The Soil Ecology Project

The Effect of Pesticides of Soil Protozoa

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Background

Protozoa are single-celled eukaryotes that play numerous beneficial roles in the soil ecosystem, and they can be found all over the earth in a variety of different terrestrial environments. The different roles that protozoa play in the soil include mineralizing nutrients so that the other soil organisms can use them, keeping the nitrogen and carbon cycles healthy, and circulating organic matter through the soil ecosystem (Johns, C., 2017). The three main types of soil protozoa are the ciliates, amoebae, and the flagellates, and they are traditionally categorized by their means of movement. Ciliates are the largest in size, consume other protozoa and bacteria, and move by waving thin strands of hair like projections called cilia. Amoebae move by a pseudopod (a temporary protrusion from the eukaryotic cell membrane), and can be further categorized by whether or not they have a shell-like covering. Finally, the flagellates, the smallest in size, use a whip-like tail called flagella in order to move (Ingham, E. R., n.d.).

All of these protozoa prefer having highly fertile soil (though the ratio of the different types can vary based on whether the soil has clay or is coarse-textured), and it is this availability of organic matter that influences the density of soil protozoa the most. Protozoa are holozoic, meaning they must internalize their nutrients in order to digest them (Yaeger,1996), and the protozoa consume primarily bacteria as their source of energy and nutrients; so they must have an abundant supply of bacteria available to eat or they die. This consumption of soil bacteria plays a key role in the nitrogen cycle because when they eat bacteria, protozoa release excess nitrogen in the form of ammonium, and this excess ammonium is then used by plants and other members higher in the food chain (Stout, 1979) to create their nucleic acids and proteins, the molecules essential for an organism’s cells to function. Hence, without nitrogen, the cells of the organism would die, and therefore, protozoa, through their intense feeding, play an essential role

in the lives of all of the other organisms living in the environment and are a key organism in the soil ecosystem. If their population becomes distorted, the soil ecosystem will become unbalanced, creating a ripple effect that affects the entire food chain in an environment.

One potential cause of a decrease in the soil protozoa population is the use of pesticides (Stout, 1979). Pesticides are any chemical that serves as insecticides, herbicides, and fungicides, and the main ingredient in most of them are chemicals known as organophosphates. These kill insects and other pests by harming the enzymes that are responsible for controlling the nerve signals in their bodies (CDC, n.d.). However, while pesticides are intended to kill large, multicellular organisms, they almost always unintentionally kill other organisms that live in an environment as well, including the microbes. Certain types of soil bacteria *can* use pesticides as a source of nutrients. But the mix of chemicals in pesticides kills the vast majority of bacteria, often harming microbes that are beneficial to the soil and environment (Muturi, Donthu, Fields, Moise, and Kim, 2017) - including potentially the protozoa.

Pesticides may also harm soil microbes not by poisoning them, but by altering soil pH. pH measures how acidic or basic something is, and the pH of soil can impact the density of microbes living there, especially the protozoa (NRCS, 1998). When pH gets to a certain level (too high or too low), cellular enzymes do not function properly, and when the enzymes fail to control the chemical reactions which causes the 4 tasks that keep cells alive, they die. The optimal pH range for enzyme function is 6-7, and healthy soil has a pH level between 6.2-7.3. The acceptable pH level that protozoa need to thrive is anywhere from five to eight, and of all the soil microorganisms, protozoa have the smallest range of acceptable pH for them to thrive (Smith & Doran, 1996). But different types of chemicals in pesticides could affect the pH of the soil, either by lowering it or raising it too much (NRCS, 1998) and therefore, soil that is too

acidic or alkaline will have protozoa whose enzymes do not function properly, causing them to die - with all the potentially catastrophic impact on the ecosystem already mentioned.

It makes sense, then, to study protozoa in our experiment. After learning about protozoa, pH levels, the nitrogen cycle, and pesticides, our problem became: Do pesticides alter the ph of the soil and does it have an impact on the number protozoa? We experimented to find out by testing 3 samples from 6 soil plots for the amount of protozoa and pH levels, both before and after we spraying pesticides on 3 out of the 6 plots and water on the remaining plots.

Experiment Outline

I. Problem: Do pesticides alter the pH of the soil in a direction that alters the density of protozoa?

II. Hypothesis: Pesticides on the soil will cause the pH in the soil to decrease and the density of protozoa to decrease.

