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Background

Liquid pollution occurs daily, and even though it is normally associated with bodies of water like lakes and streams, it can also occur on the land when people dump out their left over beverages such as soda. Since soil normally receives its water from rainfall, adding unnatural substances such as these processed soft drinks onto the soil can cause changes in the survival rate of the microorganisms living in the soil. These diverse microbes, like fungi, bacteria, and protozoa, are essential to the soil, and fungi, in particular "perform important services related to water dynamics, nutrient cycling, and disease suppression" (Ingham, n.d). As heterophic eukaryotes, they feed off of dead plants and animals through the process of decomposition (enabling them to reproduce and multiply), and in return, they release some of the decomposed nutrients back in the soil for living plants to use.

While in general, fungi cannot survive on their own without sugar and water. Water is one of the five biological molecules (along with proteins, carbohydrates, nucleic acids, and lipids), and it provides the chemical environment for the chemical reactions enabling a cell to function. Any cell that does not perform these chemical reactions can no longer survive, hence, without water fungi cannot survive and reproduce. Sugar, too, is critical to fungal survival because it is a pure energy source that help fungi transform energy (a task fungi cannot function without). Thus, sugar and water are both essential to the chemical reactions of fungal cells, and without them, there would be a problem for soil ecologically because fungi would not survive and the decomposition of organic matter in the soil, so critical to plant survival, would slow down dramatically.

This dramatic outcome could result from the common human activity of pouring liquids onto the ground. Two liquids commonly dumped out onto the ground at the RPCS campus are regular coke and diet coke. Of course, the most obvious difference between Regular and Diet coke is the sugar content. Regular coke contains high fructose corn syrup while Diet coke contains a sugar substitute, aspartame. Therefore, due to the high sugar content in Coke, the fungi, which need sugar, might enjoy the coke and it might act as a major nutrient supplier (Coca-Cola - Nutrition Connection - Products - Coca-Cola. 2009). But, Diet Coke would not because of the aspartame.

In addition to sweeteners, each of these drinks contains the ingredient potassium benzoate that could have a negative effect on the microbes such as fungi because this potassium salt of the benzoic acid is considered a food preservative that inhibits the growth of mold, yeast, and bacteria. As a preservative, it can stop the growth of these microorganisms and so dumping either regular or diet coke might harm soil fungi. But since this salt is only effective and works best in products and places with low pH levels, the chance of this ingredient stunting the growth of fungi is very low except in soils with low pH levels(Google, n.d.)

Since benzoic acid works best in places where this a low pH level, there are ingredients in these drinks that are very acidic. For example, both coke and diet coke contain carbonated water and phosphoric acid (Coca-Cola - Nutrition Connection - Products - Coca-Cola. 2009 and

Diet Coke Ingredients: Nutritional Information, Artificial Sweeteners, Health Concerns. n.d.) and therefore like all acids, they contain an excess of H^+ ions (Chem4Kids.com: Reactions: Acids and Bases). Since the concentration of H+ ions cannot go over 25% without leading to harmful chemical reactions (Carter, D., Legget, G., & Robbins, C), adding sodas that contain phosphoric acid in addition to the usual carbonic acid could prove problematic for the microbes living there and it might be an even bigger issue for them given that potassium benzoate works more effectively to stunt their growth in areas of low pH level.

Therefore, pouring regular and diet coke onto the ground could be especially dangerous because they have the potential to severely reduce the pH levels in the soil. The reason why pH levels are so important in the environment is because every living thing has an optimal pH level (Campbell, N. Reece, J. Urry, L. Cain, M. Wasserman, S. Minorsky,P. Jackson, R. 2008). When all living things remain in their optimal pH level, then the enzymes controlling their chemical reactions are able to function to generate the four tasks of life: reproduction, respiration, homeostasis, and transformation of energy. With the four tasks happening, the cells in the fungi live. But when the acidity level surrounding enzymes changes, there is a possibility that the pH level would be changed to a point where those enzymes could not perform their job, meaning that chemical reactions could not happen, preventing the four tasks from occurring. And If the four tasks do not happen, then the fungi in the soil die, with all the consequences that would have on the environment.

