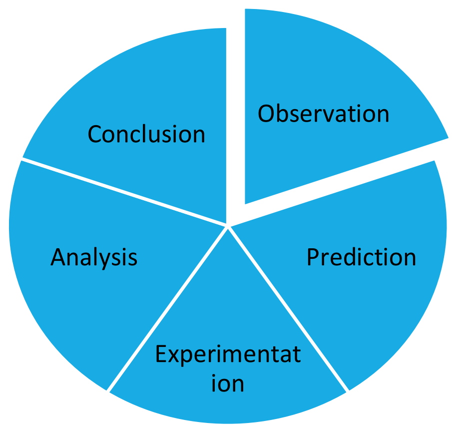
RIVERINE MICROPLASTICS FIELD LAB MANUAL

BACKGROUND INFORMATION

THE SCIENTIFIC METHOD is how scientists understand everything we know about the universe in a strategically standardized and repeatable way.

We all participate in the first step of the scientific method every day – observation.

The steps of the scientific method are as follows:



And it can be explained in an overly simplified way – a burned out flashlight.

Observation: Your flashlight isn’t working.
Hypothesis: Your flashlight’s batteries are dead.
Experiment: Replace the batteries.
Analysis: Does the light work now?

Yes?

Conclusion: Your hypothesis is accepted; the batteries were dead!

No?

Alternative Conclusion: Your hypothesis is rejected; come up with a new prediction! Perhaps it has a burned-out light bulb, or a loose electrical wire.

Congrats. You did science.

We are constantly observing the world, whether with our sense of sight, or our other senses: touch, taste, smell, sound. Environmental Scientists don’t have the luxury of being able to taste our observations, but we have developed a deep and varied suite of tools, equipment, technology, and field techniques that help us study environmental science. And sometimes all you need is a few things from your kitchen.

Plastic – Plastic products are widely used around the world, but the United Nations has called them "one of the biggest environmental challenges of this lifetime."

The first fully synthetic plastic was invented in 1907, but today's plastics aren't like their predecessor. Plastic is now made in a multitude of different types like polypropylene (PP), polyvinyl chloride (PVC), polyethylene terephthalate (PET) and Polyester. Plastic is also designed to be extremely long-lived (200 years for PE plastic bags).

The following industries account for the top uses:

- 39.5% packaging materials
- 20.1% building materials
- 8.6% automotive parts
- 5.7% electrical appliances
- 3.4% agricultural materials
- household appliances, sports equipment, and other products

World plastic production:

- 2.1 million tons in 1950
- 406 million by 2015*
    - By 2015, more than 6.3 billion tons of plastic waste had been generated
    - estimated 26 billion tons of plastic waste generated by 2050
    - Humans have produced more than 9.1 billion tons of plastic waste and plastic waste recycling rate is lower than 10% (Kaposi et al., 2014)
        - During the COVID-19 pandemic, the manufacture and distribution of plastic tremendously increased, particularly single, or limited use items like face masks, gloves, protective body suits, aprons, gowns, face shields, surgical masks, and goggles (Abhilash and Inamdar, 2022).

Types

- Primary Plastics - "intentionally manufactured to be tiny for specific purposes, such as for use in personal care products (e.g., toothpaste, facial cleanser, cosmetics and other) or as industrial raw material." (Atugoda et al. 2021)
- Secondary Plastics – formed from crushing of large plastic, UV photodegradation, or biodegradation in the environment. (Zhang et al., 2021)
- Shapes and Types: fragments (PP, PE, PET), beads, pellets, film (PP, PE, PA), fibers (PES, PET, PA), granules (PE), FOAM (PU, PVC, PS).

Global Concern

- Plastic is designed to be durable (200 years for PE plastic bags).
- Plastic is "one of the biggest environmental challenges of this lifetime" (UN environment, 2018)

EXPLORE/OBSERVE

Your group will safely explore the river's edge and areas near the river for 10-15 minutes, looking for trash and litter of any size.

