Soil Ecology Project

The Impact of Pesticides on Bacteria Population Density in Soil

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May 2014 – Ms. Riina, Honors 9 Biology

Background

Bacteria are single-cell organisms that grow and live in thin water films around soil particles and near roots. These microorganisms are prokaryotic and live on the top-most layer of the soil along with other microorganisms such as fungi, algae, and protozoa (Gerber 2006-2014). Bacteria are decomposers, which break down dead organic matter, who consume carbon compounds such as root exudates and fresh plant litter. They recycle organic matter to acquire nutrients that are useful to other organisms in the soil food web. In addition, these microbes are important for maintaining soil structure. Bacteria produce sticky substances that act as super glue to form aggregates, which are a group of organisms living closely together but less integrated than a society. They have the ability to benefit certain plant species by altering the physical properties of the soil. While it is very difficult to change soil's basic texture; bacteria can improve the soil structure by making clay more porous, and sand more water retentive (Sunset 2014). Water is retained in pores so that plants can complete the process of photosynthesis. Oxygen is also needed in pores for bacteria to do cellular respiration. Improving soil structure creates a better environment for the roots of the plants (The Living Soil 2013). Poor soil structure increases the risk of surface run-off and erosion, which prevents effective plant growth and decreases the nutrient uptake in the plant. By creating a more structured soil, bacteria facilitate the plant's process of absorption of essential nutrients so the producer population can thrive.

In the soil, bacteria can do many chemical transformations. Some transformations include degradation of organic matter, disease suppression, and nutrient transformations inside roots. One important nutrient transformation is the recycling of nitrogen. The nitrogen cycle is the

process by which nitrogen is converted between its various chemical forms to be used by different organisms. First, ammonia from dead organic matter and animal waste is converted into ammonium by nitrogen-fixing bacteria through the process of ammonification. Then, the ammonium is turned into nitrite which is then oxidized into nitrates through nitrifying bacteria. In soil, bacteria are mostly in charge of transforming inorganic constituents from one chemical to another. Their external digestion means some of the metabolites released from the use of extracellular enzymes can be used by other organism like plants. Bacteria gains nutrients and energy from these processes and then give excess nutrients to other organisms**.** (Collins 2004)

In order to keep plants as healthy and attain the highest possible crop yield, humans commonly apply pesticides to control for the damage of unwanted animals (Toxics Action Center, 2012). Chemical pesticides are substances used to control or eliminate pests on both large and small scales. As opposed to bio-pesticides or other non-toxic pesticides, (Ryan, 2014) they use toxic chemicals to kill unwanted pests by disrupting various biological processes of the pest, by poisoning the pest's nervous system, resulting in their death (Johnson, 2014). The Environmental Protection Agency notes that "pesticides physically, chemically, and biologically interfere with animals' normal behaviors and metabolisms" (NWS EPA, 2013). The route at which the pesticides are in contact with the target pest depends on the nature of the pesticide and how it is applied to the environment. Spraying, fumigating, and baiting are all common methods of application, all which are categorized as "contact pesticides", which are only effective when the pesticide is absorbed through the external body surface of the organism. No matter the type of application, many modern day pesticides act quickly and are degraded into non-toxic substances by microorganisms and other environmental processes. However, some pesticides can

continue to be effective for days, weeks, or even months after they are applied to a given area (NWS EPA, 2013).

Pesticides can not only affect the pests in the soil, they can also unintentionally harm other organisms in the environment. When pesticides seep into the soil, their residues can accumulate in drinking water and in food. When ingested, the residues can cause moderate or even fatal health risks including (but not limited to) cancer, asthma, and endocrine system disruption (Johnson, 2014). In addition, "Scientists from the University of Montreal and Harvard University released a study that found evidence that exposure to pesticide residues on vegetables and fruit may double a child's risk of attention deficit hyperactivity disorder." (Toxic Action Center, 2012) Pesticides can also cause extreme damage and eventually death to other animal species and microorganisms. In this way, pesticides can damage agricultural land by intoxicating beneficial insect species, microorganisms, and worms which maintain soil health by naturally limiting unwanted pest populations (Toxic Action Center, 2012). Also, pesticides put in the soil may have an unexpected resistance to degradation, prolonging its toxic effect on other species. Ann Hirsch, a plant molecular biologist at the University of California, Los Angeles, says that "pristine and natural interactions between bacteria and plants are being jeopardized by what we put into the soil," (Potera, 2007). Therefore, we hypothesized that the population density of bacteria will decrease when chemical pesticides are added to the soil.

