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Final Lab Report

I. Background:

Plants need a variety of nutrients in different amounts. Nitrogen, phosphate, and potassium are the most important chemicals that plants need (Fertilizer, 2005). Plants cannot carry out the four essential tasks without nitrogen (Nitrogen Fertilizer, 2005). It is an essential element in nucleic acids, proteins, etc. (The Microbial World: The Nitrogen Cycle and Nitrogen Fixation, undated). If plants cannot carry out the four tasks and do not have nucleic acids and proteins, they will die. Phosphate is a key element in energy transfer. It is a necessary ingredient in ATP, or adenine tri-phosphate, which is the energy storage device of the cell. Without ATP, a plant cell cannot carry out the four tasks in a cell because it does not have an energy supply method. Therefore, phosphate is important to the growth of a plant because "adequate P availability for plants stimulates early plant growth and hastens maturity." (The Nature of Phospherous in Soils, 2002). Potassium is the third macronutrient that plants need. It is involved in the osmosis process and water regulation in plants; as a carrier for iron, it is especially important in starchy plants (Fertilizer, 2002). Without potassium, osmosis will not work properly. Plants that cannot use osmosis will die from water loss. There are other nutrients that are needed in smaller amounts, such as sulfur, magnesium, and calcium; however, nitrogen, phosphate, and potassium are essential to the growth and health of plants. Nevertheless, these nutrients can be useless without moving through certain cycles that occur in soil.

The term “biogeochemical cycles” refers to the processes in which these and other chemicals cycle through both the geological and biological world. Four of the most important cycles to focus on when learning about the biogeochemical cycles are the water, nitrogen, carbon (and oxygen) and phosphorus cycles; each chemical possesses its own cycle which is unique from the others.

The nitrogen cycle is one of the necessary processes that soil performs twenty four hours a day, seven days a week. It begins with nitrogen in our atmosphere, which is most commonly found in air. It is essential for dozens of processes separate from that of soil. However, in the nitrogen cycle, the gas must be converted into a usable form by means of soil. This is because between every two nitrogen atoms there is a triple bond, which makes using nitrogen in its raw form difficult (The Microbial World: The Nitrogen Cycle and Nitrogen Fixation, undated). In the soil, nitrogen fixing organisms create an enzyme called nitrogenase. This combines with gaseous nitrogen and hydrogen to produce ammonia. Ammonia can also be produced by decomposers from dead material. The ammonia is then used in a process called nitrification. Bacteria oxygenate the ammonium ions to create nitrites and then turn them into nitrates. Next, through assimilation, the plants use the nitrates, which are eventually passed on to other animals through the food pyramid. The nutrients passed on to the animals provide energy for their cells to perform work, specifically the four major tasks. Nitrates can also be denitrified by certain bacteria and released back into the atmosphere to continue the cycle (Soil, 2005).

The phosphorous cycle also plays an important role in soil health. While phosphorus is dispersed in nature, it is not found alone in element form (The Nature of Phosphorus Soil, 2002). “Elemental P is extremely reactive and will combine with oxygen when exposed to the air. In natural systems like soil and water, P will exist as phosphate, a chemical form in which each P atom is surrounded by 4 oxygen (O) atoms," (The Nature of Phosphorus Soil, 2002). From the soil, plants intake phosphate, which is passed on to animals that eat the plants. Then the phosphorus returns to the soil as organic residue (The Nature of Phosphorus Soil, 2002). "Most of the Phosphorus that is used by an organism is changed into organic compounds. When plant materials are returned to the soil, this organic phosphate will slowly be released as inorganic phosphate or be incorporated into more stable organic materials and become part of the soil organic matter, " (The Nature of Phosphorus Soil, 2002). When inorganic phosphates are liberated from organic phosphates the process is called mineralization and it is triggered because of microorganisms and the breaking down of organic compounds (The Nature of Phosphorus Soil, 2002). "The activity of microorganisms is highly influenced by soil temperature and soil moisture. The process is most rapid when soils are warm and moist but well drained. Phosphate can potentially be lost through soil erosion and to a lesser extent to water running over or through the soil," (The Nature of Phosphorus Soil, 2002).

