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Ecology Report

There are many living organisms that exist in the earth's soil. One of these living organisms in the soil is bacteria, a type of microbe. The number of bacteria that are living in the soil will affect how well the bacteria will function and work within the soil. The level of bacteria in the soil is affected by many factors, one of which is the presence of fertilizer and/or the amount of fertilizer applied to the soil. When put into soil, fertilizer adds chemicals and nutrients such as nitrogen. Soil containing fertilizer may run down a hill due to erosion, thus spreading the fertilizer over the other soil. The run-off containing fertilizer will spread to regions of soil that do not need fertilizer. This causes the chemicals in the fertilizer to be transferred to these regions where the soil does not need fertilizer. Excess fertilizer can be harmful to the bacteria within the soil because it negatively tampers with the nitrogen level of the soil. Therefore, the increase of the level of nitrogen in the soil will ultimately alter the level of bacteria in the soil.

The bacteria level has a profound affect on the soil because bacteria make up the majority of the microbe population within the soil. Although bacteria are found throughout the soil, they are most abundant in or adjacent to the roots of plants. One type of bacteria is known as actinomycetes. They are thread-like filaments that are responsible for the recognizable scent of freshly exposed, moist soil. They effectively break down tough substances in the soil. Another type of bacteria, known as free-living bacteria, fix atmospheric nitrogen and add it to the soil nitrogen pool (Soil Organisms, 2003). The species of bacteria are either heterotrophic or autotrophic, but are always unicellular.

Courtney Amelung
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The bacteria have many jobs and roles in the soil such as decomposing organic

matter and lessening chemical elements in the soil. They consume organic matter and

"especially simple carbon compounds" (Soil Scientist, 2002). They also cycle nutrients,

suppress disease, and produce substances that help bind soil particles into small

aggregates which affect the soil's water movement (Soil Ecology: Background

Information, 2003). One of the functions of the bacteria is the transformation

ammonium to nitrite through symbiotic associations with plants or independently, a

process called enzymatic transformation. Another extremely important job is the

conversion of ammonium into nitrate and nitrate into nitrogen gasses (Soil Scientist,

2002). The ammonium is made of hydrogen and nitrogen. The ammonium in the soil

binds to the soil and organic matter and is changed by the bacteria into nitrate during the.

nitrogen cycle. The nitrogen cycle is important because it produces nitrate; nitrate makes

proteins, which are essential to any organism because they are needed for "body structure

and function". Nitrates are necessary to create nucleic acids, which then create DNA and

RNA (The Nitrogen Cycle, 2003). Without the nitrogen cycle, the plant would not be

able to make proteins to create chemical reactions; therefore it would not do the four

tasks: regulating the environment, synthesizing chemicals, reproduction, transforming

energy.

The bacteria play an important part in the nitrogen cycle and therefore are

beneficial to the plants. The nitrogen cycle is the process through which nitrogen is

recycled in the environment. Atmospheric nitrogen (N_2) is absorbed into the ground,

where it is transformed into nitrate (NO_3-) by nitrogen-fixing bacteria (Campbell,

Williamson, Heyden, 2004). Nitrate will then be used by plants as an important part in

Courtney Amelung
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Lisa Taber
May 27, 2004

making proteins (Nitrates, 2003). If plants are not eaten by a consumer, then they will

decompose, sending nitrogen into the ground, where saprovores (decay organisms) turn

the plant's protein into a form of nitrogen called ammonium (The Nitrogen Cycle, 2003).

When a consumer eats the plant, the nitrate from the plant is transformed into ammonium

(NH_3), a byproduct of protein metabolism, within its body. The ammonium exits the

animal's body in the form of feces (Nitrates, 2003), or it can also enter the ground when

dead animals decompose, through the same process the changes plant's protein into

ammonium. Once the ammonium is absorbed back into the ground, it undergoes

nitrification. In nitrification, bacteria (like *Pseudomonas*, *Bacillus licheniformis*,

Paracoccus and others) transform ammonium back into atmospheric nitrogen (The

Nitrogen Cycle, 2003). Ammonium transforms back into atmospheric nitrogen because

if too much ammonium was left in the soil, then it would kill everything.

However, plants can grow better in soil where fertilizer is added to the soil

because it is applied to areas where there is a deficiency of chemicals like ammonium.