References:

Binninger, J., & Allen J. (2014, January). What on Earth is Soil?

http://www.epa.gov/gmpo/edresources/soil.html

Effects of Pesticides on Soil Micro-Organism. (2014).

http://garden.lovetoknow.com/wiki/Effects_of_Pesticides_on_Soil_Micro-Organism

Johnson, S. (2014). The Effects of Herbicides & Pesticides.

http://www.ehow.com/list_6740279_effects- herbicides-pesticides.html

The Problem with Pesticides. (2012).

http://www.toxicsaction.org/problems-and- solutions/pesticides

Ryan, T. (2014). Herbicides & Pesticides.

http://www.ehow.com/info_7999925_herbicides- pesticides.html

What are pesticides and how do they work?. (2013, May).

http://www.epa.nsw.gov.au/pesticides/pestwhatrhow.htm

Potera, Carol. (2007) Agriculture: Pesticides Disrupt Nitrogen Fixation. Bethesda: National Center for Biotechnology Information

http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2137115/

Funke, B.R., Schluz, J.T. (1971) Effects of Pesticides on Soil Fertility. Fargo: North Dakota State University

http://library.ndsu.edu/tools/dspace/load/?file=/repository/bitstream/handle/10365/10205/farm_28_04_14.pdf?sequence=1

Soil Ecology Experiment – Pesticides

Problem: Do chemical pesticides increase the population density of bacteria in soil?

Hypothesis: Chemical pesticides will increase the population density of bacteria in soil.

Independent Variable: The presence of chemical pesticides in the soil

Dependent Variable: The population density of bacteria per cubic centimeter in the soil

Negative Control: the absence of chemical pesticides in soil.

Controlled Variables:

- Amount of pesticide applied to each plot
- Type of pesticide applied to each plot
- Amount of soil extracted
- Type of pesticides used
- Time of day samples extracted (for all trials)
- Time of day dilution occurs (for all trials)
- Type of pipettes used in dilution
- Location of environment
- Type of plant in environment
- Size of soil plot
- Size of culture tubes
- Type of water used in dilution
- Type of water mixed in the pesticide/water solution
- Amount of water sprayed onto each plot
- Amount of water added to the soil in each tube (during dilution)
- Amount of time one shakes the tube (during dilution)
- Amount of time allowed for the soil samples to sit before dilution
- Steepness of environment
- Amount of soil in each tube
- Size of the nutrient agar plates
- Type of nutrient agar plates
- Amount of time allowed for the bacteria colonies to grow (after dilution)

