The Impact Of Car Exhaust On pH Levels And Bacteria Population In The Soil

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Background

According to Jacobs (n.d.), most air pollution comes from cars, trucks, and other forms of transportation. The amount of cars bought and used in the United States has increased significantly over time. From 2009 to 2016, there has been a steady growth rate of cars being used every year (Auto Alliance, n.d.). As more and more people have settled in suburban areas, there has been an increase in car ownership. This then leads to an increase in air pollution as many people can no longer use public transportation to travel to work or elsewhere (GetRevising, 2016). This car growth has also impacted the growth of infrastructure, for example, “over 164,000 miles of highways in the National Highway System form the backbone of our 4-million-mile public road network” (U.S. Department of Transportation, 2019). Car exhaust lets off many chemicals into the atmosphere, including carbon monoxide, nitrogen dioxide, nitrous oxide, sulphur dioxide, suspended particles, PM10 particles, benzene, formaldehyde, and polycyclic hydrocarbons (Gislason, n.d.). Every year, “cars release approximately 333 million tons of carbon dioxide into the atmosphere” (Jacobs, n.d.). This is the reason why there is a surplus of carbon dioxide in the air. The smog, carbon monoxide, and other toxins emitted from the car exhaust, leave tailpipes at street level, which is where humans are directly breathing in the polluted air. This makes car exhaust an even bigger issue to humans then toxins emitted by industrial smoke-stacks, which release pollutants up high in the air (National Geographic, n.d.).

Transportation is one of the largest producers of air pollution. The American Lung Association has reported that car emissions in the United States kill 30,000 people annually. This air pollution causes and worsens respiratory and cardiovascular problems. Over half of Americans live in cities that fail to meet air quality standards many days a year (Jacobs, n.d.).

Along with the increasing amount of cars on the road from 1950 to the present, the amount of greenhouse gases in the air has also greatly increased (Environmental Protection Agency, n.d.). One greenhouse gas, carbon dioxide, enters the atmosphere through the burning of fossil fuels, such as car exhaust. Greenhouse gases such as carbon dioxide molecules absorb infrared radiation. The sun releases heat to the earth that is transferred through the sun's rays. When the earth absorbs these rays, it holds them and then re-radiates heat back into the atmosphere. As it goes through the atmosphere, the heat is trapped up inside carbon dioxide molecules. This then heats up the earth and causes global warming. Therefore, the more carbon dioxide that is added to the air, the more heat is trapped, in the atmosphere (Plass, 2008).

Air pollutants and particulate matter from car exhaust can get into the soil and water surfaces. Polluted water and soil enter the food chain. The food chain is the way in which animals receive their nutrients, widely affecting animals and plants. The pollution within the food chain affects the reproductive, respiratory, immune, and neurological systems of the consumers. Car exhaust also contributes to the formation of acid rain through the release of sulfur dioxide and nitrogen oxides. As these substances rise, they react with water, oxygen and other chemicals, which forms sulfuric acid and nitric acid in the precipitation (Environmental Protection Agency, n.d.). Acidic precipitation such as rain, sleet, snow, cloud vapor, and fog, changes the acidity, or pH, of waterways and soils, and it harms the organisms that live in these environments (Plass, 2008).

pH is a measurement of the acidity or alkalinity of a solution, based on a scale from 0 to 14. On this scale, 0 is the most acidic and 14 is the most alkaline, while 7 is neutral. Alkaline solutions have a high concentration of hydroxide ions, whereas acidic solutions have a high concentration of hydrogen ions. Regular rain has a pH of about 5.6, yet acid rain has a pH value

between 4.2 and 4.4 (Environmental Protection Agency, n.d.). When the soil absorbs acid rain, this lowers the pH of the soil. The low pH levels of the soil can potentially harm the organisms relying on the resources that the environment provides. Some microorganisms live in water spaces and pores of the soil, and require water to survive (King, 2018). In particular, protozoa can survive in environments with pH levels ranging from 5-8, and thrive in environments with pH levels above 7. Meanwhile, bacteria can live in a somewhat similar range of pH values from 5-9, with an optimum pH value of 7. If car exhaust causes the pH level of the soil to move out of this range, bacteria would not be able to thrive. The enzymes in organisms, including bacteria and protozoa, can only function in a certain range of pH values. If the organisms were to move into or be exposed to pH levels outside of this range, they would not be able to start and stop chemical reactions, complete any functions, and ultimately would not be able to survive in the environment.

