SOIL ECOLOGY PROJECT:

THE EFFECTS OF PESTICIDES VS. HERBICIDES ON THE POPULATION DENSITY OF SOIL BACTERIA

By Molly Beidleman, Allie Graul, and Nora Feinberg

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Soil Ecology Background

Bacteria are some of the tiniest and most abundant microbes in the Earth's soil. Living mainly in the top 10 cm where most of the organic matter is present (Reid & Wong, 2005), these prokaryotic organisms perform a wide range of chemical transformations from the degradation of organic matter to disease suppression to nutrient transformations inside of plant roots. However, the bacteria in the soil mainly transform inorganic constituents from one chemical form to another, making all of these nutrients available to the other organisms in the ecosystem (Lavelle & Spain, 2001).

Because of their many functions, soil bacteria are traditionally categorized into one of four major groups. One of these, the decomposers, consume carbon compounds and convert energy from organic matter in the soil into forms that other organisms living there can use (Ingham, 2013). To do this, they produce enzymes which start the chemical reactions that convert the biological polymers of the decaying matter into their monomers (i.e. break down the chemical compounds of the organic matter into smaller and smaller molecules), and as these enzymes break down the chemical compounds, the bacteria use some of the newly created substances for their own growth and reproduction and some of it is left in the soil for other organisms to use. Finally, when bacteria die, their own cells decompose and the nutrients within them are then also available to the plants and other soil organisms (Abbott, 2013).

Another group of bacteria, the mutualists, form partnerships with plants, and the most common of these are those associated with the Nitrogen fixing process. Certain species called nitrifying bacteria "are the microorganisms that change the nitrogen gas found in the atmosphere

into first ammonium then ultimately to nitrate, the preferred form by plants" (DeFelice, Wollenhaupt, & Buchholz, 1993) since it is more easily leached from the soil. Any excess nitrate not consumed by the plants is then reconverted to the nitrogen in the air by the denitrifying bacteria (Ingham, 2013).

Nitrogen is so vital for roots and plants as a whole because DNA, the molecule that controls all life processes, is composed of nitrogenous base pairs. DNA, in turn is transcribed into RNA and then translated into enzymes which are also made with nitrogen. These special proteins start and stop chemical reactions between the biochemicals that form living matter; hence without the nitrogen to build them and the genetic instructions for them, the proteins responsible for the chemical reactions that cause the four cellular tasks to happen (respiration, reproduction, homeostasis, and synthesis) would cease and the cells of plants (and therefore the plants) would die.

Plants, though, are important to the ecosystem as a whole because they are consumed by animals which get their nitrogen for their DNA and proteins from those plants. The animals then produce waste, and the soil bacteria feed upon this waste, converting the organic nitrogen in the waste into ammonium and then into the nitrate, which the remaining plants can use. Furthermore, once both plants and animals die, the bacteria decompose their dead bodies into ammonium as well and the cycling of the nitrogen continues. Hence, an ecosystem would collapse without bacteria.

On a smaller scale, this impact bacteria have on the environment is readily apparent in a very ordinary human activity, growing a garden. In the US, gardening is one of the most common hobbies, and in fact, 67% of American's have a garden. Yet when planting a garden, one of the

most common things people first buy is an assortment of herbicides and pesticides in order to reduce the harm other organisms can cause to their plants. Indeed, in 2000, homeowners spent 11 billion dollars on these toxic chemicals to destroy weeds and other unwanted vegetation (Niehs.nih.gov, 2012) in order to protect their hard work (Sadorf, O'Neil, 2000). Of these, herbicides are the most commonly used and usually contain a mixture of phenoxy compounds, phenyl acetic acid, benzoic acid, pthalic acid, and many other chemicals that, like pesticides, affect nuisance organisms by poisoning them (AgriInfo, 2013). The main chemical in herbicides, though, is glyphosophate which "kills plants and bacteria by inhibiting the bacterial and plant enxyme enolpyruvylshikimate-phosphate synthase, or the enzyme in the plant that allows it to grow and reproduce" (Heitkamp, Adams, and Hallas, 1992).

