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Background

Fungi are eukaryotic organisms that help carry out important roles in the ecosystem. They help recycle the elements carbon, oxygen, nitrogen, and phosphorus by breaking down organic matter, and they can be found in the air, soil, bodies of water, on plants, in food, and even in the body. There are at least 80,000 known species of fungi including yeasts, rusts, mildews, molds, mushrooms, and toadstools, and humans have even used them to make foods, such as bread, wine, beer, and cheese, as well as certain antibiotics like penicillin (Alexopoulos & Moore., 2014).

With large populations almost everywhere, fungi are very important to the health of any environment in which they live (Alexopoulos & Moore., 2014). For example, one important thing that fungi do is they hold the soil together with their hyphae (Bot & Beites, 2005). More significantly, though, fungi (along with bacteria) are the primary decomposers in an ecosystem. They are able to convert dead organic materials into chemical forms that other organisms can use, such as the nutrients plants need in order to grow (Ingham, 2014). In fact Campbell, Williamson, and Heyden (2004) say that the "plant and animals they [fungi] feed would starve because elements taken from the soil would not be returned." This is especially true for hard-to-decompose materials such as the cell walls of plants that otherwise would not be able to be broken down at all without fungi to convert the carbon trapped there into carbon dioxide for reuse in photosynthesis as part of the food chain (Horticulture, 2009). Hence, fungi are needed

for decomposition in order to maintain the movement of critical organic material through an ecosystem.

Fungi are often harmed, though, due to natural causes such as erosion. Water erosion can wash away fungi living in the topsoil, and it can do so in one of four ways: sheet erosion, rill erosion, gully erosion, and splash erosion (Rutledge et al, 1996-2004). The first of these, sheet erosion, is when a thin layer of soil is removed from a large area, while all the other forms of erosion create small channels of water. Yet, in all types of water erosion, the running water transports the soil particles until it is finished moving, and the particles are deposited in a new place. Where the soil particles end up and how far they move depends on the amount of water and how fast it is moving (Plants and Soil Science Library, 2014); all of erosion, though, displaces or removes soil from the affected area, subsequently altering the terrain there.

One such alteration could be the removal of the fungi population in that soil and the consequent negative reduction in what the fungi normally do for the ecosystem. With fewer fungi to decompose dead organism and recycle organic matter for the plants, plants would not be able to grow for two reasons. One reason being that the fungi would no longer be able to supply the plants with the critical element, nitrogen, and therefore, plants would not be able to recycle carbon dioxide through photosynthesis into the 5 biological molecules. Without nitrogen, the plants would not have DNA, RNA, or enzymes because nucleic acids, which are in DNA and RNA, are made with nitrogen. Also, nitrogen helps to make up amino acids which are the foundation for proteins, and proteins are enzymes (Campbell, Williamson, & Heyden, 2004). Without enzymes, there would be no chemical reactions, so the cells could not function or perform any kind of task. No cells could then be formed, and even if they were formed, they would not be able to work which means that no plants would be created (Brock, 2014). Without

plants, there would be no animals because they would need the plants for food. In short, if enough fungi are lost, the whole food chain would collapse.

Given the potential severity of the impact of erosion, we wanted to find out whether or not this process decreased or increased the population of fungi in the soil at the top of a hill compared to the bottom. To test this idea, we set up plots on a hillside (at the top and directly below them at the bottom) and poured water down the hill. We then took samples of the soil to count the population of fungi in the soil and compare that to the soil beforehand. We predicted that the population of the fungi at the top of the hill would decrease, and the population of the fungi at the bottom of the hill would increase.

Lab Outline

Problem: Does water erosion alter where the saprotrophic fungi populations are found on a hill side?

Hypothesis: Water erosion decreases the saprotrophic fungi population at the top of a hill and increases the saprotrophic fungi population at the bottom of the hill.

