Soil Protozoa and Acid Rain

Anahid Gregorian
Kathryn Hampton
Justine Li
Meghan Murphy

Biology 9 Honors
Mr. Brock
May 15 2003

Ani Gregorian
Kathryn Hampton
Justine Li
Meghan Murphy

There are many life forms living in the soil. All of them have specific and important roles in the soil and in the food chain. Some, like protozoa, monitor the break down of organic matter in soil; and are greatly affected by conditions such as pH of the soil. If the pH levels are too extreme, it can have damaging effects on the protozoa populations. The damaging changes in soil pH levels are caused by acid rain. The following information on protozoa, pH levels, and acid rain, lead us to our research question.

Protists, by definition are unicellular eukaryotic organisms, which includes both autotrophs and heterotrophs. This kingdom is more easily defined by what it does not include: multicellular animals and plants, fungi, and bacteria (Tatner, 2002). Since each of the other kingdoms have clear definitions, the Protista kingdom includes the rest of the living organisms. They have simplified forms of the systems that help to keep higher organisms alive. Their digestive and excretory “systems” are composed of vacuoles that transport food and waste in and out of the cell. Respiratory and circulatory “systems” are dependent on their cellular membranes (Jefferson, 2002). Protozoa also have very simple external structures. Protozoa are classified according to the appendages that they use to move. Ciliates are the largest protozoa, and they use cilia to move. Amoebae use a pseudopod to move, and Flagellates, the smallest protozoa, use flagella to move (Ingham, 2001). Protozoa structures help them perform specific and important tasks in the soil environment.

Ani Gregorian
Kathryn Hampton
Justine Li
Meghan Murphy

The protozoa's role in soil is an important one because their main purpose is to control bacteria populations and to decompose dead matter. By controlling bacteria populations, it diminishes the chance of pathogens infecting other organisms. If the bacteria population is altered, the rate of decomposition of organic matter is altered as well. The more bacteria there are to break down matter, the faster matter is broken down, and the fewer bacteria there are, the slower matter is broken down. Therefore, the protozoa are able to maintain the rate of decomposition because they help control bacteria populations. However, they are greatly outnumbered; in one mL of soil, there are approximately 72,000 protozoa and approximately 25,280,000 bacteria (Plaster, 1997). Protozoa perform very important tasks in the soil environment, but they require specific factors to perform their tasks.

To perform their tasks, Protozoa require a moist environment so that they can move which is found near the roots of plants. Larger protozoa sometimes feed on algae, fungi, and even plant debris, but mostly on bacteria. The environment where they have the greatest influence in their control of bacteria is also near plant roots. Protozoa seem to be the main predator of soil bacteria. Since bacteria eat dead matter, the energy absorbed by the bacteria is extremely low because it is at the bottom of the food chain. Therefore, a protozoon would need to eat a large number of bacteria to fulfill its energy consumption needs. At the same time, larger invertebrates are fulfilling their energy consumption needs by are eating protozoa. If the protozoa died, the invertebrates would have no food source, causing the invertebrates to die, and their predators to die as well. The entire food chain is disturbed and the major role protozoa play in the food chain becomes evident.

(Libscomb,2000). The protozoa populations, and thus the food chain, can be greatly affected by changes in the environment, such as soil pH.

