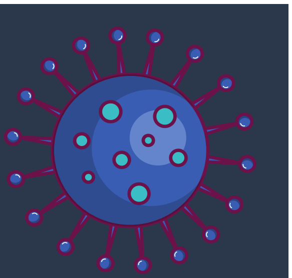
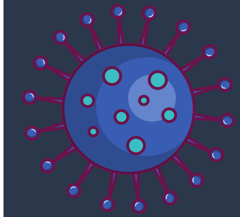
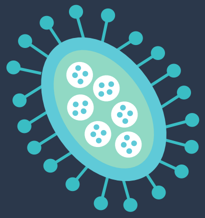
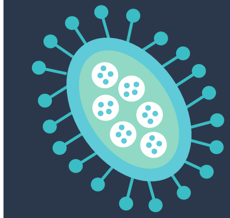
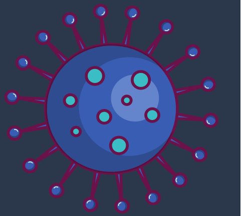



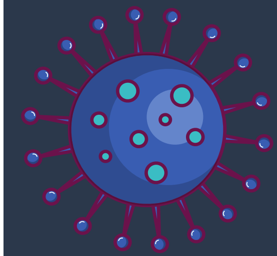
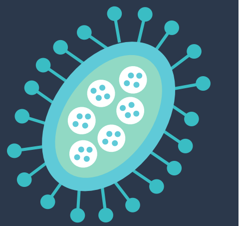
SOIL ECOLOGY PROJECT

The Impact of
Organic Insecticides
Vs.
Inorganic Insecticides
on the Population Density of
Soil Bacteria

By Lucky Hu, Kylie White
&
Maggie Hillwig

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Soil Ecology Background Information

In the Earth's soil, many micro-organisms, including fungi, algae, protozoa, and bacteria, play a variety of important roles in the soil ecosystem. These roles include transforming nutrients, breaking down organic matter, creating soil structure, and promoting plant growth. However, of all of the soil microbes, bacteria are the most abundant and play especially critical roles such as improving soil structure, recycling soil nutrients, decomposing dead organisms, and fixing nitrogen.

Bacteria that improve soil structure live around the edges of soil mineral particles, especially clay and associated organic residues. They produce a layer of polysaccharides or glycoproteins that work with organic matter, fungal hyphae, earthworm excretions, and plant roots to create micro aggregates that bind the sand, silt and clay particles of dirt together to form soil (Hoorman, 2011). These micro aggregates can withstand strong mechanical or physical forces, such as the compaction caused by humans and animals walking on it, as well as physicochemical stresses between the individual soil aggregates caused by molecular, electrostatic, and structural mechanical forces (J.R, 2005). Hence, bacteria help provides a stable structural environment for the many organisms living in the soil by creating stable spaces for them to reside and function.

One other way bacteria help generate the compounds that bind soil together is through the process of decomposition. Many types of bacteria break down the organic molecules of dead organisms by consuming simple carbon compounds, such as root exudates and fresh plant litter, releasing carbon back into the carbon cycle as CO_2 and converting energy from soil's organic matter into forms useful to the rest of the organisms in the soil food web. Decomposers are especially important in immobilizing and retaining nutrients in their cells, thus preventing the loss of nutrients, such as nitrogen, from the rooting zone (Ingham, 2009). For example, Actinomycetes, a large and important group of bacteria that give freshly turned earth its recognizable "earthy" scent, specialize in decomposing tough materials like cellulose and chitin that would otherwise remain trapped in the remains of dead plants and fungi (Roger Thorne J.D. 2010).