Fertilizer has a beneficial impact on soil by adding nutrients. However, too much

fertilizer can harm plants. If the soil has sufficient nitrogen, then adding more nitrogen

will harm the plant because it will damage the roots and prevent the plant from soaking

up any more nitrogen from the soil through the roots. While some fertilizers provide the

soil with mainly nitrogen, others provide only nitrogen, phosphorus, and potassium, and a

few others provide all macronutrients and micronutrients (Smith, 2000). Fertilizers don't

just vary in ingredients. There are many different types of fertilizer; one type is the slow-

release fertilizer. Slow- release is a very effective type of fertilizer containing a coating

that reduces the solubility of the nutrients in the soil. It is intended to allow fertilizer

Courtney Amelung
Claudia Randrup
Lisa Taber
May 27, 2004

nutrients to dissolve slowly in the soil (Motavalli, 2004). The rate of the nutrient release depends on temperature, moisture, and type of coating. Another type of fertilizer is organic fertilizer. The nutrients of an organic fertilizer must be converted to "inorganic" to enable them to be used by the soil. Organic fertilizer is valuable to the soil because it also adds to it organic matter, which benefits the soil through its natural slow-release capacity. One downfall to fertilizer is it contains salt, which will destroy the soil and plants if too much is applied. Fertilizer should only be applied if a plant needs a specific nutrient (Messick, 1997).

When fertilizer is spread to regions that do not need it, chemicals like the nitrogen (specifically nitrates) in fertilizers are spread inadvertently through run-off and may harm the soil and everything that grows in the soil. Heavy rains and an area which can experience erosion can cause soil to run down a hill, spreading into streams and other bodies of water, or areas of land, like fields. If chemicals are mixed with the soil, like a fertilizer, then those chemicals are also spread through run-off. This creates a potential problem in our school's environment. If the school uses fertilizer to encourage plant growth on campus, then the chemicals in the fertilizer (like nitrogen and salt) can be spread downhill, to the backwoods area because of high winds of heavy rain. Therefore, areas that do not need fertilizer are damaged by run-off that has fertilizer in it.

The purpose of our experiment is to explore the relationship between fertilizer run-off and bacteria levels. We will discover if, when fertilizer runs-off down a hill due to rain or high winds, bacteria levels are affected. To determine whether bacteria levels are affected, we conducted serial dilution tests as well as tests to determine the presence of ammonium, a key ingredient in fertilizer, on soil samples from different plots on a hill.

Courtney Amelung
Claudia Randrup
Lisa Taber
May 27, 2004

This will allow us to infer a relationship between the level of fertilizer, depending on the

degree of run-off, and the level of bacteria in the soil. Fertilizer may upset the balance of

the soil by altering the level of chemicals. Fertilizer adds chemicals like nitrate and

ammonium to the soil, and these chemicals affect the level of bacteria. The bacteria level

is altered by the chemical imbalance. With our experiment, we will discover how

drastically the addition of fertilizer will affect the bacteria level.

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May 27, 2004

Procedure

I. Problem: What happens to the bacteria density of the soil as fertilizer runs down the school's campus from a higher elevation to a lower elevation?

II. Hypothesis: The fertilizer that runs down to a lower elevation will increase the bacteria density.

III. Procedure:
Independent: location of soil sample on the hill at Roland Park Country School
Dependent (1): density of bacteria in soil
Dependent (2): concentration of ammonium in soil
Negative control: sample of soil from the very top of the hill (where we know fertilizer is present)
Control variable list: size of the soil sample, type of fertilizer used, incline of hill, distance between soil samples, depth at which soil sample is taken, amount of water, size of cc scoop, size of culture tube, amount of time Petri dishes are exposed to room temperature, type of Petri dish (LB), taking the soil samples on the same day at the same time, testing each sample for nitrogen and bacteria at the same time, which nitrogen test is used (LaMotte Kit)

Step-by-Step:
1. Take three soil samples at the top of a hill (This will be Plot #1- N: 39.35865 W: 076.63737) using a soil core sampler with a 2 centimeter diameter and a height of 15 centimeters Put each sample into separate plastic bag, and label the bags. Label each bag with the location (plot #) and sample (1, 2, or 3).
2. Using the same method take three samples from each the other four plots. (Make sure that you take all samples from all plots at the same time) (Plot 2: N: 39.35849 W: 0.76.63785) (Plot 3: N: 39.35848 W: 076.63772) (Plot 4: N: 39.35848 W: 076.63781) (Plot 5: N: 39.35857 W: 076.63799) Note that plot 1 is located at the top of the hill; the next plots are 4.2672 meters downhill from the one before it, etc.
3. sample 1 from plot 1testing for the presence/absence of nitrogen (ammonium) using the procedure from the LaMotte STH-4 Outfit at the same time you test it for bacteria
4. The following procedure is how to test for the density of soil bacteria using the serial dilution process.

