A Case Study

Acid Rain and Environmental Toxins: Toxicity Testing Using Bioassays

Introduction

The Applied Science IV class at Newark Valley High School, as a class research project, assumed the role of a civil engineering firm that performed environmental studies for businesses and government agencies. The Town of Newark Valley Planning Board hired the firm to research the effects of acid rain on their local economies, which included agriculture and forestry as the chief businesses.

The class was divided into work teams with one member from each team forming an Advisory Planning Committee (APC) for the project. The APC determined the course of action for the project including the data that was collected and academic content that was researched. Specific assignments were given to the work teams that included research goals and time frames for completion. Each team periodically presented the status of their assignment to the entire class or members of the APC in written and oral formats for peer review. Some of the data collected was recorded on the Cornell Environmental Inquiry web site for further peer review. As each step of the project was completed, the APC reviewed the process and provided each team with more detailed and specific instructions and tasks. Some members of the Applied Science I, II, and III classes functioned as outside teams of consultants and technicians that performed testing and Internet research assigned to them by the APC. These teams provided the APC with written reports of their results.

The APC developed a final report of their findings and submitted this report to the Newark Valley Planning Board. In addition, members of the APC presented their key findings at the Student Bioassay Congress at Cornell University.

The goal of this case study is to illustrate design and execution of an authentic research project. Students will be able to follow the process from the initial research of the current literature, to the formation and refinement of the specific research questions. The study will include two specific cases of student teams designing and executing their experimental plan, interpreting their results, and communicating their findings in a process of peer review.

Upon completion of the study, students will be able to engage in a parallel task that will allow them to address many of the nature of science questions that scientists must address as they participate in interactive research.

Background Research

The first task that the class addressed was to research current literature on the problem of acid rain in New York State. Working in groups of two or three, each team explored the Internet and library resources looking for information about the effects of acid rain on agricultural crops and forests. Pertinent information was recorded and after a week of research, each team presented their findings to the class in the form of a PowerPoint presentation.

A large volume of information was gathered, covering a vast range of topics. Some of the more important information is included in the excerpts that follow.

"Over the years, scientists, foresters, and others have watched some forests grow more slowly without knowing why. The trees in these forests do not grow as quickly as usual. Leaves and needles turn brown and fall off when they should be green and healthy."

"The ability of the soil to resist some pH change is called buffering capacity. A buffer resists changes in pH. Without buffering capacity, soil pH would change rapidly. Places in the mountainous northeast, like New York's Adirondack Mountains, have soils that are less able to buffer acids. Since there are many natural sources of acid in forest soils, soils in these areas are more susceptible to effects from acid rain."

"The acid rain causes the release of toxic substances such as aluminum into the soil. These are very harmful to trees and plants, even if contact is limited. Toxic substances also wash away in the runoff that carries the substances into streams, rivers, and lakes. Less of these toxic substances are released when the rainfall is cleaner."

"The most serious effect of surface water acidification in the northern temperate regions seems to be the decline of natural fish populations. It has become evident that aluminum, and not the H+ (proton) concentration, is the principal toxicant killing the fish."

" Today's forests are also being destroyed by the nitrogen oxides, sulfur dioxides, and hydrocarbons that are leeching the magnesium and calcium the forests need to survive."

"Acid rain does not usually kill trees directly. Instead, it is more likely to weaken the trees by damaging their leaves, limiting the nutrients available to them, or poisoning them with toxic substances slowly released from the soil. Scientists believe that acidic water dissolves the nutrients and helpful minerals in the soil and then washes them away before the trees and other plants can use them to grow."

"Aluminum is the most abundant metal on earth and has a multitude of uses in the human environment in the form of products such as beverage cans, kitchen utensils and aircraft.However, aluminum (Al 3+) is very unfriendly to agriculture as it injures plant root cells and thus interferes with root growth and nutrient uptake in crops. Aluminum's harmful effects are worst under acidic conditions where it becomes soluble; in non-acid soils it is insoluble and thus less deleterious. More than one-third of the arable land in the world suffers from soil acidity and aluminum toxicity; low agricultural productivity in acid soils is directly attributable to the effects of aluminum."

"The strategy capitalized on the fact that some plants tolerate aluminum by releasing *citric acid* which binds to the metal making it difficult to enter plant roots. Transgenic plants expressing the CSb gene from Pseudomonas aeruginosa produced up to ten-fold more citrate in their roots and released four-fold more of the compound than control plants. When grown under extremely high aluminum and acidic conditions, transgenic CSb plants showed substantially lower root growth inhibition compared to the untransformed plants. Normal seeds failed to develop roots when germinated in the presence of high aluminum while transgenic CSb seeds showed a clear tolerance. Transgenic roots contained less aluminum in their tissues, possibly because the citrate synthase produced by these plants was preventing uptake."

Upon completion of the preliminary reports, the Advisory Planning Committee (APC) created a list of topics (academic content) that they decided required further investigation and for each topic developed a set of questions that would direct the research. Each team was assigned a topic and given a week to do the research and write a report. Table A contains a list of the topics and questions.

