Radish Seed and NaCl Toxicity Bioassay

Objectives

- Conduct a radish seed toxicity bioassay to assess the effects of NaCl exposure to seed germination and radicle length.
- Practice using serial dilutions to create several different concentrations of NaCl.
- Plot toxicity data to establish dose-response curves and estimate LC50 values.

Hypothesis *(Place your prediction below)*

Key Terms

- Radicle: the part of a plant embryo that develops into the primary root
- Germination: the sprouting of a seedling from a seed
- Serial Dilutions: the stepwise dilution of a toxicant in a solution
- Dose-Response Effect: the relationship between the quantity of a toxicant and the effect or response of the organism due to exposure
- EC50: the concentration of a compound where 50% of its maximal effect is observed.

Materials

NaCl (0.5 g table salt packets)
Distilled (DI) water
10 Petri dishes with lids
~~Paper towels~~ Coffee Filter
Radish Seeds
100 ml graduated cylinder
Transfer pipettes
Tape
Ruler

Instructions

1. Initial Preparation

- Label 10 petri dishes: Control, Level 1, Level 2, … , Level 9
- Place a ~~piece of paper towel~~ coffee filter into each of the petri dishes

2. Control Treatment

- The purpose of the control treatment is to provide a standard for comparison against the other treatment groups.
- The control treatment solution consists of water only. *Do not add salt.*
- Pipette 5 ml of this DI only into the petri dish with paper towel labelled "Control"
- Cover the petri dish and put aside for later

3. Preparing Level 9

- Add 2 g of NaCl to approximately 90 ml of water in the graduated cylinder
- Mix to completely dissolve
- Bring the volume up to a final volume of 100 ml to make a 20 g/L NaCl solution
- Mix thoroughly
- Pipette 5 ml of this solution into the petri dish with paper towel labelled "Level 9"
- Cover the petri dish and put aside for later

4. Preparing Level 8

- Using your transfer pipette, discard exactly 20 ml of solution 9
- Add 25 ml of DI to the remaining 75 ml of solution 9
- This is a 25% dilution and the solution is now a 15g/L NaCl solution
- Mix thoroughly
- Pipette 5 ml of this solution into the petri dish with paper towel labelled "Level 8"

5. Serial Dilutions

- Continue to make serial dilutions using the methods outlined in step 4 and the following proportions. The solutions correspond with NaCl concentrations described in Table 1.
    - **Level 7** = 75 ml Level 8 + 25 ml water
    - **Level 6** = 75 ml Level 7 + 25 ml water
    - **Level 5** = 75 ml Level 6 + 25 ml water
    - **Level 4** = 75 ml Level 5 + 25 ml water
    - **Level 3** = 75 ml Level 4 + 25 ml water
    - **Level 2** = 75 ml Level 3 + 25 ml water
    - **Level 1** = 75 ml Level 2 + 25 ml water

Table 1. Test treatment concentrations of NaCl.

Level	NaCl Concentration (g/L)
Control	0.00
1	2.00
2	2.67
3	3.56
4	4.75
5	6.33
6	8.44
7	11.25
8	15.00
9	20.00

6. Test Initiation

- Place 10 seeds into each dish. Make sure they are evenly spaced.
- To maintain moisture, place the lids on the petri dishes and seal with parafilm. or tape.
- Position the petri dishes in a place away from direct sunlight at room temperature for 7 days.

7. Recording Data

- After 7 days, measure the radicle length in mm (Figure 1) and record data.
- After 7 days, count the number of seeds germinated in each treatment and record data.

Data Form

Radicle Length Results

Measure the radicle length in mm of each seedling (Figure 1) and record the data in Table 2.

Figure 1. The radicle of a seedling.

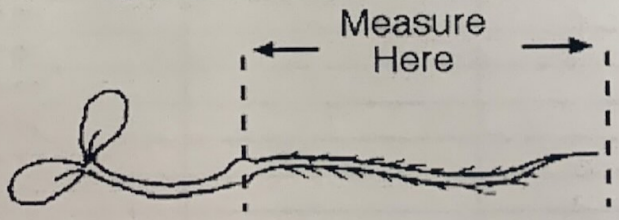

Table 2. Average radicle lengths of radish seeds exposed to a series of NaCl concentrations for a duration of 7 days.

