Julia Atwater, Natalie Hazlehurst, Maria Pasquini, Lilly Siems
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Background: Protozoa and Calcium Chloride Relation

Soil contains a diverse ecology in which millions of organisms live. The complex life cycles in soil all coexist to feed and further develop plants and animals, creating an intricate ecosystem. Knowing the contents of soil enables humans not only to maintain, but further study this environment. Protozoa, a shared name for unicellular eukaryotes missing cell walls, play key roles in soil ecology. Protozoa are the main consumers of bacteria and are the biggest in number and biomass of animals in the world (Protzoa, no date).

Protozoa belong in the kingdom Protista (unicellular simple animals that do not fall into plant and animal kingdoms easily). They feed heterotrophically, meaning that they are "an organism that cannot synthesize its own food and is dependent on complex organic substances for nutrition" (Dictionary.com). This means that protozoa break down their food from complex organisms (i.e. bacteria). Also, because protozoa are unicellular, they absorb food through their vacuoles. Protozoa have nuclei and are about .01 to .05 millimeters in size. Protozoa live in aqueous and soil environments, specifically near water, tree roots, forests, and clay, and are classified on a basis of locomotion (Protozoa, no date).

There are many types of protozoa which vary in function, location, and make-up. Flagellates are protozoa cells with one or more whip like organelles called flagella, which enable the flagellates to move around. More specifically flagella can be defined as "A long, threadlike appendage, especially a whip like extension of certain cells or unicellular

organisms that functions as an organ of locomotion" (Dictionary.com). A second type of protozoa are called amoeboids. Amoeboids move through temporary projections of the cytoplasm in cells from their pseudopods. Pseudopods are false feet on the amoeboid that function as mobility, meaning that the amoeboids flow their protoplasm (material/contents) of a living cell (Protoplasm, 2005) forward into the "foot" then bring the rest of their body into it in order to "slither" along (Sarcodina- move with pseudopodia, 2003). A third type of protozoa is apicomplexa. These protozoans however do not exist in the soil and therefore are not part of our study. The last group of protozoa are called cillates (which gets their name because of the cilia, or hair that projects from the cells surface). This group is one of the most important groups of protozoa because it can live in almost every ecosystem. Although all these groups share the name of protozoa, they are all very different (Protozoa, April 20th, 2005). Each protozoa has its own unique mobility function, feeding function, and body structure. These differences serve to equip it with the features that will enable it to best interact with its environment such as the projections on flagellates that help it move around in the soil.

Though diverse and numerous, protozoa are an essential part of the soil's ecology. They continue the fertilization cycle by regulating bacteria population, maintaining soil nutrient levels, and recycling nitrogen. The high or low levels of protozoa in the soil are dependent upon contributing factors around them such as predators, chemicals in the soil (chloride, ammonia, etc.), and the human influences (litter, dumping chemicals on the soil, etc.). Without a doubt, they are a critical and an indispensable part of the ecosystem. Protozoa are also vital to the cycles of the soil which are discussed in brief below.

The biogeochemical cycles are necessary cycles in order for there to be life present on earth. They are so important because they move around the chemicals that help form proteins, amino acids, and DNA. These three things are vital to cell life because without any of them the cell could not perform the four tasks of reproduction, synthesis of proteins, regulation, and respiration, and therefore would die. They could not perform these tasks because proteins start and stop all chemical reactions in the cell and the four tasks are the chemical reactions of a cell. Through these different cycles, various chemicals are distributed throughout the environment, making the world around us function. The different biogeochemical cycles are the water cycle, oxygen cycle, nitrogen cycle and phosphorus cycle. Both the nitrogen and water cycles will be discussed in slight to extreme detail throughout the course of this paper because protozoa play a particularly important role in each cycle (The soil ecosystem, No Date).