A factor that can affect the soil Protozoa populations is the pH level of the soil. The pH, or potential of hydrogen, is an important measurement, which determines activity in the soil. The higher the pH level, the more alkaline the soil is, and the lower the pH level, the more acidic the soil is (Beaurain, 1999). The pH level of soil often changes, and usually is not neutral, which is a level of 7.0. The most normal pH level for soil is about 6.0 (Bates and Johnston, 1991). Microbes decrease the pH level as they break down organic matter. The carbon dioxide they exhale mixes with the water in the soil to form carbonic acid, and makes the soil more acidic (MDS Harris, 2001). Certain chemicals can also change the pH level of soil. pH plays a role in controlling the activity of enzymes. Enzymes have an optimal pH at which they are most active. At the optimal pH, the greatest number of molecular collisions occurs, without changing the nature of the enzyme. The pH levels for optimal activity usually range from 6-8. A change in the amounts of acidic chemicals causes the soil pH levels to fluctuate. By manipulating the pH and causing it to be outside of its optimal range, enzymes cannot function and chemical reactions in organisms cannot take place. Organisms must perform chemical reactions for their cells to perform the four tasks: respiration, homeostasis, synthesis of chemicals, and reproduction. If cells cannot perform these four tasks they would die. When the pH level changes and enzymes cannot function, the organisms die and the food chain is disturbed as a result (Campbell, Neil A & Reece, Jane B. 2002). For example, bacteria survive in soil with a pH of around 6.0 (Bates and Johnston, 1991). If the pH level is too extreme, the bacteria will die because their enzymes cannot create chemical reactions. These changes in pH level of soil are often caused by a change in acidity of rain.

A cause of low pH levels in soil is acid rain. Humans pollute the atmosphere, and in doing so make the rain, snow and fog more acidic. The new acidic moisture that comes down is known as acid rain. Acid rain is defined as rain, fog, snow, that is polluted by acid in the atmosphere and has a damaging effect on the environment. Two common air pollutants that acidify rain are sulfur dioxide (SO_2) and nitrogen oxide (NO_X). When these substances are released into the atmosphere, they can be carried over long distances by prevailing winds before returning to earth as acidic rain, snow, fog or dust. The acids, sulfur dioxide and nitrogen oxide are released into the air through the following ways. Sulfur dioxide is generally a byproduct of industrial processes and burning of fossil fuels. Ore smelting, coal-fired power generators, and natural gas processing are the main contributors. Naturally, it is produced by forest fires, volcanic eruptions, and decaying organic matter (Kennecott Utah Copper, 2000). The other main pollutant, nitrogen oxide, is commonly diffused into the environment through emission, which is the combustion of fuels in motor vehicles, residential and commercial furnaces, industrial and electrical-utility boilers and engines, and other equipment. Burning fossil fuels, in the forms of car exhaust, industrial smokestacks, etc., also produces nitrogen oxide. The acidic particles of sulfur and nitrogen, produced in those ways, can come down with rain or snow, but they can also come down out of dry air (Kimball, 2001). After the particles come down, they combine with water in forms such as rain or snow. In the combining, sulfur dioxide becomes sulfuric acid, and nitrogen dioxide becomes nitric acid. Sometimes the environment cannot neutralize the acid being deposited, and damage

Ani Gregorian
Kathryn Hampton
Justine Li
Meghan Murphy

occurs. Acid rain damages lakes, streams, and forests. Throughout Norway and Sweden, one fifth of the lakes no longer contain any fish. In forests, the acid may cause trees to be more susceptible to disease, insects, drought, high winds, inhibit plant germination, and reproduction. As a result, tree vitality and regenerative capabilities are reduced, and in many cases cause the trees to die (*Environment Canada*, 2003).

The preceding information about protozoa, soil pH, and acid rain brought us to the research question, “Does a decrease in pH level in soil cause the protozoa population density to increase or decrease?” We will test this by pouring sulfuric acid (with a concentration of 2%) onto one of our two 50 centimeter by 50 centimeter plots, while pouring water on the other plot. We will then test the protozoa population in each and compare the difference. We predict the soil with the lower pH level will have the smaller protozoa population density. This experiment will allow us to see in what conditions the protozoa of Roland Park Country School will prosper.

