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Soil Ecology Project:

THE EFFECTS OF CAR EXHAUST ON PH AND FUNGAL DENSITY

Background

Fungi are heterotrophic microbes that live in the soil and complete many tasks essential to an ecosystem's survival. They decompose dead matter and break down organic compounds to make energy for themselves and, in so doing, provide not only for their own metabolic needs but also replenish the soil with nutrients for use by the other organisms that live there. For example, vascular plants cannot grow without symbiotic fungi because the nutrients and energy trapped in dead materials would otherwise not be available to their roots (University of Western Australia, 2004). Hence, without the fungi, soil's health rapidly decreases, a plant's life expectancy decreases, and everything dependent on the plants suffer: unhealthy soil affects all the living organisms in an ecosystem.

The main reason healthy soil needs fungi is because otherwise when plants, animals, and other organisms die, organic matter would remain trapped inside of them. This is particularly true of plants that require different varieties of fungi such as Soft Rot Fungi, Brown Rot Fungi, and White Rot Fungi to release the water, lipids, carbohydrates, nucleic acids, and proteins from their dead plant "bodies" (Featherstone, 2014). For example, Soft Rot Fungi use cellulose enzymes to release the hard to reach biological material found in dead wood, while Brown Rot Fungi use cellulose, hemicellulose, and oxidation to decompose it, and White Rot Fungi use ligninases to decompose lignins, the toughest compounds in all of nature to breakdown (Washington State University, 2004). Hence, without fungi to decompose plants, the nutrients first produced by plants in the process of photosynthesis could never be recycled back into the ecosystem (UCMP, 1998).

Another type of fungi also helps plants through decomposition, but it does so while the plant is still alive. Called mycorrhizae, these fungi connect with the roots of plants, and through this symbiotic relationship, “the fungi receive carbohydrates as energy from the host plant root while nutrients such as phosphorus and zinc are passed back into the plant roots from the soil” (University of Western Australia, 2004). The plants then use these nutrients to construct their cells and perform the functions they need to survive.

One of these nutrients, nitrogen, is especially important because if it is not recycled by the fungi and plants correctly, the entire food chain collapses. Nitrogen makes up amino acids which is a monomer of protein. Proteins are particularly important because they start and stop chemical reactions between the five biological molecules. Without these chemical reactions, the four tasks (reproduction, respiration, synthesis, and regulation of environment) would not occur in the cell which would cause the cell and any organism made up of cells (such as a plant) to die. Since a plant’s cells’ nutrients then move up the food chain to primary consumers and secondary consumers as one consumes the other (Washington State University 2004), this means that if fungi malfunction and fail to release decomposed nutrients back into the soil, there can be disastrous consequences for the entire ecosystem.

Car exhaust is one such thing that can cause this harm to occur. Most cars, trucks, trains, and airplanes run by burning some form of hydrocarbon fuel, commonly known as gas. When this fuel burns, it emits a smoky-looking substance that includes not only the soot we can see but many other chemicals as well (The American Cancer Society, 2013). Some of the chemicals in the gas are harmless. For instance, water is emitted as vapor, and even the carbon dioxide released—though a major contributor to climate change—is not directly harmful to the environment in the small quantity emitted in car exhaust (United States Environmental

Protection Agency, 2013). But the other chemicals emitted in car exhaust are quite harmful and include: “carbon monoxide, nitric oxide, nitrogen dioxide, sulfur oxides, and hydrocarbons, including polycyclic aromatic hydrocarbons (PAHs)” (American Cancer Society, 2013). Of these, the two most dangerous to the larger environment are nitrous oxide and sulfur dioxide.

While small amounts of nitrous oxide are emitted into the world naturally by the breakdown of organic matter by bacteria, the majority of this compound in the atmosphere today comes from car exhaust, and in excess, it is incredibly hazardous. Along with sulfur dioxide, when these two byproducts of car exhaust interact with the water vapor in the air, they react to produce nitric and sulfuric acid that precipitates to the ground in the form of rain, snow, or fog. This acid rain then causes major damage to buildings, forests, rivers, lakes, and soil (Stewart, 2008). For example, when the sulfuric and nitric acids seep into the soil and alter the pH, they absorb nutrients such as calcium, sodium, magnesium, and potassium which are vital to a tree’s survival and growth (Cantoria, 2012). In addition, acidic soil has a high concentration of soluble aluminum, iron, and manganese which are dangerous both to the microbes and to the plants living in an ecosystem.

Acidic soil can also negatively influence plant growth by affecting the activity of the soil’s fungi. Normally, “molds and yeast grow in wide pH range, but prefer pH between 5 and 6” (Life Sciences, n.d.). But soil which has been affected by car exhaust has a pH level of 2.5 (Bickelhaupt, 2014), and that can prevent fungi from breaking down organic matter because it can cause them to transition from their mold morphology to their yeast state. In its mold form, a fungus is performing its job of decomposing organic matter, as well as performing the tasks it needs to do for its own survival. But, if it is in the yeast form, it can only complete these latter tasks. Since soils affected by car exhaust have a pH significantly outside the normal range of 6.5-

7 (Admin, 2011), and since an organism's enzymes do not function outside of a certain pH level, the now highly acidic soil prevents a mold's enzymes from working. The mold then transforms to a yeast in order to protect itself, stopping the breakdown of organic matter, and trapping it in the inert parts of the environment (Bickelhaupt, 2014). This results in an accumulation of inert organic matter and the tie up of nutrients, particularly nitrogen, located in that matter.

