Eunice Kwon, Kendall Poling,
Theresa Statkiewicz, Beth Yancey

Background

Many things influence the soil. It is a living thing made out of many different organisms. In soil there are numerous tiny single celled organisms called microbes. They are so tiny that millions could fit into the eye of a needle (American Society for Microbiology, 2002). The microbes in the soil can be changed and altered with different types of fertilizer. The fertilizer affects the soil by giving it numerous nutrients and by providing nitrogen. By altering the nitrogen level in the soil, the amount of bacteria found in the soil will be affected. It is important to know the relationship between bacteria and nitrogen when choosing a fertilizer, along with knowing the amount of nitrogen in the fertilizer because it affects the soil quality.

There are many types of fertilizer, such as organic fertilizer and nitrogen fertilizer. Fertilizer is put into the soil to provide an ideal environment in which plants can grow. The best kind of fertilizer is organic fertilizer because the organic material that is added to the fertilizer gives plants and soil nutrients that they would not get otherwise. Most of the nitrogen found in the soil comes from the organic material that the organic fertilizer contains (Queen's Printer for Ontario, 2003). Nitrogen is one of the many elements found in fertilizer. Nitrogen is an ingredient of fertilizer because nitrogen attracts liquids (Recommendations of the Nitrogen Fertilizer Task Force on the Nitrogen Fertilizer Management Plan, 2003), thus providing the adequate amount of liquids for plant growth. Average fertilizer levels are 10-10-10 or 13-13-13, depending on the type of vegetation.

The nitrogen found in fertilizer greatly affects soil and the bacteria in the soil. A common type of nitrogen in fertilizer is ammonia nitrogen, which is a primary form of nitrogen. Ammonia reacts with water and binds tightly to soil surfaces; some microorganisms can be killed in the zone where ammonia is applied because ammonia is alkaline. However, soil microorganisms can rapidly reproduce to take advantage of nitrogen, since they reproduce through binary fission (Food Web of the Compost Pile, 2003). The bacteria in the soil use nitrogen for their other needs, too. They require nitrogen and carbon dioxide for living, and they use nitrogen to make protein, which gives the plants strength. The protein makes enzymes, which start and stop chemical reactions in the plant. These chemical reactions keep the plant alive. The protein is also used for population growth. Plants and soil cannot benefit from nitrogen in its original form, however. In order for the nitrogen in fertilizer to help soil and plants, the nitrogen must be converted to a nitrate form because plants can only use nitrogen in the nitrate form. Bacteria aid the plant in converting the nitrogen into nitrate. The nitrate moves within the soil solution (Mancl, 1987) and can reach many areas, thus reaching many plants that are spread out within a large area. In our experiment, we will use Miracle Gro fertilizer. To facilitate the growing of plants, Miracle Gro uses peat moss, plant waste, aluminum sulfate, mulch, and nitrogen.

Along with nitrogen, bacteria are also necessary for the survival of plants. Bacteria play a significant role in aiding plants in the process of obtaining nutrients. Friendly bacteria decompose crop waste by turning it into healthy soil with nutrients. The organic material that the bacteria decompose contains carbon and nitrogen. Plants can use the nitrogen found in organic fertilizer with the help of bacteria in the soil. The bacteria use carbon and nitrogen as energy and protein. When they are in a warm, moist environment, bacteria can convert ammonium to nitrate for the plant to use. This process is known as nitrification (Mancl, 1987). Bacteria also convert nitrates into nitrous oxide, nitrogen, and other nitrogen gases, and oxygen when there is little oxygen present in the soil (Manage the risks for fall-applied fertilizer, 2003). Then they use the newly created nitrogen and oxygen for respiration. This respiration produces water and carbon dioxide, which plants needs in order to survive. When little oxygen is available to the bacteria, the soil will smell like rotten eggs because of substances produced: hydrogen sulfide and ammonia-like substances. available to the bacteria, the soil will smell like rotten eggs because hydrogen sulfide and ammonia-like substances are produced.

