THE PROTOZOANS

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Protozoa Background

Protozoa -the “first animals” - are single celled eukaryotes, which can measure anywhere from 0.0002 to 0.0062 inches and are found throughout the world. There are over three hundred different species of them, and they come in four different forms: flagellates, ciliates and two types of amoeba. Flagellates have a whip like tail; ciliates have numerous small hairs, and amoeba come with shells and without them. All of these types inhabit in the soil and play various roles in helping control the soil by living off of organic material, bacteria, and fungi (protozoa forum, 2008) but mainly bacteria.

Protozoa have a strict diet, have few predators, and do both good and bad things for the soil. They live in the soil in a moist layer of water and are a major controller of bacteria levels in the soil. In fact, about 90% of the bacteria in the soil are consumed by the protozoa species (Nardi, James B. 2003), and while protozoa, being a microorganism, may be perceived as a highly vulnerable organism, they have few predators, mainly only nematodes and microarthropods. Although protozoa as a whole are beneficial to the soil, they can also be harmful. Protozoa can attack the roots of plants and cause fatal disease (Soil Protozoa. 2001). So, the role of protozoa in the soil is complex.

Protozoa can only live and thrive in the right temperatures and moisture levels; so the temperature of the soil impacts them and the growth that takes place in that environment can support a great deal. The best temperature of the soil is around 80-90 degrees Fahrenheit; However even when the soil is chilly, the temperature can still allow for the protozoa and the plants that depend on them to grow because when the soil reaches about 40 degrees the process of nitrification begins.

This cycle consists of every living thing "which encompasses the processes and chemical reactions involved in producing organic nitrogen from inorganic nitrogen and subsequently breaking down organic nitrogen back to the inorganic form" (Nitrogen Cycle- Grolier. 2008). The cycle first begins when "atmospheric nitrogen and hydrogen combine to form ammonia, NH_3; the electrical energy of lightning drives the reaction. Ammonia combines then with rain and becomes available to green plants as dilute nitric acid, HNO_3" (Nitrogen Cycle-Grolier. 2008). This compound in turn combines with photosynthesis materials to make amino acids which help create proteins. Proteins are the basis for plants because they are what enable them to grow and provide a sturdy foundation for growth. DNA uses RNA to copy itself into proteins, otherwise known as enzymes-the basis of plants; the enzymes then use the five biological molecules (lipids, carbohydrates, water, nucleic acids, proteins) to perform the four major tasks (respiration, synthesis, reproduction, regulation). Without proteins a cell could not be formed, therefore plants would be non-existent.

The second step in the nitrogen cycle is Denitrification, followed by nitrification, and lastly nitrogen fixation (Nitrogen Cycle- Grolier. 2008). Nitrification is "the conversion of ammonium (NH4+) first to nitrate (NO2-) and then to nitrate (NO3-) by the addition of oxygen," (Nardi, James B. 2003) and these processes are carried out by bacteria, which are eaten by the protozoa, releasing the nitrates to the plants. Thus, protozoa play an important role in the nitrogen cycle.

Temperature and moisture are the two most important factors when it comes to a suitable living condition for protozoa. In order to become active, protozoa requires a moist habitat (Nardi, James B. 2003). But when soil is exposed to extensive heat; it dries,

therefore protozoa become inactive and hibernate. This state is called a cyst. A place with heavy vegetation does not warm as quickly as a place with no vegetation; and a place with no vegetation, subsequently, also cools quicker (Soil Temperature. 2002). Therefore, protozoa would be comfortable living in an area that is warm and cannot cool too quickly. Moisture is a distinguishing factor of the temperature of soil. If soil is in a dry area, like a desert there will be less growth, there will also be less growth in a warmer soil, but with no moisture. There may be a cool area with colder soil, but no moisture, which would also not be suitable for protozoa life. But, if an area is moist, but too warm, the temperature will override and evaporate the water, sending the protozoa into hibernation. Temperature is just as in important as moisture. Protozoa thrives in warm soil, with moisture, according to the studies of S. M. Gittleston and T. Furguson (1970). So in conclusion, protozoa needs an environment with moisture, and a good room temperature. Putting protozoa in an incubator would send it to cyst mode, and placing protozoa in a cool place, would also make the organism inactive. Therefore, in order for successful growth, soil needs to be warm and moist.

