I. Introduction

Soil, an ecosystem of many organisms, is fifty percent air and water, while the other fifty percent is composed of minerals and organic material (DoctorDirt). In one gram of soil there can be thousands to billions of bacteria. These microbes live in small spaces between the solid matter of the soil, while water occupies the smaller areas. Soil structure is the arrangement of soil particles into naturally occurring clumps called aggregates (Nardi). By adding organic matter to the soil through living creatures, mineral particles are bound together which greatly improves the structure of the soil. Soil that loses more plant and animal materials than it gains overtime loses its structure, pores and air spaces. A fresh supply of plant and animal material is needed to keep up with the constant decomposition of material by the microbes. These organisms stir up the soil by mixing mineral matter with fresh supplies of organic material, which helps keep the soil structure from crumbling. Soil is important because it controls water movement and the interaction between chemical substances and the atmosphere.

Nitrogen “is an essential component of DNA, RNA, and proteins, the building blocks of life” (Harrison), which is why the Nitrogen Cycle is so important. In the Nitrogen Cycle, bacterium converts nitrogen, which is needed by organisms, into a usable gas during various chemical processes. Bacteria are microscopic organisms whose single cells have neither a membrane-bound nucleus nor other membrane-bound organelles like mitochondria and chloroplasts. In soil, bacteria allow nitrogen to move “between the atmosphere, biosphere, and geosphere” (Harrison). Without soil, the Carbon Cycle, “a complex series of processes through which all of the carbon atoms in existence rotate” (ThinkQuest) as well as the Nitrogen Cycle, would not occur, thus jeopardizing life-sustainment on Earth.

Bacteria can be found everywhere; a spoonful of soil contains millions of bacteria (Rainis). There are four basic bacteria shapes: spheres, rods, spirals and filaments, all of which reproduce by dividing. These bacteria have the ability to chemosynthesize, or produce useable materials from inorganic materials by use of chemical energy. Bacteria could possibly have been the first organism on Earth that allowed the nutrient cycles to occur. There are two major groups of bacteria, gram positive and gram negative. Gram negative bacteria have an extra external membrane, which is better suited to protect against the environment and is a thousand times more resistant to antibiotics than gram positive bacteria (Ingraham). One specific type of gram negative bacteria is Cyanobacteria, also known as blue-green algae. This bacterium is a major contributor to ecosystems because of its ability to photosynthesize and convert nitrogen gas into a nutrient. Bacteria use an enzyme to convert dinitrogen gas to ammonia, a gas that is compatible with plants. The amount of bacteria is greater in soil with plant roots because the roots release organic compounds that some bacteria can use as energy (Nardi). Heterotrophs and autotrophs, two classifications of bacteria, are essential to plants because they provide nutrients that the plants need. Heterotrophs transform organic substances into plant nutrients while controlling the amount of organic matter in the soil and the amount of carbon dioxide released from the soil into the atmosphere. Autotrophs make their own organic substances from carbon dioxide and can transform inorganic matter and minerals into nutrients. The bacteria in the soil are essential to life because they continually repair the Earth's soil (Ingraham).

Car emissions are “waste products produced during the combustion process” (WiseGeek), a chemical reaction between fuel and an oxidizer, resulting in heat. The gases in car emissions include carbon dioxide, carbon monoxide, nitrogen, volatile organic compounds, nitrous oxides and water vapor. These vapors are emitted while the cars are running, while the engines are still hot and while a car is sitting outside on a hot day. The pollutants from this process are possibly responsible for global warming, which is the increase of the Earth's temperature. Ever since the middle of the 20th century, when the burning of fossil fuels and forests increased, global warming has been in effect. Car emissions are creating environmental problems that could potentially be irreversible. Carbon dioxide is a greenhouse gas, a gas trapped in the atmosphere, and is one of the main contributors to global warming. Volatile organic compounds "chemically interact with heat from sunlight forming ground level ozone" (Ezine Articles) while nitrous oxides chemically react with heat from sunlight causing smog. Acid rain can damage crops, forests and wildlife populations. Not only do the gasses from the car emissions affect the environment, they affect the human body as well. Nitrous oxides can cause mucus membrane problems, discomfort, asthma and permanent lung damage. Carbon monoxide interferes with proper levels of oxygen flowing through the bloodstream. The pollutants emitted by car emissions have a great effect on human health, so they may have a great effect on the microorganisms living in nearby soil.

