SOIL ECOLOGY PROJECT

THE EFFECTS OF ACID RAIN ON BACTERIA

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Background

Soil bacteria play a still largely unknown yet vital role in the environment. Bacteria fix and release nitrogen from the atmosphere into forms other organisms can use. This nitrogen is critical because it is a necessary element in the creation of amino acids, which are necessary to create proteins, enzymes that are responsible for causing all chemical reactions in living things and the consequent chemical reactions they literally make possible. Hence, without these bacteria, there would be no life. Further, the soil bacteria also cycle elements such as sulfur, carbon, and phosphorus through decomposition, and when plants receive these nutrients through their roots or in the form of carbon dioxide in the air. Plants can then use all these chemicals to photosynthesize in order to make the five biological molecules, (water, lipids, carbohydrates, nucleic acids, and proteins). The contribution that these bacteria make enables producers (plants) to grow, creating food for primary and secondary consumers (humans). These bacteria are not just helpful, but absolutely vital to the well-being of every ecosystem. (Ben Waggoner; Brian Speer, 1995-1996).

What can ruin this perfect system of cycling biological molecules? Acid rain. Acid rain is a broad term which refers to a wet and dry deposition mixture from the atmosphere that contains higher than normal amounts of sulfuric and nitric acids. The “chemical forerunners” of acid rain result from natural sources, such as decaying vegetation, volcanoes, as well as man-made sources, such as nitrogen oxides (NO_x) and sulfur dioxide (SO_2) emissions from the burning of fossil fuels. Acid rain occurs when the various gases from there sources react in the atmosphere with oxygen, water, and chemicals to form different acidic compounds. In turn, the end result of burning fossil fuels is a light solution of nitric acid and sulfuric acid which is exactly like earlier mentioned, that and the normal wind patterns can blow the compounds across the country, sometimes for hundreds of miles (U.S. Environmental Protection Agency, 2007).

Most of the time, the impact of this rain is a negative one. The pH level that best nurtures bacteria is neutral, between 6-8, (Lavelle and Spain, 2001) and while bacteria are quite hardy (in fact, some bacteria use sulfuric acid for energy), in most cases, the pH in soil generated by acid rain produces extremely low levels of pH that can harm these microbes because acid destroys enzymes which perform chemical reactions and without those chemical reactions the microbes would die. (Science in the Real World: Microbes in Action, 1999). Hence, the more sulfuric and nitric acid that falls on the soil, the lower the bacteria density will become. (Science in the Real World: Microbes in Action, 1999). That, in turn, has consequences not only for cycling nutrients but for soil's food chains as well, leading to disruptions in all the microbe population bacteria and protozoa. Therefore, because all of the microbes work and must be in their respective numbers to function properly together in harmony, even some acid rain is disruptive to an ecosystem. (Lavelle and Spain, 2001).

In our experiment, we will be examining the effect acid rain has on soil. This will be tested by measuring the pH in soil and the density of bacteria in soil. We will be combining our knowledge of bacteria, acid rain, and pH levels to pour nitric acid, sulfuric acid, and water (as a control) on the RPCS campus to determine the possible effects of acid rain on soil and the organisms that live there. Thereby we predict that sulfuric acid will prove to be the most toxic and have the greatest effect on the soil.

Lab Outline

I. Problem: Which of the two most common types of acid rain (nitric or sulfuric acids) will alter the levels of pH and density of bacteria in the soil the most?

