Human Impact on the Sulfur Cycle

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Background Information

Biogeochemical cycles are the methods ecosystems use to cycle and recycle the critical chemical elements between the living and nonliving parts of the environment. These biogeochemical cycles involve the chemical elements carbon, phosphorus, nitrogen, sulfur, and oxygen, which are essential to living things, and each of these elements has its own cycle.

One of these cycles which humans have an impact on is the sulfur cycle. Sulfur is a part of many biological molecules which play critical roles in the living organisms in the ecosystems including proteins, multiple vitamins, and hormones. Hence its presence or absence can strongly affect an ecosystem's well being because any dramatic changes in the sulfur cycle will have an equally dramatic impact on the organisms that live there.

Normally, in the sulfur cycle, rocks and minerals break down through weathering and release sulfur into the air that reacts with the oxygen present in the atmosphere to form the compound sulfate. This sulfate then serves as a form of sulfur living things can use, and it is taken in by plants and the organisms that eat them to make many parts of their biological molecules, but most importantly in this case amino acids. Then, as organisms eat one another, they move sulfur through the food chain, and when they die and decompose, the sulfur returns to the soil where it can re-enter the geological part of the sulfur cycle (The Environmental Literacy Council, 2002).

Because humans burn fossil fuels and process metals, we release the sulfur in them into the atmosphere when it would normally remain part of the geological part of the sulfur cycle, and as a consequence, people alter the sulfur cycle significantly. Humans are responsible for 1/3 of the sulfur that reaches the atmosphere (The Environmental Literacy Council, 2002), and once there, this sulfur mixes with water, oxygen, and other chemicals in the atmosphere to create acid

rain. Acid rain then negatively affects the various ecosystems on the Earth including the soil one (United States Environmental Protection Agency, 2008) (The Environmental Literacy Council, 2002) because it significantly changes an environment's pH level.

The pH of the environment is very important because the correct balance of positively charged hydrogen ions and negatively charged hydroxide ions is vital to any of the chemical processes of life, and pH is the measurement of the concentration of the hydrogen ions in a liquid (Campbell, N. Heyden, R. Williamson, B., 2004). Having the correct amount of positively charged hydrogen ions and negatively charged hydroxide ions determines the correct structure of proteins in living things and too many or too few positively charged hydrogen ions or hydroxide ions can damage this structure. A strong change in pH, therefore, will affect the functions of the many proteins so critical to the chemical processes in living things. Enzymes in particular are susceptible to a change in pH, and since enzymes control all chemical reactions within the fundamental life of the cell, any change in an enzyme's structure will prevent any of the chemical reactions that take place in the cell from occurring, including the four essential tasks of cells: respiration, reproduction, transformation, and synthesis. If all chemical reactions cannot occur in a cell, then it would lead to death of the organism (Dr. Drage, S. and Dr. Wilkinson, D., n.d.). Normally the best pH range for most organisms is between 6 and 8, with acidic rain, the pH in an environment can drop to a range of 1 to 4, meaning acid rain can create acidic soil which can kill the organisms that live there, and that is why it is so bad (Virtual Chem Book, 2003).

One such group of organisms that are especially important is the bacteria. Bacteria are the "unsung heroes" of soil. They are responsible for changing inorganic and organic matter from one form to another, and they do so through external digestion which provides the correct

chemicals for consumption of other organisms such as plants. Bacteria are able to digest many different forms of organic and inorganic matter because of the many enzymes in their digestive metabolic pathways (The University of Western Australia, 2004). The chemicals produced from the pathways are nutrient rich and keeps the soil healthy for plant growth (ArticlesBase.com, 2008). The digestive enzymes of bacteria are made of amino acids which are found in sulfur. Without the correct amount of sulfur in the soil there would not be bacteria's digestive enzymes which would not allow them to have metabolic pathways, and without them bacteria could not produce nutrients and chemicals for the organisms in the soil. It is important to have healthy bacteria in the soil. When there are large amounts of acid in the soil from acid rain, the soil has an acidic pH level, which is unhealthy for the bacteria. Therefore when sulfur is released in the atmosphere creating acid rain which then creates low pH levels, this untimely harms the bacteria which harms the organisms that bacteria support. All of these processes contribute to bad soil and bad ecosystems.

