5.6 Phosphorus

Why is phosphorus important?

Both phosphorus and nitrogen are essential nutrients for the plants and animals that make up the aquatic food web. Since phosphorus is the nutrient in short supply in most fresh waters, even a modest increase in phosphorus can, under the right conditions, set off a whole chain of undesirable events in a stream including accelerated plant growth, algae blooms, low dissolved oxygen, and the death of certain fish, invertebrates, and other aquatic animals.

There are many sources of phosphorus, both natural and human. These include soil and rocks, wastewater treatment plants, runoff from fertilized lawns and cropland, failing septic systems, runoff from animal manure storage areas, disturbed land areas, drained wetlands, water treatment, and commercial cleaning preparations.

Forms of phosphorus

Phosphorus has a complicated story. Pure, "elemental" phosphorus (P) is rare. In nature, phosphorus usually exists as part of a phosphate molecule (PO_4). Phosphorus in aquatic systems occurs as organic phosphate and inorganic phosphate. Organic phosphate consists of a phosphate molecule associated with a carbon-based molecule, as in plant or animal tissue. Phosphate that is not associated with organic material is inorganic. Inorganic phosphorus is the form required by plants. Animals can use either organic or inorganic phosphate.

Both organic and inorganic phosphorus can either be dissolved in the water or suspended (attached to particles in the water column).

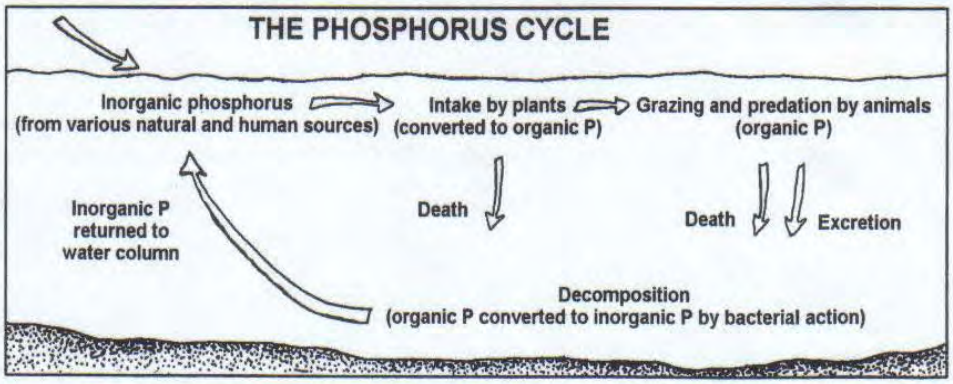
The phosphorus cycle

Phosphorus cycles through the environment, changing form as it does so (Fig. 5.12). Aquatic plants take in dissolved inorganic phosphorus and convert it to organic phosphorus as it becomes part of their tissues. Animals get the organic phosphorus they need by eating either aquatic plants, other animals, or decomposing plant and animal material.

Figure 5.12

The phosphorus cycle

Phosphorus changes form as it cycles through the aquatic environment.



As plants and animals excrete wastes or die, the organic phosphorus they contain sinks to the bottom, where bacterial decomposition converts it back to inorganic phosphorus, both dissolved and attached to particles. This inorganic phosphorus gets back into the water column when the bottom is stirred up by animals, human activity, chemical interactions, or water currents. Then it is taken up by plants and the cycle begins again.

In a stream system, the phosphorus cycle tends to move phosphorus downstream as the current carries decomposing plant and animal tissue and dissolved phosphorus. It becomes stationary only when it is taken up by plants or is bound to particles that settle to the bottom of pools.

In the field of water quality chemistry, phosphorus is described using several terms. Some of these terms are chemistry based (referring to chemically based compounds), and others are methods-based (they describe what is measured by a particular method).

The term "orthophosphate" is a chemistry-based term that refers to the phosphate molecule all by itself. "Reactive phosphorus" is a corresponding method-based term that describes what you are actually measuring when you perform the test for orthophosphate. Because the lab procedure isn't quite perfect, you get mostly orthophosphate but you also get a small fraction of some other forms.

More complex inorganic phosphate compounds are referred to as "condensed phosphates" or "polyphosphates." The method-based term for these forms is "acid hydrolyzable."

Monitoring phosphorus

Monitoring phosphorus is challenging because it involves measuring very low concentrations—down to 0.01 milligram per liter (mg/L) or even lower. Even such very low concentrations of phosphorus can have a dramatic impact on streams. Less sensitive methods should be used only to identify serious problem areas.

