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Impact of Common Beverages on Soil Protozoa

Background

Soil protozoa are single celled organisms that feed directly on bacteria and are therefore commonly found in a zone of soil surrounding the roots (Lines- Kelly, 2005) known as the rhizosphere (Ingham, 2015) where their prey live and grow in abundance (Ypsilantis, 2001). The bacteria live here in large numbers because they interact with the plants in a mutualistic relationship, and though the good bacteria are able to help plants in multiple ways (such as providing and transforming nutrients), too many bacteria can actually inhibit plant growth. Hence, because bacteria outnumber all other microbes in soil, ecosystems need a way to keep the amount of bacteria regulated to ensure healthy plants (Teague, 2010). That is the role of the protozoa: to keep the amount of bacteria regulated by eating them (University of Western Australia, 2004).

Another reason protozoa are often found in the rhizosphere is because moisture is also abundant near the roots of plants, and water is important in the presence and activeness of protozoa. Since the water is absorbed through the roots of plants, protozoa are particularly active when in contact with plant roots (Hoorman, 2011). Furthermore, while some species of protozoa may attack roots and cause diseases, many feed on root pathogens helping to reduce diseases among plants (Ypsilantis, 2001), other protozoa serve primarily as food sources in the soil food web for nematodes (Moravec, 2014) and other small organisms.

The functions and jobs of protozoa make them vital to plant life by keeping the soil ecosystem healthy. One of the key things protozoa do for plants is mineralizing soil nutrients. In order to grow, and be healthy, plants need to absorb their nutrients from the soil through their roots, and two of those primary nutrients, nitrogen and phosphate, are excreted from protozoa as products of their metabolism in the forms of ammonium and orthophosphate. This process of converting

whole or parts of organic matter into a mineral or inorganic material or structure is known as mineralizing.

Nitrogen and Phosphate are both primary macronutrients which plants use for growth and survival (NCDA, 2015), and if plants do not receive these mineralized nutrients from protozoa, and other sources, they will die. In fact, large amounts of phosphate and nitrogen are needed for a plant to survive (Laybourn- Perry, 2014) because without nitrogen, a plant cannot synthesize new material and transfer energy through photosynthesis (NCDA, 2015), and without phosphate for photosynthesis, a plant cannot form the oils, sugars, and starches it needs to grow and transform solar energy into chemical energy (NCDA, 2015). Most importantly, phosphate and nitrogen are essential to the chemical compounds of DNA, RNA, and proteins. Specifically, they are needed for amino acids and nucleotides needed for cells to function. In cells, the DNA is copied into RNA, and the RNA makes proteins, specifically enzymes that start and stop the chemical reactions that cause the four tasks of life to happen (reproduction, synthesis, transforming energy, and homeostasis). Hence, without the phosphate and nitrogen which soil protozoa help provide, the cells of plants (which like all living things are made of cells) cannot complete the four tasks of life and the plants will die. The mass loss of plants would then ruin the ecosystem because the plants are the primary source of food for all the other organisms living there.

Anything that influences the amount of protozoa in the soil influences the viability of the whole ecosystem, and one thing that can change the density of soil protozoa are changed by chemicals. Commonly, beverages are spilled on the ground. Whether this is done on purpose or accidental, the ingredients in the beverages will most likely impact the density of protozoa living in the soil because these ingredients may impact the ability of the protozoa to multiply and

reproduce. For example, Fruit Punch Gatorade contains sugar, dextrose, citric acid, natural and artificial flavors, salt sodium citrate, monopotassium phosphate, calcium silicate, modified corn starch, caramel color, color, and coconut oil (Pepsi Co, 2015); while Pepsi contains carbonated water, gluctose-fructose and/or sugar, caramel color, phosphoric acid, caffeine citric acid, and flavor (Pepsi Co, 2015). Together, all of these ingredients may impact protozoa by causing harm to the soil in which the protozoa grows. The acids, flavors, and chemicals could cause damage to the soil and take away the soil's nutrients, causing the protozoa to no longer have a place to live and grow. Acids, flavors, and chemicals will impact the soil because they are not natural to the soil nor the environment. The New York College of Environmental Science and Forestry states, "The soil pH can also influence plant growth by its effect on activity of beneficial microorganisms. Bacteria that decompose soil organic matter are hindered in strong acid soils. This prevents organic matter from breaking down, resulting in an accumulation of organic matter and the tie up of nutrients, particularly nitrogen, that are held in the organic matter." (Bickelhaupt, 2015).