Every day at Roland Park Country School, hundreds of cars drive through the carpool lane. Cars run on fuel that contains sulfur compounds. The fuel from the cars is then burned releasing sulfur oxides which then go through the atmosphere portion of the sulfur cycle caused by humans (Dr. Mohamed Al Kateeb, n.d). The resulting acidic compounds in the soil form high levels of sulfur and acidic pH level which negatively affect bacteria and the organisms bacteria support. For our experiment, we will be testing the soil near the carpool lane for sulfur levels, pH levels and the amount of bacteria. We are performing these tests in the soil near the carpool lane to see how great of an impact the car exhaust has on the R.P.C.S. campus and compare our results to a negative control. We will also demonstrate how all these processes are connected.

Katie Tutrone

Experiment

I. Problem: Does the sulfur from car exhaust increase the density of bacteria in the soil?
II. Hypothesis: The extra sulfur from car exhaust closest to the soil will increase the density of bacteria.
III. Independent variable: distance of the soil being tested from the car exhaust (carpool lane)
IV. Dependent variable: the amount of sulfur (ppm.), density of bacteria (#/cm^3) and the pH level in the soil samples.
V. Negative control: soil from the courtyard
VI. List of controlled variables:

- Amount of soil used for each test
- Kind of nutrient agar
- number of days plates grow
- Size of soil samples
- Degree of dilution (which dilutions plated)
- Amount plated on nutrient agar
- Amount of sterile water
- soil samples are taken on the same day around the same time
- Amount of soil flocculating reagent
- Size of transfer pipette
- Amount of sulfate test solution
- Size of culture tubes
- serological pipettes
- amount of Duplex indicator
- size of turbidity vial
- size of Extraction tubes
- amount of Universal Extracting Solution
- size of nutrient agar plates

Procedure:

1. Go to the RPCS front lawn, at the starting edge of grass along the carpool lane, at point latitude N 39 degrees, 29.473 minutes and longitude W 76 degrees, 33.45 minutes on the GPS. Mark this point with a flag stating “trial 1 flag 1”.
2. Place another flag that is already labeled “trial 1 flag 2”, six meters farther west of the original flag you just placed down. Make sure you are going perpendicular to the carpool lane to place the flag down.
3. Place another flag, that has already been marked with “trial 1 flag 3”, six meters west of the “trial 1, Flag 2” flag and perpendicular to the carpool lane.

