PART B: Bioassay of Ammonia on Brine Shrimp (done in groups)

Background Info.

A bioassay is a toxicity test used to determine the dose or concentration of a toxicant. In dealing with toxins, a frequent relative danger indicator is the LD50. For example the LD50 for sugar in rats is 30 grams, that is out of 100 laboratory rats, 50 would be expected to die at levels of 30 grams of sugar/kg of body weight.

A similar measure, the **LC50**, (which stands for **lethal concentration**) is often used. In this lab a small crustacean, the brine shrimp, will be used. It is normally found in brackish water and is a very hearty little organism able to tolerate high salt concentrations.

Materials

brine shrimp (from aquarium store)
brine (specifically for Brine Shrimp, mixed with aquarium water)
household non-sudsy ammonia solution
OR window cleaner such as Windex
OR Pine-Sol or some other household disinfectant
graduated cylinders
pipettes
Petri dishes (6 per group)
permanent marker, or labels for Petri dishes
test tube racks
test tubes (6 per group)
stirring rods
dissecting microscopes

Procedure

1) Label 5 test tubes as follows: 1:1, 1:10, 1:100, 1:1000, and 1:10,000. Take 11 mL of the full-strength material being tested for toxicity from the stock solution and add it to the test tube labeled 1:1.
2) Place 9 mL of brine into each of the other test tubes. Pipette 1 mL of "toxic" material from the 1:1 tube into the tube labeled 1:10. Mix well.
3) Pipette 1 mL from the 1:10 tube into the tube labeled 1:100. Mix well.
4) Pipette 1 mL from the 1:100 tube into the tube labeled 1:1000. Mix well
5) Pipette 1 mL from the 1:1000 tube into the tube labeled 1:10,000. Mix well.
6) Label six Petri dishes as follows: 1:1, 1:10, 1:100, 1:1000, 1:10,000, control. Be sure to label the *bottom of the dish, not the cover.*
7) Using a pipette, move 10 brine shrimp into each Petri dish.
8) Put 10 mL of brine in the control dish. Pour the contents of each tube into the appropriate Petri dish and observe for 10 minutes. *Be sure to add the appropriate brine solutions as quickly as possible AFTER the brine shrimp are added to the Petri dish.*
9) Using a dissecting microscope, count the number of brine shrimp alive after 10 minutes. Leave the shrimp in the dishes and determine how many are alive after 24 hours. Record your data in Data Table 2.

Data Table 2: Brine Shrimp Survival Rates

Material being tested		Number of brine shrimp alive after 10 minutes					Number of brine shrimp alive after 24 hours				
	dilution	1:1	1:10	1:100	1:1000	1:10,000	1:1	1:10	1:100	1:1000	1:10,0000
	dilution factor	10^0	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^0	10^{-1}	10^{-2}	10^{-3}	10^{-4}

Graph

1) Plot a line graph of concentration (x axis) vs. mortality (y axis). Doing the graph on the computer is recommended..
2) Determine the LC-50 from your graph.

Questions

1) What is (are) the control(s) in this experiment?
2) Based on your data in this lab, what is the safe concentration for brine shrimp (**Lowest Observable Effect Concentration** or **LOEC**?
3) 10 ppm ammonia is the standard for drinking water for humans. Can brine shrimp survive in our drinking water at this limit of ammonia?
4) Often indicator species are used to study the overall health of an ecosystem. If you were to study an ecosystem containing brine shrimp, would you use it as an indicator species? Why or Why not? Explain your reasoning.
5) What possible sources of error were present in this experiment?