I. Independent Variable: pouring water down a hill
II. Dependent Variable: number of saprotrophic fungi per cubic centimeter in soil
III. Negative Control: soil samples taken before water poured on it
IV. Controlled Variables:
    1. location of soil
    2. time of soil extraction
    3. cleanliness of materials
    4. size of culture tube
    5. amount of sterile water added to test tube
    6. amount of soil extracted
    7. how soil is extracted
    8. type of water
    9. plant life near test site
    10. concentration of serial dilutions
    11. amount of water poured down hill
    12. how water is poured
    13. force of shaking culture tubes

14. amount of soil added to culture tubes
15. amount of time culture is grown
16. how much dilution added to growth plate
17. which dilution is plated
18. type of nutrient agar
19. amount of nutrient agar
20. temperature of room
21. amount of soil in sampler
22. temperature of water poured down hill
23. material of bag soil samples are put in

Procedure:

1. Determine a 20 centimeter (cm) by 20 cm plot of soil that is on the top of a hill, at N 39˚ 21, 424 W 76˚ 38, 192, that has not been affected by erosion.
2. Mark the determined area with flags and label it #1-top. Ensure the location is not tampered with for the duration off the experiment.
3. Determine another 20 cm by 20 cm plot of soil that is at the bottom of the hill, at N 39˚ 21, 421 W O 76 ˚ 38, 195 that is 2.98 m directly downhill from site #1-top
4. Mark the determined area with flags and label it #1-bottom. Ensure the location is not tampered with for the duration of the experiment.
5. Determine another 20 cm by 20 cm plot of soil at the top of the same hill, at N 39˚ 21, 424 W 76˚ 38, 192, 1 meter to the right of #1-top when facing up hill
6. Mark the determined area with flags and label it #2-top. Ensure the location is not tampered with for the duration of the experiment.
7. Determine another 20 cm by 20 cm plot of soil that is at the bottom of the hill, at N 39˚ 21, 421 W O 76 ˚ 38, 195, that is 2.98 m directly downhill from site #2-top
8. Mark the determined area with flags and label it #2-bottom. Ensure the location is not tampered with for the duration of the experiment.
9. Determine another 20 cm by 20 cm plot of soil at the top of the same hill, at N 39˚ 21, 424 W 76˚ 38, 192, 1 meter to the right of #2-top when facing up hill
10. Mark the determined area with flags and label it #3-top. Ensure the location is not tampered with for the duration of the experiment.
11. Determine another 20 cm by 20 cm plot of soil that is at the bottom of the hill, at N 39˚ 21, 421 W O 76 ˚ 38, 195, that is 2.98 m directly downhill from site #3-top
12. Mark the determined area with flags and label it #3-bottom. Ensure the location is not tampered with for the duration of the experiment.
13. Ensure steps 14-25 are completed on the same day at the same time
14. Take 3 separate soil samples each with 2 cm diameter and 15cm deep from plot #1-top
15. Place the 3 soil samples in 3 separate plastic bags and label each #1-top-before(1), #1-top-before(2), and #1-top-before(3) respectively

16. At the same time as step 14, take 3 separate soil samples with 2 cm diameter and 15 cm deep from plot #1-bottom,
17. Place the soil 3 samples in plastic bags and label each #1-bottom-before(1), #1-bottom-before(2), and #1-bottom-before(3) respectively
18. At the same time as step 14, take 3 separate soil samples with 2 cm diameter and 15 cm deep from plot #2-top
19. Place the 3 soil samples in plastic bags and label each #2-top-before(1), #2-top-before(2), and #2-top-before(3) respectively
20. At the same time as step 14 take 3 separate soil samples with 2 cm diameter and 15 cm deep from plot #2-bottom
21. Place the 3 soil samples in plastic bags and label each #2-bottom-before(1), #2-bottom-before(2), and #2-bottom-before(3) respectively
22. At the same time as step 14, take 3 separate soil samples each with 2 cm diameter and 15 cm deep from plot #3-top
23. Place the 3 soil samples in 3 separate plastic bags and label each #3-top-before(1), #3-top-before(2), and #3-top-before(3) respectively
24. At the same time as step 14, take 3 separate soil samples each with 2 cm diameter and it 15 cm deep from plot #3-bottom
25. Place the 3 soil samples in 3 separate plastic bags and label each #3-bottom-before(1), #3-bottom-before(2), and #3-bottom-before(3) respectively
26. The day after taking the samples described above, fill 3 buckets each with 7.57082 liters of sterile room-temperature (22-25° Celsius) water
27. Pour the entirety of each of the 3 buckets of water (7.57082 liters) at a steady rate down the hill, one through #1-top, #2-top, #3-top at the same time
28. One day after pouring the water in steps 25 and 26, repeat steps 13-25 substituting "before" with "after" when labeling the bags
29. Perform steps 30-40 at the same day at the same time
30. Use a clean and new transfer pipette to add 10 milliliters (ml) of sterile water to a 15 ml culture tube and label it "10^0#1topbefore(1)".
31. Use the same pipette from step 30 to add 9 ml of sterile water to a 15 ml culture tube and label it "10^{-1}#1topbefore(1)".
32. Use the same pipette from step 30 and 11 to add 9 ml of sterile water to a 15 ml culture tube and label it "10^{-2}#1topbefore(1)".
33. Place 1 cubic centimeter (cc) of the soil from area #1-top-before into the "10^0#1topbefore(1)" culture tube
34. Cap the tube and shake forcefully.
35. Using a clean and new transfer pipette remove 1 ml of the soil/water mixture from the "10^0#1topbefore (1)" tube and place it in the "10^{-1}#1topbefore (1)" culture tube.
36. Cap the tube and shake forcefully.