One can determine the health of soil through examining the three major processes that happen underground. Nutrient cycling is one of those processes. The others are primary production, and decomposition. Primary production is simply the production of plants using the nutrients that come from soil to live. The nutrients are used to perform the 4 tasks necessary for life. Because the plant performs the 4 tasks, primary production naturally happens (reproduction). Following this, bacteria, fungi, and actinomycetes decompose plant materials and take up the resulting nutrients that have become accessible through death or senescence. The microorganisms are then consumed by fauna, producing nutrients such as nitrogen, phosphorus, and sulfur (Soil Ecology, 2004). The nutrients are spread between the soil and atmosphere through nitrification and the carbon cycle. The more these nutrients are produced, the healthier the soil will ultimately be (Soil, 2005). With healthy soil, farmers can more easily grow fruitful plants.

In today's society, we need higher yielding crops than ever. The plants that we grow for food need a constant supply of nutrients to be able to grow faster and bigger. Farming often throws off the delicate soil chemical cycles for several reasons. Processes such as ploughing aerate the soil, which can kill necessary bacteria. Farmers use pastures, not woodlands, to grow their crops and let their cattle graze, so less dead leaf matter is returned, which is also an important component in the balance. These combine to ruin the balance that is naturally found between plants and bacteria, so we have had to use alternate methods of supplying the necessary nutrients for maximum crop productivity.

Fertilizers are substances that supply nutrients to plants to supplement what is naturally available and usable in the soil. The two types of fertilizers used are organic fertilizers and chemical fertilizers (Fertilizer, 2002). The former is the more traditional method of supplementing nutrients; the latter has shifted more into use in recent times. There are distinct advantages and disadvantages to both. Chemical fertilizers are produced in factories. This careful processing ensures a balanced, predictable supply of nutrients in every container of fertilizer. The balance is useful because it ensures that plants will receive all of their nutrients necessary for faster growth (Fertilizer, 2002). This growth is also a result of the minerals used in fertilizers, which control the metabolism of plant cells. For these reasons, plants that are given chemical fertilizer grow faster and bigger than plants that are not fertilized.

However, some scientists and farmers do not support the use of chemical fertilizers. They believe that “this unnatural escalation causes watery tissues, depletes the protein quality, and becomes more susceptible to disease,” (Fertilizers/Sewage Sludge, 2004). Chemical fertilizers often use salts, which remain to harm the plants after the fertilizers have done their work, (Organic vs. Chemical, 2004), as well as acids, which can kill beneficial microbes living in the soil, “reduce the soil’s beneficial organism population and interfere with plant growth” (Chemical Fertilizer or Organic Fertilizer, 2005). In some situations, it seems that plants are better off without fertilizer. Continued use of chemical fertilizer may even result in fungus and bacterial disease “resulting from the lack of trace elements in soil regularly dosed with chemical fertilizers” (Chemical Fertilizer or Organic Fertilizer, 2005).

In addition, from an economic standpoint, the production of chemical fertilizers can also be time consuming and a drain on natural resources. Many fertilizers are made from coal, natural gas, and other non-renewable resources (Fertilizers/Sewage Sludge, 2004). Also, although chemical fertilizers have a highly beneficial short term effect, if a certain amount of dead plant matter is not returned to the soil the land will become infertile over the decades (Soil Ecology, 2004), producing an overall negative effect.

Organic fertilizers, such as compost and manure, also have advantages and disadvantages. One advantage is that organic fertilizers have a natural time release system, which results in less runoff (Fertilizer, 2002). They also contain trace elements, such as certain metals, that are not in general chemical fertilizers (Organic vs. Chemical, 2004). Organic elements improve soil physical properties such as aeration (Fertilizer, 2002). They also can renew supplies of bacteria microorganisms (Organic vs. Chemical, 2004). However, organic fertilizers can be unreliable. Because they are not produced by people, they do not have consistent chemical levels and may create imbalances of certain nutrients in the soil (Fertilizer, 2002). For example, if only one type of manure is used on a field for an extended period of time, that substance might contain a lot of one type of nutrient and not enough of another. They also can be bulky, which raises transportation and application costs (Fertilizer, 2002).