> 43a. Place 1 cc of soil sample into a culture tube containing 10 ml of sterile water; cap the tube and shake it vigorously.
> 43b. Using a serological pipette, remove 1 ml of the soil/water mixture and place into a fresh culture tube.
> 43c. Add 9 ml of fresh sterile water to this second tube; cap and shake vigorously.
> 43d. Repeat step 4b using the second tube and then repeat step 4c
> 43e. Continue step 4d with each additional tube until you have diluted the original soil/water mixture a minimum of four times (a 10^{-4} dilution). You should now have a total of five culture tubes.
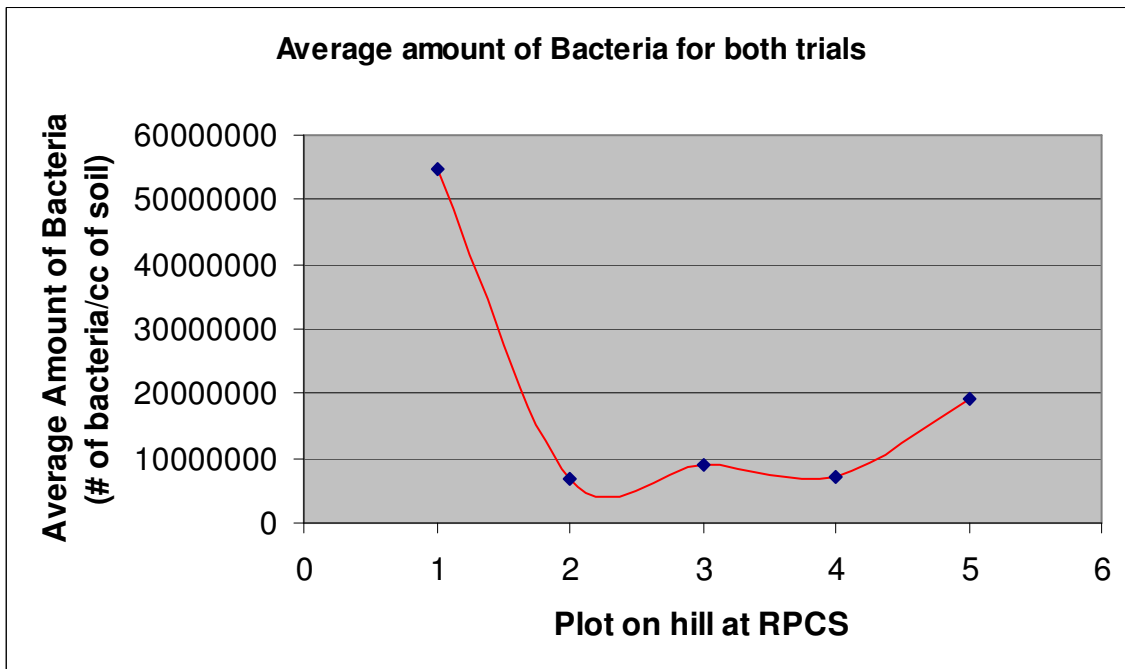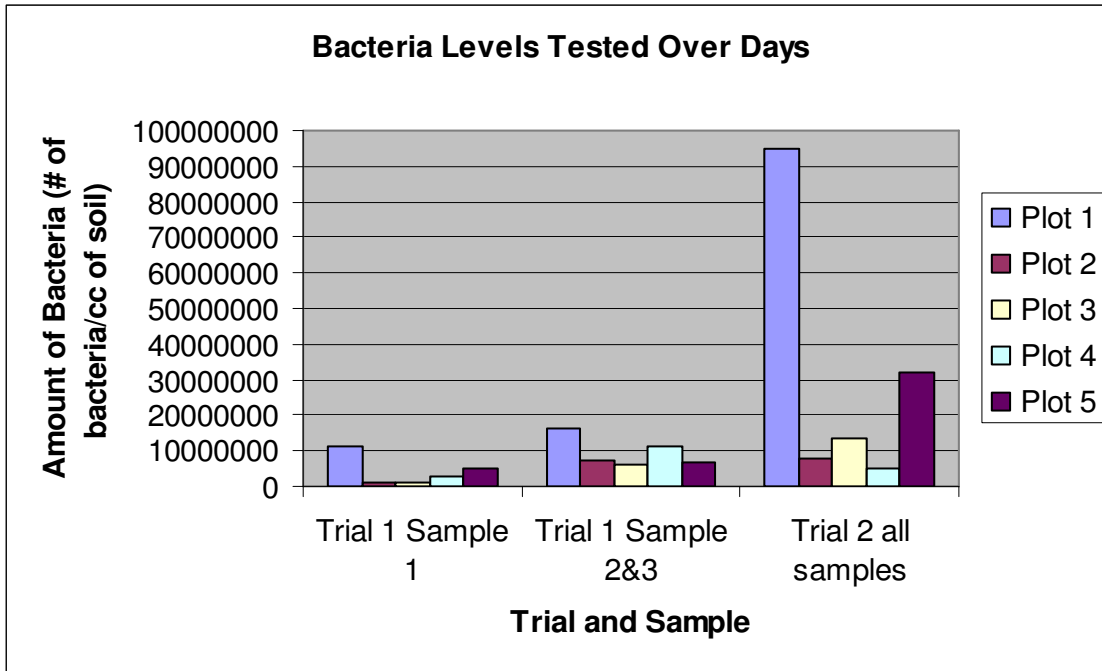
Courtney Amelung
Claudia Randrup
Lisa Taber
May 27, 2004

