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Mr. Brock

9H Biology 1

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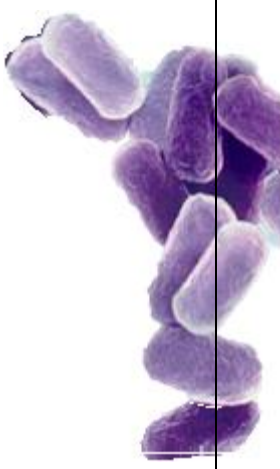
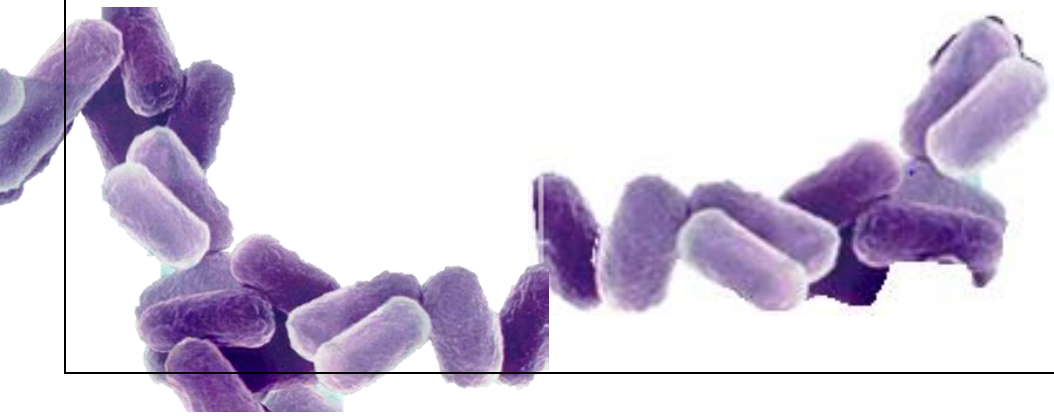


# The Effect of Soil Compaction on the Density of Soil Bacteria



## *Background*

Bacteria can be extremely beneficial, and in fact, humans depend on bacteria for their very survival. But bacteria in the soil are particularly important, and fertile soil will contain millions of bacteria per gram (Encyclopædia Britannica, 2012). Most of these bacteria live in the top 10 cm of it (Reid and Wong, 2005), but they can be found in very diverse soil environments that include much wider physical parameters than most of their fellow soil microbes. This is because, in contrast with other soil microbes, bacteria can grow and adapt faster to changing environmental conditions. As prokaryotes, their size, sturdiness, and simplicity of structure simply allow them to do so (Hoorman, 2011).

Both bacteria and other microbes are vital components of the soil because they help recycle all organisms and resources that return to the soil. Soil bacteria in particular are responsible for freeing the minerals that other organisms in the ecosystem need to survive. They do this by extracting valuable minerals from rocks by releasing enzymes that break down inorganic matter (Hornyak, 2008) and by decomposing key minerals in organic matter as well. (University of Minnesota, 2001). This category of soil microbes is especially important in the initial stages of decomposition (Ried and Wong, 2005), and soil bacteria can even break down toxins into unhazardous, useful materials. Hence it is by converting elements of their external environment and inorganic gaseous compounds into forms that can be used by plants and animals that soil bacteria can break down toxins into unhazardous, useful materials (Hoorman and Reeder, 2009).

In addition to freeing minerals, decomposing both organic and inorganic materials, and converting toxins into harmless soil components, some species of bacteria capture nitrogen from the air and add it into the soil. These nitrifying bacteria are part of a small group of aerobic

bacteria that use inorganic chemicals as an energy source, and they are crucial to the nitrogen cycle because they convert soil ammonia into nitrate for plants, often from decomposition (Encyclopædia Britannica, 2012). Nitrate, a form of chemically fixed nitrogen, enables plant growth. Nitrogen is an essential element in proteins. One of the five major biological molecules, proteins are critical to performing the four tasks (homeostasis, synthesis, respiration, and reproduction) that make life possible. Hence, without nitrogen plants and all that depend on them could not survive.

