Soil Ecology Project

The Impact of Motor Oil and Car Soap on Soil Protozoa Density

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Background

There are a lot of microorganisms in the soil which play key roles in the environment. They help produce oxygen for animals to breathe, decompose dead organic matter, and produce nutrients that plants need to thrive. They also help regulate the Earth's greenhouse gasses and temperature and provide nutrients for all higher order organisms such as producers and primary and secondary consumers. Therefore, soil microbes make it possible for all life to grow and survive (Glasener, 2013), and without them, the entire terrestrial ecosystem would fail (Noel, n.d).

One critical type of soil microbe are the protozoans. Though some cause diseases, most are very beneficial to an ecosystem (Brinch, Ekelund, and Jacobsen, 2006). They are important because certain protozoans help the fertility of soils by grazing on bacteria and thereby regulating the populations of them. This grazing improves and helps the rate at which the bacteria decompose dead organic matter (Protozoan, 2013) because it increases bacterial reproduction rates, and younger bacteria endure the heat created by the decomposition process better. In addition, the process of decomposition lowers the soil pH level and the younger bacteria can endure this change better than older bacteria. Lastly, when younger, bacteria have more energy to produce the enzymes needed to initiate the chemical reactions needed to decompose dead material in the first place (Bottle Biology, n.d). Hence, by eating the bacteria, protozoa actually aid the decomposition process in the soil.

Furthermore, the energy that the bacteria obtain by consuming the dead organic matter transfers to the protozoans once the bacteria are eaten. The protozoans then use this energy for their own metabolic needs and, in the process, excrete nitrogen and phosphorus as waste products in the form of ammonium and orthophosphate (Phosphorus, 2013).This nitrogen and phosphate can in turn be absorbed by plants through their roots, reintroducing these critical elements back into the food chain.

The reason nitrogen and phosphorus are so critical is because they are beneficial to the development of the roots, leaves, and general foliage of the plant, creating food and energy for higher order consumers who eat the producers and thereby creating a healthy environment (Nitrogen, 2013). In particular, plants use nitrogen and phosphorus to produce nucleic acids and proteins (Phosphorus, 2013), which all organisms need in order to keep their cells alive. Cells die without nucleic acids and the proteins the DNA and RNA create because enzymes control the chemical reactions which result in the four tasks which all cells (and the organisms that are made out of them) must perform to live. Thus, when higher order organisms eat producers who have absorbed nitrogen from the soil, they consume the needed proteins and nucleic acids the plants have produced, allowing their cells to perform the four tasks, allowing them to live. Thus, protozoans are a vital part of the ecosystem because they help produce the nitrogen and phosphorus the entire rest of the ecosystem needs in order to survive (Troxler, n.d).

Since these microbes are so important to the environment, anything that threatens their survival threatens the environment as a whole. One threat to these critical organisms is nonpoint source pollution (ie. water pollution which does not have a single specific origin). It can be caused by snow melting, falling rain, or any moving water that creates a flow across a hardened surface. As this liquid flows, it picks up both natural and human pollutants, spreading them around and contaminating the surrounding environment. These contaminants include substances such as road salt, fertilizers, and exhaust from cars, but one of the worst is motor oil. When motor oil leaks from vehicles and gets on roads and pavements, rain and other water sources can wash it into the soil or even into bigger bodies of water (EPA, 2013), and it takes as little as one court of motor oil to pollute nearly 250,000 gallons of water. Since American cars leak almost 180 million gallons of motor oil each year (Peirce, 2013), motor oil as a nonpoint source pollution has consequently become a major concern for the environment.

This large amount of motor oil that reaches the soil is toxic to nearly all the organisms living there because most types of motor oil contain lead. Lead decreases the soil pH level (Greene, 1993), and this drop in pH is especially problematic because it affects the enzymes that make life possible. Enzymes (as well as other proteins) are held together by positive and negative charges, and when the pH level of an environment changes, it adds and subtracts charges to an enzyme. This changes the charges that hold the enzyme together, causing it to lose its structure and change shape. But when an enzyme loses its precise shape, it becomes useless because its shape is vital to an enzyme's job of controlling chemical reactions. Therefore, when an enzyme changes shape, the chemical reaction that it makes happen changes too. So a drop in the soil pH disrupts the enzymes of the microbes and other organisms living there, and they die. Since microbes support plant life, which supports the whole ecosystem, the drop in pH causes the entire ecosystem to suffer (Zimdahl and Skogerboe, 1997). Yet if the lead in the motor oil were not harmful enough, motor oil also contains excess zinc, which interferes with the plants ability to absorb nutrients from the microbes in the first place. Here, again, no nutrients for plants causes the entire ecosystem to suffer (Northridge, 2010).

