Phosphates
(ortho- and total)

INTRODUCTION

Phosphorus is an essential nutrient for all aquatic plants and algae. Only a very small amount is needed, however, so an excess of phosphorus can easily occur. Excess phosphorus is usually considered to be a pollutant because it can lead to *eutrophication*—a condition where an overabundance of nutrients, such as phosphorus, causes increased plant and algal growth. Eutrophication can lower the levels of dissolved oxygen in the water and can render the water uninhabitable by many aquatic organisms. Phosphorus is often the limiting factor that determines the level of eutrophication that occurs.

Effects of Phosphate Levels

- High levels
  - eutrophication
    - increased algal blooms
    - increased BOD
    - decreased DO
- Low levels
  - limiting factor in plant and algal growth

Most phosphorus in surface water is present in the form of phosphates. There are four classifications of phosphates often referred to in environmental literature:

- *orthophosphates* are the inorganic forms of phosphate, such as PO_4^{3-}, HPO_4^{2-}, and $H_2PO_4^-$. These are the forms of phosphates used heavily in fertilizers and are often introduced to surface waters through runoff.
- *organically bound phosphates* are found in human and animal wastes or in decaying organic matter.
- *condensed phosphates (also called polyphosphates)*, such as $P_3O_{10}^{5-}$, are sometimes added to water supplies and industrial processes to prevent the formation of scaling and to inhibit corrosion. This is the form of phosphate that was commonly found in detergents in the past.
- *total phosphates* are the sum of all three of the forms described above. This is the most commonly reported form of phosphate concentration.

Sources of Phosphates-

- Human and animal wastes
- Industrial wastes
- Agricultural runoff
- Human disturbance of land

Phosphates are added to surface waters by a variety of means. Humans add phosphates to water through industrial and agricultural wastes. Fertilizers contain high levels of phosphates and will enter the water by means of runoff and soil erosion. In areas where land and vegetation have been disturbed, soil erosion will increase. This will lead to even more phosphates being washed out of the soil and into the water. Phosphates can also come from the excrement of animals living in or near the water.

Expected Levels

The concentration of phosphates will be expressed throughout this test in units of mg/L PO_4-P, meaning phosphorus in the form of phosphates.[1] Levels above 0.1 mg/L PO_4-P can stimulate plant growth above its natural rate. Water that receives runoff from heavily fertilized areas may have higher levels of phosphates.

[1] Note that no charge is given to the PO_4 when it is used in reporting phosphate units. Here it is being used as a generic symbol for many forms of phosphates with varying charges, such as PO_4^{3-} and HPO_4^{2-}.

A study by the U.S. Geological Survey, based on 410 sites throughout the United States, reports that in 1982, approximately 55% of the sites reported phosphate levels of greater than 0.1 mg/L PO_4-P. By 1989, this percentage had dropped to close to 40%. This decline is due in part to the reduction of phosphorus content in detergents and fertilizers.[2]

Table 1: Phosphate Levels of Selected Rivers

Site	Total Phosphates (mg/L PO_4-P)	Ortho-phosphates (mg/L PO_4-P)
Missouri River, St. Joseph, MO	0.64	0.11
Hudson River, Poughkeepsie, NY	1.60	0.02
Missouri River, Garrison Dam, ND	0.02	0.01
Rio Grande, El Paso, TX	0.41	0.07
Willamette River, Portland, OR	0.09	0.06

Summary of Methods

Both of the methods referred to below include procedures for determining levels of orthophosphates and total phosphates. The only difference between methods is the number of standards used to create the standard curve.

Orthophosphates are relatively easy to measure and will usually give a rough indication of the total level of phosphates in the water. Orthophosphate concentration is determined by means of a chemical reaction resulting in a color change dependent on the concentration of orthophosphates present. The intensity of the color is then measured with a Vernier Colorimeter.

The test for total phosphates involves *digesting,* or treating the sample with an acid and an oxidizer, and boiling for 30 minutes to convert all the phosphates into orthophosphates. The orthophosphate test is then conducted on the sample. The results are reported as total phosphates. This test is more involved than the orthophosphate test, but it is the form of phosphates most commonly reported.

Method 1: Phosphates—Colorimeter with Single Standard

A Vernier Colorimeter is used to create a 2-point standard curve of phosphate absorbance *vs*. concentration using a blank and one phosphate standard. This method is faster and easier than the multiple-standard method, but because your measurement depends upon one standard, the chances for error are somewhat higher.

Method 2: Phosphates—Colorimeter with Multiple Standards

A Vernier Colorimeter is used to create a 4-point standard curve of phosphate absorbance *vs*. concentration using a set of four phosphate standards. This method takes more time and effort than the single-standard method, but the standard curve will be based on four points, reducing the chance of error.