However, on the other hand, the acids, flavors, and chemicals could allow the soil to become a better environment for the protozoa to grow. Therefore, if the ingredients in beverages spilled on the ground alter the soil's pH in a way that damages bacteria- the food for protozoa- it will harm the protozoa also. The New York College of Environmental Science and Forestry also states, "The effect of soil pH is great on the solubility of minerals or nutrients. Fourteen of the seventeen essential plant nutrients are obtained from the soil. Before a nutrient can be used by plants it must be dissolved in the soil solution. Most minerals and nutrients are more soluble or available in acid soils than in neutral or slightly alkaline soils." (Bickelhaupt, 2015). The pH levels measure acidity and the acidity level must be correct or the enzymes will not function

properly. This important because the enzymes help the chemicals in the soil. The ingredients in the sugary beverages could allow the nutrients to grow stronger at a quicker rate which would let the protozoa live an easier life since it would no longer have to fight to stay alive. The protozoa would be able to live an easier life because they are no longer needed to produce bacteria as rapidly. Bacteria is important because it acts as a food source for many animals and organisms. The animals and organisms that do not eat the bacteria will eat animals or organisms who eat the bacteria. These organisms and animals are important to the ecosystem because they keep everything living. The ingredients could make the nutrients in the soil a better environment for protozoa growth.

The increase or decrease of the protozoa's density depending on the type of beverage will be experimented in the front lawn of Roland Park Country School (RPCS). Roland Park Country School is located in Baltimore, Maryland. The area being tested is in the front of the school with adequate sunlight and water sources to keep the soil alive and healthy. On top of the soil lies a coat of green grass. The soil will be extracted from an area with equal weather influences. The beverages will be poured on and will be soaking on the soil for forty eight hours to ensure the beverages reach and impact the soil. Since protozoa is good for the soil, the purpose of this experiment is to determine whether beverages unnatural to the ground benefit the protozoa density, disadvantage the protozoa density, or allow the protozoa density to remain neutral. Gatorade and Pepsi have a handful of the same ingredients, which might impact the protozoa density in soil.

Lab Outline

I. **Problem**: Does spilling sugary beverages increase or decrease the amount of protozoa in soil?

II. **Hypothesis:** Pepsi will decrease the amount of protozoa in soil, while Gatorade will increase the amount of protozoa in soil.

III. **Procedure:**

a. **Independent Variable**: Pouring Gatorade vs. pouring Pepsi on the soil plot

b. **Dependent Variable:** the increase or decrease of the density of protozoa in soil