4. Start a new line of flags 6 meters south of the original “trial 1 flag 1” and along the grassline. Your “trial 2 flag 1” should be at the starting edge of the grass. Repeat steps 2-3 but with the flags stating “trial 2 flag 2” or “trial 2 flag 3”
5. Start a new line of flags 6 meters south of the “trial 2 flag 1” flag and along the grassline. Your “trial 3 flag 1” flag should be the first flag at the starting edge of the grass. Repeat steps 2-3 but with the flags stating “trial 3 flag 2” or “trial 3 flag 3”.
6. For the negative control, go to the court yard, at point latitude 39 degrees, 3.4704 minutes and longitude 76 degrees, 38.4671 minutes on the GPS. Mark this point with a flag labeled “Flag 1, negative control”.
7. Six meters south of the “flag 1, negative control” flag, place another flag labeled “Flag 2, negative control”.
8. Six meters south of the “Flag 2, negative control” flag place a flag that has already been labeled “Flag 3, negative control”.
9. Each set of samples for soil for steps 10-26 should be taken on the same day and around the same time in order to control for any environmental changes.
10. Go back to trial 1, flag 1.
11. Use a soil core sampler to dig out a 15 centimeters deep and 2.5 centimeters wide sample of soil.
12. Place the soil in a plastic bag, labeling it “trial 1, flag 1, sample 1”.
13. Use a soil core sampler to dig out a 15 centimeters deep and 2.5 centimeters wide sample of soil right beside the previous “trial 1, flag 1, sample 1” hole.
14. Place the soil in a plastic bag, labeling it “trial 1, flag 1, sample 2”
15. Use a soil core sampler to dig out a 15 centimeters deep and 2.5 centimeters wide sample of soil right beside the previous “trial 1, flag 1, sample 2” hole.
16. Place the soil in a plastic bag, labeling it “trial 1, flag 1, sample 3”.
17. Repeat steps 10-16 but with flags 2 and 3 for trial 1. These flags samples must be taken immediately after doing flag 1 of trial one. For the soil that comes from flag 2, place the soil in a plastic bag reading “trial 1, flag 2, sample #”. For the soil that comes from flag 3, place the soil in a plastic bag reading “trial 1, flag 3, sample #”.
18. Repeat steps 10-17, but with the other two trials. Label the soil sample bags for each trial 2 or 3 bags corresponding to the trial number, flag number, and sample number.
19. Go back to “Flag 1, negative control”
20. Use a soil core sampler to dig out a 15 centimeters deep and 2.5 centimeters wide sample of soil.
21. Place the soil in a plastic bag, labeling it “flag 1, sample 1, negative control”.
22. Right beside the “flag 1, sample 1, negative control” hole, use a soil core sampler to dig out a 15 centimeters deep and 2.5 centimeters wide sample of soil.
23. Place the soil in a plastic bag, labeling it “flag 1, sample 2, negative control”.
24. Right beside the “flag 1, sample 2, negative control” hole, use a soil core sampler to dig out a 15 centimeters deep and 2.5 centimeters wide sample of soil.
25. Place the soil sample in a plastic bag, labeling it “flag 1, sample 3, negative control”.

26. Repeat steps 20-25 but for flag 2 and flag 3 of the negative control. Put the soil samples from these flags in bags with labels corresponding to its flag #, sample #, and negative control.
27. Before doing the soil tests, realize that all tests for steps 28-29, for each set of soil samples (e.g. all "sample 1" bags) must be performed on the same day and around the same time.
28. For each sample (e.g. all "sample 1" soils) perform the pH test and sulfate test using the LaMotte Model STH-14 Soil test kit and record the amount of sulfur (ppm.) and pH level.
29. For each sample (e.g. all "sample 1" soils), making sure that they are labeled and separated, perform the serial dilutions to test for bacteria density on the soil as follows:
    1. Label a culture tube with the sample's "trial #, flag #, sample # and 10^0". Use a clean, new transfer pipette to add 10 ml of water to the 15 mL culture tube.
    2. Use the same pipette to add 9 ml to a second 15 ml culture tube that has already been labeled with that same sample's "trial #, flag #, sample # and 10^{-1}."
    3. Repeat step 2 three more times to two additional 15 ml culture tubes, only label Them with the corresponding "trial #, flag #, sample # and 10^{-2}" and "trial #, flag #, sample # and 10^{-3}", respectively.
    4. Place 1 cc of a soil sample mentioned in step 1 into its corresponding "10^0"culture tube.
    5. Cap the tube and shake vigorously.
    6. Using a new clean pipette, remove 1 ml of the soil/water mixture from this "10^0" tube and place into its corresponding "10^{-1}" tube.
    7. Cap and shake vigorously.
    8. Using the same pipette in step 5, remove 1 ml of the soil/water mixture from this "10^{-1}" tube and place into its corresponding "10^{-2}" tube.
    9. Cap and shake vigorously.
    10. Using the same pipette in step 5, remove 1 ml of the soil/water mixture from this "10^{-2}" tube and place into its corresponding "10^{-3}" tube.
    11. Cap and shake vigorously.
    13. You should now have a total of 4 culture tubes for this one sample of soil.
    14. Place 100 µl samples from the 3rd and 4th tube (dilution 10^{-2} and 10^{-3}) onto their own separate, appropriately labeled petrifilm growth plates. The petrifilm growth plates should be labeled with their corresponding "trial #, flag #, sample #, and dilution #".
    15. Repeat steps 1-14 for all of the remaining sets of sample 1.
    16. Allow to grow for 48 to 72 hours.
    17. Examine each of the plates for each of the samples for individual bacteria colonies and choose the plate with the fewest colonies (but at least 5) to 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 $10^{\text{dilution \# at which these colonies were found}}$.

