Protozoa vs. Presence of Pesticides

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Background

Humans have a huge impact on the soil ecology. Many people have gardens and lawns that they want to be to be pest free. Pesticides are meant to kill pests including unwanted insects, weeds that infest lawns, and gardens. People, who have lawns, or gardens of some sort, often use chemicals to get rid of certain insect, or plants. Without thinking about the soil ecology, people spray chemicals to kill pest, without considering the microorganisms in the soil that they are also killing. While pesticides kill insects, it also kills important microorganisms in the soil. Microorganisms that are affected are protozoa and bacteria. Both microorganisms have an impact on the soils nitrogen levels.

Pesticides are chemicals that are used in order to kill pests, such as grubs. When pesticides are sprayed, they not only kill visible pests, but the microscopic ones that are a necessity to the health of the soil. Pesticides get rid of insects such as grubs; however they may also get rid of the protozoa in the soil. Because they are both eukaryotic organisms, the pesticides may kill not only insects, but protozoa as well, and so we chose to explore whether or not putting pesticides in the soil, would cause the number of protozoa in the soil to decrease. Based on research, we thought that the number of protozoa would decrease, thus creating a negative impact on the biochemical cycle. (Fred T. Mackenzie) When pesticides are sprayed, they become dangerous to the balance of certain things in the soil, such as the protozoa levels, the bacteria levels, and the nitrogen levels.

The active ingredient in the pesticides is Carbaryl. Carbaryl, in pesticides, enables it to either, destroy, or repel pests. When it is absorbed by the soil, it kills the pests that live in it by disabling the pest’s cells. This plays an active role in the nitrogen fixation process. Protozoa is important to the soil because it controls the amount of bacteria in the soil. Given that the protozoa number decreases due to the pesticides being sprayed, the bacteria count should increase, causing the nitrogen levels in the soil to increase. The soils food chain can be affected by a single microorganism changing in number. Protozoa eat bacteria in the soil, therefore if the protozoa level decreases then there are fewer protozoa left to eat bacteria. If there are fewer protozoa in the soil to eat bacteria, then potentially the level of bacteria will increase. If the bacteria level increases than it is harmful to the soils nitrogen cycle. Nitrogen fixation is a process which enables the nitrogen to be converted to ammonium. This process it important because it is the way in which plants and such obtain nitrogen from the atmosphere. When bacteria levels increase it throws the whole nitrogen cycle off balance by increasing the amount of nitrogen in the nitrogen cycle.

Through our background research, our group discovered the role of hypothesizing that if the pesticides kill insects, than they also kill the protozoa, thus causing the number to decrease. To test our hypothesis we had to do actually spray pesticides onto three soil plots, and water onto 3 soil plots. After doing so, protozoa must be filtered and extracted so that the number of protozoa is countable. After filtering each soil sample a few times, a microscope slide is eventually made so that the protozoa can be looked at under a microscope. The number of protozoa is counted from five different fields of the slide. After counting the protozoa the numbers have to be put in to a specific equation in order to do an analysis of the data. After the analysis is done, an actual conclusion can be made about whether or not pesticides actually kill the protozoa in the soil.

Protozoa vs. Presence of Pesticides

Problem:

Will the number of protozoa increase or decrease when we add pesticides to the soil?

Hypothesis:

If we add pesticides to the soil, then the number of protozoa will decrease.

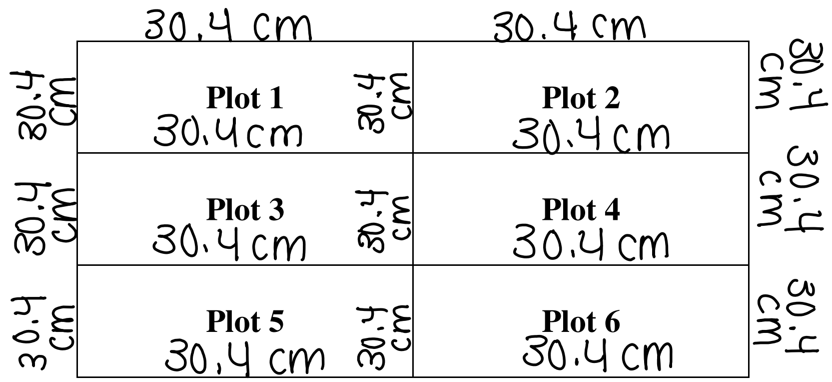
Procedure:

Independent Variable: adding pesticide to the soil
Dependent Variable: the number of protozoa we find in soil
Negative Control: soil sprayed with water instead of pesticides
Controlled Variables:

- Amount of pesticides we add to the soil
- Using the same location for collecting soil
- Type of pesticides we add to soil
- Amount of time in between soil collections
- How much soil we collect
- The time (time of day) collecting soil
- Depth of soil collected
- How many times we filter protozoa
- Amount of Methyl Green 1% Aqueous Acidified
- Amount of Filtrate
- Amount of hours Petri dishes sit (# of hours)
- Amount of distilled water
- Units of measurements
- Size of Petri dish
- Size of Filter Paper
- Beakers
- Amount of water we add to soil without pesticides
- Size of the coverslip
- Size of the Nylon Mesh Square
- Size of Nytex Nylon Mesh
- Microscope

Instructions

1. Mark 6 [30.4 x 30.4 cm] plots of land (3 for pesticides, 3 for no pesticides) for collecting soil at N 39.35803, W 076.63603. Use 12 flags to represent the location of plotting land.



2. Get 6 fresh, unused plastic bags. Label the first bag with your initials (AJK), Before, and Plot 1.
3. Label the 2nd bag with AJK, Before, Plot 2.
4. Label the 3rd bag with AJK, Before, Plot 3.
5. Label the 4th bag with AJK, Before, Plot 4.
6. Label the 5th bag with AJK, Before, Plot 5.
7. Label the 6th bag with AJK, Before, Plot 6.
8. Using the soil cylinder, 15.1 cm deep and 2.3 cm wide, place the cylinder on the ground in the top, right corner of Pot 1.
9. Twist the cylinder into the soil to the 1st mark located on cylinder.
10. After twisting soil to the 1st mark, twist the cylinder 360 degrees to collect it.
11. Pull the cylinder straight out the ground.
12. Place soil into the plastic bag titled, “Before Plot 1.”
13. Repeat steps 8-12 for the remaining plots.
14. Wear gloves when spraying pesticides.
15. Using the Bayer Advanced Complete Insect Killer for Gardens, spray plots 1,3 and 5 with 10 sprays of pesticides.
16. Using tap water, spray plots 2,4 and 6 with 10 sprays of tap water.
17. Spray 30 sprays of tap water onto each plot right after you spray plots 1, 3, and 5 with pesticides and 2, 4, and 6 with water. Allow to sit for 48 hours.
18. Get 6 fresh, unused plastic bags.
19. Label the first bag “After Plot 1 w/Pesticides.”
20. Repeat step 19 for Plots 3 and 5.
21. Label the second bag “After Plot 2 w/ Water
22. Repeat step 21 for plots 4 and 6.
23. Using the soil cylinder, collect 1 bag of soil from each plot.
24. Place the cylinder on the ground in the top, left corner of Plot 1.
25. Twist the cylinder into the 1st mark located on the cylinder.
26. Twist the cylinder 360 degrees to collect soil.
27. Pull the cylinder straight out of the ground.
28. Place soil into plastic bag titled, “After Plot 1 w/Pesticides.”
29. Repeat steps 24-28 for plots 3 and 5.
30. Place the cylinder on the ground in the top, left corner of Plot 2.

31. Twist the cylinder into the 1st mark located on the cylinder.
32. Twist the cylinder 360 degrees to collect soil.
33. Pull the cylinder straight out of the ground.
34. Place soil into plastic bag titled, "After Plot 2 w/Water."
35. Repeat steps 30-34 for plots 4 and 6.

