

Lab V-4

Soil and Water Pollution Testing

Equipment and Materials

You'll need the following items to complete this lab session. (The standard kit for this book, available from www.thehomescientist.com, includes the items listed in the first group.)

Materials from Kit

- | | |
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| <input type="checkbox"/> Goggles | <input type="checkbox"/> Reaction plate, 24-well |
| <input type="checkbox"/> Centrifuge tubes | <input type="checkbox"/> Sodium borate solution, 0.1% w/r to boron |
| <input type="checkbox"/> Hydrochloric acid | <input type="checkbox"/> Turmeric reagent |
| <input type="checkbox"/> Pipettes | |

Materials You Provide

- | | |
|---|---|
| <input type="checkbox"/> Gloves | <input type="checkbox"/> Specimens (see text) |
| <input type="checkbox"/> Desk lamp or other strong light source | <input type="checkbox"/> Water, distilled |
| <input type="checkbox"/> Paper towels | |

Background

Soil and water pollution takes many forms. It may be chemical or biological. The source may be natural—as, for example, when heavy metals leach from a crumbling rock face or scat from wild animals contaminates an apparently pristine mountain stream—or the result of human activities such as manufacturing, mining, or even simply doing a load of laundry.

Environmental scientists and technicians frequently test soil and water specimens to determine if they are contaminated by specific pollutants, ranging from specific bacteria, protozoa, or fungi to heavy metals to organic solvents to inorganic ions such as phosphates or nitrates.

These tests are often done in two phases. The first phase, called screening tests, often uses color-test reagents. Screening tests are fast, inexpensive, and can usually be done in the field. Negative results from a screening test are normally accepted as evidence that a particular pollutant is not present in a specimen at levels high enough to be of concern. A positive screening test is accepted as evidence that the pollutant in question is probably (but not certainly) present at levels high enough to be of concern. (For many pollutants, including many heavy metals, any level high enough to be detected by a screening test is by definition high enough to be of concern.) A

positive screening test is normally followed by instrumental testing, which is more sensitive and more accurate than a screening test, but is also more expensive, time-consuming, and must normally be done in a formal lab.

For example, our municipal water authority constantly tests our drinking water for a wide range of heavy metals (chromium, lead, and so on) as well as for various biological contaminants, such as *E. coli* bacteria. If a screening test indicates the presence of chromium ions at any level, that specimen is immediately subjected to instrumental testing. Similarly, a positive screening test for coliform bacteria (such as *E. coli*) is cause for immediate followup tests, including culturing and possibly DNA analysis of the culture to determine which *E. coli* variant is present.

Most biological water testing depends on *E. coli* as a proxy for microbial contamination because *E. coli* is the longest-lived among the species that commonly contaminate water supplies. If *E. coli* is absent, it's assumed that all other common microbial pollutants are also absent. If *E. coli* is present, even one of the non-pathogenic variants, it is assumed that other, pathogenic microbes may also be present. That specimen is cultured to determine the types and numbers of microbes present.

In this lab session, we'll test soil and water specimens for the presence and concentration of boron, a light element that is widely distributed in soil and water. Most people are familiar with two boron compounds: sodium borate (borax) and boric acid. Borax is widely used as a laundry supplement, and boric acid is used for purposes as diverse as making eye drops, killing small rodents, and treating insect infestations on rosebushes.

At the levels common in soil and water specimens—typically one ppm or less—boron is innocuous. In fact, as we mention in another lab session, boron is an essential micronutrient for plant growth. Nonetheless, at the much higher concentrations sometimes found in soil and water samples, boron hinders the growth of many plants. For that reason, environmental scientists may need to test boron levels in soil and water samples.

The standard screening test for boron, called the curcumin test, has been in use for more than 100 years. This test depends on the fact that boric acid reacts with an organic chemical called curcumin, which is found in the spice turmeric, to form an intensely-colored red complex called rosocyanine. The curcumin test is extremely sensitive, able to detect boron at levels of 1 part per million or less. It's also sufficiently selective to yield reliable results. (Some other chemicals yield a positive curcumin test, but none that are likely to be found in environmental samples.) Finally, the curcumin test is fast and costs only pennies per test.

The curcumin test is specific for boric acid, as opposed to boron in the form of borate salts, which yield negative results with curcumin. For this reason, the first step in the curcumin test is to acidify the specimen with hydrochloric acid, which converts any borate salts present into boric acid.

Procedure V-4-1: Obtaining soil and water specimens

In this procedure, we'll gather soil and water specimens that we'll later test for the presence and concentration of boron. Depending on your local environment, you may later find that all, some, or none of your specimens contain boron at levels that are detectable by the test we'll use.

Always wear gloves when gathering soil or water specimens. In particular, water specimens may contain pathogenic bacteria, protozoa, fungi, or other dangerous organisms.

