Soil Ecology Project

Alex Pace, Meredith Callis, Cate Wilson, Sarah Welch

Background

As a final project in ninth grade biology we were asked to do a soil ecology project. This project was to be created and tested by ourselves. We began by doing general research and decided that we wanted to address a question that had not been addressed before. The effects of fertilizer had been tested before but no one, to our knowledge, had asked the question of at what point does the fertilizer negatively affect the soil. We also began to research the vital relationship between fertilizers in the soil and the bacteria level or the amount of bacteria. We wanted to know if an overload of fertilizer would kill bacteria in the soil. After research we found the correlation between the amount of bacteria and the health of the plant. The more we researched the more we realized fertilizer may have a negative impact on the soil. Bringing us to create the hypothesis of "If double the amount of recommended fertilizer is added to a plot of land (25 by 25 centimeters) then the amount of bacteria that was originally there will decrease." We are trying to find the limit and the safest amount of fertilizer to use until it starts hurting the plant. The health of the plant will be found by looking at the nitrate nitrogen level and the amount of colonies of bacteria in the soil, before and after we adjust the conditions of the soil by adding different amounts of fertilizer.

Fertilizer (be it natural or man made) is a vital part of a plants existence. For a plant to live, it must have different chemical elements. Some of these chemical elements are

carbon, hydrogen and oxygen. These chemicals are available from air and water. Nitrogen, phosphorus, potassium are the three macronutrients and the three elements found in most fertilizers. Nitrogen plays an important role in our experiment because we use it to correlate its own relation with the number of colonies of bacteria. Sulfur, calcium, and magnesium are all secondary nutrients. Finally Boron, cobalt, copper, iron, manganese, molybdenum and zinc are all considered micronutrients. Nitrogen, phosphorus and potassium are the most important of all these chemicals, though all of them are vital to the plants existence. Nitrogen, phosphorus and potassium are important because they are necessary for amino acids, cell membranes and ATP. Amino acids, cell membranes and ATP are the "building blocks" for cells. Each chemical has a different building block to take care of. Each amino acid needs nitrogen. Just as each molecule making up every cell's membrane and each molecule of ATP contains phosphorus. Potassium makes up 1 percent to 2 percent of the weight of any plant. Potassium is the ion in cells, making it essential to metabolism. Without these chemicals a plant simply cannot live. If these micronutrients are not in the soil naturally then the growth rate of the plant will be limited. The nitrogen, phosphorous and potassium that are absent from the soil often come from other decomposing plants. "In the case of nitrogen, the recycling of nitrogen from dead to living plants is often the *only* source of nitrogen in the soil." (http://science.howstuffworks.com/question181.htm) So when plants are not growing all they need is a supply of what they are lacking. Fertilizer supplies the needed chemicals to help a plant to grow "The numbers on a bag of fertilizer tell you the percentages of available nitrogen, phosphorus and potassium found in the bag. So 12-8-10 fertilizer has 12-percent nitrogen, 8-percent phosphorous and 10-percent potassium. In a 100-pound

bag, therefore, 12 pounds is nitrogen, 8 pounds is phosphorous and 10 pounds is potassium. The other 70 pounds is known as ballast and has no value to the plants" (http://science.howstuffworks.com/question181.htm) Fertilizer has become a two sided sword. Its benefits for a plant without the adequate amount of chemicals are endless but its dangers to already healthy plant are deadly.

Bacteria come in the masses. They are simplistic and one of the oldest living forms on earth. Bacteria were here 3.5 billion years ago. In a bacterium's structure there is the capsule, the cell wall, and a cell membrane. Inside is the cytoplasm, then the endospore. There are 2.5 billion bacteria in one gram of soil, but they are found everywhere. There are five different kinds of bacteria. Bacteria are important in their ecosystem. Every living thing depends on bacteria weather that is directly or indirectly. But out of all the jobs that bacteria do decomposing is one of the most important jobs bacteria do. When plants die they decompose. Really the bacteria is releasing carbon to the atmosphere which plants use. The bacteria release vital nutrients into the air and soil. Bacteria is important earth's ecology. Bacteria also has the job of nitrogen fixing. Plants cannot use the nitrogen in the air, nor do they use atmospheric nitrogen. Nitrogen-fixing bacteria change atmospheric nitrogen into simpler substances. Those simpler substances are called nitrites. Nitrogen-fixing bacteria helps to replace the nitrogen in the soil so that plants can survive and flourish.

