How Herbicide Affects Protozoa

Biology Final Project

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"We have acted honorably."

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Background:

In our society today, a major method that is used to help prevent weeds from growing on land is the use of herbicides. Herbicides contain certain chemicals that kill all weeds and prevent them from coming back. Herbicides kill the weeds in two ways: either by contact or systemic action (also known as selective and nonselective). Systemic action kills the weed immediately through its roots where as contact just prevents the growth of weeds (it does not actually kill the weed) (Invasive Plants Association of Rhode Island Landscape Horticulture Program 1999).

Many herbicides though, contain certain chemicals that could be potentially harmful to the protozoa in the soil. This is because herbicides limit bacteria growth on a specific location of land (Invasive Plants Association of Wisconsin n.d). Since the chemicals in the herbicide kill the entire plant, the bacteria that eat-off of the weeds lose a food source, reducing the number bacteria. Fewer bacteria though, means fewer protozoa because they can no longer have their regular food source. Protozoa normally eat fungi, organic matter, but mainly bacteria. When there is a limited amount of bacteria for the protozoa to eat, the protozoa die. This is bad for our ecosystem because when the protozoa eat bacteria, they release a form of nitrogen called ammonium. Ammonium turns into nitrogen, which makes-up 78% of all worlds' matter. This nitrogen is a vital part of the plants and other foods that we eat because it makes amino acids. Amino acids are essential to our environment because they create enzymes, which start and stop chemical reactions. These chemical reactions then make or break chemical bonds. If the herbicide that is used destroys the protozoa, plants will no longer be able to create protein

enzymes. This means plants can no longer starts and stop chemical reactions, which called the cells to survive and the plant dies.

With the help of Jennifer Whalen, we discovered that a certain type of fertilizer is being used here at RPCS. This herbicide contains a chemical, called prodiamine (Scotts Miracle-Gro Company 2008). Prodiamine could be causing protozoa to be spreading and multiplying on RPCS grounds. This could be bad news because if too many protozoa are formed they will eat and spread bacteria, which is toxic to the ecosystem. Also, the protozoa spread and regulate bacteria, which cause changes in the soil. (Natural Resources Conservation Service 2008)

Specifically in our experiment, we are testing to see if herbicides have any affect on protozoa. We are testing this by getting two separate, unfertilized, plots. In one of the plats we will spray 50 mL of a herbicide and water mixture, one which contains prodiamine, and in the other we will prays 50 mL of regular tap water. By doing this, we hope to find out whether or not the number of protozoa increase or decrease due to the herbicide being present in the soil.

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Procedures:

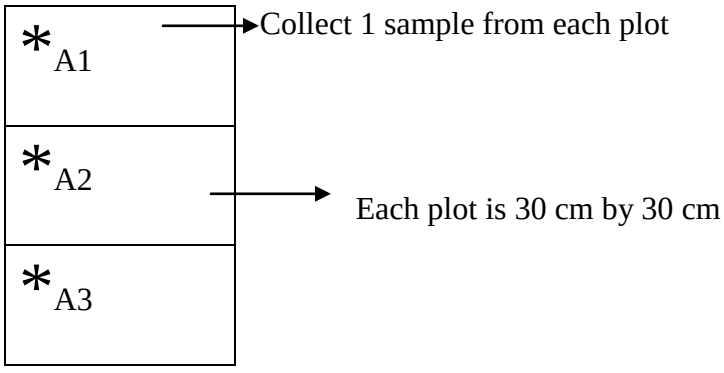
1. Question- How does a herbicide with prodiamine change the number of protozoa in the soil?