One other common nonpoint source pollution associated with cars is the soap used when washing them. This soap runs off into storm drains and soil along the way, and most car soaps also contain chemicals that harm environments and organisms when concentrated enough. A typical car soap has an average pH level of 8 while the normal pH level of soil is between 5 and

7 (Peirce, 2013). Hence, when adding the soap to the soil, it makes the pH levels in the soil rise, which can cause the same consequences for soil microbes described earlier.

However, while both motor oil and car soap can harm the environment, it is unclear which actually harms the environment more. Specifically, it is unclear which substance affects the population of protozoan more. Thus, in order to determine whether car soap or motor oil have more of a negative effect on the density of the population of protozoans, we added a proportional amount of motor oil to a specific size sample of soil, an proportional amount of car soap to a specific size sample of soil, and proportional amount of water to a specific size sample of soil. We predict that motor oil will have a greater negative impact than car soap on the density of soil protozoa.

Experiment

I. Problem: Which has a more negative impact on the density of soil protozoa, motor oil or car soap run-off?

II. Hypothesis: Motor oil will reduce the density of soil protozoa more than car soap run-off.

III. Procedure:
   A. Independent variable: type of substance added to the soil (motor oil vs. car soap)
   B. Dependent variable: density of protozoa in the soil
   C. Negative control: Adding only water to the soil
   D. Controlled variables: plant life surrounding soil (monoculture – only grass), collecting all soil on the same day at the same time, location of each trial, distance from location of flag to the spot where soil was collected, device used to collect the soil, amount of soil collected, device used to put soil in (plastic bags), device used to get location of flags (GPS), amount of substance added to each petri dish, amount of time soil sits in plastic bags, amount of time allowed for substances to interact with the soil and protozoa, amount of soil sifted into new petri dishes, amount of time allowed for soil to sit after saturated with water, amount of water used to saturate soil, time allowed for soil to filter, time allowed for second filtration of soil, amount of water added to the bottom of the new petri dishes, materials used/ way of making

uhlig extractor, type of dye added to the microscope slides, amount of dye added to the microscope slides, amount of filtrate added, equation used to find the number of protozoa per gram of soil, size of coverslip, magnification used to examined protozoa, number of fields of view counted

E. Step by step procedure:

1. One day, on the front lawn, mark a flag with "first trial" and put it at N 39 ^{0} 21.480 W076 ^{0} 38.177, a flag with "second trial" and put it at N 39 ^{0} 21.496 W076 ^{0} 38.185 location, and a "third trial" and put it at N 39 ^{0} 21.497 W076 ^{0} 38.162
2. Label 9 plastic bags, three with the number "1", three with "2", and three with "3".
3. Do the following steps, #4-6, at the same time on the same day
4. At the flag marked "first trial", collect a sample of soil 15 centimeters deep and 2 centimeters in diameter directly to the north of the flag and 7 centimeters away from the flag, a sample of soil 15 centimeters deep and 2 centimeters in diameter directly west of the flag and 7 centimeters away from the flag, and a sample of soil 15 centimeters deep and 2 centimeters in diameter directly east of the flag and 7 centimeters from the flag. Put each measurement you collected into its own plastic bag labeled "Trial 1".
5. At the flag marked "second trial", collect a sample of soil 15 centimeters deep and 2 centimeters in diameter directly to the north of the flag and 7 centimeters away from the flag, a sample of soil 15 centimeters deep and 2 centimeters in diameter directly west of the flag and 7 centimeters away from the flag, and a sample of soil 15 centimeters deep and 2 centimeters in diameter directly east of the flag and 7 centimeters from the flag. Put each measurement you collected into its own plastic bag labeled "Trial 2".
6. At the flag marked "third trial", collect a sample of soil 15 centimeters deep and 2 centimeters in diameter directly to the north of the flag and 7 centimeters away from the flag, a sample of soil 15 centimeters deep and 2 centimeters in diameter directly west of the flag and 7 centimeters away from the flag, and a sample of soil 15 centimeters deep and 2 centimeters in diameter directly east of the flag and 7 centimeters from the flag. Put each measurement you collected into its own plastic bag labeled "Trial 3".
7. On the same day, once back inside, use a pen to label one petri dish "water 1", one "motor oil 1", and one "soap 1", one petri dish "water 2", one "motor oil 2", one "soap 2", and one "water 3", one "motor oil 3", and one "soap 3".
8. Do the following steps, #9-11 at the same time, on the same day
9. Use each measurement of 15 centimeters from the "first trial" spot (the three plastic bags with 15 cm of soil in them, labeled #1) and put one plastic bag's soil

