Soil Ecology Project

HOW ROAD SALT IMPACTS BACTERIA IN THE SOIL

BROOKIE BAGLI, CAMERON LEVINE, KENNEDY WILLIAMSON, MIR MCLEAN

Background

Bacteria are prokaryotic microorganisms that can be by shape categorized into cocci, bacilli, and spirilla groups. Their dominant method of reproduction is binary fission where one cell divides to make two new cells. Most bacteria eat dead, or decaying organisms as their food source. Their main predator in the soil is protozoa. There can be as many as three million bacteria in one gram of soil (Senior, 2017). Bacteria perform many important functions in the soil ecosystem including maintaining soil structure decomposing organic matter and recycling of soil nutrients (Ingham, 2009).

Soil bacteria form microaggregates in the soil by binding soil particles together through sticky secretions. This process creates pore space in the soil, so it can store oxygen, which is necessary for cellular respiration (Ingham, 2009). These microaggregates improve soil structure by allowing for better water infiltration and water holding capacity (Ingham, 2009). Water is stored in the soil for plant growth, as water is necessary for photosynthesis. Water can be replenished by filtration, where water moves through the spaces of the soil. Without enough pore space or sufficient water filtration, the water cannot easily enter the soil. Consequently, this water can be lost through evaporation or run off. With less water, photosynthesis decreases, resulting in less organic matter in the soil and weakened soil structure that can further decrease the infiltration rate (Multimedia Capstone class at PSU, 2008).

Decomposition is when decomposers break down organic matter and extract chemicals from dead bodies or organic wastes in order to produce energy. Decomposition starts with detrivores, such as earthworms, that break down organic matter. Afterwards saprophytes, such as bacteria, continue the process with special enzymes that break organic matter into smaller pieces, eventually changing polymers into monomers. This process recycles biological molecules which

can be used in new cells. Chemical nutrients like carbon and nitrogen are also released back into the soil, to be used by organisms or released back into the air (New Hampshire Public Television, 2017). Without decomposition, nutrients in matter wouldn't be extracted and couldn't be used by organisms.

Many different types of bacteria manage the way that nitrogen, recycles in the environment (Senior, 2017). Some nitrogen-fixing bacteria can fix nitrogen gas from the air, converting it to compounds that can be used by plants growing in the same soil (Senior, 2017). When nitrogen is absorbed into the soil, different nitrifying bacteria such as nitrosomonas (Encyclopædia Britannica, Inc. 2017), help it to change states from ammonia to nitrites to nitrates so it can be absorbed by plants (Nelson, 2017). Others carry out the opposite process when there is excess nitrogen in the soil, converting nitrogen-containing compounds from decaying plants and animals into gas, which then re-enters the air (Senior, 2017). Nitrogen is so important because it is part of nucleic acids such as DNA and RNA, as well as amino acids. These biological molecules contribute to the process of protein synthesis. This is how enzymes are created. Enzymes are key to starting chemical reactions which are necessary for cells to function. Therefore, organisms cannot live without an adequate supply of nitrogen. Therefore, if bacteria populations decreased, it would consequently effect the protozoa, that consume bacteria, which means there would be a decrease in the microorganism population. Then, it would affect the plants because they don't have the support of the microorganisms, which means the plant population would decrease. Consequently, there wouldn't be enough plants for the consumers to eat, overall effecting the entire ecosystem.

Humans often use road salt in the winter to clear snow and ice from roads and walkways. Road salt is useful because it lowers the freezing point of the water, which forces the ice to melt

and prevents falling snow from freezing in the future (Tuthill, 2013). Although road salt is very effective for clearing the roads, most of it gets pushed to the sidewalks and the grass, which consequently affects the soil (New Hampshire Department of Environmental Services, 2017). Road salt doesn't just get pushed onto the sidewalks and grass, but also moves down roadside drains which lead to various waterways. Salts in ponds and lakes create a salt water layer at the bottom, trapping nutrients from aquatic plants and animals. According to (Beaudry, 2017), "High concentrations of salt in freshwater has harmful effects on the growth, reproduction, and survival of a large range on invertebrates, fish, and amphibians." High levels of chloride concentrations interfere with how animals control their intake of salt. Also, when large quantities of salt are introduced to ecosystems that can't assimilate it, it causes accumulation in the soil and water (MadSci Network. 2000). Road salt also impacts land animals when it is consumed. Because it is made at such a high concentration, it is toxic to animals such as deer or dogs and can cause dehydration, confusion, and weakness (New Hampshire Department of Environmental Services, 2017).