Protozoa balance out the soil by recycling nitrogen through the nitrogen cycle. The nitrogen cycle is one of the most important cycles for an ecosystem because nitrogen is used by living organisms to create organic molecules like amino acids, proteins, and nucleic acids. Proteins are extremely important as they are responsible for the cells tasks, chemical reactions, and life in general. The proteins start and stop the chemical reactions that are the four tasks of life. If the proteins are not there the plant will die and not only the plant but the protozoa and other microorganisms because proteins are the building blocks to all cell life. Nitrogen is thus a vital chemical in the plants life and the nitrogen is obtained in only two forms, nitrate and ammonium in a special process. The process of how plants get their nitrogen is called the nitrogen cycle (Nitrogen Cycle, 1999-2004). The nitrogen cycle is a complex process described below.

The nitrogen cycle starts with decomposed organic materials, which is where most nitrogen in ecosystems is found. Decomposers in the upper soil layer chemically modify that nitrogen from ammonia into ammonium through a process called mineralization, carried out by various bacteria and fungi. Nitrogen in this form can be held by soil collides (soil particles with negative charge, sites for cation exchange (Email from Brittany Flokstra, 2005) which is then released through a process called cation exchange. Cation exchange is the chemical trading of cations, an ion carrying positive atomic charge, between the soil minerals and organic matter with the soil solution and plant roots (Glossary of Terms, 1999-2004). On the release, the ammonium is modified again by a special type of bacteria converting the nitrite to nitrate, which is nitrogen with three oxygen's attached. That entire process is called nitrification. Much of this is returned to the atmosphere through denitrification, which provides the bacteria with the oxygen needed for respiration. The nitrate not absorbed into the atmosphere is used by the plants as a nitrogen source (Lecture 22: The Nitrogen Cycle, no date). Once the nitrogen escapes back into the atmosphere, it eventually comes back to the earth via precipitation and the cycle repeats (Nitrogen Cycle, 1999-2004). Protozoa play a major role in the nitrogen cycle completing tasks that affect all of the microorganisms.

Protozoa are an essential component to the food chain (web) of the soil. The main job of the protozoa is to eat the bacteria that are found in the soil. Both earthworms and nematodes eat the protozoa for nourishment. When the bacteria are being eaten, the protozoa lets out nitrogen into the soil thus fueling the nitrogen cycle to continue. When nutrients seep into the soil they are turned into nitrate fertilizer. Since nitrate can multiply rapidly, bacteria rushes to it and consumes the nutrients from it; protozoa is then

needed to control the growing bacteria population. If the protozoa level is too low, then the nutrients remain in the fungi and bacteria, which is not good for the plant. The protozoa levels affect the plants in different ways.

If protozoa levels are too high, then there could be inconsistent levels of nitrate and ammonium. It has been found that plants have grown more with less ammonium and grow less with more ammonium (Effect of Nitrate, 2003). This is because large concentrations of ammonium are extremely toxic. The inconsistent levels of nitrate are dangerous because nitrate is essential for building amino acids, proteins, and DNA, all vital aspects of a cells life (The Nitrogen Cycle, 2004). Protozoa levels play an important role in the life of the ecosystem but can be thrown off balance by chemicals such as a calcium chloride.

The compound called chloride plays an important part in the health of soil. Chloride is by definition any compound containing salt of hydrochloric acid. Consequently, high levels of it cause plants to die because it creates a toxic environment due to the high acidity from the high levels of hydrochloric acid in the chloride. The salt absorbed into plants also cannot be diffused out so it stays in the plant and builds up and begins to do internal harm such as clogging the cell membrane. Chloride can also be found in fertilizers, but at low levels. In one ecosystem it was found that high levels of chloride increased the bioavailability of phosphorus in peat (Projects, 2004). Phosphorus produces phospholipids which are major components in cell membranes. Phosphorus also creates chemicals vital in energy production such as the production of ATP. For protozoa this affects the forming of their cell membranes and energy production thus crippling the count of protozoa. The domino reaction of chloride levels affects the chemicals around it

which in turn affect the protozoa levels (Phosphorus, 2003). The specific chloride chemical called calcium chloride is the main focus of our experiment.

