How Does Kitchen Exhaust Alter Soil Protozoa Density?

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I. Background

Protozoa are single celled eukaryotes that vary in size and structure, and they are classified into their six phyla according to their mode of locomotion. Of the six *(Amoeba, Vorticella, Paramecium, Stylonychia, Tetrahymena, and Colpidium*), the ones living in the soil are Mastigophora, Ciliophora, Non-shelled Amoeba, and shelled Amoeba (American Society for Microbiology, 2015). These microscopic organisms are heterotrophic, obtaining their energy from the organic compounds of other living things, and one of their principal food sources in the soil are bacteria. As a consequence, they "help maintain an ecological balance in the soil….and when they graze on bacteria, protozoa stimulate growth of the bacterial population and decomposition rates and soil aggregation"(Hoorman, 2011). Furthermore "in the soil food chain, protozoa are in the third trophic level which means they are shredders, predators, and grazers" (Hoorman, 2011), providing predatory soil nematodes, soil insects, and soil microarthropods with a food source in of their own. Hence, soil protozoa play a vital role in numerous parts of the soil ecosystem.

One especially important role protozoa play as part of the microbial life in the soil is in their contributions to the biogeochemical cycles. The biogeochemical cycles are how chemical elements are moved or cycled around an environment between living and nonliving things, and protozoa help in this process by mineralizing the soil through the consumption of the bacteria living there. The ratio of carbon that protozoa need to the nitrogen in the bacteria they consume can be as much as 10:1. As a result, the protozoa release any excess nitrogen they do not need in the form of ammonium and nitrate. The ammonium and nitrate are then consumed by the plants and are beneficial to them because "nitrogen is a component of chlorophyll and therefore essential for photosynthesis. It is also the basic element of plant and animal proteins and is important in periods of rapid plant growth" (Charles Sturt University, 2015).

This process is called the nitrogen cycle (Hoorman, 2011) which is essential to ecosystems because without nitrogen there would be no amino acids to make proteins which make enzymes. Without enzymes there would be no way to start and stop chemical reactions that take place in the biological molecules (lipids, carbohydrates, proteins, nucleic acids, and water) which make and break chemical bonds using energy to create a new substance. This new substance then causes the 4 tasks to take place which are synthesis, reproduction, transforming energy, and homeostasis. If a cell can not carry out these tasks it would die and because plants are made of these cells, plants would die and animals would not be able to eat the plants and every living thing would then die. From here, waste is created from these animals, depositing nitrogen back into the ground with it. The cycle continues when the protozoa convert the nitrogen to beneficial chemicals that can be used again (Killpack & Buchholz, 1993). Then nitrogen undergoes denitrification, in which the nitrogen converts into a gas and the cycle repeats itself. Without the nitrogen cycle, all organisms would die.

Our experiment will explore protozoan's habit of obtaining carbon. The kitchen at RPCS releases exhaust from the appliances that make the food. Grease is a major part of this exhaust. Grease can be a big issue to society. Because kitchen grease is not released everywhere, the locations where the kitchen exhaust *is* released throws off the ecological balance of the nitrogen cycle. Grease contains a carbon compound called fatty acids which include saturated fats, unsaturated fats, monounsaturated fats, and polyunsaturated fats. Because bacteria are decomposers, they eat the exhaust released from the kitchen. Heterotrophs are a type of bacteria that benefit from the carbon compounds released from the exhaust pipe (Brock, 2015). “Using enzymes, these bacteria will break down complex compounds and use the nutrients to release energy”(Altidor, 2015). This energy is used for the protozoan's metabolic activity. Protozoa will

then eat the bacteria and carbon compounds by absorbing them through their cell membrane. So the expectations from our experiment is that the population of protozoa in the soil around the location closer to the kitchen exhaust will be greater than an area where there is no exhaust because there will be more carbon and bacteria for the protozoa to feed on.

II. Lab Outline

Outline of Experiment

I. Problem: Does the exhaust released from the kitchen at Roland Park Country School increase the population density of protozoa in the soil around it?

II. Hypothesis: The population density of protozoa will be greater near the kitchen exhaust than the population density farther away from the kitchen exhaust.