Road salt can be composed of 4 different chlorides: sodium chloride, calcium chloride, potassium chloride, magnesium chloride (Peter's Chemical Company, 2017). Chloride is an essential, dissolvable, non-degradable micronutrient that all crops require in small quantities. It helps plants in processes such as, photosynthesis, osmotic adjustment, and suppression of plant disease. However, excess chloride can build up in the soil from road salt, swimming pool run off, or irrigation water (University of Maryland, 2004). Therefore, too much chloride, can lead to salinity damage and toxicity (Guy, 2008). High concentrations of chloride can cause toxicity problems in crops and reduce the yield by mobilizing toxic cations such as potassium or magnesium (Guy, 2008). Chloride toxicity is most common in irrigated, dry regions, seacoast

areas, and near roads frequently treated with salt in the wintertime (University of Maryland, 2004). Chloride concentrations can also effect other organisms within the soil. If the chloride effects the soil, which the organisms live in, it will affect the way they live and get their nutrients from the soil.

Salts on the ground can also cause damage to the soil structure and ability to absorb water. When salt seeps in the soil it causes dehydration. Water molecules surround sodium ions and chloride ions to break sodium chloride down, which causes osmotic stress that affects the growth of plants (New Hampshire Department of Environmental Services, 2017). Osmotic stress is a sudden change in solute concentration around a cell, which causes a fast change in water movement across its cell membrane. Furthermore, when there is a high concentration of salt in the soil, water is drawn out of plant cells through osmosis, which causes the cells to shrivel and potentially die (Oilgae, 2017). High concentrations of soluble salts can prevent seed growth and a plant's ability to take in water. Depending on how high salt levels are, salt poisoned soil that is closest to the surface can lose its ability to support agricultural crops, native grasses, or other vegetation and can lead to surface erosion (Daily, M, Whalen, J, 2005). The importance of this experiment is to study how the application of road salt will impact the growth of bacteria in soil. Because bacteria naturally live in the soil, if road salt got into the water spaces where they live, they could potentially cause harm to the bacteria. We hypothesized based on our research that, the presence of road salt in the soil will decrease the population of bacteria.

Procedure

I. Problem: How does the amount of road salt impact the population density of bacteria in soil?

II. Hypothesis: When road salt is added to the soil, the bacteria population density will decrease.

III. Independent Variable - Application of road salt solution to soil.

IV. Dependent Variable- Population density of bacteria in 1 cc of soil.

V. Negative Control- Application of water solution to soil.

VI. Controlled variable- amount of road salt in solution, concentration of road salt solution, type of salt, amount of soil extracted and diluted, time given for bacteria to grow on plates, location of soil, type of water in the tube, amount of soil/water mixture transferred in the tube, amount soil in the tube, time given for the solutions to sink into the plots, amount of water each solution, type of pipettes, the dilution numbers plated, type of nutrient agar plates, and type of chemical test kit, how much solution is plated.

Procedure:

1. Go to N 39. 35972° W 076.63509° and set up six 20 x 20 cm plots in 2 rows and 3 columns and separate each plot using yellow flags labeled “Salt 1” “Salt 2” “Salt 3” “Water 1” “Water 2” “Water 3” at the corners (see diagram).

Salt 1	Salt 2	Salt 3
Water 1	Water 2	Water 3

2. Extract 15 centimeters of soil from "Salt 1": place the soil extractor in the appropriate plot, use a hammer to push it into the ground, then turn the soil extractor clockwise, and pull it straight out.
3. Put the first soil sample in a labeled plastic bag titled "Salt 1"
4. Repeat steps 2-3 five more times with the five other plots, labeling the bags appropriately to match the plots of the soil samples. (see diagram) Extract all samples on the same day and the same time.
5. Bring soil samples into the lab room and perform serial dilution in steps 6-18.
6. Use a clean, transfer pipette to add 10 mL of sterile water to a 15 mL culture tube. Label the tube "Salt 1 10^{0}".
7. Use the same pipette to add 9 mL of sterile water to a second 15 mL culture tube. Label the tube "Salt 1 10^{-1}".
8. Repeat step 6, 2 more times to the additional 15 mL culture tubes and label them as "Salt 1 "10^{-2}"and "Salt 1 10^{-3}".
9. Place 1 cc of Salt 1 into the "10^{0}" culture tube
10. Cap the tube and shake vigorously.

