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Soil Ecology

Impact of Pesticide and Herbicide on The Population of Bacteria

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Background

As the world's population grows considerably, agricultural needs continue to grow. To address the increasing demand for crops, the use of agricultural chemicals such as pesticides and herbicides has increased, maximizing the yield and quality of agricultural products. Herbicides are used to kill the "undesirable" weeds that steal nutrients from "desirable" plants in the soil, thereby increasing the production of crops. Pesticides are also used to increase the crop yield by killing pests that eat the crops. Both chemicals work to preserve the desired plant and maximize the total production of crops.

Pesticides are used to target insects and non-insect pests such as mosquitoes, ticks, caterpillars, and mice. Pesticides work by chemically or biologically interfering with the metabolism of pests (Environmental Protection Agency [EPA], 2017). Pesticides can be classified as contact or systemic. Contact pesticides are absorbed through the body surface of the pest, whereas systemic pesticides are absorbed from where they are applied and moved to other parts of the plant to reach their target. In this experiment, we are using "Bayer Advanced Rose & Flower Insect Killer" as our pesticide. The main active ingredient in "Rose & Flower Insect Killer" is cyfluthrin. Cyfluthrin is classified in a group of man-made insecticides called pyrethroids. Cyfluthrin acts as a stomach poison and kills the insect through direct contact. When applied, cyfluthrin attacks the nerves of insects and causes muscle spasms. That results in paralysis or starvation. This chemical is less toxic to people and mammals because their digestive system can break it down faster than insects (National Pesticide Information Center, 2015).

Instead of targeting pests, herbicides are used to destroy or suppress the growth of a weed-like plant or other unwanted vegetation (EPA, 2017). Contact herbicides kill the part of

plant that are in direct contact with the product, while systemic herbicides are absorbed by roots or leaves and travel through the plant (Lingenfelter, 2018). In this experiment, we plan to "Roundup Ready-To-Use Plus Weed & Grass Killer" as our herbicide. The main active ingredient used in "Roundup Ready-To-Use Plus Weed & Grass Killer" is glyphosate. Glyphosate limits the ability of the plants to produce certain proteins needed for growth (National Information Center, 2015). The toxicity of glyphosate is undetectable or small in organisms such as humans, rats, dogs, mice, and rabbits (Extension Toxicology Network, 1994).

Both herbicides and pesticides work to improve the crop yield. However, application of pesticides or herbicides can cause a change in the population of microbial communities in the soil, including bacteria. Bacteria live in the water space in the soil, which is near the plants' roots. Bacteria have a basic structure that includes cell wall, ribosomes, plasma membrane, DNA that is not enclosed in a membrane, and flagella for movement. Bacteria reproduce through binary fissions to produce a colony of cells that are genetically identical. Bacteria can be divided into heterotrophs and autotrophs, but bacteria found in the soil are heterotrophs because they obtain energy by eating other organisms. Also, both fungi and bacteria eat bacteria in the soil (Cambell et al., 2006).

Bacteria perform many roles in the soil, including transforming nitrogen in the nitrogen cycle and decomposing dead organic material (Juma, 1998). When living organisms die, they leave a huge amount organic material behind. Detritivores in the soil are the first to break down this material into smaller pieces. Detritivores are larger organisms, such as earthworms, maggots, and woodlice (BBC, 2014). Then bacteria and fungi, known as saprophytes, produce degrading enzymes to break down complex molecules contained in the organic material and transform them from polymers into monomers that can be reused in cells (Juma, 1998). Decomposition breaks

down organic matter into small molecules for producers to obtain nutrients for photosynthesis and produce energy. Then other consumers in the ecosystem obtain energy from the producers. As a result, decomposition helps the producers to produce energy and support consumers.