Ani Gregorian
Kathryn Hampton
Justine Li
Meghan Murphy

LAB OUTLINE

1. Problem: Does a decrease in the pH level in soil cause the protozoa population density to increase or decrease?

2. Hypothesis: The soil with the lower pH level will have the smaller protozoa population density.

3. Problem:

    a. Variables
        i. Independent Variable: pH of liquid poured on plot
        ii. Dependent Variable: population density of protozoa
    b. Controls
        i. Negative $Controls_1$: pH of soil sample on plot 1, water plot
        ii. Negative $Controls_2$: protozoa population of soil sample on plot 1, water plot
        iii. Positive Control: pH and protozoa levels of each plot before experiment
        iv. Controlled Variables:
            1. same composition of soil in both plots
            2. each watered same amount 2 liters
            3. internal and external temperature
            4. duration of experiment: 6 days
            5. number of trials: 3 samples for each plot
            6. time between watering: 4 days
            7. time between testing and extraction
            8. time of sampling
            9. same location extracted from
            10. each sample 3 cm wide
            11. each sample 15 cm deep
            12. use soil core sampler to sample
            13. location of experiment (near a tree with grass in the observed plots)
            14. same concentration of sulfuric acid (2%) poured evenly throughout plot 2
            15. ionized, uncontaminated water poured evenly throughout plot 1
            16. at all times, when using ionized water, it should be uncontaminated
            17. all petri dishes should be clean and dry before use to prevent contamination
            18. type of nylon screen or mesh, 1 mm^2
            19. type of modified Uhlig extractor, size
            20. type and diameter of 12.5 cm qualitative filter paper:

Ani Gregorian
Kathryn Hampton
Justine Li
Meghan Murphy

21. same type and size of microscope slide and 18x18 mm^2 cover slip
22. both plots fertilized equally
23. both plot with same amount of rain
24. both plots with same amount of wind
25. grass not cut during experiment on either plot
26. neither plot walked on during experiment
27. same amount of and type of run off to both plots
28. same elevation of both plots
29. both plots affected by equal amounts of pollution
30. temperature same for both plots
31. Same humidity for both plots
32. Same amount of sunlight for each plots

Procedure

1. Measure a square 50 cm by 50 cm at N 39.358121° and W 076.63627° . Mark off this square using flags at each corner and label 1 flag plot 1.
2. Adjacent to the first square, sharing a common border with it, measure and mark off one square 50 cm by 50 cm using flags at N 39.3507° and W 076.63626°. Label one flag plot 2.
3. From square 1, take 3 random* core samples 15 cm deep and 3 cm wide and put each sample in a plastic bag. Label the square number and sample number on each bag.
4. Repeat step 3 for plot 2.
5. Label 1 petri dish p 1 s 1 for plot one sample one.
6. Label another petri dish p 1 s 2.
7. Label another petri dish p 1 s 3.
8. Label another petri dish p 2 s 1.
9. Label another petri dish p 2 s 2.
10. Label another petri dish p 2 s 3.
11. Pour the soil samples in the petri dish that is labeled accordingly.
12. Allow the samples to dry thoroughly, for at least 24 hours.
13. Using the plot 1, sample 1 dish; continue to follow the Protozoa Extraction Procedure designed by Kate Brockmeyer. Place samples in the refrigerator. At the same time, follow step 14.
14. With the remaining soil from the plot 1 sample 1, at the same time as the protozoa extraction, complete the standard Test Procedure for the *La Motte Combination Soil Outfit* test kit, test the soil pH of the sample. Record data.
15. Repeat steps 13-14 for the remaining samples.
16. Remove samples from the refrigerator.
17. Place 7 µl of methyl-green stain on a clean microscope slide

