Final Project

Soil Ecology Car Exhaust Effect on Soil

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Biology 9H

Soil Ecology Project Procedure

Background

Bacteria are single-celled prokaryotic microorganisms. They can be shaped like spheres, rods, spirals, and many other shapes. Bacteria are found in many different and sometimes extreme habitats, such as hot springs, arctic environments, radioactive waste, deep sea oil seeps, deep earth crystal environments, and hypersaline ponds. In one gram of typical surface soil, there are at least 50 million bacterial colonies (Hogan; Draggan; 2012). Bacteria have four functional groups, which are decomposers, mutualists, lithotrophs, and pathogens (Ingham, 2015). Most are decomposers that break down dead organisms, animal waste, and plant litter to obtain nutrients. Bacillus subtilis and Pseudomonas fluorescens are examples of bacterial decomposers (Reid; Wong; 2005). Decomposers recycle organic waste, and release chemical elements including nitrogen, phosphorous, and carbon, which can be used as structural building blocks to build new plants and animals. Decomposers break down organic matter from polymers into monomers in order to rebuild the biological molecules. Some decomposers can break down pesticides and pollutants in soil (Ingham, 2015). Decomposers are important in immobilizing or retaining nutrients in their cells, as well as preventing loss of nutrients, such as nitrogen, from the rooting zone (Ingham, 2015).

Another group of bacteria are called lithotrophs or chemoautotrophs. These obtain energy from compounds of nitrogen, sulfur, iron, or hydrogen instead of from carbon compounds. Some

of these are important to nitrogen cycling and degradation of pollutants (Ingham, 2015). Lithotrophs perform functions in relation to water dynamics, carry out nutrient cycling, and suppress disease. Some produce substances that help bind soil particles into small aggregates. The aggregates are useful because they improve water infiltration and the soil's ability to hold water. The last group of bacteria is called the mutualists. One kind of mutualist is called nitrogen fixing bacteria (Ingham, 2015). In the part of the nitrogen cycle called ammonification or mineralization, nitrogen fixing bacteria convert organic nitrogen from dead organisms into ammonium (Boundless, 2015). After the ammonification, the next step is nitrification. In nitrification, nitrifying bacteria covert the ammonium into nitrites. Then, the nitrifying bacteria convert the nitrites into nitrates (Boundless, 2015). Nitrogen is important because it is an element in DNA, RNA, and proteins. This is important because DNA converts into RNA, which is needed to make proteins or enzymes. The enzymes are needed to start and stop chemical reactions. The four most important chemical reactions are the four properties of life. The four properties of life are reproduction, homeostasis, synthesis of new material, and transformation of energy. The four properties of life are needed for the cell to function properly. Without nitrogen, cells wouldn't be able to do what they are needed to do.

The soil food web can experience disruption through a variety of environmental impacts. The production of nitrogen oxide is one way that the soil food web can be altered and damaged. Nitrogen oxide is a gas released by car emissions, which is known to be harmful to the microorganisms within the soil as well as the larger environment (King, n.d.). Car exhaust is one of the top contributors global warming or climate change (Union of Concerned Scientists, n.d.). Global warming is caused by an excess of greenhouse gases, which trap the heat given off by the sun within Earth's atmosphere through the absorption of infrared radiation being reflected from

the Earth's surface into space. These greenhouse gases buildup around the Earth's atmosphere increasing the planet's overall climate (National Energy Information Center, 2004.). Car exhaust can contribute to this buildup through the burning of fossil fuels, releasing nitrous oxide and volatile organic compounds, two greenhouse gasses (United States Environmental Protection Agency, n.d.). Greenhouse gases such as these add to the gases that already surround Earth's atmosphere, known as the ozone layer. While the ozone layer originally ensured that the atmosphere was warm enough to support life, the addition of these greenhouse gases are over exposing the earth to radiation which excessively elevates the Earth's temperature (National Energy Information Center, 2004.). This is known as global warming. About 30% of the US's addition to global warming comes from vehicle transportation (Union of Concerned Scientists, n.d.). Car exhaust is also proven to be harmful to plants and the surrounding ecosystem (Melgarejo 2012). An experiment performed by Guadalupe in 2012 tested how long a plant could live while exposed to car exhaust. It was discovered that four of the five plants tested died in three weeks or less, and the one plant that lived had little texture and was much smaller than it was originally (Melgarejo 2012).