One of them is the synergistic effect. A synergistic effect is when one organism is affected by another. This means if pouring the acid compounds from the coke and diet coke into the soil harms the fungi, then other microorganisms that work with the fungi will also be harmed.

For example, instead of fungi and bacteria working together to decompose dead plants and other materials, bacteria might have to do the work alone. Hence, if any microorganisms are harmed in the process of pouring these chemicals onto the soil a chain reaction will happen (Campbell, N. Reece, J. Urry, L. Cain, M. Wasserman, S. Minorsky,P. Jackson, R. 2008).

Interestingly enough, there is a natural way the acidity level of soil changes that deals with fungi. This is when fungi decompose dead leaves and trees. Fungi produce acid as a result of this process, and so there is a major possibility that when coke and diet coke are poured onto the soil and the fungi gets in contact with Carbonated Water, Phosphoric Acid, and Citric acid, that these acids might make the environment more conducive for fungi to reproduce. In fact without fungi, citric acid would not exist (Fungi definition of Fungi in the Free Online Encyclopedia, n.d).

In our RPCS community this awful habit of emptying liquids has formed. Instead of pouring liquids down the drain to be properly disposed, they are dumped onto the ground. This needs to be prevented because we believe that adding these drinks could sway between positive and negative results. We have predicted that Diet Coke will destroy the soil fungi more than Regular Coke will. There is even a possibility that pouring Regular Coke could lead to more soil fungi since it contains sugar, the major nutrient. But it is pretty clear that since adding Diet Coke should change the pH level of the soil, population of the soil fungi should go down.

Experiment Procedures: Coke vs. Diet Coke

I. Question: Between Diet Coke and Coke, which liquid will decrease the density of fungi the most?

II. Hypothesis: Pouring 110 milliliters of diet coke onto the soil will decrease the density of fungi more than pouring 110 milliliters of regular coke onto the soil.

