Soil Ecology Project:
The Effect of Pesticides on Soil Bacteria Population Density

We have completed this assignment honorably:

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Background

Numerous soil microbes provide an ecosystem with beneficial and essential raw materials for the growth and survival of the other organisms living there. Among them, soil bacteria play some of the most important roles in completing the necessary tasks for an ecosystem to thrive. Cycling and recycling materials through the process of decomposition and nitrogen fixation, they utilize the enzymes they produce (such as cellulases, hemicellulases, pectinases, and phenol oxidases) to carry out the chemical reactions that break down dead organisms and their complex compounds into carbon dioxide, ammonium, and other organic compounds (Roman, A. *et al*, 2006). Bacteria are also able to chemically break down metabolic waste such as fecal material, and together all these various nutrients that bacteria release into the soil provide energy and monomers for the other organisms living there to use for their survival. For example, plants transform the nutrients produced by bacteria through decomposition into their own polymers, which are then utilized by other organisms as this material moves up the food chain and throughout the food web, and when everything dies, the cycle repeats itself, allowing the ecosystem to efficiently recycle all of its materials (Harrison, 2003).

In addition to their role in decomposition, soil bacteria also play a key role in the environmental cycles that transform chemicals in an ecosystem (such as nitrogen and phosphorus) from inaccessible to accessible forms. Nitrogen is a key component in all living organisms because it is an essential part of DNA, RNA, and enzymes (Hayat, *et al*, 2010). Nitrogen is the reason why organisms can be alive in the first place because DNA runs the cell. If DNA isn't copied into RNA then enzymes are not made and the cells of any organism can no longer function and reproduce, transform energy, synthesize, and perform homeostasis; so the organism dies.

However, in spite of the vital role nitrogen plays in all living things, the most abundant source of nitrogen is in the inaccessible form of a gas in the atmosphere. Hence, something in the ecosystem must convert this nitrogen gas into "fixed nitrogen" so that living things can use it. This process is known as the nitrogen cycle, where the element nitrogen cycles constantly between the atmosphere, biosphere, and geosphere because of the activities of different groups of soil bacteria. There are five steps to this process, and the first is nitrogen fixation. In nitrogen fixation, certain groups of soil bacteria convert the gas form of Nitrogen, N_2 , found in the atmosphere, into ammonium, NH_4^+ , which is one of the only two forms of nitrogen that plants can consume directly to create their own DNA, RNA, and enzymes. One example of bacteria that can convert nitrogen this way are the *Azotobacter*. *Azotobacter* are especially important to

plants because they live in a plant's root; so they directly give the plant fixed-nitrogen, enabling the plant to avoid expending the energy needed to absorb it actively from the surrounding soil (Harrison, 2003).

Another source of ammonium comes from the process of nitrogen mineralization. During this process, decomposers such as bacteria and fungi first convert the proteins and nucleic acids of dead organisms back into ammonium, and then protozoa release it into the soil when they eat all the bacteria. Because protozoa control the population of soil bacteria through this consumption, they ensure that a proper balance of ammonium is available for plant intake (Hoorman, 2011).

Yet regardless of whether it is produced through nitrogen fixation or decomposition, all of this ammonium undergoes further nitrification and denitrification. In nitrification, bacteria convert ammonium into nitrite, NO_2^- , and then into nitrate, NO_3^-, which is the other form of fixed nitrogen which plants can consume for their own biological needs. Then, through the process of denitrification, another group of bacteria convert any excess nitrate back into nitrogen gas, releasing it into the air. As the nitrogen gas is put back into the atmosphere, it is stored in the air to be used as organisms need it, and when it runs out, the nitrogen cycle repeats. The speed of the nitrogen cycle is dependent on the microbial life activity; so in order for the nitrogen cycle to occur at a reasonable pace, there needs to be a lot of bacteria present (Harrison, 2003).

