The Effects of Sports Drinks on Soil Acidity and Soil Protozoa

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Background Report

Soil protozoa are motile, single-celled eukaryotes that eat bacteria, other protozoa, organic matter, and fungi. They range in size from 5 to 500 micrometers in diameter, and they are classified into three groups based on how they move: Ciliates, which are the largest in size, move using hair-like projections called cilia; flagellates, the smallest in size, use a whip-like tail known as a flagella; and amoebae, which vary dramatically in size, use an arm-like projection of their cell membrane called a pseudopod. The amoebae are then divided even further into two groups: the testate amoebae, which make a shell-like covering, and the naked amoebae, which do not have this covering.

Protozoa have various important roles in the soil, and these include eating root pathogens that can attack plant roots and cause diseases, (Ingham, n.d.) providing plants carbon for the creation of new cells and energy for growth, and mineralizing nitrogen and other nutrients to make them available for plants and other organisms. However, the most significant role protozoa play is their role in the nitrogen cycle.

Because most of the nitrogen found in the environment is in the atmosphere in an inaccessible form, nitrogen gas (N_2), there are bacteria that convert this gas into inorganic nitrogen salts, such as ammonium (NH_4+) (“Nitrogen Cycle, 2018). This ammonium is then absorbed into the tissues of algae and higher plants, which are then consumed by animals. These organisms use the ammonium to make the nucleic acids and proteins that cause the chemical reactions that make cells alive. Therefore, without the ammonium form of nitrogen, the cells of plants and animals would not function, and these organisms would die.

Another source of this ammonium comes from the soil bacteria that decompose the remains and waste products of dead plants and animals through ammonification. (Hoorman, 2010). But this ammonium is often converted by other groups of bacteria into nitrites, or NO_2^-, and still other bacteria convert these nitrites into nitrates, or NO_3^-. These nitrates are also absorbed by plants and algae to make nucleic acids and proteins, and finally, denitrifying bacteria convert any excess nitrates back into nitrogen gas, and the cycle continues (Campbell, Williamson, & Heyden, 2006, p. 797).

The role protozoa play in this process is to eat the bacteria involved (Hoorman, 2010). In the cells of protozoa, the ratio of carbon to nitrogen is 10 to 1 or higher, but in the bacteria the ratio of carbon to nitrogen is 3 to 1. Thus, protozoa have a lower concentration of nitrogen than bacteria do (Ingham, n.d.), and since bacteria contain more nitrogen than the protozoa need, the protozoa release this excess nitrogen as yet another source of ammonium (Hoorman, 2010). Furthermore, although microorganisms like bacteria use most of it, some of this ammonium from the protozoa is used by plants. Hence this release of nitrogen into forms the various organisms in the soil can use make protozoa essential to the nitrogen cycle.

Since the nitrogen cycle is so important, anything that may interfere with it becomes potentially problematic, and one factor that can affect the nitrogen cycle is soil acidity. Soil naturally has some acidity, and a healthy soil has a pH above 4.8 in the subsurface and above 5.5 in topsoil. However, too much acid in soil causes vital plant nutrients to be stripped away, and as the amount of these nutrients decreases, the plants become weaker and more susceptible to diseases, increasing the possibility of dying. The major nutrients plants need, such as nitrogen, phosphorus, sulphur, potassium, manganese, and calcium, may only be available in limited quantities or not available at all in highly acidic soil.

One reason these critical nutrients become unavailable is because highly acidic soil can harm the enzymes in the protozoa. Like the enzymes in any organism, the enzymes in protozoa have a pH level where they can cause the chemical reactions cells need to function, and the optimal pH for most enzymes is between 6-8. When the acidity of the soil is outside this optimal range, the enzymes stop causing chemical reactions, and if the enzymes stop, the cells die. Therefore, the acid in soil has the potential to kill the protozoa there, and without any protozoa, the nitrogen cycle is affected since not enough ammonium will be made. This means that plants and other organisms that need nitrogen will not be able to receive the amount of nitrogen that they need to live, and the entire ecosystem is put at risk.

