What impact do car emissions have on the bacteria density in soil?

Soil Ecology Project

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Soil is an ecosystem made up of organic matter, organisms, air, water, and mineral particles (UMN, 2012). Within it, there are large and small pore spaces, and it is in these spaces where many of the soil's smallest organisms, the microbes, live. One type of these microbes are the bacteria, and the organic material that these organisms produce greatly benefits the movement of water, plant roots, and air within the soil (Nardi, 2003). Specifically, the bacteria help retain the natural nutrients in the soil as well as help breakdown various pesticides and pollutants (USDA, 2012). Thus, the overall health of soil is maintained by the natural addition of the organic matter produced by the bacteria living within it (Nardi, 2003).

One way bacteria do this is to convert nitrogen gas into usable forms for the producers. Called nitrogen fixation, it is the "process by which nitrogen-fixing bacteria [first] convert nitrogen gas to ammonia" (Campbell, N; Heyden, R; Williamson, B. 2004) and then bind the ammonia to hydrogen ions from water to form ammonium. "Nitrifying bacteria [then] convert ammonium to nitrates" (Campbell, N; Heyden, R; Williamson, B. 2004), which the producers in the ecosystem are able to absorb (along with some of the remaining ammonium) in order to build their amino acids and nucleotides. These monomers are "an essential component of DNA, RNA, and proteins, the building blocks of life" (Harrison, 2003) which is why the Nitrogen Cycle is so extremely important (ThinkQuest, 2011). Without the material provided by the bacteria to construct their enzymes, plants could not speed up the chemical reactions in their cells, enabling

them to do the four tasks of life (Campbell, N; Heyden, R; Williamson, B. 2004). Literally without nitrogen, plants could not survive.

But plants are not the only ones to benefit from the Nitrogen Cycle. After converting the nitrogen compounds for their own biological uses, plants pass them on when consumers eat these producers in order to obtain nitrogen in the form of organic molecules for the consumer's own cells. And then decomposers can release all this nitrogen in the form of ammonium from the wastes and decaying bodies of both plants and animals alike, releasing the nitrogen atoms back into the soil so that the process can start again. Thus, without the Nitrogen Cycle in the soil, there would be no terrestrial life on Earth (ThinkQuest, 2011).

Given this critical role, then, bacteria that play in ecosystems, anything that harms them could therefore have a potentially catastrophic impact on the environment. That is why even though automobiles may seem like the ideal mode of transportation, the fact that the exhaust from them has been linked to many environmental issues (Rodrigue, 2012) is highly problematic. Car exhaust contains numerous chemicals that are harmful to living things, and these include carbon monoxide, volatile organic compounds, oxides of nitrogen, and sulfur dioxides (EPA, 2000)—the latter two of which are particularly dangerous because they form acid rain that can slow down the growth of plants (Tucker, 2005; Clean Air Trust, 1999). Acid rain results when these two chemicals from car exhaust mix with precipitation in the air and fall to the ground again in the form of rain, snow, fog, or mist. The pore space in soil then allows these acidic chemicals to diffuse into the ground, and once there, they can cause damage depending on the soil's ability to buffer and neutralize some or all of the acid. But if the soil cannot buffer the acid rain it absorbs, the increasing soil acidity is harmful. The lower pH levels can denature the enzymes of the bacteria living there, and if the enzymes are unable to complete their job, then the

bacteria will die off and cannot complete the Nitrogen Cycle (Campbell; Reece; Urry; Cain; Wasserman; Minorsky; Jackson, 2008). This means that plants would not be able to receive the vital nutrients the bacteria provide. Hence, plant life in very acidic soil would grow more slowly or die (Air Quality Organization, 2012), because when soil is exposed to high levels of acidity, it is difficult for the bacteria to thrive.

Because of this significant relation between plants, bacteria, and acid rain, we plan to test the effects of chemicals in car emissions on the bacteria density in the soil near the carpool line, where they are plentiful, and compare it to the bacteria density in the soil of the courtyard. We predict that the presence of car emissions will cause the bacteria density in the soil to be lower.

