<u>The Impact Of Fertilizer On Protozoa</u>

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Mr. Brock

Protozoa or protozoans are any eukaryote that isn't an animal, fungi, or plant. They are among the simplest form of eukaryotic organism, and because they are able to eat and break down food, produce waste, and reproduce all by themselves, they may very well be the most complex eukaryotic cell (Campbell, Williamson, and Heyden 2004). Most protozoa live in bodies of water such as rivers and lakes, but protozoa on the land live in films of moisture on the particles of soil. There, their survival depends on the amount of space and growth rates of protozoa decrease as the space in the soil becomes more tightly packed together, because there is less room in the soil for the water protozoa need to survive. Also, it is best if the space in the soil be occupied with water so as to stimulate the growth of the protozoa (Lavelle, Spain 2001).

Protozoa are essential to plant life because they produce the necessary chemicals plants need to survive. One of the main chemicals is nitrogen. Nitrogen is important in plant life because it allows for the plant to produce proteins and nucleic acids; this is important because without them the four tasks (to reproduce, regulate their environment, synthesize, and respire) would not be possible. The four tasks need to happen in order to be considered a living thing and in order for the plants to be fertilized and produce more plants. The soil protozoa then eat the soil bacteria and the soil bacteria are able to convert the normally unusable atmospheric nitrogen into ammonia which the plant can use to synthesize its proteins and nucleic acids. When fertilizer is added to plants, it can make plants stay healthy for longer periods of time (Campbell, Williamson, and Heyden 2004).

Fertilizer is a substance added to the soil to improve plants' growth and population density. It is mostly made of ammonia (converted atmospheric nitrogen),

phosphorous (which provides the energy plants need to make chemical reactions happen), and other added nutrients that provide the plants with the nutrients that it needs to grow healthily. Fertilizer can create a better environment for plants than natural soil because the plants can use the excess ammonia in fertilizer to help them synthesize extra proteins.

Fertilizer can also attract bacteria, because of its nutrients, and this in turn attracts soil protozoa. The food chain between the soil bacteria and the soil protozoa therefore becomes healthier because the production of more bacteria is stimulated. This increase attracts more soil protozoa that are then able to release the atmospheric nitrogen into ammonia which plants need (Romanowski, Perry. 2007).

Because fertilizer can be so helpful, we chose to explore whether the addition of fertilizer increases or decreases the density of the protozoa found in the soil of the front lawn. Our hypothesis is that the addition of fertilizer will increase the density of protozoa found in the soil of the front lawn.

Campbell, Neil. Williamson, Brad. Heyden, Robin. (2004) Biology: Exploring Life. Pearson Prentice-Hall. http://www.pearsonsuccessnet.com/snpapp/iText/products/0-13-115075-8/text/chapter17/concept17.1.html

Lavelle, Patrick. Spain, Alister V. (2001) Soil Ecology. Springer. http://books.google.com/books?id=2Vk6roaf5HcC&pg=PA253&lpg=PA253&dq=soil+protists&source=bl&ots=AtzJeGF6l3&sig=3nGJRJRa35CeZwH3hwrLSgRfKeU&hl=en&ei=fbMISu3XEofDtwek9fSIBw&sa=X&oi=book_result&ct=result&resnum=9

Romanowski, Perry. (2007) How Fertilizer Is Made. Advameg Inc. http://www.madehow.com/Volume-3/Fertilizer.html

I. Does the addition of fertilizer increase or decrease the density of protozoa in the front lawn?

II. The addition of fertilizer will increase the density of protozoa in the front lawn.

III.

    A. The independent variable is the addition of fertilizer to certain plots.

    B. The dependent variable is the number of protozoa per grams found in the soil.

    C. The negative controlled variable is the addition of water only to certain plots.

    D. The controlled variables are the size of the plots, size of soil samples, number of samples, staying in the same latitude/longitude, amount of liquid added to each plot, type of fertilizer, concentration of fertilizer, type of water, how often we apply, how much space between plots, how long we let the ground absorb between spraying and taking samples, amount of dye, how long in Uhligs, amount of time for samples to dry, amount of distilled water added, how long the samples rehydrate, size of nylon mess, method of crushing dirt, amount of power of the microscope. `