III. Procedure:

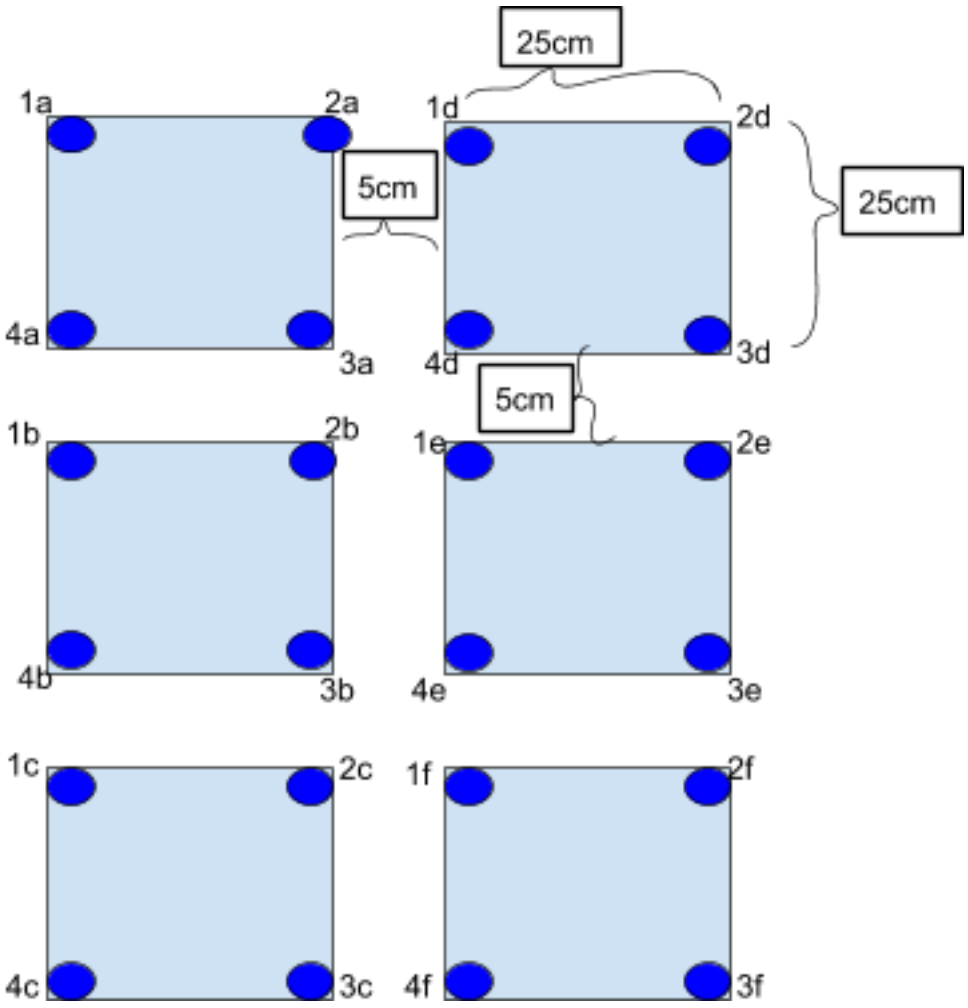
- A. Independent variable: the presence of pesticides applied to the soil
- B. Dependent Variable: the density of protozoa in the soil
- C. Positive Control: Soil samples taken before pesticides/water is added
- D. Negative Control: distilled water applied to the soil
- E. Controlled variables:
    1. Type of water poured on grass
    2. Type of water saturating the soil
    3. Type of water in Uhlig extractor
    4. Type of pesticide

5. The same amount of pesticide and the same amount of water applied
6. Location of soil
7. Amount of soil collected
8. Location of flags
9. Location of plots (coordinates)
10. Position of flags
11. Size of plots
12. Distance between plots
13. Soil collected at the same time and same day
14. Amount of soil tested
15. Magnification on microscope
16. Unit of measurement to measure plots
17. Size of holes in nylon screen or mesh
18. Size of the petri dish
19. Amount of time drying out
20. Amount of distilled water in Uhlig extractor
21. Amount of time rehydrating in distilled water
22. Type of mesh in Uhlig extractor
23. Amount of time in Uhlig extractor
24. Type of qualitative paper
25. Type of pipette
26. Size of pipette
27. Size of coverslip

