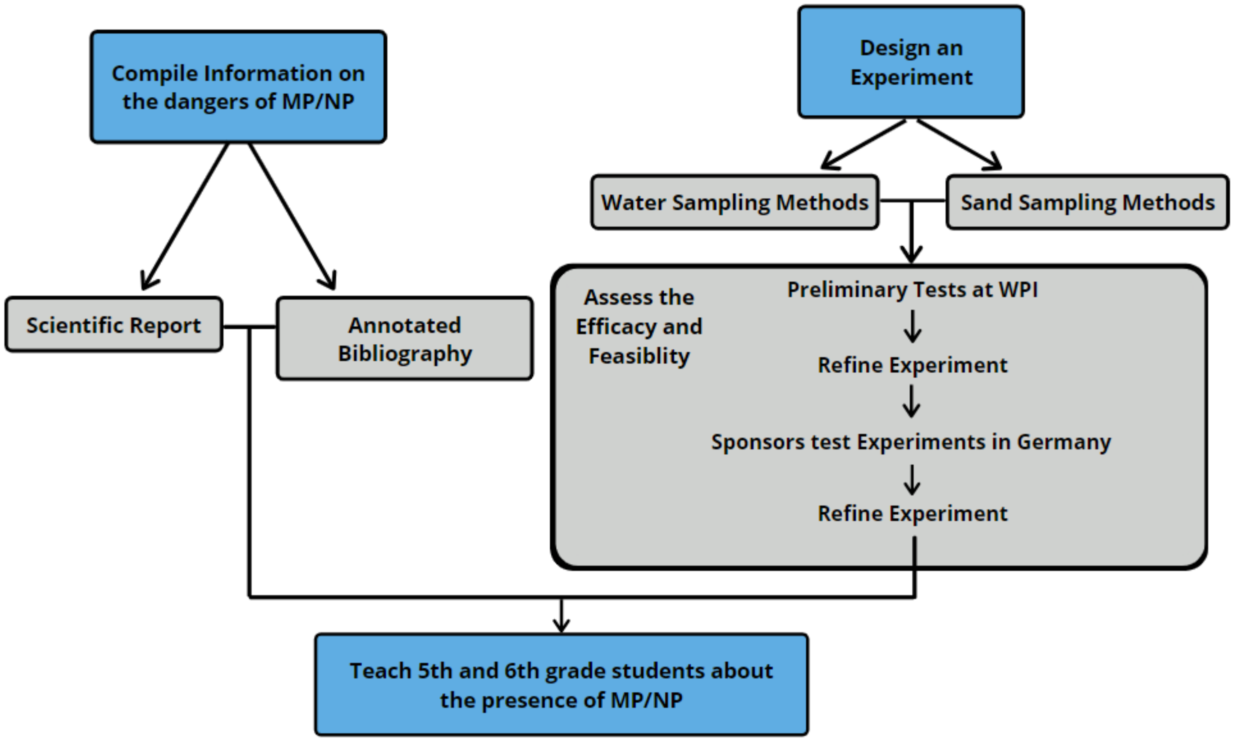


Figure 7. Project methodology roadmap.

3.1 Compiling Information on the Hazards of Microplastic and Nanoplastic

Our team consulted scholarly articles to obtain comprehensive data that could aid the IGB in understanding the biotic effects of microplastics. Using various benchmark sources that examine saltwater and freshwater, including studies done by the IGB, we gathered information on potential dangers caused by microplastic and nanoplastic pollution that aquatic biota and terrestrial biota face. We presented the material as a literature review and organized an annotated bibliography so the IGB can easily survey the articles. The research conducted on microplastic and nanoplastic pollution enables us to begin informing the next generations of their presence and the threats they pose to the planet's health.

3.2 Designing an Experiment Suitable for Students

In the experiment, we aimed to help students understand the process of data collection and analysis, as well as the importance of sample size. The principles learned in this experiment will increase the students' understanding for the world around them and challenge them to think more critically. TJP and the municipality are willing to conduct this experiment annually, which will allow for young students to get more exposure to the scientific method and experimental process, and for Treptow-Köpenick to gauge the health of their waterways.

In order to successfully design an experiment, we researched how past citizen based experiments were produced. Once the different attributes of citizen science were acknowledged, such as the need for simplicity, we investigated different experimental methods that seemed suitable for 5th and 6th grade students. These methods included several density separation, sieving, and filtration techniques, which could be conducted by these students to detect microplastic in bodies of water and shorelines.

Although we found several suitable experimental methods, we had to consider the capabilities, materials, and instruments that TJP possessed. TJP had access to light microscopes, filters, and other rudimentary lab equipment. With this in mind, we created two experimental protocols, one involving a shoreline sand sample, and the other a direct water sample.

Many shore samples involved sieving devices that either required a lot of money or a lot of time. Given these limitations and the scope of this project, the team opted to draw on a past WPI experiment that used density separation to extract microplastic from the sand. Corn syrup was used as the solvent since its density is high enough for most plastics to float while sand, rocks, and most beach debris sink. Following their protocol, we collected samples of both soil and sand for separation.

There were more water sampling methods which could be feasible for 5th and 6th grade students to perform. Ultimately we decided to use a filtration method because it was the simplest method and TJP had more instruments readily available, such as a vacuum pump. Additionally, German students will have familiarity with filtration from the NaWi program. At several locations near Worcester Polytechnic Institute (WPI), we were able to perform the experiment and conduct an efficacy study.

Upon successful completion, the experiment was sent to our sponsors to assess its feasibility for young students. Ideally, TJP and their students would complete this task, but due to SARS-CoV-2 restrictions, they were unable to do this. Therefore, our sponsors and their children volunteered to perform the experiment and gave feedback through a reflection worksheet (Appendix I). The team continued to consider the economic, technical, and operational considerations when making improvements, and due to uncertainty of where the experiment will be conducted in the future, the largest restriction was minimizing expenses. Therefore, purchases for the filtration experiment were limited to 20 USD.

In order to detect microplastics, the team researched several low-cost methods, such as the naked eye, light microscopes, and fluorescence tests. Additionally, we spliced these techniques together in an attempt to improve accuracy. Any purchased material for this step of the experiment was kept to a minimum and is discussed in the following chapter.

3.2.1 Preliminary Water Sampling Experiment

Our team first performed the experiment using samples of water gathered from waterways situated in/around the Worcester area. We performed a simple microplastic filtration experiment using plastic and glass materials to collect the water and store-bought coffee filters. We learned about the immediate difficulties of the experiment. The coffee filters took upwards of 30 minutes to filter roughly 2-4 liters of water. Not only is it time-consuming, but without careful use, the filter broke easily, forcing us to restart the filtration process. Additionally, a lot of debris was caught in the filter making it hard to determine if the sample contained microplastics.

In our secondary trial we moved from store bought coffee filters to 150 micron nylon filters and funnel, this allowed us to speed up the filtration process and increase the filter's durability. We added the use of a larger kitchen strainer before further filtration as a means of collecting larger organic debris that inhibited smaller particle visibility. Our team introduced hydrogen peroxide soaking due to its ability to digest organic material which was abundantly present in our samples. The aim of this was to increase the ability to visualize any microplastics that were obtained in the trial. In order to confirm that our materials were able to effectively collect microplastics, we synthesized a sample that contained microplastics.

We created this sample by adding facewash with primary microplastics to water and other debris. This sample enabled our team to test the effectiveness of the materials for collecting microplastics.

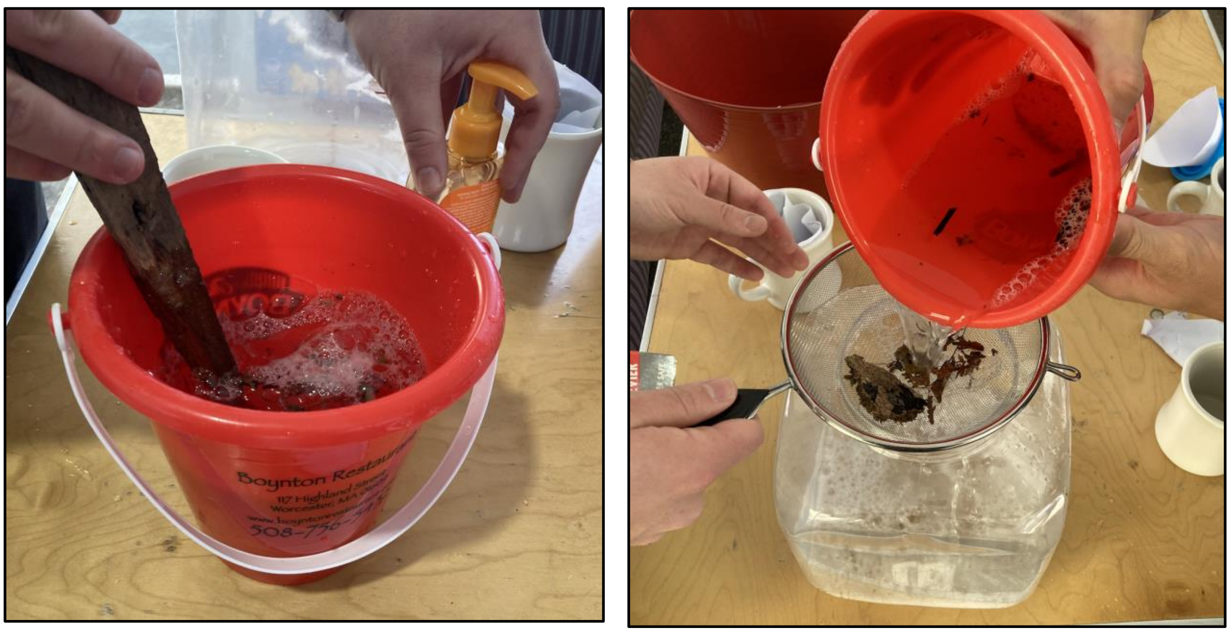


Figure 8. The first image shows us preparing a contaminated sample of water using face wash with microbeads. The second is the filtration process of the contaminated sample using a large kitchen strainer.

In our third trial, we adjusted the volume of water sampled and introduced a new 55 micron bucket filter. The addition of the bucket filter permitted our team to sample 70 liters of water in the same time it took to filter 5 liters before. This indicated the experiment can be performed on-site, increasing how quick the tests can be performed. Smaller pore size makes collecting smaller microplastics feasible. In this trail, we performed multiple hydrogen peroxide soakings to ensure sufficient organic digestion.

Figure 9. Testing finalized protocol in a local waterway using the bucket filter on-site.

The tables below indicate the pros and cons of the filters, magnification techniques and lighting options that we used throughout the experiment.

Filter Options	Paper Filter	150-micron Nylon Filter	55-micron Nylon Bucket Filter
Pros	Cheapest option	Still affordable Much faster More durable than generic paper filters	Fast Done on-site (more water) Very durable
Cons	Slow Done at lab (must transport water) Can tear/rip	Done at lab (must transport water)	Largest cost (< 20 euros)

Table 1. Comparison of the different types of filters used for the filtration experiment.

We tested each method individually and in combination with one and other when possible. Each method had its benefits, although some were better than others regarding what was most effective in identifying microplastics.

Magnification Techniques	Naked Eye (1x)	Magnifying Glass (5x)	Smartphone Microscope (523x)	Light Microscope (1000x)
Pros	Low-cost Can see entire sample	Low cost Can view sample as a whole Can distinguish larger debris	Low-cost Engaging/interactive Quicker to transition between clumps than the light microscope Can distinguish what most of the contents of the sample are	At TJP lab (no cost) Best way to determine if a piece of the sample is a microplastic
Cons	Cannot see anything except the largest microplastic (if found But usually will be smaller)	Difficult to tell what is in the sample aside from large debris	Only small samples can be observed (less than light microscope but still necessary) Purchase necessary	Difficult to analyze the entire sample Needs to be done on individual parts of sample Most time intensive

Table 2. Comparison of the four different magnification techniques used to determine if microplastics were present.

We researched several lighting methods that could be used to enhance the contrast between potential microplastics and the contents of the filter.

Light Sources	Natural Light	Blacklight	UV Light
Pros	Cost-free	Inexpensive Causes fluorescence	Inexpensive Causes fluorescence High contrast
Cons	It can be difficult to identify what is a microplastic or other debris	The fluorescence can be difficult to see on certain backgrounds Must purchase	Must purchase

Table 3. Comparison of the three different light sources we experimented with

3.2.2 Testing the Sand Sampling Method

We performed the sand/soil sampling method in tandem with the water sampling method during the first trial. We had samples of soil from the edge of a nearby river. The soil samples did not prove effective since the density of soil changes with its composition. Therefore, the sample stayed well-mixed in a dark, muddy state.

Figure 10. Results from density separation of a wet soil sample.

Since soil could not work, we gathered and tested a sand sample from a nearby beach. This proved to work much better and the density separation could be easily seen.

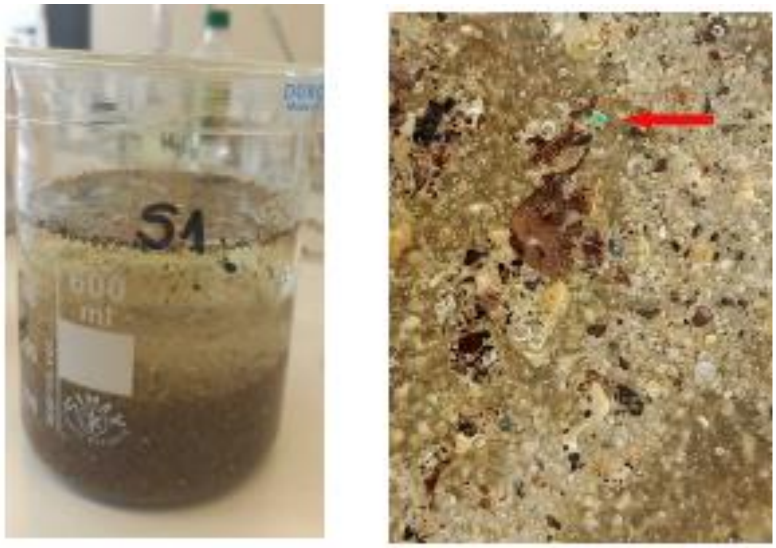


Figure 11. Results obtained from TJP's density separation of a sand sample. A blue-green microplastic is present and is indicated by the red arrow in the second picture.

3.2.3 Sponsor Trials and Feedback

We assessed the technical feasibility of our experiment when individuals from Leibniz-IGB, TJP, and associated persons performed the experiment. They made comments and suggestions to improve the clarity and functionality of the experiment and are in Appendix J. The primary concerns were over the volume of water needed to be collected and the ability to identify microplastics in the sample definitively. The comments allowed us to improve the experiment for future use with recommended adjustments, such as the bucket filter.

We purchased several products that were accessible to everyday people and would be helpful in the experiment. We tested each product, as described above. The filtration products bought were restricted to under 20 USD, or about 16.67 euros, but are not required to perform the experiment. We were unable to use a vacuum pump with a paper coffee filter. However, given the feedback from TJP, it seemed to work reasonably well and caused no problems.

3.3 Teaching 5th and 6th Grade Students through Educational Material

We developed experiments but we also know its success depends on whether students learn or not. Educational content is needed before and after the experiment is to be conducted. Using the information collected for our literature review and the designed experiment, we produced an overall lesson plan for TJP to teach 5th and 6th grade students about microplastic and nanoplastic pollution. This outlined the entire project, consisting of several visual aids and activities to assist in teaching the students, such as a slideshow presentation and infographic booklet, which were translated into German by our sponsors.

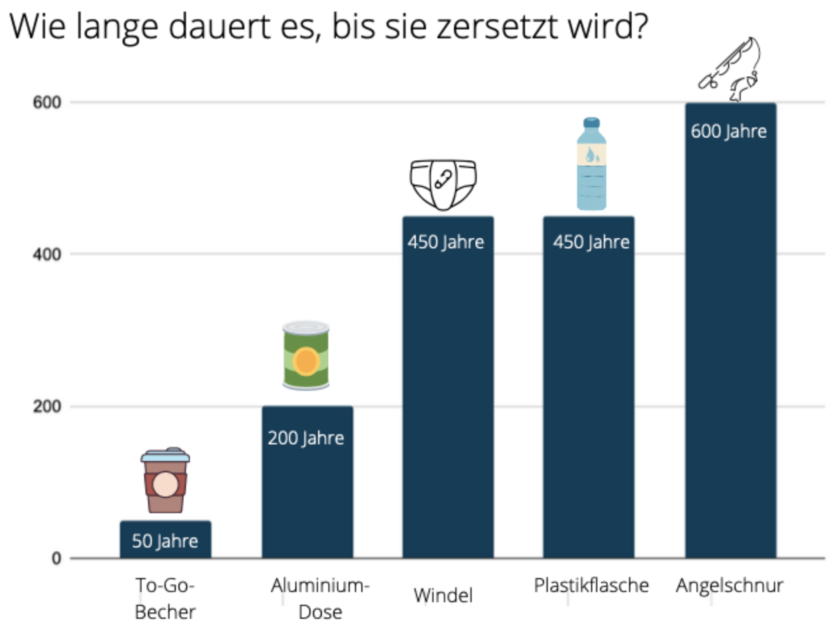


Figure 12. Example visual aid we created for the TJP student presentation. This graphic compares the average time it takes certain plastics to degrade ("Canada to Ban Single-Use Plastics as Early as 2021," 2019).

