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Background

Bacteria are one of the smallest but most common life-forms on earth, and they play a significant role in every ecosystem on the planet. One particular group of heterotrophic bacteria break down and decompose dead plant and animal cells in the soil into organic compounds, and so play a significant role in the cycling of soil nutrients.Without these bacteria, other organisms in the soil including plants wouldn't be able to live because they would have no access to certain critical elements which they need for their metabolic activities.

One of the main ways bacteria cycle nutrients is the nitrogen cycle. Plants don’t have the ability to convert the nitrogen gas in the atmosphere to make amino acids and nucleotides; so soil bacteria are essential for plants because certain groups of them, called nitrogen-fixing bacteria, are capable of converting the nitrogen gas in the air into a form of nitrogen compounds called ammonium. This ammonium can be absorbed directly by certain types of plants in order to make their proteins and DNA. Or in the next step of the nitrogen cycle (called nitrification), this ammonium can be converted into nitrite and then into nitrate, which other types of plants can absorb through their roots to assimilate into their proteins and nucleic acids. A final group of bacteria then turn any excess nitrate back into□$_2$ and oxygen through the process of denitrification, and the cycle comes full circle. (Bram, 2010)

These fixed forms of nitrogen, though, are not only essential for plants. They are essential to all organisms because all living things need nitrogen for their proteins in order to cause the chemical reactions that allow all cells to perform the four main functions of living things: reproduction, homeostasis, synthesis and transformation of energy (Schreiner, 2015). Nitrogen is a vital element used to construct amino acids first in plants and then consumed by animals up the food chain. Hence, without bacteria cycling nitrogen, there are no amino acids (the building blocks of proteins) and no nucleotides (the building blocks of DNA and RNA) available to any of the organisms in the ecosystem, and they would all die.

However, many humans behaviors can create a lot of harmful conditions which might decrease the population density of bacteria in the soil and so have a negative impact on all the things that live there. For example, chemicals people dump on the ground might prevent them from reproducing and functioning properly, significantly affecting what the bacteria do in the soil and, thereby, everything in an ecosystem that depends on the bacteria.

One such action that might be harming bacteria in soil is when people wash their cars. People who wash their cars in the driveway allow the soap used to wash the car to flow into the nearby soil, and many people might even think that washing cars on the lawn will help the environment since the ground will act as a"natural filter”(Bellon, 2014).

However, soap is made up of hydrocarbons which bacteria decompose and consume in order to grow and reproduce (Brock,2015). Therefore, exposure to car soap may cause the bacteria in the soil to grow too much, and there might be an overabundance of bacteria in the soil when soap is poured onto it. (Ballantyne, 2007). Another common ingredient in soap is triclosan. It has been found that triclosan promotes the growth of bacteria(Zaikis,2014); so it too might lead to too many bacteria in the soil. But one ingredient in soap that would not promote bacteria growth is alcohol. Alcohol can dry out and dehydrate bacteria, unfolding their proteins and killing them (Gutierrez-Mazzotti,2015).Hence car wash soap might prevent bacterial growth instead of aiding it.

Because car wash soap might help or harm soil bacteria, we tested to see if it really hurts the soil and plants that badly. Our hypothesis was that the soap would increase the bacterial population in the soil; however if we become aware that this is a big issue and is making a huge impact on the soil negatively, we can inform and convince others to not use harmful soap on their cars that hurt the soil.

Lab Outline

I. Problem: How does car washing soap impact the population density of the bacteria in the soil?

II. Hypothesis: Car washing soap will increase the density of the bacteria in the soil.

III. Procedures:

a. Independent Variable: adding ICE premium car wash and wax soap® to the soil
b. Dependent Variable: amount of bacteria in one cubic centimeter in soil
c. Negative Control: adding only water to the soil
d. Positive Control: soil samples taken before treatments applied
e. Controlled Variables:
    i. concentration of soap
    ii. amount of soap solution
    iii. amount of water
    iv. amount of soil collected
    v. amount of soil tested
    vi. samples tested on the same day at the same time