11. Using a new clean pipette, remove 1 mL of the soil/water mixture from the "10^{0}" tube and place into the "10^{-1}" tube.
12. Cap and shake vigorously.
13. Using the same pipette from step 11, remove 1 mL of the soil/water mixture from the "10^{-1}" tube, and place into the "10^{-2}" 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}" tube, and place into the "10^{-3}" tube.
16. Cap and shake vigorously.
17. You should now have a total of four culture tubes.
18. Repeat dilution steps for 7-19 for the 5 other soil samples. Dilute soil samples on the same day at the same time.
19. Plate 100 microliter samples from the 3rd and 4th tubes (dilutions "10^{-2}" and "10^{-3}") onto their own separate 3M Petrifilm™ Aerobic Count Plate labeled by their plot name and dilution number. (Ex. "Salt 1 10^{-2}").
20. Repeat this step for the other 5 samples according to their dilution number and plot name.
21. Allow bacteria to grow for 48 hours.
22. After 48 hours, count bacteria plates using a magnifying glass. Start with the dilution "10^{-3}" plate and check for at least 5 red dots or bacteria colonies. If 5 bacteria colonies aren't found proceed to the "10^{-2}" plate. Record the number of bacteria colonies and the dilution number.

23. Use the following formula: # Microbes in 1 cc of soil = # Colonies on sheet x 10^2 x $10^{|\text{dilution \# at which these colonies were found}|}$ to count the number of bacteria of soil based on the presence of salt in the solution.
24. Repeat this step for the other 5 samples according to their dilution number and plot name.
25. Perform the chloride test using the LaMotte STH-14 Chemical Test Kit in steps 24-29.
26. Use the model PWB-1 Demineralized Bottle (1155) to fill a 10 mL tube (0249) to the 10 mL line with demineralized water.
27. Use a plastic soil measure (0819) to add one level measure of the soil sample to the tube. Cap and shake vigorously for 2-3 minutes.
28. Use a piece of filter paper (0465) and a plastic funnel (0459) to filter the mixture into a second 10 mL soil tube (0249). (Fold filter paper in half and then again to form a cone which is fitted into the funnel.)
29. Use a transfer pipette (0364) to transfer five drops of the filtrate in the second tube to a flat-bottomed turbidity vial (0242).
30. Add one drop of Chloride Test Solution (5111) to the vial. Swirl gently to mix.
31. Match the turbidity or amount of precipitation against the turbidity standards on the Chloride Chart (1304). Lay the chart flat under the natural light and hold the turbidity vial one-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 grey with the 6 standard shades. The test result is read in parts per million chloride.
32. Make two 1000 mL bottles of road salt solution by adding 30 grams of sodium chloride to 1000 mL of water

33. Make two 1000 mL bottles of regular water filled at 1000 mL
34. Take the four bottles of solution outside and apply it to the plots, spreading the road salt solution back and forth equally and close to the ground on the 3 salt plots, and the water solution back and forth on the 3 water plots.
35. Allow the solutions to sit on the plots for 48 hours.
36. Repeat steps 2-3 when extracting the soil samples again labeling the bags "after" with their respective plot names
37. Repeat steps 6-23 to dilute and plate the "after" samples of the soil solutions.

