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Soil Ecology Background Research

Soil is one of the most important natural resources on the planet, second only to water. The entire food chain is dependent on the soil, and the ability of plants to grow in it and microorganisms to live in it. Many nutrients in the soil help these microorganisms survive in the soil, such as Manganese, Phosphorous, and Calcium. However, just as there are helpful nutrients, substances such as aluminum are harmful to many bacteria in the soil.

The levels of Calcium and Aluminum in the soil are both dependent on a scale called the pH scale. The term pH stands for Potential of the Hydrogen ion (Mitchell, n.d.). The scale runs from 0 through 14 and shows how acidic or alkaline a soil sample is (Mitchell, n.d.). The lower the number is on the scale, the more acidic the soil is, therefore, the higher the number the more alkaline the soil is. When the number is 7, the soil is said to be neutral, because there is an equal balance between hydrogen and hydroxide, another substance in the soil (Mitchell, n.d.). The higher the hydrogen concentration is the lower the pH and the higher the hydroxide concentration the higher the pH level (Mitchell, n.d.). When pH levels are high, calcium levels are high and aluminum levels are low, saying that pH levels and nutrients (such as calcium) have a direct relationship, where as pH levels and metallic substances (such as aluminum) have an inverse relationship (Mitchell, n.d.).

Aluminum can be very harmful to the soil at times. If the pH level of the soil is neutral or either slightly acidic or alkaline, aluminum is not harmful. However, once pH levels drop below 5.0, aluminum levels rise dramatically and can impair the growth of plant roots (“Aluminum Tolerance in Plants…” 7/5/01). Aluminum inhibits plant root growth by reacting with the microtubules in the plant root’s cytoskeleton, causing the

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plant root to swell (Blancafor, Jones, Gilroy, 1998, 159-172). As a result of this, the plants are not able to grow like they are supposed to and if this happens to enough plants, it affects the surrounding ecosystem in a negative way. Not only does aluminum then affect the plants growth, but "Bacteria are most abundant around the roots of plants." (Environmental Literacy Council, 2/18/04) By blocking plant roots, aluminum then also kills bacteria, explaining the low population density of bacteria when there are high levels of aluminum. Protozoa feed on bacteria, so as a result of a low bacteria population, there would be a low protozoa population in areas with high levels of aluminum (Environmental Literacy Council, 2/18/04). Therefore, a nutrient such as calcium could be added to the soil to increase the pH level, lowering aluminum levels.

Calcium is used to reduce the acidity of soil by transforming some of the hydrogen ions into water (H_2O) and carbon dioxide (CO_2) (Mitchell, n.d.). Since the concentration of hydrogen ions have been decreased, there is a greater concentration of hydroxide in the soil, meaning the soil is more alkaline. If added to very acidic soil, calcium would then make the soil more alkaline, increasing the protozoa population. Aluminum also directly affects the protozoa because one of its main uses is to kill bacteria and protozoa. It is often used to disinfect water in the form of aluminum sulfate (Peterson, n.d.); therefore, if there were high aluminum levels in the soil, there would be a low protozoa population. In addition to reducing the acidity of the soil calcium also has many functions in plants. Calcium helps cell walls develop properly, cell division occur correctly, and various other tasks that plant cells must perform (Anderson, n.d., *Functions*). Calcium also helps control osmosis in plants and is an essential nutrient to plant growth: "without adequate calcium storage quality suffers and with extreme

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deficiency growth points die." (Cowan, n.d., *Calcium in the Plant*) Calcium is vital in the soil not only for controlling the pH level, but also enabling plants to grow and thrive.

Protozoa are important to the soil for many reasons as well. Protozoa are "Small, usually single-celled microorganism that lives in the soil. They are non-photosynthetic and feed upon dead or live bacteria and fragments of organic matter." (Center for Study of Carbon Dioxide and Global Change, n.d., *Protozoa*) Protozoa feed on bacteria, helping to control the bacteria population in the soil. Protozoa which feed on bacteria also perform a very necessary task: they help to convert ammonia into nitrate (Ingham, n.d., *Biological Side of Soil)*. They also help with other nutrient recycling within the soil (Ingham, n.d., *Biological Side of Soil)*. Protozoa are important because of their important functions in the soil, and it is important to study them to better learn how they function and what environment they need to survive and perform their tasks. It is particularly important to study them on the Roland Park campus because previous years' research has discovered that there is something irregular with the aluminum levels on our campus. Instead of doing the inverse of what calcium levels do, in a particular part of our campus (site 1 of the backwoods) the aluminum follows what the calcium does. Because this pattern is irregular, the level of protozoa may be irregular, directly affecting our environment and the ability of plants to survive in our backwoods.