However, of all the benefits that bacteria provide to the entire ecosystem, the most important function is the role they play in the nitrogen cycle. Nitrogen is found in all living organisms in their amino acids, nucleotides and other essential molecules, but most of the nitrogen on earth is trapped in the atmosphere as nitrogen gas, a form of nitrogen inaccessible to most living things. Certain types of soil bacteria, though, can convert this nitrogen gas to ammonia (NH_3) through a process called nitrogen fixation, where it picks up another hydrogen ion from water, forming ammonium (NH_4^+), which is a form of nitrogen plants *can* absorb through their roots. Many of these nitrogen-fixing bacteria in the soil live in nodules on the roots of plants such as peas, beans, and pass this NH_4^+ directly on to their host plants in return for sugars created by the plants. But other nitrogen fixing bacteria live freely in the soil where they join with the decomposers (who release ammonia from the wastes and decaying bodies of organisms) in providing the ammonium that plants and other producers need (Brand, Jane B, and Neil A,2004). After the nitrogen-fixing bacteria complete their task, other bacteria in the soil such as ammonia-oxidizing bacteria and nitrite-oxidizing bacteria then convert any excess ammonium to nitrates NO_3^- in a process called nitrification. Nitrates are the other form the plants and other producers can also absorb to use to build their amino acids, proteins, and nucleic acids, and eventually denitrifying bacteria in the soil convert any excess nitrates back to nitrogen gas and release it into the atmosphere.

The reason accessible nitrogen is so important to plants and the rest of the organisms in the ecosystem is because DNA, the molecule that controls all life processes, is composed of nitrogenous base pairs. Furthermore, DNA is transcribed into RNA to produce enzymes and other proteins (which also contain nitrogen in their amino acids), and since enzymes start and stop the chemical reactions in cells, without the nitrogen, all the chemical reactions that cause the four properties of life (reproduction, homeostasis, transformations of energy and synthesis of new material) would stop and eventually cause the cells of the plant —and hence the plants themselves— to die. The consumers who then eat the producers to obtain their nitrogen in the form of organic monomers would consequently die, and so on up the food chain. Hence, because of the critical role that bacteria play cycling nitrogen compounds in the soil, an ecosystem would collapse without bacteria.

One potential threat to soil bacteria are pesticides. There are chemicals used in situations where people wish to kill, repel, or control certain forms of plant or animal life that are considered to be pests (hence the name), and people commonly use them when planting a garden to protect their plants and maintain high crop production. However, most pesticides are designed to affect only one specific target organism or process. Insecticides, for example, are commonly used to target insects that may be eating/destroying crops, while herbicides are used to target undesired plant life or “weeds.” Regardless of type, though, once any pesticide is sprayed onto crops, it leaves a residue filled with small amounts of harmful chemicals. While pesticides are intended to control specific fungi, insects, or other pests, their mechanisms of action are often not unique enough to prevent unintended effects, such as negatively impacting non-target microbial populations —including bacteria. Furthermore, once the pesticides kill the micro-organisms living in the soil, it can take years before the microbial populations can recover and once again live in soil that has had these toxic chemicals applied to it. Therefore, “the direct effects of all pesticide types produced, on average, negative effects for algal, protozoal, fungal and bacterial species.” (Zachery R. Valerie J. and Jason R, 2015), and since having a negative effect on bacteria means also having a negative effect on the benefits that bacteria provide to the soil ecology, pesticides are potentially a threat to the entire ecosystem.

A number of decomposers, though, have been shown to break down pesticides and pollutants in soil (Ingham, 2009), and therefore, we wanted to learn more about the impacts of pesticides on the bacteria living in the soil on our campus. We discovered that scientists have created a different formula that they call organic insecticides, which contain natural substances, including soaps, lime sulfur, and hydrogen peroxide, and we wondered whether even though these insecticides are marketed as better for the environment (less toxic) than the conventional pesticides, do they in fact have a better impact on the microorganisms, specifically focused on the bacteria that lay beneath the soil?

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Organic vs. Inorganic Insecticides Lab Report

Problem:

Will inorganic insecticides change the population density of bacteria in the soil more than the organic insecticides?

Hypothesis:

The inorganic insecticides will lead to a greater decrease in the population density of the bacteria in the soil than the organic insecticides.

Independent Variable:

Application of organic vs. inorganic insecticides to the soil plots

Dependent Variable:

Density of soil bacteria (#/cm^3)

Positive Control:

Soil samples collected before adding the pesticides and water

Negative Control:

Soil plots only with water added into to them.