III. Procedure:

A. Independent Variable: Distance of soil from kitchen exhaust

B. Dependent Variable: Population density of protozoa in the soil

C. Negative Controlled Variable: Soil taken from location completely away from kitchen exhaust at N. 39.35689° W. 076.63596°

D. Controlled Variables: Amount of soil collected, amount of soil sifted (9-10g), size of petri dish, amount of time soil dries for, size of nylon mesh, new/clean nylon mesh for each soil sample, new/clean cup for each soil sample, amount of distilled water for rehydrating the soil, amount of distilled water for Uhlig extraction, amount of time in fridge, size of uhlig extractor, size of qualitative filter paper, size of capillary tube, amount of methyl green stain, type of pipette, size of cover slip, amount of soil solution placed on microscope slide, size of microscope slide, magnification of microscope, method of collecting soil, type of marker (flag), distance in

between each row of trials, time of day and day the samples are taken from soil, type of grass near soil samples, amount of time between the collection of each trial for the soil samples, amount of time to saturate the soil, amount of time in Uhlig extractor

E. Step-by-step

1. Mark soil at N. 39.35736° W. 076.63650° (located behind the kitchen) with flag labeled "location 1 #1."
2. Mark soil 1 meter south from location 1 #1. Label flag "location 1 #2."
3. Mark soil 2 meters south from location 1 #1. Label flag "location 1 #3."
4. Mark soil 5 meters east of location 1#1 with flag labeled "location 2 #1."
5. Mark soil 1 meter south from location 2 #1. Label flag "location 2 #2."
6. Mark soil 2 meters south from location 2 #1. Label flag "location 2 #3."
7. Mark soil 10 meters east of location 1 #1 with flag labeled "location 3 #1."
8. Mark soil 1 meter south from location 3 #1. Label flag "location 3 #2."
9. Mark soil 2 meters south from location 3 #1. Label flag "location 3 #3."
10. Mark soil at N. 39.35689° W. 076.63596° (located near the swing sets) with flag labeled "location 4 #1."
11. Mark soil 1 meter south from location 4 #1. Label flag "location 4 #2."
12. Mark soil 2 meters south from location 4 #1. Label flag "location 4 #3.
13. Complete steps 14-16 all in the same day at the same time
14. From location 1 #1, use soil core extractor with a diameter of 1.8cm to extract a column of soil 15 cm deep
15. Label fresh plastic bag as "location 1 #1" and put soil sample in bag (don't reuse to avoid contamination)

16. From each other location in trial 1 (location 2 #1, location 3 #1, and location 4#1), use soil core extractor to extract 15 cm in a column with a diameter of 1.8cm sample of soil. Place each soil sample in it's own bag according to the location number and trial number
17. Repeat steps #13-16 24 hours later than the previous collection date. Label fresh plastic bags accordingly to trial number (#2) and location (e.g. Location 1 #2)
18. Repeat steps #13-16 24 hours later than previous collection date. Label fresh plastic bags accordingly to trial number (#3) and location (e.g. Location 1 #3)
19. Complete steps 20-23 all at the same day at the same time
20. Place a 15 cm sample of soil from "Location 1 #1" and place it into the bottom of a clean, empty petri dish (label petri dish "location 1 #1); and place on windowsill.
21. Repeat step 20 except with other locations from trial 1 (location 2 #1, location 3 #1, and Location 4 #1), and place the soil samples into separate, clean empty petri dishes. Make sure to put each soil sample into different petri dish. Label petri dish accordingly to the location and trial number and place and then place them all on a windowsill to dry.
22. Repeat step #20-21 except with all soil samples from trial "#2"
23. Repeat step #20-21 except with all soil samples with from trial "#3"
24. Complete steps 25-28 all on the same day at the same time
25. After letting the soil samples sit on windowsill for at least 24 hours, sift 9-10 g (record exact amount) of the soil from "location 1 #1" into a correspondingly labeled 2nd clean petri dish using a 1 square mm nylon mesh
26. Label the new petri dish accordingly
27. For each soil sample that you are sifting, use a new piece of nylon mesh and a clean cup
28. Repeat steps 24-27 for all other locations and trials (Locations 1-4 and trial # 1-4)