The lesson plan was created to reinforce the proper educational outcomes from the experimental activity. We consulted a lesson plan for 5th and 6th grade science activities to ensure clear teacher instructions in creating this. We also expanded the experiment in the lesson plan to encompass more on microplastic pollution. Our goal was to ensure students recognize the dangers of microplastic pollution regardless of their results.

We made a slideshow presentation for the students to be presented by TJP — before and after the experiment. It introduces them to the problem, engages them with interactive questions, and concludes with how they can help. Using online resources, and dialogue with our sponsors and advisors, the focus was placed on visuals and comparisons. The goal is to keep the students engaged and help them understand the dangers, and prevalence, of plastic and microplastic pollution.

In creating the infographics, we distilled the content directly from our literature review and referenced example infographics for young students provided to us by our sponsor. Again, we placed a focus on aesthetics and simplicity so we can pique the students' interests. The infographics offer students an engaging introduction to science and their local community sustainability efforts. Involving 5th and 6th grade students in local sustainability efforts could play a crucial role in improving Treptow-Köpenick's quality of life for future generations.

4 Results and Analysis

This chapter contains the results obtained from our compiled information, experimental protocol, the subsequent trials conducted to assess its efficacy and feasibility, and the overall lesson plan. The literature review summarizes the numerous effects of microplastic and nanoplastic in more depth. The annotated bibliography can assist Leibniz-IGB in gathering primary sources if desired. The team used the scientific process and engineering principles to create a final experiment that 5th and 6th grade students could complete. We created a lesson plan that TJP can follow to help teach 5th and 6th grade students learn about microplastic and nanoplastic pollution. The lesson plan contained the teacher notes, which included a collection of infographics and a presentation. The plan has several iterations of the experiment and encourages 5th and 6th grade students to engage in and make a change in their community.

4.1 Compilation of Scientific Studies on Microplastics and Nanoplastics

The technical research obtained from various literary journals led to the creation of a literature review on the immediate and potential long-term effects of microplastic and nanoplastic residence in waterways. It consisted of 30 sources from various accredited scientific journals, such as *Environmental Science & Technology* and *aquatic Environmental Research*. It allowed the team to display the diverse impacts microplastic and nanoplastic has on ecosystems, flora, and fauna. The literature review is in Appendix C. The team also created an annotated bibliography for Leibniz-IGB, which is in Appendix D. It summarizes individual articles and can direct Leibniz-IGB to the primary sources. Our literature review will hopefully enhance the knowledge and information that Leibniz-IGB can disseminate to the public and enable them to continue researching water sustainability and environmental impacts.

4.2 Student Experiment to Detect Microplastics

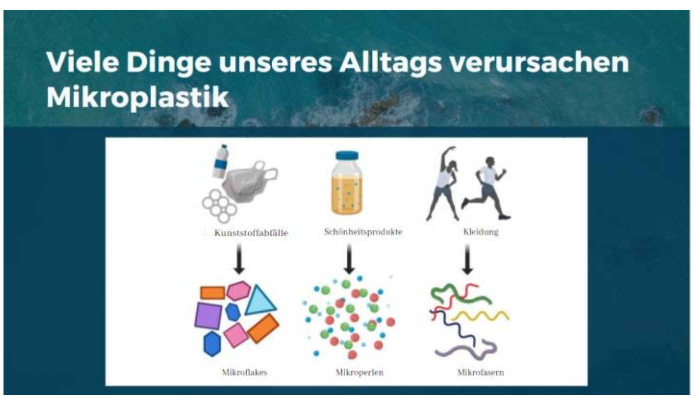
The **finalized water sampling protocol**, shown in Appendix E, would begin at the TJP lab where the students and instructors would determine the water source and location where the experiment will take place. The students will take the bucket filter, a bucket of known volume, and several smaller containers to collect water. Once at the water source, the student would proceed to clean out the smaller containers from water at the water source to get rid of debris from previous use. The students will collect water and pour it through the large kitchen strainer and then the bucket filter. Since the large bucket has a known volume, the instructors will know how much water is collected. Students will fill the large bucket until full, empty the filtered water, and repeat. Once a sufficient amount of water is collected, the bucket filter will be brought back to the lab. The students would carefully transfer their sample material by softly scraping the material from the bucket filter onto a dry filter. Using gloves and goggles for safety, these filters will be soaked in hydrogen peroxide to digest the remaining organic material. These will sit until the following day, so students will label their sample and leave them where the teacher instructs them to do so. On the second day, students should put on safety equipment again and carefully take the filter out of the hydrogen peroxide. Students can use the magnification tools and light techniques (smartphone microscope and UV light) to see if microplastics are in their sample. If they find something that they think is microplastic, they should record why in their reflection sheet and bring the sample to the instructor at TJP. The instructor will use the light microscope as a final way to identify the material in question.

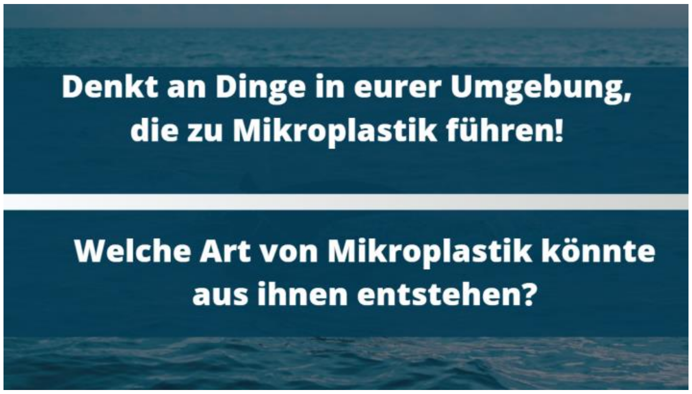
The **finalized sand sampling protocol**, shown in Appendix F, will also begin at the TJP lab where the water source and location of the sample will be determined. The students will only need a container to hold the sand in. The students and instructors will make their way to the location site, and collect several handfuls of sand from the edge of the shore. Students will take their samples back to the lab, where they will add corn syrup to the container and mix it thoroughly for two minutes. Once mixed, label the sample and leave it where the instructor wants them to sit for 24 hours. The following day, the students will look at their sample and write down what they see. Using a magnifying glass, students will try to see microplastics that are floating in the corn syrup. Similarly to the water sampling method, if students believe they have found microplastics at the top of the corn syrup, they can remove the material with tweezers. After removing the material, students will need to wash it in a small container of water before it is brought to a TJP instructor for light microscopy and final determination. The **complete water and sand experimental protocols** can be seen in **Appendix E** and **Appendix F**, respectively.

4.3 A Lesson Plan to Teach Students about Microplastics and Nanoplastics

As students, we recognize that an activity's success depends on whether the participants can learn from the experience. With this in mind, we created more educational content for TJP to use along with the experiments. We created an **overall lesson plan** for the instructors at TJP that includes additional steps to ensure the students leave knowing that microplastics are present in their lives, shown in Appendix H. The lesson plan contains a **presentation**, **experimental activity**, **infographic booklet**, **reflection worksheet**, and **supplemental videos**.

We produced a **presentation** to engage the students on the topic before and after the activity, which can be found using the link in Appendix H. We focused on comparisons to give the students perspective. The slides begin with the scale of plastic pollution. The presentation moves into microplastic presence and includes a few questions to be interactive and engage students to think critically about the role plastic plays in their lives. The experimental activities take place in the middle of the presentation. Afterwards, the remainder of the presentation will be given, which focuses on ways these students can reduce their personal plastic/microplastic waste. Figure 12, below, contains a few example slides from this presentation.

	This slide occurs in the background and gives context on the origin of different types of microplastics. Larger Plastics → Microflakes Beauty Products → Microbeads Clothing → Microfibers

<table>
<tr>
<td>
</td>
<td>We prompted several different critical thinking discussion questions for the students. Some of those including:

1. “What items around you produce microplastics?”
2. “What kind of microplastics will they produce?”</td>
</tr>
<tr>
<td>
</td>
<td>These highlighted some local initiatives to encourage students to utilize opportunities outside of the classroom to be more involved in sustainability efforts. Some initiatives include:
● Unverpackt
● GreenKayak
● a tip:tap</td>
</tr>
</table>

Figure 13. Example slides from the TJP student presentation.

In “Experimental Activity: Part 1”, the instructor synthesizes a sample of water contaminated with microplastics. This activity exposes students to the methods used in the experiment and the microplastics present in their life. The additional step in “Experimental Activity: Part 2” has students take time to clean up any plastic trash they can find in the area of consideration. Part 2 furthers the point of how widespread plastic pollution is and allows the students to perform the experiments to detect microplastics. The experimental activities can be found through the lesson plan in Appendix H.

As learning material for the students to keep after the activity, we produced an **infographic booklet**, which can be found using the link in Appendix H, using the information gathered from our research for the first objective. We distilled the content to be suitable for 5th and 6th grade students and focused on the visualization. The use of images and colors in an aesthetically pleasing way is best to keep students engaged, entertained, and informed. The infographic images in Figure 13 below are example images from the infographic collection.

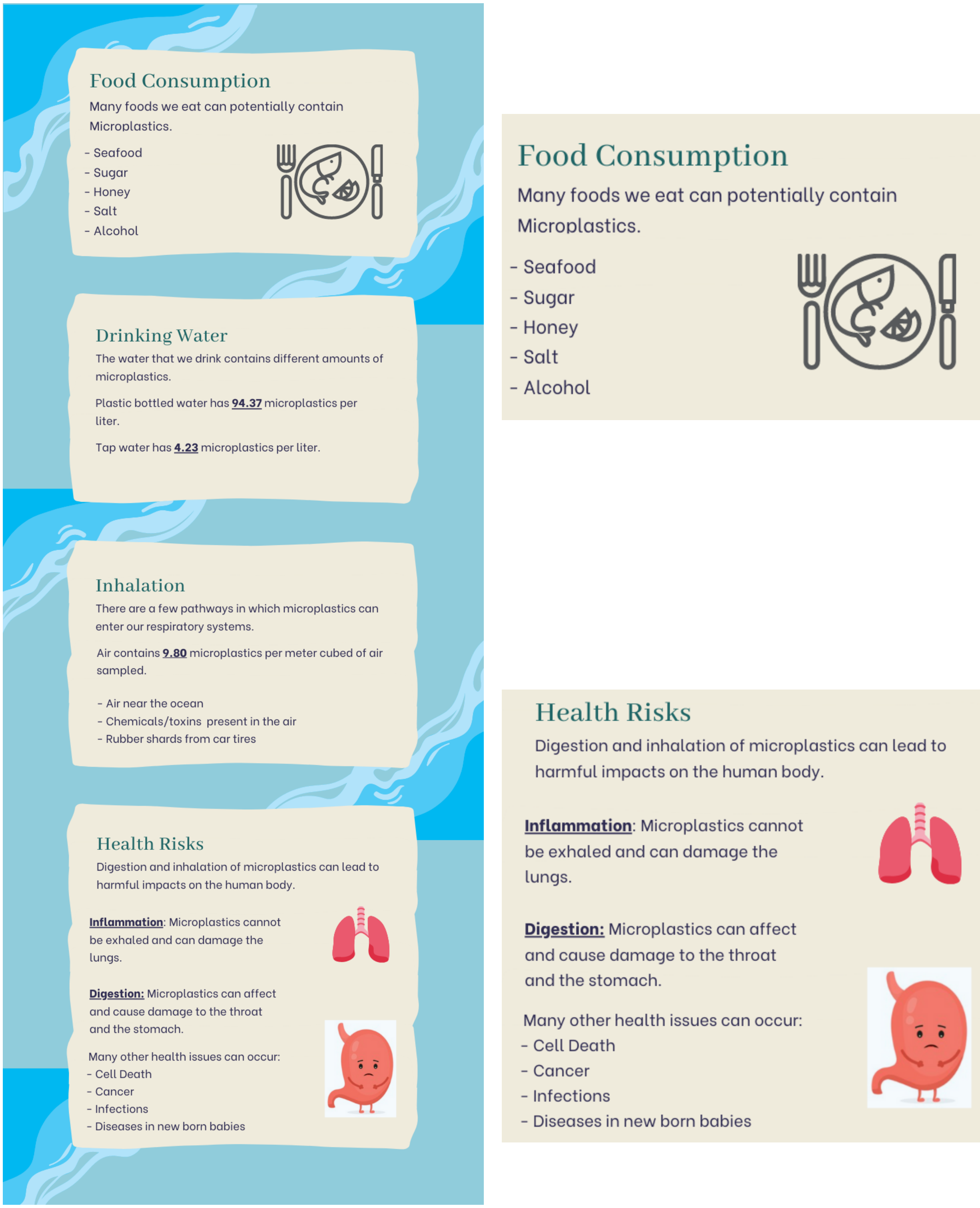
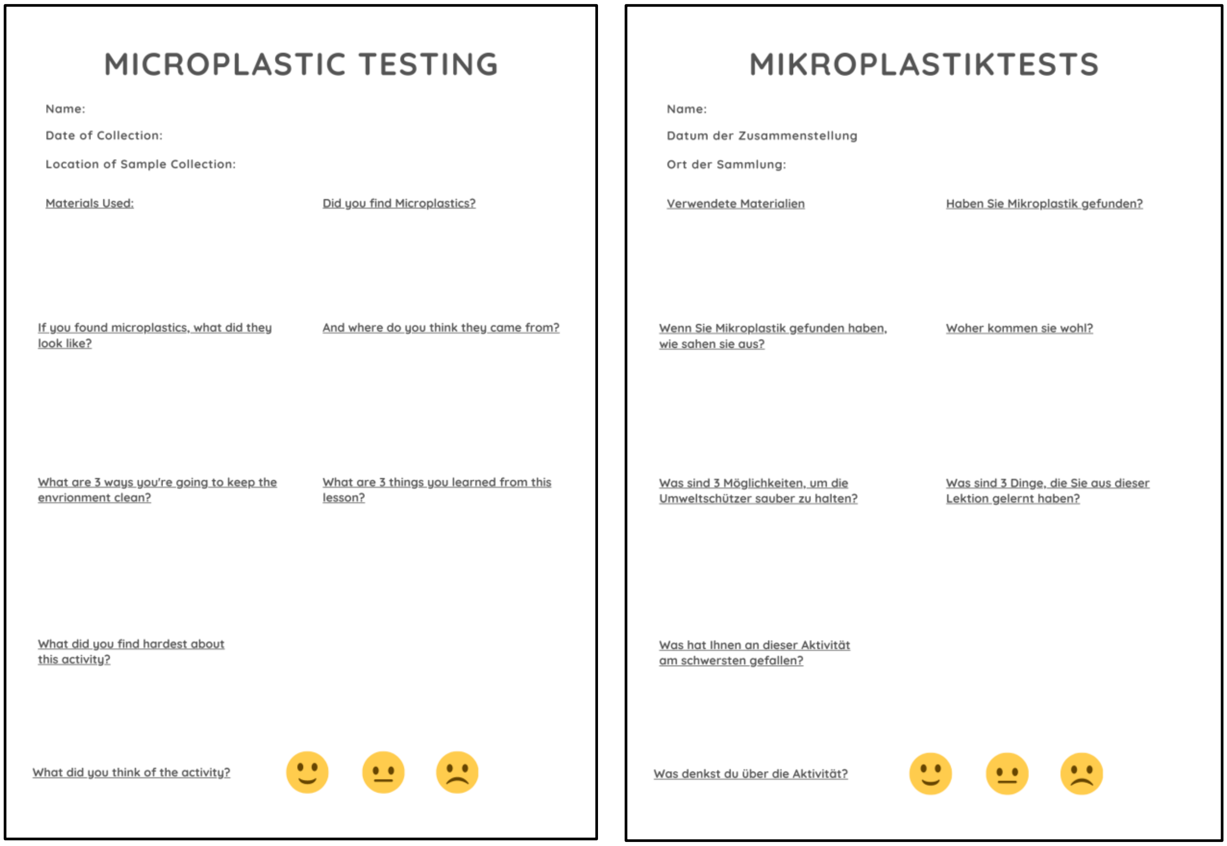


Figure 14. Example page from our infographic collection.This page shows how microplastics and nanoplastics reach humans through things such as food, water, and inhalation. They pose several health risks to humans.

