PROJECT REPORT

An Interdisciplinary Methodology to Extract Microplastics from Soil: Laying the Groundwork for a Citizen Science Project

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Abstract

The forming of microplastics in the environment continues be a global problem with damaging risks to ecosystems and human health. Currently, most microplastic studies concentrate on water and air, while research focus on terrestrial samples such as soil still lags behind. This project reports the first results of our effort to develop and implement a methodology to study microplastics in soil samples nested in a multidisciplinary teaching laboratory. Chemistry and non-chemistry students isolated and examined microplastics, typically finding blue microfibers, verified via optical microscopy. In addition, participants designed outreach activities to introduce microplastic concepts to younger students and helped refine the methodology for further use across multiple courses and community events. This project ultimately pursues the establishment of a citizen science initiative, where shipped soil samples will be processed in teaching sessions.

Introduction

Synthetic plastics are remarkable materials that offer desirable properties, such as resistance to corrosion, durability, high mechanical strength, and electrical and thermal insulating capacities; nonetheless, these qualities slow plastic waste degradation (Webb et al., 2013). The

cumulative production of plastic has reached 400.3 million tonnes (Plastics – the fast facts 2023, 2025), and its projected production is estimated to reach 1,231 tonnes by 2060. Currently, 1,014 tonnes of such plastic will not be recycled, ending up in landfills, or incinerated, mismanaged, and/or directly leaked into the environment (Organisation for Economic Co-operation and Development (OECD), n.d.). Furthermore, plastic waste accumulation is generating microplastics (Thompson et al., 2004), pieces of plastic ranging from 5 mm to approximately 1 µm in size (Hartmann et al., 2019), which are projected to reach 5.8 tonnes by 2060 (OECD, n.d.) (see Figure 1).

The harmful effects of microplastic pollution on natural ecosystems are now evident (Yadav & Mishra, 2025), and studies connecting microplastic human exposure to public health are growing. Human intake of microplastics can happen in three primary ways: (1) inhalation (Prata, 2018), (2) consumption of contaminated food (Hernandez et al., 2019), and (3) direct skin contact (Hernandez et al., 2017). Recently, literature has reported findings of microplastics in human blood (Leslie et al., 2022) and in bodily fluids such as mucus or saliva (Huang, 2022). In addition, emerging studies link human microplastic exposure to heart-related issues (Siniscalchi et al., 2024).

Historically, microplastic studies have focused on water (Desforges et al., 2014) and air (Wright et al., 2020) samples, leaving terrestrial analysis behind (He et al., 2020), possibly due to soil's intrinsic complexity and heterogeneity (Conklin, 2013). Despite such challenges, the extraction and analysis of microplastics from soil is paramount, since soil is a vital component for life on Earth. Additionally, effectively communicating microplastics research and remediation efforts remains vital. Therefore, we are pleased to present our work on developing and implementing a methodology for extracting microplastics from soil, with a dual focus on academic instruction and community engagement. This initiative enhances multidisciplinary science education and establishes the foundation for a broader citizen science project to connect environmental research with the public.

FIGURE 1. Representative Examples of Microplastics.

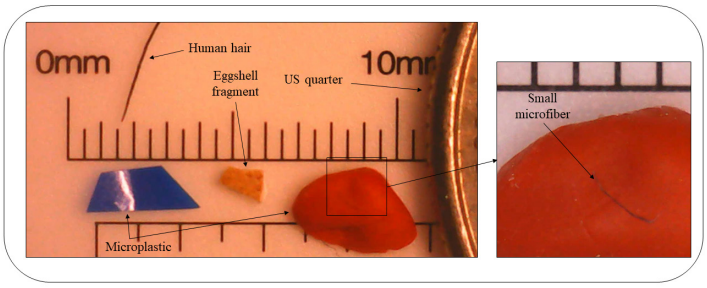
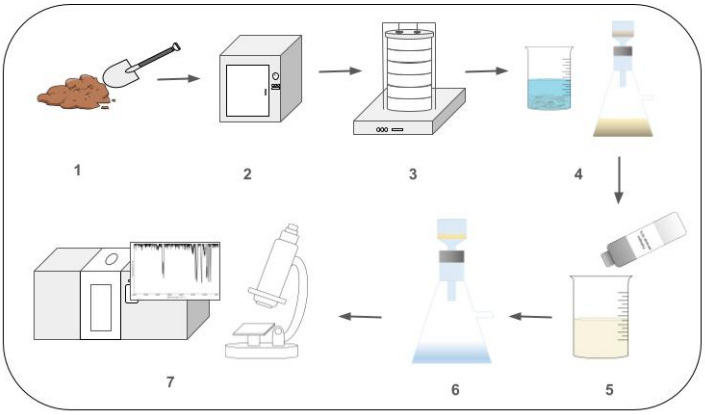


FIGURE 2. Extraction and Analysis of Microplastics



Project Description

Experimental Procedures

The extraction and analysis of microplastics uses non-plastic tools, containers, and lab supplies. The general procedure is represented in Figure 2 (Junhao et al., 2021). It encompasses (1) field sample collection, (2) sample drying, (3) physical separation via mechanical sieving, (4) floatation of light microplastics and filtration, (5) digestion of residual organic matter via chemical treatments, (6) fine filtration microplastics collection, and (7) observation and identification via optical microscopy and advanced instrumentation.

Our student participants collected samples from the banks of the Elm Fork tributaries of the upper Trinity River watershed and a local park. Samples weighed 100 g each and were stored in glass jars inside a cabinet. Before the first session, the soil samples sat uncovered in a drying oven overnight at 75° C. Drying the soil at low heat removed moisture to make it easier to handle and analyze. During the first session, the soil samples were

FIGURE 3. Microscopic Images of Blue Microfibers

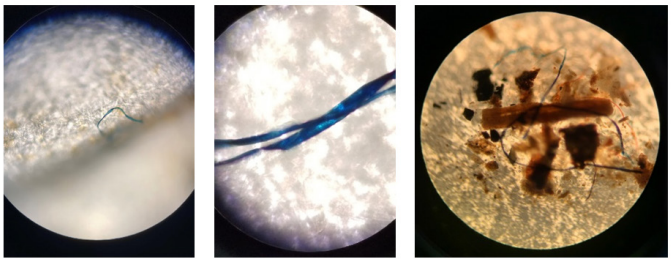


FIGURE 4. Laboratory Session, SCI 3033

crushed using a glass mortar and pestle, then sieved using metal-based meshes and an automatic shaker; the mesh aperture sizes range from 2000–25 µm. Eight-gram subsamples of the smallest particles were transferred to a glass beaker and combined with 100 mL of a saturated calcium chloride solution. This solution helps separate plastic from soil, because many plastics float easily in salty water. The mixtures were gently stirred with a glass rod, covered with a watch glass, and allowed to settle overnight.