Bacteria are prokaryotic single-celled microorganisms that live in diverse environments, performing important functions related to nutrient cycling, water dynamics, and disease suppression. Bacteria are particularly concentrated in thin water films around soil particles and near roots, in an area called the rhizosphere. With optimal food, water, and environmental conditions, bacteria thrive and reproduce rapidly. Generally, about a teaspoon of productive soil contains between 100 million and 1 billion bacteria (Ingham, n.d.). Bacteria are small, about 1 μm or 4/100,000 of an inch wide, and yet they still make up both the largest number and biomass of any soil microorganism. This small size allows them to grow and adapt more quickly to changing environmental conditions than more complex, larger microorganisms.

Bacteria are divided into four major functional groups: decomposers, mutualists, pathogens, and lithotrophs. Most bacteria are decomposers that consume simple carbon

compounds, such as root exudates and fresh plant litter. Through this process, decomposer bacteria convert energy in soil organic matter into forms useful to the rest of the organisms in the soil food web. They are particularly important in immobilizing nutrients in their cells and preventing the loss of nutrients from the rooting zone. Without bacteria immobilizing fixed nitrogen, much of it would be lost to air and water. A second group of bacteria are called mutualists, which form partnerships with plants. An example is nitrogen-fixing bacteria, which form symbiotic associations, or mutually beneficial relationships, with the roots of legumes (Lambers, Chapin, Pons, 2008). In this process, the plant supplies carbon compounds as a food source to the nitrogen-fixing bacteria, while these bacteria convert nitrogen gas into ammonium for plants to use. Next, there are bacterial pathogens, which live off of other organisms and cause diseases in plants (Ingham, n.d.). Yet, there are other bacteria, such as, actinomycetes, which produce antibiotics in order to help protect plants from other bacterial pathogens. Lastly, the fourth group of bacteria contains lithotrophs and chemoautotrophs. Rather than obtaining energy from carbon compounds, lithotrophs and chemoautotrophs use inorganic compounds from hydrogen, nitrogen, iron, and sulfur (Hoorman, 2016).

Bacteria have the ability to alter environments to benefit certain plant communities as soil conditions change. To help maintain soil structure, bacteria produce layers of glycoproteins or polysaccharides that coat the surface of soil particles. These polysaccharides can improve soil structure by cementing sand, silt, and clay particles together to form stable microaggregates. Microaggregates are groups of soil particles that bind together and create pore space for exchange and retention of both water and air (Hoorman, 2016). This increases the availability of water to plants, a vital component in photosynthesis where plants convert the energy of sunlight into the energy stored in organic molecules. This also increases the availability of air to plants.

Oxygen is a required reactant in cellular respiration, a process in which organisms acquire energy from food. Aggregates that are not stable can fall apart when struck by raindrops and release individual soil particles. This creates crusts that can potentially close pathways and pores, restricting the entry of air and water into the soil (National Soil Survey Center, 1996).

Bacteria also play a major role in the nitrogen and carbon cycles by making nitrogen and carbon accessible to other organisms. Nitrogen makes up 78% of the atmosphere, but is limited in its usable forms that help organisms grow. There are five stages in the nitrogen cycle: nitrogen fixation, nitrification, assimilation, ammonification, and denitrification (Khan Academy, 2019). In nitrogen fixation, atmospheric nitrogen gas is converted into ammonia by nitrogen-fixing bacteria. In the second stage of the nitrogen cycle, nitrification, nitrifying bacteria convert the ammonia into nitrites and nitrates. In the third stage, assimilation, as the animals eat the plants, the plants take up a certain form of nitrogen. In the fourth stage, ammonification, ammonia is formed through the breakdown of plants and waste (Thomas, n.d.). Finally, in the last stage of the nitrogen cycle, denitrification, the denitrifying bacteria take excess nitrates out of the soil and convert it into atmospheric nitrogen. The carbon cycle is another cycle where bacteria play an important role. Soil bacteria take in carbon from the organisms they decompose, and release the carbon dioxide back into the atmosphere. Plants take in carbon dioxide along with oxygen to produce energy in the process of photosynthesis. (Lagzi, Mészáros, Gelybó, Leelőssy, 2013). Having a decreased amount of bacteria in the soil, due to car exhaust, would disrupt the nitrogen cycle, and the carbon cycle (Khan Academy, 2019).

