pH Test #5: pH Affects Living Things (From a series of 5)

Adapted from: "Acid Tests" in Environmental Education in the Schools. Braus, Judy and David Wood. Peace Corps, 1993.

Grade Level: intermediate

Duration: 15 minutes

Setting: lab or classroom

Summary: Students will determine the impact that low pH levels have on either plant seeds or Daphnia in two different experiments.

Objectives: Students will recognize that low pH conditions can harm aquatic life. Students will familiarize themselves with scientific experimentation and method.

Related Module Resources:
- "pH Test #1, #2, #3, #4" Activ.
- "pH, Plants and Fish" Activity
- "pH People" Activity
- HANBOOK: p. 57-63
- FIELD MANUAL: p.33-35
- pH Information/Fact Sheet
- interactive pH scale poster [B-top]

Vocabulary: pH, biomass, bioassay

Materials (Included in Module):
- seeds (radish) [B-3-envlp]
- potting soil (some supplied in module) [B-3]
- measuring cup [B-2]
- vinegar, other acidic solutions [C-1]
- pH measuring device – pH paper, meter [A-1-MB1] or Hach pH kit (kit will take longer)[A-4]
- eyedroppers

Additional Materials (NOT Included in Module):
- distilled water
- 20 empty pint milk cartons or soda bottles, or plastic cups (DO NOT USE THE PLASTIC CUPS SUPPLIED IN THE MODULE)
- ruler
- access to window (sunlight)
- Daphnia (water fleas)
- Daphnia care supplies
- Dissecting microscope (optional)

ACADEMIC STANDARDS (ENVIRONMENT AND ECOLOGY)

7th Grade:

4.3.7.A Identify environmental health issues.
- Identify various examples of long-term pollution and explain their effects on environmental health

4.3.7.B Describe how human actions affect the health of the environment.
- explain how acid deposition can affect water, soil and air quality.

4.7.7.B. Explain how species of living organisms adapt to their environment.
- Explain how living things respond to changes in their environment.

10th Grade

4.1.10.E. Identify and describe natural and human events on watersheds and wetlands.
- Identify the effects of humans and human events on watersheds.

4.3.10.B Explain how multiple variables determine the effects of pollution on environmental health, natural processes and human practices
- Explain how human practices affect the quality of the water and soil

12th Grade

4.1.12.C Analyze the parameters of a watershed.
- Interpret physical, chemical and biological data as a means of assessing the environmental quality of a watershed

ACADEMIC STANDARDS (SCIENCE AND TECHNOLOGY)

7TH Grade

3.2.7.B Apply process knowledge to make and interpret observations.
- Describe relationships by making inferences and predictions
- Communicate, use space/time relationships, define operationally, raise questions, formulate hypotheses, test, and experiment.
- Design controlled experiments, recognize variables, and manipulate variables.
- Interpret data, formulate models, design models, and produce solutions.

3.2.7.C Identify and use the elements of scientific inquiry to solve problems.
- Generate questions about objects, organisms and/or events that can be answered through scientific investigations.
- Evaluate the appropriateness of questions.
- Design an investigation with limited variables to investigate a question.
- Conduct a two-part experiment.
- Judge the significance of experimental information in answering the question.
- Communicate appropriate conclusions from the experiment.

BACKGROUND:

pH is the measure the concentration of free hydrogen ions, which indicate whether a solution is acidic or basic. pH is measured on a scale from 1 to 14 with lower number indicating higher levels of acidity. Natural waterways should have a pH between 5 and 8.5.

All aquatic life tolerate and have adapted to a specific pH range. At high (9.6) or low (5.0) pH values, the water becomes unsuitable for most organisms. Organisms such as bacteria can tolerate the broadest range of pH levels (2-13.3). Plants are the next

tolerant, they can survive in pH levels between 6.5 and 12.5. Carp, suckers, catfish, and some insects, as well as, bass, bluegill, and crappie can exist in levels between 6 and 9.6, the latter group having a slightly narrower range. Snails, clams, and mussels have a pH range of about 7-10. Finally, the most sensitive animals include trout, mayfly nymphs, stonefly nymphs, caddisfly larvae, and many more. This group survives only in a pH range of 7-8.6. The exact minimum pH a fish can tolerate is unknown, but most fish become endangered in a pH below 5.5 and nearly all species will die in water with a pH of 4.5.

