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Brock

Biology 9

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Soil Ecology Project

Background

By Kelsey McManus, Kelsey Jackson, and Lindsay Cheek

Have you ever wondered about the soil? Have you ever thought about how important it is and what it's made of? Soil contributes to everyone's lives and plays a major role in ecosystems everywhere; however, humans alter the soil through different methods. One method we intend to examine is the way we landscape at Roland Park Country School. The fertilizer that is used in or on this soil can greatly affect the microbes residing there. One group of microbes that are affected are bacteria. These organisms are significantly diverse and are impacted by the way people treat the soil. In our experiment we plan to observe the techniques applied to these areas and distinguish whether or not humans are helping or harming them.

Kelsey Jackson, Kelsey McManus, Lindsay Cheek
Brock
Biology 9
May 25, 2007

Bacteria are one of the many microbes that are present in soil. Bacteria are one celled organisms that form in a spiral (Webster © 2006) and they do a number of jobs such as chemical transformations, degradation of organic matter, disease suppression, nutrient transformations in roots etc. There are also many kinds of bacteria found in soil, types such as decomposers, nitrogen fixers, disease suppressors, aerobes and anaerobes, Actinobacteria and sulfur oxidizers. All of these bacteria perform different jobs and play a major role in the soil. They do things such as convert sulfides to sulfates and stop diseases in the plants so they do not harm the roots. (Parsons, 2005)

One of the things that impacts soil bacteria is fertilizer. Fertilizer impacts soil and the microbes greatly. In general fertilizer increases the number of microbes found in soil because it, “increases the amount of organic substrates available to soil microbes by increasing its source”, which are plants. This means that the fertilizer actually increases the amount of resources and nourishment for microbes by increasing the number of plants. (Parsons, Jerry, 2005.) The fertilizer produces more plants which produces more resources for the microbes in the soil by providing all plants with more nitrogen and other nutrients.

Fertilizer is an important aspect of a plant's life. The major ingredients allow the growth and maturity of each plant to be to its fullest potential, and fertilizer is basically the food for the plant that provides the nutrients needed to supply things like food crops fully. In fact "experts estimate that without commercial fertilizers, the world would be without one-third of its food supply" (The Fertilizer Institute, 2007).

By far the three most important nutrients are nitrogen, phosphorus and potassium. Nitrogen helps to give the plant its vibrant green coloring and plays a role in making protein. Proteins are important because some are enzymes that start and stop chemical reactions in a plant's cells. Hence plants with an abundance of nitrogen show forceful growth, strong root systems, rich green coloring, and increased fruit and seed development. (The Fertilizer Institute, 2007)

Phosphorus is found in all living cells and is significant for all life forms because “phosphorus is a component of the complex nucleic acid structure of plants, which regulates protein synthesis. Phosphorus is, therefore, important in cell division and development of new tissue. Phosphorus is also associated with complex energy transformations in the plant.”(IANR's Communications and Information Technology, 2006). Hence the phosphorus provided to the plants is responsible for the four tasks occurring in the plants between the five biological molecules. The four tasks that occur with the help of the phosphorus are synthesizing proteins, reproduction of new cells, regulation of the plant’s environment, and transform energy.

Lastly potassium is found in all plant and animal cells. Potassium is important to plants for two major reasons: it activates enzymes that start and stop the chemical reactions for the cells to make energy for the plant to use and maintains water content for each cell. The latter is important because water is needed to cause the chemical reactions also. (The Fertilizer Institute, 2007)

Clearly, fertilizer can impact the life of plants. Yet normally the bacteria are the ones responsible for providing enough nutrients for the plant to stay alive. These microbes supply nitrogen, phosphorus, and potassium by transforming organic materials from one chemical form to another using their external digestive systems. Some of the metabolites released from the bacteria's digestive system can be used by the plants. The bacteria then get nutrients and energy from the processes that occur here and provide other organisms with forms of chemicals they need for their own processes to occur (Lavelle and Spain, 2001). When fertilizer is added, it reinforces what the bacteria do and help the plant grow bigger and better. While plants usually get the carbon, hydrogen, and oxygen and other nutrients that they need from air and soil, in some cases the air and soil don't provide enough nutrients for the plant to grow healthy. Therefore, fertilizer is used to help add nutrients to the soil.