In the following session, the mixture was carefully decanted onto a Whatman 40 filter paper set inside a ceramic Hirsh funnel and under vacuum. The portion that passed through the filter—known as filtrate—was transferred to a 250 mL Erlenmeyer flask, mixed with 50 mL of Fenton reagent (Tagg et al., 2017), and allowed to react for one hour. The Fenton process is a chemical treatment based on 30% hydrogen peroxide that degrades organic materials like plant matter without affecting microplastics. Vacuum filtration followed, using a nylon filter with a pore size of 0.25 µm, where small microplastics were collected. Figure 3 shows the microscopic images of the blue microfibers recovered.

Project Implementation

This methodology was first implemented for an interdisciplinary course, SCI 3033, Water in a Changing Environment (Spring 2022). Designed for chemistry and non-chemistry majors, it integrates real-world challenges such as plastic and microplastic pollution into its curriculum through hands-on, collaborative learning experiences. Scheme 1 shows the organizational chart, and the specific roles of all personnel involved in this project.

Gustavo A. Salazar oversaw the overall laboratory procedures while Alana K. Taylor managed personnel logistics. Graduate student Liliana Driver supervised the hands-on implementation of each laboratory technique, while each group of student participants performed the full procedure. This methodology was implemented within laboratory session times and successfully engaged all student participants. See Figure 4 for snapshots of a laboratory session.

A key component of this interdisciplinary approach involved the development of laboratory modules that support collaborative investigation of environmental pollutants. These modules gave students the opportunity to engage in the complete scientific process from literature review to dissemination of findings, while working in

SCHEME 1. Organizational Chart for SCI 3033

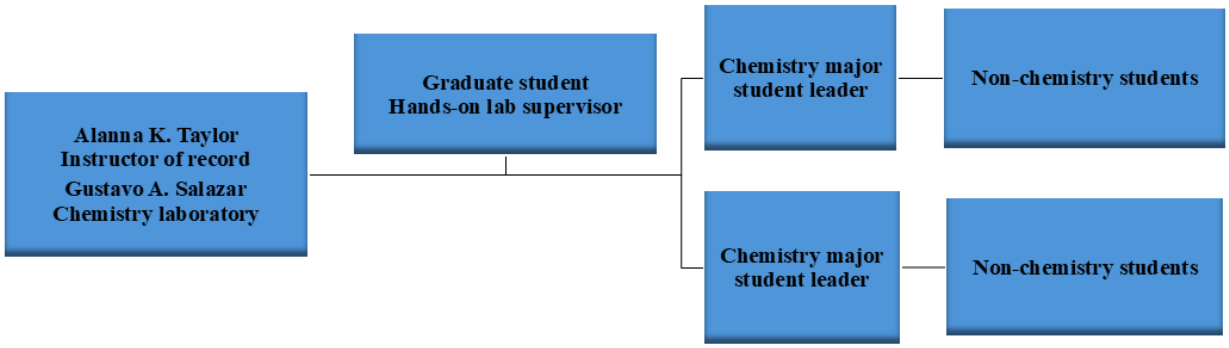


FIGURE 5. Poster Presented at 2022 Student Creative Arts and Research symposium, TWU

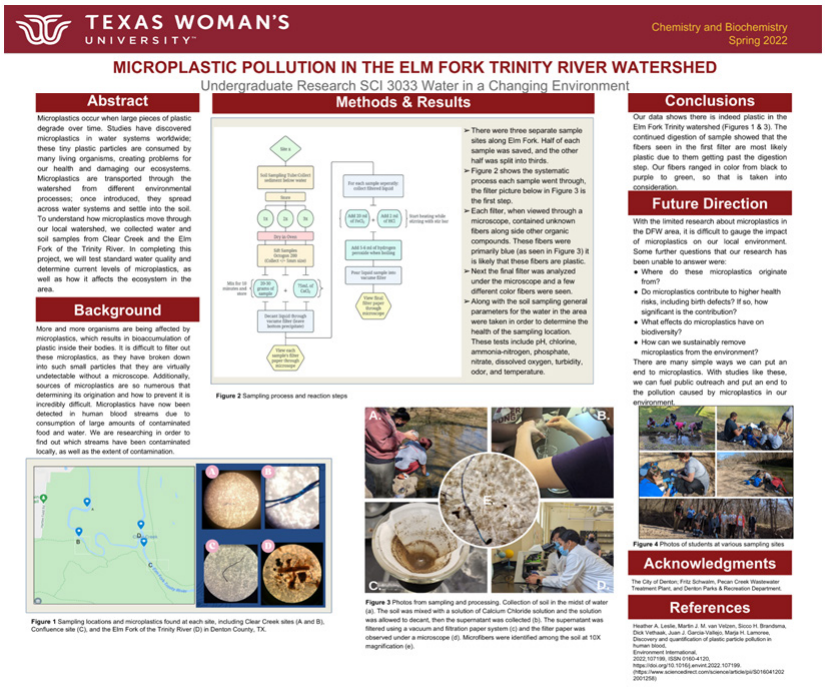


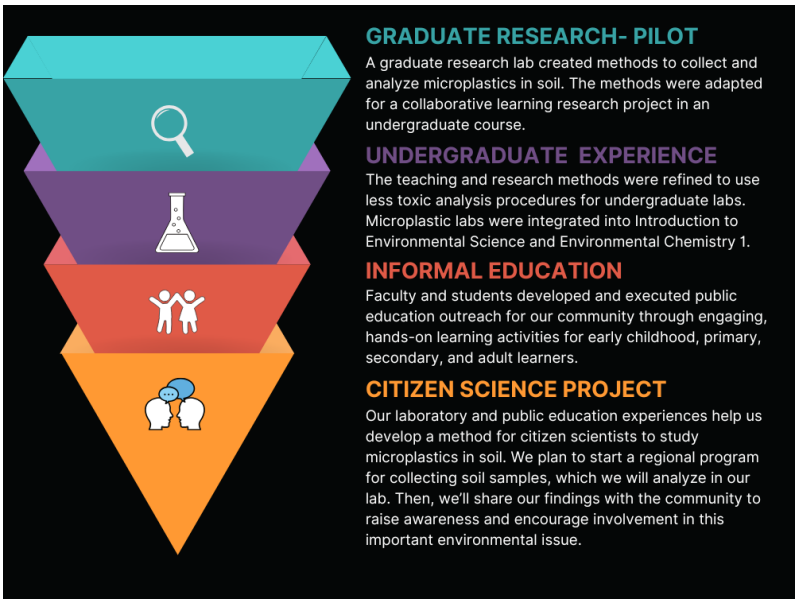
FIGURE 6. Elementary School Students Learn about Plastics in the Environment

