Baking Soda as a Fungicide

Mr. Brock-Biology 9H

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"We have acted honorably."

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Background Report:

Fungicides are chemicals that are used to kill fungi and stop fungi reproduction (Walker, 2010). They come in many different forms, one of which includes contact fungicides. These inorganic chemicals are sprayed onto surfaces to prevent (McGrath 2004) any fungi from adhering to them in the first place (Mueller 2006). Fungicides work by preventing the development of a biofilm. Normally, fungi require a thin layer of bacteria on a surface in order to adhere to it; however, contact fungicides poison this bacterial layer. Because the formation of a biofilm is prevented, the fungi are not able to access the nutrients on whatever surface they have landed on, and they die (Brock, 2006).

One important use of surface fungicides are used to protect is the leaves of plants. Normally when fungi infest a plant's surface, they begin the decomposition process, otherwise known as biodegration (Grolier Multimedia Encyclopedia: *Decomposition (biology),* 2010). The fungi release enzymes to break down the plants cells into the molecular components. The Fungi then use the plant's five biological molecules for their own growth and development. During this decomposition process, the fungi are able to multiply and spread across the surface of the leaves, consuming the plant until the plant eventually dies. Therefore, unless fungicides prevent the fungi from adhering to the surface of the plant in the first place, the plant will eventually die. (Brock, 2006).

The application of fungicides can have a major impact on the environment. When fungicides are used on the surface of plants, the fungicides also affect those organisms living in the soil due to the rain washing the remains of the fungicide into the soil. The fungicides that are used to kill fungus on the surface of plants, therefore, have the potential to also harm the three main categories of fungi which inhabit the soil, ultimately affecting the overall environment. These three categories include mutualists, decomposers and pathogens. Mutualists are a very significant type of fungi that have a symbiotic relationship with the plant. Mutualists organize together with plant roots so that the plant is able to absorb nutrients, such as phosphorus and nitrogen (Jenkins, 2006), which helps the plant to grow new leaves and branches (Fogel, 2001), from the soil. The mutualists break up these key minerals and deliver them directly to the plant. The assortments of cells in mutualists protect the roots of the plant against their predators, allowing the plant to prosper. In return, the plant provides the fungi with food and therefore, plants and mutualists have a beneficial relationship with each other. Decomposers are also a very significant type of fungi in the environment because they take the nutrients from the materials that they break down and keep them present in the soil. Therefore, if fungicides reach the fungi in the soil, they could potentially kill the fungi that help the plant and ultimately cause an unhealthy environment for other species in that ecosystem (Jenkins, 2006). Overall, fungicides, while they help the plant from being fully taken over by the fungus, potentially have a negative impact on the soil in the environment.

One alternative fungicide treatment that some claim could potentially avoid harming the environment is using a baking soda and water solution (Kuepper, Thomas and Earles, 2001). Baking soda, considered to be a “green” alternative to inorganic and chemically based fungicides, has proven successful in both preventing and killing specific species of fungi, including PM, black spot, leaf spot, anthracnose, phoma, phytophthora, scab, and botrytis. Because it does not contain the harmful chemicals that most fungicides do, baking soda has been shown not to affect the sodium levels of the soil, water runoff, or plant tissues; therefore baking soda has the potential to be an organic substitute to the more harmful artificially manufactured fungicides (Kuepper, Thomas and Earles, 2001).

However the use of baking soda and water solution to kill or prevent fungi from attacking plants may itself harm the health of the soil. Baking soda has the potential to alter the pH of the soil while pH levels can tell us much about the soil's health. The measurement of how many hydronium ions are in the soil that is present in the environment, tells us how acidic or how basic something is (Young, 2010), and this level, known as pH, is important for many reasons. Few plants survive in soil with a pH less than four or above eight, in part because the pH controls the accessibility of the phosphorous in the soil. When the acidity increases, the phosphorous becomes difficult for the plants to access and since phosphorous is an important nutrient for plants and other organisms (it is a critical component of DNA and ATP), plants can't survive if the phosphorus becomes unavailable to them (Russell, 2010). Furthermore, when the soil becomes more basic the phosphorous combines with iron making this other compound, which is critical to photosynthesis in cell respiration, difficult for the plants to access (*Part 6 the Importance of Soil pH*, 2010). Most importantly, the pH level is very critical because each enzyme has a pH level at which it performs best (Spector, 2010) and if the pH level of an environment is too high or too low, the enzymes stop working correctly, essentially altering the shape of each enzyme of the fungus. This means that a cell's chemical reactions could not occur, and therefore, that the four main tasks of life could not happen. In short, altering the pH level significantly, prevents life from existing and because soil fungi are made up of cells that require enzymes to live, a change in the pH level can significantly affect both the organisms living in the soil and the soil's health (Campbell, Reece, Urry, Cain, Wasserman, Minorsky, and Jackson, 2008). If these enzymes are harmed, the level of fungi in the soil decreases. As the decrease in soil fungi occurs, there becomes less and less life that is capable of surviving in an environment without mutualists and decomposers, the two major categories of beneficial fungi in the soil (Jenkins, 2006). Overall, if the soil fungus in the environment is removed, the entire ecosystem is affected.

