Protozoa and the Way We Affect Them

Faith Reid

Maya Jackson

Cameron Steadley

Juliet Boucher

Biology 9 Regular

Mr. Brock

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Background:

The soil is a key part any ecosystem and contains many different organisms that work together. One such group of organisms are the protozoa. "Microscopic creatures are the most abundant animals in the world in terms of numbers and biomass and are defined as single-celled eukaryotic organisms that feed heterotrophically and exhibit diverse motility mechanisms." **(NRCS n.d.)** These microbes are essential to the health of different organisms in the soil because of their bacteria consuming behavior. "As consumers of bacteria, Bacteria play a vital role in maintaining the earth as a suitable place for inhabitation by other forms of life and so protozoa play a vital role in controlling their numbers and biomass" **(Glasgow University Zoology Museum, n.d.)** But for that process to take place there have to be other key factors in the functioning of the soil and what grows in it or on it and one of those main components is pH. pH is a measure of how acidic the soil is, ranging from 0- very acidic- to 14 very basic. pH is essential because it gives the necessary conditions for soil to have a healthy balance of organisms. Normal healthy soil has a pH between 6.0- 7.0, and when the pH of the soil gets outside of this range, it can harm the organisms in the soil. There is a process called pH denaturation, this process that involves the disruption and destruction of enzymes in the organisms that live in the soil, such as protozoa. Denaturation is a process where the proteins in the soil are changed. The proteins contain acid and their electrical charges are changing from negative to positive, and back and forth. This weakens the soil because after the changing of charges the pH of the soil drops significantly past the solubility point and alters the organisms in the soil dramatically. **(Ohio State University: n.d.)** And without the enzymes in the soil the cells cannot perform the four tasks. And if the four tasks cannot function correctly chemical reactions

cannot occur. Along with chemical reactions not being able to occur the four tasks would be affected immensely by this change. During this process cells die rapidly and the number of protozoa decrease, and the level of bacteria increase. The process of Denaturation is frequently started by sulfuric acid, resulting in acid rain. Acid rain produces extremely low levels of pH and results in the number of protozoa decreasing. This controls the number of organisms living in the soil.

One of the main contributors to our acidic environment, is the high sulfate levels produced by car exhaust. Car exhaust is an omnipresent part of our way of living, and our soil and air is constantly exposed to it. Car exhaust contains many different ingredients that are pollutants to our environment. But specifically we predicted sulfuric acid or SO_2, is the cause of the decrease of protozoa in our soil at RPCS. SO_2 results in many different negative alterations in our soil but specifically the pH level and the protozoa number. Sulfuric acid, changes and mutates into acid rain, and then seeps into the soil. The pH in the soil is significant to the living environment through the different levels and different functioning's in different areas.

Acid rain then seeps into the soil, lowering the pH and killing the protozoa, and eliminating a lot of the organisms that live in the dirt. The way that the pH is affected is by the acid; the acid raises the acidity level and then lowers the pH. The lower the pH the higher the acidity. The acid in the soil that lowers the pH affects more than one characteristic of the soil. When the protozoa numbers decrease things that grow, live, and function in the soil do not function normally. Studies here have proven that in fact yes, the pH, when lower does decrease the number of protozoa living in the soil. **(Gregorian, A. Hampton, K. Li, J. Murphy, M. 2003)** What our group decided to investigate was how the soil, protozoa numbers, and pH levels

are affected by the sulfuric acid in the car exhaust around our school. We picked four different locations that gradually moved away from what we thought would be the most acidic soil samples on our school grounds, the carpool line to see whether the acidic particles spread and entered the soil, and harmed the protozoa.

References:

1. **U.S. Environmental Protection Agency. "Health and Environmental Impacts of SO_2."** (2008)http://www.epa.gov/oar/urbanair/so2/hlth1.html
2. **Spector, C. "About Soil Ph" (2001)** http://soil.gsfc.nasa.gov/soil_pH/plant_pH.htm
3. **NRCS. "Soil Biology" (n.d.)** http://www.epa.gov/oar/urbanair/so2/hlth1.html
4. **Glasgow University Zoology Museum.** "Biomedia Protozoa" **(n.d.)** http://www-biol.paisley.ac.uk/Courses/Tatner/biomedia/units/prot1.htm
5. **Gregorian, A. Hampton, K. Li, J. Murphy, M. (2003) "Little Things" Research Reports Archive.** https://faculty.rpcs.org/brockda/Little%20Things/Reports%20Archive/report%207%2003.pdf
6. **The Ohio State University. "Protein Denaturation" (n.d.)** http://class.fst.ohio-state.edu/FST822/lectures/Denat.htm

Lab

Question: Does the sulfuric acid from the car exhaust enter the soil and kill the protozoa living there?