Table A

A. Acid Rain
1. What is acid rain?
2. How is acid rain made?
3. How is acid rain different from normal rain?
4. What are the environmental effect of acid rain?
5. How is New York State affected by acid rain?

B. Aluminum Toxicity
1. What is aluminum toxicity?
2. What causes aluminum toxicity?
3. How is aluminum toxicity related to forest soils?
4. What can be done about aluminum toxicity?

C. Soil Buffering
1. What is a buffer?
2. What do buffers do?
3. What chemicals are used as buffers?
4. Can buffering agents be toxic?

D. pH
1. What is pH?
2. What is the pH scale?
3. How is pH measured?
4. How does acid rain change the pH of soil?
5. How does a buffer change the pH of soil?

E. Toxicology Terms
1. What is a bioassay?
2. Why are certain organisms used for bioassays?
3. What terms are used to communicate information about toxic materials and their effect on the environment?
4. What is synergy?

F. Soil and Soil Profiles
1. What is soil?
2. How does soil form?
3 What are the different types of soil?
4. What is a soil profile
5. What factors control the permeability of soil?
6. What factors control the porosity of soil?

G. Forest Soil and Acid Rain
1. What is forest soil?
2. What climatic conditions create a forest soil?
3. What is the relationship between forest soil and aluminum toxicity?
4. What natural buffers occur in forest soil?
5. How is forest soil related to acid rain problems in New York State?

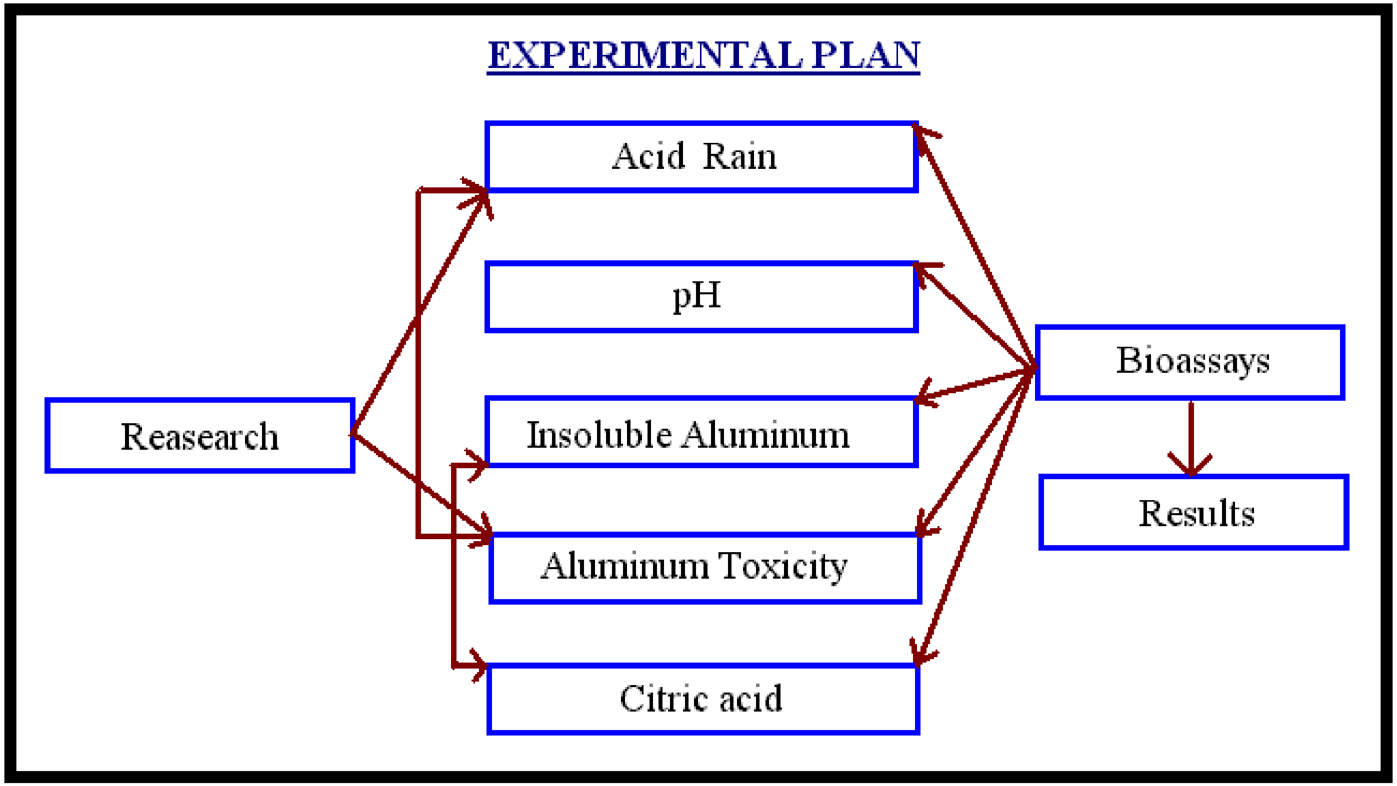
Development and Refinement of a Research Question

At the end of the week each team presented their information to the entire class in an informal question and answer format again using a PowerPoint format. From this added information, the APC then brainstormed a list of possible experimental investigations that would be required to obtain the information needed to respond to the Newark Valley Planning Board. Table B includes a list of the experimental investigations that the APC developed and Diagram 1 represents their overall experiment plan.