28. Amount of dye on the microscope slide
29. Type of dye on the microscope slide
30. Amount of filtrate on the microscope slide
31. How many fields of view observed

E. Step-by-Step Instructions

1. Label 24 flags with 1a, 2a, 3a, 4a; 1b, 2b, 3b, 4b; all the way through the letter “d” respectively.
2. At the following coordinates (N: 39° 21.399’ W: 76° 38.135’) place the flags. The squares should be in two rows of three (the first row for pesticides, and second for the negative control, water) the flags should be at each corner of the 25cm X 25cm squares, with 5 cm between each square. Plots A, B, and C will be the plots where pesticides are added and plots D, E, and F are where water will be added It should look like the following drawing:



There must be 5 cm between each plot, both ways. Each square is a plot. Each circle is a flag. Each number next to a letter is the flag's label.

3. Label 9 Ziploc bags including

A. Plot letter

B. sample number (1-3)

C. Before pesticides/ water added

Example: A1Plot Before

4. On the same day at the same time, use a soil extractor to extract 3 samples of soil that are 15cm deep and 2cm in diameter from each plot. To do this, bury the soil extractor up to the

mark in a plot of soil. Turn it clockwise twice to extract the soil. Make sure to place the 3 samples from each plot into their correspondingly labeled Ziploc bag.

5. When you are finished, there should be 18 bags with one soil sample in each.

6. Do steps 6-12 all on the same day at the same time; Using Bayer Rose and Flower Pesticide, go outside to the plots and spray plot A evenly, squeeze the nozzle of the pesticide spray bottle and hold 30 cm away from the ground, spray it 25 times.

7. Spray plot B evenly with pesticide, squeeze the nozzle of the pesticide spray bottle and hold 30 cm away from the ground, spray it 25 times.

8. Spray plot C evenly with pesticide, squeeze the nozzle of the pesticide spray bottle and hold 12 cm away from the ground, spray it 25 times.

9. Put 2 quarts of tap water in a spray bottle.

10. Once outside, spray plot D evenly with the tap water, spray it 25 times.

11. Spray plot E evenly with water, spray it 25 times.

12. Spray plot F evenly with water, spray it 25 times.

To test for soil density/protozoa:

13. On the same day at the same time, Place 15cm of soil sample of *A1*Before into the bottom of a clean, empty petri dish 9 cm in diameter and 1.5 cm deep. Do the same, each time with a new, clean petri dish, with the three soil samples from each A, B, C, D, E, and F plots; allow to dry completely.

14. Once all the soil samples are dry, sift 9-10 g of the three soil samples from each A, B, C, D, E, and F plots into a 2nd clean correspondingly labeled petri dish using a 18mm2 nylon screen or mesh, record grams of soil in each sample.

15. On the same day at the same time; add 20 mL of distilled water to each of the soil samples to saturate all three soil samples from each A, B, C, D, E, and F plots. Cover all the Petri dishes of all the soil samples from A, B, C, D, E, and F with its lid and allow to sit for 7 hours

16. Immediately after the seven hours, place each of the 18 rehydrated soil samples in its own separately correspondingly labeled modified Uhlig extractor containing 30 ml of distilled water for 24 hours. Use Nytex mesh.

17. On the same day at the same time; remove the filtrate from each Uhlig extractor and filter each sample separately a 2nd time using 12.5cm qualitative paper and refrigerate until ready to proceed

18. Using a capillary tube deposit 7 µl of methyl-green stain on a clean microscope slide (1 µl = 1 drop from a capillary tube) Then using a disposable graduated Beral-type pipette, add 18 µl (the first demarcation on the pipette) of the second filtrate of sample *A1*(from step 18 above) to stain on the microscope slide and cover with an 18 x 18mm2coverslip.

19. Examine under a microscope the four corners and the middle of the microscope coverslip at 40X observations to count the number of protozoa in that sample. Average the 5 fields of view for the equation in step 20.

20. Use the following equation to determine the population density of protozoa in the soil sample:

[(# per field of view at 40X)・(total ml of water used)・747] ÷ (grams of sifted soil)= # of protozoa per gram of soil.