II. Hypothesis: Sulfuric acid will decrease the level of pH and density of bacteria in the soil more than nitric acid.

III. Procedure:

A. Independent Variable: applications of sulfuric acid or nitric acid to the soil

B. Dependent Variable: soil pH and density of soil bacteria

C. Negative Control: the application of only water to the soil

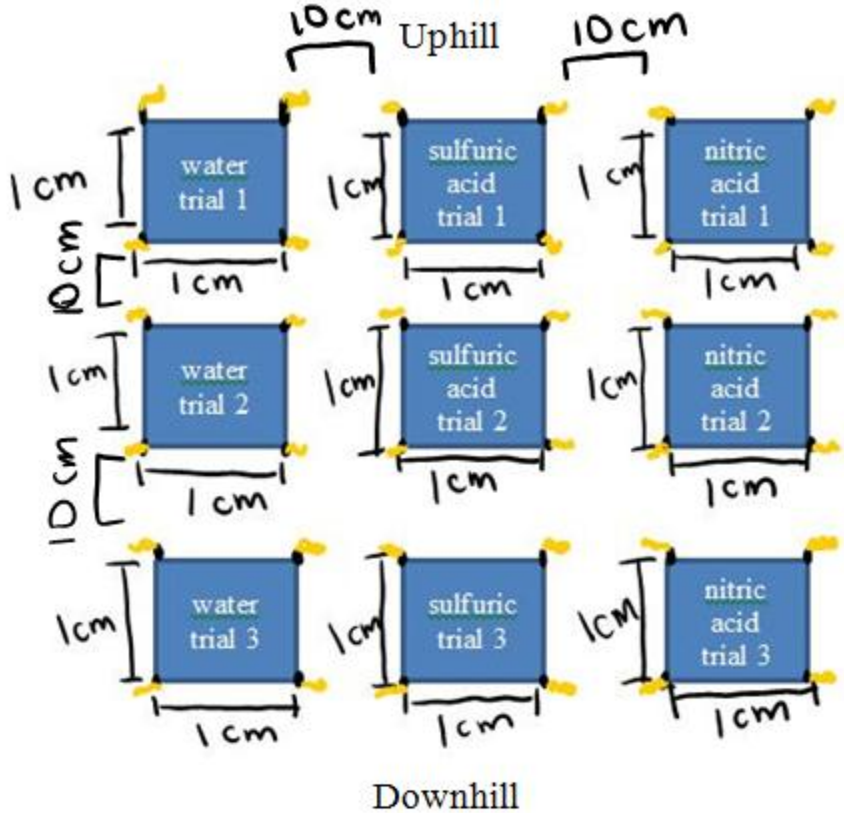
D. List of Controlled Variables:

Area of experiment, size of areas/rows and squares, shape of areas/rows and squares, number of areas/rows and squares, amount of nitric acid, amount of sulfuric acid, amount of water, concentration of nitric acid, concentration of sulfuric acid, water source, how water is distributed, how nitric acid is distributed, how sulfuric acid is distributed, amount of time of waiting for nitric acid to sink into soil, amount of time of waiting for sulfuric acid to sink into soil, amount of time of waiting for water to sink into soil, the steps in the chemical testing using the same chemical test kit to determine pH, the steps in the serial dilutions for bacteria, the amount of soil taken with the soil auger, space in between in each square, time for bacteria to grow, amount of solution plated in serial dilution, how much of the soil sample was diluted, type of soil auger used, type of growth media, and numerical dilutions we plated.

E. Step-by-Step Instructions:

1. Label three separate 1 liter bottles “S1”, “S2”, “S3” respectively. Fill each bottle with a water/sulfuric acid solution with a pH of 5.
2. Label three separate 1 liter bottles “N1”, “N2”, “N3” respectively. Fill each bottle with a water/nitric acid solution with a pH of 5.
3. Label three separate 1 liter bottles “W1” “W2” “W3” respectively. Fill each bottle with 1 liter (1000 ml) of tap water.
4. Go outside in an open area of soil which has access to plenty of sunlight and is not hindered by trees or shade and the same amount of grass coverage; the exact coordinates of this area are 39.35711˚ North and 76.63517˚ West.
5. Make a 3x3 grid on the soil; with the dimensions of each square in the grid being 30cm by 30 cm, and the space in between each square to be 10 cm. (See Figure 1)
6. Label 12 yellow flags; 4 labeled as “Water Trial 1”; 4 labeled as “Water Trial 2”, and 4 labeled as “Water Trial 3”.
7. Label 12 more flags; 4 labeled as “Sulfuric Acid Trial 1”, 4 labeled as “Sulfuric Acid Trial 2”, and 4 labeled as “Sulfuric Acid Trial 3”.
8. Label 12 more flags; 4 labeled as “Nitric Acid Trial 1”, 4 labeled as “Nitric Acid Trial 2”, and 4 labeled as “Nitric Acid Trial 3”.
9. Label grid with flags as shown in Figure 1, below. Also, see the box below Figure 1 for the scale used in Figure 1.