[2] U.S. Geological Survey, *National Water Summary 1990–91, Hydrologic Events and Stream Water Quality*, Water-Supply Paper 2400, United States Government Printing Office, 1993, 124–125.

Method 1: PHOSPHATES—COLORIMETER WITH SINGLE STANDARD

Materials Checklist

___ computer
___ Vernier computer interface
___ Logger *Pro*
___ Vernier Colorimeter
___ one cuvette
___ sampling bottle
___ 10 mL graduated cylinder
___ 25 mL graduated cylinder
___ three 50 mL Erlenmeyer flasks
___ wash bottle filled with distilled water
___ tissues (preferably lint-free)
___ Phosphate Standard (10.0 mg/L PO_4)
___ PhosVer 3 Phosphate Powder Pillows
___ 0.1 M HCl
___ * 2.63 M H_2SO_4
___ * 5.0 M NaOH
___ * hot plate
___ * Potassium Persulfate/Sulfate Powder Pillows

* needed for total phosphate test only

Collection and Storage of Samples

Important: Phosphates can adhere to glass. For best results all glassware involved in this test should be acid washed prior to use. To acid wash the glassware, soak in 0.1 M HCl for 30 minutes, then rinse thoroughly with distilled water. **CAUTION:** *Hydrochloric acid solution is very corrosive. It can cause painful burns if it comes in contact with the skin.*

1. This test is best conducted in the lab. Collect 100 mL of water, so that multiple 25 mL tests can be conducted.

2. It is important to obtain the sample water from below the surface of the water and as far away from the shore as is safe. If suitable areas of the stream appear to be unreachable, samplers consisting of a rod and container can be constructed for collection. Refer to page Intro-4 of the Introduction of this book for more details.

3. If the testing cannot be conducted within a few hours, place the samples in an ice chest or a refrigerator.

Testing Procedure

1. Obtain and wear goggles.

2. Label two Erlenmeyer flasks “A” and “B”.

3. Measure 25 mL of sample water into each flask using the 25 mL graduated cylinder.

4. Testing for total phosphates or orthophosphates.
   a. If your instructor directs you to test for *total phosphates*, go to the Data & Calculations sheet and check the “Total” box in the heading of Column C. Proceed to Step 5.
   b. If your instructor directs you to test for *orthophosphates*, go to the Data & Calculations sheet and check the “Ortho” box in the heading of Column C. Proceed to Step 6.

5. Digest the water samples. **Note:** Perform this step only if you are testing for total phosphates.
   a. Add one Potassium Persulfate/Sulfate Powder Pillow to each flask and swirl.
   b. Add 2.0 mL of 2.63 M H_2SO_4 to each flask using the 10 mL graduated cylinder and swirl. **CAUTION**: *Sulfuric acid, H_2SO_4, is very corrosive. Handle with care.*
   c. Boil the samples for 30 minutes, adding small amounts of distilled water to keep the volume near, but not above 25 mL. **CAUTION**: *Make sure you are boiling in a well-ventilated area. Do not breathe the fumes generated by this digestion.*
   d. After the 30 minute digestion period, remove the flasks from the hot plate and allow the solutions to cool.
   e. Add 2.0 mL of 5.0 M NaOH to each of the flasks using the 10 mL graduated cylinder and swirl. **CAUTION**: *Sodium hydroxide, NaOH, solution is caustic. Handle with care.*
   f. If there is less than 25 mL of liquid in any flask, replenish the volume to 25 mL with distilled water.

6. Prepare the phosphate standard solution for use.
   a. Label the third Erlenmeyer flask with an "S" for "standard" and add 25 mL of 10.0 mg/L Phosphate Standard.
   b. Add a PhosVer 3 Phosphate Powder Pillow to the flask.
   c. Swirl the solution until the powder is mostly dissolved.
   d. Continue with the testing procedure while the reaction takes place. It will take two minutes for a complete reaction to occur.

7. Position the computer safely away from the water. Keep water away from the computer at all times.

8. Plug the Colorimeter into Channel 1 of the Vernier interface.

9. Prepare the computer for data collection by opening the file "07 Phosphates" from the *Water Quality with Vernier* experiment files of Logger *Pro*.

10. Prepare a *blank* by filling an empty cuvette 3/4 full with distilled water. Seal the cuvette with a lid. To correctly use a colorimeter cuvette, remember:
    - All cuvettes should be wiped clean and dry on the outside with a tissue.
    - Handle cuvettes only by the top edge of the ribbed sides.
    - All solutions should be free of bubbles.
    - Always position the cuvette with its reference mark facing toward the white reference mark at the top of the cuvette slot on the colorimeter.

