The Effects of Soaps Used in Car Washes on the Soil

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Background

Bacteria are a very important and necessary part of the ecosystem, and they grow and live in thin water films around the soil particles near roots, in an area known as the rhizosphere (Hoorman, n.d.). A teaspoon of soil anywhere on Earth typically has bacteria whose population is between 100 million and 1 billion (Ingham, 2009), and they perform many important functions related to water movement, filtration, and nutrient cycling. They do so by creating microaggregates, or microscopic collections of particles, that are formed when soil bacteria bind soil particles together with the substances they produce, such as polysaccharides and glycoproteins, and in doing so they improve soil structure, increasing the rate of water filtration and the holding capacity of the soil (Hoorman, n.d.).

In addition to this general service that all bacteria provide for the soil, specific groups of bacteria contribute to a number of dedicated functions in the soil as well; these are the decomposers, mutualists, pathogens, and lithotrophs. Decomposers consume simple carbon compounds and have a key role in the early stages of the decomposition of organic matter, helping to provide nutrients to plants in the soil. Mutualists form actual partnerships with plants, and a common type of mutualist, the nitrogen fixing bacteria, receive carbon compounds such as sugars from the plant host in return for converting nitrogen from the air into a usable form for the plant. Bacterial pathogens can infect and kill plants, and finally lithotrophs obtain energy from nitrogen, sulfur, iron, or hydrogen compounds rather than carbon (Hoorman, n.d.); (Ingham, 2009). Without bacteria in the soil, these tasks that create the building blocks of the earth's ecosystem could not be performed.

In the part of the soil where they live, bacteria can adapt to changes rapidly because of their small size and short reproductive cycle, and certain kinds of bacteria are very tough and can

withstand harsh environmental changes. However, other types of bacteria are very fragile, and can be killed by very small changes in the soil, including changes in the acidity, salinity, moisture, as well as soil compaction ("Soil Bacteria," n.d.). Therefore many human activities that are often thought to be harmless, such as washing cars, may in fact be negatively impacting the bacteria in the soil.

Two common soaps used to wash vehicles are common dish soap and professional grade car soap. Both cleaners consist of two main components, water and surfactants, and it is the later that actually cleans and removes the dirt from the car's surfaces (Duplessie, 2012). Surfactants are chemicals made from molecules with two parts: a hydrophilic head and a hydrophobic tail, and it is the hydrophilic head that binds with water, while the hydrophobic tail binds with the grease and oil and surrounds the dirt (Disqus, 2015). The surfactant is mixed with the water and when applied to the surface of the car the hydrophobic end of the surfactant encompasses the mud or dust. This is because the hydrophobic end of the soap molecule is attracted to the oily elements in the dirt. The other end of the soap molecule, the hydrophilic head, makes a sphere like shape, called a micelle, around the dirt which keeps the dirt imprisoned and the water surrounding the dirt without directly touching it. This is because the hydrophilic head is attracted to the water and points outward forming a bubble around the dirt. Once the dirt is surrounded by the surfactant it is held in suspension by the water, allowing the dirt to be washed away easily (Disqus, 2015).

But how might these surfactants impact soil bacteria? There are a number of surfactants used in soaps to clean cars, but the main ones are anionic and nonionic (Jarkle, 2017). Anionic surfactants are surfactants that carry a net of negatively charged ions. Some examples of anionic surfactants that are in cleaners are sodium sulfate, ammonium sulfate and magnesium sulfate

(Roach, 2017). Sodium sulfate is a potentially especially detrimental surfactant to the soil because when there is an excess of sodium, it alters the physical properties of the soil making it dry and less moist, putting the 400 bacterial species that live in the soil in danger. J.E. Greaves (1922) found that when salt was added to the soil, the presence of many species of bacteria needed for plants to grow decreased in the soil, harming the plants as well as the bacteria. However, not all anionic surfactants are harmful. Bacteria are highly resistant to ammonium sulfate which shows no negative impact on soil bacteria even in large amounts (Muller & Walter, 2006).

