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May 30th, 2003

Ecology Report

There are many factors that affect the microbes of the soil. Some of these factors include pH, bacteria, nitrogen and mulch. The pH is the measure of the acidity or alkalinity of the soil. The pH level affects the activity of enzymes. Ultimately the pH controls all organisms. Bacteria consist of decomposers, mutualists, pathogens, and lithotrophs. Nitrogen affects the growth of plants. Mulch helps maintain the fertility of soil. Bacteria, pH, mulch and nitrogen are important as they affect the whole ecosystem we live in.

Mulch is any substance spread on the ground to protect plant roots from heat, cold, drought or to keep fruit clean (McCraw, 2002). It helps plants by gradually increasing soil fertility, stopping the growth of weeds, saving water, lowering waste levels, and providing organic foods.

Mulch can help acidic soils and alkaline soils become closer to a normal pH level (Homestead harvest: Preserving the Garden, 2003). Alkaline soils are found in dry climates, and acidic soils are found in moist climates. Mulches help alkaline soils become more normal by keeping water in the soil and reducing evaporation (Homestead harvest: Preserving the Garden, 2003). The mulch reduces water evaporation by protecting the soil from the sun (Plant Care: Using Mulch, 2002). Ground covering is not as effective as mulch because it differs from plant to plant. It also does not have as great an effect as mulch on the richness of the soil.

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pH is a chemical term used to express hydrogen ionization in water, which measures the acidity or alkalinity of a solution. The term refers to the breakdown of water, normally a stable molecule, into a positive hydrogen ion (H^+) and a negative hydroxide ion (OH^-). This ionization occurs when the various minerals and organic materials combine with water molecules. This forces the soil to change its properties.

The basic soil cations (Ca^{+2}, K^+ Mg^{+2}), which are positively charged ions, are gradually depleted. These ions are then replaced with cations held in microscopic soil reserves; this then leads to soil acidity because soil acidification results in an addition of hydrogen ions. Hydrogen ions are increased by the decomposing organic matter, acid-forming fertilizers, and the exchange of cations for H^+; this is done in the roots.

Soils can have a pH level from 3.5 to 11.0. (Spector, 2001) where 7.0 on the pH scale is considered neutral. The normal pH level is between 6 and 6.5. Acidic soils have a pH lower than 7, while alkaline soils have a pH greater than 7. (Spector, 2001)

pH levels also affect the complex interactions among soil chemicals. As an example, Phosphorus (P) requires a pH between 6.0 and 7.0 and becomes chemically immobile when above or below this pH level. This forms insoluble compounds with iron (Fe) and aluminium (Al) in acid soils and with calcium (Ca) in calcareous soils. To alter the pH of the soil, you would have to add limestone to increase pH or elemental sulfur to decrease the pH.

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If the pH of the soil is above 5.5, nitrogen can be dissolved and is therefore available to be absorbed by the plants. On the other hand, if the pH of the soil falls below 5.5, most major plant-nutrient minerals, such as nitrogen (N), phosporus (P), potassium (K), sulphur (S), magnesium (Mg), calcium(Ca), and some other micronutrients cannot be dissolved and therefore are unavailable for uptake by plant roots (Support Information: pH, 2003). If plants are not able to uptake these nutrients then the plants are not able to grow, thrive and fight off diseases. (Spector, 2001)

The level of pH affects the population density of bacteria, an important microbe in the soil. These bacteria in the soil can be grouped in four different ways depending on their function. The first group of bacteria in the soil is mutualists that form partnerships with plants. The second group of bacteria in the soil is the pathogens that cause gall formation in plants. The third group of bacteria in the soil is the lithotrophs or chemoautotrophs, which are important to the nitrogen cycling and degradation of pollutants. The fourth group of bacteria in the soil is decomposers that consume simple carbon compounds, such as root exudates and fresh plant litter.

The largest of the four groups of bacteria in soil are decomposers. When they decompose, bacteria convert energy in soil organic matter into forms useful to the rest of the organisms in soil food web (Soil Quality Institute, 2003). Nitrogen is one of the types of organic matter that gets converted into a different form. The plant supplies simple carbon compounds to the bacteria, and the bacteria convert nitrogen from air into a form the plants can use. (University of

Missouri Columbia, 1999). These bacteria function best when the pH level is between 6 and 6.5. If there is a low pH level then the decomposers cannot function properly and the rate at which bacteria change nitrogen into a different form is greatly reduced (University of Missouri Columbia, 1999).

Plants need nitrogen to grow, develop and produce seeds. Nitrogen is taken up by plant roots and combined into organic substances in the plant, such as enzymes, proteins and chlorophyll. Without nitrogen the plant's leaves lack chlorophyll and they lose their lush green color and the enzymes are not able to function properly. This means that the enzymes cannot control chemical reactions, which allow the cell to perform different tasks that enable it to live. Therefore the growth of the plant would be stunted.

Our problem is how far away from an area that has been mulched on the Roland Park campus does it take for the soil pH, and therefore bacteria levels, to return to normal? We did this test by collecting soil samples from three different sites that were next to a triple shredded pine mulched flowerbed. We counted the bacteria levels and found the pH levels of each soil sample and compared each one to our "normal" soil pH level and bacteria count. We found that the mulch had no affect on the pH level and bacteria levels of the soil.

