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Background

Bacteria contribute greatly to the health of the soil by completing multiple jobs. They decompose organic materials, recycling key nutrients, and they convert nitrogen into nitrate and sulfides into sulfates in order to make them usable for plants. They break down humates and humic acids, and they help suppress disease within the soil (Reid and Wong, 2005). Without the bacteria in the soil, life on land would be impossible.

Of their many activities, one especially critical way bacteria contribute to the health of the soil is through the nitrogen cycle. Through a process called nitrogen fixation, bacteria in the soil take nitrogen gas from the atmosphere and convert it into ammonia. That ammonia is then converted by bacteria into nitrites and finally into nitrates in a process called nitrification. Other sources of nitrogen come from decomposition during the process of ammonification in which dead proteins and nucleotides are broken down into ammonia that then enter the nitrogen cycle. Eventually, the excess ammonia and nitrate are drawn up by the roots of plants and thus become a part of the food chain (Thomas, n.d.). Plants use these sources of nutrients they consume to grow and develop, and in fact, they could not live without nitrogen. It plays an important part in making chlorophyll in plants, which is used in photosynthesis to make their food and energy. Then plants pass this nitrogen farther and farther up the food chain as primary consumers ingest them and then are eaten in turn by secondary consumers, passing the nitrogen along organism to organism (Paustian, 2000).

Nitrogen fertilizers impact this process because they increase the amount of ammonia within the soil. By increasing the amount of ammonia, the number of bacteria multiplies in order to process all of it, and some of this excess nitrogen is absorbed by the plants which, in turn, supply animals with extra nitrogen. The amount of ammonia and the amount of nitrates becomes unbalanced in the nitrogen cycle because of the fertilizers. This process becomes increasingly vicious when the use of fertilizer ends and thousands of bacteria end up dying because they no longer have a plentiful food source. Because the bacteria die, the ammonia can no longer be converted into nitrate. Therefore, there is no nitrate being provided to the plants, causing the plants to also die. In addition, because nitrates are salts, they dehydrate the soil (Hermary, 2007), and since bacteria depend on moisture within the soil in order to complete their tasks, the lack of water kills any bacteria that are left to provide nitrate to the plants (Lavelle and Spain, 2001).

There are, of course, benefits to using fertilizer. The major way fertilizers aid the plants is by providing them with nutrients. The thirteen mineral nutrients plants need that come from the soil are water soluble and are absorbed by the roots of the plants. However, often times, there are not enough of these nutrients within the soil and so fertilizers can deliver these necessary nutrients to plants, including the three most important ones: nitrogen, phosphorus, and potassium (NCDA&CS, n.d.).

Plants need large amounts of those three nutrients because they each aid immensely in their growth and development. Nitrogen is one of the chief elements in protein which is vital in order for the plant to grow, phosphorus provides energy to the plant in the form of ATP and

NADPH, and potassium is important for many processes such as the movement of sugars, starch formation, and pH stabilization (Frantz, Krause, Locke, Pitchay, n.d.). If the plants were unable to grow and develop into fully functional plants, malnutrition would increase around the world in humans and other animals. This would occur because animals rely upon the eating of plants in order to keep the necessary level of nitrogen within their own bodies.

Besides the amount of fertilizer used, certain kinds of fertilizer have the potential to be more harmful than others due to certain ingredients or amounts of chemicals used in a specific type. For example, chemical fertilizer may contain more nitrogen or nitrates than organic fertilizer which would make the damages discussed above more probable and more severe (U.S. EPA, 2012). Chemical fertilizers of this variety are manufactured using materials such as rock, animal, or petroleum products. These materials are then concentrated using industrial processes. A second type of fertilizer is organic fertilizer. Organic fertilizers are made of materials such as mined minerals, animal, or plant products. These materials go through no or very little processing (Savonen, 2006).

Since fertilizers do aid in the cultivation of crops and the nutrition of animals, it is important to figure out which has the lowest potential to cause harm in order to choose and use fertilizer wisely (U.S. EPA, 2012). In order to find the solution to this problem, we created an experiment testing for the effects of chemical fertilizer on bacteria compared to those of organic fertilizer. We began this experiment by marking off nine small plots of land. We then took samples of soil from each of the plots and completed serial dilutions of each sample in order to find the number of bacteria per cc of soil. We then applied each of the types of fertilizer onto their own separate plots. Next, we took more soil samples and completed more serial dilutions.

Through the completion of this experiment, we could gain an understanding for which type of fertilizer would cause more harm to the nitrogen cycle.

Procedure

I. **Problem:** Does chemical fertilizer change the population density of the bacteria within the soil more than organic fertilizer?

II. **Hypothesis:** Chemical fertilizers will decrease the population density of the bacteria within the soil more than organic fertilizer.