While there are many tests for phosphorus, only four are likely to be performed by volunteer monitors.

1. The *total orthophosphate* test is largely a measure of orthophosphate. Because the sample is not filtered, the procedure measures both dissolved and suspended orthophosphate. The EPA-approved method for measuring total orthophosphate is known as the ascorbic acid method. Briefly, a reagent (either liquid or powder) containing ascorbic acid and ammonium molybdate reacts with orthophosphate in the sample to form a blue compound. The intensity of the blue color is directly proportional to the amount of orthophosphate in the water.
2. The *total phosphorus* test measures all the forms of phosphorus in the sample (orthophosphate, condensed phosphate, and organic phosphate). This is accomplished by first "digesting" (heating and acidifying) the sample to convert all the other forms to orthophosphate. Then the orthophosphate is measured by the ascorbic acid method. Because the sample is not filtered, the procedure measures both dissolved and suspended orthophosphate.
3. The *dissolved phosphorus* test measures that fraction of the total phosphorus which is in solution in the water (as opposed to being attached to suspended particles). It is determined by first filtering the sample, then analyzing the filtered sample for total phosphorus.
4. *Insoluble phosphorus* is calculated by subtracting the dissolved phosphorus result from the total phosphorus result.

All these tests have one thing in common—they all depend on measuring orthophosphate. The total orthophosphate test measures the orthophosphate that is already present in the sample. The others measure that which is already present and that which is formed when the other forms of phosphorus are converted to orthophosphate by digestion.

Sampling and equipment considerations

Monitoring phosphorus involves two basic steps:

- Collecting a water sample
- Analyzing it in the field or lab for one of the types of phosphorus described above.

This manual does not address laboratory methods. Refer to the references cited at the end of this section.

Sample Containers

Sample containers made of either some form of plastic or Pyrex® glass are acceptable to EPA. Because phosphorus molecules have a tendency to "adsorb" (attach) to the inside surface of sample containers, if containers are to be reused they must be acid-washed to remove adsorbed phosphorus. Therefore, the container must be able to withstand repeated contact with hydrochloric acid. Plastic containers—either high-density polyethylene or polypropylene—might be preferable to glass from a practical standpoint because they will better withstand breakage. Some programs use disposable, sterile, plastic Whirl-pak® bags. The size of the container will depend on the sample amount needed for the phosphorus analysis method you choose and the amount needed for other analyses you intend to perform.

Dedicated Labware

All containers that will hold water samples or come into contact with reagents used in this test must be dedicated. That is, they should not be used for other tests. This is to eliminate the possibility that reagents containing phosphorus will contaminate the labware. All labware should be acid-washed.

The only form of phosphorus this manual recommends for field analysis is total orthophosphate, which uses the ascorbic acid method on an untreated sample. Analysis of any of the other forms requires adding potentially hazardous reagents, heating the sample to boiling, and using too much time and too much equipment to be practical. In addition, analysis for other forms of phosphorus is prone to errors and inaccuracies in a field situation. Pretreatment and analysis for these other forms should be handled in a laboratory.

Ascorbic Acid Method

In the ascorbic acid method, a combined liquid or prepackaged powder reagent, consisting of sulfuric acid, potassium antimonyl tartrate, ammonium molybdate, and ascorbic acid (or comparable compounds), is added to either 50 or 25 mL of the water sample. This colors the sample blue in direct proportion to the amount of orthophosphate in the sample. Absorbance or transmittance is then measured after 10 minutes, but before 30 minutes, using a color comparator with a scale in milligrams per liter that increases with the increase in color hue, or an electronic meter that measures the amount of light absorbed or transmitted at a wavelength of 700 - 880 nanometers (again depending on manufacturer's directions).

A color comparator may be useful for identifying heavily polluted sites with high concentrations (greater than 0.1 mg/L). However, matching the color of a treated sample to a comparator can be very subjec-

tive, especially at low concentrations, and can lead to variable results.

A field spectrophotometer or colorimeter with a 2.5-cm light path and an infrared photocell (set for a wavelength of 700-880 nm) is recommended for accurate determination of low concentrations (between 0.2 and 0.02 mg/L). Use of a meter requires that you prepare and analyze known standard concentrations ahead of time in order to convert the absorbance readings of your stream sample to milligrams per liter, or that your meter reads directly as milligrams per liter.

