

# Salinity Extremes - Version 1

**Objective:** To determine the saline concentration limits at which hypersaline organisms can live, students will modify by dilution 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 was used with some success during the July 2005 Life at the Limits: Earth, Mars, and Beyond, An Educator Workshop and Fieldtrip. Feedback is welcome!*

## Materials

For class:

- Sample of natural brine from a hypersaline environment with living, mobile microorganisms present
- 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
- 4-9 150 to 250 ml sterile Nalgene bottles with lids
- Distilled water
- Sterile microscope slides with wells
- Sterile pipettes
- Lab notebook
- Writing utensils

For each student:

- Protective eyewear
- Lab gloves

## Time

1 50 minute class period to set up

Several days for 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 within 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?*

In this experiment, the students will prepare dilutions of the natural brine to determine if the organisms can survive and thrive at lower salinities.

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

*Long term?*(an increase in the abundance of organisms in the diluted 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.

They will prepare several samples of brine, each being diluted a different increment.

Have the groups label their containers and prepare them according to the table below – or according to their own calculations if they want to use a different increment (e.g., 10% dilution increments); have them create a table that reflects their dilution plan.

Have the groups calculate the final salinities, based on the salinity of the beginning brine.

**Table 1. Example of Possible Dilution Increments**

Container	Amount of Natural Brine (STP)	Amount Distilled Water Added (STP)	Salinity
Container 1	12.5 ml	87.5 ml	(1/8)
Container 2	25 ml	75 ml	(1/4)
Container 3	50 ml	50 ml	(1/2)
Container 4	100 ml	None	Control

Have the students use gloves and protective eyewear. Caution them to add the distilled water slowly to the containers, pipette-full by pipette-full so that the environment is altered gradually for the microorganisms.

Invite the students to examine the samples that have been diluted. 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 note what they observe in each sample and 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 take 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 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.