The Effect of Zinc Nitrate and Zinc Sulfate on the Population Density of Bacteria

Kalyani Ravi
Virginia Moore
Cameron Sieber
Brooke Hartley

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Background Information

The words “soil” and “dirt” have become coincident and easily exchangeable in the modern English language. A brown, muddy, earthworm-infested substance found in the outdoors has become a definition for both. But while dirt is a lifeless material of nonliving matter, soil is something alive and active. Soil is the elixir of life, keeping the everyday workings of the Earth’s ecosystem in motion. To disturb or destroy this indispensable, essential material could have catastrophic consequences. Motor vehicles, along with other pollutant makers, have led to excess emission of sulfur and nitrogen that then enters the soil. The surplus of sulfur and nitrogen could harm living organisms in the soil, such as bacteria, and decrease the overall health of the bionetwork.

Although small, bacteria are copious in the soil and diverse in function. They are prokaryotic organisms, generally one micron in size. Bacteria consist ofs four categories: decomposers, mutualists, pathogens, and lithotrophs (Soil Quality Institute, April 2004). The groupings of bacteria are based on their role in the ecosystem. Decomposers are responsible for breaking down simple carbon compounds (Soil Quality Institute, April 2004). Decomposing bacteria, through their consumption of simple carbon compounds, provide other soil organisms with access to energy from organic matter (Soil Quality Institute, April 2004).

Bacteria play an important role in the nitrogen cycle, a type of biogeochemical cycle dealing with the relationship of the geochemistry of a region and the animal and plant life of a region. The nitrogen cycle is important because nitrogen is required to make proteins, which make DNA, a component of all living matter. In the nitrogen cycle, much of the total fixed nitrogen in the ground is made up of dead organic matter

(Physicalgeography.net, February 16, 2004). Through nitrogen fixation, bacteria turns nitrogen gas in the air to ammonium in the ground. Decomposing bacteria also release ammonium compounds through the decomposition of organic matter. Through nitrification, ammonium is then converted into nitrate by autotrophic bacteria. (Physicalgeography.net, February 16, 2004) Of the nitrogen absorbed by plants, about 90% is in the form of nitrate (University of Missouri Columbia: Nitrate in Soils and Plants, 1999). Both ammonium and nitrate are removed by plant roots and combined with organic substances. Ammonium is use to make amino acids, monomers of enzymes (Physicalgeography.net, February 16, 2004). Enzymes control chemical reactions in living cells, thus allowing them to perform their major tasks to stay a live. Nitrate is combined with organic substances such as enzymes, proteins, and chlorophyll. The absence of ammonium and nitrate forms of nitrogen lead to a lack of chlorophyll. In turn, enzymes cannot control chemical reactions. The plant would die with out the initial help of bacteria which changes nitrogen into a usable form.

Excess ammonium, however, can be potent and harmful to the soil. Unused ammonium must be returned to the air through the process of denitrification. Here, heterotrophic bacteria reduce nitrate into nitrogen (N_2) or nitrous oxide (N_2O) gas and nitrogen is circulated through the air (Physicalgeography.net, February 16, 2004). In return for being so helpful in the nitrogen cycle, bacteria benefit from their work. Through denitrification, bacteria are given the oxygen they need in order to go through respiration. (Physicalgeography.net, February 16, 2004) Also, bacteria receive carbon compounds from plants and special structures with in the roots of the plant where they

can survive in moist environments. (University of Missouri Columbia: Nitrate in Soils and Plants, 1999)

Another important biogeochemical cycle in which bacteria play a role is the sulfur cycle. The majority of the planet's sulfur exists in rocks, salts, sediments buried with in the ocean (Lenntech, 1998). Both natural and human actions are responsible for releasing sulfur into the atmosphere (Lenntech, 1998). Humans often emit sulfur as sulfur dioxide through industrial processes (Lenntech, 1998). Sulfur dioxide reacts with oxygen to produce sulfur trioxide gas (SO_3), or it reacts with water to become sulfuric acid (H_2SO_4) (Lenntech,1998). These particles then settle back onto earth. Some particles react with rain in the atmosphere and return to earth as acid deposition. (Lenntech, 1998). Bacteria are involved in the cycle because they are responsible for turning sulfur into sulfates in the ground. Plants then incorporate the sulfates into amino acids, which make up proteins. (Lenntech, 1998) The sulfur, converted by bacteria, is necessary for the plant's survival.

Humans alter the nitrogen and sulfur cycle by moving large amounts of nitrogen and sulfur compounds into the air or water. Pollutants from car exhaust and coal send excess amounts of sulfur and nitrogen into the atmosphere. (Acid Rain: Water Science for Schools April 2, 2004) The sulfur is then returned to the ground through acid rain and absorbed into the soil. When acidic water runs over land, it has an impact on the wildlife living there. According to the U.S. Environmental Protection Agency (2002), "the degree of impact depends on the chemistry and buffering capacity of the soils involved." It has been determined by scientists that the chief sources of acid rain are sulfur dioxide and nitrogen oxide and around two-thirds of sulfur dioxide comes from electric power generation depending on the burning of fossil fuels. (U.S. Environmental Protection

Agency, 2002.) These gases react in the atmosphere with oxygen and water and then come back down to Earth in the form of precipitation**.** Acid deposition causes the acidification of many sensitive forest soils and exterminates the organisms living there. (U.S. Environmental Protection Agency, 2002.) Because decomposing bacteria are only active at a high pH, they are unable to function properly when the soil becomes acidic. The plant cannot obtain nitrogen and as a result, the plant's enzymes cannot function.

