

# The Effects of Sports Drinks on the Population Density of Soil Fungi

Mr. Brock- Biology

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We will act honorable.

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### **Background**

Like clean air to breath, humans and all the other living things in ecosystems also need healthy soil to live. They need it because “[soil] provides ecosystems services critical for life...[and] is the basis of our nation’s agroecosystems which provide us with feed, fiber, food, and fuel” (Soil Society of America, 2013). Healthy soil contains an abundance of readily available elements such as carbon, nitrogen, phosphorous, potassium, calcium, and magnesium for the living things to use to complete the four tasks within their cells: respiration, homeostasis, reproduction, and synthesis. Healthy soil also has an arable texture that allows water and air to permeate it. These are both necessary because the water provides a stable environment for living things and their biochemicals to interact and react in and the air enables the organisms living in the soil to transform energy. Hence in healthy soil, all the raw ingredients are in place for the organisms living there to thrive (University of Hawai’i - College of Tropical Agriculture and Human Resources, 2014).

One such group of these organisms are the fungi. These eukaryotic microbes are responsible for decomposition which recycles the necessary chemicals back into the soil after other organisms die. Specifically, fungi complete a process called aerobic decomposition in which they break down raw organic material into compost in the presence of oxygen by releasing acids and undergoing respiration to return carbon back to the environment as carbon dioxide. It is a constant and on-going procedure that occurs whenever soil fungi break down other organic material, and through this process, the fungi

lower the high amount of carbon in the soil, returning it as carbon dioxide to the plants for photosynthesis, and recycle the low amount of nitrogen located there (Texas A&M, 2009).

One of the groups of decomposing fungi that are especially important are those that form the Mycorrhizae. These specialized plant roots form when multiple fungi combine and colonize the roots in order to connect with the plants. Ectomycorrhizal fungi, extracellular, live on the outside of root cells, while endomycorrhizal fungi, intracellular, live inside the roots (The New York Botanical Garden, 2003), but both types of mycorrhizae, exchange materials between the two organisms, allowing them to live together in harmony. In this exchange, the fungi receive carbohydrates from photosynthesis from the plants, using this sugar to grow since they cannot produce it by themselves, and in return, the fungus' hypha increases the plant's ability to absorb water and nutrients, especially phosphorus and nitrogen (Ziegler, 2014).

The reason these two elements are so important (along with the carbon dioxide the fungi release) is because these elements are critical "building blocks" for life that the plant uses for synthesis, energy transformation, and growth. Specifically, nitrogen is important because it is the basic ingredient in the amino acids that make up the protein enzymes needed to start and stop all chemical reactions in living things. Phosphorous is important because its presence is vital in order to make the DNA and RNA that create these proteins, and the carbon is important because it is used to make the basic structures of all of the different types of biological molecules. Hence, without the Mycorrhizal fungi that associate with the various types of plants, such as grass, the base of the food chain (i.e. the producers) in an ecosystem would collapse, killing off the primary, secondary, and tertiary consumers such as grasshoppers, mice, and snakes that need the plants at the base of food for their own biological molecules. The mycorrhizae, therefore, are symbiotic relationships that make life itself possible meaning that when

microbes, such as fungi, in the soil are affected by a change in the ecosystem it impacts the health of the entire ecosystem: including humans.

Unfortunately, one thing that regularly affects soil fungi is pollution, and soil pollution is becoming more and more common as the world population of humans rises (Natural Resource Conservation Service, n.d. ). According to National Digital Science Library (2010), “soil pollution is defined as the build-up in soils of persistent toxic compounds, chemicals, salts, radioactive materials, or disease causing agents, which have adverse effects on plant growth and animal health” (NDSL 2010), and in today’s world, one of the most common types of soil pollution found on school campuses are sport’s drinks. Gatorade and Propel are two versions of these popular drinks, and what makes them potential pollutants is that they both are highly acidic. Living things are very sensitive to acidity because if the acidity of the soil is too high or too low, their enzymes stop working, and as a result, a living thing cannot start or stop the chemical reactions it needs to survive would will die. Normally, fungi grow best in soil with an ideal pH of 5.0 (Hue & Ikawa, 2014). But, Gatorade has a pH of 3.3, and Propel has a pH of 4.0; therefore, it would be expected that the fungal population might decrease in soil if these sports drinks are added to the soil.