Nitrogen in fertilizers acts as plant nutrients and is important for food production. Man-made fertilizers are made to contain the same ingredients as natural fertilizers. Natural fertilizers consist of nutrients that exist naturally in the soil, atmosphere and can be found in animal manure. All plants require nitrogen, phosphorus and potassium (The Fertilizer Institute, n.d). Nitrogen helps plant with leaf growth and the building of plant materials. Phosphorus helps the plants produce seeds and grow roots. Potassium helps improve fruit quality and improve resistance to disease. All plants play a role in the environment through the N-P-K (nitrogen, phosphorus and potassium) that they are grown with.

The nitrogen cycle is one of the most important nutrient cycles in the ecosystem. In order for living things to produce organic molecules such as amino acids and proteins, the organisms need nitrogen. Nitrogen is mostly stored in the atmosphere, but it is also found in soil and oceans. (Pidwirny, 2001) Plants need nitrogen in either ammonium form or nitrate form. Most plants get their nitrogen from the soil. The nitrogen cycle is the way that nitrogen affects the environment. It begins with the breakdown from macroorganisms, or insects and beetles in the soil. (Whitelaw, 1997) What the macroorganisms don't break down is then broken down through fungal decomposition from the fungi. (Whitelaw, 1997) Then, larger organisms such as earthworms eat the fungi and bacteria that broke down the original organisms. Ammonia compounds are left when the earthworms leave droppings. Then, bacteria called nitrifying bacteria turn the nitrogen into a form that can be used by plants and then, larger organisms. (Whitelaw, 1997).

Since bacteria use plants for energy, increased amounts of nitrogen will mean increased energy for the bacteria. With increased energy, the bacteria will be able to reproduce more rapidly thus creating more bacteria in the soil. Because of the greater number of bacteria in the soil, the other secondary consumers, fungi and algae, may lose or gain energy because of the changed population density. The organisms that consume the microorganisms, collembola, nematodes, and protozoa, may get more energy because there are a greater number of microorganisms. In that sense a greater number of bacteria could be helpful.

Bacteria contribute to the balance of the soil. They act as decomposers by consuming pathogens and organic matter and producing sticky substances that act as glue to hold the soil together. ("World Under the Soil", 2003) Bacteria also provide good soil structure, allowing air and water to move through. ("World under the Soil", 2003). Bacteria help keep the soil, which therefore benefits to the survival of plants. The beneficial fungi from the microorganism breakdown eventually leads to the soluble nitrates, which leads back to the beneficial fungi, and helps feed plants. (Montavalli, Peter, 2003). Bacteria also provide good soil structure, allowing air and water to move through.

In our experiment we plan to add fertilizer that contains nitrogen in hopes of finding how it will alter the number of bacteria in the soil. At first increasing the amount of ammonia nitrogen will probably increase the amount of bacteria, for up to a certain amount. However eventually there will probably be an amount that will be too much nitrogen and the amount of bacteria will decrease.

I. **Problem**: How do increasing levels of concentration of fertilizer in soil change the population density of the bacteria in soil?

II. **Hypothesis**: In the plots with a greater concentration of fertilizer, there will be a greater amount of ammonia and therefore a larger concentration of bacteria.

III. **Experiment**:

a. **Variables**:
   - i. Independent variable 1: Concentration of fertilizer
   - ii. Independent variable 2: Levels of ammonia in soil
   - iii. Dependent variable 1: Population density of bacteria

b. **Controls**:
   - i. Negative control 1: Zero concentration of fertilizer
   - ii. Controlled variables:
     1. Size of each land plot
     2. Time samples taken and tested
     3. Location of land plots
     4. Amount of rain on each land plot
     5. Amount of sunlight on each plot
     6. Type and amount of plants on each land plot
     7. Amount of human contact on each land plot
     8. Distance of each land plot from construction/development
     9. Temperature
     10. Type of fertilizer
     11. Amount of liquid added
     12. Amount of soil that is diluted
     13. Equipment
     14. Type of test solution
     15. Contamination of micro pipettes or sterile cotton swabs
     16. Contamination of culture tubes
     17. Type of petri dishes

