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Biology 9

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Soil Ecology

Background

Bacteria are prokaryotes, which mean that they lack a nucleus and membrane bound organelles. Bacteria are unicellular microorganisms with three different shapes: bacilli (rod shaped), cocci (circular shaped), and borrelia (spiral shape). Some bacteria can make their food through photosynthesis; others engage in cellular respiration and absorb food from in their environment. Bacteria reproduce simply through binary fission, which rapidly splits one cell into two identical cells.

To start the nitrogen cycle we must start with the nitrogen element in the air. Two nitrogen oxides will always be found in the air due to the mixing with oxygen. Nitrogen only reacts with oxygen if the temperature and pressure is high (such as places where lightning has struck, power plants, and combustion engines). If the conditions are correct then nitric oxide and nitrogen dioxide will form. Over time the nitrogen dioxide has a chemical reaction with rain water which forms nitric acid. The nitrates in the nitric acid can be used by plants for nutrients. In the process of nitrogen fixation the nitrogen in the air becomes part of a biological matter by way of bacteria. Certain plants (like clover and soybeans) create nodules on their roots which a bacterium attaches to. This bacterium is undergoing nitrogen fixation in which the bacteria absorb nitrogen that is in the air and changes in into ammonia. Ammonia comes from decaying organic matter and animal waste which is converted by nitrogen fixing bacteria into ammonium. Then the ammonium is converted by nitrifying bacteria into nitrite which is later converted into nitrate. And nitrate is what the plants take up as fertilizer. Nitrogen is an extremely important part of helping plants to live because nitrogen makes up a large chunk of the cell. Nitrogenous bases are made up of nitrogen; these nitrogenous bases are what make up DNA and RNA. Nitrogen is also in amino acids which make up proteins. These proteins start and stop chemical reactions which make up the fours tasks of life, synthesis of new material, homeostasis, reproduction, transformation of energy, which make up the cell.

Nitrogen is very important to sustaining life. The body receives nitrogen it needs to grow from food. The majority of plants get their needed nitrogen supply from soil. A common human use for nitrogen is in fertilizers used from crops. This helps plants grow faster and larger. The access nitrogen eventually gets washed away by rain and end up in lakes and streams which can cause the death of cell organisms.

Decomposition is the process in which bacteria, fungi, molds, protozoa, and actinomycetes break down dead organic matter. The organic matter is then recycled into biological molecules. These saprophytic organisms can only finish this process if the conditions are correct. This means that the pH levels in the soil have to be at an optimum level to sustain life. If the pH is too low, enzymes will not function, and bacteria cannot carry out the most important process, including nitrogen fixation maintaining soil structure and decomposition.

Soil aggregates are bundles of the particles in soil that stick together because they are damp. The range in size are from 2 thousandths of a millimeter to 2 millimeters across. Because of the varying size, small spaces between them get filled with water. This water is essential for healthy soil to keep the soil aggregates moisturized. Bacteria play a key role in soil aggregates by doing on of two things. The first is by producing compounds called polysaccharides which slow down the process of decomposition for enough time to form the soil aggregates. The second is by creating small electrostatic charges which bring together the smaller soil aggregates. This creates pore space which is necessary for sustaining life in the soil which stores oxygen (caused by bacteria in cellular respiration) and water (caused by plants in photosynthesis).

Acid rain is a major environmental problem which not only affects the functioning of lakes, streams, plant life, and soil, but also humans. As acid rain seeps into the ground around forests, lakes, and streams, it can kill vital nutrients in the soil including magnesium and calcium. In addition, acid rain also releases chemicals into the ground which are problematic for plant life, including aluminum, which slows down a plant's ability to absorb water (Markets, 2004). Acid rain also kills a large quantity of bacteria in the soil. The pH level in the soil affects the enzymes which are very important. Enzymes of bacteria soil have the main task of controlling the rate at which bacteria decompose and release nutrients (Bandick 2011). Bacterial enzymes, such as glycosidase, can either speed up or slow down the decomposition process depending on the available nutrients.

Acid rain is caused by air pollution emitted through human activity including burning coal and trash. All the fossil fuels and waste that have been released into the atmosphere are combined with moisture in the atmosphere and then precipitated back down into the soil (Markets 2004). In other words, acid rain is the combination of wet deposits of materials and dry deposits of materials that come from the atmosphere and stratosphere. These deposits have a higher than usual amounts of nitric acid and sulfuric acid (Austin, 2014). As humans continue to exhibit behaviors that influence the creation of acid rain, we are effectively worsening our environment, leading to increased lung and respiratory problems in areas of high exposure to car exhaust.