Because bacteria are so highly important to a variety of processes that benefit the health of the ecosystem, anything that might compromise the health of soil bacteria could potentially compromise the health of the entire ecosystem and could seriously harm the growth and life of all the organisms living there. One such potential threat are pesticides. These are "any substance or mixture of substances intended for preventing, destroying, repelling or mitigating any pest" (Feldman, J., *et al*, 2000-2007), and when broad spectrum pesticides are applied, the chemicals in them have the potential to damage bacteria's chemical and physical structure, inhibiting them from carrying out of all of their essential roles.

For example, one of the main ingredients of pesticides, Carbaryl, has the ability to break down the cell wall of bacteria. Since bacteria are prokaryotes, they lack a nucleus and other organelles; so they automatically have to store the DNA instructions in their cytoplasm. Once Carbaryl has broken down the cell wall, the DNA in the bacteria is no longer contained, preventing the four tasks of life (reproduction, homeostasis, synthesis, and transformation of energy) from occurring, and because the bacteria cannot perform these functions, they shut down and die (Encyclopedia Britannica, 2014). Furthermore, Carbaryl has a damaging effect on

an important enzyme which bacteria produces, cholinesterase; hence even if it did not destroy the cell wall, Carbaryl would still prevent soil bacteria from operating properly.

Clearly then, the chemicals in pesticides have the potential to have a major effect on soil ecology in that they have the potential to have a major impact on the efficiency of soil bacteria and, therefore, all the organisms depending on them. Since pesticides inhibit bacterial enzymes that carry out their ability to decompose materials and provide the ecosystem with the essential building blocks for organisms, pesticides can damage the health of the entire ecosystem by interfering with the nitrogen cycle.

Therefore, after learning the effect Carbaryl in pesticides has on soil bacteria growth and efficiency, we decided to test the effect of pesticides on soil bacteria growth here at RPCS. We made a total of six plots, with half receiving pesticides and have receiving sterilized water to see if the pesticides affect the growth of soil bacteria. We hypothesize that pesticides will decrease the amount of soil bacteria present.

Outline

Problem: Do pesticides increase or decrease the population density of bacteria present in the soil?

Hypothesis: The application of pesticides to soil will decrease the population density of bacteria present in the soil.

Procedure:

Independent Variable: The application of broad spectrum pesticides to soil

Dependent Variable: Number of bacteria colonies per 1 cc of soil

Negative Control: Water only application to soil

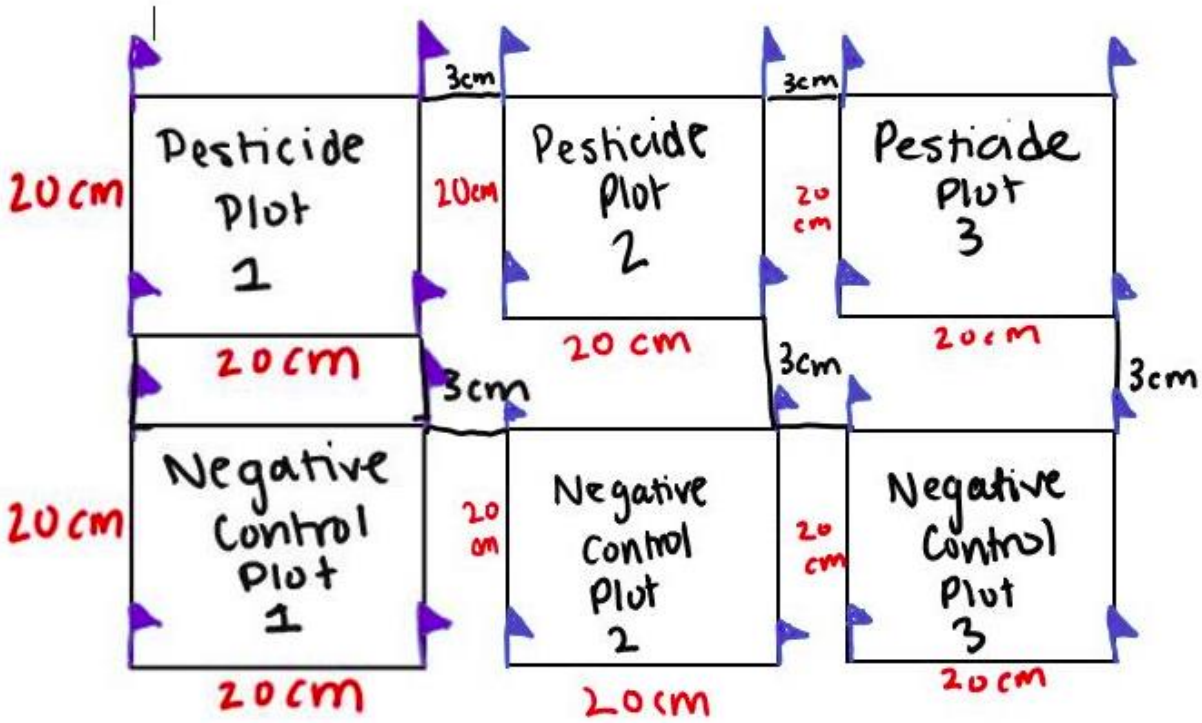
Controlled Variables: type and brand of pesticide, area from which soil is collected, amount of pesticides applied, amount of water applied, size of culture tubes, size and type of pipette, amount of nutrient agar used, type of nutrient agar, amount of time soil is exposed to pesticides, amount of time soil is exposed to water, amount of grass and plants covering the soil, type of plant covering the soil, amount of soil diluted in culture tubes, type of culture tube, size of culture tube, intensity of shaking culture tube, time and day soil is extracted from plots, time and day soil is diluted, time and day water and pesticides are applicated, size of growing plate, time bacteria on growing plate, dilution levels plated, degree to which soil is diluted, amount of soil collected

