Soil Ecology Project

The Effect of Road Salt on Bacteria Density

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Biology 9 Honors

5/31/2012

A. Background Report

Soil is made out of four basic components: air, water, organic matter and mineral particles (Pidwirny, 2010). It covers most of the land on Earth and has many different types, each with its own characteristics that determine what kinds of plants grow in it (Enchanted Learning, 2010). Soil is vital to the survival of many species on the planet, but all life in it depends on its "smallest residents" (Discovery Education, 2012), the microbes.

One of these microbes, bacteria, "power the entire ecosystem" (Discovery Education, 2012). There are millions of different types of them in the soil, and they perform many different jobs, such as increasing the solubility of nutrients, improving soil structure, fighting root diseases, and detoxifying harmful substances (BioFlora, 2012). While most bacteria are decomposers "recycling naturally occurring organic compounds and even human ones such as pesticides"(Nardi, 2003), others provide essential nutrients for plants, and together these two different groups of bacteria control the amount of organic matter in the soil, as well as the levels of CO_2 released from the soil into the air. As a consequence, they transform most of the inorganic matter containing important elements into compounds that are ultimately accessible to all of the organisms in an ecosystem (Nardi, 2003).

One specific way bacteria do this is through the nitrogen cycle. Nitrogen is found in all living things, but plants can only access it in the form of ammonium and nitrates. Through the nitrogen cycle, one type of bacteria in the soil first converts the nitrogen gas from the atmosphere into ammonium through nitrogen fixation, and then another type of bacteria converts the ammonium into nitrates in the process of nitrification. Ultimately, denitrifying bacteria will return the nitrogen into the atmosphere and the cycle will repeat, but in the meantime the various plants in the ecosystem absorb the ammonium and nitrates through their roots and transform them into amino acids and nucleotides, the building blocks for proteins and nucleic acids

(Campbell, Williamson, Heyden, 2004).These biological molecules are critical to plants because they enable plant cells to perform their four tasks of life. The consumers in an ecosystem then eat the producers, and receive their nitrogen from the amino acids and nucleotides found in the plants. Hence, the biggest benefit bacteria provide to the soil and the larger ecosystem is to provide the vital nutrients plants and other consumers need to survive. Without bacteria in the soil, an ecosystem will simply collapse.

Harming the balance of bacteria in the soil, then, impacts everything that lives in an environment, and one potential source of such harm is road salt. Composed mainly of sodium chloride and calcium chloride, it is used primarily by humans as a method to melt ice during the winter time, and though road salt might seem harmless at first, it can have a major effect on the microbes in the soil (Sohn, 2009). A high concentration of salt in the soil will cause the diffusion of water out of any cells living there since there is now a hypertonic environment surrounding the cell, and because water is the critical environment in which a cell's chemical reactions occur, the loss of it prevents a cell from performing its chemical reactions, causing it to die (Science in the Real World, 1999). Therefore, any road salt entering into the soil has the potential to kill the bacteria living there, with all the consequent harm that could result. Furthermore, in some cases excess salt might actually diffuse into a cell, causing a disruption in the electrochemical gradient which the bacteria use to "sense" their environment, making the bacteria unable to defend themselves and more vulnerable to being eaten (Campbell, Reece, 2005). Therefore because salt can be so harmful to the bacteria living in the soil and because the bacteria play such a central role in the movement of nutrients there, salt has the potential to disrupt the balance of the entire ecosystem.

To see how the use of road salt impacts the environment here at RPCS, we decided to experiment by adding sodium and calcium chloride solutions of different concentration to the soil and observe what happens to the bacteria population. Our hypothesis is that the bacteria population will decrease more and more as the concentration of the solution increases. Although you cannot get rid of salt, investigating what effect road salt has on microbes, the basis of our ecosystem, can help improve our environment.

B. Experiment

I. Problem:

Does a higher concentration of rock salt exposure decrease the population density of the soil bacteria?

II. Hypothesis:

Yes, a higher concentration of rock salt exposure will decrease the population density of bacteria in the soil.