If the pH changes even slightly, it will stress the organisms and possibly kill them. For example, the most serious consequence of increased acidity on fish is interference with the fish's reproductive cycle. Under acidic conditions, there is an overall decrease of the calcium levels in female fish, possibly to the point where she cannot produce eggs, or the egg(s) cannot pass from the ovaries. Even if the eggs continue to the stage of fertilization they might develop abnormally. Lower pH and the disruption of calcium concentrations attributes to the malfunction of the fish's osmoregulatory mechanism that controls sodium and chloride ion balance. An improper body salt level is fatal to a fish. The death of fish populations, in turn, affects animals that eat fish.

Additionally, higher acidity can increase the concentration of toxic metals in a stream, such as aluminum (Al^{+3}) and copper (Cu^{+2}). These metals were locked up in mineral matter under neutral pH levels, but become mobile when the pH lowers. Metal can clog fish gills causing breathing problems. In young fish, metals can cause deformities. It can also settle on the stream bottom filling in spaces between rocks where insects live or eggs are laid, even smothering these eggs.

Many other aquatic organisms are affected by acidic water as well. Frogs and salamanders lay their eggs in the meltwater pools of early spring. If the snow that feeds these ponds contains sufficient concentrations of sulfuric acid or nitric acid, 80% or more, their eggs could remain unhatched. In lakes where acidification is a problem, algal species diversity declines. This shift toward algae more tolerant of low pH generally destabilizes the plankton community. Also, lowering the pH of lakes may result in a decline in decomposer organisms such as bacteria and fungi. This leads to an accumulation of organic waste in water. Thus, by greatly disturbing the balance of the ecosystem, acidification causes serious problems.

An increase of acidity causes a decrease of **biomass** (the total weight of all organisms living in a particular habitat or place). Low pH levels effect not only fish and other living organisms but plants as well. Increased acidity in soils may make certain nutrients unavailable for plant use. For example, nitrogen and phosphorus for example are normally released from the soil by decomposing microbes. High levels of acidity suppress these microbes preventing the nutrients from being released and reused. This lack of nutrients makes it difficult for plants and other organisms to survive.

Toxicity testing can be used to study the effect of acidity or other pollutants on living organisms. A **bioassay** is an experimental test that measures the relative toxicity of a

water sample by using an indicator organism and seeing how it responds (either dying, stunting growth, causing accelerated heart rate, or breathing difficulties). If testing samples from a local waterway, bioassays are a useful way to determine the overall health of the water. Unlike chemical analysis that measures only one parameter at a time, a bioassay encompasses multiple parameters within the sample. If an organisms dies in the sample, then there may be harmful toxins present, but the exact toxin will not be known.

Bioassay indicator organisms can include Daphnia (water fleas), other small crustaceans, other zooplankton, minnows, and various types of seeds. These indicator organisms can be placed in varying concentrations of a water sample or known toxin (100%, 50%, 25%, 12.5%, 6.25%, 0%, etc.). If a scientist wanted to determine the amount a pollutant (such as acidity) that might be acceptable in your waterway without harming aquatic life, a bioassay is a useful tool.

Daphnia are the most popular indicator organisms in bioassays because they are sensitive to changes in water chemistry and are simple and inexpensive to raise in an aquarium. They mature in just a few days, so it does not take long to grow a culture of test organisms. Daphnia are small crustaceans that live in fresh water such as ponds, lakes, and streams and serve as an important source of food for fish and other aquatic organisms.

Plant seeds like radish and lettuce are also commonly used in toxicity experiments, do not require harming animal life, and are very useful to determine the effects of water pollution on plant growth. Acid rain impacts on plant growth can be explored using a toxicity test.

OVERVIEW: Option 1: Students conduct an experiment to determine the effects of acidity on plants. They will compare the growth and germination time of sprouting plants watered with distilled water to those watered with acidic water. Option 2: Students conduct a bioassay to determine the effects of various levels of pH on Daphnia (water fleas).

PROCEDURE:

Radish Seed Experiment

1. Label 10 planting containers as “acidic solution” and 10 as “distilled water” to identify what they will be watered with.

2. Make a solution with a pH of about 3 by mixing 1 cup of vinegar with 4 cups of distilled water. Record the pH using instructions located in the Test Kit Instruction Section of the Module Resource Guide.

3. Measure and record the pH of the distilled water.

4. Fill the 20 planting containers three-quarters full with potting soil. Plant three radish seeds in each carton.

5. Then water the seeds in the cartons labeled "acidic solution" with the vinegar solution and those labeled "distilled water" with distilled water. Measure out the amount of water given to each carton to ensure that you give each the same amount. Put the cartons in a sunny spot indoors.

6. Over the next three weeks or so, water all the seedlings with the appropriate kind of water whenever they look dry. Measure out the amount of water given to each carton to ensure that you give each the same amount.

