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Catalog No. FB1881

Publication No. 10845

Bioassay Experiment

Environmental Science Laboratory Kit

Introduction

Bioassays are scientific experiments that are commonly used to determine the effects of a substance on a living organism. In this activity, a controlled experiment on the effect of different concentrations of salt solutions on the growth of lettuce seeds will be performed to better understand bioassays.

Concepts

- Bioassay
- Toxicity
- LD_{50} values

Background

Any substance, including natural compounds obtained from plant and animal sources, can be harmful to living organisms. However, it is the dose that frequently determines the extent of the damage. Toxicologists have developed a variety of tools to evaluate the toxicity of chemicals. Acute toxicity is the immediate effect of a substance from a single dose and is relatively easy to study. Chronic toxicity results from low doses repeated over long periods of time and is much more difficult to test.

Toxicologists use LD_{50} values to estimate the acute toxicity of chemicals on humans. LD means the *lethal dose* and is expressed in milligrams of chemical per kilogram of body weight. The 50 in LD_{50} represents a mortality rate of 50% of the test animals in a toxicity study. LD_{50} values are calculated from dose–mortality curves. A typical statement reads as follows:

Aniline LD_{50} oral–rat: 250 mg/kg

This statement means that an oral dose of 250 mg of aniline per kg of body weight will kill 50% of the test sample of rats. It should be noted that no LD_{50} data exists for humans (for obvious reasons). Data from test animals is used to estimate the acute toxicity of chemicals on humans. Toxicity data should be used to understand the *relative* toxicity of chemicals and serve as a guide to asses relative danger in handling certain chemicals.

The use of a biological organism to test the toxicity of a chemical is known as *bioassay*. Bioassays are commonly used in environmental studies and by drug companies testing new products. Bioassays can help predict the possible environmental effects or damage due to agricultural runoff, drilling and mining wastes, toxic chemicals in soil, and industrial waste. They can also be used to determine the effectiveness of the cleanup of a certain contaminated site.

Experiment Overview

The purpose of this activity is to perform a method known as the dose/response experiment and determine how an organism responds to different concentrations of a potential toxin or chemical. Lettuce seeds will be used as the experimental subject and common table salt, sodium chloride, NaCl, will be used as the toxin. The effect of salt concentration on the germination rate of lettuce seeds will be measured.

Materials

Lettuce seeds, 70
Salt (sodium cloride) solutions, NaCl:
- 3%, 5 mL
- 2%, 5 mL
- 1%, 5 mL
- 0.5%, 5 mL
- 0.1%, 5 mL
- 0.01%, 5 mL

Water, distilled or deionized
Bag, plastic, resealable
Beaker, 250-mL or similar container
Bleach, sodium hypochlorite, 10 mL
Calculator
Clock or timer
Filter paper, 7 pieces
Graph paper
Marker
Petri dishes, 7
Pipet, Beral-type, 7
Tape, transparent

Safety Precautions

Bleach is a corrosive liquid that causes skin burns and is moderately toxic by ingestion and inhalation. Wear chemical splash goggles, chemical-resistant gloves, and a chemical-resistant apron. Wash hands thoroughly with soap and water before leaving the laboratory. Follow all laboratory safety guidelines.

Procedure

1. Obtain 5 mL each of the following six different concentration salt solutions—3%, 2%, 1%, 0.5%, 0.1%, and 0.01%. Also obtain 5 mL of distilled or deionized water (control solution).
2. Obtain and label seven Petri dishes with your group's initials and the salt concentration listed in step one. One Petri dish should be labeled control.
3. Place a piece of filter paper in each Petri dish.
4. Obtain approximately 70 lettuce seeds in a small beaker or similar container.
5. Completely cover the seeds with a bleach solution.
6. Allow the seeds to soak in the bleach solution for 10 minutes.
7. After 10 minutes, pour off the bleach solution and rinse the seeds well with distilled water three times.
8. Place 10 lettuce seeds on the filter paper in each Petri dish.
9. Cover the seeds in each dish with another piece of filter paper.
10. Using a clean new pipet for each salt solution, thoroughly soak the seeds in each Petri dish with the appropriate salt solution.
11. Place the lid on each Petri dish and secure with tape.
12. Place all seven Petri dishes in a plastic bag. Label the bag with your group's initials.
13. Place the bag in a dark location at room temperature for five days.
14. After five days, open the bag and count the number of seeds that germinated, the percent germination and the average length of the root on the Bioassay Worksheet.
15. Determine the log of each of the salt concentrations and record the values on the Bioassay Worksheet.
16. Graph the average length of the root versus the log of the salt concentration on graph paper.
17. Plot the percent germination versus the log of the salt concentration on the same graph. Do this by drawing a second *y*-axis on the right end of the *x*-axis.
18. Answer the *Post-lab Questions*.

Name: _____

Bioassay Worksheet

Data Table

NaCl Concentration	Number of Seeds Germinated	Percent Germinated	Root Length (mm)	Average Root Length (mm)	Log (Salt Conc.)
3%					
2%					
1%					
0.5 %					
0.1%					
0.01%					
Control (0%)					

Post-Lab Questions

1. Define LD_{50}. Describe situations where it may be used.

2. Using the percent germination results, determine the concentration of salt solution that resulted in a 50% "death" of lettuce seeds. Label this point on the graph.

3. Discuss three environmental effects of using sodium chloride as a deicing agent on highways and roads in the winter.

4. How would you determine the concentration of the solutions and the number of tests to be performed, if you were to design a bioassay of an unfamiliar potential toxin?

5. Outline the steps involved in designing a bioassay to test the toxicity of contaminated soil using lettuce seeds.

Name: _____

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