Herbicides and Their Effect on the Fungi Population in the Soil: By

Annaliese Rea, Marq Hayes, and Gina Drum

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Ms. Riina

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Background on Plants and Fungi

Fungi colonize in the roots by physically binding together to create mycelium. This is how fungi takes and receive nutrients from plants. In the soil, the soil contains yeast and molds. Fungi have two major categories: yeast and mold. The yeast is the formation of fungi that is living in a stressful environment. Yeast has a lack of resources that can make the fungi go into a stage of survival mode. This means that it only performs functions that are absolutely important to life. The mold is the formation of happy fungi that are living in a happy environment. Along with performing its own tasks, the mold performs tasks that benefit other organisms. Some fungi have a symbiotic relationship with plants these can be broken up into 2 categories: ectomycorrizhae and endomycorrizhae. Ectomycorrizhae grows around the roots of the trees whereas endomycorrizhae grow within the roots are associated with grass, crops, and shrubs (Ingham, 2014) these associations are mutually beneficial for plants and fungi. Fungi help solubilize plants by dissolving low solubility compounds that contain the plant nutrients, which the plant can't do. In return the plant feeds the Fungi photosynthetically produced sugars, so the fungi can perform its daily tasks.

Fungi perform important tasks, regulation of water flow, and recycling nutrients in the soil. A fungus relies on the plants to provide photosynthetically produced sugars from the plants, to perform its daily tasks. Fungi in return, give the plant phosphorous so that the plant can create ATP to be used in its cells for energy. Fungi also create aggregates to maintain porous soil structure. Phosphorous is a major component in ATP for the plants. Phosphorous is essential role in energy transferring process in an organism (Jones, 2009) Fungi binds soil particles together to make a mycelium web structure. This structure makes space for the water and oxygen because this too play an essential role in photosynthesis for the plants. The Water has hydrogen atoms that the plant uses to collect energy. The oxygen is a major component of photosynthesis because it comes from carbon dioxide which the plant needs to perform photosynthesis. The phosphorous is also important because it is a component of complex nucleic acids that help regulate photosynthesis.

Humans use herbicides to kill or inhibit unwanted vegetation. Forestry herbicides are used on sites for tree planting to decrease the amount of unwanted vegetation growing on the site (Henderson, 2006). Herbicides added to the site, they reduce the effect that herbaceous weeds have, so that young trees have a better chance at survival and reproduction. Herbicides are important for agriculture because it helps decrease the weed population. The weed population takes away nutrients that the crops need to live. Herbicides are most frequently used in row crop farming (Belden, 2005). Herbicides are applied to the leaves of the weed where it moves in the water filled space around the cells. Then the herbicides pass through the plasma membrane of the cell and target the important enzymes (Henderson, 2006) once the herbicides attaches to the enzymes, it starts to build up in the plant cells until the toxins destroy the weed. It is purposely made to attack the weed not the crop itself.

There are multiple applications of herbicides which kill weeds using different methods. “Contact herbicides” are herbicides that come into direct contact with the plant. “Systemic herbicides” are slower to work, but more effective as they move through the roots of the plant (Ganzel, 2009). There is also “sprayed herbicides”, which is when the herbicide chemicals are mixed with water to be sprayed on fields. Finally there are “soil-applied herbicides” which are injected into the soil to contact the roots more quickly (Ganzel, 2009). Overall, there are many different ways to use herbicides in order to get rid of unwanted vegetation. In our case, we are using Round-Up™, the active ingredient is glyphosate. Glyphosate is what specifically targets the important enzymes, in order to kill off the unwanted vegetation.

In our experiment, we are looking at how herbicides affect the population density of the fungi in the soil. We hypothesized that when the herbicide Round-Up™ is applied, the population density of fungi will decrease. Once the weeds are killed, the roots of the weeds will also die because the plants are no longer providing them with the necessary sugars to live.