Our problem asks how acidic beverages alter the density of soil protozoa. Our problem connects soil acidity to the protozoa that live in the soil, and by adding different drinks with different amounts of acid in them, we will be adding extra acid to the already acidic soil, creating an atmosphere that protozoa may have never been in before. When we pour drinks with varying levels of acidity onto the already acidic soil, we will be better informed for times in the future when the amount of acid in the soil is at an abnormal amount due to the increase in acid rain caused by the increasing amount of fossil fuels being burned every day. Hence, by doing this experiment, we will have a better understanding of how acid rain could be affecting protozoa.

Experimental Outline

I. Problem: How do acidic beverages alter the density of soil protozoa?

II. Hypothesis: Acidic drinks such as Gatorade will increase the acidity of soil and decrease the density of soil protozoa.

III. Procedure:

A. Independent Variable: Whether or not Gatorade is added to the soil.

B. Dependent Variable: Density of protozoa in the soil, soil pH levels

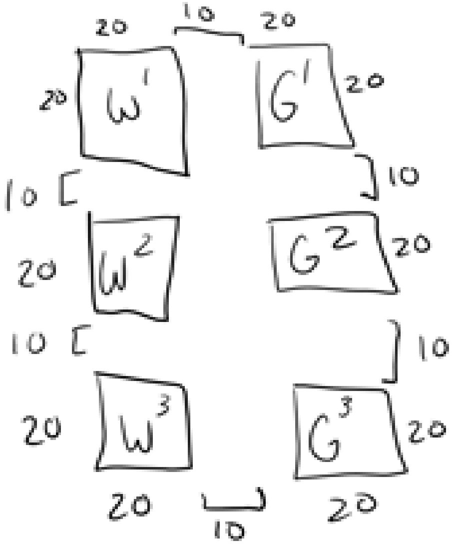
C. Negative Control: Distilled water poured onto the soil

D. Positive Control: The soil samples taken before any drinks are poured into the soil.

E. Controlled Variables: location of soil, depth of soil sampled, vegetation growing in soil, all samples taken on the same day within 30 minutes (to control for weather, moisture of soil, acidity of soil before drinks are added, temperature of soil, initial density of soil protozoa before drinks are added), amount of liquid poured onto the soil, size of soil plot, type of tool used to measure soil, size of beaker, type of beaker, temperature of liquids, number of trials, amount of soil tested, amount of soil collected, how acidity is measured, type of water, flavor of gatorade, type of flags used, location of soil plots, type of filter, size of filter, type of pipette, size of pipette, water is distilled, type of mesh used, type of balance used, size of mesh used for sifting, amount of soil sifted into petri dish, amount of water used to saturate the soil, amount of time petri dishes sit after soil is saturated, amount of water in the petri dish when the Uhlig extractor is in the petri dish, amount of time soil is in the Uhlig extractor, all samples filtered a second time, amount of stain on the microscope slide, type of stain used, size of qualitative filter paper, size of cover slip, magnification of microscope, number of fields of view examined

F. Step by Step:

1. At coordinates N 39.35812 W 76.63616, measure out six square plots of land, each 20 cm wide by 20 cm long, 10 cm apart from each other, and label them using yellow flags as shown in diagram below.



Key: "W"=water, "G"=Gatorade, number indicates which trial the plot is used for

All measurements are in centimeters

2. On the same day and at the same time as step one, immediately after labelling the plots, use a soil core extractor to obtain three soil samples from each plot of land that are 15 cm deep and have a diameter of 2 cm.

3. On the same day and at the same time as step two, place each sample into a plastic bag that is labelled in the same manner as the flag for its plot (i.e. a sample from the plot labelled "G1" goes into a ziploc bag labelled "G1"). There should be three bags for each plot of land.

4. On the same day and at the same time as step three, pour 200 milliliters of water each onto all soil plots that have been labelled with a “W”, ensuring that it is spread throughout so that all of the plot is equally saturated.

5. On the same day and at the same time as step 4, pour 200 milliliters of Glacier Freeze gatorade each onto all soil plots that have been labelled with a “G”, ensuring that it is spread throughout so that all of the plot is equally saturated.

6. Wait two days after pouring the liquid onto the plots so that the liquid can seep into the soil.

7. Immediately after two days have passed, use a soil core extractor to obtain three soil samples from each plot of land that are 15 cm deep and have a diameter of 2 cm. Obtain all soil samples on the same day at the same time.