III. Procedure

A. Independent Variable : Varying pouring diet coke vs. pouring regular coke onto soil

B. Dependent Variable: Number of microbes of fungi per cm^3 in the soil

C. Negative Control: Pouring only water onto soil

D. Controlled Variables:

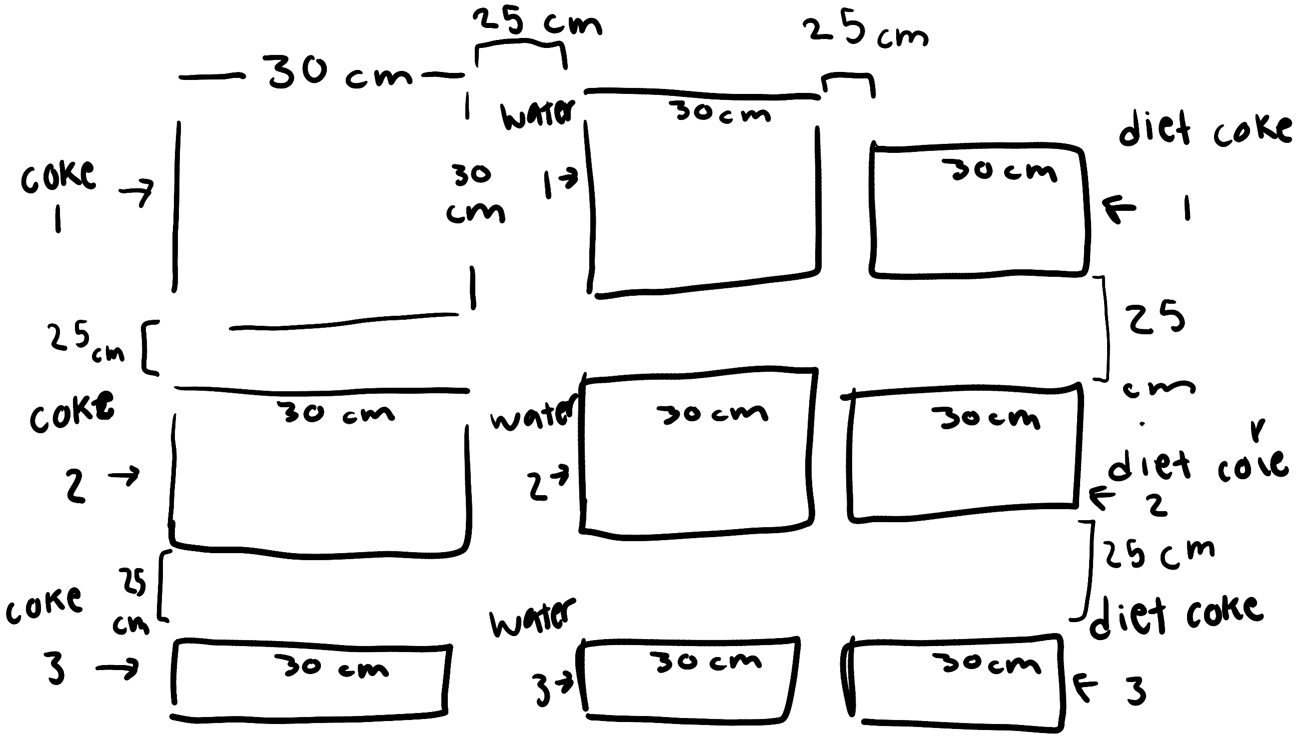
1. Amount of liquid added to soil
2. Extracting soil samples on same day at same time
3. Amount of soil taken (15cm deep with a diameter of 2cm into ground from soil auger)
4. Temperature of liquids
5. Absence of bubbles from the liquids
6. Plant life on the soil
7. Amount of water added to the soil for dilution purposes
8. Amount of soil diluted
9. Size of plotted land
10. Location of plotted soil
11. Time waited after extracting soil before performing soil test
12. Sterile Water
13. Amount Space between each plotted section
14. Time waited for fungi to grow on petri film
15. Time wait to extract soil samples after pouring liquid
16. Type of nutrient agar
17. Amount of water/soil solution plated onto petri film
18. Degree of dilutions plated
19. Size of test tubes

E. Step by Step:

1.) Designate a testing area in soil at N 39.357^0 W 76.635^0, making sure that each plot has the same exact type of plant life covering it.

2.) Take 36 metal flags. Using a marker, Label 4 flags, “Water 1”. Label 4, “Coke 1”. Label 4, “Diet Coke 1”. Label 4, “Water 2”. Label 4, “Coke 2”. Label 4, “Diet Coke 2”. Label 4, “Water 3”. Label 4, “Coke 3”. Label 4, “Diet Coke 3”.

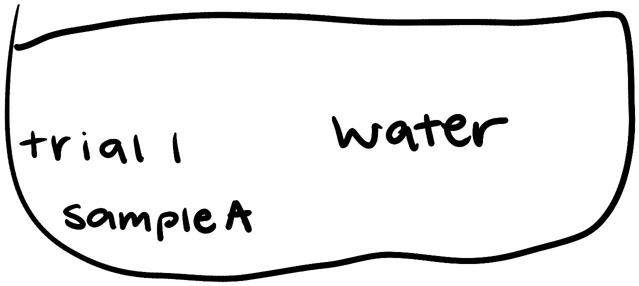
3.) Using the grouped flags, make 9 30cm by 30 cm squares. Create each section using four flags so that each square is 25 cm apart from each other. (See diagram below)



4.) Gather 18 plastic clear plastic bags. Using a marker, write “Before” on all of them. Then, label 6 with the words “trial 1”. Another 6 with the words “trial 2”. The last 6 with the words “trial 3”.

5.) On the 6 with “trial 1” have 3 with the words “sample A” and on the other three” sample B”. On the sample A bags write “coke” on one, “diet coke” on another, and “water” on the last. On the sample B bags write “coke” on one, “diet coke” on another, and “water” on the last. For example:

Before



6.) Repeat step 5 with the trial 2 and trial 3 bags.