People who work, live, attend school or drive in or near school districts have a risk of getting sick due to the car emissions caused by the traffic passing in and out of the area. The car emissions pollute the air, lowering the air quality (Nutramed). Motor vehicles release millions of tons of pollutants into the air every day. The average American citizen pollutes most while driving. Carpool lines consist of many motor vehicles which can be damaging to the development and well being of the students and whoever is nearby. The worst case scenario is a traffic jam or sitting in a carpool line with engines idle; "everyone sitting in a traffic jam is getting poisoned" (Go-Hybrid). The New York Times wrote a story on a city in Italy that decided to cut back on car emissions to prevent potential health problems and global effects.

Instead of using a bus system to get students to school, students were escorted by adults and walked to school every day. This small change decreases the amount of air pollution from car emissions. The Roland Park Country School community has about 648 cars transporting students to and from school five out of the seven days in a week, during nine months of the year (Barrs). During afternoon pick up, cars can be waiting outside with the engines running for as long as thirty minutes, emitting car emissions all the while. If the gases can affect humans, they may affect other surrounding organisms. If bacteria in the soil, which are needed in order for survival of all organisms, are affected, any life form is in danger of serious health complications. In this lab, we hope to determine if car emissions have an effect on bacterial growth and development in the soil by measuring the density of bacteria near the carpool line and farther away. In order to do this we have to test two plots of land, one close to car emissions, and one farther away from the car emissions. After we get the soil samples we will then be able to test and compare the number of bacteria in the soil. If our hypothesis, that the bacteria count will be higher in the soil farther away from the car emissions, is correct we will begin thinking of ways to reduce the amount of car emission near our school. Many ideas include more opportunities to walk and more encouragement of carpooling. The results of our experiment should positively affect our school.

II. Hypothesis- The car emissions from the carpool line of RPCS will negatively affect the bacteria density in the soil.

III. Procedure

A. **Independent Variable**- presence of car emissions

B. **Dependent Variable-** bacteria density in the soil

C. Negative control- soil that has not been exposed to car emissions

D. Controlled Variables

- **i.** Size of the plots of land
- **ii.** Location of the plots of land
- **iii.** Amount of soil tested
- **iv.** Amount of time soil is left to sit
- **v.** Human traffic
- **vi.** Slope of land
- **vii.** Sun light
- **viii.** Vegetation
- **ix.** How much chemicals and liquids we use to test the bacteria
- **x.** Date soil was sampled and tested

E. Procedure

1. Find a grass plot near the carpool line, make three squares 30cm x 30cm right next to each other using flags (use 8 flags, one on each corner of the squares)
2. Find a grass plot away from the carpool line which has the same sunlight and slope as the plot near the carpool line, make squares exactly like those described in step 1
3. Using a twisting action, embed soil core sampler into 1st mark in first plot
4. Twist the core sampler 360 degrees to isolate sample
5. Remove soil core by pulling straight up
6. Using gloves, place soil sample in a clean plastic storage bag for transport to the lab

7. Repeats steps 3-6 for 2nd and 3rd marks of the first plot, place all the soil from plot 1 in the same bag

8. Label bag baggie 1

9. Repeat steps 3-8 for plots 2-6, naming the baggies accordingly

10. Find the GPS coordinates for the Control plot and the Car Emissions plot

11. Find all necessary chemicals (if needed)

12. Put on gloves and goggles, safety is important

13. Use a clean, new transfer pipette to add 10 ml (of sterile water) to a 15 ml culture tube. Label the tube “100.”.

14. Use the same pipette to add 9 ml (of sterile water) to a second 15 ml culture tube. Label the tube “10-1.”

15. Repeat step 14 three more times for three additional 15 ml culture tubes, only label them “10-2,” “10-3", and “10-4" respectively.