21. Repeat steps 18. thru 20. on remaining 2nd filtrate samples

22. Now, using the LaMotte Model STH-14 test for pH of each of the remaining soil samples from step 14.

23. Record estimated pH level.

24. Repeat steps 4-5 and then 13-23. Using new Ziploc bags, and labeling them:

A. Plot letter

B. Sample number (1-3)

C. After pesticides/ water added

Example: A1After

IV. **Data and Analysis**:

A. **Data Table**: The Impact of Pesticides on Soil Protozoa

Sample	**Pesticides Before**	**Pesticides After**
A1	29,551	29,551
A2	609,913	609,913
A3	107,178	107,178
B1	525,173	525, 173
B2	401,106	401,106
B3	401,106	466,875
C1	459, 567	459,567
C2	155,408	155,408
C3	52,209	52,209
Average	540,154	311,887
Sample	**Water Before**	**Water After**
D1	467,071	593,583
D2	206,861	882,596
D3	204,398	207,711
E1	494,990	201,852
E2	657,684	364,445
E3	568,369	377,311
F1	618,261	531,108
F2	795,798	248,726
F3	598,403	394,509
Average	512,427	422,427

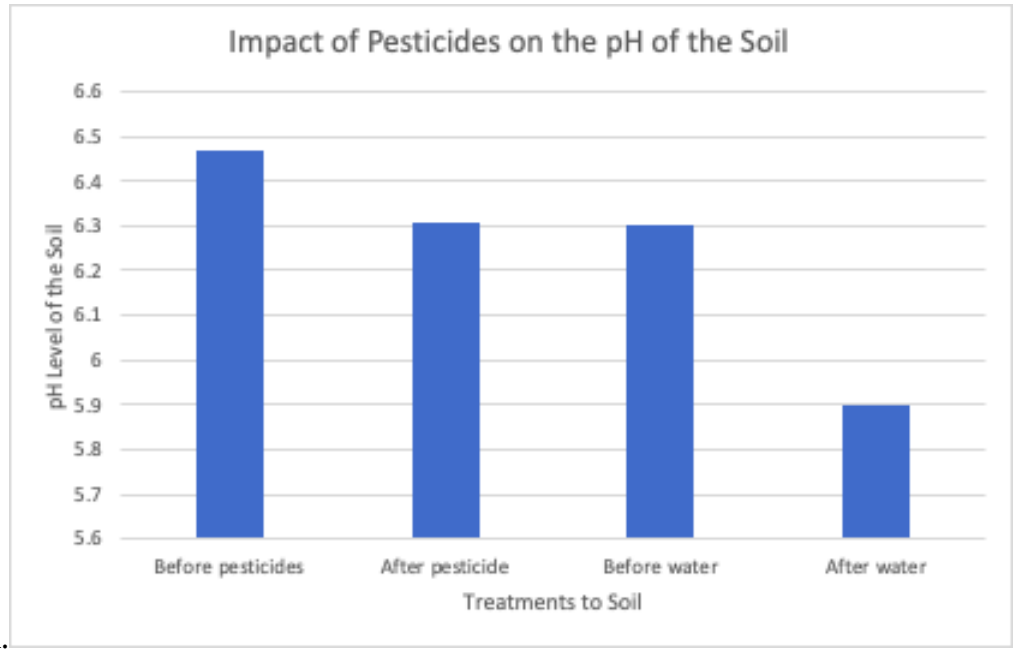
B. **Data Table**: The Impact of pesticides on the pH of the Soil

Sample	**Pesticides Before**	**Pesticides After**
A1	6.4	6.4
A2	6.4	6.8
A3	6.4	6.2
B1	6.6	6.0
B2	6.8	6.2