You may be inclined to pick up the trash to remove it from the environment, but please resist the urge at this point! You will get a chance to pick it up again at the end of the lesson.

Sketch a rough draft map of the river and the riverbank, and any other areas on the land you explore for this activity. Label the direction of the water's flow and any unique features (like a bridge, large boulders, a large tree, a dock, or any other notable feature that helps identify the lay and the scale of the location).

Map:

Once you have a general map sketched, begin to explore, and observe the location, recording the location and type of any trash or litter you find in the table below. Mark on your map where each observation occurs (using the map location number you assign it). For example, if the first location you find litter is just to the north of a dock, then you would write the number 1 on your sketched map, just to the north of a hand-drawn dock.

Also make note of how old the litter generally appeared. Do your best to accomplish this without touching it, especially if the litter is old, sharp metal. And remember, it is important at this step in the lesson to **leave the litter where you found it**.

Litter Age Key

⇒ 1 = newer
clean and/or on top of the environment

⇒ 2 = somewhat old
dirty but not weathered; may be embedded in the environment more than a newer litter item

⇒ 3 = extremely old
appearance of extreme weathering, rust, old, stamped expiration date, buried in the ground, or otherwise showing extreme weathering and exposure to the environment.

Also, as you explore and observe your location, start to think about where, if any, you would most likely find plastic pollution.

Map Location Number	Description of Litter	Estimate Age	Type of Litter/Trash Observed				
			Metal	Plastic	Wood	Paper	Other
1							

WRITING PROMPT #1

Spend a few minutes handwriting your answers to each of the following questions:

- Are there microplastics in this water? Why or why not?
- How can you test this hypothesis?
- Predict the results of this test.

RIVER SAMPLING FOR MICROPLASTICS: GRAB SAMPLES

It's time to test your hypothesis. Using what you learned from the **Explore/Observe** activity, select one location to collect a water sample to help test your hypothesis.

In addition to determining a location to sample for microplastics, your team should work together to accomplish sampling without any injuries or broken glass; and your lab manual should be filled out completely.

STEP 1.: Prepare the grab sample collection bottles.

1. Because you need to reduce the number of opportunities to contaminate your bottles with your clothes or actions, **ensure that the bottle's lids are NOT removed at this step.**

2. With a Sharpie marker, write on a strip of masking tape, and then adhere one to each bottle, the following information:

your initials; sample #1, 2, 3 or 4; the name of the river; and the date

For example:

SRG #1 CO River - 6/26/2022 SRG #3 CO River - 6/26/2022

SRG #2 CO River - 6/26/2022 SRG #4 CO River - 6/26/2022

⇒ Samples #1 and #3 will be your field blank/background samples (explained in STEP 2 of this procedure).

⇒ Samples #2 and #4 will be your grab samples (explained further in the procedures).

STEP 2.: Determine where you can best answer your research question and go to that location. (Complete the following for each sample that you collect. If it is a blank sample, write "blank" in the River/Reach field.)

Grab Sample Collection #:____________

River/Reach:_______________________________________Date:________________Time:______________

River Conditions: Calm/Mild/Intermediate/Spicy Weather Conditions:________________________

DESCRIPTION OF LOCATION:

Grab Sample Collection #:____________

River/Reach:_______________________________________Date:________________Time:______________

River Conditions: Calm/Mild/Intermediate/Spicy Weather Conditions:________________________

DESCRIPTION OF LOCATION:

STEP 3.: Obtain field blank/background sample.
A field blank (also called a background) sample is one that is collected, stored, treated, and analyzed in a manner as close to possible to your other samples to account for contaminants and other possible interferents.

So, your first sample, sample #1, is actually a sample that you'll collect at the river's edge, at the same location as your first grab sample (which will be sample #2). But instead of filling sample #1 with river water, you'll fill it with distilled or tap water that has been provided for this purpose.