In addition to anionic surfactants, the other important type of surfactant in car soap is nonionic surfactants. These are surfactants that carry no charge and they are often used as foamers and are thick liquids or syrups that are sticky to touch. Some examples of Nonionic surfactants that are in cleaners are ethoxylates and cocamide (Roach, 2017), and one experiment performed showed that ethoxylate prevents bacteria from multiplying. Therefore, the bacteria in the soil would likely not be able to multiply if this surfactant was absorbed by the soil (Moore & Denyer, 2006). Cocamide is a chemically modified version of coconut oil, and it has been recently shown to kill microorganisms as effectively as harsher disinfectants. Subjects who gargled with coconut oil for thirty days to prevent bacteria in their mouth saw a decrease in plaque that matched the decrease in plaque if a disinfectant like chlorhexidine was used (Peedikayil & Remy, 2016), and while the bacteria inhabiting the human mouth are different species than those found in the soil, it is possible that if those bacteria there were exposed to the cocamide, it might harm them as well.

While all car soaps contain surfactants, there are other ingredients that could potentially harm the bacteria in the soil that are only included in specific brands of soap. Many dishwashing

soaps, such as Palmolive Dish Soap, have as their main active ingredient an organic compound called Triclosan which possesses antibacterial properties (Cummings, n.d.). Triclosan kills both good and bad bacteria and renders medically necessary antibiotics less effective. It is toxic for some species such as algae and has even been shown to disturb hormones in animals, preventing normal development (Izlar, 2016). Palmolive Dish Soap also contains SD Alcohol 3A, which is a toxic grain alcohol that can kill bacteria ("How Does," 2009), and the ingredient DMDM Hydantoin turns into formaldehyde as it breaks down, which is an actual preservative because it kills all microbes of any kind (Chen, Djoko, Veyrier, & McEwan, 2016); (Wired Staff, 2009). These ingredients within the dish soap and car soap could be killing the necessary bacteria in the soil.

The bacteria levels, after the soaps have been absorbed by the soil, will be measured to see their effects on the health of the soil because the nutrients in it decides the stability and health of the ecosystem and the microorganism, bacteria, performs many crucial tasks for the ecosystem. At home car washes could possibly be dispersing immense amounts of pollutants from the soap into the soil, therefore damaging the ecosystem. This experiment will test whether Palmolive Ultra Antibacterial Dish Soap or Turtle Wax Ice Premium Car Care Wash and Wax car soap is more harmful to bacteria in the soil. This will answer the question whether people should wash their cars at home with car soap or dish soap in order to harm the least amount of bacteria. We predict that dish soap will decrease the density of bacteria in the soil to a greater extent than car soap.

Experiment

I. Problem: Does dish soap or car shampoo decrease the density of bacteria in the soil more?

II. Hypothesis: Dish soap will decrease the density of bacteria in the soil more than car shampoo.

III. Procedure:

a. Independent Variable: the type of soap applied to the soil plots

b. Dependent Variable: the density of bacteria in the soil (#/cc)

c. Negative Control: soil plots with only water poured on them

d. Positive Control: samples tested for density of bacteria before any solutions are added

e. List of Controlled Variables:

1. Type of dish soap used
2. Type of car shampoo used
3. Amount of soil taken in each soil sample
4. Amount of water used in diluting the soap and shampoo
5. Amount of water used as the negative control
6. Number of flags
7. Location of plots
8. Size of soil plots
9. Collect all samples on the same day at the same time
10. Amount of solutions poured into soil
11. Concentration of solutions
12. Type of water in solutions
13. The amount of time the solutions sink in