The nitrogen cycle is a complex biogeochemical cycle in which nitrogen is converted from its atmospheric molecular form (N_2) into a form that is useful in biological processes. The nitrogen cycle includes several stages: nitrogen fixation, nitrification, assimilation, ammonification, and denitrification (The Environmental Literacy Council, 2015). First, precipitation mainly deposits nitrogen into the soils and surface waters from the atmosphere. Nitrogen-fixing bacteria use an enzyme, known as nitrogenase, to break the atmospheric nitrogen molecules into individual nitrogen atoms. Then nitrogen-fixing bacteria combine hydrogen atom with an individual nitrogen atom to form ammonia (NH_3) in the process nitrogen fixation. After plants and animals die, nitrogen in the organic matter gets broken down by decomposers, such as bacteria and fungi in the soil, which returns the nitrogen back to the soil through ammonification. The next stage is nitrification, which is when nitrifying bacteria convert ammonia (NH_3) into nitrite(NO_2) and nitrate(NO_3). Plants use the nitrogen compounds, especially nitrate(NO_2), nitrite(NO_3), and ammonia, to construct DNA, RNA, and proteins in cells in the process called assimilation (Strock, 2018). Without nitrogen, living organisms lack ability to produce special proteins, called enzymes, which are needed to start chemical reactions. Chemical reactions, especially reproduction, synthesis of new material, transformation of energy, and homeostasis, are necessary to form a living cell. Therefore, nitrogen is crucial to sustain life and make new cells. Finally, remaining nitrate converts back into gaseous nitrogen through a process called denitrification, which means nitrogen goes back into the atmosphere (The Environmental

Literacy Council, 2015). Therefore, bacteria play an important role in the nitrogen cycle and transforming the atmospheric nitrogen into usable nitrogen for other organisms.

Besides fixing nitrogen, another key function of soil bacteria is improving soil structure through the formation of soil aggregates. These bacteria produce a layer of polysaccharides that coats the surface of the cell (Hoormann, 2011). These substances are important to cement sand, silt, and clay soil particles, which create stable microaggregates (Hoormann, 2011). Microaggregates "can withstand strong mechanical and physical stresses," which allows a stable soil structure to persist for several decades (Wulf et al. 2017). The improvement of soil structure allows for better water infiltration (Ingham, 2009). Water infiltration allows the water to restore within the soil, which enables plants to extract water from the soil to be used in the process of photosynthesis. If soil has poor the water infiltration, the plants will not have the necessary water supply needed for photosynthesis (Natural Resources Conservation Service, 2001). Also, the improvement of soil structure increases holding capacity of the soil (Ingham, 2009). Holding capacity of the soil means the pore space to hold resources, such as water and oxygen for organisms to use. If soil has less holding capacity, the plants also will not have necessary water supply needed for photosynthesis, and other soil organisms will not have necessary oxygen supply needed for the production of ATP.

Ideally, pesticides and herbicides would only act on target organisms and not cause a major change to the soil environment (Tvedten, 2014). However, few pesticides and herbicides function ideally. Soil bacteria can decompose some parts of herbicides and convert them into carbon sources, which would allow the bacteria population to grow. Research conducted by Sebiomo, A., et al. (2011) suggests that application of pesticides and herbicides could change the chemical composition of the soil. After the application of herbicides or pesticides, dead weeds or

pests containing the toxic chemicals from the product will remain in or on the soil. If bacteria cannot fully decompose the toxic crops and pests, these chemicals will accumulate in the soil over a long period of time and contaminate other food sources of bacteria, which could ultimately decrease the population of bacteria in soil (Lo, 2010). Studies done by Newman et. al (2016) reflected that some bacteria populations decreased as a result of glyphosate application in the long-term. Also, research conducted by Fukuto (1990) reflects that the application of pesticides decreases the population of bacteria in the soil. Based on previous research, our group predicts that both herbicides and pesticides will have negative impacts on the population of bacteria in the soil. However, herbicide seems to have a relatively low toxicity in their chemical ingredients compared to pesticides (Lorenz, 2018). We hypothesize that when herbicides are applied to the soil, the population of bacteria will decrease less than when pesticides are applied to soil.

