Soil Temperature & Yeast Levels

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Abstract:

Our group tested yeast and temperature. Our problem was: Does a higher soil temperature cause an increased yeast population in an environment with a controlled common vegetation? Our hypothesis was: a higher soil temperature will cause an increased yeast population in an environment with a controlled common vegetation. The controlled vegetation in the plots we tested in was jewelweed. Our test was very simple and ran over three days. We collected 16 samples the first day, four samples at four different times. On the second day, we collected 20 samples at five different times. On the first day, the times we collected were earlier in the day (from 10:30 a.m. to 1:30 p.m.). On our second day of testing, we collected samples later because our experiment was temperature dependant, we assumed we would get more varied data later in the day when the sun was out. After collecting the samples, we diluted one cc worth of soil from each sample, and then plated the second, third and fourth dilutions. On the third day, we counted yeast on all of the plates. Our data showed no correlation between soil temperature and yeast growth.

Introduction:

Yeast is a type of fungi that is located in many different habitats. These habitats range from the human intestine to the soil in the ground. The yeast has no chlorophyll. The main function of the yeast in the soil is to aid the decomposition of organic matter. Yeast reproduces rapidly by budding, which is a unique characteristic of yeast compared to other types of fungi ("What", undated). There are often 1,000 to 1,000,000 yeasts in one gram of soil. This is a low amount compared to the amount of other microbes in the soil (Evans, 2000).

We decided to test for yeast after we performed a survey on a microclimate and compared our results to the surveys that other members performed on the other microclimates in the same ecosystem. We did not specifically test for yeast, but we did test for fungi. Since fungi are closely related to yeast, we were able to use the fungi data to make a semi-educated guess regarding yeast. We used a faulty protocol in testing for fungi, but the amount of fungi in our specific microclimate was still considerably greater than the amount of fungi in the other microclimates. We counted thirty-seven fungi in one cc of soil which compared to the other numbers of fungi was considerably greater. The site that we decided to use to compare to our original site had 2.375 fungi. This plot obviously had considerably fewer fungi than our original plot. Based upon this data, we were able to deduce that fungi are an important aspect to look at seriously with a defined protocol. We wanted to specifically look at yeast because of there fast reproduction.

We also worked with a microbiologist named Dr. Peter Groffman. He came to analyze our survey data so that we could intelligently create a question. When he came to look at our individual, microclimate, he noticed that our plot was warmer and more

susceptible to sunlight. He also noticed that another plot had the same type of vegetation as our original plot, jewelweed. We then created our final question regarding the temperature of soil and the amount of yeast in the soil.

Materials and Methods:

Specimens examined: We took thirty-six samples each of eight centimeters cubed of soil from two areas one meter by one meter at various times over July 19th to July 20th. The first area was located at N 39.35779, W 076.635938. The other site was located at N We took four samples at each area at different times throughout the day taking four sets of samples the first day, and five sets of samples the second day. We took all of the samples at the same time so that we could compare all of the data. Half of the samples were form one area that was located out in the open. The other half of the soil was collected from an area that was located under trees. All of the samples were collected from areas covered in jewelweed so as to control that aspect of the experiment. All of the samples were kept at room temperature, and they were all plated within one hour of their collection.

Isolation and Identification: For direct isolation of the yeast cells, we plated the yeast after diluting the soil to a negative four dilution. We followed procedures given to us concerning dilutions. We filled dilution tubes using a serological pipette remembering to label the row of tubes and the pipette with the correct time and sample. We filled the first tube with 10 mL of distilled water. We filled the remaining four tubes with 9 mL of distilled water. We did this for all of the tubes using a new serological pipette for each sample. One cc of each sample was then placed into the corresponding first tube. We shook the tube for thirty seconds and began the dilution. We used the same serological

pipette that was used in the original set up for that individual sample. Using the serological pipette, we took 1 mL of the solution in the first tube and placed it in the next tube. We shook the tube until the contents were combined. We then took one meter from the that tube using the same pipette and placed it in the adjacent tube. We followed these procedures until all four of the tubes after the first tube were diluted. We then plated the dilutions. We plated the negative two, negative three, and negative four dilutions. We plated these dilutions by placing 100 μL of the dilution on a yeast petri film. We then let the films incubate at room temperature. The films finished on July 19th incubated for three days. The films finished on July 20th two days (Fell and Kurtzman, 1996; Staley, 1996).