37. Using the same pipette from step 35, remove 1 ml of the soil/water mixture from the "10^{-1}#1topbefore (1)" culture tube and place it into the "10^{-2}#1topbefore (1)" culture tube.
38. Cap the tube and shake forcefully.
39. Repeat steps 30-38 with soil samples from plots: #1-top-before (2), #1-top-before (3), #1-top-after (1), #1-top-after (2), #1-top-after (3), #1-bottom-before (1), #1-bottom-before (2), #1-bottom-before (3), #1-bottom-after (1), #1-bottom-after (2), #1-bottom-after (3), #2-top-before (1), #2-top-before (2), #2-top-before (3), #2-top-after (1), #2-top-after (2), #2-top-after (3), #2-bottom-before (1), #2-bottom-before (2), #2-bottom-before (3), #2-bottom-after (1), #2-bottom-after (2), #2-bottom-after (3), #3-top-before (1), #3-top-before (2), #3-top-before (3), #3-top-after (1), #3-top-after (2), #3-top-after (3), #3-bottom-before (1), #3-bottom-before (2), #3-bottom-before (3), #3-bottom-after (1), #3-bottom-after (2), #3-bottom-after (3), and label the culture tubes respectively
40. Plate 100 µl samples from each of the culture tubes onto their own separate 3M Petrifilm^{TM} Yeast and Mold Count Plate that are 10x4 cm containing nutrient agar with their according label (ex: no erosion"10^{0}#1topafter(1)")
41. Allow the culture to grow for 48-72 hours at room temperature (22-25° Celsius)
42. Examine each of the 3M Petrifilm^{TM} Yeast and Mold Count Plates starting with the lowest dilution and see if any yeast or mold colonies are present and record the amount of yeast or mold colonies and at what dilution level they were found
43. If a yeast or mold colony is not found in the lowest dilution, progress towards the higher dilutions, 1 dilution level at a time until a yeast or mold is found and record the amount of yeast or mold colonies and at what dilution level they were found
44. Use the provided formula to calculate the density of yeast and mold in the original soil sample

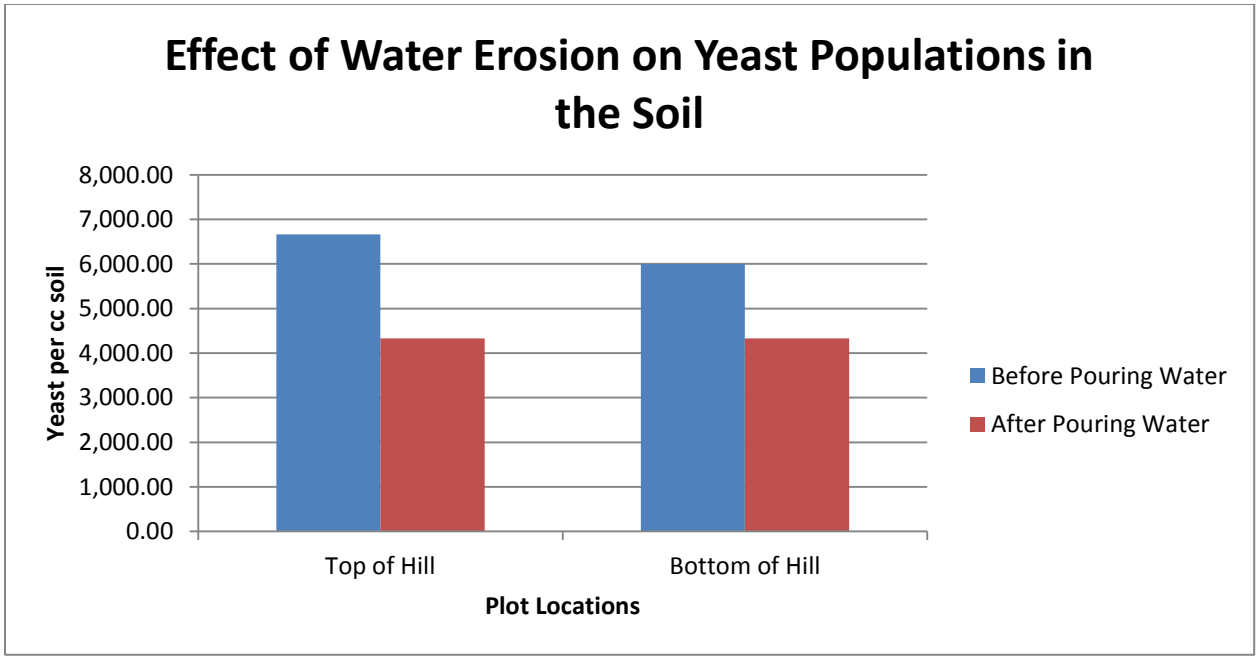
#fungi in 1 cc of soil = # Colonies on sheet x 10^{2} x $10^{|\text{dilution \# at which the colonies were found}|}$