4 Place 100 µl samples from the 4th and 5th tubes (dilutions 10^{-3} & 10^{-4}) onto their own separate, individual LB Petri plates filled with nutrient agar, spread the bacteria sample in the dish using a sterile spreading rod and allow to them incubate at room temperature for three nights. Make sure you label each plate with the plot #, soil sample #, which dilution is used, and the trial #.

5. Repeat step 4 for sample 1 of the rest of the plots. (Note: You ultimately want to perform the two processes on all the same samples of all the plots at the same time: sample 1 of plots 1-5 at the same time, sample 2 of plots 1-5 at the same time etc.)

6. Repeat steps 4 and 5 for the next two samples of all five plots.

7. After letting the Petri dishes sit out for three nights, examine each of the plates for individual bacteria colonies and choose the plate with the fewest colonies (out of the ones that are pairs) to make your estimates of the number of bacteria in the original 1 cc soil sample (number of colonies $* 10^2 * 10^{|\text{dilution factor}|}$ = # of bacteria in the cc of soil). Use this equation to find the actual amount of bacteria in the original soil scoop (1cc). Record your results for all of your samples.

8. Repeat steps 1 – 6 one more time. (You will be completing another whole experiment; therefore upon completion you will have 2 trials of the experiment).

Courtney Amelung
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Lisa Taber
May 27, 2004

IV. Data and Analysis

Plot 1 (top of hill)	Sample	# of bacteria/cc's of soil	Concentration of ammonium in soil (ppm)
Trial #1	A	11000000	0
	B	6000000	0
	C	27000000	0
Trial # 2	A	244000000	0
	B	11000000	0
	C	29000000	0
Plot 2			
(4.2672 meters downhill from Plot 1)			
Trial #1	A	1000000	0
	B	7000000	0
	C	8000000	0
Trial #2	A	7000000	0
	B	3000000	0
	C	14000000	0
Plot 3			
(4.2672 meters downhill from Plot 2)			
Trial #1	A	1000000	0
	B	7000000	0
	C	5000000	0
Trial #2	A	2000000	0
	B	7000000	0
	C	32000000	0
Plot 4			
(4.2672 meters downhill from Plot 3)			
Trial #1	A	3000000	0
	B	12000000	0
	C	11000000	0
Trial #2	A	1000000	0
	B	1000000	0
	C	14000000	0
Plot 5 (bottom of hill)			
Trial #1	A	5000000	0
	B	5000000	0
	C	9000000	0
Trial #2	A	6000000	0
	B	72000000	0
	C	18000000	0

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Bacteria Levels Tested Over Days

Average amount of Bacteria for both trials

Courtney Amelung
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Lisa Taber
May 27, 2004

