The Compost Competition

The Effects of Compost on Bacteria Density in the Soil

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I. Introduction and Problem

Soil affects life on our planet and has various essential jobs. How plants develop in the wild and how they grow and thrive depends on soil. Plants are important to the world for reasons such as, providing oxygen for many organisms to breathe, being a food source, and being used to make antibiotics that save lives. Without plants, or soil, our world would not be able to function.

Soil is the loose mineral or organic material on the outside surface of the Earth that land plants are able to grow in (“What is Soil,” 2010). Both living and nonliving things are contained in soil. Organic and inorganic materials are what make up the soil itself, and many organisms can then make it their home. Mineral particles such as clay, sand or silt from fragmented rocks are the key inorganic compounds in soil. The key organic compounds, called humus, are decomposed materials such as plants and animals ("Soil Basics," 2005). Other important players in the composition of soil are water and air because soil isn’t solid. Water and air can seep through the cracks in between soil particles and reach things, such as plant roots, that are located underneath.

Different types of soil are located in various parts of the world. This is because several characteristics of a location can affect the soil. The material in which soil forms is called the parent material. This material usually comes from multiple areas far from the place where the soil forms and can be deposited by water, ice or wind. Once soil is completed it can be manipulated by the temperature of the area and amount of rainfall. Both can influence the composition of the soil by affecting the amount of water leakage or erosion. Another factor that can greatly affect soil is the level of ground where the soil is located. In high areas, soil will most likely be thin because a lot of it has run off, and soil will most likely be thick in low areas because it can settle ("Soil Formation," 2010).

Organisms work together to produce soil by helping rocks crumble to form soil particles. After the soil is formed it stays healthy with the help of microorganisms and regular organisms. The role of microbes in soil is to break down organic material and produce more microbes (Parsons, 2005). Although there are multiple microbes in soil, some main ones are fungi, protozoa and bacteria.

Bacteria are one celled organisms that can vary in size (Ingham, 1998). Bacteria are usually around one micron in length and width, but can be different depending upon the environment that it is living in, as well as the nutrient levels. Bacteria are not visible when using the naked eye, but when looking under a microscope it is usually in the formation of a sphere (cocci) or rod (bacilli). These formations make it easier for bacteria to join together in chains or clusters ("Soil Bacteria," 2004).

The bacteria that are located in the soil are located in and between soil aggregates, normally near a decaying plant or animal debris. Another easy way to locate bacteria is when substrates are present or in the layer located just on the outside of a living root ("Soil Bacteria," 2004).

The main job of bacteria is to perform chemical transformations ("Soil Bacteria," 2004). The main transformation that bacteria perform is converting nitrogen (N2) from the atmosphere into ammonium. Ammonium is a chemical that is produced and used to help the roots of infected plants (Ingham, 1998). The process that bacteria use in order to convert the different chemicals is called external digestion ("Soil Bacteria," 2004). Another main type of bacteria is called nitrifying bacteria. Nitrifying bacteria are able to transform ammonium (NH4+) into nitrite (N02-), and then finally into nitrate (N03-) which is the preferred form for grass and row crops. This process is called nitrification. This type of bacteria is the most important and is called nitrogen fixing bacteria. Bacteria do not just convert nitrogen, but they also convert inorganic matter such as simple carbon compounds into other forms that are not only useful for the plants, but other living things in the soil. Bacteria that convert the inorganic matter into other forms are called decomposers. Bacteria decomposers also play a key role in preventing the loss of nutrients such as nitrogen by retaining them inside of their cells. A specific type of decomposing bacteria are called Actinomycetes. They decompose a wide variety of substances (Ingham, 1998).

Not only do bacteria play a key role in soil, but they also play a key role in compost. Compost is nutrient-rich mulch of organic soil that is the product that comes from the decomposition of organic material that is sanitized by heat (What is Compost, 2010). The main job of bacteria that is located in compost is to transform raw material in the compost into a rich soil. Within the soil, bacteria also transform the nitrogen in the air into other forms that are able to be used for plants. These plants are called "nitrogen fixers" ("Get Spirited," 2005).

