SEPA SCIENCE EDUCATION PARTNERSHIP AWARD
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Edvo-Kit # 926

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Nutrients in Balance: How Phosphates Help and Harm Ecosystems

Experiment Objective:

In this investigation, students will use a colorimetric assay to estimate the phosphate concentration in various water samples. They will compare results to environmental guidelines and discuss how phosphate levels affect ecosystems.

See page 3 for storage instructions.

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Safety Data Sheets can be found on our website: www.edvotek.com/safety-data-sheets

Requirements *(not included with this experiment)*

REAGENTS AND SUPPLIES

- Phosphate test kit (see below for suggestions)
- Water collection vessel (50 mL conical tube or triple-rinsed glassware with a tight fitting lid)
- 15 mL conical tubes or small triple-rinsed lab glassware
- Environmental water sample or simulated water sample for testing (see Appendix A)
- Phosphate standard (we recommend potassium phosphate)
- Fine-tip markers or labels
- Timer or smartphone stopwatch
- Paper towels
- Printed student handout and data table
- Distilled or bottled water
- Waste containers

TEST KIT SUGGESTIONS

There are no physical materials provided with this experiment. We suggest using low-range phosphate test kits to achieve the widest range of detectable color changes. Please note that while there are many kits that are commercially available, we have not specifically evaluated them with these directions.

Low Range Phosphate Test Kit 1

Low Range Phosphate Test Kit 2

Phosphate Test Kit For Freshwater And Saltwater

Background Information

WHAT ARE PHOSPHATES?

If you've ever looked closely at a bag of fertilizer or a food label, you might notice a chemical called **phosphorus** or **phosphate** listed on the label. Phosphorus is an element that is essential in many aspects of our daily lives. It's a key additive in foods such as lunch meat, cheese, and baked goods, helping to improve texture and preserve freshness. Phosphorus is an integral component in the manufacture of certain steel and metal alloys. The element also lights up the world on holidays as a key component of fireworks.

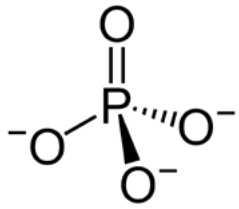

Figure 1: Stereo skeletal formula of phosphate[1].

Phosphorus isn't just important for industry and technology. It is also absolutely essential to support life on Earth, too. Most times, when scientists talk about phosphorus in living things, they are referring to the molecular phosphorus that is found in the phosphate ion. This molecule contains one atom of phosphorus that is covalently bonded to four oxygen atoms (Figure 1), and its molecular formula is written as PO_4^{3-}. The -3 in the superscript means it carries an overall negative charge, which is why we find it associated with positively charged ions like sodium, potassium, or hydrogen.

In living organisms, phosphate ions link RNA and DNA nucleotides as part of the sugar phosphate backbone. Phosphate stores and transfers energy in cells as adenosine triphosphate (ATP), and it strengthens our bones and teeth in the form of calcium phosphate. Enzymes and other proteins can be turned on or off by adding or removing phosphate groups in a process called phosphorylation. Phosphates also play an important role in ecosystems, because plants need them to grow. Without phosphorus, life as we know it could not exist!

THE EFFECTS OF PHOSPHATES ON THE ENVIRONMENT

Since phosphates play an essential role in many different aspects of life, living things need a steady supply of them. Plants, for example, absorb phosphates from the soil to grow. In farms where phosphates are not supplied, natural sources of phosphates from the soil quickly become depleted, and the plant suffers. Thus, the phosphates found in fertilizers are absorbed by the plant to help it grow faster and healthier.