Experiment Design

I. **Problem**: How will herbicides and pesticides impact the population density of bacteria in the soil?

II. **Hypothesis**: When herbicides are applied to the soil, the population of bacteria will decrease less than when pesticides are applied to soil.

III. **Procedure**:

A. Independent Variable: the presence of herbicides and the presence of pesticides on the soil respectively

B. Dependent Variable: the change in the population density of bacteria in 1 cc of soil.

C. Negative Control: the presence of water solution on the soil

D. Controlled Variables: the location of plot, the size of plot, the type of pesticides, herbicides, and water in different trials, the same number of pesticide, herbicide, and water sprays applied onto all sections, the size of the serological pipettes, the size of culture tubes, the amount of culture tubes, the same day and time to collect the soil sample, the size of scoop, the same amount of sterile water added to the tubes, the same amount of time to wait bacteria to grow on the petri plates, the same time and same day to perform the serial dilution, the same type of 3M Petrifilm™ Aerobic Count Plate, the same direction of soil core sampler is twisted, the amount of test soil, the amount of soil solution extracted, the type of caps, the type of soil core sampler, the room temperature for bacteria to grow on the petri plates, the amount of soil solution putted onto the 3M Petrifilm™ Aerobic Count Plate, the amount of the rigorousness when shake the tubes

E. Step-by-step:

1. Go to coordinate N 39.35662°, W076.63551° and make a 60 cm by 60cm square by placing a flag on each of the 4 corners.
2. Using 6 new flags, label one flag “herbicides”, one flag “pesticides”, one flag “water”, one flag “Trial 1”, one flag “Trial 2”, one flag “Trial 3”, to help designate the location of the plots.
3. Create 3 even rows, each 60cm x 20cm, with each row labelled "pesticide”, "herbicide", and "water", respectively, according to the plot structure below.
4. Create 3 even columns, each 20 cm by 60cm, with each column labelled “Trial 1”, “Trial 2”, and “Trial 3”, respectively, according to the plot structure below.

Herbicide Trial 1	Herbicide Trial 2	Herbicide Trial 3
Pesticide Trial 1	Pesticide Trial 2	Pesticide Trial 3
Water Trial 1	Water Trial 2	Water Trial 3

5. Label 9 bags with the plot name according to the diagram above. Make sure to label each bag with "before" to indicate this is before spraying any chemicals.
6. Place the soil core sampler on the surface of "Trial 1 pesticide" section and press the soil core sampler 15 cm down into the ground. Use the hammer to help move the soil core sampler down if needed.
7. Twist the soil core sampler clockwise and remove it from the "Trial 1 pesticide" section. Place the soil in the plastic bag with corresponding plot name.
8. Repeat step 6-7 for the remaining 8 plots. Make sure to place each soil sample in a plastic bag with the corresponding plot name. Also, make sure to collect the soil samples on the same day and at the same time.
9. Bring soil samples back to classroom to perform dilution procedure in steps 10 through 26.

10. Use a clean, new transfer pipette to add 10 ml sterile water to a 15 ml culture tube. Label the culture tube "Pesticide T1 (before) 10^{0}".
11. Use the same pipette to add 9 ml of sterile water to a second 15 ml culture tube. Label this "Pesticide T1 (before) 10^{-1}".
12. Repeat step 11 two more times using two additional 15 ml culture tubes, only label them "Pesticide T1 (before) 10^{-2}," and "Pesticide T1 (before) 10^{-3}" respectively.
13. Place 1 cc of the soil sample from "Pesticide Trial 1" section into the "10^{0}" culture tube by using a 1-cc scoop.
14. Cap the tube and shake vigorously.
15. Using a new clean pipette, remove 1 ml of the soil/water mixture from the "Pesticide T1 (before) 10^{0}" tube and place into the "10^{-1}" tube.
16. Cap the tube and shake vigorously.
17. Using the same pipette in step 15, remove 1 ml of the soil/water mixture from the "Pesticide T1 (before) 10^{-1}" tube and place into the "Pesticide T1 (before) 10^{-2}" tube.
18. Cap the tube and shake vigorously.
19. Using the same pipette in step 15, remove 1 ml of the soil/water mixture from the "Pesticide T1 (before) 10^{-2}" tube and place into the "Pesticide T1 (before) 10^{-3}" tube.
20. Cap the tube and shake vigorously.