Ani Gregorian
Kathryn Hampton
Justine Li
Meghan Murphy

18. Add 18 µl of the 2nd filtrate from step 6, on the Brockmeyer protocol, to the stain on the microscope slide and cover with 18 x 18 mm^2 cover slip.
19. Examine under a light microscope at 40X and count the number of protozoa in one field.
20. Repeat step 18 for 4 additional fields. Record the number of protozoa counted. Average the 5 fields and record averages.
21. Repeat steps 17-20 for remaining samples.
22. Use the following equation to determine the population density of protozoa in the soil sample: [(# per field of view at 40X) x (total ml of water used) x 747] / (grams of sifted soil) = # of protozoa per gram of soil. Record Data
23. To make the sulfuric solution, begin with 900 mL of distilled water in a flask. Add 100 mL of 1 molar solution of H_2SO_4 to the water. Take 20 mL of that solution and add 980 mL of distilled water.
24. Repeat step 23 to get a second liter of the sulfuric solution.
25. Pour both solutions made in steps 23 and 24 over plot 2 evenly, making sure to cover the entire plot. Pour the liquid out of a flask being careful not to spill outside of box. At the same time pour 2 liters of distilled water over plot 1 in the same fashion. Starting in one corner pour moving in lines back and forth.
26. Allow the solutions to stay in the soil for 4 days, untouched.
27. After 4 days, repeat steps 3-22. Record all data.

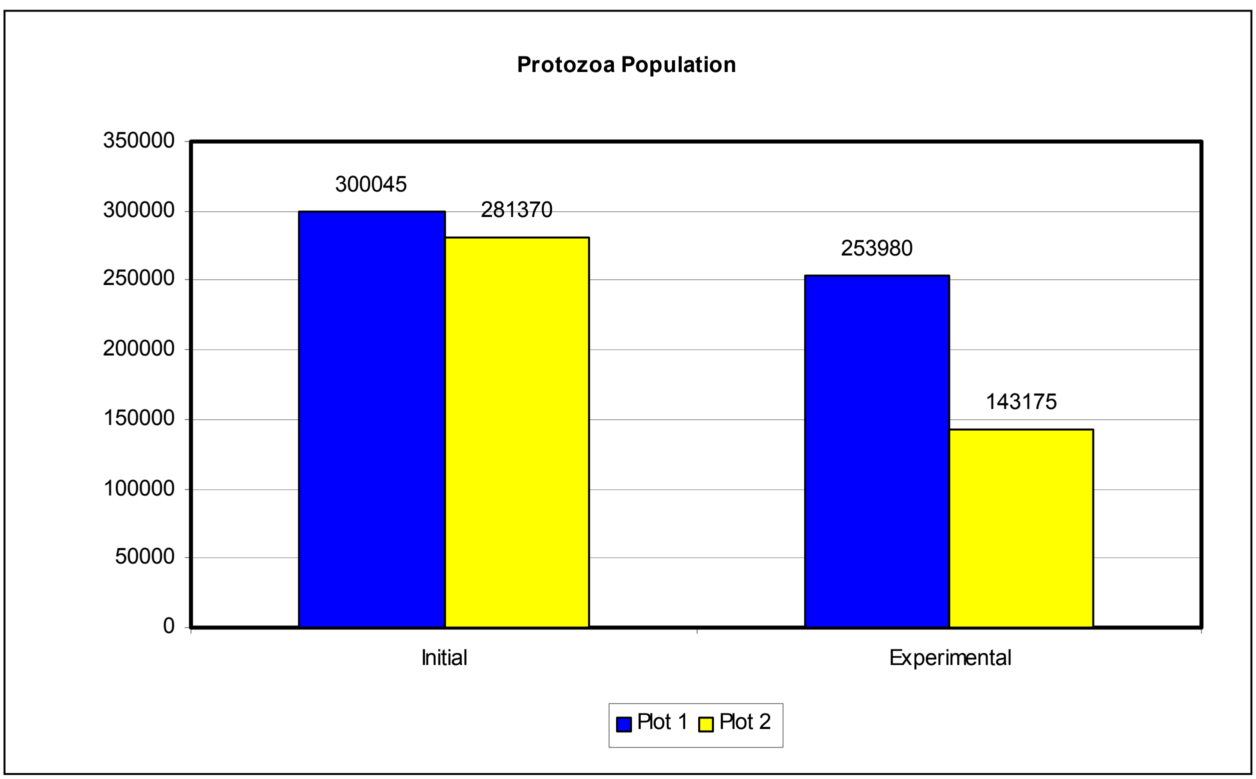
* To randomly select the locations from which the samples are taken, separate the 50 cm by 50 cm plots into 5 cm by 5 cm subplots, resulting in 100 subplots. Each subplot is labeled with a number. Using a random number generator, it generated integers between 1 and 100. 3 numbers are selected simultaneously, which will give you the plot number, which is then converted back into coordinates. If the same number is selected twice, take the 2 samples from the subplot anyway, making sure the samples do not overlap.