These two factors, soil temperature and protozoa led us to our experiment. The two factors seem to intertwine and have a relationship that we did not know about. Scientists have also proven that the presence of protozoa increases plant growth and increases the speed of which bacteria take act in decomposing non-living material. (Protozoan Importance. 2008). Therefore we thought it would be interesting and a valid cause to test the two. We thought that the soil temperature effected the growth of protozoa because the temperature effects the growth of all other plant life. We were informed that the RPCS campus has too much protozoa in some areas, and too little in

others causing decay, we decided to test this reason for the lack and flourish of protozoa growth.

Our question for our experiment was: “How does the temperature of the solid change the number of protozoa living there?” To start our experiment we decided to take soil samples from all over the RPCS campus and domesticate them in the lab. We took soil from six different plots. We took samples from a place with shade with the same plants; shade with different plants; no shade with the same plant; no shade with a different plant; no shade with mulch, and the same plant; and shade with mulch and different plants. We took samples of soil from each plot and heated half of the samples from each plot in the incubator, while the other six stayed in room temperature, to see which temperature was sufficient for protozoa life.

Background references

Protozoa forum, (2008). http://www.biologyreference.com/Po-Re/Protozoa.html

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Soil Protozoa.(2001). http://www.blm.gov/nstc/soil/protozoa/index.html

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Temperature- related occurance of protozoa. (1970)
http://www.springerlink.com/content/pj062j1678u0537x/

Lab Report

Question: How does the temperature of the soil change the number of protozoa living there?

Hypothesis: If we take two samples of soil from six different locations across the RPCS campus, and than place one sample in an incubator and one in a room temperature environment, than the soil in the warmer environment will have more protozoa.

Independent Variable: Temperature of the environment.

Dependent Variable: Number of protozoa per gram of soil

Negative Control: room temperature environment

Controlled Variables:

- Amount of soil from each area
- Time of day to take sample
- Number of days to take samples
- Number of samples taken
- Number of Petri dishes
- Size of cup
- $1mm^2$ of nylon mesh
- Amount of grams that are sifted into dishes
- Amount of water poured into the samples after shifting
- Amount of sit time in different temperatures
- Amount of distilled water poured on Petri dishes after putting them in different temperature.
- Amount of sit time in the different temperatures
- Temperature of the incubator (32 degrees Celsius)
- Amount of time filtering the sample
- Amount of time filtering the samples
- Amount of drops from the sample are placed on the slide
- Amount of methyl green dye that are put on the slides
- Amount of micro meters to filter the samples
- Size of slides and cover slips.

Getting Samples:

- Take coordinates off of GPS from 6 different plots
    - Place with shade with the same plants (N 39.35772 and W 76. 63663)
    - Shade with different plants (N 39.357870 and W 076.63634)
    - No Shade with same plant (N 39.35749 and W 076.63593)
    - No shade with different plant (N 39.35734 and W 076.63556)
    - No shade, with mulch, and same plant (N 39.35808 and W 076.63619)

    - Shade, with mulch, and different plants (N 39.35794 and W 076.63576)
- Take 15 cm by 2 cm each of two samples of soil from each plot using Augers.
- Put the samples in their own plastic bags and label on the bag which plot they were taken from and the sample number. (In order to tell them apart.)

In the Lab:

- Take each sample and do the following:
- Label Petri dishes for each sample of soil from the different plots.
- Place the samples in seperate Petri dishes and let them air dry for one day.
- Place each sample in a separate small cup and put 1mm² of nylon covering.
- "Sift" 9-10 grams of each sample into its own fresh Petri dish and record how many grams went into the Petri dish.
- Next, pour 20 ml of distilled water onto each sample.
- Take two samples from each plot and have one set of samples sit in room temp. for 7 hours and the other 6 samples sit in an incubator set at 32 degrees Celsius for 7 hours.

Filtering:

- After 7 hours put all the samples into a refrigerator until ready for next testing.
- When ready, filter the samples at the same time by doing the following:
- Label 18 fresh Petri dishes according the sample number and whether they were in the incubator or not.
- Using a graduated cylinder place 30 ml of distilled water into the bottom of the 18 new Petri dishes.
- Place each Uhlig extractor in the Petri dishes with the nylon sheet facing the bottom.
- Scoop out each sample into their own cup and allow the nylon sheet to filter the samples for 24 hours.
- Put the six incubator samples back into the incubator at about 32 degrees Celsius.
- Keep the other six samples at room temperature.
- After 24 hours take the six samples out of the incubator and the six samples in room temperature and put them in the refrigerator until further procedures.
- When ready, filter each sample a second time all at the same time.
- Use qualitative paper this time for filtering.
- Stand the glass funnels up by using the metal rods.
- Pour each sample into their own funnel with fresh qualitative paper.
- Let the liquid from the paper fall into a small cup which should be labeled as with sample number and whether placed in the incubator or not.
- After all the samples are filtered make slides by performing the following:

Making Slides:

- Using a capillary tube add 7 ul of methyl green dye to a microscope slide.
- Add 18 ul of one of the filtered samples using a graduated Beral-type pipette and cover the slide with an 18x18mm² squared cover slip.
- Do this for all samples with clean slides and new pipettes each time.