Given how fertilizer works, in the soil there are organisms that do a similar job. The nitrogen cycle is the cycle that a plants are most dependant on. This cycle allows a plant to grow, if used properly. The nitrogen cycle can harm plants if the wrong amount of ammonia is in the cycle. Nitrogen fixation is an important part of the nitrogen cycle.

This process happens naturally by a many different prokaryotes. Prokaryotes are organisms without a cell nucleus. One of these prokaryotes is bacteria. Bacteria are unicellular microorganisms. These bacteria are an significant part of the nitrogen cycle. These microorganisms are the most important part of this cycle. Ammonia can be made from the gasses in the air, from molecules of decomposing things, or the commonly used fertilizer. The most common and harmful use of ammonia comes from fertilizers. Ammonia gives food to the bacteria that then consume that food and "reproduce" creating more bacteria. Amonium is eaten by the bacteria to make its protein to live, as this happened the excess ammonium is converted into nitrates. The plants then consume In doing this, it allows more the bacteria has enough food to survive and therefore the plant does as well. However, is too much ammonia is used then the amount of bacteria will be to great, and the bacteria as well as the plant will become dependent on the fertilizer as its food. If the fertilizer was taken away, the bacteria would unable to survive due to its dependency on the fertilizer that is no longer there. This would then cause plants to die or become unhealthy, for the amount of bacteria is directly connected with the health of the plant. After the bacteria it consumes the ammonia it creates nitrates. The nitrates allow the plant to grow. Observing the change in nitrate levels according to a allotted plots of land with no fertilizer to a plot with fertilizer will allow us to see where the "border line" for a healthy amount of fertilizer is.

To address all of our hypothesis and problem we planned our experiment in a manner that would allow us to pinpoint when the fertilizer began to be detrimental to the soil. We plotted nine plots, each 25cm by 25cm and 18 cm apart. Each had a specific amount of fertilizer. Plots E1 E2 and E3 only had 700 ml of water and only 700ml of

water. R1, R2 and R3 contained the recommended amount of fertilizer along with 700 ml of water. And O1 O2 and O3 contained double the recommended amount of fertilizer along with 700 ml of water. Each plot was tested before and after the substance was added so that we could compare the nitrate nitrogen level and amount of bacteria. After collecting soil samples from each plot we tested each plot for its nitrate nitrogen level and for the number of colonies of bacteria, both before and after the substances were added so that we could compare them both. Our experiment allows us not only to prove that fertilizer can have a negative effect but it also pinpoints when the fertilizer becomes harmful.

Citations

American Society for Microbiology (2006) AVAILIBLE ONLINE AT

http://www.microbeworld.org/microbes/bacteria/

Answers Corporation Inc. (2007) AVAILIBLE ONLINE AT

http://www.answers.com/topic/bacteria

Ben Waggoner and B. R. Speer (1996) AVAILIBLE ONLINE AT

http://www.ucmp.berkeley.edu/bacteria/bacteria.html

HowStuffWorks, Inc. (1998-2007) AVAILIBLE ONLINE AT

http://science.howstuffworks.com/question181.htm

International Plant Nutrition Institute. (2007) AVAILIBLE ONLINE AT

http://www.ppippic.org/ppiweb/usanc.nsf/$webindex/article=1BDAC0F886256B8E00769AE7AFCB5843

Rachel Widenburg (1998) AVAILIBLE ONLINE AT http://www.amnh.org/nationalcenter/youngnaturalistawards/1998/bacteria.html

---

Lab Outline

I. Problem: Will an overload of fertilizer kill bacteria in the soil?

II. Hypothesis: If double the amount of recommended fertilizer is added to a plot of land (25 by 25 centimeters) then the amount of bacteria that was originally there will decrease.