For the temperature aspect of the lab, we placed four thermometers in the soil at four different places per area. We used a total of eight thermometers. We placed the thermometers in the soil and left them there so that we could have a continuous temperature. We read the temperatures trying to estimate the temperatures located in between the solid numbers on the thermometer. The thermometers went into the eight centimeters thus explaining the amount of soil we collected.

Results:

To begin the analysis of the data, we performed a t-test on the yeast counts for each site. We put all of the yeast counts for site one in the first list. We then placed all of the yeast counts for site two in list two. We then performed the t-test. The t value was 2.5796. We would like to be ninety-five percent sure. The degree of freedom is 39.4235. The t is 2.021 for 0.05. This means that the t-value is greater than the alpha-value . After

this conclusion is made, we can assume that the null hypothesis is incorrect. The t-test initially proves to us that there is a significant difference between the two populations, and since there is a difference, it is logical to see if there is a correlation between temperature and the amount of yeast in the soil.

Table 1 shows the temperature readings and the average number of yeasts at that temperature. In order to create this table, we used the temperature readings from each thermometer. If there were many yeast counts with the same temperature, we averaged the amount of yeasts in order to see if there was a correlation between the temperature of the soil and the amount of yeasts. We graphed the data points. The temperature readings were on the x-axis. The average number of yeasts per cc of the soil is located on the y-axis. Graph 1 is the actual graph of the data. As one can plainly see, there is little correlation between the data. The majority of the points were located below the 1,000,000 mark. This data creates a linear line that is constant. There are some outliers in the data. These outliers also help to confirm our assumption that the data has no correlation. In order to be mathematically sure about our analysis of the graph, we performed regression test. The linear regression test gave an equation but the coefficient of correlation was 0.059986. This is a depressing number that confirms our analysis. The graph plainly shows that there is very little correlation between the data.

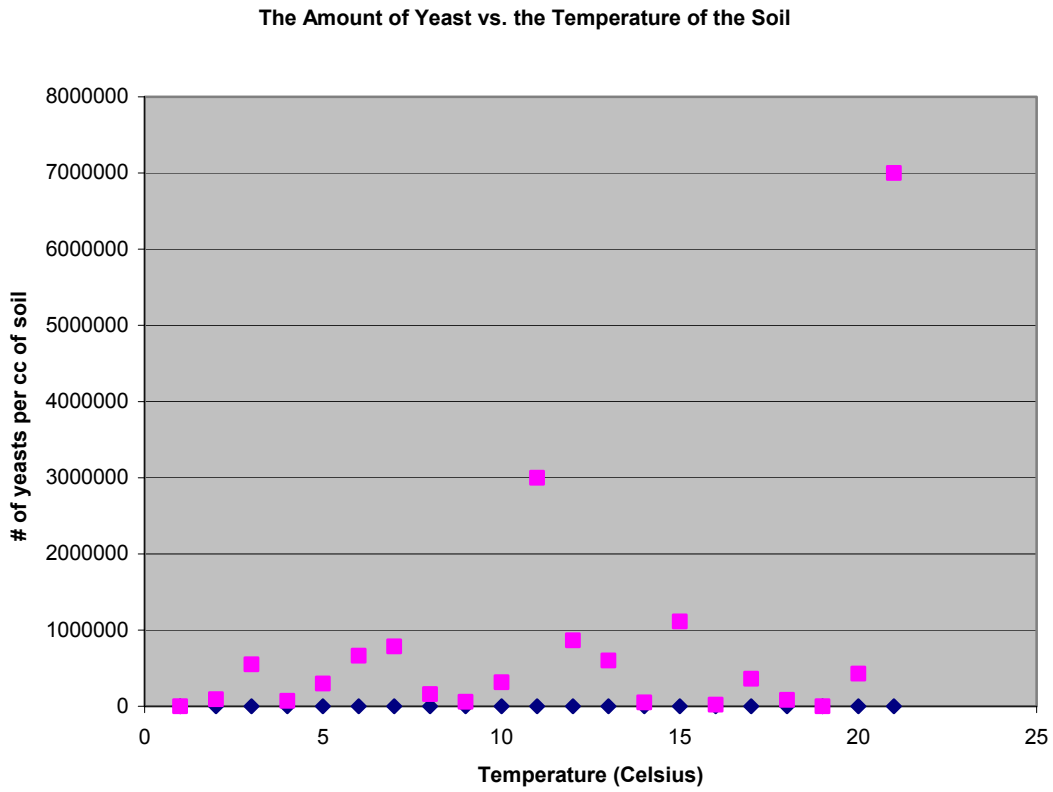
Since there was no correlation between the data, we did not have to perform t-tests between individual points and the populations that the points represent. These populations are the yeast counts that were averaged. The t-test however showed that the alternative hypothesis was correct. This information caused us to analyze our two sites again. We reevaluated the external environmental differences in the plots. The results that