8. On the same day and at the same time as step 7, place each sample into a plastic bag that is labelled in the same manner as the flag for its plot (i.e. a sample from the plot labelled “G1” goes into a ziploc bag labelled “G1”). There should be three bags for each plot of land.

9. Label 18 petri dishes in the same manner as the ziploc bags (three labelled “G1”, three labelled “G2”, etc.). The first time through label them “before” and the second time through label them “after”

10. Immediately after obtaining the samples in steps 2-3, place each of the 15cm samples of soil into the bottom of its own clean, empty petri dish that matches the label on its bag; and allow to dry completely. Perform this step on all samples at the same time on the same day.

11. Sift 9-10 g of each soil sample into its own second petri dish that has the same label as its original petri dish, using a 1 mm^2 nylon screen or mesh, and record the exact amount sifted.

12. Place the extra non-sifted soil into a petri dish and save for the pH test (see step 22)

13. Add 20 ml of distilled water to each sample to saturate the soil. Do this to each soil sample on the same day at the same time.

14. Immediately after adding the water, cover the petri dish with its lid and allow to sit for 7 hours. Do this to each soil sample on the same day at the same time.

15. Immediately after the 7 hours have passed, place each rehydrated soil sample in its own individual modified Uhlig extractor containing 30 ml of distilled water for 24 hours. Do this to each soil sample on the same day at the same time.

16. Immediately after 24 hours have passed, remove the filtrate from each Uhlig extractor and separately filter each filtrate a 2nd time using 12.5 cm qualitative filter paper. Do this to each soil sample on the same day at the same time. Refrigerate soil samples until ready to proceed to step 17.

17. Using a capillary tube, deposit 7 microliters of methyl-green stain on a clean microscope slide (1 microliter = 1 drop from the capillary tube),. Then using a disposable graduated Beral-type pipette, add 18 microliters (the first dearcation on the pipette) of the 2nd filtrate of the sample labeled W1,1 from step 16 to the stain on the microscope slide and cover with an 18 x 18 mm^2 cover slip.

18. Repeat step 17 for each remaining soil sample.

19. Examine under a light microscope at 40X at five fields of view: each of the four corners of the cover slip and the center of the cover slip. Count and record the number of protozoa in each field of view, using a click counter. Average all five fields of view and use this number in the equation in step 20.

20. Using the the following equation to determine the population density of protozoa in the soil sample:

[(#per field of view at 40X) x (total ml of water used) x 747] ÷(grams of sifted soil) = # of protozoa per gram of soil.

21. Repeat steps 9-20 for the second set of soil samples labeled “after”.

22. For the pH test, take out saved soil that was not sifted and use the LaMotte STH-14 test kit to test for the pH of all the soil samples, both the “before” and the “after” samples

IV. Data Analysis

A. Data Table

Population Density of Soil Protozoa

NOTE: W=water, G=Gatorade. Example: W1,1 would be water plot 1, first trial. So G3,2 would be Gatorade plot 3, trial 2.

	Sample	Calculations		Sample	Calculations
Before Drinks Are Added	W1,1	52589	After Drinks Are Added	W1,1	325692
	W1,2	77879		W1,2	76342
	W1,3	714964		W1,3	77010
	W2,1	49302		W2,1	227384
	W2,2	18675		W2,2	192526
	W2,3	12782		W2,3	138619
	W3,1	6723		W3,1	97110

	W3,2	11869		W3,2	84849
	W3,3	4925		W3,3	60897
	G1,1	1129555		G1,1	115038
	G1,2	4108		G1,2	321290
	G1,3	14704		G1,3	232400
	G2,1	1050133		G2,1	971593
	G2,2	1524		G2,2	381811
	G2,3	10790		G2,3	86773
	G3,1	1660		G3,1	67909
	G3,2	2310		G3,2	44277
	G3,3	31023		G3,3	6426
	Average of all water:	105523		Average of all water:	60897
	Average of all Gatorade	249534		Average of all Gatorade:	247502

