Soil Ecology Project
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Mr. Brock

The Effect of Fruit Punch Gatorade, Fruit Punch Vitamin Water, & Water on Soil Protozoa Population Density

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Background Information

According to the Soil Science Glossary (2011), "soil is the unconsolidated mineral or organic material on the immediate surface of the Earth that serves as a natural medium for the growth of land plants" (Natural Resources Conservation Services, 2011). It is formed from rocks, decaying plants and animals, protozoa, algae, fungi, and bacteria, and according to Binniger and Allen (2009), an average soil sample is 45% minerals, 25% water, 25% air, and 5% organic matter.

The main components that make up this organic matter are microbes, and one of the most common ones found in soil are protozoa. These single-celled eukaryotes feed primarily on bacteria but can also eat other protozoa, inert organic matter, and sometimes fungi, and this very large, diverse group of micro-organisms is traditionally classified into three main groups based on their shape and mode of motility. The first group are the ciliates, the largest type of protozoa, whose main source of movement is with their hair-like projections on their cell membranes. They are quite different from most protozoa because they eat the other two prominent types of protozoa, as well as bacteria. The second group of protozoa is the amoebae. These protozoa can also be large and move by *"*pseudopod," the means of a temporary "foot," that is a projection of their cell membranes. And the last major group, the flagellates, are the smallest of the protozoa (Hebert, 2012), which use their whip-like "tail" as their main method of locomotion (Natural Resources Conservation Services, 2012).

Regardless of their specific classifications, all soil protozoa share the same common purpose within the environment and live and move in the films of water and water-filled pores that occur throughout the soil. As they swim through this water, they capture their food and other

necessary items that are crucial for their survival (Diaz, 2012), including the most crucial food of all, bacteria. This is important in the soil because when the organic matter decomposes, the nitrogen is transformed by bacteria into ammonium and is absorbed by the plant roots. All of the extra ammonium gets converted by bacteria into nitrate and as protozoa eat bacteria, they release all this excess nitrogen for plants to use as well (Chemistry and Issues in the Environment, 2005). Thus, without protozoa in the soil, plants would not be able to get all the nitrogen they need to make their proteins, which would mean that the cells of the plants would be unable to perform the chemical reactions cells use to complete the four basic tasks of life (reproduction, respiration, homeostasis and synthesis). In other words, without the nitrogen released by the protozoa, plants can die. Therefore, these microbes play a critical role in the affluent life of all soils because without their role in the environment consumers would not be able to be used by plants and other members of the food web because there would be no producers, meaning that all the organisms in an ecosystem could die.

Tangential to this role that protozoa play in the soil is that they regulate bacteria populations. This is beneficial to the soil because when protozoa consume bacteria, they also stimulate the growth of the populace of the bacteria which means that there is more nitrogen which means there is more nutrients for the plants to absorb which makes the ecosystem healthier. Protozoa are an important food source for other soil organisms as well, and they help to defeat disease by competing with or feeding on pathogens (Natural Resources Conservation Services, 2012). This causes the soil food web structure to become heavily dependent and reliant on protozoa. Not only do protozoa eat bacteria and release nitrogen and other nutrients in their waste, but they can also increase the rate of decomposition by maximizing the activity of the

bacteria (Natural Resources Conservation Services, 2012). Soil protozoa are therefore essential to sustaining the soil's ecological balance (Hoorman, 2011).

Part of this balance, though, depends on the availability of water. Water is extremely necessary for soil because it dissolves the salts within the soil to create the solutions carry the food nutrients for plant growth. Soil water also regulates soil temperature; is needed in order for the metabolic activities of microorganisms; and is imperative for the process of photosynthesis (My Agriculture Information Bank, 2011). Therefore, when the ingredients in the various liquids people dump on the soil mix in with it and the organisms that live there, these beverages have the potential to impact the soil environment heavily.

