Soil Ecology Project

The Effect Curbs have on the Density of Bacteria

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The Effect Runoff has on Plant Life due to Concrete Curbs

Soil bacteria are tiny, single-celled prokaryotic microbes that perform many tasks in an ecosystem, including aiding the movement of water, cycling nutrients, and preventing disease in plants. Though small, there can be as many as 100 million to a billion of them per gram of soil, and they produce special substances to bind soil particles into larger clumps, improving the infiltration of water and air into the soil. While there are many different kinds of bacteria living in the soil, a few groups play a particularly important role in the soil, the nitrogen- fixing bacteria (Ingham, Moldenke, Edwards, 2000).

Nitrogen Fixation, also known as the nitrogen cycle, is a process where bacteria in the soil first converts atmospheric nitrogen (N_2) into ammonium (NH_4^+), a more usable form which certain plants can absorb through their roots.This part of the cycle produces so much excess ammonium that the rest of it is converted into other biologically useful forms by specialized bacteria which turn it first into nitrite (NO_2^-) and then into nitrate (NO_3^-), the other useful form of nitrogen that plants can use.

Whether it is absorbing ammonium or nitrate, all plants need this source of nitrogen because without it, plants could not manufacture nucleic acids and proteins. Nitrogen is a critical element in these biological molecules, and without it, the DNA, RNA, and enzymes need for chemical reactions to happen could not be created. If chemical reactions do not occur, cells in an organism will not function, and since the cell is the building block of life, plants could not survive without nitrogen, nor could the other organisms that depend on plants. The nitrogen in proteins and nucleic acids passes the food chain as the primary consumers eat the plants and secondary consumers eat up the primary ones (Boundless.com, 2016). Hence, via the food chain, useable forms of nitrogen spread throughout an ecosystem among other organisms until it is released into the soil by the bacteria through decomposition, the other crucial ecological process which bacteria perform.It is also the other keyway in addition to nitrogen fixation that bacteria introduce ammonium in the soil. In decomposition, bacteria consume simple carbon compounds and break down waste and dead organisms in order to cycle the important nutrients from once living back into living organisms (Watkins, 1967). They do this by converting the organic matter into carbon dioxide which they release into the air in order for the producers to use this molecule for photosynthesis and into ammonium for the producers to use in order to create the chlorophyll they need to perform their photosynthesis (Kowalski, 2014).

Bacteria are so critical to the soil that anything that alters or harms the soil can harm them and therefore the rest of the ecosystem. One activity that humans do that can harm soil is non-source point pollution caused by runoff. Runoff, “the portion of precipitation on land that ultimately reaches streams often with dissolved or suspended material” (Webster, 2016), picks up every bit of pollution that humans have dumped or unknowingly dispensed onto streets, driveways, and sidewalks (such as trash, grease, car exhaust, car oil, chemical fertilizers, etc.) and washes these chemicals downhill into streams, rivers, and even woods. This contaminated water can sink into the soil as it flows downhill, introducing its foreign chemicals into the soil where they can harm the bacteria and everything in the environment that depends on them. (Rutledge, McDaniel, Boudreau, Ramroop, Teng, Sprout, Costa, Hall, Hunt, 2011). When soil becomes polluted, it damages the rate of plant growth by harming the soil bacteria in which the plants are dependent. This means that there are less plants for animals to eat and thus unbalancing the ecosystem. Without healthy soil, farmland is threatened, threatening humanity’s food source, and the problem is only growing worse as more and more areas that produce a high amount of runoff are being built, such as parking lots and large buildings.

One proven way of keeping plant life somewhat safe is due to the use of concrete curbs. Concrete curbs can be defined as something that restrains; an enclosed border or edging; a raised edge or margin; a wall; something to strengthen, etc. Curbs have been used for centuries and have been effective with keeping excess water from sinking into the soil as well as borders between places where one can travel and where they cannot. Runoff that could potentially damage the soil is collected in a group by the curbing, as it guides this contaminated water to a local drain. This process reduces the amount of runoff available to the plant life and therefore the amount of non-source pollutants entering the soil. The lack of potential curbing in areas where plants grow and soil pertains to be healthy may be one reason as to which runoff is affecting and damaging our ecosystem (Concrete Parking, 2011).