A **reflection worksheet** was created to be filled out after the experimental activity and presentation and allows students to think about what they learned. It asks what students found difficult and calls them to list several ways to stay active in cleaning their community.



MICROPLASTIC TESTING

Name:
Date of Collection:
Location of Sample Collection:

Materials Used:
Did you find Microplastics?
If you found microplastics, what did they look like?
And where do you think they came from?
What are 3 ways you're going to keep the envrionment clean?
What are 3 things you learned from this lesson?
What did you find hardest about this activity?
What did you think of the activity?

MIKROPLASTIKTESTS

Name:
Datum der Zusammenstellung
Ort der Sammlung:

Verwendete Materialien
Haben Sie Mikroplastik gefunden?
Wenn Sie Mikroplastik gefunden haben, wie sahen sie aus?
Woher kommen sie wohl?
Was sind 3 Möglichkeiten, um die Umweltschützer sauber zu halten?
Was sind 3 Dinge, die Sie aus dieser Lektion gelernt haben?
Was hat Ihnen an dieser Aktivität am schwersten gefallen?
Was denkst du über die Aktivität?

Figure 15. English and German reflection worksheets created by our team for students to fill out after the lesson is completed.

We added **supplemental videos** regarding the topic of plastic pollution to the end of the lesson plan. This gives students another perspective aside from the presentation and infographic collection that our team produced. The videos are relatively short, but contain information on how to make environmentally friendly changes in their life. Some videos illustrate ways families are reducing their plastic use, while others focus on the science behind how microplastics and nanoplastics enter the environment and impact flora and fauna. All of these videos are to be shown at the instructor's discretion and are simply options to have an entertaining and informative conclusion to the lesson.

5 Conclusions and Recommendations

This section addresses each of the stakeholders involved in the development and completion of this project. We include conclusions on the objectives and results, and state recommendations for further education on microplastics and nanoplastics. Overall, we hope we aided Leibniz-IGB and TJP in their research and education of the district on microplastic and nanoplastic pollution. We researched the known effects of microplastic and nanoplastic on ecosystems and compiled the information in a literature review for Leibniz-IGB. An annotated bibliography was created to go along with our literature review, which allows the reader to easily navigate through the referenced research on specific impacts of microplastic and nanoplastic. Our team created a low-cost, student experiment to introduce 5th and 6th grade German students to a problem their community faces. We enhanced the students' learning outcomes of the experiment by producing a lesson plan that TJP can use to engage and enable them to make a difference in their community. The lesson plan involved informational material on microplastic and nanoplastic to help students understand their dangers. It also contained several experiments to help introduce students to laboratory techniques and get them to actively participate outside of the classroom.

5.1 Conclusions and Recommendations for the Student Experiment

We refined our project throughout the design process to ensure it was suitable, feasible and effective for students. One prevailing challenge from the trials was the transportation of water from the sampling location to the laboratory, for which we tested an experiment which can be conducted next to a body of water. Each of our created experiments are simple and easily replicable for 5th and 6th grade students. With that said, continually updating the experiment to be catered towards engaging the students at TJP and improving learning outcomes is essential to improve its efficacy each year. The feasibility of each experiment needs to continue to improve to be effective and engaging and educating students. By performing the experiments with students and getting feedback, it will be more effective than the ones we performed in this report.

5.1.1 Perform Benchmark Testing to Obtain Accurate Concentrations

We strongly recommend that **Leibniz-IGB obtains benchmark values of the microplastic concentration** of local waterways annually. A student experiment can be inaccurate, and the results may not be ideal. Therefore, by having TJP and the municipality stay in contact with Leibniz-IGB scientists, the teachers at TJP can obtain current microplastic concentrations from the Müggelsee Lake and Spree River. This data can show students how important it is to clean up their community, even if they do not see microplastic themselves. These benchmark values will be helpful when comparing the results that students obtain and Leibniz-IGB’s data will eventually be used to display the trend in microplastic pollution to the district.

5.1.2 Use Nylon or Glass Filters and 10-15% Hydrogen Peroxide

We recommend that **TJP uses nylon or glass filters**. The filtration time while using these filters is quick, even compared to vacuum filtration with paper filters. Additionally, we recommend using the bucket filter in teams for on-site testing because the students can collect the most water possible in the shortest amount of time. Our results showed that 3% hydrogen peroxide digested some organics, but there was still a lot left. In order to address this problem, we recommend that **TJP uses 10-15% hydrogen peroxide** because higher concentrations of hydrogen peroxide are more effective at dissolving organic matter. When handling hydrogen peroxide, especially in higher concentrations, it is important to use gloves, glasses and other protective equipment.

5.1.3 Perform On-site Filtration when Weather Permits

Depending on temporal boundaries, this experiment may be more or less feasible. While performing experiments in colder conditions, it was more sensible to transport collected samples and conduct them inside the facility. However, we recommend **TJP performs the bucket filtration** method when the weather is warmer. It can be done on-site and allows students to sample more water. Overall, both methods work, but they may produce different results due to the variations in the volumes of the samples.

5.1.4 Use Various Detection Techniques Together

Different detection methods that we used throughout our experiments had different benefits. Although it would be ideal to use microscopes exclusively, it is impractical. Therefore, we recommend **TJP uses of multiple detection methods together**. For example, students would identify what they believe is microplastic and bring it to the teachers to use the light microscope and make a final conclusion. If students use the smartphone microscope, it would be very engaging and interactive, it would also introduce them to altering magnifications and other microscope tools.

Our results showed that using a UV light and the smartphone microscope attachment together was the most effective. Therefore we recommend **using a UV light and a magnification tool** together. We believe that these strategies would be more educationally effective at showing students how difficult microplastic are to detect.

Many experiments use alternative detection methods. Although we could not test every variation, there may be one that works better in the future. For example, using the nile red indicator and blue light has shown promise. However, it is relatively challenging for a simple citizen based approach. Other rudimentary indication methods found online may be more suitable, such as heating a needle and causing plastics to shrink. Therefore, we recommend monitoring different ways Leibniz-IGB or other researchers have tested for microplastics.

5.2 Conclusions and Recommendations for the Lesson Plan

Microplastic and nanoplastic pollution is a developing concept in sustainability. Given plastics' durability and migration patterns to aquatic environments, penetration into freshwaters where industrial influence is not as closely related can be low. This does not negate the presence of microplastic and nanoplastic, nor does it excuse the possibility of worsening in the future. Instead, it highlights the need for continual testing and efforts to clean freshwaters and protect the organisms in the area.

Ultimately, the designed experiment is **best suited** to aid the Treptow-Köpenick municipality in engaging students, **not** collecting microplastic and nanoplastic data in their local waterways. TJP can assist the municipality by further educating the local community members on the presence and impacts of this type of pollution, but in order to obtain accurate data, the municipality will need to utilize Leibniz-IGB’s equipment.

5.2.1 Turn the Infographics into a Group Activity

The infographic booklet was created to inform students about several aspects of microplastic and nanoplastic pollution. We recommend that **TJP prints out each page of the infographic booklet** as posters and **uses them in a group activity**. The teachers at TJP can hang each one of the posters in a different area of the room, and then they can **break students up into**

small groups. Each group is assigned to one poster/page of the infographic booklet, and will be asked to share what they learned to the rest of the class. This is a simple way to introduce students to this topic, engage them through collaboration, and see what information the students attained best.

5.2.2 Work with Other Organizations in the Area

This project was specific to working with Leibniz-IGB and TJP, however we suggest that **the municipality reach out to additional organizations** in the area as well. Improving relationships with other scientific institutions and education associations, such as Forschungsverbund Berlin eV or IMWE Berlin eV, will improve the communal dynamic to combat microplastic and nanoplastic pollution. It will also enable the local government to collect data and bring awareness to different areas of the district.

5.2.3 Endorse Sustainability Initiatives

Throughout creating our lesson plan for TJP, we learned of several initiatives that work to improve sustainability in Berlin. Given the social impact they can have on the local communities, we recommend that **the municipality of Treptow-Köpenick and TJP vocally recognizes these initiatives**. Although increasing awareness of the microplastic and nanoplastic challenge in students will have a positive impact, the benefits of GreenKayak, Unverpackt, Alles Im Fluss, and other organizations should be expressed to everyone in the community. By endorsing initiatives that improve the community’s sustainability, an emphasis will be **put on eco-friendly habits** and being environmentally aware.

As we continue to address the problem of plastic pollution, whether macroplastics, microplastics, or nanoplastics, we must consider that it is our responsibility to protect, mitigate, and manage the health of our planet and surrounding ecosystems.

References

Alimba, C. G., & Faggio, C. (2019). Microplastics in the aquatic environment: Current trends in environmental pollution and mechanisms of toxicological profile. *Environmental Toxicology and Pharmacology*, *68*, 61–74. https://doi.org/10.1016/j.etap.2019.03.001

Amos, J. (2020, April 30). High microplastic concentration found on ocean floor. *BBC News*. https://www.bbc.com/news/science-environment-52489126

Annex XV Restriction Report: (1.2). (2019). European Chemicals Agency. https://echa.europa.eu/documents/10162/05bd96e3-b969-0a7c-c6d0-441182893720

Auta, H. S., Emenike, C. U., & Fauziah, S. H. (2017). Distribution and importance of microplastics in the aquatic environment: A review of the sources, fate, effects, and potential solutions. *Environment International*, *102*, 165–176. https://doi.org/10.1016/j.envint.2017.02.013

Bergmann, M., Mutzel, S., Tekman, M., Trachsel, J., & Gerdts, G. (2019). White and wonderful? Microplastics prevail in snow from the Alps to the Arctic. *Science Advances*, *5*(8). https://doi.org/10.1126/sciadv.aax1157

Campanale, C., Massarelli, C., Savino, I., Locaputo, V., & Uricchio, V. F. (2019). A detailed review study on potential effects of microplastics and Additives of Concern on Human Health. *International Journal of Environmental Research and Public Health*, *17*(4), 1212. https://doi.org/10.3390/ijerph17041212

Canada to ban single-use plastics as early as 2021. (2019). *BBC News*. https://www.bbc.com/news/world-us-canada-48477087

Cózar, A., Sanz-Martín, M., Martí, E., González-Gordillo, J. I., Ubeda, B., Gálvez, J. Á., Irigoien, X., & Duarte, C. M. (2015). Plastic Accumulation in the Mediterranean Sea. *PLOS ONE*, *10*(4), e0121762. https://doi.org/10.1371/journal.pone.0121762

De Falco, F., Di Pace, E., Cocca, M., & Avella, M. (2019). The contribution of washing processes of synthetic clothes to microplastic pollution. *Scientific Reports*, *9*(6633). https://doi.org/10.1038/s41598-019-43023-x

Dris, R., Gasperi, J., Rocher, V., Mohamed, S., & Tassin, B. (2015). Microplastic contamination in an urban area: A case study in Greater Paris. *Environmental Chemistry*, *12*. https://doi.org/10.1071/EN14167

Dris, R., Gasperi, J., Saad, M., Mirande, C., & Tassin, B. (2016). Synthetic fibers in atmospheric fallout: A source of microplastics in the environment? *aquatic Pollution Bulletin*, *104*(1), 290–293. https://doi.org/10.1016/j.marpolbul.2016.01.006

Forrest, S. A., Holman, L., Murphy, M., & Vermaire, J. C. (2019). Citizen science sampling programs as a technique for monitoring microplastic pollution: Results, lessons learned and recommendations for working with volunteers for monitoring plastic pollution in freshwater ecosystems. *Environmental Monitoring and Assessment*, *191*(3), 172. https://doi.org/10.1007/s10661-019-7297-3

Geyer, R., Jambeck, J. R., & Law, K. L. (2017). Production, use, and fate of all plastics ever made. Science Advances, 3(7), e1700782. https://doi.org/10.1126/sciadv.1700782

Gopinath, P. M., Saranya, V., Vijayakumar, S., Mythili Meera, M., Ruprekha, S., Kunal, R., Pranay, A., Thomas, J., Mukherjee, A., & Chandrasekaran, N. (2019). Assessment on interactive prospectives of nanoplastics with plasma proteins and the toxicological impacts of virgin, coronated and environmentally released-nanoplastics. Scientific Reports, 9(1), 8860. https://doi.org/10.1038/s41598-019-45139-6

Hidalgo-Ruz, V., & Thiel, M. (2013). Distribution and abundance of small plastic debris on

beaches in the SE Pacific (Chile): A study supported by a citizens science project. aquatic Environmental Research, 87-88, 12-18. https://doi.org/10.1016/j.marenvres.2013.02.015.
History and Future of Plastics. (2016, July 18). Science History Institute. https://www.sciencehistory.org/the-history-and-future-of-plastics
How plastics are made. (n.d.). Plastics Europe. Retrieved February 21, 2021, from https://www.plasticseurope.org/en/about-plastics/what-are-plastics/how-plastics-are-made
Kyba, C., Conrad, J., & Shatwell, T. (n.d.). *Research for the future of our waters*. IGB Leibniz Institutions for Freshwater Ecology and Inland Fisheries. Retrieved March 17, 2021, from https://www.igb-berlin.de/
Leibniz Institute of Freshwater Ecology and Inland Fisheries (IGB). (n.d.). https://www.igb-berlin.de/en/profile
Mani, T., Hauk, A., Walter, U., & Burkhardt-Holm, P. (2015). Microplastics profile along the Rhine River. *Scientific Reports*, *5*(1), 17988. https://doi.org/10.1038/srep17988
Microplastics and Food. (2018, January 11). Food Federation Germany.https:// www.lebensmittelverband.de/en/food/sustainability-and-environment/microplastics
Microplastic Pollution. (2019, July 1). WaterGrades. https://watergrades.com/water-blog/f/microplastic-pollution
Müggelsee. (n.d.). Berlin.De. Retrieved February 23, 2021, from https://www.berlin.de/en/tourism/lakes/4361738-6218638-mueggelsee.en.html
Municipality of BERLIN-MITTE: demographic balance, population trend, death rate, birth rate, migration rate. (n.d.). Retrieved February 23, 2021, from https://ugeo.urbistat.com/AdminStat/en/de/demografia/popolazione/berlin-mitte/11001/4
One Year of Freshwater Research: Annual Research Report 2019. (2020). IGB Leibniz Institute of Freshwater Ecology and Inland Fisheries.
Open Access Policy of the Leibniz-Institute of Freshwater Ecology and Inland Fisheries. (2019). IGB Leibniz Institute of Freshwater Ecology and Inland Fisheries.
Parker, L. (2019, June 7). *The world's plastic pollution crisis explained*. National Geographic. https://www.nationalgeographic.com/environment/article/plastic-pollution
Phosphorus Cycle. (2020, February 17). *Science Facts*. https://www.sciencefacts.net/phosphorus-cycle.html
Plastic Packaging Resins. (n.d.). American Chemistry Council. https://plastics.americanchemistry.com/Plastic-Resin-Codes-PDF/
Plastic Waste: A European strategy to protect the planet, defend our citizens and empower our industries. (2018, January 16). [Text]. European Commission. https://ec.europa.eu/commission/presscorner/detail/en/IP_18_5
Plastics Facts —Different Types of Plastics. (n.d.). Plastics Facts. Retrieved March 8, 2021, from https://www.plasticsfacts.com/new-page-1
Pusch, M., & Hoffmann, A. (2000). Conservation concept for a river ecosystem (River Spree, Germany) impacted by flow abstraction in a large post-mining area. *Landscape and Urban Planning*, *51*(2), 165–176. https://doi.org/10.1016/S0169-2046(00)00107-9
RAC backs restricting intentional uses of microplastics. (2020, June 10). European Chemicals Agency. https://echa.europa.eu/-/rac-backs-restricting-intentional-uses-of-microplastics
Roch, S., Walter, T., Ittner, L. D., Friedrich, C., & Brinker, A. (2019). A systematic study of the microplastic burden in freshwater fishes of south-western Germany—Are we searching at the right scale? *Science of The Total Environment*, *689*, 1001–1011.