As a consequence, the nutrients that the soil needs to survive are not released, and the plants that depend on them die. Therefore, the impact of the amount of yeast in soil does not simply affect the soil itself; it affects the plants that grow in that soil, the animals that eat those plants, the animals that eat those animals, and so on and so forth. Therefore, fungi play a key role in the overall health of the soil in an environment and in turn the health of an entire ecosystem.

Given this significance of soil fungi, we decided to conduct an experiment in which we would test the pH and the yeast and mold densities of six different soil locations. Three of our soil locations would be exposed to car exhaust, and the other three would be located in a courtyard that is not exposed to car exhaust. The latter three would act as our negative control because they would not come into contact with the harsh chemicals found in car exhaust. If there is a correlation between the pH of a given soil location and its yeast and mold density, we will be able to conclude that the acidity of the soil affects the density of the yeast and mold in the soil. We predict that the acidity of the car exhaust will affect the soil by decreasing the density of mold and increasing the density of yeast found in that soil.

Lab Report

- I. **Problem:** Does the acidity of car exhaust alter the form of fungi living in the soil?
- II. **Hypothesis:** The acidity of car exhaust decreases the density of mold and increases the density of yeast in the soil.
- III. **Procedures:**
 - A. Independent Variable: whether or not the location of soil sampled is exposed to car exhaust
 - B. Dependent Variable: soil pH level and density of mold and yeast per cubic centimeter of soil
 - C. Negative Control: soil samples taken from an area that is not exposed to car exhaust
 - D. Controlled Variables:
 - Make sure all soil is collected at the same time on the same day
 - Depth of soil sample taken from the ground (cm below the ground)
 - Amount of soil extracted from each location (cubic centimeters)
 - Amount of water added to each test tube (milliliters)
 - Amount of soil added to each test tube (cubic centimeters)
 - Type of water used for pH test and serial dilutions test
 - Temperature of water used for pH test and serial dilutions test (°C)
 - Type of tool used to extract soil
 - Size of test tube used
 - Type of test tube used
 - Amount of days fungi has to grow

- Type of nutrient agar
- Amount of nutrient agar
- Cleanliness of materials
- Temperature of location fungi plates are in while growing (°C)
- Moisture of the soil
- Amount of soil/water mixture placed on the nutrient agar (µl)
- Degree to which the soil is diluted each time
- How much test tubes are shaken
- How often test tubes are shaken
- Type of pH chemical test kit used

E. Step by Step:

1. Complete steps 2-8 on the same day at the same time of day.
2. Using a standard metal Soil Auger, extract a sample of soil 16 centimeters deep and 2 centimeters in diameter from location N 39.35785°, W 76.63668°. (NOTE: if the extractor does not go into ground easily, use a mallet to hammer it in)
3. Place the soil into plastic bag and label it “Courtyard: Location One”
4. Repeat steps 2-3, but this time at location N 39.35854°, W 76.03564°. (Label the plastic bag in step three “Parking Lot: Location One”)
5. Repeat steps 2-3, but this time at N 39.35766°, W 76.63673°. (Label the plastic bag in step three “Courtyard: Location Two”)
6. Repeat steps 2-3, but this time at location N 39.35914°, W 76.63555°. (Label the plastic bag in step three “Parking Lot: Location Two”)

7. Repeat steps 2-3, but this time at N 39.35778°, W 76.63659°. (Label the plastic bag in step three “Courtyard: Location Three”)
8. Repeat steps 2-3, but this time at N 39.35741°, W 76.63543°. (Label the plastic bag in step three “Parking Lot: Location Three”)
9. Return to your lab station and complete steps 10-23 with each soil sample from each location on the same day at the same time (Complete the pH test and the serial dilutions test at the same time.)
10. Complete steps 11-12 with each soil sample from each location. Make sure to use new, clean pipettes for each soil sample. Label any test tubes used during the serial dilution process as follows:
 - C1 when using soil from Courtyard Location 1
 - C2 when using soil from Courtyard Location 2
 - C3 when using soil from Courtyard Location 3
 - PL1 when using soil from Parking Lot Location 1
 - PL2 when using soil from Parking Lot Location 2
 - PL3 when using soil from Parking Lot Location 3
11. Following the model STH-14 outfit (Code 5010) perform a pH test on each of the soil samples
12. Record results in provided data table
13. Complete steps 14-34 for each soil sample location. Make sure to use new, clean pipettes for each soil sample. Re-label the test tubes in steps 14, 15, and 16 according to the following:
 - Replace C1 with C2 when using soil from Courtyard Location 2

- Replace C1 with C3 when using soil from Courtyard Location 3
 - Replace C1 with PL1 when using soil from Parking Lot Location 1
 - Replace C1 with PL2 when using soil from Parking Lot Location 2
 - Replace C1 with PL3 when using soil from Parking Lot Location 3
14. Use a clean, new transfer pipette to add 10 ml of room temperature (22-25°C) sterile water to a 15 ml culture tube. Label the tube “C1, 10⁰.”
 15. Use the same pipette to add 9 ml of room temperature (22-25°C) sterile water to a second 15 ml culture tube. Label the tube “C1, 10⁻¹.”
 16. Repeat step 15 one more time to one additional 15 ml culture tube, only label it “C1, 10⁻².”
 17. Place 1 cc of Courtyard Location 1 soil sample into the “C1, 10⁰” culture tube.
 18. Cap the tube and shake vigorously.
 19. Using a new clean pipette, remove 1 ml of the soil/water mixture from the “C1, 10⁰” tube and place into the “C1, 10⁻¹” tube.
 20. Cap and shake vigorously.
 21. Using the same pipette in step 19, remove 1 ml of the soil/water mixture from the “C1, 10⁻¹” tube and place into the “C1, 10⁻²” tube.
 22. Cap and shake vigorously.
 23. Plate 100 µl samples from the 1st, 2nd, and 3rd tubes (dilutions “C1, 10⁰” “C1, 10⁻¹” “C1, 10⁻²”) onto their own separate, correspondingly labeled 3M Petrifilm™ Yeast and Mold Count Plates. (Use the same labels on the petri plates as used in step steps 14-16)
 24. Allow fungi on the nutrient agar plates to grow for 48 to 72 hours at room temperature (22-25°C).