In our project, we are extracting soil from three different locations; a patch of land where runoff from asphalt driveways does not reach the vegetation, an area where plant life could potentially be harmed but has a concrete curb for protection, and a region of land that is crucially affected by the runoff and lacks curbing for a defense system. The importance of testing and comparing the amount of bacteria in soil samples with and without a curb is crucial to determine whether runoff is affecting the bacteria in order for plant life to grow and continue its health. If this is the case, our proposal states that concrete curbs may be a useful method of maintaining healthy soil and vegetation by reducing the amount of runoff that has potential access to the soil on the RPCS campus.

Soil Ecology Lab Report

I: Problem: How does the presence of a curb increase or decrease the density of bacteria in the soil on the RPCS campus?

II. Hypothesis: A curb will increase the density of bacteria in the soil located near it on the RPCS campus.

III: Procedure:

A. Independent Variable: Whether a curb is present or not in an area affected by runoff.

B. Dependent Variable: # of bacteria per cm^3 of soil.

C. Negative Control: Soil samples taken from an area on campus that is not affected by runoff and is covered with grass and plant growth.

D. Controlled Variables:

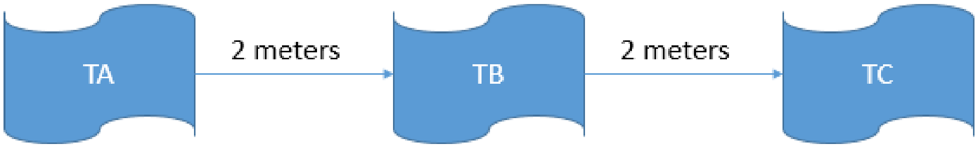
1. Amount of soil taken.
2. Taking soil sample on same day and time.
3. Type of plant life.
4. Amount of plant life.
5. Size of curb.
6. Type of pipettes used.
7. Type of water used.
8. Amount of water used.
9. Amount of soil added to water.
10. Amount of dilutions made.
11. Amount plated.
12. Amount of time allowed for bacteria to grow.
13. Dilutions used to plate on petri dish.
14. Type of sterile tip used.
15. Day of dilution.
16. Day of plating bacteria.
17. Temperature of room.
18. Light exposure in room.
19. How plates are stored.
20. Size of tubes used.
21. How much water is added to tubes.
22. How much dirt is added to tubes.
23. Type of nutrient agar

E. Step by Step Instructions:

1. Label fifteen flags with the markings:
    - TA, TB, TC.
    - BA1, BA2, BB1, BB2, BC1, BC2.
    - NA1, NA2, NB1, NB2, NC1, NC2.

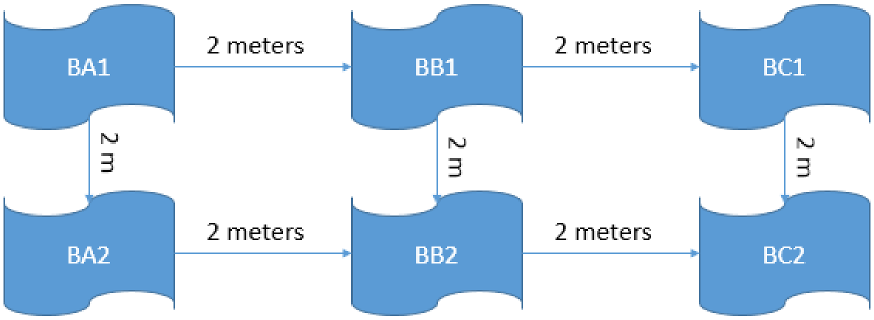
   Each flag should only have one marking on it. For example 1 flag for TA, one flag for TB, one flag for TC, etc.
2. Choose an area of land on campus that is not affected by runoff.
3. Place the flag TA at a spot of the land. (GPS: N39°21.503 W076°38.131)
4. Place the flag TB 2 meters to the left of the flag TA. (GPS: N39°21.503 W076°38.128) See Diagram 1.
5. Place the flag TC 2 meters to the left of the flag TB. (GPS: N39°21.501 W076°38.131) See Diagram 1.

Diagram 1:



6. Choose an area of land on campus that is affected by runoff, but has a curb near it.
7. Place the flags: BA1, BA2, BB1, BB2, BC1, and BC2, in a 3 by 2 grid, each flag 2 meters apart from each other. See Diagram 2.