Human's production of car exhaust contributes greatly to the effects of car exhaust on the soil. Humans rely on motor vehicles to get from place to place more than other methods of transportation. All motor vehicles release car exhaust which consists of a mixture of toxins, including: carbon monoxide, nitrous oxides, carbon dioxide, soot, sulfur dioxide, benzene, and formaldehyde. Once these chemicals are released into the environment, they lead to the production of acid rain. Acid rain is formed as the gases from car exhaust travel up into the atmosphere and mix with water oxygen and other types of chemicals to form sulfuric and nitric acids. (King, n. d). Acid rain can be measured by the pH levels.

pH measures how acidic or alkaline a substance is. It measures based upon a scale of 0 to 14. For example, a pH of 2 is ten times more acidic than a pH of 3 and 100 times (10 times 10) more acidic than a pH of 4. A pH of 7 is neutral, a pH that is less than 7 is acidic, and a pH greater than a 7 is basic. (United States Environmental Protection Agency. 2012) Acid rain decreases the pH of the water within the soil, which means the microorganisms live in an acidic environment.

A change in pH can cause vegetation problems and reproductive issues for plants. (King. N. d). The pH level determines how the enzyme works within the cell of the organism. Once the pH value goes out of a certain range, the enzyme changes the shape and function, therefore unable to complete a chemical reaction. Since the chemical reactions cannot occur, this means the four properties of life cannot be performed including: reproduction, homeostasis, transformation of energy, and synthesis of new material. Without the four properties of life, the cell will die. Therefore, organisms in the soil including bacteria and producers will be greatly impacted, which will harm the larger ecosystem. It will harm the larger ecosystem because once the producers and bacteria is greatly impacted it triggers everything else in the food web to be greatly impacted. As soon as one things is off in the food chain that is ran by a cell the whole entire ecosystem is harmed.

Our group will extract soil from three plots on our campus. Our plots contain a range of exposure to car exhaust, including: “highly exposed”, “semi-exposed” and “low exposed” soil. To test how bacteria are impacted. We hypothesize that as car exhaust increases, the pH level and bacteria population density will also decrease.

Procedure

I. Problem: How does car exhaust exposure change the pH level and the amount of bacteria in the soil?

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

III. Procedure:

A. Independent variable- The soil with high exposure (close proximity to car exhaust) and semi-exposure(some proximity) to car exhaust

B. Dependent variable- The pH level and bacteria population density in 1 cc of soil

C. Negative Control- The soil with low exposure (furthest proximity) to car exhaust

D. List of Controlled Variables- Where soil is collected, amount of soil collected, amount of time for bacteria to grow on plates, amount of soil tested in the pH test, amount of soil tested in the dilutions, type of nutrient agar plates tested on, amount of water added to culture tube, amount of soil/ water mixture to culture tube, amount of solution added to other culture tubes, amount of solution added to the agar plates, amount of demineralized water (1155) added to soil in pH test, amount of soil flocculating reagent (5643), amount of clear solution added to the large depression on the spot plate (0159), amount of duplex indicator (2221) added to the sample on the spot plate, amount of chosen range indicator added to the sample on the spot plate, color chart that the color reaction is compared against, plot size, plot location, plot environment, time of day and day extracted

from the soil, time of day and day dilution is performed, time of day and day pH test is completed.

E. Step-by- Step Instructions

1. Plot a 20x20 cm area in three locations: low exposed area at N 39° 21.535’ W 076° 38.163’; semi exposed area at N 39° 21.487’ W 076° 38.9162’; and highly exposed area at N 39° 21.470’ W 076° 38.194’ .
2. Using the soil extractor with a 2 cm diameter extract one sample of 15 cm of soil from each plot, “High exposed”, “Semi-Exposed”, “Low Exposure”, respectively and immediately place them in separate Ziploc bags and label them: “High Exposure Sample 1”, “Semi Exposure Sample 1” and “Low Exposure Sample 1”, respectively.
3. Complete the dilution in steps 5-21 for the trial 1 samples from each plot.
4. Complete the pH test, using the LaMotte STH-14 Chemical Test Kit, for all trial 1 samples on the same day at the same time in steps 22-27.
5. Use a clean, new transfer pipette to add 10 ml of sterile water to a 15ml culture tube. Label the tube “10^0 high exposure”.
6. Use the same pipette to add 9 ml of sterile water to a second 15ml culture tube. Label the tube “10^{-1} high exposure”.
7. Repeat step 6 two more times to two additional 15 ml culture tubes, only label them “10^{-2} high exposure,” “10^{-3} high exposure”, and respectively.

