The Soil Ecology Project

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The Effects of Herbicides and Pesticides on Bacteria Microbes in Soil

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Background

Within the soil, there is an entire microscopic ecosystem that is essential to the well-being of all the organisms which live above it. The tiny organisms that make up this ecosystem are called microbes, and they live in almost every soil on earth. The majority of them fall into one of four main categories: bacteria, protozoa, algae, or fungi (Boundless, 2016), and each type has its own unique function in the soil ecosystem. In general, though, they all help produce oxygen, decompose organic material, and provide nutrients for plants. A few are pathogenic and can cause disease, but the vast majority are vital for a healthy environment.

One group of microbes that plays an especially important role in the soil are the bacteria. These prokaryotic organisms work primarily as decomposers, consuming carbon compounds from their surroundings and converting these chemicals into forms more useful for the other organisms living there; hence, this group serves as the base of a healthy nutrient chain in the soil, recycling essential organic and inorganic compounds such as carbon dioxide, phosphate and sulfur dioxide (Ingham, 2000). In addition, these decomposers improve soil structure by making polysaccharides that solidify sand, silt, and clay together for stronger soil structure (Hoorman, 2016), improving water penetration and preventing erosion.

Other types of soil bacteria, though, play a significant role in the cycling of another critical nutrient, nitrogen, and there are three main groups of them involved in this complex process that are not involved in the decomposition process itself: the nitrogen fixing bacteria, the nitrifying bacteria (nonsymbiotic bacteria), the decomposers (nitrosomonas and nitrobacter), and the denitrifying bacteria (thiobacillus denitrification) . Essentially, the nitrogen cycle is a “food into waste” cycle, with different groups of bacteria converting their food into waste that is then the food for still other types bacteria, and the first in this chain are the bacteria that convert N_2

from the air into NH_3. That NH_3 is then converted by a different group of bacteria, into NH_4^+(which is one of the two forms of nitrogen plants can absorb) and the NH_4^+ is converted into NO_2^- by yet another group of bacteria before it is finally converted into NO_3^- which is released into the soil. Any remaining NO_3^- is then converted by certain anaerobic bacteria back into N_2 and released back into the atmosphere, where the cycle continues. In addition, when organisms decay, the various fungal and bacterial microbes decomposing them (such as actinomycetes) release NH_4^+ back into the soil, adding to the ammonium already fixed from the nitrogen gas from the atmosphere

What makes this cycling of nitrogen so important is because nitrogen is vital to the growth and wellbeing of plants. Plants are able to absorb either the nitrates or ammonium through their roots and they need this element because it is a main component in the chemical makeup of chlorophyll and is therefore essential to the process of photosynthesis. Nitrogen is also a large component in the chemical makeup of amino acids, which are the building blocks of proteins and nucleic acids. More importantly, without enzymatic proteins to control chemical reactions, the cells of the plant would die (and hence the plant). Without strong proteins, plants cannot grow to their full potential and end up small and shriveled. Furthermore, in the food chain, primary consumers rely on plants to get their nutrients and protein while secondary consumers get their protein from the primary consumers. Therefore, an insufficient supply of nitrogen, courtesy of the soil bacteria, will cause everything up the food chain to die, wrecking the entire ecosystem.

Because bacteria play such an important role in the health and wellbeing of earth's ecosystems, any substance or product that might harm the bacteria can have a detrimental effect on the rest of the ecosystem, and one of the methods humans use to alter the conditions of the

soil where the bacteria live is to use chemicals such as pesticides and herbicides. People use these compounds to rid themselves of unwanted or potentially harmful organisms. (Pesticides are used to kill animals, whereas herbicides target weeds) (National Pesticide Information Center, 2017). But in both cases, toxic chemicals are introduced into the environment that might harm the bacteria.