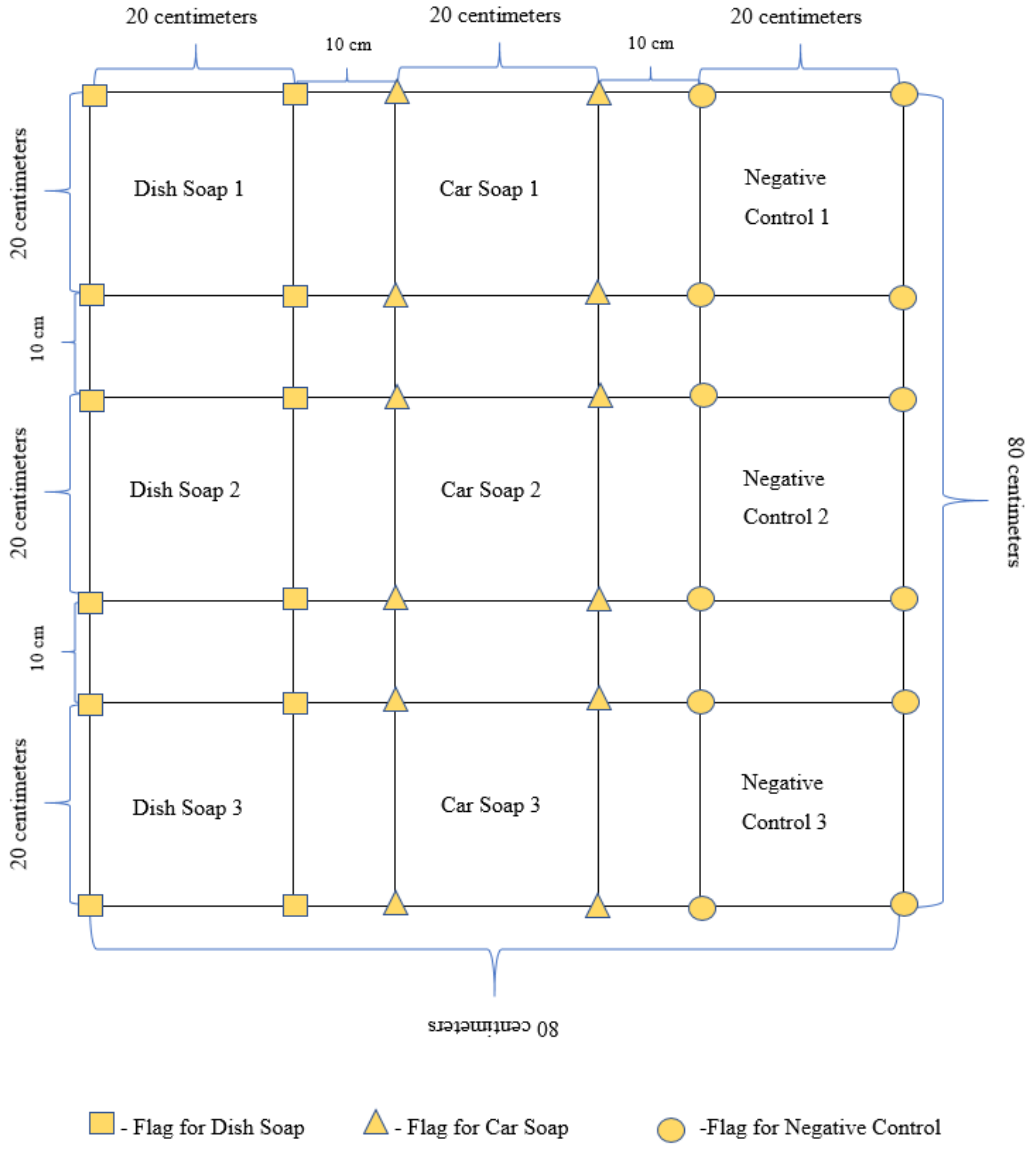
14. The soil samples without the car soap, dish soap, and water are taken on the same day at the same time
15. The soil sampled with the car soap, dish soap, and water are taken on the same day at the same time
16. Size of serological pipettes
17. Size of graduated cylinder
18. Distance between plots
19. Temperature of solutions
20. Type of water in culture tubes
21. Amount of sterile water in the different culture tubes
22. Amount of soil added to the culture tubes
23. Type of agar sheets
24. Time given for the bacteria to grow on the agar sheets
25. Amount of soil/water solution added to the agar sheets
26. Dilution of the soil/water solution added to the agar sheets
27. Time and day when serial dilutions take place
28. Time and day when soil collecting takes place
29. Size of culture tubes
30. Number of days bacteria on sheets grow
31. Degree to which the soil samples are diluted

f. Step-by-Step Instructions

1. Take 36 flags and label 4 of the flags "dish soap 1", 4 of the flags "car soap 1", 4 of the flags "n.c. 1" (for negative control). Label 4 of the flags

