The Effects of Fossil Fuel Exhaust on the Population Density of Bacteria Microbes on the RPCS Campus

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Background Information

Billions to hundreds of billions microorganisms live and inhabit any typical, garden soil. The soil provides an environment for several diverse groups of microorganisms, but the most important among them are bacteria, actinomycetes, fungi, algae and protozoa. Bacteria is neither a plant nor animal and instead it is a prokaryotic microorganism. Bacteria are tiny single-cell microorganisms that do not have a nucleus and lacks other membrane bound organelles. They are very diverse organisms that inhabit different types of environments including areas of high heat and extreme cold (Dr. Wassenaar, 2011). Two types of important bacteria that live in the soil are heterotrophic and autotrophic bacteria. Heterotrophic bacteria requires organic carbon sources in order to grow and function. Heterotrophic bacteria are important because they enhance the fertility of the soil by nitrogen fixation, which is when the nitrogen in the atmosphere is absorbed into organic compounds for growth purposes. Autotrophic bacteria uses carbon directly from the atmosphere to synthesize organic compounds by using light energy to create its own food in order to live . (Life Easy Biology, 2013).

Microorganisms in the soil break down plant and animal residue and convert these materials into plant nutrients, which help to improve the soils heath. (Dr. Elaine Ingham, 2013). This process is known as decomposition and these microorganisms convert these waste materials into organic and inorganic compounds. As a part of this process bacteria produce humic acids. In the soil, these acids chemically combine with one another to form large molecules of stabilized organic matter. Organic matter is used to improve the soil by stimulating life in the soil and providing nutrients to bacteria, fungi, earthworms, and other organisms (Mazza, 2010). This formation of large molecules is known as both a biological and a chemical process. Lastly, bacteria and fungi are responsible for performing the last type of transformation which is immobilization. Immobilization is the conversion of inorganic compounds into organic compounds (University of Minnesota, 2000).

In addition, bacteria benefits plants by providing essential substances to the plant roots. The transformation of nitrogen that is performed by bacteria in the soil is a very important part of the nitrogen cycle. The roots of legumes host nitrogen-fixing bacteria that convert vast amounts of dinitrogen (N_2) from the atmosphere into forms that plants can use. Ammonia (NH_3) is converted into nitrate (NO_3+) by nitrifying bacteria. Nitrification is a vital part of the nitrogen cycle because certain bacteria are able to transform nitrogen (produced by the decomposition of proteins) into nitrates which is essential for plant growth (University of Minnesota, 2000).

Nitrogen is important because nitrogen is major elemental component of DNA, RNA and amino acids. Nitrogenous bases of DNA make up RNA and amino acids which are used to construct enzymes. Enzymes start and stop chemical reactions which are necessary to perform the transformation of energy, homeostasis, reproduction, and synthesis of new material which are the four main tasks of life. A cell must be able to perform these tasks in order to function. Cells make up plants, if they do not function properly than the plants will not live. Plants are important to the ecosystem because they are producers. Producers are responsible for giving necessary elements to the consumers in order for the consumers to survive. (Riina, C. 2013) If there is a greater population of bacteria in the soil, then there will be a greater amount of nutrients available to sustain the health and life of plants (University of Minnesota, 2000).

Car pollution fossil fuel exhaust has negatively affected the nitrogen cycle due to release of excess nitrous oxide. Car pollution is released into the atmosphere when humans drive motorized vehicles, which use fossil fuel as power. By burning fossil fuels, it releases sulfur oxides, nitrous oxides, and other byproducts into the atmosphere. When combined with water, this forms acid rain and enhances the greenhouse gases in the atmosphere. Most notably, this is an excess of carbon, which negatively increases the amount of carbon in the atmosphere. In addition, the fossil fuels release dangerous nitrous oxide (NO_x) into the air, which the plants cannot use to make into helpful nitrates (Canyon Crest Academy, 2006). The emitted NOx is an important air pollutant, because it is critical in the photochemical oxidative reactions by which toxic ozone is formed, and because the NOx is an important source of nitrate in acidic precipitation (Ecological Society of America, 1990). Air pollutants that are released from cars can be deposited in the soil which some organisms use as a food source. These substances can affect the reproductive, respiratory and immune systems of animals, when consumed.