Step-By-Step

1. Go to the Roland Park Country School front lawn. Move to location 1 at the coordinates below:

a. Location 1
   N: 39.35801°
   W: 076.63611°

2. Measure a 20x20cm plot in Location 1. Put a flag in each corner and label plot 1
3. Now place two flags 10 cm deep in plot 1 to create two 20x20cm rectangles side by side. Connect these flag with tape down the middle of the plot to separate the two rectangles.
4. Label the left rectangle in plot 1 "negative control" rectangle and the right rectangle in plots 1 "pesticide" rectangle
5. Move to location 2 at the coordinates below:
   b. Location 2
      N: 39.35799°
      W: 076.63612°
6. Repeat steps 2-4, making sure to label each flag with "plot 2" instead of "plot 1"
7. Move to location 3 at the coordinates below:
   c. Location 3
      N: 39.35805°
      W: 076.63614°
8. Repeat steps 2-4, but label each flag with "plot 3" instead of "plot 1"
9. Refer to diagram 1 for final set-up
10. Label 1 plastic bag: "Plot 1 Negative Control Before". Label 1 plastic bag: "Plot 1 Pesticide Before". Label 1 plastic bag: "Plot 2 Negative Control Before". Label 1 plastic bag: "Plot 2 Pesticide Before". Label 1 plastic bag: "Plot 3 Negative Control Before". Label 1 plastic bag: "Plot 3 Pesticide Before".
11. Repeat step 10 for the trial 2 before samples
12. In plot 1, use a soil cylinder that is 15 centimeters in length to collect soil samples. Collect 2 different soil samples in each rectangle (negative control and pesticide) of plot 1, 2, and 3 and put each soil sample in the correctly labeled sterile Ziploc bag.
13. Complete steps 14-29 for the bacterial dilution process. Make sure you dilute each trial 1 soil sample on one day between 12:05-1:15 pm
14. Use a clean, new transfer pipette to add 10ml of sterile water to a 15ml culture tube. Label the tube "NCP1T1B 10^{0}"
15. Use the same pipette to add 9ml of sterile to a second 15 ml culture tube. Label the tube "NCP1T1B 10^{-1}"