Procedure to Create your Blank/Background Sample:

1. Remove the lid of the bottle.
2. Pour distilled or tap water provided to you into the bottle as quickly as you can without spilling it. Fill the bottle completely, then replace the lid and close it tightly.

STEP 4.: Obtain grab sample from river.

1. Collect your sample **at the water surface directly into your bottle** labeled as sample #2. Fill the bottle almost completely.

STEP 5.: Return to the field classroom/gathering point.

WRITING PROMPT #2 – DON'T ANSWER THIS UNTIL INSTRUCTED TO DO SO.

Reconsider the first writing prompt – did your answers change? Why or why not?

OBTAIN ANOTHER GRAB SAMPLE, FROM A NEW LOCATION

Rethink your testing location. This is your "second chance" to test your hypothesis. Record your sample collection in the following fields (remember, you're working with sample bottles #3 and #4 this time around).

Determine where you can best answer your research question and go to that location. **(**Complete the following for each sample that you collect. If it is a blank sample, write "blank" in the River/Reach field.)

Grab Sample Collection #:____________

River/Reach:_______________________________________Date:________________Time:______________

River Conditions: Calm/Mild/Intermediate/Spicy Weather Conditions:________________________

DESCRIPTION OF LOCATION:

Grab Sample Collection #:____________

River/Reach:_______________________________________Date:________________Time:______________

River Conditions: Calm/Mild/Intermediate/Spicy Weather Conditions:________________________

DESCRIPTION OF LOCATION:

Repeat the procedures from Steps 3 to 5, except instead of using bottles #1 and #2, you will use #3 for your new blank, and #4 for your new sample.

DENSITY SEPARATION

PREPARE THE SAMPLES FOR MICROSCOPY

To see microplastics more easily with a microscope or hand lens, it's ideal to separate the microplastics from the water.

A few studies have shown that canola oil can separate 85.8 % (mean recovery rate) of microbeads, films, fragments, filaments, foam, pellets, fibers, and spheres in the 0.02-4.4 mm particle size range, and in the following types of plastics: Post-Processing: EPS, CoPA, PA6, PE, PP, PS, PVC, PVDC, carbon; Post-Consumer: PET, and synthetic rubber.

(**Remember – to avoid contaminating your samples, it is important to remove all synthetic fabrics.** This should be done as far away from the workspace as possible, ideally 20 feet, or more. Particular attention should be paid to removing bright reds, oranges, blues, and blacks, if possible, even non-synthetic fabrics in these colors.)

Density Separation Procedure:

1. Add 10mL of canola oil to each water sample and tightly replace the lid.
2. Pick up the bottle and shake it vigorously for one minute.
3. Immediately (and carefully) pour the contents of each of the four sample bottles into four different 500 mL beakers. ***As you transfer the water from bottle to beaker, transfer the masking tape label, or if needed, apply a new one.**
4. Allow the sample to sit, undisturbed, for 15 minutes.
5. Decant the canola oil slurry into a petri dish.
6. Immediately examine the petri dish using the microscope or hand lens 40x or higher.

MICROSCOPY

Microscopy Procedure:

2. Using the dissecting microscope (or hand lens), carefully observe your sample looking for obvious objects that are bright red, bright orange, bright blue or bright, shiny black (reference the Visual ID of Microplastics chart).
3. Scan the sample in a methodical way, the same way you would mow a lawn, so that you can investigate the entire sample.
4. Use the small ruler to measure anything that appears to be a microplastic.
5. Complete the Microscope Data Sheet as you go.

MICROSCOPE DATA SHEET

Date:

Sample #	MP Type	Color (use hash marks)					Microplastics Viewed? (yes/no or maybe)
		red	orange	blue	black	other	

SKETCH & ATTEMPT TO LABEL A FEW OF THE ITEMS YOU SEE:

Data Analysis and Discussion:

How can you present your findings in a clear manner (e.g., a table, graph or other visual, etc.)

What might your findings suggest about the presence of microplastics in this location?

What might your findings suggest about your research design and your personal bias?