Table B

1. **Using the lettuce seed bioassay protocols, determine the LC_{50} levels for the following compounds:**
    a. **Aluminum chloride ($AlCl_3$), Citric acid and citric acid/aluminum chloride, Aluminum hydroxide ($Al(OH)_3$)**
2. **Using ion exchange columns, determine to toxic ion of the aluminum chloride.**
3. **Using a lettuce seed bioassay, determine the toxicity effect of pH.**
4. **Using a lettuce seed bioassay, determine the combined toxicity effect of pH and aluminum chloride.**
5. **Generate the gases that form acid rain (CO_2, SO_2, and NO_2) and for each gas determine its affect on pH in water.**
6. **Design and construct a device that that simulates the production of acid rain and its percolation through soil.**
7. **Using the acid rainmaker, perform a series of lettuce seed bioassays to determine the toxic effects of the following conditions:**
    a. **normal rain and soil**
    b. **acid rain and soil**
    c. **normal rain, soil, and aluminum hydroxide**
    d. **acid rain, soil, and aluminum hydroxide**
8. **Analyze current acid rain effects in New York State by assessing online data.**

Diagram 1.



From the list in Table B, each team selected one of the questions to study. At this point each team began performing protocol labs and or explorations in order to develop a more well-defined research question. A form of "pair review" process was used to provide feedback to help them with their task. Once the research questions were well- defined, the teams moved on to the Interactive Research, in which they designed and carried out a controlled scientific experiment with clearly defined objectives and hypothesis,

Research Team #1 (Diane, Brandi, Andrea, & Elizabeth)

Research Question: How does citric acid affect the LC_{50} for aluminum chloride ($AlCl_3$)?

Hypothesis: Based on the information we obtained from our research of the literature, we expect that the citric acid will combine with the aluminum and make it insoluble. In this form, it can't be absorbed through the roots of the lettuce seeds, making it less toxic.

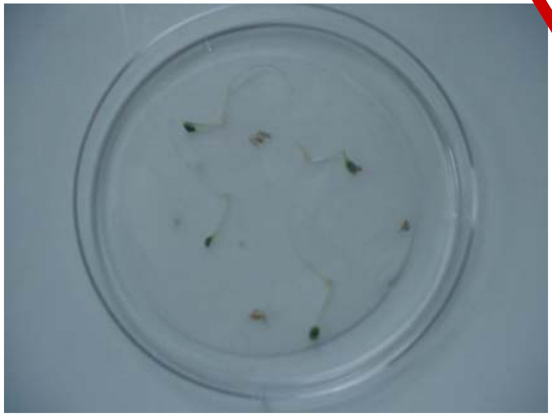
Experimental Plan: Using lettuce seeds as the bioassay organism, we will perform the bioassay protocols to determine the LC_{50} levels for aluminum chloride ($AlCl_3$) and citric acid. Once these are obtained, we will add non-toxic levels of citric acid to each of the dilutions of aluminum chloride perform the bioassay again.

Results: Data tables and graphs for germination rates and average radicle lengths were created using Microsoft Excel. The results on radicle lengths are found in the following charts.

Average radicle lengths for aluminum chloride
Average radicle lengths for citric acid
Average radicle lengths for aluminum chloride + citric acid

Conclusions: Lettuce seeds did not grow in $AlCl_3$ concentrations of 2500 mg/L or higher. The LC_{50} level for $AlCl_3$ is between 1250 - 2500 mg/L. The lettuce seeds in citric acid grew at concentrations through 2500 mg/L but did not grow at 25000 mg/L. The LC_{50} level for citric acid is greater than 2500 mg/L. The mixture of toxic aluminum (2500 mg/L) with nontoxic citric acid (250 mg/L) showed a

AlCl$_3$ (2500 mg/L)