However, too much of a good thing can often cause problems, and phosphates are no exception. When farms apply more fertilizer to a field than the crops can use, the excess nutrients don't just disappear. Instead, they can wash into rivers, lakes, or oceans during rainstorms (Figure 2). Once in the water, the same nutrients used to nourish our crops can throw ecosystems out of balance. The phosphates fuel the rapid growth of algae, leading to an algal

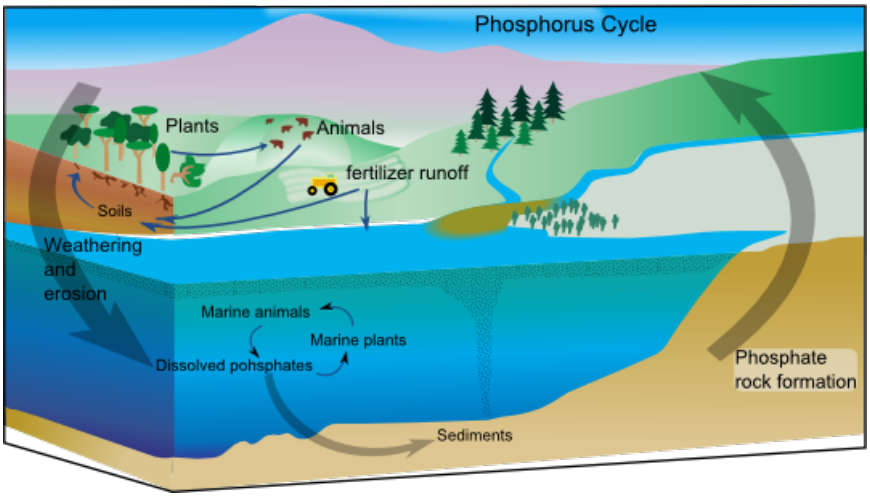

Figure 2: The Phosphorus Cycle[2].

bloom (Figure 3). When the algae eventually die and are broken down by bacteria, the decomposition uses up the dissolved oxygen in the water and creates dead zones where aquatic life cannot survive. This process, called **eutrophication**, has disastrous consequences not only for the aquatic environments, but for the surrounding land and all life that depends on healthy watersheds.

Figure 3: Example of Eutrophication[3].

Eutrophication is a massive threat to the environment which generally progresses in the following way:

1) Phosphates and other nutrients enter a watershed in unnaturally high amounts.
2) Naturally occurring algae use these nutrients to grow their population.
3) Algae populations grow to massive sizes, covering the surface of the water, which blocks sunlight for aquatic plants.
4) Algae populations outgrow themselves and die off, sinking to the bottom of the water.
5) The decomposition of algae uses dissolved oxygen in the water that fish and other aquatic organisms use to breathe, creating dead zones in the water.
6) Aquatic organisms die or are forced to leave as less and less dissolved oxygen is available.
7) Some algae release toxins into the water supply which can poison fish, shellfish, and even humans through contaminated water and seafood.
8) Terrestrial organisms which depended on the fish or aquatic life are negatively affected and their populations drop, creating a chain reaction across the food web.
9) The end result of eutrophication is a water system with little to no life. Without aquatic plant life, there's not enough dissolved oxygen available in the water system to support animal life, making "dead zones" difficult to restore.

Phosphates and other nutrients that drive eutrophication will enter the watershed in a few ways. The first and most prominent way is through the overuse of fertilizers. Farmers generally use fertilizer to supplement the soil with nutrients and help plants grow. However, when it rains, any nutrients that aren't absorbed by the plants can runoff with rain into a water system. At that point, the fertilizer spurs the growth of algae.

In cleaning products, phosphates are added to help soaps and detergents clean more effectively by softening water and breaking down dirt and grease. However, when these products are washed down the drain, the phosphates can enter rivers and lakes. There, they act like fertilizer, causing algae to grow too quickly and disrupting aquatic ecosystems. For this reason, they have largely been phased out and replacements are still being developed.

Another way nutrients can enter a water system is through animal waste. Large livestock farms create lots of animal waste which are full of nutrients for plants. This is why some people use animal waste as a fertilizer itself. This waste can runoff in the same way as artificial fertilizer, leading to algal growth and eutrophication.