8. Place 1cc of your highly exposed soil sample into the "10^0 high exposure" culture tube.
9. Cap the tube and shake vigorously.
10. Using a new clean pipette, remove 1ml of the soil/ water mixture from the "10^0 high exposure" tube and place into the "10^{-1} high exposure" tube.
11. Cap and shake vigorously.
12. Using the same pipette in step 10, remove 1ml of the soil/ water mixture from the "10^{-1} high exposure" tube and place into the "10^{-2} high exposure" tube.
13. Cap and shake vigorously.
14. Using the same pipette in step 12, remove 1ml of the soil/water mixture from the "10^{-2} high exposure" tube and place into the "10^{-3} high exposure" tube.
15. Cap and shake vigorously.
16. You should now have a total of four culture tubes.
17. Plate 100 μl samples from the second and third culture tubes (dilutions 10^{-2} and 10^{-3}) onto their own separate, labeled 3M Petrifilm^{TM} aerobic count plates containing nutrient agar.
18. Allow the bacteria to grow for 48 hours.
19. First examine the 10^{-3} plates and count the bacteria colonies. If there are less than 5 bacteria colonies, look at the 10^{-2} plates, and count

those, to make your estimates of the number of bacteria in the original 1cc soil sample using the following formula:

#Microbes in 1 cc soil = #Colonies on sheet x 10^{2}x10 $^{|\text{dilution \# at which these colonies where found}|}$

20. Record the amount of Bacteria found in 1 cc of soil from step 19 in the data table
21. Repeat the dilution test in steps 5-20 twice for the other trial 1 samples using “semi-exposure” and “low exposure” soil samples respectively.
22. Fill a test tube (0204) approximately one-third full of highly exposed soil. Use the model PWB-1 demineralizer bottle (1155) to add demineralized water to each tube, until it is one half inch from the top. Use the thumb to cap and shake until the soil is well dispersed.
23. Add five drops of soil flocculating reagent to each test tube (5643). Cap and shake to mix. Allow contents to settle before proceeding to step 23.
24. Use a 1ml pipette (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.
25. To the first sample on the spot plate, add two drops of a 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. Chose the narrow range

and indicator and appropriate chart with a mid-point that is as close as possible to the value obtained in the wide ranged test.

26. Add two drops the chosen narrow range 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.
27. Record the pH level found in 1 cc of soil from step 26 in the data table
28. Repeat steps 22-27 twice for the “semi exposed” and “low exposed” trial 1 soil samples respectively.
29. Repeat steps 2-27 for trial 2 of samples from each plot.
30. Repeat steps 2-27 for trial 3 set of samples from each plot.

IV. Data and Analysis

a. Data Tables

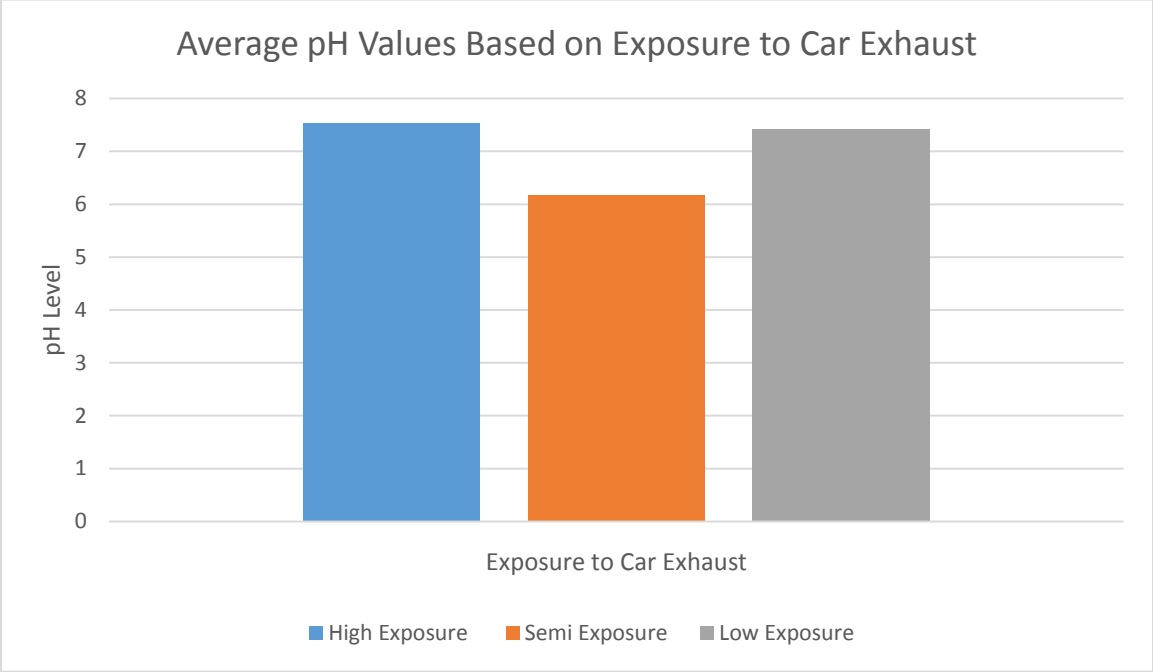
pH Values Based on Exposure to Car Exhaust