I. Problem: What impact do car emissions have on the bacteria density in soil?

II. Hypothesis: The presence of car emissions will cause the bacteria density to be lower.

III. Procedure:

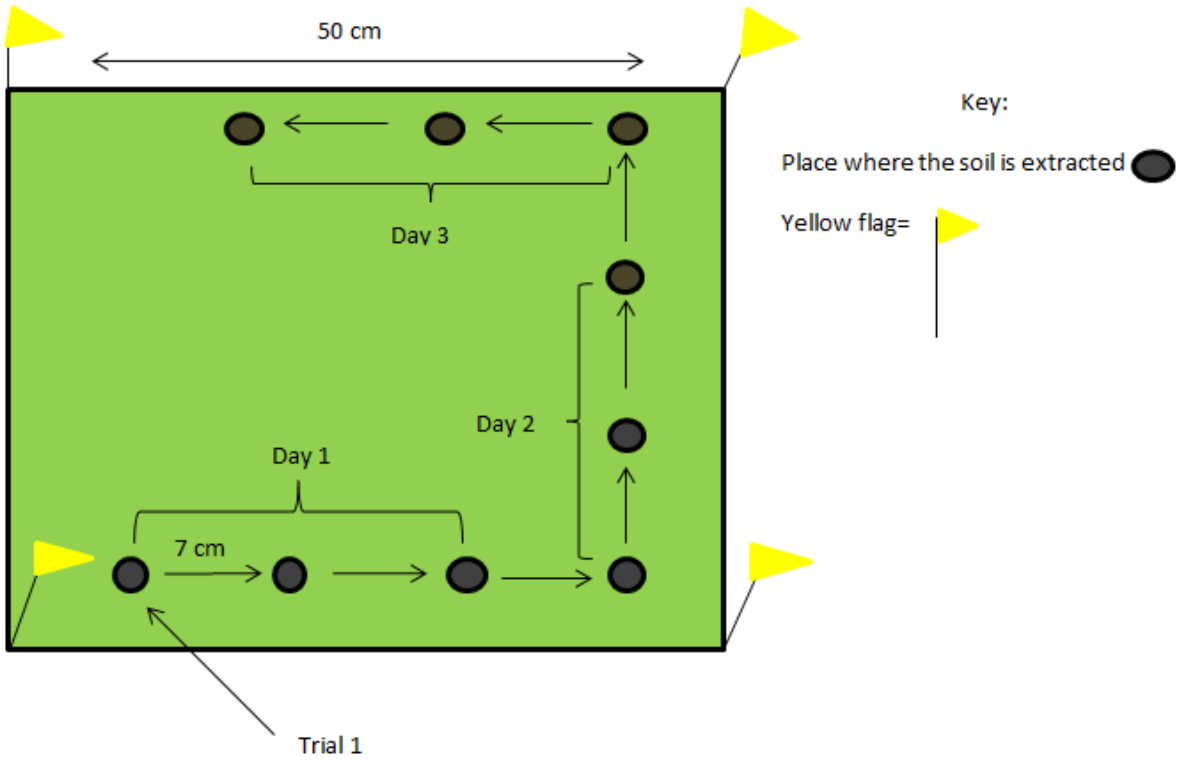
- A. Independent Variable: Location of the soil plots in relation to the amount of car emissions.
- B. Dependent Variable: The bacteria density in the soil
- C. Negative Control: The soil from the courtyard plot
- D. Controlled Variables:
  1. Size of plots of land
  2. Location of soil plot in courtyard
  3. Location of soil plot near carpool line
  4. Amount of soil tested in the dilutions
  5. Amount of soil collected from the carpool plot and courtyard plot
  6. Time the soil samples are taken

7. Number of trials taken per day
8. Human traffic
9. Slope of the land
10. Size of serological pipettes
11. Size of culture tubes
12. Size of cc scoops
13. Number of serological pipettes used
14. Amount of the soil sample in the cc scoops
15. Amount of sterile water in the 10^0 culture tube
16. Always diluting to the same degree
17. Amount of sterile water in the 10^{-1}, 10^{-2}, and 10^{-3} culture tubes
18. Use of 100 µl soil samples from dilutions 10^{-2} and 10^{-3} on the nutrient agar plates
19. Allowed bacteria to grow for 72 hours
20. Type of nutrient agar plate
21. Use of LaMotte STH Series with the basic model STH-4 Outfit kit to complete the pH test
22. Sterile water

E. Step-by-Step Instructions:

1. When extracting the soil, steps 4-9 and steps 12-17 need to be done on the same day at the same time frame for both locations.