11. Calibrate the Colorimeter.
    a. Open the Colorimeter lid.
    b. Holding the cuvette by the upper edges, place it in the cuvette slot of the Colorimeter. Close the lid.
    c. If your Colorimeter has a CAL button, Press the < or > button on the Colorimeter to select a wavelength of 565 nm (Green) for this experiment. Press the CAL button until the red LED begins to flash. Then release the CAL button. When the LED stops flashing, the calibration is complete.

12. Collect absorbance-concentration data for the blank and the phosphate standard solution. This process will create a standard curve that will be used to determine the phosphate concentrations of the samples.
    a. Click Collect to start data collection.
    b. While the blank is still in the Colorimeter, wait for the absorbance value displayed in the meter to stabilize, then click Keep.
    c. Type **0** (the phosphate concentration) in the edit box, then press ENTER.
    d. Discard the water in the cuvette. Rinse twice with the standard solution from Flask "S" and then fill it 3/4 full. Wipe the outside of the cuvette and place it in the colorimeter. After closing the lid, wait for the absorbance value displayed in the meter to stabilize, then click Keep.
    e. Type **10** (the concentration in mg/L PO_4) in the edit box, then press ENTER.
    f. Click Stop.

13. Examine the graph of absorbance *vs.* concentration. To see the relationship between these two variables, click on the Linear Fit button, . A linear regression curve will be shown for your data points. This line should pass through the data points and the on or near the origin of the graph. (Note: Another options is to choose Curve Fit from the Analyze menu, and then select Proportional. The Proportional fit, (y=Ax) has a y-intercept value equal to 0; therefore, this regression line will always pass through the origin of the graph).

14. Prepare the samples for testing.
    a. Add one PhosVer 3 Phosphate Powder Pillow to each sample flask.
    b. Swirl each solution until the powder is mostly dissolved.
    c. It will take two minutes for a complete reaction to occur. The samples will turn blue with an intensity relative to the concentration of phosphate.

15. Find the absorbance of the sample.
    a. Rinse the cuvette twice with solution from Flask A and fill it about ¾ full. Wipe the outside of the cuvette, place it into the Colorimeter and close the lid.
    b. Observe the absorbance value in the meter. When the absorbance value has stabilized, record it on the Data & Calculations sheet.

16. Use the following method to determine the unknown concentration. With the linear regression curve still displayed on your graph, choose Interpolate from the Analyze menu. A vertical cursor now appears on the graph. The cursor's x and y coordinates are displayed in a floating box. Move the cursor along the regression line until the absorbance (y) value is approximately the same as the absorbance value you recorded in Step 15. The corresponding x value is the concentration of the unknown solution in mg/L PO_4. Record this value in Column B on the Data & Calculations sheet.

17. Repeat Steps 15–16 for Flask B.

18. The values obtained in Step 16 are in units of mg/L phosphates, PO_4. Quite often, however, results for this test are reported in units of phosphorus, PO_4-P. If you wish to convert your phosphate values to phosphorus, use this equation and record the results in Column C of the Data & Calculations sheet

$$\text{phosphorus (mg/L } PO_4\text{-P)} = \frac{\text{phosphates (mg/L } PO_4\text{)}}{3.06}$$

Method 2: PHOSPHATES—COLORIMETER WITH MULTIPLE STANDARDS

Materials Checklist

___ computer
___ Vernier computer interface
___ Logger *Pro*
___ Vernier Colorimeter
___ two graduated pipets and bulbs
___ sampling bottle
___10 mL graduated cylinder
___ 25 mL graduated cylinder
___ six 50 mL Erlenmeyer flasks
___ two 100 mL beakers
___ one cuvette (or a matched set)
___ tissues (preferably lint-free)
___ 0.1 M HCl
___ wash bottle with distilled water
___ PhosVer 3 Phosphate Powder Pillow
___ Phosphate Standard (10.0 mg/L PO_4)
___ * 5.0 M NaOH
___ * 2.63 M H_2SO_4
___ * hot plate
___ * Potassium Persulfate/Sulfate Powder Pillow

* needed for total phosphate test only

Collection and Storage of Samples

Important: Phosphates can adhere to glass. For best results all glassware involved in this test should be acid washed prior to use. To acid wash the glassware, soak in 0.1 M HCl for 30 minutes, then rinse thoroughly with distilled water. **CAUTION:** *Hydrochloric acid solution is very corrosive. It can cause painful burns if it comes in contact with the skin.*

1. This test is best conducted in the lab. Collect at least 100 mL of water, so that multiple tests can be conducted.

2. It is important to obtain the sample water from below the surface of the water and as far away from the shore as is safe. If suitable areas of the stream appear to be unreachable, samplers consisting of a rod and container can be constructed for collection. Refer to Page Intro-4 of the Introduction of this book for more details.