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Lab Report

1. Problem: How many centimeters from a flowerbed that has been mulched with triple shredded pine mulch on the Roland Park campus does it take for the soil pH and therefore bacteria levels to return to normal?
2. Hypothesis: It will take 200 centimeters for the soil pH level and therefore bacteria level to return to normal from a flowerbed that has been mulched with triple shredded pine mulch on the RPCS campus.
3. Experiment
   A. Variables
      a. Independent variable 1: distance from mulched flower bed
         Independent variable 2: pH at different distances away from the mulched flowerbed
      b. Dependent variable: population density of bacteria in soil samples
   B Controls
      a. Negative control 1: 0 cm bacteria count
         Negative control 2: normal pH level of soil on the RPCS campus
      b. Positive control: normal population density of bacteria in soil.
      c. Controlled Variables: time soil samples taken, amount of soil samples taken, number of times experiment is replicated, how much soil is taken in sample, what samples are tested for, where normal levels are taken, what kind of mulch sampled, flower bed in which mulch is taken from, date soil samples taken, where in plotted area soil sample is taken from, the amount of soil from each sample used for the pH testing, amount of demineralized water is used in pH testing, amount of soil flocculating regeant used in pH testing, amount of solution transferred to spot plate, which depression used on spot plate, amount of duplex indicator used in pH testing, whether you use the duplex indicator or another indicator first, amount of soil in culture tube, amount of sterile water in each culture tube, amount of water you remove from each culture tube to the next, amount of soil dilution placed in to nutrient agar plate, the level of soil dilution tested, type of agar used.
   C. Procedure
   1. Take a soil sample in depth from the top 15 centimeters in depth and 2 cm in width of the soil using the soil test core at N:30.35807 and W: 76.63622 on a

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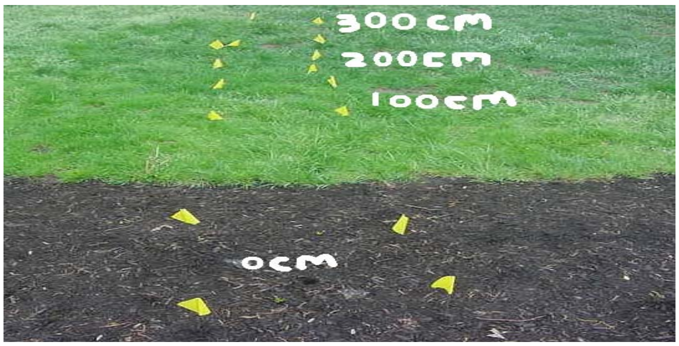
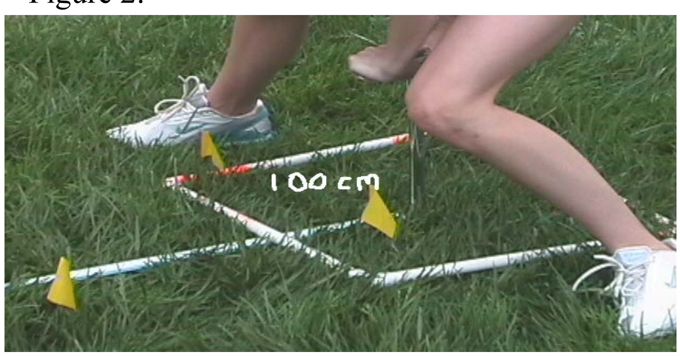
May morning. This will be used to determine the normal level of pH on the RPCS campus and the normal population density of bacteria in the soil.

2. Go to the flower bed located at N:39.35799 and W: 79.63629 on the RPCS campus that has triple shredded pine mulch in it and is surrounded by grass.
3. Take a soil sample in depth from the top 15 centimeters in depth and 2 cm in width of the soil using the soil test core 100 centimeters away from the from the edge of the grass. Refer to Figure 1.
   Figure 1:



4. Take a soil sample from the top 15 centimeters in depth and 2 cm in width of the soil using the soil test core every 100 centimeters from the flowerbed in to the grass in a straight line starting at the edge of the mulch. Refer to Figure 1.
5. Do this until you are 300 centimeters away from the triple shredded mulched flowerbed.
6. Around each place that you took a soil sample, mark the corners with flags in order to identify the area next time. See figure 2.

Figure 2:



7. Repeat steps 2-8 two more times at flowerbeds located at N: 39.35846 and W: 76.63597 and N: 39.35808 and W: 76.63628.

8. Repeat steps 2-8 two more times at flowerbeds located at N: 39.35846 and W: 76.63597 and N: 39.35808 and W: 76.63628.
9. Using the pH testing procedure from the LaMotte testing kit determine the pH level of the soil. Using the serial dilution technique determine the number of

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bacteria in each of the soil samples diluted ten to the negative forth (10^{-4}). Be sure to do both test simultaneously. Record all of the collected data.