Some pollutants, like nitrogen oxides and sulfur oxides, are major contributors to acid rain, which changes the pH, or acidity, of waterways in the soil and can harm any organisms that rely on these resources. Acid rain’s downpour of acid is what increased the percentage of acid in bodies of water. pH affects the growth and function of bacteria because it changes the functioning of the enzymes within the bacteria, because enzymes can only work properly in a particular range of pH that is not so prominently acidic. This means the bacteria will not be able to perform chemical reactions properly, since enzymes are responsible for starting and stopping chemical reactions (Amy, W. 2012). If bacteria are not able to perform chemical reactions, than they cannot perform the four tasks of life, which means they will not be able to provide nitrates to the ecosystem. Once bacteria die, they cannot provide nitrates to the plants, which could potentially cause the ecosystem to collapse because the bacteria decomposers support the producers, 1st degree consumers, and all other members of an ecosystem. (Riina, C. 2013) . When there is too much nitrogen, the bacteria cannot turn it all into nitrates and the plants will die from the excess nitrogen. Plants need to live as a food source for humans, as well as a producer of oxygen for all living beings. People need oxygen in order to breathe, and it keeps their hearts beating and bodily systems in check. Additionally, people need food for energy, and plants provide the majority of nutrients necessary for a human to thrive (Leonard, 2009).

To conclude, we hypothesize that there should be a greater density of bacteria in areas closer to a fossil fuel source because the plants will need more bacteria to match the greater amount of nitrates. The more bacteria there are in the soil, the more nitrogen there is for the plants. Hopefully, mutated bacteria will have developed closer to the fossil fuel source in order to combat the effects of fossil fuels. Natural selection will have created a stronger new breed of bacteria to fuel the soil, and these mutated bacteria will have reproduced significantly, causing to increased populations. There must be more bacteria to help process the excess chemicals from the fossil fuels, and so the soil must have adapted to include these bacteria.

The Effects of Fossil Fuel Exhaust on the Population Density of Bacteria Microbes on the RPCS Campus

Question: Does the proximity to fossil fuel exhaust from the Roland Park carpool increase or decrease the population density of bacteria microbes in the soil on the RPCS campus?

Hypothesis: The population density of bacteria microbes will be highest in areas of RPCS that have the closest proximity to fossil fuel exhaust.

Variables

i. Independent variable: The proximity of the Island and Front Lawn locations to the fossil fuel exhaust source (carpool lane)

ii. Dependent variable: The population density of bacteria microbes in the soil sample

iii. Controlled variables: Variety and amount of fertilizer used on soil samples, time of day/day soil samples were collected, depth of soil sample from the ground, amount of soil tested, culture tube size, amount of time allowed for bacteria to grow, size of petri film, amount of soil in culture tubes, amount of sterile water in culture tubes, amount of soil/water mixture put onto petri plates, variety of nutrient agar, number of dilutions performed per sample, which dilutions are plated, amount of exhaust exposed to for each trial of a location

iv. Negative control: The soil plots farthest away from the exhaust source; lack of fossil fuel exhaust in the "Courtyard" location

Step-By-Step Instructions

1) Collect the samples detailed in steps 2-7 at the same time on the same day in order to ensure the maximum control of the environment. Trial 1 from every site must be

collected on the same day and time, and the same applies to Trials 2 and 3. A diagram of the sample locations can be viewed in Diagram I.

2) Using a soil core that is 2 centimeters in diameter with a depth of 15 centimeters, collect 3 soil samples from Location 1 at exactly N39’21.489° W76’38.135°, on the “Island” of the RPCS campus (as shown in Diagram I). Each soil sample should be exactly 10 centimeters away from one-another, to the right of one-another, and 15 centimeters deep. This is "Maximum Exposure to Fossil Fuels".
3) Put the three trials of collected soil samples into three separate, sterile ziploc plastic bags labeled "Maximum Exposure to Fossil Fuels" samples 1, 2, and 3 respectively.
4) Using the same soil core, repeat step 2 at Location 2 at exactly N39’21.482° W76’38.181°, on the Front Lawn of the RPCS campus (as shown in Diagram I). This is "Increased Exposure to Fossil Fuels".
5) Put the three collected soil samples into three separate, sterile plastic bags labeled "Increased Exposure to Fossil Fuels" samples 1, 2, and 3 respectively.
6) Using the same soil core, repeat step 2 at Location 3 at exactly N39’21.470° W76’38.197° at the Courtyard of the RPCS campus (as shown in Diagram I). This is "Minimum Exposure to Fossil Fuels".
7) Put the three collected soil samples into three separate, sterile plastic bags labeled "Minimum Exposure to Fossil Fuels" samples 1, 2, and 3 respectively.
8) Transport the samples into the lab environment and complete each dilution process (detailed in steps 9 through 23) simultaneously for the 3 respective samples from Maximum, Increased, and Minimum Exposure to Fossil Fuels. Trial 1 from every site