Data

Number of Bacteria in 1cc of soil based on presence of salt in solution

	Before Application	After Application
Water one	70,000 colonies per 1cc of soil	10,000 colonies per 1cc of soil
Water two	100,000 colonies per 1cc of soil	30,000 colonies per 1cc of soil
Water three	360,000 colonies per 1cc of soil	180,000 colonies per 1cc of soil
Salt one	490,000 colonies per 1cc of soil	40,000 colonies per 1cc of soil
Salt two	60,000 colonies per 1cc of soil	60,000 colonies per 1cc of soil
Salt three	50,000 colonies per 1cc of soil	10,000 colonies per 1cc of soil

Average number of bacteria in 1 cc soil based on presence of salt in solution

Averages	Before Application	After Application
Water	176,666 colonies per 1cc of soil	73,333 colonies per 1cc of soil
Before	200,000 colonies per 1cc of soil	36,666 colonies per 1cc of soil

Amount of chloride (ppm) in soil based on presence of salt

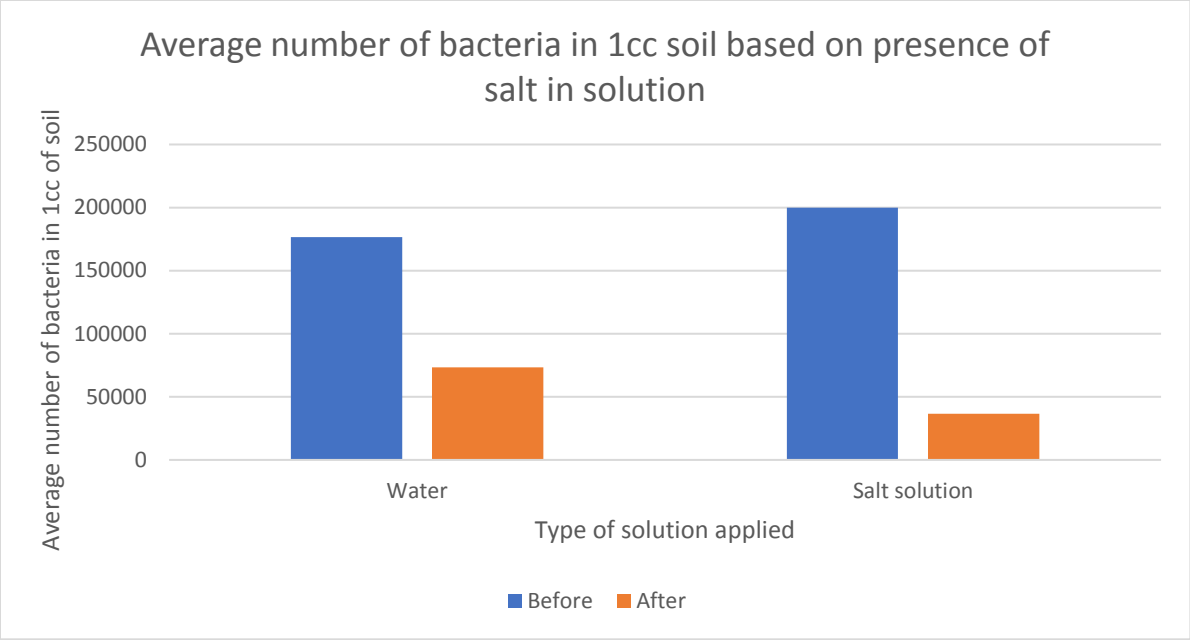
	Before Application	After Application
Water 1	50 ppm	200 ppm
Water 2	500 ppm	200 ppm
Water 3	100 ppm	50 ppm
Salt 1	200 ppm	200 ppm
Salt 2	100 ppm	50 ppm
Salt 3	25 ppm	200 ppm