2. Stand at the location 39.35775° N and 76.63655° W which is on the Roland Park Country School courtyard near the Knott Lobby and plot a 50 cm by 50 cm square on the ground at this location.
3. Place four yellow flags in the four corners of the plot. (See diagram)



4. Position the 48 cm long soil extractor (with a 2 cm diameter), on the ground at least 5cm away from any yellow flag. (See diagram)
5. Push soil extractor into the ground and twist it clockwise. If needed, hit the top of soil extractor with rubber mallet to push the extractor further into the ground.
6. Continue to push the soil extractor into the ground until it reaches the first mark or 15 cm.
7. Pull the soil extractor upwards and place soil into plastic bag labeled “Courtyard trial # 1” and the date.

8. Place soil extractor 7 cm on the ground to the right (see diagram above) from the first soil extraction spot and repeat steps 4-6 with the bag labeled “Courtyard trial # 2” and the date.
9. Place soil extractor 7 cm on the ground to the right (see diagram above) from the second soil extraction spot and repeat steps 4-6 with the bag labeled “Courtyard trial # 3” and the date.
10. Stand at the location 39.35800° N and 76.63567° W which is by the parking lot in the carpool line and plots a 50 cm by 50 cm square on the ground at this location.
11. Place four yellow flags in the four corners. (See diagram)
12. Position soil extractor on the ground at least 5cm from any yellow flag. (See diagram)
13. Position the 48 cm long soil extractor (with a 2 cm diameter), on the ground at least 5cm away from any yellow flag. (See diagram) Then push soil extractor into the ground and twist it clockwise. If needed, hit the top of soil extractor with rubber mallet to push the extractor further into the ground.
14. Continue to push the soil extractor into the ground until it reaches the first mark or 15 cm.
15. Pull the soil extractor upwards and place soil into plastic bag labeled “Carpool Line trial # 1” and the date.
16. Place soil extractor 7 cm on the ground (See diagram) to the right from the first soil extraction spot and repeat steps 13-16 with the bag labeled “Carpool Line trial # 2” and the date.
17. Place soil extractor 7 cm on the ground (See diagram) to the right from the second soil extraction spot and repeat steps 13-16 with the bag labeled “Carpool Line trial # 3” and the date.

18. Repeat steps 4-9 and 12-18 each day at the same time, with the second trial being taken 3 days after the first, and the third trial taken 6 days after the first.
19. Steps 20-36 must be done on the same day during the same time frame.
20. After extracting the soil, complete the pH test at the same time frame on the same day as the serial dilution test for bacteria for all of the bags of soil.
21. Use a clean, new transfer pipette to add 10 ml of sterile water to a 15 ml culture tube. Label the tube "10^0" and also have "Courtyard Trial 1, day 1" on it.
22. Use the same pipette to add 9 ml of sterile water to a second 15 ml culture tube. Label the tube "10^{-1}" and also have "Courtyard Trial 1, day 1".
23. Repeat step 22 three more times to three additional 15 ml culture tubes, only label them "10^{-2}," and "10^{-3}" respectively, along "Courtyard Trial 1, day 1".
24. Place 1 cc of your Courtyard Trial 1, Day1 soil sample into the "10^0"culture tube.
25. Cap the tube and shake vigorously.
26. Using a new clean pipette, remove 1 ml of the soil/water mixture from the "10^0" tube and place into the "10^{-1}" tube.
27. Cap and shake vigorously.
28. Using the same pipette in step 26, remove 1 ml of the soil/water mixture from the "10^{-1}" tube and place into the "10^{-2}" tube.
29. Cap and shake vigorously.
30. Using the same pipette in step 26, remove 1 ml of the soil/water mixture from the "10^{-2}" tube and place into the "10^{-3}" tube.
31. Cap and shake vigorously.
32. You should now have a total of four culture tubes.

33. Plate 100 µl samples from the 3rd and 4th tubes (dilutions 10^{-2} & 10^{-3}) onto their own separate 3M Petrifilm™ Aerobic Count Plates. (one labeled Courtyard, Day 1, Trial 1 10^{-2} and another labeled Courtyard, Day 1, Trial , 10^{-3})
34. Allow to grow for 48 to 72 hours.
35. Examine each of the plates for individual bacteria colonies and choose the plate with the fewest colonies (but at least 5) at the lowest dilution level 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 $10^{|\text{dilution \# at which these colonies were found}|}$.
36. Using the LaMotte STH Series with Model STH-4 Outfit kit, complete the pH test on the soil from "Courtyard Trial 1 day 1".
37. Repeat steps 19- 36 (which includes the serial dilution test and pH test) using each of the remaining 17 soil samples previously extracted.