Level	Radicle Length (mm)										Average Length (mm)
Control											
1											
2											
3											
4											
5											
6											
7											
8											
9											

Growth Inhibition Analysis

Calculate growth inhibition for the average radicle lengths (Equation 2) and record the results in Table 3. Plot growth inhibition and NaCl concentration (Figure 2). Indicate the EC50 value for growth inhibition on the graph.

$$\text{Growth Inhibition (\%)} = \frac{RL_c - RL_{ti}}{RL_c} \times 100\% \qquad \text{Equation 2}$$

RL_c = Mean Control Radicle Length (mm)

RL_{ti} = Mean Treatment Radicle Length (mm)

Table 3. Percent growth inhibition of radish seeds exposed to a series of NaCl concentrations over the duration of 7 days.

Level	NaCl concentration (g/L)	Average Length (mm)	Growth Inhibition (%)
Control	0.00		
1	2.00		
2	2.67		
3	3.56		
4	4.75		
5	6.33		
6	8.44		
7	11.25		
8	15.00		
9	20.00		

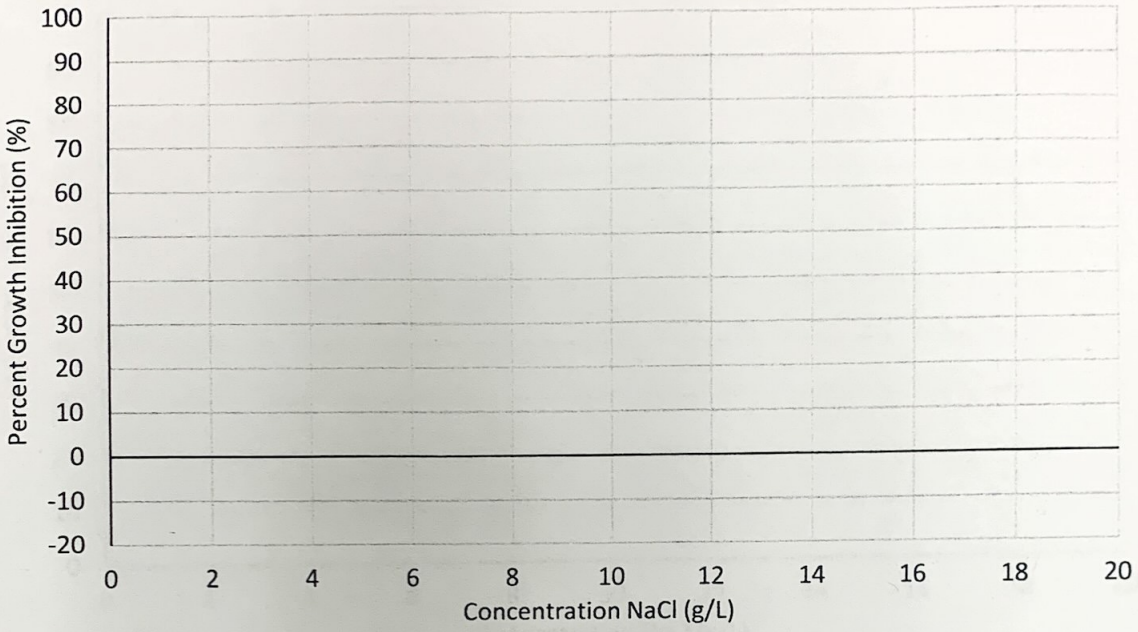

Figure 2. Percent growth inhibition of radish seeds exposed to a series of NaCl concentrations over the duration of 7 days.

