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Edvo-Kit #

EDVO-KIT# 929

Aquatic Detectives: Solve the Mystery of the Contaminated Waterway

Experiment Objective:

Water pollution has wide-reaching impacts on both wildlife and human populations. Contamination can enter the water supply from factories that discharge waste directly into a body of water. In this experiment, students will test simulated water samples to determine what contamination is present, and which factories contribute.

See page 3 for storage instructions.

ersion 929.240603

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Experiment Components

COMPONENTS Store this experiment at room temperature.	Check (🔨)	
 A PBS Buffer B Simulated Lead Solution C Simulated BPA Solution D Simulated Copper Solution E Simulated Lead Indicator F Simulated BPA Indicator G Simulated Copper Indicator 		Experiment #929 is designed for 10 groups. This experiment can be stored at room temperature.
SUPPLIES		
 Large Snap top tubes (5 mL) Small Snap top tubes (1.5 mL) Large transfer pipet Small transfer pipet Tube lables 		PLEASE NOTE: This experiment is a simu- lation and intended solely for educational purposes. The reagents included in this kit will not identify contamination in environ- mental samples.

Requirements

- Automatic micropipettes with tips (optional)
- Safety goggles and disposable laboratory gloves
- 100 mL beakers or flasks
- Distilled or deionized water

All experiment components are intended for educational research only. They are not to be used for diagnostic or drug purposes, nor administered to or consumed by humans or animals.



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Background Information

Clean water is essential for maintaining the health and vitality of the planet. Aquatic ecosystems like rivers, lakes, oceans, and wetlands are home to a vast array of species and provide suitable living conditions and abundant food sources for both aquatic and terrestrial organisms (Figure 1). Freshwater and saltwater ecosystems are home to more than just fish; they also include a diverse array of invertebrates, algae, and plants that form the foundation of the aquatic food web. Biodiversity within aquatic ecosystems also provides various services, like water purification, nutrient cycling, and flood control. These services not only benefit aquatic life but also have far-reaching effects on terrestrial ecosystems and human societies. They make the ecosystem more resilient to environmental changes and disturbances, helping to buffer against the impacts of climate change, invasive species, and other stressors.

"Water quality" refers to the chemical, physical, and biological characteristics of water. The quality of water is usually in relation to its suitability for a specific purpose, like drinking, swimming, fishing, or supporting aquatic life.

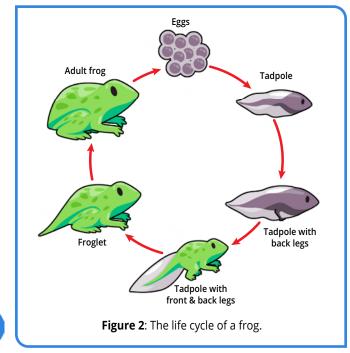


Figure 1: Aquatic Biomes are diverse ecosystems that support a vast array of plant and animal life. A. A rural pond¹. B. A coral reef². C. An urban river³.

Water pollution has had severe and wide-reaching impacts on both wildlife and human populations. This includes habitat degradation, with pollution altering and destroying aquatic habitats, making them unsuitable for many species. Contamination like heavy metals, pesticides, and nutrient runoff can disrupt the balance of aquatic ecosystems, threatening the survival and growth of the plants and animals therein. For example, amphibians like frogs depend on clean water for reproduction (Figure 2). Polluted water can hinder the development of their eggs and larvae, potentially lead-

ing to population declines. Similarly, migratory fish species need clean and unobstructed waterways to complete their life cycles and sustain their populations. The successful reproduction of these aquatic species contributes to increased biodiversity within these ecosystems.

"We forget that the water cycle and the life cycle are one." – Jacques Yves Cousteau, French oceanographer, filmmaker, and inventor known for his underwater explorations





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Industrial discharges, agricultural runoff, and urban development can lead to habitat degradation through sedimentation, nutrient enrichment, acidification, and chemical pollution. Contaminants like heavy metals, pesticides, and industrial chemicals have toxic effects on aquatic life, leading to deformities, reproductive issues, and population decline in fish, amphibians, and invertebrates. Excessive nutrient pollution, particularly from agricultural runoff and sewage discharges, can lead to algal blooms (Figure 3). These blooms can deplete oxygen levels, creating "dead zones" where fish and other aquatic life struggle to survive. Some pollutants can accumulate in the food chain, a phenomenon called bio-accumulation. Predators at the top of the food chain, including humans, can consume prey with concentrated toxins, leading to health issues and disruptions in ecosystems.