    E. Step-By-Step:

        1. Get 24 flags and separate them into 6 groups of 4.

        2. Label 4 flags with group 1 and mark group with the word fertilizer as well.

        3. Label 4 flags with group 2 and mark group with the word fertilizer as well.

        4. Label 4 flags with group 3 and mark group with the word fertilizer as well.

        5. Label 4 flags with group 4 and mark group with the word water as well.

        6. Label 4 flags with group 5 and mark group with the word water as well.

        7. Label 4 flags with group 6 and mark group with the word water as well.

8. Go to grassy flat location at North 39° 21.480' and West 076°38.166'.

9. Take flags and make six plots that are each 25 by 25 centimeters that are 25 centimeters apart for the previous plot.
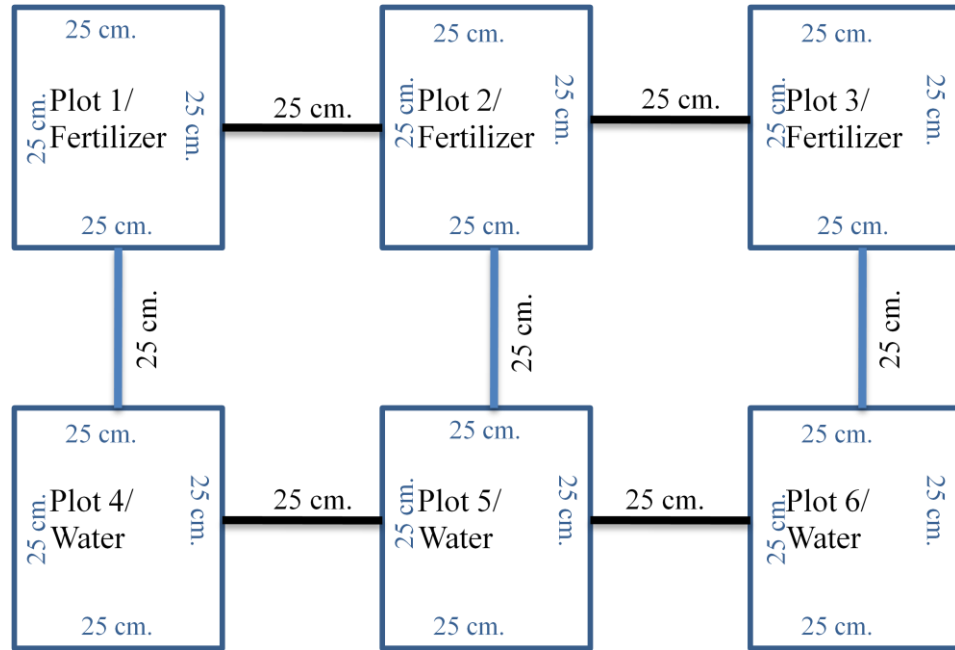
10. Get one sample from each plot using Soil Test Core Sampler that is 15 centimeters deep and 2 centimeters in diameter (make sure to use separate Soil Test Core Sampler for each plot or make sure to wash Soil Test Core Sampler each time to avoid contamination). Put each soil sample into its own separate labeled plastic bags.

11. Make sure you take all samples on the same day at the same time.

12. Make sure to label each bag with the number of the plot either 1, 2,3,4,5 or 6 in which the sample came from.

13. Then test for the amount of protozoa in all of the 6 soil samples.

B. Protozoa Extraction:

14. Place each of the samples collected in step 11 into its own clean, empty petri dish bottom, and allow each sample to dry completely (usually 24 hours).

15. Next, place each soil sample in its own a 3-oz plastic cup (you may wish to use a clean mortar and pestle to help crush up the dirt; just be sure to wash and dry thoroughly between each sample to avoid contamination) and cover the top with its own square piece of 1 mm^2 nylon mesh, using the rubber band to secure the mesh in place.

16. Using the balance, sift 9-10 g of each soil sample through the mesh into its own 2nd clean petri dish and be sure to record the final amount of each soil sample in your data chart.

17. Add 20 ml of distilled water to each sample of sifted soil, cover the petri dish with its lid and allow to sit for at least 7 hours.

18. Place 30 ml of water into a clean, empty petri dish (you may use the washed and dried one from step 1) and set the Uhlig extractor upright in the water. Repeat this step for all soil samples.

19. Scoop the rehydrated soil from each sample into the bottom its own Uhlig extractor and allow them all to sit for 24 hours.

20. Clean, dry beakers. Using filter paper a second time (This liquid now contains the protozoa you will examine under the microscope.) Repeat for all samples using different Uhlig extractors, funnels, filters and petri dishes.

21. Using a capillary tube, deposit 7 µl of methyl-green stain on a clean microscope slide.

22. Then using a new clean pipette, add 18 µl (the first demarcation on the pipette) of the filtrate from the first soil sample to the stain and cover with an 18x18 mm^2 cover slip. Repeat for all samples.

23. Examine all slides individually under the microscope on the 60X power and count the number of protozoa in 5 different fields of view.

24. Average of these 5 fields in the following equation to determine the population density of protozoa for each soil sample:

    [(# per field of view at 60X) (total ml of water used) 2165] ÷ (grams of sifted soil) = # of protozoa per gram of soil

25. Record results in lab.

26. Take six plastic bottles of the same size to make the fertilizer solution in.

27. Fill each bottle with 500 ml. of water.

28. Then measure out 1 g. of fertilizer and pour it into one of the bottles.

29. Measure out 1g. of fertilizer and add it to another bottle filled with water that does not have fertilizer in it.

30. Measure out another gram of fertilizer and pour it in one of the bottles full of water that also has not fertilizer in it.

