Anne Brockmeyer, Lauren Sless,
Racquel Tillman and Kerry Weir
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BACKGROUND

Two kinds of runoff that affect our soil today are erosion and chemical runoff. Runoff is damaging to the soil and the environment, altering the form and ability of organisms in the ecosystem to perform their 4 tasks to survive. In erosion, the powerful water moves the soil and washes away important organisms, which alters the environment. Chemical runoff carries chemicals over the soil, causing the chemicals to absorb into the ground. This damages the necessary fungi and other organisms needed in the soil. Hence, in our project, we decided to research the difference in biodiversity of fungi in the soil caused by the chemical runoff of fertilizer. We expect the fertilizer will decrease the biodiversity of fungi as it is added to unaffected soil. We predicted this outcome because the fertilizer will add to the soil an excess amount of nitrogen and phosphoric acid, bathing the fungi in their own byproducts. We believe this will cause the diversity of fungi to eventually diminish in the ecosystem.

Runoff in the world today causes many problems with the ground. The water from rain or general precipitation falls from the sky and lands on the ground. The water either seeps into the ground or, when it reaches hills, falls down the slope, taking valuable microorganisms and nutrients with it to the bottom of the hill. Runoff carries nitrogen and phosphoric acid in fertilizer, which seeps into the soil, degrading the soil's quality. This chemical-rich runoff goes into the streams and lakes. The harmful chemical runoff inherent in urban life is much greater because of the excess concrete and asphalt that is in the cities. This is because there is less soil to absorb the chemicals, causing the runoff to

flow directly over the pavement, eventually collecting in excess amounts. However, all runoff, no matter how excessive, results from a group of common causes.

It is easy for us to create runoff because there are many characteristics that produce/affect runoff. Runoff is affected by: land use, vegetation, soil type, and drainage area. Other affects are: basin shape, elevation, Topography, drainage patterns, and bodies of water in the basin (Nevada Division of Water Planning, 2003). Toxic chemicals consist of pesticides, fertilizers, motor oil, gasoline, antifreeze and other things. In order to get to the waterways these chemicals must travel through the ground (Nevada Division of Water Planning, 2003). The chemicals that are most commonly washed away due to runoff are fertilizers and pesticides. Pesticides kill bugs and insects, and damage ecosystems, creeks, rivers, lakes and oceans. Fertilizers help gardens grow, and add nutrients to water causing excessive plant and algae growth. However, fertilizer may damage the quality and amount of fungi in the soil. As these chemicals travel through the ground they inevitably affect each type of fungi living in the ground.

Two common fungus phyla are *Zygomycota* and *Ascomycotina.* Zygomycetes (such as rhyzopus) use zygospores to reproduce, and are very commonly found in terrestrial habitats such as soil and decaying leaves and plants. Rhizopus, specifically, is classified as a contaminant and plant pathogen when describing its role in the environment and soil. *Ascomycetes* (such as *Aspergillus*, *Arthrinium, Aureobasidium, Bipolaris, Chaetomium, Curvularia, Fusarium, Penicillium, and Ulocladium*) are found in rotting wood and leaves; mostly in terrestrial environments. Many of these examples are found in decaying plant and organic material, breaking down dead plants and returning the nutrients to the soil (Dooley, 2002). Most of the other species are

considered plant contaminants and pathogens, causing harm to some vegetative material around them depending on the environment. Even more produce mycotoxins (toxins released from most molds) (EMSL Analytical, Inc., 2003). Mycotoxins are almost all cytotoxic, and disrupt cell processes in other organisms. They make molds formidable competitors with the rest of the organisms in the environment (Mold-Help, 2003).

However, despite the various effects that fungi have on the immediate environment in which they live, they are part of a much bigger picture. Fungi break down any decaying organic matter, producing the required chemicals to sustain the life processes, and therefore life itself. This entire process will be explained in detail later, but basically, the fungi return every nutrient to the soil that they consume plus more. They benefit the environment by distributing the needed reactants to nearby vegetation for the production of energy, food (photosynthesis), and environment regulation (Brock, 2005).

