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Background Report

The purpose of our experiment is to find if the sodium chloride in SSS ice melt affects the bacteria in soil. Every winter, SSS ice melt is used on the sidewalks of our school to melt the snow, and some of this spreads onto the soil. Although SSS ice melt is supposed to be better for the plants than other ice melt, the sodium chloride may still affect the bacteria count, and even though there are so many in the soil it is important to keep the soil healthy.

There are an enormous number of microbes that live in the soil, and they all serve different purposes. Some microbes are beneficial to the soil, and some are harmful, but they nonetheless are all important. The two most influential of these many variations of microbes in the soil are soil beneficial bacteria (actimomycetes) and fungi (mycelial fungi). The beneficial soil bacteria serve many purposes. First, the bacteria make more room in the soil structure for air and water by making microscopic spaces for them (Ball, 2003). This is vital to the life of any organism in the soil. Without air and water in the soil, it would be impossible for life to exist there. Second, the bacteria decompose organic matter and some pesticide residues in the soil (Ball, 2003). This helps for the overall health and life of organisms in the soil. Because many of the other organisms in the soil feed on residues for nutrients, if they feed on pesticide residues then they could die. Third, the bacteria suppress soil pathogens that could cause disease in plants (Ball, 2003). This too also helps keep the organisms in the soil healthy and alive. The relationship between the plants and the beneficial microbes is a give-take relationship; the plants supply energy from the air and sun, and the microbes keep the plants healthy by suppressing the pathogens in the soil.

The mycelial fungi and actimomycetes serve many important purposes as well. They help the natural growth hormones in the plants and also help to suppress the soil pathogens that could cause disease in the plants as well. The natural growth hormones in the plants are what allow them to grow healthily. This enables the plants to transport various nutrients among other healthy, enriched areas later in life (Sims, 1990).

All in all, these two kinds of microbes in plants and the soil are extremely important, and without them, all producer life would cease to exist. This is why it is important not to kill these things off by using salt melt and other harmful things to the bacteria in the soil. With the salt in the soil, it not only makes it impossible for bacteria and microbes to live there, but in the long scheme of things it makes it so that people and animals can no longer live either. It changes the food chain by eventually causing the plants to die. If the plants die, there will be no other food for humans and other animals to live and all life would die off. All of these can be affected by SSS salt melt, but the important question is, what ingredient is causing this problem?

There are four main ingredients used in the SSS ice melt which is used at Roland Park Country School. There is sodium chloride, potassium chloride, Alpha-D-Glucopyranoside, and urea (Triple S, 2007). Potassium Chloride is composed of potassium and chlorine. It is commonly used as an ingredient in fertilizers. The growth of plants is often stunted by the lack of potassium. This is why it's in many fertilizers (The Columbia Electronic Encyclopedia, 2004). Alpha-D-Glucopyranoside is what gives the ice melt its blue color and is also used in many fertilizers (J Clin Microbiol, October, 1996); so it does not affect the plants and bacteria in the soil. Urea is also used in many fertilizers. It releases few pollutants into the environment (Curtis J. Overdahl, George W. Rehm and Harvey L. Meredith, 1991) and therefore is not harmful to many of the bacteria. However, sodium chloride causes the salt to dry the water in the soil up, which affects the environment. The environment is then no longer hypotonic, but hypertonic. This causes the microbes to dehydrate and die (or at the least stop reproducing) and harms the food chain, which was stated earlier.

Therefore, out of sodium chloride, potassium chloride, Alpha-D-Glucopyranoside, and urea, (the only four ingredients in the SSS ice melt), we think it is the sodium chloride that is causing the bacteria count to decrease. Since the sodium chloride dries up and decreases the amount of water, it makes it difficult for the bacteria to survive.

The pH scale commonly ranges from 0 to 14, and is an expression for the effective concentration of hydrogen ions in a solution. Solutions with a pH less than seven are considered acidic, but those with a pH greater than seven are considered basic (Omega, 2006). Something with the pH of 7 is considered neutral because it is the pH of pure water. The pH of something usually depends on the activity of hydrogen ions, but for very pure dilute solutions the molarity may be used as a substitute with some sacrifice of accuracy (Malibu Water Resources, 2007). Molarity is the measure of concentration of the solution which is usually measured in moles of solute per liter of solution. It is sometimes difficult to determine an accurate value for the pH of a solution because the pH of something is dependent on the bacteria's activity, and that is sometimes hard to measure. An accurate pH measurement is necessary to the study of many chemical processes. In order to research a chemical you need to know the pH at which a chemical reaction proceeds at it's fastest in order to understand the reaction. If the pH is lower or higher than 7, the enzymes in the soil are deformed, which hinders them from performing chemical reactions vital to the life of the cell. The death of cells in the soil on a much larger scale could lead to the death of plants, or the primary consumers in the food chain, which in an extremely exaggerated situation could lead to food shortages and the death of humans.