However, these sports drinks also contain high amounts of sugar and sodium. Sugar and sodium are known to, “boost the microbial population, thereby speeding up the rate at which nutrients become available” (Hall, 2011). Since Gatorade has 35.492g of sugar and 270.422g of sodium while Propel has 0g of sugar and 194.366g of sodium, one would expect Gatorade might cause more of an increase in the population density of the fungi than the Propel. Hence while the acidity of the drinks may decrease the population of soil fungi, the sugar and sodium might increase the population by providing the fungi with a food source.

In our experiment, we are testing the effect that these two different types of sports drink will have on the soil. All of these liquids are very commonly found in and around the Roland Park Country School's campus, especially outside on the sports fields. RPCS does a very good job of recycling the plastic bottles used on campus, but in order to recycle the bottle, they have to be empty. This leads to humans pouring the liquid remaining in their bottles out on to the soil and potentially damaging the habitat of the fungi and the living things that depend on them. We hypothesized that Gatorade will have the greatest impact on the population density of the soil fungi because, even though the extra acidity might kill some of the fungi living there, the large amount of sugar and sodium will increase the overall fungal population in the soil.

### **Lab Outline**

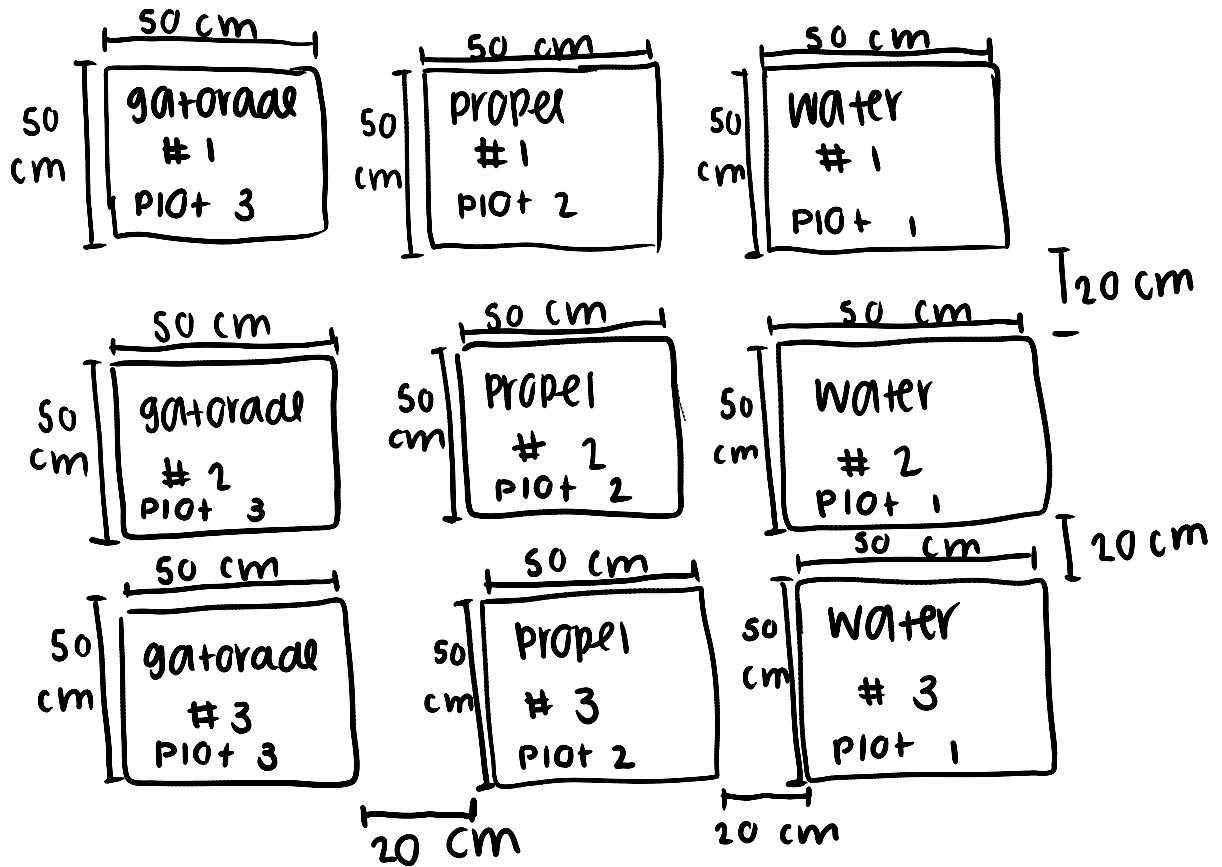
- I. Problem: Between "Fruit Punch" Gatorade and "Berry" Propel, which energy drink will increase the fungi population density in the soil the most?
- II. Hypothesis: Pouring 600 milliliters of "Fruit Punch" Gatorade onto the soil will increase the population density of fungi more than pouring 600 milliliters of "Berry" Propel into the soil.
- III. Procedure:
  - A. Independent Variable – Type of sports drink added to the soil
  - B. Dependent Variable – Population density of fungi in the soil
  - C. Negative Control – Pouring only water into the soil
  - D. Controlled Variables –
    1. Temperature of liquid added to the soil
    2. Amount of liquid added to the soil
    3. Sample sizes of soil taken
    4. Degree to which soil is diluted