Because nitrogen fixation by these bacteria is so critical, anything that can interfere with it, such as soil compaction, can decrease soil fertility. Nitrifying bacteria are especially important in the initial stages of decomposition, and they function best when soil moisture is high (Reid and Wong, 2005). However, soil compaction decreases soil moisture (University of Minnesota, 2001). Soil compaction occurs when soil particles are pressed tightly together under external pressure. Sources of compaction can include heavy rain (Hoorman, Sá, and Reeder, 2009), wheels, feet, sidewalks, a lack of crop rotation, and plowing at the same depth without variation (University of Minnesota, 2001). A lack of active plant growth can also lead to soil compaction (Hoorman, 2009). Soil particles packed together restrict water drainage by reducing the space for large pores. Pores normally act as channels for water and oxygen to penetrate the soil, and larger pores allow more water and oxygen to reach the plant roots and soil microbes living in the soil, but with compaction the pores become smaller and there is less oxygen and water reaching plant roots and soil microbes. Furthermore, soil compaction makes it more difficult for the roots of plants to break through the top and reach down into the soil because it increases "the ability of soil to resist being moved by an applied force" (University of Minnesota, 2001).

Other than soil compaction's negative effects on plants, soil compaction also negatively effects bacteria. Although bacteria, as a species, are very resilient, soil bacteria prefer tilled soil because they thrive on high levels of oxygen and release nutrients in organic matter from tillage (Hoorman, 2009), therefore they do not flourish in a soil-compacted environment (Hoorman, Sá, and Reeder, 2009). This is important for humans to understand, because the compaction of soil can indirectly threaten the lives of not just the bacteria, but all the organisms in an ecosystem. By changing the soil environment for bacteria, humans may be harming plant growth, thus endangering all other organisms. All terrestrial organisms depend on plants, for they represent the base of a food chain. Plants provide the organisms above them in the food chain with energy from the sun. Without plants, none of the animals would be able to survive. Since plants rely on a healthy soil environment to grow and develop, we must look to the soil to determine the actual health of an ecosystem.

Our experiment will test the effects of soil compaction on bacteria populations within the Roland Park Country School campus. By examining the population density of bacteria from soil environments with different levels of soil compaction, we are seeking to determine the relative health of the bacteria populations that we sample. We will take bacteria cultures from areas that we hypothesize are less heavily compacted, such as areas near sidewalks, and areas that are less heavily compacted, such as sites with increased distance from sidewalks. Our data will provide us with a measure of the impact of compaction on the density of bacteria. We hope to be able to assess the influence of our school's sidewalks on the bacterial population in the soil next to them. We hypothesize that soil bacterial populations are denser in the soil farther from the sidewalks with less compaction.

