INVESTIGATION

29

Lab

Bioassay Experiment

PURPOSE

- Conduct a controlled experiment to test the toxicity of salt on the growth of lettuce seeds
- Apply the experimental results to environmental problems
- Design a bioassay experiment

INTRODUCTION

The use of a biological organism to test the toxicity of a chemical compound is termed **bioassay**. In this method it is assumed that a test organism will react in a predictable way to increasing amounts of a particular chemical compound. Bioassay has been used by drug companies to test new products on laboratory animals before humans. Bioassays are also used in environmental testing. They can determine the degree of harm to be expected from toxic soil, industrial effluents, agricultural runoffs, dredge spoils, and drilling and mining wastes, as well as to test for the effectiveness of the clean-up of a contaminated site.

In this investigation you will perform what is called a **dose/response experiment**. This method requires you to increase the dose of a chemical incrementally and record how the organism responds to the exposures. For a test organism, you will use lettuce seeds, the Buttercrunch variety if it is available. Lettuces are commonly used in bioassays, along with millet, because their root growth, rather than just germination rate, is especially sensitive to many chemicals. For a variety of reasons salt solutions will be the toxin. Salt is inexpensive and safe to use, and, as you learned in your investigation of soil salinization, it is a widespread environmental problem for plants.

Materials

- lettuce seeds (preferably Buttercrunch)
- salt
- graduated beaker (1 per group)
- petri dishes (10 per group)
- bleach
- filter paper
- distilled or deionized water
- sealable bag (1 large)
- semi-log graph paper

Optional:

- Excel spreadsheet

Procedure

Step 1 Make salt water solutions that are the following percents by volume:
3% 2.5% 2% 1.5% 1% 0.5% 0.1% 0.05% 0.01%
Each lab group can make 100 mL of one solution and share with the other groups.

Step 2 Label 10 petri dishes with your name and the salt concentration. Nine will be test solutions and one will be the control with distilled or deionized water.

Step 3 Place a piece of filter paper on the bottom of each petri dish. (You can also use a piece of fresh paper towel cut to size.)

Step 4 Soak your lettuce seeds in a solution of bleach for 10–15 minutes to kill off any fungus that may interfere with the results of the experiment. Rinse the seeds thoroughly 3–5 times in distilled or deionized water.

Step 5 Place 10 lettuce seeds on the paper in the bottom of each petri dish. Make sure there is a separation between each seed.

Step 6 Cover the seeds with another piece of filter paper or paper towel.

Step 7 Soak the seeds in each petri dish with the appropriate salt solution. Be careful not to let standing liquid solution accumulate on the bottom of the dish. Add just enough solution to moisten the paper tops and bottoms.

Step 8 Add distilled or deionized water to the control petri dish.

Step 9 Seal all 10 petri dishes in a large sealable bag labeled with your name. Put the bag in a dark place at room temperature for 5 days.

Step 10 Make a table of data to include

- the number of seeds that germinated,
- the percent germination,
- the average length of the root, called the **radicle**.

Step 11 After 5 days, open the sealable bag and start to collect your data. Count how many seeds in each petri dish germinated and measure the radicle in millimeters. Be sure that you measure only the root, from the seed remnant to the tip of the root, not the shoot and beginnings of leaves.

Step 12 Calculate the average radicle length for each petri dish and record these data in your data table.

Step 13 Graph the average length of the radicle against salt solution strength on semi-log graph paper. The salt solutions will be plotted logarithmically. (This can be done with pencil and paper or using a spreadsheet like Excel.)

Step 14 On the same graph, plot the percent germination versus the salt solution strength. To do this, make another *y*-axis on the right end of the *x*-axis.

1a. What is meant by the term ***threshold of toxicity***? In what other contexts have you seen this term used?

b. On your graph of percent germination vs. solution strength, label the Threshold of Toxicity.

2a. What is meant by ***LD-50***? Describe some situations in which it is used.

b. Label LD-50 on your graph of percent germination vs. solution strength.

3. Discuss the three environmental effects of using sodium chloride (NaCl) on roads and highways during ice and snow storms.

4. A common homeowners' substitute to sodium chloride for de-icing driveways and sidewalks is calcium chloride, $CaCl_2$. Using your background knowledge of ***colligative*** properties from chemistry, explain why calcium chloride should be a more effective de-icing agent.

Problems

Problems

5. Water solutions of toxins are usually dilute, making it difficult to use them in a bioassay. Sediments, especially silty ones, make a very good test medium because they can be thousands and sometimes millions of times more contaminated than water. Outline a bioassay procedure to test the toxicity of a contaminated sediment using lettuce seeds as the test organism.

6. Suppose you had to design the bioassay of a potentially toxic material with which you are not familiar. How would you decide on the number and intervals of concentrations to test? How would you determine the starting and final concentrations?