Soil pH Levels

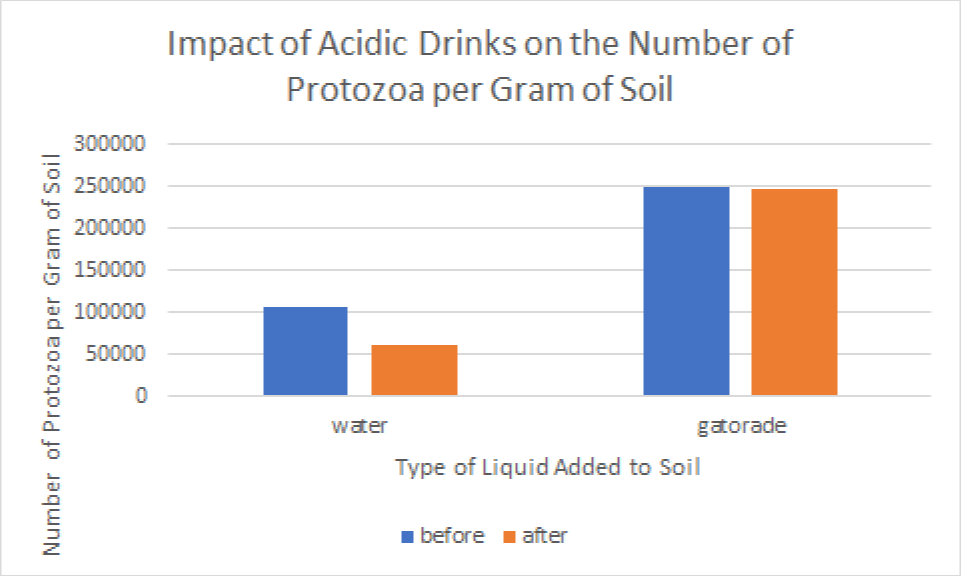
NOTE: 1,1 stands for plot 1, trial 1. This means that 2,3 stands for plot 2, trial 3.

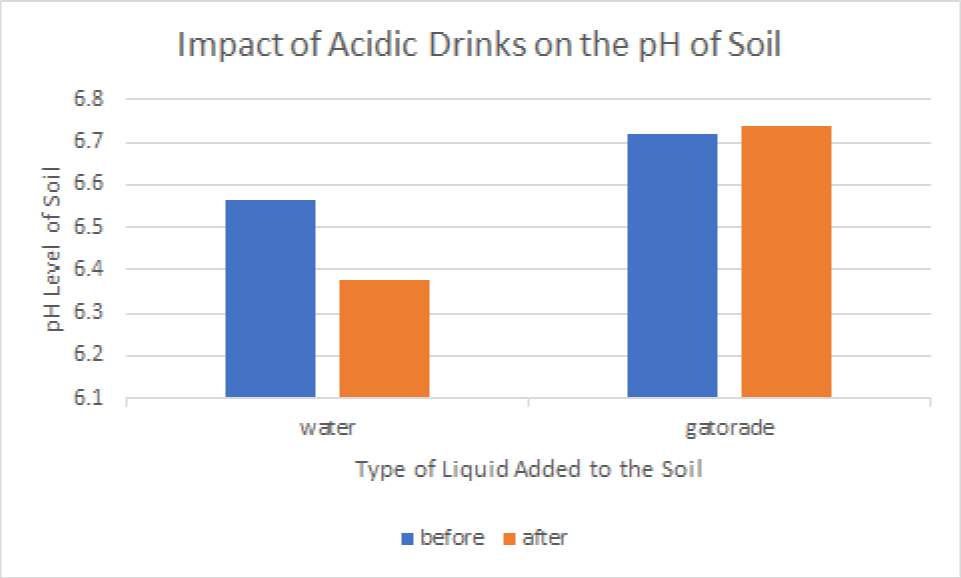
Soil Sample	Trial	pH Before Adding Drinks	pH After Adding Drinks