Although yeast and mold are both fungi, they each have a different effect on soil. They both fall under the same category of fungi, which includes mildews, yeasts, large mushrooms, and mold. When yeast is added to the soil of a dying plant, the nitrogen and phosphates, found in the soil, are added to the plant's soil, jumpstarting the production of energy and food. Yeast consumes sugar for energy and releases carbon dioxide. This adds to the carbon cycle, which we will talk about later. Not only does the carbon add to the carbon cycle but it also helps in the process of photosynthesis. The carbon dioxide goes into the leaf and becomes carbohydrates for the leaf, with energy from the sun. When the sun is absorbed by the chloroplasts, chlorophyll produces a color change in the leaf because the yeast are feeding the microbes (Paskvan, 2002). After this

oxygen is released from the plant and goes into the air. Yeast increases the microbial populations and activity in your soils; however, mold breaks down organic materials. Mold is typically found in moist, damp areas where humans exist. The more moisture there is in the soil the faster the rate is at which mold reproduces. While yeast creates oxygen through photosynthesis, mold takes care of plants and other organisms after they die. Yeast and mold are the most common fungi found in soil and are very different in their purpose with soil.

A major role yeast and mold population's play in the soil is in biogeochemical cycles. A biogeochemical cycle is the movement or cycling of matter through a system (Apache, 2003). The biogeochemical cycles are: geological cycle, carbon cycle, carbonate- silicate cycle, nitrogen cycle, and phosphorus cycle. Related to soil, the most important cycles are: geological cycle (hydrologic), carbon cycle, nitrogen cycle, and phosphorous cycle. In our case, we are focusing on the carbon, nitrogen, and phosphorus cycles.

The mold and yeast are part of the carbon cycle because they release carbon compounds as byproducts of decomposition. The carbon (carbon dioxide) is released into the air to be absorbed by plants in respiration, producing glyceraldehydes phosphates through the process of photosynthesis. Glyceraldehydes phosphates are what sustain the life of everything in the world; they are the building blocks for all living things. When an animal eats the plant it is kept alive because of the nutrients. The animal then releases its waste and the mold and yeast in the soil decompose it. From this, the carbon cycle starts again. Essentially, the carbon cycle keeps organisms living, however, without the nitrogen and phosphorus cycle that is impossible.

The Nitrogen Cycle is the most necessary cycle in the ecosystem needed for it to function. The Nitrogen Cycle produces the primary nutrient for green plants. The nitrogen is found in the air as N_2. The nitrogen can also be used for mineralization by bacteria, fungus, and actinomycetes in the upper level of the soil. Yeast and mold are considered part of the fungus family, so one can believe that yeast and mold use the nitrogen cycle to mineralize the upper level of the soil. Also, yeast and mold help with the biodegrading and decomposing of organic matter in the soil. Plants get nitrogen from nitrogen compounds dissolved in the soil. Animals get nitrogen from eating these plants (Utah State, 1999). The Nitrogen cycle is the decomposing of nitrogen compounds. Proteins produce organic nitrogen compounds that return to the environment by excretion(Kimball, 2004). the Proteins are also considered organic matter and yeast decomposes organic matter. This demonstrates that yeast helps in the nitrogen cycle to decompose the nitrogen compounds. The nitrogen cycle, however, could not continue without the phosphorus.

The phosphorus cycle goes on in the soil in liquid form and is never in a gaseous state. Runoff causes the rocks to release phosphorus which are required in fertilizers to increase growth in plants. Phosphorus is essential for all living organisms. The purpose of phosphorus is the provide nutrients for plants and gives a plant the energy it needs to grow. A fungus causes phosphates to be released and it goes back into the environment to be reused. All of theses cycles are important to the big picture of our project.