Herbicides and Their Effect on Soil Lab Report

I. Problem: Does the presence of herbicides change the population density of fungi in the soil?
II. Hypothesis: Herbicides will decrease the population density of fungi in the soil.
III. Procedure:
    a. Independent variable- presences of herbicides on soil
    b. Dependent variable- population density of fungi in 1 cc soil
    c. Negative control – presence of water on soil

d. Controlled variables- amount of herbicides used, type of soil tested, type of herbicides used, where the samples are extracted from, amount of herbicides used, amount of soil tested, size of culture tubes used, size of serological pipettes, amount of water used to dilute samples, type of water used in dilution, type of plate used, amount of time herbicides are left on soil, amount of time water is left on soil, amount of time that is given for the fungi to grow

e. Step-by-step-

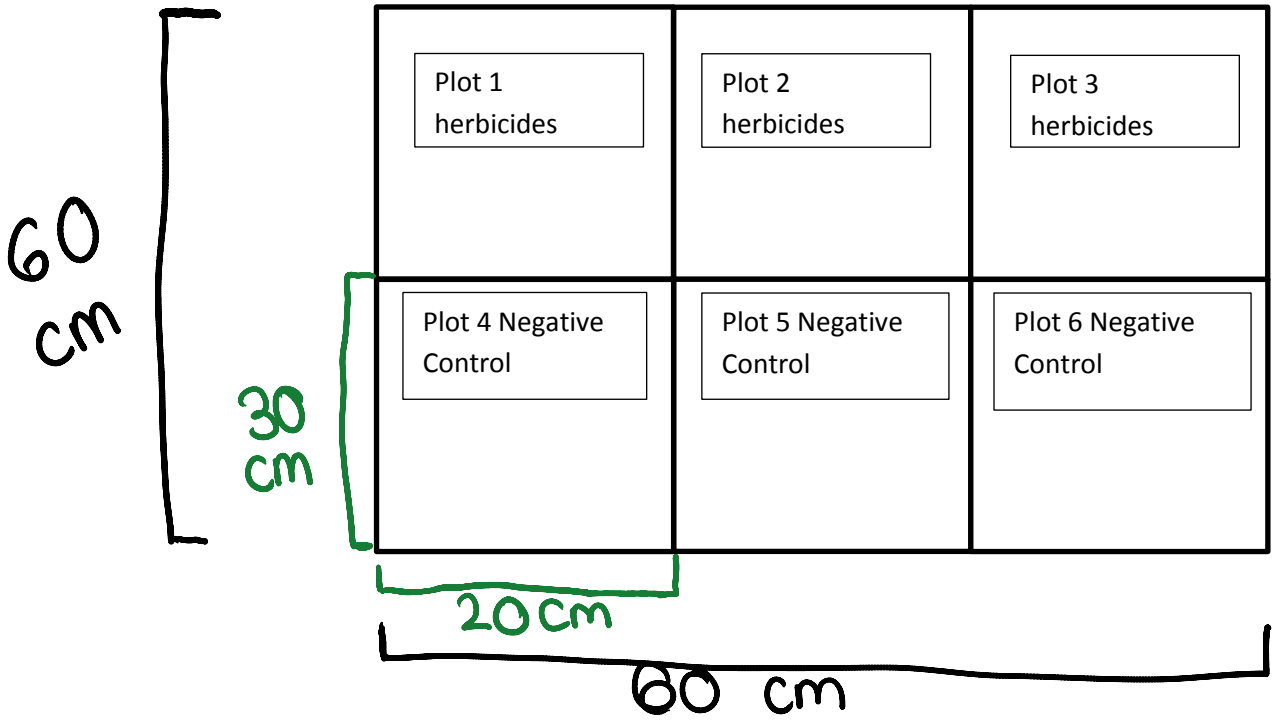
1. Make a 60 cm by 60 cm square at a location with coordinates of N: 39.35811^{o} W: 075.63625^{o}
2. Split the square into six equal 20 cm by 30 cm plots (see Diagram 1)
3. Extract 15 cm of soil from plot 1 by taking a soil extractor (with a diameter of 2 cm) and a hammer and sticking the soil extractor into the ground. Hit it with the hammer until you get to the 15 cm into the soil, and then pull it up from the ground.
4. Put the soil that you extracted from the ground into a zip lock bag and label it “before plot 1”
5. Complete step 3-4 for samples from plots 2-6 and label them “before” and the respective plot number
6. In steps 7-18 perform dilution procedure on the same day at the same time for all “before” samples
7. Put 10 ml of sterile water into a 15 ml culture tube and label it “10^{0} plot 1”