Because of the information we found through our extensive research, we decided to perform an experiment that would test the effects of SSS ice melt on the soil of our school campus. To do so, we found an area of soil close to a sidewalk, and made 6 plots with the same areas. It was important that the soil samples came from near a sidewalk, because realistically, during the winter, the ice melt is placed on the sidewalks, so it would have a better chance of spreading on an area near a sidewalk. We took soil samples from all 6 plots of soil, and found the individual pH levels and bacteria counts for each plot of soil. We then added an SSS ice melt solution to 3 of the 6 plots, and water to the other 3 plots, and let the liquids sit in the soil for 48 hours. After those 48 hours, we went back and took samples yet again from each plot of soil, and performed the pH tests and serial dilution tests on every individual bag of soil. We then recorded the bacteria counts and pH levels for each soil sample, before and after we added either SSS ice melt or water. We performed t-tests on our results to find how our experiment was, and were extremely successful. Our experiment overall went very well, and we were able to find something that was beneficial to our school and our knowledge.

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Lab Report

Problem – Does SSS ice melt decrease the bacteria count in the soil at Roland Park Country School?

Hypothesis – If SSS ice melt is added to soil, then the bacteria count will decrease.

Procedure –

Independent variable – Addition of salt solution to the soil
Dependent variables – Number of bacteria in the soil and pH level of the soil
Negative control – Water added to the soil
Controlled variables –

Area being tested
When the area is tested
How long ice melt is in the soil
How diluted ice melt is
Amount of soil taken
Size of area being tested
How long water is left in the soil
Size of the soil sample
How much water is added to the soil

Controlled Variables for serial dilution test –

How vigorously the tubes are shaken
The amount of water added
Type of water that is used (sterile water, dirty water)
The level the soil is diluted to
How much dirt is added
Plates used after serial dilution
Time we allow petrifilm plates to incubate
Amount put on petrifilm plates

Controlled variables for pH testing -

What color sheet is used
How much soil is used
How long soil sets
How long tube is shaken
How many drops of chemicals used

Step by step instructions –

1. Collect all materials – including sterile plastic bags, SSS ice melt, soil, 18 flags, serial dilution test kit, a scale, micro pipette, sterile water, goggles, gloves, pH test kit, alcohol, 80ml beaker.
2. Select a area of 36 X 36cm on a piece of land that could realistically have SSS ice melt on it in the winter (for example an area near a sidewalk) and use the GPS coordinator to find the latitude and longitude of the location of your piece of land
3. Insert flags into the four corners around the square.
4. Grid out the square, so that there are 6 even rectangles (18cm X 12cm).
5. Label each plot with numbers 1-6.

Plot 1	Plot 4
Plot 2	Plot 5
Plot 3	Plot 6

6. In all boxes, take a sample of soil by inserting the soil core sampler into the ground and take a core sample of 14cm long X 2cm in diameter. Place all samples into separate bags, labeled appropriately with number grid and "before"
7. Mix 143 grams of salt with 1000 ml of water.
8. Shake and mix the water and salt until all of the salt is dissolved.
9. For plots 4, 5, and 6 of the grid add about 333ml of salt solution to each box.
10. For plots 1, 2, and 3 of the grid add about 333ml of water.
11. Let solution sit in the soil for approximately 48 hours.
12. Using the same technique described in step 6, take a soil sample from each plot and place into separate sterile plastic bags – labeled with the number, "after" and either salt or water accordingly.
13. Perform pH test on soil so that you know whether the increase in pH level is causing the bacteria to decrease. (For each soil sample, the pH and serial dilution tests must be performed at the same time. For example, you must perform the serial dilution and pH tests for soil sample 1 at the same time, as well as soil samples 2, 3, 4, 5, and 6)

Note: You must perform this test twice on every individual soil sample, once for the sample before you add anything to it, and once for after you add either the SSS ice melt or the water to the sample