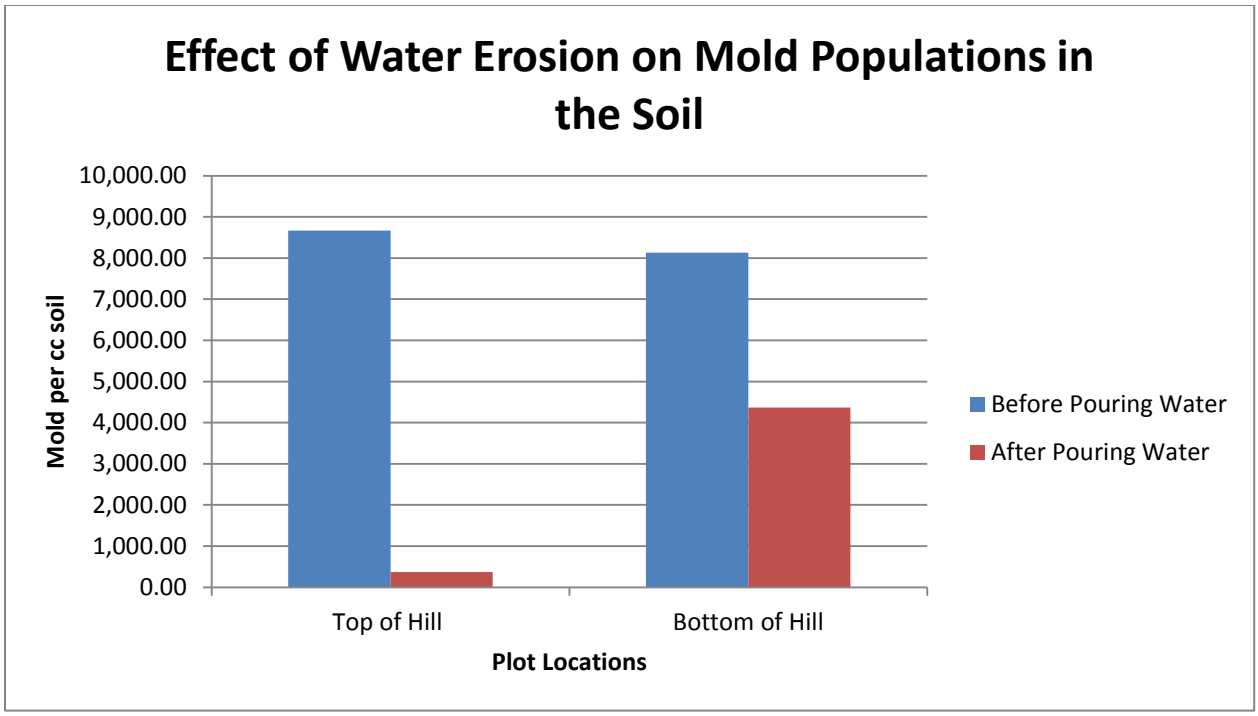
Soil Fungi Densities Before and After Simulated Erosion