8. Put 9 ml of sterile water in another 15 ml culture tube and label it "10^{-1} plot 1"
9. Repeat step 8 and label the culture tube "10^{-2} plot 1"
10. Put 1 cc of soil from plot 1 into the "10^{0} plot 1" culture tube and cap this and shake
11. Use a clean serological pipette remove 1 ml of mixture from the "10^{0} plot 1" culture tube and add it to "10^{-1} plot 1" culture tube
12. Cap and shake hard
13. Keeping the same serological pipette from step 11, and remove 1 ml of the mixture out of "10^{-1} plot 1" culture tube and add it to "10^{-2} plot 1" culture tube
14. Cap and shake hard
15. label three 3M Petrifilm™ yeast and mold count plates "plot 1 10^{0}", "plot 1 10^{-1}" and "plot 1 10^{-2}"
16. Plate 100µL of "10^{0} plot 1" solution onto the fungi dish that says " plot 1 10^{0}" using P200 micropipette
17. Repeat step 16 for 10^{-1} and 10^{-2} dilution respectively from plot 1
18. Complete steps 7- 17 with soil from plots 2-6 on the same day at the same time
19. Allow the fungi to grow for 48 hours
20. Examine the "10^{-2}" plate first and find one yeast colony and one mold colony. The yeast colonies look like sharp dots and the mold colonies look lake large fuzzy green spots. If you cannot find yeast or mold

colonies on "10^{-2}" plate then move to the "10^{-1}" plate. If there are still no yeast or mold colonies look at the 10^{0}" plate.

21. Record the dilution number on a data table and then record the number of yeast and mold colonies for each plot sample
22. At the same time complete steps 20-21 with soil from plots 2-6 on the same day and time # Microbes in 1 cc of soil = # colonies on sheet x 10^{2} x $10^{|\text{dilution number at which the colonies were found}|}$
23. Repeat steps 3-22 with labeling it "trial 2 before" and then with the respective plot numbers
24. Spray 26 sprays of Round Up™ Herbicides onto plots1-3
25. Spray 26 sprays of tap water onto plots 4-6
26. Let the plots sit for 48 hours
27. Repeat steps 3-23 labeling all samples "after" and respective plot and trial number

Diagram 1:



IV. Data and Analysis:

Number of Yeast and Mold Colonies in 1 cc of Soil

Soil Sample Number	Number of yeast (fungi) colonies on plate	Number of mold colonies on plate	Total number of fungi in 1 cc of soil
Plot 1 trial 1 before	300	3000	3300
Plot 2 trial 1 before	3000	1000	400
Plot 3 trial 1 before	400	1000	1400
Plot 4 trial 1 before	100	2000	2100
Plot 5 trial 1 before	30000	3000	33000
Plot 6 trial 1 before	200	100	300
Plot 1 trial 2 before	200	400	600
Plot 2 trial 2 before	600	100	700
Plot 3 trial 2 before	700	300	1000
Plot 4 trial 2 before	300	100	400
Plot 5 trial 2 before	100	100	200
Plot 6 trial 2 before	300	200	500
Plot 1 trial 1 after	900	400	1300
Plot 2 trial 1 after	700	100	800
Plot 3 trial 1 after	1300	500	1800
Plot 4 trial 1 after	600	200	800
Plot 5 trial 1 after	300	100	400