III. Independent Variable: The amount of Fertilizer added to each plot

IV. Dependent Variable: Quantity of Bacteria in each plot and the amount of nitrate in the soil

V. Negative Control: Absence of Fertilizer and the presence of water on the 3 plots without fertilizer (row one)

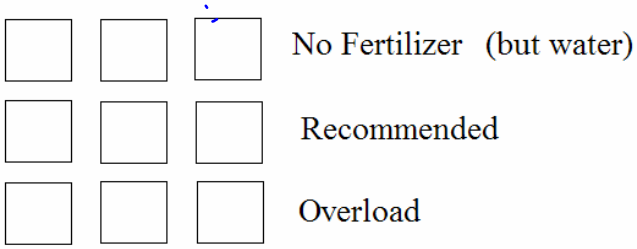
VI. List of controlled Variables:

- Where your plots are located
- The type of fertilizer
- The organism you are testing
- The spacing and how big each plot is

- When you do each test: the dilution and nitrate test must be done at the same time
- The chemicals you use
- Where you put each soil sample
- Size of soil samples
- Control for the nitrate test by following the LaMotte STH Series test kit
- Sterility of your water
- Sterility of your pipette tips
- The amount of time you shake it for
- The amount of soil you put in each
- Each cap goes with its own test tube
- Make sure to put the disposable tip of the micro pipette immediately goes into the alcohol solution
- Type of plates
- How much water you put in the tubes
- How much solution goes on each plate

VII. Step-by-step:

1) Make a 3*4 plot scheme, each plot 25 by 25 centimeters, with 18 centimeters of separation. Put each plot in the location of the following coordinates: N 39.357, W 76.636 Measurements



2) In the middle of each plot, push the soil corer (diameter _2 cm__) into the ground (Push the soil corer exactly 15 centimeters into the ground). If the ground is too hard, use a rubber mallet to bang and to help the soil corer to go far into the ground.
3) Then pull the soil corer out of the ground, and if needed, scrape the soil out of the soil corer using a metal scoop
4) Put each soil sample in its own plastic bag with each bag labeled. It should be labeled according to the trial, the plot number and the amount of fertilizer in that specific plot.
5) You must complete this nitrate test and the serial dilution test (step 6) on the same day, at the same time on the same bag for each soil sample. Use the LaMotte STH Series test kit to test for nitrate levels
6) While doing step 5, at the same time and on the same bag of soil, test for the amount of bacteria using the Serial Dilutions

Serial Dilutions:

a. Take 1 culture tube and fill it with 10 mL of sterile water and then fill 4 other culture tubes with 9 mL of sterile water using a pipet. Label the tubes as follows: 10^0, 10^{-1}, 10^{-2}, 10^{-3}. The 10^0 tube got the 10 mL
b. Place 1 cc of your soil sample into the 10^0 culture, cap and shake vigorously
c. Using a serological pipette, remove 1 ml of the soil/water mixture and place into the culture tube labeled 10^{-1} with 9 mL of sterile water, cap and shake vigorously.
d. Repeat step c. but place 1 mL of the 10^{-1} tube in the 10^{-2} tube
e. Continue step d. with each additional tube (going from the 10^{-1} tube to the 10^{-2} tube to the 10^{-3} tube) until you have diluted the original soil/water mixture 3 times (10^{-3} dilution).
f. Plate 100 µl samples from the 3rd and 4th tubes (dilutions 10^{-2} & 10^{-3}) onto their own separate, labeled Petrifilm Aerobic Count Plate and allow to incubate at room temperature over night.
g. Examine each of the plates for individual bacteria colonies and choose the plate that is the most dilute with at least 5 colonies to make your estimates of the number of bacteria in the original 1 cc soil sample (# colonies on plate * 10^2= # of bacteria in dilution tube; # of bacteria in dilution tube $10^{|\#\text{of dilutions}|}$= # of bacteria in original sample tube).

7) When you get results from tests, record in data tables.
8) Collect 9 containers with caps to mix water and the fertilizer

9) In the first 3 containers for plots 1, 2, and 3 for "Empty" put 700 ml of water with NO fertilizer added into each container. Make sure you label all 3 containers EMPTY and according to their plot.