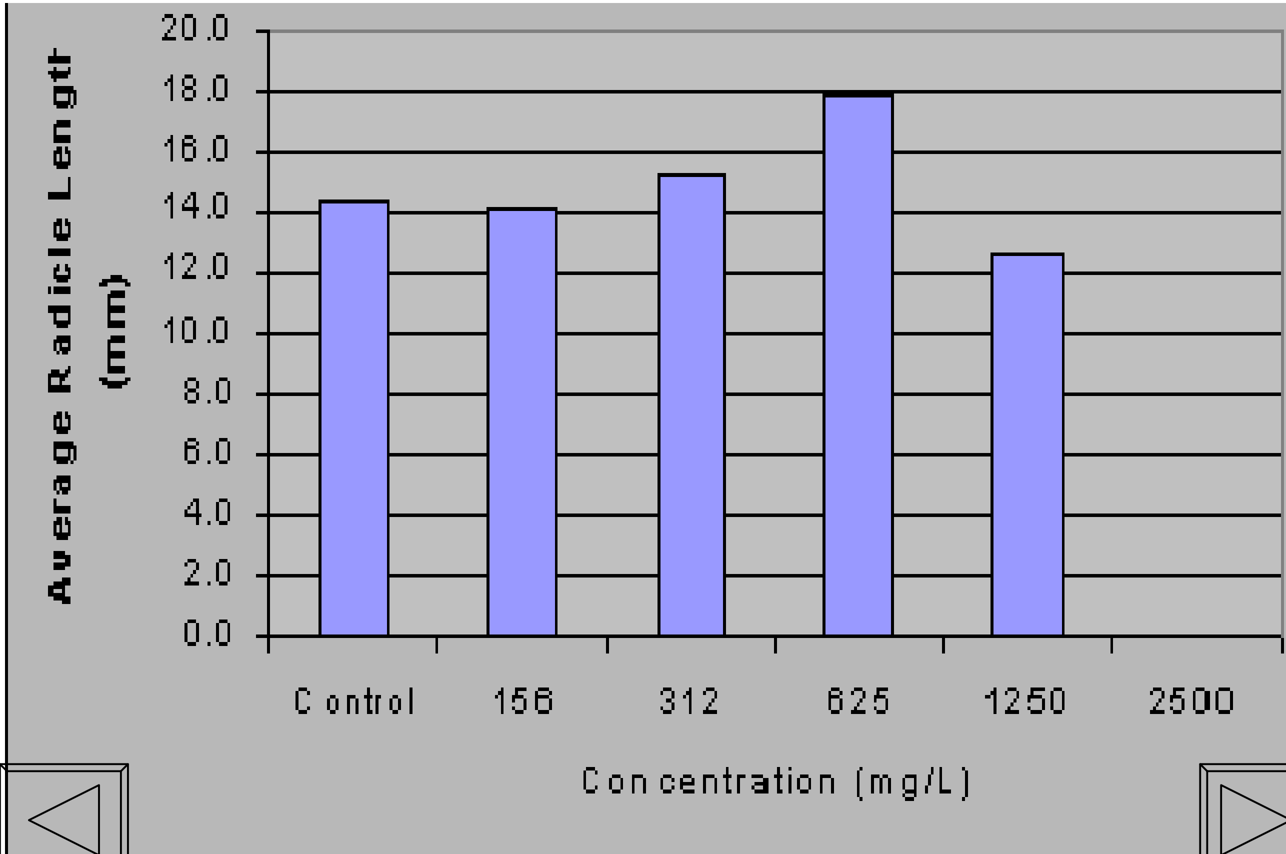


Citric acid (250 mg/L)

Citric acid + AlCl$_3$

Aluminum Chloride

Concentration (mg/L)	Radicle Length (mm)															Length (mm)
Control	3	5	24	22	21	19	17	10	15	2	16	25	7			14.3
156	16	18	22	19	18	14	7	10	9	13	17	7	14			14.2
312	9	14	8	23	20	16	19	17	16	20	17	19	15	0		15.2
625	22	24	20	12	19	13	8	15	24	21						17.8
1250	14	15	16	15	7	21	15	20	19	4	4	16	7	4		12.6
2500	0	0	0	0	0	0	0	0	0	0	0	0				0.0



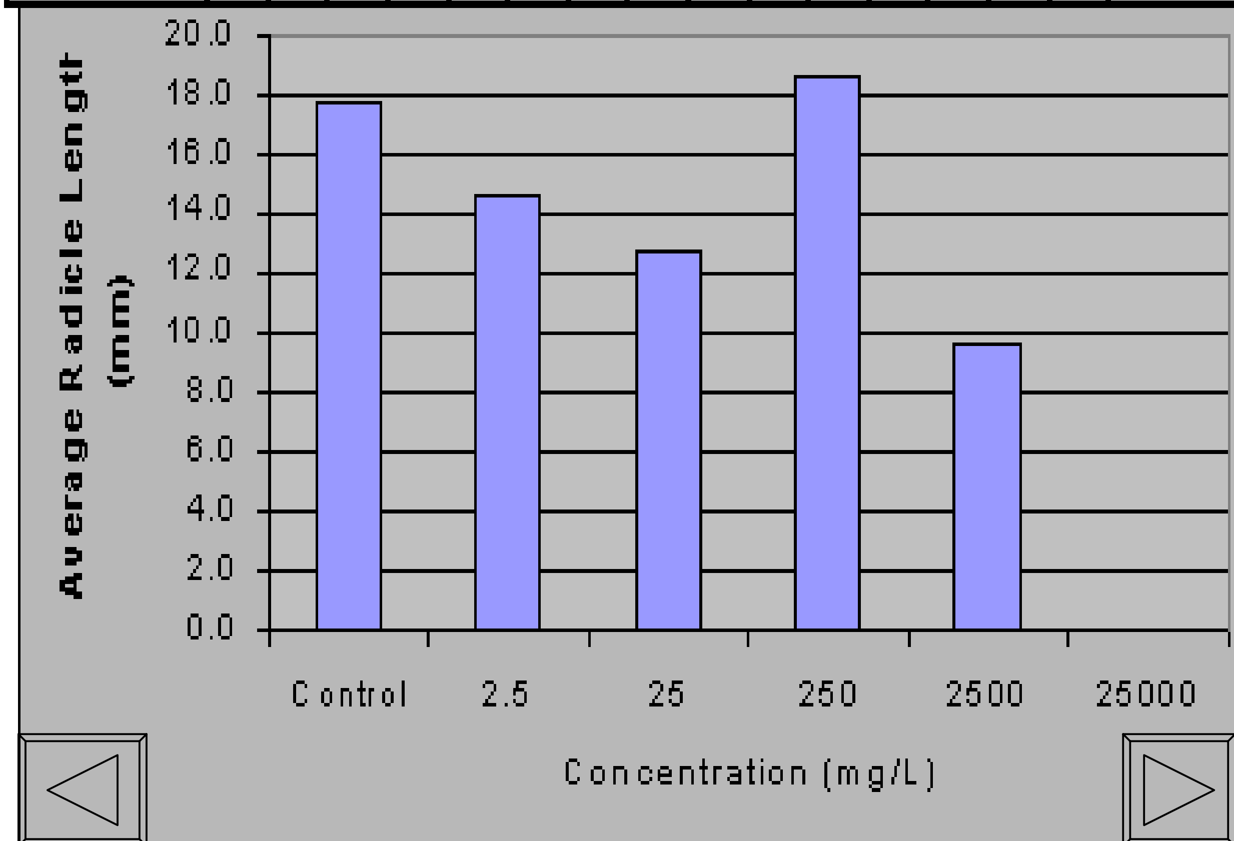
AlCl$_3$ (2500 mg/L)