III. Procedure:

A. Independent Variable:

Concentration of rock salt in the solution applied to the soil

B. Dependent Variable:

Population density of the soil bacteria

C. Negative Control:

Water added to the soil instead of rock salt solution

D. Controlled Variables:

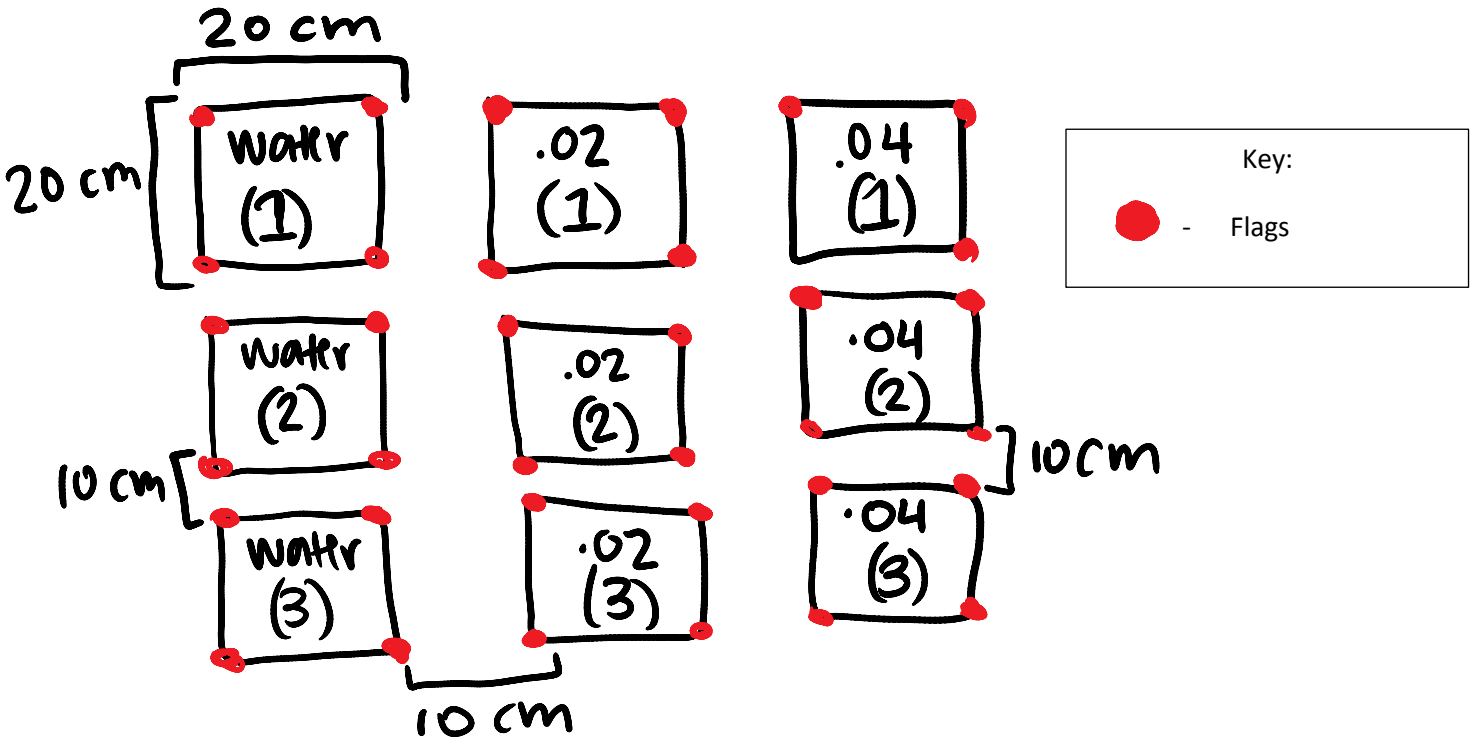
Amount of soil tested, area where soil is taken from, time waiting for solution to sink into soil, microorganism counted for (bacteria), type of solution used, type of tools used, size of land plots,

same process used to count number of soil bacteria (i.e.: type of water used in the dilution process [sterile]), amount of dilution placed on the petri plates, where the water in the solutions is from, how the solution is distributed in the soil, amount of solution added to the soil, time of day and day soil is taken from the ground, depth in the soil sample, length and width of soil samples, number of samples taken before and after the solution is added to the soil, number of land plots, number of land plots for each concentration, amount of soil diluted, degree to which diluted, type of nutrient agar, how long bacteria grew.

E. Step-By-Step Procedure

Step 1: Find a flat area of grass 80cm X 80cm at the coordinates 39.35834° north and 76.63610° west, located on the RPCS front lawn about 13 meters from the front entrance.

Step 2: Separate the area into 9 plots of land each 20 cm X 20 cm, with 10 cm between each plot (see diagram).



Step 3: Label 4 flags as water (1) and put them on the corners of the plot where the first plot with just water added will be (see diagram in step 2).