10) In the next 3 containers, for plots 1, 2, and 3, of "Recommended" mix 700 ml of water along with 50 grams of fertilizer in each container. Label these containers "Recommended" and according to which plot

11) For the last 3 containers for plots 1, 2, and 3 of "Overload" mix 700 ml of water in each along with 100 grams of fertilizer in each container. Label these containers "Overload" and according to which plot.

12) Carry the 9 containers down to the plots, and make sure that you get the right container for the right plot. According to what the label on each container says, after you thoroughly shake it for 30 seconds, pour all of the water or fertilizer into it's specific plot. Do not pour it in one spot, make sure you pour over the whole area each plot covers

13) Wait 2 days (one class period) before you take the samples again

14) In the middle of each plot, push the soil corer (diameter _2 cm__) into the ground (Push the soil corer exactly 15 centimeters into the ground. If the ground is too hard, use a rubber mallet to bang and to help the soil corer to go far into the ground.

15) Then pull the soil corer out of the ground, and if needed, scrape the soil out of the soil corer using a metal scoop

16) Put each soil sample in its own plastic bag with each bag labeled. It should be labeled according to the trial, the plot number and the amount of fertilizer in

that specific plot. This time, the fertilizer has already been added so these samples are the samples that include the fertilizer.

17) Repeat nitrate test and serial dilutions of these new soil samples. Follow steps 5-6 at the same time on each soil sample with the fertilizer added

18) Record all the data

Data Charts/ Observations

EMPTY

Amount of Bacteria and Nitrate Nitrogen Before Fertilizer

Plot Label	Number of bacteria per cm^3	Nitrate Nitrogen in ppm
E1	820000	30 ppm
E2	170000	10 ppm
E3	2200000	20 ppm
Average	1063333	20 ppm

Amount of Bacteria and Nitrate Nitrogen After Fertilizer

Plot Label	Number of bacteria per cm^3	Nitrate Nitrogen
E1	170000	15 ppm
E2	1600000	7.5 ppm

E3	1600000	15 ppm
Average	1123333	12.5 ppm

RECOMMENDED

Amount of Bacteria and Nitrate Nitrogen Before Fertilizer

Plot Label	Number of bacteria per cm^3	Nitrate Nitrogen
R1	220000	15 ppm
R2	120000	15 ppm
R3	100000	10 ppm
Average	146666	13.333 ppm

Amount of Bacteria and Nitrate Nitrogen After Fertilizer

Plot Label	Number of bacteria per cm^3	Nitrate Nitrogen
R1	21500000	5 ppm
R2	5300000	10 ppm
R3	30500000	10 ppm
Average	19100000	8.333 ppm

OVER LOAD

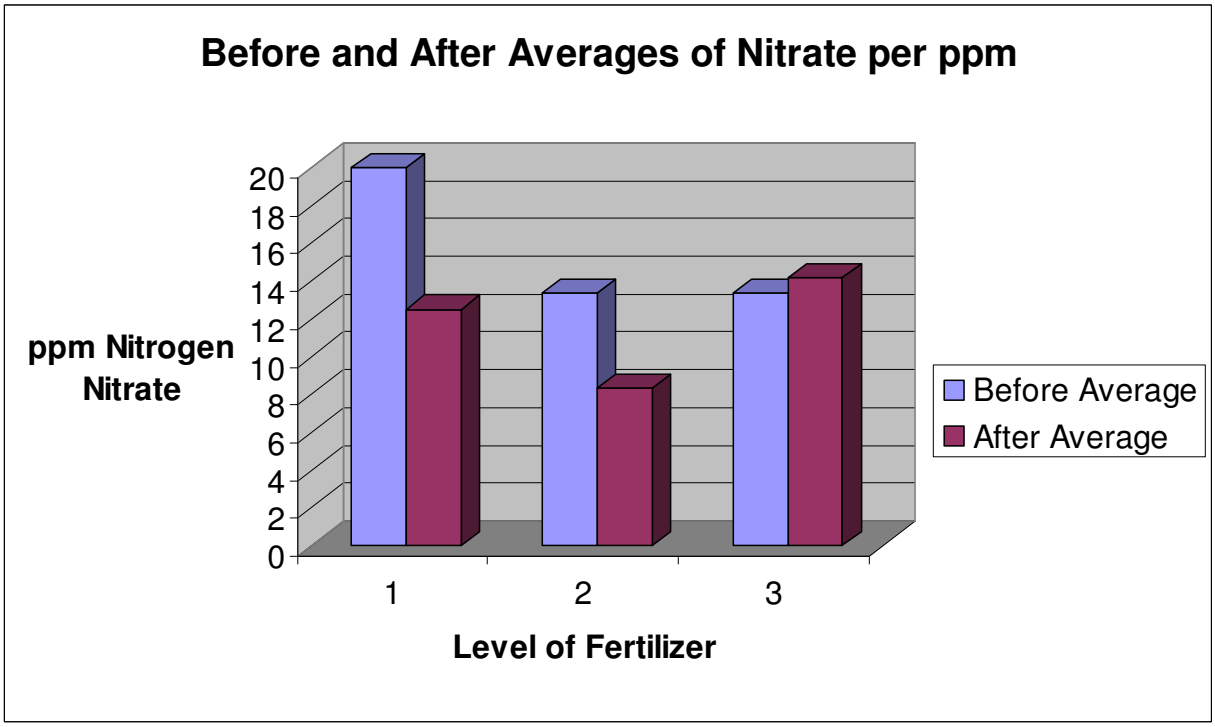
Amount of Bacteria and Nitrate Nitrogen Before Fertilizer

