Soil Ecology Report

The Effect of Acid on Bacteria Levels

By: Meghan Doyle, Georgia Fish, Autumn Johnson, and Cydney White

We will complete this assignment honorably.

Cydney White

Georgia Fish

autumn Johnson

Meghan Doyle

Georgia Fish, Meghan Doyle, Autumn Johnson, Cydney White

Mr. Brock

Biology 9H

Background

Bacteria are prokaryotic microorganisms that reside in places as diverse as the soil where plants grow to the bread people eat, and there are four major groups that live in the environment: pathogens, decomposers, mutualists, and lithotrophs. The decomposers, lithotrophs, and mutualists are beneficial to ecosystems because they recycle dead organisms into the soil, converting them into forms useful to the rest of the organisms in the soil food web (Ingham, n.d.); they degrade pollutants; and they form partnerships between plants and the rest of the soil ecosystem. However, large amounts of pathogenic bacteria can be not only harmful to the soil but to the humans who come in direct contact with it as well (Baumgardner, 2012). All four groups of bacteria live in small clumps called microcolonies that attach to the soil surfaces, and the amount of them can change with fluctuations in levels of moisture, temperature, and substrate availability in the soil (Turco, 2014).

Many very important tasks occur in the soil, and bacteria are involved in nearly all of them: taking in electrons and energy from the sun, decomposing plant and animal residue, and converting nitrogen into the nitrogen cycle. It is this latter that is one of the most important tasks bacteria perform. It is so critical because nitrogen is a required element for organisms, such as plants, to form organic molecules such as amino acids (which are the foundation of proteins) and nucleic acids (DNA and RNA) (Pidwirny, 2010). DNA, RNA, and proteins are important because they are the building blocks of all living organisms since without them there would be no chemical reactions in plants. Furthermore, since plants are the foundation of the foodchain. Without their cells, there simply would be nothing alive to form an ecosystem in the first place. Hence, all ecological processes need a source of nitrogen to build and run the cells of living things, and the method for generating this source is the nitrogen cycle.

During the nitrogen cycle, certain soil bacteria fix atmospheric nitrogen into a usable form for nitrification. Nitrification is the process which converts the ammonia into nitrite ions which the plants can take in as nutrients. Other soil bacteria, convert the ammonium ions into nitrate ions through the process of nitrification Decomposing bacteria also convert the nitrogen-rich waste compounds of dead organisms into the simpler one of ammonium. Finally, through the process known as denitrification another group of bacteria convert the excess nitrate in the soil back into nitrogen gas (N2), which is then released back into the atmosphere to begin the cycle again. (McGraw, n.d.)

Another very important cycle in which bacteria play a vital rule is the carbon cycle. Carbon is a finite resource that cycles through the Earth in many forms. The carbon cycle is a process that takes place as carbon is exchanged throughout the earth's ecosystems, oceans, geospheres, and the atmosphere. This makes carbon available to all living organisms. It is important because carbon is the core building blocks for biological molecules that are crucial to the many chemical reactions living things need carbon in order to survive. Bacteria play a critical part in cycling this carbon because they break down the carbon compounds in dead animals and plants, releasing the CO_2 from this process for plants to use in photosynthesis to create the complex organic molecules that the plants (and those that depend on them) need in order to live. (Carbon Cycle Science, n.d.).

One thing that can upset both the nitrogen and carbon cycles is pollution, and the world's leading cause of pollution is the burning of fossil fuels, especially the emission of car exhaust. Due to the extremely blistering temperatures in the engines of the vehicles, nitrogen and oxygen from the air can combine to form nitrogen oxide, which is a severe irritant that can contribute to the formation of acid rain (Romano, 2016). The level of acid in substances is determined by the pH scale (1-14) which measures acidity. Acidity is the level of hydrogen ions in substances such as water and soil, and altering soil pH is harmful because plants that grow in certain environments can only handle certain levels of pH (Soil pH and its Effects on Biodiversity, n.d.). pH determines how efficiently the enzymes of a plant's cells function and engage in chemical reactions, and therefore, how efficiently cells work. If the pH conditions of the enzymes are changed, their efficiency will decrease leading to a decrease in the ability of cells of