## *Procedure*

I. Problem: Are soil bacteria populations denser in soil with high or low levels of compaction?

II. Hypothesis: Soil bacterial populations are denser in soil with less compaction.

III. Procedure:

A. Independent Variable: distance North away from the sidewalk to vary the amount of soil compaction

B. Dependent Variable: density of bacteria per cubic centimeter of soil

C. Negative Control: soil sample taken farthest from the sidewalk

D. List of Controlled Variables:

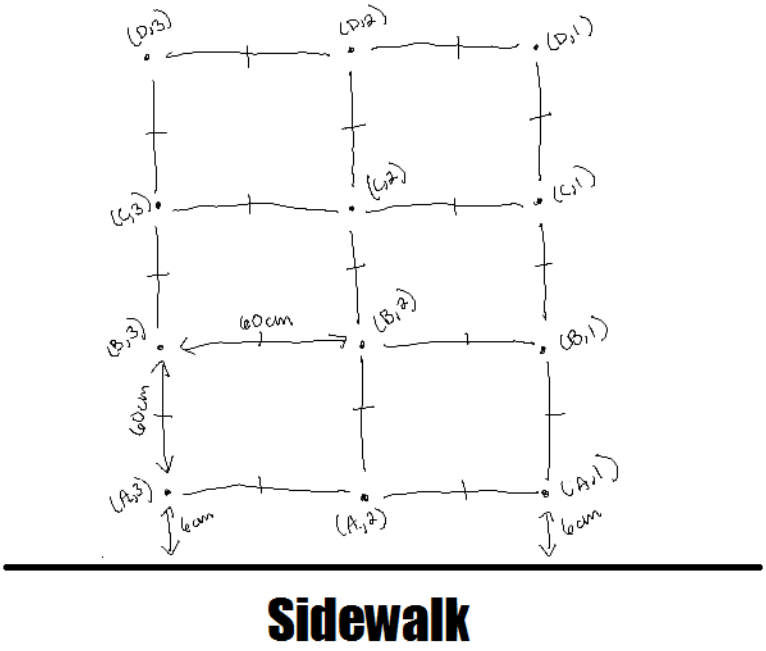
- Surrounding plant species
- Location of soil plots on campus (front lawn of RPCS)
- Amount of soil extracted
- Amount of soil collected from each plot
- Volume of sterile water used in each culture tube (10 ml)
- Amount of soil in each culture tube
- Same day and time of day soil collected
- Amount of time each culture is allotted to grow
- Type of water (sterile water)
- Size of scoop (1-cc)
- Size of serological pipette (10 ml)
- Size of culture tube (15 ml with cap)
- Type of nutrient agar plates (Petrifilm ™ Aerobic Count Plate)
- Amount of bacteria solution plated (100 ul)
- Size of soil core tester
- Angle soil extractor is put into the soil (perpendicular to the ground)
- How much diluted each time

E. Step-by-Step Instructions:

1. At GPS coordinates N 39.35806°, W 76.63618° on the RPCS campus, 6 cm north away from the northern sidewalk, use a tape measure to measure 6 60cm x 60cm square plots. At each corner of the square plots place a yellow plotting flag. Label the yellow plotting flags with letters (A, B, C, and D) and numbers (1, 2, and 3). Flags

in row A should be closest to the sidewalk, and flags in row D should be farthest west from the sidewalk. Numbers represent the column of the flag. Flags in column 1 are farthest to the right, and flags in column 3 are farthest to the left. See diagram below for the plotting plan and how to properly label the plotting flags.

Diagram:




2. Perform steps 2-7 on the same day and at the same time of day.
3. 1 cm North of the plotting flag "A,1" (behind the plotting flag), perpendicular to the ground, place a soil extractor with a 2 cm. diameter 15 cm. into the soil (until the soil surface reaches the 1st dotted line of the soil extractor)
4. Rotate the soil extractor 360° clockwise
5. Pull the soil extractor straight out from the ground
6. Place the soil taken from the soil extractor into a clean zip lock plastic bag labeled with a permanent marker "A,1", in accordance with the plotting flag it was behind when extracted from the soil and write the time the soil was extracted. Lightly tap the side of the extractor against the sidewalk to release the soil into the labeled clean zip lock plastic bag.
7. Repeat steps 3-5 1 cm north of the other plotting flags (behind the plotting flags), in order by column first ("B,1", "C,2", "D,1"; "A,2", "B,2", "C,2", "D,2"; "A,3", "B,3", "C,3", "D,3")
8. Once you have finished collecting all of your soil and placing it in the properly labeled clean zip lock plastic bag, bring the soil samples in their bags back to the science room.

**Serial Dilution for Bacteria**

In the science room, **perform the Serial Dilutions (steps 1 through 16) for Bacteria protocol for all soil samples in each trial at the same time.** See instructions below for the Serial Dilutions for Bacteria protocol.

1. Use a clean, new transfer pipette to add 10 ml of sterile water to a 15 ml culture tube. Label the tube "A,1" in accordance with the soil sample and "$10^{o}$" in accordance with the dilution value.
2. Use the same pipette to add 9 ml to a second 15 ml culture tube. Label the tube "A,1" in accordance with the soil sample and "$10^{-1}$" in accordance with the dilution valued.
3. Repeat step 2 three more time to three additional 15 ml culture tubes, only label them "$10^{-2}$, "$10^{-3}$" respectively with "A,1" to stand for the soil sample.
4. Place 1 cc of your soil sample ("A,1") into the "A,1 $10^{o}$" culture tube.
5. Cap the tube and shake vigorously.
6. Using a new clean pipette, remove 1 ml of the soil/water mixture from the "A,1 $10^{o}$" tube and place into the "A,1 $10^{-1}$" tube.
7. Cap and shake vigorously.
8. Using the same pipette in step 6, remove 1 ml of the soil/water mixture from the "A,1 $10^{-1}$" tube and place into the "A,1 $10^{-2}$" tube.
9. Cap and shake vigorously.
10. Using the same pipette in step 6, remove 1 ml of soil/water mixture from the "A,1 $10^{-2}$" tube and place into the "A,1 $10^{-3}$" tube.