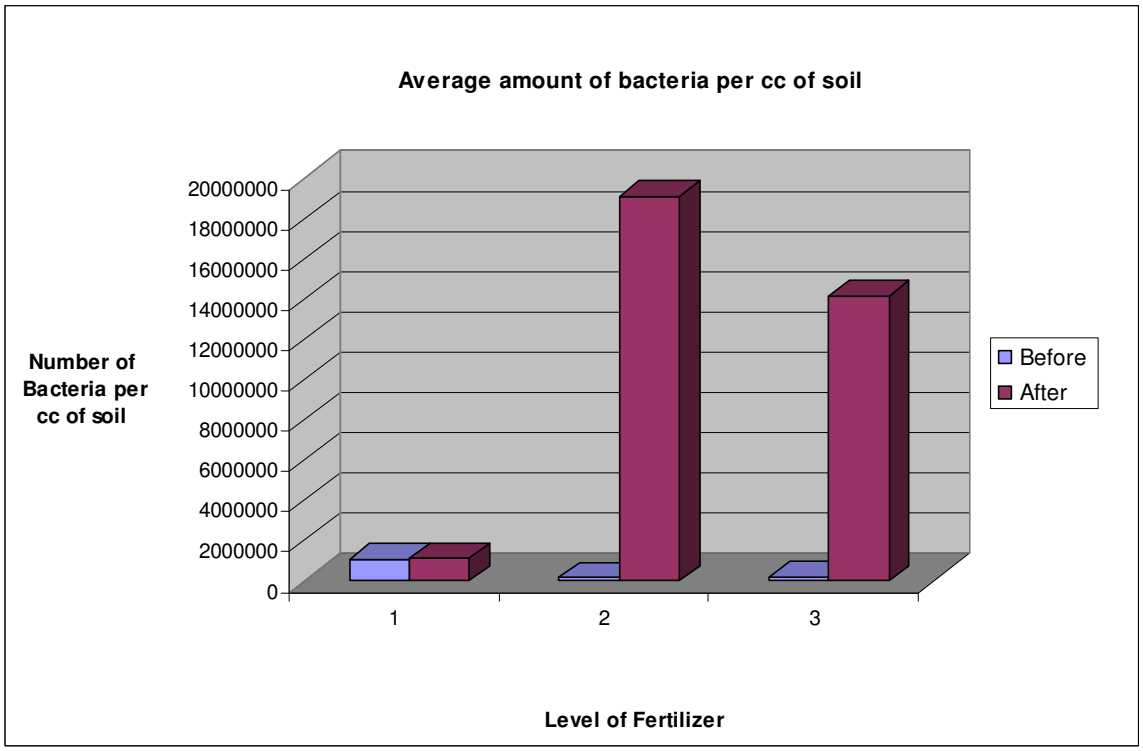
Plot Label	Number of bacteria per cm^3	Nitrate Nitrogen
O1	500000	10 ppm
O2	50000	20 ppm
O3	40000	10 ppm
Average	196666	13.333 ppm