IV. Data and Analysis:

A. Data Table:

pH Levels and Bacteria Density From Soil Exposed to Varying Degrees of Car Emissions

Sampling Day	Location	Trial	pH Level of Soil	Bacteria Density (#/cc of soil)
1	Courtyard	1	6.4	700000
		2	6.4	1300000
		3	6.4	1300000
	Carpool	1	6.2	3600000
		2	6.4	2000000
		3	6.0	1600000
2	Courtyard	1	6.4	1100000
		2	6.4	1200000
		3	6.4	1400000
	Carpool	1	7.2	1700000
		2	6.4	1500000
		3	6.4	900000
3	Courtyard	1	6.4	600000
		2	6.6	1500000
		3	6.2	1200000
	Carpool	1	6.4	700000
		2	6.4	800000
		3	6.4	800000

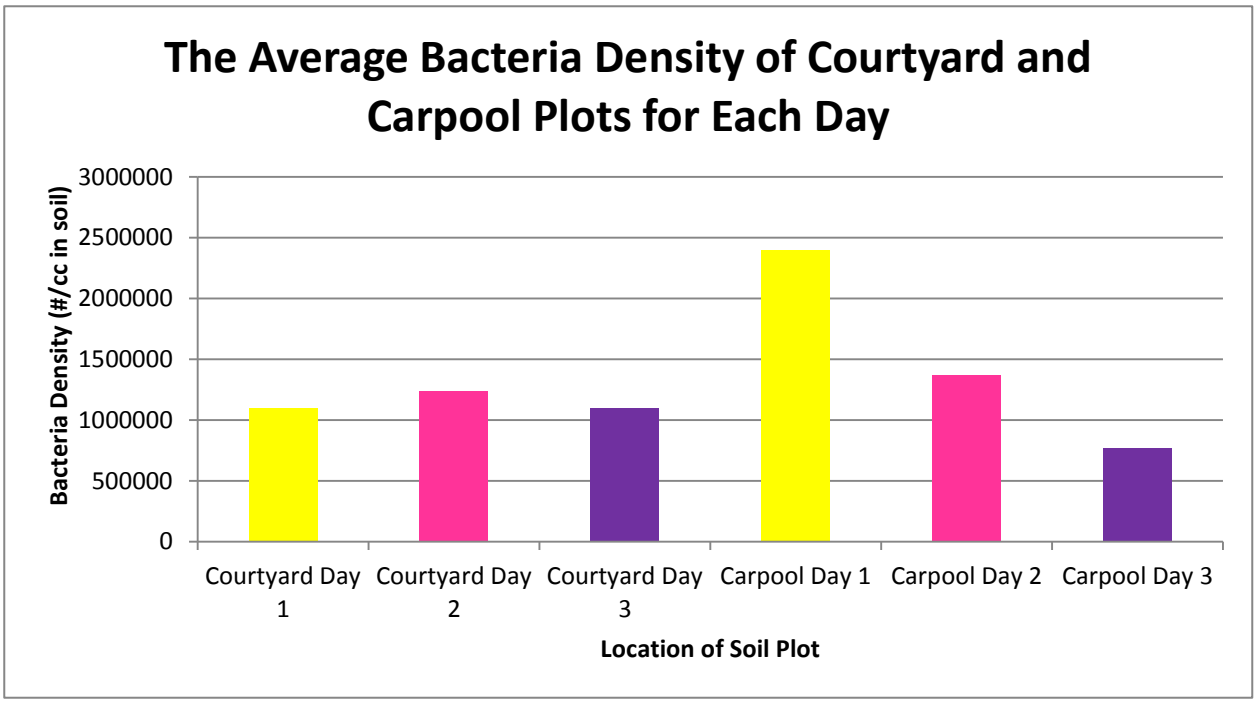
Average pH Levels and Bacteria Density from Soil Exposed to Varying Degrees of Car Emissions