Yet, while the chemicals in pesticides and herbicides are known to harm their intended recipients in gardens, there is some debate about their impact on the bacteria living in the soil. According to Martin T.K. Tsui and L.M. Chu (2003), "bacteria should be much more tolerant to the toxicity of the IPA salt of glyphosophate" found in most herbicides, and the Annie Apple Seed project cites Rick Holley, a professor of food microbiology at the University of Manitoba in Canada, claiming that "what we found was that in four of those pesticides, including at least one in each category, the bacteria in 24 hours increased 1,000-fold, and if we held it longer it increased 10,000-fold" (Fonfa, 2012).

What is more, all these pesticides and herbicides may also be having other unintentional consequences; such they could be potentially disrupting the nitrogen cycle. Therefore, some species of soil bacteria are already fragile and can be killed by very slight changes in the environment of the soil, including differences in soil temperature, carbon substrate, or moisture

that can cause the bacteria population to grow or shrink in just a few days (Reid & Wong, 2005). Since "the chemical content of pesticides is known to be harmful to bacteria and other microorganisms in the soil" (Niehs.nih.gov, 2012), the possible disruptions by the application of pesticides and herbicides could have enormous consequences. Without the bacteria to convert the inorganic nitrogen for the plant's use, plants can die, causing all the consequent disruptions to the ecosystem as a whole.

That includes here at Roland Park Country School. Roland Park applies pesticides and herbicides in order to maintain the lush front lawn and knowing that, we decided to test the effects of pesticides and herbicides on the soil in that front lawn. Specifically, we focused on the effects of pesticides and herbicides on soil bacteria. Although not necessarily the targeted subject, we wanted to see what, if any, harmful effect the herbicides and pesticides might have on the bacteria living in the front lawn. We set up 9 plots 3 of which would act as our negative control or the soil not treated with an herbicide or pesticide, 3 plots which would be treated with an herbicide, and 3 plots with a pesticide, and we tested the soil from these for bacterial density. We hypothesized that pesticides and herbicides will increase the growth rate of bacteria but that the pesticide will stimulate the growth rate faster than that of the herbicide.

Lab Report

Problem- Which will lead to a greater increase in the population density of bacteria in soil, pesticides or herbicides?

Hypothesis – Pesticides will lead to a greater increase in the population density of bacteria in the soil.

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Procedure:

A. Independent variable – type of chemical the soil is exposed to (herbicides or pesticides.)
B. Dependent variable – the amount of bacteria per cubic centimeter in soil.
C. Negative control – the soil without a chemical present and only water added.
D. Controlled variables –
    - Amount of chemical added to each soil plot
    - Type of herbicide
    - Type of pesticide
    - Amount of soil extracted
    - Type of GPS device
    - Size of pipettes for serial dilutions
    - Size of soil plot
    - Soil samples taken from the RPCS lawn at N39^{o} 21.494^{o}, W076^{o} 38.167 on the same day at the same time.
    - Size of culture tubes
    - Type of nutrient agar
    - 100 μL of solution dilution placed on agar plate
    - Size of petri dishes
    - Time allowed for nutrient agar plates to grow
    - Amount of soil sample put in the first dilution tube
    - Amount of water in the different dilution tubes
    - The level to which diluted (10^{-4}) and which dilution plated.

E. Step by step procedures –

1. Locate a 90 cm x 90 cm plot of soil on the RPCS lawn at N39^{o} 21.494, W076^{o} 38.167.
2. Section it off into 30 cm x 30 cm sections according to the following diagram and mark each section with one flag directly in the center of each plot, labeling according to the following diagram.