Figure 1:



<u>Helpful Information about Drawing Above</u>

1) The scale of the drawing of the squares in each row above is 1 cm: 30 cm.

2) There is not a lot of room to show it, but there will be 10 cm in between each square.

3) The yellow sticks in the drawing represent the yellow flags in each corner of each square. Each yellow flag will have the label of the square on it. However, it is hard to show the label on the tiny yellow sticks, so the label of each square is in the square for the drawing.

4) The “uphill” and “downhill” show the directions of the hill which our square plots are on.

10. Obtain nine plastic re-sealable, unused sandwich bags.
11. Label three separate plastic re-sealable bags: “before trial 1 Water” “before trial 2 Water” “before trial 3 Water” respectively.
12. Label three more separate plastic re-sealable bags: “before trial 1 Sulfuric Acid”, “before trial 2 Sulfuric Acid”, and “before trial 3 Sulfuric Acid” respectively.
13. Label three more separate plastic re-sealable bags: “before trial 1 Nitric Acid”, “before trial 2 Nitric Acid”, and “before trial 3 Nitric Acid” respectively.
14. Place these plastic bags in their respective soil plots at the same time on the same day.
15. Make sure that steps 16 and 17 below take place at the same time on the same day.
16. Now, use the soil auger/soil core sampler to get a 15cm deep and 2cm wide soil sample for each square in the grid. (On the soil auger, there is sometimes a line at the 15cm mark, otherwise, carry a metric ruler along with you.)
17. Put each soil sample inside its correspondingly labeled plastic bag at the same time on the same day.
18. Make sure that steps 19-48 below also take place at the same time on the same day, and that trials for all test substances are tested together (e.g. “Before Trial 1 Water”, “Before Trial 1 Sulfuric Acid”, and “Before Trial 1 Nitric Acid” are all tested at same time on same day.)
19. Use the model STH-14 test kit to perform the pH test on each of the “before” soil samples at the same time on the same day.
20. While this is going on, also perform the serial dilutions for bacteria.

21. For the serial dilutions test for bacteria, label the test tubes with what is being tested, e.g. NT1 for the Nitric Acid Trial One.
22. Use a clean, new transfer pipette to add 10 ml of sterile water to a 15 ml culture tube. Label the tube "10^0 W1", standing for Water Trial 1.
23. Use the same pipette to add 9 ml to second 15 ml culture tube. Label the tube "10^{-1} WT1".
24. Repeat step 23 three more times to three additional 15 ml culture tubes, but labeling them "10^{-2} WT 1," "10^{-3} WT 1", and "10^{-4} WT 1" respectively.
25. Place 1 cc of soil from the "Before Water Trial 1" bag into the "10^0 WT 1" culture tube.
26. Cap the tube and shake vigorously.
27. Using a clean pipette, remove 1 ml of soil/water mixture from the "10^0 WT 1" tube and place into the "10^{-1} WT 1" tube.
28. Cap and shake vigorously.
29. Using the same pipette as in step 27, remove 1 ml of the soil/water mixture from the "10^{-1} WT 1" tube and place into the "10^{-2} WT 1" tube.
30. Cap and shake vigorously.
31. Using the same pipette in step 19, remove 1 ml of the soil/water mixture from the "10^{-2} WT 1" tube and place into the "10^{-3} WT 1" tube.
32. Cap and shake vigorously.
33. Using the same pipette in step 27, remove 1 ml of the soil/water mixture from the "10^{-3} sulfuric acid trial 1" tube and place into the "10^{-4} sulfuric acid trial 1" tube.