Hypothesis: The soil in the area with the least amount of exposure to car exhaust will have the most protozoa.

Independent Variable: The distance of soil from car exhaust

Dependent Variable:

- Number of protozoa/g of soil
- Amount of sulfate(ppm) in the soil
- pH of the soil

Negative Control: Grassy area where there is no car exhaust present (the courtyard soil)

Controlled Variables:

- amount of soil tested
- how much soil we extract
- distance between points
- collection time of sample
- same soil test kit
- same amount of water placed into petri dishes
- same amount of demineralized water added to soil samples
- same amount of Soil Flocculating Reagent added to soil samples
- same amount of solution added to spot plates
- same amount of Duplex Indicator added to soil samples
- same amount of Universal Extracting Solution added to soil samples
- same amount of soil added to samples
- same type of filter paper used
- same funnel used for soil samples
- same amount of general soil added to turbidity vial
- same amount of Sulfate Test Solution added to soil samples
- same amount of distilled water added to soil samples
- same amount of time soil samples are set out
- same amount of time soil samples are in refrigerator
- same amount of methyl-green stain added to microscope slides
- Size of cover slips
- Magnification of microscope
- Amount of 2^{nd} filtrate on each slide

Procedures:

1. On the RPCS campus go to the point where the GPS of North 39^{o}21.492 and West 076^{o}38136 and put a flag in that spot. Label the Flag; Flag 1, point 1.
2. Measure 2 ½ meters South from the first flag point and place a flag in this spot. They should be in a straight line. Label it Flag 2, point 1.

3. Repeat step 2 with the final flag and label it Flag 3, point 1.
4. Make sure you do these following steps 5-9 on the same day around the same time.
5. Go back to the Flag 1, point 1.
6. Use a soil core sampler and dig it into the ground where the flag is and extract 15.24 cm core of the soil with a 2 cm diameter. Place the soil sample into a plastic baggie and label the area Location 1 point 1.
7. Measure24 meters further west from the first flag to the next point. At this point repeat step 5 but label it Location 1 plot 2.
8. Next go 24 meters west from the point in step 7 and repeat step 5 but label it Location 1 plot 3.
9. Finally go into the RPCS Courtyard at the GPS of North 39^{o}21.467 and West 076^{o}38.197 and repeat step 5 but label it Location 1 plot 4.
10. Next repeat steps 6-9 but with:
    a. The flag labeled Flag 2 location 2, plot 1. The 24 meters away from that location 2 plot 2. Then for the next location 2 plot 3 and finally the courtyard location 2 plot 4.
    b. For the third flag label the first one location 3 plot 1, then the next one location 3 plot 2, next location 3 plot 3 and finally the courtyard location 3 plot 4.
11. Label 15 individual petri dishes with what the label says on the bag you got it from.
12. Take all 15cmx2cm of all soil samples and place them each into their own bottom of clean, empty Petri dishes and allow them to dry completely. Place them in the ones labeled after what bag it's from. (This should take about 24 hours.)
13. After they all are completely dry, sift 9-10 grams of each of the soil into a 2^{nd} Petri dish labeled the same from the first Petri dish using $1mm^2$ nylon screens or mesh for each soil sample. Make sure you know what Petri dish is for what soil sample from the label and record the amount of soil per grams for each Petri dish.
14. For the remaining soil from each sample, test each sample for Sulfate and pH levels using the Model STH-14 Outfit.
15. For the soil that was sifted before in step 13 follow these procedures:
    a. Add 20 ml of distilled water to each of the 15 petri dishes.
    b. Cover all the Petri dishes with their lids and allow them to sit for 7 hours.
    c. Place the soil samples in their own modified Uhlig extractor containing 30 ml of distilled water for 24 hours.
    d. Remove the filtrate from the petri dishes and filter the soils a 2^{nd} time using 12.5 cm qualitative filter paper.
    e. Using a capillary tube, deposit 7 µl of methyl-green stain on a separate clean microscope slide for each soil sample. Then using a different disposable graduated Beral-type pipette for each soil sample, add 18 µl of the 2^{nd} filtrate from step 6 to the stain on the microscope slide and cover with an 18 x18 mm^2 cover slip.