Nitrogen is defined as "a nonmetallic element that makes up about 78 percent of the atmosphere by volume, occurring as a colorless, odorless gas. It is a component of all proteins, making it essential for life, and it is also found in various minerals" (The free dictionary 2010). Nitrogen, in its different forms, is key to sustaining life because it allows organisms to build amino acids, proteins, and nucleic acids (Concept 36.3 2004). Nucleic acids are important to life because they store genetic information. Amino acids are crucial to sustaining life because they are what make up proteins. Proteins are key to life because they provide defense, they allow cells to function, they provide nutrient storage, control cell signaling, and control chemical reactions in cells. Overall amino acids, nucleic acids, and proteins are all critical in maintaining life which is why nitrogen is also key to life.

Nitrogen is present all around us, but most organisms cannot use it in its original form. For example, dinitrogen gas (N2), a form of nitrogen, makes up three fourths of all of the air that organisms breathe, but very few creatures can use this nitrogen from the air. Therefore, animals depend on other animals and plants for their nitrogen source. However, there are only two forms of nitrogen that plants can use, ammonia which is a combination of nitrogen and hydrogen, and nitrates which is a combination of nitrogen and oxygen (Nordi 2003, p. 12). For nitrates to be formed the ammonia in the soil must pick up another hydrogen ion from water. This produces ammonium which other bacteria can convert to nitrates in a process called nitrification (Concept 36.3 2004). For the ammonia to be created a process must occur called nitrogen fixation. Nitrogen fixation is when specialized bacteria such as actinomycetes convert dinotrogen gas (N2) into ammonia. (Nordi 2003, p. 12) This means that if there are more bacteria in a soil plot, more nitrogen fixation can occur and therefore more ammonia can be made to sustain life in that area.

Compost is nutrient-rich mulch of organic soil that is the product that comes from the decomposition of organic material that is sanitized by heat (What is Compost, 2010). Compost helps plant growth (What is Compost, 2010). It is produced by mixing compostable manure, food, and other organic materials (What is Compost, 2010). In the first stage of compost, mesophilic bacteria break down compounds (Trautmann and Olynciw, 1996). In the second stage of compost, the temperature rises to higher than 40 degrees Celsius (Trautmann and Olynciw, 1996). This is when thermophilic, or heat-loving, bacteria take over (Trautmann and Olynciw, 1996). These bacteria are able to survive the heat because they make endospores, which are walls that resist heat (Trautmann and Olynciw, 1996). In the third stage of compost, the temperature reaches above 55 degrees Celsius (Trautmann and Olynciw, 1996). This is when most pathogens are killed because they cannot survive in the heat (Trautmann and Olynciw, 1996). In the final stage of compost, most high-energy compounds, which are proteins, fats, and complex carbohydrates, become absent from the compost, and this causes the temperature to decrease, allowing mesophilic bacteria to flourish again (Trautmann and Olynciw, 1996). There are not many bacteria that can live above 65 degrees Celsius, so by mixing the compost and keeping it in fresh air, composters keep the compost below this temperature (Trautmann and Olynciw, 1996).