"dish soap 2", 4 of the flags "car soap 2", and 4 of the flags "n.c. 2". Label 4 of the flags "dish soap 3", 4 of the flags "car soap 3", and 4 of the flags "n.c. 3".

2. Go to 39°21.349’ North and 76°38.128’ West. Using two meter sticks, measure an 80 cm by 80 cm square for reference. Leave the metric rulers on the grass.



3. Using the diagram above as a reference, measure out and mark the plots with the appropriately labeled flags
4. Take 27 plastic medium bags and divide them into 9 groups of three. (these bags are to collect the soil samples before the car soap, dish soap, and water are poured into the soil [positive control]).
5. Label the bags according to the following criteria: whether or not the bag is positive control- (PC), which plot the sample is from, (Dish Soap, Car Soap, or Negative Control [NC]), and which trial number the sample is from (1, 2 or 3), and which sample number the sample is (.1, .2, or .3).
6. For example, label the first plastic bag in the first group "PC dish soap 1.1", the second bag "PC dish soap 1.2", and the third bag "PC dish soap 1.3". PC is for positive control, dish soap for the plot, and 1.3 for the first trial and the third sample.
7. All soil samples for the positive control, steps 8-12, must be taken on the same day and in the same time period.
8. In the same time period, on the same day, using a metal soil core extractor, the hammer, and the first set of 27 bags, extract a 15 cm deep, 2 cm in diameter, soil core in the plot marked by the "PC dish soap 1". Place your soil sample in the correspondingly labeled plastic bag, it should be the bag that says "PC dish soap 1.1".
9. Repeat this process two more times in the same "dish soap 1" plot varying the placement of the soil sample and placing the soil in the corresponding

plastic bag marked with “pc dish soap (trial number and number of soil core sample)”.

10. Repeat steps 8-9 in the plot marked with the "car soap 1" flags and collect the soil samples in the correspondingly labeled plastic bags marked with “pc car soap (number of soil core sample)”.
11. Repeat steps 8-9 in the plot marked with the "n.c. 1" flags and collect the soil samples in the correspondingly labeled plastic bags marked with “pc n.c. (number of soil core sample)”.
12. Repeat steps 8-11 in the soil plots labeled “dish soap 2”, “car soap 2”, “n.c. 2”, “dish soap 3”, “car soap 3”, and “n.c. 3”. Make sure to put the soil sample in the correspondingly labeled back. The bags used in this part of the experiment should all have “pc (either dish soap, car soap, or n.c.) (trial number) (number of soil core sample)” marked on the bag.
13. Store the bags of soil samples in the same location until ready to measure the bacteria.
14. On the same day and in the same time period, combine all of the soil samples from each plot into one bag. For example, "PC dish soap 1.1", "PC dish soap 1.2", and "PC dish soap 1.3" should all be combined into the first bag ("PC dish soap 1.1"). Thoroughly mash up and combine the soil in the bags. Repeat this step with the soil samples in the remaining 8 plots and be sure to keep each set of 3 soil samples separate from the other soil samples from the other plots.