7.) Take all bags outside on the same day.

8.) The amount of soil that should be extracted is 15cm deep of the metal soil auger with a diameter of 2 cm. Extract two soil samples from each section at the same time on the same day and place each separate the soil sample into its corresponding bag.

9.) Take all soil samples back to lab on the same day at the same time to do Serial Fungi Dilution Test.

10.) Make sure for the following steps (11-30) that they are all done on the same day at the same time.

11.) Gather 27 15 ml culture tubes.

12.) Divide the 15 ml culture tubes into 9 sections, each having three tubes.

13.) For one section , Label one test tube “10^{0} before trial 1 sample A water”. Label a second tube “10^{-1} before trial 1 sample A water” . Label the third tube “10^{-2} before trial 1 sample A water”. You should now have three test tubes for the “before trial 1 sample A water” bag.

14.) Use a clean, new transfer pipette to add 10 ml of sterile water to the “10^{0}” 15 ml culture tube labeled “10^{0} before trial 1 sample A water”.

15.) Use the same transfer pipette to add 9 ml of sterile water to the “10^{-1}” 15 ml culture tube labeled “10^{-1} before trial 1 sample A water”.

16.) Repeat step 14 one more time to the “10^{-2}” 15 ml culture tube labeled “10^{-2} before trial 1 sample A water”.

17.) Using the plastic scooper, Place 1 cc of the soil from the bag labeled “Before trial 1 sample A water” into the “10^0” culture tube.

18.) Cap the test tube and shake vigorously.

19.) Using a new clean pipette, remove 1 ml of the soil/water mixture from the “10^0” tube labeled “10^0 before trial 1 sample A water” and placed into the “10^{-1}” tube labeled “10^{-1} before trial 1 sample A water”.

20.) Cap and shake vigorously.

21.) Using the a new transfer pipette, remove 1 ml of the soil/water mixture from the “10^{-1}” tube and place into the “10^{-2}” tube labeled “10^{-2} before trial 1 sample A water”.

22.) Cap test tube and shake vigorously till soil has been thoroughly mixed.

23.) Plate separate 100 µl samples from the second and third tubes (tubes labeled 10^{-1} and 10^{-2}) onto their own separate, correspondingly labeled “3M Petrifilm™ Yeast and Mold Count Plate”

24.) Repeat steps 12-23 for all remaining sections using the same labeling system making sure that all samples go in their corresponding labeled tubes and Petrifilm™ plates.

25.) Wait 144 hours before examining each of the petri films for individual fungi.

26.) In the following steps 27-30 make sure they are performed on the same day at the same time.

27.) Choose the petri film with the lowest dilution value and examine for yeast and mold.

28.) While examining, know the solid dots are yeast and the fuzzy shapes are mold.

29.) To count the fungi first look for yeast and mold using a magnifying glass.

30.) Record the number of yeast on it, the number of mold on it, and the film’s dilution value.

31.) If none or either yeast or mold are not found on the lowest dilution value move up to the next lowest dilution value and repeat step 27.

32.) Repeat the counting of yeast and mold described in steps 27-29 until all petri film are all counted for.

33.) Use the following formula to calculate the amount of fungi in each soil test.

a. # Microbes in 1 cc of soil= # Colonies on sheet x 10^2 x $10^{|\text{dilution \# at which these colonies were found}|}$

34.) Record data in chart.

35.) Take 36 more plastic bags. Using a marker, write “After” on all of them. Then repeat the labeling instructions stated in step 4-6.

36.) On the same day at the same time go outside to perform steps 37-42

37.) In the row of three 30cm by 30 cm squares designated for Diet Coke, evenly distribute 110 milliliters of fresh, room temperature, flat Diet Coke onto each plot with a “diet coke” on the flag.

38.) In the row of three 30cm by 30 cm squares designated for Coke, evenly distribute 110 milliliters of fresh, room temperature Regular Coke into each plot with the “coke” written on the flag.

39.) In the row of three 30cm by 30cm squares designated for water, evenly distribute 110 milliliters of room temperature tap water into each plot with “water” written on the flag.