Step-by-Steps:

01. Walk to coordinates listed below covered with buttercup plants:

a. N: 39°21.411'
b. W: 76°38.177'

02. Reference to diagram below step 9 for steps 3-9.
03. Measure a 20 by 20 cm soil plot and mark the area with flags on each corner
04. Write on these flags: “Pesticide Plot 1 Trial 1”
05. Measure another 20 by 20 cm plot of land 3 cm behind the first plot, this plot is the place to perform the negative control
06. Mark the plot of land with flags in each corner
07. Write on the flags: “Negative Control Plot 1 Trial 1”
08. Repeat steps 3-7 for Plot 2, but write on the flags, “Pesticide Plot 2 Trial 2” and “Negative Control Plot 2 Trial 2” and make sure each pesticide plot is 3 cm to the right of the last one, and make sure each negative control plot is 3 cm behind its corresponding pesticide plot.
09. Repeat steps 3-7 for Plot 3, but write on the flags, “Pesticide Plot 3” and “Negative Control Plot 3 Trial 3”and make sure pesticide plot is 3 cm to the right of the last one, and make sure each negative control plot is 3 cm behind its corresponding pesticide plot.



10. Label 3 plastic bags: The first one reading “Pesticide Plot 1 Trial 1 Before Sample A.” The second one reading “Pesticide Plot 1 Trial 1 Before Sample B.” the third one reading “Pesticide Plot 1 Trial 1 Before Sample C”
11. Label another 3 plastic bags: The first one reading “Negative Control Plot 1 Trial 1 Before Sample A.” The second one reading “Negative Control Plot 1 Trial 1 Before Sample B.” The third one reading “Negative Control Plot 1 Trial 1 Before Sample C”
12. Repeat steps 10 and 11 for plot 2 and 3, but change the number of the plot on the bag corresponding with the correct plot and trial number on the flags.
13. Make sure that you collect the soil from each plot at the same time and on the same day in step 14
14. Collect 3 samples of soil using a 45.5 cm soil core extractor and go 10 cm deep and 2 cm wide, between 9:30: and 10:15 am. Collect soil samples from all 3 plots at the same time, 3 each from negative control and pesticide plots and place each soil sample in its corresponding bag
15. Complete the serial dilution process in steps 16-30 at the same and the same day.
16. Use a clean, new transfer pipette to put 10ml of sterile water into a 15ml culture tube and label this tube “NCP1T1B 10^{0}”
17. Use the same pipette to add 9 ml of sterile water to a second 15 ml culture tube and label this tube “NCP1T1B 10^{-1}”
18. Repeat step 17 two more times to two additional 15 ml culture tubes, only label them “NCP1T1B 10^{-2} and NCP1T1B 10^{-3}
19. Place 1 cc of your “negative control plot 1” trial 1 soil sample into the NCP1T1B 10^{0}.
20. Cap the tube and shake vigorously.
21. Using a new clean pipette, remove 1 ml of the soil/water mixture from the “NCP1T1 10^{0}” tube and place it into the “NCP1T1B 10^{-1} tube.
22. Cap and shake vigorously.
23. Using the same pipette in step 21, remove 1 ml of the soil/water mixture from the “NCP1T1B 10^{-1} tube and place into the “NCP1T1B 10^{-2}” tube.
24. Cap and shake vigorously.
25. Using the same pipette in step 21, remove 1 ml of the soil/water mixture from the “NCP1T1B 10^{-2}” tube and place into the 10^{-3} tube.
26. Cap and shake vigorously.
27. You should now have a total of four culture tubes.