Calcium chloride is commonly used for defrosting ice on roads, control dust, and as a preservative for food. It is very soluble in water and is crystalline, lumpy, or flaky, and usually white. Calcium Chloride rapidly absorbs water and dries gases by passing them through it (Calcium Chloride, 2005). In another test it was found that calcium chloride enhanced more ammonium N, potassium, phosphorus, magnesium, copper, and zinc in the crops being tested (Calcium More than Just Limestone, 2004). It has been proven that chemicals like phosphorus and magnesium are harmful to the lives of protozoa (Calcium More than Just Limestone, 2004). Therefore if the calcium chloride in our plots enhances these chemicals as well, then the protozoa will die. Calcium chloride has a rather large impact on the soil ecosystem.

The locations we chose for use in our experiment are 15cm by 15cm plots that vary slightly in elevation. The plots are tested for existing calcium chloride and protozoa. Then they are manipulated with a calcium chloride solution to see the effects it has on protozoa count. When the calcium chloride is poured on the soil we expect the protozoa count to die due to the harmful chemical. However if too much calcium chloride is added it may kill the grass increasing the protozoa count. The protozoa would feed off of the bacteria from the decaying plant material and thus have a larger count. The calcium chloride throws off other chemicals in the nitrogen cycle by causing an imbalance thus having a direct affect on protozoa population. Our experiment tests the effects of calcium chloride on protozoa.

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Experiment:

I. Problem: What does the addition of calcium chloride do to the population density of soil protozoa?
II. Hypothesis: If calcium chloride is added to soil, then the population density of soil protozoa will decrease.
III. Experiment:

a) Independent Variable: The presence of added calcium chloride in the soil.
b) Dependent Variable #1: The population density of protozoa in the soil
c) Dependent Variable #2: The amount of chloride in the soil
d) Negative Control #1: The plot of soil with no calcium chloride added
e) Negative Control #2: The protozoa and chloride count in the soil before calcium chloride is added
f) Controlled Variable: amount of soil taken for each soil sample, number of different locations that the soil samples are taken from, number of samples taken at each location, time of sample taken, amount of calcium chloride solution added to plot, concentration of calcium chloride in solution, amount of space between plots, terrain, size of soil sample, size of plots, simultaneously doing chloride and protozoa extraction, amount of time allowing the soil to dry, amount of distilled water added to each sample, amount of methyl green stain, amount of demineralized water added to the soil, amount of chloride test solution for each sample, amount of time calcium chloride solution is left in the plot, which plot

calcium chloride is added to, amount of water added to each plot, length of time between adding water and calcium chloride and taking soil samples

g) Procedure: (The Kate Brockmeyer method of protozoa extraction and The LaMotte STH series of professional soil testing outfits Code 5029)
    a. Go to the GPS coordinates of North 39.35701 and West 076.63625.
    b. In this area mark two separate plots of soil, each being 15 cm by 15 cm in width and length (Each plot should be 3 cm away from each other). Make sure you label one of the plots 'PLOT 1' and the other 'PLOT 2'. The combination of 'PLOT 1' and 'PLOT 2' makes AREA 1 (when you replicate the experiment this will be AREA 2 or AREA 3)
    c. Next use the soil corer and extract 10 cm (with a diameter of 2 cm) deep of soil from AREA 1, PLOT 1, SAMPLE A. Put this into a plastic bag and label it accordingly. Do this for two additional samples in the same plot (label the two other samples SAMPLE B and SAMPLE C and put each into a separate bag). When you have extracted three soil samples in one plot move on to the next plot (PLOT 2). Repeat everything you did for PLOT 1 for PLOT 2
    d. Mix 10.4 grams of calcium chloride with 1 liter of water and evenly pour over PLOT 1
    e. Pour 1 liter of water evenly onto PLOT 2
    f. Leave the calcium chloride solution on the plot for 24 hours
    g. Repeat step c but label (for plot 1: AREA 1, PLOT 1, SAMPLE A2 - AREA 1, PLOT 1, SAMPLE B2 - AREA 1, PLOT 1, SAMPLE C2 and for plot 2: AREA 1, PLOT 2, SAMPLE A2 - AREA 1, PLOT 2, SAMPLE B2 - AREA 1, PLOT 2, SAMPLE C2)
    h. Dump each sample into the bottom of separate clean, empty Petri dishes (label each Petri dish according to what each plastic bag says); and allow the soil to dry completely for at least 24 hours. (Leave the tops of the Petri dishes off until the soil is dry)
    i. Sift 9-10 grams of each sample of soil into separate 2^{nd} clean Petri dishes using a plastic cup, rubber band, and $1mm^2$ nylon screen for each sample. Make sure you record the amount of soil that you sift and save the extra soil just in case something goes wrong.
    j. Add 20 ml of distilled water to each sample to saturate the soil
    k. Cover the Petri dishes with their lids and allow them to sit for 7 hours.
    l. After 7 hours, place each soil sample (separately) into modified Uhlig extractors (when you do this make sure that each Uhlig extractor is placed in an open, clean Petri dish that is labeled according to the soil sample that is being put in). Pour 30 ml of distilled water in the bottom of each of the Petri dishes with the Uhlig extractors in them. Let sit for 24 hours at room temperature.
    m. While doing steps n-q, simultaneously do steps r-v to each of the soil samples
    n. For each soil sample, remove the filtrate and filter a 2^{nd} time using 12.5 cm qualitative filter paper

