SEP

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Lab Report: Protozoa Density

Car exhaust is hazardous. It contains large amounts of carbon monoxide, nitrogen dioxide, sulfur dioxide, particulate matter, benzene, formaldehyde, and polycyclic hydrocarbons, all of which are highly toxic to living things when exposed to them in any form. For example, particulate matter can impair the respiratory system, can cause lung damage and even cancer, and millions of people and animals each year can suffer premature deaths due to inhaling these particles (Bloch, 2008). Even touching car exhaust can fatally impact any living thing with which it comes in contact (Bloch, 2008 & ThinkQuest, n.d).

The chemicals produced in car exhaust are worse still when mixed with moisture. Nitrogen oxide and sulfuric dioxide from the exhaust interact with the moisture in the atmosphere to produce sulfuric and nitric acid, which then fall to earth in the form of acid rain. Within this precipitation, the acid levels can be so high that it severely damages anything it comes in contact with, and in fact, when the acid rain is a concentrated enough, it can destroy plants and even buildings (Ophardt, 2003 & Upper Midwest Aerospace Consortium, 2006).

The acid in the rain can also kill the microorganisms that live in the soil; so the more acidic it is in the soil, the fewer microorganisms are present there (Ophardt, 2003). This is because when the pH level of an environment is very high or very low, it causes the enzymes in living things to stop working, and when the enzymes stop working, the consequences for living things are dire (Worthington Biochemical Corp, 2007). Enzymes start chemical reactions that then make and break chemical bonds to create a new substance, and it is these reactions that enable a cell to reproduce, synthesize, maintain homeostasis, and complete respiration. Consequently, if there are no functional enzymes because the pH level is too high or too low, then the cells of an organism will stop working and die, including those that inhabit the ground such as mosses, fungi, bacteria, and algae (ThinkQuest, n.d).

One group of soil organisms that are especially at risk because they live only in the water found in the soil are protozoa. These unicellular eukaryotes have evolved into three different types (protozoa with flagella, protozoa covered with cilia and 2 types that are amoebae, and their main niche or role in the soil is being a microbe predator (Nardi, J. 2003 & DulceCorazon, 2009). Protozoa mainly hunt and feed off of bacteria, but they can also eat some fungi and even other smaller protozoa, and while some species of protozoa are harmful to plants, the majority are helpful, preferring to live next to or near plant roots because that is where their main source of food- bacteria and other organic matter- resides (DulceCorazon, 2009 & Basic-info-4-organic-fertilizers.com, n.d.).

As the protozoa consume their prey, they actually perform numerous functions that help the plants. One such role is to help make nutrients and nitrogen available for plants and other soil organisms. (Ypsilantis, B, Hilty, J, mineralize nutrients, making them Davis, S, McCluskey, C. 2001). The protozoa start an elaborate process in the soil by eating bacteria. After the protozoa

have eaten the bacterial, they then have nutrients and nitrogen for their own use, but then they release some of the excess nitrogen and other nutrients which thye got from eating the bacteria into the soil. Since the protozoa are usually near the roots of plants, all the nitrogen and nutrients from the soil are then absorbed by the plants and used by them to make their own enzymes for running the chemical reactions that the plants need to perform. So this whole process is keeping not only the protozoa alive but the plants as well. Furthermore, if some kind of living thing eats the plants, then that thing will get the nutrients and nitrogen it needs to make its enzymes to keep it alive, hence the protozoa in the soil are crucial to the survival of all living things.

There are many roles for protozoa to do in the soil besides providing nitrogen to help keep other living things alive. Protozoa are a large part of the soil food chain and they help sustain the soil food web by eating bacteria in the soil. (Ypsilantis, B, Hilty, J, Davis, S, McCluskey, C, 2001). This is because when protozoa eat the bacteria it promotes the growth of bacteria populations, and in so doing, the protozoa are promoting an increased rate of decomposition (Ypsilantis, B, Hilty, J, Davis, S, McCluskey, C, 2001. & Basic-info-4-organic-fertilizers.com, n.d.). There are also some pathogenic protozoa in the soil that attack the roots of plants and can cause disease in them, but most soil protozoa reduce the amount of plant disease by feeding off the bacterial and fungal pathogens that attack plants. (Ypsilantis, B, Hilty, J, Davis, S, McCluskey, C, 2001).