plants and other organisms to function and therefore a decrease in life. Car exhaust can cause this change in the pH in the environment by combining the sulfur dioxide and nitrogen oxides released with water in the atmosphere to form acid rain. When acid rain reaches the earth, it flows across the surface and runoff water enters the water system and sinks into the soil, therefore, robbing the soil of essential nutrients and releasing aluminum into the soil which makes it harder for trees/plants to absorb the water they need to survive.

Car exhaust causes air pollution and this might reach into the soil on the ground. If it reaches to the ground, this will cause the population of the bacteria to decrease. If the pollution can travel as far as 1.5 miles, does it just stop there? We are trying to see if whether the distance away from the car exhaust changes the bacteria levels in the soil.

Experiment Procedure

- Problem: Does the distance away from car exhaust increase or decrease the bacteria density in the soil?
- Hypothesis: The closer soil is to a source of car exhaust, the lower the bacteria density will be in the soil.
- Independent Variable: Location of the soil tested (distance away from the street)
- Dependent Variable: Bacteria density in the soil of the three different locations. The pH levels of the soil at the three different locations.
- Negative Control: Soil from a location where there is no exposure to car exhaust
- Controlled:
    - Amount of soil taken for each sample
    - Size of pipettes
    - Size of culture tubes with caps
    - Size of scoop of soil for diluting
    - Amount of sterile water
    - Size of micro pipette
    - Size of plastic bag
    - Time and day you take the samples
    - The amount of clear solution put in the culture tubes for the bacteria dilution test.
    - The amount of soil put in the culture tubes.
    - The amount of soil and clear solution mixture taken with the micro pipette from the 10^{-3} and 10^{-4} labeled culture tubes.
    - The 10^{-3} and 10^{-4} dilution plated each time
    - Type of agar used to grow
    - How long the bacteria grew
    - The same soil test kit for pH
- Step-by-Step:

1. Pick one plot of soil located N 39.35894◦ and W 076.63538◦ for plot 1. Pick another plot located N 39.35827◦ and W 076.63611◦ for plot 2. Finally pick your last plot of soil located N 39.35771◦ and W 076.3667◦ for plot 3.
2. Label three different flags with plot 1 for the 1st location 3.45 meters away from Roland Ave, plot 2 for the 2nd location 91.44 meters from Roland Ave, and plot 3 with the last location not being exposed to any car exhaust.
3. Place the three corresponding labeled flags randomly, for each location, in their corresponding soil plot locations
4. Complete step 5-6 all on the same day at the same day.
5. Use a hammer and soil extractor to get 3 separate samples of 15 cm deep of soil with a 2 cm diameter from each flag location. Combine the 3 separate samples into 1 bag.
6. Repeat step 5 with the 2nd location and with the 3rd location.
7. Complete step 8 for the pH test and steps 10-26 for the serial dilutions test on the same day at the same time.
8. To find the pH level of the soil samples, use the LaMotte STH-14 test kit on the plot 1 soil bag.
9. Complete step 8 over with plot 2 and 3.
10. Use a clean, new transfer pipette to add 10 ml to a 15 ml culture tube. Label the tube "Plot 1 tube 10^0.".
11. Use the same pipette to add 9 ml to a second 15 ml culture tube. Label the tube "Plot 1 10^{-1}."
12. Repeat step 2 three more times to three additional 15 ml culture tubes, only label them "Plot 1 10^{-2}," "Plot 1 10^{-3}", and "Plot 1 10^{-4}" respectively.
13. Place 1 cc of the Plot 1 soil samples into the "Plot 1 10^0" culture tube
14. Cap the tube and shake vigorously
15. Using a new clean pipette, remove 1 ml of the Plot 1 soil/water mixture from the "Plot 1 10^0" tube and place it into the "Plot 1 10^{-1}" tube.
16. Cap the tube and shake vigorously
17. Using the same pipette in step 15, remove 1 ml of the soil/water mixture from the "Plot 1 10^{-1}" tube and place into the "Plot 1 10^{-2}" tube.
18. Cap and shake vigorously.
19. Using the same pipette in step 15, remove 1 ml of the soil/water mixture from the "Plot 1 10^{-2}" tube and place into the "Plot 1 10^{-3}" tube
20. Cap the tube and shake vigorously
21. Using the same pipette in step 15 remove 1 ml of the soil/water mixture from the "Plot 1 10^{-3}" tube and place into the "Plot 1 10^{-4}" tube.
22. You should now have a total of five culture tubes
23. Using the micro pipette, collect the soil/water mixture separately from the Plot 1 10^{-3} & Plot 1 10^{-4}
24. Place 100 ul samples from the 4th and 5th tubes (dilutions 10^{-3} & 10^{-4}) onto their own separate correspondingly, labeled 3M Petrifilm™ Aerobic Count Plate.
25. Using the presser, push down on the water/soil mixture to spread out the mixture.
26. Using the combined soil samples from plots 2 and 3 repeat steps 10-25
27. Allow all plates to grow for 48 to 72 hours
28. Examine the plate with the lowest dilution, 10^{-4}, and look for individual bacteria colonies. If there is at least 5 colonies on the 10^{-4} plate, eliminate the 10^{-3} dilution plate. If there are less than 5 colonies, examine the 3M Petrifilm™ Aerobic Count Plate labeled 10^{-3}. Count the number of bacteria colonies on the 3M Petrifilm™ Aerobic Count Plate chosen. To make your estimates of the number of bacteria in the original 1 cc soil sample use the following formula:
29. Repeat steps 4- 29 2 more times for a total of 3 trials

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

Analysis

a. Data Tables

pH level of Soil Based on Plot			
	Plot 3: Negative Control	Plot 2: Middle	Plot 1: Next to Street
Day 1	6.4	7	7.4
Day 2	7.4	6.4	7.4
Day 3	6.4	6.6	7.7

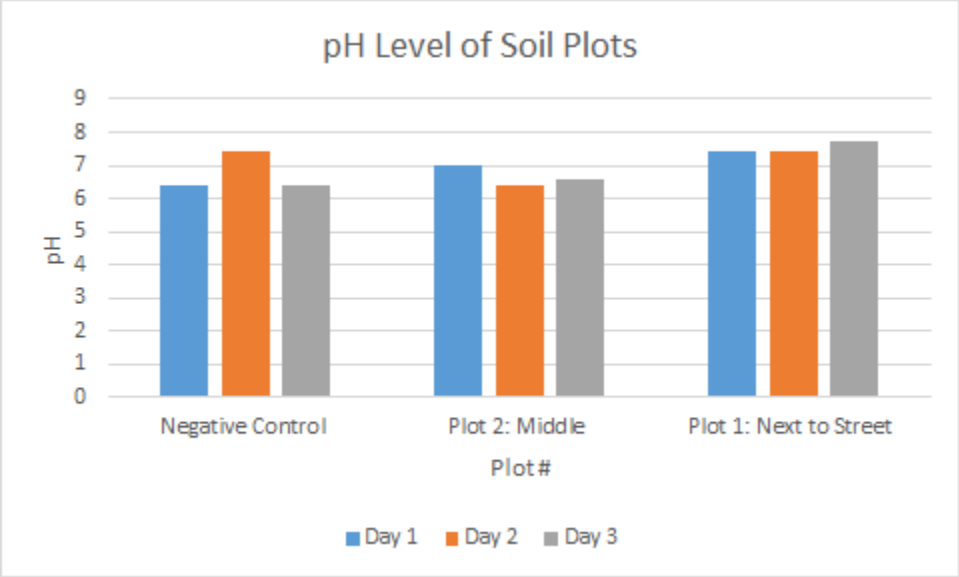
Average Level of pH			
	Plot 3: Negative Control	Plot 2 : Middle	Plot 1: Next to Street
Average	6.73	6.67	7.5