The method of using a baking soda and water solution has proven to be effective against eight different signs of fungi on the surface of plants; however the impact of the baking soda that runs into the soil has not yet been discovered (Kuepper, Thomas and Earles, 2001). According to Kuepper G., Thomas R., and Earles R. (2001), it is possible that one might need to add nutrients to their plant's soil. Why would one need to have nutrients added to the soil when there are organisms which provide the benefits of these nutrients? This speculation may lead to the question of what the baking soda is doing to these organisms. All of this information could lead one in the direction of questioning whether or not this "solution" is really better for the environment. Therefore our topic for the Soil Ecology Project adds on to the unanswered questions of these researchers (Kuepper, Thomas and Earles, 2001). Good amounts of fungi in the soil are what actually make a plant healthy, however, if fungicides run into the soil, we would like to test whether or not the fungicide, in our case: baking soda, actually affects the soil's amounts of fungi, both yeasts and molds. Although the exact process that baking soda performs to kill the fungi is unknown, we would like to find out if the amount of fungi in the soil is affected by this fungicide. We will test for the amount of fungi through taking soil samples, one from each of the six different treatment plots, and by performing multiple serial dilution tests which will be plated onto “Petrifilm^{tm} Yeast and Mold Count Plates”. These plates will grow the fungi until the fungi colonies are evident enough to count. It is known that when a fungus senses that the environmental conditions are not ideal, they transform from their mold to the yeast form due yeast being more likely to survive in harsher environments (although there is no change in their DNA). Therefore the amount of yeasts and molds that are counted will also have an impact on our conclusions to this experiment. We would also like to investigate whether or not the soil is left unhealthy after the solution is used, and we will do this by testing the pH levels (Ingham, 2009).

Procedure:

I. **Problem**: Does baking soda have an impact on the density of fungi and the pH level in the soil?

II. **Hypothesis**: Baking soda decreases the density of fungi and increases the pH level in the soil.

III. **Procedure**:
   A. **Independent Variable**: whether the baking soda treatment is applied to the soil

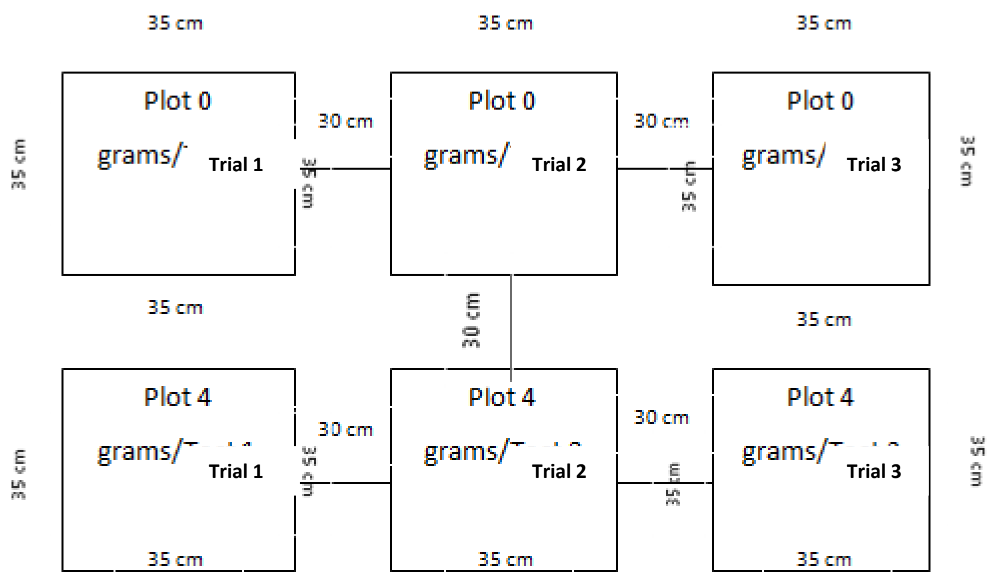
   B. **Dependent Variable**: density of fungi and pH level in the soil samples