Citric acid (250 mg/L)

Citric acid + AlCl$_3$

Citric Acid

Concentration (mg/L)	Radicle Length (mm)															Length (mm)
Control	-	-	22	23	24	-	-	11	14	8	-	-	23	17	-	17.8
2.5	19	15	23	11	15	15	12	21	6	0	20	18	17	20	8	14.7
25	14	9	15	-	-	6	9	14	17	13	26	17	7	6	-	12.8
250	15	26	18	10	-	37	23	12	9	-	17	19	19	-	-	18.6
2500	5	-	-	-	-	7	6	-	-	-	13	9	14	13	-	9.6
25000	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-



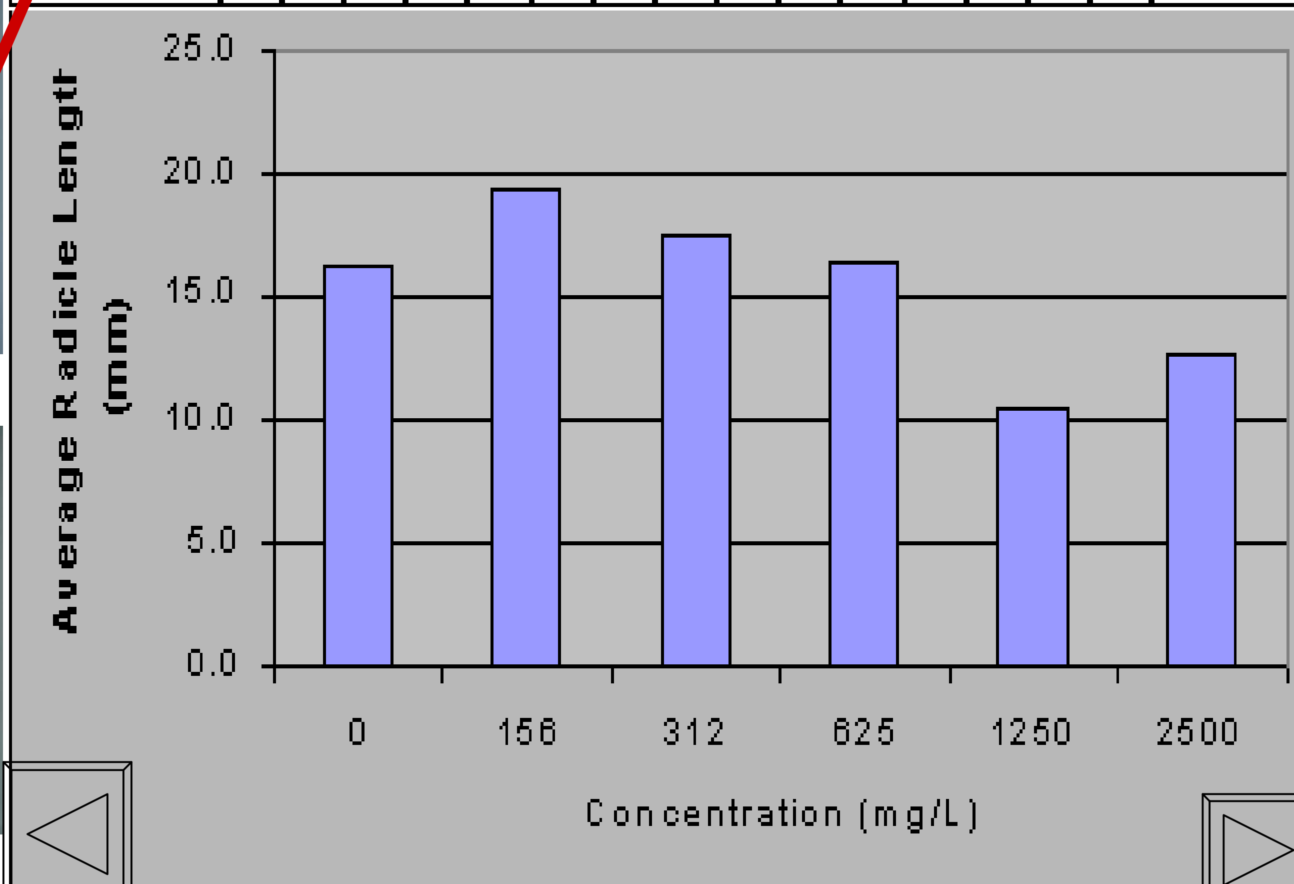
AlCl$_3$ (2500 mg/L)