Protozoa Extraction

1. Get 12 Petri dishes.
2. Label the 1st Petri dish "Before Plot 1."
3. Repeat step 2 for plots 2-6.
4. Label the 7th Petri dish "After Plot 1."
5. Repeat step 4 for plots 2-6.
6. Place all of the sample soil from Plot 1 into the bottom of a clean Petri dish labeled "Before Plot 1" and let it dry for 24 hours.
7. Sift 9-10 grams of the soil into a 2nd Petri dish labeled "Before Plot 1 Sifted" using a 1 ml square nylon mesh screen.
8. Record the number of grams for Plot 1 soil.
9. Add 20 ml of distilled water to the soil of Plot 1, to saturate it.
10. Cover the Petri dish labeled "Before Plot 1" with its lid, and allow to sit for 7 hours.
11. After the 7 hours, place the same soil in a "modified" Uhlig extractor containing 30 ml of distilled water; allow to sit for 24 hours.
12. Remove the filtrate and filter for the 2nd time using 12.5 cm quantitative filter paper.
13. Deposit 7 µl of methyl-green on a clean microscope slide. Using a disposable graduated beral-type pipette, then add 18 ul of the 2nd filtrate from step 12 to the stain on the microscope slide and cover with an 18 x 18 mm² cover slip.
14. Examine slide under a light microscope at 40x for the quantitative data of the number of protozoa living in the soil.
15. Using the counter, count the number of protozoa.
16. Use the following equation to determine the population density of protozoa in the soil sample:

[(# per field of view at 40X) • (total ml of 2nd filtrate) • 747] / (grams of sifted soil) = # of protozoa per gram of soil

24. Record results in data charts.
25. Repeat steps 6-16 for the remaining plots.
26. Repeat steps 6-16 for protozoa extraction, only using the "After" samples of soil with pesticides and water added.

Data and Analysis

Before Adding Pesticides and Water

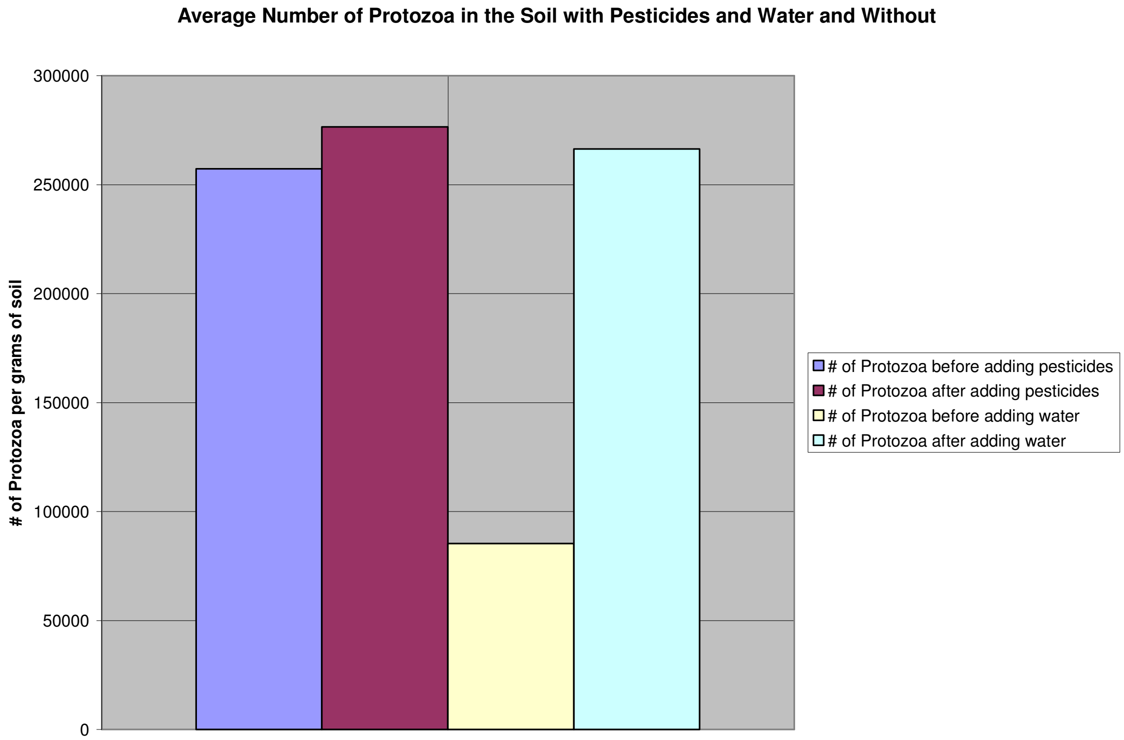
Plot #	**Number of Protozoa per gram of soil, before we add pesticides**	**Plot #**	**Number of Protozoa per gram of soil, before we add the water**
Plot 1	381,201	**Plot 2**	116,467
Plot 3	259,484	**Plot 4**	104,419
Plot 5	131,340	**Plot 6**	35,015