25. Organize the 3M Petrifilm™ by locations
26. Count the number of mold colonies on 3M Petrifilm™ labeled 10^{-2} for a specific sample and location. If mold is found record the number of mold and this dilution value in the data table and do not complete steps 27-28 unless no mold is found.
27. If mold is not found at dilution 10^{-2} , count the number of mold colonies on 3M Petrifilm™ labeled 10^{-1} for a specific sample and location. If mold is found record the number of mold and this dilution value in the data table and do not complete step 28 unless no mold is found.
28. If mold is not found at dilution 10^{-1} , Count the number of mold colonies on 3M Petrifilm™ labeled 10^0 for a specific sample and location. If mold is found record the number of mold and this dilution value in the data table
29. Count the number of yeast colonies on 3M Petrifilm™ labeled 10^{-2} for a specific sample and location. If yeast is found record the number of yeast and this dilution value in the data table and do not complete steps 30-31 unless no yeast are found.
30. If yeast is not found at dilution 10^{-2} , count the number of yeast colonies on 3M Petrifilm™ labeled 10^{-1} for a specific sample and location. If yeast is found record the number of yeast and this dilution value in the data table and do not complete step 31 unless no yeast are found.
31. If yeast is not found at dilution 10^{-1} , Count the number of yeast colonies on 3M Petrifilm™ labeled 10^0 for a specific sample and location. If yeast is found record the number of yeast and this dilution value in the data table
32. Determine the estimate of mold and yeast in 1 cc of soil with the formula:

Mold or Yeast in 1 cc of soil = # Colonies on sheet x 10² x 10^{|dilution # at which these colonies were found|}.

- 33. Record results in the provided data table.
- 34. Thoroughly clean the lab station with alcohol.
- 35. Repeat steps 1-34 at least two more times with new soil from the same locations mentioned in steps 2-8.

IV. Analysis

Data Tables:

Possible Impact of Car Exhaust on pH Levels in the Soil

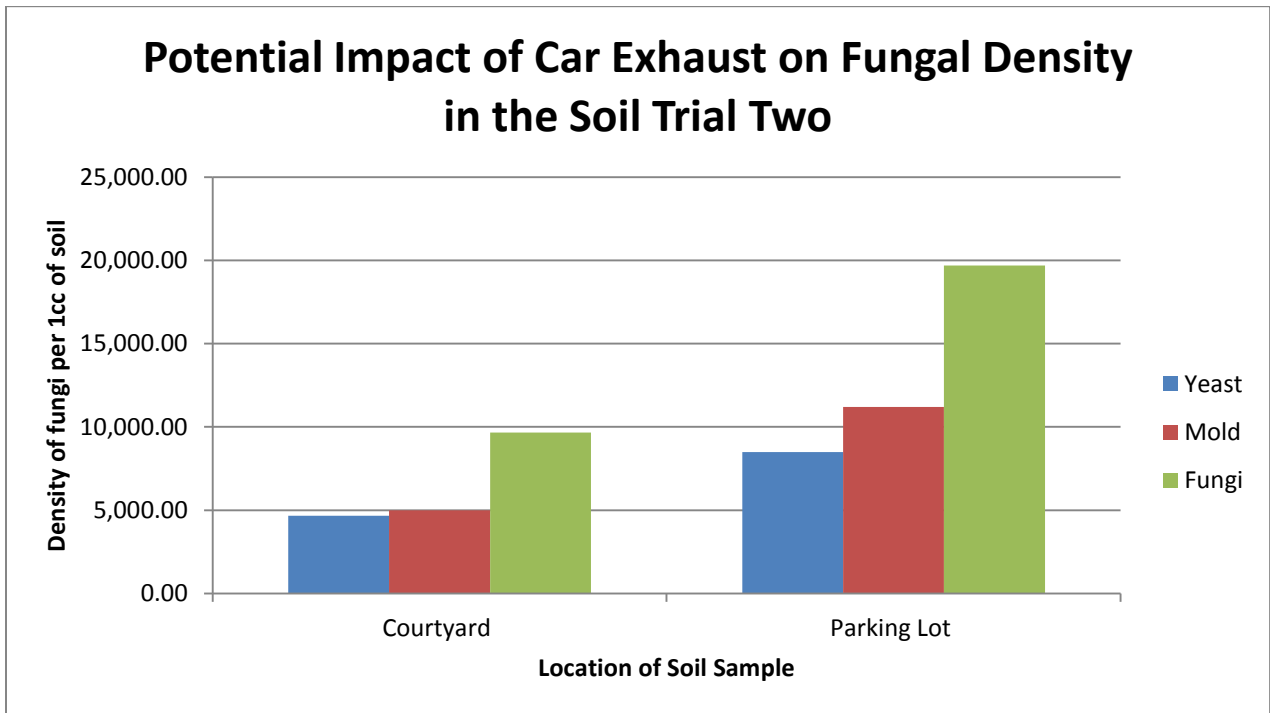
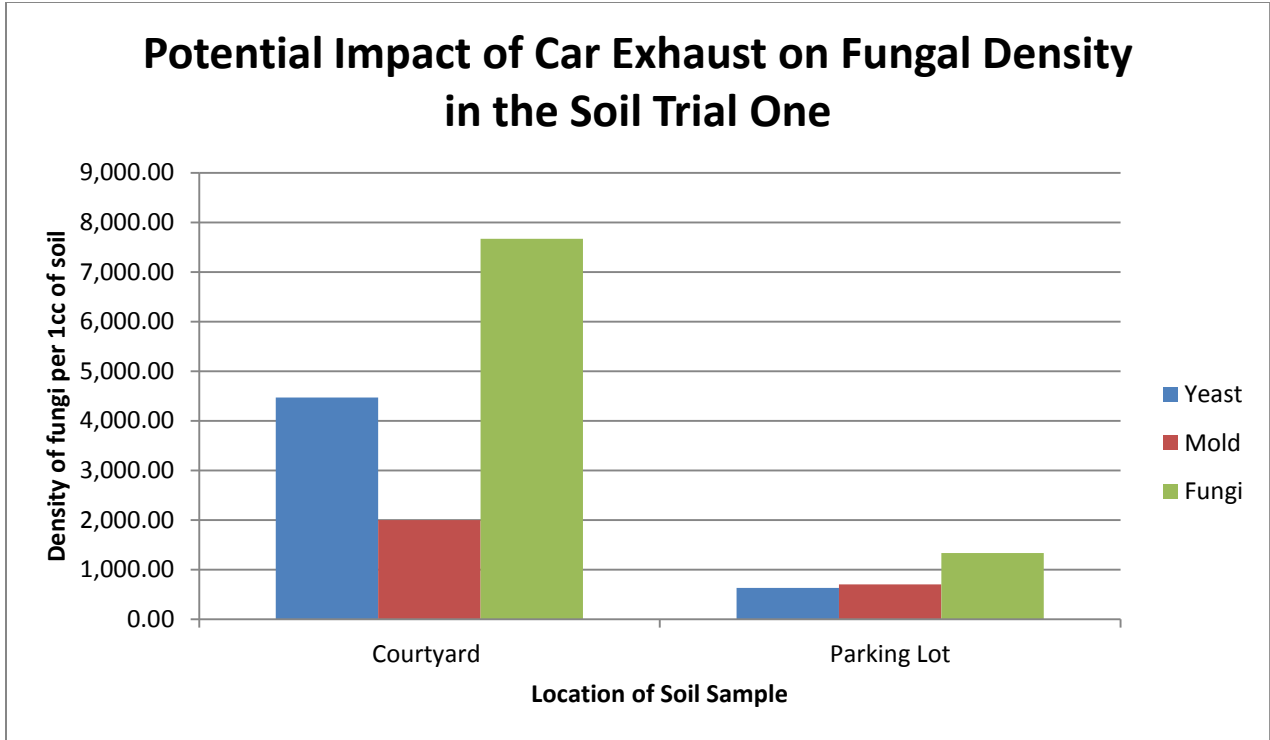
Location where Soil Samples are taken	Acidity of Soil (pH)		
	Trial 1	Trial 2	Trial 3
Courtyard Location One	8.0	8.0	8.2
Courtyard Location Two	7.4	7.4	8.2
Courtyard Location Three	7.4	5.8	6.8
Average Courtyard	7.6	7.067	7.733
Parking Lot Location One	7.2	7.4	7.4
Parking Lot Location Two	7.0	7.4	6.6
Parking Lot Location Three	7.0	8.2	7.6
Average Parking Lot	7.067	7.667	7.2