Figure 3: A Coastal algal bloom⁴.

Humans are also susceptible to the pathogens and toxic chemicals found in polluted water. Contaminated water can carry bacteria, viruses, and parasites that lead to illnesses including cholera, dysentery, and typhoid. Furthermore, exposure to heavy metals such as lead, arsenic, and mercury in drinking water can cause neurological and developmental problems. This is especially dangerous for vulnerable populations, such as infants and children, pregnant people, and the elderly. Contaminated water can lead to severe health issues, including stunted growth, developmental delays, and complications during pregnancy and childbirth. Providing access to clean water for washing, drinking, and cooking is one of the most effective means of reducing the burden of waterborne diseases, particularly in developing regions where access to healthcare is limited. Due to the effects of contaminated water on human development and guality of life, clean water is a cornerstone of public health policy.

Water pollution is a critical global issue that requires international collaboration for effective remediation. The diverse sources of this pollution—including industrial discharges, agricultural runoff, urban sewage, and chemical spills—pose severe risks to biodiversity, human health, and water usability (Figure 4). For example, the Ganges River in India, considered sacred to many, faces severe pollution due to industrial and domestic waste discharge. In the 1950's, Lake Karachay in Russia served as a dumping site for nuclear waste and had to be filled in completely due to the dangerous levels of radioactivity. In Sao Paulo, the Tiete River is severely polluted by urban runoff and untreated sewage, impacting water quality and local communities (Figure 5). To address these widespread challenges, comprehensive and coordinated multinational strategies are essential to protect and restore our water sources.

In the United States, Ohio's Cuyahoga River became infamous for its pollution beginning in the 1860's, with industrial discharges and oil slicks causing the river to catch fire multiple times. The fire of 1969 led to the



Figure 4: Contamination can enter water supplies in two ways:

- Point source water pollution is any contaminant that enters a body of water from a single, readily identifiable source, such as a factory or a recycling plant.
- Non-point source water pollution cannot be traced to a single, identifiable source and often results from everyday activities like septic tank overflow, soil erosion, and run-off from farms that contain animal waste and/or fertilized.



Figure 5: Foam as a result of pollution on the Tiete River in Sao Paulo⁵.

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creation of the U.S. Environmental Protection Agency (EPA) and the Clean Water Act in the early 1970s. Water safety in the United States is not limited to public water systems or bodies of water. According to the Center for Disease Control and Prevention (CDC), about one in eight U.S. residents rely on private well water which is not monitored in the same way. Pathogens, heavy metals, agricultural run-off, and by-products of fracking can be found in these water sources.

Due to frequent testing and remediation, drinking water in the United States is generally safe. Recently, the water pollution crisis in Flint, Michigan, gained international attention and revealed a disturbing failure in public health and infrastructure management. In 2014, the city switched its water source from the Detroit water and sewerage department to the Flint River without properly treating the water to prevent lead contamination. The corrosive nature of the river water caused lead to leach from aging pipes into the drinking water supply, exposing residents to dangerous levels of lead. Robust water monitoring and adherence to high-quality standards are essential for safeguarding drinking water reserves and aquatic ecosystems, enabling life on Earth to flourish.

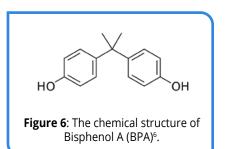
SCENARIO: BECOMING AN ENVIRONMENTAL INVESTIGATOR

There is something contaminating the major river in Dresmark, causing widespread fish death, loss of species diversity and tainted drinking water. Residents and children of the town have reported headaches, fatigue, abdominal pain, as well as vomiting and yellowing of the skin. The mayor of Dresmark has asked the EPA to open an investigation. Three factories sit along the riverbank: a plastic factory, battery recycling plant, and a copper processing plant. These factories have been identified as potential sources for point source pollution.