We know that both nitrogen and sulfur, combined with H_2O to form acid rain, decrease pH levels in the soil, thus disenabling the role of decomposing bacteria and also decreasing the bacteria population. Given that nitrogen and sulfur are emitted by cars at the Roland Park Country School campus, we wanted to look at which negatively charged ion (sulfate or nitrate), put in the soil by way of acid rain, is more harmful to the population density of bacteria in the soil. We plan to solve this problem by manipulating the amount of zinc sulfate and zinc nitrate compounds that different portions of soil receive. We believe that the zinc does little in the soil and will not affect soil bacteria population, therefore we will be able to look solely at the effects of the sulfate and nitrate. We know that increasing levels of nitrate will provide bacteria with carbon compounds and an environment where bacteria can grow and reproduce. We believe nitrate is more beneficial to bacteria than sulfate, and therefore, sulfate will be more harmful to the population of bacteria in the soil.

Lab Report

I. Problem: Which negatively charged ion (nitrate or sulfate) put into the soil by way of acid rain is more harmful to the population density of bacteria in the soil?

II. Hypothesis: Sulfate will be more harmful to the population density of bacteria in the soil than Nitrate.

III. Problem

A. Variables

Independent Variable: The type of negative ion found in different types of acid rain (nitrate or sulfate) added to the soil.

Dependent Variable$_1$: Population density of bacteria in soil
Dependent Variable$_2$: Amount of sulfate in the soil
Dependent Variable$_3$: Amount of nitrate in the soil

B. Controls

Negative Control$_1$: Putting no additional zinc sulfate or zinc nitrate into the ground (only putting water)
Negative Control$_2$: Taking samples before applying zinc sulfate, zinc nitrate, and water to the different plots

Control Variable List:

1. amount of time zinc sulfate and zinc nitrate sit in soil (48 hours)
2. same positively charged ion being used (zinc),
3. method of extracting soil
4. time soil samples are taken
5. size of soil sample extracted from ground (2.5 cm diameter X 10 cm height)
6. distance between samples taken
7. longitude and latitude at which samples are taken at
8. distances each plot are from each other
9. amount of zinc sulfate and zinc nitrate being added to the soil
10. distribution of zinc sulfate and zinc nitrate on soil
11. concentration of zinc sulfate and zinc nitrate added to the soil
12. weather conditions during which soil samples are taken
13. testing a given set of samples all at the same time
14. placing of each sample within each plot
15. balance used to weigh zinc sulfate and zinc nitrate
16. size of soil core sampler
17. LaMotte Kit used and supplies inside used for tests
18. size of soil sample used in serial dilutions (1 cc)
19. size of transformation tubes in serial dilutions
20. size of serological pipette used in serial dilutions
21. amount of soil/water mixture removed in each dilution (1 mL)
22. size of bacteria sample placed on Petrifilm® (100 µl)
23. amount of time before counting bacteria on Petrifilm (48 hours)

C. Procedure

Plot Set-up:
1. Choose three plots of grass in the school parking lot at location: 39.35817^{o} North and 076.6355^{o} West. Mark off each plot (preferably with a flag at each corner of the soon to be made square) so that its dimensions are 20 X 20 centimeters. Maintain 5 centimeters between each plot, making sure they are in a straight row.
2. Designate each of these plots to be the control and experimental plots
    Plot 1: Control
    Plot 2: Experimental-Zinc Sulfate
    Plot 3: Experimental-Zinc Nitrate

Extracting Soil:
3. Position yourself so that the plots are in a row in front of you. Be sure that Plot 1 is on the left, Plot 2 is in the middle, and Plot 3 is on the right. Find a soil core sampler with a diameter of 2.5 cm. Take the sample at the point of 5 cm from the left side (do not pretend that your left is the plots right, your left is the plots left) of the plot and 6 cm from the side of the plot farthest away from you. Twist the sampler until it is10 cm deep into the ground so that the sample is 10 cm of soil from Plot 1. Place the soil core sample in a clean, plastic Ziploc bag and label it as 1-1B (for plot 1, trial 1, before adding chemicals).
4. Extract a second sample of the same size (2.5 cm in diameter and 10 cm deep) in the same fashion as in step two placed at the point of 10 cm from the left side of the plot and 6 cm from the side of the plot farthest away from you. Place the soil sample in a clean, plastic Ziploc bag and label it as 1-2B (for plot 1, trial 2, before).
5. Extract a third sample of the same size (2.5 cm in diameter and 10 cm deep) at the point of 15 centimeters from the left side of the plot sample and 6 cm from the side of the plot farthest away from you. Place the soil sample in a clean, plastic Ziploc bag and label it as1-3B (for plot 1, trial 3, before).
6. Repeat step 3-5 for Plots 2 and 3. Change the labels on the plastic bags accordingly so that they are 2-1 B, 2-2B, 2-3B, 3-1B, 3-2B, and 3-3B. Make sure all samples from steps 2-5 are taken on the same day at the same time.

Testing for Sulfate, Nitrate, and Bacteria: ***note: Testing for sulfate, nitrate, and bacteria for any given sample must be done at the same time***
7. Use the *LaMotte STH Series* of professional soil test. Perform the Sulfate test on each of the soil samples taken at each plot (1-1B, 1-2B, 1-3B) to find the amount of sulfate in each soil sample. Record data.
8. Use the *LaMotte STH Series* of professional soil test. Perform the Nitrate test on each of the soil samples taken at each plot (1-1B, 1-2B, 1-3B) to find the amount of nitrate in each soil sample. Record data.
9. Using a 1 cc soil scoop, take a 1 cc soil sample from the 1-1B bag. Place this sample into a 15 mL transformation tube. Add 10 mL of sterile water; cap the tube and shake vigorously.

