Salinity Extremes – Version 2

Objective: To determine the saline concentration limits at which hypersaline organisms can live, students will modify by dilution and salinity concentration several brine samples from a natural hypersaline environment.

This activity is intended to be a "quick and dirty" field analysis;
it does not follow standard microbiological procedures.

There are many variations on this activity that can be attempted in the classroom.
This version has not been tested … but may have merit. Feedback is welcome!

Materials

For class:

- Sample of natural brine from a hypersaline environment with living, mobile microorganisms present
- Salts from hypersaline environment
- 2 or more scales for measuring salt (postal scale can be used)
- Refractometer or hydrometer within the range of the natural brine
- A few laser pointers (optional)

For each group of 4 students:

- ~500 ml of brine
- 8 or more 150 to 250 ml sterile Nalgene bottles with lids (depending on experiment)
- Weighing paper
- Small spatula / scoopula
- Distilled water
- Sterile microscope slides with wells
- Sterile pipettes
- Lab notebook and writing utensils

For each student:

- Protective eyewear
- Lab gloves

Time

1 50 minute class period to set up
Several days to allow samples to culture
1 50 minute class period for observations and discussions

National Science Education Standards

Science as inquiry. Content Standard A
Students will ask questions, plan and conduct an experiment, employ simple equipment and tools to gather data, analyze and interpret results to construct a reasonable explanation, communicate investigations and explanations.

K-4. Life Science. Content Standard B. Organisms have basic needs. Behavior of individual organisms is influenced by internal and external queues. An organism's patterns of behavior are related to the nature of that organism's environment.

5-8. Life Science. Content Standard B.
All organisms must be able to obtain and use resources, grow, reproduce, and maintain stable internal conditions while living in a constantly changing environment. Regulation of an organism's internal environment involves sensing the internal environment and changing physiological activities to keep conditions with in the range required to survive. Biological adaptations include changes in structures, behaviors, or physiology that enhance survival and reproduction in a particular environment.

9-12. Life Science. Content Standard B. Most cell functions involve chemical reactions. Cell functions are regulated. Organisms can both cooperate and compete in ecosystems.

Procedure

Day 1. Examine the brine by placing a few drops in the well of a microscope slide and observing the sample under the microscope.

Are there microorganisms present?

In what abundance?

Are they mobile?

Have the students sketch some of the microbes for later possible identification.

What is the salinity of the natural brine?

Could they – the students - live if they were swimming in the brine?

What would happen if they were freshwater fish – would they be able to survive?

What special mechanisms might organisms that live in a hypersaline environment have?

Does the environment where the sample was collected change during the year? In what ways? Are the organisms present prepared to adjust to these changes? How might they adjust?

The organisms living in high salinity environments (hypersaline) are called “halophiles” (salt loving). Some halophiles can live in salinities up to 35% (“normal” sea water is about 3.5%).

Halophiles have to maintain the chemistry of the water in their cells in order to live – salinity inside the cell can’t be as high as the water in which they live – so they face a challenge (how would you feel if you drank saltwater?). To maintain the chemical balance of the water in their cells, halophiles must have an osmotic potential equal to their external environment. Osmosis is the process in which water moves from an area of high concentration to an area of low concentration. So some organisms build special cell walls to exclude intake of salt. Others use different types of solutes in their cells, which act like salt and allow the balance to be maintained. Some have pumping systems that constantly eject salt that is taken up.

Invite the students to prepare their experiment.

How might they test the limits of salt concentration at which these organisms can live?

They will use several samples of the natural brine to determine if the organisms can survive and thrive at different salinities. Some of the samples they will dilute, some they will concentrate.