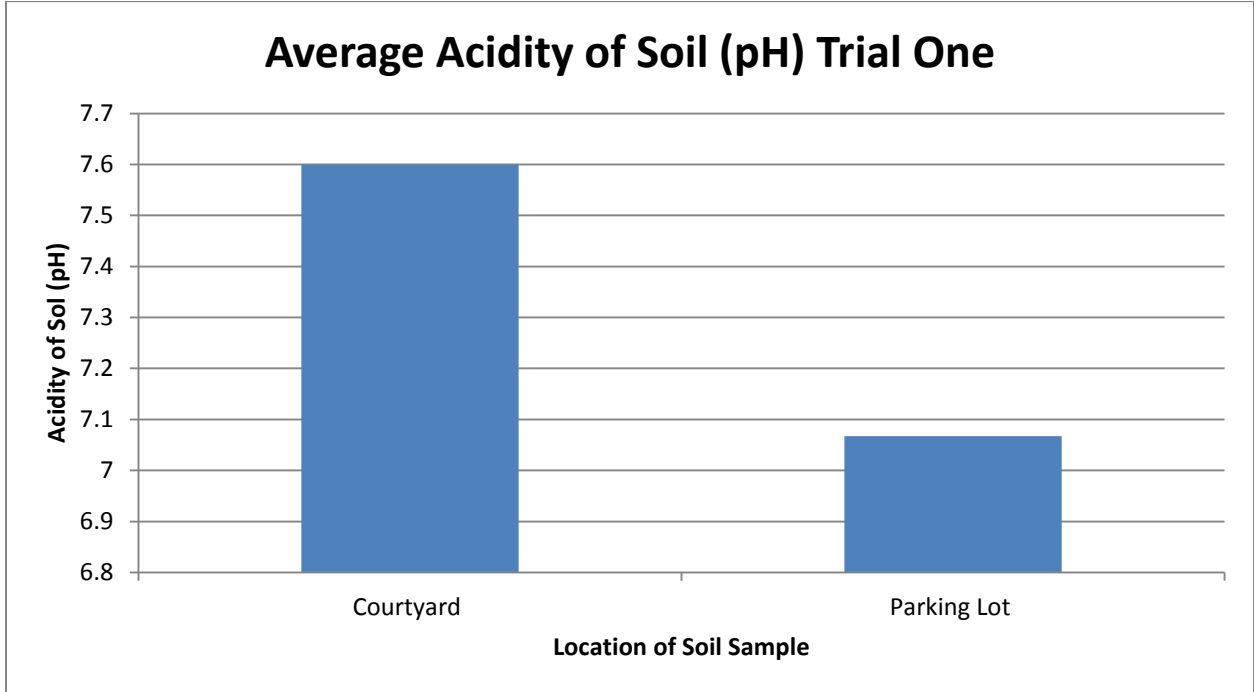
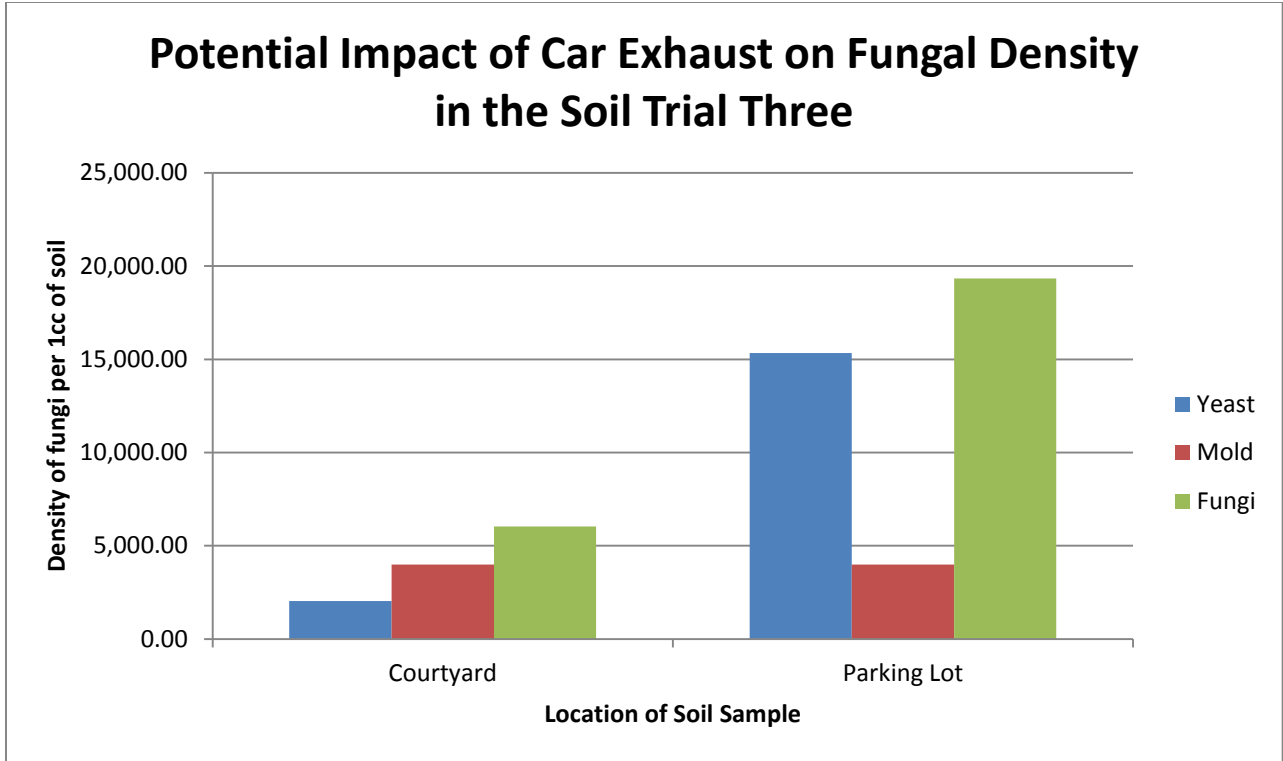
Possible Impacts of Car Exhaust on Fungal Density in the Soil