10. Using a serological pipette, remove 1 mL of the soil/water mixture from the first transformation tube and place into a fresh, second transformation tube.
11. Add 9 mL of fresh sterile water to this second tube; cap and shake vigorously.
12. Repeat step 10 using the second, diluted tube and then repeat step 11 with a third tube.
13. Continue with step 12 with each additional tube until you have diluted the original soil/water mixture a minimum of four times (a 10^{-4}) dilution. You should now have a total of five culture tubes.
14. Place 100 µl samples from the 4^{th} and 5^{th} tubes (dilutions 10^{-3} and 10^{-4}) onto their own Petrifilm® and allow to sit at room temperature for 48 hours.
15. After 48 hours, examine each of the plates for individual bacteria colonies and choose the plate with the fewest colonies to make your estimates of the number of bacteria in the original 1 cc soil sample. Use the following formula to find the number of bacteria in the original soil sample:

$$\#\text{ colonies on plate} \times 10^{2} \times 10^{[\#\text{ of dilutions}]}$$

16. Repeat steps 7-15 with all other soil samples from Plots 2 and 3.

Making Zinc Sulfate and Zinc Nitrate Solutions:s
17. Fill three plastic bottles with 1 liter of water each, labeling the bottles as P1Control, P2Sulfate, and P3Nitrate.
18. Use a balance and a weigh boat to hold material being weighed. Place weigh boat on scale and zero out. The balance used must be able to go into the hundredths of a gram. Using a scoop, carefully pour the Zinc Sulfate powder into the weigh boat so that its weight equals .02g.
19. Use the same balance and a new weigh boat to weigh the Zinc Nitrate powder. Place weigh boat on scale and zero out. Using a scoop, carefully transfer the Zinc Nitrate powder into the weigh boat so that its weight equals .01g.
20. Pour the .02g of Zinc Sulfate powder into the P2Sulfate bottle with 1 liter of water. Close the bottle and shake vigorously for forty-five seconds.
21. Pour the .01g of Zinc Nitrate powder into the P3Nitrate bottle with 1 liter of water. Close the bottle and shake vigorously for forty-five seconds.
22. Pour the contents of each bottle directly into the center of its corresponding plot of soil.s
23. Wait 48 hours.

Extracting Soil After:
24. Repeat step 3 for Plot 1 with some exceptions. Take the sample at the point of 6 cm from the side of the plot closest to you and 5 cm from the left side of the plot. Place the soil sample in a clean, plastic Ziploc bag and label it 1-1A (for plot 1, trial 1, after adding chemicals).
25. Extract a second sample in the same fashion as in step 3, placing it at the point of 6 cm from the side of the plot closest to you, and 10 cm from the left side of the plot. Place the soil sample in a clean, plastic Ziploc bag and label it 1-2A (for plot 1, trial 2, after).
26. Extract a third sample in the same fashion as in step 3, placing it at the point of 6 cm from the side of the plot closest to you, and 15 cm from the left side of

the plot. Place the soil in a clean, plastic Ziploc bag and label it 1-3A (for plot 1, trial 3, after).
27. Repeat step 24-26 for the second and third plots. Change the labels on the plastic bags accordingly so that they are 2-1A, 2-2A, 2-3A, 3-1A, 3-2A, and 3-3A.
28. Repeat steps 6-14 with all samples from the three plots after adding zinc sulfate solution and nitrate solution and just water.

29. Make 3 new plots near the first three. Set plots up at location 39.35816^{o} North and 076.6356^{o} West. Mark off each plot (preferably with a flag at each corner of the soon to be made square) so that its dimensions are 20 X 20 centimeters. Maintain 5 centimeters between each plot, making sure they are in a straight row. Repeat steps 2-28. Mark off each plot (preferably with a flag at each corner of the soon to be made square) so that its dimensions are 20 X 20 centimeters. Maintain 5 centimeters between each plot, making sure they are in a straight row.
(Time allowing, repeat steps 1-28 to get as many possible trials. For each additional repetition, use three new plots to take samples)

NOTE
The tests for Nitrate and Sulfate are from the Lamotte STH Series of professional soil testing outfits.

IV. Data and Analysis

Negative Control – Plot # 1 Before and After Adding Water

Trial #	Sample # Before	Bacteria Pop. Dens. In Soil (cfu/cm^3)	Nitrate (ppm)	Sulfate (ppm)	Sample # After	Bacteria Pop. Dens. In Soil (cfu/cm^3)	Nitrate (ppm)	Sulfate (ppm)
1	1-1B	5×10^6	10	250	1-1A	1.1×10^7	10	250
1	1-2B	8×10^6	20	500	1-2A	5×10^6	5	100
1	1-3B	9×10^6	10	200	1-3A	5×10^5	20	100
2	1-1B	1.3×10^7	5	100	1-1A	9×10^6	2.5	250
2	1-2B	5×10^6	2.5	250	1-2A	5×10^6	10	250
2	1-3B	6×10^6	2.5	100	1-3A	8×10^6	5	100