10. To determine the amount of bacteria take 100 μl of the soil sample diluted ten to the negative forth (10^{-4}), and put it in an easy gel agar. Swirl the two together. Pour the solution into its own nutrient agar plate. Wait until the liquid has turned into a solid, and then flip the nutrient agar plate over.
11. After three days count the number of bacteria colonies in the nutrient agar plate.
12. Count the number of bacteria by using this formula: number of bacteria colonies multiplied by 10^2 multiplied by 10^{-4}. This will give you the total number of bacteria in the original 100 μl the soil sample.
13. Repeat steps 1-5 and 7-12 two more times on a May morning, 30 centimeters away to the left or right from where you took the first soil sample in each of the sites when you are standing directly in the line of the previously taken soil sample of that plot.

Data:

Ecology Data

TRIAL 1

		0 cm	100 cm	200 cm	300 cm	negative control
Site 1						
	PH level	7.4	7.2	6.4	6.4	6.6
	Number of Bacteria	2,000,000	117,000,000	16,000,000	24,000,000	11,000,000
Site 2						
	PH level	7.4	6.8	6.4	7.2	
	Number of Bacteria	20,000,000	11,000,000	24,000,000	19,000,000	
Site 3						
	PH level	7.2	6.7	6.8	7	
	Number of Bacteria	16,000,000	10,000,000	12,000,000	140,000,000	

TRIAL 2

		0 cm	100 cm	200 cm	300 cm	negative control
Site 1						
	PH level	7.3	7.4	7.2	6.6	6.4
	Number of Bacteria	13,000,000	18,000,000	14,000,000	27,000,000	7,000,000
Site 2						
	PH level	7.4	7.2	7	6.2	
	Number of Bacteria	11,000,000	12,000,000	8,000,000	9,000,000	
Site 3						
	PH level	7.4	7.2	7.5	7.4	
	Number of Bacteria	24,000,000,	8,000,000	264,000,000	50,000,000	

TRIAL 3

		0 cm	100 cm	200 cm	300 cm	negative control
Site 1						
	PH level	7.4	7.4	7.2	7.6	6.2
	Number of Bacteria	23,000,000	15,000,000	121,000,000	674,000,000	8,000,000
Site 2						

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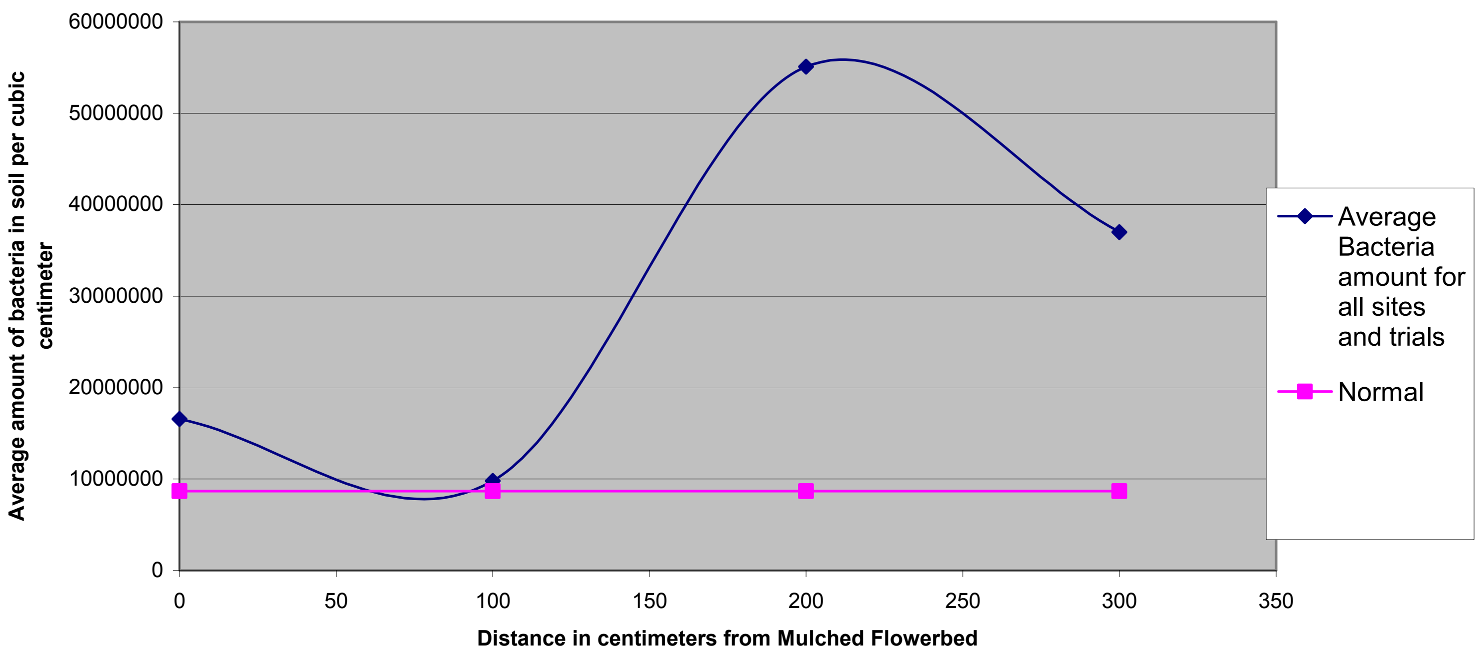
	PH level	7.6	7.4	7.2	6.2
	Number of Bacteria	15,000,000	5,000,000	6,000,000	17,000,000
Site 3					
	PH level	7.4	7.2	7	7.4
	Number of Bacteria	25,000,000	27,000,000	31,000,000	24,000,000
Average Bacteria					