Citric acid (250 mg/L)

Citric acid + AlCl$_3$

Citric Acid + Aluminum Chloride

Concentration (mg/L)	Radicle Length (mm)															Length (mm)
0	19	22	24	17	17	21	4	7	22	2	19	20	18	16	-	16.3
156	30	28	20	16	22	24	11	16	13	12	17	24	-	-	-	19.4
312	27	16	26	16	20	20	19	19	13	13	15	7	-	-	-	17.6
625	15	13	3	9	11	22	25	25	21	20	-	-	-	-	-	16.4
1250	9	14	14	6	10	15	19	7	2	9	-	-	-	-	-	10.5
2500	24	21	14	13	8	9	8	9	7	22	8	17	5	-	-	12.7



Poster Presentation: The pictures below illustrate the poster presentation the team gave share their findings with the class. Diagram 2 represents a sample of the feedback the group received from the peer review process.

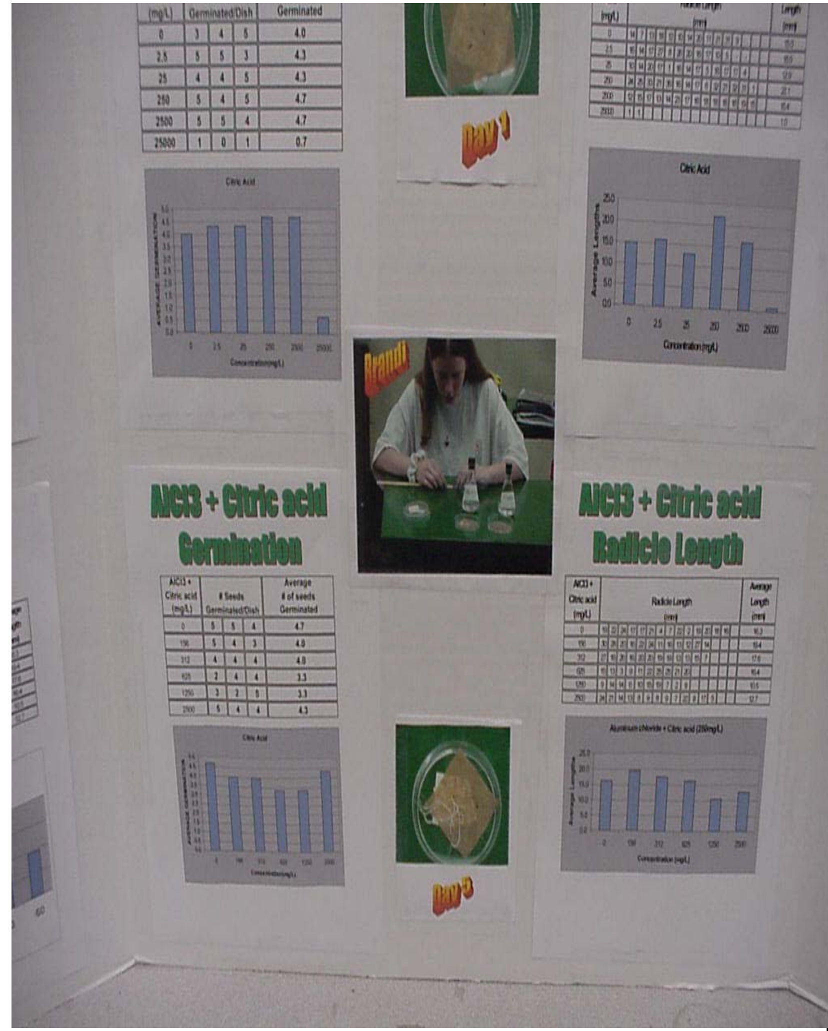


Diagram 2.