Diagram 2:



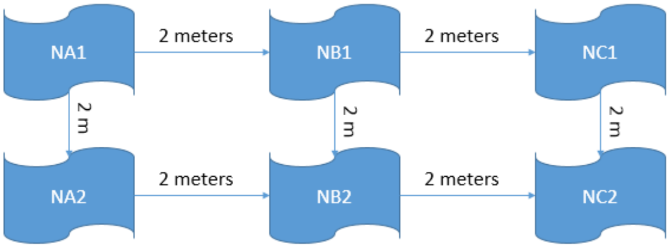
10. Find and write down the coordinates for each flag. BA1, BA2, BB1, BB2, BC1, and BC2.

Coordinates:
BA1 (N39°21.403 W076°38.132)
BA2 (N39°21.402 W076°38.132)
BB1 (N39°21.401 W076°38.132)
BB2 (N39°21.400 W076°38.127)
BC1 (N39°21.401 W07638.192)
BC2 (N39°21.400 W076°38.127)

11. Choose an area of land on campus that is affected by runoff, but has a no curb near it.

12. Place each flag: NA1, NA2, NB1, NB2, NC1, and NC2, in a 3 by 2 grid, each flag 2 meters apart from each other. See Diagram 3.

Diagram 3:



13. Find and write down the coordinates for each flag. NA1, NA2, NB1, NB2, NC1, and NC2.

Coordinates:
NA1 (N39°21.429 W076°38.192)
NA2 (N39°21.427 W076°38.193)
NB1 (N39°21.428 W076°38.191)
NB2 (N39°21.427 W076°38.194)
NC1 (N39°21.427 W076°38.195)
NC2 (N39°21,427 W076°38,196)

15. Label 15 plastic bags to correspond to the labels the each of the flags.

16. Keep in mind that you must obtain all the soil from steps 17- 20 at roughly the same time on the same day.

17. Use a soil extractor and obtain soil from each flag plot that is a 13.5 cm tall and 2.5 cm diameter chunk of soil.

18. Take the soil extractor and place it directly next to one of the flags.

19. Extract soil from each flag.

20. Place soil in labeled bag that matches flag.

21. The next steps 22-36 should be completed on the same day at the same time.

22. Label five culture tubes with the same flag labels. There should be 5 culture tubes for each flag.

23. Use a clean, new transfer pipette to add 10 ml of sterile water to a 15 ml culture tube. Label the tube “10^{0}”and TA1. Make sure this also has a label for its flag.

24. Use the same pipette to add 9 ml of sterile water to a second 15 ml culture tube. Label the tube “10^{-1}” and TA1. “”

25. Repeat step 2 three more times to three additional 15 ml culture tubes, only label them “10^{-2}”, “10^{-3}” and “10^{-4}” respectively. All labeled TA1 as well.

26. Place 1 cc of your soil sample into the “10^{0}” TA1 culture tube.

27. Cap the tube a shake vigorously.

28. Using the same pipette as in step 4, remove 1 ml of the soil/water mixture from the TA1 “$10^{0”}$ tube and place it into the “10^{-1}” tube.

29. Cap and shake vigorously.

30. Using the same pipette as in step 4, remove 1 ml of the soil/water mixture from the “10^{-1}” TA1 tube and place it into the “10^{-2}” TA1 tube.