Sulfate Plot- Plot #2 Before and After Adding Zinc Sulfate

Trial #	Sample # Before	Bacteria Pop. Dens. In Soil (cfu/cm^3)	Nitrate (ppm)	Sulfate (ppm)	Sample # After	Bacteria Pop. Dens. In Soil (cfu/cm^3)	Nitrate (ppm)	Sulfate (ppm)
1	2-1B	1.2×10^7	5	250	2-1A	4.2×10^6	20	100
1	2-2B	1.15×10^6	20	100	2-2A	4.1×10^6	15	250

1	2-3B	1.5×10^7	10	100	2-3A	5×10^5	10	100
2	2-1B	5×10^6	5	250	2-1A	5×10^6	7.5	150
2	2-2B	1.2×10^6	5	500	2-2A	2.7×10^6	7.5	500
2	2-3B	8×10^5	5	100	2-3A	1.9×10^6	5	100

Nitrate Plot – Plot #3 Before and After adding Zinc Nitrate

Trial #	**Sample # Before**	**Bacteria Pop. Dens. In Soil (cfu/cm^3)**	**Nitrate (ppm)**	**Sulfate (ppm)**	**Sample # After**	**Bacteria Pop. Dens. In Soil (cfu/cm^3)**	**Nitrate (ppm)**	**Sulfate (ppm)**
1	3-1B	6×10^6	30	100	3-1A	2×10^7	20	100
1	3-2B	9×10^6	30	250	3-2A	1.2×10^7	20	100
1	3-3B	7×10^6	30	250	3-3A	1×10^6	15	100
2	3-1B	6.6×10^6	10	500	3-1A	5.2×10^6	5	150
2	3-2B	1.2×10^6	5	250	3-2A	5×10^5	10	150
2	3-3B	9×10^6	10	250	3-3A	1.8×10^7	5	250

Average Data for Negative Control Plot (Plot #1)

When Sample Taken (before or after adding water)	**Bacteria Number**	**Nitrate (Parts/Million)**	**Sulfate (Parts/Million)**
Before	7666666	8	233
After	7.75×10^6	9	175

Average Data for Sulfate Plot (Plot # 2)

When Sample Taken (before or after adding zinc sulfate)	**Bacteria Number**	**Nitrate (Parts/Million)**	**Sulfate (Parts/Million)**
Before	5858333	8	217
After	3066666	11	200

Average Data for Nitrate Plot (Plot #3)

When Sample Taken (before or after adding zinc nitrate)	**Bacteria Number**	**Nitrate (Parts/Million)**	**Sulfate (Parts/Million)**
Before	6466666	19	267
After	9.45×10^6	12.5	141

Average Amount of Nitrate in Soil for Plots 1-3 Before and After Adding Water, Zinc Sulfate and Zinc Nitrate

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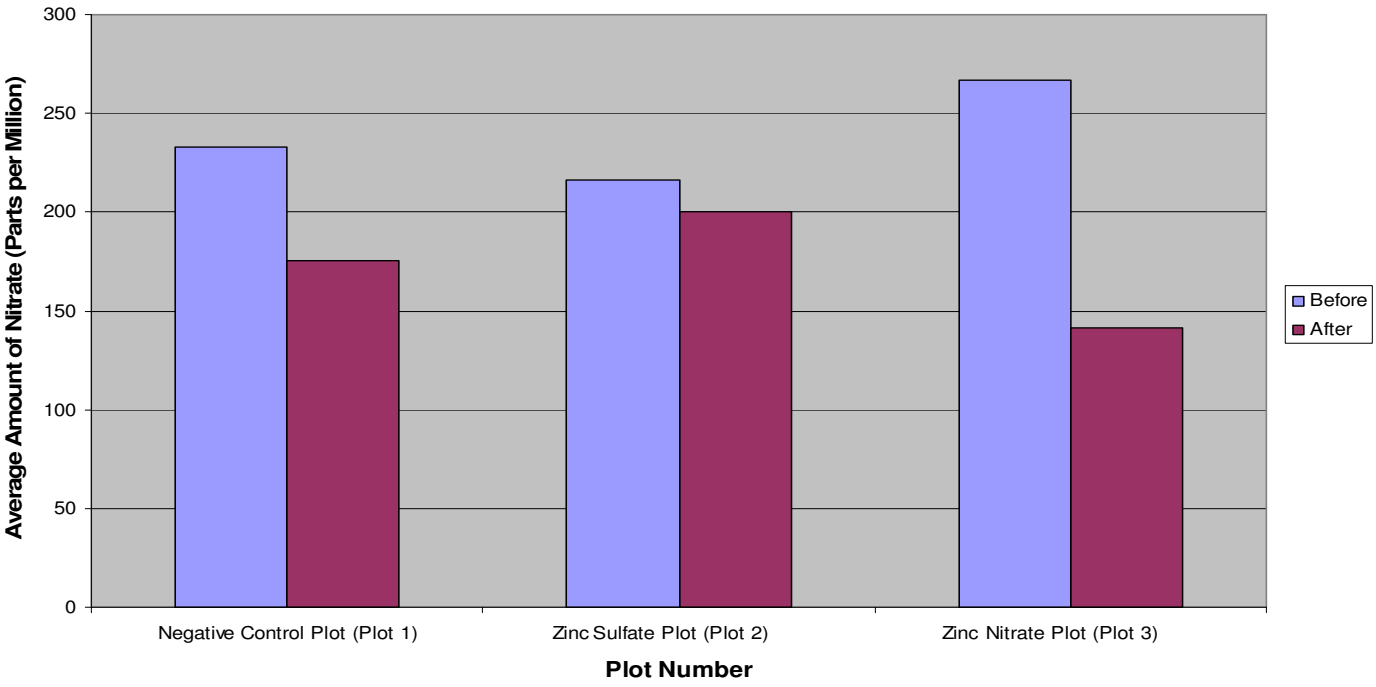