team-based settings. As outlined in Appendix 1, students conducted authentic research that included field sampling, laboratory analysis, data interpretation, and public presentation of their work. For example, during week 2 students participated in fieldwork at a local waterway (Clear Creek and Elm Fork of the Trinity River), where they collected soil samples and analyzed water quality. Subsequent weeks' laboratory sessions allowed students to engage in multistep analytical techniques to quantify and characterize microplastic pollution. Students then synthesized their findings into scientific posters, which were presented at the 2022 Student Creative Arts and Research Symposium held at our university (see figure 5).

In parallel with our formal curriculum, we developed informal educational labs and activities designed to reach younger audiences and promote science literacy regarding microplastics and environmental stewardship. This initiative has inspired student-led public outreach and education efforts within the community. For elementary-aged audiences—a Montessori school in our case—we created a microplastic "glitter" lab tailored for second-grade students and scout troops. The informal lesson, "Plastic in the Environment," introduced the young students to basic Earth science concepts, including the Earth's spheres, the water cycle, and the watershed (see Figure 6).

These foundational concepts were used to explain how litter travels through the environment and how plastics and microplastics can infiltrate soil and water systems. The session also covered the fundamentals of plastic materials, the formation of microplastics, and their potential impact on ecosystems. Following the discussion, students engaged in a hands-on glitter lab activity, which modeled microplastic contamination using glitter as a stand-in for microplastic fibers. Each group received a digital scale, digital microscope, two glass petri dishes, a control sample (no glitter), and a prepared glitter-contaminated sample. Students began by forming a hypothesis, then observed both soil samples, first with the naked eye and later through microscopes, recording their findings throughout. The activity concluded with a reflection and discussion where students shared their conclusions based on their observations. The glitter lab allowed young learners to experience the scientific process in a tangible and age-appropriate way while building early awareness of environmental issues related to plastic pollution. To connect classroom learning with real-world science, the presentation concluded with photos of Texas Women's University students conducting fieldwork, analyzing

DIAGRAM 1. Structure of Multidisciplinary Teaching Laboratory



samples in the lab, and presenting their results at a research symposium. This provided the elementary students with relatable role models and a glimpse into environmental research in action.

Discussion

We have implemented an experimental methodology into a multidisciplinary teaching laboratory that successfully isolated microplastics from soil. Chemistry and non-chemistry student participants collaborated and performed all procedures within the teaching laboratory timeframe. Furthermore, student participants and the project members developed educational material and demonstrations for elementary schools and the community in a general setting, laying the foundation for a citizen science project (see Diagram 1).

The SCI 3033 Water in a Changing Environment course provided students with an interdisciplinary platform to examine the complexities of global water issues, emphasizing local environmental concerns. In this course, students developed and delivered an informal environmental education lesson focused on watersheds and the issue of microplastic pollution to children aged four to six at a local Montessori school. Students read age-appropriate books and used hands-on demonstrations, such as building a simple watershed model, to illustrate how microplastics travel through water systems and affect the environment. This effort displayed the students' ability to synthesize course content into engaging, accessible formats for younger audiences while reinforcing their own learning through teaching. Additionally, this experience illustrates how student engagement can bridge classroom learning with community-based microplastic research.

Examples of citizen science projects in microplastic pollution do exist, yet they focus on water bodies (Forrest et al., 2019) or nearby areas (Nel et al., 2020), larger microplastics (Lots et al., 2017; Adventure Scientists, 2024), or a larger geographical area with limited analysis (The Big Microplastic Survey, n.d.; Barrows et al., 2018). Some published methodologies could lead to implementations into citizen science projects (Camins et al., 2020; Scircle et al., 2020; Doyen et al., 2019); however, procedures could become impractical for citizen scientists. Our team is pursuing the development of a simple yet effective methodology that could be reproducible in the teaching laboratory by a diverse population of students and would process shipped samples from citizen scientists. The potential for a citizen scientist to analyze and categorize the characteristics of microplastics in soil is important, since it can lead to a more informed strategy for remediation development. Including students with diverse majors helps to better understand the dynamic between citizens with different career paths.

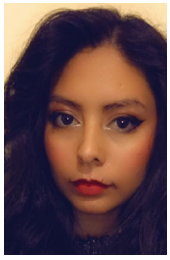
Conclusions and Future Work

A methodology to study microplastics in soil has been developed and implemented in a multidisciplinary laboratory session. This project helped engage a diverse class, connecting chemistry and non-chemistry students. Qualitatively, the first experiments have isolated blue microfibers from samples collected locally. Student participants presented their findings in a university-wide symposium. Student participants also developed simplified adaptations of this methodology for educational purposes at the K–12 level and for the community in general. Finally, we are continuing our efforts to develop logistics for a citizen science project where shipped samples could be analyzed in a laboratory teaching session.

About the Authors

Gustavo A. Salazar is an assistant professor in the Chemistry and Biochemistry division at Texas Woman's University. He received his PhD in Chemistry from the University of North Texas, with a focus in synthetic chemistry and luminescence spectroscopy. He worked on the microwave-assisted synthesis of novel ambipolar polyimine ligands and their tricarbonyl rhenium complexes; he was particularly interested in the photoluminescent phenomenon "luminescence rigidochromism" present in a mononuclear rhenium complex. Salazar has transferred his laboratory and instrumentation experience to Texas Woman's University, where he teaches Environmental Chemistry I and General Chemistry I and II. His current research interests are in microplastic pollution and related topics.