in a petri dish labeled, “water 1”, a different plastic bag’s soil in a petri dish labeled “car soap 1”, and soil in the last plastic bag into a petri dish labeled “motor oil 1”

10. Use each measurement of 15 centimeters from the “second trial” spot (the three plastic bags with 15 cm of soil in them, labeled #2) and put one plastic bag’s soil in a petri dish labeled, “water 2”, a different plastic bag’s soil in a petri dish labeled “car soap 2”, and soil in the last plastic bag into a petri dish labeled “motor oil 2”
11. Use each measurement of 15 centimeters from the “third trial” spot (the three plastic bags with 15 cm of soil in them, labeled #3) and put one plastic bag’s soil in a petri dish labeled, “water 3”, a different plastic bag’s soil in a petri dish labeled “car soap 3”, and soil in the last plastic bag into a petri dish labeled “motor oil 3”
12. On the same day, after you have put all the soil in the petri dishes, do steps 13-22 at the same time.
13. Add 2 ml of tap water to the petri dish labeled “water 1”
14. Add 2 ml of Ice Premium Care car wash 2 in 1 (clean and shine) car soap to the petri dish labeled “soap 1”
15. Add 2 ml of SAE5W-30 motor oil to the petri dish labeled “motor oil 1”
16. Add 2 ml of tap water to the petri dish labeled “water 2”
17. Add 2 ml of Ice Premium Care car wash 2 in 1 (clean and shine) car soap to the petri dish labeled “soap 2”
18. Add 2 ml of SAE5W-30 motor oil to the petri dish labeled “motor 2”
19. Add 2 ml of tap water to the petri dish labeled “water 3”
20. Add 2 ml of Ice Premium Care car wash 2 in 1 (clean and shine) car soap to the petri dish labeled “soap 3”
21. Add 2 ml of SAE5W-30 motor oil to the petri dish labeled “motor oil 3”
22. Put a lid over each petri dish and put parafilm around each one, but leave a little crack for air
23. Let the petri dishes sit for 48 hours so that the substances can interact with the soil
24. Exactly 48 hours later, take the lid off of each petri dish
25. Let all soil samples sit for 48 hours in order to air dry
26. Get 9 small cups and label them respectively “water 1”, “soap 1”, “motor oil 1”, “water 2”, “soap 2”, “motor oil 2”, “water 3”, “soap 3”, “motor oil 3”
27. After 48 hours, on the same day at the same time, put each of the dried soils into its own mortar and pestle and grind up the soil
28. Then pour the soil from the mortar and pestle into the corresponding small cup that is labeled correctly for the correct soil.
29. Once each soil sample is in its own small cup, on the same day at the same time, cover it with a 1 mm^2 nylon mesh in order to sift 9-10 g into the 9 new clean petri

dishes labeled “water 1 new”, “soap 1 new”, “motor oil 1 new”, “water 2 new”, “soap 2 new”, “motor oil 2 new”, “water 3 new”, “soap 3 new”, “motor oil 3 new”. Make sure to match the added substance and trial number from the small cup with the added substance and trial number of the petri dishes, putting the correct sifted soil into the correct petri dish. Record the mass of the soil. After you finish using the small cups, wash them out so that you can use them for the second filter.