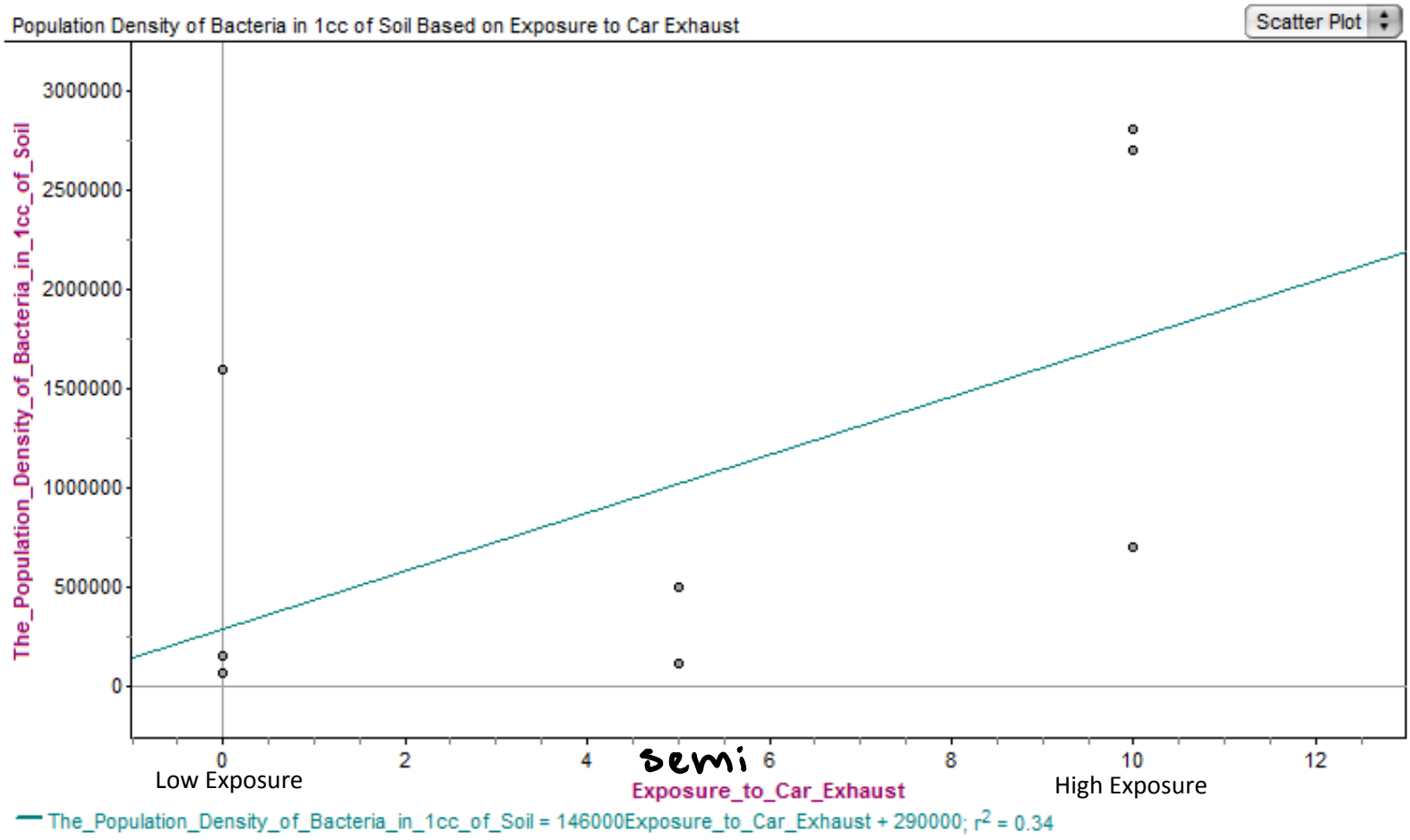
		Trial 1	Trial 2	Trial 3	Average
High Exposure	pH	7.5	7.7	7.4	7.53
Semi Exposure	pH	6.1	6.5	5.9	6.17
Low Exposure	pH	7.4	7.4	7.5	7.43