The cycles start with fungi, as decomposers, existing in soil and feeding off of everything that dies on the ground above. The waste of the decomposers is then

released further into the soil. These wastes are: carbon, nitrogen, and phosphorus. Each biochemical has a match with the three byproducts: proteins with the nitrogen, lipids with the carbon, and nucleic acids with the phosphorus. These matches are important because each create a chemical reaction which starts each cycle; nitrogen, carbon and phorphorus. The combination of this each produces the three biogeochemical cycles we are talking about. When an animal dies the fungi decomposes it and the cycle begins again. However if fertilizer is added, the fungi is either reduced in number or completely killed because fertilizer kills important fungi that decompose dying plants. When there are not enough fungi it does release its byproducts; which causes the cycling of life. This process causes the ecosystem to die out.

When discussing the biodiversity of mold and yeast in the soil, many factors are taken into account. It is important to recognize the use of fertilizers and pesticides because they can greatly affect the production of mold and yeast. As fertilizers and pesticides help gardens grow and protect insects they are also very harmful to the ecosystem. Mold and yeast are important aspects of the ecosystem and should not be weeded out because they are a part of the biogeochemical cycles. Without mold and yeast, in the ecosystem it would be difficult to survive.

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Experiment

Problem: Does fertilizer increase or decrease the biodiversity of yeast and mold in the soil?

Hypothesis: As fertilizer is added to the soil it decreases the biodiversity of yeast and mold in the soil.

Experiment:

A. Independent Variable: the addition of the Miracle-Gro solution

B. Dependent Variable: the density of yeast and mold colonies

C. Negative Control: the soil spots without Miracle-Gro solution added during the experiment, and the soil spots before experiment was started

D. Controlled Variables: Depth that the soil cylinder inserted into soil, size of sample, day and time soil is collected, how far apart the soil samples are, amount of dilution from each test tube, size of dilution samples, same type of plates, same sized plates, location of the soil samples on the plates, time soil samples are taken, type of fertilizer, amount of fertilizer, size of spots, how far spots are away from each other.

E. Procedure:
    1. Find a spot near a curb side (N39.35824; W076.63554); measure out a 163 x 100 cm piece of land in that area and mark it off (this is the area that your experiment will be done in)
    2. In that area mark off two 15 cm x 15cm spots with little flags (these spots should be 10 cm away from each other); label one with chemicals and the other with non-chemicals (alternate these markings); do this for two more spots in the area that you marked off
    3. Label the plastic bags that you are going to use to carry the soil samples (not the before samples) to the lab in by writing chemical or non chemical spot 1a.b.c,2 a.b.c, or 3a.b.c on the front of the bag
    4. All samples should be taken on the same day and once you are done taking any samples place them in a clean plastic bag (do not reuse bags for fear of contamination)
    5. Take your first samples from the soil that hasn't been touched yet (before samples); only take 1 sample (6cm of soil in the cylinder) from each of these spots; do this by using10-15 cm diameter soil cylinders and twisting the soil cylinder until soil is filled inside of it to the first mark; twist 360 degrees to isolate sample; remove soil core by pulling straight up.