34. You should now have a total of five culture tubes.
35. Plate separate 100 μl samples from the 2nd and 3rd tubes onto separate correspondingly labeled 3M Petrifilm™ Aerobic count plates.
36. Repeat steps 21-35 for all remaining soil samples, being sure to label dilution tubes to match their corresponding soil samples.
37. Allow samples from step 35 to grow on the 3M Petrifilm™ Aerobic count plates for 48 to 72 hours.
38. Count the number of bacteria colonies on the plate labeled with 10^{-3}, (which is the more diluted plate), make sure that there are at least 5 bacteria colonies on the plate. Record the number of bacteria and the dilution label which was observed.
39. If there are not at least 5 bacteria colonies on the plate labeled 10^{-3}, then count the number of bacteria colonies on the plate labeled 10^{-2}, (which is the less diluted plate). Make sure to count all of the bacteria colonies on the plates with magnifying glasses.
40. To get the end result/ the number of microbes in 1 cc of soil, use the following formula:

    # Microbes in 1 cc of soil = # Colonies on sheet x 10^2 x $10^{\text{l dilution \# at which these colonies were found l}}$

41. Collect the data and put in data table.
42. Obtain nine plastic re-sealable, unused sandwich bags.

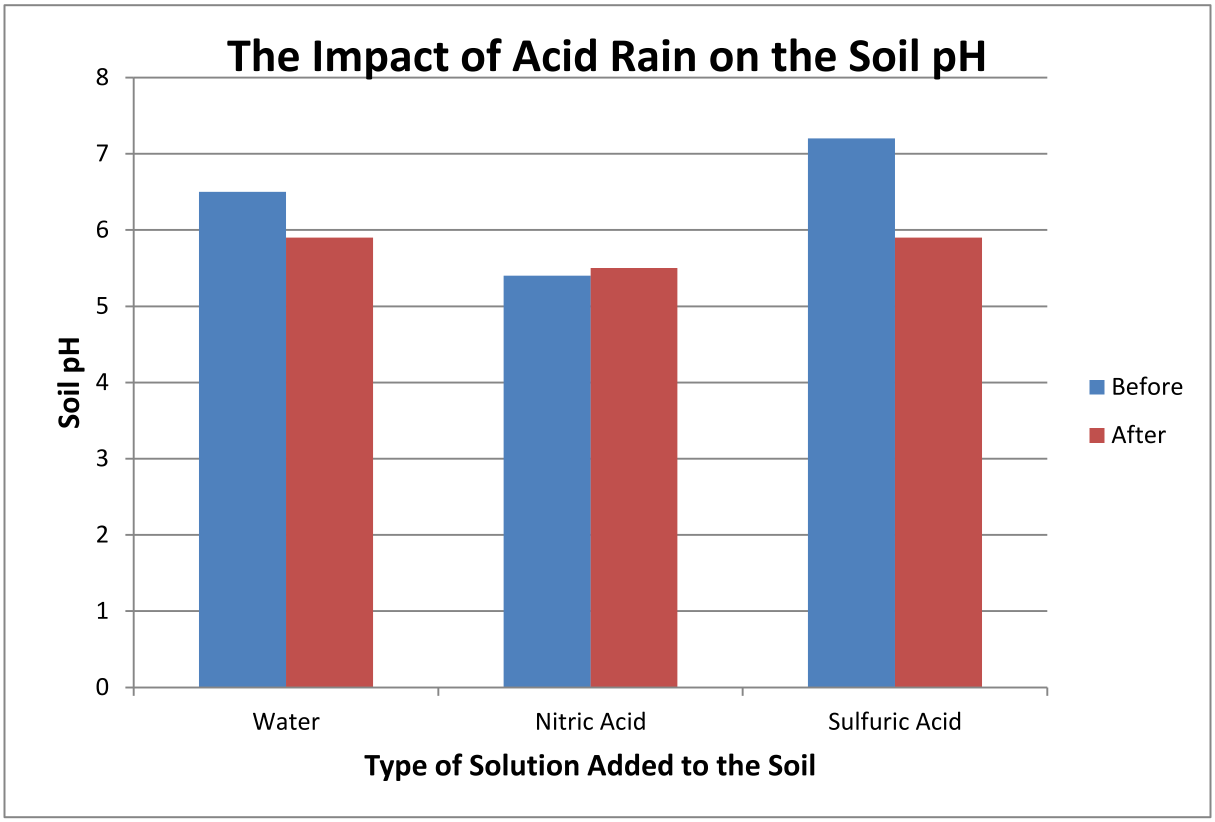
43. Label three separate plastic re-sealable bags: “after trial 1 Water” “after trial 2 Water” “after trial 3 Water” respectively.
44. Label three more separate plastic re-sealable bags: “after trial 1 Sulfuric Acid”, “after trial 2 Sulfuric Acid”, and “after trial 3 Sulfuric Acid” respectively.
45. Label three more separate plastic re-sealable bags: “after trial 1 Nitric Acid”, “after trial 2 Nitric Acid”, and “after trial 3 Nitric Acid” respectively.
46. Pour 1 liter of the sulfuric acid, nitric acid, and water (from steps 1-3) from each of the 1 liter bottles onto each corresponding square of the each row; pour evenly on soil. Wait at least 24 hours for the sulfuric acid, nitric acid, and water to sink into the soil.
47. Then repeat steps 14-41, but do these steps for the “after” data and change the labels on any materials that previously had “before” written on it to have “after” written on it.
48. Clean up all materials used.