The causes of these various influences are from the different ingredients within the drinks. However, when the ingredients from Fruit Punch Gatorade and Fruit Punch Vitamin Water combine with the H_2O and moisture that keep the protozoa in the soil alive and healthy, the preservatives in these beverages may prove to be harmful. Preservatives are intended to prevent decomposition and therefore are usually harmful to the organisms that decompose materials in the soil. Fruit Punch Vitamin Water has no preservatives, but Red dye 40 is an ingredient found in Fruit Punch Gatorade that when broken down, separates into toluene and benzene. Toluene, also known as methyl benzene, is a colorless, flammable liquid that in small amounts is not harmful to soil because the bacteria there decompose it. But, Benzene is a highly flammable liquid that can contaminate groundwater (Department of Sustainability, Environment, Water, Population and Communities, 2012) and therefor potentially harm soil protozoa. Fruit Punch Gatorade, on the other hand, contains two preservatives known as Sodium Citrate and Monopotassium Phosphate, both of which could hurt the protozoa as well.

Despite these potentially damaging elements in them, Fruit Punch Vitamin Water and Fruit Punch Gatorade are two types of sports drinks that are very popular and often sold here at RPCS in our school store. Because this store is located in a very central and convenient location, numerous students frequently visit it to buy drinks during the course of the school day. Due to the extensiveness of the school, students are mostly allowed to drink these beverages anywhere they would like. When students are done drinking these drinks, they are advised to recycle them in one of the many recycling bins located around the school. Unfortunately, that is not always the case, and many times the left over liquids in these sports drinks are simply poured out onto the various grass areas around the school. In fact, this situation occurs so frequently that we have decided to which liquid will cause a greater change in the population density of soil protozoa in the soil: Fruit Punch Gatorade, Fruit Punch Vitamin Water, or Water. Because soil protozoa rely so heavily on water and areas with high levels of moisture, we predict that while pouring both Fruit Punch Gatorade and Vitamin Water onto the grass, it will change the population of protozoa found in those soils; the addition of Fruit Punch Gatorade to the soil will cause the greatest change in the population density of protozoa in the soil. We think this because Gatorade is the least organic out of the three liquids; therefore it will change the environment of the protozoa the most and cause the most drastic change in their population.

Experiment

I. Problem:

Which liquid causes a greater change in the population density of soil protozoa: Fruit Punch Gatorade, Fruit Punch Vitamin Water, or Water?

II. Hypothesis:

The Fruit Punch Gatorade will cause a greater change in the population density of soil protozoa.

III. Procedure:

a. Independent variable: Type of liquids poured onto soil (Fruit Punch Gatorade, Fruit Punch Vitamin Water or Water)

b. Dependent Variable: Population density of protozoa in the soil

c. Negative Control: Water poured on the soil

d. Positive Control: Population density of protozoa in the soil before adding liquids to it

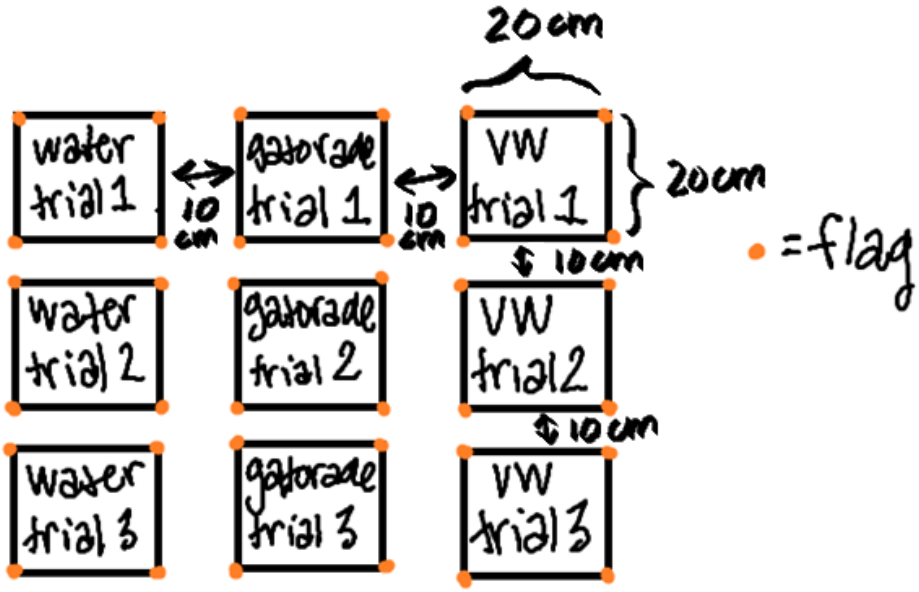
e. Controlled Variables:

- Amount of liquid added to soil
- Extracting all soil samples for a given trial on same day at same time
- Amount of soil taken (15cm deep with a diameter of 2cm into ground from soil auger)
- Temperature of liquids
- Size of plotted land
- Location of plotted soil and plant life there
- Time waited after extracting soil before drying soil

- Amount of space between each plotted section
- Season
- Size of pipette
- Size of nylon screen
- Amount of time allowed for drying
- Amount of time allowed for water to saturate
- Amount of distilled water used to saturate soil
- Size of petri dish
- Size of Nytex © mesh in Uhlig
- Amount of water added to Uhlig extractor
- Amount of time allowed for soil to sit in Uhlig extractor
- Second filtrate filtered at the same time
- Size of microscope slide
- Strength of magnification protozoa observed at
- Type of microscope
- Type of dye
- Amount of dye added to the microscope slide
- Size of cover slip
- Amount of filtrate added to microscope slide

f. Step-by-Step Instructions:

1. Go to the flat plot of grass that is near the parking lot where there is eco-friendly car parking that is located at RPCS and is at N 39.35799˚ and W 076.63544˚
2. Take 36 metal flags and label them. There will be 4 labeled “water trial 1”, 4 labeled “water trial 2”, 4 labeled “water trial 3”, 4 labeled “Gatorade trial 1”, 4 labeled “Gatorade trial 2”, 4 labeled “Gatorade trial 3”, 4 labeled “Vitamin Water trial 1”, 4 labeled :”Vitamin Water trial 2” and 4 labeled “Vitamin Water trial 3”
3. Make nine 20cm by 20cm squares with 10 centimeters between each square (See Diagram Below).
4. Mark each corner of every 20cm by 20cm square by sticking a flag in the ground with the designated liquid and the trial. (See Diagram Below).



5. Collect 9 clear plastic bags and using a marker, label each of them with "Before". Along with that, label three of the bags with "trial 1", three of them with "trial 2" and three of them with "trial 3"
6. On the 3 bags labeled "trial 1" write "water" on one, "Gatorade" on another, and "Vitamin Water" on the last (See Diagram).

7. Repeat step 6 for the trial 2 and trial 3 "Before" bags respectively.
8. Do the following steps, 9- 11, all at the same time on the same day
9. Take all of the bags outside
10. The amount of soil that you will extract is very precise. The amount is 15 centimeter deep of the metal soil auger with a diameter of 2 cm. Take one soil sample from each of the plotted squares from step 4 and place them in their corresponding labeled bags.
11. Take all of the bags back to the lab, and then proceed to do the Protozoa Extraction Test.

12. All at the same time, label nine separate petri dishes with its liquid, trial number, and "Before," and place each 15cm sample of soil into the bottom of its own separate clean, empty, petri dishes labeled corresponding to each of the soil samples taken, and allow it to dry completely for 24 hours.
13. Using a $1mm^2$ nylon screen, sift 9-10 grams of each of the soil samples from step 12 into its own separate new, clean petri dishes, which should again be labeled with the corresponding liquid and trial number as well as "before."
14. All at the same time, add 20 ml of distilled water to each separate petri dish to saturate the soil and cover each petri dish with its lid and allow it to sit for 7 hours.
15. Then all at the same time, place each of the soil samples from step 14 in their own separate modified Uhlig extractor containing 30 ml of distilled water for 24 hours
16. All at the same time, remove the filtrate of each sample and filter it separately a 2^{nd} time using 12.5 cm qualitative filter paper.
17. Complete the following instructions for each of the soil samples in step 16 at the same time, matching each soil sample to its corresponding separately labeled microscope slide
    - Using a capillary tube, deposit 7µl of methyl-green stain on a microscope slide labeled "B trial 1 water" (1 µl=1 drop from the capillary tube).
    - Then, using a disposable graduated Beral-type pipette, add 18 µl (the first demarcation on the pipette) of the 2^{nd} filtrate of "B trial 1 water"

from step 16 to the stain on “B trial 1 water” soil microscope slide and cover it with an 18 x 18 mm^2 cover slip.