16. Repeat step 15 two more times to two additional 15 ml culture tubes, only label them "NCP1T1B 10^{-2} ", "NCP1T1B 10^{-3}"
17. Place 1 cc of your negative control plot 1 trial 1 soil sample into the "NCP1T1B 10^{0} "culture tube.
18. Cap the tube and shake vigorously
19. Using a new clean pipette, remove 1 ml of the soil/water mixture from the "NCP1T1B 10^{0} " tube and place into the "NCP1T1B 10^{-1} " tube
20. Cap and shake vigorously

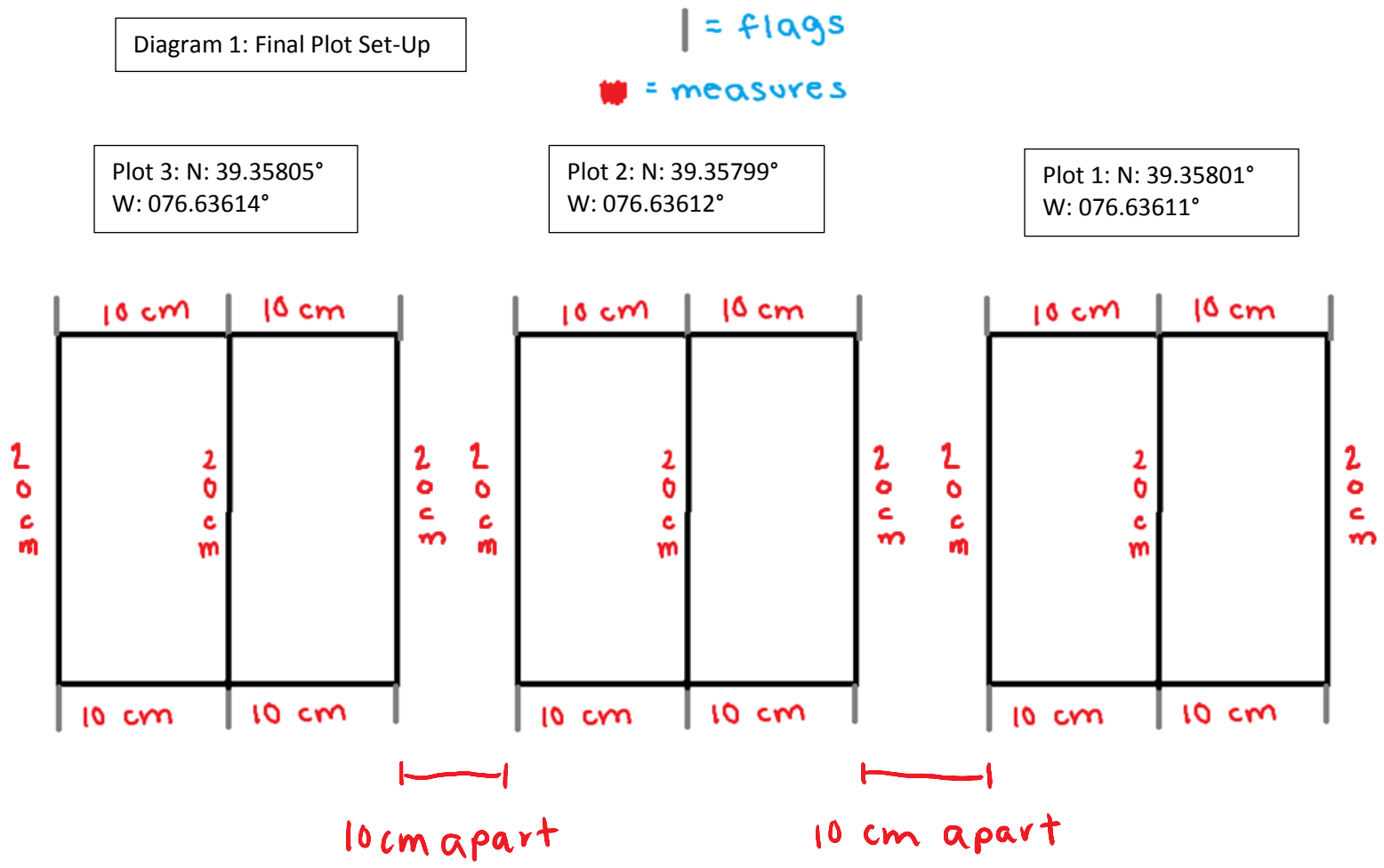
21. Using the same pipette in step 19, remove 1 ml of the soil/water mixture from the "NCP1T1B 10 $^{-1}$" tube and place it into the "NCP1T1B 10 $^{-2}$" tube.
22. Cape and shake vigorously
23. Using the same pipette in step 19, remove 1 ml of the soil/water mixture from the "NCP1T1B 10^{-2}" tube and place into the "NCP1T1B 10^{-3}" tube.
24. Cap and shake vigorously.
25. You should now have a total of four culture tubes.
26. Plate 100 µl samples from the 3rd and 4th tubes (dilutions NCP1T1B 10^{-2} & NCP1T1B 10^{-3}) using the P200 Micro pipette onto their own separate, labeled 3M Petrifilm™ Aerobic Count Plate petri plates containing nutrient agar.
27. Repeat steps 14-26 for negative control plot 2 trial 1 (labeled NCP2TIB), negative control plot 3 trial 1 (NCP3T1B), pesticide plot 1 trial 1 (PEP1TIB), pesticide plot 2 trial 1 (PEP2TIB), and pesticide plot 3 trial 1 (PEP3TIB) respectively. All the "before" samples must be diluted on the same day at the same time.
28. Allow the bacteria colonies to grow on the plates for 72 hours
29. Examine each of the plates for individual bacteria colonies which can be seen as small red dots. Start by looking at the 10^{-3} agar plate and see if there are at least 5 bacterial colonies. If not, examine the 10^{-2} plate. Estimate the number of bacteria colonies in the original 1 cc soil sample using the following formula (shown below). Record in the data table the number of microbes in 1 cc of the soil, the number of colonies on the sheet, and the dilution number at which these colonies were found in the data table.