Over the course of our research, we used previous data from two sites on the RPCS campus, one modeling the regular relationship between calcium and aluminum (site 3) and one modeling an irregular relationship on our campus (site 1). Here as aluminum rose, so did calcium, where regularly calcium does the opposite of what aluminum does (see data table):

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Data from E.S.S.R.E. Microclimate Databases:

	Site 1	Site 2	Site 3	Site 4
2001				
Aluminum Levels	150	121	4	5
Calcium Levels	2312.5	2625	1025	1000
2002				
Aluminum Levels	17	N/A	125	20
Calcium Levels	404.13	N/A	850	1300
2003				
Aluminum Levels	N/A	125	66.67	71.25
Calcium Levels	N/A	362.5	275	1458.33
Protozoa Levels 2002	Quadrat 1: 10051.3 Quadrat 2: 17336.1 Quadrat 3: 7603.9 Quadrat 4: 14590.5		Quadrat 1: 231479.78 Quadrat 2: 138352.46 Quadrat 3: 102889.99 Quadrat 4: 136654.02	

This irregular relationship between the calcium and aluminum then led to our question:

Do the protozoa in site 1 follow the expected pattern with aluminum or calcium?

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Soil Ecology Problem

I. **Problem**: What happens to the protozoa density in certain locations in the RPCS backwoods when the relationship between calcium and aluminum in the soil does not exhibit the normal inverse relation?

II. **Hypothesis**: The relationship between protozoa and aluminum will be as expected while the relationship between calcium and protozoa will be inverse.

III. **Procedure**:

a. Variables

i. Independent variable: location of sample- E.S.S.R.E Microclimate site 1 (North 39.35794 West 076.63618) vs. E.S.S.R.E Microclimate site 3 (North 39.35817 West 076.63900)[1]

ii. Dependent variable1: level of calcium in soil

iii. Dependent variable 2: level of aluminum in soil

iv. Dependent variable 3: density of protozoa in soil

b. Controls

i. Negative control: samples taken from E.S.S.R.E Microclimate site 3 (North 39.35817 West 076.63900)

ii. Controlled variables list: date/time soil collected, amount of soil collected, depth soil sample taken from, method used for extracting protozoa, method used to mix solutions (by hand), method used for measuring time, method used to produce uhlig extractors,

[1] http://faculty/brockda/ESSRE%20Locations.htm. General Description of the E.S.S.R.E. Survey Site 1 (North 39.35794 West 076.63618) and Site 3 (North 39.35817 West 076.63900)

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refrigerator used to place soil samples in suspended animation until ready for chemical testing, amount of universal soil extraction solution used in each chemical test, amount of time for soil and universal soil extraction solution to mix together in soil sample tube, amount of aluminum test chemical, amount of calcium test chemical.

c. Step-by-step

1. Using a soil core sampler (2 cm wide), collect a soil sample 10 cm deep into the soil from 2 different locations (E.S.S.R.E microclimate site 1 located at North 39.35794 West 076.63618 and site 3 located at North 39.35817 West 076.63900)
2. First travel to E.S.S.R.E Microclimate site 1 (North 39.35794 West 076.63618) and place soil core sampler in the soil, push down and collect soil sample.
3. Place each of the 3 soil samples in separate clean plastic bags and label appropriately.
4. Next travel to E.S.S.R.E microclimate site 3 located at (North 39.35817 West 076.63900) and place soil core sampler in the soil, push down and collect soil sample
5. Place each of the 3 soil samples in separate clean plastic bags and label appropriately.
6. Bring each soil sample into the classroom. Empty each plastic bag into a different Petri dish (labeled appropriately) and line up all samples along windowsill. Allow soil samples to dry out completely for twenty four hours.
7. Sift 9 to 10 grams of the soil (after being completely dried for twenty four hours) into a second clean Petri dish using a 1 mm^2 nylon screen or mesh
8. Record number of grams of soil in each soil sample.
9. Use LaMotte STH test kit method to create a universal extraction solution from the samples of dried, sifted soil.
10. Test the extraction solutions for the level of aluminum and the level of calcium using the LaMotte STH test kit.
11. Record results of the level of aluminum and calcium for each of the 6 tests in lab notebook.
12. Test for protozoa.
    a. Add 20 mL of distilled water to the dried samples to saturate the soil.
    b. Cover the Petri dish, and allow to sit for 7 hrs
    c. Place soil sample in a modified Uhlig extractor containing 30 mL of distilled water for 24 hrs
    d. Remove filtrate and filter a second time using 12.5 cm qualitative filter paper