21. Place 100 µl samples from the 3rd and 4th tubes (dilutions 10^{-2} & 10^{-3}) onto their own separate, 3M Petrifilm™ Aerobic Count Plate labeled with the corresponding plot name.
22. Repeat steps 10 through 21 for the soil samples from the other 8 plots. Make sure to perform the serial dilution for all samples on the same day at the same time.
23. Allow the bacteria to grow on the plates for 48 hours at room temperature.
24. Use the magnifying glass to examine the bacteria colonies on 10^{-3} plate and count the number of red dots that signify bacteria colonies. If there are less than 5 bacteria colonies on 10^{-3} plate, count the number of bacteria colonies on the 10^{-2} plate. Record the number of red dots on the plate and the dilution number.
25. Make estimations of the number of bacteria in the original 1 cc soil sample by using the following formula:

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

26. Record the calculated number of colonies for each section in the data table.
27. Spray 11 sprays of the "Roundup Ready-To-Use Plus Weed & Grass Killer" onto the "Herbicide Trial 1" section, 11 sprays of the "Rose and Flower Insect Killer" onto the "Pesticide Trial 1" section, and 11 sprays of water onto the "Water Trial 1" section.

28. Repeat step 27 for the remaining 6 sections. Make sure to spray all of the solutions onto their corresponding plots on the same day and at the same time. Wait 24 hours.
29. Label 9 plastic bags with 9 plot names respectively referring to the plot diagram. Make sure to label “after” on the bags to indicate after spraying chemicals.
30. Place the soil core sampler on the surface of “Trial 1 pesticide” section and press the soil core sampler 15 cm down into the ground. Use the hammer to help move the soil core sampler down if needed.
31. Twist the soil core sampler clockwise and remove it from the “Trial 1 pesticide” section. Place the soil in the plastic bag with corresponding plot name.
32. Repeat step 30-31 for the remaining 8 plots. Make sure to place all soil samples in a plastic bag with the corresponding plot name. Also, make sure to collect the soil samples on the same day and at the same time.
33. Repeat steps 10-26 to dilute and plate all extracted soil samples after spraying the “Roundup Ready-To-Use Plus Weed & Grass Killer”, “Rose and Flower Insect Killer”, and water.

Data Table and Analysis

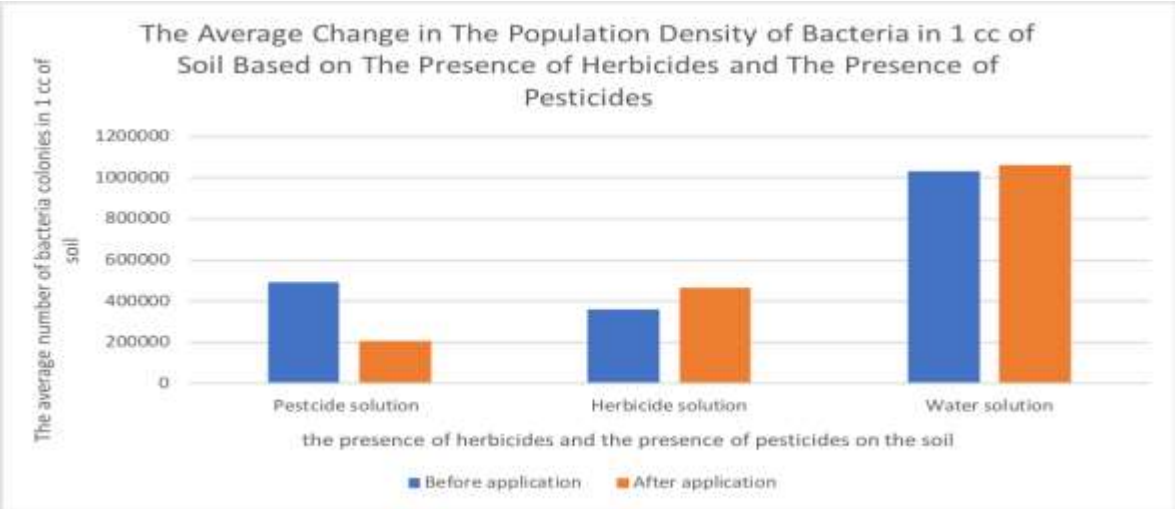
Table 1: The Change in the Population Density of Bacteria in 1 cc of Soil Based on the Presence of Herbicides and the Presence of Pesticides