It doesn't take much phosphate to cause problems in a body of water. While having some phosphate in water is important to nourish plants, even a small increase can lead to algal blooms. In fact, the U.S. Environmental Protection Agency (EPA) recommends less than 40 parts per million in drinking water, or 0.00042118M. That's an incredibly tiny amount!

The best way to fight the problem of eutrophication is to prevent excess nutrients from entering the watershed in the first place. On farms, strategies like planting buffer strips of grass or trees along riverbanks can trap excess fertilizer before it washes into the watershed. Crop rotation and careful fertilizer use can prevent the buildup of nutrients in the soil. Managing animal waste properly by collecting it in covered facilities and composting it also keeps harmful bacteria out of streams. Together, these strategies protect the flora and fauna that rely on clean water, while ensuring people have safe water to drink and to use for recreation.

HOW DO WE MEASURE PHOSPHATES?

Phosphorus is a nutrient that all living things need. In small amounts, it supports healthy plant and animal growth. However, when phosphorus enters lakes, rivers, or streams in excess, it can create major ecological problems. This process, called eutrophication, causes thick algae growth, blocks sunlight, and reduces the oxygen available for fish and other aquatic life. Understanding phosphate levels in local water sources helps communities protect water quality and restore ecosystem balance.

In professional laboratories, scientists measure phosphate using specialized equipment called spectrophotometers, which detect how much light a solution absorbs. While this provides highly accurate results, it also requires reagents, instruments, and precise handling.

Figure 4: Gathering water from a lake polluted with algae[4].

In this experiment, you'll measure phosphate using colorimetric assays—the same kind often used in aquariums or environmental field testing. Each strip contains a small area with chemical reagents that react with phosphate ions in the water. As the strip reacts, it develops a color that corresponds to the phosphate concentration. You'll compare the developed color to a reference chart to estimate the phosphate level of your sample.

This approach provides semiquantitative results (meaning it gives a range, not an exact number), but it's quick, safe, and reliable enough to reveal patterns in water quality. You'll use your results to discuss how different water sources—tap, bottled, pond, or garden runoff—can vary in phosphate levels and what those differences mean for the health of local ecosystems.

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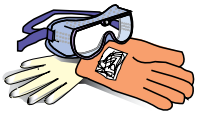
Experiment Overview

EXPERIMENT OBJECTIVE

In this investigation, students will use a colorimetric assay to estimate the phosphate concentration in various water samples. They will compare results to environmental guidelines and discuss how phosphate levels affect ecosystems.

LABORATORY SAFETY

1. Gloves and goggles should be worn routinely as good laboratory practice.
2. DO NOT MOUTH PIPET REAGENTS - USE PIPET PUMPS.
3. Always wash hands thoroughly with soap and water after handling reagents or biological materials in the laboratory.



Wear gloves and safety goggles

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.

Module I: Water Sample Collection by Students (Optional)

This optional module allows students to participate in collecting water samples for the activity. Students will gather real-world samples, label them carefully, and bring them back to the lab for analysis. It can be completed as an in-class field exercise or as a supervised homework assignment. Remember, plan to test within 24 hours for best results. (If delayed, refrigerate overnight.)

ENVIRONMENTAL WATER COLLECTION PROCESS

1. **CHOOSE** water sources (school fountain, pond, garden hose, tap, etc.) A good class goal is 10–12 total unique water samples to share. Collect only from safe, accessible areas. Never enter deep water, climb fences, or trespass.

   COLLECT samples from a variety of sources such as:
   - Tap water (cold first draw)
   - Bottled or distilled water (control/zero sample)
   - School fountain or sink
   - Garden hose or irrigation runoff
   - Local pond, stream, or puddle (with teacher permission)
   - Aquarium water

2. **LABEL** each sample vessel clearly with name, date, time, and site description. Use masking tape or a waterproof label and fine-tip marker.