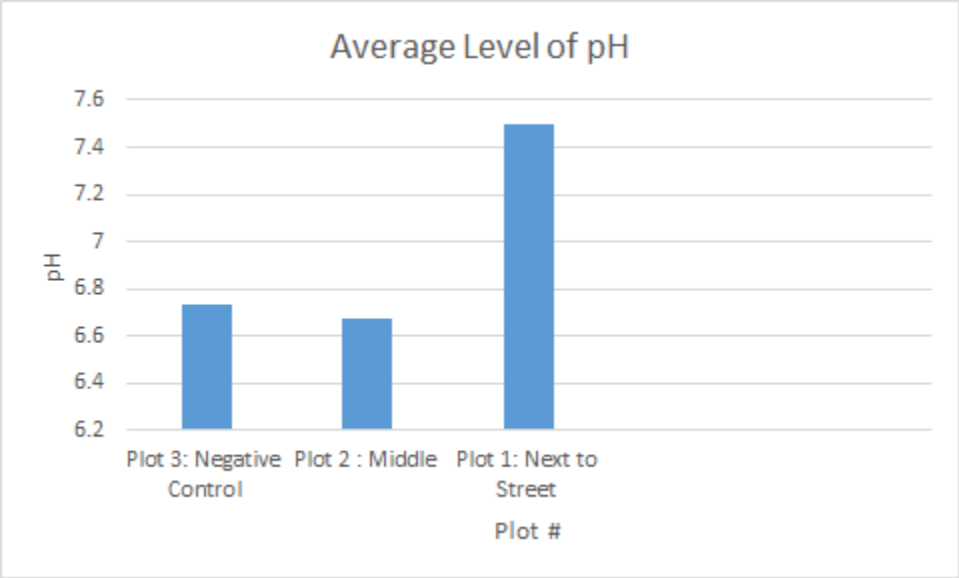
Bacteria Levels Based on Plot in cm^3			
	Day 1	Day 2	Day 3
Negative Control	22,000,000	19,000,000	52,000,000
Plot 2: Middle	44,000,000	1,300,000	125,000,000
Plot 1: Next to street	2,400,000	35,000,000	11,000,000

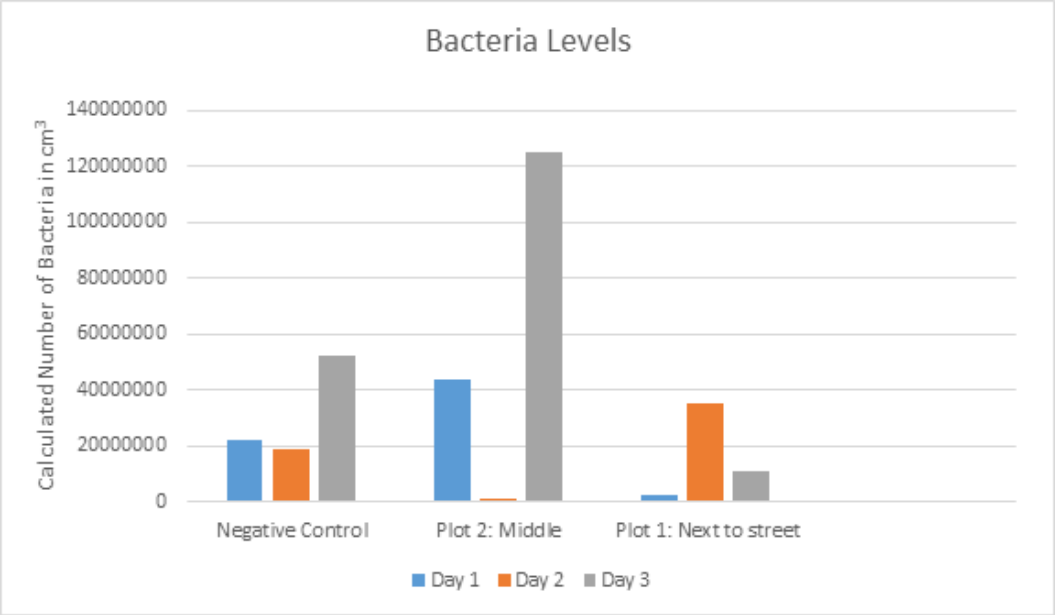
Bacteria Level Averages in cm^3			
	Negative Control	Plot 2: Middle	Plot 1: Next to Street
Average	31000000	56766666.7	16133333.3