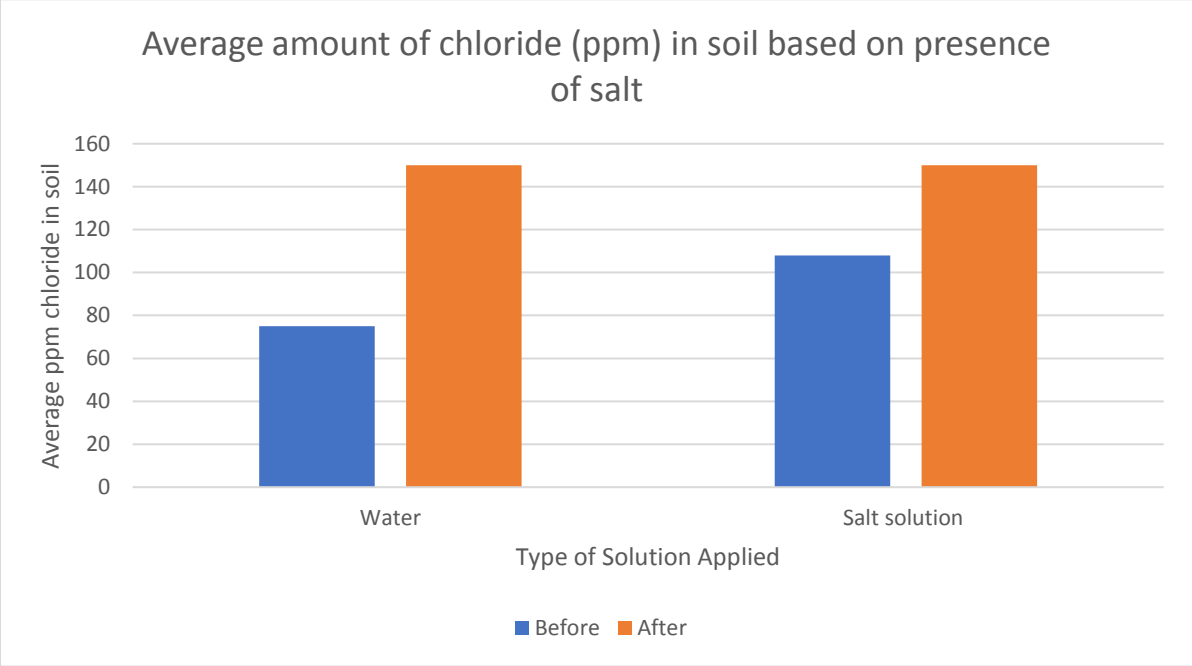
Average amount of chloride (ppm) in soil based on presence of salt

Averages	Before Application	After Application
Water	216 ppm	150 ppm
Salt	108 ppm	150 ppm

Average amount of chloride (ppm) in soil based on presence of salt (Without outlier)

Averages	Before Application	After Application
Water	75 ppm	150 ppm
Salt	108 ppm	150 ppm





Conclusion

In conclusion, our hypothesis stated, when road salt is added to the soil, the bacteria population density will decrease. Our hypothesis was not supported as shown through our results. The amount of bacteria in the negative control plots before adding water was 176,666 per cc of soil. After adding water, the amount of bacteria in the negative control plots was 73,333 per

cc of soil. The amount of bacteria in the soil decreased by 41%. The amount of bacteria in the salt plots before adding the salt solution was 200,000 per cc of soil. After our group added the salt solution, the amount of bacteria in the salt plots was 36,666 per cc of soil. The amount of bacteria in the soil decreased by 81%. Because our group's negative control was water, the bacterial population after the water solution was added, was not expected to change in the water plots. Environmental factors could have affected our negative control and salt plots because both had a different percentage decrease. The amount of chloride in the negative control plots before adding water was 75 ppm of chloride in the soil and the amount of chloride after adding water was 150 ppm of chloride in the soil. The amount of chloride in the salt plots before adding salt solution was 108 ppm of chloride in the soil and the amount of chloride in the salt plots after adding the salt solution was 150 ppm of chloride in the soil. The amount of chloride increased 100% for the negative control plots, which means it doubled, and the amount of chloride increased 39% for the salt plots. The chloride content for the negative control plots was not supposed to change at all because there is no chloride in water. This means that there was some type of environmental factor that occurred in the soil. From our group's results, we can say that both the water plots and the salt plots had a decrease in their bacteria population and both group's chloride concentration increased as well. Because both the chloride concentration increased and the bacteria population decreased for the water plots and the salt plots, our group cannot determine if the independent variable (application of salt solution) had an impact in the soil. In order to determine the salt solution, we would need to see the negative control (application of water solution) stay the same throughout the experiment. When we put our solutions into the plots, it rained over the weekend. This means that the rain could have washed the salt solution into the water plots. There was not a significant change of chloride content for

the salt solution. Because the chloride concentrations were roughly the same, the environment in the salt water plots was not inherently different from the environment in the water plots. There is no way to prove which plots are the salt plots or water plots because their concentrations of chloride are more or less the same. There was not a major difference in the chloride concentration, so we could not evaluate the differences in the bacteria population between the salt and the water plots. Our group could not determine why the bacteria populations decreased by the amounts they did, but we do know that the chloride between the water and salt plots was not different. The chloride concentration of the salt and the water plots did not change, even though we put them in different locations and put different solutions into them. The salt plots and the water plots did not have a large difference in their chloride concentration.