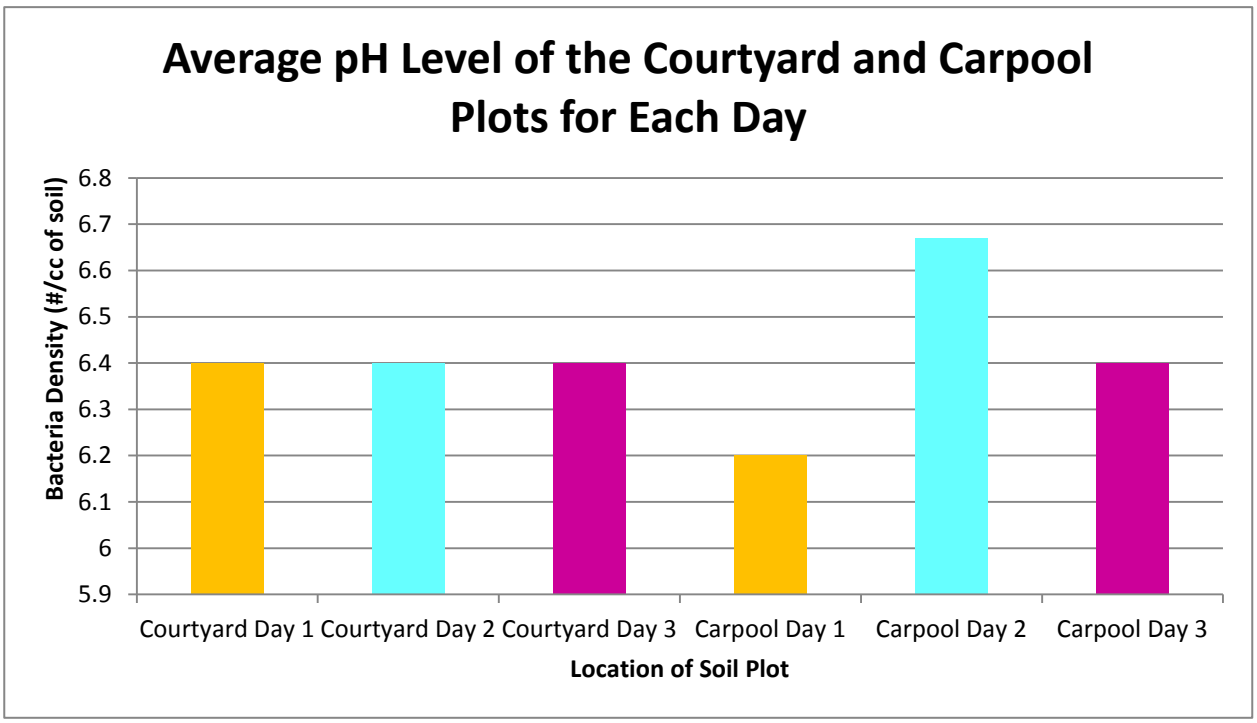
Sampling Day	Location	Average for the pH level of Soil	Average Bacteria Density in Soil (#/cc of soil)
1	Courtyard	6.4	1100000
	Carpool	6.2	2400000
2	Courtyard	6.4	1233333
	Carpool	6.7	1366667
3	Courtyard	6.4	1100000
	Carpool	6.4	766667