https://doi.org/10.1016/j.scitotenv.2019.06.404
Rummel, C. D., Jahnke, A., Gorokhova, E., Kühnel, D., & Schmitt-Jansen, M. (2017). Impacts of biofilm formation on the fate and potential effects of microplastic in the aquatic environment. *Environmental Science & Technology Letters*, *4*(7), 258–267. https://doi.org/10.1021/acs.estlett.7b00164
Society, M. (n.d.). *Nitrogen cycle | Microbes and the outdoors*. Retrieved February 23, 2021, from https://microbiologysociety.org/why-microbiology-matters/what-is-microbiology/microbes-and-the-outdoors/nitrogen-cycle.html
Structural constituency data Berlin-Treptow-Köpenick—The Federal Returning Officer. (n.d.). Retrieved February 23, 2021, from https://www.bundeswahlleiter.de/en/bundestagswahlen/2017/strukturdaten/bund-99/land-11/wahlkreis-84.html
Sulphate in River Spree and Lake Müggelsee | IGB. (n.d.). Retrieved March 7, 2021, from https://www.igb-berlin.de/en/project/sulphate-river-spree-and-lake-muggelsee
Technischer Jugendbildungsverein in Praxis (TJP). (n.d.). http://www.tjp-ev.de/start/
THE 17 GOALS | Sustainable Development. (n.d.). Retrieved February 23, 2021, from https://sdgs.un.org/goals
The Giant Issue of Microplastics. (2019, August 20). Water Pollution. https://www.water-pollution.org.uk/microplastic/
The Nitrogen Cycle | Animates Pet Supplies. (n.d.). Retrieved March 8, 2021, from https://www.animates.co.nz/articles/the-nitrogen-cycle
Tu, C., Chen, T., Zhou, Q., Liu, Y., Wei, J., Waniek, J. J., & Luo, Y. (2020). Biofilm formation and its influences on the properties of microplastics as affected by exposure time and depth in the seawater. *Science of The Total Environment*, *734*, 139237. https://doi.org/10.1016/j.scitotenv.2020.139237
Tyree, C., & Morrison, D. (n.d.). *Plus Plastic: Microplastics Found in Global Bottled Water*. Orb. Retrieved February 21, 2021, from http://orbmedia.org/stories/plus-plastic/
US EPA, O. (2020, November 6). *Plastic Pollution* [Overviews and Factsheets]. US EPA. https://www.epa.gov/trash-free-waters/plastic-pollution
Welle (www.dw.com), D. (n.d.). *Fridays for Future: The #Climatestrike movement comes of age | DW | 15.03.2019*. DW.COM. Retrieved February 23, 2021, from https://www.dw.com/en/fridays-for-future-the-climatestrike-movement-comes-of-age/a-47938623
Willkommen—TJP e.V. (n.d.). Retrieved February 23, 2021, from http://www.tjp-ev.de/start/
Yang, Y., Liu, W., Zhang, Z., Grossart, H.-P., & Gadd, G. M. (2020). Microplastics provide new microbial niches in aquatic environments. *Applied Microbiology and Biotechnology*, *104*(15), 6501–6511. https://doi.org/10.1007/s00253-020-10704-x

Appendices

Appendix A: Sponsor Description

Leibniz-IGB was founded in 1992 by the merger of three predecessor non-university research institutions. The three predecessor institutions were: *The Institute for Inland Fisheries (IfB) in Berlin-Friedrichshagen, The Department of Experimental Limnology Neuglobsow* of the Central Institute for Microbiology and Experimental Therapy, and *The Department of Hydrology at Mügggelseedamm 260*, a branch of the Institute for Geography and Geoecology. It was headquartered in Müggelsee and had an experimental station in Stechlinsee. To date, the institution has several locations throughout Germany and has obtained its present name: "*Leibniz Institutions for Freshwater Ecology and Inland Fisheries*", after the inclusion of the Leibniz Association in 2000. The institute has undergone changes in administration as recently as 2020, when they introduced a new director: Luc De Meester, a Belgian aquatic ecologist and evolutionary biologist (Leibniz-Institut für Gewässerökologie und Binnenfischerei, 2019).

The Leibniz-IGB mission statement, **"**Research for the future of our freshwaters," exemplifies the purpose of their union. They strive to research and obtain a vast understanding of the complex relationships between bodies of water and their biota. Their research aids in predicting responses to "natural and human-induced environmental changes and to develop measures for sustainable water management" (Kyba). Additionally, Leibniz-IGB's mission is to make their research findings, develop solutions, and recommendations for conservation and management of inland waters publicly available, and ultimately support decision-making processes at the local, national and international levels. This is why they support an Open Access Policy, which works to "provide unlimited and free access to quality-controlled academic information" (Leibniz-Institut für Gewässerökologie und Binnenfischerei, 2019) by removing technical, financial, and legal barriers. This is promoted by making any legally eligible publications by the academic staff openly accessible, providing its staff funding opportunities for publishing in open access journals, and other means of implementation. This policy was agreed on by the Board of Management in December 2019.

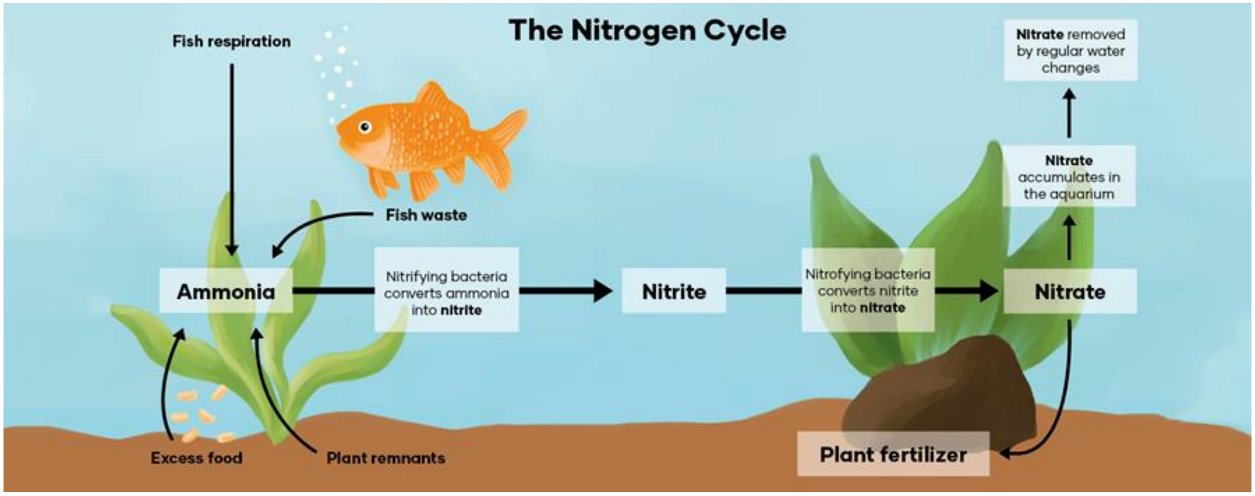
The Leibniz-IGB's research division is made up of six departments: Ecohydrology, Ecosystem Research, Experimental Limnology, Biology and Ecology of Fishes, Ecophysiology and Aquaculture, and Chemical Analytics and Biogeochemistry. These six departments conduct research on 38 subtopic groups. Additionally, the company works across departmental boundaries on three cross-cutting research domains: Aquatic Biodiversity, Aquatic Fluxes under Global Change, and Human-Aquatic Ecosystem Interactions. The company works in close cooperation with universities and research institutions worldwide. A few specific freshwater biodiversity projects have been protecting insects through environmentally friendly lighting, tracking the consequences of invasive species, and fitness training for sturgeons (an endangered fish species). As an institution, Leibniz-IGB is passionate about the scientific community and its diversity. It even co-organizes Soapbox Science events in Berlin, which work to promote more female role models in science and break existing stereotypes (Leibniz-Institut für Gewässerökologie und Binnenfischerei, 2019).

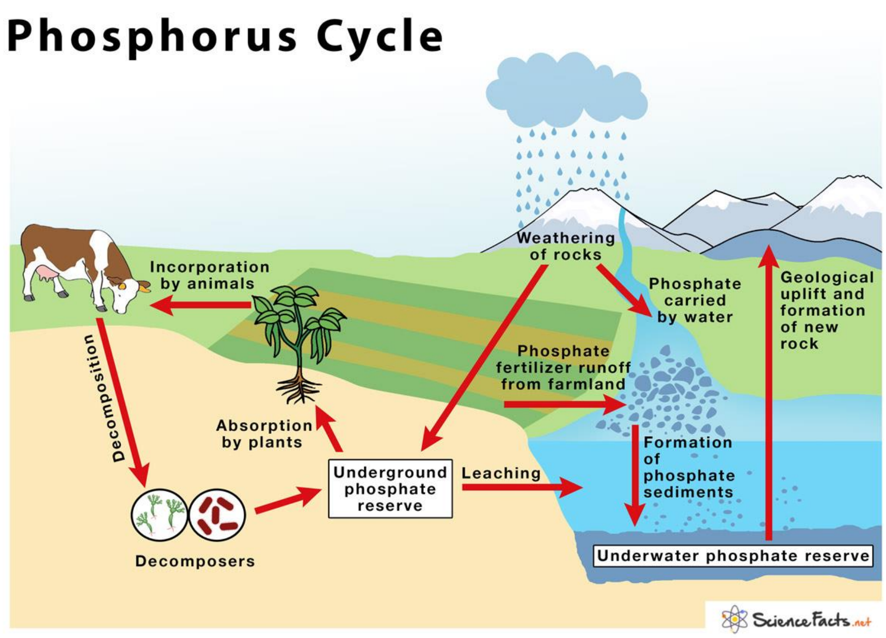
Leibniz-IGB is Germany's largest international research center for freshwaters, with personnel compiling hydrologists, microbiologists, and ecologists, among many other professions. Leibniz-IGB produced 345 publications, including 291 peer-review journals, in 2019. The institution's employment in 2019 consisted of 148 scientists (including 53 postdoctoral scientists and 37 doctoral students), 92 science-supporting staff, along with two apprentices and many involved from third parties. That same year, Leibniz-IGB had federal funding amounting to 17,357,400 € in addition to 11,518,327 € from external grants for an overall budget of 28,875,726 € (Leibniz-Institut für Gewässerökologie und Binnenfischerei, 2019). Due to the Leibniz-IGB development since their last evaluation in 2011, the Senate of the Leibniz Association recommended, in early 2019, continued funding by the Federal Government and the Länder of the Leibniz-IGB for the next seven years.

One of the Leibniz-IGB's goals is to have better management of inland waters, including reducing contaminants like microplastics which threaten both human and aquatic life. Leibniz-IGB has intensely studied the development of microbial biofilms on microplastics and nanoplastics and its effects on the ecosystem. Microplastic and nanoplastics pose several dangers to the ecosystem that are currently being explored, they are not only vectors in the transportation of toxic chemicals, but are toxic in nature. Composed of large amounts of nitrogen and carbon, plastics will affect water quality, environmental blooming, fish and amphibian bioprocesses, and lead to adjacent problems (Kyba). Aligning with their mission to manage the aquatic environmental impacts caused by humans, Leibniz-IGB's research has set the foundation to remedy this problem.

Through Leibniz-IGB's research it is known that water and environmental changes are inexplicably tied to one another. Analysis shows the sensitive reactions of aquatic life to environmental changes, as well as the opposite, where water stores and releases large quantities of pollutants and greenhouse gases to plankton and other eukaryotes. Leibniz-IGB's commitment to conducting research on the future of our fresh water sources has led to multiple research projects focused on pollution. Many performed by the institution in 2019 examined greenhouse gases in inland waters. These projects included new insights into carbon cycling in flowing waters, assessing fungicides as an underestimated hazard for freshwater organisms, and analyzing remote sensing data as a way to make it easier to monitor and protect lakes in the future. (Leibniz-Institut für Gewässerökologie und Binnenfischerei, 2019). Analysis of water and matter cycles connects the impact of contaminated sediment to impacts on the complex physical, hydrological, biological, and chemical interactions of the biosphere. We are excited to assist in a sustainability project with an esteemed, environmentally focused research organization such as Leibniz-IGB.

Appendix B: Nitrogen and Phosphorus Cycles





Appendix C: Literature Review

Microplastics and Nanoplastics: A Literature Review

Global Presence

It is no surprise that microplastics and nanoplastics pollution is continuing to spread globally, penetrating aquatic environments. Microplastics and nanoplastics can enter the environment through several methods, including inadequate waste disposal, agricultural drainage, and degrading human-made infrastructure (Horton & Dixon, 2017). After entering marine ecosystems, microplastics and nanoplastics have reached regions like the Arctic, mid-ocean islands, and deep-sea, despite little human contact (Zarfl & Matthies, 2010 & Alimba & Faggio, 2019). A study from 2010 estimates that the flux of plastic to the Arctic is around 16,200-1.9 million tons/year depending on location, human activity, natural conditions, and sampling approach (Zarfl & Matthies, 2010). Essentially, microplastics are present in all components of marine environments, including organisms, due to their high mobility (Li et al., 2018).

Current Concentrations

The behaviors of microplastics and nanoplastics have resulted in our current situation facing plastic pollution. A review conducted in 2017 discussed many concentrations worldwide and estimated that plastic makes up 80-85% of marine litter (Auta et al., 2017). Furthermore, given the likelihood of microplastics and nanoplastics accumulating on beaches, it is estimated that they are 3.3% of sediment weight on heavily impacted beaches. A worldwide review compiled microplastics and nanoplastics concentrations in many countries and most continents. Among those, was Germany with a concentration of 5000-7000 items/m^3 on urban beaches and 150-700 items/m^3 on rural beaches (Van Cauwenberghe et al., 2015). Aquatic concentrations can range from about 0 to millions of items per m^3, depending on currents and migration. Microplastics and nanoplastics have penetrated drinking water and can even be present in bottled and tap water (Koelmans et al., 2019).

`

Physical and Chemical Behavior

Over the past few decades, researchers have been able to analyze how microplastics behave in water. A study produced in 2016 compiled many behavioral commonalities for how microplastics and nanoplastics act in saltwater. Microplastics' physical and chemical behaviors were analyzed in fresh and saltwater, and no difference was observed. Physically, the migration of microplastics and nanoplastics occurs through waves, currents, tides, and wind, along with gradients within the body of water depending on temperature and salinity (Wang et al., 2016). Given the size, the bulk motion of water itself is the main driving force of plastic transportation. However, plastics spread due to concentration differences. Deep-sea currents are primarily responsible for transporting microplastics and nanoplastics at lower depths and cause plastics to collect on the bottom of waterways. Typically, microplastics and nanoplastics float in water due to their lower density. However, as they spend time in marine environments, additional material can adhere to them, causing their density to increase. This phenomenon is called biofouling and is a common occurrence in marine environments, where organisms such as barnacles or algae grow on the surface of MP. Several freshwater studies conducted over the last decade concluded the same observations, with the exception of salinity causing migration of microplastics and nanoplastics and ionization of metals onto the microplastics and nanoplastics (Li et al., 2018 & Wagner et al., 2014). A study conducted in 2021 proved this while researching the effects of biofilms, colonies of bacteria on the surface of MP in freshwater environments. Although initially floating, they found that as biofilms formed on MP, it changed the buoyancy and caused the MP to sink (Miao et al., 2021). As a result of the change in physical behavior, microplastics and nanoplastics can accumulate on the waterbed as sediment. Although sedimentation is common, humans more readily observed plastics that collect on shores and beaches.

Oceanographers and researchers have begun mapping circulation models to determine where accumulation will occur in different bodies of water. Collection can not only happen in the environment but in organisms as well. The buildup of microplastics and nanoplastics can have detrimental effects on biota, which will be discussed later. However, it is essential to note that they migrate in the body and are transported through animals (Wang et al., 2016). There is a direct correlation between global plastic production and plastic detected by the public eye in recent years. Although worldwide temporal accumulation continues to occur, Europe has remained stagnant in plastic pollution due to the harmful impacts plastics have on the environment.

Once microplastics and nanoplastics enter waterways, they are subject to chemical behaviors consisting of two main components: degradation and adsorption. Under degradation, several chemical properties of microplastics and nanoplastics change due to the influence of environmental factors, such as heat, light (visible and ultraviolet(UV)), erosion, abrasion, and chemicals. Researchers can classify degradation according to the method used to break down plastics. Examples include photo-oxidative, thermal, ozone-induced, mechanochemical, catalytic, or biodegradation (Singh & Sharma, 2008). As microplastics and nanoplastics degrade, their size, shape, color, and tensile strength are among the first properties to change. UV radiation causes a majority of the plastic breakdown in aquatic environments; however, it is a slow process. Once the buoyancy of microplastics and nanoplastics change, degradation is slowed (increased depth causes less light penetration) (Wang et al., 2016).