must be diluted on the same day at the same time. This same rule applies to Trials 2 and 3 respectively.

9) Use a clean, new transfer pipette to add 10 ml sterile water to a 15 ml culture tube. Label the tube “Courtyard 1 10^{0}.”
10) Use the same pipette to add 9 ml of sterile water to a second 15 ml culture tube. Label the tube “Courtyard 1 10^{-1}.”
11) Repeat step 10 two more times for two additional 15 ml culture tubes, only label them “Courtyard 1 10^{-2}” and “Courtyard 1 10^{-3}”, respectively.
12) Place 1cc of soil sample from the Courtyard Sample 1 in the 10^{0} culture tube.
13) Cap and shake vigorously.
14) Using a clean new serological pipette, remove 1 ml of the soil/water mixture from the 10^{0} tube and place into the 10^{-1} tube.
15) Cap and shake vigorously.
16) Using the same pipette as in step 14, remove 1 ml of the soil/water mixture from the 10^{-1} tube and place into the 10^{-2} tube.
17) Cap and shake vigorously.
18) Using the same pipette in step 14, remove 1 ml of the soil/water mixture from the 10^{-2} tube and place into the 10^{-3} tube.
19) Cap and shake vigorously.
20) Repeat steps 9-19 with the samples from Front Lawn 1 and Island 1, placing each sample in their respectively labeled culture tubes.
21) Plate 100 µl samples from the 3rd and 4th tubes (dilutions 10^{-2} and 10^{-3}) of each of the three locations (Front Lawn 1, Island 1, Courtyard 1) onto their own separate, labeled (by

site, sample, and dilution number) 3M Petrifilm™ aerobic count plates containing nutrient agar. Use a spreader to flatten sample.

22) Allow plates to grow for exactly 48 hours.

23) Examine the Courtyard 1 10^{-3} petrifilm plate for individual bacteria colonies and, if it has at least 5 colonies, use it make your estimates of the number of bacteria in the original 1 cc soil sample using the following formula:

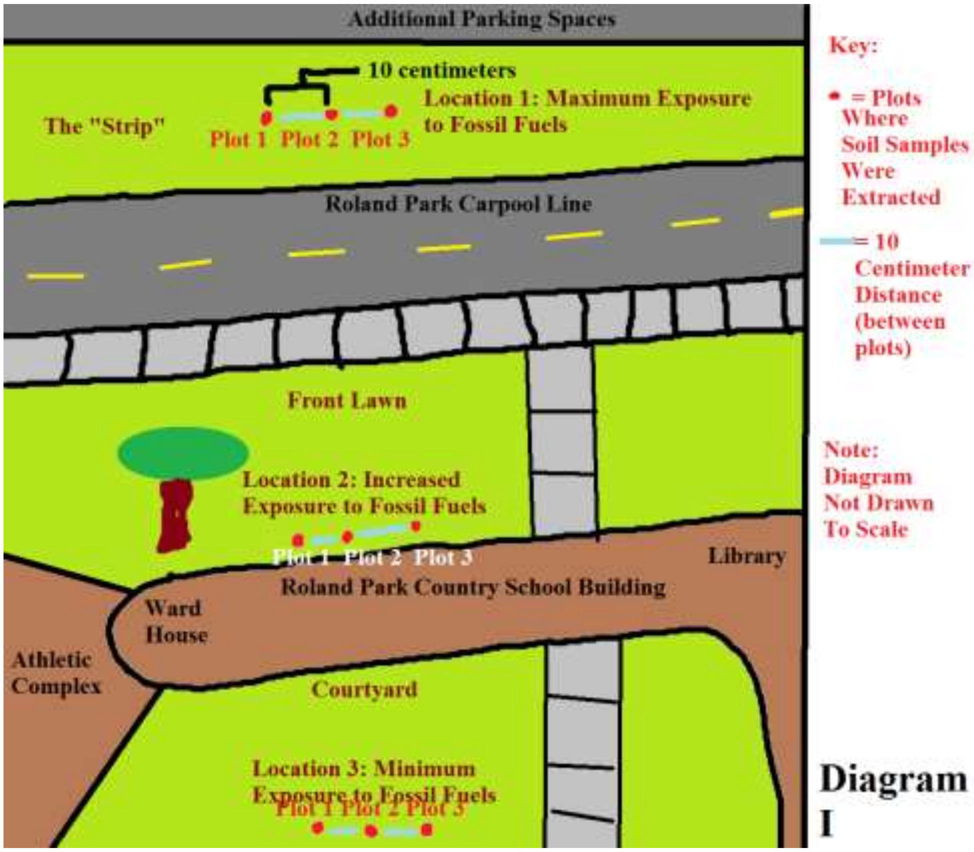
    a. # Microbes in 1 cc of soil = # Colonies on sheet x 10^2 x $10^{|\text{dilution \# at which these colonies were found}|}$