According to the bar graph showing the average amounts of nitrate in the soil for plots one, two, and three before and after adding water/zinc sulfate/zinc nitrate, the negative control plot had an average of 233 parts per million of nitrate before adding water. After adding water, this plot had an average of 175 parts per million of nitrate. 233 parts per million is not equal to 175 parts per million, so it was necessary to do corrected differences. Corrected differences were necessary because when the negative control changed, it meant that something else in the environment caused the nitrate to change in amount. In order to accurately compare experimental results to the negative control, the negative control must not change. If it does change, then the negative control must have a corrected difference, which applies the amount of change from the negative control to the other variables. In this situation, there is a corrected difference of 58 parts per million. It was necessary to add 58 parts per million to the average amount of nitrate after applying zinc sulfate in plot 2 and the average amount of nitrate after applying zinc nitrate in plot 3. Before adding zinc sulfate to plot two, there was an average of 216 parts per million of nitrate in the plot. After adding zinc sulfate to plot two, there was an average 19.4 % (42 parts per million) increase of nitrate and there was an average of 258 parts per million of nitrate in the plot. Before adding zinc nitrate to plot three, there was an average of 267 parts per million of nitrate in the plot. After adding zinc nitrate to plot three, there was an average 25.46 % (68 parts per million) decrease of nitrate and there was an average of 199 parts per million of nitrate in the plot. With correctional differences, the graph shows that adding zinc sulfate in plot 2 increased the average amount of nitrate in parts per million while adding zinc nitrate in plot 3 decreased the average amount of nitrate in parts per million.

Average Amount of Sulfate in Soil for Plots 1-3 Before and After Adding Water, Zinc Sulfate, and Zinc Nitrate

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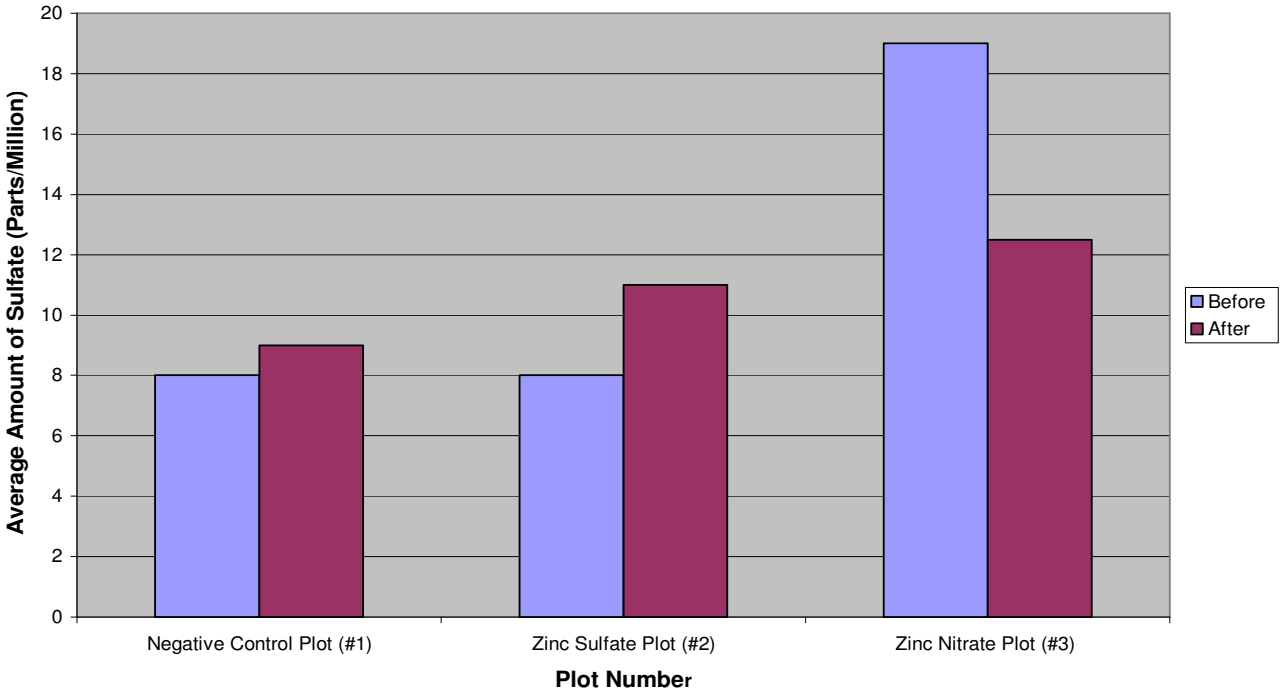

According to the bar graph showing the average amount of sulfate in the soil for plots one, two, and three before and after adding water/zinc sulfate/zinc nitrate, the negative control plot had an average of 8 parts per million of sulfate in it before adding water. After adding water, the negative control plot had an average of 9 parts per million of sulfate in it. Therefore, it was necessary to do corrected differences. There was a corrected difference of 1 part per million. In this situation, it was necessary to subtract 1 part per million from the average amount of sulfate after applying zinc sulfate in plot 2 and from the average amount of nitrate after applying zinc nitrate in plot 3. In plot two, before adding zinc sulfate, there was an average of 8 parts per million of sulfate in the soil. After adding zinc sulfate, the sulfate content increased by an average of 25 % (2 parts per million), and there was an average of 10 parts per million of sulfate in the soil. Before adding zinc nitrate to plot three, there was an average of 19 parts per million of sulfate in the soil. After adding zinc nitrate to plot three, the sulfate content decreased by an average of 39.47 % (7.5 parts per million) and there was an average of 11.5 parts per million of sulfate in the soil. With correctional differences, the graph shows that adding zinc sulfate in plot 2 increased the average amount of sulfate in parts per million while adding zinc nitrate in plot 3 decreased the average amount of sulfate in parts per million.