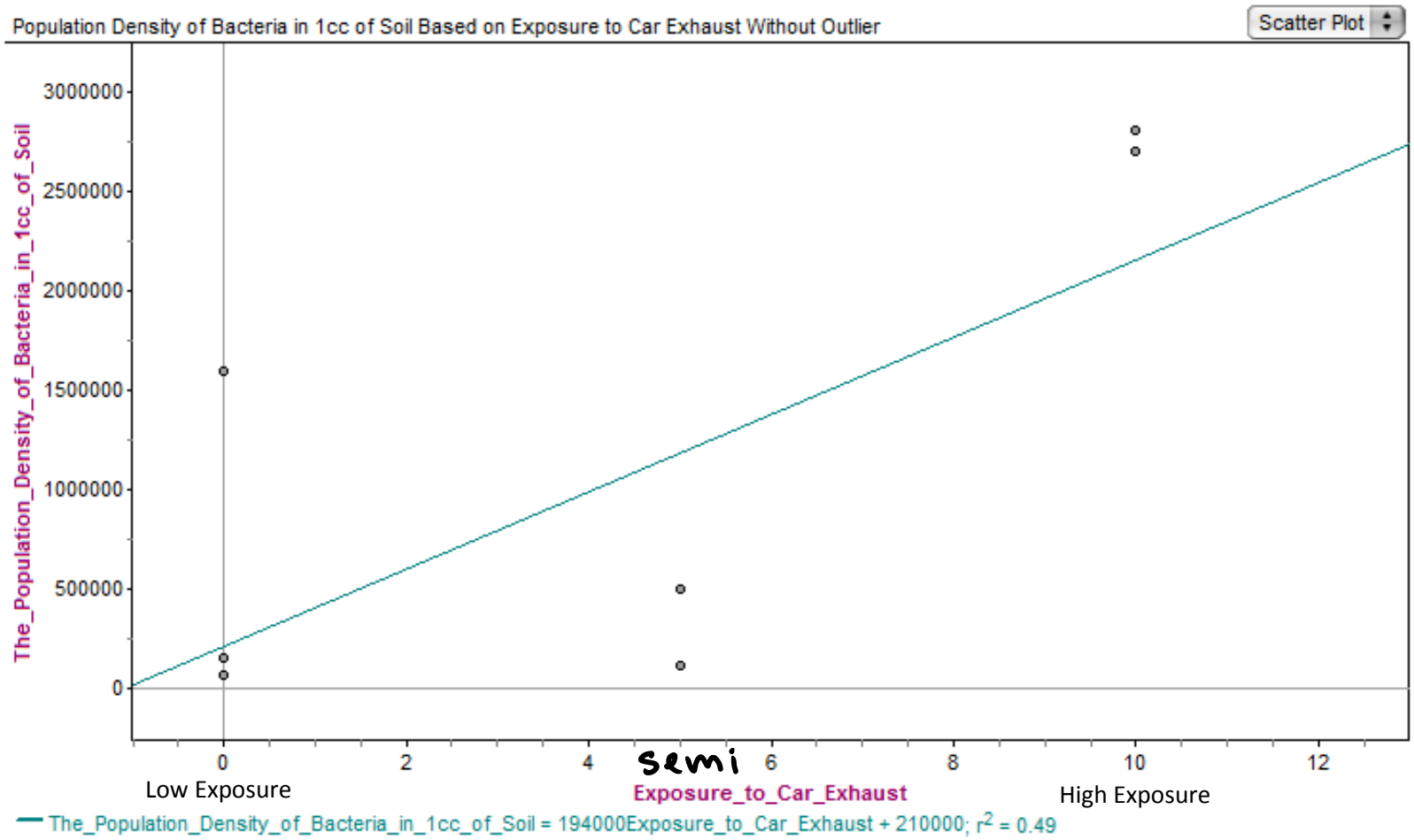
Population Density of Bacteria in 1 cc of Soil Based on Exposure to Car Exhaust