Plot 6 trial 1 after	1200	400	1600
Plot 1 trial 2 after	1000	100	1100
Plot 2 trial 2 after	1000	1000	2000
Plot 3 trial 2 after	1000	1000	2000
Plot 4 trial 2 after	1000	1000	2000
Plot 5 trial 2 after	1000	300	1300
Plot 6 trial 2 after	10000	1000	11000

Average number of Fungi in Soil Before and After Herbicides in 1 cc of Soil- Trial 1

	Average number of fungi colonies in 1 cc of soil before herbicides	Average number of fungi colonies in 1 cc of soil after herbicides
	1700	5233
Negative Control	11800	933

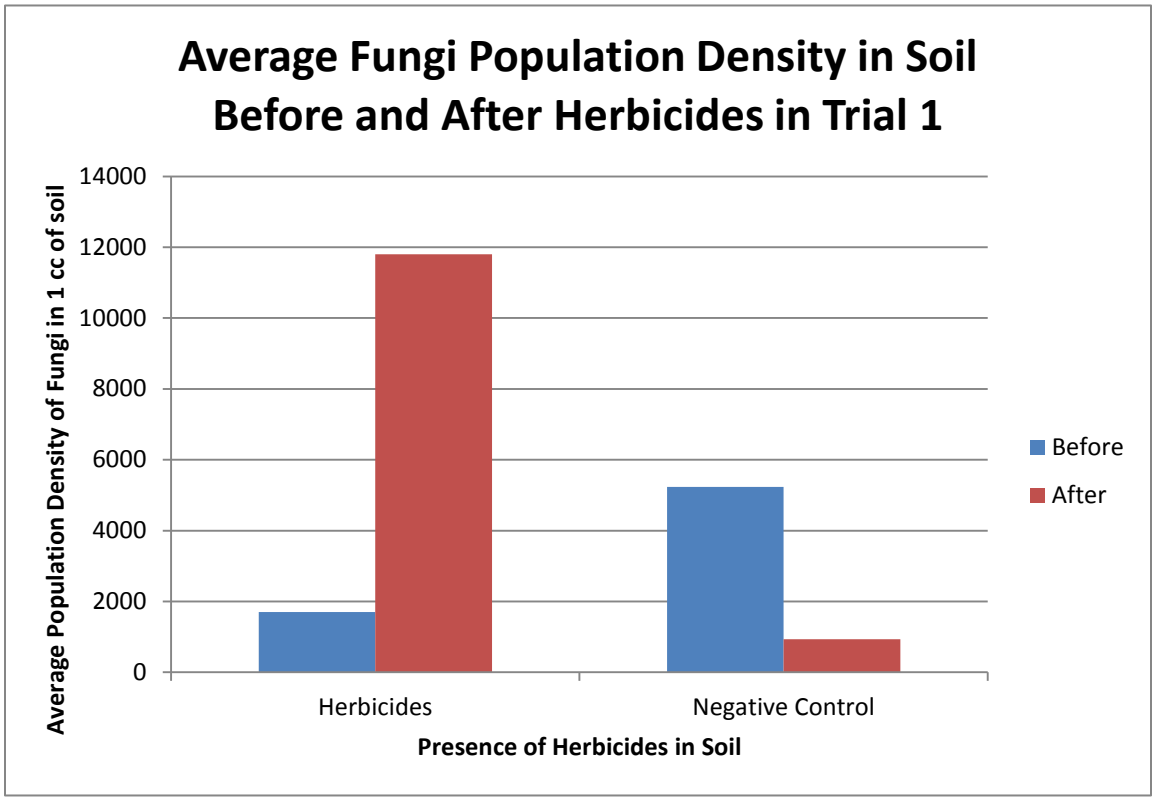
Average number of Fungi in Soil Before and After Herbicides in 1 cc of Soil- Trial 1