- Repeat these instruction for each filtrate

18. Examine each microscope slide under a light microscope at 60X and observe 5 fields of view, one at the upper left corner of the cover slip, one at the upper right corner, one at the bottom left corner, one at the bottom right corner, and one in the middle.
19. Count how many soil protozoa there are in each of those fields of view and record that value.
20. Using the following equation to determine the population density of the protozoa in the soil sample: [(# per field of view at 60X) · (total ml of water used) · 2165] ÷ (grams of sifted soil) = # of protozoa per gram of soil.
21. Record number of soil protozoa in chart
22. All on the same day at the same time, go outside to perform steps 23-25.
23. In the three 20 cm by 20 cm squared plots with labeled flags that read water trial 1, water trial 2, and water trial 3, evenly distribute 150 mL of fresh, room temperature, tap water onto each respective plot with a flag labeled water.
24. In the three 20 cm by 20 cm squared plots with labeled flags that read trial 1 Gatorade, trial 2 Gatorade, and trial 3 Gatorade, evenly distribute 150 mL of fresh, room temperature, fruit punch Gatorade onto each respective plot with a flag labeled Gatorade.
25. In the three 20 cm by 20 cm squared plots with labeled flags that read trial 1 Vitamin Water, trial 2 Vitamin Water, and trial 3 Vitamin Water, evenly

distribute 150 mL amount of fresh, room temperature, fruit punch Vitamin Water onto each respective plot with a flag labeled Vitamin Water.

26. Wait 2 days before using the metal soil auger to take the new soil samples from the soil.
27. Collect 9 more clear plastic bags and using a marker, label each of them with "After". Along with that, label three of the bags with "trial 1", three of them with "trial 2" and three of them with "trial 3"
28. Repeat steps 8-21, completing each step at the same time for each of the new soil samples making sure to label the petri dishes accordingly, and always labeling the materials with "After."

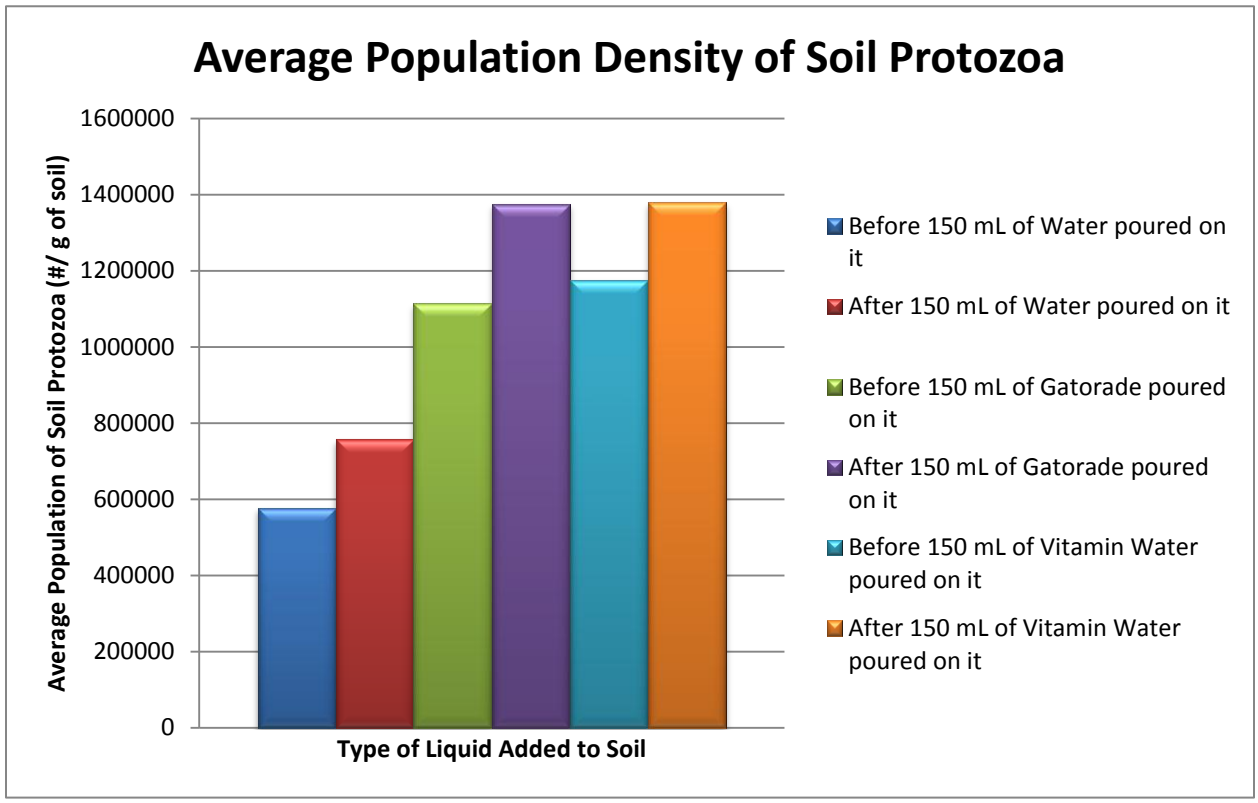
IV. Data and Analysis:

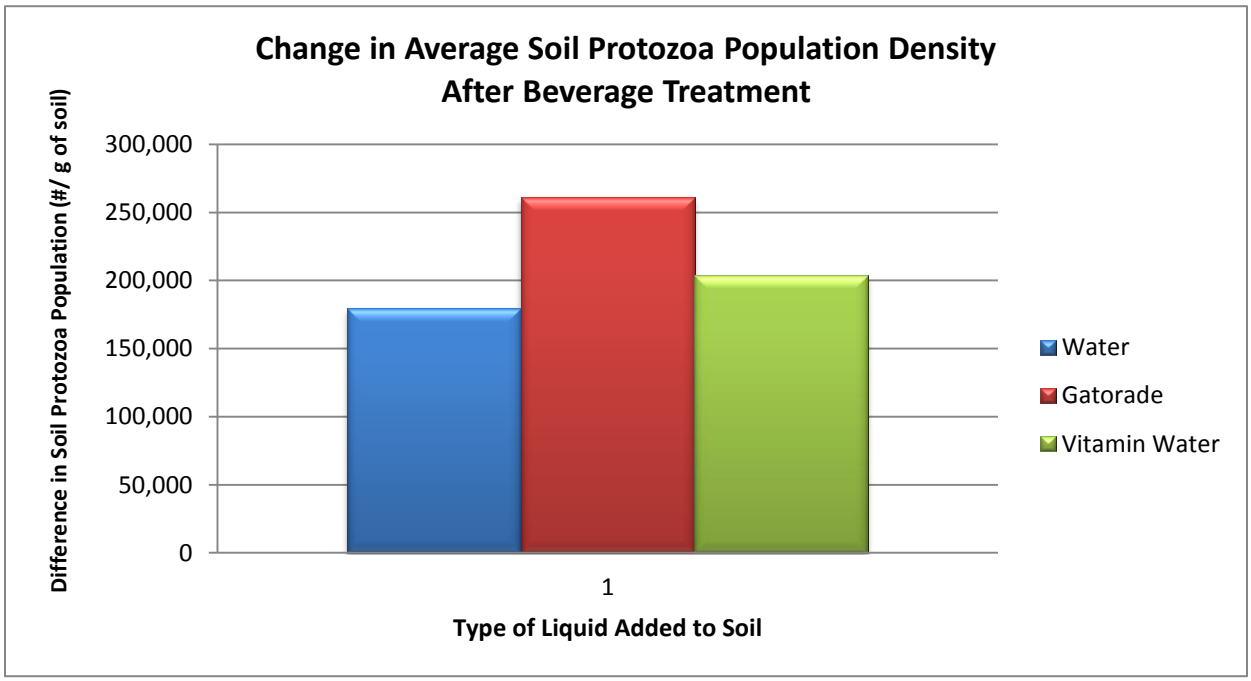
a. Data Table:

Population Density of Soil Protozoa (#/ g of soil)						
Type of Liquid Added to Soil						
	Soil with 150 mL of water poured on it		Soil with 150 mL of Fruit Punch Gatorade poured on it		Soil with 150 mL of Fruit Punch Vitamin Water poured on it	
	Before Application	After Application	Before Application	After Application	Before Application	After Application
Trial 1	611, 434	159,526	1,725,234	932,793	1,937,105	1,194,098
Trial 2	778,047	1,439,495	777, 121	1,734,209	142,146	1,393,431
Trial 3	342,423	672,052	840,665	1,458,526	1,447,132	1,549,684
Average	577,301	757,024	1,114,340	1,375,176	1,175,461	1, ,379,071