After Adding Pesticides and Water

Petri Dish #	**Number of Protozoa per gram of soil, w/ pesticides**	**Petri Dish #**	**Number of Protozoa per gram of soil, w/ just water**
Plot 1	270,296	**Plot 2**	213,543
Plot 3	233,190	**Plot 4**	324,078
Plot 5	325,794	**Plot 6**	261,374

Average Number of Protozoa with Pesticides and with just Water

Number of Protozoa w/ pesticides		**Number of Protozoa w/ just water**	
Before	***After***	***Before***	***After***
257, 341	276,426	85, 300	266,331



Conclusions/Statistical Analysis

Statistical Analysis:

We found that the increase in protozoa where water was present produced a p-value of 0.01 when we conducted a t-test. We also found that the increase in protozoa where pesticides were present produced a p-value of 0.82.

Conclusion:

Our hypothesis was incorrect. When we added the pesticides to the soil the amount of protozoa increased. The p-value that we got (0.01) means that there is an 18% chance that adding the pesticides caused the increase in protozoa; therefore replicating the experiment would be helpful in making sure the data is accurate. Also if you look at the data tables with the averages you will find that in the data that we compared the

"After" data was higher than the "Before" data for both adding pesticides and adding water to the soil. In fact, when we added pesticides to the soil our "After" amount of protozoa was 3.6% more than the "Before" amount of protozoa. This means that the increase in protozoa from before we added pesticides to after we added pesticides increased by about 3.6%. Since there was only a 3.6% increase our group may consider replicating the experiment to see if we could get data that was more unambiguous. When we added just the water to the soil there was a 51.5% increase in the amount of protozoa that were in the soil from before we added water to after we added water. An explanation for this figure is that the protozoa use the water for hydration; therefore and increased amount of it would make the protozoa increase. The same data is represented in graph form. If you look at the graph you will see that the bar telling us the number of protozoa after we added the pesticides is a little bit higher than the bar telling us the amount of protozoa before adding the pesticides; therefore representing our 3.6% increase. The same applies for adding water to the soil. In the graph the bar telling us the number of protozoa after adding water is about 51.5% higher than the bar telling us the number of protozoa before adding water to the soil. For further research our group could consider the question "If we add pesticides to the soil will the amount of bacteria increase or decrease?" This would be a logical question to ask for further research because the bacteria are the protozoa's food source; therefore if the food source is killed by pesticides then chances are that the protozoa would die too. However if the bacteria multiply as a result of the pesticides then the protozoa would multiply as well. From our conclusions with this experiment, that adding pesticides to the soil make the protozoa increase, a rational hypothesis to the bacteria question would be that the amount of bacteria would increase after adding the pesticides. This is a reasonable hypothesis because chances are if the protozoa are increasing because of the pesticides it is because their food source is also increasing because of the pesticides.

Bibliography

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