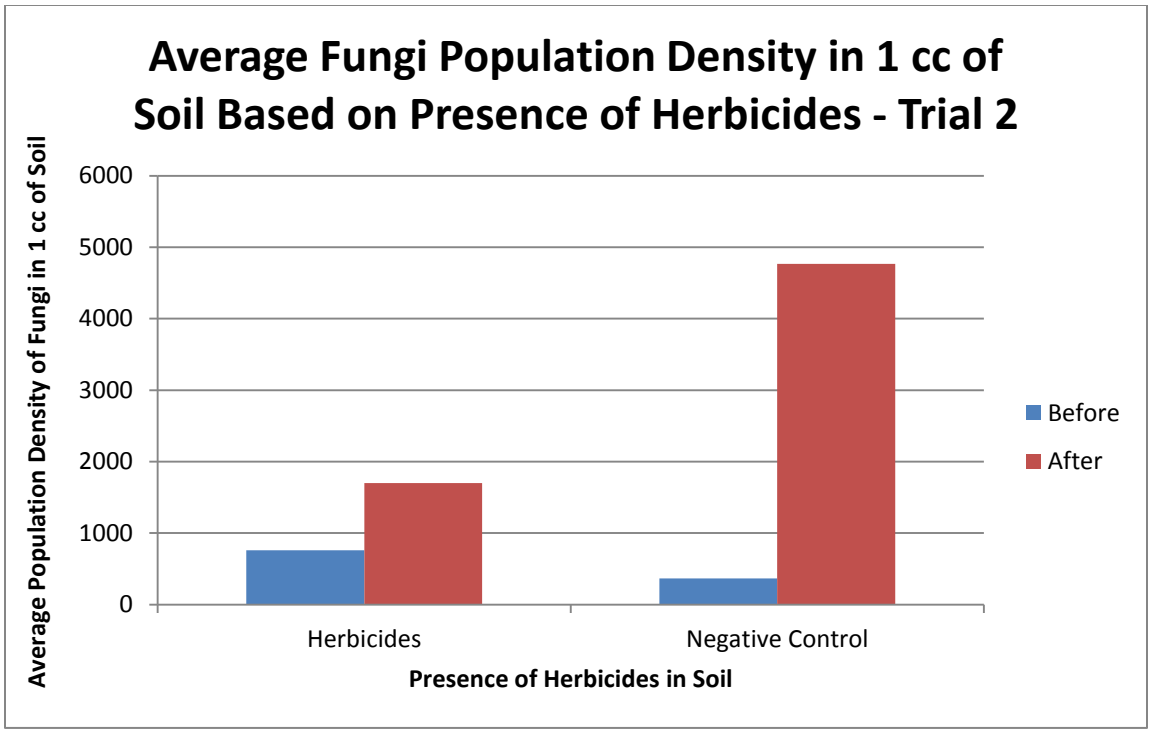
	Average number of fungi colonies in 1 cc of soil before herbicides	Average number of fungi colonies in 1 cc of soil after herbicides
	766	1700
Negative Control	366	4766