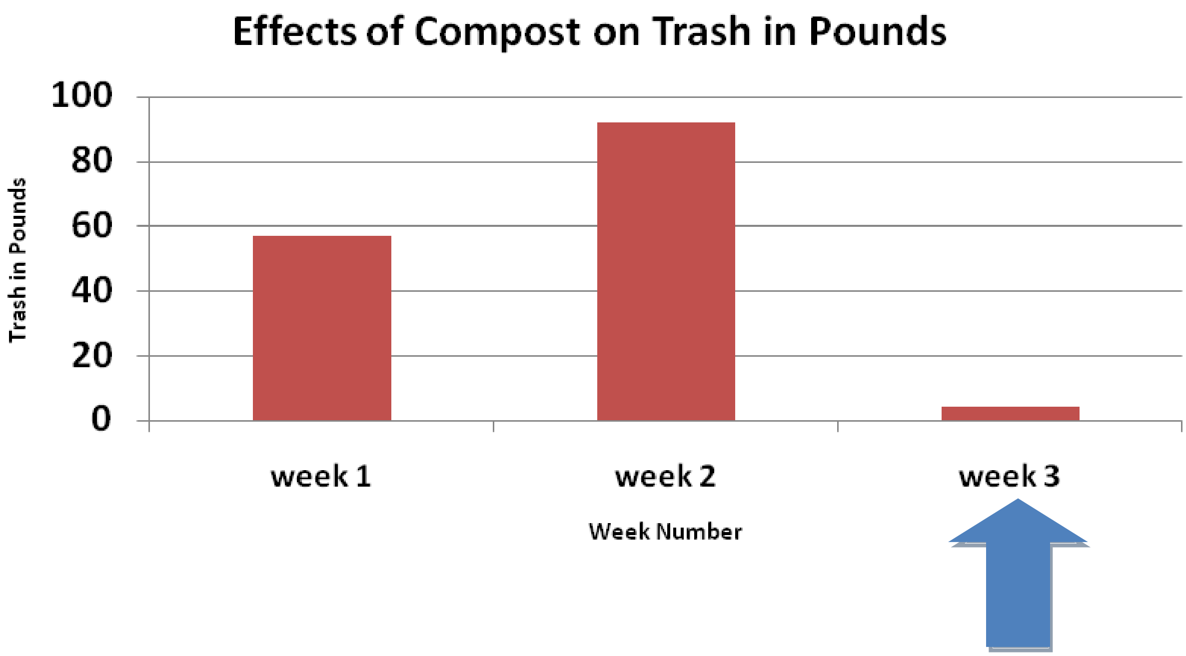
A compost heap that is in an advanced stage is excellent for soil (Soil Basics- Creating Fertile, Healthy Soil, 2005). The advanced stage is when the compost is dark and does not smell anymore (Soil Basics- Creating Fertile, Healthy Soil, 2005). It increases the amount of microorganisms in the soil because soil particles group into one and make aggregates (Soil Basics- Creating Fertile, Healthy Soil, 2005). Aggregates enhance drainage by making spaces in the soil.

There are benefits and detriments of composting. One of the benefits is that it reduces trash (Barss April 28, 2010). By putting some food and utensils that used to be trash in the compost, we reduce trash, thus helping the environment (Barss April 28, 2010). We attend Roland Park Country School, a private school located in Baltimore City. The trash production at RPCS has gone down significantly. Before we started composting, the school produced 144 pounds of trash (Barss April 28, 2010). Through composting, the school has reduced the trash to 4.5 pounds (See figure 1) (Barss April 28, 2010)! The compost also serves as a fertilizer for soil, and this helps keep the soil healthy (Soil Basics- Creating Fertile, Healthy Soil, 2005). Additionally, composting helps RPCS beat Bryn Mawr in the Green Challenge (Barss April 28, 2010)! There are negatives to composting, as well. The compost has an odor that causes the hallways to have a foul smell. Composting also costs the school money (Barss April 28, 2010).

We buy compost from Leafgro. This compost is $5.00 for a bag of 42.5 liters of compost. It also takes time and effort to sort the trash and the compost.

We are doing this experiment in order to measure bacteria density in soil after adding compost. Bacteria perform nitrogen fixation, and nitrogen fixation is essential for life. More amino acids, proteins, and nucleic acids can be made if there are more bacteria in the soil to produce nitrogen in different forms. This will allow more organisms to live in the soil. By doing this experiment we will see if using compost as a fertilizer allows more bacteria to live in the soil. To do this we will have six plots of soil. Three plots will contain only soil, and our next three plots will have compost added to the soil. We are also going to test the number of bacteria in just compost so that we can see how many bacteria we are adding to the soil by adding the compost. Then we are going to wait a week and test to see the number of bacteria in each group of plots. If the number of bacteria in the soil and compost mixture is more than the amount of bacteria in just the soil and just the compost combined then we can prove that compost will increase the density of bacteria. Our school is currently using compost as fertilizer on the lower school garden, and this experiment will determine if compost increases microbial density in soil.