Step 4: label 4 flags water (2) and put them on the corners of the plot where the second plot with just water added will be (see diagram in step 2).

Step 5: Label 4 flags water (3) and put them on the corners of the plot where the second plot with just water added will be (see diagram in step 2).

Step 6: Label 4 flag .02 (1) and put them on the corners of the plot where the first plot in which the solution with a concentration of .02% will be added.

Step 7: Label 4 flags .02 (2) and put them on the corners of the plot where the second plot in which the solution with a concentration of .02% will be added.

Step 8: Repeat step 8 but label the flags .02 (3) and put them on the corner of the plot where the third plot in which the solution with a concentration .02% will be added.

Step 9: Repeat steps 7-8 but instead of labeling the flags .02 (1), .02 (2), and .02 (3), label the flags .04 (1), .04 (2), and .04 (3) and place them where the first, second, and third land plots in which .04% concentration will be added.

Step 10: Label 9 plastic bags as you labeled your flags (this is where your soil samples will go).

Step 11: Take one soil sample from each plot 15cm in length and 2cm in diameter on the same day and at the same time of day. Place each soil sample from each separate land plot into its separate corresponding and labeled bag, based on the flag labels.

Step 12: Acquire the materials necessary for a serial dilution; make sure you do the serial dilutions on each soil sample for all the samples at the same time.

Step 13: Use a clean, new transfer pipette to add 10 ml to a 15 ml culture tube. Label the tube "10^0 Water (1)".

Step 14: Use the same pipette to add 9 ml to a second 15 ml culture tube. Label the tube "10^{-1} water (1)".

Step 15: Repeat step 14 one more time to one additional 15ml culture tube, only label it "10^{-2} water (1)".

Step 16: Place 1 cc of soil sample "water (1)" into the "10^0 water (1)" culture tube.

Step 17: Cap the tube and shake vigorously.

Step 18: Using a new clean pipette, remove 1 ml of the soil/water mixture from the "10^0 water (1)" tube and place into the "10^{-1} water (1)" tube.

Step 19: Cap and shake vigorously.

Step 20: Using the same pipette in step 18, remove 1 ml of the soil/water mixture from the "10^{-1} water (1)" tube and place into the "10^{-2} water (1)" tube.

Step 21: Cap and shake vigorously.

Step 22: You should now have a total of three culture tubes.

Step 23: Plate 100 µl samples from the 2nd and 3rd tubes, dilutions 10^{-1} water (1) & 10^{-2} water (1) onto their own separate, correspondingly labeled petri plates containing nutrient agar.

Step 24: Repeat steps 13-27, but instead of using the sample from the water (1) plot, use the sample from water (2). Also, label your tubes water (2) (ex: 10^0 water (2); 10^{-1} water (2)).

Step 25: Repeat steps 13-25, but instead of using the sample from the water (1) plot, use the sample from water (3). Also label your tubes water (3) (ex: 10^0 water (3); 10^{-1} water (3)).

Step 26: Repeat steps 13-25, but instead of using the sample from the water (1) plot, use the sample from the .02 (1) plot. Also label your tubes .02 (1) (ex: 10^0 .02 (1)).

Step 27: Repeat steps 13-25, but instead of using the sample from the water (1) plot, use the sample from the .02 (2) plot. Also label your tubes .02 (2) (ex: 10^0 .02 (2)).

Step 28: Repeat steps 13-25, but instead of using the sample from the water (1) plot, use the sample from the .02 (3) plot. Also label your tubes .02 (3) (ex: 10^0 .02 (3)).

Step 29: Repeat steps 13-25, but instead of using the sample from the water (1) plot, use the sample from the .04 (1) plot. Also label your tubes .04 (1) (ex: 10^0 .04(1)).

Step 30: Repeat steps 13-25, but instead of using the sample from the water (1) plot, use the sample from the .04 (2) plot. Also label your tubes .04 (2) (ex: 10^0 .04(2)).

Step 31: Repeat steps 13-25, but instead of using the sample from the water (1) plot, use the sample from the .04 (3) plot. Also label you tubes .04 (3) (ex: 10^0 .04(3)).

Step 32: Allow your samples to grow for 48 hours.

Step 33: 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 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 $10^{0\ |\ \text{dilution \# at which these colonies were found}|}$.

Step 34: Record all of the population observations in the data table.

Step 35: Fill two 1000mL bottles with tap water. Label one .02% solution and the other .04% solution.

Step 36: Measure one gram of sodium chloride using a balance, and pour it into the bottle labeled .02% solution.