Humans help to create acid rain by emitting car exhaust and other fossil fuels in the air. When the primary pollutants of car exhaust, including nitrous oxides and sulfur oxides, are combined with moisture in the atmosphere, nitric and sulfuric acids are precipitated back into the soil. Acid rain occurs in many forms, including wet deposition and dry deposition. Wet deposition is when the wind blows the acidic chemicals into wet climates and the acid falls in the form of rain, sleet, fog, snow, or mist, when it gets absorbed in the ground it can affect a large number of plants, animals, and aquatic life. Dry deposition happens when the wind blows the chemicals into dry climates and the acid falls in the form of dust or smoke, which sticks to the ground and other surfaces. The acidity of the rain is determined by pH level. Normal rain is slightly acidic (pH range 5.2 – 6.0) because carbon dioxide and water react together in the air to form a weak acid called carbonic acid. Rain will become acid rain when the pH level of the rain falls below this range. (Conserve Energy Future, 2014)

Based on the information that we have learned throughout the experiment we hypothesized that acid rain will decrease the level of pH in the soil, thereby decreasing the population density of bacteria in the soil.

Experiment Outline

I. **Problem:** Does acid rain change the population density of bacteria based on pH levels in the soil?

II. **Hypothesis**: Acid rain will decrease the level of pH in the soil, thereby decreasing the population density of bacteria in the soil.

III. **Procedure:**

   a. <u>Independent Variable:</u> Application of sulfuric acid solution (acid rain) to the soil
   b. <u>Dependent Variable:</u> Change in the population density of the bacteria in 1cc soil
   c. <u>Negative Control:</u> Application of water to the soil
   d. <u>Controlled Variables:</u> Acidity of sulfuric acid solution, amount sulfuric acid solution, environment, type of soil, population density, time exposed to sulfuric acid, amount of soil, type of indictor, amount of indicator, type of chemicals, amount of soil flocculating reagent, amount of clear solution, amount of demineralized water, amount of universal extracting solution, amount of extracting solution, site location, weather conditions, moisture of the soil, amount of water, size of plots, clean/new pipette, type of pipette, sterile water, type of tube, amount of soil/water mixture, how much of the samples placed on the petri plates, how long the bacteria has grown on plates, amount of indicators, type of indicators, type of plate used
   e. <u>STEP-BY-STEP:</u>

1. Go to N- 39^{o} 21.488’ and W0-86^{o} 38.134’ coordinates to make following plots.
2. Make a box 91.44 cm across, by 60.96 cm down. Section off into three sections across by putting a flag at the following points; 0cm, 30.48cm, 60.96cm, 91.44cm. Next, section off three sections down by putting a flag at the following points: 0cm, 30.48cm, 60.96cm. Also section a flag at 30.48cm across by 30. 48cm down, 30. 48cm across by 60.96cm down, 60.96cm across by 30.48cm down, 60.96cm across by 60.96cm down, 91.48cm across by 30.48cm down, 91.48cm across by 60.96cm down.
3. Collect 15 cm of soil from Plot 1 by pushing the soil extractor 15 cm into the ground, then turn clockwise while pulling the soil extractor out of the ground.
4. Repeat step 3 to extract soil from Plots 2, 3, 4, 5, and 6 respectively to collect all "before” samples.
5. Take the "before" soil samples back to the lab to the pH chemical test in steps 6-19 using the LaMotte Combination Soil Model STH-14 Code 5010-01.” All soil samples must be tested on the same day at the same time.
6. Fill a test tube 1/3 full of soil, from "Plot 1 before"
7. Use the Model Demineralizer Bottle to add demineralized water to the tube, until it is one-half inch from the top.
8. Cap and shake until the soil is well dispersed.
9. Add 5 drops of Soil Flocculating Reagent.
10. Cap and shake to mix.
11. Allow contents to settle before proceeding to step 12.
12. Use a 1 ml pipet to transfer 1 ml of the clear solution above the soil to one of the large depressions on a spot plate.
13. Transfer a second 1 ml sample to the other large depression on the spot plate.
14. To the first sample on the spot plate, add two drops of *Duplex Indicator
15. Compare the resulting color reaction against the Duplex Color Chart.
16. The wide range pH rest result indicates which narrow range indicator and color chart should be selected to perform a more precise pH test.
17. Choose the narrow range indicator and appropriate chart with a mid-point that is as close as possible to the value obtained in the wide range test.
18. Add two drops of the chosen narrow range indicator to the second sample on the spot plate.
19. Compare the resulting color reaction against the appropriate color chart to obtain a precise soil pH reading.
20. Repeat steps 6-19 for plots 2,3,4,5 and 6 "before" respectively.
21. On the same day as the pH test, perform the serial dilution on all six "before" soil samples using steps 22-37.
22. Use a clean, new transfer pipette to add 10 ml of sterile water to a 15 ml culture tube. Label the tube "Before Plot 1-10^{0}."