Car exhaust decreases the pH levels in the soil, which lowers the amount of bacteria colonies in the soil ecosystem. If there is not enough bacteria in the soil, protozoa will lose a major food source, dead organisms will not be broken down through decomposition, and the soil

structure will suffer. Protozoa are the nutrient source for larger soil invertebrates and they get nutrients from bacteria. When these invertebrates die, the carbon and nitrogen in their bodies goes into the soil which causes an abundance of dead organic matter with fewer decomposers. All organisms depend on the soil food web for nutrition, so if the plant population (the producers) decreased, than each trophic level would encounter a food shortage; this would cause the entire ecosystem to be negatively impacted (Ingham, n.d.). Plants need nitrogen and carbon to thrive, so if these biochemicals cannot be converted into usable forms, plant growth will be reduced, and the soil environment will be less fertile. These changes to the ecosystem reduce the efficiency of carbon and nitrogen cycles, which ultimately affect the sustainability of the ecosystem.

In this experiment, we are testing how different levels of exposure to car exhaust will impact pH levels and population density of bacteria in the soil. We hypothesize that as the exposure to car exhaust increases, the pH level of the soil will decrease and the population density of bacteria in the soil will increase. We hypothesize this because of how pH levels indirectly affect bacteria. Bacteria are eaten by protozoa, which live in the water pores and spaces in the soil. The pH of the soil most directly impacts the water spaces. When the pH level lowers from 5-8, due to car exhaust, the protozoa cannot thrive. If the protozoa population decreases due to the effects of car exhaust, then bacteria will have fewer predators, allowing the bacteria population to increase.

Experimental Design:

I. Problem: How does the exposure to car exhaust impact pH levels and population density of bacteria in the soil?

II. Hypothesis: As the exposure to car exhaust increases, the pH level of the soil will decrease and the population density of bacteria in the soil will increase.

III. Procedure:

A. Independent Variable: the exposure level of the soil to car exhaust

B. Dependent Variable: pH level of the soil and population density of bacteria colonies in 1 cc of soil

C. Negative Control: lowest level of exposure of soil to car exhaust

D. Controlled Variables: Type of water for dilution, amount of chemical solution in pH test, depth of soil extracted (15 cm), amount of soil used in pH test, amount of soil in dilutions, type of chemical pH test kit used, number of soil plots, the distance between each soil plot, location of plots, size of culture tube, the temperature of the water, placement of flags, number of flags, type of flags, size of micropipette, size of culture tube, time given for bacteria to grow on plates, date and time of pH test, size of plot, date and time of soil extraction, date and time of dilution, amount of dilution tubes, time bags sit before dilution, length of time dilution tubes were shaken, amount of water in each test tube, amount of soil in each test tube, amount of soil and water solution transferred from one test tube to the next, type of tool used for soil extraction, size of pipette, size of pipette, type of culture tubes, intensity of shaking test tubes in soil dilution, intensity of shaking test tubes in soil pH test

E. Step-by-Step:

    1. Plot a 20x20 cm plot using 4 flags labeled “low proximity” and place flags at N 39.35793° W 076.63651°.
    2. Plot a 20x20 cm plot using 4 flags labeled “semi-proximity” and place flags at N 39.35907° W076.63607°.
    3. Plot a 20x20 cm plot using 4 flags labeled “far proximity” and place flags at N 39.35798° W 076.63651°.

4. At each plot using a soil core sampler with a 2.5 cm diameter, extract the soil by pushing the soil core sampler into the ground at a depth of 15 cm and then turn clockwise and pull up straight. Place soil from the close proximity plot in a sandwich size plastic bag and label, “close proximity,” then repeat this extraction process with the other two plots “semi-proximity,” and “far proximity” accordingly. Extract these three samples on the same day and at the same time.
5. Bring samples into the lab and begin dilution and pH test in steps 6 - 27. Complete both of these tests on the same day at the same time.
6. Use a clean, new serological pipette to add 10 ml of sterile water to a 15 ml culture tube. Label the tube “10^{0} high exposure”
7. Use the same pipette used in step 6 to add 9 ml of sterile water to a second ml culture tube. Label the tube “10^{-1} high exposure”
8. Repeat step 7 two more times to two additional 15 ml culture tubes, only label them “10^{-2} high exposure,” and “10^{-3}high exposure,” respectively.
9. Place 1 cc of your high exposure soil sample into the “10^{0} high exposure” culture tube.
10. Cap the tube and shake vigorously.
11. Using a new clean serological pipette, remove 1 ml of the soil/water mixture from the “10^{0} high exposure” tube and place into the “10^{-1}high exposure” tube.
12. Cap and shake vigorously.