For future research, there can be multiple things that our group can change, in order to have different results in our experiment. Our group used sodium chloride. If we used a different type of salt, including potassium chloride, calcium chloride, and magnesium chloride, there might be a different bacteria population size in our soil and a different amount of chloride content as well. Also, if our group added an increased or decreased amount of road salt into the soil, that might change the amount of bacteria in the plots. Even though our group couldn't predict what the weather might be like, the rain did have an impact on our end results. The rain may have washed the salt solution into the water plots and if it didn't rain, our group may have had different end results. For the future, our group can separate the water plots and the salt plots from each other. The plots will stay the same size, but if they are apart from each other, there can be a greater chance that the salt solution will not enter the water plots.

Works Cited

Agvise Laboratories. (2017) Water Holding Capacity

https://www.agvise.com/educational-articles/soil-water-holding-capacity/

Beaudry, F. (2016) The Environmental Effects of Road Salt

https://www.thoughtco.com/environmental-effects-of-road-salt-1204123

Daily, M, Whalen, J. (2005) Bureau of Environmental remediation/remedial section guidance

http://www.kdheks.gov/ber/policies/BER_RS_013A.pdf

Encyclopædia Britannica, Inc. (2017) Nitrifying bacterium

https://www.britannica.com/science/nitrifying-bacterium

Encyclopædia Britannica, Inc. (2017) Nitrogen-fixing Bacteria

https://www.britannica.com/science/nitrogen-fixing-bacteria

Guy, S. (2008) Chloride

http://www.smart-fertilizer.com/articles/chloride

Ingham, E. R. (2017). Bacteria. Retrieved from Natural Resources Conservation Services

website: https://extension.illinois.edu/soil/SoilBiology/bacteria.htm

Ingham, E.R. (2009) Soil Biology Primer. Ankey IA: Soil & Water Conservation Society

http://ohioline.osu.edu/factsheet/anr-36

MadSci Network. (2000) Joe McLeod, Staff, Advanced Services, Charter Communications

http://www.madsci.org/posts/archives/2000-11/974375486.Es.r.html

Multimedia Capstone class at PSU. (2008) What is infiltration and why is it important?

http://ecomerge.blogspot.com/2010/05/what-is-infiltration-and-why-is-it.html

Nelson, Ken. (2017). Science for Kids: Nitrogen Cycle. Ducksters. Retrieved from http://www.ducksters.com/science/ecosystems/nitrogen_cycle.php

New Hampshire Department of Environmental Services. (2017) Environmental, Health and Economic Impacts of Road Salt

https://www.des.nh.gov/organization/divisions/water/wmb/was/salt-reduction-initiative/impacts.htm

New Hampshire Public Television (2017)

http://www.nhptv.org/natureworks/nwep11b.htm

Oilgae. (2017) Osmotic Shock

http://www.oilgae.com/ref/glos/osmotic_shock.html

Peters Chemical Company. (2017)

http://www.peterschemical.com/break-the-ice-comparison-of-ice-melting-chemicals/

Senior, K. (2017) Where Do Bacteria Live?

http://www.typesofbacteria.co.uk/where-do-bacteria-live.html

Serchan, S, Jones, D. (2009) The Baltimore Ecosystem Study Virtual Tour

http://www.beslter.org/virtual_tour/Chloride.html

Tuthill, S. (2013) Why is salt used on icy roads? Boston: AccuWeather, Inc

http://www.accuweather.com/en/home-garden-articles/earth-you/icy-sidewalks-and-roadways-can/5631019

University of Maryland (2004)

https://extension.umd.edu/hgic/chlorine-toxicity-trees-and-shrubs