Some of the common chemicals found in pesticides include aldrin/dieldrin, atrazine, chlordane, chlordecone, endosulfan, endrin aldehyde, hexachlorobenzene, and methylene chloride, (New South Wales EPA, 2016) but one very common pesticide contains the active ingredient cyfluthrin. This drug is an extremely harmful chemical that is potent to many different organisms in nature, including organisms as diverse as fish and bees. Cyfluthrin is a neurotoxin that causes hyperexcitation of the nervous systems, causing neurons to fire at a rate much higher than normal. It induces changes in nerve membranes, causing abnormal potassium and sodium flows. The repetitive discharges from the neurons causes blockages to further nerve impulses. The calcium concentration in nerve tissue is also harmed by cyfluthrin because cyfluthrin suppresses an enzyme that is involved in transporting calcium. Organisms that come into prolonged content with cyfluthrin often experience symptoms such as stinging skin, tremors, convulsions, decreased blood pressure, labored breathing, weight loss, kidney inflammation, vomiting, diarrhea, and a decrease in body temperature. Even a small exposure can result in lethargy and an inability to gain weight (Cox, 1994). This ultimately leads to convulsions and death. (Gilbert, 2014).

Plants, though, do not have nervous systems. Therefore, herbicides must use a different method to target undesired plant life. These chemicals must be taken in by the leaf and move through cells until they finally reach an enzyme target (Martin, 2004). Some chemicals

commonly used in to do this include acetochlor, arsenic, diquat, glyphosate, metolachlor, and propanil.

One common active ingredient in herbicide is 2,4-D, dimethylamine salt. This chemical comes from a parent acid and exists in both ester, acid, and salt form, but in herbicides it is most commonly used in its salt form. 2,4-D, dimethylamine works by causing uncontrollable cell division in vascular tissues. After exposure to 2,4-D, dimethylamine, abnormal increases in cell wall plasticity, biosynthesis of proteins, and production of ethylene occur, and it causes the cells in plants that carry water nutrients to grow and divide without stopping, killing the plant. This chemical is designed to target and control terrestrial and aquatic weeds in both agricultural and non-agricultural setting, but it can also be an irritant to eye tissue in animals and humans. Extreme level of exposure can cause damage to the eye, thyroid, kidney, adrenals, and ovaries or testes. (NPIC, (n.d.))

Although pesticides and herbicides are used to get rid of unwanted things, and they may be convenient for farmers, they can cause harm to people and organisms that are actually beneficial to our environment (Gilbert, 2014) and therefore have an impact on the environment that is a largely negative one. For example, pesticides and herbicides can cause short term and long term diseases in humans, and along with their designated targets, pesticides and herbicides can harm important insect species and microorganisms, including the possibly bacteria responsible for nitrogen and keeping all the other organisms in the soil alive.

Because of the possible harmful consequences of using pesticides and herbicides, we are comparing the effects of herbicides and pesticides on bacterial population in the soil in our experiment. We wanted to see if pesticides or herbicides had a negative impact on bacterial population numbers, and if so, which one had a greater effect. We predict that pesticides reduce

population density of bacteria more than herbicides do, because pesticides contain more potent chemicals than herbicides.

Experiment Outline

I. Problem- How do pesticides and herbicides change the population density of bacteria in soil?

II. Hypothesis- Pesticides in the soil reduce population density of bacteria more than herbicides in the soil do.

III. Procedure

a. Independent Variable- Application of herbicide or pesticide to soil plots

b. Dependent Variable- Population density of bacteria in the soil (#/ cm^3)

c. Positive Control- Population density of bacteria in each soil plot before independent variable is applied