Step 37: Measure one gram of calcium chloride using a balance, and pour it into the bottle labeled .02% solution.

Step 38: Vigorously shake the bottle until the sodium and calcium chloride are dissolved.

Step 39: Measure two grams of sodium chloride using a balance, and pour it into the bottle labeled .04% solution.

Step 40: Measure two grams of calcium chloride using a balance, and pour it into the bottle labeled .04% solution.

Step 41: Vigorously shake the bottle until the calcium and sodium chloride are dissolved.

Step 42: Take one last 1000mL bottle, label it water, and fill it with tap water.

Step 43: Pour 200mL of the .02% solution on the plot labeled .02 (1).

Step 44: Pour 200mL of the .02% solution on the plot labeled .02 (2).

Step 45: Pour 200mL of the .02% solution on the plot labeled .02 (3).

Step 46: Pour 200mL of the .04% solution on the plot labeled .04 (1).

Step 47: Pour 200mL of the .04% solution on the plot labeled .04 (2).

Step 48: Pour 200mL of the .04% solution on the plot labeled .04 (3).

Step 49: Pour 200mL of the water on the plot labeled water (1).

Step 50: Pour 200mL of the water on the plot labeled water (2).

Step 51: Pour 200mL of the water on the plot labeled water (3).

Step 52: Wait 48 hours for the solutions to sink into the ground and then repeat steps 11 and 12 at the same time you took the samples before the solutions were added (label the bags the same way but write “after” on each bag).

Step 53: Repeat steps 13-36 (when you label be sure to add an “A” to all things you label as to symbolize your “after” results).

Step 54: Be sure to record all of you bacteria populations in the data table.

C. Data/Analysis

Bacteria Colonies in Soil

Soil Treatments		Number of bacteria in 1 cc of soil	
Solution	**Sample #**	**Before Treatment**	**After Treatment**
0.0% NaCl/KCl	1	490,000	230,000
	2	150,000	1,250,000*
	3	330,000	110,000
0.02% NaCl/KCl	1	840,000	250,000
	2	350,000	270,000
	3	190,000	200,000
0.04% NaCl/KCl	1	290,000	3,040,000*
	2	700,000	120,000
	3	90,000*	280,000
0.0% NaCl/KCl	Average	323,333	530,000
0.02% NaCl/KCl	Average	460,000	240,000
0.04% NaCl/KCl	Average	360,000	1,146,666

*****Note: The starred data are considered outliers and are not used in the final averages because they are 10 times less or more than the normal range of data. This happened because of errors made when using the micropipette and petri-film plates. We dropped these data points because when they are used, they disprove our hypothesis, but when they are not used, they prove it. Our hypothesis, which was supported by previous research, states that as the levels of salinity in the soils go up, the levels of bacteria go down.