Figure 1 *



Composting Started at RPCS

* Taken from Martha Barss, Environmental Education and Sustainability Coordinator at RPCS

II. Hypothesis

Adding compost to the soil will increase bacteria density in the soil.

III. Procedure

a. Independent Variable

Presence of compost

b. Dependent Variable

Density of bacteria in soil

c. Negative Control

The three plots without compost

d. List of Controlled Variables

1. Amount of sunlight that the plots of soil receive
2. Amount of artificial light that the plots of soil receive (none)
4. Amount of water that plots of soil receive (only the rain)
5. Amount of shade that plots of soil receive (no shade)
6. Number of trees within 25 feet of the plots
7. Type of plot (just soil, just compost, soil and compost)
8. Amount of contact between plots and humans (no contact)
9. Slope of the plots (all on flat ground)
10. Amount of soil plots and soil/compost plots (three of each)
11. Amount of chemicals/fertilizer added to plots (no chemicals/fertilizer added to plots)
12. Place where plots are located (behind patio outside of RPCS cafeteria)
13. Day and time that soil was collected
14. Amount of samples collected from each plot
15. Time that positive control samples are tested (9:25-10:45 on May 5, 2010)
16. Time that experimental data samples are tested
17. Type of water used for testing (sterile)
18. Amount of compost added to each compost plot (500 ml, 1500 ml total)
18. Amount of compost added to negative control (no compost added)
19. Protection of compost with saran wrap
20. Type of gloves
21. Use of gloves
22. Protection of pipettes from dust using saran wrap

e. Step-by-Step Instructions

1. Make six plots: three with just soil, and one with compost on top of the soil
2. Flag off these six plots to eliminate people traffic
3. Using a soil cylinder, use twisting action to embed soil core sampler to 1st mark (rotate clockwise)
4. Twist 360 degrees to isolate sample
5. Place soil core sample in clean plastic storage bag for transport to the lab
6. Repeat steps 3-5 until three samples are taken from each plot, but keep three samples from one plot in one bag
7. Use these samples as positive control
8. Add 500 ml of compost to the three compost plots
6. Use a clean, new transfer pipette to add 10 ml to a 15 ml culture tube. Label the tube “100.”.
7. Use the same pipette to add 9 ml to a second 15 ml culture tube. Label the tube “10-1.”
8. Repeat step 2 three more times to three additional 15 ml culture tubes, only label them “10-2,” “10-3", and “10-4" respectively.
9. Place 1 cc of your soil sample into the “100”culture tube.
10. Cap the tube and shake vigorously.
11. Using a new clean pipette, remove 1 ml of the soil/water mixture from the “100” tube and place into the “10-1” tube.
12. Cap and shake vigorously.
13. Using the same pipette in step 5, remove 1 ml of the soil/water mixture from the
14. “10-1” tube and place into the “10-2” tube.
15. Cap and shake vigorously.
16. Using the same pipette in step 5, remove 1 ml of the soil/water mixture from the
17. “10-2” tube and place into the “10-3” tube.
18. Cap and shake vigorously.
19. Using the same pipette in step 5, remove 1 ml of the soil/water mixture from the
20. “10-3” tube and place into the “10-4” tube.
21. You should now have a total of five culture tubes.
22. Plate 100 µl samples from the 4th and 5th tubes (dilutions 10-3 & 10-4) onto their own separate, labeled petri plates containing nutrient agar (NOTE: on your first sample, plate ALL 5 dilutions to determine which two dilution values will give you the best data; dilutions 10-3 & 10-4 are only the most probable ones).
23. Allow to grow for 48 to 72 hours.
24. Examine each of the plates for individual bacteria colonies and choose the plate with the fewest colonies (but at least 5) to make your estimates of the number of bacteria in the original 1 cc soil sample using the following formula: # Microbes in 1 cc of soil = # Colonies on sheet x 102 x 10 | dilution # at which these colonies were found|.
25. If there are not individual colonies but still a “lawn” at the 10-4 dilution, repeat the dilution adding a 5th (10-5) & 6th (10-6) dilutions, etc. as necessary until individual colonies are observed.
26. Repeat steps 3-26 for negative control and experimental data (compost and soil plots)
27. Get GPS coordinates (N: 39 degrees 21.425, W 076 degrees 38.162)
28. Formula for bacteria count: # of colonies • $10^2 \bullet 10^{|\text{dilution factor}|}$