III. **Procedure:**

a. **Independent Variable:** the application of chemical or organic fertilizer to the soil

b. **Dependent Variable:** the population density of the bacteria in the soil

c. **Negative Control:** the application of only water to the soil

d. **Controlled Variables:**

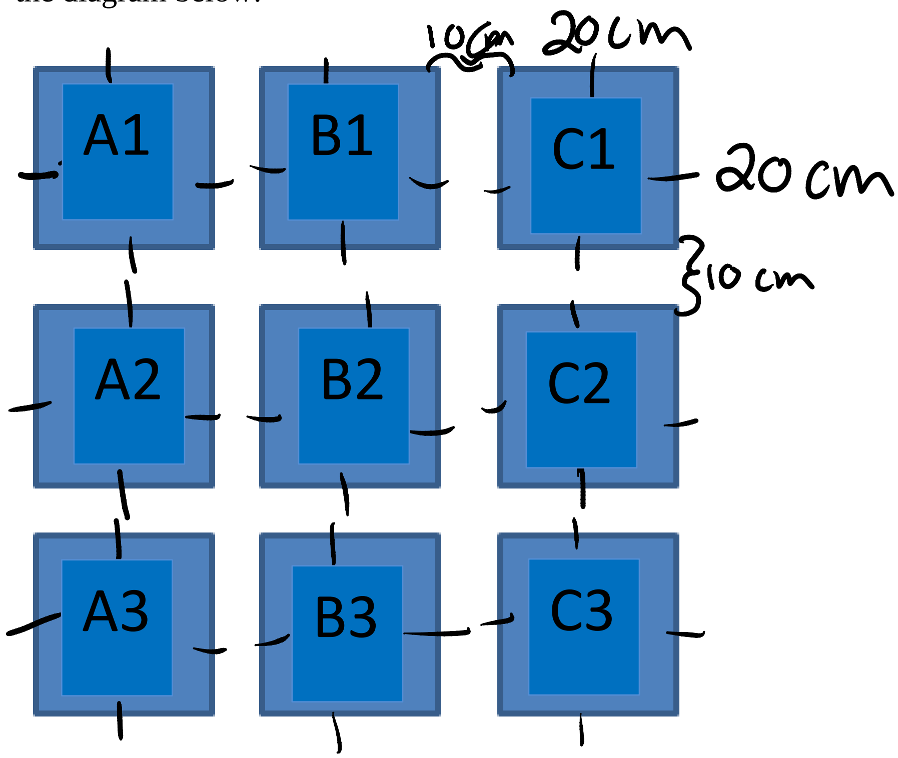
- Time when samples are taken
- Locations where soil samples are collected
- Amount of soil collected
- Amount of fertilizer used
- Size of the test sites
- Given time for fertilizer to set in the soil
- Type of solution (sterile water)
- Amount of sterile water
- Amount of dirt put in each tube
- Amount of time before counting bacteria on nutrient agar plates
- Size of test tubes

- Size of pipette
- Size of nutrient agar plates
- Type of nutrient agar plates
- Amount of dilution put on nutrient agar plates
- Amount of nutrient agar

e. Step-by-Step:

1. Mark off three areas each 20 cm by 20 cm with 10 cm in between each one. Place the first of the areas so that it has the coordinates of North 39.35803º West 076.631612º (label it "A1"), the second so that it has coordinates of North 39.035803º West 076.63605º (label it "B1"), and the third so that is has coordinates of North 39.35804º West 076.63611º (label it "C1"). See diagram in step 4
2. Mark off three more areas each 20 cm by 20 cm with 10 cm in between each one. Place the first of the areas so that it has the coordinates of North 39.35818º West 076.63654º (label it "A2"), the second so that it has coordinates of North 39.35817º West 076.63657º (label it "B2"), and the third so that it has coordinates of North 39.35820º West 076.63651º (label it "C2"). See diagram in step 4
3. Mark off three more areas each 20 cm by 20 cm with 10 cm in between each one. Place the first of the areas so that it has the coordinates of North 39.35824º West 076.63612º (label it "A3"), the second so that it has coordinates of North 39.35822º West 076.63612º (label it "B3"), and the third so that it has coordinates of North 39.35821º and West 076.63612º (label it "C3").see diagram in step 4

4. Note that once you are done marking off your areas, your plots should look like the diagram below:



5. On the same day at the same time collect three separate soil samples 20 cm deep into the soil and 2 cm wide from each location. Put soil in plastic bag labeled “trial 1 A1”, “trial 1 B1”, “trial 1 C1”, “trial 2 A2”, and so until you have “trial 3 C3”. You should end up with 27 bags of soil samples
6. Spread 0.6 grams of chemical fertilizer on each site, “A1”, “A2”, and “A3”.
7. Spread 4 grams of organic fertilizer on each site, “B1”, “B2”, and “B3”.
8. And put nothing on locations “C”, “C2”, and “C3”.
9. Pour 333 1/3 ml of water on each site.
10. Let the fertilizer set in the soil for 48 hours.
11. Take the bags of soil that you have already collected and do steps 12-29 on the same day at the same time.

