Nitrate: Its Importance in Water and Its Measurement

When nitrogen (N_2) occurs in the atmosphere it is relatively inert and not available to most plants. When nitrogen combines with other elements to form a compound it is called “fixed nitrogen.” In combined form nitrogen is an essential plant nutrient and is a major component of most fertilizers. The term nitrate is used to refer to nitrogen in the form NO_3^-. While the presence of some fixed nitrogen in water is not a problem, excessive concentrations of nitrate (and phosphate, another essential plant ingredient) can cause serious environmental water problems. But these are problems that can be addressed, which is why the monitoring of nitrate levels is important.

Although the pre and post discussions are necessary to provide important information about each topic, it is the activity that is most vital to this unit. Be sure to allow plenty of time to complete the activity.

Pre-Activity Discussion (Answers can be found in the Background Information section below)
Before beginning the activity, ask students:
1) What are nitrates and why is the level of nitrate in the water important?
2) How does it affect living things?
3) How does nitrate in water cause oxygen depletion?

The Activity

THINK SAFETY! Wear safety goggles when working with these chemicals and wash your hands after the experiment. These chemicals are poisonous; don’t taste them.

Equipment needed
--Small table
--Hach Nitrates kit, Model Ni-14 cadmium reduction method
--Jug of deionized or distilled water (needed for the 0 – 10 mg/L test only)
--Scissors to open powder pillows in the Hach kit
--A watch that can measure seconds
--Safety goggles (2 pr.)
--Rubber gloves for leader and student using chemicals
--Long-handled dipper, if needed, to sample from a steep bank
--Work/data sheets
--Wastewater jug for disposal of spent toxic solutions.
--Data form and clipboard

Site selection and set-up
Choose a site with room for a table and easy access to the stream. Be sure it is upstream from activities that may cause turbidity (BMI collection, stream speed) and free of obstructions. Avoid sites with nettles, thorny plants or poison ivy. The water should be deep enough to collect samples without getting sediment from the stream bottom.

Procedure
1. Collect a clear water sample from the selected site along the stream.
2. Rinse both of the color viewing tubes three times with the sample water.

3. Fill one color viewing tube to the 5 mL mark (the lowest ring on the tube) with sample water. (low range; 0 – 1.0 mg/L. For 0 – 10 mg/L test see below.)
4. Put on your safety goggles. Give the student volunteer assistants safety goggles. Appoint timekeepers.
5. Open a NitraVer 6 nitrate reagent powder pillow and add its contents to the sample in the tube. Stopper the tube and shake for 3 minutes. Allow the sample to stand undisturbed for 30 seconds. Particles of cadmium metal will remain in the sample and settle to the bottom of the tube.
6. Carefully pour this prepared sample into the second color viewing tube. Be sure to keep the cadmium particles in the first tube.
7. Open a NitriVer 3 nitrite reagent powder pillow and add its contents to the sample. Stopper the tube and shake for 30 seconds. A red color will develop if nitrate is present. Allow at least 10 but not more than 20 minutes to elapse before proceeding.
8. Insert the tube containing the prepared sample into the right top opening of the color comparator.
9. Rinse out the cadmium metal from the other tube and place it in the hazardous waste jug. Fill the tube to the mark with the original (untreated) water sample, and place it into the top left opening of the color comparator.
10. Hold the comparator up to a light source (sky, window, lamp) and view through the openings in front. Rotate the disc to get a color match between the sample and the disk and when it matches a number will be visible in another opening on the comparator. This number is *N*, the concentration of nitrate as N. To convert *N* to the concentration of NO_3^-, multiply by 4.4.

Nitrate concentration as N = ___________mg/L

Nitrate concentration as NO_3^- = _____________mg/L

Nitrate N concentrations < ~ 3 mg/L are not a problem. Nitrate as N concentrations above 10 mg/L indicate the water is significantly impaired. In this area nitrate concentrations are generally quite a bit less than 1 mg/L.

Post-activity discussion questions:

1. On the basis of the nitrate reading *N*, what can you conclude about the water in the stream? Are there any visible signs in the stream of high nitrate levels?
2. What are some ways that nitrate get into the watershed? Why are farming practices so critical? Lawn care?
3. How can people keep the amount of nitrate in a stream to a healthy level?