IV. Data and Analysis

A. Data Table

Samples 1, 2, 3 are no compost plots, samples 4, 5, 6 are compost plots

Positive Control

Bacteria Sample	Number of Colonies	Bacteria Density
1: 10^{-3}	13	1,300,000
2: 10^{-3}	23	2,300,000
3: 10^{-4}	13	13,000,000
Average Bacteria Density for Just Soil	16 and 1/3	5,533,333
4: 10^{-3}	22	2,200,000
5: 10^{-3}	23	2,300,000
6: 10^{-3}	30	3,000,000
Average Bacteria Density for Soil with added Compost	25	2,500,000

Formula for bacteria count: # of colonies • 10^2 • $10^{|\text{dilution factor}|}$

Bacteria Density in Compost Bin

Bacteria Sample	Number of Colonies	Bacteria Density
Just Compost: 10^{-4}	70	70,000,000

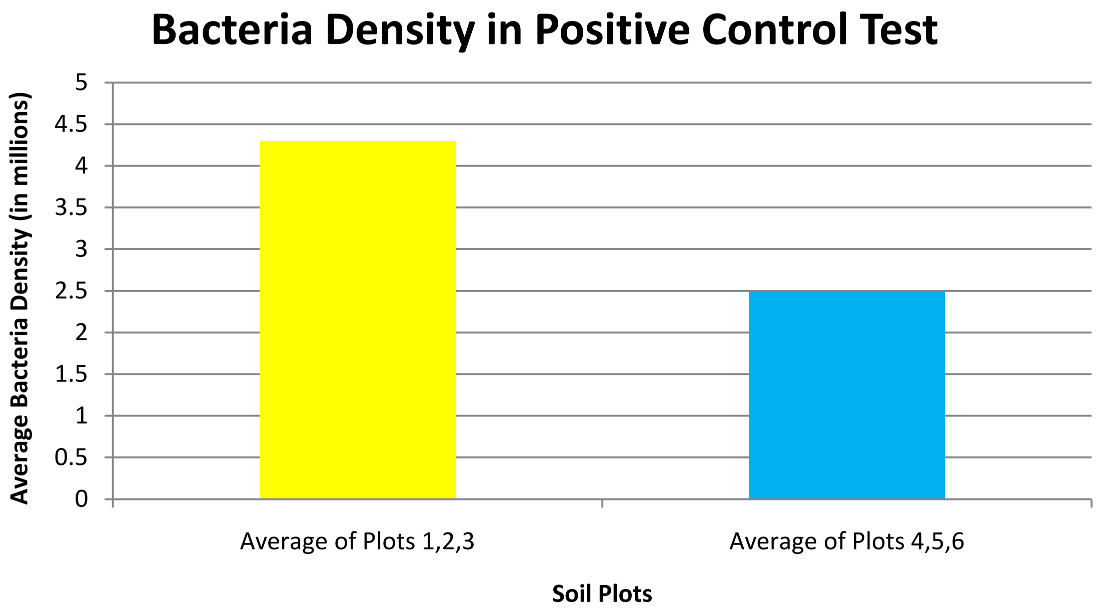
Formula for bacteria count: # of colonies • 10^2 • $10^{|\text{dilution factor}|}$