Our school often applies chemical fertilizer to the athletic fields and lawn. We wonder whether the copious amount of fertilizer applied is having the anticipated effect. We intend to test bacteria populations, an indicator of soil health, in areas where we have supplemented soil nutrients with the chemical fertilizer Miracle-Gro®. We will also test for phosphate to determine if the fertilizer is creating an imbalance of chemicals in the soil. Both of these tests will indicate whether or not the fertilizer we used helped create a balanced soil environment. We will compare the bacteria populations and the phosphate levels with the populations and chemical balances in an area where we have not applied chemical fertilizer to see whether or not the fertilizer had subtler effects. Our group predicts that the fertilizer may decrease the bacteria population because of chemical poisoning.

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I. Lab Outline

Problem: How does the addition of fertilizer change the density of bacteria in soil?

Hypothesis: Fertilizer decreases the density of bacteria.

Experiment:

Independent Variable: The independent variable is the presence of fertilizer.

Dependent Variable: The dependent variables are the number of bacteria and the amount of phosphate.

Negative Control: The negative controls are the soil samples from an unfertilized area and the test results of the pre-experiment phosphate tests and serial dilutions (on the soil from all plots).

Controlled Variables:

- Time when soil is sampled
- Type of fertilizer
- Same location for each group of samples
- Amount of soil taken
- Amount of fertilizer applied
- Amount of water
- Time when soil is tested for phosphate
- Time when soil is serial diluted
- Time when certain samples are used
- Size of agar plate
- Time bacteria colonies were given to grow on agar plate
- Amount of water in serial dilution tube
- Amount of soil diluted
- Distribution of soil in test tubes (they were shaken)
- Amount of soil on agar plate
- Type of agar plate (PetriFilm aerobic count plate)
- Amount of Phosphate Reagent #2 used
- Amount of Phosphate Reagent #3 used
- Amount of soil extract used in phosphate test
- Amount of water used in phosphate test
- Distance between plots
- Size of plots
- Concentration of fertilizer
- Amount of soil used in testing
- Distribution of fertilizer

- Sterilization of water

Step-by-Step Procedure

1. Find an area of grass and mark ten plots (20 centimeters apart) that are 15 cm x 15 cm.
2. Using a GPS, pinpoint the location and record the longitude and latitude of the plots. (N 39.35803°, W 76.63626°.)
3. Split the ten plots into two group of five (named group A and group B). In each group, label the plots numbers 1-5. B will be the fertilized group.
4. Take 1 sample (ten centimeters deep, two centimeters wide) from the center of each plot (using a soil cylinder). Place the samples in separate airtight Ziploc bags.
5. Apply 1 liter of tap water to each plot in group A. Make sure to cover all areas of the plot.
6. Apply 1 liter of half strength Miracle-Gro® all-purpose fertilizer to each plot in group B. Make sure to cover all areas of the plot.
7. Let the plots absorb the fertilizer for 4 days.
8. After 4 days, take 1 sample of soil (10 cm deep, 2 cm wide) from each plot using a soil cylinder.
9. Put each sample in a separate Ziploc bag and seal tightly.
10. Afterwards, put 1 cc of a soil sample into a culture tube containing 10 ml of sterile water; cap the tube and shake vigorously.
11. Using a serological pipette, remove 1 ml of the soil/water mixture and place into a fresh culture tube.
12. Add 9 ml of fresh sterile water to this second tube; cap and shake vigorously.
13. Repeat step 11 using the second, diluted tube and then repeat step 12 with this third tube.
14. Continue step 13 with each additional tube until you have diluted the original soil/water mixture a minimum of four times (a 10^{-4} dilution). You should now have a total of five culture tubes.
15. Plate 100 µl samples from the 4th and 5th tubes (dilutions 10^{-3} & 10^{-4}) onto their own separate, individual PetriFilm aerobic count plates filled with bacteria agar and allow to incubate at room temperature over night.
16. Examine each of the plates for individual bacteria colonies and choose the plate original 1 cc soil sample (# colonies on plate x 10^{2} = # of bacteria in dilution tube; # of bacteria in dilution tube x $10^{[\# \text{ of dilutions}]}$ = # of bacteria in original sample tube).
17. If there are not individual colonies but still a “lawn” at the 10^{-4} dilution, repeat steps 6-11, adding a 5th dilution, 6th dilution, etc. as necessary until individual colonies are observed.
18. Repeat steps 7-15 for every soil sample and record the number of bacteria; be sure to label everything to avoid confusion.