Alana K. Taylor is a lecturer in the Chemistry and Biochemistry division at Texas Woman's University. She is passionate about science and education and has significantly contributed to the field through her innovative teaching methods. She earned her master's degree from the University of North Texas, where she focused on enhancing STEM education for non-majors. Alana recognized the importance of making science accessible to all and concentrated on developing strategies to engage and inspire students from diverse backgrounds. Currently pursuing a PhD in Education and Organizational Leadership, Alana's research interests lie at the intersection of communities, education, and environmental science. Her doctoral work centers on studying community resiliency to climate change, aiming to identify practical solutions for building sustainable and adaptive communities facing environmental challenges.



Liliana A. Driver earned a Bachelor of Science in Biochemistry from Texas Woman's University. Her research focuses on the removal, isolation, and analysis of microplastics in soil using physical and chemical techniques. By employing advanced methodologies, Liliana aims to enhance detection accuracy and develop effective strategies for mitigating microplastic contamination in terrestrial environments.

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LAB: Detecting Microplastics in Soil and Sediment, and Analyzing Water Quality in our Local Watershed

Purpose:

Isolate and quantify microplastics and microfibers from creek soil and water samples collected in the local watershed in order to gain experience performing scientific research, assess local environmental pollution.

Learning Objectives:

- Describe the sources, types, and environmental impact of microplastics in aquatic and terrestrial environments.
- Conduct field sampling of water and sediment in a local watershed according to scientific protocols.
- Apply techniques to isolate and identify microplastics and microfibers in soil and water, using methods such as sieving, density separation, chemical digestion, and microscopy.
- Conduct water quality assessments using portable water testing kits.
- Analyze and interpret environmental data on the presence of microplastics and microfibers and water quality parameters.
- Communicate findings in written and visual formats, including a final research poster.

Project Overview:

Week 1: Introduction & Literature Review

- **Date: Jan. 20**
- **Location: Classroom**
- Activities:
    - Guest speaker (expert in environmental pollution or microplastics).
    - Overview of plastics, microplastics, and local watershed issues.
    - Assign literature review topics; introduce TWU Library database tools.
- Assignments:
    - Literature review summary (due Feb. 10).
- Learning Focus:
    - Understanding sources and implications of plastic pollution.
    - Practicing academic research and synthesis.

Week 2: Field Sampling and Water Quality Monitoring

- **Date: Feb. 3**
- **Location: Clear Creek Natural Heritage Center**
    - Sample locations:
        - Clear Creek
        - Elm Fork
        - Confluence
    - *Store Samples at TWU*
- Activities:
    - Divide into sampling teams (Clear Creek, Elm Fork, Confluence).
    - Collect water and sediment samples.

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    - Conduct in-field water quality tests (pH, turbidity, DO, nitrates, etc.).
- Learning Focus:
    - Hands-on experience with sample collection and environmental fieldwork.
    - Interpreting real-time water quality data.

Week 3: Initial Screening and Density Separation

- **Date: Feb. 17**
- **Location: Chemistry Lab**
- Activities:
    - Dry, grind, and sieve soil samples.
    - Conduct density separation using $ZnCl_2$.
    - Create control samples.
- Learning Focus:
    - Understand and apply principles of physical separation based on particle size and density.
    - Practice contamination prevention methods.

Week 4: Filtration and Microscopy (Part I)

- **Date: Mar. 3**
- **Location: Chemistry Lab**
- Activities:
    - Perform vacuum filtration on the supernatant from the density separation.
    - Visualize solids under a stereomicroscope.
    - Prepare and apply the Fenton reagent to digest organic materials.
- Learning Focus:
    - Master laboratory filtration techniques.
    - Recognize microfibers and microplastics under the microscope.

Week 5: Filtration and Microscopy (Part II)

- **Date: Mar. 24**
- **Location: Chemistry Lab**
- Activities:
    - Final vacuum filtration.
    - Visualize and quantify the remaining microplastics and microfibers.
    - Record and analyze class data.
- Learning Focus:
    - Compare controls and samples.
    - Begin synthesizing the data for the final poster presentation.

Week 6: Poster Session & Reflection

- **Date: Mar. 31**
- **Location: Classroom**
- Activities:
    - Finalize and present research posters.

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    - Group reflections and peer feedback.
- Learning Focus:
    - Scientific communication.
    - Critical thinking on environmental implications and potential solutions.

Poster Guidelines:

Title: Clear, descriptive, and relevant to the local watershed.

Sections:

- Introduction
- Methods (sampling, separation, filtration)
- Results (tables, graphs, micrograph images)
- Discussion (patterns, pollution sources, significance)
- References

Visuals: Required—graphs, photos from field/lab, microscopy images.

Presentation: Present a 5–10-minute summary of work.

Rubric:

Component	Weight
Literature Review Summary	**15%**
Fieldwork Lab Sheet & Observations	**15%**
Lab Notebooks/Worksheets (Lab 3 & 4)	**25%**
Final Filter Analysis and Class Data	**15%**
Final Research Poster	**30%**

Introduction:

Human-made pollution comes in many forms, and one of the most prevalent in modern society is plastic. Plastics are synthetic or semi-synthetic organic polymers used in numerous ways in modern society. From the packaging at the local grocery store to the clothes we wear and the toys that many of us grew up playing with, we are surrounded by plastics. The affordability, versatility, and water imperviousness of plastic polymers provide much of their appeal. They are typically made from petroleum products, with repetitive carbon-carbon and carbon-hydrogen bonding patterns punctuated by cross-linkable functional groups (see Figure 1, which shows the structural formulas of polyethylene [bottom] and polyester [top]).

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APPENDIX

However, other types of polymers can also be made from more renewable, biological materials, such as cellulose. Plastic materials are remarkably resistant to biodegradation upon exposure to a wide variety of conditions. This is one of the primary advantages of plastics from both a manufacturer's and consumer's perspective, but also a significant disadvantage in terms of environmental sustainability. For example, most plastics do not dissolve in water, and many are also impervious to strong acids, bases, and oxidants. Plastic materials tend to persist in one form or another in the environment for a very long time. Although pure plastics are usually biologically inert and are considered non-toxic to living organisms, many compounds that leach from plastics during their breakdown are carcinogenic or endocrine disruptors, and other toxic environmental pollutants tend to "stick" to plastics in the environment.