15. Measure the bacteria in the positive control soil samples in the same time period. Steps 16-29 should be completed during the same time period on the same day.
16. To measure the bacteria, use a clean, new 10 ml serological pipette to add 10 ml of sterile water to a 15 ml culture tube. Label the tube “Car Soap 1 10^{0} ”.
17. Use the same pipette to add 9 ml of sterile water to a second 15 ml culture tube. Label the tube “Car Soap 1 10^{-1}.”
18. Repeat step 17 two more times to two additional 15 ml culture tubes, only label them “Car Soap 1 10^{-2},” and “Car Soap 1 10^{-3}”.
19. Using a 1 cubic centimeter scoop, measure 1 cc of your soil sample and add it into the “Car Soap 1 10^{0} ” culture tube.
20. Cap the tube and shake vigorously.
21. Using a new 10 ml serological pipette, remove 1 mL of the soil/water solution from the “Car Soap 1 10^{0} ” tube and add it into the “Car Soap 1 10^{-1}” tube
22. Cap the tube and shake vigorously.
23. Using the same pipette from step 21, remove 1 mL of the soil/water solution from the “Car Soap 1 10^{-1}” tube and place into the “Car Soap 1 10^{-2}” tube.
24. Cap and shake vigorously.

25. Using the same pipette from step 21, remove 1 ml of the soil/water solution from the "Car Soap 1 10^{-2}" tube and place into the "Car Soap 1 10^{-3}" tube.
26. Cap and shake vigorously.
27. You should now have a total of four culture tubes.
28. Plate 100 µL samples from the 3rd and 4th tubes (dilutions 10^{-2} & 10^{-3}) onto their own separate 3M Petrifilm™ Aerobic Count Plate sheets, which have been labelled corresponding to the label on the culture tube.
29. Repeat steps 16-28 eight more times with each of the remaining sample bags.
30. Allow the bacteria colonies on the AC nutrient agar sheets to grow for 72 hours.
31. Examine each of the sheets for individual bacteria colonies and choose the plate at 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. Record the data from the positive controls in the data table.
33. Using a 100 milliliter graduated cylinder, measure out 150 milliliters of room temperature tap water into a 500 ml plastic solution bottle.
34. Using a 10 mL serological pipette, measure 1.2 milliliters of the Palmolive Ultra Antibacterial Dish Soap and deposit it into the solution bottle.

35. Secure the bottle with the lid and gently swirl the solution bottle. Label the solution bottle "dish soap” and set it aside.
36. Repeat steps 33-35, using a new 500 ml solution bottle and a new 10 mL serological pipette, replacing the dish soap with the Turtle Wax Ice Premium Car Care Wash and Wax car soap, and labelling "car soap" on the bottle.
37. Using a 100 mL graduated cylinder, measure out 150 milliliters of room temperature tap water into a 2 L plastic pitcher.
38. On the same day and during the same time period, use a new 100 mL graduated cylinder to measure out 50 mL of the dish soap solution and pour it evenly across the whole "dish soap 1" plot. Then, measure out another 50 mL of the dish soap solution and pour it evenly across the entirety of the "dish soap 2" plot. Measure out another 50 mL of the dish soap solution and pour it evenly across the "dish soap 3" plot. Repeat this using the car soap and the plain tap water in their respective designated plots.
39. Wait 1 day to let the soil absorb the liquids.
40. Take 27 medium, plastic bags and label them the same way as the first set, except DO NOT include PC on the labels (these bags are for your soil samples after the car soap, dish soap, and water are poured into the soil.)
41. Do steps 41- 44 in the same time period and on the same day. Using a metal soil core extractor, the hammer, and the second set of 27 bags, extract a 15 cm deep and 2 c.m. in diameter soil core in the plot marked by

the "dish soap 1". Collect your soil sample in the correspondingly labeled plastic bag, it should be the bag that says "dish soap 1.1".

42. Repeat this process two more times in the same "dish soap 1" plot, varying the placement of the soil sample and collecting the soil in the corresponding plastic bag.
43. Repeat steps 41-42 in the remaining 2 dish soap plots (“dish soap 2” and “dish soap 3”) and putting the samples in the correspondingly labeled bags.
44. Repeat steps 41-43 in the plot labeled “car soap” and “nc” plots and collect the soil samples in the correspondingly labeled plastic bags.
45. On Day 3 after collecting the soil samples from steps 41-44 repeat steps 16-32 for all the (trial #).1 samples.
46. On Day 6 after collecting the soil samples from step 41-42 repeat steps 16-32 for all (trial #).2 samples.
47. On Day 9 after collecting the soil samples form steps 41-42 repeat steps 16-32 for all (trial #).3 samples.