However, if it has less than 5 colonies, count the number of bacteria on the Courtyard 1 10^{-2} plate.

24) Repeat step 23 for the Front Lawn 1 and Island 1 plates, respectively.

25) Repeat steps 9-24 for trials 2 and 3 of the Courtyard, Island, and Front Lawn locations.

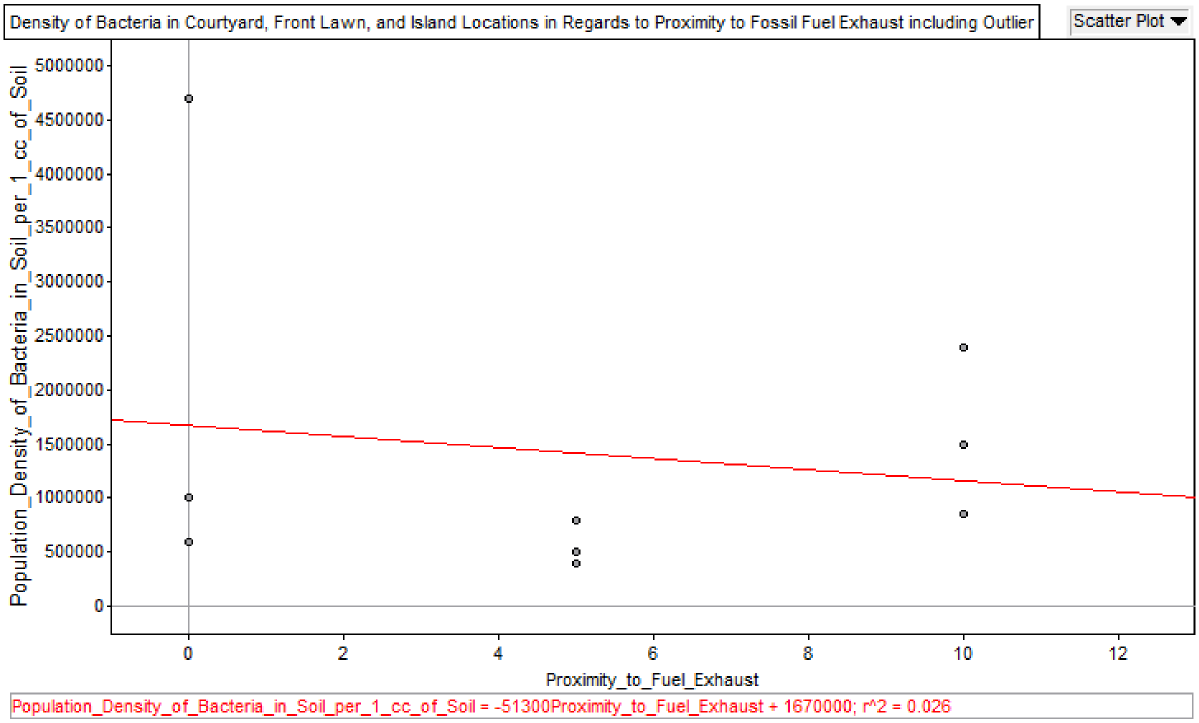
DIAGRAM I

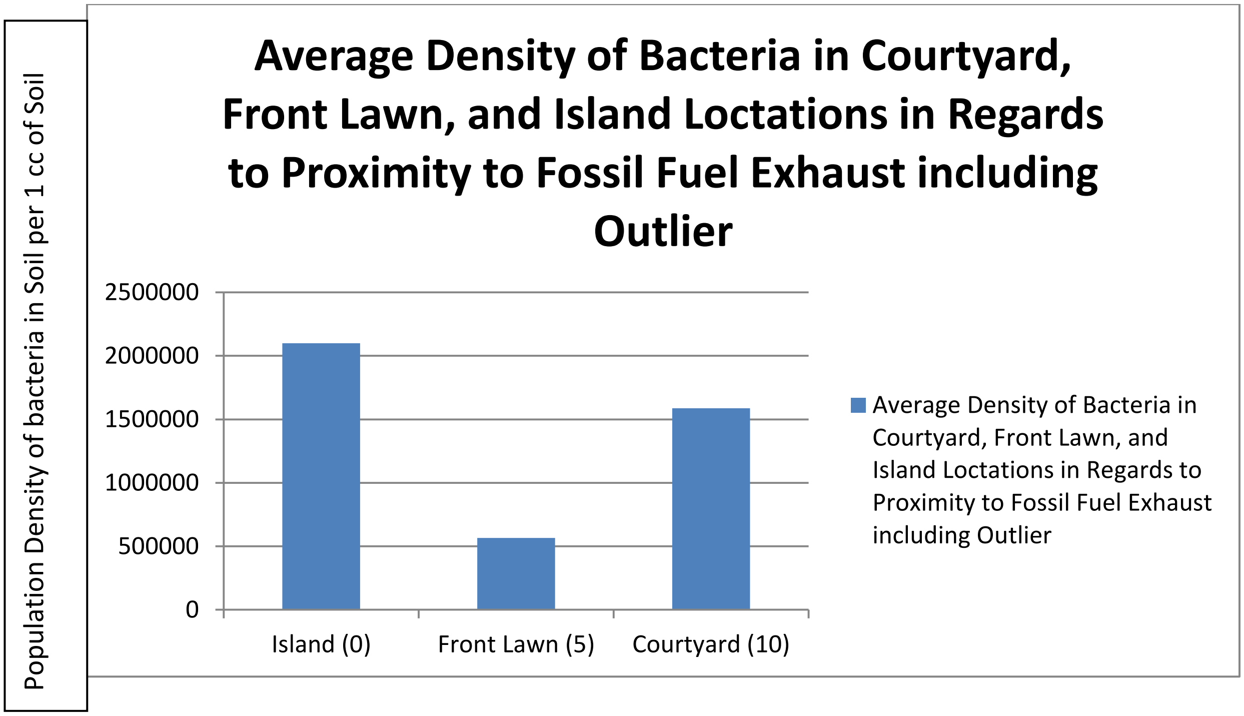


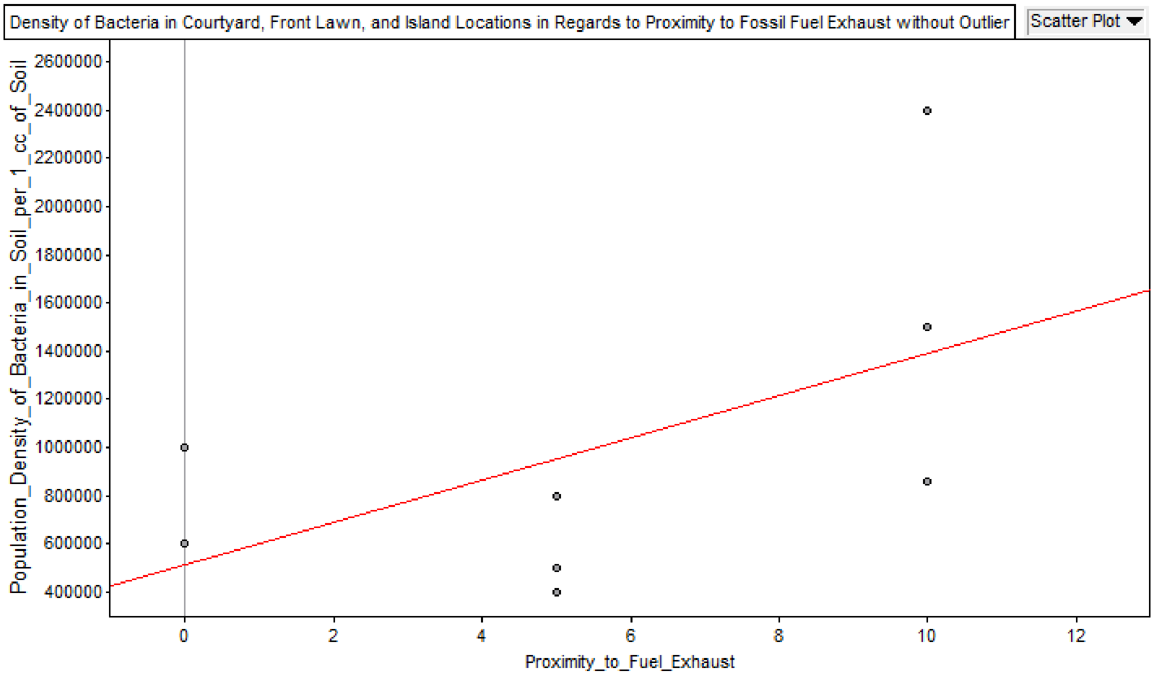
Density of Bacteria in Courtyard, Front Lawn, and Island Locations in Regards to Fossil Fuel Exposure

Soil Sample	Dilution Number	# Bacteria on Plate	# Bacteria in 1cc of Soil	Average # of Bacteria in 1 cc of Soil
Courtyard 1	10^{-2}	86	860,000	1,586,667
Courtyard 2	10^{-3}	15	1,500,000	
Courtyard 3	10^{-3}	24	2,400,000	
Front Lawn 1	10^{-3}	8	800,000	566,667
Front Lawn 2	10^{-3}	5	500,000	
Front Lawn 3	10^{-2}	40	400,000	
Island 1	10^{-3}	6	600,000	2,100,000
Island 2	10^{-3}	47	4,700,000	
Island 3	10^{-3}	10	1,000,000	