5. Amount of soil we test with in a serial dilution
6. Size of plotted land for soil
7. Plant life on top of the soil
8. Amount of time between pouring liquid and collecting soil again
9. Area of land between each plotted land site
10. Amount of time to wait for fungi to increase on nutrient agar
11. Size of test tubes
12. Sterile water
13. Which dilutions are plated
14. Amount of nutrient agar
15. How much of the dilution is plated on nutrient agar
16. Type of nutrient agar

E. Step-by-step –

1. Find a testing area in the soil at N 39° 21.506 and W 76° 38.154, splitting the soil areas up into 9 different sections, each 50 cm. by 50 cm. squares that are 20 cm. apart from one another (See diagram below)
2. Make sure each 50 cm. by 50 cm. section has the same plant life
3. Take 36 metal flags. Take 4 flags and label them: “Gatorade Trial 1, Plot 3”
4. Take four more flags and label them: “Gatorade Trial 2, Plot 3”
5. Take four more flags and label them: “Gatorade Trial 3, plot 3”
6. Take four more flags and label them: “Propel Trial 1, Plot 2”
7. Take four more flags and label them: “Propel Trial 2, Plot 2”
8. Take four more flags and label them: “Propel Trial 3, plot 2”
9. Take four more flags and label them: “Water Trial 1, Plot 1”
10. Take four more flags and label them: “Water Trial 2, Plot 1”
11. Take four more flags and label them: “Water Trial 3, Plot 1”

12. Take each group of 4 flags and set up the plots as shown in the diagram:



13. Take 27 clear plastic bags. Mark each bag with "Before"

14. Take 3 bags and label them: "Trial 1, Plot 3/Gatorade" bags and then 1, 2 and 3 respectively

15. Take 3 bags and label them: "Trial 2, Plot 3/Gatorade" bags and then 1, 2 and 3 respectively

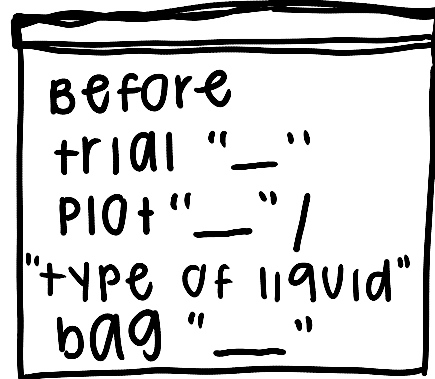
16. Take 3 bags and label them: "Trial 3, Plot 3/Gatorade" bags and then 1, 2 and 3 respectively