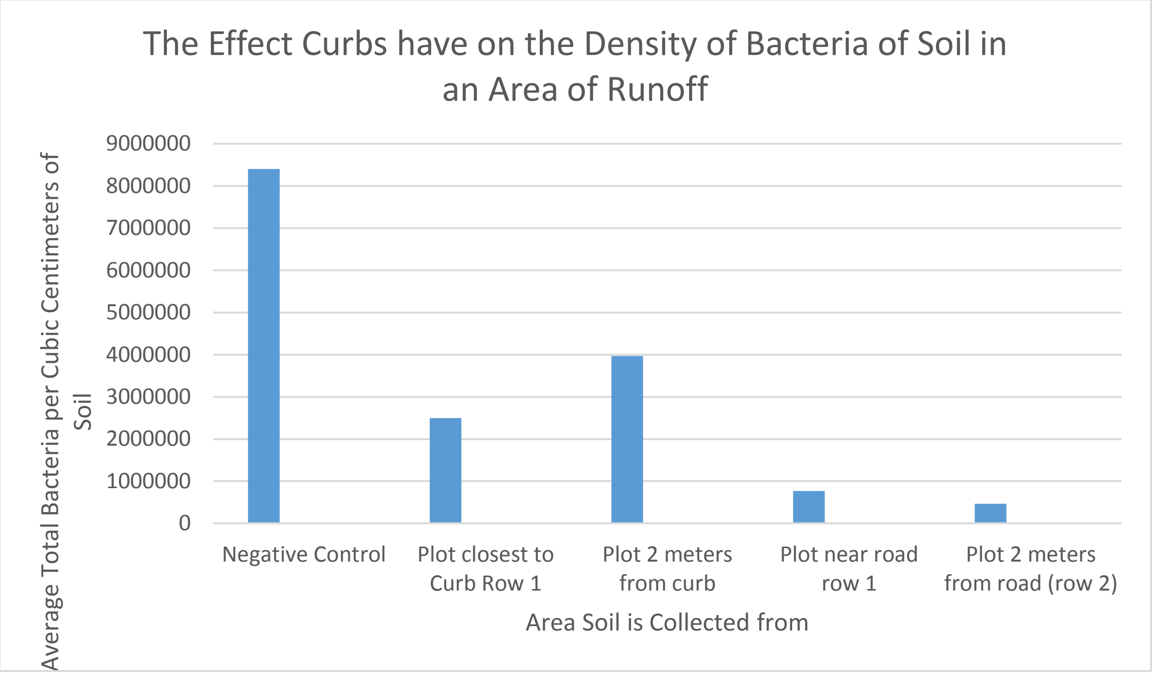
31. Cap and shake vigorously.

32. Using the same pipette as in step 4, remove 1 ml of the soil/water mixture from the TA1 “$10^{-2”}$tube and place it into the TA1 “10^{-3}” tube.

33. Cap and shake vigorously.
34. Using the same pipette as in step 4, remove 1 ml of the soil/water mixture from the TA1 “10^{-3}” tube and place it into the TA1 “10^{-4}” tube.
35. You should now have a total of five culture tubes.
36. Plate 100µl samples from the 4th and 5th dilution tubes (dilutions 10^{-3} and 10^{-4}) onto their own separate, labeled petri plates containing nutrient agar
37. Allow to grow for 48 to 72 hours.
38. Complete steps 21-38 14 more times with all of the plots of soil. For example complete this again, but this time with the TA2 soil and labeling the tubes with TA2 instead of TA1.
39. Examine each of the plates for individual bacterial colonies and choose the plate with the fewest colonies (but at least 5, this is usually the 5th dilution, but in some instances the 4th) to make your estimates of the number of bacteria in the original 1cc soil sample using the following formula:
40. # Microbes in 1 cc soil = # colonies on sheet x 10^{2} x $10^{|\text{dilution \# at which these colonies were found}|}$
41. Complete this test with a soil sample from each flag.
42. Observe and record data.

The impact of the Presence or Absence of a Curb on the Density of Bacteria in the Soil

SOIL SAMPLE AREA	**TOTAL BACTERIA PER CUBIC CENTIMETER**
Negative Control A	25,000,000
Negative Control B	150,000
Negative Control C	60,000
Plot Closest to Curb row 1 column A	6,000,000
Plot Closest to Curb row 1 column B	800,000
Plot closest to curb row 1 column C	700,000
Plot 2 meters from curb (row 2 column A)	700,000
Plot 2 meters from Curb (row 2 column B)	1,200,000
Plot 2 meters from curbs (row 2 column C)	10,000,000
Plot near road row 1 column A	1,200,000
Plot near road row 1 column B	800,000
Plot near road row 1 column C	300,000
Plot 2 meters from road (row 2 column A)	400,000
Plot 2 meters from road (row 2 column B)	500,000
Plot 2 meters from road (row 2 column C)	500,000
Average	
Negative Control	8,403,333.3333
Plot closest to curb row 1	2,500,000
Plot 2 meters from curb (row 2)	3,966,666.67
Plot closest to road row 1	766,666.67
Plot 2 meters from road (row 2)	466,666.67