29. Complete steps 30-31 all on the same day at the same time
30. Add 20 ml of distilled water to each of the 12 soil samples individually in order to saturate the soil
31. Cover the petri dishes with their lids and allow to sit for 7 hours
32. After the samples have sat for 7 hours, place them in a refrigerator until you can start step 34
33. Complete steps 34-35 all at the same day at the same time
34. Place the soil sample from “location 1 #1” in a modified correspondingly labeled Uhlig extractor containing 30 mL of distilled water for 24 hours at room temperature
35. Repeat step #34 for all other soil samples (Locations 1-4 #1-4) individually
36. Complete steps 37-38 all on the same day at the same time
37. Remove filtrate from all locations and filter a 2nd time using 12.5cm qualitative filter paper into its own correspondingly labeled cup
38. Place the cups in the refrigerator until you can start step 39
39. Complete steps 40-43 all on the same day at the same time
40. Using a capillary tube, deposit 7 µl of methyl-green stain on a clean microscope slide (1µl is equivalent to 1 drop from the capillary tube)
41. Then using a disposable graduated Beral-type pipet, add 18 µl (the first demarcation on the pipette) of the filtrate from “Location 1 #1” on the microscope slide and cover with an 18x18 square mm cover slip
42. Examine under a light microscope at 40X to count the protozoa (Protozoa will appear translucent and slightly bluer than their surroundings. Make sure to adjust the light so that the maximum number of protozoa that are visible, and if needed use a counter.)

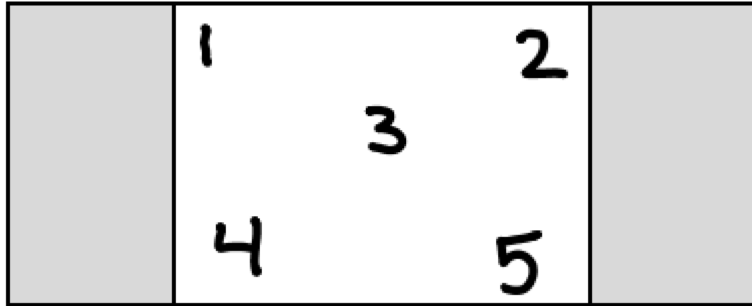


Figure A

43. Observe microscope slide in order as shown in *Figure A* and record the amount of protozoa in each location (1-5)

44. Average the 5 different amounts of protozoa for each location and trial and record in data table

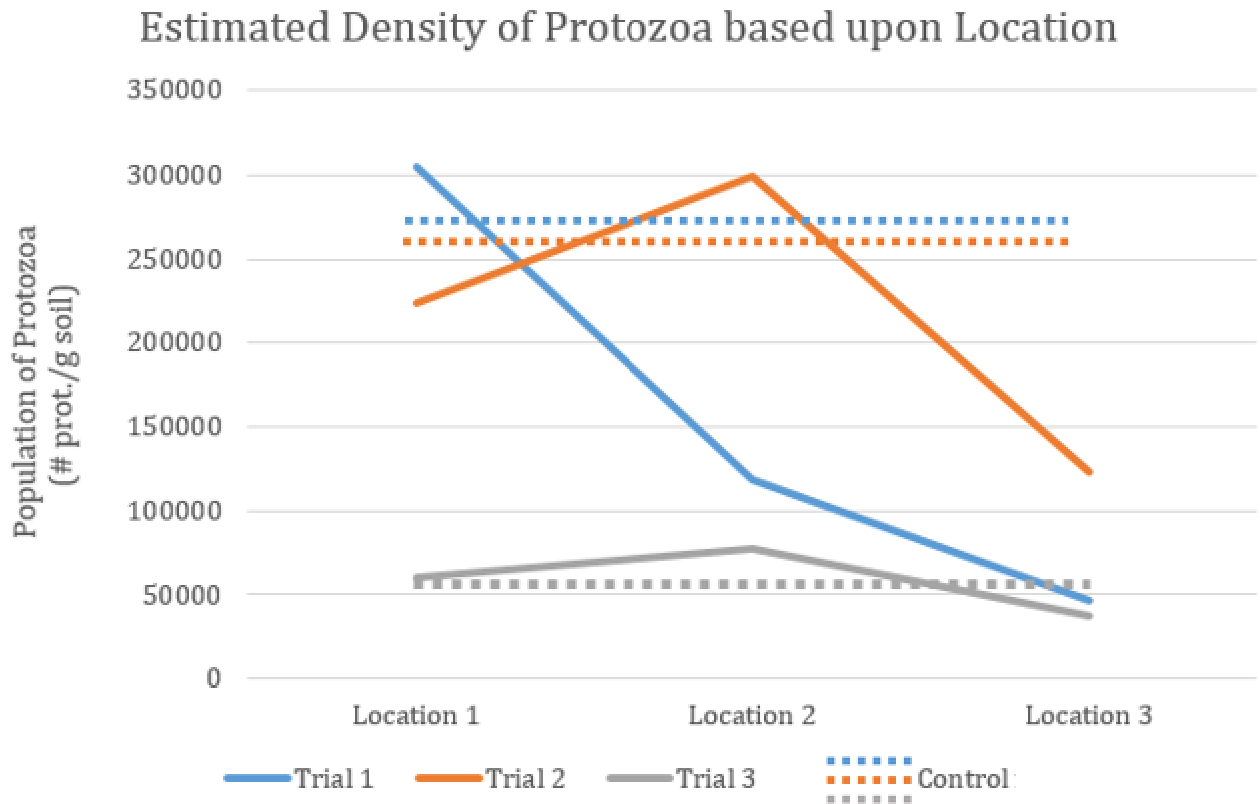
45. Repeat steps 40-43 with all other locations and trials (Locations 1-4, Trial # 1-4) individually