16. Place 1 cc of your soil sample into the “100”culture tube.

17. Cap the tube and shake vigorously.

18. Using a new clean pipette, remove 1 ml of the soil/water mixture from the “100” tube and place into the “10-1” tube.

19. Cap and shake vigorously.

20. Using the same pipette in step 18, remove 1 ml of the soil/water mixture from the “10-1” tube and place into the “10-2” tube.

21. Cap and shake vigorously.

22. Using the same pipette in step 18, remove 1 ml of the soil/water mixture from the “10-2” tube and place into the “10-3” tube.

23. Cap and shake vigorously.

24. Using the same pipette in step 18, remove 1 ml of the soil/water mixture from the "10-3" tube and place into the "10-4" tube.

25. Repeat steps 13-24 for soil from baggies 2-6

26. You should now have a total of 30 culture tubes.

27. Plate 100 µl samples from the 4th and 5th tubes of each soil sample(dilutions 10-3 & 10-4) onto their own separate labeled petri plates containing nutrient agar (NOTE: on your first sample, plate ALL 5 dilutions to determine which two dilution values will give you the best data; dilutions 10-3 & 10-4 are only the most probable ones).

28. You should now have 12 petri plates

29. Allow to grow for 48 to 72 hours.

30. Examine each of the plates for individual bacteria colonies and choose the plate with the fewest colonies (but at least 5) for each soil sample to make your estimates of the number of bacteria in the original 1 cc soil sample using the following formula:

 # Microbes in 1 cc of soil = # Colonies on sheet x 10^2 x $10^{|\text{dilution \# at which these colonies were found}|}$

31. If there are not individual colonies but still a "lawn" at the 10-4 dilution, repeat the dilution adding a 5th (10-5) & 6th (10-6) dilutions, etc. as necessary until individual colonies are observed.

F. Equipment

i. 4 10ml serological pipettes

ii. 2 Glass stirring sticks
iii. 30 15ml culture tubes with caps
iv. 6 l-cc cup scoops
v. Sterile water
vi. 12 Nutrient agar plates
vii. 6 Disposable dropper or pipettes
viii. 2 P200 micro-pipette with 12 tips
ix. Soil core samplers
x. 6 Plastic bags
xi. GPS
xii. Camera/cell phone
xiii. Gloves
xiv. Goggles
xv. Sharpies
xvi. 16 flags
xvii. Colored tape
xviii. Paper towels
xix. Bleach water
xx. Beaker for contaminated 1-cc cup scoops
xxi. 3 culture tube stands
xxii. 2 magnifying glasses

IV. Data Table and Graph

A. Data Table

Soil Samples	Dilution Number	# of bacteria colonies	Bacteria Count	Averages
Control 1	10^{-3}	0	0	7.33×10^6
Control 2	10^{-4}	16	16,000,000	
Control 3	10^{-4}	6	6,000,000	
Car Emission 1	10^{-3}	25	2,500,000	2.67×10^6
Car Emission 2	10^{-3}	16	1,600,000	
Car Emission 3	10^{-3}	39	3,900,000	