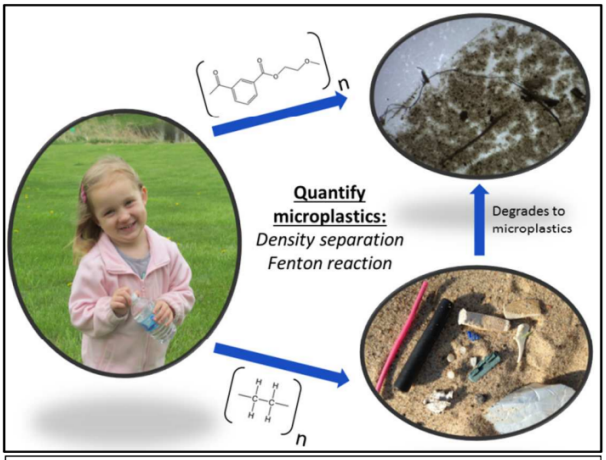

Figure 1: Plastics, such as polyethylene, are used in many items such as water bottles. These large items can degrade into smaller and smaller pieces, eventually yielding microplastics with a diameter of less than 5 mm. Many fabrics, such as the fleece jacket shown on the child in this figure, are made of polyesters. These polyester fabrics shed a great deal of plastic microfibers during their wear and washing.

Although plastic pollution, especially in waterways and oceans, has been studied for years and is well known to many people, a lesser-known problem is microplastic pollution. **Microplastics are plastics less than 5 mm in diameter and are a class of emerging pollutants of concern due to their widespread presence in water and soil.** Their effect on ecosystems and food chains is largely unknown, but a variety of organisms readily ingest them. Although microplastics have been around for decades, it is only recently that many research groups have established their ubiquitous presence in waterways, oceans, and surrounding areas.

Microplastics come from primary and secondary sources and include both particles and microfibers. Primary microplastics are intentionally produced small-sized microplastics that are added to personal care products and as industrial scrubbers (this addition to products began in the 1990s), whereas secondary microplastics form from the degradation and fragmentation of larger plastic items. Regulatory efforts are already underway to curb the use of primary microplastics, with legislation such as the Microbead-Free Waters Act of 2015 being enacted in the United States. States are making efforts to diminish microplastic pollution in the future.

A thorough understanding of where microplastic pollution is coming from is currently not complete. **This laboratory experiment will focus on isolating and quantifying microplastics found in soil and water from our local watershed, Clear Creek and the Elm Fork tributary of the Upper Trinity River** (Figure 2).

This lab will analyze how many microplastics are being retained in the creek soil and the adjacent water quality. During sampling, water quality will be monitored using a water quality kit. In the laboratory, samples collected will be dried, ground, sieved, and separated according to density, and digested and filtered in order to isolate microplastics from the natural materials. These microplastics will then be visualized with a

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stereomicroscope and quantified in terms of microplastics and microfiber size, shape, and color. Students will compare and plot samples, and after tabulating their results will assess whether or not the sampling sites are a significant source of microplastics.

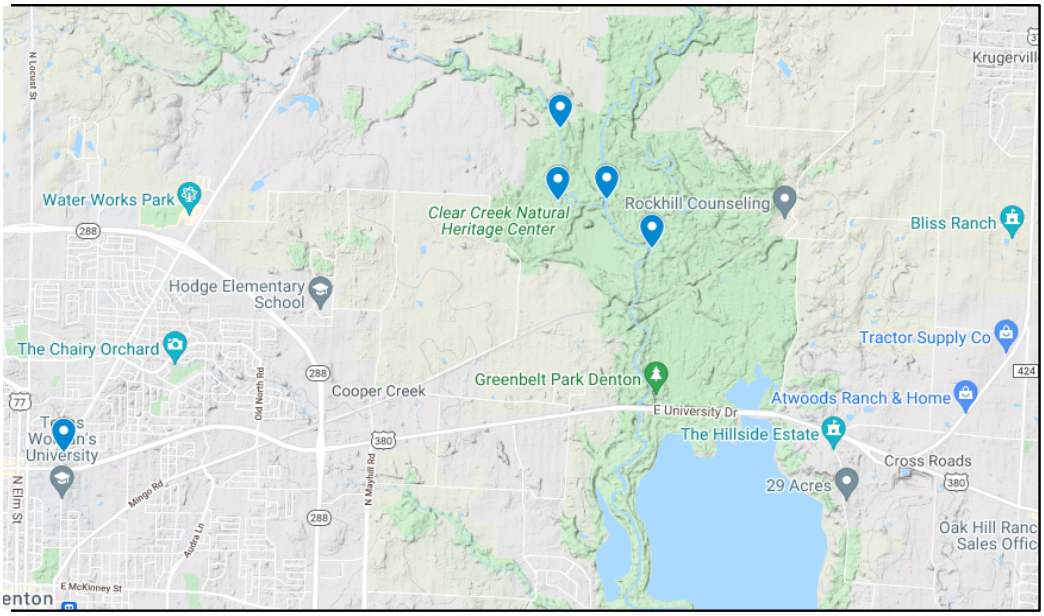

Figure 2: Sampling Site, blue unlabeled markers indicate sampling site. Map created on Google Maps.

Assignments + Procedures:

Jan. 20, Lab 1: Begin Microplastic Research Project

- ➢ Guest Speaker
- ➢ Discussion
- ➢ Overview of project
- ➢ Begin Literature Review

Project Planning

- **Literature Review:** As a class, we will be compiling a literature review. Individually, choose one topic to write a two- to three-paragraph summary on. Use the TWU Library electronic database to find your articles. *If you need assistance finding a paper to read, you can contact Suzi Rumohr, our content librarian.* Be sure to cite your sources and references. ***The summary is due on February 10 as a discussion post in Canvas.***
  - Literary Review Topics:
    - Microplastics
    - Bioaccumulation (of microplastics)
    - Water Quality
    - Local Watershed (Upper Trinity)
    - Microbead-Free Waters Act of 2015
    - *A topic of interest to you (but still related to the research project/investigation).*

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- *How to write a summary*
- *What is a Literature Review?*
- *How to write a Literature Review (we will do this as a class).*

Feb. 03, Lab 2: Fieldwork: Clear Creek Natural Heritage Center

- Collect Samples + Fieldwork:
  - Sample locations:
  - Clear Creek
  - Elm Fork
  - Confluence
- Store Samples at TWU
- Fieldwork Lab Sheet

Collect Samples + Water Monitoring at Various Locations.