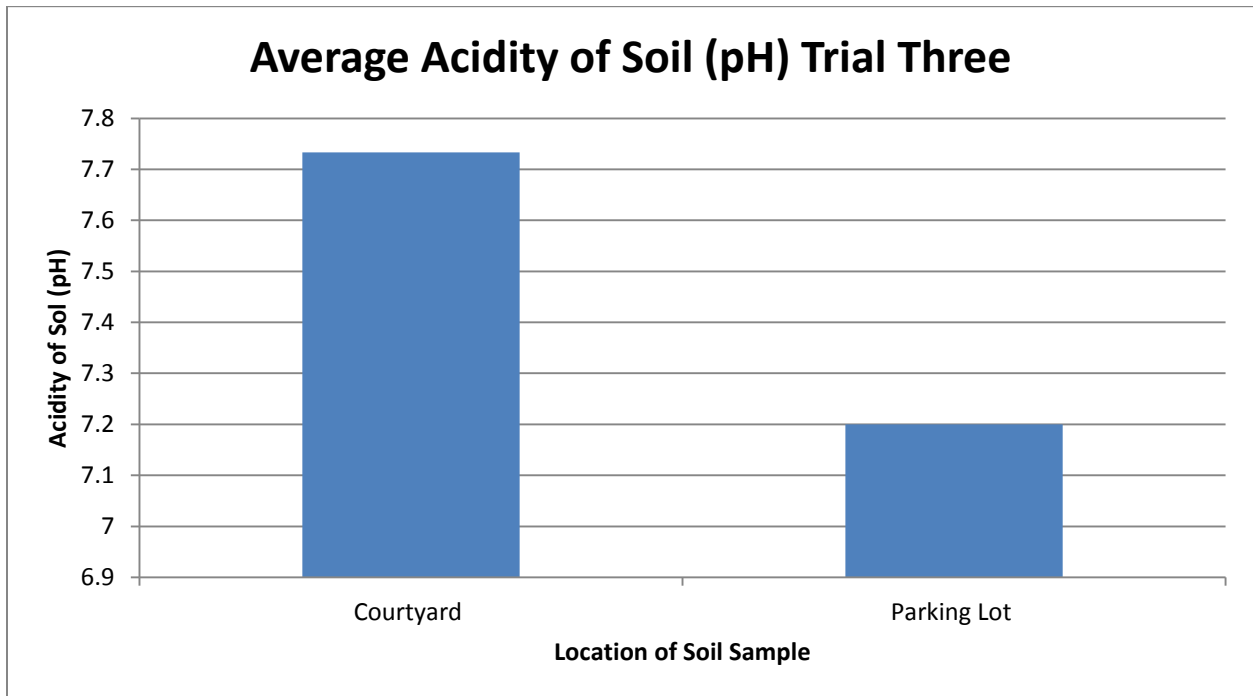
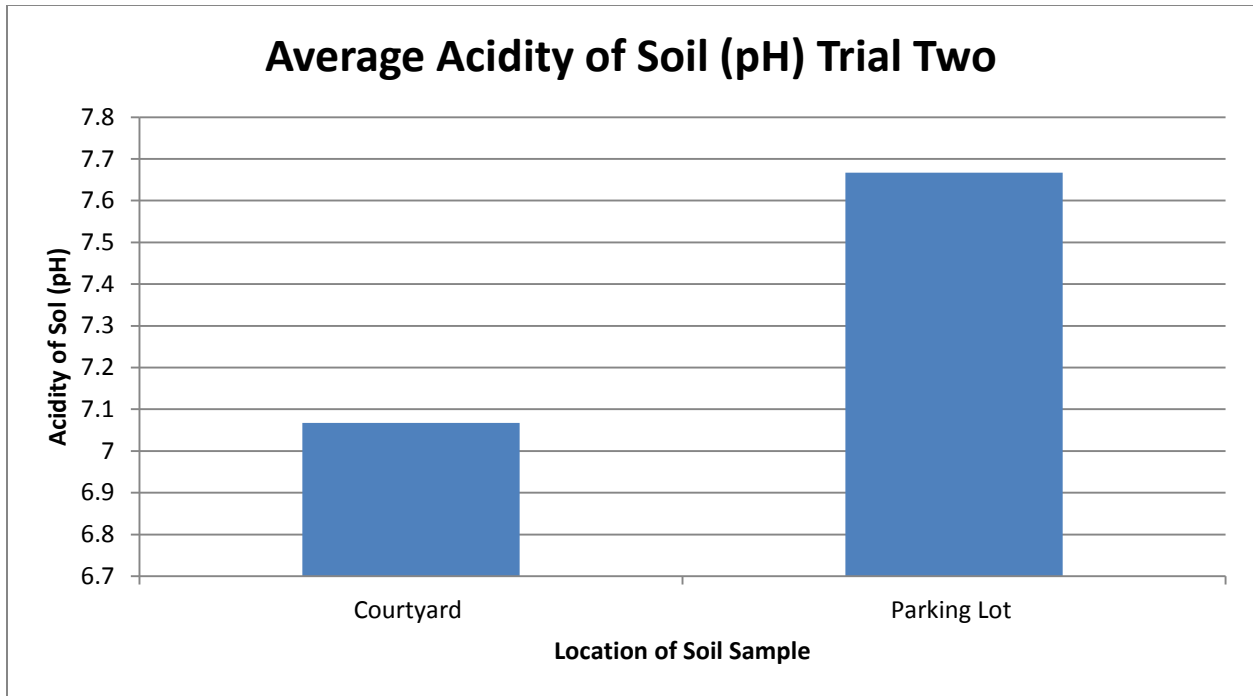
Trial	Location where Soil Samples are taken	Density of Yeast Per 1cc of Soil	Density of Mold per 1cc of Soil	Total Amount of Fungi per 1cc of Soil
Trial One	Courtyard One	3,000	2,000	5,000
	Courtyard Two	400	2,000	2,400
	Courtyard Three	10,000	2,000	12,000
	Parking Lot One	400	1,000	1,400
	Parking Lot Two	1,000	1,000	2,000
	Parking Lot Three	500	100	600
Trial Two	Courtyard One	2,000	3,000	5,000
	Courtyard Two	2,000	2,000	4,000
	Courtyard Three	10,000	10,000	20,000
	Parking Lot One	20,000	30,000	50,000
	Parking Lot Two	5,000	3,000	8,000
	Parking Lot Three	500	600	1,100
Trial Three	Courtyard One	1,000	1,000	2,000
	Courtyard Two	1,100	1,000	2,100
	Courtyard Three	4,000	10,000	14,000
	Parking Lot One	6,000	4,000	10,000
	Parking Lot Two	30,000	7,000	37,000
	Parking Lot Three	10,000	1,000	11,000