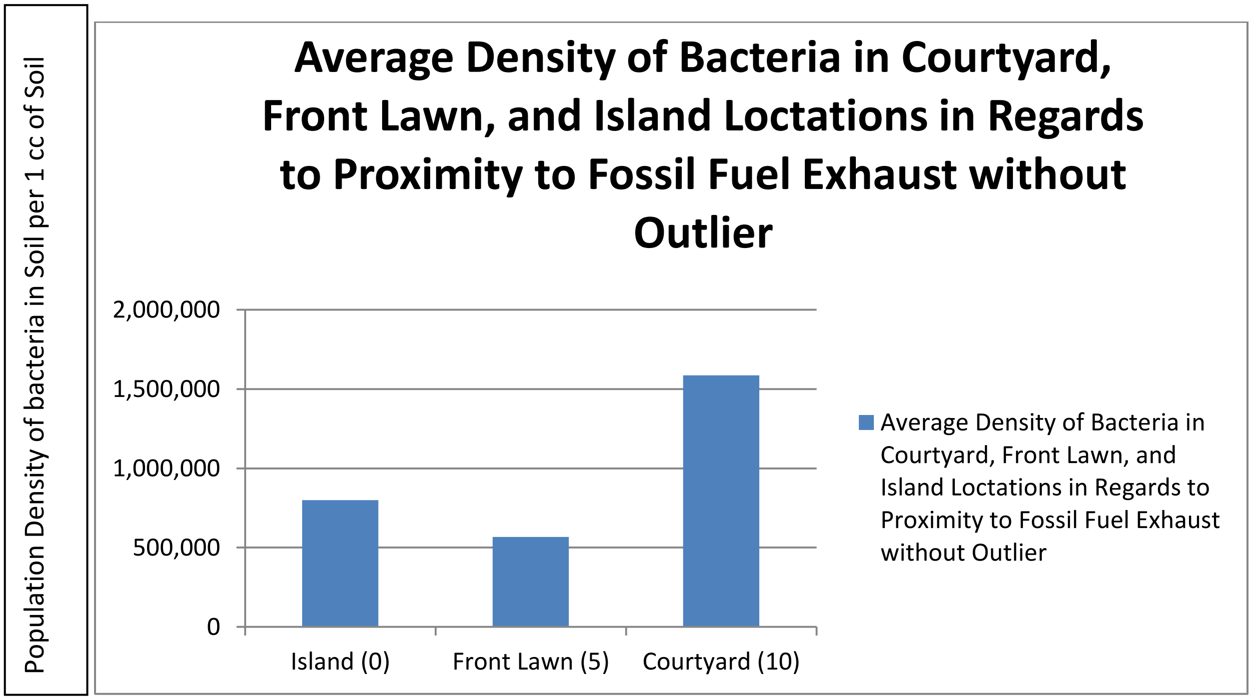
Graphs


Density of Bacteria in Courtyard, Front Lawn, and Island Locations in Regards to Proximity to Fossil Fuel Exhaust including Outlier
Scatter Plot
Population_Density_of_Bacteria_in_Soil_per_1_cc_of_Soil
5000000
4500000
4000000
3500000
3000000
2500000
2000000
1500000
1000000
500000
0
0
2
4
6
8
10
12
Proximity_to_Fuel_Exhaust
Population_Density_of_Bacteria_in_Soil_per_1_cc_of_Soil = -51300Proximity_to_Fuel_Exhaust + 1670000; r^2 = 0.026


Average Density of Bacteria in Courtyard, Front Lawn, and Island Loctations in Regards to Proximity to Fossil Fuel Exhaust including Outlier
Population Density of bacteria in Soil per 1 cc of Soil
2500000
2000000
1500000
1000000
500000
0
Island (0)
Front Lawn (5)
Courtyard (10)
Average Density of Bacteria in Courtyard, Front Lawn, and Island Loctations in Regards to Proximity to Fossil Fuel Exhaust including Outlier



Population_Density_of_Bacteria_in_Soil_per_1_cc_of_Soil = 88300Proximity_to_Fuel_Exhaust + 510000; r^2 = 0.31



Conclusion to Population Density of Bacteria

In the hypothesis of our experiment, we stated how the population density of bacteria microbes will be highest in the areas of RPCS that have a closer proximity to fossil fuel exhaust in contrast to areas that are further from the source. Our experiment refuted our hypothesis, as we discovered that the density of bacteria was highest in the location furthest from the fossil fuel exhaust, when not counting the major outlier in our data. This idea is validated by the concept that the healthiest soil is most likely the furthest from a pollutant fossil fuel source, and therefore has the most bacteria. To find our data, we gathered 3 trials of soil from 3 locations on the RPCS campus: the island, the front lawn, and the courtyard. The island was closest to the fossil fuel exhaust, as it was between the carpool and a parking lot on the RPCS campus. The front lawn was right in front of the school, a few dozen meters away from the carpool line. The courtyard, however, was completely secluded from the fuel exhaust in the carpool line. It was the furthest away from fossil fuel exhaust on campus.