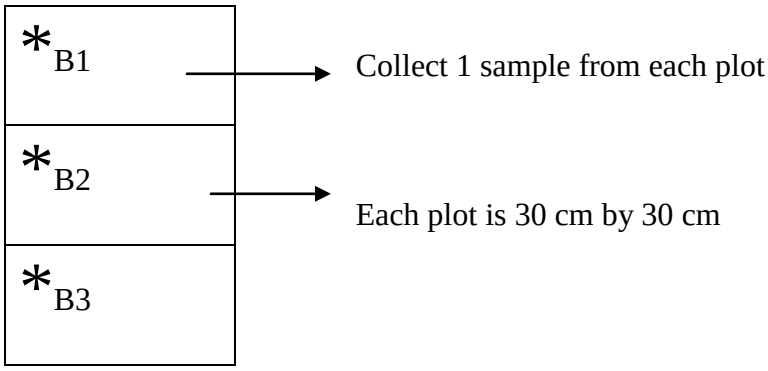
2. Hypothesis- When adding herbicide to soil, the number of protozoa will decrease.

3. Procedure-
    - independent variable: application of herbicide to one of the 2 plots
    - dependent variable: numbers of protozoa per grams found in each plot sample
    - negative control: the plot with only water applied
    - controlled variables: the amount and type of herbicide, amount of water, location of soil, amount of soil for each side (same size plots), the time of the experiment (collect each set of soil at the same times), temperature, amount of distilled water, hours you allow the soil to sit to dry, type of filter paper, amount of dye, measurement of cover slip, number and size of petri dishes, size of meshes, amount of water, types of liquids, amount of liquids, the amount of magnification, amount of soil sample, same size bags, the same size and amounts of liquid in the spray bottles, size of soil sample, how long the application sits before collecting the "after" soil samples

Step by Step instructions-
*NOTES: when collecting the soil samples, make sure **all** of them are collected at the same time or complete one set at the same time.

1. Find an unfertilized location of soil (GPS COORDINATES: W 76° 38.159' N 30° 21.45'- we used the area behind the school- towards the backwoods because we knew the land was not fertilized especially because we saw weeds growing) and measure out 30cm by 30cm plots with 10 cm in-between each plot. For each trial, there will be 2 plots (A plots and B plots)





* The "A" plots will be sprayed with water and the "B" plots will be sprayed with herbicide.

2. Mark the flags of the plots that you will spray with the herbicide and water mixture A1, A2, and A3. Mark the flags of the plots that you will spray with water B1, B2, and B3.
3. Make sure that the "B" plots are far enough away from the "A" plots so that nothing gets contaminated. Our A plots were about 3 meters away from the B plots.
4. Using the soil core sampler, take soil from each of your plots (use the soil core sampler that is 15cm by 2cm so all of your soil samples are the same size)
5. While taking samples use a metal probe to help get the soil sample out of the soil core sampler.
6. Put each soil sample into it's own correctly labeled sandwich size plastic bag
7. Bring the soil samples inside and gather several Petri dishes (one for each soil sample)
8. Put the soil samples in separate Petri dishes (labeled the Petri dishes by color coating them so we could tell which sample belonged to which sample) and let them sit out to dry until there is no moisture left in them (about 48 hours)—these will be your "before" soil samples.
9. Make the herbicide solution by putting 300 ml of regular water in a spray bottle. In a second spay bottle put 300 ml of water and 6 ml of the herbicide (Weed-b-gon max). Be careful not to get the spray bottle confused so label them so limit the confusion.
10. Spray all A plots (A1, A2, and A3) with 50 ml each of the herbicide. Spray all B plots (B1, B2, and B3) with 50 ml of water, **not** the herbicide.
11. Wait about 48 hours until returning to plot and collect the after soil samples. BE SURE TO COLLECT THE SAMPLES ON THE SAME DAY! The after soil samples should be gathered the same way as the before soil samples (repeat steps 4-7)