c. **Procedure**
   - i. Plot 5 0.5 x 0.5 meter plots of land with a stake at each corner and at every .25 meter points.
     1. Our Global Positioning System was:
        - a. Plot 1- North 39.35708, West 76.69057
        - b. Plot 2- North 39.35699, West 76.63956
        - c. Plot 3- North 39.35694, West 76.6359
        - d. Plot 4- North 39.35693, West 76.63590
        - e. Plot 5- North 39.35691, West 76.63588
   - ii. On Day 0, mix the following amounts of fertilizer with 150 mL of water.
     1. Plot 1- No fertilizer added to water
     2. Plot 2- 5 g. of fertilizer
     3. Plot 3- 10 g. of fertilizer
     4. Plot 4- 15 g. of fertilizera
     5. Plot 5- 20 g. of fertilizer

iii. Stir with stirring rod until fertilizer dissolves.
iv. Pour the fertilizer solution so that the entire plot of land is covered. Do this efficiently by pouring the solution in a snake like motion: from the beginning of the first flag to the second flag.
v. On Day 1 of the experiment (wait 4 days after applying fertilizer), take 3 samples of 15 cm deep with a diameter of 2 cm from each plot of land using a soil test core.
vi. Put the 15 soil samples in separate plastic bags that are labeled with the plot land and the sample number. For example, for the plot 1, sample 1 bag, the bag should be labeled "Plot 1 Sample 1".
vii. Place the bags in room temperature.
viii. On Day 2, perform the serial dilution test (through the plating of the bacteria), while performing the LaMotte ammonia nitrogen test for each of the soil samples.
ix. For the serial dilution test, plate only the 10^{-3} dilution onto a gelled petri dish.
x. Using the ammonia nitrogen test chart, record results
    1. Very low- 0
    2. Low- 1
    3. Medium- 2
    4. High-3
xi. On Day 3 of the experiment, count the number of bacteria colonies on each petri dish.
xii. Then, multiply the number of colonies on the plate by 10^2, which equal the number of bacteria in dilution tube. Then, multiply the number of bacteria in the dilution tube by 10^4.
xiii. Record the number of bacteria.