40.) Wait 72 hours before using the metal soil auger to take the new soil samples from the ground.

41.) Repeat steps 8-9. Be careful to insure you are using the bags labeled “after”.

42.) Take out 36 test tubes.

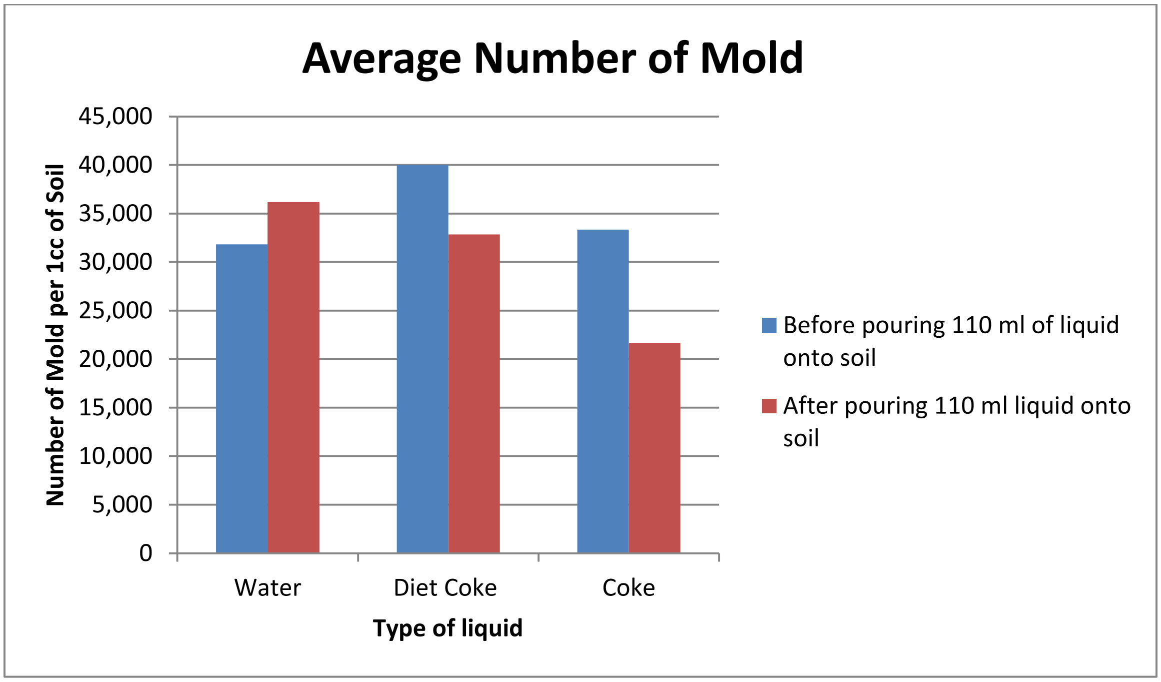
43.) Repeat steps 10-35 for each bag following the same labeling fashion only changing “before” to “after”. Also use the same counting system described as well.