	0	100	200	300
	16555556	9777778	55111111	37000016
Normal	8666667	8666667	8666667	8666667

Average amount of Bacteria taken at different distances from the mulched flowerbed compared to the normal Bacteria Level



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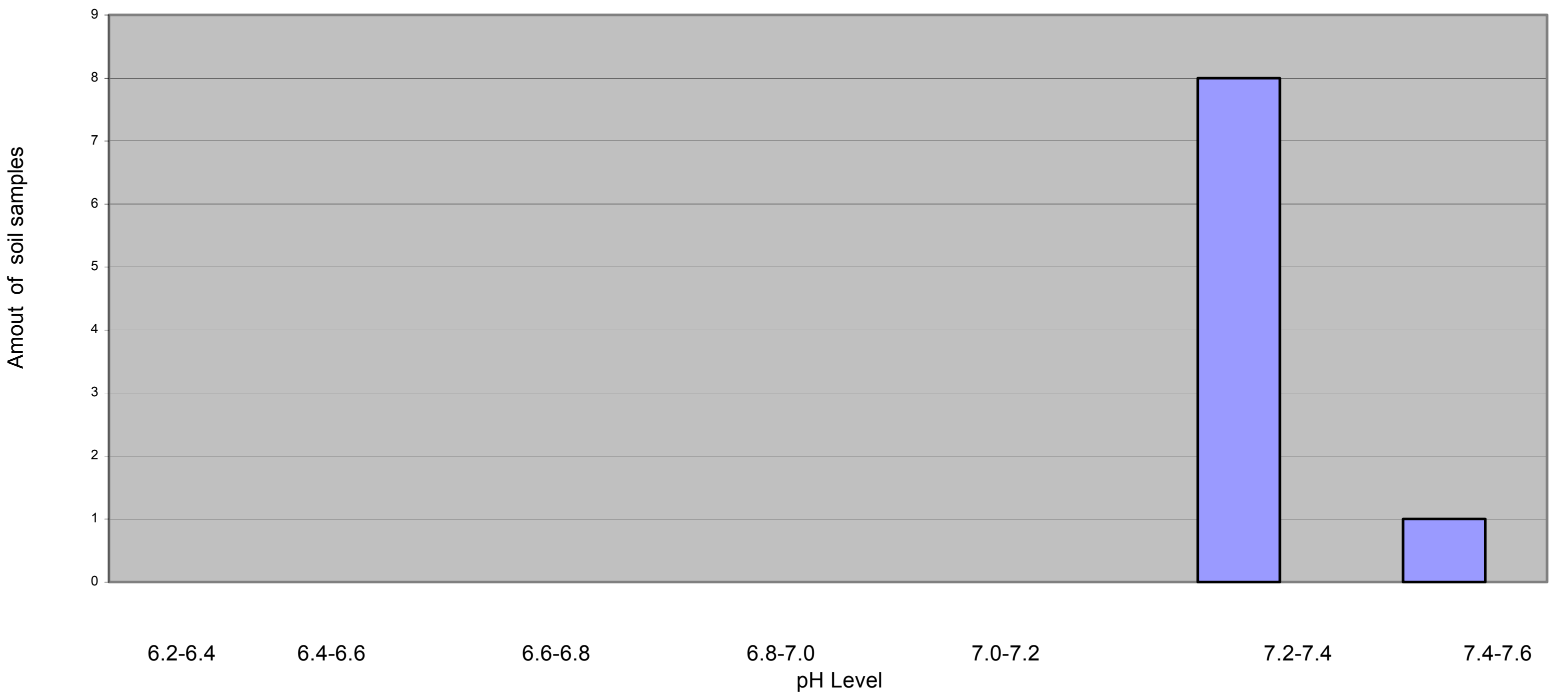
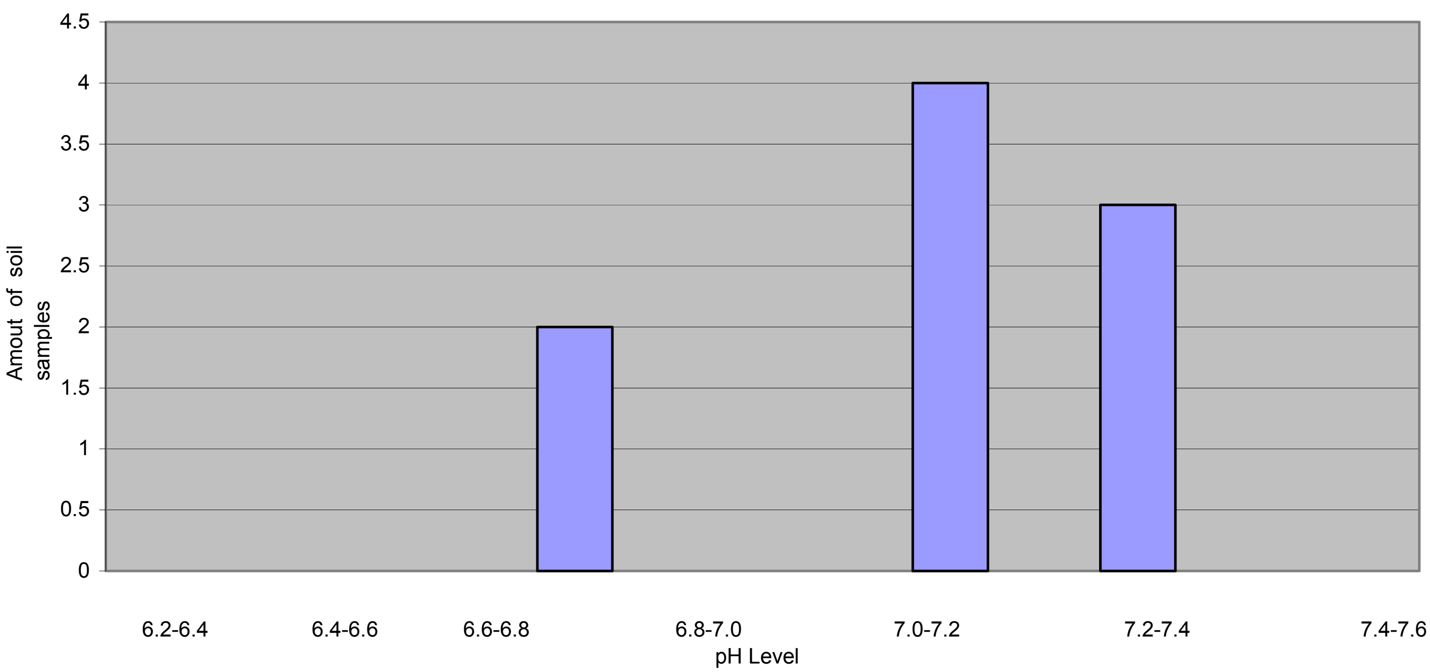
Amount of Soil Samples 0 cm Away from Mulched Area at Various pH Levels