	Pesticides Solution		Herbicides Solution		Water Solution	
	Before application	After application	Before application	After application	Before application	After application
Trial 1	700,000 bacteria colonies in 1 cc of soil	40,000 bacteria colonies in 1 cc of soil	800,000 bacteria colonies in 1 cc of soil	460,000 bacteria colonies in 1 cc of soil	500,000 bacteria colonies in 1 cc of soil	590,000 bacteria colonies in 1 cc of soil
Trial 2	500,000 bacteria colonies in 1 cc of soil	350,000 bacteria colonies in 1 cc of soil	100,000 bacteria colonies in 1 cc of soil	910,000 bacteria colonies in 1 cc of soil	1,700,000 bacteria colonies in 1 cc of soil	1,900,000 bacteria colonies in 1 cc of soil
Trial 3	280,000 bacteria colonies in 1 cc of soil	240,000 bacteria colonies in 1 cc of soil	190,000 bacteria colonies in 1 cc of soil	30,000 bacteria colonies in 1 cc of soil	900,000 bacteria colonies in 1 cc of soil	700,000 bacteria colonies in 1 cc of soil

Table 2: The Average Change in the Population Density of Bacteria in 1 cc of Soil Based on the Presence of Herbicides and the Presence of Pesticides

Pesticide Solution		Herbicide Solution		Water Solution	
Before application	After application	Before application	After application	Before application	After application
493,333 bacteria colonies in 1 cc of soil	210,000 bacteria colonies in 1 cc of soil	363,333 bacteria colonies in 1 cc of soil	466,666 bacteria colonies in 1 cc of soil	1,033,333 bacteria colonies in 1 cc of soil	1,063,333 bacteria colonies in 1 cc of soil

Graph: The Average Change in Population Density of Bacteria in 1 cc of Soil Based on the Presence of Herbicides and the Presence of Pesticides



Conclusion

Our hypothesis, when herbicides apply to the soil, the population of bacteria would decrease less than when pesticides apply to the soil, is not supported. Our results show that the average population density of bacteria in 1 cc of soil before applying the pesticide solution is 493,333, and the average population density of bacteria in 1 cc of soil after applying the pesticide solution is 210,000. In total, when pesticides apply to soil, the population of the bacteria decreased 57.43%. Also, the average population density of the bacteria in 1 cc of soil before the application is 363,333, and the population density of the bacteria in 1 cc of soil after the application is 466,666. So, when herbicides apply to soil, the population of bacteria increase 28.44%, which disproves our hypothesis.

In the negative control group, the average population density of bacteria in 1 cc of soil before and after applying the water solution are 1,033,333 and 1,063,333. So, when water apply to soil, the population of bacteria increases 2.9%. The data does not show a dramatic population density change, which guarantees that when we apply a solution to the soil, it will not impact the population density of bacteria.

Herbicides have a relatively low acute toxicity in the active ingredient, such as glyphosate used in this experiment, which means that herbicides possess a relatively high biodegradability. As a result, soil bacteria can decompose the herbicides and convert it into usable carbon sources for their growth. On the other hand, pesticides have a relatively high acute toxicity in their active ingredients, such as Cyfluthrin used in this experiment, which means that pesticides possess a relatively low biodegradability. As a result, dead pest containing toxic

pesticides cannot be decomposed by soil bacteria and will accumulate in the soil environment, which later contaminates the usable food sources for the bacteria. As a result, the application of pesticides will ultimately lead to the decrease of the bacteria population in the soil.