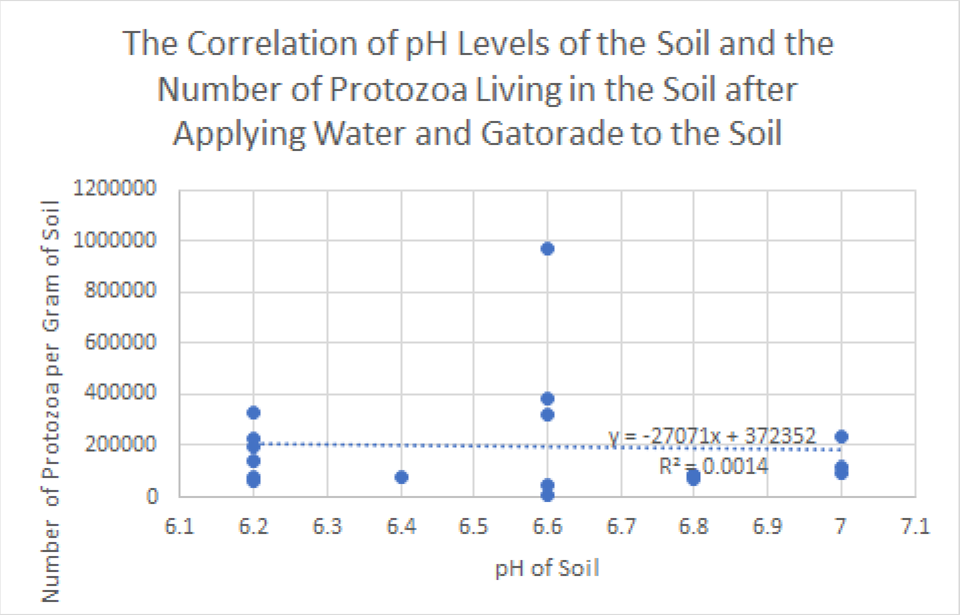
Gatorade	1,1	7.2	7.0
Gatorade	1,2	7	6.6
Gatorade	1,3	6.4	7.0
Gatorade	2,1	6.2	6.6
Gatorade	2,2	7	6.6
Gatorade	2,3	7	6.8
Gatorade	3,1	6.2	6.8
Gatorade	3,2	6.4	6.6
Gatorade	3,3	7	6.6
Water	1,1	6.3	6.2
Water	1,2	7	6.2
Water	1,3	6.2	6.4
Water	2,1	6.2	6.2
Water	2,2	6.8	6.2
Water	2,3	7	6.2
Water	3,1	6.2	7.0
Water	3,2	7	6.8
Water	3,3	6.4	6.2

Average of all Gatorade:		6.7172	6.7374
Average of all water:		6.5657	6.3737

V. Graph:







V. Conclusion:

Our hypothesis stated that acidic drinks, such as Gatorade, would increase the acidity of soil and decrease the density of soil protozoa. Our hypothesis was wrong because the acidity of the soil plots onto which we poured Gatorade became more basic rather than more acidic, and the density of soil protozoa increased instead of decreasing. This is seen in our data because the average initial pH for the plots we would later pour water onto was 6.5657, but the average acidity after we poured the water onto the soil plots was 6.3737. Since the pH level decreased, the soil became more acidic. However, the opposite thing happened for our Gatorade plots. The average starting pH for the plots we poured Gatorade onto was 6.7172, and after we poured the Gatorade onto the soil plots it increased to 6.7374. Since the pH level increased, the soil became more basic. On average, the number of protozoa per gram of soil on the water plots before we added the water was 105,523. After we poured the water onto the soil plots, the average number of protozoa per gram of soil was 60,897. Since the number of protozoa decreased so much, a natural occurrence must have happened while our experiment was going on, causing the number of protozoa to decrease, because water does not have any properties that would cause the population of the protozoa to decrease. In contrast, before we poured Gatorade onto our plots, the average number of protozoa per gram of soil was 249,534 protozoa. We hypothesized that the number of protozoa per gram of soil would decrease; it did, but it did not decrease as significantly as the average number of protozoa per gram of soil in our water plots. Because the number of protozoa in the Gatorade plots did not decrease as much as the number of protozoa in the water plots, we can see that the Gatorade in fact helped the protozoa instead of hurting them. The Gatorade would have counteracted whatever natural occurrence caused the number of protozoa in the water plots to decrease by causing more protozoa to reproduce, increasing the population density of protozoa per gram of soil. By causing more protozoa to reproduce, the number of protozoa per gram of soil would rise, making it look like the number of protozoa stayed the same in our results. There must be a reason that we did not get the results that we expected.