pH Level

Amount of Soil Samples 100 cm Away from Mulched Area at Various pH Levels



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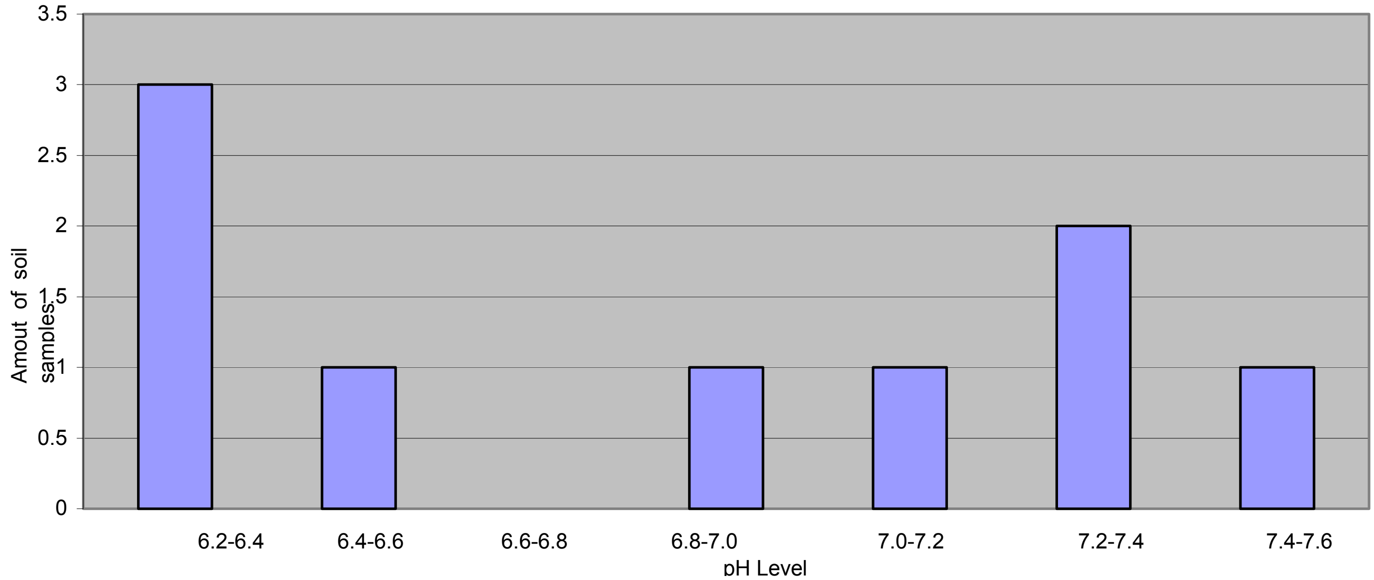
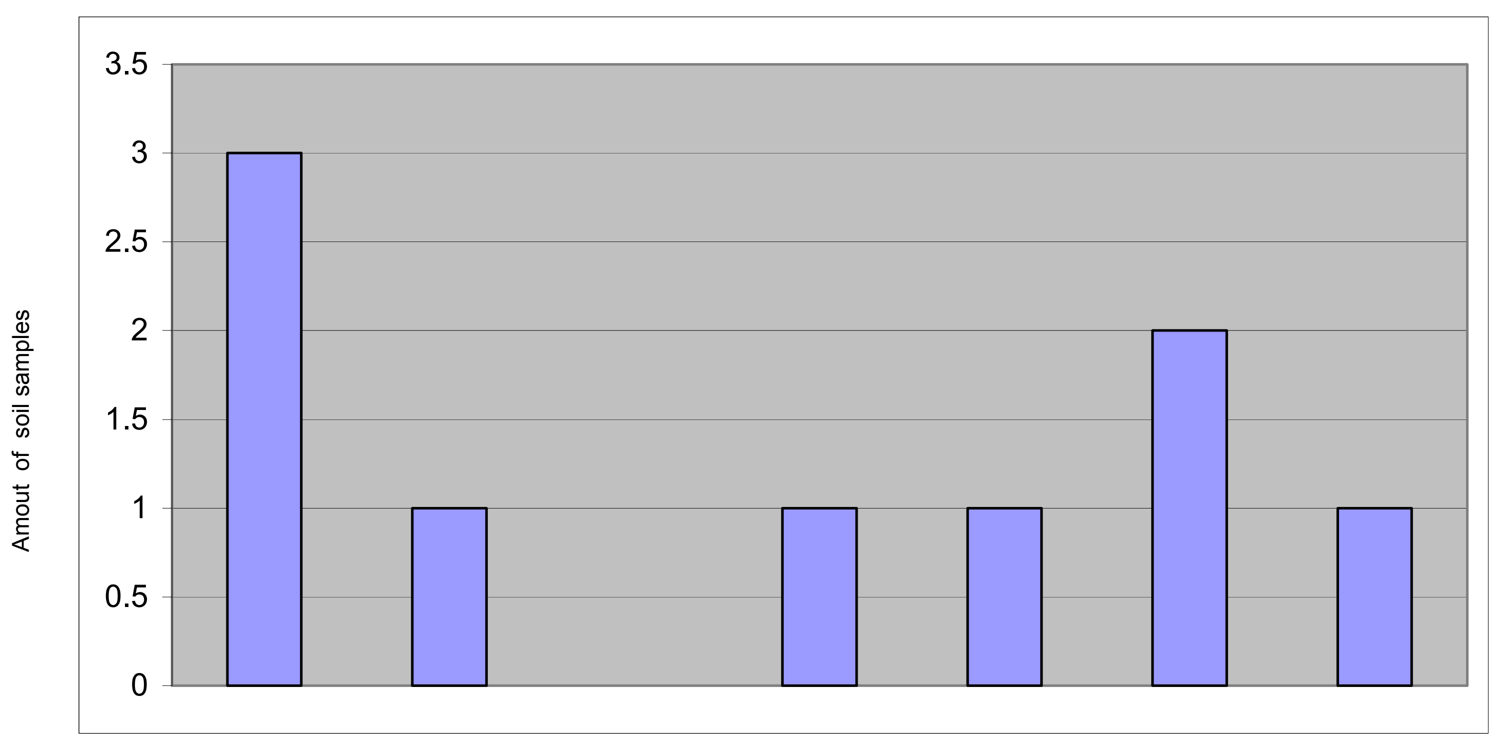
pH Level