Regardless to what role the protozoa play in the environment the number of the in the soil will be affected, if there is an excess amount of car exhaust near the soil resulting in extremely high or low pH levels, the number of protozoa in that soil sample will be affected. Too much car exhaust near the soil could result in low pH levels, and the number of protozoa in that soil would

decline. But if there are too few protozoa, the important roles that they carry out would not be fulfilled, and the entire ecosystem would suffer as a conspirer.

Because of all the great things that protozoa do for all living things and now knowing that they can be harmed, we decided to make an experiment to see if the car exhaust would affect the amount of protozoa in the soil on the RPCS campus. What we are doing is testing if being closer to the carpool line will affect the number of protozoa that are in the soil there. We are testing this because we think that the car exhaust will produce more acid rain on the soil near the carpool line. So what we think will happen is that the closer the soil is to the carpool line the less number of protozoa will be in the soil.

Experiment:

I. Problem: What impact does car exhaust have on the density of soil protozoa?

II. Hypothesis: If the soil is closer to the carpool lane, then the density of protozoa in soil will be less than the density of soil protozoa farther from the carpool lane.

III. Procedure:

   a. Independent Variable: distance of the location of the soil samples from the carpool line

   b. Dependent Variable: pH level and density of protozoa in the soil sample

   c. Negative Control: soil samples from the cherry tree courtyard

   d. Controlled variables: size of soil samples, how many soil samples, how long soil left out to dry, size of nylon or mesh screen to sift with when sifting soil, size of petri dish, size of filter paper, same test kit for pH test, same size cover slip for microscope, same amount and type of dye for microscope, same kind of

microscope slide, microscope magnification kept at 60X, same kind of microscope, same equation to find number of protozoa in soil, same type of Beral-type pipette, same time hydrating, same time in Uhlig filtering, how far apart soil samples are, same amount of filtrate on slide to look at

e. Step by Step Instructions:
    i. For steps i-xi the soil needs to be collected on the same day around the same time
    ii. Label three separate plastic bags: sidewalk 1, sidewalk 2, sidewalk 3 respectively
    iii. The first soil sample should be taken 4.88 meters away perpendicular from the carpool lane at the GPS coordinates N: 39 21.485 WO: 76 39.149, the second should have the GPS coordinates N: 39 21.485 WO: 76 38.148, the third should have the GPS coordinates N: 39 21.484 WO: 76 38.150, the three different samples are 15 centimeters apart from each other running parallel to the sidewalk
    iv. Push the 2.5 cm wide soil auger 15.5 cm into the ground, and place each soil sample in its proper corresponding bag
    v. Label three more plastic bags: 4.88 meters 1, 4.88 meters 2, 4.88 meters 3 respectively
    vi. Get three more samples 4.88 meters farther along the same perpendicular line. These three samples are 15 centimeters apart from each other running parallel to the sidewalk also. The first soil sample 4.88 meters on the same perpendicular line from the first sample should be taken at the GPS

coordinates N: 39 21.484 WO: 76 38.155, the second soil sample should be taken at the GPS coordinates N: 39 21.488 WO: 76 38.153, and the third soil sample should be taken at the GPS coordinates N: 39 21.485 WO: 76 38.151

vii. Push the 2.5 cm wide soil auger 15.5 cm into the ground, and place each soil sample in its proper corresponding bag

viii. Label three more new plastic bags: courtyard 1, courtyard 2, and courtyard 3 respectively

ix. Go into the cherry tree courtyard and get three more samples using soil auger like before, each sample should be 15 centimeters away from each other in a line horizontally. The first soil sample in the cherry tree courtyard should be taken at the GPS coordinates N: 39 21.467 WO: 76 38.198, the second soil sample should be taken at the GPS coordinates N: 39 21.465 WO: 76 38.199, the third soil sample should be taken at the GPS coordinates N: 39 21.470 WO: 76 38.202

x. Push the 2.5 cm wide soil auger 15.5 cm into the ground, and place each soil sample in its proper corresponding bag

xi. Bring in the plastic bags and label 9 separate empty petri dishes: 1 courtyard, 2 courtyard, 3 courtyard, 1 sidewalk, 2 sidewalk, 3 sidewalk, 4.88m 1, 4.88m 2, 4.88m 3 respectively

xii. Place each soil sample in its own separate correspondingly labeled petri dish and allow it to dry completely for a minimum of 24 hours

xiii. Label a second set of petri dishes (the same way as labeled in step x) but add “sifted” to the title.