d. Negative Control- Soil plots with only water applied to them

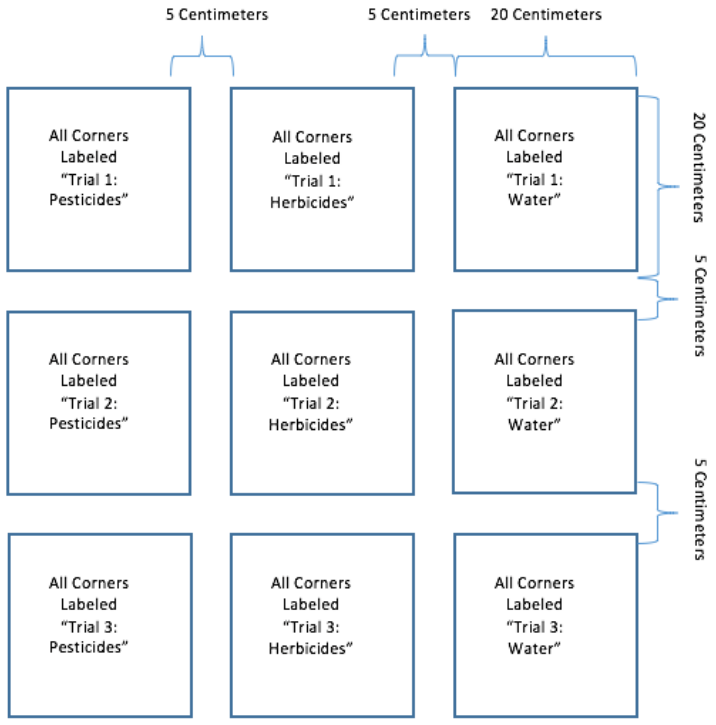
e. Controlled Variables- Location of soil plots, plant life in all soil plots, typography of land, sunlight exposure on soil plots, size and type of micropipette, size of culture testing tubes and caps, size of soil plots, unit of measurement, distance between each soil plot, climate of plots, order in which plots are placed, layout of soil plots, method of taking soil samples, method of mixing soil samples, type of soil, brand of pesticide, brand of herbicide, where soil samples are taken from, amount of soil in the sample taken, amount of herbicide applied, amount of pesticide applied, method of testing for bacteria population density, size of pipettes, amount of soil sample placed into 15ml culture testing tube, method and

amount of time 15ml test tube is shaken for, how hard the 15 ml test tube is shaken, amount of time samples grow for, number of culture tubes made, amount of material diluted between culture tubes, method of making culture tubes, amount of time soil plots sit with the pesticides, herbicides, or water, method of applying herbicides, pesticides, or water, amount of sterile water added to culture tubes, amount of soil placed into 10^0 15ml culture test tube, degree of serial dilution, amount of pipettes used during serial dilution, amount of material diluted between culture tubes, type of water (tap water) used to spray water plots, type of applicator for herbicides, pesticides, and water, dilution of herbicides and pesticides, distance from soil plot and applicator, type of nutrient agar, amount of nutrient agar, size of agar plate, amount of diluted liquid applied to plate, temperature nutrient agar is grown at, location where nutrient agar grows, amount of hours the nutrient agar grows for

A. Step-by-Step Procedure

1. Outside, on the Roland Park Country School campus, at N 39°21.397' W 76 °38.119 find a plot of land with only grass growing on the plot in a mostly shady area
2. Measure, with a meter stick, a 20 centimeter by 20 centimeter box on the grassy area, marking each corner of this box with a small, metal, flag. Each of these 4 flags labeled "Trial 1: Pesticides"
3. Next to this plot, leave a 5 centimeter space in between the next plot, and plot another 20 centimeters by 20 centimeters with a flag at each corner, this time labeled "Trial 1: Herbicide" (see diagram below)

4. Next to this plot, leave a 5 centimeter space in between the next plot and plot another 20 centimeters by 20 centimeters with a flag at each corner, this time labeled “Trial 1: Water” (see diagram)
5. Below this row of 3 soil plots, make two more rows of three soil plots, marking all corners with flags. Although, this time, mark the second row “Trial 2”, and the 3rd row “Trial 3” (diagram below)



6. From each of these 9 soil plots that have been sectioned off, begin to collect soil samples from each one (steps 7-8). These samples must all be taken on the same day at the same time.
7. Do this by using a mallet to hammer a soil core extractor with a 2 cm diameter into the soil. Take a core that is 15.5 cm deep with a diameter of 2 cm. This is a ‘before’ soil sample.

8. Take 3 soil extractions from each of the 9 soil plots, resulting in a total of 27 soil extractions. Place each of these soil samples into separate plastic bags, labeled with the same titles as their marker flags (example: Trial 1: Water)
9. At the same time on the same day do steps 9 and 10. Combine the 3 soil samples from each of the 9 different plots (for example: combine the three soil samples from "Trial 1: Pesticides"). You should have a total of 9 plastic bags filled with soil.
10. Mix the soil samples with clean hands until the soil has the consistency of wet sand. Wash hands between mixing each of the 9 soil plot samples.
11. Now use the soil samples to dilute for bacteria by doing the follow steps. These following steps (11a through 11o) must be done on the same day at the same time.