A **battery recycling plant** is dedicated to the process of collecting and processing used batteries to recover valuable materials and properly handle potentially hazardous substances. These facilities serve several important purposes, including reducing the need for raw materials, minimizing environmental impacts, and reducing the accumulation of toxic waste. One of the most common types of battery that is recycled at this plant are lead-acid batteries, which are commonly found in vehicles and uninterruptible power supply (UPS) systems. These rechargeable batteries have been used for a wide range of applications due to their reliability, durability, and relatively low cost. Recycling lead-acid batteries is crucial for environmen-

tal and safety reasons, as these batteries contain hazardous materials like lead and sulfuric acid. The recycling process involves several steps to recover valuable materials while minimizing environmental impact, and if the steps are not done correctly there are major risks for lead to enter the waterways. Lead is a toxic metal that can have severe health consequences (Figure 7), especially when it contaminates drinking water sources and is ingested or otherwise absorbed by individuals.

Plastic factories are industrial facilities where various plastic products are manufactured through the processing of plastic resins and other raw materials. These factories produce a wide range of plastic products that serve numerous purposes



in our daily lives, like toys, storage containers, automotive components, and filament for 3D printing. One chemical often used in the manufacture of plastic is Bisphenol A (BPA), a synthetic chemical compound present in polycarbonate plastics (Figure 6). It was widely used in consumer products, including plastic water bottles, food storage containers, and the lining of canned goods. Over time, these plastics can break down, especially when exposed to heat, sunlight, or acidic or basic conditions. When this happens, BPA can leach into the surrounding environment, including water. BPA pollution is a cause for concern because it is an endocrine-disrupting chemical, which means it can interfere with hormone systems in both humans and aquatic life. It has been associated with various adverse health effects, including reproductive and developmental issues. Recently, BPA has been detected in water samples, aquatic organisms, and even drinking water supplies.

The **copper processing plant** on this riverbank extracts copper from mined ore in a process called smelting (Figure 8). During the process of smelting, physical grinding, high temperatures, and chemical reactions are performed to separate



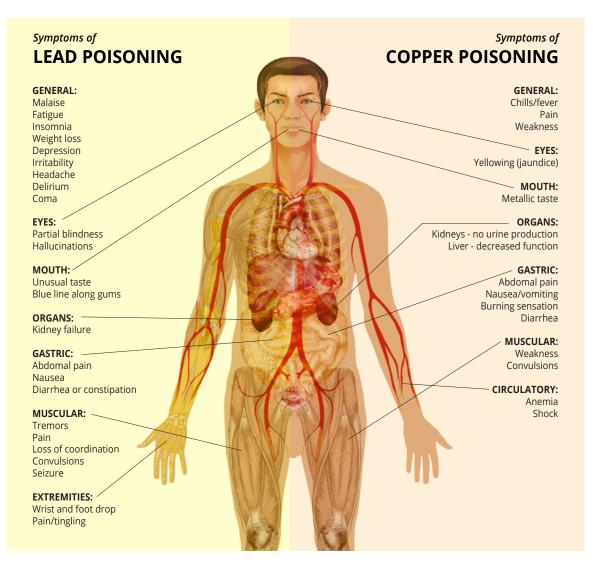
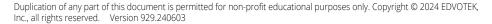


Figure 7: Symptoms of Lead and Copper Poisoning⁷.

the copper from other minerals and impurities. Copper is an essential metal, used in the electronics, construction, and telecommunications industry, among others. However, the smelting process can have significant environmental impacts due to the discharge of process water during the smelting process. Large quantities of water are used for cooling, dust control, and as a medium for transporting crushed ore and concentrates. This process water can become contaminated with chemicals, heavy metals, and pollutants used in the smelting process. If not properly managed and treated, this contaminated water can be released into nearby waterways, causing pollution. Copper is highly toxic to aquatic organisms, particularly fish and invertebrates like crustaceans and mollusks. It interferes with their respiratory and osmoregulatory systems, affecting their ability to breathe and maintain internal water balance.



Figure 8: Copper smelter⁸



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To determine whether factories on the riverside are to blame for the decline in the river's species diversity and drinking water quality, the EPA investigators identified four different river sites to be sampled and tested, as follows (Figure 9):

- Site 1: Upstream from the factories
- Site 2: Downstream the battery recycling plant
- Site 3: Downstream the plastic factory
- Site 4: Downstream the copper processing plant

Each sample will be tested for BPA, copper, and lead, the three suspected contaminants. The identification of the contaminant will not only help conclude the investigation but also facilitate the cleanup of the river to restore aquatic life and ensure the safety of the town's drinking water.