6. Take 9.8 ml of Miracle-Gro plant food and add it to 2 liters of water; (this will be your fertilizer solution); add this to all the spots marked with chemicals; then take 3 samples from each spot these (spots marked with chemical and non-chemical) do this using the same method described in step 4
7. Once all these samples are taken carry them back to the lab
8. Use a microcentrifuge tube to create a 1 cc soil sample for each location.
9. Collect a 1 cc sample of the soil from each bag and place it in a 15 ml transformation tube; do this for every soil sample bag you gathered (make sure all serial dilution in done on the same day); mark the test tubes and Petrifilm plates that you transfer the soil into according to the bag you pulled it from
10. There should be one transformation tube with soil in it from every bag so far; for every transformation tube now put 2 other transformation tubes next to it mark them with 10^{0}, 10^{-1}, 10^{-2}
11. Add 10 ml of sterile water to the 10^{0} transformation tube and 9ml of water to the 10^{-1}, and the 10^{-2} cap it, and shake vigorously
12. Remove a 1 ml sample from the 10^{0} transformation tube and place it in the 10^{-1} transformation tube; cap it, shake vigorously. Take 1 ml sample from the 10^{-1} transformation tube and place it in the 10^{-2} transformation tube. This process is the dilution of the soil samples (the dilutions should only be done to the 10^{0}, 10^{-1}, 10^{-2}).
13. Remove a separate 100 ul sample from the 10^{-1}, and the 10^{-2} transformation tubes
14. Plate the 10^{-1}, and the 10^{-2} 100 ul samples on separate Petri film plates; mark the plates according the bags it came from and if its either the 10^{-1} or the 10^{-2}
15. Allow the plates to grow at room temperature for 2 days.
16. Examine each plate from a dilution series to find the ones with between 5-30 colonies; count the colonies on only those plates and use the formula to calculate the density of bacteria in the original cc of each soil sample; Counts in cfu/cm^{3} formula= # of colonies x 10^{2} x $10^{\text{dilution factor}}$

Data and Analysis

A) Data

1- first replication
2- second replication
3- third replication
A- first dug sample
B- second dug sample
C- third dug sample
(-)- non-chemical
(+)- chemical

Before

Soil Samples (bag #)	**Colors**	**Total Fungi/cm^3 (cc)**	
		Yeast	**Mold**
1-	Black	----------	6000
	Green	----------	2000
	Blue/Green	11000	----------
1+	Black	----------	4000
	Green	----------	4000
	Blue/Green	40000	----------
2-	Black	----------	29000
	Green	----------	40000
	Blue/Green	11000	----------
2+	Black	----------	28000
	Green	----------	2000
	Blue/Green	70000	----------
3-	Black	----------	90000
	Green	----------	3000
	Blue/Green	110000	----------
3+	Black	----------	40000
	Green	----------	3000
	Blue/Green	11000	----------

After the Addition of Fertilizer (+) or Water (-)

Replication 1

Soil Samples (bag #)	**Colors**	**Total Fungi/cm^3 (cc)**	
		Yeast	**Mold**
1A-	Black	----------	14000
	Green	----------	3000
	Blue/Green	9000	----------
1A+	Black	----------	26000
	Green	----------	3000
	Blue/Green	9000	----------
1B-	Black	----------	6000
	Green	----------	1000
	Blue/Green	8000	----------
1B+	Black	----------	5000
	Green	----------	2000
	Blue/Green	3000	----------
1C-	Black	----------	60000
	Green	----------	40000
	Blue/Green	7000	----------
1C+	Black	----------	4000
	Green	----------	1000
	Blue/Green	3000	----------

Replication 2

Soil Samples (bag #)	**Colors**	**Total Fungi/cm^3 (cc)**	
		Yeast	**Mold**
2A-	Black	----------	100000
	Green	----------	20000
	Blue/Green	10000	----------
2A+	Black	----------	90000
	Green	----------	30000
	Blue/Green	40000	----------
2B-	Black	----------	5000
	Green	----------	2000
	Blue/Green	2000	----------
2B+	Black	----------	14000
	Green	----------	6000
	Blue/Green	25000	----------
2C-	Black	----------	13000
	Green	----------	2000
	Blue/Green	5000	----------
2C+	Black	----------	140000
	Green	----------	1000
	Blue/Green	90000	----------

Replication 3

Soil Samples (bag #)	Colors	Total Fungi/cm^3 (cc)	
		Yeast	**Mold**
3A-	Black	----------	110000
	Green	----------	2000
	Blue/Green	80000	----------
3A+	Black	----------	15000
	Green	----------	1000
	Blue/Green	11000	----------
3B-	Black	----------	130000
	Green	----------	40000
	Blue/Green	50000	----------
3B+	Black	----------	5000
	Green	----------	0
	Blue/Green	3000	----------
3C-	Black	----------	60000
	Green	----------	3000
	Blue/Green	60000	----------
3C+	Black	----------	1000
	Green	----------	1000
	Blue/Green	4000	----------