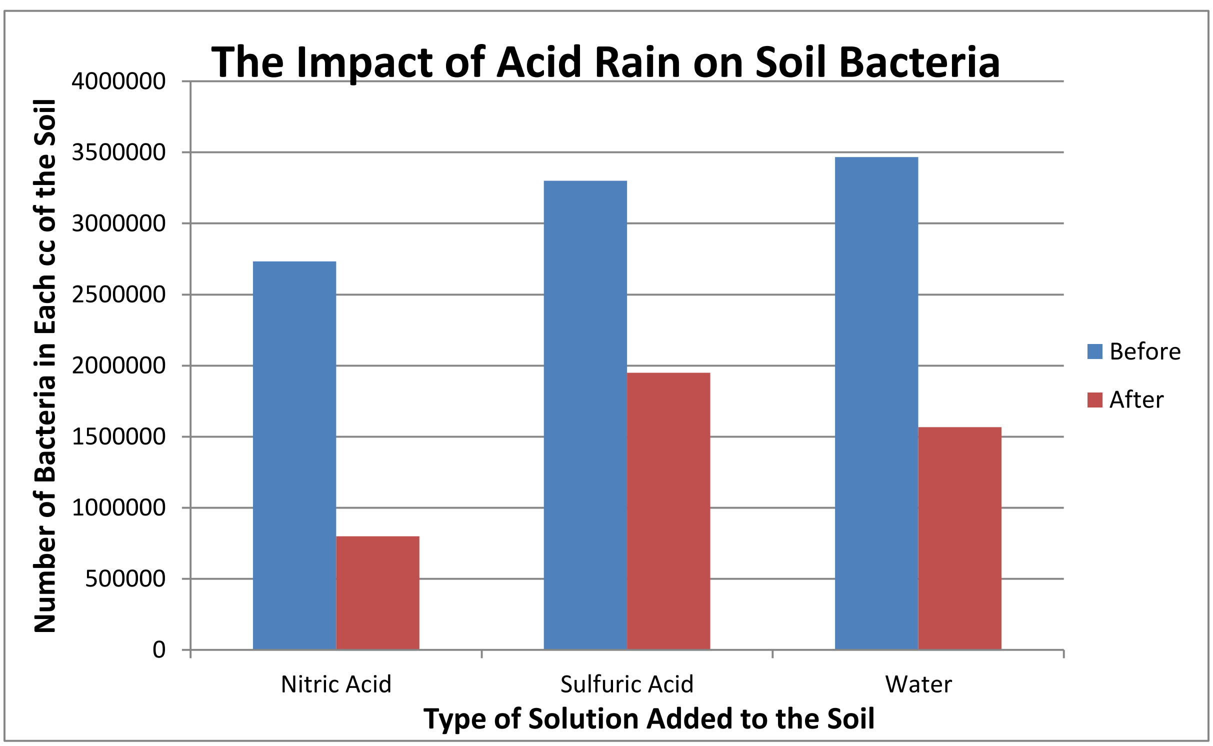
Data/Observations

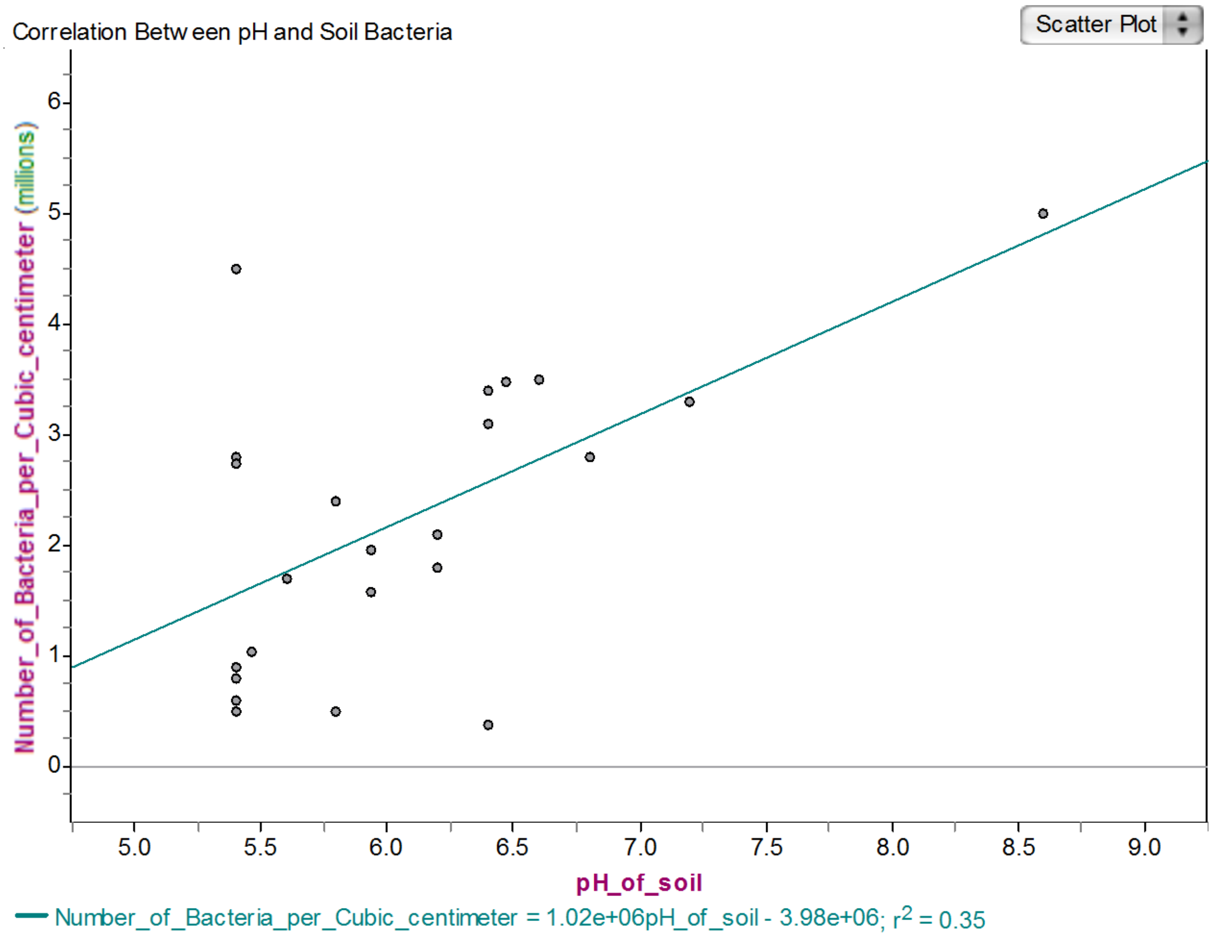
A. Data Table: It is below on a separate page (see page 11.)