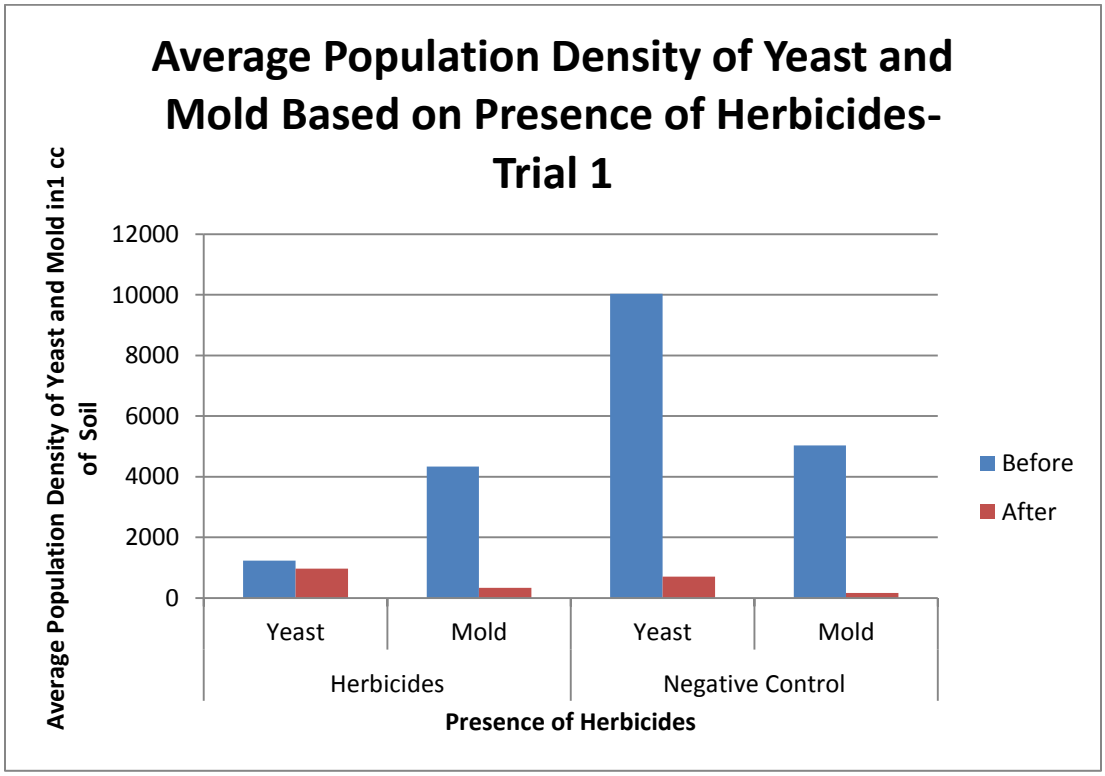
Average Number of Yeast and Mold Before and After Herbicides in 1 cc of Soil

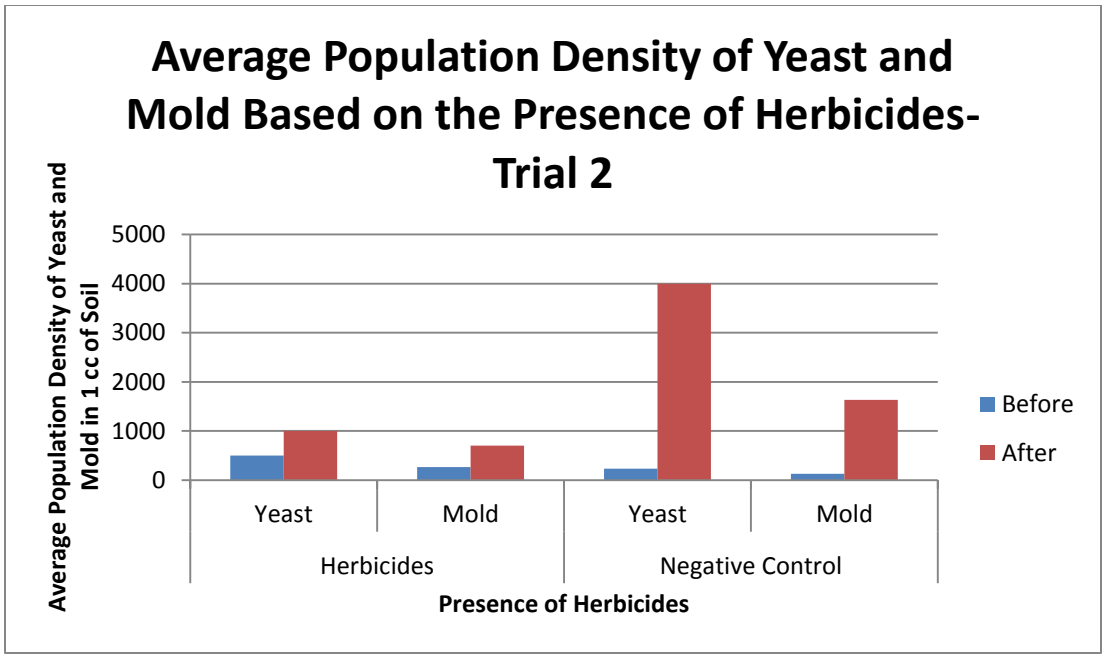
	Average number of yeast colonies in 1 cc of soil				Average number of mold colonies in 1 cc of soil			
	Before herbicides		After herbicides		Before herbicides		After herbicides	
		Negative Control		Negative Control		Negative Control		Negative Control
Trial 1	1233	10040	966	700	433	5033	333	166
Trial 2	500	233	1000	4000	266	133	700	1633