Change in Average Soil Protozoa Population Density After Beverage Treatment (#/ g of soil)			
Type of Test	Soil with 150 mL of water poured on it	Soil with 150 mL of Fruit Punch Gatorade poured on it	Soil with 150 mL of Fruit Punch Vitamin Water poured on it
	179,723	260,836	203,610

b. Graph


Average Population Density of Soil Protozoa
Average Population of Soil Protozoa (#/ g of soil)
1600000
1400000
1200000
1000000
800000
600000
400000
200000
0
Type of Liquid Added to Soil
Before 150 mL of Water poured on it
After 150 mL of Water poured on it
Before 150 mL of Gatorade poured on it
After 150 mL of Gatorade poured on it
Before 150 mL of Vitamin Water poured on it
After 150 mL of Vitamin Water poured on it


Change in Average Soil Protozoa Population Density After Beverage Treatment
Difference in Soil Protozoa Population (#/ g of soil)
300,000
250,000
200,000
150,000
100,000
50,000
0
1
Type of Liquid Added to Soil
Water
Gatorade
Vitamin Water

V. Conclusion

Our hypothesis was correct because, as we predicted, out of Fruit Punch Vitamin Water, Fruit Punch Gatorade, and water, the addition of Fruit Punch Gatorade to the soil was the liquid that had the greatest change on the population density of soil protozoa. After completing our experiment, we found that our hypothesis was true because the average change in the soil protozoa population density after the beverage treatment of Fruit Punch Gatorade, was 260,836 protozoa per gram of soil, while after the beverage treatment of water and Fruit Punch Vitamin Water, the average change in the soil protozoa population was 179,723 and 203,610 protozoa per gram of soil, which can be seen in the second graph. This shows that the addition of Fruit Punch Gatorade to the soil caused the most change in the average soil protozoa population because it is the largest number out of the three; therefore it caused the difference of the average soil protozoa population per gram of soil before and after to be the most drastic.

The first graph shows that, on average, each plot of soil with water, Fruit Punch Vitamin Water, and Fruit Punch Gatorade added to it, received a boost that caused their soil protozoa population to increase and was mostly caused from something in the outside environment. As seen in the second graph, the soil with the addition of Fruit Punch Vitamin Water, on average, had 23,887 more protozoa than the soil with water added to it, whereas the soil with the addition of Fruit Punch Gatorade, on average, had 81, 113 more protozoa than the soil with water added to it. Because the difference between the average soil protozoa population density in the soil with Vitamin Water added to it and the soil with water added to it was extremely smaller than the average soil protozoa population density in the soil with Gatorade added to it and the soil with water added to it, we believe that is an indication that some

element within the Fruit Punch Gatorade gave the protozoa population a significant boost. Because of this finding, we believe that the small boost that the soil with water and Fruit Punch Vitamin water did have, was merely because of the amount of water found within them both. This increase probably occurred because protozoa greatly rely on water and moisture to help them move around and survive within the soil, so if there were high amounts of water within those specific plots of soil, it most likely caused the protozoa to become more healthy, therefore able to reproduce more often, causing their population to have a bit of an increase. We also noticed that Fruit Punch Gatorade caused a significantly greater change in protozoa population; therefore we went back and looked at the ingredients within both Fruit Punch Gatorade and Fruit Punch Vitamin Water to observe their differences and specific amounts of various ingredients. We did this as a way to try to figure out what exactly within the Fruit Punch Gatorade was causing this enormous upsurge in protozoa population. Upon examining their ingredient lists, we found that the ingredient that was causing the soil protozoa population to increase by such a great amount was its sugar. This drastic boost most likely had to do with the fact that Fruit Punch Gatorade has 34 total grams of carbohydrates in it and all of them are sugars. Whereas in Fruit Punch Vitamin Water, it has 32 total grams of carbohydrates and only 31 of the grams are sugars, one gram being a more complex carbohydrate. Based on this discovery, for further research, we would investigate whether varying amounts of sugar solutions would affect the population density of soil protozoa in the soil. We would do this by deliberately putting different amounts of sugar solutions into different plots of soil within a specific area. Because the ingredients in Fruit Punch Gatorade and Fruit Punch Vitamin Water are extremely similar, their differences in amounts of sugar would be the only element of the drinks that would be logical to further test. We hypothesize

that the greater amount of sugar solution added to the soil, the more soil protozoa there would be, because there would be more food for the protozoa to eat, therefore they would be able to reproduce more and survive longer.

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