B. Graphs: There are three of them. They are below the data table, (see page 12 and the top of page 13.)

The Impact of Acid Rain on Soil Ecology												
Trial Number	Water Treatment				Sulfuric Acid Treatment				Nitric Acid Treatment			
	The Number of Bacteria Per Cubic Centimeter of Soil		Soil pH		The Number of Bacteria Per Cubic Centimeter of Soil		Soil pH		The Number of Bacteria Per Cubic Centimeter of Soil		Soil pH	
	Before Added	After Added	Before Added	After Added	Before Added	After Added	Before Added	After Added	Before Added	After Added	Before Added	After Added
1	3,800,000	500,000	6.4	5.8	2,100,000	500,000	6.2	5.4	4,500,000	600,000	5.4	5.4
2	3,500,000	2,400,000	6.6	5.8	5,000,000	3,400,000	8.6	6.4	900,000	1,700,000	5.4	5.6
3	3,100,000	1,800,000	6.4	6.2	2,800,000	n/a	6.8	6	2,800,000	800,000	5.4	5.4
Average:	3,466,667	1,566,666	6.5	5.9	3,300,000	1,950,000	7.2	5.9	2,733,333	800,000	5.4	5.5


The Impact of Acid Rain on the Soil pH
Soil pH
0
1
2
3
4
5
6
7
8
Water
Nitric Acid
Sulfuric Acid
Type of Solution Added to the Soil
Before
After


The Impact of Acid Rain on Soil Bacteria
Number of Bacteria in Each cc of the Soil
0
500000
1000000
1500000
2000000
2500000
3000000
3500000
4000000
Nitric Acid
Sulfuric Acid
Water
Type of Solution Added to the Soil
Before
After



Conclusions

Our hypothesis was not proven right through our testing; sulfuric does not, nor does nitric acid, affect the density of bacteria in the soil and the soil pH. We tested the effect of the two types of acid, (nitric acid and sulfuric acid) on the soil to see which type is most toxic and acidic to the soil and to the bacteria that live in the soil. We did this by setting up plots outside, 3 plots for water, 3 plots for nitric acid, and 3 plots for sulfuric acid. Then, we poured the water, nitric acid, and sulfuric acid onto each of their correspondingly labeled plots at the same time on the same day. Our data after we poured the solutions confirmed the falseness of our original hypothesis. The average soil pH for the water plots before water was actually added was 6.5. The average soil pH for the nitric acid plots before the nitric acid was actually added was 5.4. The average soil pH for the sulfuric acid plots before the sulfuric acid was actually added was 7.2. After we added the solutions to their correct plots, our data changed. The average soil pH for the water plots after the water was added was 5.9. The average soil pH for the nitric acid plots after the nitric acid was added was 5.5. The average soil pH for the sulfuric acid plots after the sulfuric acid was added was 5.9. The soil pH difference between the beginning and after samples of the nitric acid plots was minor; the soil pH went from 5.4 to 5.5, which is not a huge change. The small increase in soil pH once nitric acid was added indicates that the nitric acid made the soil slightly less toxic and acidic. The soil pH difference between the beginning samples and after samples of the sulfuric acid plots was drastic (and the biggest difference of all the plots); the soil pH went from 7.2 all the way down to 5.9. This major decrease in soil pH once sulfuric acid was added indicates that the sulfuric acid may have made the soil acidic and toxic, but other data conflicts with this inference. The soil pH difference between the beginning samples and after samples of the water plots was rather odd; the soil pH went from 6.5 to 5.9. The data shows that the water plots actually became more toxic and acidic when water was poured on them. This is not fully true. As one would gather, water is not typically toxic, but in our experiment we used water as a negative control. The fact that the water changed without acid shows that some other factor was changing the pH in the soil, not particularly acid. From the various soil pHs we collected, we can conclude that so far acid is not a truly major factor to the toxicity of the soil.