Understanding the adsorption of different materials to microplastics and nanoplastics is essential to understanding its impacts. Microplastics and nanoplastics adsorb persistent organic pollutants (POPs) and metals due to their affinity for hydrophobic surfaces (plastic is more hydrophobic than water). Studies indicate that weathering and residence time positively correlate with adsorption. Many say this is the case because it increases the surface area, increases polarity, and allows more foulants to attach to plastic debris. Of the hundreds of thousands of adsorbents, some of the most notable are toxic compounds and heavy metals. Each of these is widely recognized for its harmful effects on humans and the environment. Microplastics and nanoplastics acting as transportation for these toxic compounds pose severe risks for both the aquatic environment and the biota living in or around them (Wang et al., 2016, Alimba & Faggio, 2019 & Wagner et al., 2014).

Toxicity

Microplastics and nanoplastics have chemical, physical, and biological factors that threaten aquatic life. While ingestion is typically accidental, a study done in southern Germany detected microplastics in 1,160 fish ranging from more than 20 species in six lakes. Roughly a fifth of the fish tested had ingested microplastics, and it is believed that smaller nanoplastics were present but could not be detected (Roch et al., 2019). Additionally, nanoplastics can be absorbed into the bloodstream. Once in the blood, nanoplastics can travel throughout the body and target several different areas, including the nervous system (Campanale et al., 2020).

Aside from the toxic effects within an organism's body, microplastics and nanoplastics are harmful in local environments. Once in contact with water, microplastics and nanoplastics form an organic layer on their surfaces almost instantly. These biofilms are dynamic structures that can be affected by several factors, including the surface texture of the plastic, the plastic's chemical makeup, the polarity/hydrophilicity of the plastic, porosity, and the charge of the plastic (Wang et al., 2016). With the number of microplastics and nanoplastics that are in water sources, colonization is inevitable. Bacteria/biofilms pose dangers to animals through accidental ingestion and can disturb the flow of nutrients in bodies of water, posing a threat to the overall health of the animal (Tu et al., 2020).

Bacteria that form on microplastics and nanoplastics heavily influence nutrients in aquatic environments by interrupting the nitrogen and phosphorus cycles. In the nitrogen cycle, bacteria are responsible for converting nitrogen into usable products for plants to absorb. The concentration of bacteria that forms on microplastics has been shown to cause total nitrogen in the system to increase the concentration of usable nitrogen. However, the bacteria also carried out a denitrification process, which converts usable nitrogen to atmospheric nitrogen, and lowers nitrogen concentration in the water. Similarly, the bacteria affect the phosphorus cycle through sorption, substances physically and chemically bonding. The concentration of nitrogen and phosphorus facilitates the growth of algae and other microorganisms in the ecosystem, impacting the overall health of the oceans and other water bodies (Rummel et al., 2017).

Biofilms are not the only material that adsorbs to the surface of microplastics and nanoplastics. Heavy metals, persistent organic pollutants (POPs), and toxins also adhere to their surface, making them even more toxic to aquatic life when consumed. Several hydrophobic toxins, such as dioxins, polychlorinated biphenyls, and dichlorodiphenyltrichloroethane (DDT), can adhere to microplastic's carbon chains. To this effect, the two most commonly used plastics, polyethylene, and polypropylene can collect up to 10 times more pollutants than other types of microplastics (Katsnelson, 2015). Similarly, heavy metals adhere to the surface of microplastics. Microplastics act as vectors, allowing these toxins to pass into an organism through ingestion. Some of these metals are inherently dangerous, including silver (Ag), uranium (U), lead (Pb), and mercury (Hg), among others. Not only can metals and POPs adsorb to the MP, but the adhered material can also be released into different biomes. As plastics break down in the water, they can release harmful monomers, additives, and chemical byproducts (Wang et al., 2016). As MP accumulate waterborne toxins, they allow pollutants to be transferred to different environments and between water and biota (Wagner et al., 2014). These toxins pose the most severe threat to aquatic environments and organisms because they create a significant disruption in the ecosystem's balance.

Effects on Aquatic Organisms

Much of the research conducted on the effects of microplastics and nanoplastics pollution focuses on smaller aquatic organisms. Due to their small size and environment, these aquatic organisms are at risk of hazardous health impacts from microplastics.

One significant concern is how microplastics can impact aquatic food chains. Bioaccumulation occurs when higher trophic organisms eat smaller organisms that contain MP. This phenomenon is part of the reason why various research groups have discovered these plastics in the digestive tracts of over 100 different species, including oysters, fish, and zooplankton (Katsnelson, 2015). Some research considers the interactions between microplastics and aquatic organisms, including a 2016 study on copepods, a common, tiny crustacean. According to this study, copepods feed near the sea surface level, where they can promote transportation of microplastics through fecal matter, which sinks lower in the water (Clark et al., 2016). Organisms such as zooplankton consume microplastics, mistaking them for food, and promote biomagnified effects (Rochman et al., 2013). Once ingested, translocation of microplastics inside the organism can occur. For example, ingested microplastics can be retained in the digestive tract and then line the gut or relocate to nearby tissues (Wang et al., 2016). Consumed microplastics and nanoplastics also have harmful physical impacts on the organism.

The most prominent animal of consideration has been fish. Microplastics can alter the food consumption and energy allocation patterns of marine species, which affects their fitness and reproduction. Plastic ingestion can also cause gut blockage, oxidative stress, and endocrine disruption. "This reallocation of energy reserves from reproduction to maintenance, with resulting reductions in reproductive success, is a recurring theme emerging from chronic exposure studies with microplastics" (Galloway & Lewis, 2016). Fish with exposure to small plastic debris also result in pathological and oxidative stress and liver inflammation (Rochman et al., 2013). The liver is crucial in metabolism and detoxification; damaging the liver may hinder these biological mechanisms in fish.

Much research also considers microplastic effects on smaller aquatic organisms, which have shown similar results. For example, one study on oysters exposed to polystyrene found that their stress responses were activated in exposed digestive tissues. Dynamic energy budget (DEB) models predicted a shift of energy allocation from reproductive output to structural growth and maintenance. "A disturbance in individual energetics revealed by DEB modeling suggested that micro-polystyrene particles have threatened the physiological integrity of oysters and consequently increased the maintenance costs, as described in response to various stresses and species" (Sussarellu et al., 2016). Essentially, oysters require more energy to maintain homeostasis. This was demonstrated explicitly with reduced activity in the oyster's insulin pathways. "The insulin pathway plays a crucial role in mobilizing reserves during gametogenesis and has an essential role in germinal cell proliferation and maturation. We thus hypothesize that micro-polystyrene exposure negatively impacts cell proliferation and differentiation processes in gonads through the down-regulation of genes responding to insulin signaling" (Sussarellu et al., 2016). The effects go beyond the individual, as some studies of other stressors, such as ocean acidification, show that effects in oyster larvae can maintain their impact later in life, reducing settlement success, population growth, and productivity (Galloway & Lewis, 2016). These effects are present in other organisms as well. A 2013 study of marine worms found that worms raised in sediment containing 5% PVC ate less, had more inflammation, and had a 50% drop in energy reserves (Wright et al., 2013).

The demonstrated issues that microplastics can cause within aquatic organisms are of great concern, especially since microplastics are seldom talked about in day-to-day life. These nearly invisible pollutants could have lasting impacts on aquatic species populations if kept unchecked. They could even affect humans, and consuming these exposed aquatic organisms is one potential source of risk for humans.

Health Risks to Humans

Microplastic consumption ranges are alarming due to the immense amount of adverse effects they can have on the human body. Particle characteristics such as size and shape are influential in how the body reacts to said particles. For example, thinner fibers are respirable regarding the respiratory system, whereas thicker fibers cause more toxicity to pulmonary cells. Larger fibers cause the lungs to ineffectively clear fibers of 15-20 μm or larger by alveolar macrophages and a mucociliary escalator (Wright & Kelly, 2017). Similarly, acute inflammatory response, oxidative stress, and macrophage function are consequences of microplastic inhalation exposure. Oxidative stress with nanoparticles of quartz and TiO_2 origin is reported to cause inflammation and intestinal fibrosis, a disease caused by intestinal inflammation (Wright & Kelly, 2017). The biopersistence of microplastics could lead to various other biological responses, including genotoxicity, apoptosis, and necrosis. If these conditions are sustained, a range of outcomes can ensue, including tissue damage, fibrosis, and carcinogenesis (Wright & Kelly, 2017).

Microplastics are not only harmful themselves, but the chemicals they attract pose health risks as well. Chemical effects could be established due to the composition of the polymer itself, the leaching of unbound chemicals, or unreacted residual monomers (Wright & Kelly, 2017). These effects can lead to endocrine disruption, congenital disabilities, immune system complications, child development delays, and cancer. A study from 2020 highlights exposure to phthalates at immune system developmental stages and the correlation to certain types of cancer. Most current testing for cancer patterns due to microplastics are being conducted on small mammalian animals such as mice, but further testing is said to occur in the coming years (Madhu, et al., 2020). Microplastic may not be the cause of these cancers, but they can carry carcinogenic chemicals. The chemicals that foster on these plastics, such as BPA, have been linked to statistical cancer patterns (Exposure to Chemicals in Plastics, 2020). This study also claims that when considering that cancer incidence has increased as countries moved forward into industrialization, environmental toxin exposure constitutes a relevant public health concern worldwide. Microplastic consumption and its effects on human health are continuously being researched as microplastic pollution becomes a more pressing global issue.

Human Exposure

Humans face exposure to microplastics and nanoplastics every day, whether it is through the food they eat, the water they drink, or even the air they breathe. A study evaluating 15% of common American caloric intake estimated that a person's annual microplastic consumption ranges between 39,000 and 52,000 particles per year (Cox et al., 2019). Dietary exposure pathways can begin with consuming many types of fish, honey, sugar, beer, and even salt (Wright & Kelly, 2017). A 2019 study done by Cox, reported the following final microplastic concentrations of the foods: seafood (1.48 MPs/g), honey (0.10 MPs/g), sugar (0.44 MPs/g), alcohol (32.27 MPs/g), and salt (0.11 MPs/g). Over time, microplastics and nanoplastics can accumulate in the body, causing several adverse effects. Ocean water is commonly associated with microplastics. However, few people know that varying concentrations of microplastics exist in bottled and tap water. Bottled water can increase a human's annual MP consumption by 90,000 MPs/g, and tap water increases MP consumption by 4,000 MPs/g (Cox et al, 2019). Aside from direct consumption, exposure stems from sea-salt aerosol, wastewater treatment byproducts, tires, and other sources that can cause humans to inhale microplastics and nanoplastics (Wright & Kelly, 2017). This kind of exposure can increase the annual consumption to 74,000 to 121,000 MPs/g (Cox et al. 2019). Caloric microplastic consumption amounts are high on their own, and when combined with water consumption and inhalation, those amounts only increase.

Ultimately, the emergence of microplastics and nanoplastics has displayed diverse effects on the surrounding ecosystem. These tiny pollutants have worked their way into every crevice worldwide in the last couple of decades. As microplastics and nanoplastics reside in water, they cause disruptions in the flow of nutrients in the ecosystem and organisms. Harmful chemicals and toxins build up on these particles and are transmitted to animals when ingested. Once eaten, microplastics and nanoplastics work up the food chain, even reaching humans. By continuing research on microplastics and nanoplastics, their prolonged effects can be further understood and communicated.

Appendix D: Annotated Bibliography for the Literature Review

Alimba, C.G., & Faggio, C. (2019). Microplastics in the aquatic environment: Current trends in environmental pollution and mechanisms of toxicological profile. *Environmental Toxicology and Pharmacology, 68,* 61-74. https://doi.org/10.1016/j.etap.2019.03.001

- This is a review which focuses on the current toxicological consequences of microplastics on aquatic biota. Microplastics are capable of absorbing organic contaminants, metals, and pathogens, which can induce greater toxic effects. Numerous studies listed (Wagner et al., 2014 and Barletta et al., 2019) show that plastic debris is the same in benthic, pelagic, and shores of all aquatic ecosystems. It gives a brief history and trend of plastic pollution, giving a timeline of the evolutionary history. Plastic ingestion is most prevalent among surface-feeding organisms, mainly focusing on seabirds, sea turtles, and fishes as indicators and discussing problems with each specific species. This article is especially good for researching the specific ways plastics affect different species, and how the induced toxicity of microplastics affects aquatic life (section 4.2). It strongly addresses the problem of poor management of plastics and how that will cause aquatic ecosystem problems. This will be important for us discussing the impact of microplastics on aquatic life, and the potential long term effects. It does a good job of addressing the small scale problem by using the visible large scale problems of plastics.

Auta, H.S., Emenike, C.U., & Fausiah, S.H. (2017). Distribution and importance of microplastics in the aquatic environment: A review of the sources, fate, effects, and potential solutions, *Environment International, 102,* 165-176. https://doi.org/10.1016/j.envint.2017.02.013

- This is a review of the sources, fates, effects, and potential solutions to the microplastic problem. It mainly focuses on the most important sources and fates, but possible follow ups to remediate microplastics that can be adopted. Main sources of microplastics are primary (manufactured for particular industrial or domestic applications) and secondary (larger plastic debris that fragment over time into smaller particles). Although microplastic effects on certain species, the fate inside aquatic organisms offers a new perspective on the challenge and its manifestation in animals. Possible solutions include using microbes that feed on microplastics, management guidelines, and recycling efforts. This article is not as in depth, but it gives us a good step to jump off point for where they come from, and the overall lifecycle in water. Specifically, the use of microbial organisms to clean up waste is interesting, and I think it would be quite an innovative way for companies to fight this issue. This article would help form the background and recommendations of our project, and also help us diagram the path of plastics in the ecosystem.

Capdevila, A., Kokimova, A., Ray, S., Avellan, T., Kim, J., & Kirsche, S. (2020). Success factors for citizen science projects in water quality monitoring. *Science of The Total Environment*, *728*, Article 137843. https://doi.org/10.1016/j.scitotenv.2020.137843

- This study reviews successful factors used in the facilitation of citizen science projects pertaining to water quality monitoring in previous sustainability efforts. The motivation for this study is the increasing sustainability efforts are highlighting data gaps, pushing the need for more citizen involvement on local, national and global levels. Although citizen data collection could address the gap in temporal and spatial data, some fear citizen data may be skewed, selective or even biased. The success of citizen science projects refers to various aspects, including an increase of highly reliable water quality data, the use of data for research outputs and water quality policies, as well as, broader societal effects of data gathering processes such as raising awareness, ability development, and societal interaction. The methodology of this study was mainly a literature review of previous scholar experiments involving citizen science projects, which was used in the comparison of successful citizen science projects.

Clark, J. R., Cole, M., Lindeque, P., Fileman, E., Blackford, J., Lewis, C., Lenton, T. M., & Galloway, T. S. (2016). aquatic microplastic debris: A targeted plan for understanding and quantifying interactions with aquatic life. Frontiers in Ecology and the Environment, 14(6), 317–324.

- This study presents the problem of identifying sources and sinks for microplastic pollution, and the challenges for quantifying it. Microplastic pollution is an issue of increasing environmental and ecological concern, and the current understanding of it is extremely limited. However, observations suggest that interactions with aquatic life play a significant role. Patterns in ocean currents and biological productivity suggest that interactions are most likely to occur in coastal environments close to the source of plastic contamination. An example of one such interaction is with copepods, a common, tiny crustacean. They feed near the sea level and their fecal matter sink which allows for the spread of sediments lower in the water.

Cox, K. D., Covernton, G. A., Davies, H. L., Dower, J. F., Juanes, F., & Dudas, S. E. (2019). Human consumption of microplastics. Environmental Science & Technology, 53(12), 7068–7074. https://doi.org/10.1021/acs.est.9b01517

- This study goes into human consumption of microplastics, which is not well known. They analyzed data from studies across various nations that researched microplastic presence in food types, and used them to determine the extent that humans are consuming microplastics on a recommended diet, based on the daily recommended intake of each food type. They concluded that humans may be consuming between 39,000 and 52,000 microplastic particles per year. These estimates do not include inhalation, and drinking a recommended water intake out of only plastic bottles can increase it by 90,000 particles, compared to an increase of 4,000 from only drinking tap. While it is acknowledged that these will be extremely varied, and there were alot of limitations, it is an interesting model of human microplastic consumption.