When looking back on our experiment, we realized that we had made a critical mistake that affected the results we got. Distilled water has a neutral pH of 7. This means that when distilled water is added to soil, the acidity of the soil should not change. The pH of the water plots most likely became more acidic because there was a lot of rain during the time that the liquids were soaking into our plots, and rain is acidic. Glacier Freeze Gatorade, the type of Gatorade that we poured onto our soil plots, has a pH of 3.5. This means that the drink is acidic and therefore should have made the soil more acidic, but it did not. The soil naturally became more acidic because of all the rain we got during our trial period, but the soil that we added Gatorade to still became more basic in the end. This may have happened because the main ingredient of Gatorade is water. As stated above, water neutralizes the naturally acidic pH of the soil. This means that the water in the Gatorade made the soil more basic, counteracting the acidity that the soil gained from the rainfall. The water in the Gatorade is not the only reason that the acidity of the soil in the Gatorade plots became more basic. The type of acid in Gatorade is citric acid. Unlike sulfuric acid and nitric acid, the two acids in acid rain, citric acid is a weak acid. This means that the hydrogen ion, which is what acid is measured in, does not release from the chemical it is bound to as easily it does in stronger acids, like sulfuric and nitric acid. Since the hydrogen ion does not release as easily, you need a larger amount of citric acid to have the same effect on the soil as a smaller amount of sulfuric or nitric acid. Because there was a lot of rain during our experiment, there may have been a lot of sulfuric and nitric acid that entered the soil. These large amounts of stronger acids could have had a bigger impact on the pH of the soil than the 200 mL of Gatorade that we poured onto the soil, which contained some citric acid. The design flaw that we made is that we did not pour enough Gatorade onto each soil plot to make a difference in the pH, especially since there was a lot of rain during our experiment that could have affected the pH of the soil. Now that we know of this design flaw, we could redo our experiment with a larger amount of Gatorade and see if we prove our hypothesis. We could also do further research.

One way to think about what may have caused our results is to realize that we had a design flaw, but another way is to think about it is other elements in the soil. Our linear regression plot has an r^2 value of -0.0014. This number is very close to 0, which indicates that there is no correlation between soil acidity and the number of protozoa per gram of soil. This is how we realized that something else happened that caused our results. Our graphs show that the number of protozoa per gram of soil in our Gatorade plots only decreased by a little, and that the pH of the soil became more basic. We already know that the rain we got during our experiment could have made the soil more acidic, creating an environment that was bad for the protozoa living in the soil. However, our graphs show us that the Gatorade that we added created an environment where the protozoa could excel and thrive. We can infer this because the number of protozoa in the water plots, which were our negative control, decreased by a greater amount than the number of protozoa per gram of soil decreased in our Gatorade plots. Since water is neutral and does not have any properties that would cause it to kill protozoa, we can infer that the number of protozoa in our Gatorade soil plots also went down, but that something caused the number to increase again. We also know that the Gatorade made the pH of the soil more basic, but only by a little. One possible reason for these two things could have to do with bacteria.

The role of bacteria in the nitrogen cycle is that they make ammonia. Ammonia reacts with water in the soil to make ammonium. Ammonium makes the pH of the soil become more basic. Gatorade has a lot of sugar in it, which is food for bacteria. By pouring Gatorade into the soil, we put a lot of sugar in the soil that the bacteria could have consumed. The more food bacteria have, the more they reproduce and the faster they do so. A greater amount of ammonium be produced due to the increase in bacteria causing a rise in pH in the soil. Additionally, bacteria are also food for protozoa. If the bacteria start rapidly reproducing, there are going to be more for the protozoa to eat. The more food that is available for the protozoa, the faster and more often the protozoa are going to reproduce. Because all these protozoa could be reproducing more often and more quickly, the population of the protozoa would increase, so the overall decrease in protozoa in the Gatorade plots would not be as large. Protozoa, like bacteria, make ammonium. If there is also an increase in protozoa in the soil, the level of pH would become more basic because both protozoa and bacteria would be making ammonium.

Because of this data, some further research we could do would have to do with nitrogen and bacteria. In order to see if this is true, we could test for the amount of ammonium in the soil. We could also test for the number of bacteria in the soil to see if that number increased after the Gatorade was poured onto the soil. This would be a complex experiment so the easiest option to do first, would be to just redo the whole experiment with a large increase in the amount of Gatorade poured onto the soil plots, so that we do not have the same design flaw as before.

Therefore, the data obtained in the experiment proves that small quantities of Gatorade have a positive effect on soil protozoa and cause their population density to increase, while also causing soil acidity to become more basic.

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