7. Record the date each seed sprouts and measure the heights of the seedlings every few days. Use the table provided at the end of this activity for your data.

Daphnia (Water Flea) Experiment

Teacher Preparation:

You will need to order Daphnia from a science supply catalog or rear your own from those collected from a pond. They reproduce quickly and for this experiment, very young Daphnia (neonates) should be used because they are more sensitive to toxic substances. Daphnia care and culturing instructions are enclosed with this activity.

Student Experiment:

1. You can test as many different pH levels as you want to prepare. Possibilities could include a set of 3.5, 5, 6, 7, 8, 9, and control or 4,5.5,7, 8.5, and control. Add enough acid to approximately 100mL distilled water in each container until a desired pH is reached. Basic water can include spring water or water from a groundwater source (no chlorine though). The control should only have distilled water in it and it should be close to pH 7. Water levels in all containers should be similar if possible.

2. Label the containers with the pH level that were created for each container and also label the control.

3. Using a widebore pipette, randomly select and carefully place ten Daphnids into each container. Place the pipette tip below the surface and gently expel each Daphnid individually into the container.

4. Using a dissecting microscope, measure and record mortality and survival of the Daphnia at one hour and then at 24 and 48 hours. Record this data in the table provided at the end of this activity. You may also want to record the pH of each container after 48 hours to see if how much the pH levels changed.

5. Scientists are often interested in determining which concentration caused 50% mortality (denoted at the LC50 in science write-ups) after 48 hours.

DISCUSSION:

Discuss the results with the students. *The seeds watered with the acidic solution should have sprouted later than those watered with the distilled water or may not have sprouted at all. If they did sprout, they might have yellow or stunted leaves. The Daphnia mortality should have been more likely in the lower pH levels.*

Ask the students why a number of seeds or Daphnia were used for each solution. *If the students don't know, explain that this is to reduce the likelihood of any one seed or Daphnia skewing the results because of disease, other problems or individual differences.* Also, ask them why they had to water some seeds with distilled water or allow some Daphnia to live in the distilled water (pH 7). *These plants and Daphnia were the control – they allow the experimenter to measure their results against what would have happened normally.*

Discuss what affects acid rain might have on plant life or aquatic life based on the experiment. Keep in mind that acid rain is different in composition and generally is less acidic with a pH of about 4. Even though these were radish seeds (a terrestrial plant), do the students think that the same type of effects would occur to aquatic plants. *(acid rain does affect aquatic plant health).* Would acidic conditions affect other types of aquatic creatures? *Yes, the background section and other various visual aids, handouts, and fact sheets discuss the impact low pH levels have on aquatic life.*

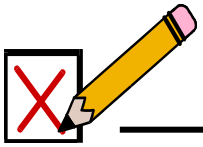
EVALUATION:

- Explain how acidic water can harm aquatic plants and animals.
- Have students explain why certain steps are used in the activities experimental design.
- Correctly completed data sheets or graphs created from the data.

EXTENSIONS AND MODIFICATIONS:

- Conduct the experiments using water collected from a waterway suspected to have pollution in it to see how it affects the seeds or indicator organisms.
- Research pH ranges that aquatic life can tolerate
- Have students do research on the Internet on other toxicity tests or bioassays that could be completed.

NOTES (TEACHERS, PLEASE WRITE ANY SUGGESTIONS YOU HAVE FOR TEACHERS USING THIS ACTIVITY IN THE FUTURE):



DATA SHEET: pH TEST #5: pH AFFECTS LIVING THINGS

Student Name _____ Date _____

RADISH SEED EXPERIMENT:

pH of distilled water:_____ pH of acidic water:_____

Container #	Treatment	Height or sprouting information on day (mark X if not sprouted):												
		3	5	7	9	11	13	15	17	19	21	23	25	27
1	Water													
2	Water													
3	Water													
4	Water													
5	Water													
6	Water													
7	Water													
8	Water													
9	Water													
10	Water													
11	Acidic water													
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16	Acidic water													
17	Acidic water													
18	Acidic water													
19	Acidic water													
20	Acidic water													

DATA SHEET: pH TEST #5: pH AFFECTS LIVING THINGS

Student Name _____ Date _____

DAPHNIA (WATER FLEA) EXPERIMENT:

After 1 Hour:

PH Level	Survived	Died

NOTES:

After 24 Hours:

PH Level	Survived	Died

After 48 Hours:

PH Level	Survived	Died

At what pH level did 50% mortality occur (LC50)?

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