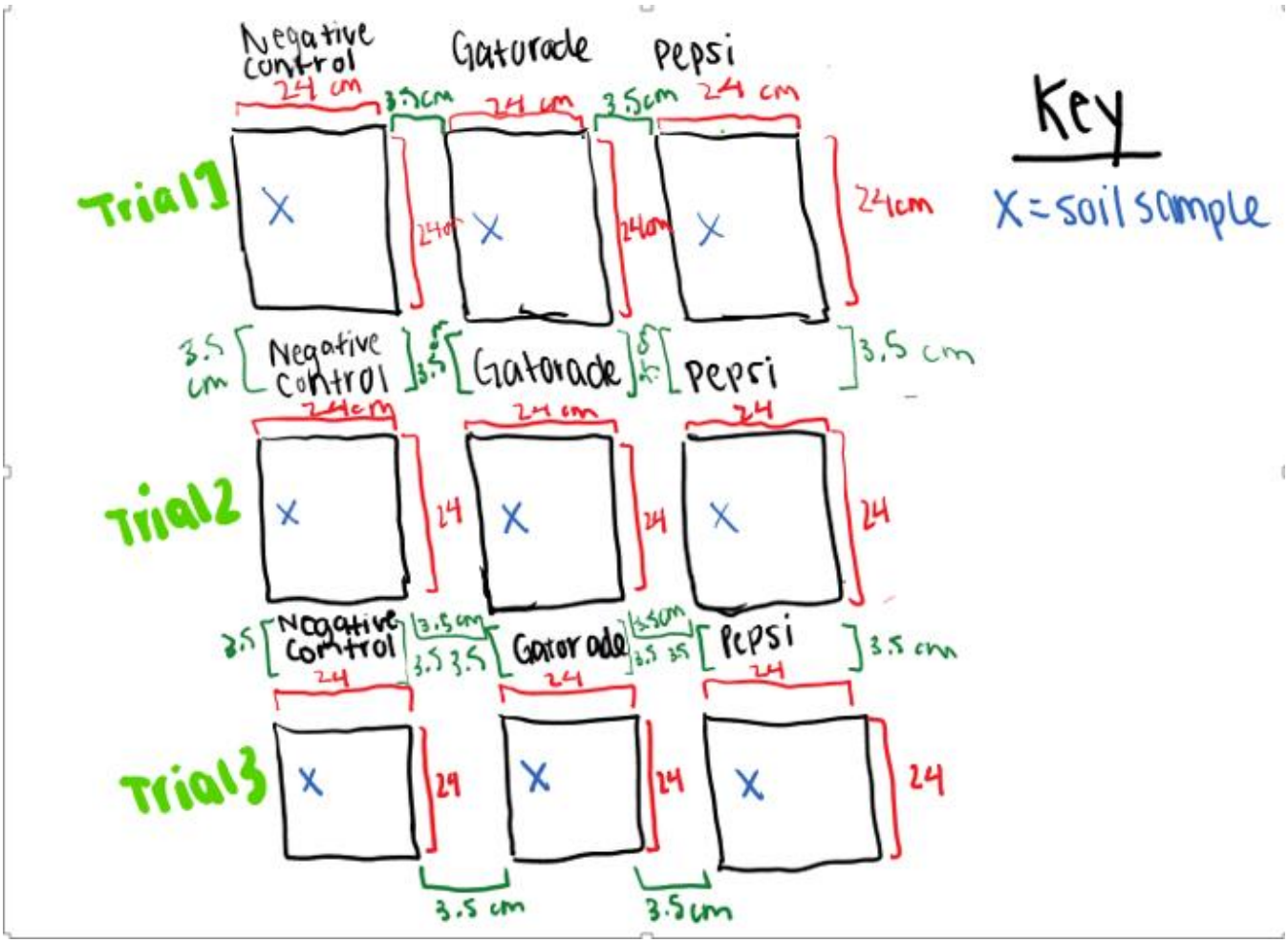
c. **Negative control**: Pouring only water on the soil sample

d. **Controlled Variables:** type of Gatorade, type of Pepsi, type of water, amount of liquid on the soil plot, amount of soil collected, amount of soil tested, temperature of drinks, amount of time allowed for absorption, size of petri dishes, amount of soil sifted, amount of distilled water poured on sifted soil, size and material of screen used for sifting, amount of methyl- green stain, amount of filtrate placed on microscope slide, size of microscope slide, size of cover slip, type of microscope, magnification, equation used for finding amount of protozoa in soil sample, type of pipette used for methyl green stain, size of extractor, number of fields of view counted, amount of time spent being rehydrated, amount of time spent in uhlig extractor, amount of time beverages set into the soil plots, amount of time soil takes to dry out, all samples tested same day same time, all samples taken same day same time

e. **Step by Step Instructions:**

1. Go out to the RPCS front lawn, to N 39 degrees 21.49’ WO 76 degrees 38.163’ location, being sure to mark the location with a GPS.

2. Make 9 24 x 24 cm plots with 3.5 cm in between each plot, separating one from another. 3 plots per trial, each trial consisting of distilled water (negative control), Pepsi (beverage 1), and Gatorade (beverage 2, as shown below:



Make sure to label flags to indicate which plot is for which beverage.