Amount of Bacteria and Nitrate Nitrogen After Fertilizer

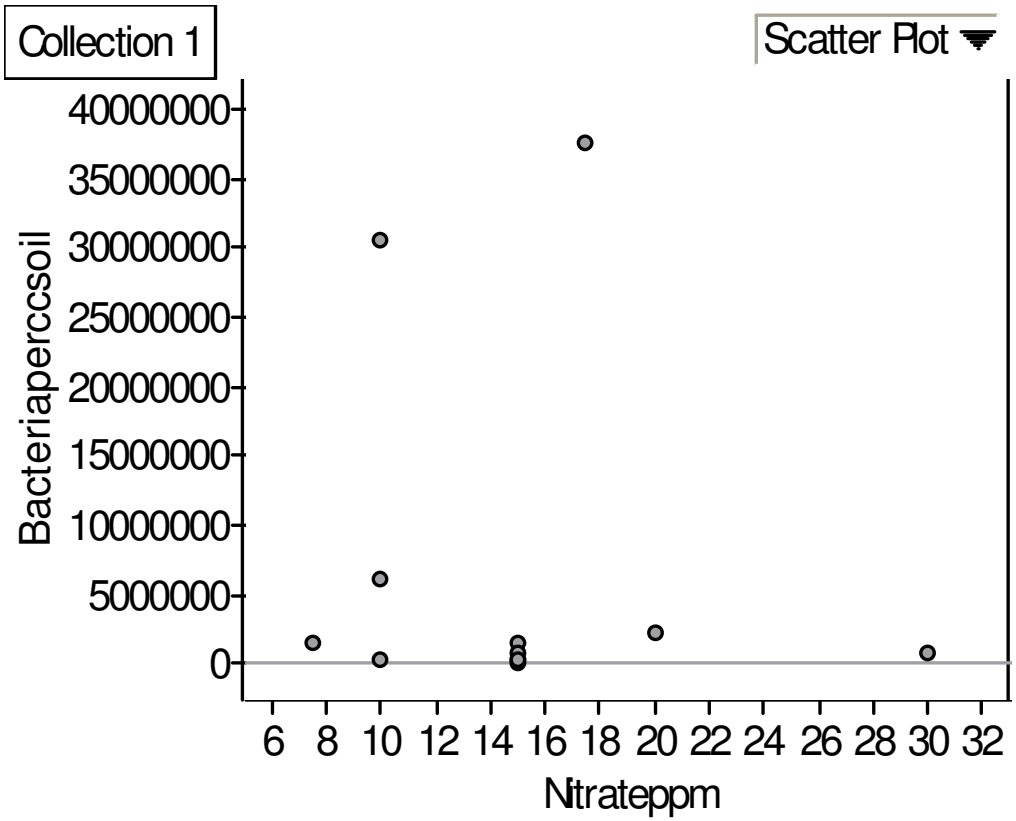
Plot Label	Number of bacteria per cm^3	Nitrate Nitrogen
O1	37500000	17.5 ppm
O2	700000	15 ppm
O3	6100000	10 ppm
Average	14166666	14.166 ppm

Analysis


Before and After Averages of Nitrate per ppm
ppm Nitrogen Nitrate
20
18
16
14
12
10
8
6
4
2
0
1
2
3
Level of Fertilizer
Before Average
After Average


Average amount of bacteria per cc of soil
Number of Bacteria per cc of soil
20000000
18000000
16000000
14000000
12000000
10000000
8000000
6000000
4000000
2000000
0
1
2
3
Level of Fertilizer
Before
After

Scatter Plot of Nitrate Nitrogen Level and Bacteria Count



Collection 1

	Nitrateppm	Bacteriaperccsoil
1	30	820000
2	10	170000
3	20	2200000
4	15	170000
5	7.5	1600000
6	15	1600000
7	15	120000
8	10	30500000
9	17.5	37500000
10	15	700000
11	10	6100000
12	15	220000

Estimate of Collection 1

Linear Regression

Independent attribute (continuous): Nitrateppm
Dependent attribute (continuous): Bacteriaperccsoil

Independent attribute: **Nitrateppm**
Dependent attribute: **Bacteriaperccsoil**
Sample count: **12**

Equation of least-squares regression line:
Bacteriaperccsoil = **-203355 Nitrateppm + 9858700**
Correlation coefficient, r = **-0.093609**
r-squared = **0.0087626**, indicating that **0.87626**% of the variation in **Bacteriaperccsoil** is accounted for by **Nitrateppm**.