vii. size of culture tubes

viii. coordinates of the original soil

ix. time soap left on soil

x. how we pour the soap on the soil

xi. amount of surface area of the soil covered by water and soap solution

xii. type of soap (Ice Premium Car Care Wash and Wax)

xiii. time water left on soil

xiv. time soap solution left on soil

xv. size of plots

xvi. distance between each plot

xvii. size/type of culture tubes

xviii. size/type of testing tubes

xix. size of micropipette with tips

xx. size of soil core sampler

xxi. size/type of nutrient agar plates

xxii. amount of liquid on the agar plates

xxiii. amount of liquid in the culture tubes

xxiv. which dilutions plated on the agar plates

xxv. degree to which dilutions were done

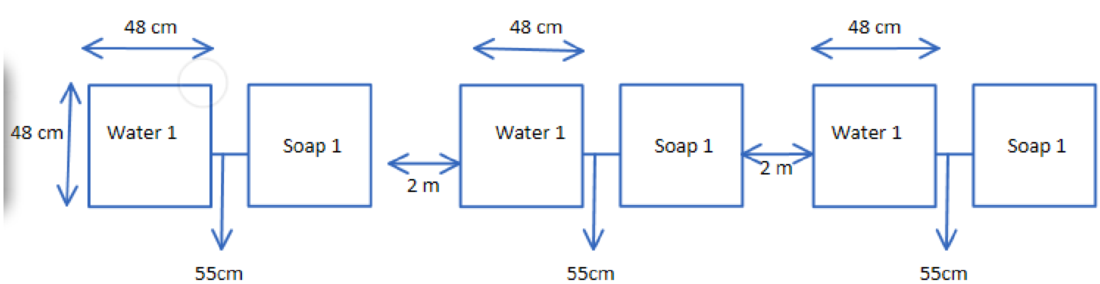
xxvi. amount of time solution let to grow on agar plates

f. Step-by-Step Instructions:

1. Label 4 stakes “water 1”.
2. Repeat step 1, 6 times, replacing the labeling each time with

- "soap 1", "water 1", "water 2", "soap 2", "water 3", "soap 3"
- each set should consist of four flags, and there should now be six sets of flags.

3. Label 18 plastic bags respectively with labels of "water1a", "water 1b", "water 1c", "soap1a", "soap1b", "soap1c", "water2a", "water2b", "water 2c", "soap2a", "soap2b", "soap2c", "water3a", "water 3b", "water 3c", "soap 3a", "soap 3b", and "soap 3c".
4. Take the stakes and bags outside to a grassy land position along Deepdene Road with the coordinates N 39.35671667 and W 76.63592167
5. Put the 4 stakes labeled "water 1" onto a flat grassy land area in a square formation with a width of 48 cm and length of 48 cm. The plots should be 55 cm from each other. (see diagram below)
6. repeat step 5 with "soap 1 flags" for one square
7. repeat step 5 with "water 2 flags" for a different square
8. repeat step 5 with "soap 2 flags" for a different square
9. repeat step 5 with "water 3 flags" for a different square
10. repeat step 5 with "soap 3 flags" for a different square



11. Collect every soil sample listed in step 3 on the same day at the same time. Using the soil core sampler with a width of 2 cm take 3 samples from each plot going into the ground 15 cm deep and put all 18 of the soil samples into their correspondingly labeled, separate plastic bags as follows:

Soil Sample	Bag Number
water 1, sample 1	water 1a
water 1, sample 2	water 1b
water 1, sample 3	water 1c
soap 1, sample 1	soap 1a
soap 1, sample 2	soap 1b
soap 1, sample 3	soap 1c
water 2, sample 1	water 2a
water 2, sample 2	water 2b
water 2, sample 3	water 2c
soap 2, sample 1	soap 2a
soap 2, sample 2	soap 2b
soap 2, sample 2	soap 2c
water 3, sample 1	water 3a
water 3, sample 2	water 3b
water 3, sample 2	water 3c
soap 3, sample 1	soap 3a
soap 3, sample 2	soap 3b
soap 3, sample 3	soap 3c

12. do steps 13-28 on the same day at the same time

13. Use a clean, new transfer pipette to add 10 ml sterile water to a 15 ml culture tube. Label the tube 10^{0} water 1."