11. Cap and shake vigorously.
12. You should now have a total of four culture tubes.
13. Place 100 µ samples from the 3rd and 4th tubes dilutions ("A,1 $10^{-2}$" and "A,1 $10^{-3}$") onto their own separate 3M Petrofilm™ aerobic count plates containing the nutrient agar each labeled with "A,1" to represent the soil samples and "$10^{-2}$" or "$10^{-3}$" according to the dilution valued.
14. Repeat steps 1-13, this time labeling the 3M Petrofilm™ aerobic count plates and the culture tubes with the letter and number of each of the corresponding separate soil sample ("B,1", "C,1", "D,1"; "A,2", "B,2", "C,2", "D,2"; "A,3", "B,3", "C,3", "D,3").
15. Allow all plates to grow for 48 to 72 hours.
16. Examine each of the plates for individual bacteria colonies and choose the plate with the fewest colonies (but at least 5 colonies) and the lowest dilution value to make your estimates of the number of the bacteria in the original 1cc soil using the following formula:

# microbes in 1 cc of soil = # of colonies on sheet $x10^{2} \times 10^{|\text{dilution number at which these colonies were found}|}$

## *Data and Analysis*

A. Data Table

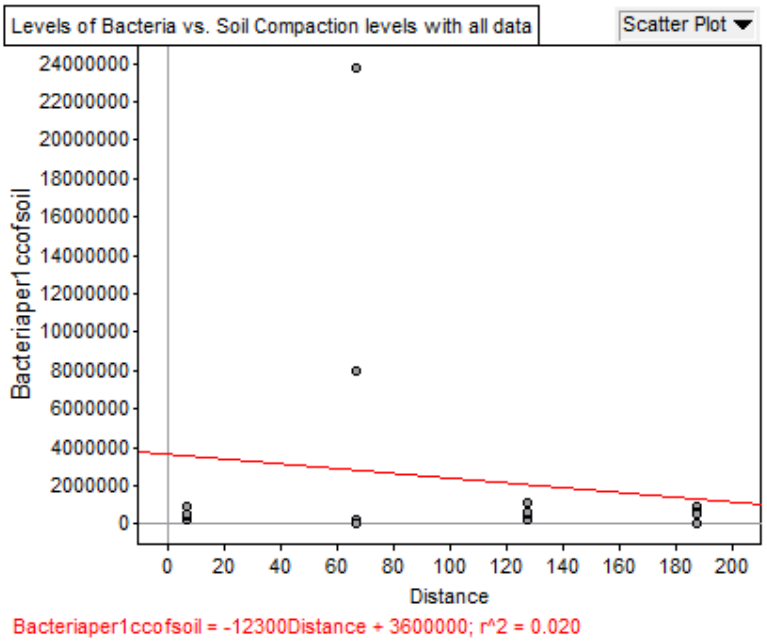
**Density of Bacteria in Different Levels of Soil Compaction**

| Trial Number | Distance from sidewalk | Density of bacteria per 1 cc of soil |
|---|---|---|
| Trial #1 | A (7 cm.) | 180,000 |
| | B (67 cm) | 23,800,000* |
| | C (127 cm) | 1,100,000 |
| | D (187 cm) | 930,000 |
| Trial 2 | A (7 cm) | 900,000 |
| | B (67 cm) | 220,000 |
| | C (127 cm) | 210,000 |
| | D (187 cm) | 600,000 |
| Trial #3 | A (7 cm) | 470,000 |
| | B (67 cm) | 50,000* |
| | C (127 cm) | 480,000 |
| | D (187 cm) | 60,000* |
| Average | A (7 cm) | 516,667 |
| | B (67 cm) | 220,000 |
| | C (127 cm) | 596,667 |
| | D (187 cm) | 765,000 |