   C. **Negative Control**: applying plain water to the soil.

   D. **Controlled Variables**:
   - Amount of deminerilized water
   - Amount of sterilized water
   - Amount of tap water
   - Size of plot
   - Amount of soil tested
   - Amount of soil taken for sample
   - Distance between plots
   - Location of plots
   - Size of test tubes
   - Type of chemicals used

- Amount plotted on each agar plate
- Type of nutrient agar plates
- Time and date of soil collection
- Time between applying baking soda solution and taking soil samples
- Amount of time for fungi to grow on agar plates
- Degree to which the samples were diluted
- Plating all dilutions
- Timing of fungus and pH experiment
- Amount of baking soda in the solutions

1. Step by Step procedure
    1) Find a grassy area on the RPCS campus with soil that is not polluted with pesticides, fungicides, etc. To find the exact location use a GPS, the coordinates should be North: 39.35711°, West: 076.63709°
    2) Then mark six plots with four flags in each corner. Each plot should be 35×35 cm with 30 cm between each plot, as shown in the diagram below. Label each plot directly on the flags depending on the plot treatment and trial number. For example, Plot 0 grams Test 1, Plot 4 grams Test, etc. depending on the test.



    3) Now take 3 separate soil samples from the center of each plot using the soil sampler to take soil samples each 15 centimeters deep and 2 centimeters wide. Make sure to record the date and time of the sample collecting. Take all the samples at the same time on the same day.
    4) Put each of these samples in their correspondingly labeled "Before" bag, with its label including the treatment of the plot, trial number, and test number, as well as the date and time (e.g. Plot 0 grams, Trail 1, Test 1, 8:30 A.M. 5/2/10 "Before").

5) All experiments for both pH and fungi should be tested at the exact same time on the exact same day, in other words, do both the pH and fungi tests simultaneously.
6) Now use the LaMotte Model STH-14 Outfit Series pH test kit to test the pH level of each of the "Before" samples before the solutions are added to the soil.
7) Now record your results in the "pH before solution" column of the data table.
8) To test for Fungi simultaneously first place 3 15 ml culture tube test tubes into a white test tube rack and gather 3 lids which are placed beside the rack.
9) Label the 1st test tube "10^{0} Plot 0 grams Trial 1"
10) Label the 2nd test tube "10^{-1} Plot 0 grams Trial 1"
11) Label the 3rd test tube "10^{-2} Plot 0 grams Trail 1"
12) Use a new pipette to add 10 ml of water to the first 15 ml culture tube labeled "10^{0} Plot 0 grams Trial 1"
13) Use the same pipette to add 9 ml of water to the second 15 ml culture tube labeled "10^{-1} Plot 0 grams Trial 1"
14) Use the same pipette to add 9 ml of water to the third 15 ml culture tube labeled "10^{-2} Plot 0 grams Trial 1"
15) Place 1 cc of your "Before Plot 0 grams Trial 1" soil sample into the "10^{0} Plot 0 grams Trial 1" culture tube
16) Cap the tube and shake vigorously.
17) Using a new clean serological pipette pump, remove 1 ml of the soil/water mixture from the "10^{0} Plot 0 grams Trial 1" test tube and place into the "10^{-1} Plot 0 grams Trial 1" tube.
18) Cap and shake vigorously.
19) Using the same serological pipette pump in step 17, remove 1 ml of the soil/water mixture from the "10^{-1} Plot 0 grams Trial 1" tube and place into the "10^{-2} Plot 0 grams Trial 1" tube.
20) Cap and shake vigorously.
21) You should now have a total of 3 culture tubes each with a different dilution of soil.
22) Gather 3 Petrifilmtm "Yeast and Mold Count Plates" and label the 1st plate "10^{0} Plot 0 grams Trial 1", the 2nd plate "10^{-1} Plot 0 grams Trial 1" and the 3rd plate "10^{-2} Plot 0 grams Trail 1"
23) Plate 100 μl onto the "10^{0} Plot 0 grams Trial 1" Yeast and Mold Count Plate"
24) Repeat steps 22-23 for both the "10^{-1}" test tube, releasing this one onto the "10^{-1} Plot 0 grams Trial 1", "3M Petrifilmtm Yeast and Mold Count Plate", and also repeat for the "10^{-2}" test tube, releasing this one onto the "10^{-2} Plot 0 grams Trial 1" "3M Petrifilmtm Yeast and Mold Count Plate". Be sure to change the tips each time.
25) You should now have 3 Petrifilmtm "Yeast and Mold Count Plates" each with a different dilution step on it.
26) Repeat steps 8-25 on the soil from the bags labeled "Plot 0 grams Trial 2", "Plot 0 grams Trial 3", "Plot 4 grams Trial 1", "Plot 4 grams Trial 2", and "Plot 4 grams Trial 3".