14. Fill a test tube (0204) approximately one-third full of soil (the soil sample you use will change depending on which soil sample you are testing.) Use the model PWB-1 Demineralizer Bottle (1155) to add demineralized water to the tube, until it is one-half inch from the top. Cap and shake until the soil is well dispersed.
15. Add 5 drops of Soil Flocculating Reagent (5643). Cap and shake to mix. Allow contents to settle before proceeding to step 3.
16. Use a 1 mL pipet (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.
17. To the first sample on the spot plate, add two drops of *Duplex Indicator (2221). Compare the resulting color reaction against the duplex color chart (1313). NOTE: The wide range indicator and color chart should be selected to perform a more precise pH test. Choose the narrow range indicator and appropriate chart with a mid-point that is as close as possible to the value obtained in the wide range test. (see table)

18. Add two drops of 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.
19. Perform serial dilution test (For each soil sample, the pH and serial dilution tests must be performed at the same time. For example, you must perform the serial dilution and pH tests for soil sample 1 at the same time, as well as soil samples 2, 3, 4, 5, and 6)

Note: You must perform this test once on every individual soil sample

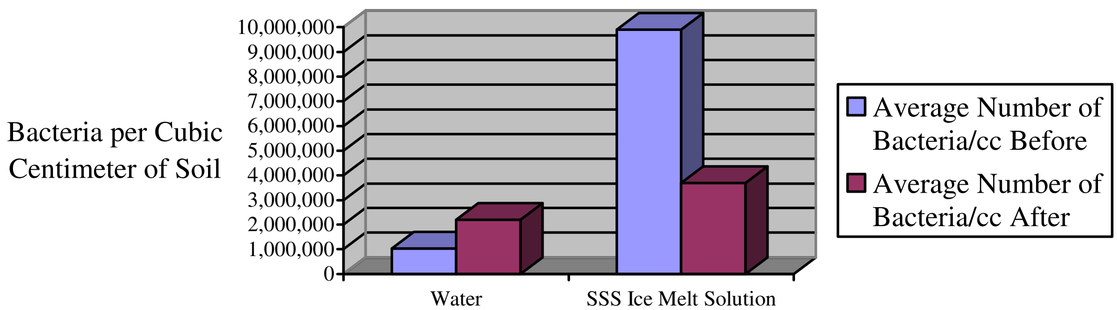
20. Label 4 culture tubes 10^{-0}, 10^{-1}, 10^{-2}, and 10^{-3}
21. Put 10 ml of sterile water into the culture tube labeled 10^{-0} and put 9 ml into the rest of the culture tubes
22. Take 1 cc of soil (the soil sample you use will change depending on which soil sample you are testing) and put into culture tube labeled 10^{-0} and shake vigorously.
23. Using a serological pipette take 1 ml of the solution in tube 10^{-0} and place into culture tube 10^{-1} and shake vigorously
24. Using a serological pipette take 1 ml of the solution in tube 10^{-1} and place into culture tube 10^{-2} and shake vigorously
25. Using a serological pipette take 1 ml of the solution in tube 10^{-2} and place into culture tube 10^{-3} and shake vigorously
26. Plate 100 micro liters from the 3rd and 4th tubes (10^{-2} and 10^{-3}) onto their own separate plates. Label each plate with the soil sample number, whether it is the soil sample before you add water or SSS ice melt solution, and the dilution number
27. Allow to incubate at room temperature for at least 48 hours
28. Examine each of the plates for individual bacteria colonies and choose the plate with the fewest colonies (but there must be at least five)
29. Count the number of colonies on the plates
30. Find (# colonies on plate X 10^2)($10^{|\text{# of dilutions}|}$) to learn how many bacteria were originally in the cc of soil
31. Record the number of bacteria colonies in 1 cc of soil and the pH level.