    18. Record the density of soil for each sample.
30. Repeat steps 28-29 for the other two sample sets ("sample 2" soils and "sample 3" soils)

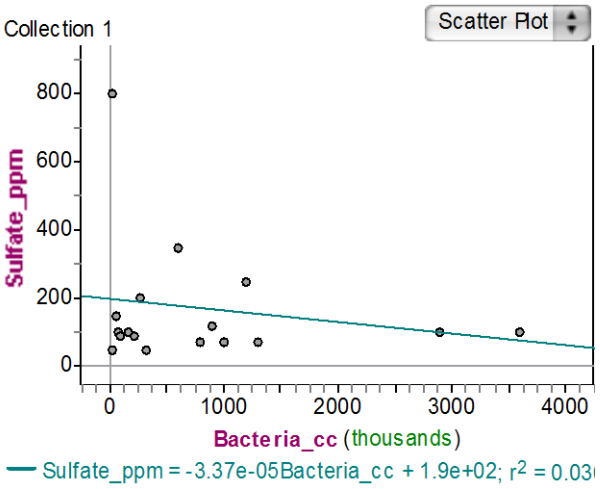
Melina Liebler

Data Table:

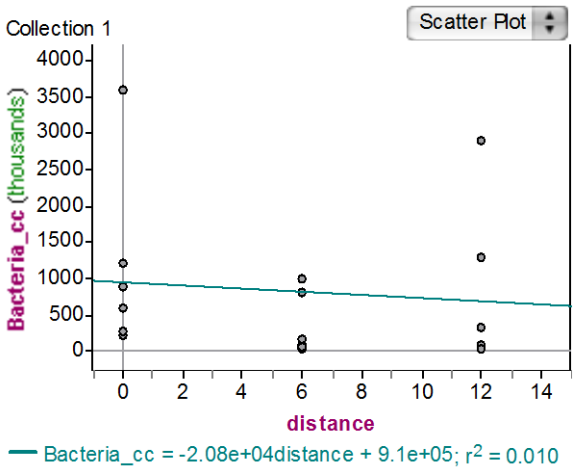
Sample & Trial	**Flag Location**	**Soil pH Levels**	**Soil Sulfate Levels (ppm)**	**Soil Bacteria Density (cc)**
Sample 1; Trial 1	Flag 1 (0m)	8.2	100	3600000
Sample 2; Trial 1	Flag 1 (0m)	7.4	250	1200000
Sample 1; Trial 2	Flag 1 (0m)	7.2	120	900000
Sample 2; Trial 2	Flag 1 (0m)	7.6	200	270000
Sample 1; Trial 3	Flag 1 (0m)	7.2	90	220000
Sample 2; Trial 3	Flag 1 (0m)	8.2	350	600000
Average		**7.63**	**185**	**188611**
Sample 1; Trial 1	Flag 2 (6m)	8.2	100	80000
Sample 2; Trial 1	Flag 2 (6m)	5.3	100	170000
Sample 1; Trial 2	Flag 2 (6m)	5.2	800	20000
Sample 2; Trial 2	Flag 2 (6m)	6.9	70	100000
Sample 1; Trial 3	Flag 2 (6m)	6.0	150	50000
Sample 2; Trial 3	Flag 2 (6m)	5.4	70	800000
Average		**6.16**	**215**	**31889**
Sample 1; Trial 1	Flag 3 (12m)	6.2	70	1300000
Sample 2; Trial 1	Flag 3 (12m)	6.2	50	320000
Sample 1; Trial 2	Flag 3 (12m)	7.2	100	2900000
Sample 2; Trial 2	Flag 3 (12m)	6.4	90	90000
Sample 1; Trial 3	Flag 3 (12m)	7.0	100	140000
Sample 2; Trial 3	Flag 3 (12m)	7.2	50	20000
Average		**6.7**	**76.6**	**795000**
Sample 1	Negative Control	8.2	100	420000
Sample 2	Negative Control	8.2	70	60000
Average	**Negative Control**	**8.2**	**85**	**240000**