11a. Use a clean, new transfer pipette to add 10 ml to a 15 ml culture tube. Label the tube "10^0 Pesticides Positive Control Trial 1"

11b. Use the same pipette to add 9 ml to a second 15 ml culture tube. Label the tube "10^{-1} Pesticides Positive Control Trial 1"

11c. Repeat 11b three more times to two additional 15 ml culture tubes, only label them "10^{-2} Pesticides Positive Control Trial 1", "10^{-3} Pesticides Positive Control Trial 1", respectively.

11d. Place 1 cc of the soil in the pesticides positive control trial 1 plastic bag into the "10^0 Pesticides Positive Control Trial 1" culture tube".

11e. Cap the tube and shake vigorously.

11f. Using a new clean pipette, remove 1 ml of the soil/water mixture from the "10^{-1} Pesticides Positive Control Trial 1" tube and place into the "10^{-2} Pesticides Positive Control Trial 1" tube.

11g. Cap the tube and shake vigorously.

11h. Using the same pipette from step 11f, remove 1 ml of the soil/water mixture from the "10^{-1} Pesticides Positive Control Trial 1" tube and place into the "10^{-2} Pesticides Positive Control Trial 1".

11i. Cap the tube and shake vigorously.

11j. Using the same pipette from step 11f, remove 1 ml of the soil/water mixture from the "10^{-2} Pesticides Positive Control Trial 1" tube and place into the "10^{-3} Pesticides Positive Control Trial 1"

11k. Cap the tube and shake vigorously.

11l. You should now have a total of four culture tubes.

11m. Plate 100µl samples from the 3rd and 4th tubes (dilutions "10^{-2} Pesticides Positive Control Trial 1" and "10^{-3} Pesticides Positive Control Trial 1") onto their own separate, correspondingly labeled, 3M Petrifilm™ Aerobic Count Plate

11n. Repeat steps 11a through 11m for each of the 9 soil samples, labeling each 15 ml culture testing tube $10^{\#}$, Type of Test (Pesticides, Herbicides, Water), Positive Control, Trial Number

11o. Allow all 18 agar plates to grow for 48 to 72 hours in the same location at the same temperature (room temperature).

11p. First, examine the plate with the lowest dilution value for individual bacteria colonies and determine if there are 5 of more bacteria colonies on the plate. If this plate does not have at least 5 bacteria colonies, move to the next highest dilution plate. Make your estimates of the number of bacteria in the original 1 cc soil sample using the following formula:

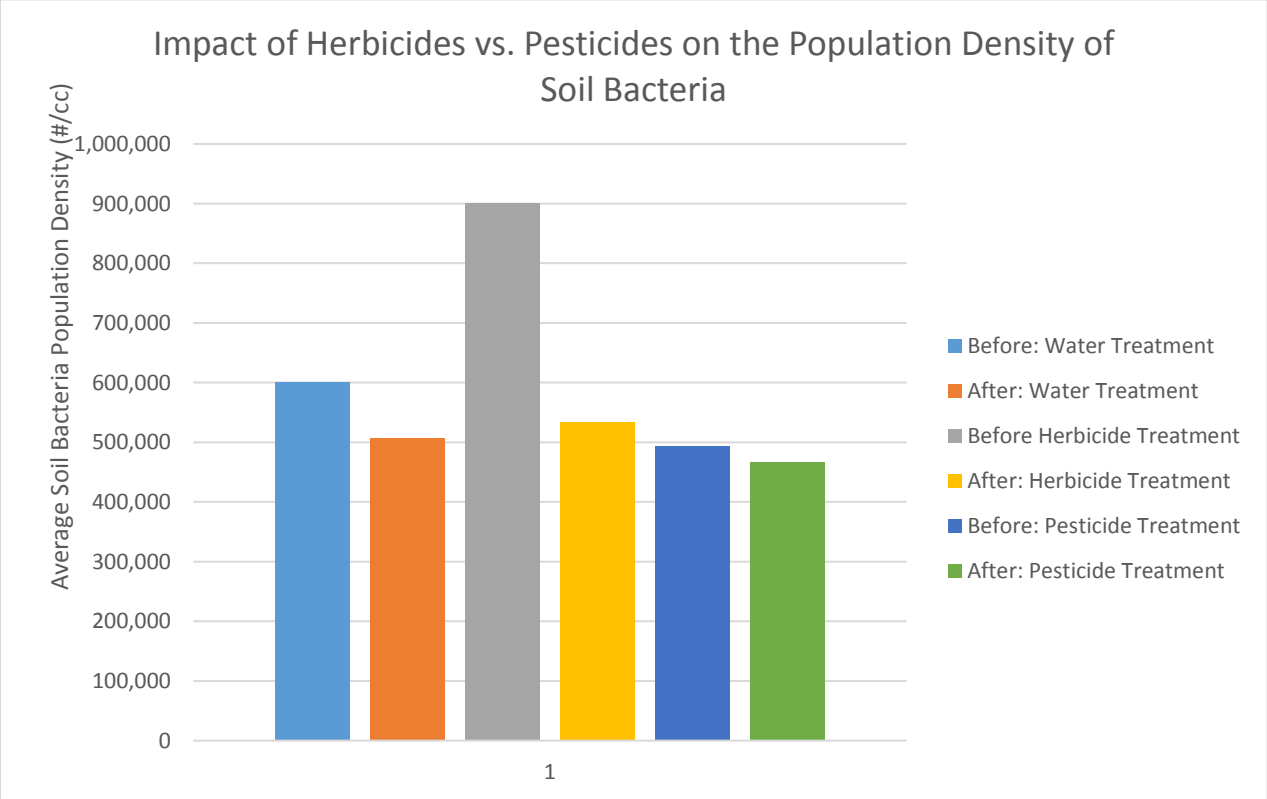
Microbes in 1 cc of coil = # Colonies on sheet x 10^2 x $10^{|\text{dilution \# at which these colonies were found}|}$

12. Steps 12-14 should take place on the same time on the same day. 20 centimeters above the ground, apply 7 sprays of Bayer Advanced Rose & Flower Insect Killer pesticide to each of the 3 soil plots labeled with "Trial #: Pesticides"
13. 20 centimeters above the ground, apply 7 sprays of Roundup Weed & Grass Killer herbicide to each of the 3 soil plots labeled with "Trial #: Herbicides"
14. 20 centimeters above the ground, apply 7 sprays of tap water to each of the 3 soil plots labeled with "Trial #: Water"
15. Let these plots sit for 34 hours
16. After 34 hours, repeat steps 6-10 to collect soil samples from each of the 9 soil plots
17. Repeat step 11-11p, labeling each 15 ml culture test tube: $10^{\#}$, Type of Test (Pesticides, Herbicides, Water), Trial Number. Make sure to record all information in data table.

Data Analysis

Impact of Herbicides vs. Pesticides on the Population Density of Soil Bacteria (#/cm^3)

Trials	Water Treatment		Herbicides Treatment		Pesticides Treatment	
	Before water was applied	After water was applied	Before herbicide was applied	After herbicide was applied	Before pesticide was applied	After pesticide was applied
1	900,000	900,000	900,000	600,000	310,000	600,000
2	800,000	430,000	800,000	800,000	70,000	200,000
3	280,000	190,000	1,000,000	200,000	1,100,000	100,000
Averages	600,000	506666.667	900,000	533333.3	493333.3	466666.667