27) Allow for all 18 of the "Petrifilm™ Yeast and Mold Count Plates" to grow for 72 hours.
28) After 72 hours have passed, use a magnifying glass to count the amount of colonies of mold and the amount of colonies of yeast on the 10^{-2} plate for each trial of each treatment. If there is no yeast or mold on level 10^{-2} for a specific treatment trial, move to the next level up e.g. the 10^{-1} plate.
29) Record your results in the "Before" data table in the # of Yeast or # of mold column of the data table.
30) Then use the equation: # of microbes in 1 cc of soil = # of colonies on sheet × $10^2 \times 10^{|\text{dilution \# at which these colonies were found}|}$, for the yeast and mold count from each treatment plot, and trial number. Record this information in the "Before" data table in the # of Yeast per 1 cc of soil column for the yeast and the # of Mold per 1 cc of soil for the mold. Add these two figures together for the total # of Fungi in 1 cc of soil and recording this in a separate column. .
31) Now you will have the results in a data table for both the pH and fungi levels before adding the baking soda and water solution.
32) Now you are ready to start the initial experiment.
33) Make three tap water solutions. In three plastic bottles pour 500 ml of plain tap water into each bottle and label them 0 grams of baking soda.
34) Make three baking soda and water solutions. In three other plastic bottles pour 500ml of tap water and then mix 4 grams of baking soda into each plastic bottle. Label these three bottles 4 grams of baking soda.
35) Now put aluminum foil over top of the opening of the bottle and secure with a rubber band. Now use a pencil to poke three holes in the foil to simulate a watering can.
36) Pour the "0 grams" bottles onto the plot 0 grams Trial 1, 2 and 3 distributing evenly, using a different 500 ml bottle for each separate plot.
37) Then pour the "4 grams of baking soda" bottles onto each plot labeled 4 grams Trial 1, 2 and 3 distributing evenly, using a different 500ml bottle for each separate plot.
38) Take all the samples at the same time on the same day.
39) Wait 1 full day for the solution and water to soak in. Go back and take 3 separate soil samples from the center of each plot using the soil sampler to take a soil sample 15 centimeters deep and 2 centimeters wide each. Put each of these samples in their correspondingly labeled "After" bag, with its label including the treatment of the plot, trial number, and test number, as well as the date and time (e.g. Plot 0 grams, Trail 1, Test 1, 8:30 A.M. 5/14/10 "After").
40) You must again test for pH and fungi density on the same day at the same time.
41) Do the pH tests by using the LaMotte Model STH-14 Outfit Series pH test kit to test the pH level of each of the "After" samples.
42) Record the pH level in the "pH after the solution" column.
43) To test for the amount of fungi after adding the solution follow steps 8-31.
44) Now, you should have gathered the levels of pH before, the amount of fungi in 1cc of soil before the solutions, the pH level after the solutions, and lastly, the

amount of fungi in 1 cc of soil after the solutions were added to the soil. Compare the results.

Data and Analysis:

Data Tables:

Fungi Tests and pH Before Baking Soda Solution Added to Soil

Plot treatment/trial number/test number	pH Level	Number of yeast per cc of soil	Number of mold per cc of soil	Total amount of fungi per cc of soil
4 grams of baking soda/Trial 1/Test 1	8.2	15,000	19,000	34,000
4 grams of baking soda/Trial 2/Test 1	8.2	80,000	60,000	140,000
4 grams of baking soda/Trial 3/Test 1	8.2	10,000	7,000	17,000
0 grams of baking soda/Trial 1/Test 1	8.2	60,000	30,000	90,000
0 grams of baking soda/Trial 2/Test 1	8.2	30,000	60,000	90,000
0 grams of baking soda/Trial 3/Test 1	8.2	120,000	20,000	140,000

Average Table Before Baking Soda

Plot treatment/trial number/test number	**pH Level**	**Number of yeast per cc of soil**	**Number of mold per cc of soil**	**Total amount of fungi per cc of soil**
4 grams of baking soda	**8.2**	**35,000**	**28,667**	**63,667**
0 grams of baking soda	**8.2**	**70,000**	**36,667**	**106,667**