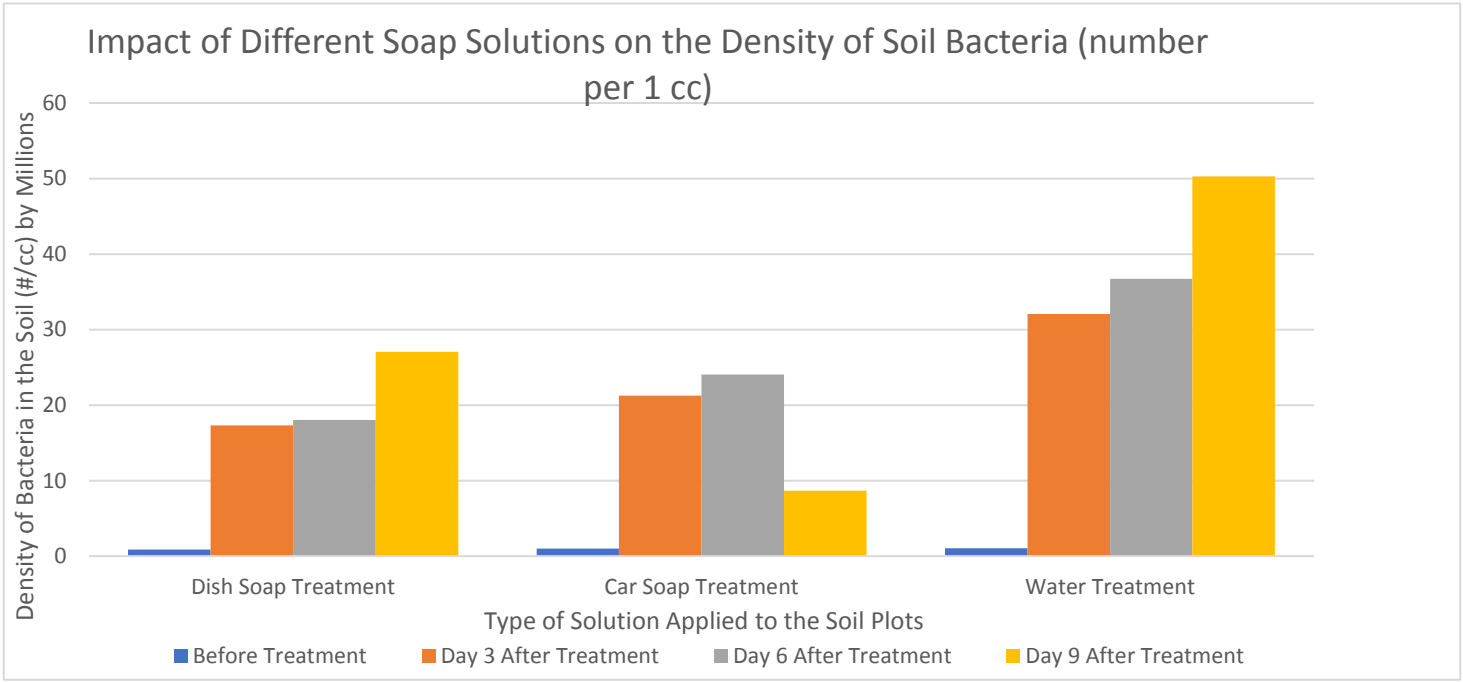
Data

a. Data Table

Impact of Different Soap Solutions on the Density of Soil Bacteria (number per 1 cc)