However over fertilizing can cause many problems and is bad for the bacteria and the rest of the environment. The fertilizer stunts the growth of the plant's roots. This is mainly where the bacteria reside. The bacteria use the roots as their way of communication with the plant. Also the roots given the bacteria anything they need from the plants. So when the fertilizer is applied to the plants the roots are the main area affected and because of this the bacteria can not always survive (Groffman, 2007). Overall, fertilizer may help the microbes live because "soil microbes depend on plants for their nourishment and fertilizers that nourish plants also nourish the biology of the soil" (Bugg, Robert, 1990).

The purpose of our experiment then, is to determine whether or not the humans are assisting the bacteria diversity by using fertilizer and how much fertilizer should be applied. We will test the soil on our campus where it is not

Kelsey Jackson, Kelsey McManus, Lindsay Cheek
Brock
Biology 9
May 25, 2007

chemically treated and collect samples. Once the numbers of different species from these areas are determined, we will apply different amounts of fertilizer to these same areas and take more samples. We will then observe the samples under a magnifying glass after being diluted and grown on Petri Plates to distinguish the difference in species.

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Kelsey Jackson, Kelsey McManus, Lindsay Cheek

Brock

Biology 9

May 25, 2007

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Lab Report

Problem: How does the amount of fertilizer used affect the biodiversity of bacteria in the soil?

Hypothesis: If the amount of fertilizer applied to a plot of grass increases, then the biodiversity of bacteria in the soil will increase.

Independent Variable: amount of fertilizer applied to the grass.

Dependant Variable: the different types of bacteria in the soil.

Kelsey Jackson, Kelsey McManus, Lindsay Cheek

Brock

Biology 9

May 25, 2007

Negative control: the plots with only water added.

List of Controlled Variables:

- type of plants on the plot of land
- size of the plot of land being tested
- type of fertilizer
- location of plots
- amount of water
- time of day soil samples are taken
- length of time fertilizer is left on plot
- type of Petri film used
- time when dilution is performed
- amount of soil taken for soil samples
- amount of soil diluted
- to what level the soil is diluted
- amount of soil solution applied to petri film
- amount of sterile water in each culture tube
- process for identifying different bacteria

Procedure:

1. Mark off 3 25 cm. by 25 cm. squares side by side at N 39.35774^0 and W 76.63528^0, marking the first square with flags that say 40 g. of fertilizer, the second square with flags saying 80 g. of fertilizer and the third square saying 0 g. of fertilizer; make sure all of the flags have letter “A” on them, to indicate which test
2. repeat step 1 two other times in the same general area as the first 3 squares, making sure that the next set of three plots are marked with “B” and the last set of plots are marked with “C”

Kelsey Jackson, Kelsey McManus, Lindsay Cheek

Brock

Biology 9

May 25, 2007

3. using a soil core sample, wood and hammer push the soil sampler which is 2 cm. in diameter into the ground until it reaches 15 cm. , collect one sample from each plot and place in the bag marked with the plot that the soil is from, be sure to take all of the samples at the same time on the same say (there should be A, B, and C bags that have 40 g. of fertilizer soil in them, there should be A, B, and C bags that have 80 g. of fertilizer soil in them and there should be A, B, and C bags that have 0 g. of fertilizer soil in them)
4. make sure that the soil samples collected together are also tested together
5. to begin the serial dilutions on the samples get a test tube rack, 18 culture tubes and caps, sterile water, 3 1-cc scoops, smoother, 19 bacteria Petri film, 10 10 ml serological pipettes, serological pipette pumps, micro pipette, micro pipette tips, sterilizing solution, alcohol
6. label 1 culture tube with the number 10^0 at the top, the number 80 at the bottom and one with the letter A, the top number indicates the stage of serial dilution and the bottom number indicates the amount of fertilizer in grams that will be applied to that soil sample and the letter on the bottom, next to the amount of fertilizer shows which test the soil belongs to, A, B or C
7. label 1 culture tube with the number 10^0 at the top, and 40 and A at the bottom
8. label 1 culture tube with the number 10^0 at the top, and 0 and A at the bottom
9. label 1 culture tube with the number 10^{-1} at the top, and 80 and A at the bottom
10. label 1 culture tube with the number 10^{-1} at the top, and 40 and A at the bottom
11. label 1 culture tube with the number 10^{-1} at the top, and 0 and A at the bottom
12. label 1 culture tube with the number 10^0 at the top, and 80 and B at the bottom
13. label 1 culture tube with the number 10^0 at the top, and 40 and B at the bottom