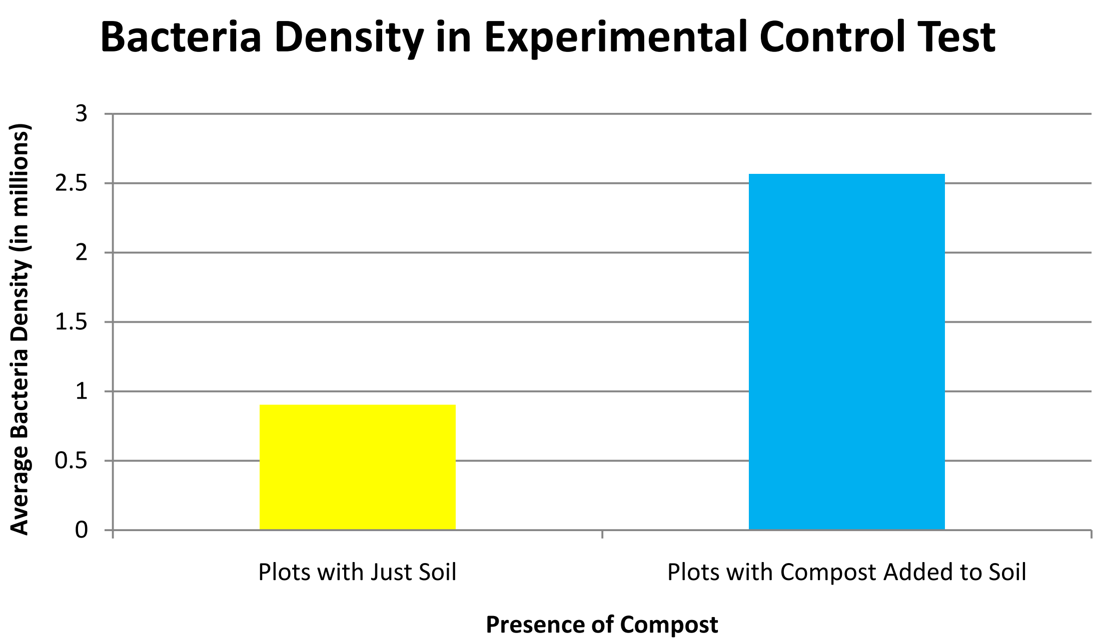
Experimental Data

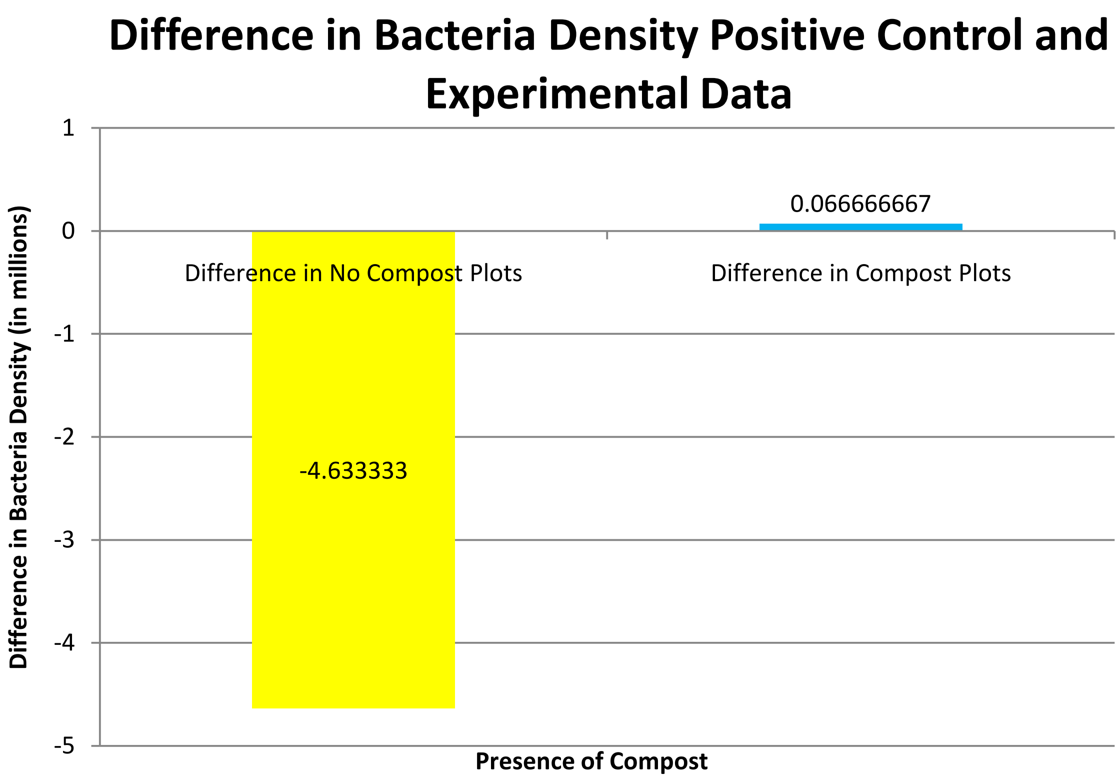
Bacteria Sample	Number of Colonies	Bacteria Density
1: 10^{-3}	7	700,000
2: 10^{-3}	14	1,400,000
3: 10^{-3}	6	600,000
Average Bacteria Density for Just Soil	9	900,000