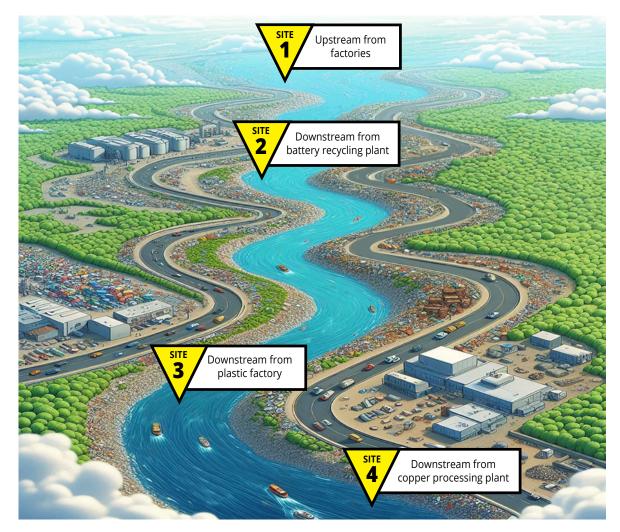


Figure 9: River sites to be tested

Background Information Image Attribution:

- 1. Figure 1A https://commons.wikimedia.org/wiki/File:Epping_Forest_High_Beach_Essex_England_-_spring_pond_08.jpg
- 2. Figure 1B https://commons.wikimedia.org/wiki/File:Arrecifes_de_Cozumel_National_Park.jpg
- 3. Figure 1C https://commons.wikimedia.org/wiki/File:MariaValeriaBridge.jpg
- 4. Figure 3 https://www.pexels.com/photo/coastline-with-algal-bloom-water-5006877/
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Experiment Overview

EXPERIMENT OBJECTIVE

Water pollution has wide-reaching impacts on both wildlife and human populations. Contamination can enter the water supply from factories that discharge waste directly into a body of water. In this experiment, students will test simulated water samples to determine what contamination is present, and which factories contribute.

LABORATORY SAFETY

- 1. Gloves and goggles should be worn routinely as good laboratory practice.
- 2. Exercise extreme caution when working with equipment that is used in conjunction with the heating and/or melting of reagents.
- 3. DO NOT MOUTH PIPET REAGENTS USE PIPET PUMPS.
- 4. Exercise caution when using any electrical equipment in the laboratory.
- 5. Always wash hands thoroughly with soap and water after handling reagents or biological materials in the laboratory.

LABORATORY NOTEBOOKS

Scientists document everything that happens during an experiment, including experimental conditions, thoughts and observations while conducting the experiment, and, of course, any data collected. Today, you'll be documenting your experiment in a laboratory notebook or on a separate worksheet.

Before starting the Experiment:

- Carefully read the introduction and the protocol. Use this information to form a hypothesis for this experiment.
- Predict the results of your experiment.

During the Experiment:

- Record your observations.
- Record any challenges faced while performing the experiment.

After the Experiment:

- Interpret the results does your data support or contradict your hypothesis?
- If you repeated this experiment, what would you change? Revise your hypothesis to reflect this change.





Testing the Water Samples

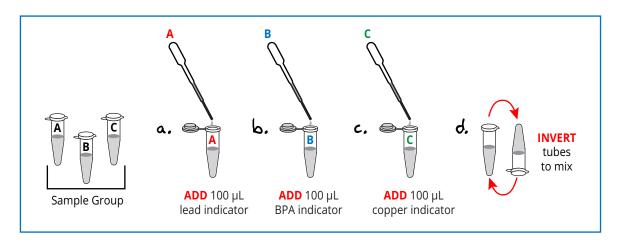
- 1. **COLLECT** water samples, microcentrifuge tubes, pipettes, and testing reagents.
- 2. Using a marker, **LABEL** all of the microcentrifuge tubes with the sample identity and the assay reagent, as indicated in the Table 1, below. The samples were collected at four separate locations (1-4) along the river (see Figure 9 on page 8), plus there are positive (P) and negative (N) controls. Each sample and the controls will be tested for the presence of Lead (A), BPA (B), and Copper (C).