o. Put 7µl of methyl-green stain on a clean microscope slide. Then add 18µl of the second filtrate to the stain on the microscope slide and cover with an 18 x 18 mm^2 cover slip (do this separately for each soil sample)
p. Examine each sample separately under a light microscope at 40X for protozoa. Examine 5 different fields of view on the slide and count the protozoa from each of the 5 areas on the slide. Average the number of protozoa taken at the 5 different areas. Put the average into the equation in the next step.
q. Use the following equation to determine the population density of protozoa in the soil samples: [(#per field of view at 40X) x (total ml of 2^{nd} filtrate) x 747] ÷ (grams of sifted soil) = # of protozoa per gram of soil.
r. Add water to each filtrated soil sample using the model PWB-1 Demineralizer Bottle to fill 5 mL soil tube to the 5 mL line with demineralized water.
s. Use the plastic soil measure to add one level measure of the soil sample to the tube. Cap and shake vigorously for 2-3 minutes (Do this separately for each soil sample)
t. Use a piece of filter paper and a plastic funnel to filter the mixture into a second tube to a flat-bottomed turbidity vial (Do this separately for each soil sample)
u. Add one drop of Chloride Test Solution to the vial for each sample. Swirl gently to mix
v. For each sample, match the turbidity or amount of precipitation against the turbidity standards on the Chloride Chart. Lay the chart flat under natural light and hold the turbidity vial one-half inch above the black strip in the middle of the chart. View the black strip down through the turbid sample and compare the resulting shade of gray with the six standard shades. The test result is read in parts per million chloride.
w. Do steps b-v to at least two more areas

Data:

*Plot 1 represents soil samples with 10.4 grams of chloride and 1 liter of water on them
*Plot 2 represents soil samples with 1 liter of water on them

Data Tables before chloride and water is added:
Area 1 Data Table:

Location of Soil Sample	Number of Protozoa per gram of soil	Amount of Chloride in the Soil in parts per million
Area 1 Plot 1 Sample A	26184 Protozoa	150 ppm
Area 1 Plot 1 Sample B	6872 Protozoa	150 ppm
Area 1 Plot 1 Sample C	8232 Protozoa	50 ppm
Area 1 Plot 2 Sample A	31453 Protozoa	150 ppm
Area 1 Plot 2 Sample B	86622 Protozoa	75 ppm
Area 1 Plot 2 Sample C	5976 Protozoa	75 ppm

Area 2 Data Table:

Location of Soil Sample	Number of Protozoa in Soil on average	Amount of Chloride in the Soil in parts per million
Area 2 Plot 1 Sample A	45273 Protozoa	200 ppm
Area 2 Plot 1 Sample B	69364 Protozoa	75 ppm
Area 2 Plot 1 Sample C	37735 Protozoa	100 ppm
Area 2 Plot 2 Sample A	38482 Protozoa	150 ppm
Area 2 Plot 2 Sample B	159111 Protozoa	150 ppm
Area 2 Plot 2 Sample C	27918 Protozoa	75 ppm

Area 3 Data Table:

Location of Soil Sample	Number of Protozoa in Soil on average	Amount of Chloride in the Soil in parts per million
Area 3 Plot 1 Sample A	23157 Protozoa	150 ppm
Area 3 Plot 1 Sample B	59013 Protozoa	75 ppm
Area 3 Plot 1 Sample C	33539 Protozoa	200 ppm
Area 3 Plot 2 Sample A	44073 Protozoa	150 ppm
Area 3 Plot 2 Sample B	70965 Protozoa	200 ppm
Area 3 Plot 2 Sample C	76224 Protozoa	200 ppm

Data Tables after chloride and water is added:

Area 1 Data Table:

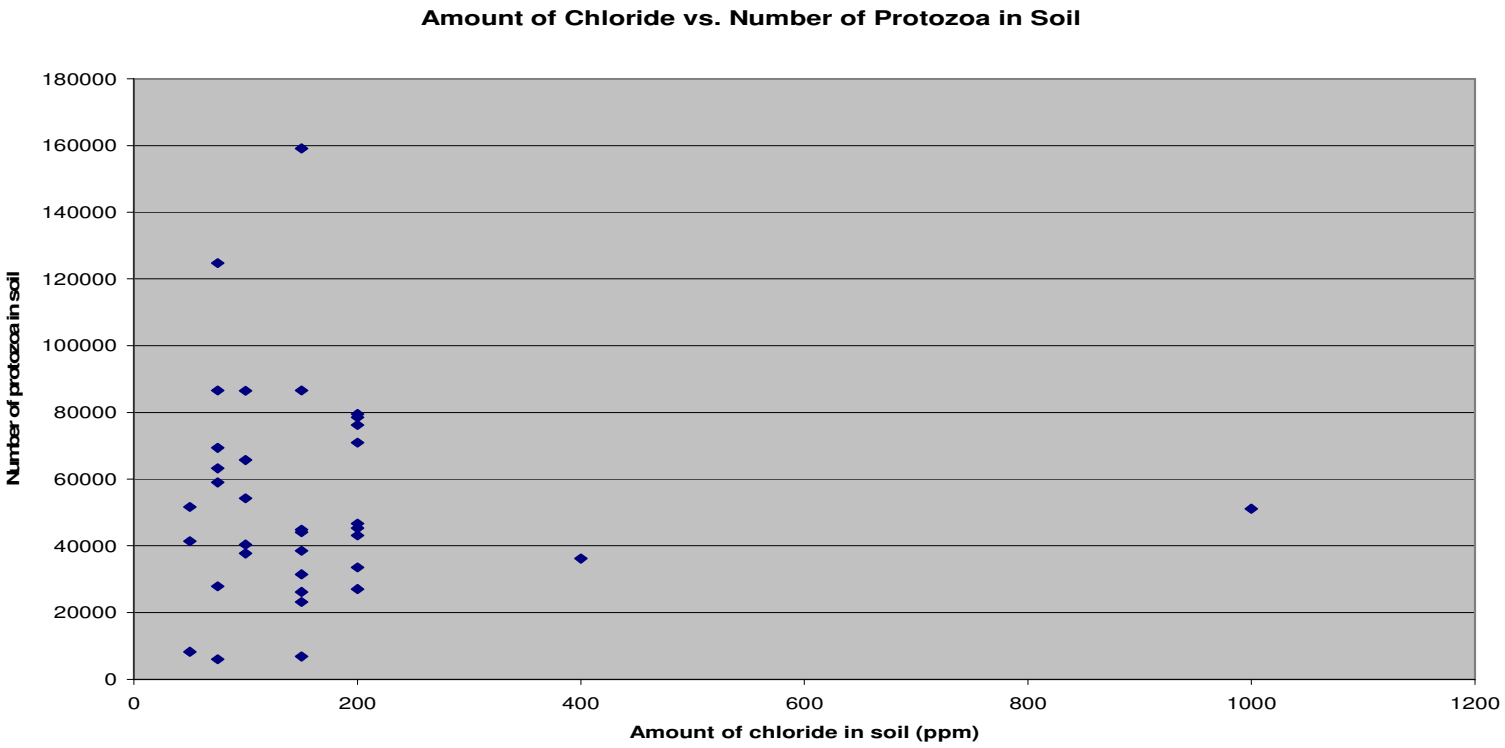
Location of Soil Sample	Number of Protozoa per gram of soil	Amount of Chloride in the Soil in parts per million
Area 1 Plot 1 Sample A	65736 Protozoa	100 ppm
Area 1 Plot 1 Sample B	63266 Protozoa	75 ppm
Area 1 Plot 1 Sample C	51654 Protozoa	50 ppm
Area 1 Plot 2 Sample A	54256 Protozoa	100 ppm
Area 1 Plot 2 Sample B	51111 Protozoa	1,000 ppm
Area 1 Plot 2 Sample C	86620 Protozoa	150 ppm