As a class we will split into groups to collect soil and water samples and to collect data from our sample sites. The locations we will be collecting from are Clear Creek, Elm Fork, a tributary of the Upper Trinity River, and the point where they converge together aka the Confluence. *Click this link to see a map of our sites*.

Procedures:
During all procedures be sure to record important observations on your Lab Sheet.
Collect Samples and Water Quality Test

Purpose: Collect sample water and soil samples for use in the lab and perform water quality testing on-site. Test the water for chlorine, pH, nitrates, phosphates, ammonia, dissolved oxygen, and temperature.
Materials:

- 120 cm Turbidity Tube
- LaMotte Water Pollution Kit
- Glass jars
- Phone with a camera

Step 1: Collect Soil + Water Samples
Throughout the procedure, ensure your hands are clean, keep clothing away from samples, and cover materials as much as possible to reduce contamination. All glassware should be cleaned with deionized or filtered water before going to the field.

1. Take a picture of your site and record your observation on your Lab Sheet.
2. You will collect a water and a sediment sample for your site. Obtain glass jars to collect the samples.
3. Record the location of your site on the jar.
4. Carefully pack the sample to take back to the laboratory.

Checkpoint: At this point, you should have one sample of sediment and one sample of water.

Step 2: Water Quality Test
1. Get a turbidity reading using the turbidity tube.

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2. Follow the directions on the LaMotte Water Pollution Kit and test for chlorine, pH, nitrates, phosphates, ammonia, dissolved oxygen, and temperature.

Checkpoint: At this point, you should have data for your site.
Disposal: Follow the guidelines outlined in the LaMotte Water Pollution Kit.

Feb. 17, Lab 3: Initial Screening and Density Separation

- Chem Lab: Screening + Density and Filtration + Visualization
- Lab Sheet

Initial Screening and Density Separation

Introduction:
The first step in this laboratory is to pulverize and separate particles by size in dried soil using sieves, which are available with different pore sizes. The soil you will use has been dried in an oven before use for accurate mass determination and to facilitate the sieving process. Wet, muddy soil will not pass through the sieves easily. A mortar and pestle is used to break up the soil particles into fine pieces, as this low level of mechanical force is unlikely to break up any microplastics. The larger particles will not be analyzed because this laboratory is focusing on microplastics analysis, which by definition must be smaller than 5 mm in diameter.

Following this sieve separation, transfer a 20–30 g sample of the dried, sieved soil into a 250 mL beaker and use the density of the microplastics as the next means of separation from the natural materials. Recall that density is equal to mass/volume, and different substances have different densities. Pure water, for example, has a density of 1.0 g/mL. Anything with a density less than 1.0 g/mL, such as Styrofoam or oil, will float on water. In contrast, anything with a greater density than 1.0 g/mL will sink in water (such as a piece of lead or honey). Many plastics float in water, indicating a density of less than 1.0 g/mL. However, the density of some plastics falls within the range of 1.0–1.3 g/mL and they will therefore sink in water. We will create a solution with a density of around 1.3 g/mL to ensure that all the microplastics float or suspend in the solution and can be separated from heavier solid particles.

Throughout this lab, it is important to keep your solutions and filter paper covered. The reason for this is to reduce contamination of your samples, since microfibers are ubiquitous and readily shed from certain types of clothing. For Part A, you will complete the mechanical sieving process of your soil and the density separation. For Part B and in the following weeks, you will filter the liquid portion of your solution, visualize the components present on the filter paper using a stereomicroscope, and degrade the natural components in your sample using the Fenton reagent. Lastly, you will again filter the liquid portion of your sample and use a stereomicroscope to visualize and quantify the number of microplastics and microfibers found in your original sample.

Procedures:
During all procedures, be sure to record important observations on your Lab Sheet as the experimental steps are proceeding.

Screening of soil samples and density separations

Purpose: Isolate soil that has a soil particle size of less than approximately 5 mm using a sieve. Separate solid particles with a density of 1.3 g/mL and lower from heavier particles using density separation and a solution with a density of approximately 1.3 g/mL.

Materials:

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- #4 sieve (4.75 mm)
- hot plate
- scoopula
- mortar and pestle
- 2, 150 mL beaker
- 2, 250 mL beakers with lids/watch glass (or foil)
- zinc (II) chloride tetrahydrate
- 10 mL graduated cylinder
- magnetic stir plate with magnetic stir bar

Step 1: Screening of soil.
Throughout the procedure, ensure your hands are clean, keep clothing away from samples, and cover materials as much as possible to reduce contamination. All glassware should be cleaned with deionized or filtered water.

1. You will use oven-dried soil samples. Obtain approximately 40 g of the dried soil using a clean 150 mL beaker from either sample A or B, and determine its mass. Be sure to record the mass of the beaker before adding any soil. Record the exact mass and your sample location in your Lab Sheet. Cover the beaker.
2. Transfer the soil to a mortar and pestle, and pulverize it to break up clumps of dirt and other materials.
3. Sieve the 40 g soil sample and collect the soil with particle sizes of approximately 5 mm or smaller into a 250 mL beaker. Soil with particle sizes below 5 mm is the soil that goes through the sieve. Keep the sample covered as much as possible with foil, and keep your clothing away from the sample.
4. If some clumps maintain integrity throughout the separation process, remove the soil from the sieve into a mortar, and use the pestle to break up the clumps. Add the ground soil back into the sieve and repeat the process.
5. Transfer 20–30 g of your soil to a clean 250 mL beaker and cover it with a lid. Record the exact mass of the sieved soil in the beaker on your Lab Sheet, making sure to record the mass of the beaker before adding the soil. Record this mass of dry soil on all Lab Sheets, as you will need it for your final analysis.
6. At this point, create a sample control (or blank) by obtaining a clean 150 mL beaker. Cover the beaker with foil or a watch glass, just as you did with the beaker containing the soil.