Amount of Soil Samples 200 cm Away from Mulched Area at Various pH Levels



Amount of Soil Samples 300 cm Away from Mulched Area at Various pH Levels



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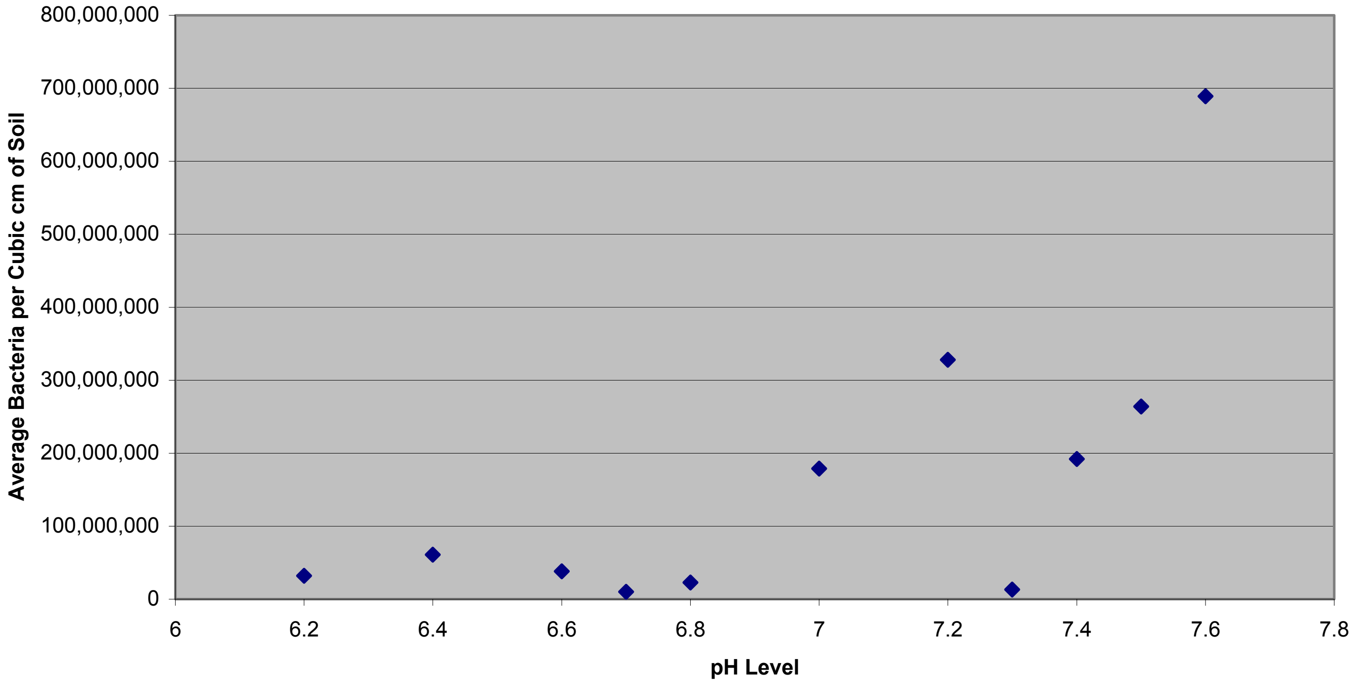
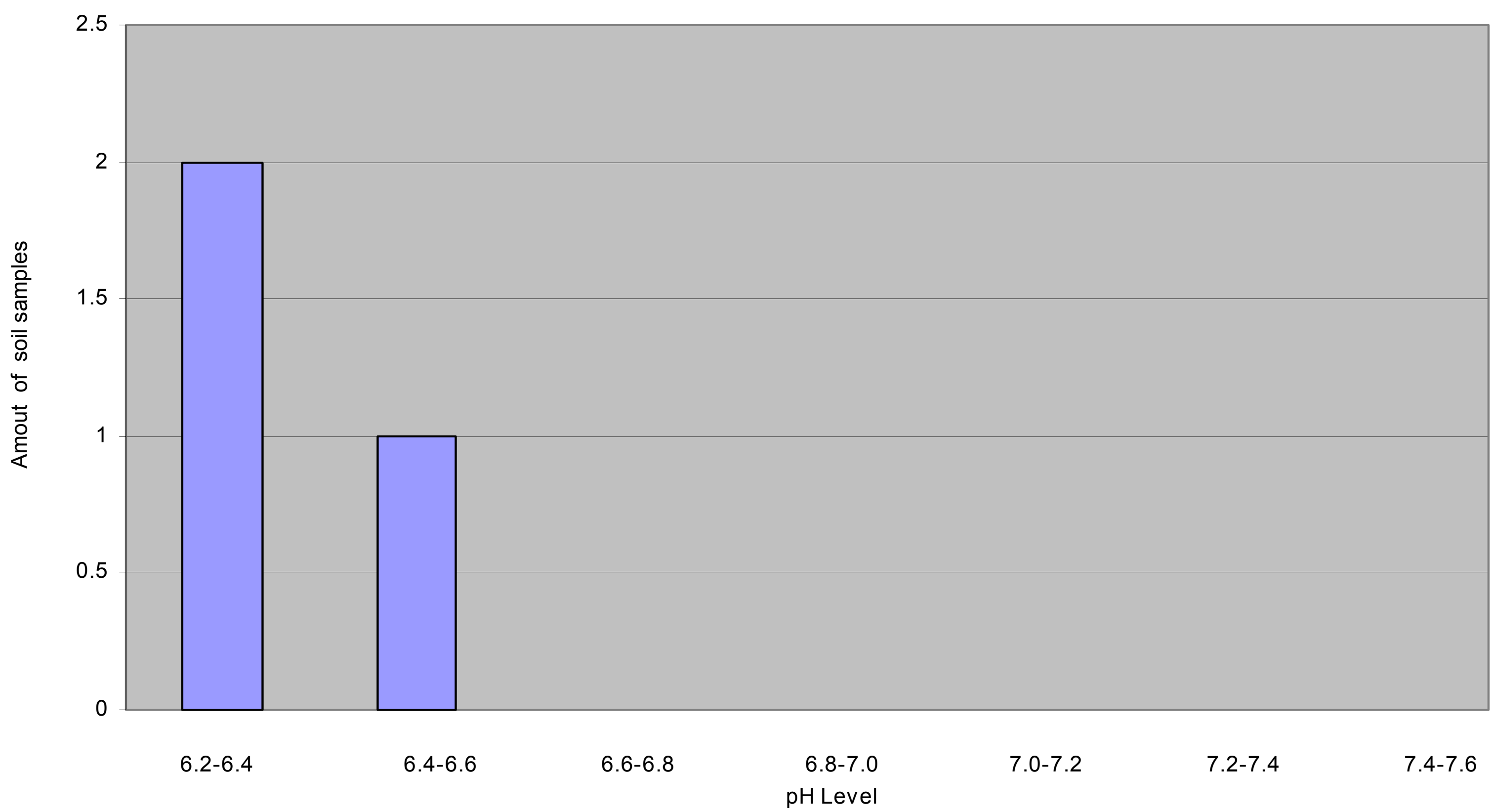
Average Bacteria at Different pH Levels