28. Place 100 µl samples from the NCP1T1B 10^{-2} tube on a 3M petri film aerobic count plate and label the plate correspondingly and use a spreader to spread the diluted soil water on the plate.
29. Place 100 µl samples from the NCP1T1B 10^{-3} tube on a 3M petri film aerobic count plate and label the plate correspondingly use a spreader to spread the diluted soil water on the plate.
30. Repeat the serial dilution process in steps 16-30 for the each of the soil samples using the labels defined below in the same time period and on the same day as the NCP1T1B sample.

- pesticide plot 1 trial 1 before “PEP1T1B”
- negative control plot 2 trial 2 before “NCP2T2B”
- pesticide plot 2 trial 2 before “PEP2T2B”
- negative control plot 3 trial 3 before “NCP3T3B”
- pesticide plot 3 trial 3 before “PEP3T3B”

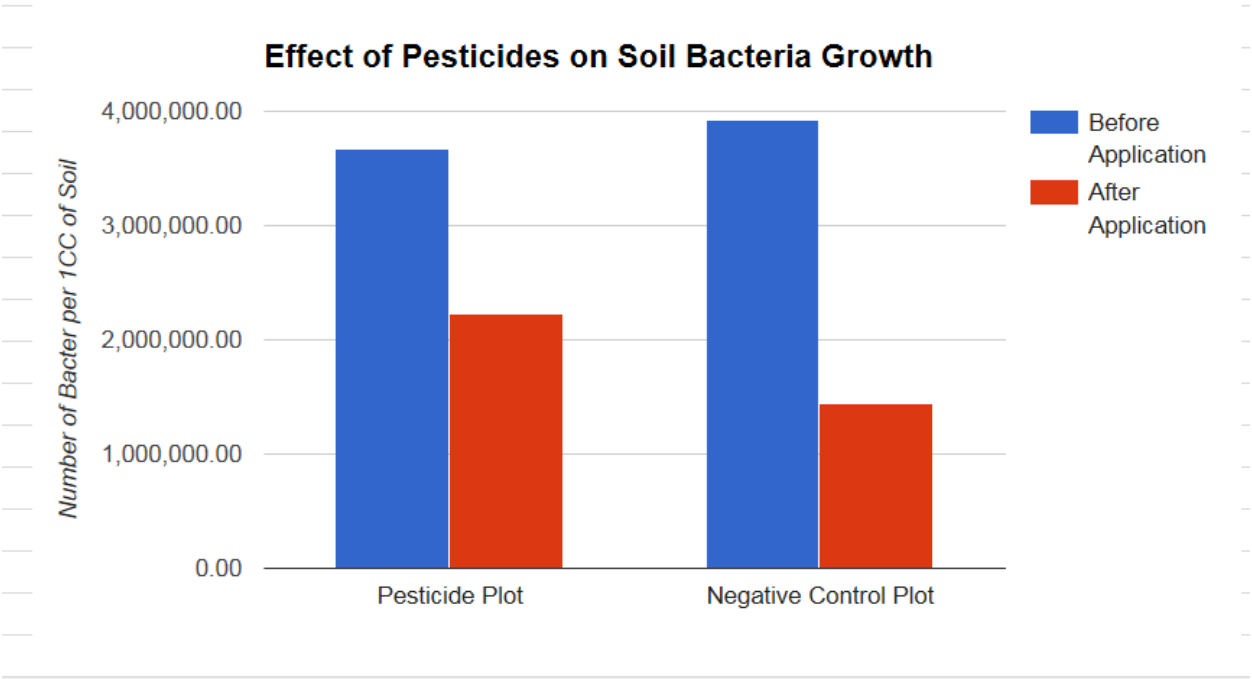
31. Allow all the bacteria colonies from each sample to grow for 48 hours
32. Examine each of the plates for individual bacteria colonies and choose the plate with the fewest colonies and the lowest dilution (but at least 5) to make your estimates of the number of bacteria in the original 1 cc soil sample using the following formula: # Microbes in 1 cc of soil = # Colonies on sheet x 10^2x $10^{|\text{ dilution \# at which these colonies were found}|}$.
33. Collect 150 ml of sterile water in a spray bottle.
34. Get Bayer Advanced Complete Insect Killer for Gardens spray bottle pesticide in a spray bottle
35. Complete steps 36 and 37 for all 3 plots at the same time and on the same day.
36. Spray the Broad spectrum pesticide on pesticide plots 1, 2, and 3 20 times until wet.