14. Use the same pipette to add 9 ml sterile water to a second 15 ml culture tube. Label the tube 10^{-1} water 1".

15. Repeat step 14 to two additional 15 ml culture tubes, only label them "10^{-2} water 1" "10^{-3} water 1" respectively

16. Place 1 cc of your water 1 soil sample into the "10^{0} water 1" culture tube.

17. cap the tube and shake vigorously.

18. Using a new clean pipette, remove 1 ml of the soil/water mixture from a new tube labeled "10^{0} water 1" and place it in another tube labeled 10^{-1}water 1".

19. Cap the 10^{-1}water 1" tube and shake vigorously.

20. Using the same pipette in step 18, remove 1 ml of the soil/water mixture from the tube labeled10^{-1}water 1" and place into the tube labeled 10^{-2}water 1".

21. Cap and shake vigorously.

22. Using the same pipette in step 18, remove 1 ml of the soil/water mixture from the tube labeled 10^{-2}water 1" and place into the tube labeled 10^{-3} water 1".

23. cap the 10^{-3}water 1" tube and shake vigorously.

24. you should now have a total of 4 culture tubes.

25. Label 18 separate 3M Petrifilm □□ Aerobic Count agar plates as follows:

Culture Tube Label	Agar Plate Label
Water 1, 10^{-3}	Water 1, 10^{-3}
Water 1, 10^{-2}	Water 1, 10^{-2}
Soap 1, 10^{-3}	Soap 1, 10^{-3}
Soap 1, 10^{-2}	Soap 1, 10^{-2}
Water 2, 10^{-2}	Water 2, 10^{-2}
Water 2, 10^{-3}	Water 2, 10^{-3}
Soap 2, 10^{-2}	Soap 2, 10^{-2}
Soap 2, 10^{-3}	Soap 2, 10^{-3}
Water 3, 10^{-3}	Water 3, 10^{-3}
Water 3, 10^{-2}	Water 3, 10^{-2}
Soap 3, 10^{-3}	Soap 3, 10^{-3}
Soap 3, 10^{-2}	Soap 3, 10^{-2}

26. Plate 100 µl samples from the 10^{-2}water 1” and 10^{-3}water 1” on the 15 ml onto corresponding agar plates.
27. Repeat steps 14-26, five more times with all trials of all soil samples: soap 1, water 2, soap 2, water 3, and soap 3.
28. Allow all plates to grow for 48 to 72 hours.
29. Examine each of the plates for individual bacterial colonies and choose the plate with the fewest colonies (at least 5 or more) and the lowest dilution value to make your estimates of the number of bacteria in the original 1cc soil sample using the following formula:

Bacteria in 1 cc of soil = # Colonies on sheet x 10^2 x 10 ^|dilution # at which colonies were found|

30. Mix 10 mL of ice premium car care wash and wax soap® (car wash soap) with 1 liter of water.
31. Do step 30 two more times, so you have a total of three 1L soap-water solutions on the same day at the same time
32. On the same day at the same time pour one liter of the soap water mixture we made in step 31 on each of the soap plots evenly covering all areas. On the same day and same time when we pour the soap solution, pour 1 liter of distilled water on each of the water plots evenly covering all areas (water 1, water 2, water 3).
33. Wait 48 hours to let both solutions soak into the ground.
34. Repeat step 11 once the treatment with the water and soap solution is done.
35. Repeat steps 12-29 after treatment is done soil samples.
36. Record the data that you have gotten from the soap samples samples.