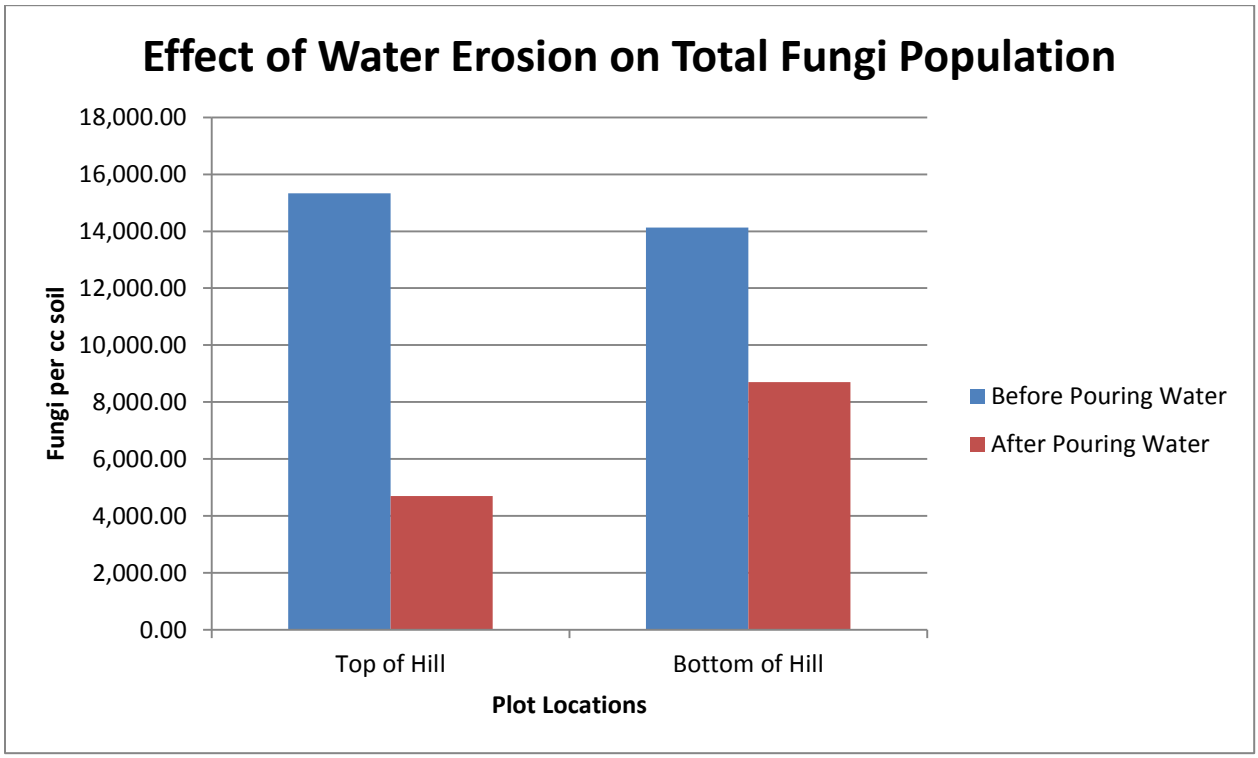
Before Pouring Water			
Location	# yeast/cc soil	# mold/cc soil	Total # of Fungi/cc soil
Site 1 Top of Hill- Before Pouring Water	10,000 yeast per cc	20,000 mold per cc	30,000 fungi per cc
Site 1 Bottom of Hill -Before Pouring Water	10,000 yeast per cc	400 mold per cc	10,400 fungi per cc
Site 2 Top of Hill- Before Pouring Water	10,000 yeast per cc	5,000 mold per cc	15,000 fungi per cc
Site 2 Bottom of Hill- Before Pouring Water	6,000 yeast per cc	14,000 mold per cc	20,000 fungi per cc
Site 3 Top of Hill- Before Pouring Water	0 yeast per cc	1,000 mold per cc	1,000 fungi per cc
Site 3 Bottom of Hill- Before Pouring Water	2,000 yeast per cc	10,000 mold per cc	12,000 fungi per cc
Averages Fungi Densities Before Pouring Water			
Location	# yeast/cc soil	# mold/cc soil	Total # of Fungi/cc soil
Top of Hill- Before Pouring Water	6,666.67 yeast per cc	8,666.67 mold per cc	15,333.33 fungi per cc
Bottom of Hill- Before Pouring Water	6,000 yeast per cc	8,133.33 mold per cc	14,133.33 fungi per cc