13. Using the same pipette in step 11, remove 1 ml of the soil/water mixture from the "10^{-1}high exposure" tube and place into the "10^{-2} high exposure" tube.
14. Cap and shake vigorously.
15. Using the same pipette in step 11, remove 1 ml of the soil/water mixture from the "10^{-2} high exposure" tube and place into the "10^{-3} high exposure" tube.
16. Cap and shake vigorously.
17. There should now be a total of four culture tubes.
18. Plate 100 µl samples from the 3rd and 4th tubes (dilutions 10^{-2} high exposure & 10^{-3}high exposure) onto their own 3M Petrifilm™ Aerobic Count Plate. The plates should be labeled according to their plot name, dilution number, and the trial number.
19. Repeat step 6-18 two more times with the low and semi exposure soil samples respectively. Label the tubes by replacing "high exposure" with "semi-exposure" and "low exposure" respectively. Complete these tests on the same day as the high exposure test.
20. Using a LaMotte STH-14 Chemical Test Kit, fill a test tube (0204) approximately one-third full of the same "close-proximity" soil used in the soil dilution. Use the Model PWB-1 Demineralizer Bottle (1155) to add demineralized water to the tube, until it is one-half inch from the top. Cap and shake until the soil is well dispersed.

21. Add 5 drops of Soil Flocculating Reagent (5643). Cap and shake to mix. Allow contents to settle before proceeding to Step 22.
22. Use a 1 mL pipet (0354) to transfer 1 mL of the clear solution above the soil to one of the large depressions on a spot plate (0159). Transfer a second 1 mL sample to the other large depression on the spot plate.
23. To the first sample on the spot plate, add two drops of *Duplex Indicator (2221). Compare the resulting color reaction against the Duplex Color Chart (1313). NOTE: The wide range pH test result indicates which narrow range indicator and color chart should be selected to perform a more precise pH test. 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.
24. Add two drops of the chosen narrow indicator to the second sample on the spot plate. Compare the resulting color reaction against the appropriate color chart to obtain a precise soil pH reading.
25. Repeat steps 20-24 two more times, with the other two soil samples from the soil dilution, “semi-proximity” and “far-proximity”, on the same day, and at the same time respectively. Record all pH values on the data tables as you discover the number.
26. Allow bacteria plates to grow for 96 hours
27. Examine each of the plates for individual bacteria colonies and observe the 10^{-3} plate. If there are 5 or more bacteria colonies, then count the number of colonies on this plate, if not count the colonies on the 10^{-2}plate

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 $10^{|the\ dilution\ number\ at\ which\ these\ colonies\ were\ found|}$