Average Amount of Bacteria in Soil (Plots 1-3) Before and After Adding Water, Zinc Nitrate, and Zinc Sulfate

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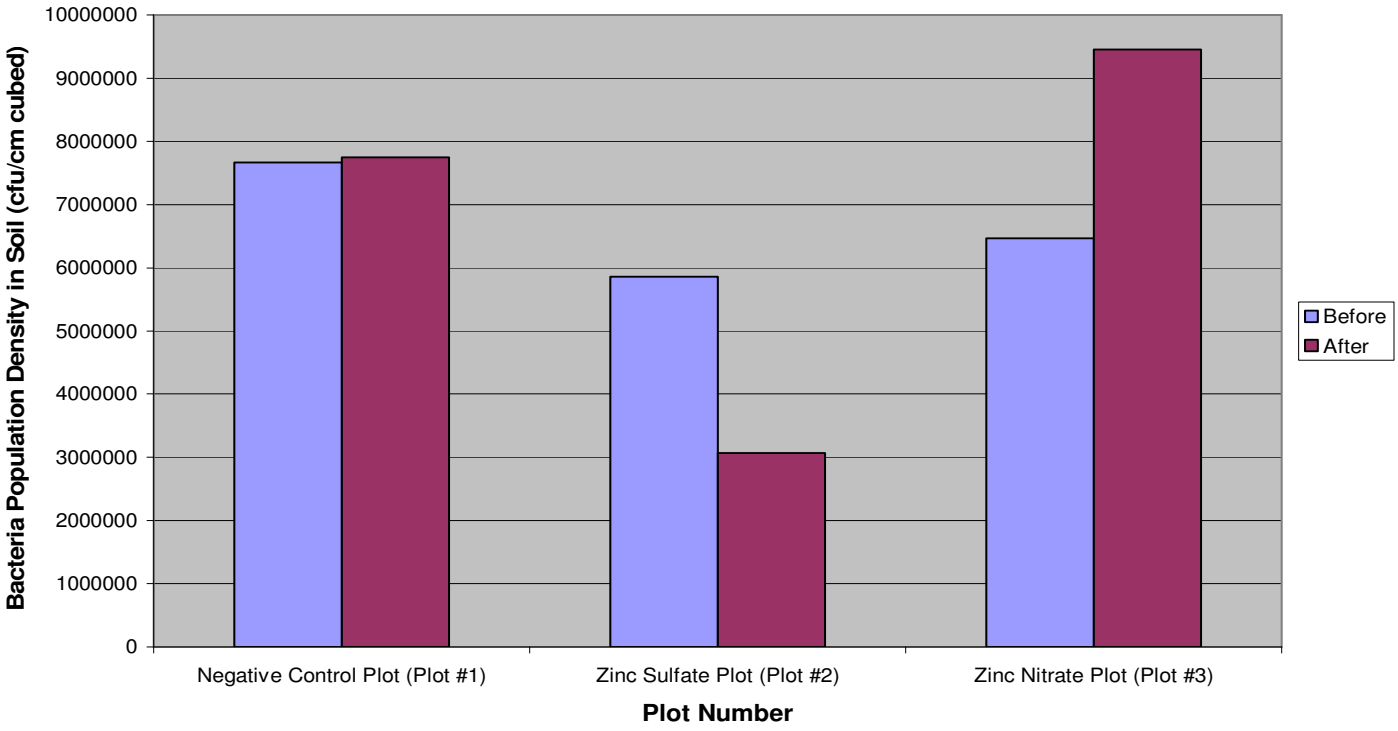
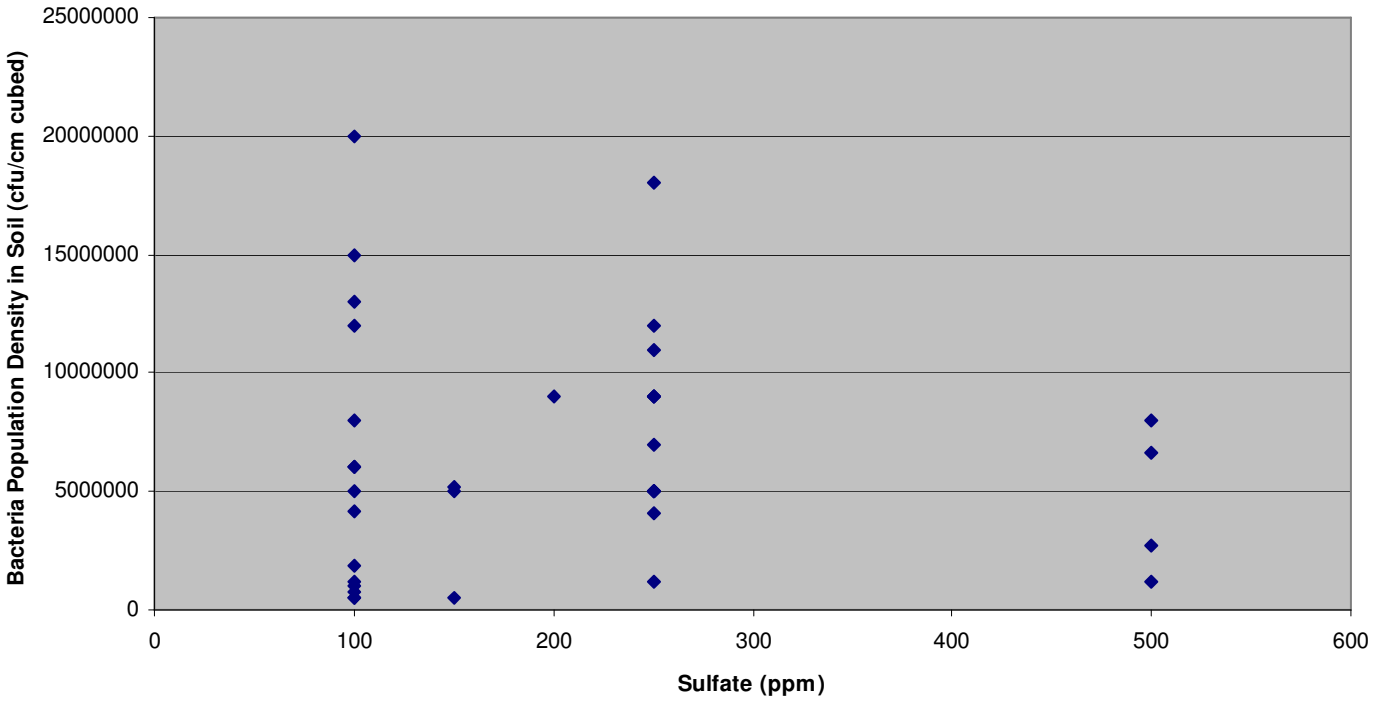
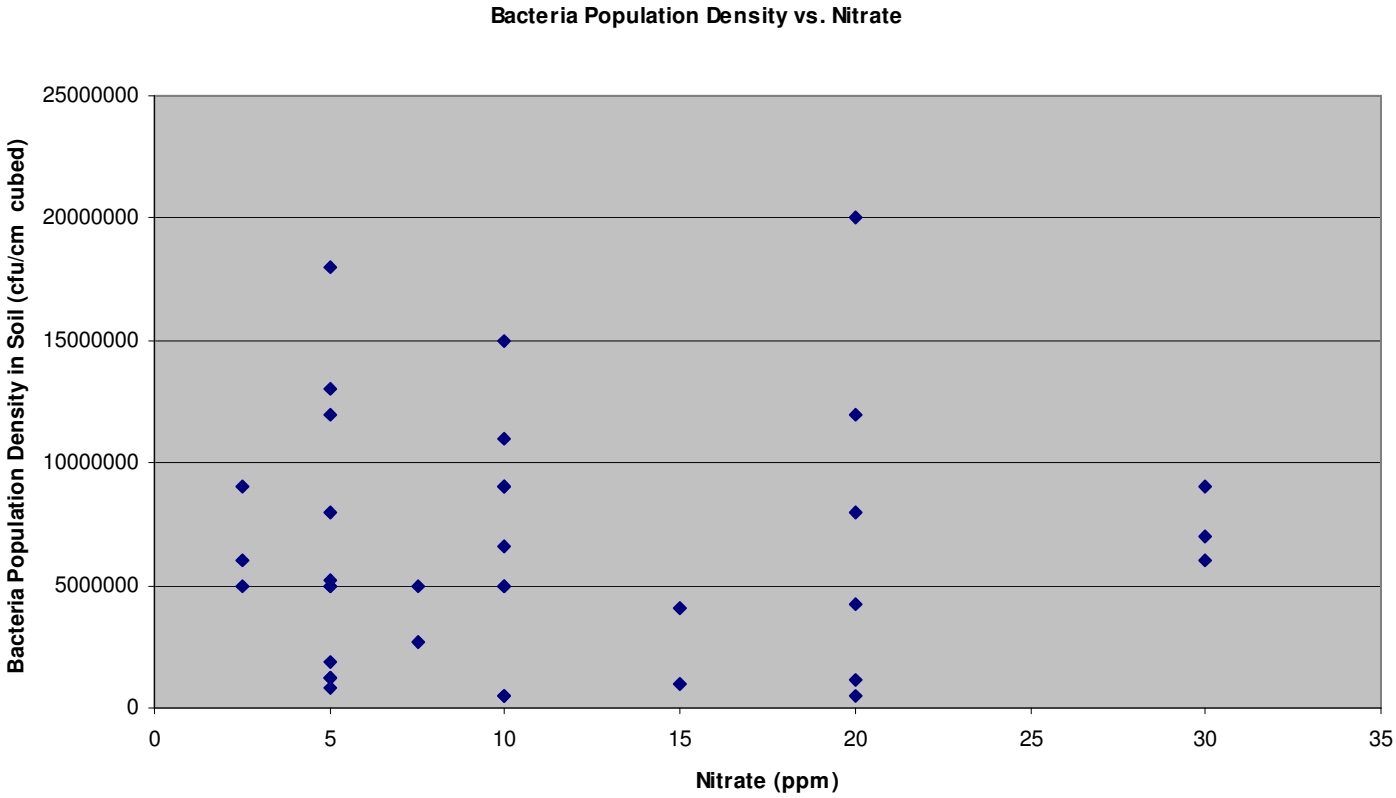


