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Soil Ecology Project

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Background

In one teaspoon of soil, there are usually more than 100 million bacteria. Bacteria are one of the most important microorganisms in the soil. Bacteria are a prokaryotic, which means that they do not have a distinct nucleus or membrane bound organelles. Bacteria can be categorized in many different ways. Bacteria can be sorted based on its shape; they are either in the form of a spiral shape, rod or coccus which is spherical. They can also be categorized into aerobic bacteria, organism that require an oxygenated environment and anaerobic bacteria; do not use oxygen to live in their environment. This microorganism can also be classified as gram positive in bacteria, or gram negative bacteria based on their difference in cell wall structure (Business ,2014). Lastly, bacteria can be either autotrophic, organisms that produce their own food, or heterotrophic, organisms that acquire food from other sources.(Hyperphysics, 2000) (James J. Hoorman, 2011) Bacteria play a major role in maintaining soil structure, decomposition and in the nitrogen cycle.

The nitrogen cycle is critical to all organisms in order to recycle one of the essential elements in order for organisms to live and grow. Nitrogen is a major component of the bases used to construct DNA and RNA and is also used to make amino acids. (John Harrison, 2003) In the nitrogen cycle, ammonia enters the ground through animal waste and dead organic matter. (Britannica, 2014).The nitrogen-fixing bacteria converts ammonia to ammonium, which feeds the nitrifying bacteria, who ammonium convert into nitrites. Then, the same nitrifying bacteria will make nitrites into nitrates, which is the useable form of nitrogen for plants (Harlan

Bengtson, 2010). Nitrates in the soil directly benefits plant growth by constructing DNA and enzymes. In all cells, the DNA is transcribed into RNA, which is then translated into enzymes, a special protein which starts and stops chemical reactions. In a cell this allows the four tasks of life to be accomplished which include homeostasis, reproduction, transformation of energy and synthesis of new material. Without nitrogen, no organism could live, so this is why it is so critical for the element to be recycled. (Britannica, 2014).

Bacteria are a key part in plant and animal tissue decomposition in the soil. The result of decomposition is that the polymers are broken down into monomers so that biological molecules including proteins, lipids, carbs, water and nucleic acids can be reformed in new cells. Bacteria are aquatic organisms that live in the water-filled pores of soil. Their activities are directly dependent on relatively high soil water contents. (The University of Western Australia, 2004) There are four functional soil bacteria groups which include decomposers, which are bacteria that consume simple sugars and simple carbon compounds, such as root exudates and fresh plant litter. (James J. Hoorman, 2011)

Humans use rock salt predominantly in the winter when roads or walkways are treacherous when covered in ice, snow, or slippery surfaces, which happens when the sodium in the rock salt interacts with the ions of calcium and magnesium particles. Rock salt can seriously harm soil microbes, and can cause them to lose potassium and phosphorus. (Gold, 2013) This is due to the high amount of sodium in the rock salt. (Gold, 2013) Sodium can cause soil to clog, making it harder for water and air to collect in the soil. (Swan,2014) As well as sodium, other chemicals found in road salt, such as calcium and chloride can also negatively affect the soil, causing metals in soil to deteriorate.(Maxisalt, 2010) While salt provides some nutrients for plants, to grow. (Dara, 2012) Salt is an important chemical that plants need, however too much

salt is very dangerous to a plants health.(Hannick, 2005) Salt can hinder metabolic processes, such as when a plant takes in energy form the sun the plant uses the energy received form the sun as well as the chlorophyll to photosynthesis. (Nemours, 1995)Toxic levels of salt can create the death of plants. More than 0.2% of sodium or more than 0.5% of chloride can cause salt toxicity with in plants. (Dara, 2012) When a plant comes in contact with a vast amount of salt, the leaf area decreases, making it a smaller area for light to hit the leaf, which is essential in photosynthesis. (Hannick, 2005) In addition, when a cell is in a salty environment, and the concentration of water in the solution is less than the inside of the cell, the water will leave the cell which dries it out. (Newton,1991)

