

Toxic! Or Is It?

One of the big questions about any type of pollution is, "Will it hurt plants, animals, or humans?"

How can we find out? We don't want to risk harming lots of living organisms to see whether a substance is toxic, especially if the organisms are humans!

Scientists investigating pollution often use a technique called "bioassay" to test for toxicity. A bioassay is a measurement of the effects of a substance on living organisms. Tests for toxic substances use certain species called "indicator species," which may be juvenile fishes, plant seeds, microscopic animals, or even bacteria! Several individuals of an indicator species are exposed to the substance being tested for a certain period of time, and then compared to another group of the same species that was not exposed to the substance (the second group is called the "control group"). If the organisms in the test group show effects such as slow growth, reduced movement, or death and these effects are not seen in the control group, this is considered to be an indication that the substance being tested is toxic.

Bioassays usually do not indicate which specific substances cause toxicity, or the amounts of toxic substances present. But they do provide a good indication of the total toxicity of samples that may contain more than one toxic substance, and they are a quick and inexpensive way to test for toxicity in many samples.

Here is a simple bioassay that you can use to test for toxicity in runoff water that you suspect may be contaminated.



Beach pollution at Bay St. Louis, MS after Hurricane Katrina. Courtesy NOAA.

What You Will Need

- 12 or more ziplock plastic bags
- One paper towel for each plastic bag
- Ten radish seeds for each plastic bag
- Permanent marker for labeling sample jars and plastic bags
- Household bleach, about 1/4-cup
- Distilled water, about one quart
- Ruler graduated in millimeters (mm)
- Tweezers or forceps
- Glass containers for collecting samples; baby food jars or similar size are perfect
- Clean glass jar for mixing bleach solution, about one pint
- Measuring cup that is not used for food
- Protective gloves and safety glasses, one set for each person participating in this activity

What You Will Do

Use a radish seed bioassay to test for toxicity

Warnings

- Do this activity with an adult partner!
- Always check with an adult before collecting samples that may be contaminated with harmful substances.
- Wear protective gloves and safety glasses when handling sample solutions!
- Do not use glasses, dishes, or any container that is used for food!
- Wash your hands after handling sample solutions, paper towels, and seeds!

How to Do It

1. Collect water samples to be tested. You may want to test water from puddles, streams, or ponds. Do this **ONLY** with an adult partner! Wear protective gloves and safety glasses! Collect each sample in a clean glass jar, and label the jar with a number.
2. Put on protective gloves and safety glasses, then mix one ounce of household bleach with nine ounces of distilled water in a clean glass jar.
3. Put the radish seeds in a clean glass container, and add about two ounces of the bleach solution from Step 2. The bleach solution kills fungi, which could interfere with seed germination.
4. Bioassays are carried out in zip-lock plastic bags containing a paper towel saturated with the test solution. Saturated means

that the towel is damp all over, but isn't dripping. Three separate tests (called "replicates") will be done for each solution. Prepare three plastic bags for each sample to be tested, plus three more bags for the control solution (distilled water). Label each bag with the sample number, plus a letter to identify the three replicates (so for sample number 1, there would be three bags labeled "1-A," "1-B," and "1-C"). Put a folded paper towel in each bag.

5. Pour enough sample solution into each bag to saturate the filter paper. Use the same amount of sample in all tests. Saturate the paper towels in the control bags with the same amount of distilled water.
6. Using tweezers or forceps, place ten seeds on the paper, evenly spaced, in each bag. Seal the top of the bag.
7. Put the bags on a tray or shallow box where they can remain for five days. The bags should be at room temperature, but do not place them in direct sunlight! Each day, record the number of seeds that germinated (split open) in each bag, and measure (to the nearest mm) the length of the root that has emerged from each germinated seed.
8. At the end of five days, calculate the average number of seeds that germinated in the three bags for each sample and for the

controls. Also, calculate the average length of the roots among the germinated seeds in the three bags. If fewer seeds germinated in bags containing test samples, or if the roots grew more slowly than in the control bags, this is an indication that the samples may contain toxic substances.

Want to Do More?

1. For more information about simple bioassays, see "A Simple Bioassay Using Lettuce Seeds," by Joe Rathbun, in *The Volunteer Monitor* volume 8, number 1 (available online at <http://www.epa.gov/owow/monitoring/volunteer/newsletter/volmon08no1.pdf>); and "Students Test for Toxics," by Mark Mitchell, in *The Volunteer Monitor* volume 5, number 1 (available online at <http://www.epa.gov/owow/monitoring/volunteer/newsletter/volmon05no1.pdf>).
2. Visit <http://www.epa.gov/owow/nps/eduinfo.html> for more information about polluted runoff.

This activity was adapted from The Seeds Tell the Story (21 pages, pdf, 660 Kb) by Mel Goodwin, The Harmony Project, Charleston, SC; part of NOAA's National Ocean Service Nonpoint Source Pollution Discovery Kit [http://oceanservice.noaa.gov/education/kits/pollution/lessons/pollution_seeds.pdf].