Background Information

Nitrogen occurs as N_2 in the atmosphere and is a relatively inert gas that is not available for use by most plants. As “fixed nitrogen” (when the nitrogen is chemically combined in the form of ammonia or ammonium salts, urea, or nitrates) this element is an essential plant nutrient. Fixed nitrogen is a major component of most fertilizers. The term nitrate is used to refer to nitrogen in the form NO_3^-

When microbes break down ammonia, urea, or proteins, the nitrogen combines with oxygen to form NO_3^-. This reduces the dissolved oxygen level in the water and in extreme cases can cause dead zones for all animals that rely on oxygen to survive. Also, ammonia is quite toxic to aquatic life. High concentrations of fixed nitrogen in lakes and streams may also be responsible for excessive growth of aquatic plants, including algae blooms. These problems can also be caused by phosphate, as is the case in Ford and Belleville Lakes. A large algae bloom also occurred in Lake Erie near Toledo in 2014 contaminating the drinking water with a toxin from blue-green algae for almost half a million people. Excessive concentrations of nitrate (>10 mg/L) cause an illness, methemoglobinemia, in infants.

Common sources of fixed nitrogen in our waters include fertilizer runoff from fields, lawns, golf courses, etc; domestic sewage and septic tank leachate; slaughterhouse and some food processing wastes; runoff from Concentrated Animal Feeding Operations (cattle feedlots, pig and poultry operations).

Common forms of fixed nitrogen (N) are as follows: organic (proteins, polypeptides, urea), NH_3 (ammonia), NO_2^-(nitrite), and NO_3^- (nitrate) .

By and large the elemental N_2 in the atmosphere is not available to plants. Only a relatively few plants can use N_2 Legumes such as alfalfa, clover, vetches and beans, utilize it via root nodules of N-fixing bacteria. A few blue-green algae species also can use N_2 in the atmosphere.

Where there's oxygen available, the reactions $NH_3 + O_2 \rightarrow NO_2^-$ and $NO_2^- + O_2 \rightarrow NO_3^-$ occur. One can also have denitrification under some anaerobic circumstances, in which process nitrate is converted to inert N_2 gas. Bacteria are involved in all of these reactions.

The fixed N in fertilizers is generally in the form of ammonia or ammonium salts, nitrate, or urea [$OC(NH_2)_2$]. Ammonia and urea N are present in very high concentrations in livestock feeding (feedlot, Concentrated Animal Feeding Operation) wastes.

For this and all other units, advanced level information is available if desired. Contact the HRWC and request an electronic version of the unabridged manual.

Notes on the experimental process and analysis of NO_3^-.

Analysis of NO_3^-. Cadmium reduction method.

(1) Reduce the NO_3^- to NO_2^- by reacting it with cadmium metal;

$$NO_3^- + Cd + 2H^+ \rightarrow NO_2^- + Cd^{+2} + H_2O.$$

(2) This nitrite in acid solution is reacted with sulfanilic acid [4-aminobenzene-sulfonic acid, $H_2N\text{-}C_6H_4\text{-}SO_3H$] to produce a diazonium salt, $HSO_3\text{-}C_6H_4\text{-}N{\equiv}N^+$, which then reacts with N-(1-naphthyl)-ethylenediamine dihydrochloride to produce a red-colored azo dye.

The limit of detection with this kit is 0.01 mg NO_3^- per L when it is used in the 0 – 1.0 mg/L mode as described here and in the kit instructions. Dispose of the waste as hazardous because of the cadmium. If the test needs to have a higher range (0 – 10 mg/L), modify Step 3 in the above procedure as follows:

Step 3 Modified: Rinse one of the color viewing tubes with distilled water 3 times. Rinse the dropper in the kit 3 times with sample water, then put in 0.5 mL of sample (lowest mark on dropper) into the color viewing tube. Then add distilled water to bring the water level in the tube up to the 5 mL mark (the lowest mark). Go to Step 4 above.

Step 10: After carrying out Step 9 above, multiply your answers by 10 to get the nitrate as N and the nitrate concentrations in the water sample.

Environmental chemistry of nitrogen

Common forms of fixed nitrogen (N) are as follows: organic (proteins, polypeptides, urea), NH_3 (ammonia), NO_2^-(nitrite), and NO_3^- (nitrate). The fixed N in fertilizers is generally in the form of ammonia or ammonium salts, nitrate or urea [$OC(NH_2)_2$]. Where there's oxygen available, the reactions $NH_3 + O_2 \rightarrow NO_2^-$ and $NO_2^- + O_2 \rightarrow NO_3^-$ occur. One can also have denitrification under some anaerobic circumstances, in which process nitrate is converted to inert N_2 gas. All these reactions require the action of bacteria.

Nitrate Data Sheet

When you have completed the measurement, record your data.