Conclusion

Our hypothesis, that pesticides in the soil reduce population density of bacteria more than herbicides in the soil do, was proven to be false. Before herbicides, pesticides, and water were added to our soil plots, the average density of bacteria in the water treatment plots was 600,000 bacteria per cubic centimeter. After testing the after samples of the water treatment plots, the average density of bacteria in the water treatment plots was 506,666.667 bacteria per cubic centimeters, a 15.55% decrease in average density of bacteria per cubic centimeter. Because water should have no impact on population density of bacteria, this shows that there is a naturally occurring factor in the environment that is negatively impacting the survival of bacteria. In samples from the pesticides and herbicides plot, we should have seen corresponding percentage of decrease in population numbers. However, this was not the case. When samples were taken from the pesticide treatment soil plots before applying pesticides, the average density of bacteria was 493,333.3 bacteria per cubic centimeter. After applying pesticides and waiting 36 hours, the average number of bacteria per cubic centimeter was 466,666.667. This creates a 5.41 % average decrease in population. The percentage of decrease was less than when only water was added to the soil plots. This means that something within this environment helped to grow the bacteria population when pesticides were applied. On the other hand, in herbicide treatment soil plots, the average population density of bacteria per cubic centimeters before applying herbicides was 900,000. After applying herbicides and waiting 36 hours, the average number of bacteria per cubic centimeters was 533,333.3. This creates a 40.74 % decrease in population. This shows that some factor in herbicides is heavily negatively impacting the population density of bacteria at a significantly higher rate than the rate of decrease in the negative control (water treatment soil plots).

Though our group did not test for protozoa levels in soil, we compared with another group within our class (Taylor, *et al* 2017) who did test effects of herbicides on population density of protozoa. Through their experiment, they found that applying herbicides to the soil increased the average protozoa population density by 50.59%, which is an extremely high increase compared to their negative control of water, in which the protozoa population density remained stable. The two soil plots, while from a microbial perspective were far apart, shared extreme similarities in the ecosystem and environment. The plots had similar plant life, sun exposure levels, precipitation levels, temperature, and were within 10 meters of each other on the same hillside. Their findings lead us to believe that when herbicides were added to our soil, the chemicals of the herbicides could have killed the plant life. This possible increase in dead plant material may have provided a food source for the bacteria, and therefore might have increased the population of bacteria, as they decomposed and reproduced. Protozoa and bacteria have a predator to prey like relationship, in which protozoa eat bacteria. The sudden population spike in bacteria may have attracted additional protozoa to consume these bacteria. Therefore, as a consequence, this could explain the significant drop in the bacterial population density seen in our herbicide treatment soil plot data.

While looking at this same data (Taylor, *et al* 2017), although the herbicide trial soil plot data does not support our hypothesis, it brings up an interesting observation about the environment. Similarly, to the herbicide treatment plots, the applied pesticide chemicals could have provided bacteria with ample material to decompose, and therefore grown the bacteria population by causing them to reproduce. As previously stated, it is believed that a rise in bacteria population could possibly lead to a rise in protozoa, which feed off of bacteria. However, as previously shown above, the bacteria population did not decrease, compared to the

negative control (water treatment) plots, when pesticides were applied. A possible reason for this is that pesticides, which contain a neurotoxin called cyfluthrin, are affecting the protozoan's ability to survive. Cyfluthrin causes hyperexcitation of the nervous systems, causing neurons to fire at a rate much higher than normal. It induces changes in nerve membranes, causing abnormal potassium and sodium flows. The repetitive discharges from the neurons causes blockages to further nerve impulses. (Cox, 1994). This ultimately leads to death. (Gilbert, 2014). Similarly, it is possible that the same blockage in the membrane of a protozoan could take place when the protozoan is exposed to pesticides. This could possibly lead to death of that protozoan. If this is correct, and the pesticides did cause the population of protozoan to decrease, this would explain the growth of bacteria in the pesticide treatment soil plots. With less protozoan in the soil, the bacteria had a smaller amount of predators to face and therefore could more easily survive and reproduce. If this was true, we would not see the same amount of decrease in bacteria population density in the pesticide treatment soil plots than we did in the herbicide treatment soil plots.

We could further our research in multiple ways. One way we could do this is by testing the protozoan population density in the soil when both pesticides and herbicides are applied. We could also test multiple types of pesticides with different cyfluthrin levels to see which type of pesticide most effects protozoan survival rates. A third way to further our research would be to test the amount of living, as well as decomposing, amount of plant life in correspondence to the population density of bacteria in soil. All of these future experiments could lead to more understand of microbes in the soil.

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