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e. Using a capillary tube, deposit 7 µl of Methyl green stain on a clean microscope slide one µl = 1 drop from the capillary tube. Then using a disposable graduated beral type pipette add 18µl (the first demarcation on the pipette) of the 2nd filtrate from step 14 to the stain on the microscope slide and cover with and 18x18 mm^2 cover slip
f. Examine under a light microscope at 40X the amount of protozoa living in 5 fields of view. Average the count of protozoa into 1 field of view.
g. Use the following equation to determine the population density of protozoa in the soil sample. [(# of protozoa per field of view) x (total mL of 2nd filtrate) x 747] / grams of sifted soil) = # of protozoa per gram of soil.
h. Record results of number of protozoa per gram of soil.

13. Repeat this entire experiment 2 additional times.

IV. **Data and Analysis**:

a. Data tables:

Trial	Sample	Aluminum for Site 1(in ppm)	Aluminum for Site 3 (in ppm)	Calcium for Site 1 (in ppm)	Calcium for Site 3 (in ppm)	# of Protozoa in Site 1 (per 1 gram of soil)	# of Protozoa in Site 3 (per 1 gram of soil)
1	A	125	10	1400	1000	100491.16	84207.27
1	B	80	5	700	1000	65106.95	135413.62
1	C	80	5	700	1000	72436.36	17558.35
2	A	125	10	1400	1400	103969.92	95072.72
2	B	125	10	700	1400	96245.05	52448.94
2	C	80	10	700	1400	103944.26	111583.125
3	A	125	10	700	1400	95978.18	72183.79
3	B	80	5	700	1400	41123.51	34758.37
3	C	125	5	700	1400	128857.5	161538.75
Average	------	105	8	856	1267	89794	84974

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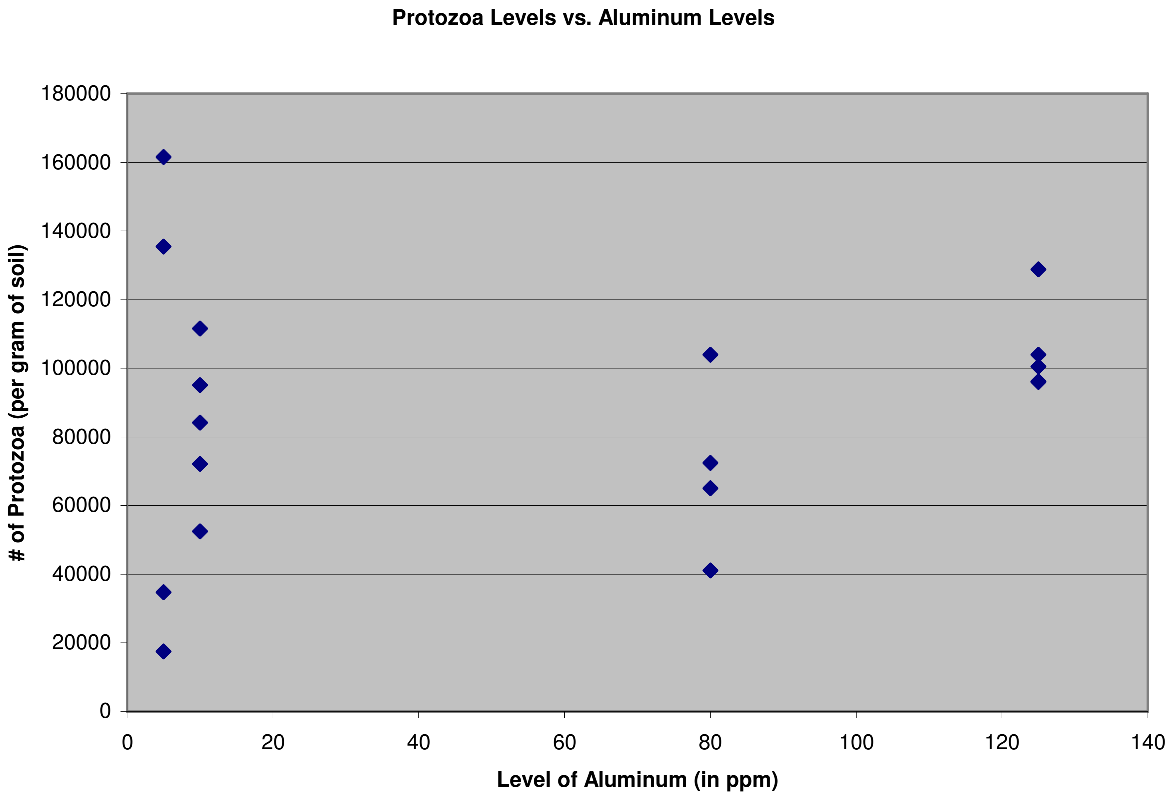
b. Graphs