3. Collect one 18 cm deep by 1 cm diameter, vertical soil sample from every one of the plots being used, and place each soil sample into its own plastic bag labeled with which plot it came from, (e.g. “Water 1” for the bag that has the sample from the 1st Negative control Plot, and “Pepsi 2” for the bag that has the sample from the 2nd Pepsi plot, and so on). Make sure all 9 of these samples are all collected on the same day, at the same time.

4. Within the same day as the soil was collected, Place each 18 cm deep by 1 cm diameter sample of soil into the bottom of its own, labelled, clean, empty petri dish. Be sure to label each petri dish with the proper markings for each soil sample to symbolize which plot the soil came from (e.g. “Water 1” for the bag that has the sample from the 1st Negative control Plot, and “Pepsi 2” for the bag that has the sample from the 2nd Pepsi plot, and so on).

5. Still within the same day, place the soil in a spot near sunlight, and allow it to sit for at least 24 hours, allowing it to dry completely.

6. After the 24 hours of waiting have been completed, separately sift 9-10 grams of each dried samples of soil into its own, separate, 2nd clean petri dish using a 1mm squared nylon screen or mesh. Be sure to label these new petri dishes with the corresponding markings that symbolize which plot the soil sample came from.

7. Add 20 ml of distilled water to each sample to saturate the soil. Be sure this step is completed for all soil samples within the same day, at the same time.

8. Cover each petri dish with its own lid to and allow to sit for exactly 7 hours. Be sure this step is completed for all soil samples within the same day, at the same time.

9. Place each rehydrated soil sample in its own, separate modified Uhlig extractor containing 30 ml of distilled water, and allow to sit for exactly 24 hours. Be sure that this step is completed for all soil samples within the same day, at the same time.

10. After the 24 hours of waiting are completed, remove the filtrate from each Uhlig extractor and filter each liquid a 2nd time into their own, clean petri dish using 12.5 cm qualitative filter paper, labelling the petri dish (e.g. “Water 1” for the sample from the 1st Negative control Plot, and “Pepsi 2” for the sample from the 2nd Pepsi plot, and so on). Be sure this step is completed for all soil samples within the same day, at the same time.

11. Using the capillary tube, deposit 7 µl of methyl- green stain on a clean microscope slide (1 µl = 1 drop from the capillary tube). Do this for each of the samples. Be sure that this step is completed for all the soil samples within the same day, at the same time.

12. using a disposable graduated Beral- type pipette, add 18 µl (the first desecration on the pipette) of the 2nd filtrate from step 10 to the stain on the microscope slide and cover with an 18 x 18 mm squared cover slip. Do this for each of the samples on separate microscope slide. Be sure this step is completed for all the soil samples within the same day, at the same time.

13. Examine each sample under a light microscope at 40x power and observe of the various protozoa living in the soil. Adjust accordingly to be able to view the protozoa which will be a slightly darker blue color than of what surrounds it. Be sure this step is completed for all soil samples on the same day, at the same time.

14. Examine the protozoa for each sample in each of the four corners as well as the center of the microscope slip, and then average those five numbers together to get the number per field of view of the population density of the protozoa, which is the number that will be used in the equation.

15. Use the following equation to determine the population density of the protozoa in each soil sample, being sure to keep track of which sample is being calculated:

[(Average # per field of view at 40x) • (total ml of liquid used) • 747] / (grams of sifted soil) = # of protozoa per gram of soil.

16. Record found number of population density of protozoa.

17. Pour 111 ml of Fruit punch Gatorade each onto the plots for beverage one, trial one; beverage one, trial two; beverage one, trial three.

18. Pour 111 ml of Pepsi each onto the plots for beverage two, trial one; beverage two, trial two; beverage two, trial three.

19. Pour 111 ml of distilled water each onto the plots for Negative control, trial one; Negative control, trial two; Negative control, trial three.