   RECORD the following information in your lab notebook:
   - Location name (e.g., "Lodi Park Pond – near dock")
   - Date and time
   - Nearby land use
   - Initials of collector
   - (OPTIONAL) Photograph and/or GPS coordinates

   INCLUDE these observations on your data sheet or in a notebook:

Site Name	Date/Time	Weather	Water Appearance	Nearby Land Use	Notes

3. **COLLECT** approximately 50 mL of water per sample in a clean plastic bottle or tube.
   - Collect ~50 mL of each sample. Use small clean bottles, 2 oz plastic vials, or 50 mL collection tubes at the site.
   - Rinse each container three times with the sample source water before filling.
   - Label containers clearly with location, date, and initials or group number.
   - Cap immediately to prevent contamination or evaporation.
   - Wash hands after handling natural water.

4. **STORE** samples in a cooler or refrigerator until testing.
   - Avoid heat or direct sunlight.
   - Gently invert vessels before aliquot into smaller containers for testing. (We recommend 10 mL of sample in a 15mL conical tube or small flask)
   - Test within 24 hours for best results.

Module II: Testing Water Samples

PRACTICE TESTING AND CALIBRATION (10 MIN.)

1. **REVIEW** the instructions on the test container.
2. **FILL** one 15 mL conical tube with approximately 10 mL of distilled or bottled water.
3. **PERFORM** the assay as described by the manufacturer's directions.
4. **START** timing immediately.
5. **READ** the assay at the correct time (for example, 30 seconds) and RECORD the value in Table 1 on page 10. NOTE: This is your control sample and should read near zero.
6. **REPEAT** the assay with phosphate control samples provided by your instructor.

TESTING LOCAL WATER SAMPLES (30 MIN.)

If your instructor is providing pre-filled test tubes, skip to step 3

1. **LABEL** your conical tubes with the sample sources (e.g., Tap, Pond, Garden Runoff).
2. **FILL** each tube with 10 mL of sample water.
3. **PERFORM** the assay as described by the manufacturer
4. **START** timing immediately.
5. **READ** the result at the correct time according to the kit instructions.
6. **MATCH** the color to the chart and **RECORD** all results in Table 1 on page 8.

TESTING HIGH PHOSPHATE SAMPLES

If time and extra test reagents are available, students may investigate any samples that appear darker than the highest color block on the chart.

1. To estimate the concentration more accurately, **MIX** 1 part of your sample with 3 parts distilled water.
2. **RETEST** the diluted sample by repeating the assay.
3. **MULTIPLY** the new reading by 4 to estimate the undiluted concentration.
   Example: Diluted sample reads 2.0 mg/L → Actual sample ≈ 8.0 mg/L
4. **RECORD** both the dilution ratio and the new color reading.

Study Questions

1. In your own words, describe what eutrophication is and how it affects aquatic ecosystems.

2. Why do plants and animals both need phosphorus to survive?

3. How can too much phosphate harm lakes, ponds, and streams?

4. Why might fertilizer runoff increase phosphate levels in nearby water?

5. What types of water sources do you predict will have the highest phosphate levels? Why?

6. How can simple color-based tests (like test strips) still provide useful scientific data, even if they aren't as precise as laboratory instruments?

7. If one of your samples was "off the chart," what could you do to estimate its concentration more accurately?

CHALLENGE QUESTION:

8. How could communities use phosphate testing to protect local water quality or identify pollution sources?

Instructor's Guide

Notes to the Instructor

UNDERSTANDING PHOSPHATE UNITS

Phosphate test kits measure orthophosphate (PO_4^{3-}), the form of phosphorus that dissolves in water and can fuel algae growth. Different products show results in different units, but they mean the same thing chemically — they're just different ways of expressing concentration.