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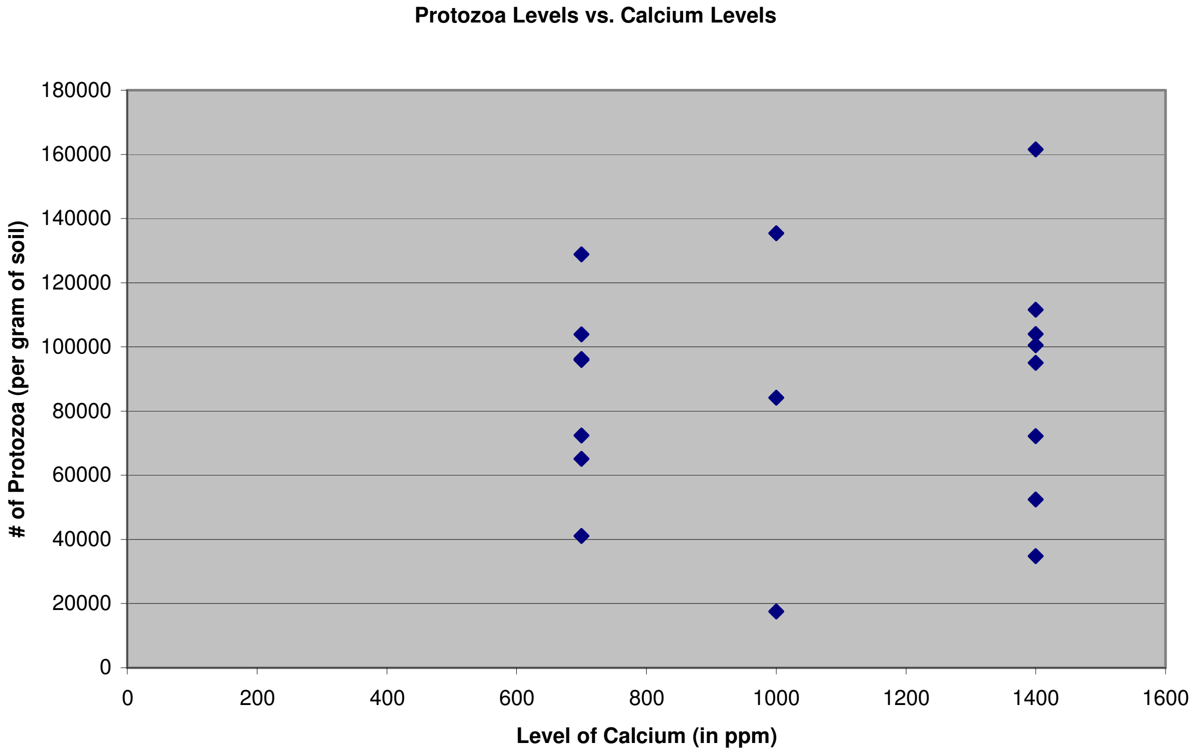