37. Spray the sterile water on negative control plots 1, 2, and 3 20 times until the plot is wet
38. Allow plots to sit for 48 hours.
39. Label three plastic bags: The first one reading “Pesticide Plot 1 Trial 1 after Sample A.” The second one reading “Pesticide Plot 1 Trial 1 after Sample B.” The third one reading “Pesticide Plot 1 Trial 1 after Sample C.”
40. Label three plastic bags: The first one reading “Negative Control Plot 1 Trial 1 after Sample A.” The second one reading “Negative Control Plot 1 Trial 1 after Sample B.” The third one reading “Negative Control Plot 1 Trial 1 after Sample C”
41. Repeat steps 39 and 40 for plot 2 and 3, but change the number of the plot and trial corresponding with the correct plot and trial number on the flags.

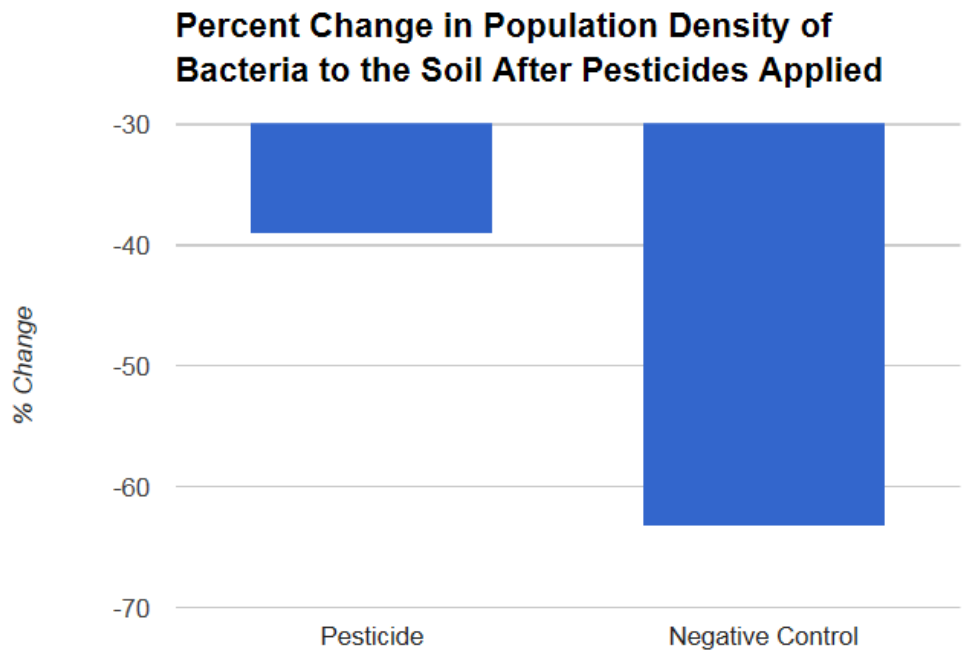
42. In steps 43, remember that all soil samples should be collected at the same time on the same day.
43. After 48 hours, collect 3 samples of soil from each pesticide and negative control plot by going 10 cm deep and 2 cm wide between 9:30 and 10:15 am using the 45.5 cm soil core extractor, and place each soil sample in its corresponding plastic bag.
44. In step 45, remember that all soil samples should be diluted at the same time.
45. Test the population density of bacteria after water and pesticides being applicated from each of the plots during 9:30 and 10:15am by repeating steps 16-30 for the after soil samples which had pesticides and water applicated to them, but make sure to change all the “before” labeling on the tubes (B) before pesticides and water were applicated to “after” labeling on the tubes (A) after they are applied.
46. Repeat steps 31 and 32, for all of the soil samples.