<u>Equation</u>

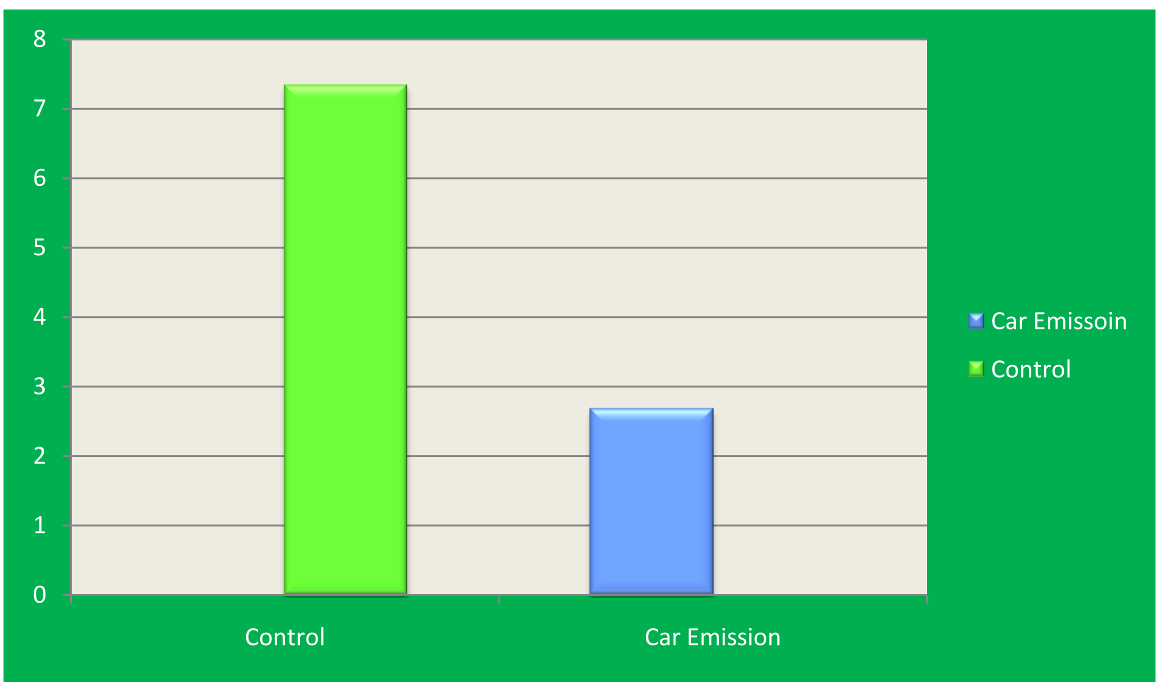
Microbes in 1 cc of soil = # Colonies on sheet x 10^2 x $10^{|\text{dilution \# at which these colonies were found}|}$

B. Graph

<u>Number of Bacteria vs. Location of Soil</u>

Number of Bacteria

(in millions)



Type of soil (Car Emission Effected vs. Non Car Emission Effected)

V. Conclusion

Through our in depth studies and observations obtained from our experiment, we concluded that car emissions do in fact affect bacterial population in soil negatively, thus proving our hypothesis correct. Our experiment showed an enormous decrease in the amount of bacteria which resided in soil near the carpool line of Roland Park Country School. In our data the bacteria population in the non car emission effected soil was five million more than that of the car emission effected soil. Our observations made previous to our experiment, on our plots led us to believe our hypothesis was correct, before we even began testing. The grass located near the carpool lane was dried up and dead looking while the plots near the Fitness Center, which were farther away from the carpool line, were green and healthy. Between the observations we obtained from the plot itself and the bacteria population; it is safe to conclude that car emissions are the reason for a decline in the health of microorganisms.

There are many components in car emissions that may have been the cause of the decrease in the bacterial population. One component could possibly be the carbon monoxide in car emissions. Carbon monoxide, "produced by the incomplete burning of various fuels" (CPSC), is blamed by many researcher's for global warming and the poisoning of organisms. From our experiment we cannot conclude exactly what component in car emissions is affecting the bacteria, but it is safe to conclude that car emissions are killing the bacteria. Ultimately, the effects of car emissions could be irreversible, not only for humans but for the bacteria in the soil.

Although cutting out transportation all together is unrealistic, people can make some changes in order to make a smaller footstep on the planet. Whether it is riding a bike, buying an energy efficient car or using public transportation, anything which limits the amount of car emissions is beneficial for the health of the planet. In the Roland Park community, cars idle in the carpool line for many minutes at a time waiting for the children, which is continuously poisoning the air and killing the bacteria in the soil. If every family carpools with another, the number of cars, 648, would be cut in half. This slight change could make a large impact on the health of the soil in Roland Park Country School. Planet earth and its inhabitants are all important to the survival of humans and people should make an effort to protect them. If many positive changes are made to Roland Park Country School's care for the environment, it can begin to nurse the planet back to health and set an example for other schools. It is time to make drastic changes for the health of the planet.

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