B) Analysis

Average Before Data Table for Total Fungi/cc

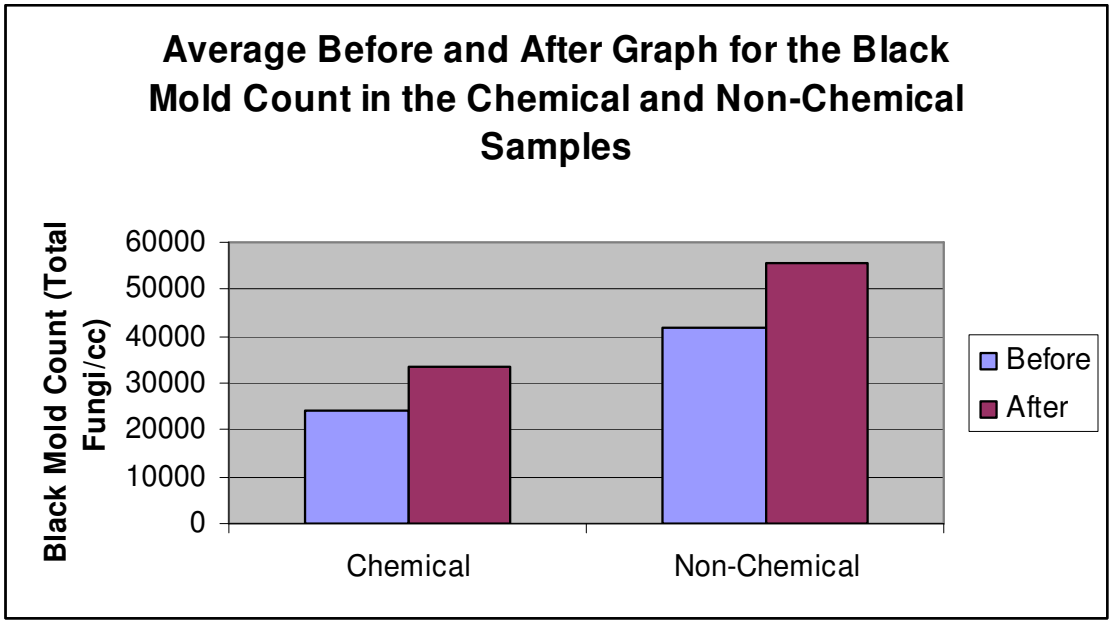
	Colors	Average Total Fungi/cm^3 (cc)	
		Yeast	**Mold**
Chemical (+)	Black	----------	24000
	Green	----------	3000
	Blue/Green	40333	----------
Non- Chemical (-)	Black	----------	41667
	Green	----------	15000
	Blue/Green	11000	----------