4: 10^{-3}	7	700,000
5: 10^{-4}	5	5,000,000
6: 10^{-3}	20	2,000,000
Average Bacteria Density for Soil with added Compost	10 and 2/3	2,566,666 and 2/3

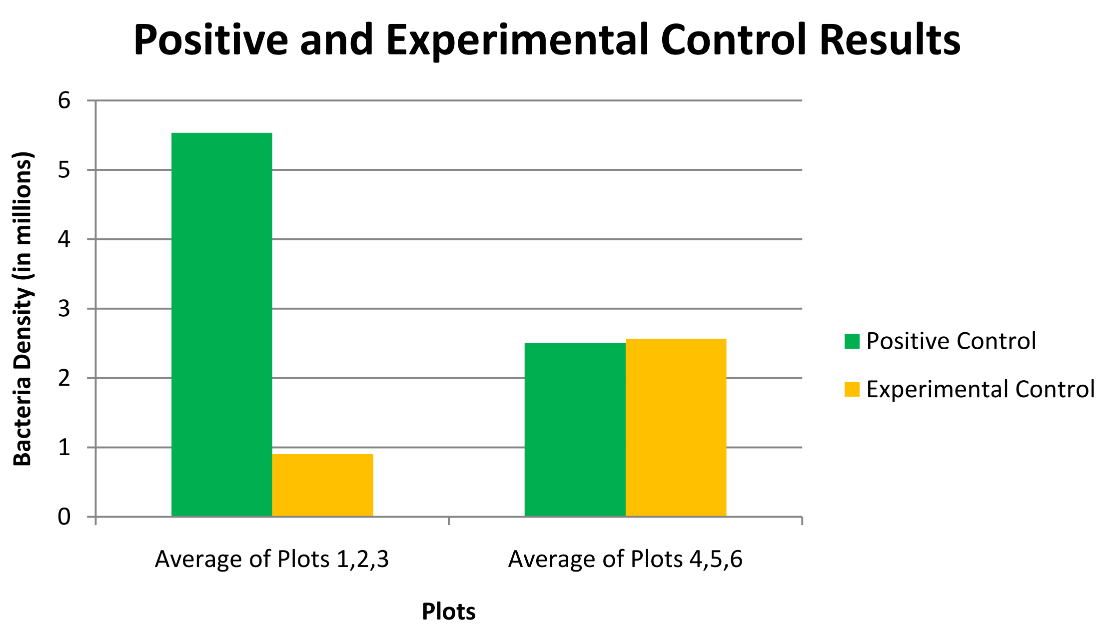
Formula for bacteria count: # of colonies • 10^2 • $10^{|\text{dilution factor}|}$