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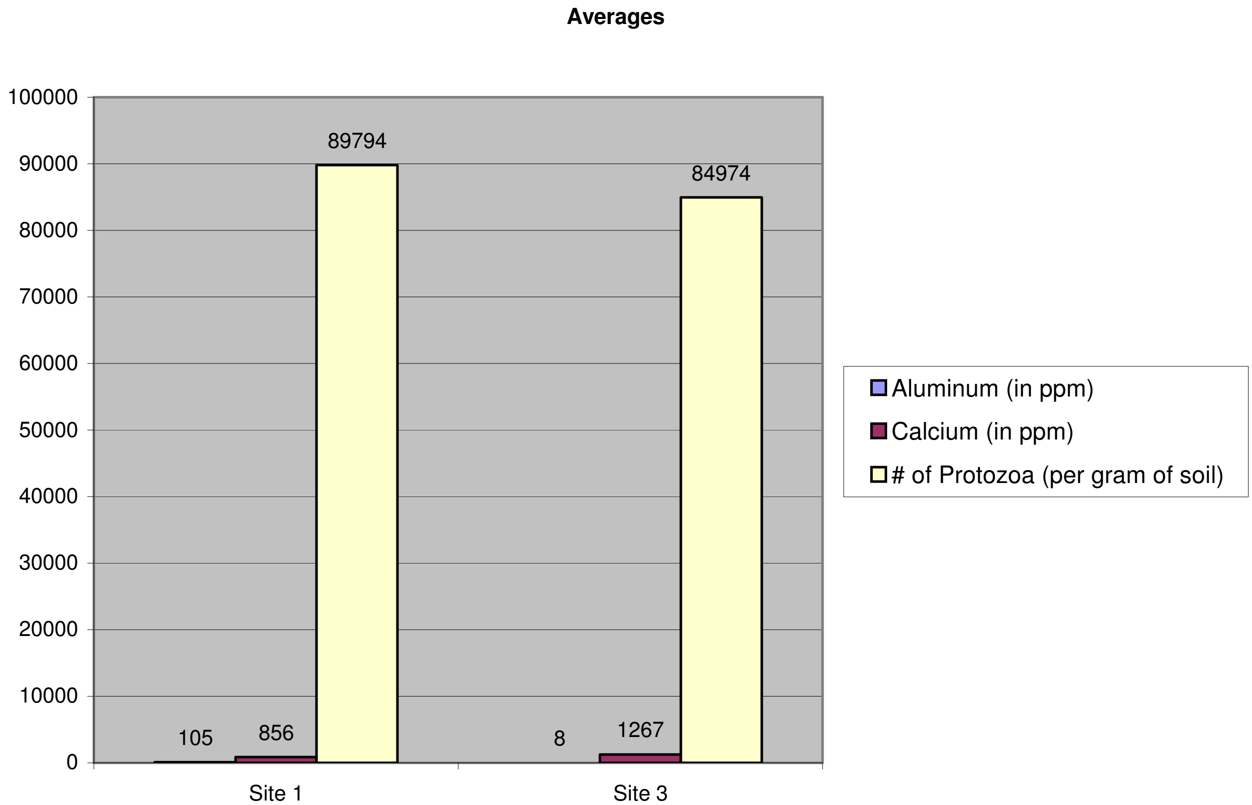

V. **Conclusion**:

In conclusion, our hypothesis was incorrect. In site 1, in comparison to the data from 2002, the aluminum levels rose dramatically as did the calcium levels. In 2002, the average aluminum level in site 1 was 17 and rose to 105 this year. The average calcium level was 404.13 in 2002 and rose to 855.56 this year. We hypothesized that the protozoa would follow their regular pattern with the aluminum and have an inverse relationship with the calcium. In this case, if our hypothesis were correct, the protozoa levels would have dropped as the aluminum levels rose and the calcium levels dropped. However, the protozoa levels also rose, establishing that the relationship between the calcium and protozoa followed the regular pattern and the relationship between protozoa and aluminum was inverse. In 2002 the average protozoa population density per gram of soil was 12,395 and this year, the average population density was 89,794. The level of every component we tested for rose in comparison to 2002, when regularly two should rise and one should drop. This is modeled in site 3, because in comparison to 2002,

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aluminum levels dropped dramatically and calcium and protozoa levels rose dramatically. The aluminum dropped from 125 parts per million to 8 parts per million. The calcium levels rose from 850 to 1267. The protozoa levels decreased, which is not what should have happened in the control site. This could have been because the aluminum in site 3 has a greater effect on the protozoa than the calcium, therefore the protozoa would still decrease as the aluminum decreased, despite the fact that the calcium was rising. The aluminum may have overpowered the calcium, explaining why the protozoa levels do not fit the expected pattern. The number of protozoa changed from an average of 152344 (in 2002) to an average of 84974 per gram of soil. The protozoa levels in site one increased from an average of 12395 (in 2002) to an average of 89794 protozoa per gram of soil. The aluminum level, calcium level, and protozoa level all rose, contradicting our hypothesis stating that the aluminum level would decrease if the calcium and protozoa levels both rose. IN the future, we could research other chemicals in the soil to see what their influence on the protozoa population was. This could explain why the protozoa levels followed an irregular pattern. We could also research the specific type of protozoa in the sites of the backwoods and see how they react with aluminum and calcium.