The change of bacteria population will influence the ecosystem by impact the biomass in ecosystem. Bacteria as type of decomposers break down organic matter and recycle nutrients, which means that decomposers, such as bacteria and fungi, support the energy production of producers through photosynthesis. As a result, other consumers are able to obtain energy from producers. So, if the population of bacteria increases, more organic material breaks down by the bacteria. Then producers are able to obtain more nutrients and produce more energy to support other consumers. As a result, the total stored energy in ecosystem, the biomass, will increase, which reflects a healthy ecosystem. However, if the population of bacteria decreases, less bacteria engage in the decomposition of organic matter. Then producers are not able to obtain enough nutrients to produce energy to support other consumers. As a result, the biomass will decrease in the ecosystem, which reflects an unhealthy ecosystem.

In this experiment, we only waited for the herbicide, pesticide, and water solution to react with bacteria in the soil 24 hours, so the data only shows the population change of bacteria in a short time. We can improve the experiment by extracting soil samples multiple times at different time periods after spraying solutions, which would help us to see if the population change after a long time. Then we can compare the result with this experiment. As a result, we can see if the population of bacteria decreases after the application of pesticide not only in a short time period but also in a long-term. In this way, we can also see that if the population of bacteria increases after the application of herbicide not only in a short time period but also in a long period.

In our experiment, our data may have been affected by the weather, as it did rain. The rain might run off the pesticide, herbicide, and water solution applied on the soil, which might result in an unprecise result. To improve the experiment, we could have sprayed solutions and taken the 'after' soil in a period when it would not rain, which would prevent other weather factors to influence our experiment. After we improve our experiment in this way, we expect a decrease of bacteria population after the application of herbicides. The toxicity of herbicides will decrease and the biodegradability of the chemicals in the herbicides will increase after the rain dilute part of herbicides. As a result, herbicides decompose by bacteria into usable carbon source and increase the population of bacteria. However, if rains do not dilute the herbicide solution, chemicals will not be decomposed by bacteria and contaminates other food source of bacterial, which ultimately leads to the population decrease. Moreover, we expect a greater decrease of bacteria population after the application of pesticides. When the rain does not dilute pesticides and decrease the toxicity of pesticide, the toxic chemicals in the pesticides will contaminate more food source for bacteria, which leads to a greater decline of bacteria population.

First, in the future research, we can choose other types of pesticides and herbicides that contain different active ingredients, such as acephate in pesticides, bendiocarb in pesticides, and Triclopyr in the herbicides, and apply them onto the soil. Moreover, we can use the same procedure to extract soil samples, perform the serial dilution and examine the bacteria population; we can see if different kinds of active ingredients have a similar or a more drastic impact on the population density of bacteria. As a result, we will know which type of active ingredient that has less impact on the bacteria population but kills the pest or weeds effectively as the primary type of pesticide or herbicide. It will give the agricultural product company

informed information to decide which active ingredient they should choose to use in pesticides and herbicides.

Second, we can choose to spray more or less numbers of pesticide and herbicide solution onto the soil. In this experiment, we spray 11 sprays of pesticide and herbicide solution onto 20 * 20 cm of soil, which results in a decrease of bacteria population after applied the pesticide solution and an increase of bacteria population after applied the herbicide solution. In the future, we can spray less number of pesticide onto 20 *20 cm of soil and repeat the experiment with less spray numbers. From the result, we can predict which number of pesticides will not impact or increase the bacteria population and kill the pests. Furthermore, we can spray more number of herbicide onto 20 *20 cm of soil and repeat the experiment with more spray numbers. From the result, we can predict which number of herbicide sprays can greatly increases the bacteria population as well as kills the weed. By varying the numbers of spray and comparing the results, we can inform the farmers how many sprays of agricultural product are better to get a higher agricultural production.

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