	Dish Soap Treatment				Car Soap Treatment				Water Treatment			
Trial	Before Treatment	Day 3 After Treatment	Day 6 After Treatment	Day 9 After Treatment	Before Treatment	Day 3 After Treatment	Day 6 After Treatment	Day 9 After Treatment	Before Treatment	Day 3 After Treatment	Day 6 After Treatment	Day 9 After Treatment
Plot 1	600,000	18,000,000	21,600,000	26,400,000	1,200,000	13,300,000	23,400,000	9,500,000	1,100,000	23,300,000	36,600,000	41,200,000
Plot 2	280,000	15,400,000	16,000,000	25,200,000	1,100,000	32,400,000	25,600,000	11,800,000	1,000,000	35,400,000	36,000,000	43,000,000
Plot 3	1,700,000	18,500,000	16,500,000	29,600,000	700,000	18,000,000	23,100,000	4,700,000	1,000,000	37,500,000	37,600,000	66,600,000
Average	860,000	17,300,000	18,033,333.3	27,066,666.6	1,000,000	21,233,333.3	24,033,333.3	8,666,666.6	1,033,333.3	32,066,666.6	36,733,333.3	50,266,666.6

b. Graph



Conclusion

The general trend of our data shows that dish soap decreases the density of bacteria in the soil to a greater extent than car soap. In the hypothesis, it was predicted that the dish soap would decrease the density of bacteria in the soil more than the car shampoo. Therefore, the majority of the evidence collected proves our hypothesis correct. The evidence states that the amount of bacteria in 1 cc of soil in the negative control plots, or the water treatment area, on average was 1,033,333.3 particles of bacteria. This was before the water was poured in the plots. Three days after the water was absorbed by the soil, the amount of bacteria particles in 1 cc of soil increased to 32,066,666.6. Six days after the water was absorbed by the soil, the amount of bacteria particles in 1 cc of soil increased to 36,733,333.3. The final data from the positive control was collected nine days after the water was absorbed by the soil; the amount of bacteria particles in 1 cc of soil had increased to 50,266,666.6. This demonstrates that the bacteria population increased as the amount of time increased that followed the water treatment. Bacteria thrive in wet environments because bacteria use water to dissolve food for its energy and growth (Fraser, n.d.). More water in the soil accommodates the bacteria's ability to consume food, and with more food, the bacteria will thrive in their environment because they can reproduce faster. The conditions that the soil was in were wet and hot as well, conditions that are ideal for the swift reproduction of bacteria. A rise in temperature typically increases the enzyme activity within bacteria ("Physical Factors," n.d.), and enzyme function is essential in the reproduction of bacteria.

The evidence states that, within the dish soap plots, before the Palmolive Ultra Antibacterial Dish Soap was poured in the plots, the amount of bacteria in 1 cc of soil was, on average, 860,000 particles of bacteria. Three days after the dish soap was poured into the ground and absorbed by the soil, the amount of bacteria particles in 1 cc of soil increased to 17,300,000.

Six days after the dish soap was absorbed by the soil, the amount of bacteria particles in 1 cc of soil increased to 17,300,000. Nine days after the dish soap was absorbed by the soil, the last dish soap data was collected, where the amount of bacteria particles in 1 cc of soil had increased to 27,066,666.67. This data proves that as more time passes after the dish soap is absorbed by the soil, the larger the bacteria's population becomes. This may be due to the fact that three days after the dish soap is poured into the soil, the density of the bacteria is at its lowest because the bacteria cannot defend itself against the substances in the dish soap. As time passes, the substances in the car soap degrade and the bacteria can recover and resist the toxic ingredients in the soap. By the ninth day since the dish soap was absorbed by the soil, the bacteria have gone through natural selection in which the successful phenotypes, those that have not been killed by the dish soap, live and reproduce, and the unsuccessful phenotypes die before having the chance to reproduce. The combination of natural selection and the gradual soap degradation may be why the amount of bacteria drastically increases on the ninth day following the dish soap's absorption by the soil.