*This information is being omitted from the averages and from one of the scatter plots due to the fact that it is highly improbable for these levels of bacteria to exist in soil

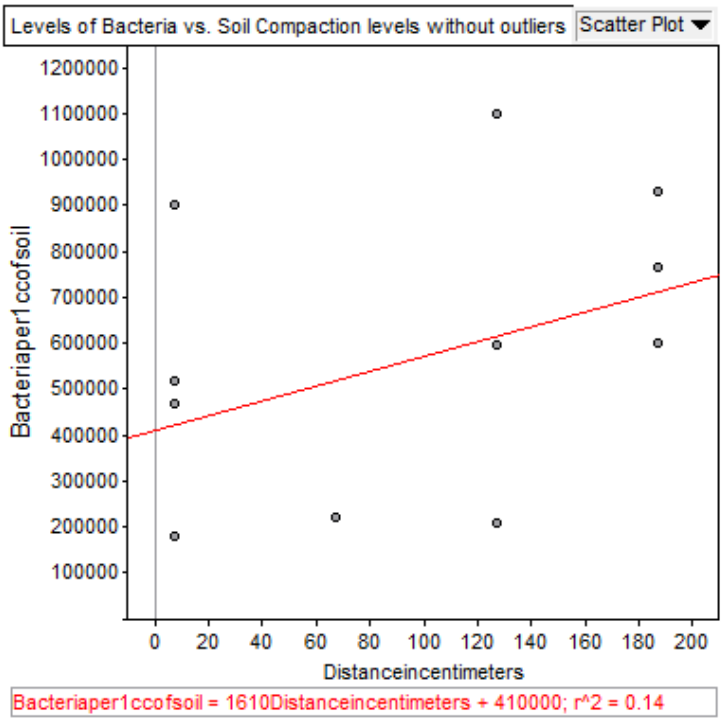
B. Graphs

Scatter Plot 1



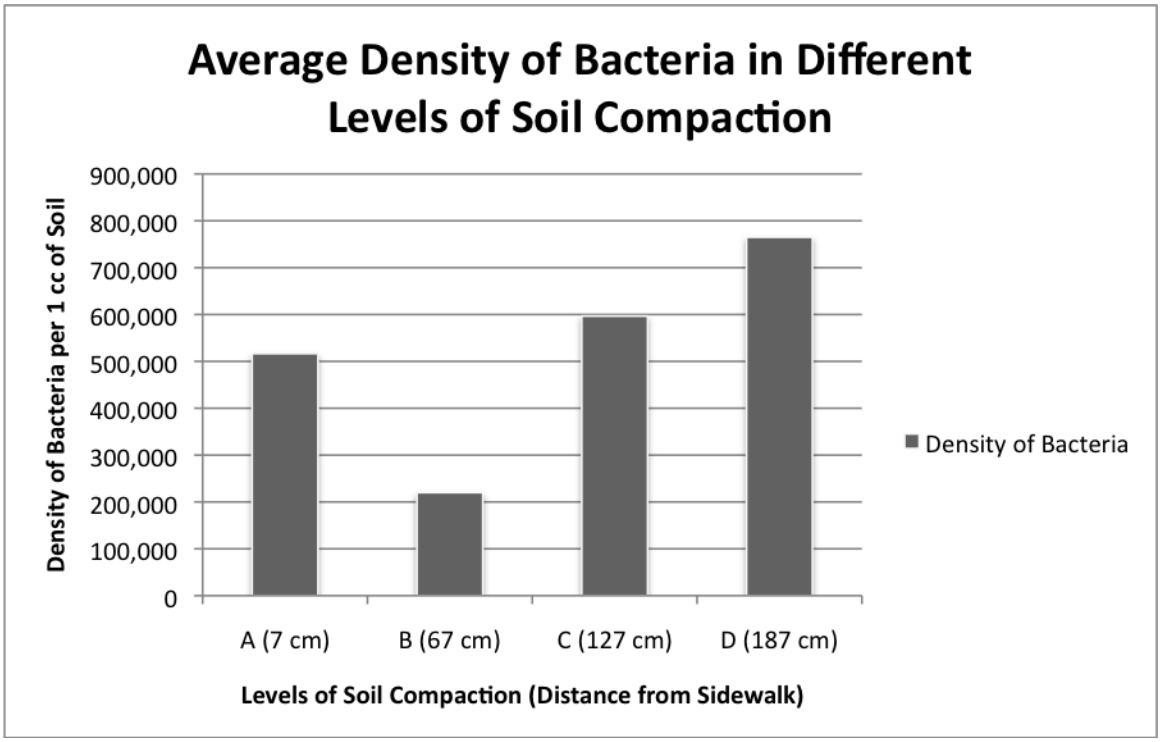

*This scatter plot includes the outlier points.

Scatter Plot 2




*This scatter plot omits the outlier points.

Bar Graph 1




* This bar graph contains the average density of bacteria per 1 cc of soil in different levels of soil compaction, omitting the same information from the data table (23,800,000, 50,000, and 60,0

## *Conclusion*

Our hypothesis was proven correct. The bacteria do appear to prefer soil that is not compacted or is less compacted.

As shown in the data table, there are three outliers in the data (23,800,000, 50,000, and 60,000), which were omitted from the average densities of bacteria per 1 cc of soil. These outliers were omitted from the averages and from one of the scatter plots because it is highly unlikely that these densities of bacteria exist in soil. The cause of these outliers is most likely a flaw in our soil extraction, dilution process, or plating the bacteria. Two of the three outliers

were taken from 67 cm away from the sidewalk which explains why the average density of bacteria per 1 cc of soil from 67 cm away from the sidewalk is 220,000, a significantly smaller number than any of the other averages.

According to our averages with these data points omitted, bacteria prefer soil that is less compacted as opposed to soil that is more compacted. The average number of bacteria for the "A" plots (or the plots that were 7 cm away from the sidewalk) is 516,667 bacteria per 1 cubic centimeter (cc) of soil. For the "C" plots or the plots that were 127 cm away from the sidewalk the average was 596,667 bacteria per 1cc of soil and for the "D" plots or the plots that were 187 cm away from the sidewalk the average was 765,000 bacteria per 1 cc of soil. Looking at these averages, it seems that the further away from the sidewalk and therefore the less compacted the soil is, the more the bacteria thrive. The only average that appears to go against these finding