12. Use a clean, new transfer pipette to add 10 ml sterile water to a 15 ml culture tube. Label the tube “A1 10^{0}”.
13. Use the same pipette to add 9 ml of sterile water to a 15 ml culture tube. Label the tube “A1 10^{-1}”.
14. Repeat step 13 two more times to two additional 15 ml culture tubes, only label them “A1 10^{-2}”, and “A1 10^{-3}” respectively.
15. Repeat steps 12-14 but use the letter B1 instead of the letter A when labelling.
16. Repeat steps 12-14 once more but use the letter C1 instead of the letter A when labelling.
17. Place 1 cc of the soil sample from location A1 into the “A1 10^{0}” culture tube.
18. Using a new clean pipette, remove 1 ml of soil/water mixture from the “A1 10^{0}” tube and place into the “A1 10^{-1}” tube. Cap and shake vigorously.
19. Using the same pipette “A1” as used in step 18, remove 1 ml of the soil/water mixture from the “A1 10^{-1}” tube and place into the “A1 10^{-2}” tube. Cap and shake vigorously.
20. Using the same pipette “A1” as used in step 18 and 19, remove 1 ml of the soil/water mixture from the “A1 10^{-2}” tube and place into the “A1 10^{-3}” tube. Cap and shake vigorously.
21. Place 1 cc of the soil sample from location B1 into the “B1 10^{0}” culture tube.
22. Repeat steps 18-20 but using the “B1” samples and test tubes.
23. Place 1 cc of the soil sample from location C1 into the “C1 10^{0}” culture tube.
24. Repeat steps 18-20 but using the “C1” samples and test tubes.
25. There should now be a total of 4 culture tubes for each letter.

26. Plate 100 µl samples from the A1 3rd and A1 4th tubes (dilutions A 10^{-2} and A 10^{-3}) onto their own separate, labelled 3M Petrifilm™ Aerobic Count Plate. Label one 1A1 10^{-2} label the other 1A1 10^{-3}.
27. Repeat step 31 but label one 1B1 10^{-2} and the other 1B1 10^{-3}.
28. Repeat step 31 but label one 1C1 10^{-2} and the other 1C1 10^{-3}.
29. Repeat steps 12-28 with the remaining soil samples.
30. Allow to grow for 48 to 72 hours.
31. Separately for the “A1”, “B1”, and “C1” tubes, examine each of the plates for individual bacteria colonies and choose the plate at the lowest dilution, 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 10^2 x $10^{|\text{dilution \# at which these colonies were found}|}$.

32. Collect three separate soil samples with the fertilizer 20 cm deep into the soil and 2 cm wide from each location on the same day so you have 27 bags. Look back at step 5 to see how to label them.
33. Repeat steps 11-31 with the newly collected soil samples.

Data Table(s):

Impact of fertilizers on bacteria density in the soil

Trial 1

Plot label	Number of bacteria per cubic centimeter of soil before fertilizer application	Number of bacteria per cubic centimeter of soil after fertilizer application
Site with chemical fertilizer A1	1,200,000	100,000
Site with chemical fertilizer A2	5,600,000	1,100,000
Site with chemical fertilizer A3	4,000,000	270,000
Site with organic fertilizer B1	1,600,000	800,000
Site with organic fertilizer B2	1,700,000	700,000
Site with organic fertilizer B3	440,000	1,300,000
Site with water C1	1,300,000	3,000,000
Site with water C2	1,600,000	800,000
Site with water C3	40,000	50,000

Impact of fertilizers on the soil in density of bacteria

Trial 2

Plot label	Number of bacteria per cubic centimeter of soil before fertilizer application	Number of bacteria per cubic centimeter of soil after fertilizer application
Site with chemical fertilizer A1	700,000	60,000
Site with chemical fertilizer A2	530,000	180,000
Site with chemical fertilizer A3	230,000	110,000
Site with organic fertilizer B1	600,000	900,000
Site with organic fertilizer B2	180,000	430,000
Site with organic fertilizer B3	700,000	600,000
Site with water C1	640,000	1,400,000
Site with water C2	120,000	480,000
Site with water C3	90,000	160,000