The best estimate for the slope is **-203355** +/- **1.52394e+06** at a **95** % confidence level. (The standard error of the slope is **683952**.)

When **Nitrateppm** = **0** , the predicted value for a future observation of **Bacteriaperccsoil** is **9.85866e+06** +/- **3.8697e+07**.

Conclusion

Our hypothesis was, if the doubled amount of recommended fertilizer is added to a plot of land (25 by 25 centimeters) then the amount of bacteria from the original plot will decrease. The level of nitrogen after we added the fertilizer supports our hypothesis. For the average of the empty plots, the level of nitrogen decreases. For the recommended amount of fertilizer plots, the average also decreases. However, for empty the average nitrate level decreases by 7.5 ppm which makes sense because the water that we poured into the empty pot diluted the nitrate level. The average of the recommended plots with fertilizer added only decreases by 5 ppm. This means that although the nitrate level decreased, it decreased by a smaller amount which proves the recommended fertilizer added more nitrate to the soil. Then for the 'overload' plots, the nitrate level increased by .833. This data proves that by adding more fertilizer each time along with water, the nitrogen in the fertilizer helped increase the level of nitrate in the soil. Therefore, our data from the average nitrogen level in the soil before and after the added amount of fertilizer, supports our hypothesis. For our average amount of bacteria after the fertilizer

was added is somewhat inconclusive. For the empty plots the amount of bacteria after we added the water show that the bacteria did not change a lot at all. To be exact it only increased by 60000 bacteria per cm^3 in the soil. When we added 50 grams of fertilizer to the 3 recommended plots, the average out of the 3 plots increased by 18903334 bacteria cm^3 in the soil. Since we predicted that the recommended is good for the bacteria, so far our hypothesis is correct. However, for the average 3 overload plots, there was an increase of 13970000 bacteria per cm^3 in the soil. Although this is an increase which does go against our hypothesis, 4933334 is the amount of bacteria per cm^3 that got killed from the recommended plots to the overload plots. The more that is added after the recommended amount is going to harm the amount of bacteria, which is shown in the graph. Meaning if we were to carry out our experiment and do more tests with a bigger amount of fertilizer, that would keep decreasing the amount of bacteria per cm^3 in the soil. Since our hypothesis states if the doubled amount of recommended fertilizer is added to a plot, then the bacteria will decrease, then since that is our hypothesis that means we are correct. As we added more fertilizer, the percentage of bacteria did in fact decrease. If we were to do another test it would be more valid. To support this we found out the percentage of certainty that the fertilizer in fact had an effect on the plots. Our first plot had a 6% chance. This meant that the change from before and after with adding no fertilizer was 6% certain. Although this may be a low percentage, it strengthened our hypothesis because the negative control is not changing in this plot, so the low percentage portrays this and supports that there was in fact a small change. Next we found that for our second plot, recommended amount, that it was 88% certain that it had something to do with the adding of fertilizer. Lastly we found for our final plot that we could be 67%

certain that the fertilizer had something to do with the change in bacteria. These percentages support are hypothesis that if 100 grams of fertilizer is added to a plot of land it will harm or kill the bacteria. In conclusion, because out of the tests that we did take, our hypothesis was correct because the amount of bacteria did decrease the more the fertilizer we added. If we did do more tests it would show even more a decrease in bacteria when we add more fertilizer. Overall, our hypothesis, if the doubled amount of recommended fertilizer is added to a plot of land (25 by 25 centimeters) then the amount of bacteria from the original plot will decrease.

Citations

The Honor Code

We have acted honorably.

Alex Pace, Meredith Callis, Cate Wilson, Sarah Welch