Graphs:

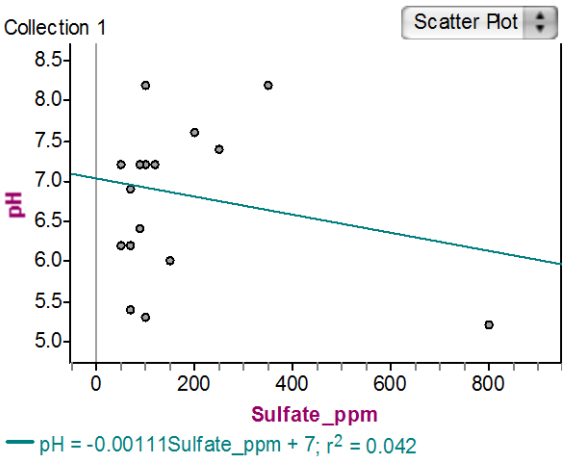
Bacteria Density vs. Soil Sulfate Levels



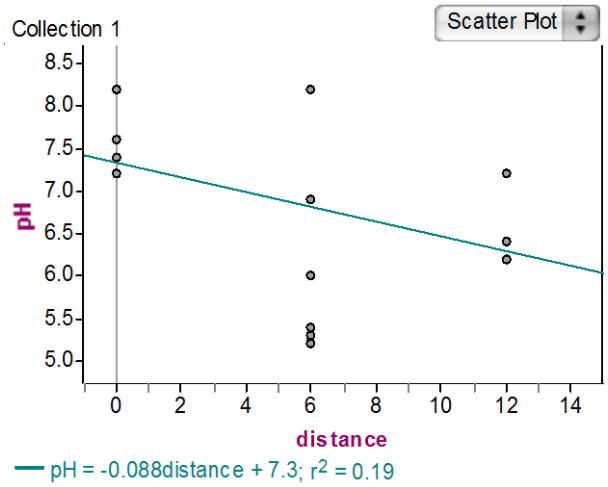
Distance from Carpool Lane vs. Bacteria Density



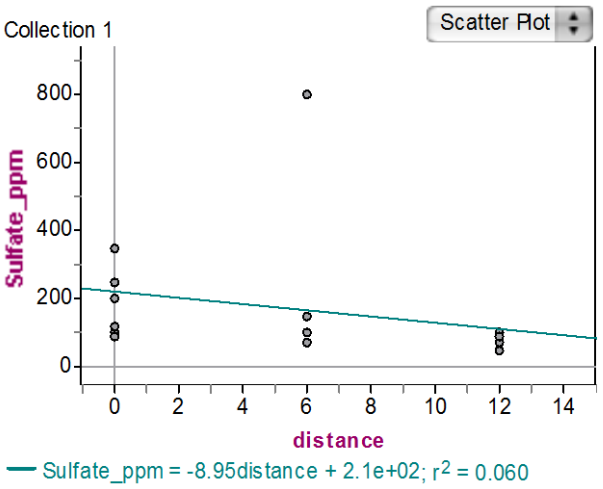
Soil Sulfate Levels vs. Soil pH Levels



Distance from Carpool Lane vs. Soil pH Levels



Distance from Carpool Lane vs. Soil Sulfate Levels



The *t*-Test

p-levels for Soil pH Levels

Flag 1 (0m) ->	Negative Control	p= 0.03
Flag 2 (6m) ->	Negative Control	P=0.008
Flag 3 (12m) ->	Negative Control	p=0.64910 ^-4
Flag 1 (0m) ->	Flag 2 (6m)	p=0.03
Flag 2 (6m) ->	Flag 3 (12m)	p=0.36

If you look at the first two p-values from Flag 1 and 2 to the Negative Control, the p-values are less than 0.05, which means that there is a statistically significant difference, so you can tell that there is a difference! Flag 3 has still a change, but it is not that significant than from Flag 1 and 2, because the p-value is closer to 0.45, which means that true means were equal.

p-levels for Sulfate Levels:

Flag 1 (0m) ->	Negative Control	p= 0.07
Flag 2 (6m) ->	Negative Control	P=0.32
Flag 3 (12m) ->	Negative Control	p=0.69
Flag 1 (0m) ->	Flag 2 (6m)	p=0.82
Flag 2 (6m)->	Flag 3 (12m)	p=0.29

If you look at the first two p-values from Flag 1 the Negative Control, the p-values are less than 0.05, which means that there is a statistically significant difference, so you can tell that there is a difference! Flag 2 and Flag 3 have still a change, but it is not that significant than from Flag 1, because the p-value is closer to 0.45, which means that true means were equal. Also the Flag 1 p-levels to these of Flag 2 differ from the p-levels for the Soil pH Levels. In this case the true means were equal and there was no significant change.

p-levels for Soil Bacteria Density:

Flag 1 (0m) ->	Negative Control	p= 0.16
Flag 2 (6m) ->	Negative Control	P=0.54
Flag 3 (12m) ->	Negative Control	p=0.31
Flag 1 (0m) ->	Flag 2 (6m)	p=0.03
Flag 2 (6m) ->	Flag 3 (12m)	p=0.36

If you look at the first p-value from Flag 1 and the values from Flag 3 to the Negative Control, the p-values are less than 0.05, which means that there is a statistically significant difference, so you can tell that there is a difference! Flag 2 has still a change, but it is not that significant than from Flag 1 and 3, because the p-value is closer to 0.45, which means that true means were equal.
In this case the Flag 2 p-values to these from Flag 3 are closer to 0.45 and more than 0.05, which means again that the true means were equal and that there was no significant change.

Erin Weinstock

Conclusion

Our hypothesis was incorrect. We thought that the farther from the carpool lane an area was the lower the sulfate level would be. We believed that the sulfate released into the soil from the fumes in the exhaust from the cars would cause there to be more bacteria near the carpool lane. The amount of sulfate in the soil decreased as the distance from the carpool lane increased. For instance, during trial 1 of the second sample the sulfate levels decreased 250 ppm to 100 ppm to 50 ppm, from flag 1 to 2 to 3 respectively. There was also a decrease in the amount of

bacteria as the distance increased. For example, in trial 2 of sample 2, flag one had 270,000 bacteria per cubic centimeter. At flag two of the same trial and sample the number of bacteria decreased to 100,000 per cubic centimeter and at flag 3 the number of bacteria decreased yet again to 90,000 per cubic centimeter. However the key was with the pH. As the distance from the road increased, the pH of the soil became more acidic, despite the decrease of sulfur (an acidic element). The pH levels in trial 2 of sample 1 were as follows, 7.2 for flag 1, 5.2 for flag 2, and 7.2 for flag 3. The very acidic pH levels indicate that something other than the car exhaust is affecting the pH in those areas. The acidic pH of the soil is killing bacteria in the soil, creating a gradual decrease in the amount of bacteria as the pH increases. The decrease in sulfate levels is probably caused by bacteria that are releasing sulfate as they decompose matter. There are more bacteria near the road, so there is more sulfate released. This would also explain some inconsistencies in the data where there were larger amounts of bacteria and higher sulfate levels at some of the flag 2 areas than at the flag 1 areas.

The interesting relationships between distance, bacteria, sulfate, and pH are further shown by the R squared values. The R square value for bacteria's relationship to sulfate was only 0.03. This means the sulfate is responsible for about 3% of the changes in the amount of bacteria. The R squared value for the pH's relationship to the sulfate was similar to its value for the bacteria. It was about 0.04 showing sulfate is responsible for 4% of the changes in pH. The similarity between these values shows that the sulfate has little effect on either the pH of the soil or the bacteria in the soil. The R squared value of the pH and distance relationship is much higher at 0.19. This also supports the idea that the acidic pHs of the soil, not the sulfate levels, are lowering the number of bacteria within it.

Further research on this project could be conducted by investigating the causes of change in pH. This could help us better understand what causes the radical changes in pH in this area and therefore what causes the changes in the bacteria and sulfate levels.

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Honor Code: We have acted honorably.