Fungi and pH Tests After Baking Soda Solution Added to Soil

Plot number/trial number/test number	pH Level	Number of yeast per cc of soil	Number of mold per cc of soil	Total amount of fungi per cc of soil
4 grams of baking soda/Trial 1/Test 1	4.4	12,000	7,000	19,000
4 grams of baking soda/Trial 2/Test 1	8.2	80,000	30,000	110,000
4 grams of baking soda/Trial 3/Test 1	8.0	12,000	8,000	20,000
0 grams of baking soda/Trial 1/Test 1	8.2	60,000	10,000	70,000
0 grams of baking soda/Trial 2/Test 1	8.2	6,000	6,000	12,000
0 grams of baking soda/Trial 3/Test 1	8.2	4,000	4,000	8,000

Average Table for After Baking Soda

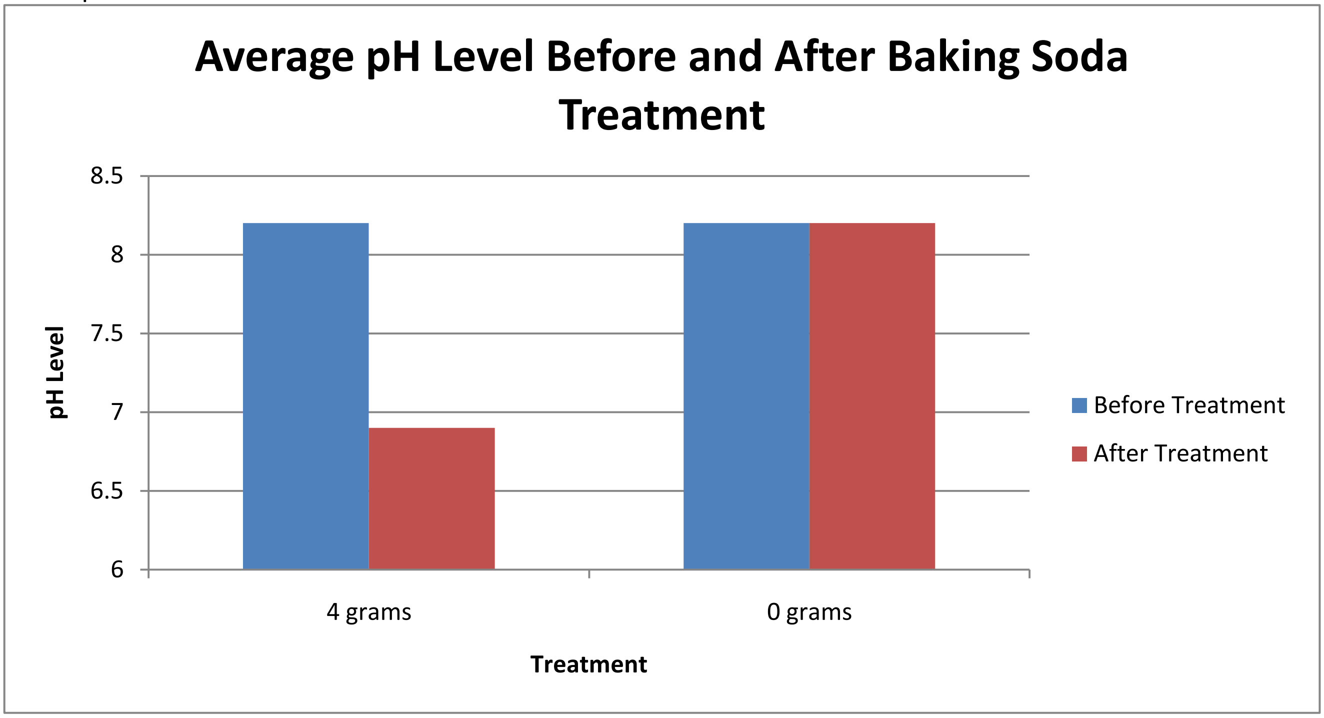
Plot treatment/trial number/test number	**pH Level**	**Number of yeast per cc of soil**	**Number of mold per cc of soil**	**Total amount of fungi per cc of soil**
4 grams of baking soda	**6.9**	**34,667**	**15,000**	**49,667**
0 grams of baking soda	**8.2**	**23,333**	**6,667**	**30,000**