In plants, salt-induced dehydration is seen clearly through foliage harm osmotic tension that damages root growth. (Lake, 2014) In addition, salt can destroy the chemistry of soil in which it filters. (New Hampshire, 2014) This causes weak and broken down soil which loses its qualities of sponginess and firmness when it has been dried out. This drastically changes the structure of the soil and prevents plants from accessing water. In addition, salt is known to drop the soils pH level of the soil making it more acidic. (New Hampshire, 2014) The pH level of the soil shows the acidity or basicity of the liquids in the soil, which depends on the concentration of hydrogen ions. pH is measured on a scale of 0-14, 0-6 being the acidic ,7 being the neutral or balanced level of pH, and 8-14 being the basic. (Clean Air Markets Divisions, 2012) When the extremes of acidity or alkalinity arise, a variety of earthworms and nitrifying bacteria vanish (Lake, 2000).

Bacteria will also be affected by dehydration, reduction of soil quality. Salt removes water from microorganisms if the salt concentration internally is high enough. We hypothesized

that The higher the amount of road salt, the higher the chloride level in the soil, and the lower amount, the lower population density of bacteria.

Lab Outline

Problem: Does road salt change the chloride levels in the soil, and how would this change the population density of bacteria?

Hypothesis: The higher the amount of road salt, the higher the chloride level in the soil, and the lower amount, the lower population density of bacteria.

Independent Variable: presence of sodium chloride solution

Dependent Variable: population density of bacteria in 1cc soil; chloride level in the soil

Negative Control: absence of sodium chloride solution on the soil presence, presents of water

Controlled Variables: grams of sodium chloride in solution, amount of soil taken from the ground, size of the plot, mL of water used in salt solution, size of plot, location of plot, size of soil extractor, size of culture tube, amount of soil diluted, amount of diluted soil on the petri plate, which dilutions are plated, type of nutrient plates, amount of salt solution poured on plots, day and time extracting samples, amount of sterilized water, amount of demineralizer

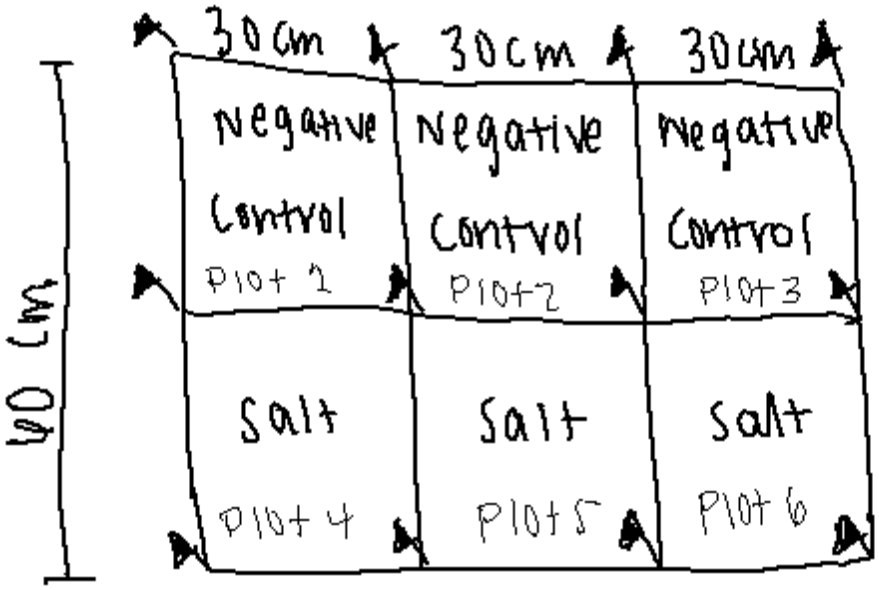
Procedure:

1. Go to a flat surface on RPCS campus at N=39° 21.401’ WO= 76° 38.117’ and make a plot 60cmx90cm
2. Get 12 flags (label with group initials) to mark off smaller plots. Divide plot into six 20x20 cm boxes using flags at the corners of the square. The three squares in the first row are labeled “negative control”, the three squares in the second row are labeled “salt”. See Diagram 1
3. Extract ALL samples from Trail 1 on the same day at the same time
4. Push the soil core sampler 15 cm down into the soil of Plot 1 and turn clockwise, then pull it out of the ground. Empty the soil out into a plastic bag labeled “before Plot 1”
5. Repeat step 4 for Plots 2, 3, 4, 5, and 6 and label plastic bag with their respective plot names
6. Perform dilution procedure for all samples in steps 7-20. Make sure that all six “before” samples are diluted on the same day at the same time.
7. Use a clean, new transfer pipette to add 10mL of sterile water to a 15mL culture tube. Label the tube “10^0 before Plot 1”
8. Use the same pipette to add 9mL of sterilized water to a second 15ml culture tube Label the tube “$10^{-}1$ before Plot 1”

9. Repeat step 8 two more times to two additional 2mL culture tubes Label them "10^{-2} before Plot 1" and "10^{-3} before Plot 1"
10. Place 1cc of your soil sample into the "Plot 1 before 10^{0}" culture tube
11. Cap the tube and shake vigorously
12. Using a new pipette, remove 1 mL of the soil/water mixture from the "10^{0}" tube and place into the "Plot 1 before 10^{-1}" tube
13. Cap and shake vigorously
14. Using the same pipette in step 12, remove 1 mL of the soil/water mixture from the "10^{-1}" tube and place into the "Plot 1 before 10^{-2}" tube
15. Using the same pipette in step 14, remove 1 mL of the soil/water mixture from the "10^{-2}" tube and place into the "Plot 1 before 10^{-3}" tube
16. Cap and shake vigorously
17. You should now have a total of 4 culture tubes
18. Plate 100 µL samples from the 3rd and 4th tubes (dilutions 10^{-2} and 10^{-3} Plot 1 before) onto their own separate 3M Petrifilm ™ Aerobic Count Plate
19. Allow bacteria colonies to grow for 48 hours
20. Examine each of the plates for individual bacteria examine 10^{-3} first, then 10^{-2} colonies and choose the plate with the fewest colonies (but at least 5) to make your estimates of the number of bacteria in the original 1 cc soil sample using the following formula:
    # Microbes in 1cc of soil= # colonies on sheet x 10^{2} x $10^{|\text{dilution \# at which these colonies were found}|}$
21. Repeat dilution steps 8-21 for Plots 2, 3, 4, 5, and 6 respectively
22. Complete the chloride test for all plots in steps 23-29 on the same day at the same time as the dilutions for the "before" samples
23. In order to see the chloride increase levels, use the Model PWB-1 Demineralizer bottle (1155) to fill a 20 mL soil tube (0249) to the 10 mL line with demineralizer water
24. Use the plastic soil measure (0819) to add one level measure of the soil sample to the tube. Cap and shake for 2 minutes
25. Use a piece of filter paper (0465) and a plastic funnel (0459) to filter the mixture into a second 5 mL soil tube (0249). (fold filtered paper in half and the in half again to form a cone which it fitted into the funnel)
26. Use a transfer pipette (0364) to transfer 5 drops of the filtrate in the second tube to a flat bottomed turbidity vial (0242)
27. Add 1 drop of *chloride test solution (5111) to the vial. Swirl gently to mix
28. Match the turbidity or amount of precipitation against turbidity standards on the chloride chart (1304). Lay the chart flat under natural light and hold the turbidity vial 1 half inch above the black strip in the middle of the chart. View the black strip down through the turbid sample and compare the resulting shade of gray with the 6 standard shades. The test result is read in parts per million chlorides. Then multiply the test result by 2 to get the actual ppm for the soil sample.
29. Repeat chloride test in steps 22-28 for all other before samples (Plots 2-6)