Kelsey Jackson, Kelsey McManus, Lindsay Cheek

Brock

Biology 9

May 25, 2007

14. label 1 culture tube with the number 10^0 at the top, and 0 and B at the bottom
15. label 1 culture tube with the number 10^{-1} at the top, and 80 and B at the bottom
16. label 1 culture tube with the number 10^{-1} at the top, and 40 and B at the bottom
17. label 1 culture tube with the number 10^{-1} at the top, and 0 and B at the bottom
18. label 1 culture tube with the number 10^0 at the top, and 80 and C at the bottom
19. label 1 culture tube with the number 10^0 at the top, and 40 and C at the bottom
20. label 1 culture tube with the number 10^0 at the top, and 0 and CB at the bottom
21. label 1 culture tube with the number 10^{-1} at the top, and 80 and C at the bottom
22. label 1 culture tube with the number 10^{-1} at the top, and 40 and C at the bottom
23. label 1 culture tube with the number 10^{-1} at the top, and 0 and C at the bottom
24. place the tubes marked 10^0 with A on the bottom from most amount of fertilizer to the least amount
25. place the tubes marked 10^{-1} with A on the bottom next to the tubes marked 10^0 from most amount of fertilizer to the least amount
26. place the tubes marked 10^0 with B on the bottom from most amount of fertilizer to the least amount
27. place the tubes marked 10^{-1} with B on the bottom next to the tubes marked 10^0 from most amount of fertilizer to the least amount
28. place the tubes marked 10^0 with C on the bottom from most amount of fertilizer to the least amount
29. place the tubes marked 10^{-1} with C on the bottom next to the tubes marked 10^0 from most amount of fertilizer to the least amount

Kelsey Jackson, Kelsey McManus, Lindsay Cheek

Brock

Biology 9

May 25, 2007

30. fill the culture tubes with a 10^0 at the top with 10 ml of sterile water using a serological pipette
31. fill the culture tubes with the number 10^{-1} on the top with 9ml of sterile water
32. fill the tubes with 10^0 on the top and 80 on the bottom with the soil samples from those plots, being sure to use either a different 1-cc scoop or to wash it out in between the different samples, make sure the soil samples from A plots go in the tubes marked with A on the bottom and so on and so forth for B and C
33. fill the tubes with the 10^0 on the top and 40 on the bottom with the soil samples from those plots, make sure the soil samples from A plots go in the tubes marked with A on the bottom and so on and so forth for B and C
34. fill the tubes with the 10^0 on the top and 0 on the bottom with the soil from those plots, make sure the soil samples from A plots go in the tubes marked with A on the bottom and so on and so forth for B and C
35. place the caps on the tubes marked 10^0 and shake the tubes before the 1 ml is taken to place in the tubes marked 10^{-1}
36. using a separate serological pipette for each soil sample, take 1 ml of solution from tubes marked 10^0 and place it in the tubes marked 10^{-1}
37. then place a cap on all the tubes marked 10^{-1} and shake
38. prepare 18 petri film pieces marking them the same as the culture tubes, indicating the amount of fertilizer
39. then using a micro pipette, making sure to change the tip in between each sample, place 100 µl of each solution from the culture tubes on their corresponding petri film pieces
40. empty and wash all of the culture tubes, caps and scoops in water and alconox
41. wash the work station with alcohol
42. allow the petri film to sit at room temperature for about 2 days