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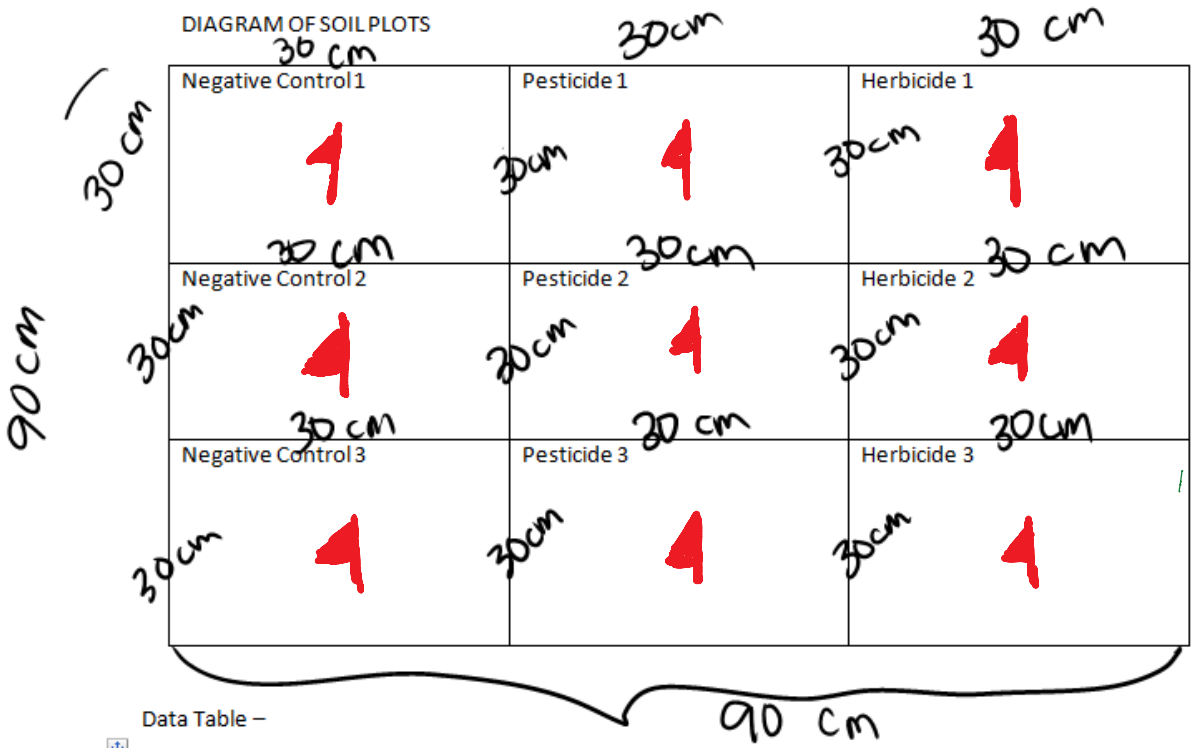

3. Steps 4-7 should be done on the same day at the same time.
4. Put the soil extractor 15 ½ centimeters deep and 2 centimeters wide into the first soil plot, twisting clockwise, and bring the soil up.
5. Put the soil sample in a plastic bag, labeled according to the diagram noting that this sample has been taken before the experiment and with an "A" to show that it is the first sample from this specific plot (e.g. "before Pesticide 1A").
6. Repeat steps 4-5 twice on the same soil plot, writing a "B" the second time and a "C" the third time instead of an "A"
7. Repeat steps 4-6 on the eight remaining soil plots.
8. Perform the serial dilutions for bacteria on all 27 samples, in order to find how much bacteria is located in the soil before the experiment.
9. In order to perform the serial dilutions, perform these instructions:

    A. All of B-R steps must be performed on the same day at the same time for all soil samples.
    B. Begin by testing negative control sample 1A