17. Take 3 bags and label them: "Trial 1, Plot 2/Propel" bags and then 1, 2 and 3 respectively

18. Take 3 bags and label them: "Trial 2, Plot 2/Propel" bags and then 1, 2 and 3 respectively

19. Take 3 bags and label them: "Trial 3, Plot 2/Propel" bags and then 1, 2 and 3 respectively

20. Take 3 bags and label them: “Trial 1, Plot 1/Water” bags and then 1, 2 and 3 respectively
21. Take 3 bags and label them: “Trial 2, Plot 1/Water” bags and then 1, 2 and 3 respectively
22. Take 3 bags and label them: “Trial 3, Plot 1/Water” bags and then 1, 2 and 3 respectively



23. On the same day at the same time, take three separate soil samples 20 cm. deep into the soil from each plot with the metal soil auger that has a diameter of 2 cm. Place each soil sample into its correspondingly labeled bags.
24. Take all soil samples back into the lab on the same day
25. Complete the serial dilution tests on the same day at the same time as one another. See steps 26-41:
26. For one of the sections label a test tube “ $10^0$  before trial 1 plot 3/Gatorade”, Label a second test tube “ $10^{-1}$  before trial 1 plot 3/Gatorade”, Label the third test tube “ $10^{-2}$  before trial 1 plot 3/Gatorade”; Now having 3 test tubes for the “before trial 1 plot 3/Gatorade”.
27. Use a clean, new transfer pipette to add 10 ml of sterile water to a 15 ml culture tube labeled “ $10^0$  before trial 1 plot 3/Gatorade”.
28. Use the same pipette to add 9 ml of sterile water to a second 15 ml culture tube labeled “ $10^{-1}$  before trail 1 plot 3/Gatorade”.
29. Use the same pipette to add 9 ml of sterile water to a third 15 ml culture tube labeled “ $10^{-2}$  before trail 1 plot 3/Gatorade”.



30. Place 1 cc of your “Before trial 1 plot 3/Gatorade” soil sample into the 15 ml culture test tube “ $10^0$  before trial 1 plot 3/Gatorade” by using a plastic scooper.
31. Cap the tube and shake hard
32. Using a new clean pipette, remove 1 ml of the soil/water mixture from the “ $10^0$  before trial 1 plot 3/Gatorade” tube and place it into the “ $10^{-1}$  before trial 1 Plot 3/Gatorade” tube
33. Cap and shake hard
34. Using the same pipette as in step 32, remove 1 ml of the soil/water mixture from the “ $10^{-1}$  before trial 1 plot 3/Gatorade tube and place it into the “ $10^{-2}$  before trial 1 plot 3/Gatorade tube.
35. Shake the tube making sure all soil has been mixed
36. Label the nutrient agar plates with their respective trial number, plot number and tube degree.
37. Put a cap on the microfilm pipette tip
38. Plate 100  $\mu$ l samples from the 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> tubes ( $10^0$ ,  $10^{-1}$ ,  $10^{-2}$ ) onto their own separate “3M Petrifilm™ Yeast and Mold count plate” using the micropipette
39. Pop off the micropipette tip into sterile water
40. Using the “3M Petrifilm™” disk push down on the nutrient agar plate
41. Repeat steps 25-40 with each “Before” soil sample. Change the label on the tubes and nutrient agar plates to match the corresponding plot and trial number labeled on the sample bag.
42. Allow the fungi to grow for 48-72 hours in a room temperature room.
43. For each nutrient agar plate complete step 44 using a magnifying glass count the number of yeast; look for solid dots on the nutrient agar.
44. Start by looking at the “ $10^{-2}$ ” dilution value. Determine if there is any yeast. If there is record the number of yeast and dilution number. If there is not any yeast move to the next dilution number, “ $10^{-1}$ ” and determine if there is any yeast. If there is record the number of yeast and dilution number. If there is

not, again move to the last dilution number, “10<sup>0</sup>” and determine if there is any yeast. If so, record the number of yeast and dilution number.