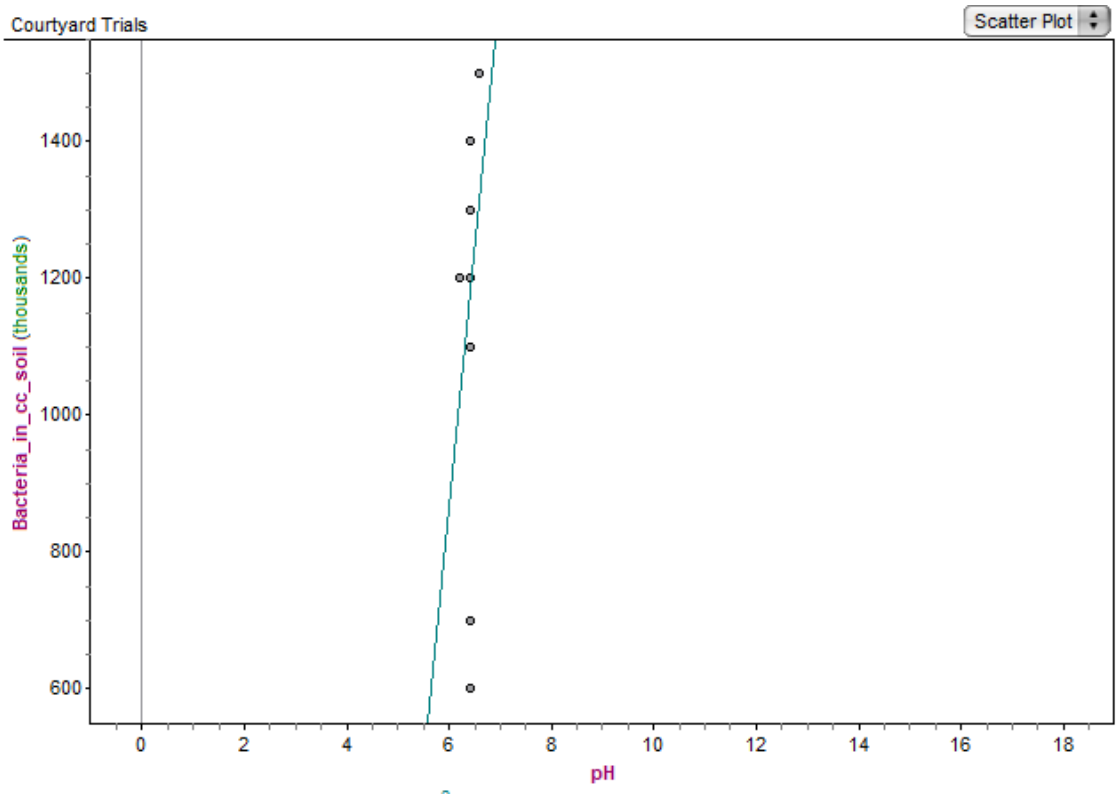
Total Average pH Levels and Bacteria Density from Soil Exposed to Varying Degrees of Car Emissions

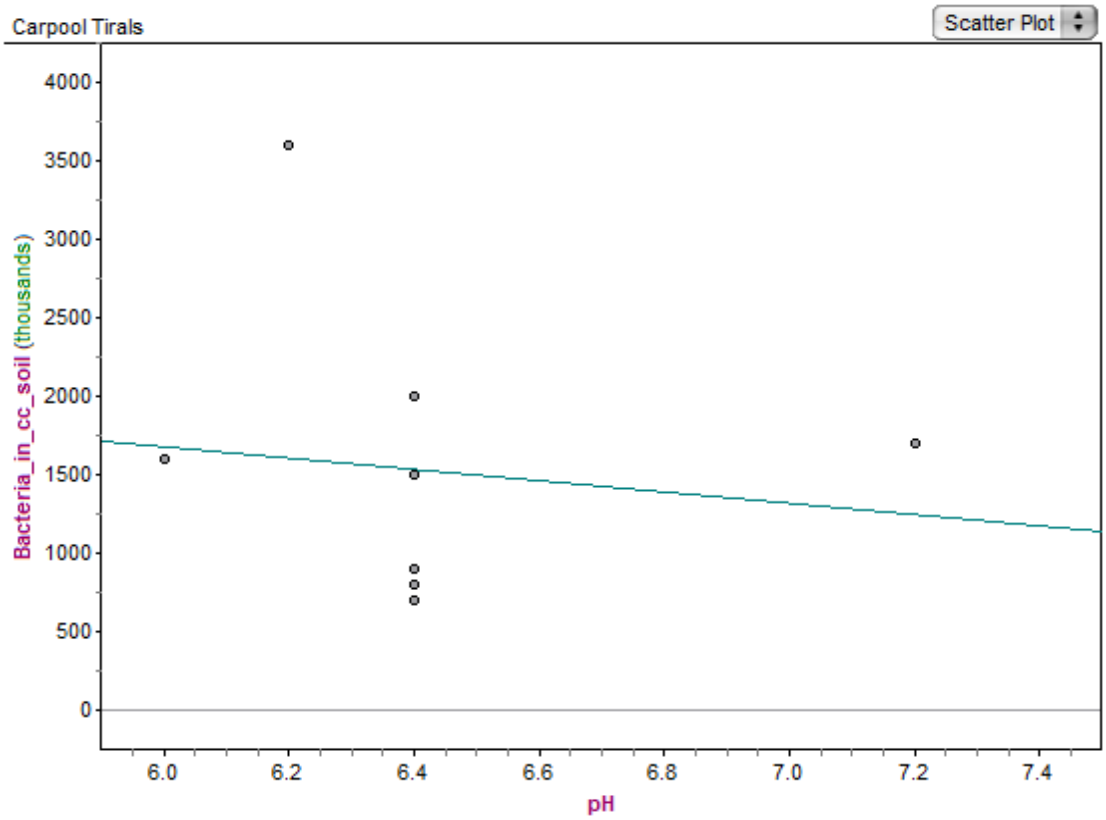
Location	Total Average for the pH level of Soil	Total Bacteria Density (#/cc of soil)
Courtyard	6.4	1144444
Carpool	6.4	1511111