B. Graph or Summary of Analysis






Difference in Bacteria Density Positive Control and Experimental Data
Difference in Bacteria Density (in millions)
1
0
-1
-2
-3
-4
-5
0.066666667
-4.633333
Difference in No Compost Plots
Difference in Compost Plots
Presence of Compost


Positive and Experimental Control Results
Bacteria Density (in millions)
6
5
4
3
2
1
0
Average of Plots 1,2,3
Average of Plots 4,5,6
Plots
Positive Control
Experimental Control

V. Conclusion

Our hypothesis was supported. We predicted that compost would increase bacteria density in the soil, and bacteria density in the compost plots increased by about 66,667 bacteria, or about 2.6 percent. The bacteria density increased in these plots because the compost alone contained over 70 million bacteria. When the bacteria-rich compost was added to the soil, the amount of bacteria in the soil increased. However, it didn’t increase by much because of water deficiency. In the week that we allowed the plots to sit without touching them, there was no rainfall and we did not water the soil. Because of this, bacteria density went down in the soil without compost by about 4,633,333 million bacteria. Bacteria cannot grow without water because water is something they need to survive (Bacteria, 2010). The plots with compost added however, were able to increase in bacteria density even though there wasn’t water added. This is because the compost, which was so full of bacteria, made up for the lack of water when added to the soil. When we first measured bacteria density in the soil for our positive control, there was not a water deficiency because it had rained the day before. By the time that we did our experimental control and negative control, which was one week later, the soil had dried out and bacteria density had decreased.

If we were to do this experiment again, we would water each plot with 200 ml of water each day to ensure that water deficiency would not cause bacteria to decrease. At the beginning of our experiment we thought about watering the plots, but we decided that it would be unnecessary because the plots would get water from the rain. Since there was no rain, however, the plots did not get any water. If we were to do the test again, we would also let the compost sit in the plot for two weeks instead of one week. This would allow the compost to have more of an effect on the bacteria growth in the soil. Thirdly, if we had more time, we would do many experimental tests on different days. This would also help us get more accurate results.

In addition to water, bacteria need to be in an environment of a certain temperature in order to survive. Most bacteria thrive at a temperature of about 98.6 degrees Fahrenheit, but a good temperature really depends on what type of bacteria is living in the area. If we had more time and a larger budget, we could have made our own environment in which it was a set temperature all the time. This would control the temperature of the area that the bacteria were living in and allow the bacteria to increase as much as possible. Bacteria also thrive at a certain pH level. Most bacteria have the highest survival rate in a pH of about 6.7 to 7.5. We did not test our soil to see what the pH was. The bacteria may not have grown well due to the pH not being at a suitable level for bacteria. What pH is best for bacteria also depends on the type of bacteria that is in the soil. If we were to do this experiment again, we would test the pH of the soil to see if it had the pH that allows bacteria to grow. (Bacteria, 2010)

Roland Park Country School can use this information for the school community. We have recently begun composting at RPCS, and we now know that adding compost to soil does increase bacteria density in the soil. This is important because bacteria are what convert nitrogen into different forms that plants can use. Nitrogen is crucial to sustaining life because it allows organisms to build amino acids, nucleic acids, and proteins. Most organisms cannot use nitrogen in its original form. Therefore, nitrogen fixation must occur, the process in which bacteria convert dinitrogen gas into ammonia. Ammonia is the type of nitrogen that organisms can use to build nucleic acids, amino acids, and proteins. Therefore, bacteria are an essential factor in sustaining plant life in the soil.

Since we now know that compost increases bacteria density in the soil, we should continue to add compost to our RPCS garden. We also know that watering the compost plots is extremely important. Additionally, we know the pH and temperature that bacteria like to live in. We can share this information with the RPCS community so they could make changes to our soil and hopefully continue to help plants grow in the soil!

Our hypothesis was supported. We predic

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