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C. Use a clean, new transfer pipette to add 10 ml of water to a 15 ml culture tube. Label this tube "10^0 before negative control 1A."
D. Use the same pipette to add 9 ml of water to a second 15 ml culture tube. Label the tube "10^{-1} before negative control 1A."
E. Repeat step C three more time to three additional 15 ml culture tube, only label them "10^{-2} before negative control 1A.", "10^{-3} before negative control 1A."
F. Using a 1 cc scoop, place 1 cc of negative control 1A sample into the "10^0 before negative control 1A" tube
G. Cap the tube and shake vigorously.
H. Using a new clean pipette, remove 1 ml of the soil/water mixture from the 10^0 before negative control 1A" tube with a serological pipette and place it in the "10^{-1} before negative control 1A."
I. Cap and shake vigorously.
J. Using the same pipette in step H, remove 1 ml of the soil/water mixture from the "10^{-1} before negative control 1A" tube and place into the "10^{-2} before negative control 1A."
K. Cap and shake vigorously.
L. Using the same pipette in step H, remove 1 ml of the soil/water mixture from the "10^{-2} before negative control 1A" tube and place into "10^{-3} before negative control 1A" tube.
M. Cap and shake vigorously.
N. You should now have a total of four culture tubes.
O. Repeat steps A-N with the negative control samples 2A and 3A, the pesticide samples 1A, 2A, and 3A, and the herbicide samples 1A, 2A, and 3A, labeling accordingly, for a total of nine soil samples.
P. Repeat steps A-O with all the B and C samples.
Q. Label 3M Petrifilm™ Aerobic Count Plates to correspond to the labeled 10^{-2} and 10^{-3} dilution tubes for all soil samples
R. Plate 100 microliter samples from the 3rd and 4th tubes (dilutions "10^{-2} before negative control 1A" & "10^{-3} before negative control 1A") onto their own separate correspondingly labeled 3M Petrifilm™ Aerobic Count Plate.
S. Allow plates to grow for 48 to 72 hours.
T. Examine the 10^{-2} and 10^{-3} plates for individual bacteria colonies. If the 10^{-3} plate has at least 5 colonies 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 $10^{|\text{dilution\# at which these colonies were found}|}$

U. If the 10^{-3} plate does not have at least 5 colonies, use the10^{-2} plate

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10. After the amount of bacteria in soil is found, record the data.
11. To make 1/5 liter of the "Ortho Weed B Gon" herbicide solution, combine 4 ml of herbicide solution with 204 ml of water in a bottle, shake, and label herbicide
12. Pour 1/5 liter of Bayer pesticide into a bottle and label it pesticide.
13. Pour 1/5 liter of distilled water into a bottle and label it water.
14. Go back out to plots.
15. With a pipette, place 9 ml of herbicide solution directly in the center, where the flag is, on each 900 cm^2 plot marked herbicide.
16. With a different pipette, place 9ml of Bayer pesticide directly in the center, where the flag is, on each of the other designated 900 cm^2 plots marked pesticide.
17. With a different pipette, place 9ml of the water directly in the center, where the flag is, on each of the other designated 900 cm^2 plots marked negative control.
18. Let stand for 48 hours.
19. After 48 hours, return to the soil plot and repeat steps 3-7 with all of the "After" samples.
20. Repeat step 9 with these samples omitting the word "before" on your labels and add the word "after"

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DATA AND ANALYSIS:

Impact of Herbicides and Pesticides on the Density of Soil Bacteria

Trial	Trial #	Negative	Herbicide	Pesticide
Trial A before	1	19000000 #/ cc of soil	14900000 #/cc of soil	10700000 #/cc of soil
	2	5900000 #/ cc of soil	15000000 #/ cc of soil	72000000 #/cc of soil
	3	17900000 #/ cc of soil	16700000 #/ cc of soil	10900000 #/cc of soil
Trial A after	1	7900000 #/ cc of soil	12400000 #/ cc of soil	11600000 #/cc of soil
	2	10800000 #/ cc of soil	73500000 #/ cc of soil	4800000 #/ cc of soil
	3	26000000 #/ cc of soil	7700000 #/ cc of soil	10200000 #/ cc of soil
Trial B before	1	16800000 #/ cc of soil	6300000 #/ cc of soil	12100000 #/ cc of soil
	2	64000000 #/ cc of soil	16700000 #/ cc of soil	35000000 #/ cc of soil
	3	24000000 #/ cc of soil	1800000 #/ cc of soil	14000000 #/ cc of soil
Trial B after	1	37600000 #/ cc of soil	21000000 #/ cc of soil	25100000 #/ cc of soil
	2	18600000 #/ cc of soil	15100000 #/ cc of soil	7800000 #/ cc of soil
	3	19200000 #/ cc of soil	10100000 #/ cc of soil	35000000 #/ cc of soil
Trial C before	1	30700000 #/ cc of soil	13300000 #/ cc of soil	44900000 #/ cc of soil
	2	4100000 #/ cc of soil	20800000 #/ cc of soil	39400000 #/ cc of soil
	3	28100000 #/ cc of soil	31500000 #/ cc of soil	21400000 #/ cc of soil
Trial C after	1	27800000 #/ cc of soil	33200000 #/ cc of soil	29500000 #/ cc of soil
	2	22400000 #/ cc of soil	46400000#/ cc of soil	5300000 #/ cc of soil
	3	52400000 #/ cc of soil	38700000#/ cc of soil	37200000 #/ cc of soil