Impact of fertilizers on bacteria density in the soil

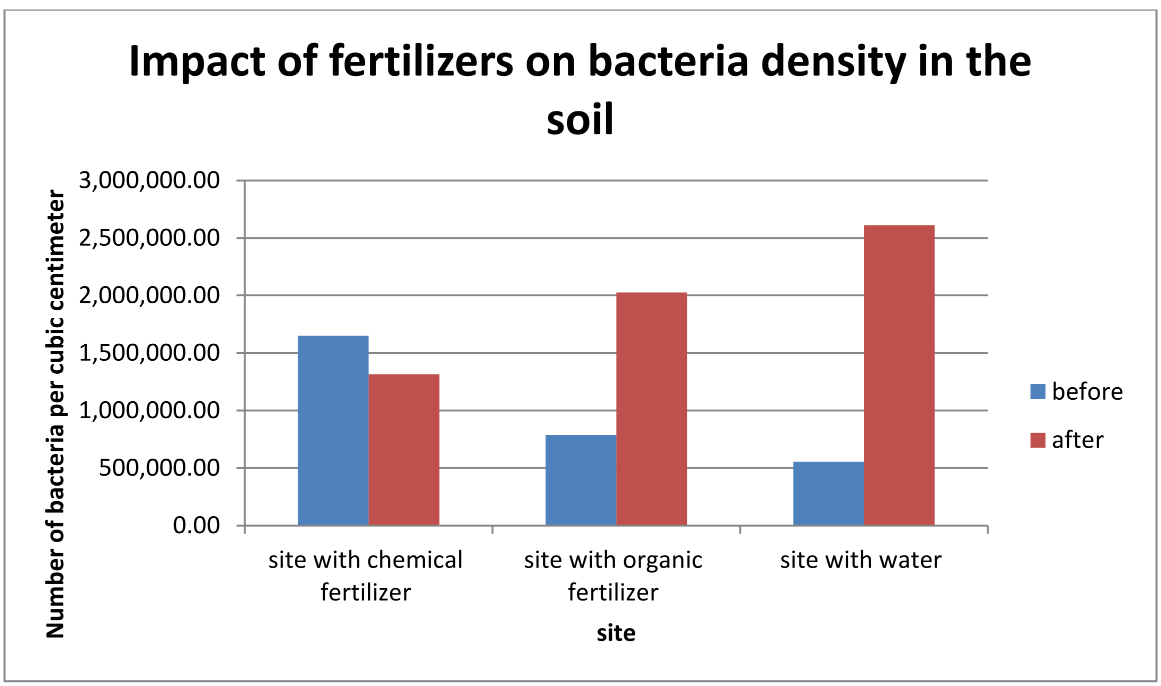
Trial 3

Plot label	Number of bacteria per cubic centimeter of soil before fertilizer application	Number of bacteria per cubic centimeter of soil after fertilizer application
Site with chemical fertilizer A1	1,000,000	1,100,000
Site with chemical fertilizer A2	700,000	7,200,000
Site with chemical fertilizer A3	900,000	1,700,000
Site with organic fertilizer B1	1,100,000	3,700,000
Site with organic fertilizer B2	700,000	2,800,000
Site with organic fertilizer B3	50,000	7,000,000
Site with water C1	800,000	13,600,000
Site with water C2	260,000	1,300,000
Site with water C3	150,000	2,700,000

Impact of fertilizers on bacteria density in the soil

Average table

Plot label	Number of bacteria per cubic centimeter of soil before fertilizer application	Number of bacteria per cubic centimeter of soil after fertilizer application
Site with chemical fertilizer A1	1,651,111.111	1,313,333.333
Site with organic fertilizer B1	785,555.556	2,025,555.556
Site with water C1	555,555.555	2,610,000



Conclusion

Our hypothesis stated that chemical fertilizers decrease the population density of the bacteria within the soil more than organic fertilizer. Through the completion of our experiment, we have concluded that our hypothesis is correct. After averaging the number of bacteria colonies from three trials of the experiment, we found that the number of bacteria per cubic centimeter of soil increased by 1,240,000 after organic fertilizer was applied, but decreased by 337,777.778 after chemical fertilizer was applied. The evidence shows that chemical fertilizers decrease the population density of bacteria more than organic fertilizer does.

In terms of future research, there is a lot more work we could complete to grasp a true understanding of which type of fertilizer causes more damage. Through lots of research we found the following information: while some of the excess nitrogen provided by fertilizers is absorbed by the plants which also supply animals with extra nitrogen, the amount of ammonia and the amount of nitrates unbalances the nitrogen cycle. This process becomes increasingly vicious when the use of fertilizer stops and thousands of bacteria end up dying because they no longer have a bountiful food source. Therefore, for future research, we would stop using fertilizer and test to see if on average the population density of bacteria decreases even further.

Citations

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