20. Let each plot sit for at least 24 hours.

21. After the 24 hours of waiting have been completed, Collect one 18 cm deep by 1 cm diameter, vertical soil sample from each plot, and place each soil sample into its own plastic bag labeled with which plot it came from (e.g. "Water 1"` for the bag that has the sample from the 1st Negative control Plot, and "Pepsi 2" for the bag that has the sample from the 2nd Pepsi plot, and so on). Make sure all 9 of these samples are collected all on the same day, at the same time.

22. For each sample, repeat steps 4- 15, and record the number of population density per gram of soil for each beverage sample.

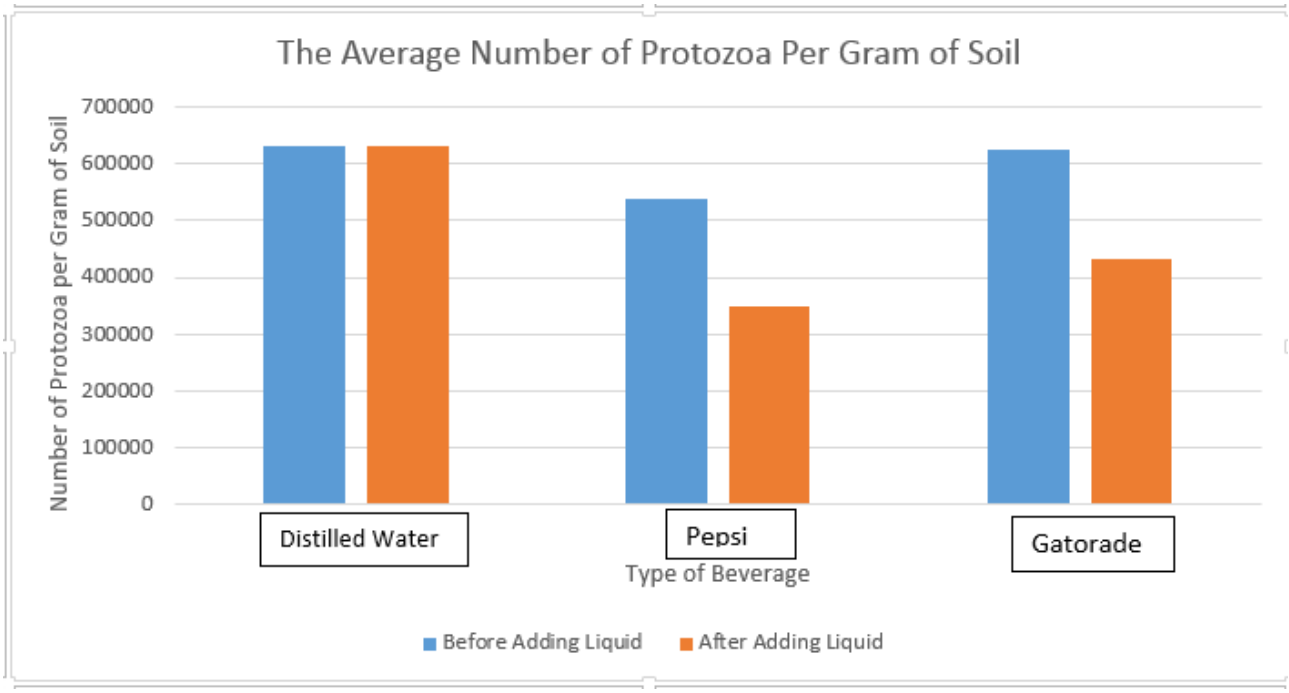
IV. Data and Analysis:

a. Data Table:

The effect of Beverages on the Density of Soil Protozoa

Beverages	**Plots- Negative Control: Distilled Water**		**Plots- Beverage 1: Pepsi**		**Plots- Beverage 2: Fruit Punch Gatorade**	
Trials	*Density of Protozoa per gram of soil Before*	*Density of Protozoa per gram of soil After*	*Density of Protozoa per gram of soil Before*	*Density of Protozoa per gram of soil After*	*Density of Protozoa per gram of soil Before*	*Density of Protozoa per gram of soil After*
Trial 1	696396.7742	655530.612	466096.875	337354.839	453019.3548	574306.452
Trial 2	634556.8421	462061.856	640847.3694	386491.304	591104.3478	482676.923
Trial 3	563061.2903	423808.163	503081.6327	321845.745	830631.5217	239527.174
Average	631338.3023	513800.2103	536675.2924	348563.9627	624918.4081	432170.183