		Trial 1	Trial 2	Trial 3	Average
High Exposure 10	Bacteria Number in 1cc of soil	2,800,000	2,700,000	700,000	2,066,666.67
Semi Exposure 5	Bacteria Number in 1cc of soil	500,000	120,000	500,000	373,333.33
Low Exposure 0	Bacteria Number in 1cc of soil	70,000	160,000	1,600,000	610,000

b. Analysis


Average pH Values Based on Exposure to Car Exhaust
8
7
6
5
4
3
2
1
0
pH Level
Exposure to Car Exhaust
High Exposure
Semi Exposure
Low Exposure


Population Density of Bacteria in 1cc of Soil Based on Exposure to Car Exhaust
Scatter Plot
The_Population_Density_of_Bacteria_in_1cc_of_Soil
3000000
2500000
2000000
1500000
1000000
500000
0
0
2
4
6
8
10
12
Low Exposure
semi
Exposure_to_Car_Exhaust
High Exposure
The_Population_Density_of_Bacteria_in_1cc_of_Soil = 146000Exposure_to_Car_Exhaust + 290000; r2 = 0.34


Population Density of Bacteria in 1cc of Soil Based on Exposure to Car Exhaust Without Outlier
Scatter Plot
The_Population_Density_of_Bacteria_in_1cc_of_Soil
3000000
2500000
2000000
1500000
1000000
500000
0
0
2
4
6
8
10
12
Low Exposure
semi
Exposure_to_Car_Exhaust
High Exposure
The_Population_Density_of_Bacteria_in_1cc_of_Soil = 194000Exposure_to_Car_Exhaust + 210000; r2 = 0.49

V. Conclusion

In this experiment, we tested how exposure to car exhaust changes the pH level and the amount of bacteria in the soil. Our hypothesis was, as exposure to car exhaust increases, the pH level and bacteria population density will decrease. This hypothesis was proven incorrect. The data shows that as the exposure to car exhaust increased, the bacteria amount also increased. The average for the bacteria population in the low exposure soil trials was 610,000. The average for the bacteria population in the semi exposure soil trials was 373,333.33. The average for the bacteria population in the highly exposure soil trials was 2,066,666.67. When the bacteria colony data was put on a line of best fit graph there was an r^2 value of 0.34, which is a very poor correlation. Looking at the graph you could identify an outlier in the high exposure bacteria population density, so we created another line of best fit graph in which we removed this outlier. With this outlier removed, the r^2 value increased to 0.49. This is still a low correlation but is an improvement from the previous graph. Meanwhile, the pH level was about the same for the high and low exposure to car exhaust plots, but the pH level for the semi exposure to car exhaust plot was lower than the others. The average for the pH level in the low exposure soil trials, was 7.43 and the average for the pH level in the high exposure soil trials was 7.53. Meanwhile the average for the pH level in the semi exposure soil trials, was 6.17. These results could be due to the location of the soil plots. The highly exposed plot was placed in an area that was more recently exposed to car exhaust, which could have affected the amount of exposure it received. In the future if we performed this test again we would place this plot in an area that has been exposed to car exhaust for a longer amount of time, which could change the results. The semi exposed plot was in close proximity to many big trees. These trees absorb a lot of the water from the soil,

taking it away, and causing the soil to be more acidic. If we were to do this experiment over again, we would place this plot in an area with less trees around, so that the soil could have a more balanced amount of water. This balanced amount of water could change the amount of pH and bacteria in the soil. In the future would keep the low exposure plot in the same place. It was not in close relation with any big trees or exposure to car exhaust, making it a good location for a plot.

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