23. Use the same pipette (from step 22) to add 9 ml of sterile water to a second 15 ml culture tube. Label the tube “Before Plot 1- 10^{-1}."
24. Repeat step 23, two more times to two additional 15 ml culture tubes. Only label them "Before Plot 1- 10^{-2}," and "Before Plot 1- 10^{-3},” respectively.
25. Place 1cc of your "Before Plot 1" soil sample into the "10^{0}" culture tube.
26. Cap the tube and shake vigorously.
27. Using a new clean pipette, remove 1ml of the soil/water mixture from the "10^{0}” tube and place into the "10^{-1}" tube.
28. Cap and shake vigorously.
29. Using the same pipette in step 27, remove 1 ml of the soil/water mixture from the "10^{-1}" tube and place into the "10^{-2}" tube.
30. Cap and shake vigorously.
31. Using the same pipette in step 27, remove 1 ml of the soil/water mixture from the "10^{-2}" tube and place into the "10^{-3}" tube.
32. Cap and shake vigorously.
33. You should now have a total of four culture tubes.
34. Plate 100 µL samples from the 3rd and 4th tubes (dilutions 10^{-2} & 10^{-3}) onto their own separate, labeled 3M of Petrifilm™ Aerobic Count plate. Label the plate “Before Plot1 10^{-2}” & “Before Plot1 10^{-3}”
35. Allow bacteria to grow for 48 hours.
36. Examine each of the plates for individual bacterial colonies and choose the plate with the fewest colonies (but at least 5). First look at the 10^{-3} plate and check if there are at least 5 colonies. If there are then count how many colonies are found and record data. If there are less than 5 colonies, look at then look at the 10^{-2}plate, check to see if there are more than 5 colonies; count how many colonies are found and record data. Make your estimates of the number of bacteria in the original 1 cc soil sample using the following formula: # Microbes in 1 cc of soil = # Colonies on sheet x 10^{2} x $10^{|\text{dilution \# at which these colonies were found}|}$.
37. Record number of colonies on the plate, the dilution at which the colonies were found, and the estimated number of microbes in 1 cc of soil in the Data Table.
38. Repeat steps 22-37 on each "before" sample for Plots 2, 3, 4, 5, and 6 respectively.
39. To make the "acid rain" solution, add 900 ml of distilled water to 100 ml of 1 molar solution of sulfuric acid
40. Take 20 ml of the solution from step 39 and add 980 ml of distilled water in a separate container to make 1 liter of "acid rain" solution.
41. Repeat steps 39 & 40 to make a second liter of the acid rain solution.
42. Pour the 1.5 liters of "acid rain" solution over plots 1, 2 and 3 evenly and make sure to cover the whole plot.
43. Pour 1.5 liters of water over plots 4, 5 and 6 evenly and make sure to cover the whole plot.

44. To extract, dilute, and test the "after" samples, repeat steps 3-37 this time, labeling the plastic bags, culture tubes, and petri plates as "after" samples with their respective plot numbers.