1. Nitrate concentration as N = ___________mg/L.
2. Multiply this result by 4.4 to obtain the nitrate concentration as NO_3^- = _____________mg/L

   Nitrate N concentrations < ~ 3 mg/L are not a problem. Nitrate N concentrations above 10 mg/L indicate the water is significantly impaired. (Mitchell and Stapp, p. 68)

3. Does your nitrate N concentration indicate that this water has a nitrate problem?___________
4. Why is the level of nitrates in this water important?

5. How do people contribute to this problem?

6. What can people do to reduce the amount of nitrates in the river?

Nitrates Lesson Narrative

Intro. 5 – 8 minutes. The mission is to make these points: Nitrate amounts can vary. Too little and plants can't live in the water. Too much and excessive plant growth, such as algae blooms, can be a problem, and may make it impossible for animals to live in the water. Human activities can add too much nitrate or nitrate-forming material to the water: fertilizers, domestic sewage, pet waste and farm animal wastes are top sources.

1. My name is _________ and I'm a volunteer with the Nitrates station. Please tell me your names. (Go around)
2. What do you already know about nitrates and streams?
3. Nitrogen is necessary for plants and animals to live. We need plants because only plants can make food from light. How do plants get what they need to make their bodies? We know they use water and carbon to make sugar for food but they also need a few extra chemicals. One of them is nitrogen. There is a lot of nitrogen in the air, but not in a form that plants can use to make their bodies. So people use fertilizers. Fertilizers usually contain phosphates and nitrates. At this station we will focus on nitrates.
4. Where do nitrates come from? (Fertilizers and animal wastes are two big sources. Another is human waste from failing septic systems, or wastewater treatment systems that are not equipped with a denitrification stage.)
5. It is OK if there is some nitrate in the streams, but the concentration should not exceed 3 mg/L. Too much can cause an overgrowth of water plants. When the plants die, their decay can use up the dissolved oxygen in the water. This kills fish and other aquatic animals.

The Activity. 10 – 15 minutes. Assign the roles. Collect water. Test it. Repeat with 2 tubes of water, if time.

6. Explain that the group is going to measure the amount of nitrates in the stream. Explain and assign the different ways that students will help.
7. If poison ivy is present, show it to students or pick a spot to access the stream that doesn't have any poison ivy.
8. Ask the students to look at the stream. Can we tell if there are nitrates in the water? No, the water-soluble nitrates are colorless. So to find out we have to use a chemical test.
9. One student collects water. The volunteer or a student can rinse the color viewing tubes with river water, or the container of drinking water.
10. Put on your safety goggles. Give a student volunteer assistant a pair of safety goggles. Appoint a second and third as time keepers.
11. Rinse both color viewing tubes three times with sample water. Then fill one of the color viewing tubes to the 5 mL mark (the lowest ring on the tube) with sample water.

REACT WITH NITRATES

12. Use the scissors to open a NitraVer 6 nitrate reagent powder pillow and add its contents to the sample in the tube. Stopper the tube and shake for 3 minutes. Allow the sample to stand undisturbed for 30 seconds. Particles of cadmium metal will remain in the sample and settle to the bottom of the tube.
13. Carefully pour this prepared sample into the second color viewing tube, keeping the cadmium particles in the first tube.

MAKE A COLORFUL COMPOUND

14. Open a NitriVer 3 nitrite reagent powder pillow and add its contents to the sample in the second tube. Stopper the tube and shake it for 30 seconds. A red color will develop if nitrate is present. Allow at least 10 but not more than 20 minutes to elapse before doing Steps 14-16.
15. Insert the tube containing the prepared sample into the right top opening of the color comparator.
16. Rinse out the cadmium metal from the other tube (hazardous waste), fill the tube to the mark with the original (untreated) water sample, and place it into the top left opening of the color comparator.

MEASURE NITRATES BY COMPARING TO A STANDARD COLOR

17. Hold the comparator up to a light source (sky, window, lamp) and view through the openings in front. Rotate the disc to get a color match and read the nitrate as N concentration through the scale window. To convert to NO_3^-, multiply by 4.4.
18. Nitrate concentration as N = ____________mg/L
19. Nitrate concentration as NO_3^- = ______________mg/L
20. Nitrate N concentrations < ~ 3 mg/L are not a problem. Nitrate N concentrations above 10 mg/L indicate the water is significantly impaired. In this area nitrate N concentrations are generally well below 1.0 mg/L.
21. Have the recorder read aloud and fill in questions 1 and 2 on the data sheet.
22. Have the recorder read aloud question 3. Have students answer.
23. Repeat for questions 4 and 5.
24. Thanks for visiting with me today. It’s time for you to go to your next station ______________.