Step 2: Density separation.
1. Prepare 100 mL of a 3.6 M ZnCl2 solution, which will create a solution with a density of 1.2–1.3 g/mL. Use a clean 150 mL beaker to prepare this solution and keep it covered as much as possible. Record your calculations on your Lab Sheet. If the solution does not dissolve immediately, add a magnetic stir bar to the beaker, place it on the magnetic stirrer, and stir the solution until the solid has completely dissolved. If after 10 minutes of stirring, your solid has still not fully dissolved, transfer the beaker to a hot plate for a few minutes.
2. Test the density of 5.0 mL of the zinc chloride solution. Add 85 mL of the zinc chloride solution to your 250 mL beaker containing the sieved soil, and pour the last 10 mL into the control beaker. To test the density, remember that d = m/v, and the units of density are typically g/mL. Therefore, use a 10 mL graduated cylinder to obtain 5.0 mL of the zinc chloride solution. Then, determine the mass of this solution in grams by weighing the graduated cylinder before and after adding the 5.0 mL of zinc chloride solution. Record your calculations on your Lab Sheet.
3. Add a clean magnetic stir bar to the soil and mix it with the zinc chloride solution. Using a magnetic stirrer, stir the solution thoroughly for 10 minutes, keeping it covered with foil as well as possible. Remove the stir bar from the solution when you are finished.
4. Clean the stir bar and add it to your control beaker. Stir the solution in the beaker for a minute, and then remove the stir bar. Keep the beaker covered as much as possible.
5. *If needed, store both beakers:* the one containing the soil and zinc chloride solution, and the control beaker. Make sure they are covered.
6. Clean the stir bar, mortar, pestle, and sieve, and rinse each with deionized water.

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Checkpoint: At this point, you should have one beaker of sieved soil in a 3.6 M zinc chloride solution covered with foil and a covered control beaker.
Disposal: All soil that is not placed in a 250 mL beaker can be disposed of in the regular garbage.

Mar. 03, Lab 4: Filtration and Microscopy (Part I)

- Chem Lab: Vacuum Filtration
- ASSC 267: Stereomicroscope
- Lab Sheet

Introduction:

The soil solution will be filtered to separate the solids that are suspended or floating in the liquid from the solids at the bottom of the beaker. We will use a method of filtration called vacuum filtration to collect microplastics and other particles with a density of less than 1.3 g/mL on a piece of filter paper. We will use a nylon filter membrane to collect the microplastics in this step, which is resistant to the strong oxidant used in this procedure. The filter paper, which will contain any microplastics and a variety of other materials at this point, will then be viewed using a stereomicroscope.

This filter paper will then be transferred to another beaker, and the contents will be oxidized to remove natural material. Since natural substances, such as plant materials and cotton fibers, may still be present at this point, the next step is to digest these natural fibers using an oxidant. An oxidant is a substance that removes electrons from different species during a redox reaction. The oxidant is formed from the Fenton reagent, which works best at a pH of 2–3 and can digest a wide range of organic molecules, macromolecules, and macroinvertebrates. Plastics, however, are not digested or decomposed by this oxidation process.

In the Fenton reagent reaction, ferrous iron (Fe2+) is added to a solution of hydrogen peroxide, and iron (II) acts as a catalyst for the production of a strong oxidant that can oxidize various organic matrix compounds in the solution. More specifically, this reaction creates many free radicals, which are powerful and non-selective oxidants, as shown below, where the dot after a chemical formula represents a free radical. This reaction is highly exothermic and has been used over the years to oxidize contaminants in soil or wastewater.

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + HO^{\cdot} + OH^{-}$$

$$Fe^{3+} + H_2O_2 \rightarrow Fe^{2+} + HOO^{\cdot} + H^{+}$$

The HO• and HOO• are the free radicals that react with and digest the organic matrix present in the sample. These secondary reactions often have water and carbon dioxide (gas) as a product, which is why you can see a great deal of "foaming" or "bubbling" during this reaction, as this is the carbon dioxide gas escaping the solution. You will store your oxidized filter paper until next week, at which point you will re-filter your solution and visualize your filter paper one last time.

Procedures:

During all Part B procedures, be sure to record important observations on your Lab Sheet as the experimental steps proceed.

Filtration and visualization followed by Fenton reagent exposure to digest natural material.

Purpose: Isolate the floating and suspended particles by decanting and filtering. View the filter paper under the microscope. Subject the collected solids to the Fenton reagent to digest the natural materials.

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Materials:

- hot plate with a magnetic stirrer and stir bar
- vacuum filtration apparatus
- 30% (v/v) hydrogen peroxide
- 0.0075 M FeCl2 solution
- 0.1 M HCl
- glass stir rod
- forceps
- 10 mL and 100 mL graduated cylinder
- squirt bottle with deionoized water
- pH paper
- nylon filter paper, .45 µm–5 µm pore size

Step 1: Filter the liquid and visualize the filter paper.

1. Obtain your soil/solution mixture, but take care not to disturb it! You want to keep the solids on the bottom of the beaker and the liquid on the top separate. Carefully remove it from your lab drawer and do not shake it unnecessarily. You will pour out the liquid portion into a vacuum filtration filter fitted with a piece of nylon filter paper. Your instructor will help you with this vacuum filtration step. This process is called decanting the liquid.
2. When your liquid mixture is almost gone, stop decanting to ensure that you don't add the solid to your filter paper. Use a water squirt bottle to rinse any solids that have stuck to the insides of the filtration cup onto the filter paper.
3. Shut off the vacuum. Remove the glass top from the vacuum filtration setup and break the vacuum by unplugging the vacuum hose, if necessary. Using tweezers, remove your filter paper from the funnel and put it on a watch glass. Add a second watch glass to the top of your original watch glass as a cover for your filter paper. Be sure to flip the covering watch glass so that the glass does not compress the sediment on the filter paper.
4. Thoroughly rinse the filter funnel and filter holder, and vacuum filter the contents of the control beaker. Follow the same procedure for rinsing the filter and removing the filter paper. Use a new filter paper for the control beaker.
5. View each filter paper under the magnification of the stereomicroscope and record your observations. Try to identify microfibers or microplastics in your samples. Some samples may not contain any microplastics or microfibers at all. You must remove the cover watch glass before microscope visualization.

Step 2: Subject the material on your filter paper to the Fenton reagent to degrade natural materials.

The following steps should be completed under a fume hood, wearing gloves and goggles, due to the use of concentrated hydrogen peroxide and the potential for rapid gas production.