xiv. Now sift 9-10 g of each soil sample from step xi into its correspondingly labeled 2nd clean petri dish, use a 1mm^2 nylon screen or mesh, do this for each petri dish of dried soil

xv. Record how many grams of soil are now in each 2nd petri dish

xvi. With the soil leftover in the first petri dish you will perform the pH test

xvii. Using the LaMotte STH-14 Series test for pH of each soil sample by using the soil leftover in the first petri dish (not the sifted soil), and record pH of each soil sample

xviii. For steps xvii-xxvi, each step must be fully done to each sample on the same day at the same time, you must be able to completely work through these steps or find stopping points where you are able to put the samples in the refrigerator so it suspends the protozoa

xix. Add 20 ml of distilled water to each sifted soil petri dish at the same time, on the same day preparing for the protozoa test

xx. Cover each petri dish with its lid and allow it to sit for 7 hours

xxi. Place each soil sample in its own individual modified Uhlig extractor containing 30 ml of distilled water and filter for 24 hours

xxii. Remove the filtrate from each petri dish and filter each soil sample a 2nd time using 12.5 cm qualitative filter paper

xxiii. Label nine separate clean microscope slides like you have labeled the plastic bags and petri dishes

xxiv. Using a capillary tube deposit 7 ul (1 ul = 1 drop from the capillary tube) of methyl-green stain on each clean microscope slide

xxv. Using a disposable graduated Beral-type pipette, add 18 ul (the first demarcation on the pipette) of the 2nd filtrate from step xxi to the stain on each microscope slide and cover each microscope slide with an 18 x 18 mm^2 cover slip [be sure to change pipette for each different soil sample]

xxvi. Examine each slide under a light microscope at 60X observation.

xxvii. Do steps xxviii-xxix to each soil sample

xxviii. Take 5 observations with the microscope five fields of view, one picture at the upper right corner of the slide, one picture at the lower right corner of the slide, one picture at the lower left corner of the slide, one picture at the upper left corner, and one picture in the middle of the slide

xxix. Count the number of protozoa on each picture, and average out to get the average number of protozoa per field of view on the slide, use this number in the equation and put into the spot that says # per field view at 60x

xxx. Use the following equation to determine the density of protozoa in the soil sample

[(# per field view at 60X) x (total ml of water used) x 2165] / (grams sifted soil) = # of protozoa per gram of soil

xxxi. Record number of protozoa for each soil sample

IV. Data and Analysis

a. Data Table

Protozoa Density and pH Level of Different Soil Samples

Location	**Trial #**	**# Protozoa per gram of soil**	**pH level**
Courtyard Soil	1	31573	8
	2	43300	8.2
	3	188294	8.2
	Average	87722	8.1
Sidewalk Soil	1	102670	6.8
	2	492253	6.7
	3	617025	6.6
	Average	403983	6.7
4.88 M from Sidewalk Soil	1	120328	6.4
	2	541250	6.2

	3	846515	6.8
	Average	502698	6.5

Figure 1:

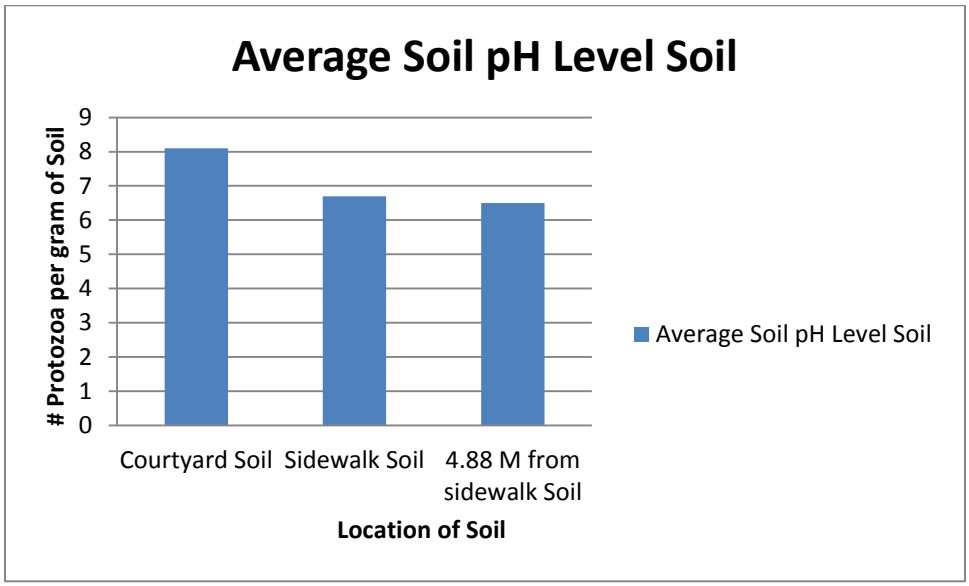


Figure 2:

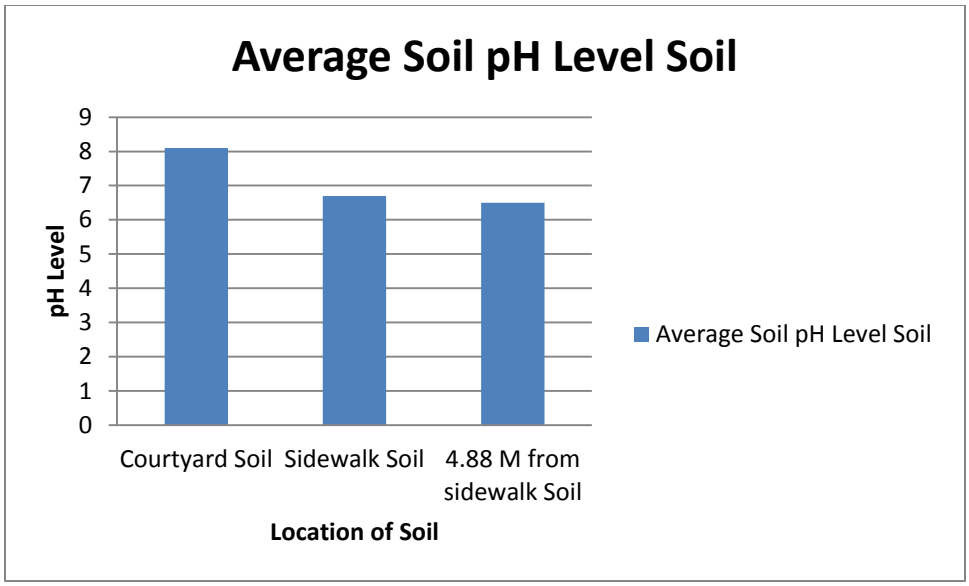

Average Soil pH Level Soil
pH Level
9
8
7
6
5
4
3
2
1
0
Courtyard Soil
Sidewalk Soil
4.88 M from sidewalk Soil
Location of Soil
Average Soil pH Level Soil

Figure 3:

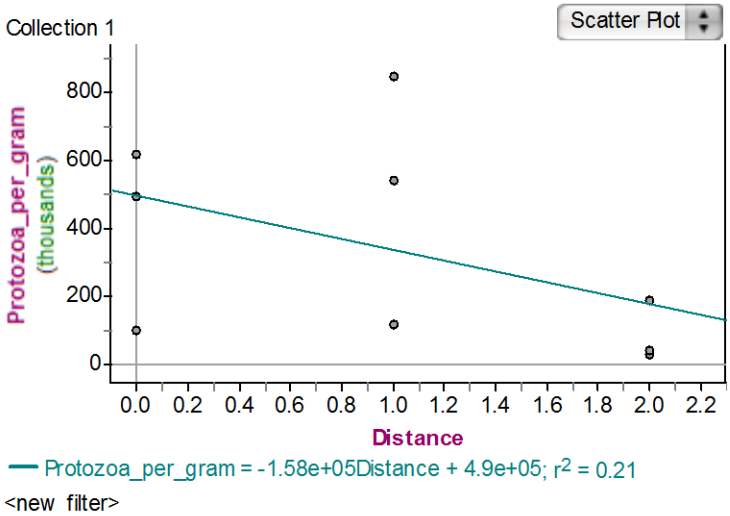

Collection 1
Scatter Plot
Protozoa_per_gram (thousands)
800
600
400
200
0
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
1.8
2.0
2.2
Distance
Protozoa_per_gram = -1.58e+05Distance + 4.9e+05; r2 = 0.21
<new filter>