Controlled Variables:

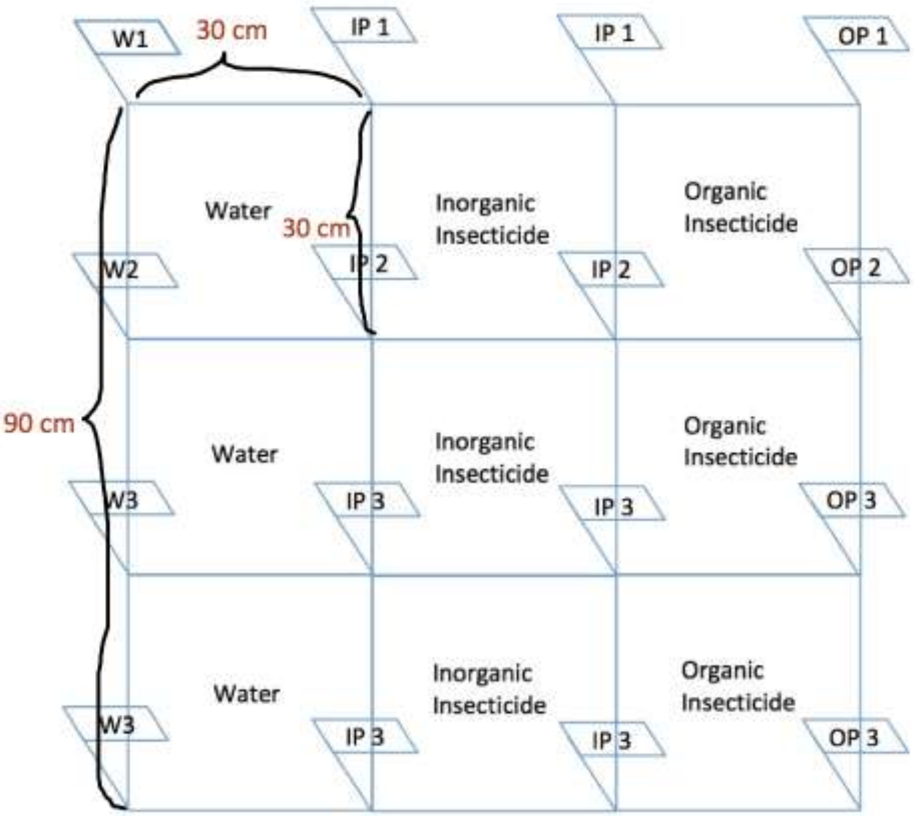
- Type of organic insecticides
- type of inorganic insecticides
- Amount of soil extracted
- Area of each of the soil plot
- Soil samples taken, and the diluting process performed at same day and same time
- the size of the culture tubes with caps
- 10 of sprays of organic insecticides added into the OP soil plot
- 10 of sprays of inorganic insecticides added into the IP soil plot
- 10 of sprays of water added into the W soil plot
- 1 cc size of scoop soil sample put in the 10^0 dilution tube
- 10 ml of sterile water in the 10^0 dilution tube, 9 ml of sterile water in each of the 10^{-1}, 10^{-2} and 10^{-3}dilution tubes
- Degree to 10^{-3} sample diluted.
- the dilution samples put on the plate
- The amount of time let bacteria to grow
- type of bacteria nutrient agar
- amount of solution dilution on agar plate
- 72 hours allow solution dilution on plates to grow
- location of soil plot

space between each soil plot

Procedure:

1. Get 16 flags and label them to correspond with where they will be placed on your plots of land.
2. Label 8 with "IP" (inorganic pesticides), 4 with "OP" (organic pesticides), and 4 with "W" (water).
3. Locate a 90 cm x 90 cm plot of soil on the RPCS lawn at N 39°21.418, W076°38.184.
4. Section it off into 30 cm x 30 cm plots and each one with the correctly labeled flags, as shown in the Figure 1 (each flag should either be labeled W (1,2,3), IP (1,2,3), OP (1,2,3):

#Figure 1



5. Steps 6- 9 should be done on the same day at the same time.
6. Once you have made your plots, collect a soil sample with a 3 cm diameter, that is15 cm deep from W1.
7. Put the soil into a plastic bag that labeled "W1A".
8. Repeat steps 6-7 twice more in the same soil plot, writing a “W1B” the second time and a “W1C” the third time.