46. Use the following equation to then determine the population density in each of the 12 soil samples: [(average # per field of view at 40X) * (total ml of water used) * 747] / (grams of sifted soil) = # of protozoa per gram of soil .

47. Record population density from each sample into data chart

III. Data and Observations

Approx. Population Estimate of Protozoa near Kitchen Exhaust (# prot./g soil) on the RPCS Campus				
	Location 1 (1 meter from exhaust)	Location 2 (6 meters from exhaust)	Location 3 (11 meters from exhaust)	Negative Control
Trial 1	304652.78	118897.5	46235.37	270233.4
Trial 2	223464.26	299416.08	123417.4	264100.65
Trial 3	60369.8	77623.04	37674.8	54888.26



IV. Conclusion

Our hypothesis stated that out of our three locations, the protozoa population would be most dense in the location closest to the kitchen exhaust. However, our experiment results do not support this hypothesis. Trials 2 and 3 were taken in the late day after the soil had been exposed to more kitchen exhaust. In these trials, we concluded that location 2 had the greatest population density. In addition to collecting soil from 3 locations based on the proximity from the kitchen exhaust, each location was 5 meters away from each other. For our negative control, we collected soil from the opposite side of the campus from the kitchen exhaust. We did this because it allowed us to compare areas with more exhaust, to an area that has not been exposed to the kitchen exhaust at

all. In trial one, which was collected around 9:30 AM, location 1 had the most densely populated protozoa with approximately 304652.78 protozoa per gram of soil. Location 2 had the second largest amount with approximately 118897.5 protozoa per gram of soil and location 3 had approximately 46235.37 protozoa per gram of soil. This trial all together had the highest amount of protozoa in each location. The negative control for trial 1 was approximately 270233.4 protozoa per gram of soil. Although trial 1 supports our hypothesis, trials 2 and 3 show different results. Both trials 2 and 3, location 2 had the most protozoa. Trial 2 was collected around 1:30PM. Location 1 had approximately 223464.26 protozoa per gram of soil, location 2 had approximately 299416.08 protozoa per gram of soil, and location 3 had approximately 123417.4 protozoa per gram of soil. The negative control for trial 2 was approximately 264100.65 protozoa per gram of soil. In trial 3, which was collected around 3:15PM, location 1 had approximately 60369.8 protozoa per gram of soil, location 2 had approximately 77623.04 protozoa per gram of soil, and location 3 had approximately 37674.8 protozoa per gram of soil. The negative control for trial 3 was approximately 54888.26 protozoa per gram of soil. We believe that these results depend on both the time of day the soil was collected, as well as where the exhaust from the kitchen was falling. As shown on the graph, the negative control for trial 1 was very high. This shows that there were a lot of protozoa located all around the campus. In trial 2, the negative control decreased slightly and in trial 3, it decreased even more. This shows that over time, the population of protozoa got smaller. There are many possible reasons, but we think it is because as time went on, the soil became more and more dry due to a lack of rain. This would cause protozoa to die because they need moisture to survive. Although the negative control continued to go down in trials 2 and 3,

location 2 had more protozoa than the negative control in both trials. We believe that the reason that the largest amount of protozoa was found in location 2 for trials 2 and 3 was because of the wind. We believe that rather than the exhaust just falling off the side of the building into location 1, it blows farther east and lands in location 2. From our data, we concluded that our hypothesis was incorrect because in 2 out of 3 trials, the location closest to the kitchen did not have the most densely populated protozoa.

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