Figure 4:

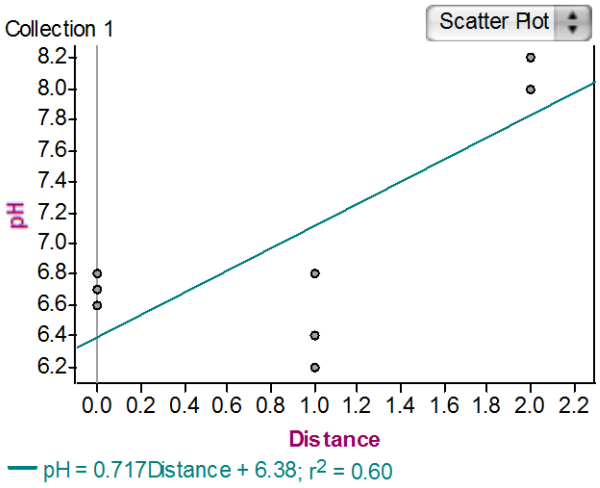
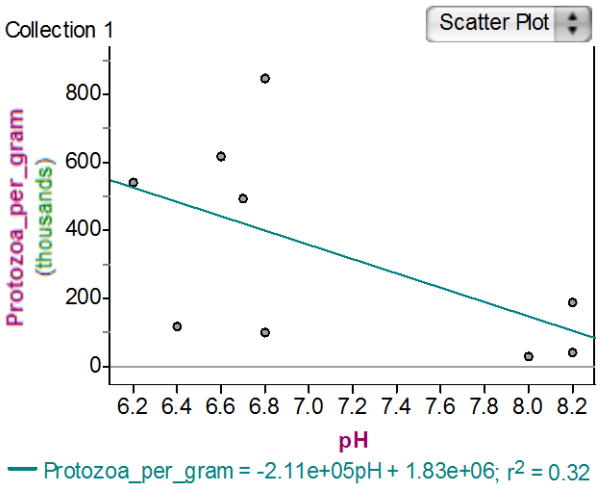


Figure 5:



V. Conclusion:

Our hypothesis was that the farther you got away from the carpool lane there would be a larger amount of protozoa in the soil. Our hypothesis was proven wrong because the

courtyard has the least amount of protozoa. Figure 1 shows the average number of protozoa; you can see that the courtyard has the least average amount of protozoa, while the 4.88 meters has the most average amount of protozoa. Figure 3 shows that the further from the sidewalk, the fewer protozoa there will be. These two graphs prove our hypothesis wrong.

Although our hypothesis was proven wrong, our reasoning behind our hypothesis was proven correct by our data. We reasoned that there would be a higher acidity level the closer you got to the carpool lane, which would cause a decrease in protozoa. The sidewalk soil and the 4.88 meter soil were both more acidic than the courtyard soil. Referring to Figure 4 that compares pH and distance, you can see that as you move further away from the carpool lane the pH level gets larger and less acidic. Figure 5 shows that when the pH is neutral there is an increase of protozoa, but as the pH becomes less acidic the protozoa number decreases. Figure 5 illustrates that the normal process between protozoa and the environment is happening. Figure 1 shows us that the protozoa increase from the sidewalk soil to the 4.88 meter soil, which is what we predicted. The courtyard soil though, shows a large decrease in number of protozoa which brings up the idea that there may be an unrecognized variable in the courtyard soil which is also affecting the number of protozoa and thus proves our hypothesis wrong.

For further research this experiment could be changed so that we would have more soil plots on the front lawn, and be able to recognize if the courtyard had an unrecognizable variable. To test this we would take another samples 4.88 more meters away from where the second sample was taken, and then a fourth sample 4.88 more meters further from the third soil plot on the front lawn. We would keep the plot in the courtyard the same. It would make more sense to add two more plots out on the front lawn and not in the courtyard because our

data supported that from the sidewalk to second plot the protozoa density went up. Therefor by changing the experiment by adding two new plots we would be able to tell if the soil in the courtyard had an unrecognizable variable or not.

Citation:

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