Kelsey Jackson, Kelsey McManus, Lindsay Cheek
Brock
Biology 9
May 25, 2007

43. then examine the petri film with a magnifying glass and count how many red large colonies there are, red small colonies, pink large colonies and pink small colonies
44. don't forget that if there are any other different colonies that do not fit one of those four descriptions, add them to the table also
45. record data in tables
46. place the petri film in the biohazard bag
47. to make the fertilizer solutions for the plots with 40 g. of 34-0-10 fertilizer measure out 500 ml of water and using a scale, measure out 40 g. of fertilizer, then add the water and fertilizer together and mix, be sure to make 3 separate solutions with 40 g. of fertilizer
48. then make the fertilizer solutions for the plots with 80 g. of 34-0-10 fertilizer by measuring out l L of water and using a scale to measure out 80 g. of fertilizer, then add the water and fertilizer together and mix, be sure to make 3 separate solutions with 40 g. of fertilizer
49. then measure out 1 L of water 3 times and put in different containers for the plots with 0 g. of fertilizer
50. apply the .5 L of 40 g. fertilizer solution to the 40 g. plots and the 1 L of 80 g. fertilizer solution to the 80 g. plots and the 1 L of water to the plots with 0 g. of fertilizer
51. allow the fertilizer to sit on the plots for 4 days then repeat steps 3-46 for the soil samples with fertilizer on them

Kelsey Jackson, Kelsey McManus, Lindsay Cheek

Brock

Biology 9

May 25, 2007

Data:

BEFORE FERTILIZER	**0 g. of Fertilizer** (# of different types of bacteria)*				**40 g. of Fertilizer** (# of different types of bacteria)*						**80 g. of Fertilizer** (# of different types of bacteria)*			
	LR	SR	LP	SP	LR	MR	SR	LP	MP	SP	LR	SR	LP	SP
PLOT A	**28**	**40**	**7**	**30**	**4**	**61**	**88**	**4**	**45**	**109**	**0**	**0**	**0**	**97**
PLOT B	**4**	**9**	**1**	**23**	**0**	**0**	**216**	**0**	**0**	**720**	**0**	**36**	**0**	**66**
PLOT C	**0**	**22**	**3**	**56**	**25**	**101**	**108**	**20**	**75**	**65**	**83**	**101**	**54**	**27**
TOTALS	**32**	**71**	**11**	**109**	**29**	**162**	**412**	**24**	**120**	**894**	**83**	**137**	**54**	**190**

Kelsey Jackson, Kelsey McManus, Lindsay Cheek
Brock
Biology 9
May 25, 2007

AFTER FERTILIZER	0 g. of Fertilizer (# of different types of bacteria)*						40 g. of Fertilizer (# of different types of bacteria)*						80 g. of Fertilizer (# of different types of bacteria)*						
	LR	MR	SR	LP	MP	SP	LR	MR	SR	LP	MP	SP	LR	MR	SR	LP	MP	SP	MB
PLOT A	**15**	**25**	**62**	**21**	**12**	**28**	**12**	**6**	**791**	**9**	**13**	**217**	**0**	**0**	**0**	**0**	**21**	**0**	**22**
PLOT B	**37**	**0**	**30**	**17**	**0**	**52**	**18**	**0**	**0**	**90**	**0**	**432**	**0**	**0**	**38**	**0**	**0**	**51**	**0**
PLOT C	**150**	**0**	**55**	**69**	**0**	**63**	**118**	**0**	**203**	**16**	**0**	**109**	**47**	**0**	**30**	**77**	**0**	**254**	**0**
TOTALS	**202**	**25**	**147**	**107**	**12**	**143**	**148**	**6**	**994**	**115**	**13**	**758**	**47**	**0**	**68**	**77**	**21**	**305**	**22**