Area 2 Data Table:

Location of Soil Sample	Number of Protozoa in Soil on average	Amount of Chloride in the Soil in parts per million
Area 2 Plot 1 Sample A	43126 Protozoa	200 ppm
Area 2 Plot 1 Sample B	46688 Protozoa	200 ppm
Area 2 Plot 1 Sample C	Protozoa	ppm
Area 2 Plot 2 Sample A	44820 Protozoa	150 ppm
Area 2 Plot 2 Sample B	36218 Protozoa	400 ppm
Area 2 Plot 2 Sample C	86495 Protozoa	100 ppm

Area 3 Data Table:

Location of Soil Sample	Number of Protozoa in Soil on average	Amount of Chloride in the Soil in parts per million
Area 3 Plot 1 Sample A	78511 Protozoa	200 ppm
Area 3 Plot 1 Sample B	27019 Protozoa	200 ppm
Area 3 Plot 1 Sample C	41410 Protozoa	50 ppm
Area 3 Plot 2 Sample A	40399 Protozoa	100 ppm
Area 3 Plot 2 Sample B	124765 Protozoa	75 ppm
Area 3 Plot 2 Sample C	79519 Protozoa	200 ppm



AVERAGE DATA TABLES

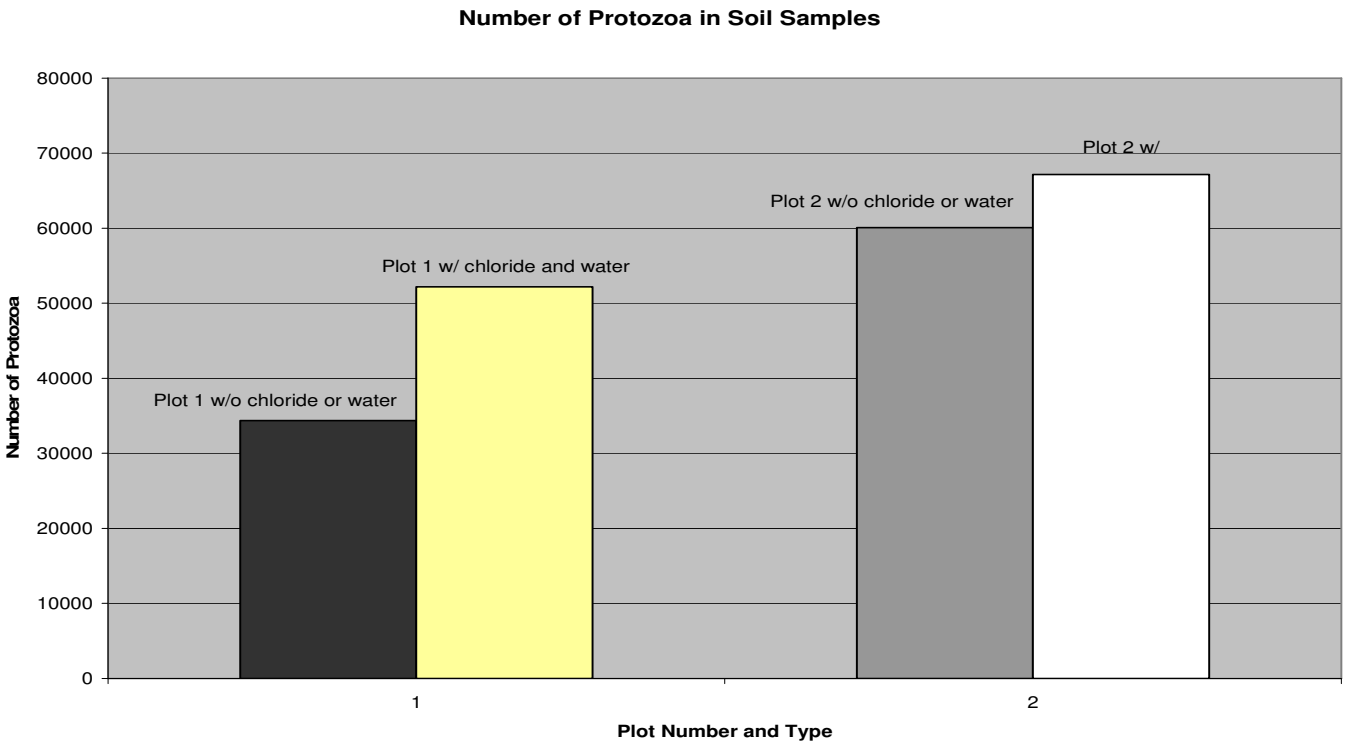
Protozoa

Plot 1 with out chloride or water
34374 protozoa

Plot 1 with chloride and water
52176 protozoa

Plot 2 with out chloride or water
60092 protozoa

Plot 2 with chloride and water
67134 protozoa



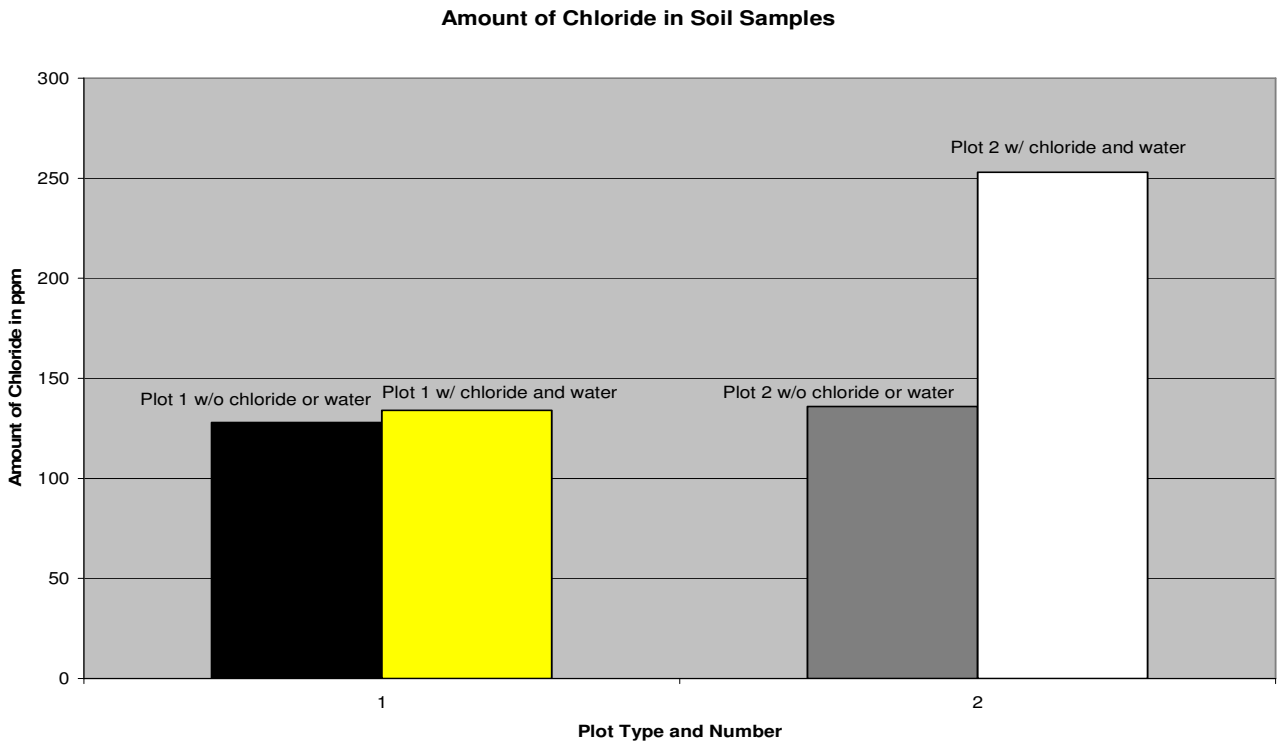
Chloride

Plot 1 with out chloride or water
128 ppm

Plot 2 with out chloride or water
136 ppm

Plot 1 with chloride and water
134 ppm

Plot 2 with chloride and water
253 ppm



Analysis

Chloride Graph

Throughout the experiment, the amount of chloride that was initially found in the earth was about the same number. However, when additional chloride (and water in the 2nd bar in Plot 2) was added, one plot had a significant increase in the amount of chloride found within the soil sample, while one experienced such a minor change that it was like a change never occurred in the first place.

In order to factor in environmental changes, both of the plots with chloride and water added would have to decrease by 86%.

Protozoa Graph