Essel, R. Engel, L. Carus, M., & Ahrens, R. H. (2015). *Sources of microplastics relevant to aquatic protection in Germany*. Umweltbundesamt. https://www.umweltbundesamt.de/sites/default/files/medien/378/publikationen/texte_64_2015_sources_of_microplastics_relevant_to_aquatic_protection_1.pdf.

- While this article focuses primarily on the areas of waste and pollution in Germany it offers great Insight into the formation of microplastics. The increase in microplastics as pollutants in the oceans around the world are discussed. The comparison between microplastics as pollutants in different decades was discussed as well. Potential sources for plastic pollution were identified. This article does not have a large amount of data on the concentration of microplastics in inland sources. The article does present interesting information on how different sizes of microplastics affect different organisms. This source will help us with educating individuals on how they can make changes in pollution.

Exposure to Chemicals in Plastic. 2020 Breastcancer.org. https://www.breastcancer.org/risk/factors/plastic.

- This article was produced by a breast cancer website, and it highlights the link between plastic and plastic toxins with cancer statistics. Plastics have the potential to leach harmful chemicals, research suggests that high exposure levels of certain chemicals can alter cellular structure ultimately affecting cell function. BPA is a weak synthetic estrogen found in many plastics, and due to it being a hormone obstructor it can potentially lead to breast and ovarian cancer. The article states that breast cancer patients are told to stray away from known BPA carriers. Lastly, this article lists methods and ways in which breast cancer patients could reduce their exposure.

Galloway, T. S., & Lewis, C. N. (2016). aquatic microplastics spell big problems for future generations. Proceedings of the National Academy of Sciences of the United States of America, 113(9), 2331–2333.

- This source is a depiction of how microplastics are being found to be harmful for aquatic life. There is little legislation about aquatic plastic debris because there is not much evidence demonstrating its problems. These problems include the microplastics ability to disrupt aquatic feeding habits, reproductive success, energy allocation, increase oxidative stress, and potentially house toxins. This source connects different studies to define the ways in which aquatic life can be affected by microplastics. However this is not the direct source of these studies.

Gorman, D., Moreira, F. T., Turra, A., Fontenelle, F. R., Combi, T., Bícego, M. C., & de Castro Martins, C. (2019). Organic contamination of beached plastic pellets in the South Atlantic: Risk assessments can benefit by considering spatial gradients. *Chemosphere*, 223, 608–615. https://doi.org/10.1016/j.chemosphere.2019.02.094

- This study analyzed the organic toxicity of microplastics along a beach in Brazil's South Atlantic coastline. 14 stations were sampled along a 39km stretch of coast between Pontal de Sul and the beaches of Matinhos. Organic contaminants were analyzed according to the standard methods outlined by the United Nations Environmental Program, which the study goes into exact detail for. These specified contaminants were polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs). The resulting concentrations of PAHs found were between 1,454 and 6,002 ng/g and the concentrations of PCBs found were between 0.8 and 104.6 ng/g. In general there appeared to be a North to South decline in contaminant concentration.

Horton, A.A., and Dixon, S.J. (2017). Microplastics: An Introduction to Environmental Transport Processes. *WIREs Water*, vol. 5. *Wiley Online Library* doi:https://doi.org/10.1002/wat2.1268.

- Larger plastic items can enter the freshwater environment through inadequate waste disposal, either through littering or loss from landfill and transported from land via wind or surface runoff. In addition to macroplastics, there are significant direct inputs of microplastics to freshwater systems. Agricultural drainage and runoff from farmland can result in input of agricultural plastics or sewage-sludge derived fibers and microbeads.

Katsnelson, A. (2015). News Feature: Microplastics present pollution puzzle. *Proceedings of the National Academy of Science of the United States of America*, 112 (18). https://doi.org/10.1073/pnas.1504135112

- The article gives a good overall summary of the dangers of microplastics and nanoplastics in relation to aquatic organisms. It details the two most commonly used plastics, polyethylene, and polypropylene, which can collect up to 10 times more pollutants than other types of microplastics. As well it includes information on the accumulation of plastics in different animals' internal organs. The study also stresses the importance of the issue and its potential dangers in the coming years.

Koelmans, A.A., et al. (2019). Microplastics in Freshwaters and Drinking Water: Critical Review and Assessment of Data Quality. *Water Research*, vol. 155. doi:10.1016/j.watres.2019.02.054.

- This study assessed the quality of fifty studies done in researching microplastics in drinking water sources. These water sources include: rivers, lakes, groundwater, tap water and more. The concentration of microplastics in the samples spanned ten order of magnitude (1 x 10^2 to 10^8 #/m^3. Tested for were the most commonly used microplastics polymers known in commercial and industrial industries in the current age.

Out of 50 samples tested only four samples tested positive for all proposed quality criteria. Water was tested by pumping buckets with water then sieving to isolate particles of the desired range. Samples had a minimum limit of 500L sample volume to ensure more accuracy in testing water samples. In surface water, > 300 µm, microplastic concentration span concentrations of 1 x 10^-3 to 10 particles per litre. Ethanol and formalin have been shown not to affect polymer characteristics. This study used Raman microscopy as the main means of polymer detection.

Li, J., Liu, H., & Chen, P. (2018). Microplastics in freshwater systems: A review on occurrence, environmental effects, and methods for microplastics detection, *Water Research, 137*, 362-374. https://doi.org/10.1016/j.watres.2017.12.056

- This article reviews the occurrences which cause microplastic accumulation, environmental impacts, and methods to detect microplastics in freshwater. Key factors which affect plastic motion are wind, geostrophic circulation, turbulence, and oceanographic effects, as well as density, size, and shape of the physical plastic. The article includes the potential of microorganisms colonizing new environments and thus causing disruptions and changing the biofilms communities. Several methods are used to sample microplastics, including volume-reduced and bulk sampling, and after each sample is taken, they explain methods to determine the contents. Finally, once the microplastics are removed, they discuss how to identify and quantify them. This article has a general overview of freshwater testing techniques which, including a streamlined way to collect, separate, and quantify microplastics. This is helpful for a younger demographic, and will be useful for designing our own experiment for the students in Berlin. This article focuses on freshwater systems, and so this is more relevant to our project, which focuses on inland bodies of water.

Madhu, D., Elanjickal, A.I., Mankar, J.S., Krupadam, R.J. (2020). Assessment of cancer risk of microplastics enriched with polycyclic aromatic hydrocarbons. *Journal of Hazardous Materials*, vol. 398 *www.sciencedirect.com*, https://doi.org/10.1016/j.jhazmat.2020.122994

- This study highlights the toxic transfer of pollutants from plastics to aquatic life and humans. The study reports the hazardous nature in which plastics affect aquatic health and the link with plastic pollients and cancer statistics. Microplastics were absorbed using N2 adsorption isotherms and analysed using a surface analyzer. Test allowed for assessing PAH absorbance on differing size microplastics and that relation to cancer statistics. PAH consumption is higher in children than adults, so children are at a higher risk of certain cancers due to their dietary consumption. While there is not enough evidence to link microplastic consumption to cancer, this study recommends that it is a topic that should be explored and investigated further as their study did have fragments of conclusive results.

Mani, T., Hauk, A., Walter, U., & Burkhardt-Holm, P. (2015). Microplastics profile along the Rhine River. *Scientific Reports,* 5(1), Article 17988. https://doi.org/10.1038/srep17988

- This article is about the role that rivers have in carrying pollutants into the ocean. It mentioned that there has not been a single large river that has been studied for microplastics loads along its length. Through testing 11 locations over 820 km of river, microplastics were found at every location. This article then discusses the average number of particles in km^-2. As well, the article mentions the dangers of microplastics as toxins to several different animals. The relation of inland bodies of water to oceans is a unique fact that has not been presented in any other article read so far. The numerical data of particles per km^-2 is a shocking discovery and again, is a key factor in expressing the severity of microplastics in water. This article will help us relate the microplastic crisis globally, as many bodies of water are interconnected.

McCormick, A.R., Hoellein, T.J., London, M.G., Hittie, J., Scott, J.W., Kelly, J.J. (2016). Microplastic in surface waters of urban rivers: concentration, sources, and associated bacterial assemblages. *Ecosphere - Wiley Online Library, 7*, https://doi.org/10.1002/ecs2.1556

- More conformational evidence on the presence of microplastics in water. It goes into depth on how they get there and more specifically how they are able to go through water treatment plants. The source also specifies the two main specific dangers the plastics have, their ability to block organs when consumed and the biofilm that is present when they enter water. Overall this source has very useful information on the location of mp in the water and the dangers that they pose. They proposed their own study in 2014 by suspending a 333 micron net in the water of a river and letting the stream flow through it and catch microplastic.

Miao, L., Gao, Y., Adyel, T.M., Huo, Z., Liu, Z., Wu, J., Hou, J. (2021). Effects of biofilm colonization on the sinking of microplastics in three freshwater environments, *Journal of Hazardous Materials, 413*, https://doi.org/10.1016/j.jhazmat.2021.125370

- A study of microplastic in freshwater systems in China analyzing the impact of biofilm which accumulates on microplastic. They tested 3 microplastic and their buoyancy, concluding that biofilm affects the sinking of microplastic in freshwater and therefore influences the behavior and distribution characteristics of microplastic. Analysis of each biofilm was conducted and suggested compositions were obtained based on properties of each. Due to the density changes of the microplastic, it changed the buoyancy and therefore caused the typical transportation characteristics to change. This can cause future potential concerns in aquatic ecosystems.

Roch, S., Walter, T., Ittner, L. D., Friedrich, C., & Brinker, A. (2019). A systematic study of the microplastic burden in freshwater fishes of south-western Germany - Are we searching at the right scale?. *The Science of the Total Environment*, *689*, 1001–1011. https://doi.org/10.1016/j.scitotenv.2019.06.404

- Researchers conducting experiments on microplastics in aquatic life in Baden Wurttemberg, (southern) Germany. They found microplastics in over 1,160 fish ranging more than 20 species of fish across six lakes. The number of microplastics in each fish that tested positive was concluded. An estimation that 70% of particles were so small that they could be suitable for translocation in tissues was made. The lack of a standardized detection method for 95% of microplastics hurts the accuracy of the study. The study focuses on microplastics as toxins and not their potential with relation to bacterial microfilms. The study allows us with the education of the dangers of microplastics on wildlife.

Rochman, C. M., Hoh, E., Kurobe, T., & Teh, S. J. (2013). Ingested plastic transfers hazardous chemicals to fish and induces hepatic stress. Scientific Reports, 3(1), 3263. https://doi.org/10.1038/srep03263

- This study aimed to analyze the risk associated with microscopic plastic litter and its ingestion by aquatic species. It demonstrates the effects that exposure to a mixture of polyethylene and chemical pollutants sorbed from the environment. Fish that were fed virgin polyethylene fragments exhibited hepatic stress, although less stress than those fed aquatic polyethylene fragments. The liver toxicity and pathology shown in this study give a baseline regarding bioaccumulation and associated health effects from plastic ingestion in fish.

Rummel, C. D., Jahnke, A., Gorokhova, E., Kühnel, D., & Schmitt-Jansen, M. (2017). Impacts of biofilm formation on the fate and potential effects of microplastic in the aquatic environment. *Environmental Science & Technology Letters*, *4*(7), 258–267. https://doi.org/10.1021/acs.estlett.7b00164

- The dangers of microplastics and bacteria are highlighted by this article. The breeding ground for bacteria that microplastic presents is utilized in minutes to hours upon contact of the microplastics to water. This then produces a biofilm, which when consumed by an animal can cause damage to its internal organs. Several factors such as the chemical make-up of the plastics, along with its surface can change the effectiveness of the biofilm. The depth in which this article explains the formation of the biofilm is a great platform to teach the reader about their claim, the dangers of microplastics. As a reader I would have liked to hear whether or not different bacteria thrived on the surface of the microplastics as some bacteria are more of a threat than others. This paper gives us insight into the dangers of microplastics and bacterial microfilms.

Segovia-Mendoza, M., Nava-Castro, K.E., Palacios-Arreola, M.I., Caray-Canales, C., Morales-Montor, J. (2020). How Microplastic Components Influence the Immune System and Impact on

Children Health: Focus on Cancer.*Birth Defects Research*, vol. 112. *Wiley Online Library*, doi:https://doi.org/10.1002/bdr2.1779.

- Talks about BPA and Phaltetes found in and on microplastics that are being tested to show increased sicks of cancer, currently in small mammalian rodents and further testing on humans is currently being worked on.

Singh, B., Sharma, N. (2008). Mechanistic implications of plastic degradation, *Polymer Degradation and Stability, 93,* https://doi.org/10.1016/j.polymdegradstab.2007.11.008

- This paper investigates the mechanisms for degrading microplastics, and allows the reader to better understand how removing plastic and other pollution can improve overall global health. The article goes into types of plastics and how different types of degradation, such as photo-oxidative degradation, thermal degradation, biodegradation, and ozone-induced degradation, can impact the lifecycle of a plastic polymer. It dives into initiation and propagation of the degradation process, and overall effects of plastic degradation/potential effects on the environment. Additionally, different factors, such as chemical composition and molecular weight affect how polymers degrade, and the article dives into those a little.

Sussarellu, R., Suquet, M., Thomas, Y., Lambert, C., Fabioux, C., Pernet, M. E. J., Goïc, N. L., Quillien, V., Mingant, C., Epelboin, Y., Corporeau, C., Guyomarch, J., Robbens, J., Paul-Pont, I., Soudant, P., & Huvet, A. (2016). Oyster reproduction is affected by exposure to polystyrene microplastics. Proceedings of the National Academy of Sciences, 113(9), 2430–2435. https://doi.org/10.1073/pnas.1519019113

- This study aimed to assess the impact of microplastic pollution on filter-feeder organisms. They analyzed the impact of polystyrene microspheres on the physiology of Pacific aquatic oysters by exposing adult oysters for two months during their reproductive cycle. The results showed that microplastic exposure had physical effects to the oysters digestive mechanisms, reproductive output (larval development), and endocrine system.

Van Cauwenberghe, L., Devriese, L., Galgani, F., Robbens, J., Janssen, C.R. (2015). Microplastics in sediments: A review of techniques, occurrence and effects, *aquatic Environmental Research, 111,* https://doi.org/10.1016/j.marenvres.2015.06.007

- This is an in-depth analysis of literature regarding microplastic in sediments. Various extraction techniques were used to observe and record the occurrences and distribution within sand and other sediment. It compiles information from beaches in most large countries around the world, and allows us to see how one country, let's say Germany, compares to other countries. Urban beaches of Germany have 5000-7000 items/m^3 and rural beaches have 150-700 items/m^3.

Wagner, M.,Scherer, C., Alvarez-Munoz, D., Brennholt, N., Bourrain, X., Buchinger, S., Fries, E., Grosbois, C., Klasmeier, J., Marti, T., Rodriguez-Mozaz, S., Urbatxka, R., Vethaak, A.D., Winther-Nielsen, M., & Reifferscheid, G. (2014). Microplastics in freshwater ecosystems: what we know and what we need to know, *Environmental Sciences Europe, 26,* https://doi.org/10.1186/s12302-014-0012-7

- This is an early compilation of articles regarding microplastic and their impact on freshwater systems. Clearly, not as much is known for microplastic in freshwater, but they admit the growing concern because of its high mobility and permeability. They cite a few impacts of microplastic on freshwater species, but many problems are how microplastic is a vector for other contaminants, such as metals, bioaccumulation, and toxic compounds. Additionally, they connect microplastic to European water policy evolution over the past couple decades; microplastic poses risks to several aquatic litter concerns not only for their effects, but their ability to act as vectors. Although older with respect to how new this topic still is, it shows many of the gaps in knowledge are still present today.

Wang, J., Tan, Z., Peng, J., Qiu, Q., & Li, M. (2016). The behaviors of microplastics in the aquatic environment. *aquatic Environmental Research, 113,* 7-17. https://doi.org/10.1016/j.marenvres.2015.10.014

- This paper seeks to understand the physical, chemical, and biological behaviors of microplastics, and further investigates the impacts of microplastics on aquatic life. The paper discussed why plastics are so prevalent in aquatic litter, how it moves, what can impact where it accumulates, and the potential physical impacts. Chemically, the article discusses why plastics are even more dangerous, citing how plastics have the tendency to adsorb other pollutants and metals (which make the impacts even worse). Additionally, the authors expand the impacts of microplastics to mechanical, chemical, and additional effects of ingestion, thus giving us a more persuasive argument for why this needs to be taken care of and why humans should care what we are doing with plastics. This article uses a lot of sources which can lead us to more specific methods and impacts of microplastics. One important scope is the effects on multiple organisms, and in the bio behaviors, it directs us to several other sources which discuss several smaller sea biota which have been researched.