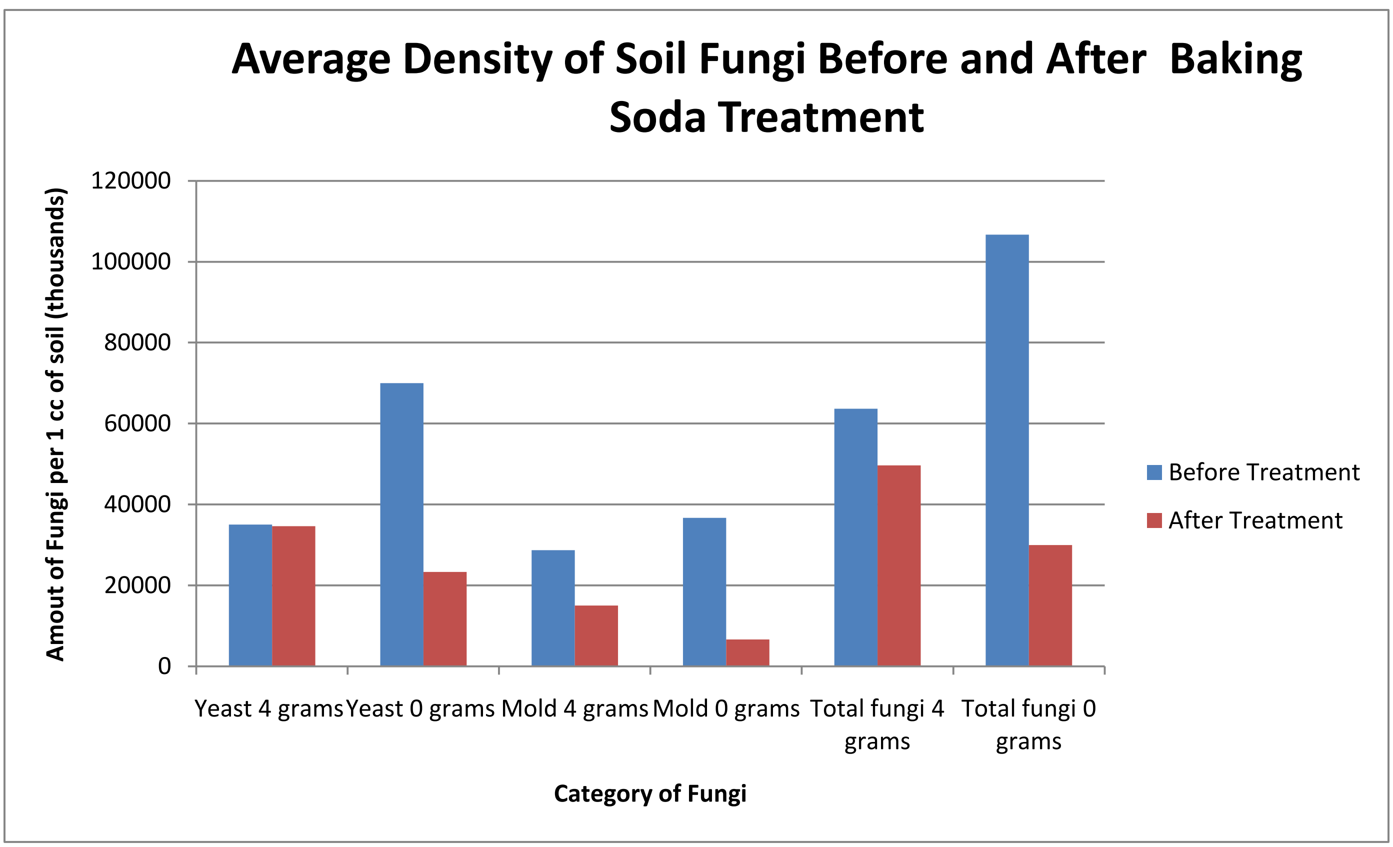
P-Values Table

	Before 4 Grams of baking compared with After 4 grams of baking soda	Before 0 grams of baking soda compared with After 0 grams of baking soda	After 4 grams of baking soda compared with After 0 grams of baking soda
Yeast	0.99	0.23	0.71
Mold	0.49	0.12	0.38
Total Fungi	0.78	0.044	0.61

***P-values were calculated between the different experimental circumstances and the different types of fungi, including yeast, mold and total fungi.