Data Table

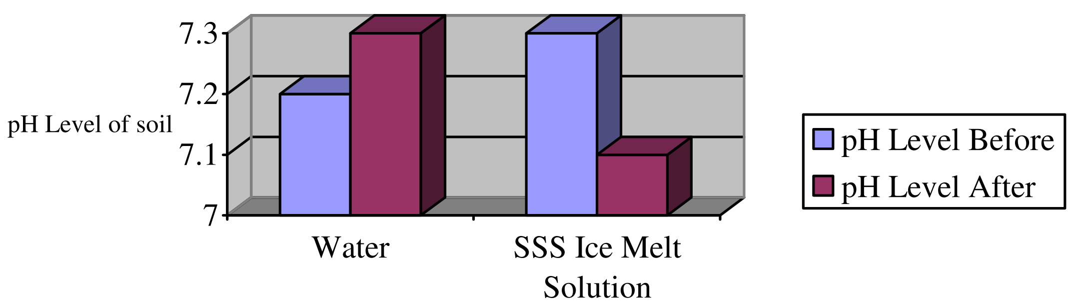
	Water				SSS Ice Melt Solution			
	Before	After	Before	After	Before	After	Before	After
	Num. of bacteria/ cc of soil	Num. of bacteria/ cc of soil	pH level of soil	pH level of soil	Num. of bacteria/ cc of soil	Num. of bacteria/ cc of soil	pH level of soil	pH level of soil
Sample 1	500,000	500,000	7.2	7.4	NA	NA	NA	NA
Sample 2	1,200,000	4,200,000	7.2	7.2	NA	NA	NA	NA
Sample 3	1,400,000	1,900,000	7.2	7.4	NA	NA	NA	NA
Sample 4	NA	NA	NA	NA	4,200,000	7,600,000	7.3	7.0
Sample 5	NA	NA	NA	NA	14,200,000	1,800,000	7.2	7.2
Sample 6	NA	NA	NA	NA	11,300,000	1,700,000	7.3	7.2
Average	1,033,333	2,200,000	7.2	7.3	9,900,000	3,700,000	7.3	7.1

Data

Number Bacteria Before and After Adding SSS Ice Melt Solution or Water



pH Level Before and After Adding SSS Ice Melt Solution or Water



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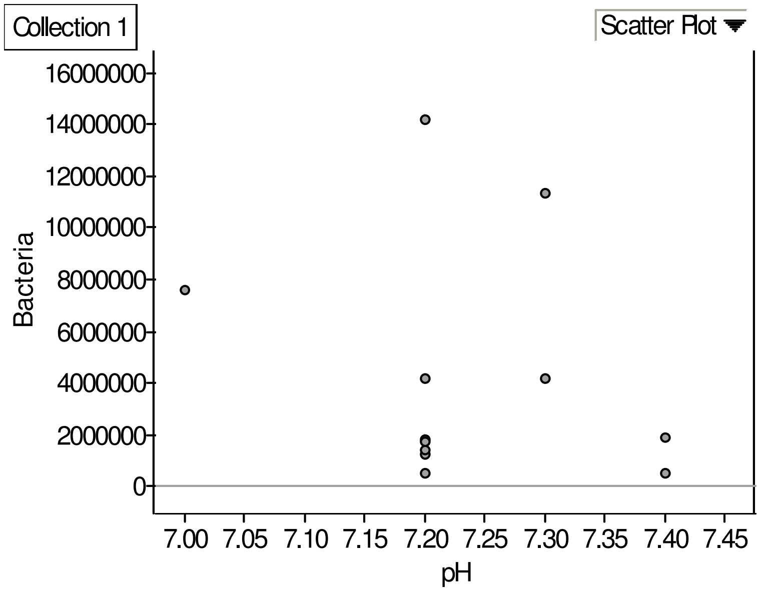
Bacteria Versus pH



$r^2 = .048632$

T-Test Results

Before and after bacteria counts with water: p = .39
pH before and after with water: p = .39
Before and after bacteria counts with SSS ice melt solution: p = .05
pH before and after with SSS ice melt solution: p = .17

Conclusion – In conclusion, our hypothesis was that if SSS ice melt is added to soil, then the bacteria count will decrease. According to our pH test and pH versus Bacteria graph, our hypothesis proved correct. Although the pH did not have anything to do with the bacteria count, the SSS ice melt did decrease the bacteria count in the soil greatly. The average number of bacteria in the soil plots before we added the SSS ice melt solution was 9,900,000 and the average number of bacteria in the soil after we added the solution was 3,700,000. This means that the average bacteria count decreased by 6,200,000, which is an astonishing amount of bacteria. The p values that we gathered based on the before and after bacteria values with water (.39), pH before and after with water (.18), before and after bacteria values with the SSS ice melt solution (.05), and pH before and after with SSS ice melt solution (.17) were low enough to allow us to conclude that our hypothesis was valid and that we should build off of our experiment. If we could redo our experiment we would be able to draw the same conclusions, but we would try to find a different environmental state other than pH that could affect or that has correlation with the pH.

WE HAVE ACTED HONORABLY

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