TABLE 1: Labeling of Sample Tubes				
Sample	Lead (A)	BPA (B)	Copper (C)	
Site #1 Upstream	1A	1B	1C	
Site #2 Recycling plant	2A	2B	2C	
Site #3 Plastic factory	3A	3B	3C	
Site #4 Scrap metal plant	4A	4B	4C	
Negative control	NA	NB	NC	
Positive control	PA	PB	PC	

NOTE:

A negative result for lead is red. A positive result for lead is yellow. A negative result for BPA is clear. A positive result for BPA is purple. A negative result for copper is clear. A positive result for copper is aqua.

- 3. Using a marker, LABEL the transfer pipets: "A" (Lead), "B" (BPA), and "C" (Copper).
- 4. **DISPENSE** the river water by transfering 700 μL of the "Site 1" water into each of the three site #1 tubes (labeled 1A, 1B, 1C). **REPEAT** the process to prepare the samples from sites 2, 3, and 4, and the positive and negative control samples.



- 5. **IDENTIFY** the 3 tubes designated **SITE 1** (1A, 1B, 1C). Use the appropriate transfer pipette to **ADD** the indicators.
 - a. ADD 3 drops/100 µL of lead indicator into tube 1A.
 - b. **ADD** 3 drops/100 µL of BPA indicator into tube 1B.
 - c. ADD 3 drops/100 μL of copper indicator into tube 1C.
 - d. Gently invert the tubes to **MIX** the sample and indicator thoroughly. **RECORD** results and observations in Table 2.



Testing the Water Samples, continued

- 6. **IDENTIFY** the 3 tubes designated **SITE 2** (2A, 2B, 2C). Use the appropriate transfer pipette to **ADD** the indicators.
 - a. ADD 3 drops/100 µL of lead indicator into tube 2A.
 - b. ADD 3 drops/100 µL of BPA indicator into tube 2B.
 - c. **ADD** 3 drops/100 µL of copper indicator into tube 2C.
 - d. Gently invert the tubes to **MIX** the sample and indicator thoroughly. **RECORD** results and observations in Table 2.
- 7. **IDENTIFY** the 3 tubes designated **SITE 3 (**3A, 3B, 3C). Use the appropriate transfer pipette to **ADD** the indicators.
 - a. ADD 3 drops/100 µL of lead indicator into tube 3A.
 - b. ADD 3 drops/100 µL of BPA indicator into tube 3B.
 - c. **ADD** 3 drops/100 µL of copper indicator into tube 3C.
 - d. Gently invert the tubes to **MIX** the sample and indicator thoroughly. **RECORD** results and observations in Table 2.
- 8. **IDENTIFY** the 3 tubes designated **SITE 4** (4A, 4B, 4C). Use the appropriate transfer pipette to **ADD** the indicators.
 - a. ADD 3 drops/100 µL of lead indicator into tube 4A.
 - b. ADD 3 drops/100 µL of BPA indicator into tube 4B.
 - c. ADD 3 drops/100 µL of copper indicator into tube 4C.
 - d. Gently invert the tubes to **MIX** the sample and indicator thoroughly. **RECORD** results and observations in Table 2.
- 9. **IDENTIFY** the 3 tubes designated **NEGATIVE CONTROL** (NA, NB, NC). Use the appropriate transfer pipette to **ADD** the indicators.
 - a. ADD 3 drops/100 µL of lead indicator into tube NA.
 - b. **ADD** 3 drops/100 µL of BPA indicator into tube NB.
 - c. **ADD** 3 drops/100 µL of copper indicator into tube NC.
 - d. Gently invert the tubes to **MIX** the sample and indicator thoroughly. **RECORD** results and observations in Table 2.
- 10. **IDENTIFY** the 3 tubes designated **POSITIVE CONTROL** (PA, PB, PC). Use the appropriate transfer pipette to **ADD** the indicators.
 - a. ADD 3 drops/100 µL of lead indicator into tube PA.
 - b. **ADD** 3 drops/100 µL of BPA indicator into tube PB.
 - c. ADD 3 drops/100 µL of copper indicator into tube PC.
 - d. Gently invert the tubes to **MIX** the sample and indicator thoroughly. **RECORD** results and observations in Table 2.
- 11. **COMPARE** results to positive and negative samples to determine who is responsible for each contaminant.