45. Repeat step 44 for each nutrient agar plate now looking for mold using magnifying glass; look for fuzzy dots or shapes on the nutrient agar.

46. Use the following formula to calculate the dilution # at which these colonies are found:

$$\# \text{ of yeast or mold in 1 cc of soil} = \# \text{ of Colonies on sheet} \cdot 10^2 \cdot 10^{\text{dilution factor}}$$

47. Record the data in the data table

48. Take 27 more plastic bags. Repeat steps 13-22 for labeling instructions just now instead of “Before” write “After”.

49. On the same day and at the same time go outside to perform the following steps (50-52):

50. In each designated section for “Fruit Punch” Gatorade pour 300 milliliters of room temperature “Fruit Punch” Gatorade per plot

51. In each designated section for “Berry” Propel pour 300 milliliters of room temperature “Berry” Propel per plot

52. In each designated section for water pour 300 milliliters of room temperature water per plot

53. Perform step 54 on the same day at the same time.

54. Wait 6 days before taking three separate soil samples 20 cm. deep into the soil from each plot with the metal soil auger that has a diameter of 2 cm. Place each soil sample into its correspondingly labeled bags.

55. Make sure you are using the “After” bags

56. Take out 27 new 15 ml culture test tubes

57. Repeat steps 24-47 but for each labeling replace “Before” with “After”

### Data Tables

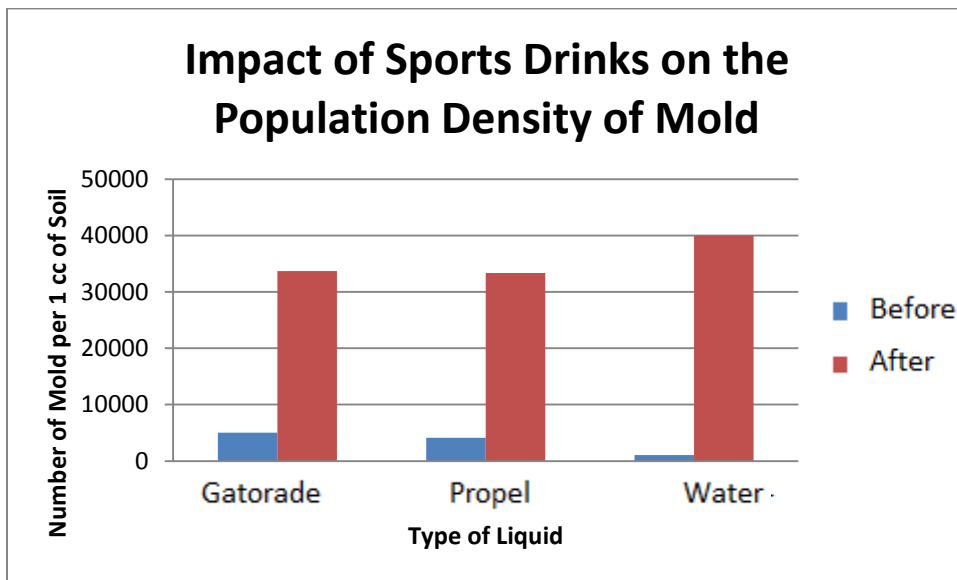
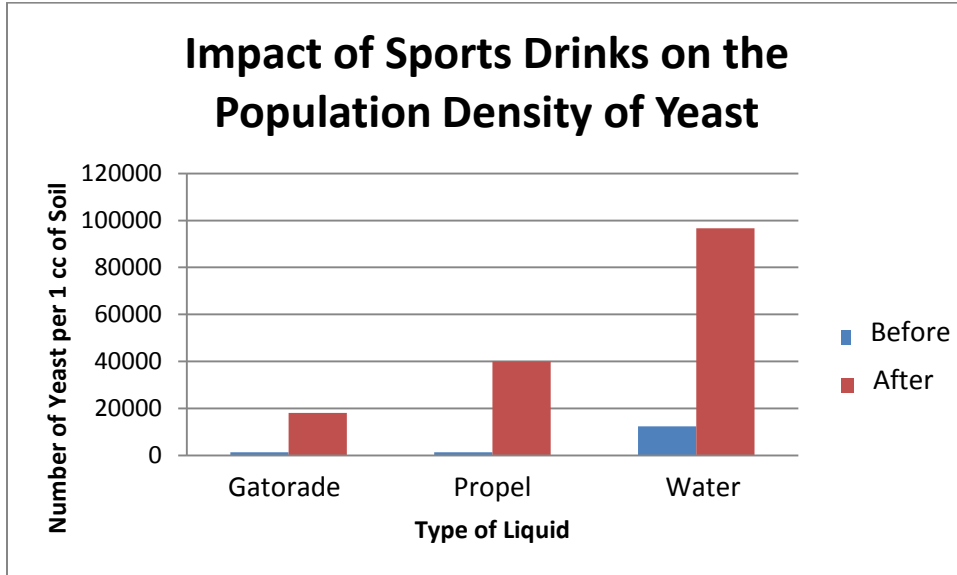
#### Population Density of Fungi in the Soil before Pouring Sports Drinks Into the Soil