28. Repeat steps 4 - 27 four more times for a total of five trials, collecting new soil before testing again.

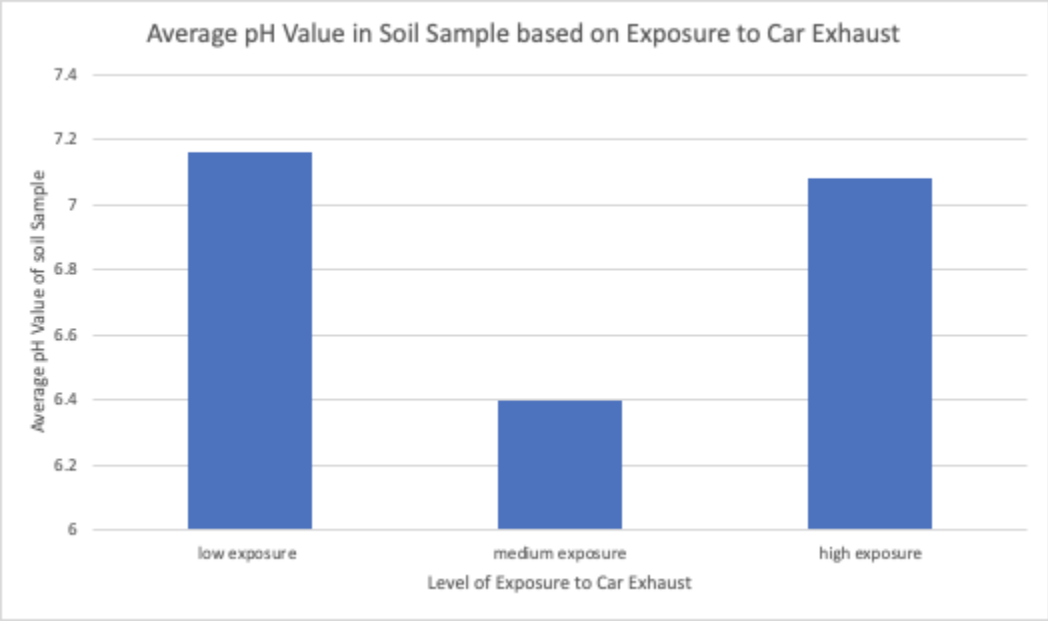
IV. Data and Analysis

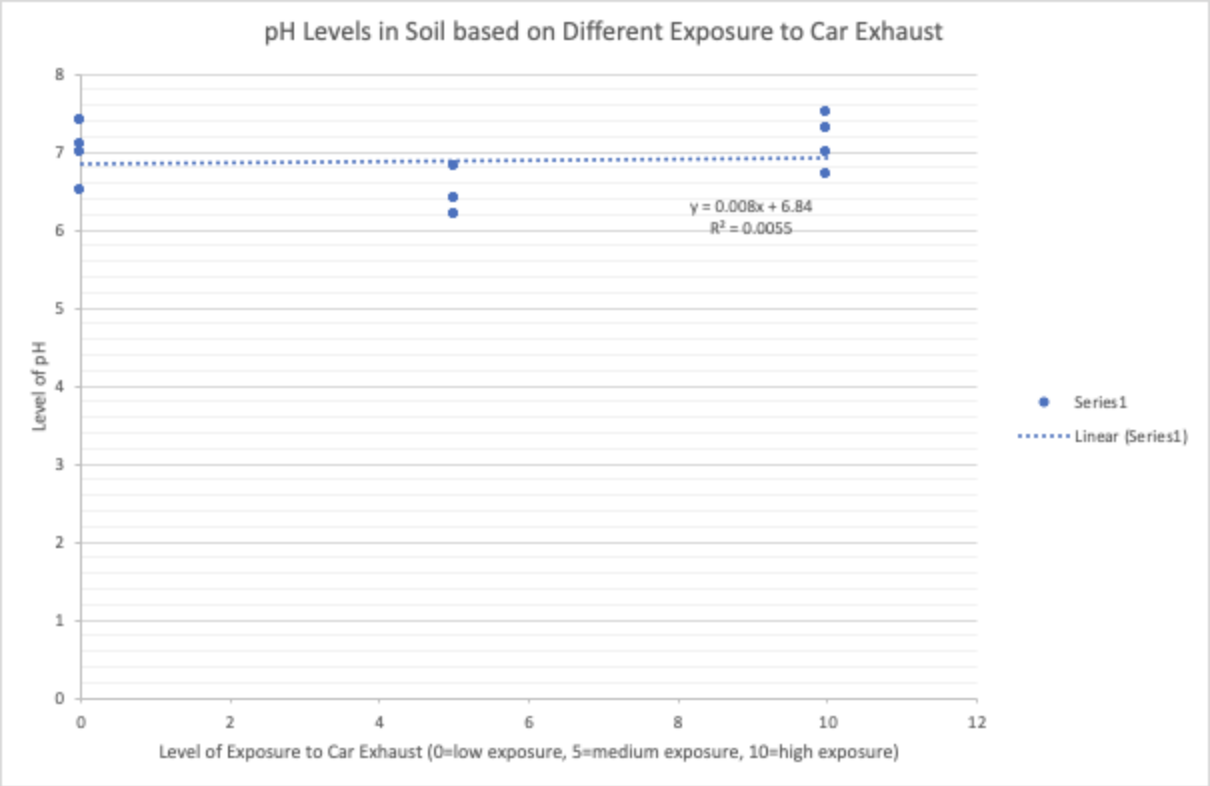
pH Levels in Soil based on Different Exposures to Car Exhaust