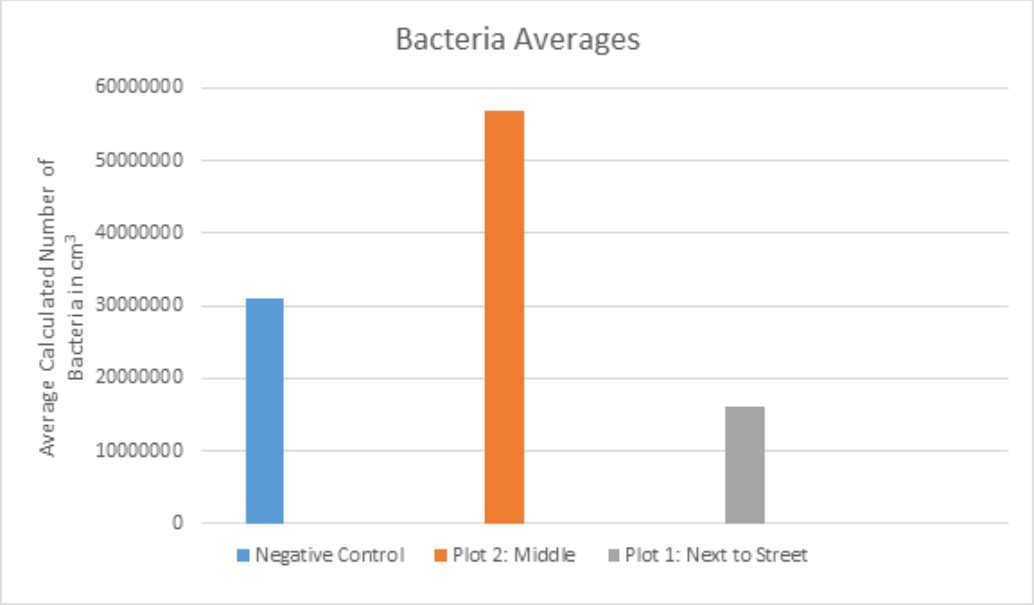
Bacteria in Soil	
Samples	Calculated Number of Bacteria/cm^3
Plot 1 Trial 1	2400000
Plot 2 Trial 1	44000000
Plot 3 Trial 1	22000000
Plot 1 Trial 2	35000000
Plot 2 Trial 2	1300000
Plot 3 Trial 2	19000000
Plot 1 Trial 3	11000000
Plot 2 Trial 3	125000000
Plot 3 Trial 3	52000000

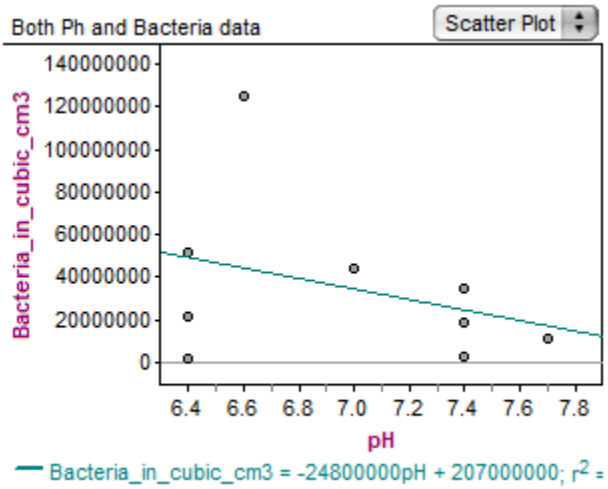
b. Graphs


pH Level of Soil Plots
9
8
7
6
5
4
3
2
1
0
pH
Negative Control
Plot 2: Middle
Plot 1: Next to Street
Plot #
Day 1
Day 2
Day 3