#Microbes in 1 cc of soil = # Colonies on sheet x 10^2 x 10 $^{|\text{dilution \# at which these colonies were found}|}$

30. Repeat steps 14-29 for all of the trial 2 before samples from the negative control and pesticide rectangles from plots 1, 2, and 3. Label each plate according to the plot location and dilution number: for negative control plot 1 trial 2 (NCP1T2B) negative control plot 2 trial 2 (labeled NCP2T2B), negative control plot 3 trial 2 (NCP3T2B), pesticide plot 1 trial 2 (PEP1T2B), pesticide plot 2 trial 2 (PEP2T2B), and pesticide plot 3 trial 2 (PEP3T2B) respectively. All the trial 2 "after" samples must be diluted on the same day at the same time.
31. Collect 2 ml of water using the serological pipette
32. Put the 2 ml of water in the middle of the negative control plot 1 rectangle (left side of plot 1)
33. Collect 2 ml of "Bayer Advanced -- Complete Insect Killer for Gardens" using a different serological pipette
34. Put 2 ml of "Bayer Advanced – Complete Insect Killer for Gardens" in the middle of the pesticide plot 1 rectangle
35. Repeat steps 31-34 for plots 2 and 3 respectively
36. Wait 48 hours for pesticide and water to soak in

37. Label 1 plastic bag: “Plot 1 Negative Control Trial 1 After”. Label 1 plastic bag: “Plot 1 Pesticide Trial 1 After”. Label 1 plastic bag: “Plot 2 Negative Control Trial 1 After”. Label 1 plastic bag: “Plot 2 Pesticide Trial 1 After”. Label 1 plastic bag: “Plot 3 Negative Control Trial 1 After”. Label 1 plastic bag: “Plot 3 Pesticide Trial 1 After”.
38. Repeat step 37 with the trial 2 after samples
39. Repeat step 12 two times to extract both trial 1 and 2 “after” samples on the same day at the same time
40. Repeat steps 14-29 for each trial 1 after soil sample from the negative control and pesticide rectangles from plots 1, 2, and 3. Label each plate according to the plot location and dilution number: for negative control plot 1 trial 1 (NCP1TIA) negative control plot 2 trial 1 (labeled NCP2TIA), negative control plot 3 trial 1 (NCP3T1A), pesticide plot 1 trial 1 (PEP1TIA), pesticide plot 2 trial 1 (PEP2TIA), and pesticide plot 3 trial 1 (PEP3TIA) respectively. All the trial 1 “after” samples must be diluted on the same day at the same time.
41. Repeat steps 14-29 for each trial 2 after soil sample from the negative control and pesticide rectangles from plots 1, 2, and 3. Label each plate according to the plot location and dilution number: for negative control plot 1 trial 2 (NCP1T2A) negative control plot 2 trial 2 (labeled NCP2T2A), negative control plot 3 trial 2 (NCP3T2A), pesticide plot 1 trial 2 (PEP1T2A), pesticide plot 2 trial 2 (PEP2T2A), and pesticide plot 3 trial 2 (PEP3T2A) respectively. All the trial 2 “after” samples must be diluted on the same day at the same time.

Diagram 1: Final Plot Set-Up



Number of bacteria colonies in 1cc of soil before adding pesticides	
Soil sample	#bacteria colonies in 1cc soil
Plot 1 negative control trial 1 before	3,800,000
Plot 2 negative control trial 1 before	48,000,000
Plot 3 negative control trial 1 before	1,360,000
Plot 1 pesticides trial 1 before	1,000,000
Plot 2 pesticides trial 1 before	2,200,000
Plot 3 pesticides trial 1 before	20,400,000
Plot 1 negative control trial 2 before	4,500,000
Plot 2 negative control trial 2 before	1,600,000
Plot 3 negative control trial 2 before	1,700,000
Plot 1 pesticide trial 2 before	1,300,000
Plot 2 pesticide trial 2 before	4,600,000
Plot 3 pesticide trial 2 before	1,900,000

