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**Environmental Science Education Title:** Nutrients in Surface Water

**Overview:** Nitrogen and phosphorus are essential plant nutrients found in aquatic ecosystems, and both are monitored as a part of water quality testing, because in excess amounts, they can cause significant water quality problems.

Nitrogen in water is measured as the common form nitrate (NO3-) that is dissolved in water and readily absorbed by photosynthesizers, such as algae. The common form of phosphorus measured is phosphate (PO43-), which is strongly attracted to sediment particles as well as dissolved in water. In excess amounts, both nutrients can cause an increase in aquatic plant growth (Figure 1) that can disrupt the light, temperature, and oxygen levels in the water below, which leads to eutrophication and hypoxia (low dissolved oxygen in water), forming a “dead zone” of no biological activity. Sources of nitrates and phosphorus include wastewater treatment plants, runoff from fertilized lawns and agricultural lands, faulty septic systems, animal manure runoff, and industrial waste discharge.

**Principles:** Nitrate and phosphate concentrations can be measured in water samples using known chemical reagents that cause the sample to change color when in the presence of a specific nutrient, with increasing color intensity indicating an increased concentration of the nutrient. To ensure release of any phosphate molecules that are bonded to sediments in the water, phosphorus samples are digested chemically and with heat to release phosphate bonds for a measure of total phosphate in the sample.

The reagent used for analyzing nitrate is a modification of the Cadmium Reduction Method, using gentisic acid in place of 1-naphthylamine. The necessary cadmium, sulfanilic acid, and gentisic acid have been combined into a single stable powder called NitraVer 5. The cadmium metal is used to reduce nitrates (NO3-) to nitrites (NO2-) (reaction 1).

NO3- + Cd +2H+ → NO2- + Cd2+ + H2O

NO2- + sulfanilic acid + 2H+ → HO3S + diazonium salt

diazonium salt + gentisic acid → amber-colored species

Next, the nitrite ions react in an acidic medium with sulfanilic acid to form an intermediate diazonium salt, which forms an amber color when coupled with gentisic acid.

Organic phosphates in the sample are converted to orthophosphates through hydrolysis achieved by mixing samples with potassium persulfate and heating in a COD reactor with acid and persulfate. The COD reactor is an instrument for incubating glassware (up to 25 vials at one time) containing hazardous ingredients at high temperatures.

Orthophosphates can then react with sodium molybdate in an acid medium to produce a mixed phosphate/molybdate complex.

12MoO3 + H2PO4- → H2PMo12O40

Ascorbic acid is used to reduce the complex causing an intense molybdenum blue color. Potassium pyrosulfate, ascorbic acid, and sodium molybdate are contained in one stable powder reagent, PhosVer 3.

reduction

H2PMo12O40 ------------------> blue-colored species

ascorbic acid

To quantify the color intensity produced by the reagent, a spectrophotometer is used to measure the specific wavelength of light that corresponds with each color caused by the nutrients and their reagents (nitrates, green; phosphates, blue). The spectrophotometer then sends a beam of light through each sample to measure the amount of light absorbed by the color (absorbance). The darker the color, the higher the absorbance. The spectrophotometer then converts the absorbance to a displayed nutrient concentration (mg/l) based on known concentration assays.

**Procedure:**

1. **Measure Nitrogen in Sample**
   1. On the spectrophotometer, find the program for nitrate (with user manual or instrument menu) and enter the program number.
   2. Measure out 10 mL of the water sample into a graduated cylinder. Pour this into one of the sample tubes.
   3. Repeat for a second sample tube.
   4. Add the contents of one NitraVer 5 reagent powder pillow to one sample tube.

* 1. Cap both sample tubes.
  2. On the spectrophotometer, press timer and enter to start a reaction period for the reagent. Place the tube between thumb and forefinger and shake the sample vigorously until the reaction time is over and the timer beeps. Sample will begin to turn a shade of green.
  3. Press enter. A second five minute reaction period will begin.
  4. After the timer beeps the second time, wipe off the outside of the two sample tubes with a lint-free paper towel.
  5. Place the sample tube without reagent (blank) into the spectrophotometer.
  6. Tightly cover the cell with the instrument cap to ensure ambient light is blocked.
  7. Zero the spectrophotometer with the blank for a reading of 0.0 mg/L NO3-N.
  8. Remove the blank cell and place the sample cell with reagent into the cell holder. Tightly cover the sample cell with the instrument cap.
  9. Press READ. The cursor will move to the right, then the results in mg/L NO3-N will be displayed.