In the courtyard location, our three respective trials measured 860,000, 1,500,000, and 2,400,000 bacteria per 1 cc of soil. We measured this through our dilutions. The average of amount of bacteria for the courtyard was 1,586,667. In the front lawn location, our three respective trials measured 800,000, 500,000, and 400,000 bacteria per 1 cc of soil. The average of this location was 566,667 bacteria per 1 cc of soil, which was less than the courtyard, showing that the courtyard had more bacteria. However, our island location did not follow this trend set by the courtyard and front lawn. Whereas the amount of bacteria increased with the amount of proximity from the courtyard to the front lawn, the island had the most bacteria of all. Our three trials from the island had 600,000, 1,000,000, and 4,700,000 bacteria per 1 cc of soil, respectively. This averaged to 2,100,000 bacteria per 1 cc soil. 4,700,000 (Island 3) revealed itself to be a true outlier, as it was 2,500,000 bacteria greater than our other greatest piece of data (Courtyard Trial 3) and 3,700,000 bacteria greater than the greatest piece of island data (Island 2). This outlier skewed our lines of best fit to reveal that the bacteria density decreased with proximity to the fossil fuel exhaust, forcing us to think critically of our data. We resolved to also take into consideration the average of the island data without the outlier, which was merely 800,000 bacteria per 1 cc of soil. This made much more sense with our experiment, and so we heightened its importance in contrast to the outlier. This outlier may have come from a contaminated test tube or a recently deceased bug in that soil, which may have increased the bacteria as it decomposed.