Amount of Normal Soil Samples at Various pH Levels



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Conclusion

Our hypothesis was that it will take 200 centimeters for the soil pH level and therefore bacteria level to return to normal (pH- 6 to 6.5, bacteria- 8666666.67 per cubic cm of soil) from a flowerbed that has been mulched with triple shredded pine mulch on the RPCS campus. Our hypothesis was wrong; neither the pH nor bacteria levels returned to normal at 200 cm away from the mulched flowerbed.

The bacteria was closest to returning to the normal bacteria level at 100 centimeters away from the mulched flowerbed as the bacteria count was 9777777.788 per cubic cm and the normal bacteria count was 8666666.67 per cubic cm. The bacteria level at 0 centimeters away from the mulched flowerbed was the second closest distance to returning to the normal level as the bacteria count in that soil sample of 1 cubic cm was 16555555.56. The bacteria level at 300 centimeters away from the mulched flowerbed was third closest to the normal level, as the bacteria count was 37000015.55. per cubic cm. The bacteria level at 200 centimeters from the mulched flowerbed was furthest away from the normal bacteria level as the bacteria count was 55111111.1 per cubic cm. Therefore any effects the mulch had on the pH did obviously not affect the bacteria level, as there was no pattern in our data.

There was no relationship between the pH level and the bacteria level. When the pH level was at 6.2, the bacteria count was 32,000,000 bacteria per cubic cm. When the pH level was at 6.7 the average bacteria count was 10,000,000 bacteria per cubic cm. When the pH level was 7.3 the average bacteria count was 13,000,000 bacteria per cubic cm. When the pH level was at 7.6, the average bacteria count was 689,000,000 bacteria per cubic cm. The levels do not increase or decrease at a steady rate, clearly showing that

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the pH has no effect on the number of bacteria in the soil. Also, the only times that there was any significant increase or decrease was when the pH level was 7 or above. If the pH had an affect on the bacteria, there would be consistent increases or decreases of the amount of bacteria from one pH level to another pH level. Bacteria are flourishing when the pH level is at 7 and above, but not at a pH levels from 6 to 6.5, which is when bacteria are supposed to be the most plentiful.

There was no relationship between the pH level and the distance from the mulched flowerbed. In the graph that showed the amount of soil samples with a certain pH level at 0 centimeters, which is in the mulched flowerbed, 8/9 of the soil samples had a pH level of 7.2-7.4; the other soil sample had a pH of 7.4-7.6. In the graph that showed the amount of soil samples with a certain pH level in the normal plots, 2/3 soil samples had a pH of 6.2-6.4 while 1/3 soil samples had a pH of 6.4-6.6. If the distance away from the mulched flowerbed had a relationship with the pH level, then the father the distance away from the mulched area was, the more number of soil samples closer to 6.2-6.4 there would be. This was true with out data from the plots from 0-100 cm away from the mulched area, however it wasn't true for our data from 200-300 cm away from the mulched area. 8/9 soil samples at taken from 0 cm away from a mulched flowerbed had a pH level of 7.2-7.4. In the soil samples taken from 100 cm away from a mulched flower bed, only 4/8 samples had a pH level of 7.0-7.2; 3/9 had a pH level of 7.2-7.4, and 2/9 soil samples had a pH of 6.6-6.8. From the 0cm away from a mulched flowerbed soil samples to the 100 cm away from a mulched flowerbed soil samples there was an increase of two soil samples with a pH of 6.6-6.8. The amount of soil samples per pH level taken from the 200 cm and 300 cm away from a mulched flowerbed were the exact

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same. Given this, the amount of soil samples per a certain pH level did not change due to distance away from a mulched flowerbed.

Something is happening 100 centimeters away from the mulched flowerbed that is affecting the pH level and bacteria levels. We did not have many sources of error, however we do recommend counting the bacteria in the nutrient agar plates after two days. For some of our samples we didn't count the number of bacteria colonies until 4 days, and they had begun to grow mold. Two days gives the bacteria colonies time to settle and grow, yet not enough time to grow mold, making it hard to count. For future research on factors which could alter pH levels of soil, and therefore bacteria levels in soil, we recommend testing other environmental factors besides mulch, because it was not a really a huge factor, especially the further out we took samples from the mulched flowerbed.

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