1. Transfer each filter paper to a 150- or 250-mL beaker and add 2 mL of 0.1 M HCl and 20 mL of 0.0075 M FeCl2 solution to the beaker containing the filtered sediment. To the control beaker, add 1 mL of HCl and 10 mL of 0.0075 M FeCl2 solution. Stir the contents of each beaker slowly with the magnetic stir bar to avoid damage to the filter paper. The pH should be 2–3. Test the pH with pH paper after adding the HCl and FeCl2 and allowing the solution to stir for approximately 1 minute. If the solution is not acidic enough, add HCl dropwise with stirring, checking the pH after every few drops, until a pH of 2–3 is reached.
2. Begin warming the mixture on a hot plate under the hood. The mixture should only be heated to about 70 °C, not to boiling! Carefully, use a thermometer to monitor the liquid's temperature. If the temperature goes above 70 °C then remove your beaker from the hot plate, turn down the temperature of the hot plate, and wait for it to cool down. While heating, slowly add 5–6 mL of 30% hydrogen peroxide (H_2O_2) to the sediment mixture. Add 3 mL of the H_2O_2 to the control beaker. It takes several minutes for the reaction to reach its maximum reactivity. The maximum reactivity is determined by maximum "foaming."
3. Keep a bottle of deionized (DI) water nearby. If the reaction begins to froth excessively, to the point where it may spill over the sides of the beaker, remove the beaker from the heat and add DI water to calm the reaction. After adding all the hydrogen peroxide, continue stirring for an additional 15 minutes. If you use it, remove the magnetic stir bar before storing

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your solution. After the foam has subsided, use the markings on the side of your beaker to determine and record the volume of the final solution on your Lab Sheet. Cover each beaker with foil and store them until next week.

Checkpoint: The Fenton reagent reaction needs to be complete (no more bubbling) before you can store your solution. Make sure the beakers are covered with foil or a watch glass until next week.
Disposal: Leftover liquid from the Fenton reagent exposure may be discarded into the container labeled "Zinc Chloride Waste" in the hood. Soil solution and Fenton reagent solution that you no longer need to use can be disposed of in the regular garbage, but solid soil should not be disposed of directly down the drain as it may cause plumbing blockages.

Mar. 24, Lab 5: Filtration and Microscopy (Part II)

- Chem Lab: Vacuum Filtration
- ASSC 267: Stereomicroscope
- Lab Sheet

Introduction:

After the Fenton oxidation reaction last time, there should not be any natural fibers left in the solution that will confuse your microscopic identification of microfibers and microplastics. This week the solution will be filtered again using vacuum filtration, and you will then view your filter paper using the stereomicroscope and analyze the microfibers and microplastics. You will identify the microplastics and microfibers on your filter paper. The larger chunks of microplastics will usually be more obvious to identify, but microfibers also have a characteristic appearance shown in Figure 2.

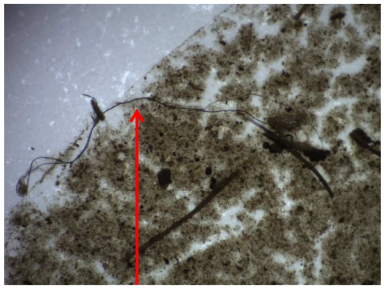

Figure 2: Image showing microfiber on a filter paper that was processed similar to your filter paper. The microfiber has a distinctive appearance, compared to the fragmented organic matrix (red arrow points at microfiber).

You must move your filter paper through the viewing area of the microscope to analyze the entire area of your filter paper. Depending on where your soil sample was taken, you may or may not be able to identify microfibers. Count and record the number of microfibers and microplastics you find. This visual inspection of samples is what researchers currently use to assess microplastic and microfiber contamination in both soil and water samples. An additional confirmation step that is often performed, which we will not do in this lab, is subjecting the larger particles to a spectroscopy technique after visual identification. This technique can confirm that a particle or fiber is in fact plastic and can identify the specific type of plastic by exploiting the fact that different chemical compounds and polymers will absorb different wavelengths of the electromagnetic spectrum in a way that is characteristic of that specific compound or material.

Procedures:

During all procedures, be sure to record important observations on your Lab Sheet as the experimental steps are proceeding.

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Second vacuum filtration to isolate particles and final microscopic viewing.

Purpose: Final filtration to isolate the synthetic particles and identify and count the number of microplastics and microfibers remaining on your filter paper using a stereomicroscope.

Materials:

- vacuum filtration apparatus
- nylon filter paper, 0.45 µm–5.0 µm pore size
- stereomicroscope
- forceps
- water wash bottle

Step 1. Vacuum filtration

1. Use a water wash bottle to clean off the filter paper, and then, using forceps, remove it from the beaker. If your filter paper is still intact, you can reuse it by adding it to the funnel of the vacuum filter apparatus. If your filter paper is not still intact, wash off the remaining pieces of the old filter paper with the water bottle and discard them. Use a fresh nylon filter in the vacuum filtration apparatus if your original filter paper must be discarded. Pour the liquid mixture from the beaker into the vacuum filter funnel to collect the solid particles that remain after exposure to the Fenton reagent. Rinse your beaker with deionized water and add the rinse to the filter funnel. Repeat if some of your mixture remains in the beaker. Rinse the inside of the vacuum cup to remove any solid material stuck to the inside of the vacuum cup onto the filter paper using DI water in a squirt bottle. Your instructor will help you with the vacuum filtration process.
2. Shut off the vacuum. Remove the glass top of the vacuum filtration set-up and break the vacuum by unplugging the vacuum hose if necessary. Your instructor will help you with this part. Using forceps, remove the filter paper from the funnel and place it on a watch glass or a petri dish. Add a second watch glass to the top of your original watch glass as a cover for your filter paper, flipping the top watch glass so that it does not compress the filter paper on the bottom watch glass.
3. Repeat the above procedure for the contents of the control beaker, keeping the control filter paper on a separate watch glass.
4. View each filter paper under the stereomicroscope's magnification and record your observations.

Step 2. Stereomicroscope observations

1. View all parts of your filter paper using the stereomicroscope, counting and recording the number of microplastics and microfibers on each part. Record these numbers and sketch the shapes on your lab report.
2. Carefully place identified microplastics into a small vial if you can remove them from the filter paper with tweezers.
3. On the class board, record the total number of microplastics and microfibers you found in your sample, and where your sample location site was. Also share the initial mass of the dried soil you tested (this value was determined in the first week, and should be between 20–30 g). Also, record the number of microfibers or microplastics present on the filter from your control sample.
4. Collect the entire class data to complete questions on the Lab Sheet.

Disposal: All filter papers may go in the regular garbage, and the beakers can be rinsed in the sink.

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