Average Density of Yeast and Mold in Soil

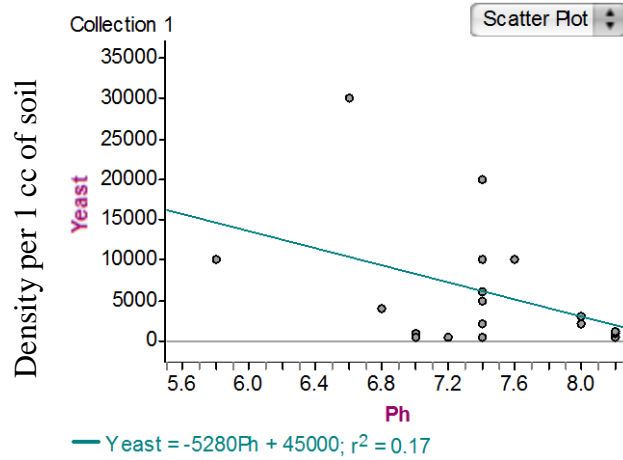
Trial	Location where Soil Samples are taken	Average Density of Yeast per 1cc of Soil	Average Density of Mold per 1cc of Soil	Average Total of Fungi per 1cc of Soil
One	Courtyard	4,466.667	2,000	7,666.667
	Parking Lot	633.333	700	1,333.333
Two	Courtyard	4,666.667	5,000	9,666.667
	Parking Lot	8,500	11,200	19,700
Three	Courtyard	2,033.333	4,000	6,033.333
	Parking Lot	15,333.333	4,000	19,333.333



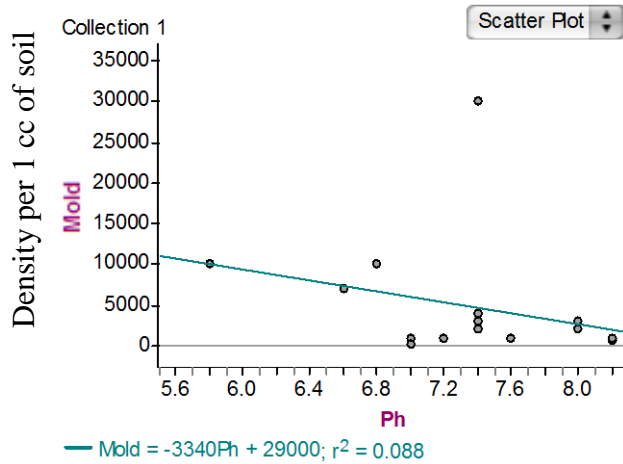




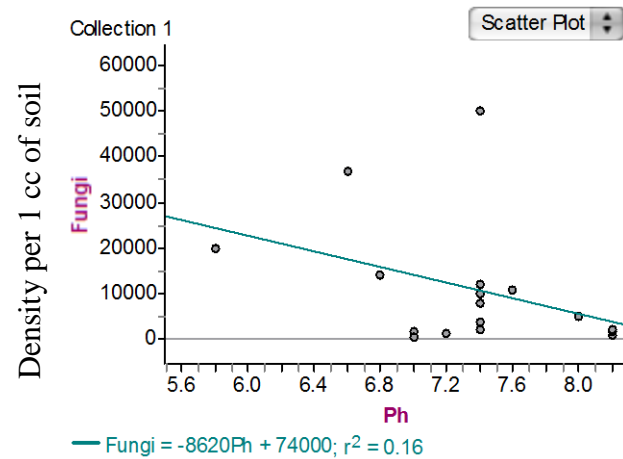
The Correlation between pH and Yeast



The Correlation between pH and Mold



The Correlation between pH and Fungi



V. Conclusion

Our hypothesis, “The acidity of car exhaust decreases the density of mold and increases the density of yeast in the soil” was disproven in this experiment. Based on our scatterplot graphs, we observed that the fungi experienced normal behavior in relation to the acidity (pH) of the soil. There was a negative correlation between the fungi and the pH levels which is what we would expect because the fungi prefer to live in pH ranges of 5-6 (Life Sciences n.d.) The slope of the line on the graph relating the density of yeast per 1cc of soil and the pH of the soil was -5,280 meaning that as the pH increases by 1, the density of yeast per 1cc of soil decreased by 5,280. The slope of the line on the graph relating the density of mold per 1cc of soil and the pH of the soil was -3,340, meaning that as the pH increases by 1, the density of mold per 1cc of soil decreased by 3,340. The slope of the line on the graph relating the density of fungi per 1cc of soil and the pH of the soil was -8,620 meaning that as the pH increases by 1, the density of fungi per 1cc of soil decreased by 8,620. Because these correlations follow the expected behavior of fungi in different pH levels, we are able to conclude that the research we found was not affected by any extreme circumstances in the ecosystem of Roland Park Country School; therefore we can use our data meaningfully to determine whether our hypothesis was right or wrong.

Our pH values also display the expected trend over the course of a few days. The average pH in the Courtyard on the first day we tested (trial one) was 7.6. The average pH in the Courtyard on the second day we tested (trial two) was 7.067 which is 0.533 less than the average pH for trial one. The average pH in the Courtyard on the third day we tested (trial three) was 7.733 which is 0.666 more than the average pH for trial two. The average pH in the Parking Lot on the first day we tested (trial one) was 7.067. The average pH in the

Parking Lot on the second day we tested (trial two) was 7.667 which is 0.6 more than the average pH for trial one. The average pH in the Parking Lot on the third day we tested (trial three) was 7.2 which is 0.467 less than the average pH for trial two. This fluctuation of pH over the course of a few days is the expected trend, which means that natural ecological processes are taking place in this environment's pH. This means that we should see a similar trend in our fungi levels; however, this is not the case.