Ammonia Levels vs. the Amount of Bacteria

Courtney Amelung
Claudia Randrup
Lisa Taber
May 27, 2004
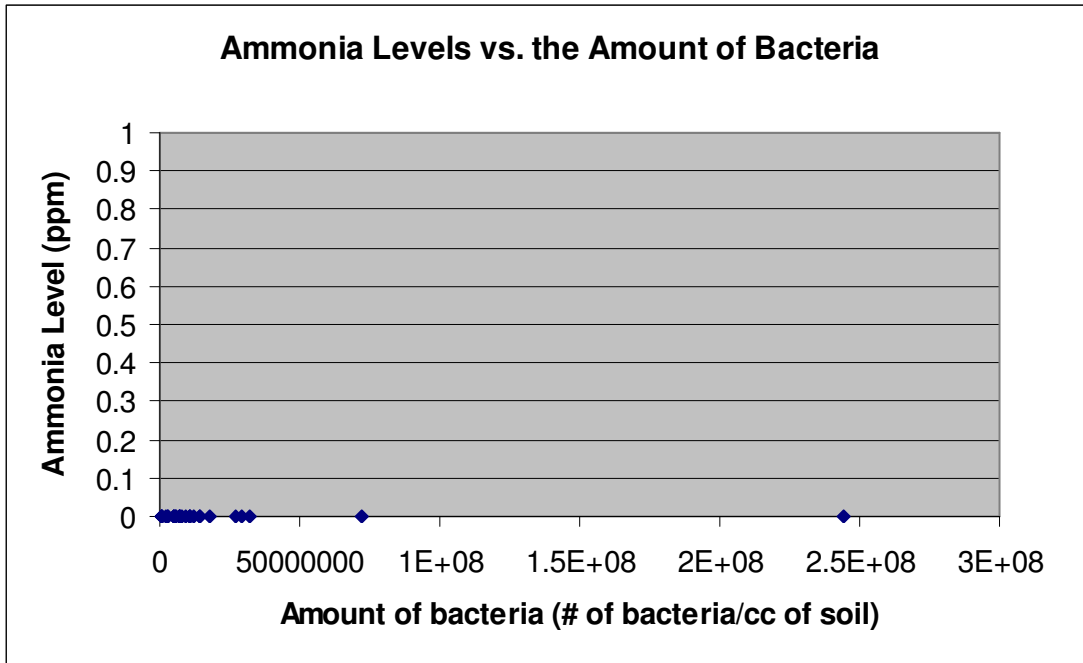
V. Conclusion

After performing the experiment, we have not acquired enough information to prove or disprove our hypothesis. We cannot determine if our hypothesis was correct from our data. We could not determine the presence of fertilizer by testing for ammonium in the soil. Therefore, we cannot make any conclusions from our data regarding the relationship between presence of fertilizer and level of bacteria.

We tested for ammonia to determine the level of fertilizer. Testing for ammonium was our second source of error. We discovered, after testing, that the presence of ammonium was not an accurate way to test for the presence of fertilizer. Soon after the fertilizer is applied to the soil, the ammonium is used. Therefore, there would be none remaining from the fertilizer for us to find, regardless of how much fertilizer was applied. Instead of testing for ammonium, we should have tested for nitrate to determine the presence/absence of fertilizer. Nitrate remains in the soil, unlike ammonium. The level of nitrate in the soil would be able to give us enough information to determine the level of fertilizer within the soil. We did not have the appropriate information to be able to infer a relationship between the level of fertilizer and the level of bacteria within the soil. The ammonia level was exactly zero for all samples, trials, and plots throughout the experiment.

We also conducted serial dilutions to find the level of bacteria in the soil. When examining the average amount of bacteria for both trials, the largest amount was found in Plot 1, at the top of the hill. The average amount of bacteria for Plot 1 was 54666667 bacteria (# of bacteria/cc of soil). The average amount for Plot 2 was 6666667; Plot 3 was 9000000; Plot 4 was 7000000; and finally Plot 5 was 19166667. It is interesting that

Courtney Amelung
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May 27, 2004

even when we tested different samples on different days, Plot 1 continuously had the

larger amount of bacteria. We tested Trial 1 Sample 1 on a different day then we tested

Samples 2 and 3. The results for Trial 1 Sample 1 showed us that Plot one 1 had the most

bacteria, with 11000000 bacteria in that sample. Continuing down the hill the amount of

bacteria (in # of bacteria/cc of soil) was: 1000000 (in Plot 2), 1000000 (in Plot 3),

3000000 (in Plot 4), and 5000000 (in Plot 5). Another source of error we had in our

experiment is that we only allowed the Trial 1, Sample 1 Petri dishes incubate at room

temperature for one night while we let Petri dishes of the remaining samples of Trial 1

and all samples of Trial 2 incubate at room temperature for three nights. This is a

potential reason we received the results we did for Trial 1 Sample 1. We tested Trial 1,

Samples 2 and 3 at the same time. When averaged the data, the total amount of bacteria

is consistently larger in Plot 1. At Plot 1, the average amount for both samples is

16500000 per cc of soil. The average of Plot 2 was 7500000, Plot 3 was 6000000, Plot 4

was 11500000, and Plot 5 was 7000000. After letting them incubate at room temperature

for three nights, Plot 1 had the largest amount of bacteria. The average of all trials for

Plot 1 was 94666667 bacteria.

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May 27, 2004

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