How to prepare standard concentrations

Note that this step is best accomplished in the lab before leaving for sampling. Standards are prepared using a phosphate standard solution of 3 mg/L as phosphate (PO_4). This is equivalent to a concentration of 1 mg/L as Phosphorus (P). All references to concentrations and results from this point on in this procedure will be expressed as mg/L as P, since this is the convention for reporting results.

Six standard concentrations will be prepared for every sampling date in the range of expected results. For most samples, the following six concentrations should be adequate:

0.00 mg/L	0.12 mg/L
0.04 mg/L	0.16 mg/L
0.08 mg/L	0.20 mg/L

Proceed as follows:

1. Set out six 25-mL volumetric flasks—one for each standard. Label the flasks 0.00, 0.04, 0.08, 0.12, 0.16, and 0.20.
2. Pour about 30 mL of the phosphate standard solution into a 50 mL beaker.
3. Use 1-, 2-, 3-, 4-, and 5-mL Class A volumetric pipets to transfer corresponding volumes of phosphate standard solution to each 25-mL volumetric flask as follows:

Standard Concentration	mL of Phosphate Standard Solution
0.00	0
0.04	1
0.08	2
0.12	3
0.16	4
0.20	5

Note: The standard solution is calculated based on the equation: A = (B x C) ÷ D

Where:

A = mL of standard solution needed

B = desired concentration of standard

C = final volume (mL) of standard

D = concentration of standard solution

For example, to find out how much phosphate standard solution to use to make a 0.04-mg/L standard:

A = (0.04 x 25) ÷ 1

A = 1 mL

Before transferring the solution, clear each pipet by filling it once with the standard solution and blowing it out. Rinse each pipet with deionized water after use.

4. Fill the remainder of each 25 mL volumetric flask with distilled, deionized water to the 25 mL line. Swirl to mix.
5. Set out and label six 50-mL Erlenmeyer flasks: 0.00, 0.04, 0.08, 0.12, 0.16, and 0.20. Pour the standards from the volumetric flasks to the Erlenmeyer flasks.
6. List the standard concentrations (0.00, 0.04, 0.08, 0.12, 0.16, and 0.20) under "Bottle #" on the lab sheet.
7. Analyze each of these standard concentrations as described in the section below.

How to collect and analyze samples

The field procedures for collecting and analyzing samples for phosphorus consist of the following tasks:

TASK 1 **Prepare the sample containers**

If factory-sealed, disposable Whirl-pak® bags are used for sampling, no preparation is needed. Reused sample containers (and all glassware used in this procedure) must be cleaned (including acid rinse) before the first run and after each sampling run by following the procedure described in Method B on page 128. Remember to wear latex gloves.

TASK 2 **Prepare before leaving for the sample site**

Refer to page 19-21 for details on confirming sampling date and time, safety considerations, checking supplies, and checking weather and directions. In addition to sample containers and the standard sampling apparel, you will need the following equipment and supplies for total reactive phosphorus analysis:

- Color comparator or field spectrophotometer with sample tubes for reading the absorbance of the sample
- Prepackaged reagents (combined reagents) to turn the water blue
- Deionized or distilled water to rinse the sample tubes between uses
- Wash bottle to hold rinse water
- Mixing container with a mark at the recommended sample volume (usually 25 mL) to hold and mix the sample
- Clean, lint-free wipes to clean and dry the sample tubes

Note that prepackaged reagents are recommended for ease and safety.

TASK 3 **Collect the sample**

Refer to page 128 for details on how to collect water samples using screw-cap bottles or Whirl-pak® bags.

TASK 4 **Analyze the sample in the field (for total orthophosphate only) using the ascorbic acid method.**

If using an electronic spectrophotometer or colorimeter:

1. "Zero" the meter (if you are using one) using a reagent blank (distilled water plus the reagent powder) and following the manufacturer's directions.
2. Pour the recommended sample volume (usually 25 mL) into a mixing container and add reagent powder pillows. Swirl to mix. Wait the recommended time (usually at least 10 minutes) before proceeding.
3. Pour the first field sample into the sample cell test tube. Wipe the tube with a lint-free cloth to be sure it is clean and free of smudges or water droplets. Insert the tube into the sample cell.
4. Record the bottle number on the field data sheet.
5. Place the cover over the sample cell. Read the absorbance or concentration of this sample and record it on the field data sheet.
6. Pour the sample back into its flask.
7. Rinse the sample cell test tube and mixing container three times with distilled, deionized water. Avoid touching the lower portion of the sample cell test tube. Wipe with a clean, lint-free wipe. Be sure that the lower part of the sample cell test tube is clean and free of smudges or water droplets.