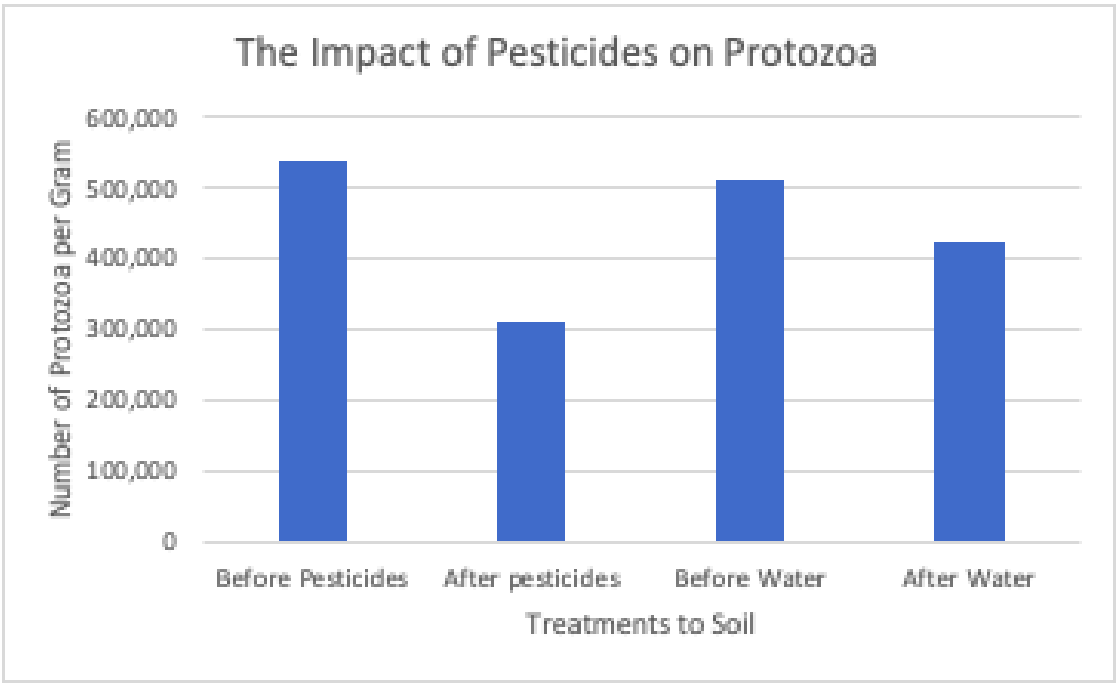
B3	6.8	6.8
C1	6.2	6.2
C2	6.4	6.0
C3	6.2	6.2
Average	6.5	6.3
Sample	**Water Before**	**Water After**
D1	7.0	6.0
D2	6.6	6.2
D3	6.6	5.6
E1	5.8	5.8
E2	6.2	6.0
E3	6.0	5.6
F1	6.2	5.4
F2	6.4	6.6
F3	6.2	5.8
Average	6.3	5.9

V. Graphs

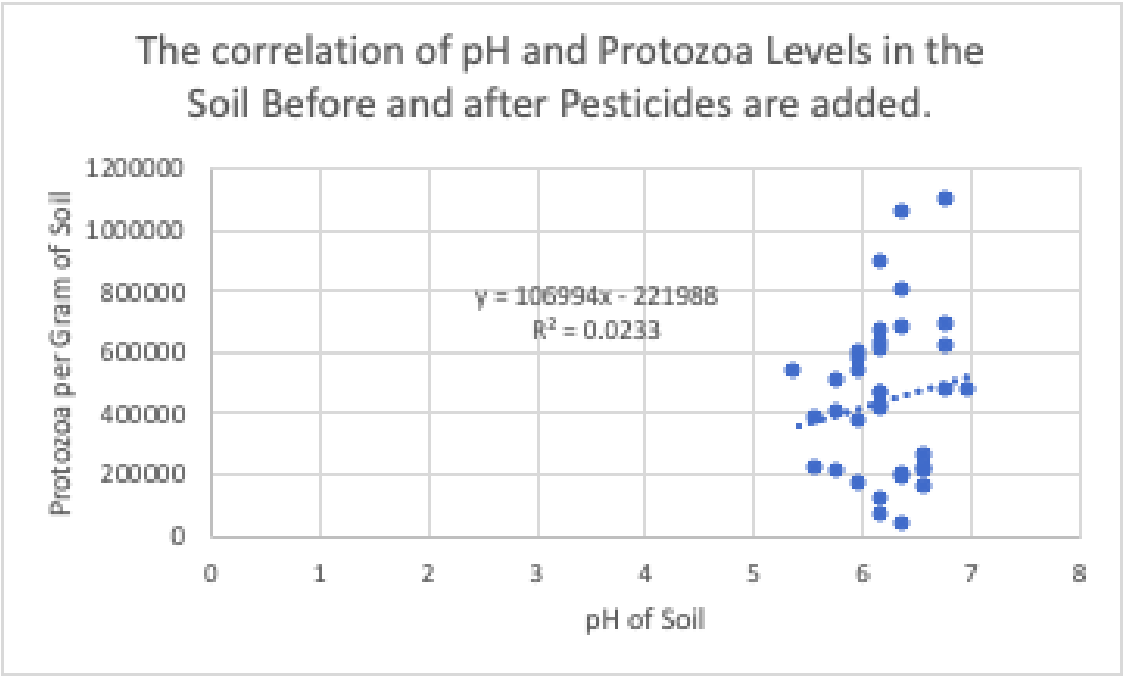
A.