Disregarding the outlier, we were able to conclude that bacteria density in soil does indeed increase with proximity away from a fossil fuel source, as the average courtyard bacteria density was 1,586,667, the average front lawn density was 566,667, and the average island density without outlier was 800,000. When given a line of best fit, there is a clear correlation between the numerical data and distance from the fossil fuel exhaust in the carpool. Soil bacteria are part of the foundation to every ecosystem and are responsible for supporting all of the producers and consumers within the ecosystem because they decompose organic materials for producers to use. Then the cycle of consumers consuming the producers and other consumers is able to take place only because of this soil. The more bacteria there are within the soil, the more nutrients that are available to support and sustain plants so they are able to survive within the ecosystem. Bacteria also supply the majority of fixed nitrogen to the environment. Bacteria are able to make nitrogen within the air usable to plants. Bacteria convert ammonium into plant-usable nitrates, which otherwise would have been useless and possibly harmful to the plants, and are necessary to the environment to allow humans to breathe healthy air and for plants to grow.

The repercussions of fossil fuel deprive the environment of its necessary nutrients from bacteria in the soil. The lack of health in the soil causes bacteria to die from the fossil fuels, whereas soil not as close to fossil fuels can thrive. Humans should consider carpooling or biking in order to reduce their fossil fuel footprint and help preserve the population of bacteria in the soil.

Should we choose to ever redo our experiment, there are a few changes we would make. A fault in our experiment was the idea that the car exhaust that exposed the island to fossil fuels did not provide a dangerous amount of excess nitrogen to the soil. A more profound set of data might have come from beside a busy highway or with controlled exposure to the tailpipe of a fuel source. Also, we could have had taken a larger amount of soil samples from different locations that were more dispersed to get better data that showed a correlation between the location and the amount of exposure the soil had to fossil fuel exhaust. The outlier in our data proved to throw off our correlations, and so we would want to prevent it in further samples by increasing our amount of samples per location. Within one of our island samples, there was an outlier that made the average amount of bacteria much greater than the other locations. This could have happened because of a recently deceased organism, such as a bug, in that sample. So because it had to be decomposed, in that sample there was an unusually increased amount of bacteria. This brings forth the idea of why bacteria were supported more in some locations compared to other. Without the outlier, which was the large amount of bacteria in one of the island samples, there was almost a direct correlation between the amount of bacteria and the proximity to fuel exhaust. So because the courtyard and front lawn were not very exposed to fuel exhaust, they were more able to support bacteria within their environment compared to the island. However, that one spike in bacteria for one of the island samples threw off our data.

In conclusion, in this experiment, our data refuted our hypothesis that the population density of bacteria would be highest in those areas of RPCS that have the closest proximity to fossil fuel exhaust. We were able to suggest the opposite, that the population density of bacteria was highest in those areas of RPCS furthest from the fossil fuel source. This is supported by the idea that fossil fuel provides the soil with hostile conditions, brought upon by the nitrous oxide. These hostile conditions stall bacteria from growing and set them into a dormant stage, which will prevent them from reproducing. Bacteria with pro-adaptive mutations might compete better in these hostile conditions, and so this lesser population of mutated bacteria would be the set closer to the fossil fuel. In further experiments, it would be suggestive to research the different varieties of bacteria in each location, in regards to traits. Mutated bacteria, through natural selection, might become the dominant species due to the fossil fuels that the modern age is rooted in. It would also be interesting to research whether the lack of bacteria in the fossil-fuel contaminated soil affected humans significantly, by measuring trends in city parks versus open country. Perhaps these trends might predict the future of human dependence on fossil fuels in regards to their equal dependence on clean air.

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