What signs might they look for to determine if the organisms survive over the short term? (organisms living and mobile before the salinity is altered are living and mobile after the alteration)

Long term?(an increase in the abundance of organisms in the altered brine; this can be determined by examining samples under the microscope and also by an increase in the turbidity of the samples)

Turbidity is defined as a decrease in the transparency of a solution due to the presence of suspended material. In the case of this experiment, what's suspended is microorganisms. If the organisms survive the decrease in salinity, the population may grow (an indication that the organisms not only survived, but are able to *thrive* at the new salinity). As the population increases, the turbidity of the water increases.

Have the students test the salinity of their original brine sample. They will use this to calculate how much salt to add to the samples with increased salinity.

Have the groups label their containers and prepare them according to the table below (note that the quantities may need to be altered based on salinity of the beginning brine). Alternatively, invite them to make their own calculations if they want to use a different increment of dilution / concentration (e.g., 10% increments); have them create a table that reflects their plan. Have the groups calculate the final salinities, based on the salinity of the beginning brine.

Table 1. Example of Possible Experiment Increments

Container	Amount of Natural Brine (STP)	Amount of Distilled Water (STP)	Amount of Salt to Add	Salinity	Actual Salinity % or ppt
Container 1	12.5 ml	87.5 ml	None	(1/8)	
Container 2	25 ml	75 ml	None	(1/4)	
Container 3	50 ml	50 ml	None	(1/2)	
Container 4	100 ml	None	None	Control	
Container 5	100 ml	None	_______ ml	(2x)	
Container 6	100 ml	None	_______ ml	(4x)	
Container 7	100 ml	None	_______ ml	(8x)	

The students should use gloves and protective eyewear.

After determining how much salt to add to their samples to reach the desired salinities, the students can use a scale to prepare the appropriate quantities of the salt.

The use of salt from the hypersaline environment in which the brine was collected is the best approach, although it should be noted that the salt may be different from the salt in the brine (different salts precipitate at different brine salinities, leaving the remaining brine altered). It may be possible to substitute salt used in home marine aquariums, or even table salt - but this is experimental. The type of salt used should be reflected upon in the discussion of the final experiment outcomes.

Caution students to add the distilled water slowly to the containers, pipette-full by pipette-full so that the environment is altered gradually for the microorganisms. The salinity should be increased gradually as well.

Invite the students to examine the samples. Each sample should be taken with a clean pipette and placed in a separate well on a microscope slide.

Are there living microorganisms present?

In what abundance?

Have the students sketch some of the microbes for later possible identification.

Seal each container with its lid, backing the lid off by one turn. This will allow gas to be exchanged but will reduce evaporation and will keep airborne particles from getting into the samples.

Place the containers in a location where they will receive indirect sunlight (if photosynthesizing organisms are present), but will not be disturbed, and are away from heat and cold. Watch the containers over several days to weeks; *does the color change in any of them? The turbidity?*

Final Day. After several days to a few weeks, Invite the students to examine their containers. What do they observe?

Has the color changed in any of them? The turbidity?

Invite the students to examine a sample from each container, using a clean pipette and placing the sample in a separate well on a microscope slide.

Are there living microorganisms present?

In what abundance?

Are the same types of microorganisms present in each of the samples, or have some not adjusted to the changed salinity?

Do some of the containers not contain any living microbes? What does this tell you about the upper and/or lower limits of salinity at which these organisms can adapt?

Why might some of the organisms thrive in the containers of diluted brine, but not thrive in another natural environment of similar conditions with this lesser salinity?

Microbial populations grow at an exponential rate. Some students may argue that the differences in turbidity are due to the original dilution, after several days of exponential growth of a microbial population, there should be little difference between the turbidity of the different containers – if they contain thriving populations.

Extension

The students can “measure” turbidity by assessing the relative amount of laser light that can pass through each of their samples and onto a sheet of paper behind the samples (the samples will have to be placed in clear containers, rather than typical Nalgene containers). If the water is completely clear, the laser beam will pass through the sample. The laser beam will be increasingly impeded with increasing turbidity. If the water is completely turbid, the laser beam will be scattered and little light will pass through the sample.