Next, for the number of bacteria per cubic centimeter for soil, we saw clear changes between sulfuric acid, nitric acid, and water. The average number of bacteria per cubic centimeter for the water plots before any water was poured on them was 3,466,667. The average number of bacteria per cubic centimeter for the nitric acid plots before any nitric acid was poured on them was 2,733,333. The average number of bacteria per cubic centimeter for the sulfuric acid plots before any sulfuric acid was poured on them was 3,300,000. After we added the solutions to their correct plots, our data changed. The average number of bacteria per cubic centimeter for the water plots after the water was poured on them was 1,566,666. The average number of bacteria per cubic centimeter for the nitric acid plots after the nitric acid was poured on them was 800,000. The average number of bacteria per cubic centimeter for the sulfuric acid plot after the sulfuric acid was poured on them was 1,950,000. The difference between the before and after averages of the number of bacteria per cubic centimeter for the water plots may be puzzling at first. However, this really is not puzzling because it just shows that again there is another factor decreasing the number of bacteria and that the acids are playing a minor role, if any. Therefore this data proves that the acid had little to no impact on the bacteria and that some other environmental component is affecting our experiment.

The bacteria data also shows another interesting twist. From the bacteria graph, we can see that there was already an original problem with the soil causing bacteria to die. The sulfuric acid seemed to in a way help the soil a bit, and the nitric acid seemed to hurt it. So therefore, whatever was happening to the soil already was being helped by the sulfuric acid and was being worsened by the nitric acid.

Looking back at the data one can clearly see that the water changed dramatically in each test. This odd change shows that the acids (nitric acid and sulfuric acid) weren't changing the soil components, but something else was changing it. The scatter graph we made also helps display that all of the data we collected reflects the real-world situation of acid rain in the environment. This is true because as the pH becomes more neutral the bacteria number increases. This is to be expected because higher levels of pH are less toxic which allow enzymes to start and stop chemical reactions (function) which in turn allows bacteria to function. That's why we know that normal ecological processes were taking place in the soil we were testing. This data shows that acid rain does not have a truly damaging impact on the soil. From all of our data, we can conclude that the acid in our small controlled experiment had little to no effect on the bacteria or soil pH. This is surprising to us because these acids are famously toxic and dangerous to all organisms. However, it may be that the acid rain didn't affect the soil in our experiment drastically.

Now that we see and know that these acids are not very threatening to bacteria, which are so vital to our world, we need to look elsewhere to find answers. We see there is another factor already affecting the soil that the sulfuric acid is helping and the nitric acid is worsening. A logical step would be to find exactly what that factor is. Along with that, we need to find out why the sulfuric acid helps the soil, and the nitric acid hurts the soil. With this data, we have discovered something possibly greater than acid rain. What it could be is what we are going to find out.

Works Cited

Homeplanet. (2011) Soil Types and Testing. http://www.rain.org/global-garden/soil-types-and-testing.htm.

Lavelle, P. and Spain, A.V. (2001) *Soil Ecology*, pp 201-356 Kluwer Academic Publishers, The Netherlands. http://www.soilhealth.see.uwa.edu.au/components/bacteria.

Oracle Education Foundation. (1997) The Nitrogen Cycle. Oracle Education Foundation: http://library.thinkquest.org/11353/nitrogen.htm

Science in the Real World: Microbes in Action. (1999) Science in the Real World: Microbes in Action. Science in the Real World: Microbes in Action: http://www.umsl.edu/~microbes/pdf/introductiontobacteria.pdf.

Speer, B; Waggoner, B. (2006) Bacteria: Life History and Ecology. University of California: http://www.ucmp.berkeley.edu/bacteria/bacterialh.html

State University of New York College of Environmental Science and Forestry. (2011) Soil pH: What it Means. State University of New York College of Environmental Science and Forestry: http://www.esf.edu/pubprog/brochure/soilph/soilph.htm

U.S. Environmental Protection Agency. (2007) What is Acid Rain? U.S. Environmental Protection Agency: http://www.epa.gov/acidrain/what/

U.S. Environmental Protection Agency. (2007) What is pH? U.S. Environmental Protection Agency: http://www.epa.gov/acidrain/measure/ph.html.

Vasquez M.; Espejo R. T. (1997) Chemolithotrophic Bacteria in Copper Ores Leached at High Sulfuric Acid Concentration. Appl Environ Microbiol: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1389111/