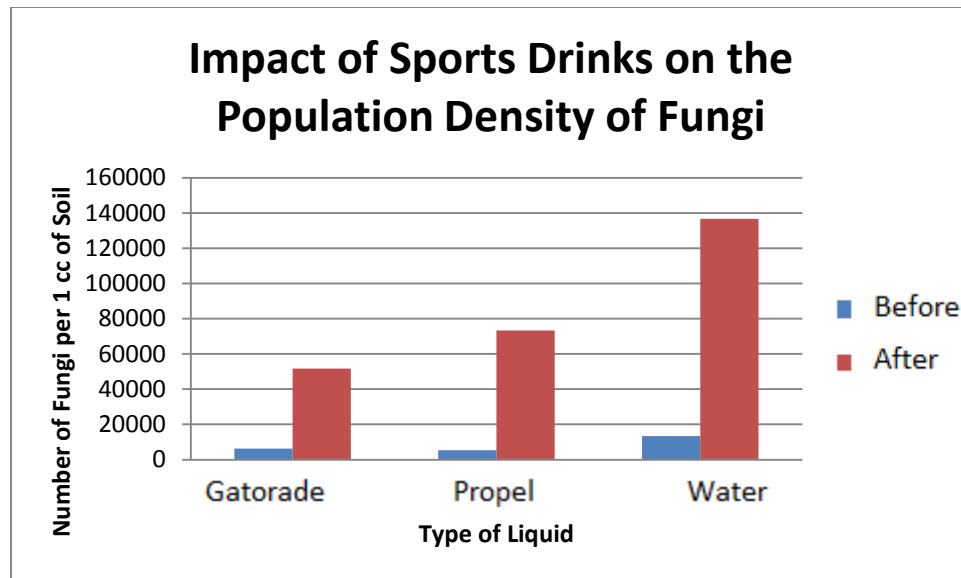
Trial number	Before pouring 600 ml of Gatorade in the soil			Before pouring 600 ml of Propel in the soil			Before pouring 600 ml of Water in the soil		
	# of Yeast/cc	# of Mold/cc	# of total fungi/cc	# of Yeast/cc	# of Mold/cc	# of total fungi/cc	# of Yeast/cc	# of Mold/cc	# of total fungi/cc
Trial 1	2000	4000	6000	2000	10000	12000	7000	100	7100
Trial 2	1000	10000	11000	900	300	1200	20000	2000	22000
Trial 3	900	1000	1900	1000	2000	3000	10000	1000	11000
Average	1300	5000	6300	1300	4100	5400	12333	1033	13367