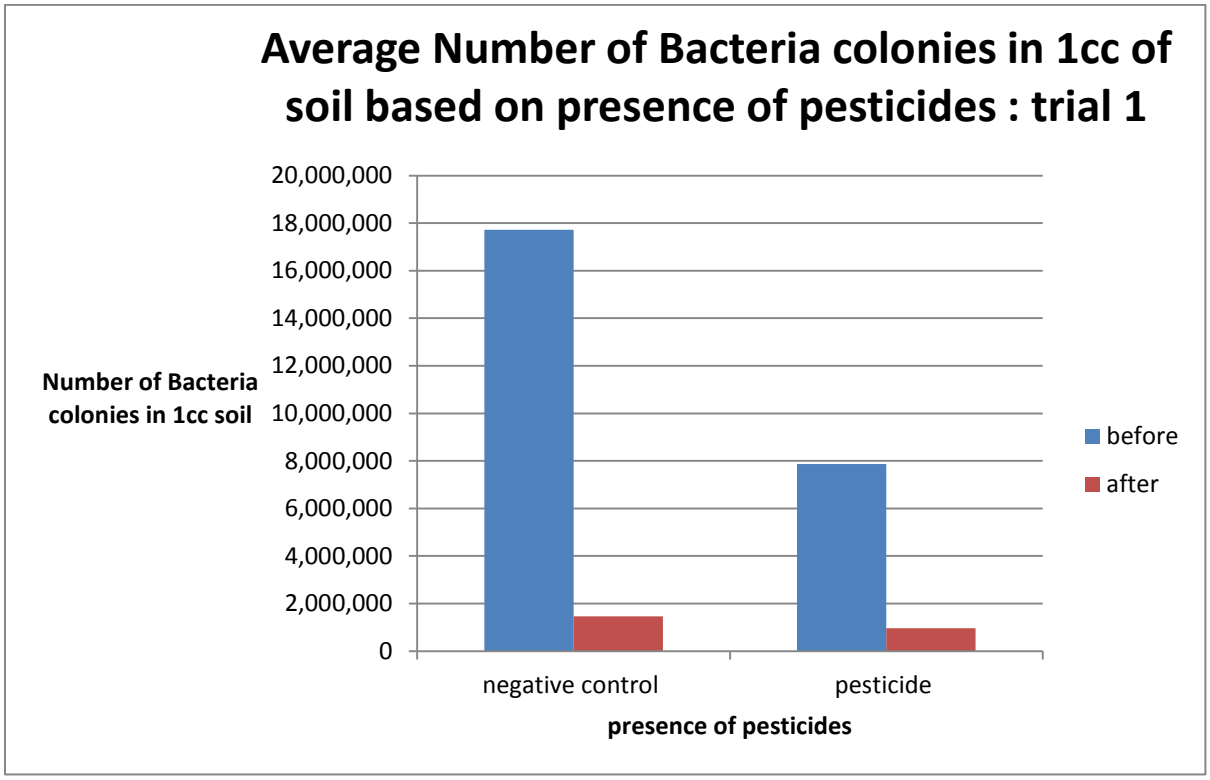
Number of bacteria colonies in 1cc of soil after adding pesticides	
Soil sample	# bacteria colonies in 1cc soil
Plot 1 negative control trial 1 after	1,600,000
Plot 2 negative control trial 1 after	1,200,000
Plot 3 negative control trial 1	1,600,000
Plot 1 pesticide trial 1	900,000
Plot 2 pesticide trial 1	700,000
Plot 3 pesticide trial 1	1,300,000
Plot 1 negative control trial 2 after	25,000,000
Plot 2 negative control trial 2 after	12,500,000
Plot 3 negative control trial 2 after	3,500,000
Plot 1 pesticide trial 2 after	15,300,000
Plot 2 pesticide trial 2 after	4,400,000
Plot 3 pesticide trial 2 after	5,700,000

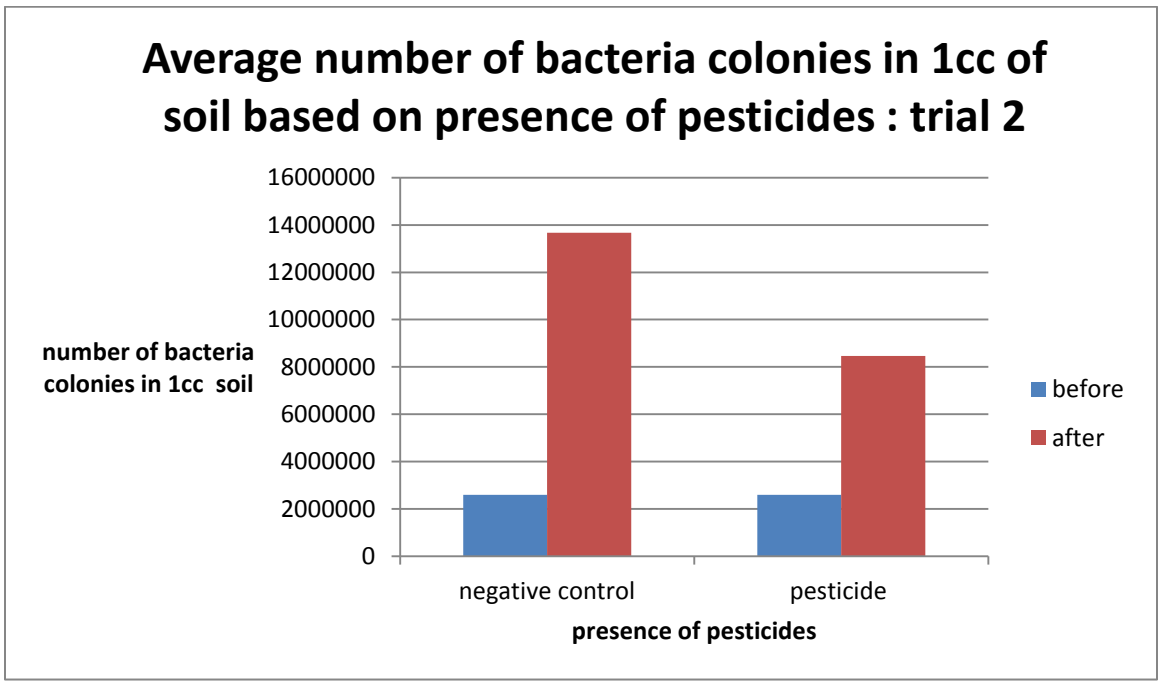
Average number of bacteria colonies in 1cc of soil based on presence of pesticides trial 1