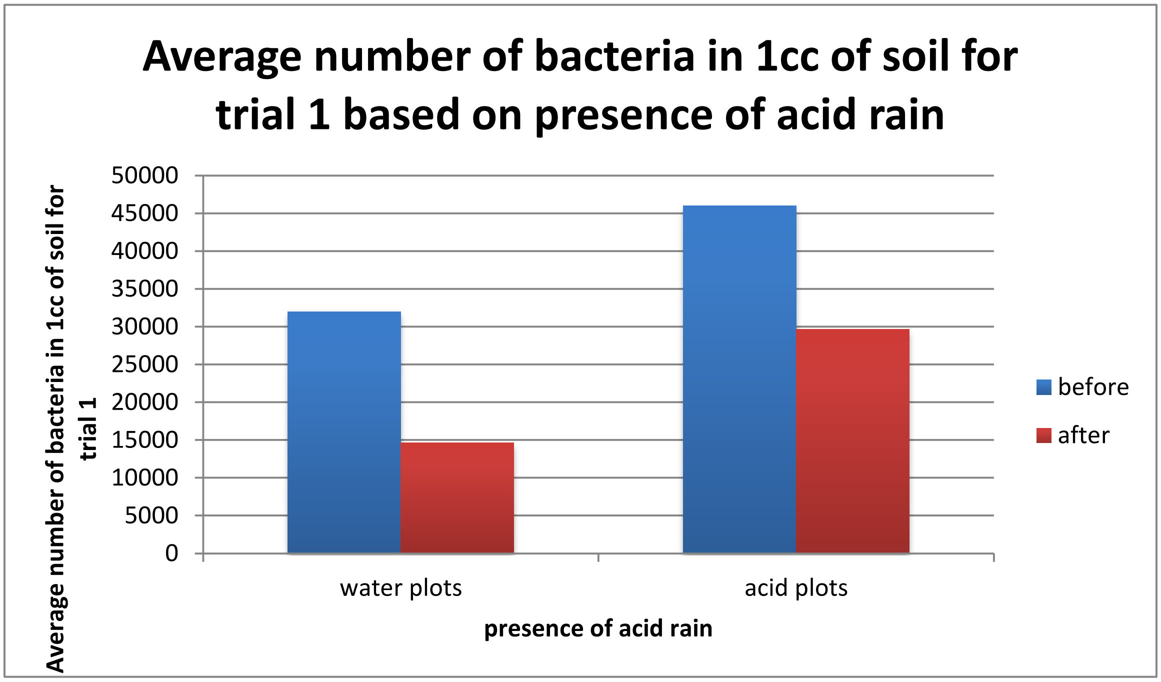
Diagram 1:

Data

❖ Bacteria colonies

Soil sample	Number of bacteria In 1 cc soil of soil
Water 1 Before 10-3	22000
Water 2 Before 10-3	14000
Water 3 Before 10-3	60000
Acid 2 before 10-3	60000
Acid 3 before 10-3	32000
Water 1 After 10-3	18000
Water 2 After10-3	7000
Water 3 after 10-2	19000
Acid 1 after 10-3	25000
Acid 2 after 10-3	39000
Acid 3 after 10-3	25000
Water before average	32000
Water after average	14666
Acid before average	46000
Acid after average	29666

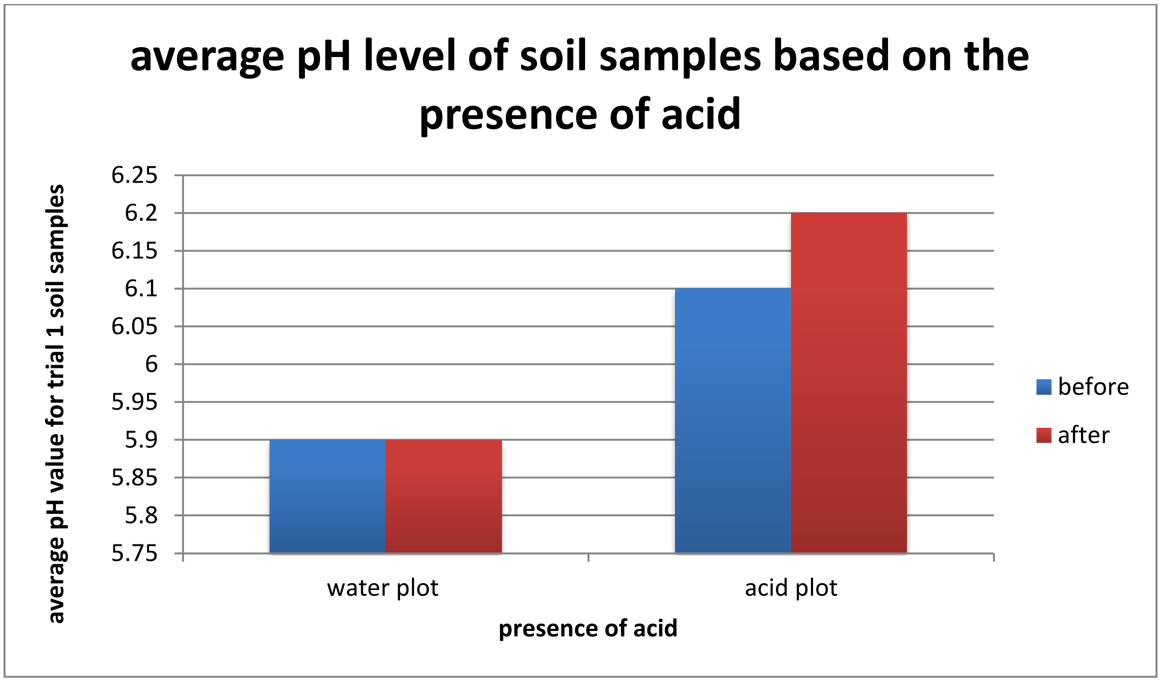
- Graph



- pH levels

Soil sample	pH level	
	Before acid rain or water	After acid rain or water
water plot 1	5.8	6.2
Water plot 2	6.0	5.4
Water plot 3	5.8	6.1
Acid plot 1	6.1	6.2
Acid plot 2	6.1	6.2
Acid plot 3	6.1	6.2
Average water plot	5.9	5.9
Average acid plot	6.1	6.2