9. Repeat steps 6-8 on the eight remaining soil plots labeled differently (W2A, B, C/ W3A, B, C/ IP1A, B, C/ IP2A, B, C/ IP3A, B, C/ OP1A, B, C/ OP2A, B, C/ OP3A, B, C)
10. Mix all three samples (A, B, C) from each plot (W1, W2, W3, IP1, IP2, IP3, OP1, OP2, OP3) into one plastic bag, one soil plot one plastic bag (total amount of 9 bags).
11. To perform serial dilutions for bacteria on all 9 samples at same day and same time, to find how much bacteria are in each soil plot before the experiment. Perform instructions a-s at same day and same time:
    a. Use a clean, new transfer pipette to add 10 ml of sterile water to a 15 ml culture tube. Label this tube "10^0 W1."
    b. Use the same pipette to add 9 ml of water to a second 15 ml culture tube. Label the tube "10^{-1} W1."
    c. Repeat step b two more time to three additional 15 ml culture tube, only label them "10^{-2} W1", "10^{-3} W1."
    d. Using a 1 cc scoop, place 1 cc of W1 soil sample into the "10^0W1" tube
    e. Cap the tube and shake vigorously.
    f. Using a new clean pipette, remove 1 ml of the soil &water mixture from the "10^0 W1" tube with a serological pipette and place it in the "10^{-1} W1" Tube.
    g. Cap and shake vigorously.
    h. Using the same pipette in step f, remove 1 ml of the soil/water mixture from the "10^{-1} W1" tube and place it into the "10^{-2} W1" tube.
    i. Cap and shake vigorously.
    j. Using the same pipette in step f, remove 1 ml of the soil/water mixture from the "10^{-2} W1" tube and place into the "10^{-3} W1" tube.
    k. Cap and shake vigorously.
    l. You should now have a total of four culture tubes.
    m. Repeat steps a-l with the negative control water samples W2 and W3, the inorganic insecticides IP1, IP2, and IP3, and the organic insecticides samples OP1, OP2, and OP3, labeling accordingly, for a total of nine soil samples.
    n. Label 3M PetrifilmTM Aerobic Count Plates to correspond to the labeled 10^{-2} and 10^{-3} dilution tubes for all soil samples. (total amount of 18)
    o. Plate 100 microliter samples from each the 10^{-2} and 10^{-3} dilution tubes onto their own separate correspondingly labeled 3M PetrifilmTM Aerobic Count Plate.
    p. Allow plates to grow for 72 hours.
    q. 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 $10^{|\text{dilution\# at which these colonies were found}|}$
    r. If there are not individual colonies but still a "lawn" at the 10^{-3} dilution, repeat the dilution adding a 4th (10^{-4}) & 5th (10^{-5}) dilutions, etc. as necessary until individual colonies are observed.
    s. Record the data in the data table.
12. Make your own organic pesticides by using: 350 ml of water, 15 grams of olive oil, 15 grams of Dawn dish soap original scent, 15 grams of cayenne pepper, and 1 garlic clove, crushed.

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13. Put all these ingredients in 400 ml spray bottle
14. Shake the bottle vigorously, pour the mixture you have made into a spray bottle.
15. Fill a spray bottle up with tap water
16. Obtain a bottle of Bayer Advanced Rose and Flower Insect killer
17. Once you have the organic pesticides, inorganic pesticides, and sterile water, go outside and spray 10 times of organic pesticides on to the organic pesticides plot.
18. While one person is spraying the organic pesticides have the other two people spray the water on the water plots and the inorganic pesticide on the inorganic plots. Doing this all at the same time.
19. Label 27 plastic bags to correspond with the soil sample that will be kept in it.
20. After 48 hours, collect a soil sample with a 3 cm diameter, that is15 cm deep from each plot on the same day and same time.
21. Repeat step 11 diluting process on the new samples on the same day and same time.
22. Record data, compare result of the new samples to the negative control samples result from step 11.