Ani Gregorian
Kathryn Hampton
Justine Li
Meghan Murphy

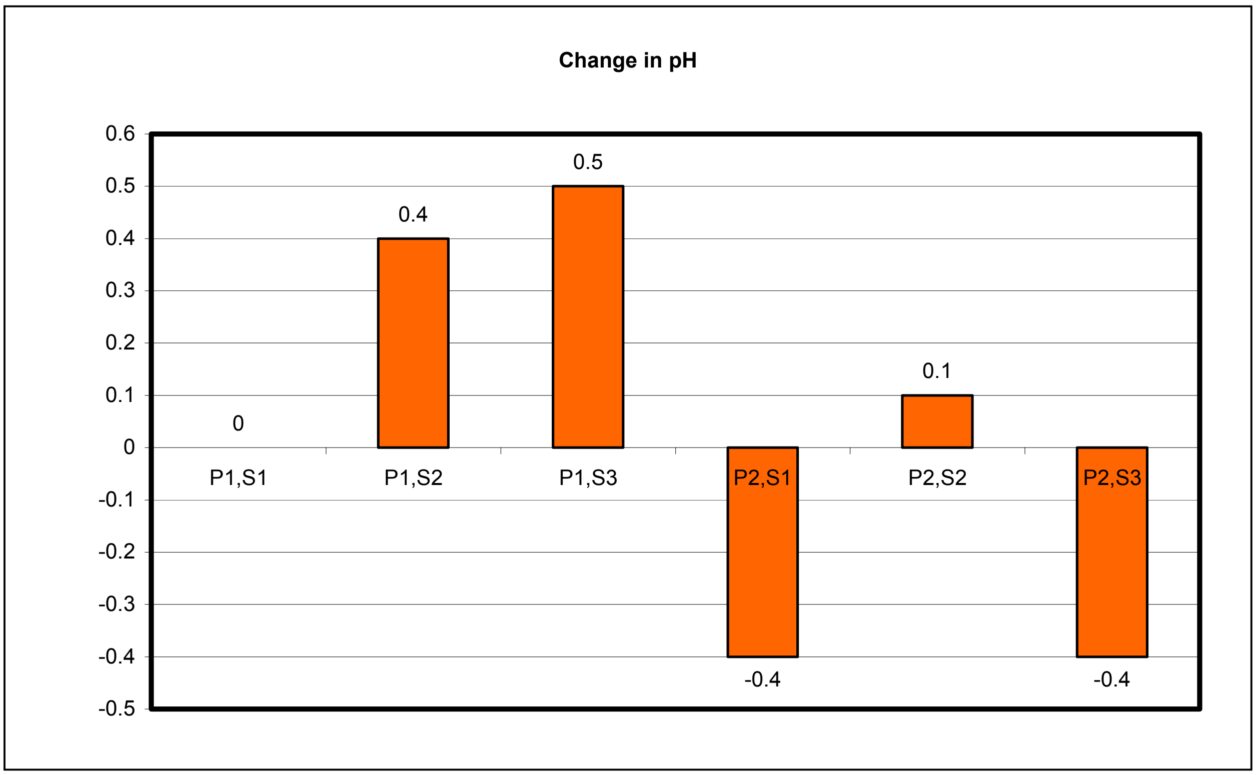
DATA/ OBSERVATIONS

Data

Condition	Plot #	Sample #	Protozoa	pH
Initial	1	1	336150	7.8
		2	283860	7.5
		3	280125	7.5
		Average	300045	7.6
	2	1	265185	7.4
		2	276390	7.1
		3	302535	7.0
		Average	281370	~7.2
0% Sulfuric Acid	1	1	197955	7.8
		2	291330	7.9
		3	272655	8.0
		Average	253980	7.9
2% Sulfuric Acid	2	1	70965	7.0
		2	287595	7.2
		3	70965	6.6
		Average	143175	~6.9



Ani Gregorian
Kathryn Hampton
Justine Li
Meghan Murphy

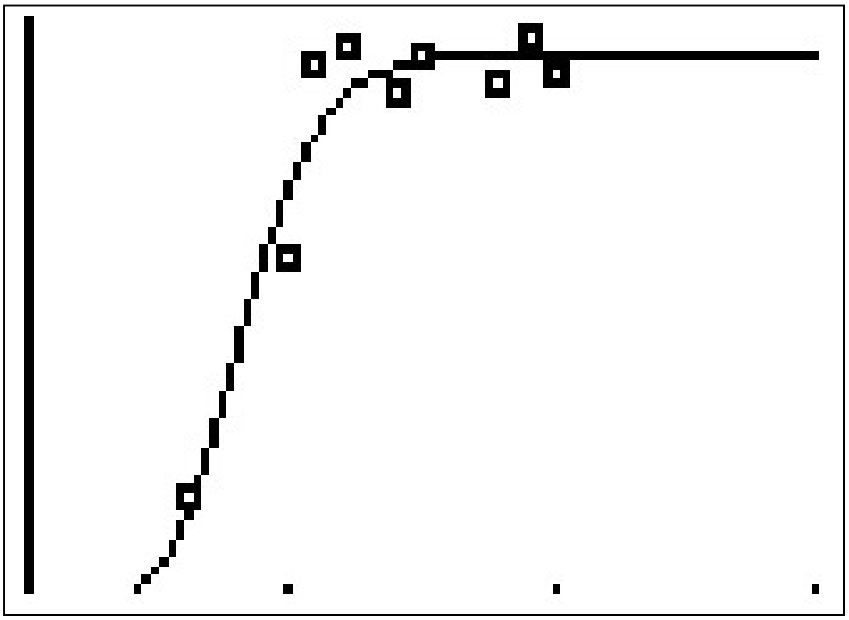


The protozoa population graphs are based on the averages on the 3 subplots of each plot. From the data, we can see that there was a decrease in protozoa populations in both plots.

Initially, the protozoa population is regular. There is only a small variation between the two pieces of data. After the experiment, there was a significant difference in the protozoa populations between the plots.

We can see that in Plot 2, there was a general decrease in pH, while in Plot 1, there was a general increase. This was our positive control. ☺

Ani Gregorian
Kathryn Hampton
Justine Li
Meghan Murphy



By plotting pH vs. protozoa levels (pH being the independent variable), we can see that there is a relationship between the 2. the logistic function being used to accurately model the collected data is

Y= 282202.25519004/(1+7.9871630699586E18e^(-6.4056327718732X))

This equation is shown above on the graph. (Each mark is 1.0, starting from 6.0).

Ani Gregorian
Kathryn Hampton
Justine Li
Meghan Murphy

CONCLUSION

From the data shown, we are able to conclude that our hypothesis was correct. PH affects protozoa population greatly. The graph which displays the correlation between pH and protozoa shows that as the pH values increase, it appears that the number of protozoa increases as well. The decrease in the protozoa population in Plot 2 is expected because Plot 2 was the experimental plot where we changed the pH deliberately by adding sulfuric acid. This was also the soil which had a lower pH. From the graph of pH vs. Protozoa, population we can see that the optimal range for protozoa levels is between 7.0 and 8.0. Since we did not collect data from the other end of the pH range, we cannot determine whether the optimal range is only between pH values of 7.0 and 8.0 or if there is another lower range as well.