Wright, S. L., & Kelly, F. J. (2017). Plastic and human health: A micro issue? Environmental Science & Technology, 51(12), 6634–6647. https://doi.org/10.1021/acs.est.7b00423

- This is a review article of various scientific literature to discuss and evaluate the effect of microplastics on human health. They analyzed major literature, through 2016, of accumulation, particle toxicity, and chemical and microbial contaminants. If inhaled or ingested, microplastics may accumulate and induce an immune response from localized particle toxicity. Chronic exposure is expected to be a larger concern due to accumulation effects that can take place.

Wright, S., Rowe, D., Thompson, R., & Galloway, T. (2013). Microplastic ingestion decreases energy reserves in aquatic worms. Current Biology, 23(23), R1031–R1033. https://doi.org/10.1016/j.cub.2013.10.068

- The purpose of this study was to observe aquatic worms maintained in sediments spiked with microscopic unplasticised polyvinyl chloride (UPVC) at concentrations overlapping those in the environment. This is due to the lack of sufficient studies on the biological effects of microscopic plastics in water. Microplastics are increasing in abundance in aquatic environments, and they can act as a substrate for the adherence of hydrophobic contaminants, deposition of eggs, and colonization by unique bacterial assemblages. This study found that deposit-feeding aquatic worms in this environment had significantly depleted energy reserves, by up to 50%. The results suggest that this reserve depletion comes from combining reduced feeding activity, inflammation, and longer gut residence times of ingested material.

Wurm, F., Spierling, S., Enes, H., & Barner, L. (2020). Plastics and the environment—Current status and challenges in Germany and Australia. *Macromolecular Rapid Communications.*, *41*(18), Article 2000351. https://doi.org/10.1002/marc.202000351

- This report analyzes current waste management and plastic waste management of Germany and Australia. This report was conducted due to the important role that polymers and plastics play in modern life. They are necessary for some health related fields, (most recently PPE equipment), but they also create immense waste that may be even more harmful to the health of humans and wildlife. This study goes into detail about the applications of plastics in Germany and Australia, and also implemented sustainability programs in hopes to manage waste. The German Packaging Act started January of 2019, was an effort to reduce negative effects of packaging waste and increase recycling rates. Australia has implemented a PS scheme to manage the impacts of plastic products and materials on human life and health. The study goes further into littering reports, aquatic life statistics and general information on macromolecules and microfibers. This study concludes that although polymers and plastics have been crucial to advancements in many fields, their harmful effects need to be addressed with urgency in every country.

Zarfl, C., Matthies, M. (2010). Are aquatic plastic particles transport vectors for organic pollutants to the Arctic? *aquatic Pollution Bulletin, 60(10), 1810–1814*. https://doi.org/10.1016/j.marpolbul.2010.05.026

- This paper analyzes the impact of microplastics as a transport mechanism to remote locations, like the Arctic. Microplastics are persistent and can have organics sorbed into

them, allowing for a route to the Arctic through biomagnification and ocean currents. The extent to which this occurs, and its significance, is not quantified. The objective of this study was to do just that; estimate plastic and pollutant flux to the Arctic. The plastic flux they found to be between 62,000 and 105,000 tons per year. The resultant PCB flux was 1g to 1.6kg per year. It is acknowledged that there is considerable uncertainty in pollution routes to the Arctic, including atmospheric, meaning these estimates are likely an underrepresentation.

Appendix E: Water Sampling Experimental Method

Note: There are two options for filtration in the water sampling method. The first is transporting volumes of water to the lab and using nylon filters and a funnel. The second is using a larger bucket filter and performing the filtration on-site.

Filtering at the Lab

Materials:

- Container with known volume (Around 2L)
- 150 micron Filter and Funnel
- Large Kitchen Strainer
- Hydrogen Peroxide
- Container with sample
- Secondary Container
- Waste Container
- Cup

Protocol at the water source:

1. Proceed to the water source and mark exactly where you are going to collect water.
2. Clean out your container using water from the water source. Do this by filling a bit of the container, shaking it, then emptying it 3 times.
3. Once rinsed, move about 5m upstream and collect water from the source by filling the container with a known volume.

Protocol at the lab:

4. Hold the kitchen strainer over the secondary container and pour the water through the strainer into the container. Like it shows here:

5. When all the water is now in the secondary container, look at the strainer to see if you caught anything that could be plastic. It will be mostly leaves, sticks, or twigs, but there could be larger plastic pieces.
6. Place the filter paper into the funnel. Hold the funnel (with the filter in it) over the waste container and pour the water from the secondary container through the filter into the container. Like it shows here:

7. When the filter is done draining you may look at the filter to see if you can spot microplastics. Carefully take the filter paper out of the funnel and place it into the cup.

8. Soak the filter in hydrogen peroxide, and make sure you fill the cup just enough to cover your sample. Like it shows here:

9. Leave it to soak overnight.
10. Carefully take the filter out and place it on a dry work surface
11. Look at the filter and see if you can find microplastics

Filtering on-site

Materials:

- 55-micron bucket filter is recommended
- Large bucket with known volume
- Kitchen strainer
- Smaller container to collected water
- Hydrogen Peroxide
- Cup

Protocol:

1. Proceed to the water source and mark exactly where you are going to collect water.
2. Clean out your smaller container using water from the water source. Do this by filling a bit of the container, shaking it, then emptying it 3 times.
3. Once rinsed, move about 5m upstream and set up the bucket filter over the large bucket with known volume.
4. Collect water from the source by filling the smaller container
5. Pour the water through the kitchen strainer and bucket filter, into the large bucket. Like it shows here:

6. Repeat step 5 until the large bucket is full of water
7. Once the bucket is full of water, pull the filter out and dump the bucket of water back into the water source.
8. Repeat steps 4-7 until an adequate amount of water has been filtered (Ideally 50-70L).

9. Bring the filter back to the lab.
10. Soak the filter in hydrogen peroxide, and make sure you fill the cup just enough to cover your sample.
11. Leave it to soak overnight.
12. Carefully take the filter out and place it on a dry work surface
13. Look at the filter and see if you can find microplastics

Appendix F: Sand Sampling Experimental Method

Note: This sampling method's results vary depending on the composition of the shore (sand vs. soil makes a difference). You should do this with SAND and NOT SOIL. Furthermore, the sand should (ideally) be thoroughly dried but MUST be at the edge of the waterbank.

Materials:

- 4 hand-scoops of sand
- Container
- Corn Syrup
- Mixing Tool

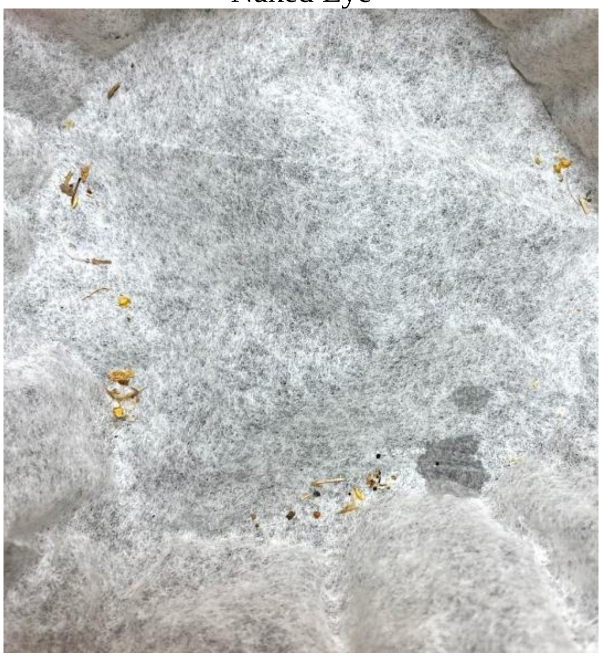
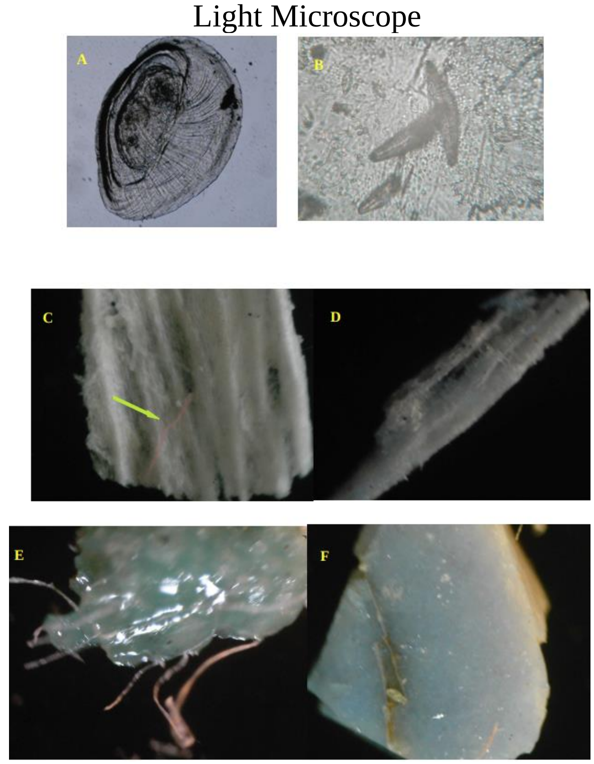
Procedure:

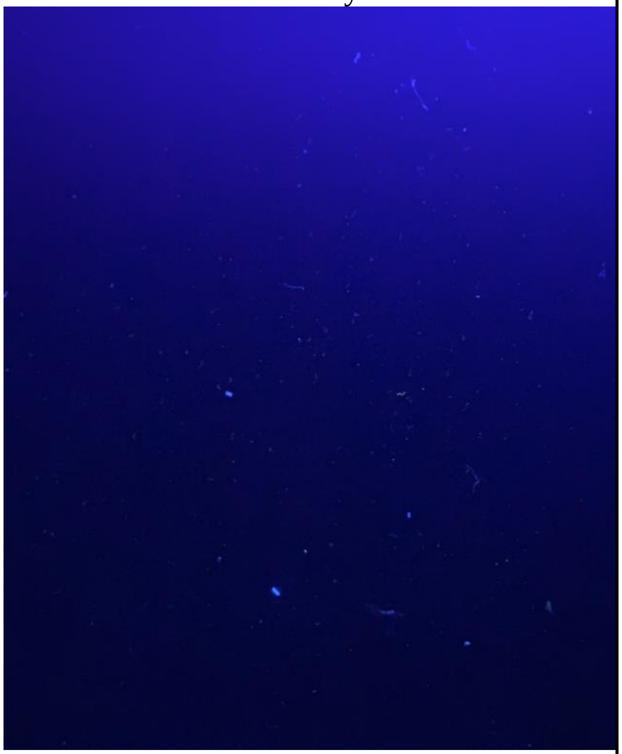
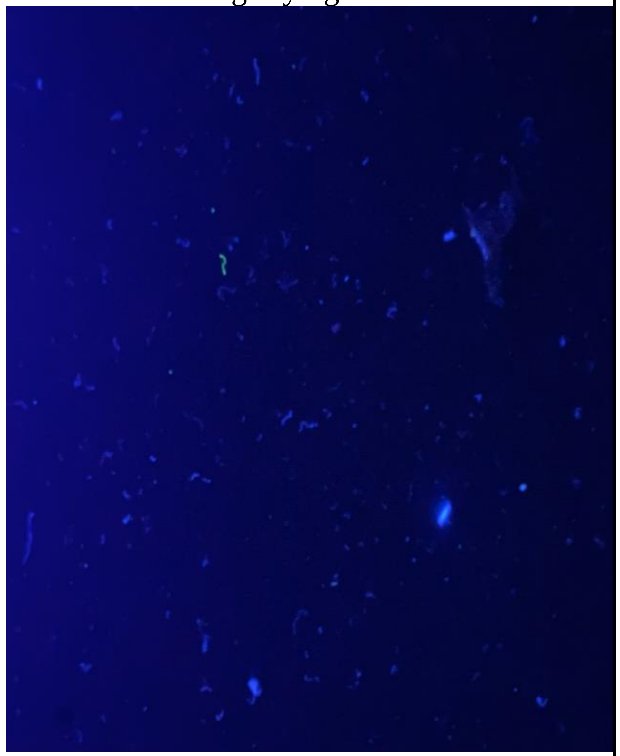
1. Find a favorable shoreline with sand to test from and mark exactly where you are going to collect sand.
2. Collect at least 4 handfuls of sand from DIRECTLY NEXT TO the water source and place them in a container. If you are collecting multiple samples, space out the sampling locations by about 10m.
3. Into the container with the sand sample, pour enough corn syrup to completely cover the sand by about 4cm.

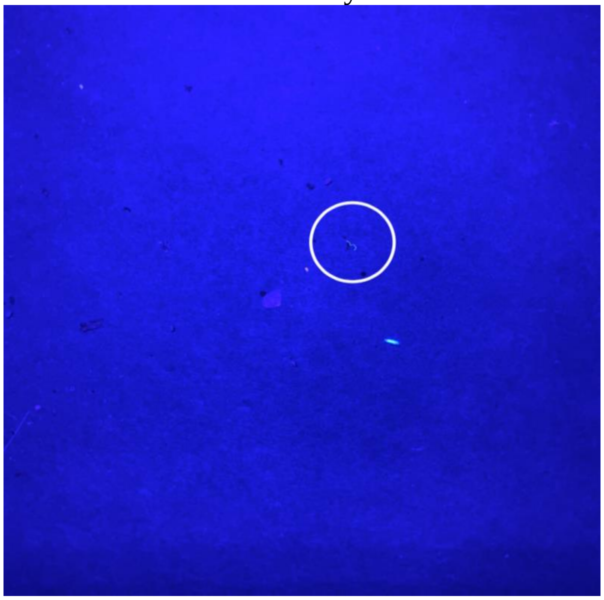
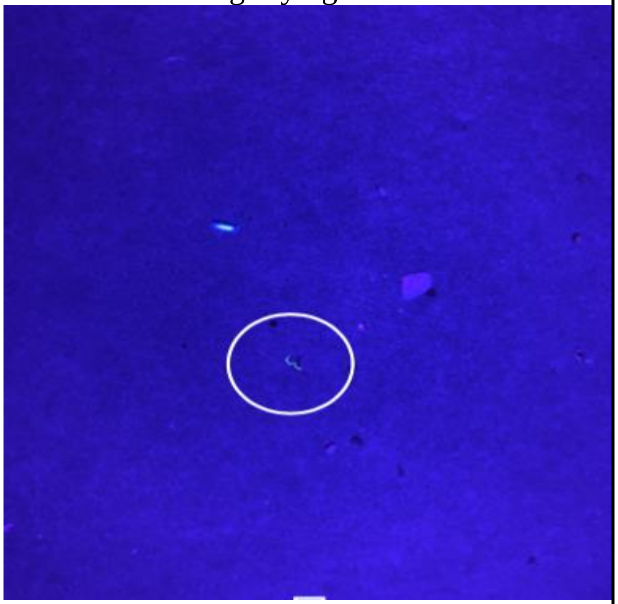
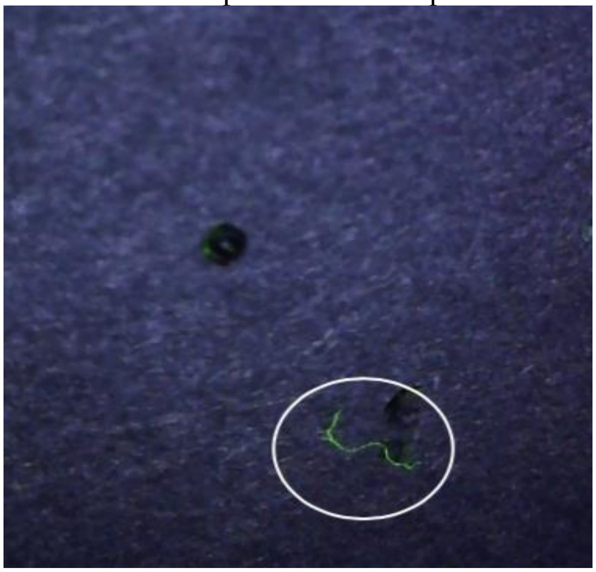
4. Use a spoon or other mixing tool to thoroughly mix the corn syrup and sand for 2 minutes. Stir slowly so you don't agitate the mixture, as this can result in the presence of bubbles in the solution.