Unit Type	Symbol	Meaning	Conversion
Parts per billion	ppb	micrograms of phosphate per liter of water	1000 ppb = 1 mg/L
Milligrams per liter	mg/L	milligrams of phosphate per liter of water	1 mg/L = 1000 ppb
Milligrams per liter as phosphorus	mg/L as P	amount of phosphorus element only (not oxygen)	1 mg/L as P = 3.07 mg/L as PO_4^{3-}

Quick Conversions

- 1000 ppb = 1 mg/L (as PO_4^{3-})
- 1 mg/L (as PO_4^{3-}) = 0.33 mg/L (as P)

PreLab Preparations

BEFORE CLASS

1. **Gather Materials**
   - Phosphate test kits (at least 1 per sample to be tested)
   - 15 mL conical tubes (one per sample)
   - Distilled or bottled water (for the control)
   - Labels, permanent markers, and disposable cups for temporary sample holding
   - Gloves, paper towels, and waste container

2. **Prepare Water Samples**
   - Collect water samples from diverse sources (tap, bottled, pond, runoff, hose, rain barrel, fountain, etc.).
   - Collect ~50 mL from each source using clean containers.
   - Before the lab, aliquot 10 mL per tube for class testing.
   - Label tubes clearly (e.g., Pond A, Tap B, Control).
   - If desired, create a series of samples using the potassium phosphate powder.

3. **Set Up Controls**
   - Use distilled or bottled water as the "zero" phosphate control.
   - Optional: include a known fertilizer runoff sample for comparison.

4. **Organize Group Materials**

   Students will need:
   - Labeled tubes (water samples to be tested + 1 control)
   - Assay reagents
   - Data table sheet
   - Waste cup
   - Safety goggles and gloves

5. **Optional Prep**
   - Prepare a few extra test kits for groups that may perform a dilution trial (Part C).
   - If time allows, test all samples yourself once beforehand to ensure they yield varied color results.

AFTER CLASS

1. Have students share their results to build a classwide comparison chart.
2. Store unused kits sealed and dry for future activities.
3. Dispose of water samples down the drain if safe to do so.

EXTENSION IDEAS

1. Data Map: Have students mark sampling sites on a classroom or local map.
2. Mini Research: Compare phosphate strip results to online data from local water quality reports.
3. Cross-school Collaboration: Share class averages with other schools or community groups.

Troubleshooting

Issue	Likely Cause	Solution
Color too faint	Underdeveloped or expired strips	Retest with new strip; verify timing window
Color too dark / off-scale	Phosphate above strip range	Dilute 1:3 with distilled water and multiply by 4
Patchy color	Dipped too long or uneven wetting	Dip briefly (1 second) and hold horizontal during development
Hard to match chart	Poor lighting	Read in bright, natural light against white background

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

Appendix A:
Water Sample Collection Guide

COLLECTING MULTIPLE SAMPLES FROM ONE SITE (E.G., A POND)

If your class wants to explore how phosphate levels vary within a single location, follow these tips:

1. **Create Distance Between Sampling Points**
   - Space samples at least 2–3 meters (6–10 feet) apart along the shoreline or around the perimeter.
   - Note each location on a simple map or sketch of the site (Label: Site A, Site B, Site C).
   - Collect all samples within 10–15 minutes so environmental conditions stay similar.

2. **How Many to Collect**
   - 3–5 samples per site is plenty to see small variations.
   - For example: "Pond – near inlet," "Pond – center edge," "Pond – shady area."

3. **Sample Consistency**
   Collect each sample the same way:
   - Rinse container 3 times in the water.
   - Submerge just below the surface (about 5–10 cm deep).
   - Avoid disturbing mud or algae.
   - Cap underwater if possible to minimize air bubbles.

4. **Recording Site Information**

Include these observations on your data sheet or in a notebook:

Site Name	Date/Time	Weather	Water Appearance	Nearby Land Use	Notes

Reflection Prompt:
After testing, discuss:
- Did phosphate levels differ between your sampling sites? What local features (runoff, plants, shade, nearby lawns) might explain those differences?