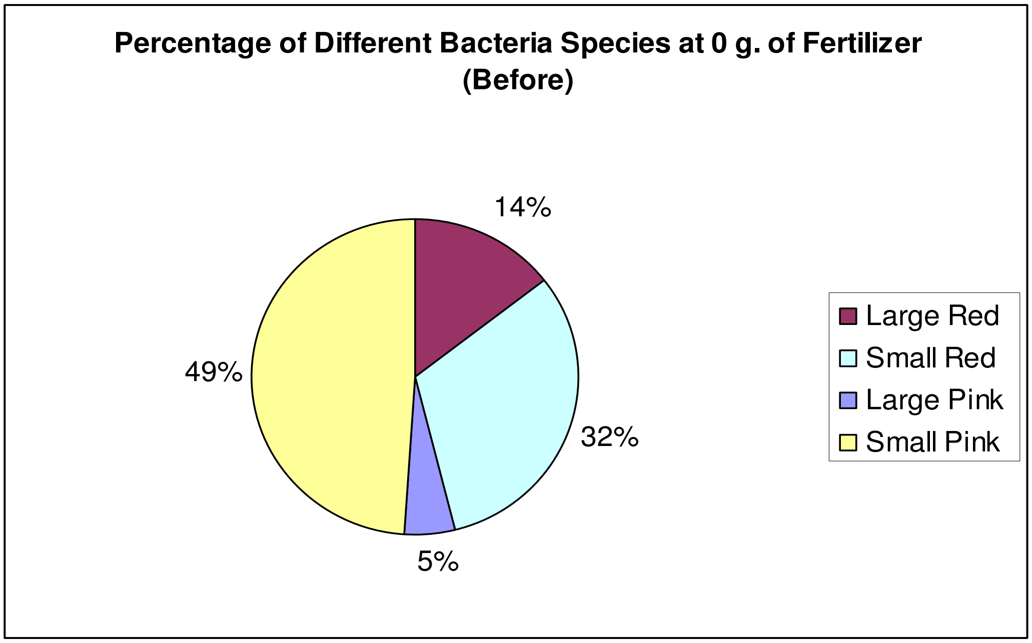
*** LR= large red, MR= medium red, SR= small red, LP= large pink, MP= medium pink, SP= small pink, MB= medium beige**

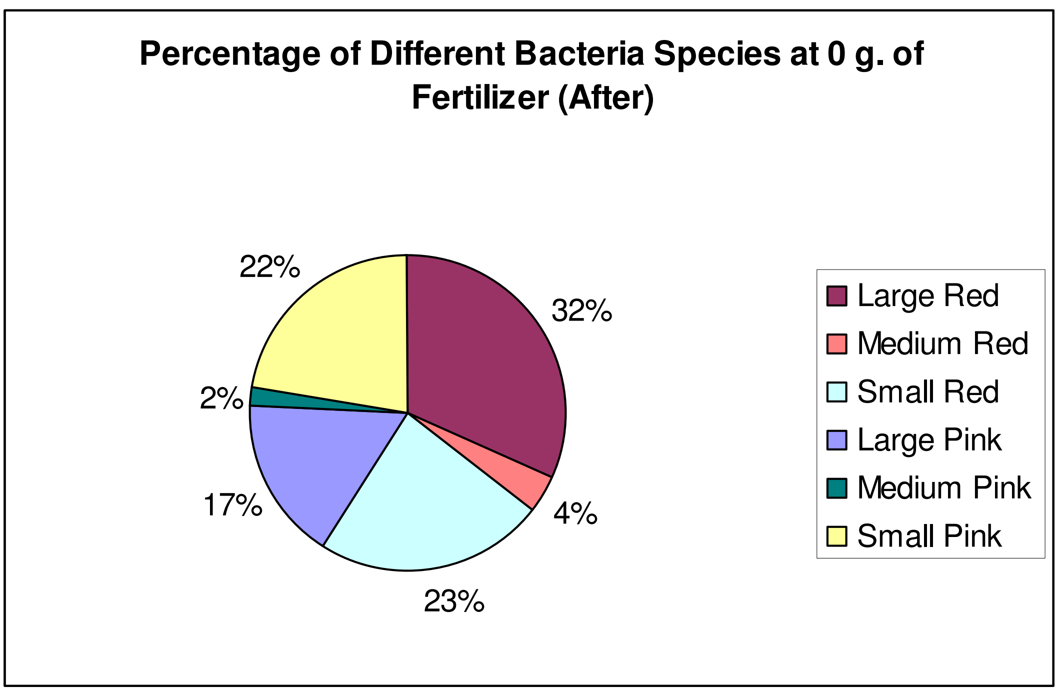
Kelsey Jackson, Kelsey McManus, Lindsay Cheek
Brock

Biology 9

May 25, 2007

Graphs:



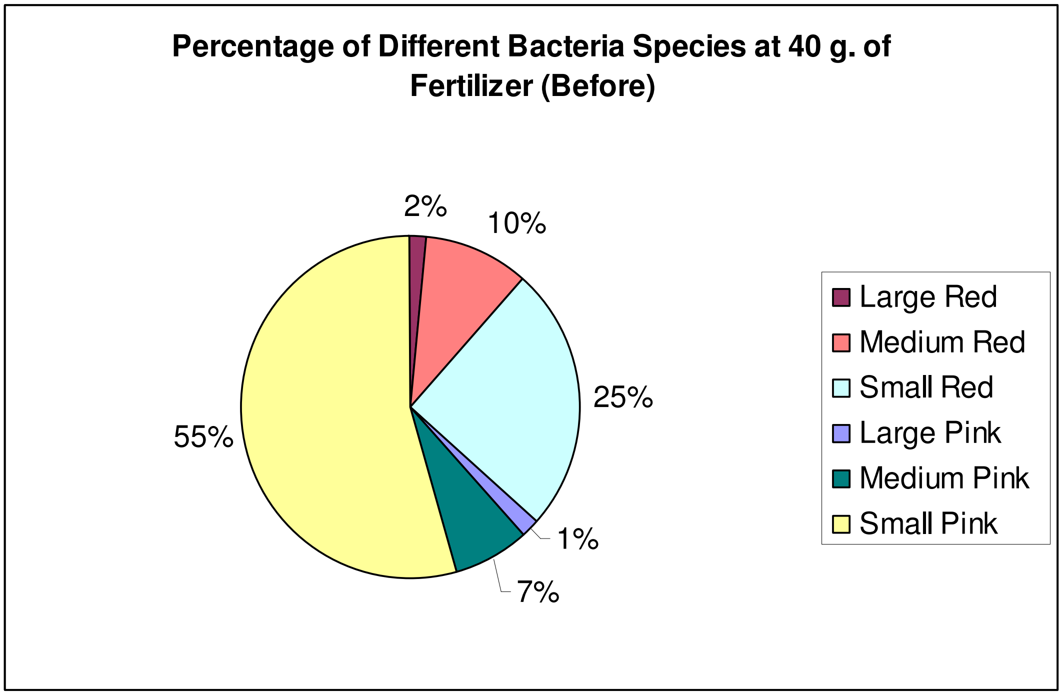


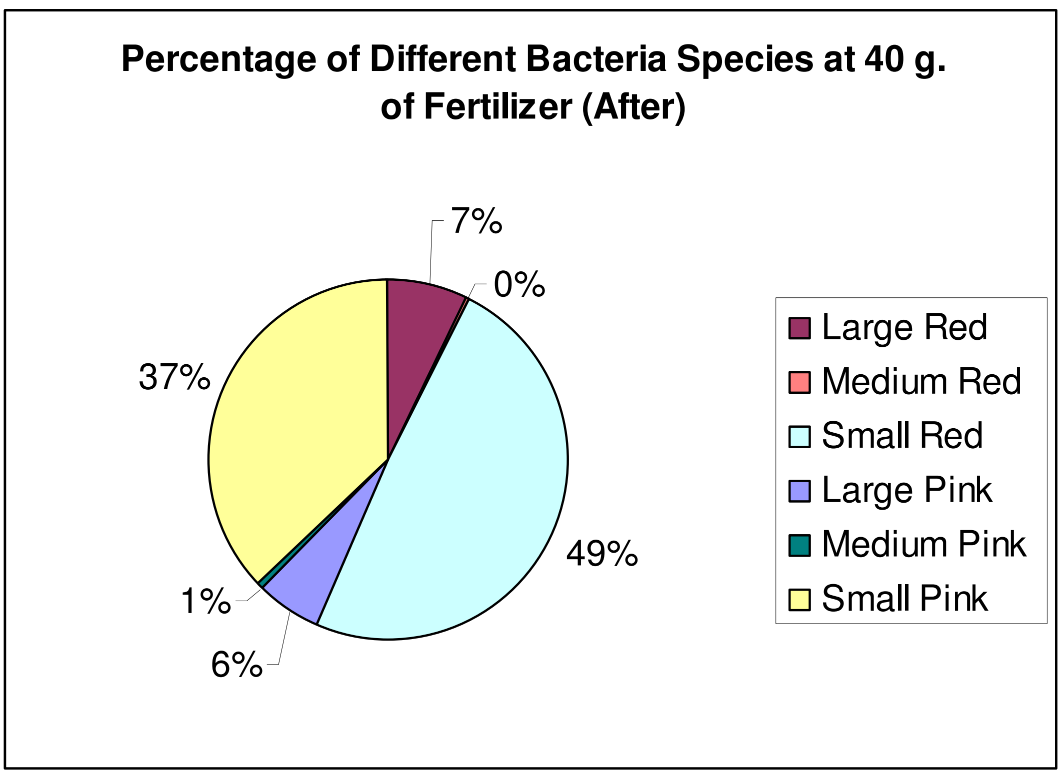
Kelsey Jackson, Kelsey McManus, Lindsay Cheek