The initial amount of protozoa found in the plots where only chloride was added varied dramatically. Plot 1 had a small number of protozoa while Plot 2 had a large number. However, when chloride (and water in the 2nd bar in plot 2) was added, Plot 1 had a large increase in the number of protozoa in the soil, while Plot 2 experienced a small increase.

In order to factor in environmental changes, both of the plots with chloride and water added would have to decrease by 12%.

Protozoa and Chloride Graph

This scatter plot shows that the direct relationship between chloride and the protozoa population is a negative one.

Conclusion

Originally, we believed that the more calcium chloride that we put in the soil, the more the protozoa population would decrease. That is why we initially set aside plot 2 to pour additional chloride into. However, instead of decreasing the population of protozoa in the soil, the calcium chloride actually did the opposite. In our hypothesis we stated that if calcium chloride was added to soil, then the population density of protozoa in the soil would decrease, however, according to our data, this did not prove to be the case.

The main way to describe our experiment was to talk about our problems which were abundant, even from the beginning of our experiment. When we first were introduced to this project we had no idea at all what we were going to do. For the longest time we had no idea what we were going to do, and when we finally figured something out, it was too general to work. However, we were unaware of this for the moment, and spent one full class taking GPS locations when we should have been figuring out what we wanted to do. It wasn't until Mr. Graffman came that we even had an idea of what we were going to do, and by then we were already way behind all of the other groups.