1. Obtain at least two or three water specimens from different sources, such as a pond, stream, ditch, and so on. Label a centrifuge tube with the date and source of the specimen. Simply fill the tube with the specimen and recap it.
2. Obtain at least two or three soil specimens from different sources. Simply transfer about a tablespoon of soil to a labeled and dated centrifuge tube and recap the tube.

If you run out of centrifuge tubes, substitute clean soda bottles, plastic bags, or similar containers.

3. When you return home, rinse each of the specimen containers thoroughly in running tap water to remove any external contamination and wash your hands thoroughly.
4. Put on goggles and gloves.
5. Uncap each water specimen container and add two drops of hydrochloric acid. Recap the container and invert it several times to mix the solutions.
6. Uncap each soil specimen container, fill it nearly full of tap water, and add two drops of hydrochloric acid. Recap the container and invert it several times to mix the solutions. Allow the solids to settle.

If you have a balance, you can obtain quantitative results by weighing the soil specimens and measuring the volume of water added. For example, you might use 10 g of soil and 40 mL of water and subsequently determine that the boron concentration in the water is 20 ppm. Assuming that all of the boron in the soil has dissolved in the water, you can calculate that a boron concentration of 20 ppm in 40 g of water means that the boron concentration in your 10 g of soil must be 80 ppm.

7. Place the containers aside until you are ready to test the specimens.

Procedure V-4-2: Testing the reagents

Before analyzing specimens, careful scientists test their reagents to make sure that they perform as expected. Before proceeding to specimen analysis, we need to verify that our curcumin reagent reacts as expected with a specimen known to contain boron.

1. If you have not already done so, put on your goggles and gloves. Review the hazards of each chemical you will use.
2. Place the 24-well reaction plate on a white paper towel under a desk lamp or other strong light source.
3. Transfer 1 mL of distilled water to each of wells A1, B1, C1, and D1.
4. Transfer 5 drops of the sodium borate solution to each of wells B1 and D1.
5. Transfer 1 drop of 6 M hydrochloric acid to each of wells B1 and C1.

At this point, well A1 contains only distilled water, well B1 contains sodium borate and hydrochloric acid, well C1 contains hydrochloric acid, and well D1 contains sodium borate but no hydrochloric acid. By observing which well or wells exhibit a color change, we can determine which of the chemicals or combination of chemicals causes that color change.

6. Add two drops of the turmeric reagent to each of wells A1, B1, C1, and D1.
7. Observe the color changes, if any, in each of the wells, and record your observations in your lab notebook.

Procedure V-4-3: Making boron concentration comparison standards

In this procedure, we'll set up a two 3X3 arrays in the reaction plate. We'll later use the center wells of these arrays for testing actual specimens. Each of the eight surrounding wells will have a different concentration of boron ions, so we can easily compare the center specimen well with each of the eight adjacent wells in each array.

1. If you have not already done so, put on your goggles and gloves. Review the hazards of each chemical you will use.
2. Place the 24-well reaction plate on a white paper towel under a desk lamp or other strong light source.
3. Using a graduated pipette, transfer, as accurately as possible, 1.0 mL of distilled water to each of wells A2, A3, B1, B2, B3, C1, C2, and C3.
4. Transfer, as accurately as possible, 1.0 mL of the sodium borate solution to well A1 and another 1.0 mL of the sodium borate solution to well A2. Mix the solution in well A2 thoroughly by stirring it with the tip of the pipette and by drawing up and expelling the solution several times.

At this point, we have 1.0 mL of sodium borate solution in well A1. That solution is 0.1% with respect to boron, which can also be stated as 1,000 parts per million (ppm) boron. Well A2 contains 2.0 mL of solution that is 500 ppm with respect to boron.

5. Draw up 1.0 mL of the solution in well A2 and transfer it to well A3. Mix the solution thoroughly. Well A2 now contains 1.0 mL of 500 ppm boron, and well A3 2.0 mL of 250 ppm boron.
6. Draw up 1.0 mL of the solution in well A3 and transfer it to well B3. Mix the solution thoroughly. Well A3 now contains 1.0 mL of 250 ppm boron, and well B3 2.0 mL of 125 ppm boron.
7. Draw up 1.0 mL of the solution in well B3 and transfer it to well C3. Mix the solution thoroughly. Well B3 now contains 1.0 mL of 125 ppm boron, and well C3 2.0 mL of ~63 ppm boron.
8. Draw up 1.0 mL of the solution in well C3 and transfer it to well C2. Mix the solution thoroughly. Well C3 now contains 1.0 mL of ~63 ppm boron, and well C2 2.0 mL of ~31 ppm boron.
9. Draw up 1.0 mL of the solution in well C2 and transfer it to well C1. Mix the solution thoroughly. Well C2 now contains 1.0 mL of ~31 ppm boron, and well C1 2.0 mL of ~16 ppm boron.
10. Draw up 1.0 mL of the solution in well C1 and transfer it to well B1. Mix the solution thoroughly. Well C1 now contains 1.0 mL of ~16 ppm boron, and well B1 2.0 mL of ~8 ppm boron. To keep the solution level in all wells equal, draw up 1.0 mL of the solution in well B1 and discard it.