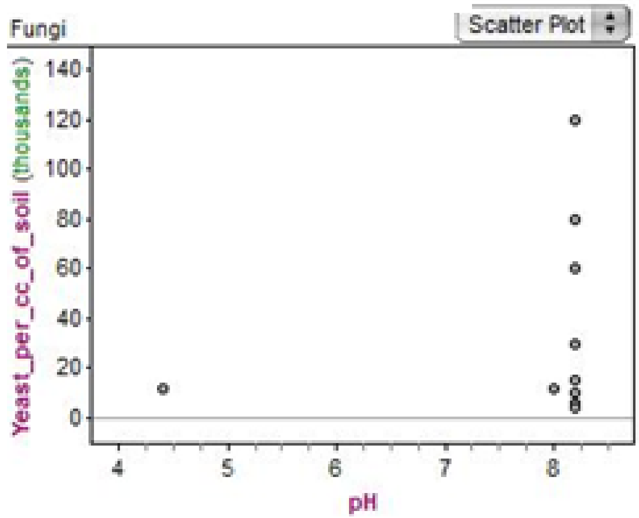
Graphs:


Average pH Level Before and After Baking Soda Treatment
pH Level
8.5
8
7.5
7
6.5
6
4 grams
0 grams
Treatment
Before Treatment
After Treatment

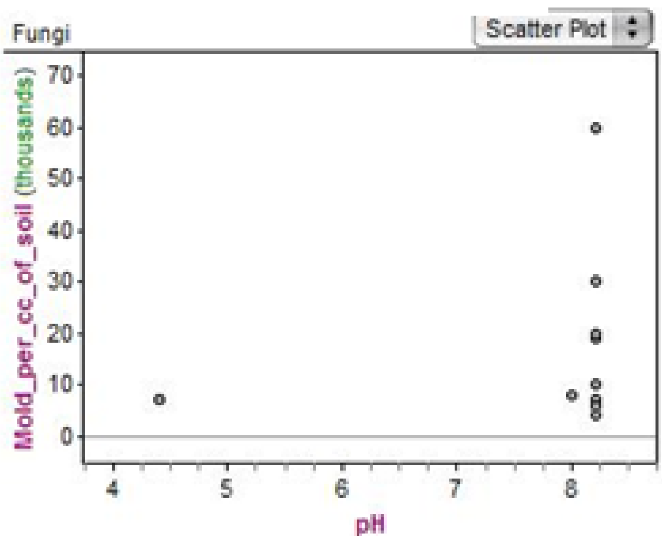

Average Density of Soil Fungi Before and After Baking Soda Treatment
Amout of Fungi per 1 cc of soil (thousands)
120000
100000
80000
60000
40000
20000
0
Yeast 4 grams
Yeast 0 grams
Mold 4 grams
Mold 0 grams
Total fungi 4 grams
Total fungi 0 grams
Category of Fungi
Before Treatment
After Treatment

Plot Conditions

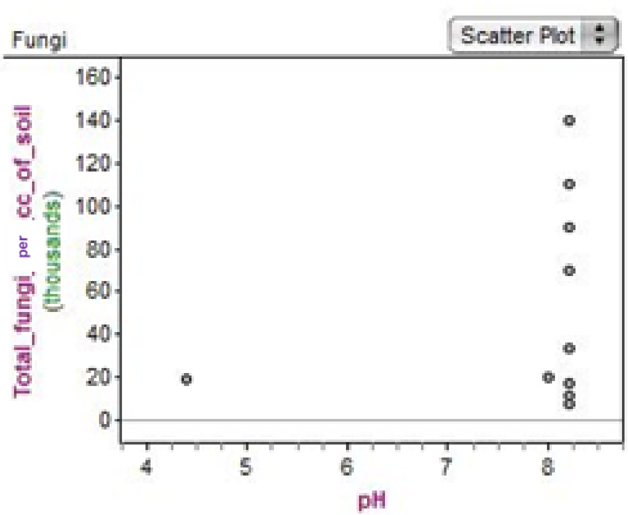
pH vs. Yeast Density in the Soil



pH vs. Mold Density in the Soil



pH vs. Total Fung Density in the



Conclusion:

The data that we have gathered in our Soil Ecology Project has proven our hypothesis, of baking soda decreasing the density of fungi and increasing the pH level in the soil, wrong. We decided to hypothesize this because baking soda is known to decrease the acidity levels in substances (ARM & HAMMER®, 2008), and acts as a fungicide on the surface of plants (Kuepper G, Thomas R, and Earles R, 2001). Therefore, we questioned whether or not the fungicide would also kill the fungi in the soil after washing off of the surface of the plant after it rain. When we tested this hypothesis, many of our results did not support our hypothesis.

One of the results that did not support our hypothesis was the drop in pH, meaning the increase in acidity, in the plot where we added the solution of four grams of baking soda mixed with 500 ml of tap water. In one of the 4 grams plots the pH was 8.2 before we added the baking soda solution, and after we added the solution, the pH decreased to 4.4. In another one of the 4 grams plots, the pH was 8.2 before the solution was added, and it stayed at 8.2 after we added the 4 grams of baking soda. The last 4 grams plot had a pH of 8.2 before the baking soda solution as well, and decreased to 8.0 after we added the solution. The average pH level for the plot 4 grams before the baking soda solution was added was 8.2, and it dropped to 6.9 after the solution was added, on average. In the plots where we only added tap water, or 0 grams of baking soda, the pH level stayed consistent at 8.2, before and after the baking soda was added. This also meant that we had an average pH level of 8.2 for the 0 grams plots both before and after the solution. Since the average pH for the plot 4 grams decreased, and got more acidic, it proves our hypothesis, that it would increase the pH levels and make it more basic, wrong.