Data table

Impact of Inorganic Insecticides vs. Organic Insecticides

on the Density of Soil Bacteria (# / cc)

	Water Treatment Plots		**Inorganic Insecticide Treatment Plots**		**Organic Insecticide Treatment Plots**	
Trials #	Before adding water	After adding water	Before adding inorganic insecticide	After adding inorganic insecticide	Before adding organic insecticide	After adding organic insecticide
#Trial 1	2,100,000	21,000,000	2,600,000	4,500,000	1,800,000	9,000,000
#Trial 2	4,700,000	7,500,000	33,700,000	12,000,000	13,700,000	7,600,000
#Trial 3	8,600,000	13,500,000	7,600,000	15,900,000	2,290,000	16,500,000
Average	5,133,333	14,000,000	14,633,333	10,800,000	5,930,000	11,033,333

Graph

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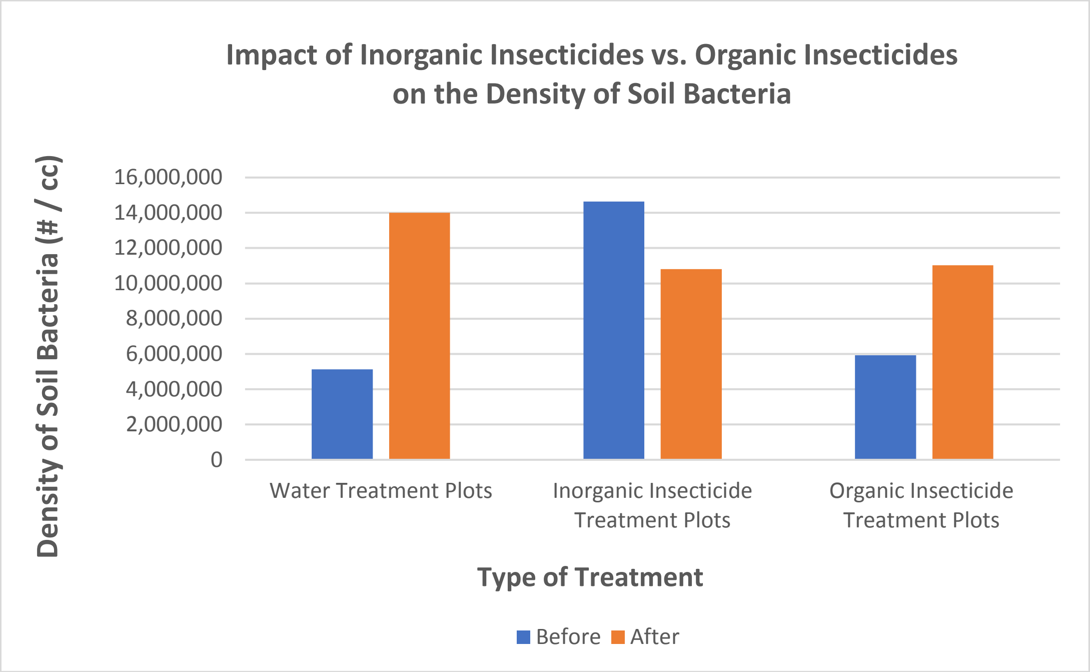
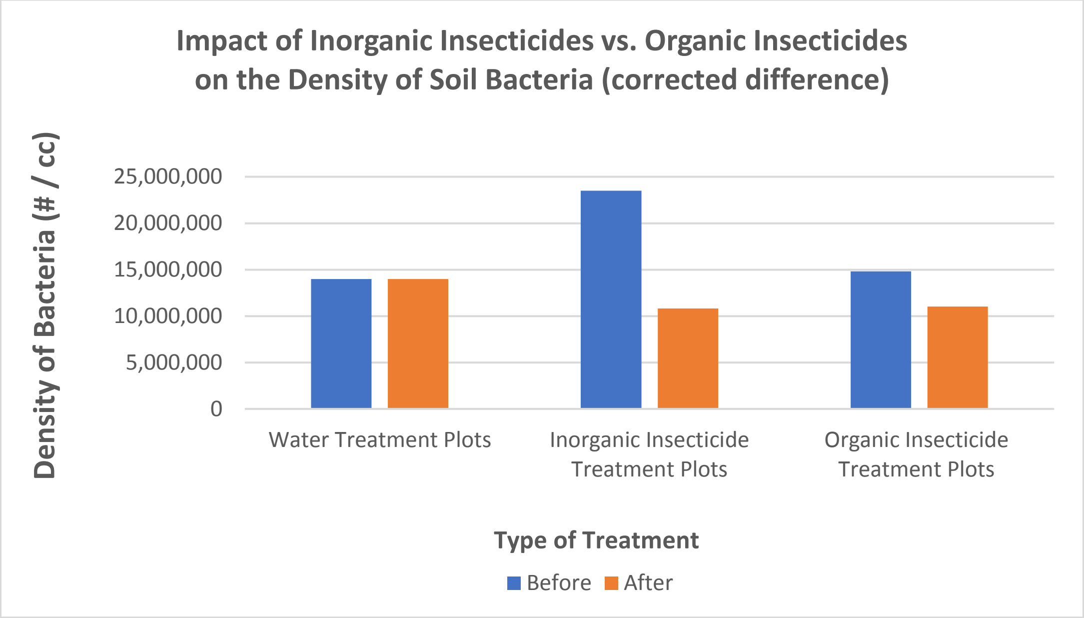
#Graph No.1