In our first trial, the average amount of total fungi in the Courtyard was 7,666.667 per 1cc of soil and the average amount of total fungi in the Parking Lot was 1,333.333 per 1cc of soil. Therefore, there were 6,333.333 less total fungi per 1cc of soil in the Parking Lot than in the Courtyard. We would expect fewer fungi in the Parking Lot than in the Courtyard if the pH in the Courtyard was closer to the preferred range for fungi which is 5-6 (Life Sciences, n.d.) However, this did not occur. For trial one, the average pH level in the Courtyard was 7.6, and the average pH level in the Parking Lot was 7.067. This means that on average for the first trial, the Parking Lot soil was more acidic than the soil from the Courtyard by 0.533; therefore, the pH level in the Parking Lot was closer to the ideal range for fungi than in the Courtyard. This data disproves our hypothesis because the amount of total fungi in the Courtyard is greater than the amount of total fungi in the Parking Lot, which is the opposite of what we would expect. The fungi should not be thriving in the Courtyard environment based on the pH because fungi prefer to live in soil with a pH range of 5-6 and the pH in the Courtyard was 7.6. Instead, they should be thriving in the Parking Lot because the pH was closer to the ideal range.

In our second trial, the average amount of total fungi in the Courtyard was 9,666.667 per 1cc of soil and the average amount of total fungi in the Parking Lot was 19,700 per 1cc of

soil. Therefore, there were 10,033 more total fungi per 1cc of soil in the Parking Lot than in the Courtyard. For trial two, the average pH level in the Courtyard was 7.067, and the pH level in the Parking Lot was 7.667. This means that on average for the second trial, the Courtyard was more acidic than the Parking Lot by 0.6; therefore, the Courtyard's pH level was closer to the ideal living range for fungi than in the Parking Lot. This disproves our hypothesis because the pH in the Courtyard is closer to the ideal range for fungi than the Parking Lot, and the amount of total fungi in the Courtyard is less than the amount of total fungi in the Parking Lot.

In our third trial, the average amount of total fungi in the Courtyard was 6,033.333 per 1cc of soil and the average amount of total fungi in the Parking Lot was 19,333.333 per 1cc of soil. Therefore, there were 13,300 more total fungi per 1cc of soil in the Parking Lot than in the Courtyard. For trial three, the average pH level in the Courtyard was 7.733, and the average pH level in the Parking Lot was 7.2. This means that on average for the third trial, the Parking Lot was more acidic than the Courtyard by 0.533; therefore, the pH level of the Parking Lot was closer to the ideal living range for fungi than the Courtyard. This supports our hypothesis because on average, there are more total fungi in the Parking Lot than in the Courtyard, and the Parking Lot's average pH level was closer to the ideal living range for fungi, so this is the trend we would expect to occur. Although this trial supports our hypothesis, we cannot conclude that our hypothesis is correct because the first and second trial disprove our hypothesis. Because the fungi did not follow the expected trend, and the pH did, it is evident there must be a different aspect of the environment that is causing the fluctuation of the fungal density.

In the future, it would be interesting for us to investigate what other possible aspects of the environment could be causing the fluctuation of fungal density in the soil since the car exhaust did not have an effect. We realized that the plant life around our chosen soil locations differs from one location to the next. Based on this observation, it would be reasonable to perform further experiments in which we would test to see whether or not the type of plant life in a soil's environment affects the fungal densities in the soil.

Works Cited

Admin. (2011). pH Levels of Garden Soil. World Press.

Alan Watson Featherstone. (2014) Decomposition and Decay. Tree of Life. Forres Scotland.

http://www.treesforlife.org.uk/forest/ecological/decomposition_decay.html

The American Cancer Society. (2013) Diesel Exhaust. Oklahoma City: The American Cancer Society.

<http://www.cancer.org/cancer/cancercauses/othercarcinogens/pollution/diesel-exhaust>

Bickelhaupt, D(2014) Soil pH: What is Means. College of Environmental Studies and Forestry.

<http://www.esf.edu/pubprog/brochure/soilph/soilph.htm>

Cantoria, C. (2012) Learn about the Effects of Acid Rain on Plants and Trees. Bright Hub Inc.

<http://www.brighthub.com/environment/science-environmental/articles/63082.aspx>

Norrell, K. (2010) The Effects of Air Pollution on Plants and Animals. Bryn Mawr, Bryn Mawr

College.<http://serendip.brynmawr.edu/exchange/node/8250>

Stewart, R. (2008) Acid Rain and Acid Deposition. Department of Geosciences, Texas A&M University. <http://oceanworld.tamu.edu/resources/environment-book/acidrain.html>

UCMP (1998) Introduction to the Fungi. <http://www.ucmp.berkeley.edu/fungi/fungi.html>

United States Environmental Protection Agency. (2013) All About Carbon Dioxide. Washington:

DC: United States Environmental Protection Agency

<http://epa.gov/climatestudents/basics/today/carbon-dioxide.html>

United States Environmental Protection Agency. (2013) Sulfur Dioxide. Washington, DC;

United States Environmental Protection Agency

<http://www.epa.gov/airquality/sulfurdioxide/health.html>

The University of Western Australia.(2004) Soil Fungi. CRICOS.

<http://www.soilhealth.see.uwa.edu.au/components/fungi>

Washington State University. (2004). Tree Fruit Soil and Nutrition. Tree Fruit Research and

Extension Center. Wenatchee. WA.

<http://soils.tfrec.wsu.edu/webnutritiongood/soilprops/soilpH.htm>