19. At the same time that the serial dilutions are happening, have another group member test for phosphate in the sample using a LaMotte Model NF Test Kit (Code 5090).**
20. Make
21. After making a general soil extract, use a transfer pipette (0364) to a fill a "Phosphorous B" Tube (0244) to the mark with a general soil extract.
22. Add 6 drops of *Phosphate Reagent #2 (5156). Cap and shake vigorously.
23. Add one *Phosphorous Reagent #3 Tablet (5157). Cap and shake until dissolved.
24. Immediately compare the color that develops in the test tube against the Phosphorus Color Chart. Hold the tube about one inch in front of the white surface in the center of the color chart. View the chart and sample under natural light for optimum color comparison. The test result is read in pounds per acre Available Phosphorus.
25. Convert each pound per acre in ppm by dividing in half. Record the data.
26. Repeat steps 10-25 for every soil sample; label everything to avoid confusion.

***Warning**: Reagents marked with a * are considered hazardous substances.

** Taken from the instruction manual for the LaMotte Model STH-4 Outfit (used to test soil for pH, nitrate nitrogen, phosphorus, and potassium).

II. Data and Analysis

Phosphate Levels (ppm) in Fertilized Plots

Plot Number	Pre-fertilizer (ppm)	Post-fertilizer(ppm)
1	32.5	100
2	5	25
4	12.5	100
5	5	12.5

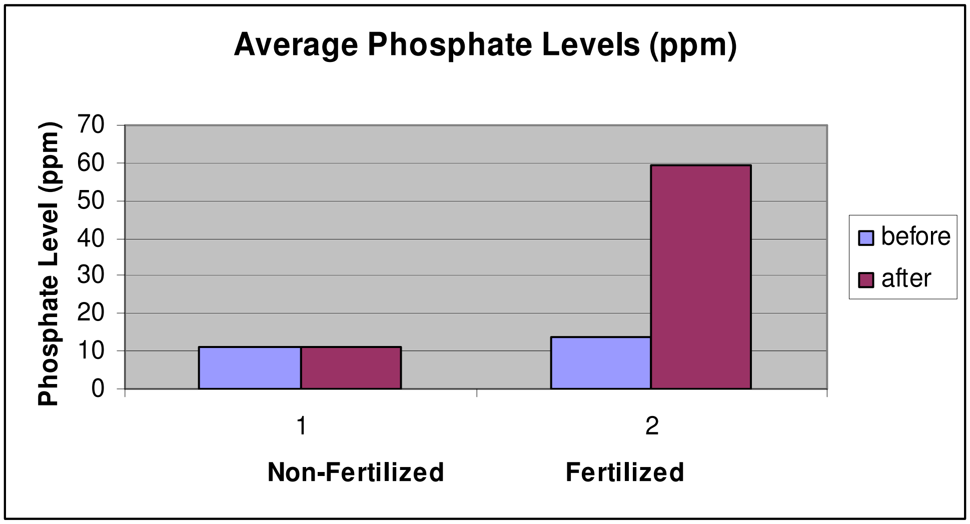
Number 3 has been omitted due to a lost sample.

Phosphate Levels (ppm) in Non-fertilized Plots

Plot Number	Pre-water (ppm)	Post-water(ppm)
1	5	5
2	12.5	12.5
3	12.5	12.5
4	12.5	12.5
5	12.5	12.5

Average Phosphate Levels (ppm)

Plot Type	Before (ppm)	After (ppm)
Fertilized	13.75	59.375
Non-fertilized	11	11



Without fertilizer, phosphate levels did not change. However, after the addition of fertilizer, phosphate levels changed by an average of 45.625 ppm