❖ Graph:



❖ Conclusion:

Our hypothesis was incorrect. Our hypothesis stated that Acid rain will decrease the level of pH in the soil and decrease the population density of bacteria in the soil. The data we found from the change in pH levels showed that the acid rain solution caused the pH levels to increase slightly, but by doing so it made the soil more basic and less acidic. Our water plots started out with a pH level of 5.9, and our acid plot soil started out with a pH level of 6.1. After adding our negative control, (water) the pH level in the water plot stayed at an average of 5.9, which is what we want. We know the acid rain caused the pH level to slightly increase, because our data showed that after adding the acid rain solution to the soil plots the pH level in the soil went from an average of 6.1 to an average of 6.2. It is a very small change so the acid rain solution really had no effect on the soil so our hypothesis was wrong concerning acid rain decreasing the pH levels in the soil. Also, our bacteria colony data shows that the acid rain solution caused the population to the density of the bacteria in the soil. Before we added our negative or independent controls, our water plot soil had an average of 32000 bacteria colonies in 1cc of soil and the acid plot soil had an average of 46000 colonies in 1cc of soil. Then after adding our negative control (water) to the water plots, the number of bacteria colonies in the soil decreased from an average of 32000 colonies to an average of 14666 colonies in 1cc of soil. After adding the acid rain solution to the acid plots the number of bacteria colonies in the soil dropped from an average of 46000 to an average of 29666 colonies in 1cc of soil. Even though our bacteria colonies did decrease like we predicted, our negative control also changed. This means that there was another outside factor in the environment that was causing the population density to decrease. Therefore, there is no way for us to know if there was a decrease in the bacteria population, because of the acid rain solution or because of that other outside factor; so again we were disproven in our hypothesis.

In terms of future experiments, we think it would be a good idea to move our soil plots further to the back woods. We also think it would allow us to see a bigger change in our data if we make our acid solution stronger by not diluting it as much. We think moving our plots to the back woods would improve our experiment, because in our experiment, we previously had our plots placed near a parking lot with many cars running each day, so we believe that the exhaust and gasses from the cars could have affected the outcome of our experiment. We also believe that making our acid solution stronger would help, because after adding the solution in our previous experiment, the pH levels in the soil did not change. By making the acid solution stronger it would be more likely that the soil pH level would decrease.

Bibliography

Chemistry and Issues in the Environment. Last modified November 25, 2006. Accessed May 28, 2014. http://www.elmhurst.edu/~chm/onlcourse/chm110/outlines/nitrogencycle.html.

Clean Air Kids. Last modified August 11, 2007. Accessed May 18, 2014. http://www.clean-air-kids.org.uk/acidrain.html.

Davidoff, Ansel. "Acid Rain Effects." EPA. Last modified February 26, 2014. Accessed May 8, 2014. http://www.epa.gov/acidrain/education/site_students/whatcauses.html.

Decomposition. Last modified March 7, 2010. Accessed May 18, 2014. http://www.countrysideinfo.co.uk/decompos.htm.

Earth Kind Landscaping. Last modified June 29, 2008. Accessed May 22, 2014. http://aggie-horticulture.tamu.edu/earthkind/landscape/dont-bag-it/chapter-1-the-decomposition-process/.

Kids Crossing. Last modified May 10, 2011. Accessed May 22, 2014. http://eo.ucar.edu/kids/green/cycles7.htm.

Princeton EDU. Last modified May 12, 2004. Accessed May 18, 2014. http://www.princeton.edu/~achaney/tmve/wiki100k/docs/Soil_pH.html.

Sheenan, John. "Acid Rain." Adirondack Council. Last modified July 8, 2004. Accessed May 8, 20014. http://www.adirondackcouncil.org/page/privacy-policy-8.html.

Soil Quality. Last modified September 19, 2011. Accessed May 18, 2014. http://soilquality.org/indicators/soil_enzymes.html.

Tech Alive. Last modified January 1, 2000. Accessed May 8, 2014. http://techalive.mtu.edu/meec/module10/Decomposition.htm.

U.S Environment Protection Agency. Last modified December 4, 2012. Accessed May 15, 2014. http://www.epa.gov/acidrain/what/.