At this point, we have eight wells that contain boron solutions, with each well half as concentrated as the preceding well. The concentrations in this first 3X3 array range from 1,000 ppm down to about 8 ppm. The next step is to create a second 3X3 array of wells that contain concentrations ranging from 4 ppm down by halves. To avoid small fractions, we'll shift to using ppb (parts per billion) to specify concentrations for this second array. A concentration of 4 ppm is the same as 4,000 ppb, so that's where we'll start.

11. Transfer, as accurately as possible, 1.0 mL of distilled water to each of wells A4, A5, A6, B4, B5, B6, C4, C5, and C6.
12. Transfer, as accurately as possible, 1.0 mL of the 8 ppm sodium borate solution from well B1 to well A4 to produce a solution that's about 4 ppm (4,000 ppb) with respect to boron. Mix the

solution in well A4 thoroughly by stirring it with the tip of the pipette and by drawing up and expelling the solution several times.

13. Repeat the serial dilution procedure to produce concentrations of ~2,000 ppb (A5), ~1,000 ppb (A6), ~500 ppb (B6), ~250 ppb (C6), ~125 ppb (C5), ~63 ppb (C4), and ~31 ppb (B4).
14. Add one drop of turmeric reagent to each of the 18 populated wells in the two arrays. The center wells (B2 and B5), which contain only distilled water, provide a reference to compare the other wells against. Those two wells should have a noticeable yellow color, which is the natural color of the reagent. Depending on lighting conditions, the yellow in those wells may be too pale to see clearly. If that's the case, add one more drop of the turmeric reagent to wells B2 and B5. If that's sufficient to show the contents of those wells as clearly yellow, also add one more drop of the turmeric reagent to each of the other populated wells.
15. Record the concentrations of the wells and your observations of any color changes that occurred (and how quickly or slowly they occurred) in your lab notebook. Include a sketch of the layout and concentrations of the wells.

At this point, you can continue immediately with the following procedure, testing environmental specimens. If you delay performing that procedure for a significant length of time, carefully cover the reaction plate with its lid to prevent evaporation.

Procedure V-4-4: Testing specimens for boron

In this procedure, we'll test our environmental specimens for the presence and concentration of boron.

1. If you have not already done so, put on your goggles and gloves.
2. Transfer 1 mL of your first specimen to well B5 and add the number of drops of turmeric reagent that you decided was optimum in the preceding procedure. Note any color change and the intensity of the color relative to the comparison wells, and record your observations in your lab notebook.

If the color of the specimen is intermediate between the colors of two comparison wells, estimate the concentration of the specimen based on interpolation. For example, if the color of the specimen is about midway between the 4 ppm and 2 ppm comparison wells, you might record the concentration of the specimen as about 3 ppm. If the color is between 2 ppm and 4 ppm but closer to 4 ppm, you might record the concentration of the specimen as about 3.5 ppm.

3. If the specimen showed no color change, or if the color is within the range of the comparison wells surrounding well B5, proceed to the next step. If the color is more intense than any of the comparison wells, repeat the preceding step, starting with 1 mL of the specimen in well B2.
4. Use a pipette to withdraw the liquid from well B5 (and B2, if you used it) and discard it. Use the corner of a paper towel to absorb the last few droplets. Fill the well with distilled water, stir the contents, and empty the well with a pipette. Repeat this rinse twice and then dry the well with the corner of a paper towel. The well is now ready for testing the next specimen.

Incidentally, don't be disappointed if none of your environmental samples contain detectable levels of boron. Some do; some don't. In science, negative results are often as useful as or more useful than positive results.

Review Questions

??? RBT: review questions/answers and add additional material after completing the labs.

??? Production: Please strip the answers (formatted as comments) from the final layout. RBT

1. We used acidified tap water to solubilize any boron salts present in the soil specimens. What potential error does this introduce? What change in procedure might we make to eliminate this potential error?

It's possible that tap water contains sufficient boron to be detectable with our reagent. We could eliminate this potential error by using distilled water. Alternatively, we could test the tap water (after acidifying it), and use it if it yields a negative test for boron.

2. Did you detect boron in any of your water or soil specimens? If so, at what level?

Answers will vary. Boron is widely distributed in the environment, but often at levels that are below the detection threshold of the turmeric reagent. The most common source of detectable boron levels is soil from flower beds or gardens that have been treated with boric acid or borax as an insecticide. Borax is also widely used as a laundry supplement and for buffering swimming pool water, so any body of water into which these sources flow may contain detectable boron.

3. Describe an experiment to determine the effect of elevated boron levels on plant growth.

Answers will vary, but one possible experiment would be to grow seedlings in foam cups filled with sand or vermiculite and with their bottoms punched for drainage. Water the seedlings regularly with fertilizer solutions that contain 0, 1, 10, 100, and 1,000 ppm boron and observe the effect, if any, of each boron level on the corresponding seedlings.