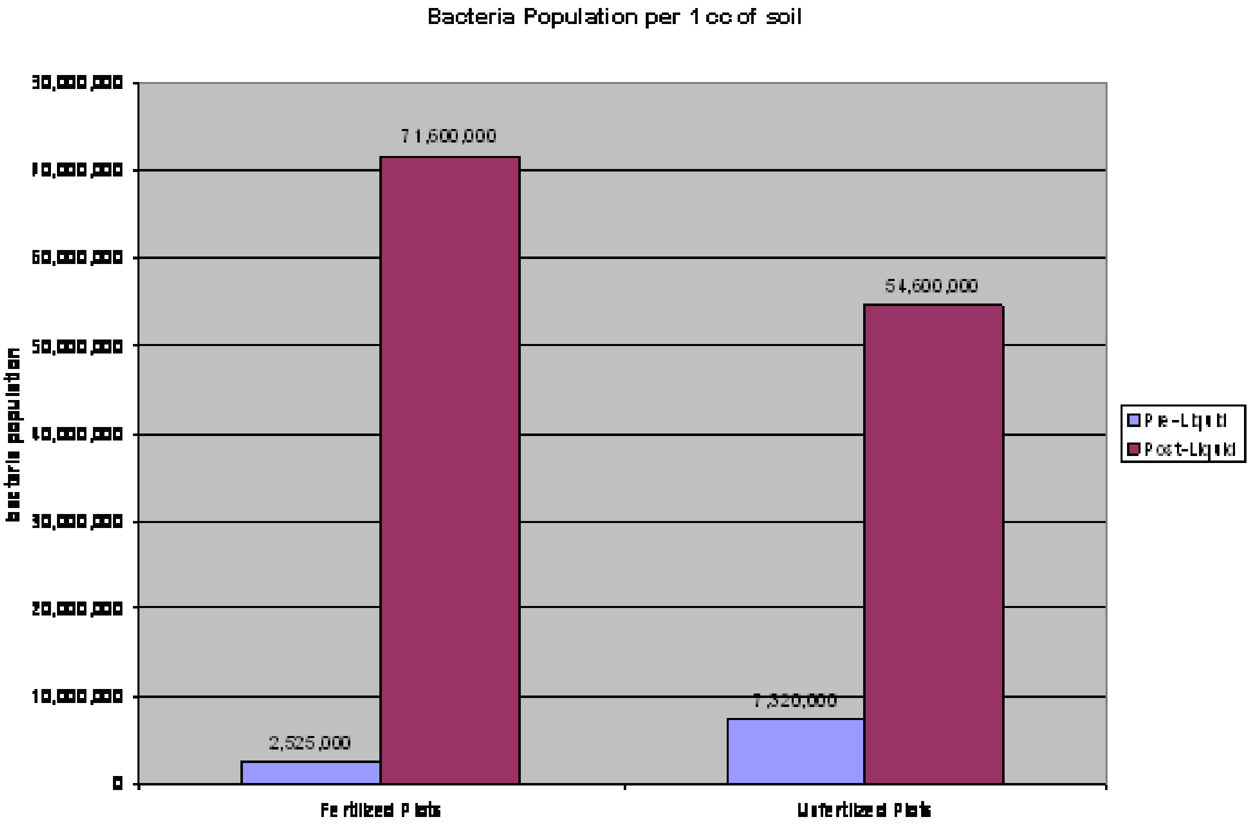
Bacteria populations per 1 cc in fertilized Plots