According to the graph showing the average amount of bacteria in soil for plots one, two, and three before and after adding water/zinc sulfate/zinc nitrate/, the bacterial population density was 7,666,666 colonies per one cubic centimeter of soil before adding water to the negative control plot. After the water was added, the bacteria population density increased by an average of .01% (83,334 colonies) and there was an average of 7,750,000 colonies per one cubed centimeter of soil in the negative control plot. A .01% increase is so small that the population density of bacteria stayed almost equal (therefore there was no need for a correctional difference). Before adding zinc sulfate to plot 2, the population density of bacteria was an average of 5,858,333 colonies per one cubic centimeter of soil. After the zinc sulfate was added to the plot, the population density of bacteria, which decreased by an average of 46.65% (2,791,667 colonies), was an average of 3,066,666 colonies per one cubic centimeter of soil. Before adding zinc nitrate to plot 3, the population density of bacteria was an average of 6,466,666 colonies per one cubic centimeter of soil. After the zinc nitrate was added to the plot, the population density of bacteria, which increased by an average of 46.13% (2,983,334 colonies), was an average of 9,450,000 colonies per one cubic centimeter of soil. The graph shows that adding zinc sulfate in plot 2 decreased the average population density of bacteria in the soil while adding zinc nitrate in plot 3 increased the average population density of bacteria in the soil.



According to the scatter plot comparing Bacteria Population Density versus the amount of Sulfate in the soil, there was a trend. We believe that there is a negative correlation between sulfate and bacteria population. This trend is as the sulfate increases in parts per million, the population density of bacteria (cfu/cm^3) decreases. We believe this because statistically, the r^2 value was .0013.



According to the scatter plot comparing Bacteria Population Density versus the amount of nitrate in the soil, there was a trend. We believe that there is a positive correlation between nitrate and bacteria population. This trend is as the nitrate increases in parts per million, the population density of bacteria (cfu/cm^3) increases as well. We believe this because statistically, the r^2 value was .0042.

V. Conclusion

Our hypothesis that zinc sulfate was more harmful than zinc nitrate to the population density of bacteria was correct. Looking at the average levels of nitrate in the soil, adding zinc sulfate increased the average amount of nitrate from 216 ppm to 258 ppm (a 42 ppm increase). Looking at the average levels of sulfate in the soil, adding zinc sulfate increased the average amount of sulfate from 8 ppm to 10 ppm (a 2 ppm increase). Looking at the average population density of bacteria per cfu/cm^3, adding zinc sulfate decreased the average population density of bacteria from 5,858,333 colonies per one cubic cm of soil, to 3,066,666 colonies per one cubic cm of soil (a 2,791,667 cfu/cm^3 decrease). Our scatter plot supports the average decrease in bacteria with the addition of zinc sulfate because the correlation between sulfate levels and bacteria was negative (as the zinc sulfate increased, the bacteria population decreased). We can conclude that increased amounts of zinc sulfate (beyond what is already in the soil) are harmful to bacteria because they caused a dramatic population decrease of bacteria in the soil. We believe that because bacteria were dying, average levels of nitrate rose due to the fact that decomposing bacteria provide the soil with raised levels of nitrate. We believe that the average levels of sulfate rose in correspondence with the fact that the level of sulfur was increased by pouring zinc sulfate on the plot.

Looking at the average levels of nitrate in the soil, adding zinc nitrate decreased the average amount of nitrate from 267 ppm to 199 ppm (a 68 ppm decrease). Looking at the average levels of sulfate in the soil, adding zinc nitrate decreased the average amount of nitrate in the soil from 19 ppm to 11.5 ppm (a 7.5 ppm decrease). Looking at the average population density of bacteria per cfu/cm^3, adding zinc nitrate increased the average population density of bacteria from 6,466,666 colonies per one cubic centimeter of soil, to 9,450,000 colonies per one cubic cm of soil (a 2,983,334 cfu/cm^3 increase). We can conclude that increased amounts of zinc nitrate (beyond what is already in the soil) help the growth of bacteria. We believe that because bacteria were increasing, nitrate levels decreased, due the fact that nitrate was being used for carbon compounds and carbohydrates to supply the bacteria with energy. We also believe that sulfate levels decreased because sulfate was being used to make proteins which allowed the bacteria to reproduce and increase as they did. The decreased levels of sulfate and nitrate after zinc nitrate was poured on the soil make sense as what would be expected given the sulfur and nitrogen cycles. Sulfur and Nitrogen enter the ground, are converted to usable forms, and then used by bacteria, plants, and other organisms for proteins and energy. Pouring zinc nitrate in the ground would supply bacteria with more energy from the nitrate. With the increase in bacteria, sulfate and nitrate levels would decrease because they were used by the bacteria.

From our data we can conclude that sulfur was indeed more harmful to the population density of bacteria in the soil than nitrate. Given this information, we can

further conclude that the sulfur component of car emissions at Roland Park Country School is harmful to bacteria population, while the nitrate component of car emissions is helpful. Our next step in further research would be to investigate why excess amounts of added sulfate are so harmful to bacteria population density given the fact that sulfate is usually helpful in creating proteins for living organism. More specifically, we could look at what component of the sulfate is harming the bacteria.

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