TABLE 2				
Sample	Lead (A)	BPA (B)	Copper (C)	Results
Site #1 Upstream				
Site #2 Recycling plant				
Site #3 Plastic factory				
Site #4 Scrap metal plant				
Negative control				
Positive control				

RECORD all results and observations in Table 2, below.

After comparing the results, who is responsible for each contaminant?



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Study Questions

- 1. What are the three main characteristics used to determine water quality?
 - A) Taste, color, and smell
 - B) Temperature, turbidity, and hardness
 - C) Chemical, physical, and biological
 - D) pH level, salinity, and alkalinity
- 2. What is the primary purpose of testing water quality in a river?
 - A) To determine its color
 - B) To check its temperature
 - C) To evaluate its suitability for drinking, swimming, fishing, or supporting aquatic life
 - D) To measure its flow speed
- 3. Which type of pollution cannot be traced to a single, identifiable source?
 - A) Point source pollution
 - B) Non-point source pollution
 - C) Industrial pollution
 - D) Agricultural pollution
- 4. Which of the following is NOT provided by aquatic ecosystems?
 - A) Water purification
 - B) Nutrient cycling
 - C) Air quality improvement
 - D) Flood control
- 5. What is bioaccumulation?
 - A) The accumulation of nutrients in water bodies
 - B) The buildup of pollutants in an organism's body over time
 - C) The increase of water volume in rivers during floods
 - D) The process of sedimentation in lakes and rivers



Short Answer Questions

1. What are the main functions of aquatic ecosystems?

2. What are some health impacts of consuming water contaminated with heavy metals such as lead, arsenic, and mercury?

3. What led to the creation of the EPA and the Clean Water Act?

Thought Questions

1. Explain the concept of bioaccumulation and how it affects aquatic ecosystems and human health. Identify and discuss some real-world examples of the effects of water pollution.

2. Describe the relationship between the terrestrial and aquatic ecosystems. How does the health of one impact the other?



Instructor's Guide

Educator Overview

This experiment is designed to support the concepts and content of the Next Generation Science Standards, through its connections to specific performance expectations and scientific practices. This kit covers multiple NGSS disciplinary core ideas and topics as described by the Middle and High School Science Standards. The lesson would be appropriate in middle or high school biology, environmental science, earth science, or chemistry classes and electives. These materials have not been endorsed or reviewed by NextGenScience or WestEd.

MIDDLE SCHOOL STANDARDS

Disciplinary Core Ideas include:

- MS-ESS3-3; through the design and use of the monitoring assays for different water contaminants.
- MS-ESS3-4; through an exploration of human usage of water, a critical natural resource.
- MS-LS2-4; through the investigation of the consequences of water pollution on ecosystems, particularly from industrial activities.

Crosscutting Concepts include:

- Cause and effect; through the identification of sources of pollution and their effects on water quality.
- Stability and change; through contamination in the water disturbing the balance of the local ecosystem.

Science and Engineering Practices include:

- SEP1: Asking Questions and Defining Problems, through critical reading of the provided background information on water quality testing and the environmental scenario and recognizing
- SEP3: Planning and Carrying Out Investigations, through developing a hypothesis, performing water quality testing, and evaluating experimental data.

HIGH SCHOOL STANDARDS

Disciplinary Core Ideas include:

- HS-ESS3-1; through an exploration of human usage of water, a critical natural resource, and the impact of poor water quality on humans and ecosystems.
- HS-LS2-6; through the analysis of the role of clean water in supporting biodiversity in an ecosystem, and the ecological and human health consequences of poor water quality.
- HS-LS2-7; through the evaluation and development of water testing strategies for identifying bodies of water in need of remediation.
- HS-ETS1-2; through the design of the investigation to identify sources of water contamination, and critical reading of the background to propose remediation strategies.

Crosscutting Concepts include:

- Cause and effect; through the identification of sources of pollution and their effects on water quality.
- Stability and change; through contamination in the water disturbing the balance of the local ecosystem.