f. Examine under a digital light microscope at 40X and examine each corner and the center of the microscope slide.
g. Use the following equation to determine the population density of protozoa in the soil samples:

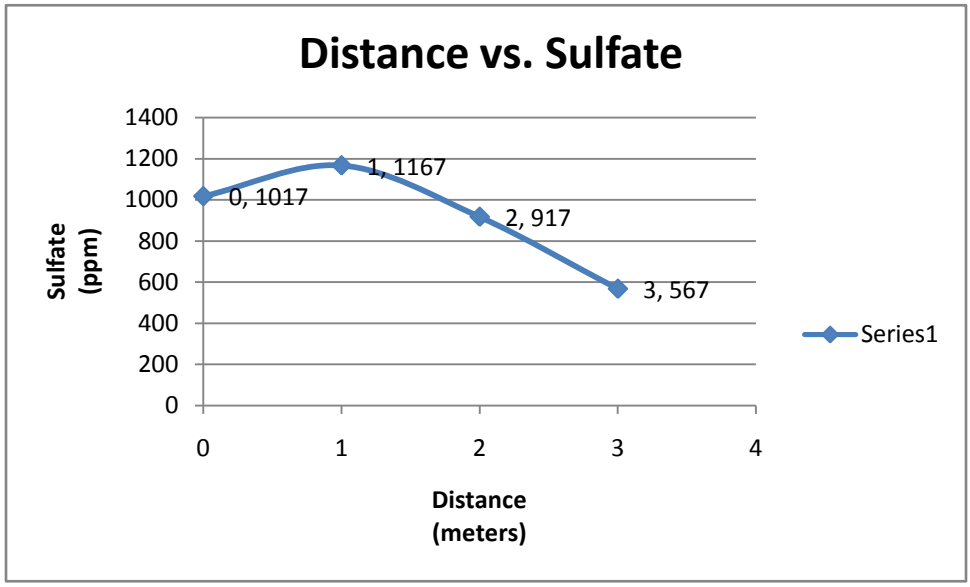
[(# per field of view at 40X) • (total ml of water used) •747]÷ (grams of sifted soil) = # of protozoa per gram of soil.

Analysis and Data Graphs:

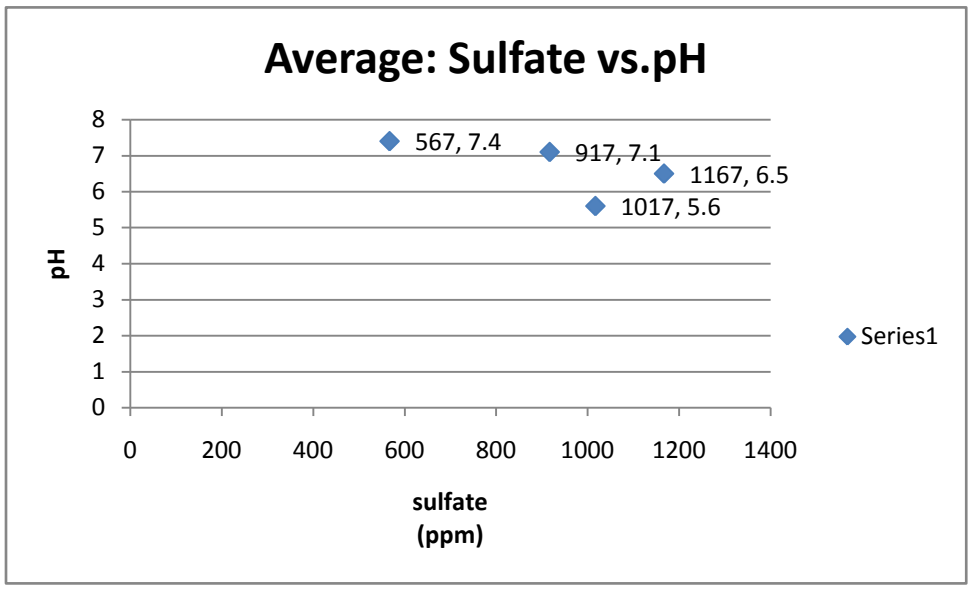
The Effects of Car Exhaust on the Soil:

Location	**Trials**	**pH**	**Sulfate (ppm)**	**# of Protozoa/ gram**
Plot 1	Location 1	5.4	1500	945422
	Location 2	5.4	150	1204839
	Location 3	6.1	1500	741190
	Average	**5.6**	**1017**	**963817**
Plot 2	Location 1	6.4	2000	1684723
	Location 2	6.9	500	499311
	Location 3	6.3	1000	1027125
	Average	**6.5**	**1167**	**1070386**
Plot 3	Location 1	7.3	1000	222527
	Location 2	7.1	750	801531
	Location 3	6.9	1000	1168694
	Average	**7.1**	**917**	**730917**
Plot 4	Location 1	7.2	2000	187152
	Location 2	7.5	1000	1362659
	Location 3	7.4	500	1292005
	Average	**7.4**	**567**	**947272**

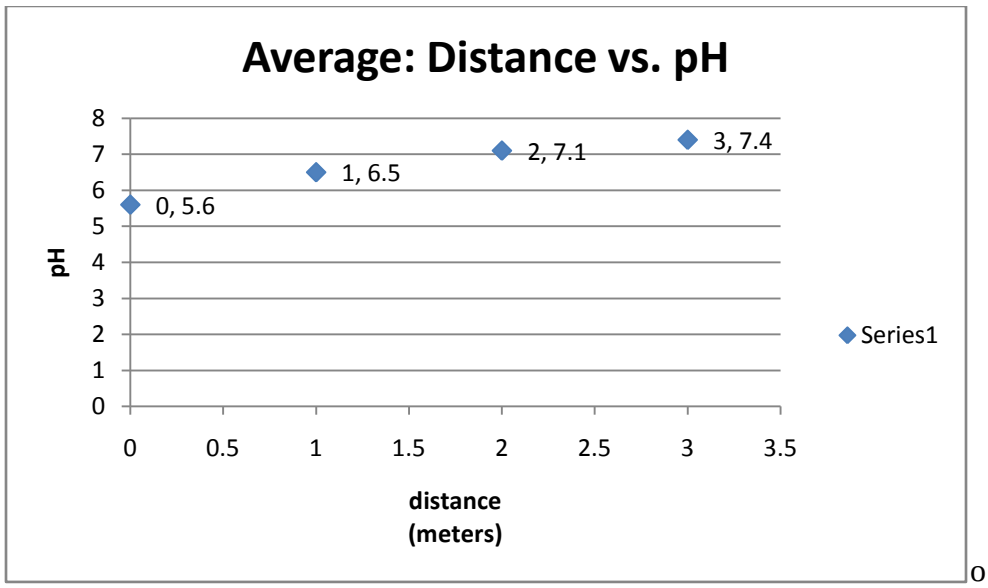
Distance vs. Sulfate:



Averages: Sulfate vs. pH



Distance vs. pH:



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Conclusion

To conclude our soil ecology project experiment our group's hypothesis was invalid. The statement of our hypothesis was "The soil in the area with the least amount of exposure to car exhaust will have the most protozoa." We were wrong because when we looked at the protozoa farthest away from the car exhaust, the protozoa was decreasing instead of increasing like we predicted. To prove this from our data the plot one average, closest to the car exhaust, contains 963,817 protozoa. The plot four averages which was the farthest away from the car exhaust was 947,272. In order for our hypothesis to be correct the plot one average would need be lower and the plot four averages would need to be higher, but in this case it was not.

However, our hypothesis actually should have been correct. Because all of the data we collected before counting the protozoa, lead us to believe that it was all correct. We predicted that the acid rain in the car exhaust was affecting the levels of sulfate and pH in the soil. All of

our data for the Sulfate and pH supports our hypothesis. The closer the soil was to the car exhaust, the higher the sulfate was. To prove this the closer we were to the driveway at plot one the sulfate level was at 1,167 parts per million compared to the farthest away plot four had the sulfate level of 567 parts per million.

With the lower amount of sulfate levels we figured that the pH levels would be higher the farther away that the plots were from the car exhaust. To prove this, our data shows that at the plot farthest away from the car there was a pH level of 7.4; plot one, the plot closest to the car exhaust there was a pH level of 5.6. With this information our group made a ground breaking discovery that the sulfuric acid in the car exhaust is actually affecting the pH and Sulfate levels in soil.

Finally, some things that our group could have done in order for our hypothesis to become valid. For further research we could figure out how and why pH is having no impact on protozoa. In order for our hypothesis to become valid for further research skills there could be more distances in between our plots. Secondly instead of taking twelve soil samples we could have had more plentiful samples. Finally instead of counting the three spots on protozoa slides we could have counted all five, and taken all of the slide counts into consideration in the total number of protozoa.