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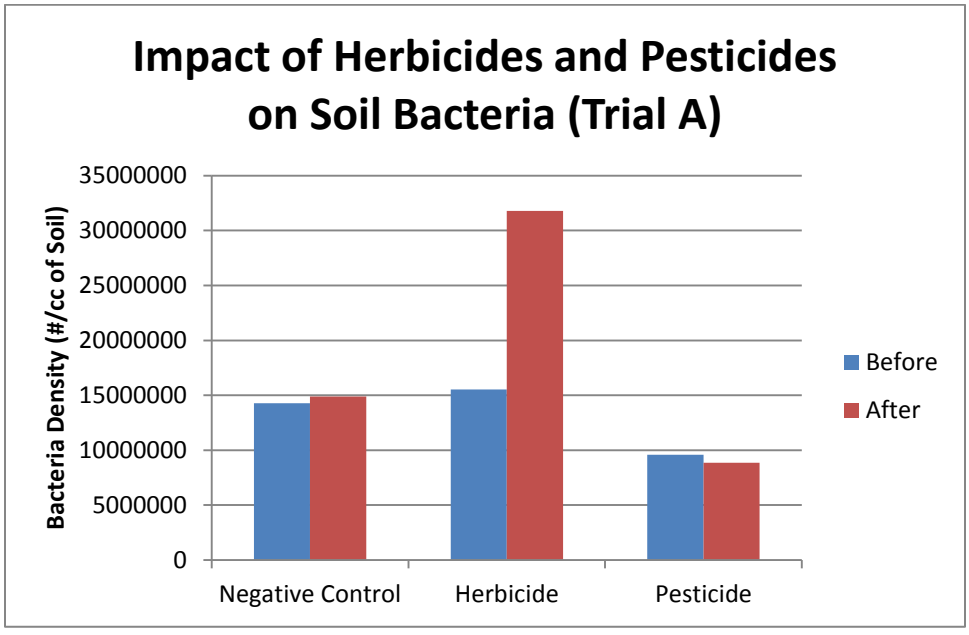
Average Impact of Herbicides and Pesticides on Soil Bacteria

Trial	Negative Control	Herbicide	Pesticide
Trial A Before	14266667 #/ cc of soil	15533333 #/ cc of soil	9600000 #/ cc of soil
Trial A After	14900000 #/ cc of soil	31800000 #/ cc of soil	8866667 #/ cc of soil
Trail B Before	15733333 #/ cc of soil	8266667#/ cc of soil	9866667 #/ cc of soil
Trial B After	25133333 #/ cc of soil	39466667 #/ cc of soil	22633333 #/cc of soil
Trial C Before	20966667 #/ cc of soil	21866667 #/ cc of soil	35233333 #/ cc of soil
Trial C After	34200000 #/ cc of soil	39433333 #/ cc of soil	24000000 #/ cc of soil

Percent Change in Population Density of Soil Bacteria

	Negative control	Herbicide	Pesticide
Trial A	4.44%	104.72%	-7.64%
Trial B	59.76%	377.21%	129.39%
Trial C	63. 12%	80.34%	-31.88%
Averages	42.44%	187.42%	29.97%
Corrected Differences	0.00 %	144.98%	-12.47%