B. Graphs:


The Average Bacteria Density of Courtyard and Carpool Plots for Each Day
Bacteria Density (#/cc in soil)
3000000
2500000
2000000
1500000
1000000
500000
0
Courtyard Day 1
Courtyard Day 2
Courtyard Day 3
Carpool Day 1
Carpool Day 2
Carpool Day 3
Location of Soil Plot


Average pH Level of the Courtyard and Carpool Plots for Each Day
Bacteria Density (#/cc of soil)
6.8
6.7
6.6
6.5
6.4
6.3
6.2
6.1
6
5.9
Courtyard Day 1
Courtyard Day 2
Courtyard Day 3
Carpool Day 1
Carpool Day 2
Carpool Day 3
Location of Soil Plot


Courtyard Trials
Scatter Plot
Bacteria_in_cc_soil (thousands)
1400
1200
1000
800
600
0
2
4
6
8
10
12
14
16
18
pH


Carpool Tirals
Scatter Plot
Bacteria_in_cc_soil (thousands)
4000
3500
3000
2500
2000
1500
1000
500
0
6.0
6.2
6.4
6.6
6.8
7.0
7.2
7.4
pH

V. Conclusion:

After following through with this experiment, we concluded that our hypothesis was incorrect. This is made clear, as the bacteria density in the soil of the carpool line was in fact lower than that of the courtyard. The average bacteria density in the soil of the carpool line was 1511111 microbes/cc of soil, while the average bacteria density in the soil of the courtyard was 1144444 microbes/cc of soil. Based on our data, there was a significant difference in the total bacteria density of both plots. The average pH level in our soil plot located in the carpool line was 6.4, the same as the pH level in the courtyard soil. According to an interpretation of pH reading from the LaMotte STH Series with the basic model STH-4 Outfit kit, both of these soil plots are considered to be slightly acidic. These results show that the levels of pH are not affecting the bacteria density in the courtyard soil. However, the pH levels are affecting the bacteria density in the soil of the carpool line. In the carpool line, the pH levels were unstable, which therefore made the enzymes dysfunctional, lowering the bacteria density. We cannot prove that the car exhaust was the cause of the lowering of the pH levels and bacteria density. This lowering could be caused by an outside factor, completely unrelated to car exhaust. Our data clearly proves our hypothesis wrong. In order for our hypothesis to have been correct, the bacteria density in the carpool line would be lower than the density in the courtyard.

Our research that was collected before we began our experiment, told us that the bacteria density should have been lower in the presence of car exhaust. This research tells us that our hypothesis should have been accurate, but our data proved us otherwise. In order to securely prove or disprove our hypothesis, we need to conduct more experiments using soil in different locations. While conducting our experiment, we realized that we had a major design flaw. Our flaw was that we did not allow our collected soil to sit in a controlled environment, for the same

time from where we collected the soil until we tested it. Because of this mistake our data may have been incorrect. We know now that if we were to do this experiment again, after extracting the soil, we would allow it to sit in a controlled environment for a set amount of time before testing both the pH levels and the bacteria density.

If our hypothesis was proven correct and car exhaust does in fact unbalance the natural order of soil life. A way to help keep the soil healthy is to try and use fossil fuels less. Seeking alternative power sources and doing simple actions like walking to school, riding your bike, or carpooling with others, would produce less harmful chemicals into the air. Without the production of these chemicals, we could possibly increase the density of bacteria in our soil, thus improving the overall health of our environment.

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