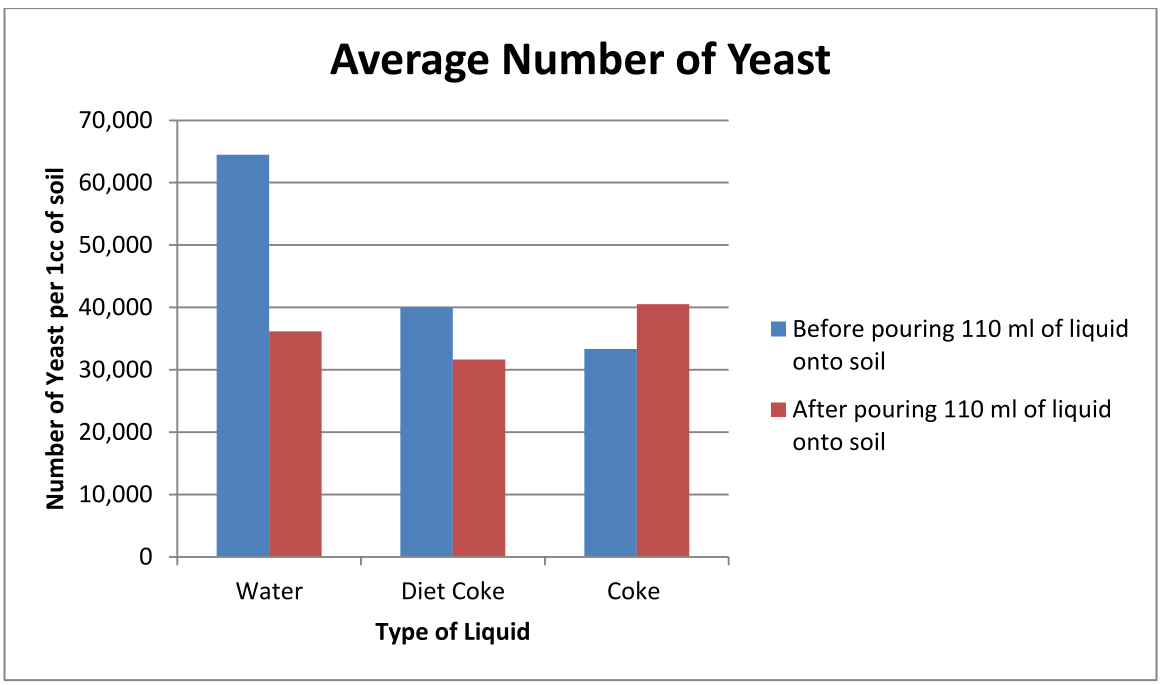
Data Tables

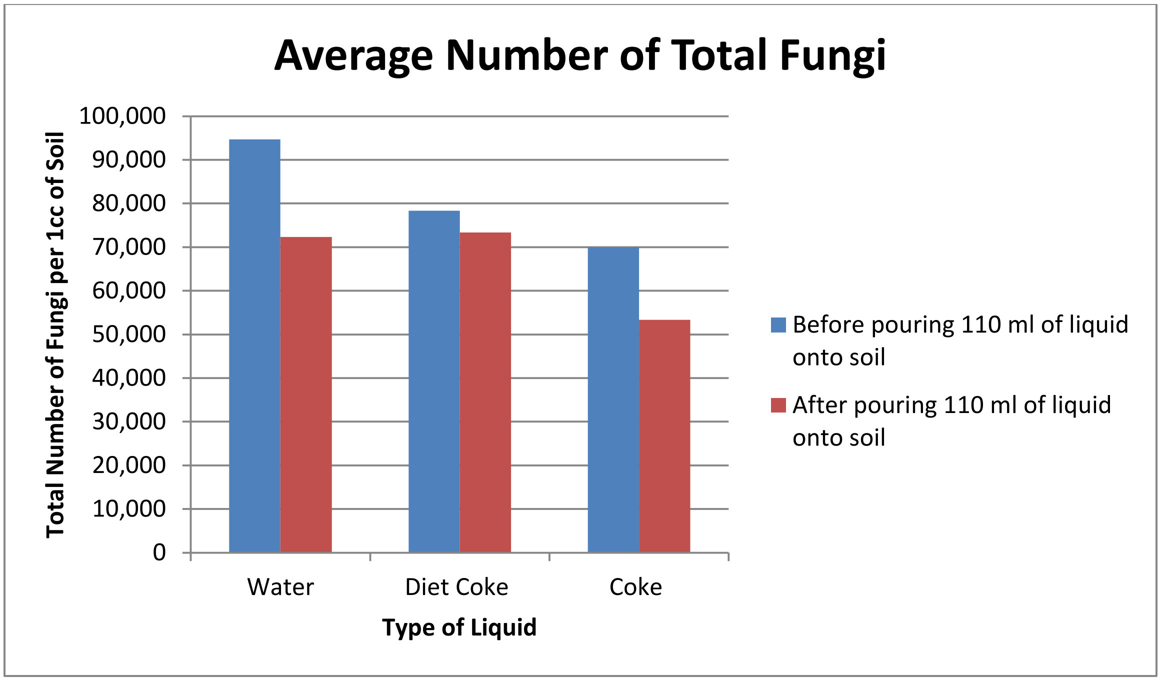
Quantity of Soil Fungi before pouring the liquids into the soil									
Type of test	Pouring 110 milliliters of Water			Pouring 110 milliliters of Coke			Pouring 110 milliliters of Diet Coke		
Type of Fungi	Number of Yeast per 1cc of soil	Number of Mold per 1cc of soil	Total number of all fungi per 1cc of soil	Number of Yeast per 1cc of soil	Number of Mold per 1cc of soil	Total Number of all fungi per 1cc of soil	Number of Yeast per 1cc of soil	Number of Mold per 1cc of soil	Total Number of all fungi per 1cc of soil
Trial 1	Sample A: 20,000	Sample A: 30,000	Sample A: 50,000	Sample A: 40,000	Sample A: 70,000	Sample A: 110,000	Sample A: 50,000	Sample A: 40,000	Sample A: 90,000
	Sample B: 280, 000	Sample B: 90,000	Sample B: 360,000	Sample B: 10,000	Sample B: 10,000	Sample B: 20,000	Sample B: 70,00	Sample B: 50,000	Sample B: 120,000
Trial 2	Sample A: 12,000	Sample A: 8,000	Sample A: 20,000	Sample A: 60,000	Sample A: 20,000	Sample A: 80,000	Sample A: 30,000	Sample A: 30,000	Sample A: 60,000
	Sample B: 5,000	Sample B: 3,000	Sample B: 8,000	Sample B: 30,000	Sample B: 30,000	Sample B: 60,000	Sample B: 20,000	Sample B: 50,000	Sample B: 70,000
Trial 3	Sample A: 50,000	Sample A: 40,000	Sample A: 90,000	Sample A: 60,000	Sample A: 50,000	Sample A: 110,000	Sample A: 40,000	Sample A: 40,000	Sample A: 80,000
	Sample B: 20,000	Sample B: 20,000	Sample B: 40,000	Sample B: 20,000	Sample B: 20,000	Sample B: 40,000	Sample B: 30,000	Sample B: 20,000	Sample B: 50,000
Average	64, 500	31,833.333	94,666.6667	36,666.6667	33,333.333	70,000	40,000	38,333.333	78,333.333