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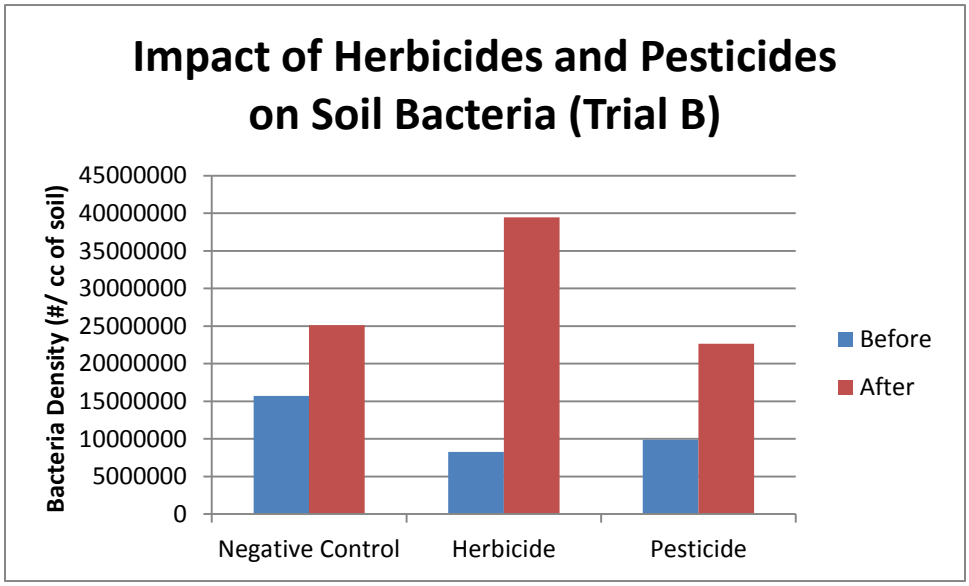