Brock

Biology 9

May 25, 2007



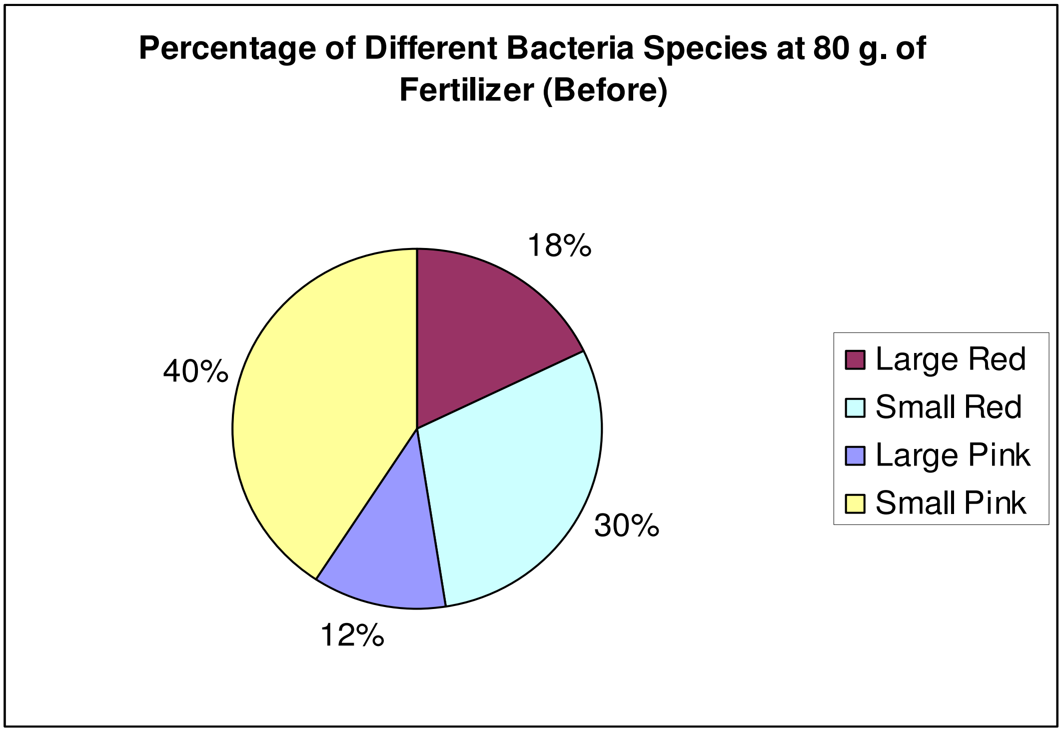


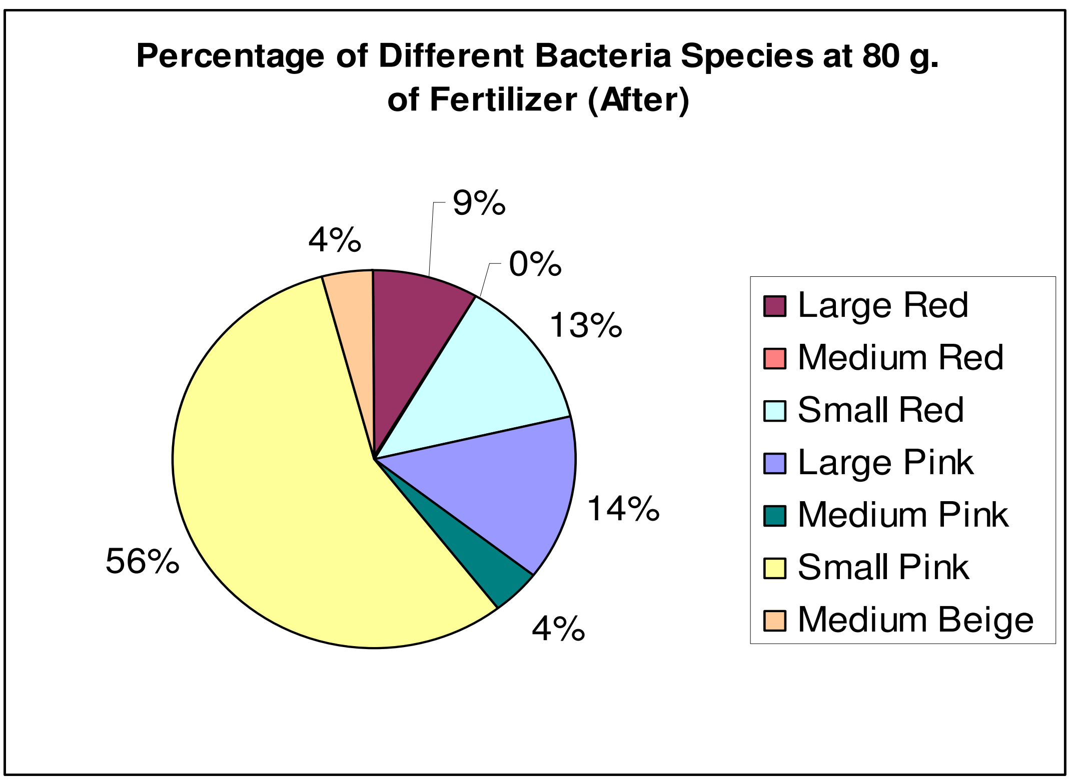
Kelsey Jackson, Kelsey McManus, Lindsay Cheek

Brock

Biology 9

May 25, 2007





Kelsey Jackson, Kelsey McManus, Lindsay Cheek

Brock

Biology 9

May 25, 2007

We preformed a statistical analysis of our data and the following P- values were found:

	0 g. of Fertilizer						**40 g. of Fertilizer**						**80 g. of Fertilizer**						
	LR	MR	SR	LP	MP	SP	LR	MR	SR	LP	MP	SP	LR	MR	SR	LP	MP	SP	MB
P- VALUES	.28	.03	.26	.14	.01	.3	.05	.05	.38	.04	.04	.45	.13	0	.2	.13	.02	.49	.02

Kelsey Jackson, Kelsey McManus, Lindsay Cheek
Brock
Biology 9
May 25, 2007

Conclusion:

In conclusion our hypothesis was incorrect. We predicted that if the amount of fertilizer applied to a plot of grass increases, then the biodiversity of bacteria in the soil will increase. According to the data the water was actually the reason the biodiversity of the bacteria increased. By looking at the graphs of before and after plots of 0 grams of fertilizer you can see that the before graph had 4 species and the after graph had 6 species. The plots of grass without fertilizer; however had 1 liter of water, increased by 2 species. If you look at the pie graphs for .5 liter of water and 40 grams of fertilizer the numbers of species did not increase. On the graphs of plots that had 1 liter of water and 80 grams of fertilizer the species also increased by 2; the before graphs showed 4 species and the after graphs showed 6 species. When looking at the results of the experiment the question of if the water or after a certain amount of fertilizer affects the biodiversity of bacteria. The data leads to conclude that it was the water that actually affected the biodiversity of bacteria. The percentages on the after graph with 0 grams of fertilizer show higher percentages than the 80 grams of fertilizer. There are percentages such as: 22%, 32%, 23%, etc on the after graph with 0 grams of fertilizer. There are percentages such as: 13%, 9%, 14%, 56%, etc on the after graph of 80 grams of fertilizer. The percentages from the graphs without fertilizer are bigger and the percentages from the graphs with 80

Kelsey Jackson, Kelsey McManus, Lindsay Cheek

Brock

Biology 9

May 25, 2007

grams of fertilizer vary more. To see which set of data was most accurate we looked at the p-values. The p-values of the plots with 0 grams of fertilizer are lower than the p-values of the plots with 80 grams of fertilizer. Therefore, by looking at the percentages of the graphs and the p values; the water was what affected the biodiversity of the bacteria to increase

Kelsey Jackson, Kelsey McManus, Lindsay Cheek

Brock

Biology 9

May 25, 2007