#Graph No.2



Conclusion

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As one can see from the data, the hypothesis was supported. We hypothesized that the inorganic insecticides led to a greater decrease in the population density of the bacteria in the soil than the organic insecticides. According to the data collected from the experiment, the average density of soil bacteria before adding water treatment (the negative control) to the soil plots is 5,133,333/ cc of soil. However, the average density of soil bacteria after adding water is 14,000,000/ cc of soil, which shows that the average density of soil bacteria increased by 8,866,667/ cc of soil. Graph No. 1 further supports the data by clearly showing the water treatment soil plot increased the average density of bacteria. This data shows the environment for bacteria to live became better between the first time collected soil samples and the second time collected soil samples. we hypothesized that the rain from those days caused the bacteria population in the soil to increase because the similar research has shown the soil bacteria population will have an increased effect after rain. In the rain plot, bacterial numbers doubled within 3 days and declined during the following period of drought (Johan Schnürer, Marianne Clarholm, Sven Boström, Thomas Rosswall, 1986).

The purpose of having a negative control is to know the change in the average density of bacteria without the independent variable affecting it. The environment is the only factor that could have changed the negative control. If there is an increase in average population density of bacteria in negative control plots, it means the same increase will happen in the inorganic insecticide treatment plots and organic insecticide plots. After corrected the difference of both inorganic insecticide treatment plots and organic insecticide plots bars in Graph No.2, the average population density of bacteria decreased significantly by 12700000/ cc of soil. The graph clearly shows a huge drop in the average population density of soil bacteria under the inorganic insecticide treatment compared to the negative control. The decrease of population density of bacteria shows that inorganic insecticide has a very harmful impact on the population density of the bacteria in the soil. The average bacteria population density from the organic insecticide treatment plot also had a decrease, but not as much as the inorganic insecticide. There is a decrease of 376334/ cc of soil in average population density of soil bacteria in the organic insecticide treatment plot. Graph No.2 shows that it did not decrease as much as in the inorganic insecticide treatment plot. Based on the data collected in this investigation, it can clearly be concluded that the inorganic insecticide will lead to a greater decrease in the population density of the bacteria in the soil than the organic insecticides.

The result that inorganic insecticides have led to a greater decrease in the population density of the bacteria in the soil than the organic insecticides makes sense because the active ingredient cyfluthrin that is contained in the inorganic insecticide will not be found in the organic insecticide. Cyfluthrin has a specific interaction with the sodium channels of the nerve membranes, and this takes place, increasing neuronal excitability (Appel and Gericke 1993; Bradbury and Coats 1989). Even though bacteria do not have a nervous system, they do have a sodium channel, which means cyfluthrin will have an interaction to the sodium channels in the bacteria. Therefore, further research could answer the question of what specific interaction the cyfluthrin has with the sodium channels in bacteria.

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