is the "B" plot or the plot 67 cm away from the sidewalk, which has an average of 220,000 bacteria per 1 cc of soil. The "B" plot's average is smaller than the "A" plot's average, despite the fact that is farther away from the sidewalk. However, due to the outliers omitted, there was only one trial to support the "B" plot's average and the other averages appear to support this trend.

When looking at Scatter Plot #2 of our data, we see this same trend appearing. After the points are placed onto the graph, we then must find the line of best fit. This line of best fit has an upward slope. Our x-axis has the distance from the sidewalk, and our y-axis is labeled with the levels of bacteria. This upward slope shows that the further from the sidewalk the plot is – the less compacted the soil is – the higher the bacteria levels are. When the outliers are not omitted from the data, as shown on Scatter Plot #1, the line of best fit slopes slightly downward but it is nearly flat, and so it does not go directly against our previous findings. Furthermore, the reasons

for these outliers are unknown but along with our mistakes in plating and diluting it may also be that certain other soil conditions could factor into the density of bacteria besides soil compaction.

According to our averages and our scatter plot, we can draw the conclusion that bacteria prefer soil that is less compacted. This is probably because soil that is freer has more oxygen in it. Soil that is freer also has more water because compacted soil does not provide space for water. Bacteria need oxygen and water to survive. Therefore, they would flourish in less compacted soil.

There are further experiments that can be conducted based on our own experiment. From our experiment, an experiment can be designed ascertaining which factors of soil compaction compact the soil the most. Another experiment that could be performed could be which species of bacteria are most affected by soil compaction. Also, our experiment never directly measured the level of soil compaction. This experiment could be redone where the compaction level of the soil was accurately measured.

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