We also know that pH level had no affect on the fungi density. When we look at the before and after pH of the 0 grams plots, it stayed the same at 8.2. The total fungi of the before and after 0 grams plots decreased by 76,667 fungi/cc in the soil. We then can conclude that the pH level had no effect on the density of the fungi.

The average density of yeast in the plots with the 4 grams of baking soda treatment dropped only 333 yeast/cc soil from before the treatment to after the treatment. The p-value of 0.99 shows that there was no significant change from the average density of yeast in the before the 4 grams of baking soda treatment was added from the density of yeast after the 4 grams of baking soda treatment was added. The average density of yeast in the plots with the 0 grams of baking soda treatment dropped significantly at 46,667 yeast/cc soil from before and after. The p-value of 0.23 soil shows that there was a significant change from the average density of yeast in the before treatment than the average density of yeast in the after treatment.

The average density of mold in the plots with the 4 grams of baking soda treatment dropped 13,667 mold/cc soil from before the treatment to after the treatment. The p-value of 0.49 mold/cc soil shows that there was a noteworthy drop between the before treatment and after treatment in the plots with a 4 grams of baking soda treatment. The average density of mold in the plots with the 0 grams of baking soda treatment dropped 30,000 mold/cc soil from before and after the treatment. The p-value of 0.12 mold/cc soil shows that there was a prominent drop between the before and after in the plots with a 0 grams of baking soda treatment in the density of mold.

The average amount of total fungi in the plots with the 4 grams of baking soda treatment dropped 14,000 total fungi/cc soil from before and after the 4 grams of baking soda treatment. The p-value of 0.78 total/cc soil shows that there was a distinguished drop between the before and after the 4 grams treatment in the total fungi. The average amount of total fungi in the plots with the 0 grams of baking soda treatment dropped 76,667 total fungi/cc soil which is a significant amount. The p-value of 0.049 total/cc soil shows there was an extremely drastic change between the before and after in the total fungi in the plots with the 0 grams of baking soda treatment.

The average density of yeast dropped 11,334 total yeast/cc soil from the average amount of yeast after the 4 grams of baking soda was added to the soil, compared to the average amount of yeast after the 0 grams of baking soda added to the soil. The p-value of 0.71 shows that there was a moderate change between the average 0 grams of baking soda treatment plot after the baking soda and water solution, and the average 4 grams of baking soda treatment plot after the solution. The average density of mold dropped 8,333 mold/cc soil from the average after 4 grams of baking soda was added to the soil, compared to the average after the 0 grams of baking soda was added to the soil. The p-value of 0.38 shows that there was a large difference between the average density of mold after the 4 grams of baking soda was added to the soil, compared to the average density of mold after the 0 grams of baking soda was added to the soil. The average density of the total fungi decreased by 19,667 total fungi/cc soil between the average of total fungi after the 4 grams of baking soda was added to the soil and the average total fungi after the 0 grams of baking soda was added to the soil. The p-value of 0.61 shows that the density of the total fungi had a slight decrease between the average total fungi after the 4 grams of baking soda was added to the soil and the average total fungi after the 0 grams of baking soda was added to the soil.

We also know that there was another environmental factor that was not preferable to the fungi. We know this because the plot with only tap water added decreased in the amount of yeast, mold, and total fungi. The yeast dropped down from 70,000 yeast/cc before we added the solution to 23,333 yeast/cc. The mold decreased from 36,667 molds/cc molds to 6,667 molds/cc. The total amount of fungi went down from 106,667 total fungi/cc to 30,000 total fungi/cc. In this case the baking soda added to the 4 grams plots actually helped more of the fungi to survive the other environmental problem that was making it harsh for the fungi in the soil to live. Even though the mold level went down in the four grams plot, we know that the baking soda helped the yeast survive because the density stayed the same before and after. This tells us that the mold in the 4 grams plot might have gone into the yeast form because the acidity level made the environment harsh. This caused more of the fungi in total to survive. If we were to further our research we would try to figure out what the other environmental problem was that caused the 0 gram plots to go down in the amount of fungi as well.

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