Seed Germination Results

Record the number of seeds germinated per test treatment and calculate the germination failure percent (Equation 1) and record the results in Table 4. Note, germination failure is defined as a seed with a radicle less than 5 mm in length. Plot germination failure and NaCl concentration in Figure 3. Indicate the EC50 value for germination failure on the graph.

$$\text{Germination failure (\%)} = \frac{\text{Failed Seeds}}{\text{Total number of Seeds}} \times 100\% \qquad \textbf{Equation 1}$$

Table 4. Percent germination failure for radish seeds exposed to a series of NaCl concentrations over the duration of 7 days.

Level	NaCl concentration (g/L)	Germination (seeds/dish)	Germination Failure (%)
Control	0.00		
1	2.00		
2	2.67		
3	3.56		
4	4.75		
5	6.33		
6	8.44		
7	11.25		
8	15.00		
9	20.00		

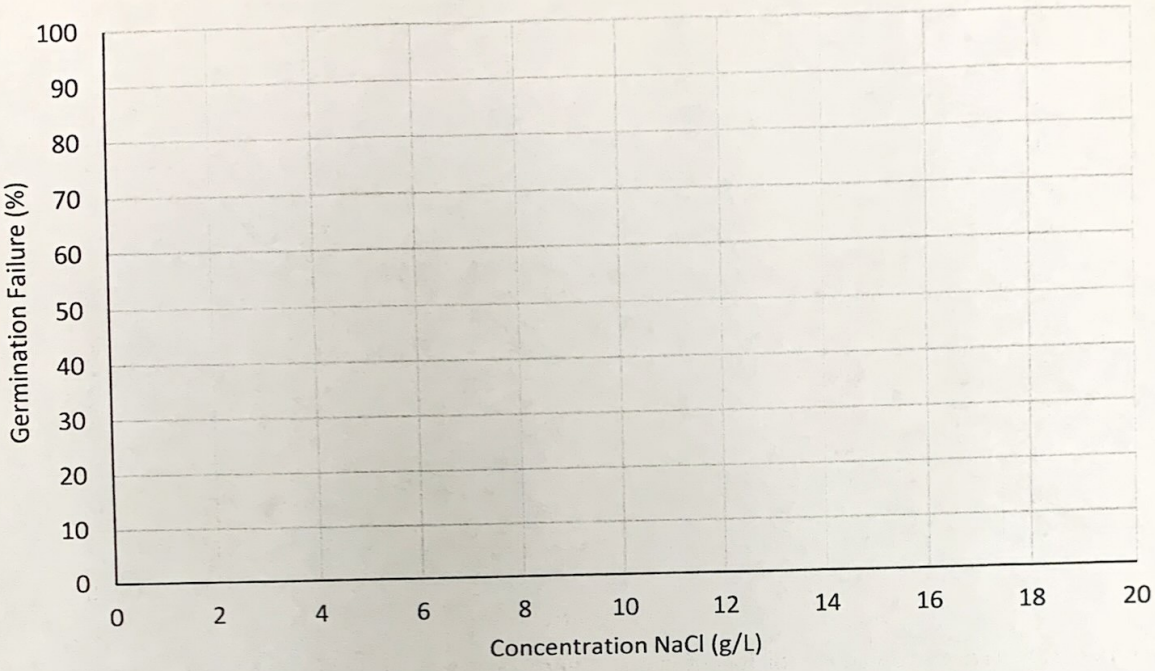

Figure 3. Percent germination failure for radish seeds exposed to a series of NaCl concentrations over the duration of 7 days.

Discussion Questions

- How does your control results compare to your test treatments? What would poor germination success or small radicle lengths in your control indicate?
- Describe the germination and radicle length trends. Where your hypotheses correct?
- *Hormesis* occurs when a favorable biological response results from at very low exposures of toxins or stressors. In other words, hormetic compounds stimulate at low doses and inhibit at high doses. Do you observe this effect in your experiment?
- What is the difference between the term EC50 and LC50?
- Estimate the germination EC50 and radicle length EC50 for your data.
- Which endpoint is more sensitive to NaCl? Use the EC50 values to explain your answer.
- Are there differences in the trends between these two indicators of toxicity?
- What is the significance of assessing the toxicity of NaCl in the environment?