B.



C.



VI. Conclusion

Our hypothesis was: pesticides on the soil will cause the pH in the soil to decrease and the density of protozoa to decrease. After completing our experiment and reviewing our data, we are able to theorize that our hypothesis was incorrect. The pH level of soil measures how acidic

or basic the soil is. The pH of the soil can have a substantial impact on the number of microbes living in the soil, such as the protozoa in the soil (NRCS, 1998). The average number of protozoa in the soil before pesticides was sprayed was 540,154. The average number of protozoa after the pesticides were sprayed was 311,887. The average number of protozoa in the soil before the water was sprayed was 512,427. After the water was sprayed, the amount dropped to 422,427. The graph showing the connection between the pH level and protozoa amount shows an increase in protozoa when the pH is higher or closer to 7.

The pH level of the soil that pesticide was added to remained higher than the pH level of the soil that water was added to. This is telling because it shows that the pesticide actually protected the pH level of the soil from dropping too drastically. When pH gets to a certain level (too high or too low,) cellular enzymes do not function properly, and when the enzymes fail to control the chemical reactions which cause the 4 tasks that keep cells alive, they die. The optimal pH range for enzyme function is 6-7, and healthy soil has a pH level between 6.2-7.3. Additionally, the acceptable pH level that protozoa need to thrive is 5-8 (Smith & Doran, 1996).

Our data tables and graphs show that the average pH level of the soil plots before pesticide was sprayed was 6.5. The average pH level of the soil plots after the pesticide was sprayed was 6.3. Before water was sprayed on the soil, the average pH was 6.3, and after water was sprayed, the average pH decreased to 5.9. One reason why the pH might have decreased when pesticides were introduced to the soil was because of the natural environment. Additionally, we can theorize that weather decreased the soil pH because there was a big storm after we added pesticide and water to the soil plots. The downpour and humidity may have caused the pH level to decrease substantially, because rain can often be acidic. In humid weather with heavy rainfall, the pH of the soil decreases due to the process of soil acidification. The

chemicals in pesticides must have a certain factor that can prevent soil acidification, which might be why the pH did not drop as drastically as the water-sprayed plots. The reason why the average pH decreased after water was added could be that the water did not have factors that could prevent soil acidification in the plots. A way to prove this theory is by creating another experiment that tests the pH of the soil before and after there is a rain storm, without added pesticides or water to see if there is a change in pH. Another theory is that there are certain types of bacteria that can live off pesticides. These bacteria continue to produce more nitrogen and the soil becomes more alkaline. These are two theories that could lead to further research to prove why soil pH dropped more than the pesticide plots' soil pH. While part of the outcome of the experiment was what we predicted in our hypothesis (pesticide caused protozoa levels to decrease,) we cannot theorize that the pH level of soil decreased due to the pesticide that was sprayed on the soil. Instead, we can theorize that the pesticide protected the pH of the soil from dropping too drastically. We can form theories about why the pH level dropped so substantially in our negative control plots. We can also form theories about why the pesticide decreased the numbers of protozoa in the soil.

If we were to redo this experiment, we should test soil plots that are in a flat area, so if it were to rain, the plots would get a somewhat equal amount of rain and weather exposure. Therefore, if there was a runoff, it would get equally distributed to all of the plots. Our plots were at the bottom of the hill and the pesticide plots were closer to the hill. Therefore, the runoff distribution would be uneven. We would also test the bacteria and the nutrients of the nitrogen.

References

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