Throughout the actual course of our experiment was where we saw the most problems. It all started with the fact that it took us a long time to get our soil samples because we were not good at getting the right amount of soil out. In addition, the fact that for certain tests we had to wait 7 or 24 hours before going on proved to be a problem because we couldn't find times when at the end of those 7 or 24 hours we would be there. At the time we weren't really into the whole idea of working extra hours, so if it wasn't really convenient, we would definitely not be doing it.

However, at a certain point something switched on inside of us and we began to actually work. Then we encountered what was perhaps the biggest problem of them all: chemical testing. It was a time consuming process and sadly, we did not have unlimited time. Once our teacher left, that was it. Consequently, we spent the majority of our time in the biology room, which caused stress levels to skyrocket and nerves to shatter. Instead of taking time off from school, we were doing just the opposite. Also, at that time the entire group was not making these sacrifices. Sometimes they would have to leave, sometimes they would just not come, and sometimes even though they were there it seemed like it would have been better if they weren't.

Even though we had our fair share of problems, and at some times it really seemed unbearable, we managed to have a lot of fun with the project and go from being behind and having no clue, to actually finishing our experiment and coming to a successful conclusion, although not the one we had expected.

Although our experiment proved to be interesting and provided us with logical results, further testing could lead could prove our new hypothesis: calcium helps to boost the population of protozoa. Further testing that we would choose to do is while we were testing for protozoa count and chloride levels, we could also test for calcium levels. This way we could see whether the soil had more chloride or more calcium and relate that to the protozoa level to fully finish our ideas.