Data Tables

Effect of Pesticides on Soil Bacteria Growth

Trial	**Type of Treatment: Negative Control- Sterilized Water (# of Bacteria per 1 CC)**	**Type of Treatment: Negative Control- Sterilized Water (# of Bacteria per 1 CC)**	**Type of Treatment: Pesticide (# of Bacteria per 1 CC)**	**Type of Treatment: Pesticide (# of Bacteria per 1 CC)**
	Before Treatment	**After Treatment**	**Before Treatment**	**After Treatment**
1	4,800,000	420,000	5,300,000	2,500,000
2	2,000,000	1,600,000	1,100,000	2,800,000
3	5,000,000	2,300,000	4,600,000	1,400,000
Average	3,933,333.333	1,440,000	3,666,666.667	2,233,333.333


Effect of Pesticides on Soil Bacteria Growth
4,000,000.00
3,000,000.00
2,000,000.00
1,000,000.00
0.00
Number of Bacter per 1CC of Soil
Pesticide Plot
Negative Control Plot
Before Application
After Application


Percent Change in Population Density of Bacteria to the Soil After Pesticides Applied
-30
-40
-50
-60
-70
% Change
Pesticide
Negative Control

Conclusion

Based off of our results, our hypothesis exploring whether broad spectrum pesticides had a negative effect on soil bacterial populations was incorrect, for the application of broad spectrum pesticides to soil did not have a significant and true negative impact on population density of bacteria in 1 cc of soil. This is evident because of the positive control; by conducting a positive control before applying the negative control of sterile water or pesticides to the soil plots, we are able to observe the amount of bacteria colonies in soil in the regular environment without any experiment being tested. We were able to obtain a standard amount of bacteria colonies in 1 cc of soil in the environment, which we were able to use to observe any positive or negative change the negative control of water and the pesticides had on the number of bacteria colonies in 1 cc of soil.

We see by the negative relationship between the positive and negative control, that there was a negative impact on the number of bacteria colonies after the application of sterile water contributing to the conclusion that there were additional environmental factors that had a negative impact on our experiment. Because of additional negative environmental factors, we observe that both the number of bacteria colonies in 1 cc of soil applied with pesticides and applied with water, decreased; before applying sterile water on the negative control plots, the average number of bacteria colonies in 1 cc of soil of 3 plots was 3,933,333.33. After applying the sterile water on the negative control plots, the average number of bacteria colonies in 1 cc of soil was 1,440,000 displaying a 63.389% decrease in the population density. Also, we see a negative impact on the bacteria colonies after the application of pesticides; the average number of colonies in 1 cc of soil of 3 plots was 3,666,666.667. After applying the pesticides on the pesticide plots, the average number of bacteria colonies in 1 cc of soil was 2,233,33.333 displaying a 39.09% decrease in population density of bacteria colonies. However, this decrease in bacterial population after the application of pesticides was due to a negative environmental factor and not truly because of the pesticides; we see this because our application of pesticides compared to the application of water, in fact aided on the survival of bacteria colonies against whichever environmental factor played a role in our experiment. This is shown by the smaller percent decrease in the number of bacteria colonies from the positive control to after the application of pesticides compared to after the application of water. We see that the negative percent change for the bacteria colonies in 1 cc of soil from the positive control to after the pesticides were applied was 39.09% which was 24.299% less of decrease compared to the negative percent change of 63.389% from the positive control to after the application of water.