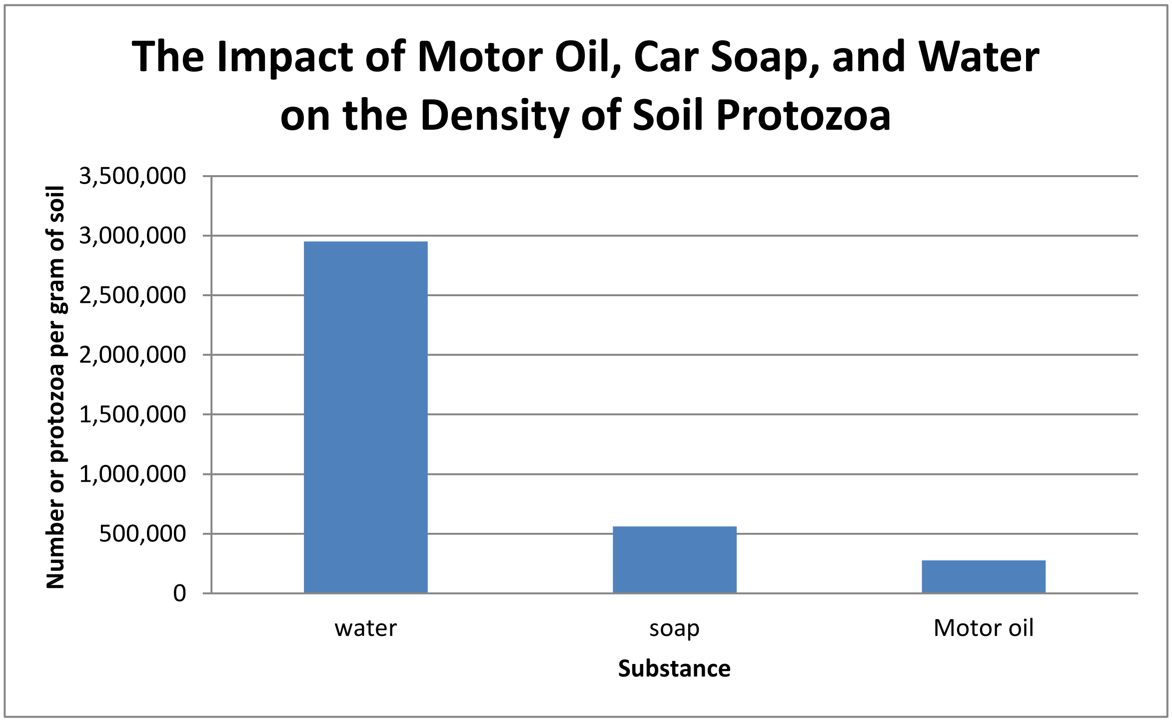
30. On the same day as step 29, saturate each sample of soil with 20 ml distilled water all at the same time and put the lid on each petri dish. Make sure to leave the petri dishes in a place where they will not get knocked around or moved.
31. Allow samples to sit for 7 hours at room temperature
32. After 7 hours, put each one in the refrigerator until ready to perform steps 35-39.
33. Make nine modified Uhlig ciliate sandy sediment separators out of plastic cups & 2 sheets of nylon bridal veil
34. Do the following steps, #35-38 on the same day at the same time
35. Add 30 ml of distilled water to the bottom of each of nine new 100x15 mm petri dishes, that are labeled respectively “water 1”, “soap 1”, “motor oil 1”, “water 2”, “soap 2”, “motor oil 2”, “water 3”, “soap 3”, “motor oil 3”
36. At the same time, place each Uhlig extractor into a petri dish
37. Scoop each rehydrated soil sample into its corresponding extractor
38. If the water does not touch the uncut part of the Uhlig extractor, then add more water until it does, and record the extra amount of water that you added to each petri dish.
39. Allow all soil samples to filter for 24 hours at room temp.
40. After 24 hours, put a lid over each petri dish and put it in the refrigerator until ready to perform steps 42-47
41. Do steps 42-47 on the same day at the same time
42. Filter each of the soil samples a second time using qualitative filter paper
43. On the same day and all at the same time, label 9 microscope slides, one with “water 1”, one with “motor oil 1”, one with “soap 1”, one with “water 2”, one with “motor oil 2”, and one with “soap 2”, one with “water 3”, one with “motor oil 3”, one with “soap 3” in order to prepare microscope slides for viewing from the second filtrate.
44. Using a capillary tube, add seven drops of methyl green dye to each of the nine microscope slide (1 ul = 1 drop from the tube)
45. Add 18 ul of each of the nine filtrates, “water 1”, “motor oil 1”, “soap 1”, “water 2”, “motor oil 2”, “soap 2”, “water 3”, “motor oil 3”, “soap 3” using a graduated Beral-type pipette (the first demarcation) to their respective microscope slides and cover each slide with an 18 x 18 mm^2 cover slip