30. Combine the 1000 mL of distilled water with 5 grams of sodium chloride to make a salt solution. Apply across plots 4, 5, and 6 spread evenly. Then apply 1000mL across plots 1, 2, and 3.
31. Repeat steps 4-29 to extract, dilute, and test all "after" samples from all six plots. Also, label Ziploc bags, culture tubes and petri plates with "after" instead of "before"

DIAGRAM #1



Data/Observations

Amount of bacteria in 1cc soil before adding salt

Sample	# of bacteria in 1cc soil
Plot 1, row 1 (negative control)	540,000
Plot 2, row 1 (negative control)	1,300,000
Plot 3, row 1 (negative control)	1,100,000
Plot 4, row 2 (salt)	390,000
Plot 5, row 2 (salt)	800,000
Plot 6, row 2 (salt)	780,000

Amount of bacteria in 1cc soil after adding salt

Sample	# of bacteria in 1cc soil
Plot 1, row 1 (negative control)	3,800,00
Plot 2, row 1 (negative control)	6,400,000
Plot 3, row 1 (negative control)	8,000,000
Plot 4, row 2 (salt)	2,800,000
Plot 5, row 2 (salt)	7,800,000
Plot 6, row 2 (salt)	2,300,000

Amount of chloride in the soil (in ppm) before adding salt

Sample	Chloride Test (in ppm)
Plot 1, row 1 (negative control)	50 ppm
Plot 2, row 1 (negative control)	50 ppm
Plot 3, row 1 (negative control)	50 ppm
Plot 4, row 2 (salt)	50 ppm
Plot 5, row 2 (salt)	50 ppm
Plot 6, row 2 (salt)	50 ppm

Amount of chloride in the soil (ppm) after adding salt

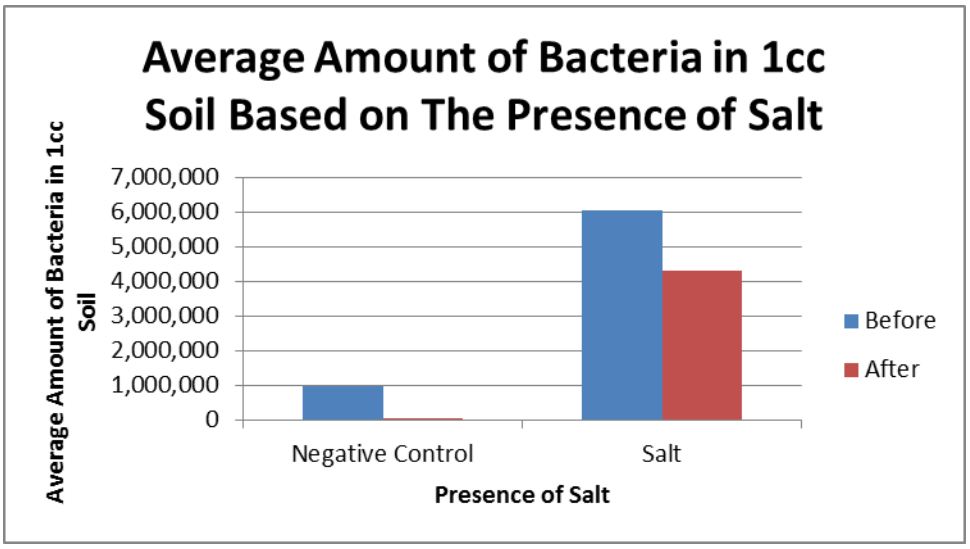
Sample	Chloride Test (in ppm)
Plot 1, row 1 (negative control)	50 ppm
Plot 2, row 1 (negative control)	50 ppm
Plot 3, row 1 (negative control)	100 ppm
Plot 4, row 2 (salt)	50 ppm
Plot 5, row 2 (salt)	100 ppm
Plot 6, row 2 (salt)	100 ppm