Peer Review Form

1 - Very Clear
2 - Mostly Clear
3 - Somewhat Clear
4 - Largely Unclear

Clear statement of the research question	1	Comments: Research problem was clearly printed out at the beginning of the poster.
Clear expectations of the experimental results	3	Comments: I think I know what they mean. They were not really clear what they were doing with the citric acid.
Experimental procedures and data collecting methods clearly defined	2	Comments: They said they used the same procedures that we all used to do the bioassays. How much citric acid did they add to the Al?
Data clearly represented and compared to predicted results	1	Comments: Graphs looked great. I quess the conclusion agreed with results. I didn't really look too close.
Clear statement of support or no support for the hypothesis	2	Comments: The pictures of the lettuce seeds show that the seeds grew better with citric acid and aluminum.

Notes:

Peer Review - On Line: **Upon completion of their research, the group posted their results on the Cornell Environmental Inquiry site (http://ei.cornell.edu/toxicology/peerreview/). Their goal was to receive feedback that would better prepare them to attend the Bioassay Congress at Cornell University. The information posted was as follows:**

What is the title of your bioassay?

Acid rain and aluminum toxicity

What is your research question?

How does citric acid affect the LC_{50} for aluminum chloride ($AlCl_3$)?

Briefly describe your experiment. What bioassay organisms did you use? What substances did you test?

We used lettuce seeds to determine the toxicity level of the following: pH, aluminum chloride, citric acid, and aluminum chloride plus citric acid.

Summarize your data here.

Click here to see data on aluminum chloride.
Click here to see data on citric acid.
Click here to see data on aluminum chloride + citric acid.

What conclusions can your reach?

The LC50 value for the aluminum chloride was about 2500 mg/L. When citric acid was added to that amount of aluminum chloride the seeds grew very well. The citric acid seemed to neutralize the toxic effect of the aluminum. The effect of acid rain on the aluminum is included in a second report.

What would you change if you carried out the experiment again?

Try adding citric acid to all of the substances and try and raise the pH to 6-14 and see if they grow.

Research Team #2 (Will and Steve)

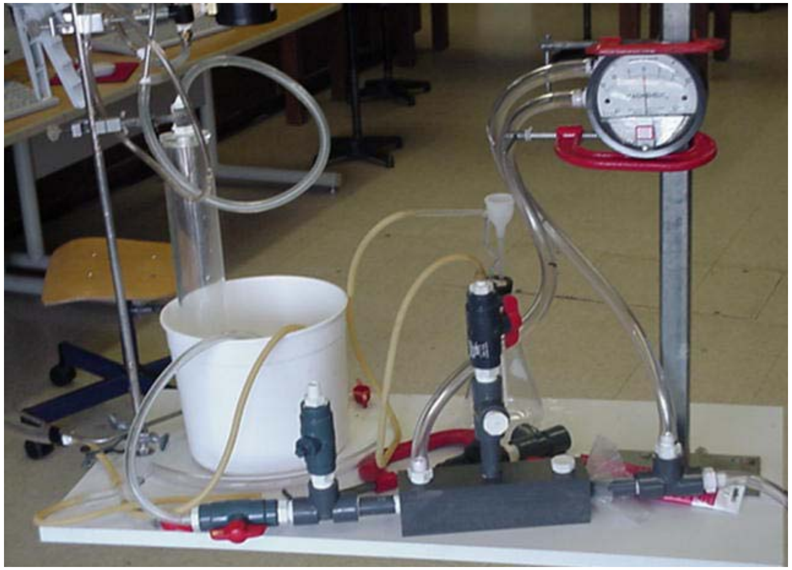
Will

Steve

Research Design Problem: Design and construct a device that that simulates the production of acid rain and its percolation through soil.

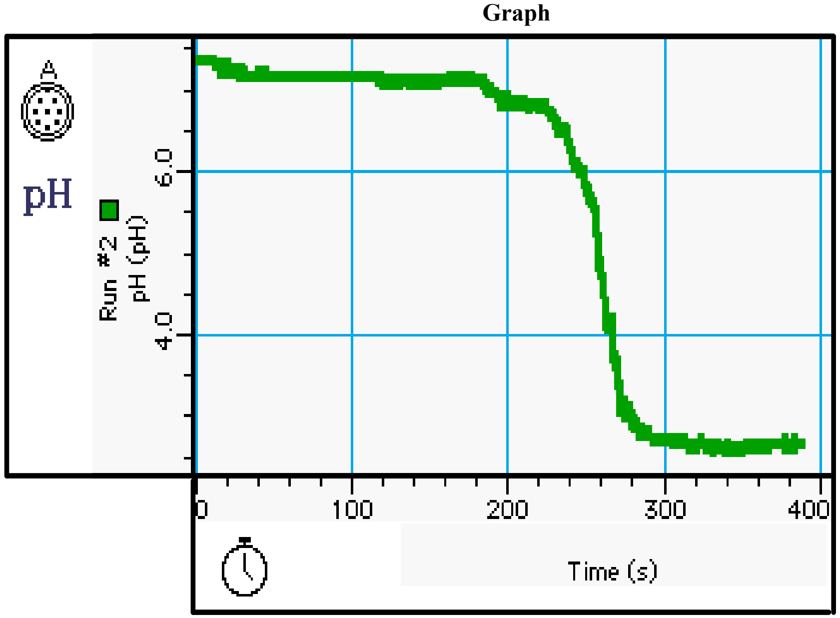
Design and Experimental Plan: The gases that form acid rain are toxic and have a strong odor. The device needed to produce the acid rain must be a closed system that confines the gas to a system of sealed tubes from the time it is generated to the time it is added to water. Once in water these gases are dissolved and will not escape into the air. A pumping system will be needed to move the gases through the system. Pressure gauges will be needed to determine that the gases are moving in the correct direction. Once the device is made, we will use it to generate acid rain by adding sulfur dioxide and nitrogen dioxide to water. We'll measure the pH using a computer probe. Once it is working, other students will use it to sprinkle the acid rain on soil and perform bioassays.