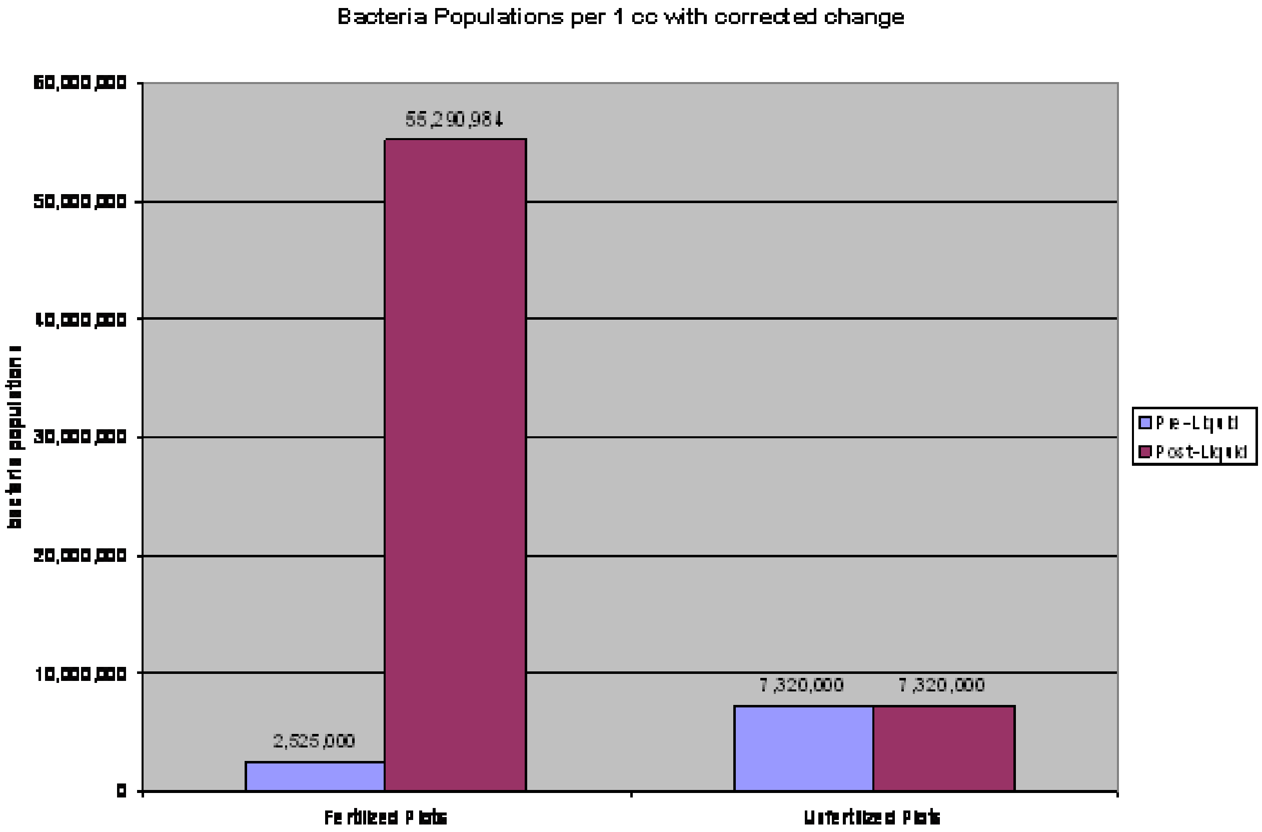
Plot #	Pre-Fertilizer	Post-fertilizer
1	3,500,000	24,000,000
2	1,700,000	70,000,000
3		8,000,000
4	3,800,000	241,000,000
5	1,100,000	15,000,000
Average	2,525,000	71,600,000
Corrected Change	2,525,000	55,290,983.61

Bacteria populations per 1 cc in unfertilized plots

Plot #	Pre-Water	Post-Water
1	5,000,000	40,000,000
2	6,000,000	106,000,000
3	1,600,000	61,000,000
4	10,000,000	10,000,000
5	14,000,000	56,000,000
Average	7,320,000	54,600,000