Quantity of Soil Fungi after pouring the liquids into the soil									
Type of test	Pouring 110 milliliters of Water			Pouring 110 milliliters of Coke			Pouring 110 milliliters of Diet Coke		
Type of Fungi	Number of Yeast per 1cc of soil	Number of Mold per 1cc of soil	Total number of all fungi per 1cc of soil	Number of Yeast per 1cc of soil	Number of Mold per 1cc of soil	Total Number of all fungi per 1cc of soil	Number of Yeast per 1cc of soil	Number of Mold per 1cc of soil	Total Number of all fungi per 1cc of soil
Trial 1	Sample A: 70,000	Sample A: 110,000	Sample A: 180,000	Sample A: 40,000	Sample A: 30,000	Sample A: 70,000	Sample A: 60,000	Sample A: 80,000	Sample A: 140,000
	Sample B: 10,000	Sample B: 20,000	Sample B: 30,000	Sample B: 40,000	Sample B: 20,000	Sample B: 60,000	Sample B: 30,000	Sample B: 20,000	Sample B: 50,000
Trial 2	Sample A: 7,000	Sample A: 7,000	Sample A: 14,000	Sample A: 20,000	Sample A: 20,000	Sample A: 40,000	Sample A: 90,000	Sample A: 50,000	Sample A: 140,000
	Sample B: 80,000	Sample B: 10,000	Sample B: 90,000	Sample B: 40,000	Sample B: 20,000	Sample B: 60,000	Sample B: 13,000	Sample B: 7,000	Sample B: 20,000
Trial 3	Sample A: 30,000	Sample A: 20,000	Sample A: 50,000	Sample A: 40,000	Sample A: 30,000	Sample A: 70,000	Sample A: 20,000	Sample A: 20,000	Sample A: 40,000
	Sample B: 20,000	Sample B: 50,000	Sample B: 70,000	Sample B: 10,000	Sample B: 10,000	Sample B: 20,000	Sample B: 30,000	Sample B: 20,000	Sample B: 50,000
Average	36,166.66667	36,166.66667	72,333.33333	31,666.666667	21,666.66667	53,333.33333	40,500	32,833.333333	73,333.33333