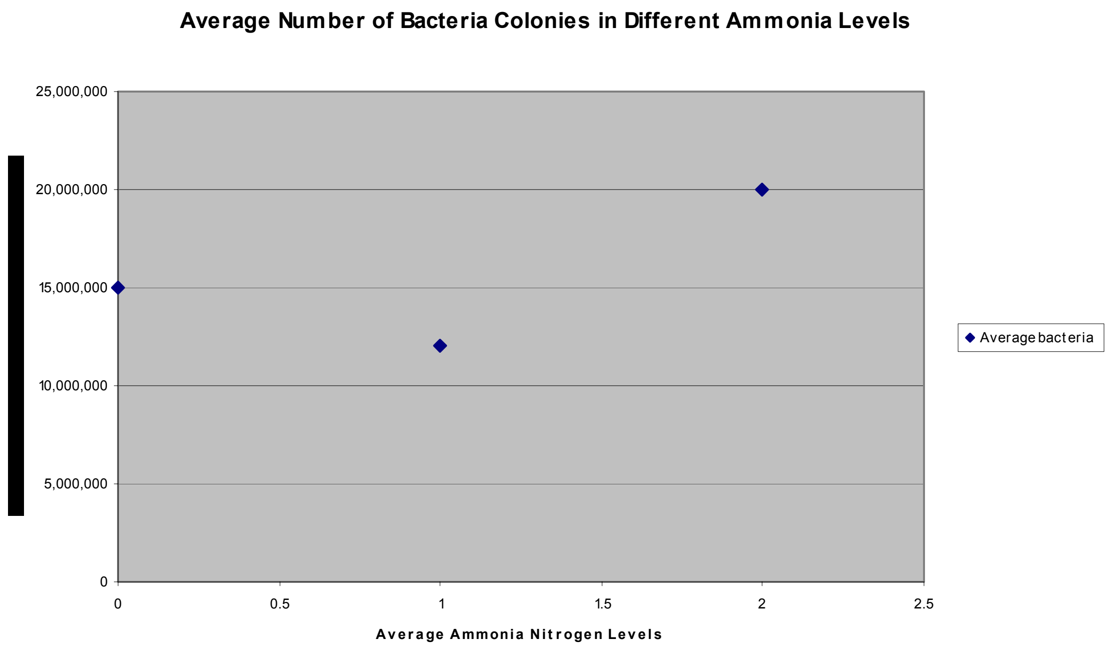
IV. Data and Analysis

Key
Very Low- 0
Low- 1
Medium- 2
High-3

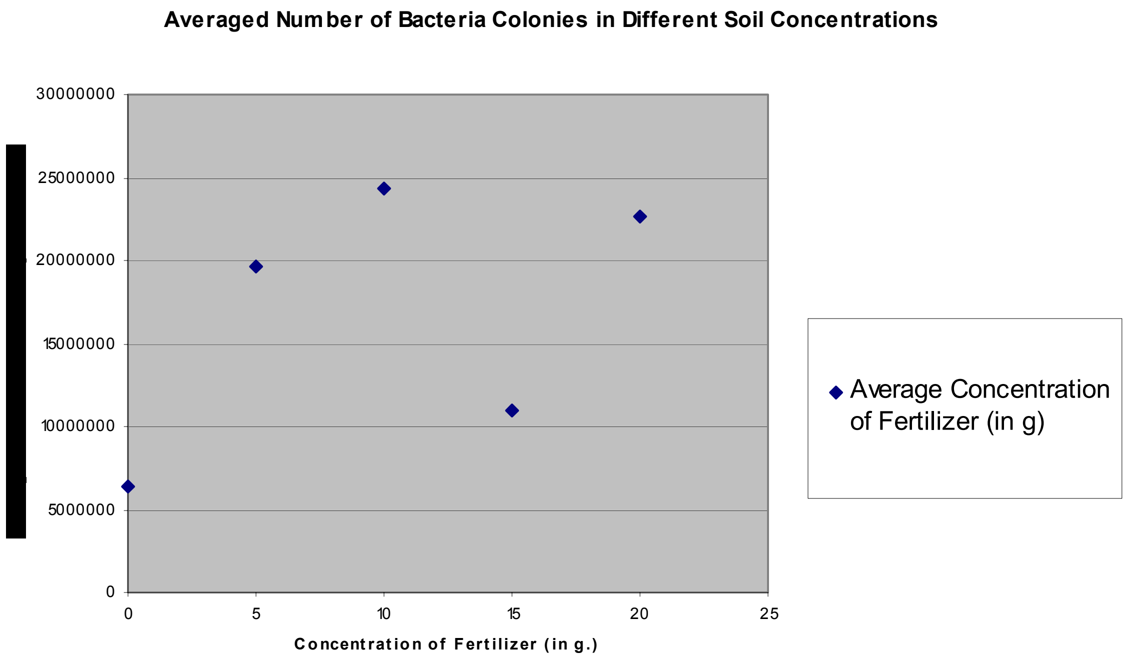
		Number of Bacteria Colonies in Original Sample Tube	Ammonia Nitrogen Levels in Different Plots
Plot 1			
	Sample 1	2000000 per cubic cm	0

	Sample 2	15000000 per cubic cm	1
	Sample 3	2000000 per cubic cm	0
	Average	6333333.33 per cubic cm	0.33
Plot 2			
	Sample 1	44000000 per cubic cm	1
	Sample 2	9000000per cubic cm	1
	Sample 3	6000000 per cubic cm	1
	Average	19666666.66 per cubic cm	1
Plot 3			
	Sample 1	51000000 per cubic cm	0
	Sample 2	9000000 per cubic cm	1
	Sample 3	13000000 per cubic cm	1
	Average	24333333.33 per cubic cm	0.66
Plot 4			
	Sample 1	7000000 per cubic cm	0
	Sample 2	6000000 per cubic cm	1
	Sample 3	20000000 per cubic cm	2
	Average	11000000 per cubic cm	1
Plot 5			
	Sample 1	13000000 per cubic cm	0
	Sample 2	28000000 per cubic cm	1