3. Samples should be stored in a refrigerator until measurements are made.

Testing Procedure

1. Obtain and wear goggles.

2. Label two Erlenmeyer Flasks “A” and “B”.

3. Measure 25 mL of sample water into each flask, using the 25-mL graduated cylinder.

4. Testing for total phosphates or orthophosphates.
   a. If your instructor directs you to test for *total phosphates*, go to the Data & Calculations Sheet and check the “Total” box in the heading of Column C. Proceed to Step 5.
   b. If your instructor directs you to test for *orthophosphates*, go to the Data & Calculations Sheet and check the “Ortho” box in the heading of Column C. Proceed to Step 6.

5. Digest the water samples. **Note:** Perform this step only if you are testing for total phosphates.
   a. Add one Potassium Persulfate/Sulfate powder pillow to each flask and swirl.
   b. Add 2.0 mL of 2.63 M H_2SO_4 to each flask using the 10 mL graduated cylinder and swirl. **CAUTION**: *Sulfuric acid, H_2SO_4, is very corrosive. Handle with care.*
   c. Boil the samples for 30 minutes, adding small amounts of distilled water to keep the volume near, but not above 25 mL. **CAUTION**: *Make sure you are boiling in a well-ventilated area. Do not breathe the fumes generated by this digestion*.
   d. After the 30-minute digestion period, remove the flasks from the hot plate and allow the solutions to cool.
   e. Add 2.0 mL of 5.0 M NaOH to each of the flasks using the 10 mL graduated cylinder and swirl. **CAUTION**: *Sodium hydroxide, NaOH, solution is caustic. Handle with care.*
   f. If there is less than 25 mL of liquid in any flask, replenish the volume to 25 mL with distilled water.

6. Prepare the set of phosphate standards.
   a. Add 75 mL of 10.0 mg/L Phosphate Standard to a 100 mL beaker.
   b. Add 75 mL of distilled water to another 100 mL beaker.
   c. Label four Erlenmeyer flasks with the numbers 1–4.
   d. Pipet 5, 10, 15, and 20 mL of 10.0 mg/L Phosphate Standard into Flasks 1–4, respectively. With a second pipet, deliver 20, 15, 10, and 5 mL of distilled water into Flasks 1–4, respectively. Volumes and concentrations for each flask are summarized below.
   e. *Thoroughly* mix each solution by swirling each flask.

Flask Number	10.0 mg/L PO_4 (mL)	Distilled H_2O (mL)	Concentration (mg/L PO_4)
1	5	20	2.0
2	10	15	4.0
3	15	10	6.0
4	20	5	8.0

7. Prepare the set of phosphate standards for use.
   a. Add one PhosVer 3 Phosphate Powder Pillow to each of the four flasks.
   b. Swirl each solution until the powder is mostly dissolved.
   c. Continue with the testing procedure while the reaction takes place. It will take two minutes for a complete reaction to occur. The sample will turn blue with an intensity relative to the concentration of phosphate.

8. Position the computer safely away from the water. Keep water away from the computer at all times.

9. Connect the Colorimeter to the Vernier interface.

10. Prepare the computer for data collection by opening the file "07 Phosphates" from the *Water Quality with Vernier* experiment files of Logger *Pro*.

11. Prepare a *blank* by filling an empty cuvette 3/4 full with distilled water. Seal the cuvette with a lid. To correctly use a colorimeter cuvette, remember:

- All cuvettes should be wiped clean and dry on the outside with a tissue.
- Handle cuvettes only by the top edge of the ribbed sides.
- All solutions should be free of bubbles.
- Always position the cuvette with its reference mark facing toward the white reference mark at the top of the cuvette slot on the colorimeter.

12. Calibrate the Colorimeter.
    a. Open the Colorimeter lid.
    b. Holding the cuvette by the upper edges, place it in the cuvette slot of the Colorimeter. Close the lid.
    c. If your Colorimeter has a CAL button, Press the < or > button on the Colorimeter to select a wavelength of 565 nm (Green) for this experiment. Press the CAL button until the red LED begins to flash. Then release the CAL button. When the LED stops flashing, the calibration is complete.