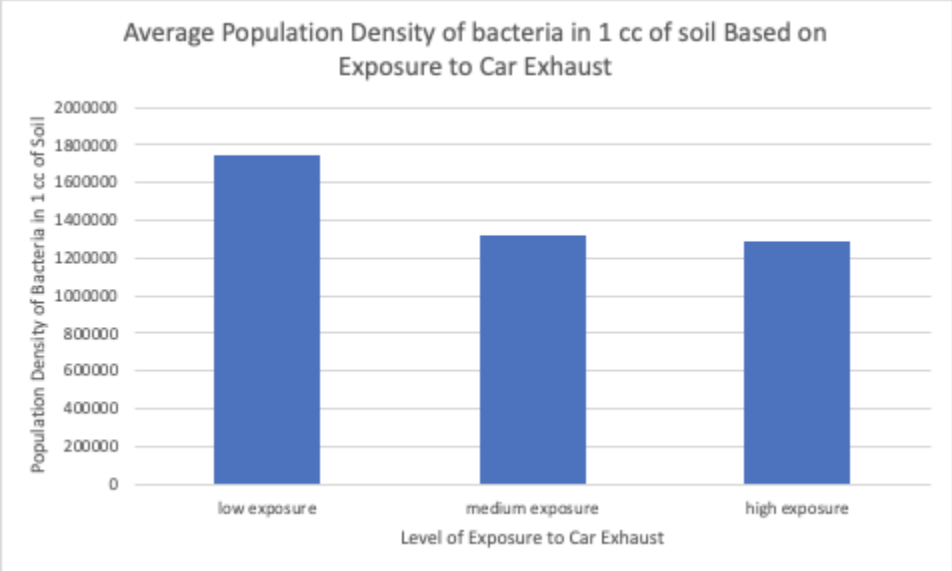
		Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Average
High Exposure	pH Levels on a Scale from 0-14	7.0	6.5	7.4	7.1	7.4	7.08
Medium Exposure	pH Levels on a Scale from 0-14	6.4	6.4	6.2	6.8	6.2	6.4
Low Exposure	pH Levels on a Scale from 0-14	6.7	7.3	7.5	7.0	7.3	7.16

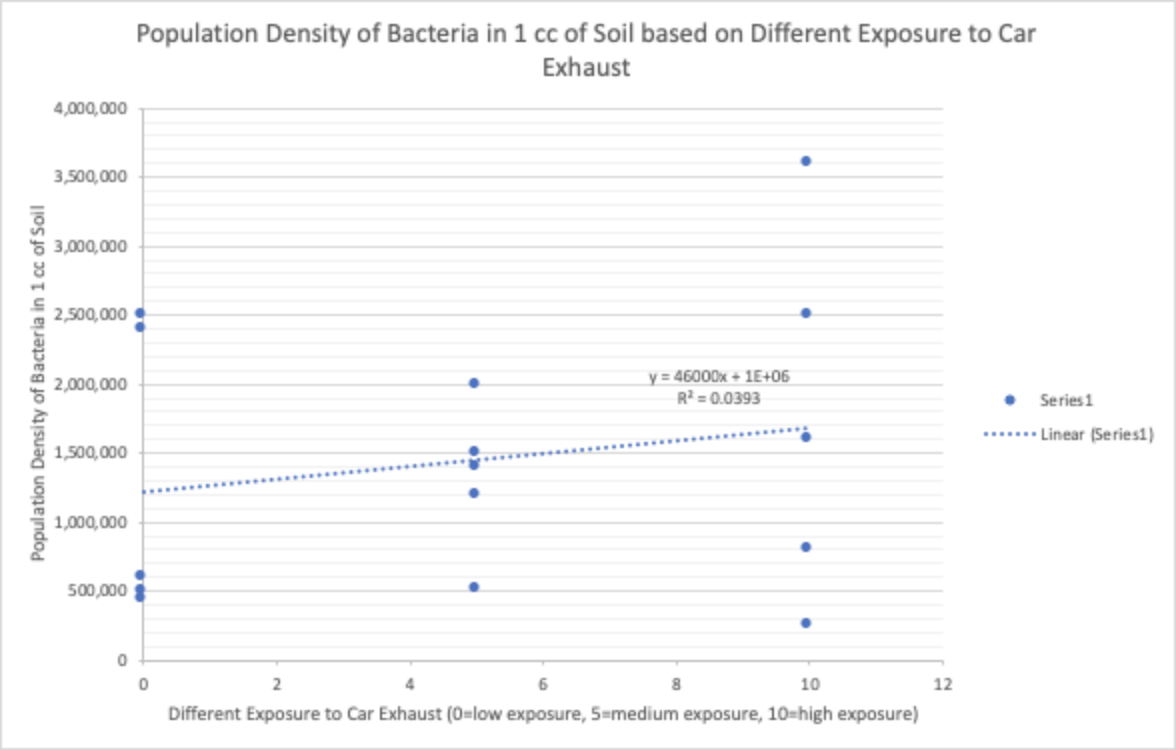
Number of Bacteria in 1 cc of soil with Different Exposure to Car Exhaust

		Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Average
High exposure	Number of bacteria in 1 cc of soil	500,000	2,400,000	600,000	450,000	2,500,000	1,290,000
Medium exposure	Number of bacteria in 1 cc of soil	1,500,000	2,000,000	520,000	1,400,000	1,200,000	1,324,000
Low exposure	Number of bacteria in 1 cc of soil	2,500,000	3,600,000	250,000	800,000	1,600,000	1,750,000




pH Levels in Soil based on Different Exposure to Car Exhaust
Level of pH
Level of Exposure to Car Exhaust (0=low exposure, 5=medium exposure, 10=high exposure)
y = 0.008x + 6.84
R² = 0.0055
Series1
Linear (Series1)


Average Population Density of bacteria in 1 cc of soil Based on Exposure to Car Exhaust
Population Density of Bacteria in 1 cc of Soil
low exposure
medium exposure
high exposure
Level of Exposure to Car Exhaust



V. Conclusion:

We hypothesized that as the exposure to car exhaust increased, the pH level of the soil would decrease and the population density of bacteria in the soil would increase. Our hypothesis was not supported by our data. Our average pH level for soil at a high exposure to car exhaust was 7.08, our average pH level for a medium exposure was 6.4, and our average pH level for a low exposure was 7.16. Our bar graph illustrates that our highest pH level was for low exposure to car exhaust and our lowest pH level was for a medium exposure to car exhaust. In our line of best fit graph, our equation for the line is R^2= 0.0055, which shows us that the pH value did not show a consistent pattern or strong correlation. In our bacteria experiment our average number of bacteria in 1 cc of soil for high exposure to car exhaust was 1,290,000. Our average number of

bacteria in 1 cc of soil for a semi-exposed area was 1,324,000. Our average number of bacteria in 1 cc of soil for low exposure to car exhaust was 1,750,000. Our bar graph of averages for the amount of bacteria in 1 cc of soil supports our hypothesis, but the line of best fit graph does not. In the line of best fit graph, we see trial data versus averages. Our trial data shows that it was very inconsistent and varied more, which weakens the support of the bacterial populations increasing as the exposure to car exhaust increases. The scatter plots around the line of best fit, especially around high exposure to car exhaust, show how much the data varied. The lack of correlation in our data helps us conclude that the Roland Park environments, in which we extracted our soil, was not affected to any extreme by car exhaust.

We would be interested in the data we would collect if we altered aspects of our experiment. If we were to perform this experiment again, our group would have used different sites to collect soil from. We would use an empty park around no cars for our low exposure, we would use a normal, but not popular road for a medium level of exposure, and we would use a popular city road that is driven by frequently every day for our high exposure. We would change our sites in order to potentially observe a more distinctive impact of car exhaust on the environment. We would also add more proximities to car exhaust that are in between our high, low, and medium exposures. This would allow us to see more of a pattern as the amount of data we collect would increase. In addition, when extracting the soil, we would assure that the soil does not have a clay factor to it in order to make our color test more efficient. Since protozoa and bacteria are closely tied together, it may be necessary and helpful to test protozoa as well. Since protozoa live in water spaces in the soil, it is important that the proximity to plants and trees is far. Plants absorb a large amount of moisture out of the ground which could change our data. Ultimately, changing these variables may help us in finding a definite solution to our problem.

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