For this experiment, there was an extra variable that was added due to negligence. When taking the pH values and extracting protozoa from the samples, we only added water for the protozoa extraction, but did not do the same for the pH levels, which may or may not have altered the results. We had an additional source of error in the pouring of the liquids throughout the plots. The chemicals should have been poured directly into the middle of each plot therefore allowing both chemicals to diffuse into the soil evenly. Directions in which we could further our research is to find another way in which pH affects the soil or the organisms in the soil. We could test pH above the optimal range, instead of below. We could also cause a greater change in the pH of the soil to see if that caused different results. To pursue our research, we could cause the change in pH using a different chemical or a different dilution of the chemical that we used.

Ani Gregorian
Kathryn Hampton
Justine Li
Meghan Murphy

References

Bates, T.E. and Johnston R.W (1991, November) *Soil Acidity and Liming*. Retrieved May 4, 2003 from http://www.gov.on.ca/OMAFRA/english/crops/facts/91-086.htm#c1

Beaurain, Bill.(1999). Soil Acidity…How to Adjust the Soil pH in Your Garden. In *The Garden Helper*. Retrieved May 4, 2003, from http://www.thegardenhelper.com/acidsoil.html

Campbell, Neil A & Reece, Jane B. (2002). *Biology Sixth Edition*. San Fransico, CA: Benjamin Cummings

Environment Canada. (2003). The Green Lane™, Environment Canada's World Wide Web site. Retrieved May 20, 2003, from http://www.ec.gc.ca/acidrain/

Gunn, Cullen (2001), *soil acidification*. Retrieved May 4, 2003 from http://www.netc.net.au/enviro/fguide/soilacid.html

Ingham, Elaine R. (2001). Soil Protozoa. In *The Soil Biology Primer* (chap. 5). Retrieved May 4, 2003, from http://soils.usda.gov/sqi/SoilBiology/protozoa.htm

Jefferson Road Elementary School. (May 2002). *Protozoa*. Retrieved May 3, 2003 from http://pittsford.monroe.edu/jefferson/calfieri/Animals/PROTOZOA.html

Kennecott Utah Copper (2000). *Sulfuric Acid.* Retrieved May 8, 2003 from http://www.kennecott.com/sulfuricacid.html

Kimball, John W., Dr. (2001, December) *Acid Rain*. Retrieved May 8, 2003 from http://users.rcn.com/jkimball.ma.ultranet/BiologyPages/A/AcidRain.html

Libscomb, D. (2000, September) *Protozoa*. Retrieved May 17, 2003, from http://biology.usgs.gov/s+t/noframe/m2083.htm

MDS Harris (2000- 2001) *Soil PH* Retrieved May 4, 2003, from http://www.ag.mdsharris.com/AgriKnowledge/Soil%20pH.htm

Plaster, E. J. (1997). *Soil Science and Management*. Retrieved May 18, 2003 from www.agronomy.psu.edu/courses/soils101/labs/ecology.html

Tatner, Paul. (2002). Protozoa. In *Biomedia*. Retrieved May 5, 2003, from http://www-biol.paisley.ac.uk/courses/Tatner/biomedia/units/prot1.htm

Trahktenberg, Izolda. (2001). *About Soil pH*. Retrieved May 5, 2003, from http://ltpwww.gsfc.nasa.gov/globe/soil_pH/plant_pH.htm

Ani Gregorian
Kathryn Hampton
Justine Li
Meghan Murphy

Whitelaw, Mark (1997) *pH and Your Soil's Health*. Retrieved May 20, 2003, from http://markw.com/phsoil.htm

Ani Gregorian
Kathryn Hampton
Justine Li
Meghan Murphy