31. Shake the three bottles with the fertilizer in them with the caps on for 5 minutes.

32. Then pour the first three plots with its own 500 ml of fertilizer solution. Pour 500 ml with water onto the other 3 remaining plots.

33. Then after 24 hours of letting the ground absorb the fertilizer and water repeats steps 10-24 one time.

Mr. Brock (2009) Materials Needed Per Class for Bacteria Serial Dilutions:

https://faculty.rpcs.org/brockda/Little%20Things/Little%20Things%20Handout%2005a.pdf

Data and Analysis

Data/Averages Table

Number of Protozoa Per Gram

Plot #	Before	After	Difference
1	4,795,475	1,524,811.828	- 3,270,663.1721
2	2,565,054.348	1,451,263.736	- 1,113,790.612
3	776,770.8333	3,291,683.673	+ 2,514,912.84
Averages	**1,858,288.394**	**2,089,253.079**	**- 623,180.3147**
4	3,626,932.99	3,099,368.421	- 527,564.569
5	926,262.8866	1,839,086.022	+ 912,823.1354
6	3,280,637.755	582,884.6154	- 2,697,753.14
Averages	**2,611,277.877**	**1,840,446.353**	**- 770,831.5245**

Averages Graph
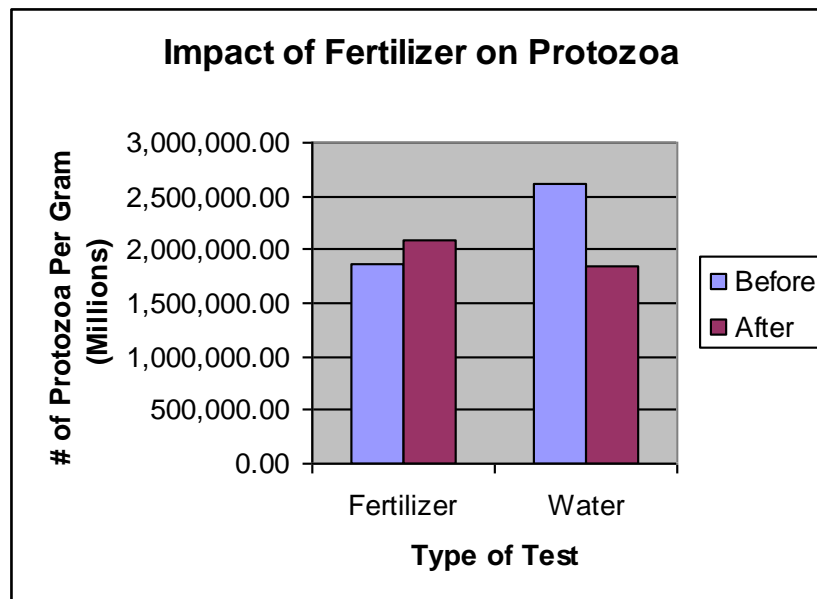
Concluding our experiment, we found that our hypothesis was indeed correct. Our

hypothesis was "would the addition of fertilizer increases or decreases the density of the

protozoa found in the soil of the front lawn." The fertilizer increased the density of the protozoa

found in the soil because the average of the density of the protozoa increased by 230,964.685

after fertilizer was added. The density of the protozoa actually decreased for the plots where

fertilizer was not added; density decreased by 770831.524. Therefore, the addition of fertilizer

positively affected the density of the soil protozoa found on the front lawn. During the process of

our experiment, we had a few setbacks but we were able to overcome them. After we put flags

down next to our plots, some of the maintenance did not realize that they could not mow the

lawn where our plots were. So when we came back to check on our plots the next day, our plot

flags were all rearranged and some were even in another groups plot area. However, we just re-

measured our plot squares and put them back into place without too much trouble. Additionally,

our entire experiment was in jeopardy after we forgot to put our soil samples into the refrigerator; once again we managed to fix the problem and overcome the challenge. Both of these challenges could have altered our entire experiment because we may have replaced our flags in the wrong place and/or our soil samples would have been ruined when we came in the next day to test them for protozoa. For further research, our group would consider the question "Would the addition of different types of fertilizer have the same affect on the density of the protozoa?" This question is a reasonable question to research because although the fertilizer we used affected the density of the protozoa positively, other fertilizers may not do the same because they're nitrogen levels may be different than that of the fertilizer we used. Judging from this, a rational hypothesis to this question would be that the addition of different types of fertilizer would increase the density of the protozoa. This hypothesis makes sense because although the levels of nitrogen may vary, ultimately the added nutrients would attract more protozoa.

We have acted honrally

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