46. You will be looking at the protozoa from five different field views, to find the number of protozoa per field view, and then use that information to do the following step. Use the microscope at view 60X to look at each field of view. You will take the average number from all five fields of view for each trial of each substance. Use this average in the equation in step 47.
47. In order to count the protozoa, use the equation : (# per field of view at 60X) • (total ml of water used) • 2165] ÷ (grams of sifted soil) = # of protozoa per gram of soil

Data and Analysis

The Impact of motor oil, car soap and water on the density of soil protozoa

Trials	Number of protozoa per gram of soil after		
	water	soap	Motor oil
1	1,002,507	517,272	323,307
2	6,909,768	607,932	300,821
3	945,079	562,900	207,748
Average	2,952,451	562,701	277,292



Conclusion

Our hypothesis is correct; motor oil did reduce the density of soil protozoa more than car soap runoff. We know this because the average density of protozoa per gram of soil after adding an appropriate amount of car soap for a specific amount of time is 562,701 while there is only an average of 277,292 protozoa per gram of soil after adding the motor oil with the same constraints. Therefore the motor oil decreases the density of protozoa in the soil more than car soap. We are almost 99% certain that the different substances in the soil are what affected the population of protozoa because the p value between the data collected about the density of protozoans exposed to motor oil verse the data collected about the density of protozoans exposed to car soap is 0.004. However, we know that both substances negatively affected the population density of protozoa in the soil because the average density of protozoa per gram of soil when tap water (the negative control) was added is 2,952,451, which is significantly greater than the average density of protozoa per gram of soil when either motor oil or car soap was added. We are almost 70% sure that the difference between the populations of protozoa exposed to motor oil and populations of protozoa exposed to tap water is due to the different substances being present because the p value between the data collected about the density of protozoans exposed to motor oil verse the data collected about the density of protozoan exposed to tap water is 0.309. We are almost 65% sure that the difference between the populations of protozoans exposed to car soap and the populations of protozoans exposed to water is due to the different substances being present because the p value between the data collected about the density of protozoans exposed to car soap verse the data collected about the density of protozoans exposed to tap water is 0.351.

Furthermore, protozoans are vital to the entire ecosystem because they produce nitrogen and phosphorus, helping plants thrive because they help the plants produce proteins and nucleic acids which are responsible for initiating chemical reactions. These chemical reactions then result in the 4 main tasks all cells (and therefore plants) need to survive, thereby creating the foundation food source for most ecosystems (producers). Therefore people must be more concerned about motor oil leaking and take more steps to prevent motor oil from entering the soil than car soaps because motor oil has a larger capacity to be detrimental to the protozoa populations. However, strides should also be taken to reduce the amount of car soaps entering the soil because they also have a significant negative impact on the density of protozoans.

It can then be concluded that both car soap and motor oil decrease the population density of the protozoans by altering the pH level in the soil, further negatively affecting the protozoans. This would harm protozoans because the different pH levels change the charges and shapes of enzymes, making them unable to perform vital chemical reactions for protozoan life. To see if the motor oil truly affects the population of protozoans primarily by changing the soil pH level more than car soap, one could try this same experiment again, but additionally test continuously for the pH levels in the soil. However, if it is seen that the motor oil and the car soap affect the soil pH levels equally, one could repeat this experiment and observe if the two substances change other certain chemical compounds vital to protozoans. One could also test for zinc levels because motor oil contains zinc, another harmful chemical for protozoans which most car soaps do not possess. By doing so, once could determine what makes motor oil more dangerous to protozoans than car soap.

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