#### Population Density of Fungi in the Soil after Pouring Sports Drinks Into the Soil

Trial number	After pouring 600 ml of Gatorade in the soil			After pouring 600 ml of Propel in the soil			After pouring 600 ml of Water in the soil		
	# of Yeast/cc	# of Mold/cc	# of total fungi/cc	# of Yeast/cc	# of Mold/cc	# of total fungi/cc	# of Yeast/cc	# of Mold/cc	# of total fungi/cc
Trial 1	20000	60000	80000	10000	30000	40000	100000	50000	150000
Trial 2	30000	40000	70000	60000	40000	100000	170000	40000	210000
Trial 3	4000	1000	5000	50000	30000	80000	20000	30000	50000
Average	18000	33667	51667	40000	33333	73333	96667	40000	13667

## Graphs





#### Conclusion

Our hypothesis “Pouring 600 milliliters of “Fruit Punch” Gatorade onto the soil will increase the population density of fungi more than pouring 600 milliliters of “Berry” Propel into the soil” was proven incorrect. In our experiment, the sports drink “Fruit Punch” Gatorade had the least increase of total fungi in the soil in comparison to the sports drink “Berry” Propel and water. We found that after pouring 600 mL of the sports drink “Fruit Punch” Gatorade onto the soil, the average population density of the fungi in 1 cc of soil grew by 45,366.67. After pouring 600 mL of the sports drink “Berry” Propel onto the soil, the average population density of the fungi in 1 cc of soil grew by 67,933.33. After pouring 600 mL of water onto the soil, the average population density of the fungi in 1 cc of soil grew by 123,300. Because of the recognizable growth in all of the fungi, we looked at the before and after ratios with the negative control plot, which tested for water. The ratios compared the number of yeast per 1 cc and number of mold per 1 cc. The ratio of before yeast and mold was 12,333.33yeast:1,033.33mold, and the ratio of after yeast and mold per 1 cc was 96,666.67yeast:40,000mold. These proportions show

that the number of yeast in the environment was always greater than the number of mold; therefore, the location that we performed our tests on had soil that is challenging for fungi to grow in. Regardless of the harsh environment, there was an environmental factor between the time that we collected our before samples and the time we collected our after samples that caused both the population density of yeast and the population density of mold to increase because the fungi was able to grow and reproduce. Based on our data, the sports drinks had little to no influence on the mold, and we suspect that statistical analysis, such as p values, would show the closeness in population density of the mold with Gatorade, Propel, and water.

When analyzing the population density of the yeast, the presence 600 mL of water in the soil cause the population density of the yeast in 1 cc of soil to increase by 84,333.333. The increase in population density of the yeast that were exposed to water was much larger than the population of the yeast that were exposed to Propel or Gatorade. After pouring 600 mL of Gatorade onto the soil, the average population density of the yeast in 1 cc of soil grew by 16,700, and after pouring 600 mL of Propel onto the soil, the average population density of the yeast in 1 cc of soil grew by 38,700. While the positive environmental factor was still present (allowing growth and reproduction), it is obvious that the Gatorade and Propel were inhibiting the yeasts' ability to grow and reproduce because the population growth of the Gatorade and Propel were much smaller than the population growth of the water. The most threatening sports drink was the Gatorade because some factor within the drink was preventing the population growth of the yeast. We predict that pH is the factor that is inhibiting the growth of the yeast because both Gatorade and Propel have high acidities, but Gatorade has a lower pH which is causing a lesser population increase.

Given that Gatorade has a pH of 3.3 and Propel has a pH of 4.0, it is logical for us to predict that the acidity of the drinks is harmful to the yeast. Therefore, in future research, we would create three solutions. The first solution, our negative control, would be water that has a pH of 7.0. The second solution would be water that resembles the Gatorade and has the same pH of 3.3 without all the ingredients in the sports drink. The third solution would be water that resembles the Propel and has the same pH of 4.0 without all the ingredients in the sports drink. By conducting this experiment to find further research on this topic, we would be able to either confirm or eliminate pH as the factor in Gatorade and Propel that inhibits the growth of yeast.

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