a. Graph-




Average Fungi Population Density in 1 cc of Soil Based on Presence of Herbicides - Trial 2
Average Population Density of Fungi in 1 cc of Soil
6000
5000
4000
3000
2000
1000
0
Herbicides
Negative Control
Presence of Herbicides in Soil
Before
After


Average Population Density of Yeast and Mold Based on Presence of Herbicides- Trial 1
Average Population Density of Yeast and Mold in1 cc of Soil
12000
10000
8000
6000
4000
2000
0
Yeast
Mold
Yeast
Mold
Herbicides
Negative Control
Presence of Herbicides
Before
After



V. Conclusion:

Our fungi are categorized into 2 categories: yeast and mold which are the formation of fungi that live in certain environments. In our case the yeast is the formation of fungi that is growing in a stressful environment. Making the fungi go into survival mode and only perform the tasks it needs to survive. The mold, is the formation of fungi that is living in a happy environment where it makes the tasks it needs to survive, and also can perform other tasks to benefit other organisms. If there is more mold colonies than yeast colonies, then they are living in a healthy environment. If there are more yeast colonizes than mold colonies, then there is something in the environment that is causing some of the fungi to become stressed. Our data shows that the environment that they are living in has something that is making the fungi stressful. Our hypothesis was that once the herbicides were applied to the soil, the population would decrease; however in this case the herbicides caused the population of the fungi colonize to increase for trail one

before herbicides, the ratio of yeast to mold colonies was 3:1. For the after herbicides the ratio for yeast to mold was 3:1 also. The before negative control yeast to mold colonies was 2:1 and the after yeast to mold was 4:1. for trial 2 the before herbicides yeast to mold was 2:1 and the after was1:1. There was an increase in the yeast population compared to the mold population, so some outside factor is stressing out the fungi making them live in an unhealthy environment. So in a way the after herbicides form trial 2 benefitted from the herbicides in trail 1. For the before negative control the ratio was 2:1 and the after was also 2:1. Average number of fungi colonies before herbicides are 1233 colonies per cc of soil. The average numbers of fungi colonize after herbicides are 1500 colonies per cc of soil. For the negative control, the average number of fungi colonies before applying water are 6083 colonies per cc of soil and the after water are 2850 colonies per cc of soil. This decreased in fungi population must have been caused by an outside factor because we only applied water.

A future experiment that could be performed as a follow up experiment to the one we conducted would be to test a specific brand of herbicide and its effect on the fungi population around trees compared to the fungi population around shrubs and herbaceous weeds. We only used how the herbicide Round Up™ affected the fungi population in the soil under the grass in different plots. This experiment would be useful because then you would be able to see if herbicides affect different fungi population equally or if a specific herbicide kills one type of fungi population more than another based on if its located under trees or shrubs.

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