The soil before treatment had the least amount of bacteria in it because after the dish soap was poured in the soil, there was an increase of rain and heat in the environment. The amount of rain and heat increased between the time that the positive control samples were collected and the times the dish soap plot samples were collected. Additionally, even after the positive control sample was taken, and before the solutions were deposited, the bacteria had time to reproduce. As said previously, bacteria thrive in a wet and hot environment, and are able to reproduce more quickly and efficiently.

The evidence from the soil samples taken before the Turtle Wax Ice Premium Car Care Wash and Wax car soap was poured on the car soap plots demonstrates that the amount of

bacteria in 1 cc of soil was, on average, 1,000,000 particles of bacteria. Three days after the car soap was absorbed by the soil, the amount of bacteria particles in 1 cc of soil increased to 21,233,333.3. Six days following the soil's absorption of the car soap, the amount of bacteria particles in 1 cc of soil increased to 24,033,333.3. This data also proves that as more time passes after the car soap is absorbed by the soil, the more the bacteria's population grows. Once again, this is due to the fact that three days after the dish soap is poured into the soil, the density of the bacteria is at its lowest because the bacteria does not know how to defend itself against the substances in the dish soap. As time passes, the substances in the car soap degrade, and the bacteria can recover and resist the toxic ingredients in the soap. This data also shows that the amount of bacteria in the soil before treatment is less than the amount of bacteria after the car soap is absorbed. This is because the environment was warmer and wetter prior to the time the samples were taken. Finally, when all the data is looked at together, it can be seen that the bacteria density in the car soap plots after the car soap was added is larger and recovers faster than the bacteria density in the dish soap plots after the dish soap was added. The reason why the dish soap killed more bacteria than the car soap is because it contains an antibiotic ingredient known as Triclosan, which is used to kill all bacteria, good and bad. Car soap does not contain this ingredient, and its main purpose is focused on removing dirt and bacteria rather than killing it.

The final set of car soap data was collected was nine days after the car soap solution was absorbed by the soil. The data shows that the amount of bacteria particles in 1 cc of soil decreased to 8,666,666.6. This piece of data did not fit the pattern of the rest of the data and created a new question; why did the amount of bacteria suddenly decrease? After much contemplation, a new problem arose. Do the fungi and protozoa density increase when the car

soap is added to the soil? This new problem was prompted from the idea that the fungi and protozoa continued, to a greater extent, to thrive in their environment when the car soap was added to the soil. Protozoa and fungi perform a type of cellular respiration called anaerobiosis in order to reproduce properly. This requires fungi to use a considerable amount of sulfate (Johnson, McConnell, Robinson, & Waire, 2016). The car soap used in this experiment contained sulfate. The more sulfate the fungi has, the faster it can reproduce. In an experiment called *The Influence of High Sulfate Levels on Soil Fungi Reproduction*, the organisms, fungi thrive when there is more sulfate in the soil. As time passes, the sulfate in the soil increases and the amount of fungi in the soil also increases (Johnson, McConnell, Robinson, & Waire, 2016). The amount of fungi increasing in the soil would decrease the amount of bacteria in the soil because fungi eat bacteria. Protozoa also eat bacteria as their primary food source which would decrease the bacteria population and increase the protozoa population (Ingham, n.d.). Protozoa thrive in damp environments and therefore reproduce more. Since there was heavy rain when the soil samples were taken there could may have been an increase in the population of protozoa in the soil. This means that there is more bacteria that are being eaten because there are more protozoa (Nardi, 2003, p. [53]). Due to this new research, a new hypothesis was formed that predicted that the fungi and protozoa density would increase when the car soap was added to soil. This hypothesis can be tested by pouring a certain amount of car soap into a plot of soil and measuring the protozoa and fungi levels in that soil at the same time as you measure bacteria levels.

From the evidence it can be concluded that the dish soap decreased the density of the bacteria in the soil to a greater extent than the car soap did, but both the car soap and the dish soap, when compared to the water, or negative control, decreased the density of the bacteria in

the soil significantly. To continue our research, we will measure the levels of bacteria, fungi, and protozoa when these soaps are added.

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