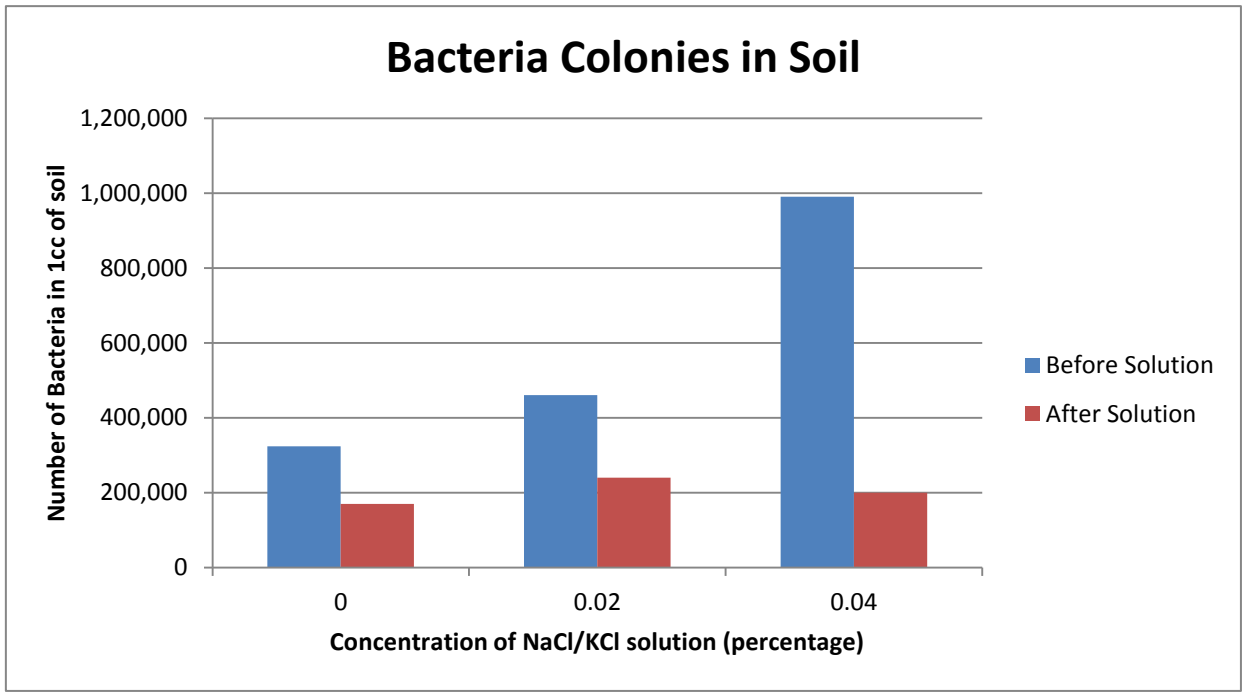
Corrected Averages

0.0% NaCl/KCl – (Before) 323,333 and (After) 170,000

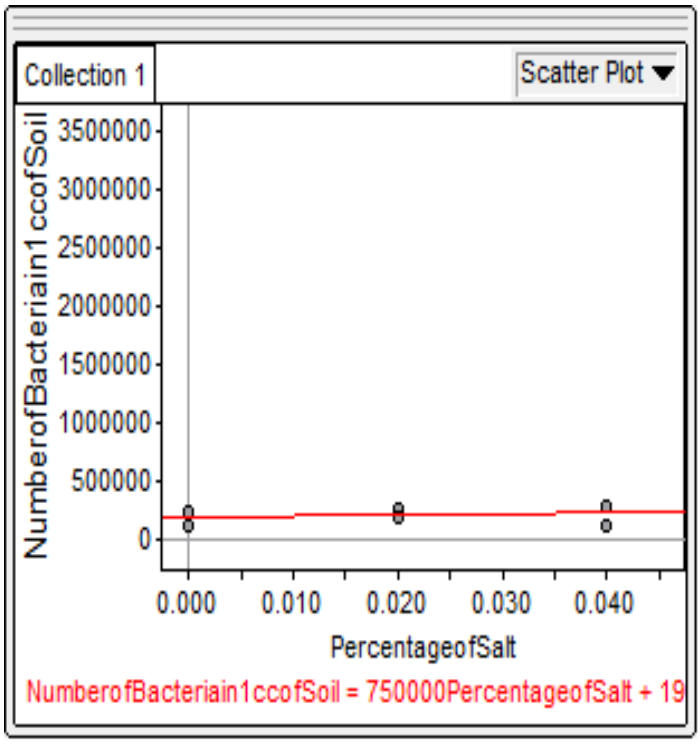
0.02% NaCl/KCl – (Before) 460,000 and (After) 240,000

0.04% NaCl/KCl – (Before) 990,000 and (After) 200,000

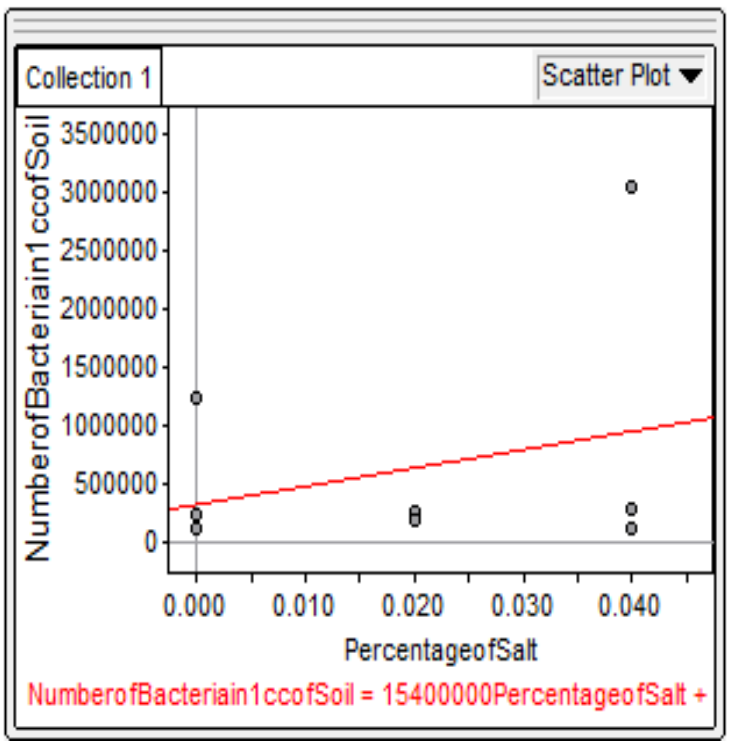
Analysis:



Corrected Data of Bacteria Colonies



Raw Data of Bacteria Colonies



D. Conclusion

Our hypothesis was that a higher concentration of a sodium and calcium chloride solution decreases the population density of the soil bacteria. When using our raw and corrected data, our hypothesis is incorrect because in all tests of 0.00%, 0.02%, and 0.04% NaCl/KCl solution, the bacteria populations decreased significantly after the application of the solutions, but there were environmental factors that further caused the drop in bacteria population.

For the 0.00% NaCl/KCl solution, the average bacteria population before the solutions were poured on the plots was 323,333. After the application of the 0.00% NaCl/KCl solution, the bacteria population decreased to 170,000. Because the 0.00% NaCl/KCl solution is water, which shouldn't affect the growth of bacteria, this signifies that there is a component in the natural environment that is changing the bacterial population. The bacteria population was 460,000 before the application of the 0.02% NaCl/KCl solution. After the application of the 0.02% NaCl/KCl solution, the average bacteria population was 240,000. When looking at the first graph of corrected data, there is hardly any difference in the bacteria count for the 0.00% and 0.02% plots. This shows that both samples were nearly identical in their counts, showing that the environmental factor played a larger role than the saline solution in the bacteria dying. In the plot of 0.04% NaCl/KCl solution, before it was applied, the average bacteria population was 990,000. After the application of the 0.04% NaCl/KCl solution however, the bacteria population dropped to 200,000. When the drop in bacteria from the 0.00% plot due to the environment is taken away from the 0.04% plot count after the solution was poured shows that the salinity of the 0.04% solution greatly impacted the bacteria population. This may have happened due to where we poured the solution and where we took the sample from in relation to where the liquids were poured and the samples were taken from in the other plots. Also, our

plots were not an even distance from the sidewalk on both sides, so that may have contributed to the anomaly. One last possible factor would be that there were plants and roots that were effecting the compaction of the soil, thus affecting the starting numbers of bacteria populations. When looking at the regression line in the first scatter plot, the r2 value is 0.031, meaning that there is only a 3.1% chance that the sodium and calcium chloride are what caused the change in bacteria populations.

If we used the raw data collected, our experiment would disprove our hypothesis. This happened because we made an error in using the micropipettes and petri-film plates. Because of the outlying data, the averages are significantly different than they would be without the outlying data. The soil bacteria test in the third plot before the solutions were poured on was one of the outlying data we collected. We counted 90,000 bacteria in this one sample, and the next smallest sample was 150,000. This most likely happened because we did not put 100mL of the serial dilution on the petri-film plate. There is a significant enough difference between the 90,000 bacteria and the rest of our data that it is considered an outlier. Another set of outlying data was in the second plot of our 0.00% NaCl/KCl after pouring on the solution. After using the equation, we calculated 1,250,000 bacteria when the rest of our samples were fewer than 300,000. The first plot of 0.04% NaCl/KCl after the solution had been poured on also had a questionable amount of bacteria. We counted 3,040,000, which is not realistic at all. This most likely occurred because we put more than 100mL of the dilution on the petri-film plate. When looking at the second scatter plot, the r2 value is 0.076, meaning that there is a 7.6% chance that the sodium and calcium chlorides were in fact the reason the bacteria populations changed. These data, it would prove our hypothesis wrong because there was an “increase” in the number of bacteria counted in two of our tests.

For further research, we would test to see if the plant that was in the vicinity of the soil plots affected the diversity in the bacteria populations. Our hypothesis would be that the roots of the plants change the bacteria population density in the soil. If we were to perform this experiment, there would be further instructions included to ensure that the data is reasonable and accurate. Ways of accomplishing this would include measuring the distance from the sidewalk on either side of our plots and further assessing the area to make sure that there are no plants or roots that could be impacting the bacteria. By doing so, the chances of getting inaccurate data would be decreased. Another factor that should be controlled is where the solution is poured on the plots and where the samples are taken from within the plots. This will even out the amount of NaCl/KCl solution the bacteria are exposed to, making it a more even distribution, and therefore a more accurate result when testing.

E. References:

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