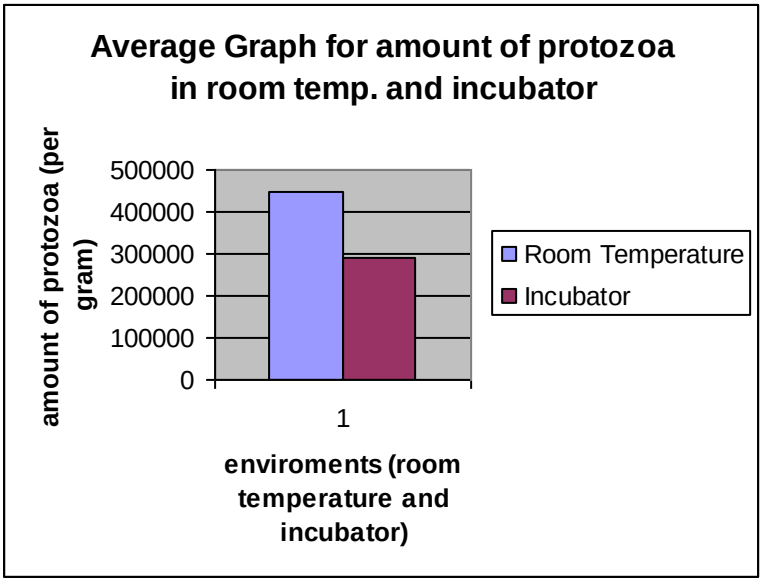
- Observe the slide by viewing at a magnification size of 60X power.
- Take a snap shot of what is seen in each slide by clicking the snap shot button, then click the arrow pointing to the left, and finally press export to save the picture onto the computer.
- Count all the protozoa that are in the snap shots of each individual sample.
- After all the pictures of the slides have been saved to the computer and protozoa have been counted in each snap shot, perform the density equation with each sample.
- (# per view of field at 60X) x (total ml of water used 50 ml) ÷ (grams of soil sifted) = # of protozoa per gram of soil.

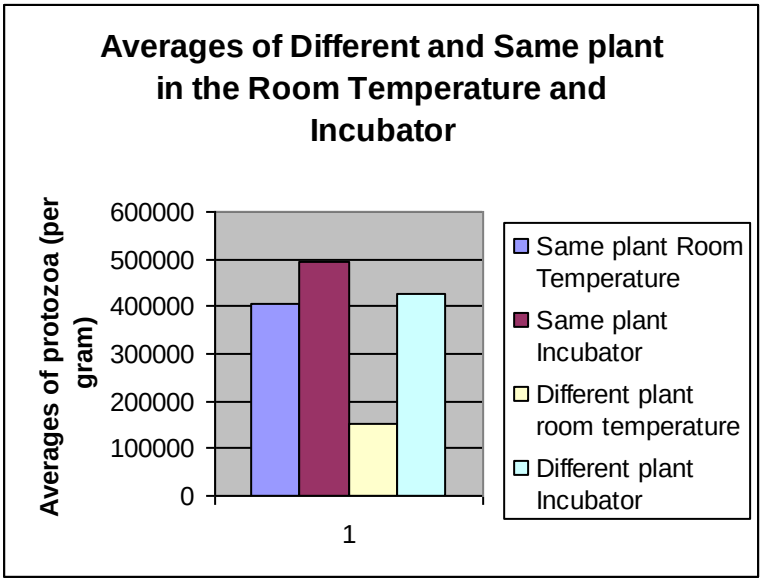
Citation:
http://www.greencastonline.com/SoilTempMaps.aspx