Final Graphs- Yeast, Mold, and Total Fungi






Average Number of Total Fungi
Total Number of Fungi per 1cc of Soil
100,000
90,000
80,000
70,000
60,000
50,000
40,000
30,000
20,000
10,000
0
Water
Diet Coke
Coke
Type of Liquid
Before pouring 110 ml of liquid onto soil
After pouring 110 ml of liquid onto soil

Conclusion

Our hypothesis, "Pouring 110 milliliters of diet coke onto the soil will decrease the density of fungi more than pouring 110 milliliters of regular coke onto the soil" is incorrect based on the data discovered during this experiment. We discovered that after pouring coke onto the soil the yeast on average of three trials and two samples increased by 33,500.003 soil fungi and that mold on average of three trials and two samples decreased by 11666.663 soil fungi. The total number of fungi decreased by 16666.6667. On the other hand, after pouring diet coke on the soil, the yeast increased on average of three trials with two samples by 500 and the mold on average of three trials and two samples decreased by 5500. The total number of fungi decreased by 5000. Clearly, a decrease of 5000 total fungi is smaller than a decrease of 16666.6667 total fungi. Since coke's total number of fungi decreased more than diet coke's total number of fungi than our hypothesis was proven wrong.

There are many other things this data suggests about these two liquids. First, when we poured only water onto the soil on average of three trials and two samples the number of yeast went down 28,333.333 and the number of mold went up 4,333.3337. This shows that when fungi are in a normal environment the mold goes up and the amount of yeast goes down. This is also supported with the knowledge that fungi in the form of mold this means that the fungi are comfortable in their environment and are going about their normal routine. Thus, when fungi are the form of yeast then they are stressed and are in their protective mode. The ideas of yeast representing stressed fungi and mold representation comfortable fungi also helps support the data found from the coke and diet coke data. Hence, the fact that pouring coke made the mold decrease and the yeast increase shows the presence coke made the soil fungi stressed. Also, pouring diet coke made both yeast and mold decrease. This data proves that the presence of diet coke did not allow fungi to reach a stage of comfort but it also did not force fungi into its protective zone.

Reflecting on our data, we noticed that something in the environment was destroying all the soil fungi, proven by the average total number of fungi during water test decreasing by 22,333.334. The water test should not have had such a drastic drop. Since the Coke's test decrease of soil fungi was close to the total number of fungi decreased during the water test, we can guess that water and coke work similarly with soil fungi. Both were not able to help with soil fungi against this mystery predator in the environment. Interestingly enough, some ingredients in Diet Coke were able to protect the fungi from the dangerous force in the environment preventing such a huge drop in the total amount of fungi. For example, potassium benzoate and aspartame found especially in Diet Coke could be in charge from keeping the environment from killing the soil fungi. There is also a possibility that the potassium benzoate acted as a preserver towards this force in the environment, stunting its growth enabling it to consume and destroy the soil fungi. We believe these ingredients halted the environment the most.

For further research, we would want to research the individual, different ingredients in Diet Coke, such as Potassium Benzoate and Aspartame. To research this information we would see what happens to soil fungi when just the potassium benzoate is added to the soil. Potassium Benzoate is possibly responsible for preventing the unknown attacker in the environment from hurting the fungi. The next experiment would emphasize the importance of chemicals and unnatural substances being added to the soil, stressing again the major issue of students adding their waste or liquids into the soil. But this further research would specify the issue and categorize the harmful substances.

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