b. Graph



Conclusion

Our Hypothesis States that the pouring of Pepsi will decrease the amount of protozoa in the soil, whereas the pouring of Fruit Punch Gatorade will increase the amount of protozoa in the soil. Our hypothesis was proven to be only half correct, and the data we collected shows that. We completed 3 trials of tests as well as a before and after trial, otherwise known as a positive control, to indicate whether or not the spilling of sugary beverages increases or decreases the amount of protozoa in the soil. We collected samples from our soil plots prior to pouring the three beverages, Fruit Punch Gatorade, Pepsi, and Distilled water, our negative control, onto the plots. We found the amount of protozoa per gram of soil for each plot. The average amount of protozoa per gram of soil for the Negative control (Distilled Water) plots was 631338.3023. The Average amount of protozoa per gram of soil for the Beverage 1 (Pepsi) plot was 536675.2924. The

average amount of protozoa per gram of soil for the Beverage 2 (Gatorade) plot was 624918.4081. After we poured the Pepsi on the Beverage 1 Plots, Fruit Punch Gatorade on the Beverage 2 Plots, and Distilled Water on the Negative Control Plots, we found the amount of protozoa per gram of soil on the new altered soil samples we collected from each plot which we also did three trials of tests. The before and after had to be the same amount of trials in order to have accurate results. The average number of protozoa per gram of soil for the distilled water soil was 513800.2103. The amount of protozoa per gram of soil for the Pepsi soil was 348563.9627. The amount of protozoa per gram of soil in the Fruit Punch Gatorade soil was 432170.183. The Distilled water, with it being our negative control, had hardly any effect on the soil, as shown by the previous data from the soil with no alteration. The Fruit Punch Gatorade had the most impact on the soil, causing the amount of protozoa per gram of soil to decrease by 192748.225 protozoa per gram of soil. The Pepsi caused the amount of protozoa per gram of soil to decrease by 188111.33 protozoa per gram of soil. Both the Pepsi and the Gatorade caused the amount of protozoa per gram of soil to decrease significantly; however it was the Gatorade that decreased the amount of protozoa the most. Therefore, In conclusion, both Fruit Punch Gatorade and Pepsi decreased the amount of protozoa per gram of soil significantly, proving that Gatorade did not increase the amount, but Pepsi did decrease the amount, showing that our hypothesis was not 100% correct. In order for us to make our experiment more effective, changes will need to be made so that the future may have more intricate results. One way to modify this experiment in order for this to happen is to do more trials of tests. More trials give more information, therefore making the data found more accurate. For example if one were to complete 7 trials of tests as opposed to 3

trials, more data would be found on the subject, thus more to work with, thus more accuracy. Another way to modify this experiment would be to use different beverages to pour onto the soil. Beverages that have an extreme contrast in ingredients would provide a possible large difference in results, because we would be able to pinpoint exactly what it was that made the difference. Perhaps Fruit Juice and Milk for example. Similar beverages made by different companies may also provide a different scenario of data found, because of the similarity in ingredients, yet the way they are made would make the difference. Perhaps Pepsi vs. Coke for example. Similar beverages made by the same company may also provide different results, due to the fact that they have almost all the same ingredients, except for the odd one or two that differentiate one from another. Perhaps different flavors in Gatorade for example. Another factor that could contribute to more intricate results could be different kinds of soil to collect samples of, pour the beverages on, and test. A number of trials could be completed with soil from a treated lawn, some from a field where crops grow, some from soil in the woods. This would impact the experiment because the amount of protozoa per gram of soil could differ due to its habitat. If data is collected from different locations, or different types of soil, this could also have an impact on whether or not more results could be found for this particular experiment. Future modifications to this experiment include, more trials completed, different beverages tested, and different locations or types of soil, all of which would impact the experiment to give more intricate data and accurate results.

Citations:

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