1. **Measure Phosphorus in Sample**
   1. Measure out 5.0 mL of the water sample using a graduated cylinder.
   2. Pour measured water into a sample tube.
   3. Add the contents of one potassium persulfate powder pillow for phosphonate to the sample tube.
   4. Cap the tube tightly and shake to dissolve.
   5. Label the top of the tube cap, and place the tube in a COD reactor (in a chemical hood) and heat for 30 minutes.
   6. Place it in a test tube rack and allow to cool until room temperature.
   7. Using a graduated cylinder, measure out 2 ml of 1.54 N sodium hydroxide.
   8. Pour this into the sample tube. Cap and mix.
   9. On the spectrophotometer, find the program number for phosphate (with user manual or instrument menu) and enter the program number.
   10. Clean the outside of the sample tube with a lint-free paper towel.
   11. Place the test tube so that any label on the glass is facing the front of the instrument.
   12. Place the cover on the test tube and press ZERO button. The display should show 0.00mg/L PO43-.
   13. Take out test tube and add the contents of one PhosVer 3 phosphate reagent powder pillow to the test tube.
   14. Cap tightly and shake for 10 – 15 seconds.
   15. Press timer and then enter. A 2-minute waiting period will begin.
   16. After the timer beeps, clean the outside of the test tube with a lint-free paper towel.
   17. Place the test tube into the instrument with any labels facing the front of the instrument.
   18. Place the cover over the test tube.
   19. Press READ. The display will show the results in mg/L.

**Representative Results:** A sample analyzed for nitrates after mixed with NitraVer 5 reagent (left) and a sample for phosphate after being mixed with PhosVer 3 reagent (right).

Graph comparing nitrates from 5 sampling locations along the Chicago River North Branch (Figure 2).

Average nitrate concentrations compared upstream and downstream from a water treatment plant (Figure 3). The downstream measurement represents the discharge from the treatment, which is low in nitrates due to the removal of organic material during the water treatment process.

Graph of phosphorus for different locations along the Chicago River (Figure 4).

Average phosphate concentrations compared upstream and downstream from a water treatment plant (Figure 5). The downstream measurement represents the discharge from the treatment, indicating possible phosphorus inputs near the discharge area, possibly from riparian lawn fertilizer that could contribute to eutrophication in Lake Michigan.

**Applications:** High concentrations of nitrates and phosphorus can stimulate eutrophic conditions in water by causing algal bloom that negatively affects other water quality factors including dissolved oxygen, temperature, and other indicators. Excess nitrates can lead to hypoxic water (low levels of dissolved oxygen) no longer able to support aerobic life creating a “dead zone,” where non-mobile species mass die-off and mobile species move away to other waters. Dead zones are occurring globally in coastal regions where large amounts of high-nutrient runoff and wastewater converge, and aquatic life is most highly concentrated (Figure 6). Two of the largest dead zones are in the Baltic Sea, where on average 49,000 km2 of water contained less than 2 mg/L of dissolved oxygen, and the northern Gulf of Mexico, with a dead zone measured at 17,353 km2.

**Legend:**   
Figure 1: Algal Bloom.

Taken in 2011, the green scum shown in this image was the worst algae bloom Lake Erie has experienced in decades. Record torrential spring rains washed fertilizer into the lake, promoting the growth of microcystin producing cyanobacteria blooms. Vibrant green filaments extend out from the northern shore.

Figure 2: Graph comparing nitrates between different land use types (undeveloped, agricultural, and urban).

Figure 3: Average nitrate concentrations compared upstream and downstream from a water treatment plant. The downstream measurement represents the discharge from the treatment.

Figure 4: Graph of phosphorus for different locations along the Chicago River.

Figure 5: Average phosphate concentrations compared upstream and downstream from a water treatment plant. The downstream measurement represent the discharge from the treatment.

Figure 6: Marine dead zones world-wide.  
Red circles show the location and size of many dead zones. Black dots show dead zones of unknown size. Darker blues in this image show higher concentrations of particulate organic matter, an indication of the overly fertile waters that can culminate in dead zones. The size and number of marine dead zones — areas where the deep water is so low in dissolved oxygen that sea creatures can’t survive — have grown explosively in the past half-century. It’s no coincidence that dead zones occur downriver of places where human population density is high (darkest brown).