we received showed us that there is a lack of correlation between temperature and yeast counts. The results, however, also confirmed that there was indeed a difference between the two plots.

Table 1

Temp.	average amount of fungi at that temp.
18	93333.3
18.5	550000
18.6	70000
18.8	300000
19	665000
19.5	785000
19.6	160000
19.7	60000
19.8	315000
19.9	3000000
20	864444.4
20.1	600000
20.2	50000
20.25	1115000
20.3	20000
20.5	360000
20.6	85000
20.8	0
21	430000
21.25	7000000

Graph 1



Discussion:

In this experiment, data disproving the original hypothesis was found. The original hypothesis was that the higher the temperature, the more yeast i.e. a positive correlation between temperature and yeast counts.

We found that there was no correlation between temperature and yeast counts. The graph shows this both visually and mathematically. Visually, it is fairly evident that there is no correlation. Mathematically, the regression line had a coefficient of correlation less than 0.1. This data gave us a fairly good idea that in this case, there was no relationship between temperature and yeast. Using our analysis, however, we are sure

that the alternative hypothesis was correct. The populations of yeast in the different sites were in fact different. This difference is not because of temperature as seen in the graph and regression. There are many different reasons that could possibly create this difference.

We attempted to find some logical external reasons that could affect the differences in population. We were not able to look at specific internal reasons for the differences in population because of time. This would be, however, a valuable aspect to consider when creating an intelligent conclusion. There are many possible external reasons for the differenced. Both sites are located by water, but the amount of water that the soil tested interacted with is unknown and if it is different, this could cause a difference in population counts of yeast. Wee attempted to control the vegetation by choosing two sites that have jewelweed. We did not, however, carefully consider soil litter or the amount of jewelweed in the different plots. These two variable could have a considerable impact on the soil. These are just a few differences that we were able to find in the limited amount of time available to us.

The external reasons for differences would be valuable aspects to research. There are many possible research questions that could come from this experiment. One concerns the reasons for the differences in the population. Another possible question concerns the relationship of yeast and temperature in alternative environments.

References:

Evans, Erv. “Soil Life.” Available [online] http://www.ces.ncsu.edu/depts/hort/consumer/quickref/soil/soillife.html; June 2000.

Fell, Jack W. and Cletus P. Kurtzman. (1996). “Yeasts.” *Methods for the Examination of Organismal Diversity in Soils and Sediments*; ed. G.S. Hall. Paris: CAB INTERNATIONAL. Pp 103-108.

Staley, James T. (1996). “Heterotrophic Bacteria: the Cultivation Approach.” *Methods for the Examination of Organismal Diversity in Soils and Sediments*; ed. G.S. Hall. Paris: CAB INTERNATIONAL. Pp 1-10.

“What are Yeasts?.” Available [online] http://genome-www.stanford.edu/Saccharomyces/VL-what_are_yeast.html; undated.