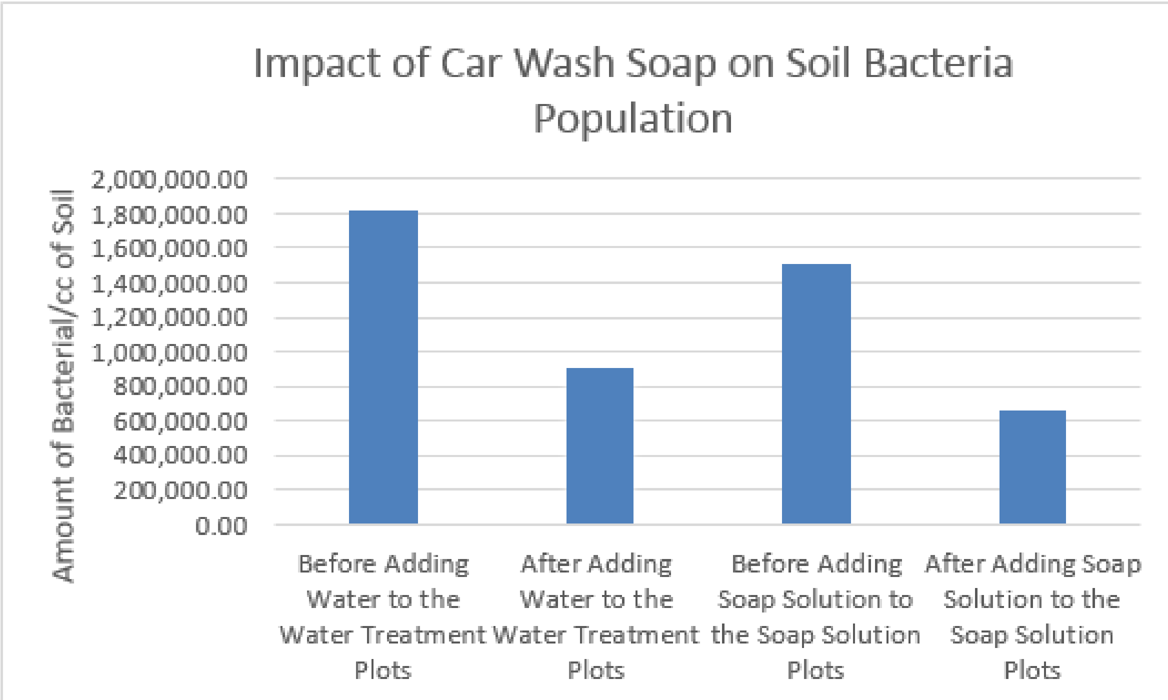
IV. Data and Analysis

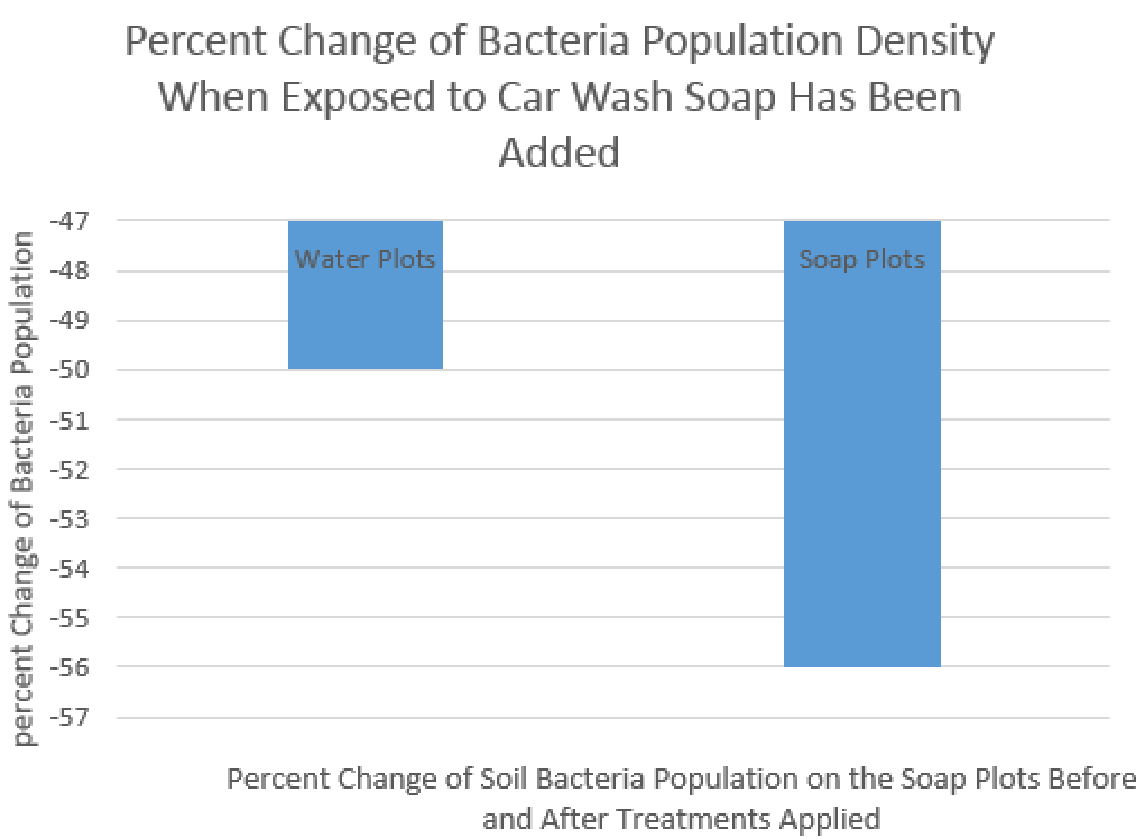
a. Data Table

Impact of Soap on Bacterial Population:

	Estimated Number of Bacteria/cc of soil in Water and Soap treatment plots			
	Before Soap Solution Is Present on treatment plots	After Soap Solution Is Present on treatment plots	Before Soap Solution Is Present on treatment plots	After Soap Solution Is Present on treatment plots
Trials	Water treatment plots	Water treatment plots	Soap treatment plots	Soap treatment plots
1	500,000	1,000,000	190,000	1,500,000
2	4,700,000	800,000	4,300,000	120,000
3	250,000	900,000	50,000	360,000
Average	1,816,666.667	900,000	1,513,333.333	660,000

b. Graph


Impact of Car Wash Soap on Soil Bacteria Population
Amount of Bacterial/cc of Soil
2,000,000.00
1,800,000.00
1,600,000.00
1,400,000.00
1,200,000.00
1,000,000.00
800,000.00
600,000.00
400,000.00
200,000.00
0.00
Before Adding Water to the Water Treatment Plots
After Adding Water to the Water Treatment Plots
Before Adding Soap Solution to the Soap Solution Plots
After Adding Soap Solution to the Soap Solution Plots


Percent Change of Bacteria Population Density When Exposed to Car Wash Soap Has Been Added
percent Change of Bacteria Population
-47
-48
-49
-50
-51
-52
-53
-54
-55
-56
-57
Water Plots
Soap Plots
Percent Change of Soil Bacteria Population on the Soap Plots Before and After Treatments Applied

c. Conclusion:

In conclusion our hypothesis, car washing soap will increase the density of the bacteria in the soil, was not supported. We discovered through our data that there was a decrease of 56% of the population of bacteria for the soap and a decrease of 50% for the water. Therefore the soap had a more negative impact on the bacteria in the soil. Even though there was an environmental shift causing the plots with water's bacteria population to decrease, we still see a larger decrease in the population of bacteria when soap was applied to the plots. We thought that the soap would have a positive impact on the bacteria by making it grow in population because of the glycerol, and triclosan in the soap. In our background research, we found bacteria feed off glycerol and turn the it into carbohydrates, and triclosan often increases the population of bacteria .

In the future we can experiment to see if the alcohol in the car wash soap actually did have a negative impact on the soil bacteria. We could test this by changing the independent variable of the experiment to an alcohol solution. We can also experiment to see if glycerol does in fact bring the population of soil bacteria up by changing the independent variable to glycerol but keep the rest of the procedure the same. We could have different locations of the soil plots to see if it wasn't just a shift in the bacteria that caused the population to fall.

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