Clearly, we see that there was a negative environmental factor that influenced the negative impact on the number of bacteria colonies in 1 cc of soil after the application of water as well as pesticides. However, despite this environmental factor's overall negative impact on bacteria population density, the application of broad spectrum pesticides aided the bacteria population to survive against this environmental factor shown by its smaller decrease of bacteria populations when compared to no pesticides being applied on the negative control plots, but instead the application of water which resulted in a greater decrease in bacterial populations.

Possible environmental factors that negatively impacted our experiment can be further explored to more accurate data of the effect of pesticides on soil bacteria colonies. The most likely environmental factor that could have had a negative role in our experiment could be credited to the heavy rainfall between the time period of collecting positive control to application of water and pesticides and collection of after application soil. When collecting the positive control soil, there had not been any rainfall for a week. However, once we applied the pesticides and water on the soil plots, there was heavy amounts of rainfall during the 48 hours dedicated to the absorption of the pesticides and water (NOAA, 2015). The rainfall's most probable effect on our experiment was its possible impact on the increase of protozoa in the soil. Since protozoa such as acanthamoeba and paramecium, reproduce in water, the excess amount of water could have been favorable to the presence of these organisms in our experiment (Microbe World, 2014). This increase in protozoan presence in the soil could have negatively impacted the number of bacteria colonies in our soil plots because of the relationship between protozoa and bacteria. Protozoa who are higher up on the food chain act as hunters of bacteria for bacteria is a food source of protozoa. Since bacteria convert nitrogen into ammonium for plants utilization, yet the bacteria utilize the ammonium before the plants' roots can use them, protozoa eat bacteria to control their population growth (Hoorman, 2011). However we see that the pesticides helped the bacteria population survive this probable increase of protozoa. This could be credited to certain chemicals in pesticides, for they may have contributed to the greater population density of bacteria after the application of pesticides. Chemicals in pesticides such as Carbaryl, not only harm bacteria cell walls, but can. Carbaryl has such negative effects on bacteria contributing to the formulation of our hypothesis of pesticides having a negative effect on bacteria populations; however, the application of pesticides and its chemicals like Carbaryl not only would have negatively affected the bacteria populations, but the its predator of its increased protozoan populations due to the excess rainfall. These chemicals having an obvious effect on a variety of microbes because of its toxicity, not only had a negative impact on bacteria population density but on its predator of protozoa, contributing to result of pesticides indirectly

aiding the survival of bacteria populations despite its probable negative chemical effect on bacteria. In the future, we could conduct an experiment to explore this linear effect of excess water on bacteria population. We could conduct a similar experiment under the same weather conditions, however along with testing the effect of broad spectrum pesticides on population densities of bacteria colonies, we could also test the probable greater protozoa populations compared to bacteria, along with the effect of the pesticides on the protozoa populations. By collecting this data, it is probable that we would confirm that there would be an increase in protozoa explaining this environmental factor's negative impact on bacterial populations. However by collecting data about protozoa populations, we could also confirm any decrease on protozoa populations the chemicals in pesticides would have caused, contributing to our result of a smaller decrease in bacterial populations after the application of pesticides because of the probable negative effect pesticides had on the predator, protozoa, allowing there to be a greater amount of bacteria survival. By conducting this experiment, we can observe the chain effect made by the presence of excess water and the broad spectrum pesticides' effect on those excess protozoa populations and bacteria populations' bacterial populations to further the accuracy of our results

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