After Pouring Water			
Location	# yeast/cc soil	# mold/cc soil	Total # of Fungi/cc soil
Site 1 Top of Hill- After Pouring Water	2,000 yeast per cc	800 mold per cc	2,800 fungi per cc
Site 1 Bottom of Hill-After Pouring Water	2,000 yeast per cc	100 mold per cc	2,100 fungi per cc
Site 2 Top of Hill- After Pouring Water	10,000 yeast per cc	0 mold per cc	10,00 fungi per cc
Site 2 Bottom of Hill - After Pouring Water	10,000 yeast per cc	3,000 mold per cc	13,000 fungi per cc
Site 3 Top of Hill - After Pouring Water	1,000 yeast per cc	300 mold per cc	1,300 fungi per cc
Site 3 Bottom of Hill - After Pouring Water	1,000 yeast per cc	10,000 mold per cc	11,000 fungi per cc
Averages Fungi Densities After Pouring Water			
Location	# yeast/cc soil	# mold/cc soil	Total # of Fungi/cc soil
Top of Hill- After Pouring Water	4,333.33 yeast per cc	366.67 mold per cc	4,700 fungi per cc
Bottom of Hill- After Pouring Water	4,333.33 yeast per cc	4,366.67 mold per cc	8,700 fungi per cc

Graphs




Effect of Water Erosion on Mold Populations in the Soil
10,000.00
9,000.00
8,000.00
7,000.00
6,000.00
5,000.00
4,000.00
3,000.00
2,000.00
1,000.00
0.00
Mold per cc soil
Top of Hill
Bottom of Hill
Plot Locations
Before Pouring Water
After Pouring Water


Effect of Water Erosion on Total Fungi Population
18,000.00
16,000.00
14,000.00
12,000.00
10,000.00
8,000.00
6,000.00
4,000.00
2,000.00
0.00
Fungi per cc soil
Top of Hill
Bottom of Hill
Plot Locations
Before Pouring Water
After Pouring Water

Conclusion

Our hypothesis was disproven. We hypothesized that water erosion decreases the saprotrophic fungi population at the top of a hill and increases the saprotrophic fungi population at the bottom of the hill. We predicted that erosion would decrease the fungi population in the soil at the top of the hill because we researched the effects of erosion, and it is said to displace the topsoil of the affected area and deposit the soil where it stops moving (Plants and Soil Science Library, 2014). We assumed the water would stop moving at the bottom of the hill and deposit particles of the topsoil, in which the fungi live, from the top of the hill. From this assumption, we then suspected that the fungi population would increase at the bottom of the hill. We were incorrect because, as the graph indicates, the fungi population decreased at the top of the hill, and at the bottom of the hill after the erosion. The top plots originally had an average of 15,333.333 fungi per cc, and the bottom plots originally had 14,133.333 fungi per cc. After pouring the water on the hill, the top plots had an average of 4,700 fungi per cc, while the bottom plots had an average of 8,700 fungi per cc. Both the top and the bottom plots' fungi populations decreased, but the bottom plots were not as severely impacted. The yeast and mold populations decreased as well. The top plots before pouring water originally had an average of 6,666.667 yeast per cc and after pouring the water, the top plots had an average of 4,333.333 yeast per cc. Before the pouring of the water, the top plots has an average of 8,666.667 mold per cc. After the pouring of the water, the top plot had an average of 366.667 mold per cc. The bottom plots before pouring water originally had an average of 6,000 yeast per cc and after the pouring of the water, they had on average 4,333.333 yeast per cc. The bottom plots before the pouring of the water had on average 8,133.333 mold per cc and after the pouring of the water, they had the average of 4,366.667 mold per cc. From this data it is apparent that the yeast and mold

populations decreased. The mold populations had a very dramatic decrease, and the fungi populations had a slightly less dramatic decrease. The yeast population remained stable before and after the pouring of the water which indicates that there was another environmental factor that made both the yeast populations decrease other than erosion. We know that the erosion did not decrease the yeast population because when the mold population goes up, the yeast population should decrease (Brock, 2014); yet, our data shows that the mold and yeast populations both went down. Our experiment showed that water erosion decreased the fungi populations in soil at the top of the hill and at the bottom of the hill and it showed that there was another environmental factor affecting the molds, causing their population to have a very dramatic decrease.

For future research, we hope to discover the environmental factor that caused the yeast populations to decrease. We would test to see if the temperature of the soil could be the possible environmental factor (AgriInfo, 2011). Our first step would be to take soil samples, and then we would expose the soil to various temperatures. We would test the soil at 25°C for the negative control, and count the yeast and mold populations. Next, we would expose the soil to temperatures such as 0°C and 60°C, and again count the yeast and mold populations. After counting the yeast and mold populations for all three temperatures, we would be able to calculate the total fungi population. Using the yeast population count, we would then be able to determine if the temperature of the soil was the environmental factor that caused the yeast population to decrease at the stable rate it did in our original erosion experiment.

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