Science and Engineering Practices include:

- SEP1: Asking Questions and Defining Problems, through critical reading of the provided background information on water quality testing and the environmental scenario and recognizing
- SEP3: Planning and Carrying Out Investigations, through developing a hypothesis, performing water quality testing, and evaluating experimental data.



Pre-Lab Preparations

PREPARING THE WATER SAMPLE SOLUTIONS

Water samples can be prepared up to seven (7) days before class.

- 1. Label the large, 5 mL microcentrifuge sample and testing reagent tubes using the provided labels.
 - a. 10 "Neg" for Negative Control
 - b. 10 "Pos" for Positive Control
 - c. 10 "#1" for Sample 1
 - d. 10 "#2" for Sample 2
 - e. 10 "#3" for Sample 3
 - f. 10 "#4" for Sample 4
- 2. Label four (4) small beakers or flasks as follows: Sample 1/Neg Control, Sample 2, Sample 3, Sample 4/Pos Control.

3. Prepare the river water samples.

a. Sample 1/Negative Control:

Dilute the entire volume of PBS Buffer (Component A) into 65 mL distilled or deionized water.

b. Sample 2:

Dilute 2 mL of Simulated Lead Solution (Component B) in 48 mL distilled or deionized water.

c. Sample 3:

Dilute 2 mL of Simulated Lead Solution (Component B) and 2 mL of Simulated BPA Solution (Component C) in 46 mL distilled or deionized water.

- d. **Sample 4/Positive Control:** Dilute 3 mL of Simulated Lead Solution (Component B), 3 mL of Simulated BPA Solution (Component C), and 4 mL Simulated Copper Solution (Component D) in 65 mL distilled or deionized water.
- 4. Mix all samples well by gently stirring or swirling the beakers.
- 5. Using the large transfer pipet, dispense 3 mL of each water sample into the appropriately labeled tubes.

PREPARING THE INDICATOR SOLUTIONS Indicator solutions can be prepared up to seven (7) days before class.

- 1. Label the small, 1.5 mL microcentrifuge sample and testing reagent tubes using the provided labels.
 - a. 10 "L.I." for Lead indicator
 - b. 10 "B.I." for BPA indicator
 - c. 10 "C.I." for Copper indicator
- 2. Prepare the "simulated copper indicator" by adding the entire mass of Component G to 50 mL of distilled or deionized water.
- 3. Using the large transfer pipet, dispense 1 mL of each indicator solution into the appropriately labeled tubes.

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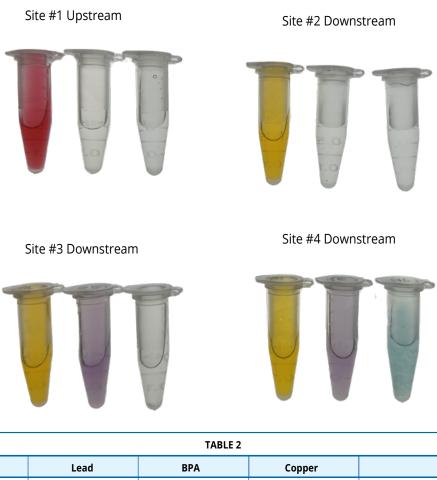
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Each group should receive:

- 1 Negative Control
- 1 Positive Control
- 1 Sample 1
- 1 Sample 2
- 1 Sample 3
- 1 Sample 4
- 1 Lead indicator
- 1 BPA indicator
- 1 Copper indicator
- 12 Unlabeled 1.5 mL microcentrifuge tubes
- 3 Small transfer pipettes

Experiment Results and Analysis



Sample	Lead	BPA	Copper	Results
Site #1 Upstream	Red	Clear	Clear	No contamination
Site #2 Downstream	Yellow	Clear	Clear	Positive for lead
Site #3 Downstream	Yellow	Purple	Clear	Positive for lead and BPA
Site #4 Downstream	Yellow	Purple	Aqua	Positive for lead, BPA, and copper
Negative control	Red	Clear	Clear	No contamination
Positive control	Yellow	Purple	Aqua	Positive for lead, BPA, and copper

All of the factories are responsible for contaminating the water and will have to partake in remediation and in improving their policies for dealing with hazardous waste.



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Please refer to the kit insert for the Answers to Study Questions