5. Leave this mixture for about 24 hours, giving time to separate the plastics from the sand.
6. Observe the plastics extracted. They should be floating in the corn syrup.

Appendix G: Images of Detection Methods

Natural Light	
Naked Eye 	Magnifying Glass 
Smartphone Microscope 	Light Microscope 

<table>
<tr><th colspan="2">Blacklight</th></tr>
<tr><td>Naked Eye
</td><td>Magnifying Glass
</td></tr>
<tr><td>Smartphone Microscope
</td><td>Light Microscope</td></tr>
</table>

UV Light	
Naked Eye 	Magnifying Glass 
Smartphone Microscope 	

Appendix H: Lesson Plan

Berlin Microplastics Lesson Plan

Educational Objective

The purpose of this project is to increase the students' awareness of microplastics and the dangers they pose while implementing the scientific method. While the experimental activity may not produce conclusive results that show a presence of microplastics in the water sample used, this lesson accomplishes a couple things. First, the students get exposure to scientific experimentations and protocols, hopefully engaging their curiosity. Additionally, the students will learn the potential effects of microplastics and get exposed to this relatively new source of pollution. The supplemental activities, like cleaning macroplastics from the shores, are to further the point that plastic pollution is a big problem. The goal at the end of the lesson is for the students to understand the issue of microplastic pollution and learn ways they can help clean the environment while getting exposed to scientific procedures.

Pre-Experiment Educational Material

- The teacher will give a presentation on pollution and microplastics using the attached link and hand out the infographic collection for the students to keep and take home with them
    - The focus of this presentation is the scale of plastic pollution, and to get the students to understand how much of a problem it really is
    - See the speaker notes for these slides on the next page
    - After the speaker notes there are links to potential videos to play at the end of the presentation

Attachments

Slideshow Presentation (English):
https://www.canva.com/design/DAEdpmcha3Q/nZcyKX3vqqnFKiJmmBotcg/view?utm_content=DAEdpmcha3Q&utm_campaign=designshare&utm_medium=link&utm_source=sharebutton

Slideshow Presentation (German):
https://www.canva.com/design/DAEc-lA0jqg/m2ye7rdeG09OahOnb5raFg/view?utm_content=DAEc-lA0jqg&utm_campaign=designshare&utm_medium=link&utm_source=sharebutton

Speaker Notes for the Presentation
https://docs.google.com/document/d/18JpRV1QcbEikI_wIL6teP_-A-7gNqQW7cDEIij5zioI/edit?usp=sharing

Infographic Collection (English):
https://www.canva.com/design/DAEdufUsFEY/nzYfouN_O_tX0fSEGV_SsQ/view?utm_content=DAEdufUsFEY&utm_campaign=designshare&utm_medium=link&utm_source=sharebutton

Infographic Collection (German):
https://www.canva.com/design/DAEeGKGBGBo/hRHDv2rhNDXqCd1sCMbWzg/view?utm_content=DAEeGKGBGBo&utm_campaign=designshare&utm_medium=link&utm_source=sharebutton

Student Protocol Handouts:
https://drive.google.com/drive/folders/1Byq4ssnNTalNxNfOWjKyEoA5EWw4Rrwm?usp=sharing

Student Results/Reflection Assignment (English):
https://www.canva.com/design/DAEdplY1HKg/MGpx9J7SSNz47LMF_5SakQ/view?utm_content=DAEdplY1HKg&utm_campaign=designshare&utm_medium=link&utm_source=sharebutton

Student Results/Reflection Assignment (German):
https://www.canva.com/design/DAEeGKGBGBo/hRHDv2rhNDXqCd1sCMbWzg/view?utm_content=DAEeGKGBGBo&utm_campaign=designshare&utm_medium=link&utm_source=sharebutton

<u>Experimental Activity</u>

<u>Part 1</u>

In this activity, the students will be performing the experiment using a sample contaminated with primary microplastics to familiarize them with the method, and the presence of microplastics in everyday life. Students will be partnered up (or in groups of 3) to perform this experiment.

Note: For the identification, it can be difficult to tell what is microplastic, therefore it is recommended to have the students use magnifying glasses in an attempt to see more clearly and potentially a microscope with supervision.

Day 1

Materials for Teacher Prep:

- Hydrogen Peroxide (around 10%)
- Large container (enough volume for all the kids to perform the experiment later)
- Product with primary microplastics (also called microbeads)
    - Could be face wash, toothpaste, makeup, etc. Just some quick research into a product is necessary to ensure it has some form of microplastic/microbead.
- Organic material (soil, leaves, twigs, etc.) to simulate a real sample

Teacher Prep:

1. Combine water (any source) with the product containing microplastics in the large container
2. Mix in the collected organic material to create a "dirty" water sample
3. Give each partnership/group of students a copy of the "Student Protocol Part 1"

Materials for Students:

- 150 micron Filter and Funnel
- Large Mesh Kitchen Strainer
- Container with known volume (Around 2L)
- Secondary Container
- Waste Container
- Cup
- Safety Equipment: (For Step 6)
    - Gloves
    - Safety Goggles

Student Protocol for Part 1:

1. Collect some of the water samples using a container with known volume.
2. Have one partner hold the kitchen strainer over the secondary container and the other partner pour the water through the strainer into the container. Like it shows here:

3. When all the water is now in the secondary container, look at the strainer to see if you caught anything that could be plastic. It will be mostly leaves, sticks, or twigs, but there could be larger plastic pieces.
4. Place the filter paper into the funnel. Have one partner hold the funnel (with the filter in it) over the waste container and the other partner pour the water from the secondary container through the filter into the container. Like it shows here:

5. When the filter is done draining you can look at the filter to see if you can spot microplastics but we aren't done yet! Carefully take the filter paper out of the funnel and place it into the cup. Make sure you don't spill any of the sample.
6. Put on your safety equipment and soak the filter in Hydrogen Peroxide (provided by your teacher), and make sure you fill the cup just enough to cover your sample. Like it shows here:

7. Label your sample and leave it where your teacher says

Day 2

8. Put on your safety equipment and carefully take the filter out of the cup and place it on a dry work surface
9. Look at the filter and see if you can find microplastics.

Part 2

In this activity, students will go to the water source of interest. To begin they will search for all types of plastics on the shoreline. Reinforce the point that by finding and properly disposing of macroplastics, they are preventing those plastics from degrading into micro- and nanoplastics that pollute the waterways. Then the students will perform the experiment, slightly differently depending on if you are filtering at the lab or on-site. There is also the addition of an optional sand sampling method using a density separation in an attempt to identify microplastics along the shoreline of the water source.

Teacher Prep:

1. Decide whether you will be filtering on-site of the water source or take a water sample back to the lab
2. Decide on a major water source to test (Likely Muggelsee Lake or Spree River).
3. Give each partnership/group of students a copy of the "Student Protocol Part 2" (Depending on the location of filtration)
4. To further the point of plastic pollution being an issue, it is recommended to include cleaning up the shore of large pieces of plastic. This would be done by giving the kids trash bags and gloves and having them look around the area and help prevent these macroplastics from becoming microplastics

Filtering at the lab

Day 1

Materials for Students at the water source:

- Container with known volume (Around 2L)

Student Protocol at the water source:

1. Proceed to the water source and mark exactly where you are going to collect water.
2. Clean out your container using water from the water source. Do this by filling a bit of the container, shaking it, then emptying it 3 times.
3. Once rinsed, move about 5m upstream and collect water from the source by filling the container with a known volume.

Materials for Students at the lab:

- 150 micron Filter and Funnel
- Large Kitchen Strainer
- Hydrogen Peroxide
- Container with sample
- Secondary Container
- Waste Container
- Cup
- Safety Equipment: (For Step 5)

    - Gloves
    - Safety Goggles
- Blacklight
- Microscope

Student Protocol at the lab:

4. Have one partner hold the kitchen strainer over the secondary container and the other partner pour the water through the strainer into the container. Like it shows here:

5. When all the water is now in the secondary container, look at the strainer to see if you caught anything that could be plastic. It will be mostly leaves, sticks, or twigs, but there could be larger plastic pieces.
6. Place the filter paper into the funnel. Have one partner hold the funnel (with the filter in it) over the waste container and the other partner pour the water from the secondary container through the filter into the container. Like it shows here:

7. When the filter is done draining you can look at the filter to see if you can spot microplastics but we aren't done yet! Carefully take the filter paper out of the funnel and place it into the cup. Make sure you don't spill any of the sample.
8. Put on your safety equipment and soak the filter in Hydrogen Peroxide (provided by your teacher), and make sure you fill the cup just enough to cover your sample. Like it shows here:

9. Label your sample and leave it where your teacher says

Day 2

10. Put on your safety equipment and carefully take the filter out of the cup and place it on a dry work surface
11. Look at the filter and see if you can find microplastics

<u>Filtering on-site</u>

The purpose of filtering on site is to make it easier and more feasible to use larger volumes of water. We recommend filtering around 50-70 Liters of water for sampling. That could mean, depending on the size of your large bucket, filling, emptying, and refilling it multiple times.

Day 1

Materials for Students at the water source:

- 55-micron bucket filter is recommended
- Large bucket with known volume
- Smaller container to collected water
- Large kitchen strainer

Student Protocol at the water source:

1. Proceed to the water source and mark exactly where you are going to collect water.
2. Clean out your smaller container using water from the water source. Do this by filling a bit of the container, shaking it, then emptying it 3 times.
3. Once rinsed, move about 5m upstream and set up the bucket filter over the large bucket with known volume. Be sure it is secure at the edge of the water.
4. Collect water from the source by filling the smaller container
5. Have one partner hold the kitchen strainer over the bucket and the other partner pour the water through the strainer and bucket filter into the large bucket. Like it shows here:

6. Repeat step 5 until the large bucket is full of water
7. Once the bucket is full of water, have one partner pull the filter out and hold onto it. The other partner will dump the bucket of water back into the water source.

8. Bring the filter back to the lab.

Materials for Students at the lab:

- Hydrogen Peroxide
- Filter with sample
- Dry, new filter paper
- Cup
- Safety Equipment: (For Step 7)
    - Gloves
    - Safety Goggles

Student Protocol at the lab:

9. Carefully transfer the sample onto a dry filter paper and place that filter paper in a cup
10. Put on your safety equipment and soak the filter in Hydrogen Peroxide (provided by your teacher), and make sure you fill the cup just enough to cover your sample. Like it shows here:

11. Label your sample and leave it where your teacher says

Day 2

12. Put on your safety equipment and carefully take the filter out of the cup and place it on a dry work surface
13. Look at the filter and see if you can find microplastics

<u>Sand Sampling (Optional)</u>

This is an optional method to identify microplastic presence in the sand at the shoreline of the water source of interest. This method is a density separation using corn syrup which is dense enough for most plastics to float but rocks, sand, and other things will sink to the bottom.
Note: The sample must be sand, as it is a density separation experiment and soils have different densities depending on their composition.

Day 1

Materials for Students at the water source:

- Container

Protocol for Students at the Water Source:

1. Proceed to the water source and mark exactly where you are going to collect water.
2. Clean out your smaller container using water from the water source. Do this by filling a bit of the container, shaking it, then emptying it 3 times.
3. Once rinsed, take it to the edge of the shoreline, right where it touches the water.
4. Collect at least 4 handfuls of dry sand from the shoreline, and place it directly into the container.
5. Bring the container with your sample back to the lab.

Materials for Students at the lab:

- Container with sample
- Corn Syrup
- Mixing tool
- Magnifying glass

Protocol for Students at the lab:

6. Fill the container with enough corn syrup to cover the sample, plus about a few centimeters on top.
7. Have one partner hold the container down and keep it secure. The other partner will take the mixing tool and mix the sample and corn syrup for about 2 minutes, until it is all well-mixed together.
8. Label your sample and leave it where your teacher says

Day 2

9. Take a look at your sample and see if you can see any microplastics that should be floating on top. Use a magnifying glass to help identify if there are microplastics.

Post-Experiment Educational Material

- The students should complete part of the Results/Reflection Assignment
  - Part of it is to be completed after ending the presentation
- The teacher should finish the presentation with the rest of the slides. These focus on ways the students can do their part to reduce their personal plastic waste

Appendix I: Reflection Worksheet Template

1. What age were the experimenters?

2. Were the instructions simple/clear?

3. Was the quantity of water we asked you to get sufficient? Too much? Not enough? Was it difficult to obtain and/or transport?

4. What was your resulting concentration? (It's okay if you were unable to measure)

5. Were you surprised by your results?

6. Was the experiment engaging?

7. Did you enjoy it?

8. Did you run into any difficulties? If so, how did you overcome them?

9. Comments, Questions, Concerns?

Appendix J: Sponsor Feedback on Experiment

TJP e.V. Reflection Worksheet

1. What age were the experimenters?
Adults, members of TJP

2. Were the instructions simple/clear?
Instructions for sampling are clear and easy to follow. In the same way, primary school children will be able to follow them, to collect required samples.

3. Was the quantity of water we asked you to get sufficient? Too much? Not enough? Was it difficult to obtain and/or transport?
The chosen sampling day was not favorable in terms of the weather, strong wind and low temperatures made sampling difficult.
The type of filter paper we used made filtering very slow, so we decided to carry it out in the laboratory. This fact reduced the amount of water we were able to transport, only 2 liters, it is an insufficient quantity for the purpose of this trial.
Another difficulty that arose, due to the shallow depth of the shore, was to gather the required amount of water without getting wet in the attempt. The presence of a breakwater facilitated the gathering of water, but maybe not safe enough for school children.
It was not easy to collect 2 L of water, a quantity like 15 L or higher as subjected was not feasible due to the characteristics of the sampling place and the weather conditions described above.
Next time we should choose a place with more suitable characteristics for sampling.

4. What was your resulting concentration? (It's okay if you were unable to measure)
In our 2 L water sample, we were unable to find or identify any particles of microplastic. Anyway it was very difficult to discern.
From the observation through the first extracted debris (large one) we couldn't find any plastic particles (large or small), it was very difficult to discern from the organic residues.
In the second filtration we were not able to ensure the presence of microplastics. After two consecutive 24 hours digestions with 12% and 30% Hydrogen Peroxide solutions, apparently, visually, we were not able to distinguish microplastic from the rest of debris. By using a blacklight we identify fluorescence, but neither we can be sure if it was due to microplastic or a cross-contamination from the used filter paper.

5. Were you surprised by your results?
The results were in line with our expectations

6. Was the experiment engaging?
For us both parts of the experimental process were very appealing. Both the sampling part, despite the bad weather, and the post-treatment and analysis of the samples in the laboratory. I can imagine that for the restless and curious nature of a child, this activity will be equally or even more attractive. Additionally, being part of a real scientific study will be an extra motivation for school children.

7. Did you enjoy it?
A lot! The whole process.

8. Did you run into any difficulties? If so, how did you overcome them?
Some of difficulties we found have been described, like slow filtration rate, transport of samples or water gathering. But the most difficult was to identify microplastic particles.

9. Comments, Questions, Concerns?
The result may lead schoolchildren to believe that the problem of microplastics in Müggelsee does not exist.
The trip to Müggelsee (Geocaching) and the observation of litter on the beach and on the shore is a good method to introduce children to this issue, but then if they are not able to find any microplastic in the samples it can lead to wrong conclusions.
It would be useful to complete this trial with simple laboratory experiments, in which commons cosmetics will be examined to determine the presence of microplastics in their composition. This fact could help children to understand that microplastic are also a part of their everyday life and they can contribute to finding solutions to the issue.
Besides we also brought some sand samples, as you suggested at first. We found two blue/greenish potential microplastic particles. One we found by visual observation of the sample and one in a density test with syrup (we made syrup ourselves out of sugar). They were not fluorescent, but looked like microplastic under the microscope to us.

Angelina (IGB) Reflection Worksheet

- The water quantity was no problem, we filtered the water directly on the shore.
- We tried normal coffee filters. One filter is quite suitable for 2 litres of water, after that it runs through too slowly and you have to replace the filter.
- We couldn't find any plastic parts in the kitchen filter, but we could find microplastics along the direct shore line. Maybe it makes sense to include that (e.g. collect plastic along two metres of shoreline up to 30cm inland).
- As for the amount of organic material, there are certainly periods that are better and worse (spring algal bloom). The less organic material, the faster and better the filtering process.

- In the coffee filter we found a few individual small white particles that could possibly be microplastics (hard to tell).
- When experimenting with hydrogen peroxide, the instructions should perhaps include gloves and possibly protective goggles for the children.