Soil sample	# bacteria colonies in 1cc soil
Negative control before	17,720,000
Pesticide before	7,866,666
Negative control after	1,466,666
Pesticide after	966,666

Average number of bacteria colonies in 1cc soil before pesticides and after pesticides trial 2

Soil sample	# bacteria colonies in 1cc soil
Negative control before	2600000
Pesticide before	2600000
Negative control after	13,666,666
Pesticide after	8,466,666


Average Number of Bacteria colonies in 1cc of soil based on presence of pesticides : trial 1
Number of Bacteria colonies in 1cc soil
20,000,000
18,000,000
16,000,000
14,000,000
12,000,000
10,000,000
8,000,000
6,000,000
4,000,000
2,000,000
0
negative control
pesticide
presence of pesticides
before
after


Average number of bacteria colonies in 1cc of soil based on presence of pesticides : trial 2
number of bacteria colonies in 1cc soil
16000000
14000000
12000000
10000000
8000000
6000000
4000000
2000000
0
negative control
pesticide
presence of pesticides
before
after

Conclusion

Our hypothesis was disproven because there was a natural fluctuation of bacteria and protozoa in the soil because protozoa eat bacteria, eventually die off, and then the bacteria reproduce (and the cycle restarts). The pesticides had no significant effect on the bacteria populations in the soil based on our data. Instead of the pesticides decreasing the bacteria populations in the soil, natural variables caused our data to fluctuate and change majorly. Since we did not get the result witch support our hypothesis, we compared the numerical evidences of all 8 averages of our lab. The first graph of trail 1 shows the decrease of the population density of bacteria in our negative control and pesticide group after 48 hours. But then in trail 2, we saw the opposite result as the population density of bacteria in negative control and pesticides both increase after 48 hours. In the first trail, we got 17,720,000 bacteria colonies in 1cc soil of negative control before we placed them for 48 hours, and we got 7,866,666 bacteria colonies in 1 cc soil of pesticide rectangles before we placed them for 48 hours. After 48 hours, our bacteria colonies were decreasing in both negative control and pesticides rectangles---they had 1,466,666 bacteria in 1cc soil and 966,666 bacteria in 1cc soil. In the graph of trail 2, the numbers of bacteria colonies in 1cc of soil after we placed it for 48 hours were more than before we placed them. In the data table of trail 2, we got the same numbers--- 2,600,000 bacteria colonies in 1cc soil of negative control and pesticide rectangle before we placed them. After 48 hours, the negative control soil got 13,666,666 bacteria colonies in 1cc soil, and the pesticide rectangle got 8,466,666 bacteria colonies in 1cc soil. The data of two trials gives us two different graph and result. This could have resulted from the weather during our “48 hours”---it was raining at those days, the pesticides might be washed away by the rain. Also, the bacteria population in trial 1was decreasing because of protozoa eating them right before our first dilutions, and soon after the protozoa populations died because we let the trial 2 soil sit for a longer period of time before dilution. That means the bacteria in the trial 2 soil was reproducing by themselves, and increased the total population. So therefore, our trial 1 and trial 2 graphs display a

natural fluctuation between protozoa and bacteria populations. In conclusion we saw that pesticides had no major effect on the population density of bacteria in the soil.

For the future, we decided to perform more trials to get more data. Because we have no way of changing the protozoa populations in the soil, we thought that if we do more trials, the accuracy of our data would be higher and higher. Also, there was another reason that caused our data accuracy---weather. Because of raining, our pesticide was washed away and we cannot test our soil correctly. We'll make sure that it will not rain during the 48 hours that the pesticides sit in the soil. And we could also have multiple application of pesticide to make sure the pesticide successfully enter the soil.