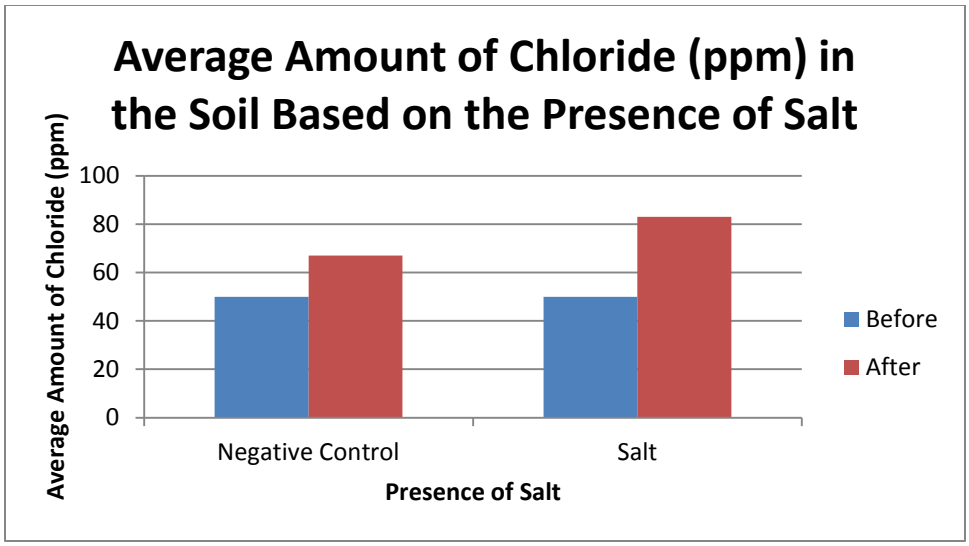
Average amount of bacteria in 1cc soil

	Negative Control	Salt
Before	980,000	6,066,666
After	65,666	4,300,000

Average amount of chloride in the soil (in ppm)

	Negative Control	Salt
Before	50 ppm	50 ppm
After	67 ppm	83 ppm





Conclusion

For our soil ecology project we wanted to study the effects of road salt on bacterial populations in the soil. Through our extensive research on bacteria, and its role in the soil web, as well as the study of negative effects of road salt, we were able to form our hypothesis. "The higher the amount of road salt the higher the chloride level in the soil, and the lower the population density of bacteria" Our claim was proved through the information from our two soil dilution tests, and our two chloride tests.

The information we gathered showed that there were more bacterial colonies in the first (non-salt) experiment and less in the salt experiment. This was true for both the negative control plots and the salt plots. Originally, the negative control plots had an average of 980,000 cc. in the soil, and ended with 65,666 cc. in the soil. The end results of the negative control plots were surprising, since they had no salt solution applied to them. The salt plots had a similar turn-out; they started with 6,066,666 cc. in the soil and after treated with salt solution, ended with an average of 4,300,000 in the soil. This shows that the bacterial colonies were negatively affected

after treated with salt solution. There was an apparent decrease in colonies, after applied with salt solution. Our data proved that when the soil had a high amount of salt the soil colonies decreased, just as our hypothesis states.

As for the chloride test, the negative control plots and the salt plots had the same outcome for the first test. Both had an average of 50 ppm when tested for amount of chloride. After we applied salt to the salt plots, the chloride levels increase significantly, and increased to an average of 83 ppm. Our negative control plots, strangely also had an increase in chloride levels, with an average of 67 ppm. However, the increase was not as great as the increase the salt plots experienced. The data from this test proves that the level of chloride, or salinity in the soil, increased after the salt solution was applied.

The data concludes that the higher the amount of chloride, or salt present in the soil; the higher the decrease of soil colonies, just as our hypothesis proves. Through all our tests and data, our hypothesis was proved.

Although we were able to successfully prove our hypothesis, to have a more conclusive experiment, in the future we should perform additional experiments. For example, since the experiment was based on road-salt, which is toxic, a pH test would help in gaining further information on the amount of substances that negatively affect the soil. In our original idea for the experiments, we included a pH test. However, based on timing this test was left out of the project. A pH test would allow further evidence on the negative effects road salt has on soil bacteria. Through this set of tests and experiments, we hope collect conclusive data, to fully prove our hypothesis.

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