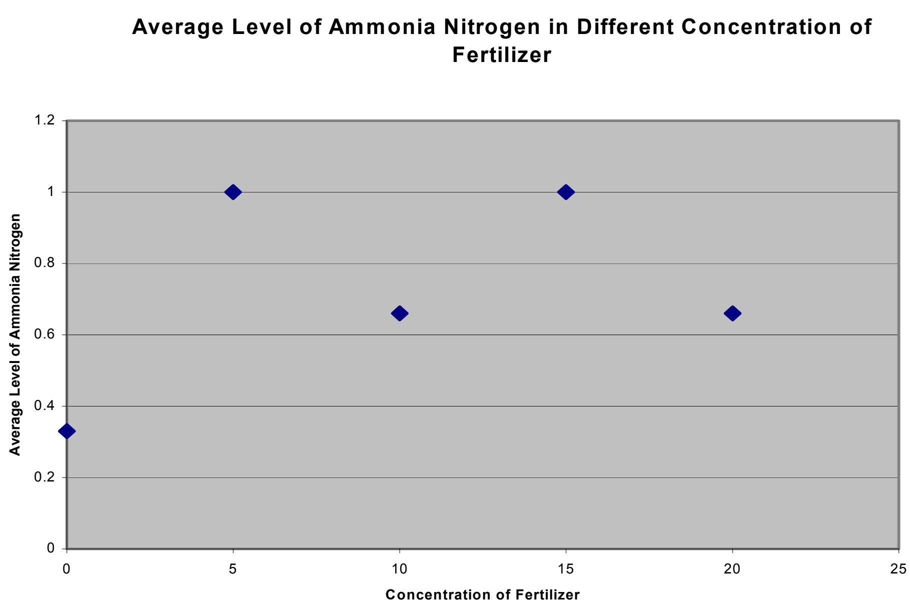
	Sample 3	27000000 per cubic cm	1
	Average	22666666.66 per cubic cm	0.66



Key
Very Low- 0
Low- 1
Medium- 2
High-3



Key for Ammonia Nitrogen
Very Low- 0
Low- 1
Medium- 2
High-3



V. Conclusion

Our hypothesis for our experiment, in the plots with a greater concentration of fertilizer, there will be a greater amount of ammonia and therefore a larger concentration of bacteria, was proven incorrect. All of the plots had about the same amount of ammonia nitrogen, regardless of the amount of fertilizer we put on. The ammonia nitrogen level for plot 1, sample 1 was very low, plot 1, sample 2 was low, and the third sample from plot 1 was very low. All three samples we took from plot 2 had a low ammonia nitrogen concentration. The first sample from plot 3 had a very low concentration, and the second and third samples both had low concentrations. In plot 4, the ammonia nitrogen levels differed for each sample we took. The first sample had a very low concentration, the second had a low concentration, and the third sample had a medium concentration. The amount of ammonia nitrogen concentration for plot 5 sample 1 was very low, and the second and third samples from plot 5 were both low. We expected the amount of ammonia nitrogen concentration to increase from plot 1 to plot 2 and from plot 2 to plot 3, etc, with plot 5 having the highest concentration. The more fertilizer we put in each plot, the higher the concentration of ammonia nitrogen we expected to have. We expected the number of bacteria to increase as we increased the concentration of the fertilizer, however it did not. The average number of bacteria in plot 1 was 6333333.33 per cubic centimeter and increased to 19666666.66 bacteria per cubic centimeter for the average of plot 2. The number of bacteria continued to increase in plot 3 with an average of 24333333.33 bacteria per cubic centimeter. The number of bacteria in plot 4 was significantly lower than the bacteria in plot 3, there were 11000000 bacteria per cubic centimeter. The number of bacteria increase from plot 4 to plot 5; there were 28000000 bacteria per cubic centimeter in plot 5. Our hypothesis was proved wrong as we expected the ammonia nitrogen level and the number of bacteria per cubic centimeter to increase from plot 1 to plot 5.

There are two changes that we would make to our experiment if we were to do it again in the future: we would allow more time between applying the fertilizer and testing the samples and apply the fertilizer to the plots more evenly. This would give the fertilizer more time to change the soil and we would have more varying results for Ammonia Nitrogen test. Applying the fertilizer the plots more evenly would make all of the soil in each plot almost the same. We had a few sources of error. The first was that we did not have a positive control. The second was that we did not pour the fertilizer on the plots in an orderly fashion so every part of the plot had a different amount of the fertilizer on it. To have more accurate results to being with we should have wait more days after we put the fertilizer on the plots to take soil samples. Waiting only two days after putting the fertilizer on the plots did not allow for enough change in the bacteria and ammonia Nitrogen levels. We realized this after we tested all of the samples that we took on Day 1 of our experiment.

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