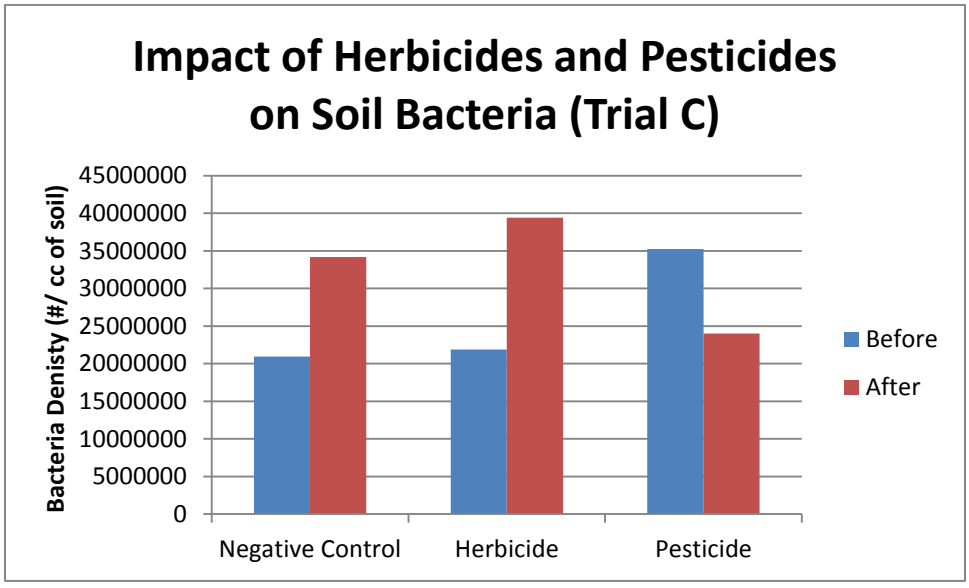

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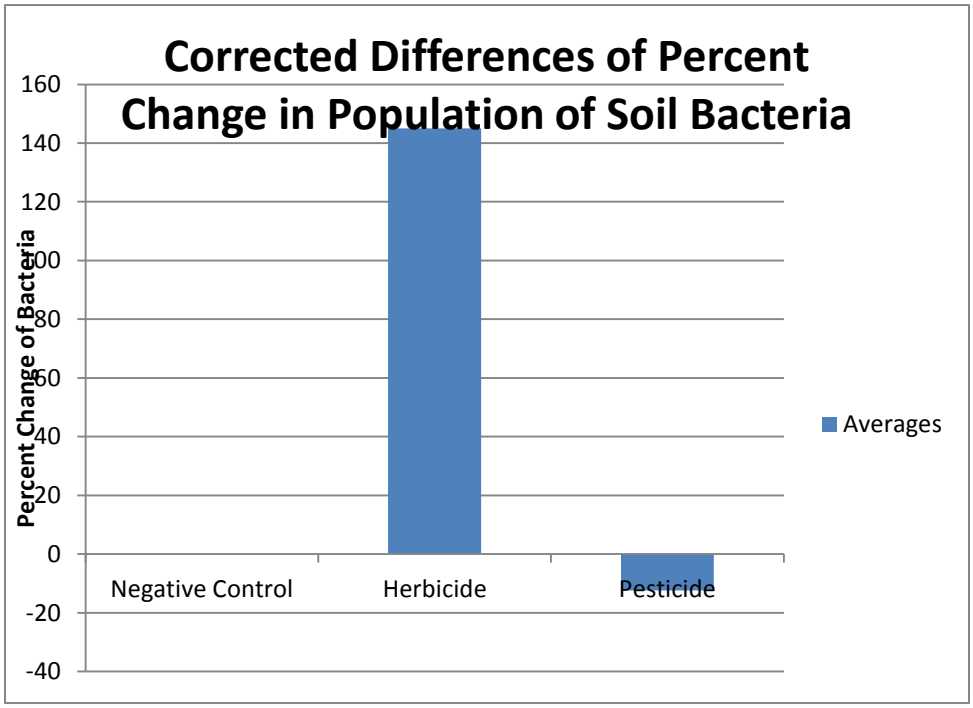

Conclusion-

Our Hypothesis that "Pesticides will lead to a greater increase in the population density of bacteria in the soil" was proven incorrect. Instead, our conclusive data showed that herbicides had a positive effect on the population density of soil bacteria, while pesticides caused a negative effect. The average percent change for our negative control was 42.44%. This tells us that from the beginning of our experiment something changed for the benefit of the environment. Perhaps, the rain caused the bacteria population in the soil to increase. The purpose of the negative control is to control for environmental changes. If there is an increase in population density in the negative control plots it also means that we would expect to see the same increase in the plots with herbicides and pesticides. Therefore, by finding the corrected differences for the averages of all three substances, we can determine the effect of each chemical on the bacteria's population. While the plots with pesticides showed a net increase of 29.97% in bacteria population, by using

the corrected differences format, we see that the use of pesticides actually decreased 12.47% from the naturally increased average population of 42.44%. Because the corrected difference of pesticide shows that the population of bacteria was not as high as it was in the negative control plots, we can conclude that the pesticides actually have a negative impact on the population density of soil bacteria. In contrast, the net increase in population for herbicides was 187.42%, increasing a whopping 144.98% from the naturally increased population of 42.44%. The increase of population from the negative control shows that herbicides have a positive impact on the amount of bacteria in the soil.

The fact that herbicides increased the population density of the soil bacteria makes sense because the main chemical in herbicides is glyphosate. According to Martin T.K Tsui and L.M Chu (2003), "bacteria should be much more tolerant to the toxicity of the IPA salt of glyphosate". Also, it is conceivable that pesticides rather decreased the population of the soil bacteria because "the chemical content of pesticides is known to be harmful to bacteria and other microorganisms in the soil" (Neihs.nih.gov, 2012). We can conclude from our experiment that since pesticides have a negative impact on the amount of bacteria in the soil, they could furthermore be having a negative impact on our ecosystem. By decreasing the bacteria in the soil, pesticides have the potential to disturb essential processes like the nitrogen cycle. Therefore, a logical follow-up experiment could be examining the effects of pesticides on the nitrogen cycle.

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