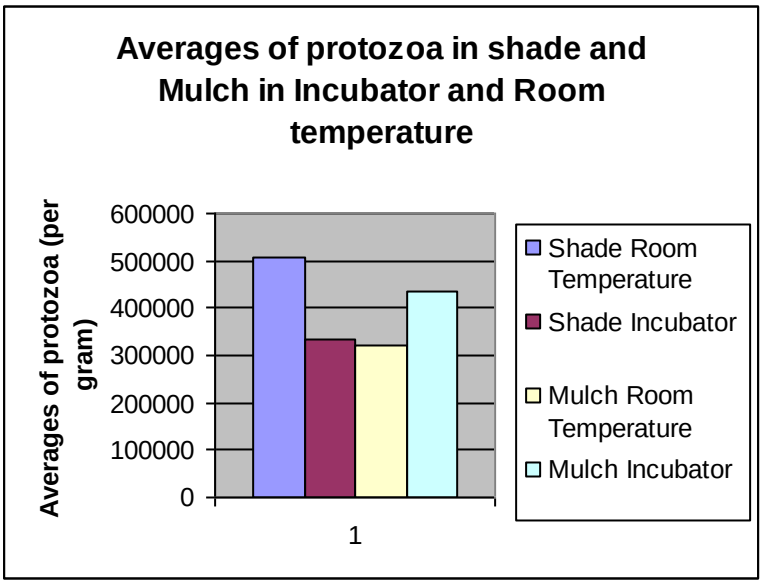
Data Tables for Density of Protozoa and Average of Protozoa in Room Temperature and Incubator

Plot descriptions	Protozoa Density Room Temperature (23 degrees Celsius)	Protozoa density in Incubator (32 degrees Celsius)
1 (10^{th} grade courtyard)- N 39.35772 and W 76.63663	459242 per gram	95165 per gram
2 (Front yard by entrance)- N 39.357870 and W 076.63634	465541 per gram	368511 per gram
3 (Middle Courtyard- by trashcan)- N 39.35749 and W 076.63593	709638 per gram	36083 per gram
4 (Middle Courtyard by basketball court)- N 39.35734 and W 076.63556	420972 per gram	372861 per gram
5 (Front yard)- N 39.35808 and W 076.63619	43737 per gram	328030 per gram
6 (front entrance by benches)- N 39.35794 and W 076.63576	600082 per gram	541250 per gram

Average of Protozoa found in Room Temperature environment	Average of protozoa found in Incubator environment
449877 per gram	290317 per gram


Average Graph for amount of protozoa in room temp. and incubator
amount of protozoa (per gram)
500000
400000
300000
200000
100000
0
1
enviroments (room temperature and incubator)
Room Temperature
Incubator


Averages of Different and Same plant in the Room Temperature and Incubator
Averages of protozoa (per gram)
600000
500000
400000
300000
200000
100000
0
1
Same plant Room Temperature
Same plant Incubator
Different plant room temperature
Different plant Incubator



Conclusion:

After testing the eighteen soil samples our hypothesis we thought to be right was incorrect. There are many alternatives that can lead to the explanation why our hypothesis was false. When our group first had the idea that heat would increase the amount of protozoa we never thought beyond that. After further research in trying to understand what went wrong we figured out that protozoa actually need moisture in order to stay alive and grow. There are many possibilities that when putting the chosen six samples in the incubator, the protozoa felt like they were suffocating from the heat. Moisture actually turned out to be a large factor of the growth and health of protozoa. Another alternative in explaining our incorrect hypothesis is the home of the protozoa in the beginning of the whole process. There is a good chance that where the soil came from

affected the life of the protozoa that were living in the ground in that location. The plants that were taken from the shaded areas, with the same plants, and placed into the incubator, actually could not survive in the extreme temperature change. The plants were adapted to the colder areas and when placed in the incubator they could not handle the heat because of the lack of moisture. Also, by looking at the graphs many signs show that our hypothesis could have been correct. In looking further into the interesting analysis from our data we discovered that soil that was originally in sunny areas and placed in the incubator actually had a higher amount of protozoa than the ones placed in room temperature. This is most likely because the plants were already adapted to the warmer temperatures so they felt at home being in the warm incubator. Even though some of the data showed that the our hypothesis being that more protozoa would grow more when placed in an incubator was very possible, it all came down to the final averages of the amount protozoa in room temperature and the amount protozoa in the incubator. In the end it made out to be that the average amount of protozoa in the samples placed in room temperature was 449877, and the average of all protozoa in the samples placed in the incubator was 290317. Although it was disappointing to see that our hypothesis was wrong, the concluding data made our experiment a lot more fascinating than expected to be. From this experiment we have gained more knowledge about the growth of protozoa. We figured out that temperature and moisture does in fact affect the health of protozoa. For further testing we would test different situations of moisture level to see whether protozoa does indeed grow more in an environment with a high amount of moisture.