Design Results: The picture at the right illustrates our completed "acid rainmaker."



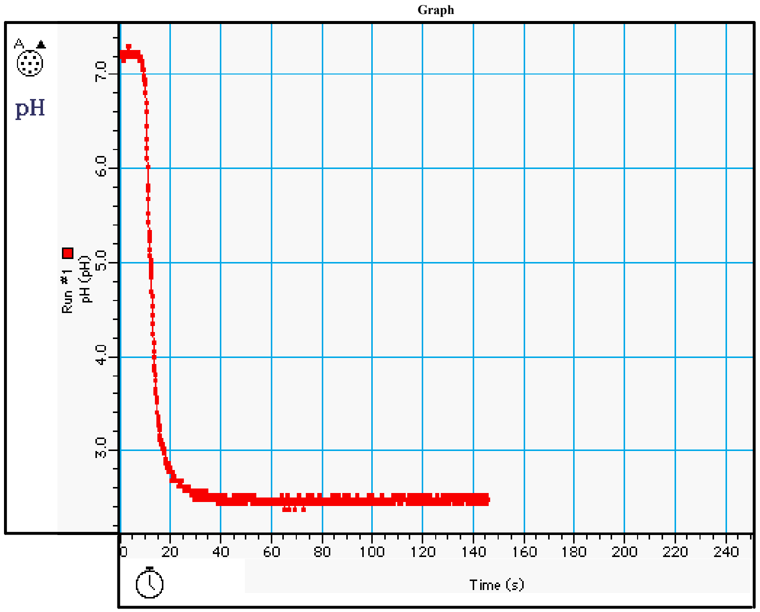
Experimental Results: NO_2 and SO_2 were generated in closed Erlenmeyer flasks, pushed through the "rainmaker" with a pressure differential and bubbled into water. The pH for the water was recorded with a computer probe. The results are shown in graphs 1 and 2. The pH of the water for NO_2 was 1.5 and the pH for the SO_2 was 2.5

Graph 1.



SO_2

Graph 2.



NO_2

Conclusions: The "acid rainmaker" is a closed system that safely allows for the production of acid rain by adding sulfur dioxide, nitrogen dioxide, and carbon dioxide. One or more gases can be added during one trial. This device will allow other students to perform their research.

Peer Review - In Class: Diagram 3 represents a sample of the feedback the group received after a presentation of their design project to the class. During the presentation, the team explained their design, described the purpose of each part, and demonstrated how the device worked.

Presentation showed planning, practice, and organization.

Very clear		**Warm comments:** They worked hard on the project and deserve a good grade. Great use of technology.
Mostly clear	X	
Somewhat clear		**Cool Comments:** The group did a very good job, but when they were talking, they didn't talk loud enough. I don't think they shared the work.
Largely unclear		

Notes

Presenters showed good understanding of experimental design and results.

Very clear	X	**Warm comments:** The rainmaker looks very scientific. It seems to work very well. Wow. What a device. I wish I could do that.
Mostly clear		
Somewhat clear		**Cool Comments:** Everything seems to work but I don't understand why you need all of the pressure gauges.
Largely unclear		

Notes

Advisory Planning Committee
Paradise Valley Planning Board
Peaceville, USA 11111 March 1, 2001

Dear APC,

I am Project Director of *Innovative Investigators, Inc.*, a civil engineering firm that does environmental studies for businesses and government agencies, and I need your help! I was browsing the Internet recently, hoping to find the name of an organization that has expertise in analyzing data from environmental studies. I was intrigued by your website that describes your use of bioassays in researching the effects of acid rain on your local economies, particularly your chief businesses of agriculture and forestry.

Our research lab has acquired quite a comprehensive quantity of data taken in a community similar to yours, and we were hoping we could hire you to help analyze the data, draw conclusions and make recommendations for remediation. Some of the preliminary recommendations made to us by another lab are unsupported by the data. Accompanying this letter is a list of questions we are hoping to answer, along with the raw data we would like you to analyze.

As you may have several research teams involved in this project, we have created a website where you can post your data analyses, conclusions, questions, or recommendations. Please call if you have any questions. If there are no problems, please read and sign the proposed agreement. I look forward to hearing from you soon.

Sincerely,

Dr. Ellen Earthy
Innovative Investigator, Inc.