13. You are now ready to collect absorbance-concentration data for the phosphate standard solutions. This process will create a standard curve that will be used to determine the phosphate concentrations of the samples.
    a. Click Collect to start data collection.
    b. Empty the water from the cuvette. Using the solution in Flask 1, rinse the cuvette twice with ~1 mL amounts and then fill it ¾ full. Wipe the outside with a tissue and place it in the Colorimeter. After closing the lid, wait for the absorbance value displayed on the computer screen to stabilize.
    c. When the absorbance value has stabilized, click Keep.
    d. Type **2** (the phosphate concentration value) in the edit box, then press ENTER.
    e. Discard the cuvette contents as directed by your teacher. Using the solution in Flask 2, rinse the cuvette twice with ~1 mL amounts and then fill it 3/4 full. After closing the lid, wait for the absorbance value displayed on the computer screen to stabilize.
    f. When the absorbance readings have stabilized, click Keep.
    g. Type **4** (the phosphate concentration) in the edit box, the press ENTER.
    h. Repeat for Flask 3 (6 mg/L PO_4) and Flask 4 (8 mg/L PO_4).
    i. Click Stop when you have finished.
    j. Discard the standard solutions, as directed by your instructor.

14. Examine the graph of absorbance *vs.* concentration. To see if the curve represents a direct relationship between these two variables, click on the Linear Fit button, . A best-fit linear regression line will be shown for your data points. This line should pass near or through the data points and the origin of the graph. (Note: Another options is to choose Curve Fit from the Analyze menu, and then select Proportional. The Proportional fit, (y=Ax) has a y-intercept value equal to 0; therefore, this regression line will always pass through the origin of the graph).

15. Prepare the water samples for testing.
    a. Add one PhosVer 3 Phosphate Powder Pillow to Flask A and one to Flask B.
    b. Swirl the solutions until the powder is mostly dissolved.
    c. Again, it will take two minutes for a complete reaction to occur.

16. Find the absorbance of the sample.
    a. Rinse the cuvette twice with solution from Flask A and fill it about ¾ full. Wipe the outside of the cuvette, place it into the Colorimeter and close the lid.
    b. Observe the absorbance value in the meter. When the absorbance value has stabilized, record it on the Data & Calculations sheet.

17. Use the following method to determine the unknown concentration. With the linear regression curve still displayed on your graph, choose Interpolate from the Analyze menu. A vertical cursor now appears on the graph. The cursor's x and y coordinates are displayed in a floating box. Move the cursor along the regression line until the absorbance (y) value is approximately the same as the absorbance value you recorded in Step 16. The corresponding x value is the concentration of the unknown solution in mg/L PO_4. Record this value on the Data & Calculations sheet.

18. Repeat Steps 16−17 for Flask B.

19. The values obtained in Step 17 are in units of mg/L phosphates, PO_4. Quite often, however, results for this test are reported in units of phosphorus, PO_4-P. If you wish to convert your phosphate values to phosphorus, use this equation and record the results in Column C of the Data & Calculations sheet

$$\text{phosphorus (mg/L } PO_4\text{-P)} = \frac{\text{phosphates (mg/L } PO_4\text{)}}{3.06}$$

DATA & CALCULATIONS

Phosphates

Stream or lake: _____ Time of day: _____

Site name: _____ Student name: _____

Site number: _____ Student name: _____

Date: _____ Student name: _____

Column	A	B	C
Flask	Absorbance	❑ Total ❑ Ortho Phosphates Concentration (mg/L PO_4)	❑ Total ❑ Ortho Phosphorus Concentration (mg/L PO_4 - P)
A			
B			
Average			

Column Procedure:

A. Record the absorbance value from the computer.
B. Record the concentration of phosphates as determined by interpolation of the standard curve.
C. Calculate the concentration of phosphorus, using the formula:

$$\text{phosphorus (mg/L } PO_4\text{-P)} = \frac{\text{phosphates (mg/L } PO_4\text{)}}{3.06}$$

Field Observations (e.g., weather, geography, vegetation along stream) _____

_____

_____

_____

Test Completed: _____ Date: _____

Vernier Lab Safety Instructions Disclaimer

THIS IS AN EVALUATION COPY OF THE VERNIER STUDENT LAB.

This copy does not include:

- **Safety information**
- **Essential instructor background information**
- **Directions for preparing solutions**
- **Important tips for successfully doing these labs**

The complete *Water Quality with Vernier* lab manual includes 16 water quality tests and essential teacher information. The full lab book is available for purchase at: http://www.vernier.com/cmat/wqv.html

Vernier Software & Technology
13979 S.W. Millikan Way • Beaverton, OR 97005-2886
Toll Free (888) 837-6437 • (503) 277-2299 • FAX (503) 277-2440
info@vernier.com • www.vernier.com