Average Level of pH
7.6
7.4
7.2
7
6.8
6.6
6.4
6.2
pH
Plot 3: Negative Control
Plot 2 : Middle
Plot 1: Next to Street
Plot #


Bacteria Levels
Calculated Number of Bacteria in cm3
140000000
120000000
100000000
80000000
60000000
40000000
20000000
0
Negative Control
Plot 2: Middle
Plot 1: Next to street
Day 1
Day 2
Day 3


Bacteria Averages
Average Calculated Number of Bacteria in cm3
60000000
50000000
40000000
30000000
20000000
10000000
0
Negative Control
Plot 2: Middle
Plot 1: Next to Street



Conclusion

In conclusion, our hypothesis stating, the closer soil is to a source of car exhaust, the lower the bacteria density in the soil will be, was incorrect. In this experiment we tested how car exhaust, specifically acid affects the level of bacteria in soil. Our data showed that ecosystem of our school functions pretty well. As normal, when the pH levels increase, bacteria levels decrease, and when pH levels decrease, bacteria levels increase. Our graph comparing both the pH levels and bacteria level shows this to be true. We had three different plots placed on the Roland Park Country School Campus. The first plot was located next to Roland Ave, highly exposed to car exhaust. The second plot was located on the front lawn, in a halfway point between the street and courtyard, moderately exposed to car exhaust. The third plot was in the courtyard exposed to no car exhaust. We learned that bacteria like to dwell in soil that is slightly acidic, so we hoped to find higher bacteria levels in plot 1 and plot 2. We collected three soil samples from each plot on three different days. After collecting the soil each day, we combined each soil sample for a specific plot together in one bag, and used half of the soil for a pH test and the other half for a sterile dilution test. Since we were testing for both the presence of acid and the bacteria level of the soil these two tests had to be performed at the same time, using soil from the same plot. To rate the level of pH we used the chart from the LaMotte STH-14 test kit.

Interpretation of pH reading:

If pH is	Then the soil is
Below 5.5	Strongly Acid
5.5-6.0	Moderately Acid
6.1-7.0	Slightly Acid
Above 7.0	Alkaline

For plot 1 we discovered the average level of pH was 7.5, alkaline; meaning the soil had no acid in it. The bacteria level average for plot 1 was 16133333.3 cm^3. This shows that although there was no acid found in plot 1's soil, another factor is decreasing bacteria in soil located near the street. For plot 2 the average level of pH was 6.67, slightly acidic. The bacteria level average for plot 2 was 56766666.7cm^3. This shows that although the soil from plot 2 was not highly exposed to car exhaust, it still had acid it in, making the bacteria levels rise. For plot 3, our negative control, the average level of pH was 6.73, also

slightly acidic. The bacteria level average for plot 3 was 31000000 cm^3. This certain plot confused us, because this specific plot was not exposed to car exhaust at all, so there must have been another factor around it causing there to be acid in it. These levels for pH and bacteria make sense, because bacteria prefers to grow and live in soil that is slightly acidic. However if plot 1 was highly exposed to car exhaust, why did it not have any acid? These results show why our hypothesis was wrong. The acid of car exhaust was not the factor that impacted the bacteria. For future work, it is necessary to complete more research about car exhaust in order to determine what specific element in car exhaust is impacting the bacteria levels. We would have to determine if it was car exhaust or the other many factors impacting the soil, such as location, compaction, or weather.

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