Conclusion

In conclusion, our hypothesis was proven correct, stating that a curb will increase the density of bacteria in soil located near it on the RPCS campus. The plots that were located closest to the curb had the highest density of bacteria. The plots closest to the curb on average had 2,500,000 bacteria per cubic inch, and the plots 2 meters from curb had on average 3,966,666.67 bacteria per cubic inch. While the plots near the road and not a curb had a much smaller amount of bacteria, the ones closest to the road contained an average of 766,666.67 bacteria per cubic centimeter, and the plots 2 meters from the road have an average of 466,666.67 bacteria per cubic centimeter. The fact that our data shows a smaller accumulation of bacteria in the area without a curb affected by runoff, proves that curbs have a lasting effect on the soil quality and the bacteria lifespan in a certain area of land. What really sets the data apart is the obvious differences in the amount of bacteria growth between each plot of soil. First is the difference between the top plots of soil 2 meters away from the curb and the plots of soil that are next to the curb that is a barrier between the immediate soil and the runoff. This data can easily prove that runoff does indeed affect that amount of bacteria growth. The difference between the plot of land not affected by runoff and the plot of land closer to the curb was 5903333.3333 total bacteria per cubic centimeter. This meaning that the plot of land that was not affected by runoff had around 5903333.3333 more total bacteria per cubic centimeter then the plot of land closest to the curb. The difference between the plot 2 meters away from the curb and the plot right near the curb is 1466666.67 total bacteria per cubic centimeter, meaning that there were 1466666.67 more bacteria per cubic centimeter in the plot further away from the curb. These two differences prove that runoff actively decreases the amount of bacteria in a certain plot of soil. The next two differences prove that a curb increases the amount of bacteria in a certain plot of soil and that our hypothesis was correct. The difference between the plot of soil closest to the curb and closest to the road is 1,733,333.33 total bacteria per cubic centimeter. This means that there are 1,733,333.33 more bacteria per cubic centimeter on the plot of soil closer to the curb then the plot of soil closest to the road. Keep in mind that the plot of soil closest to the road has no curb by it. The difference between the soil closest to the road and the plot of soil two meters from the road is 300,000 total bacteria per cubic centimeter. These points of difference prove that not only does runoff decrease the amount of bacteria per cubic centimeter on a plot point, but it also proves that a curb increases the amount of bacteria per cubic centimeter in a plot of soil. These two points mean that curbs are extremely useful in keeping the bacteria in soil alive and keeping the soil healthy.

Bacteria needs to be in the soil in order for it to be healthy. Bacteria is a big part in the nitrogen cycle. It converts nitrogen into a more usable form, the nitrogen is then able to be used by plants so they can create nucleic acids and proteins. Nitrogen also is an important element in biological molecules and if not present, DNA, RNA, or enzymes would not be created and chemical reactions would not take place, if chemical reactions do not take place cells could not be created which is needed for an organism to function. This whole process creates a more efficient ecosystem which leads to a healthier environment. As concrete curbing is not only useful for driving, is also acts as a barrier to runoff and anything else that would travel downhill on the RPCS campus. The limited amount of runoff from the streets that has access to the soil in order for plant life to maintain its health is proven to be a product of having concrete curbing. In order for the rest of the vegetation on the campus to maintain its health, our proposal concludes that curbs should be added to block out the toxins and runoff that could potentially harm the soil.

Citations

Conserve Energy Future (2009) Conserve Energy Future http://www.conserve-energy-future.com/causes-and-effects-of-soil-pollution.php

Ingham, R, Moldenke, R, Edwards, A, (2000) Soil Biology Conservation Society.

Kalkaska Country Community Center (2003) "Surface Water - Threats." <http://www.kalkaskacounty.net/planningeduc0052.asp>.

Lal, R (2001) " *Land Degradation: An Overview*. Natural Resources Conservation Service. <http://www.nrcs.usda.gov/wps/portal/nrcs/detail/soils/use/?cid=nrcs142p2_054028>.

McCarthy (2016) The Sources and Solutions: Agriculture. Environmental Protection Agency <https://www.epa.gov/nutrientpollution/sources-and-solutions-agriculture>.

Moisset, B (2011) Native Plants and Wildlife Gardens

http://nativeplantwildlifegarden.com/how-chemicals-affect-your-soil/

National Geographic Society (2016) National Geographic Society

http://nationalgeographic.org/encyclopedia/air-pollution/

National Ready Mixed Concrete Association (2011) National Ready Mixed Concrete Association

http://concreteparking.org/index.html

Net Industries (2016) Net Industries

http://science.jrank.org/pages/1967/Decomposition.html

Perlman, H (2016) The USGS Water Science School

http://water.usgs.gov/edu/runoff.html

The Shodor Education Foundation, Inc. (1998) The Shodor Education Foundation, Inc.

https://www.shodor.org/master/environmental/water/runoff/RunoffApplication.html