12. Put these soil samples in Petri dishes (which are also labeled) as well and let them out to dry just like the before soil samples (repeat steps 7-8). Color coat the Petri dishes so you know how to tell them apart
13. When all soil samples are dried out and ready to test, sift about 9-10 grams of each soil sample into it's own 2^{nd} clean Petri dish (label it like its soil sample) using a 1mm squared nylon screen or mesh. After sifting, label the weight on the Petri dish because you will use it later in the experiment.
14. Complete step 13 to all soil samples.
15. Then add 20 ml of distilled water to saturate the soil. Complete this to each Petri dish (20 ml goes in each Petri dish)
16. Cover the Petri dishes with their lids and let them sit for 7 hours
17. After the soil samples have sat for 7 hours, put the samples in the refrigerator to allow the protozoa to go back to sleep
18. Put 30 ml of distilled water in the bottom of a clean and labeled Petri dish (do this for all of the samples but use a different Petri dish for each sample)
19. Next, you need filter the samples with uhlig extractor. DO ALL SAMPLES AT THE SAME TIME
20. Put each uhlig extractor in a Petri dish with 30 ml of distilled water in it
21. Complete steps 18-21 to all samples being careful not to contaminate anything
22. Put each soil sample in a different uhlig extractor being careful not to contaminate anything
23. Let them filter for 24 hours
24. Filter each sample separately a second time by using the funnels. Fold filter paper in half and then in half again and put it in the funnels. FILTER THE SAMPLES AT THE SAME TIME!
25. Put the liquid from each Petri dish into a different funnel
26. Wait until all of the liquid is drained to remove the cups. (Label the cups so you know which cup belongs to which sample)
27. Using a capillary tube deposit 7 µl of methyl-green stain on a clean microscope slide.
28. Then using disposable graduated beral-type pipette, add 18 µl of the 2^{nd} filtrate from 1 sample to the stain on the microscope slide (label the slides so you know which one belongs to each sample) and cover with an 18x18 mm^2 cover slip. This step will be done to each sample so repeat steps 27-28 for all samples.
29. Put soil under a 60X magnification
30. Use the digital blue to take the picture of the soil sample so its easier to begin counting protozoa
31. Count the numbers of protozoa in the field of view of the microscope

32. Use the equation to count the amounts of protozoa: [(number per field of view at 60X)·(total ml of water used) ·2165]/(grams of sifted soil)= number of protozoa per gram of soil.
33. Compare and contrast the difference in the numbers of protozoa between the "before" and "after" samples

DATA TABLES:

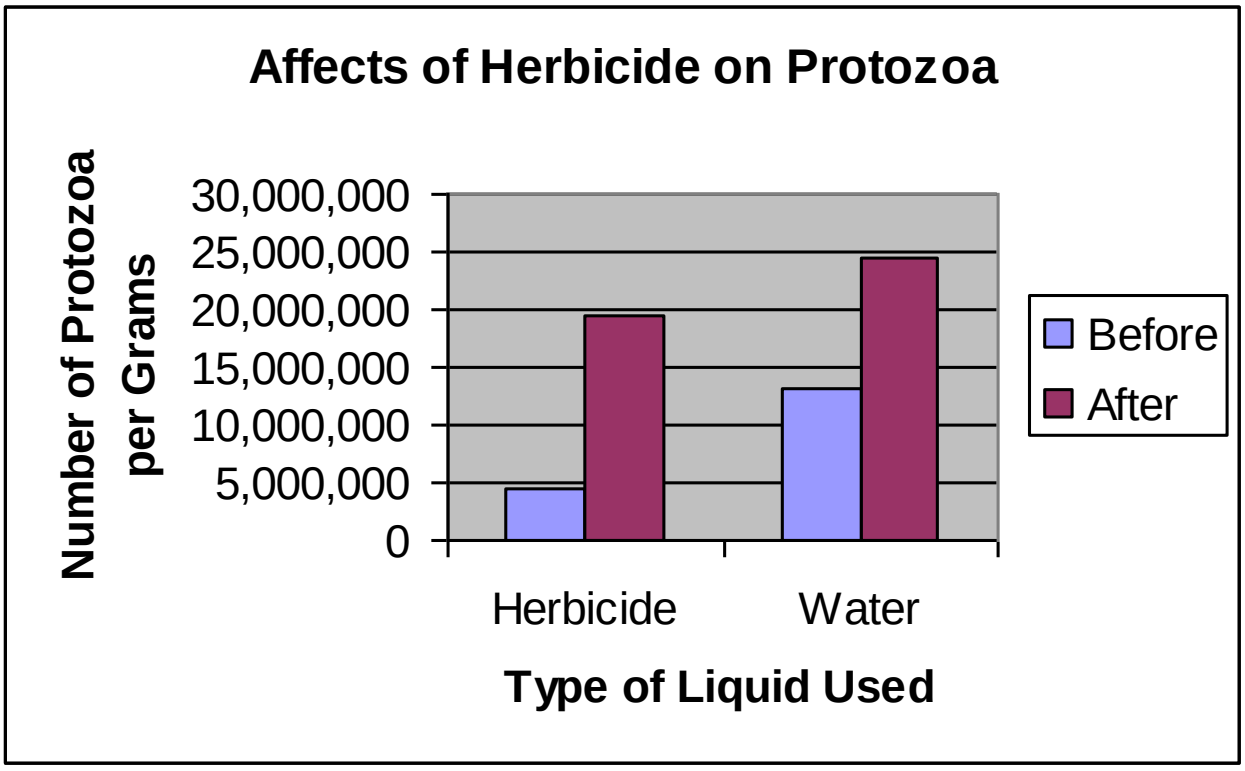
Protozoa Extraction Lab

	Condition of Plot	Number of protozoa per grams BEFORE	Number of Protozoa per grams AFTER
1 A	Before: no liquid After: herbicide and water mixture	4,102,105	3,418,421
2 A	Before: no liquid After: herbicide and water mixture	7,520,526	28,866,666
3 A	Before: no liquid After: herbicide and water mixture	1,435,969	6,836,842
1 B	Before: no liquid After: water	56,421,212	16,237,500
2 B	Before: no liquid After: water	1,513,172	9,571,579
3B	Before: no liquid After: water	705,978	47,490,323

Averages Table- Protozoa Extraction Lab

	Condition of Plot	Average Number of Protozoa per grams BEFORE	Average Number of Protozoa per grams AFTER
"A" Plots	Before: no liquid After: herbicide and water mixture	4,352,866	13,040,643
"B" Plots	Before: no liquid After: water	19,546,790	24,433,134

GRAPH:



CONCLUSION:

Our hypothesis was tested incorrect in the protozoa extraction lab. For this final project, our group chose to test protozoa. We were looking to see if herbicide (we chose to use weed-b-gon max) affects the amount of protozoa in a certain area of soil. In our

hypothesis, we stated that when adding herbicide to a plot with soil, the number of protozoa will decrease. From the data, we can prove that both the "A" and "B" plots increased the number of protozoa from their before to after. The "B" plot (the plot that we sprayed water on) increased by about 5 million protozoa as it went from 19,546,790 to 24,433,134. Then our "A" plot (the plot we sprayed with the herbicide and water mixture) more than tripled in number of protozoa, going from 4,352,866 to 13,040,643. The data that we have found, though we proved our hypothesis incorrect, proves that an herbicide helps increase the number of protozoa much quicker (and more effectively) than just water does.

For our experiment, if we were to test it again, there are a few things that we would change. First, we would have changed our independent variable. It would have been interesting to find-out how other types of herbicides affect the soil. Also, it would have been interesting to know how the herbicides react to different types of soil locations. Secondly, we could have changed our negative control. Our group could have used a different type of liquid, other than water, to compare to the herbicide. Finally, we could have made our plots a different size and see how the different size plot affects the amount of protozoa that we found. There are many directions that we could take our experiment to test it in different ways. This could help further both student and scientist's research to find a true answer to our problem.