Corrected Change	7,320,000	7,320,000





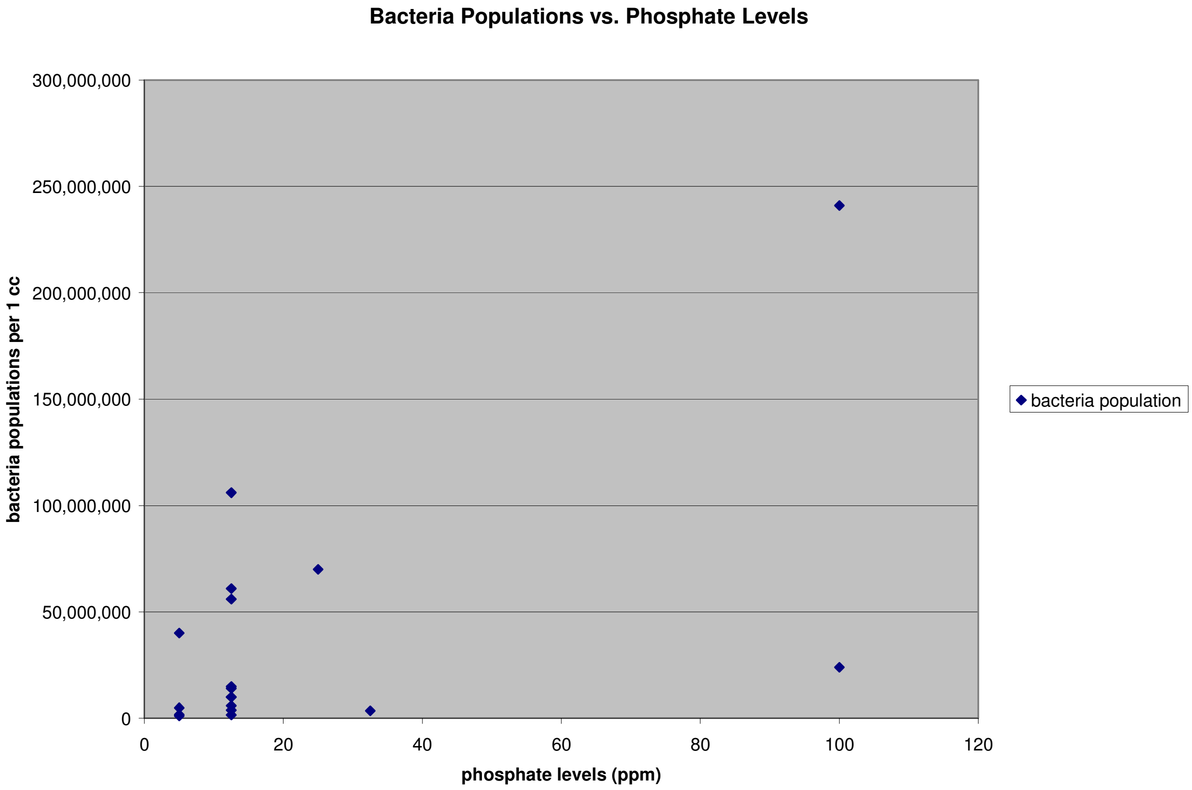
In both the fertilized and the unfertilized plots, the bacteria populations dramatically increased. The unfertilized plots' populations increased by 645.90%. When this is factored into the fertilized plots' bacteria populations, the corrected change for the post-fertilization figures is 55,290,984 bacteria. The density of bacteria increased after the addition of fertilizer increased by 52,765,983.61 colonies. This is still a significant increase. Therefore, the serial dilutions show that, even with the corrected change, the bacteria populations in the fertilized plots increased after the plots were fertilized.

Average Phosphate Level and Bacteria Density in Unfertilized Plot

Dependent Variable	Before	After
Bacteria Density (# of colonies)	7,320,000	54,600,000
Phosphate Level (ppm)	11	11

Average Phosphate Level and Bacteria Density in Fertilized Plot

Dependent Variable	Before	After
Bacteria Density (# of colonies)	2,525,000	71,600,000
Phosphate Level (ppm)	13.75	59.375



Least squared regression line has an r squared value of .34808, which indicates that 34.808% of the change in bacteria populations can be accounted for by phosphate levels. In other words, this graph shows that bacteria population does go up as phosphate levels go up (to a certain extent)

III. Conclusion

Our hypothesis was proved incorrect. The density of bacteria in the fertilized plots, with corrected change, increased by 20.9% after the addition of fertilizer (The density of bacteria increased after the addition of fertilizer increased by 52,765,983.61 colonies). The phosphate levels in our soil after the addition of fertilizer increased by 45.625 parts per million. This outcome shows a positive correlation between phosphate levels and bacteria populations, meaning that, in our experiment, when we added fertilizer both the phosphate levels and the bacteria populations went up. This makes sense because fertilizer contains phosphate, which accounts for the increased phosphate levels. Phosphate is an essential part of energy storage and release, because it is part of the molecule ATP. Therefore, the phosphate in the fertilizer probably had a double positive effect on the bacteria. First, it gave the bacteria more phosphate supplies for greater energy, which would allow them to reproduce, carry out the life processes faster, and make bacteria populations rise. The phosphate also helps the plants grow, which in the long run create more nutrients for the bacteria to use. The bacteria could have thrived because we used fertilizer in moderation.

In further experimentation, it would be beneficial to repeat the tests. We did not replicate properly, which gives less validity to our results. Instead of taking at least 3 samples from each plot, we only took 1. We could also make more plots in different locations in order to see if the change is uniform and experiment with different strengths of fertilizer. The different strengths could help to determine whether it is possible to poison microbes if they have too much or if they will continue to thrive off of the fertilizer. Also, we could apply a solution that had just phosphate in it to the soil, unlike the fertilizer we used, which contained other nutrients as well. This would prove that it was phosphate causing the change in bacteria populations, not other elements of the fertilizer.