Be sure to use the same sample cell test tube for each sample. If the test tube breaks, use a new one and repeat step 1 to "zero" the meter.

If using a color comparator:

1. Follow the manufacturer's directions. Be sure to pay attention to the direction of your light source when reading the color development. The light source should be in the same position relative to the color comparator for each sample. Otherwise, this is a source of significant error. As a quality check, have someone else read the comparator after you.
2. Record the concentration on the field data sheet.

TASK 5 **Return the samples (for lab analysis for other tests) and the field data sheets to the lab/drop-off point.**

Samples for different types of phosphorus must be analyzed within a certain time period. For some types of phosphorus, this is a matter of hours; for others, samples can be preserved and held for longer periods. Samples being tested for orthophosphate must be analyzed within 48 hours of collection. In any case, keep the samples on ice and take them to the lab or drop-off point as soon as possible.

TASK 6 **Analyze the samples in the lab.**

Lab methods for other tests are described in the references below (APHA. 1992; Hach Company, 1992; River Watch Network, 1992; USEPA, 1983).

TASK 7 **Report the results and convert to milligrams per liter**

First, absorbance values must be converted to milligrams per liter. This is done by constructing a "standard curve" using the absorbance results from your standard concentrations.

1. Make an absorbance versus concentration graph on graph paper:
   - Make the "y" (vertical) axis and label it "absorbance." Mark this axis in 0.05 increments from 0 as high as the graph paper will allow.
   - Make the "x" (horizontal) axis and label it "concentration: mg/L as P." Mark this axis with the concentration of the standards: 0, 0.04, 0.08, 0.12, 0.16, 0.20.
2. Plot the absorbance of the standard concentrations on the graph.
3. Draw a "best fit" straight line through these points. The line should touch (or almost touch) each of the points. If it doesn't, make up new standards and repeat the procedure.

Example: Suppose you measure the absorbance of the six standard concentrations as follows:

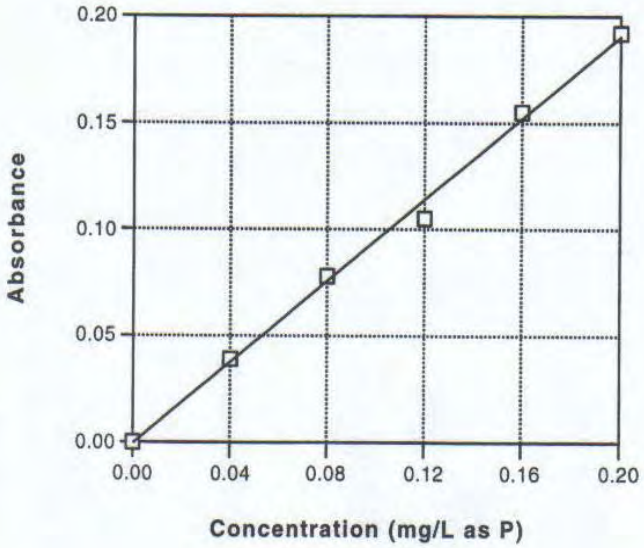
Concentration	Absorbance
0.00	0.000
0.04	0.039
0.08	0.078
0.12	0.105
0.16	0.155
0.20	0.192

The resulting standard curve is displayed in Fig. 5.13.

4. For each sample, locate the absorbance on the "y" axis, read horizontally over to the line, and then more down to read the concentration in mg/L as P.
5. Record the concentration on the lab sheet in the appropriate column. NOTE: The detection limit for this test is 0.01 mg/L. Report any results less than 0.01 as "<0.01." Round off all results to the nearest hundredth of a mg/L.

Figure 5.13

Absorbance of standard concentrations, when plotted, should result in a straight line



Results can either be reported "as P" or "as PO_4." Remember that your results are reported as milligrams per liter—weight per unit of volume. Since the PO_4 molecule is three times as heavy as the P atom, results reported as PO_4 are three times the concentration of those reported as P. For example, if you measure 0.06 mg/L as PO_4, that's equivalent to 0.02 mg/L as P. To convert PO_4 to P, divide by 3. To convert P to PO_4, multiply by 3. To avoid this confusion, and since most state water quality standards are reported as P, this manual recommends that results always be reported as P.

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