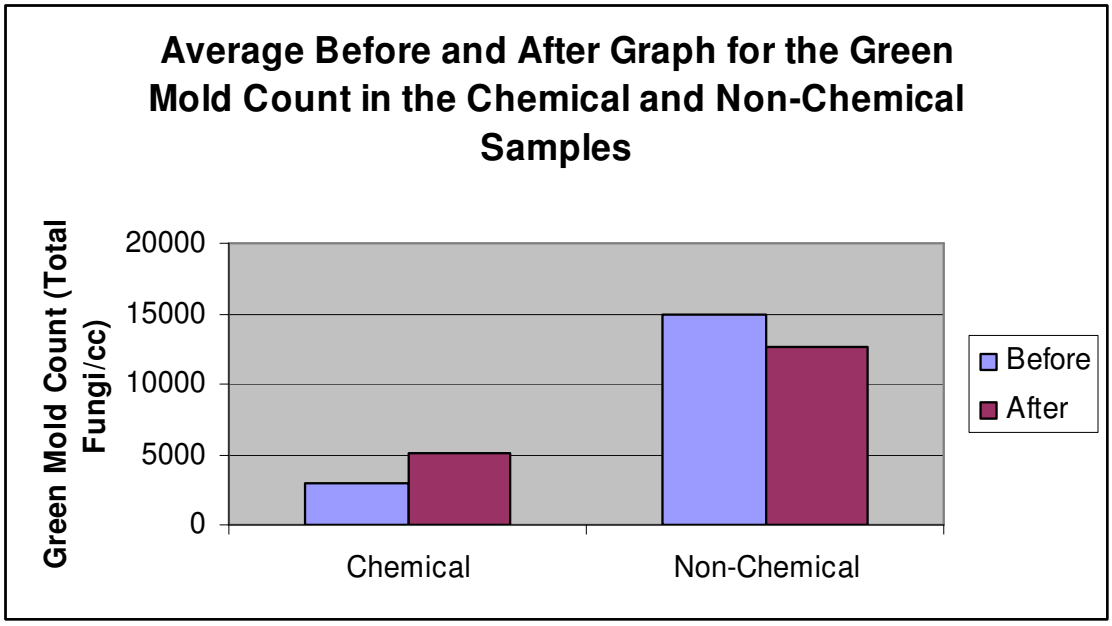
Average After Data Table for Total Fungi/cc

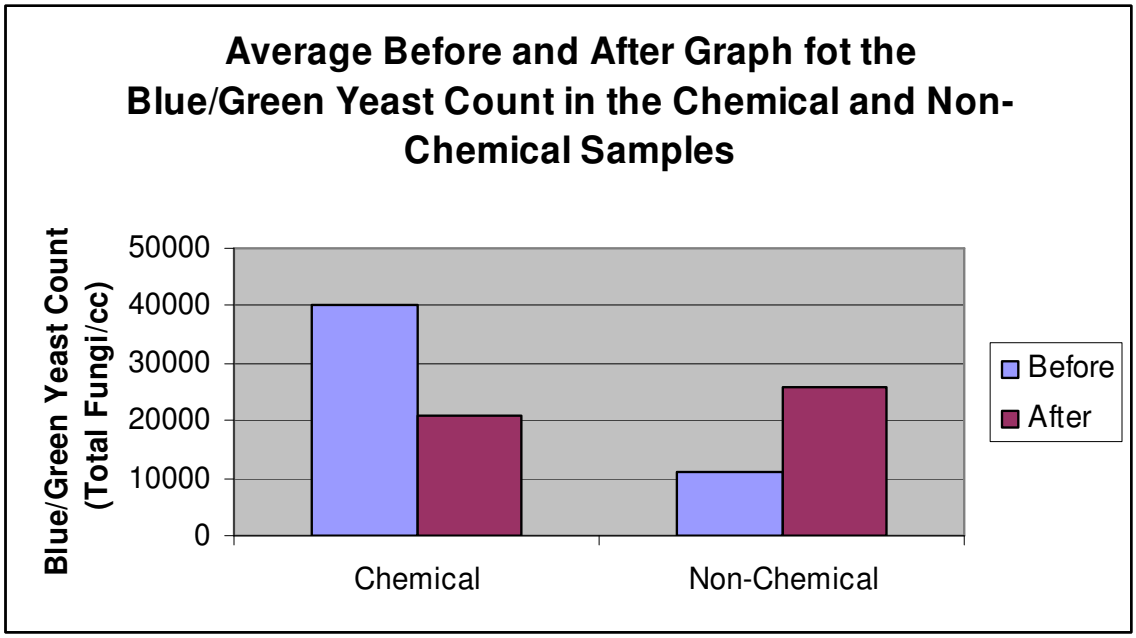
	Colors	Average Total Fungi/cm^3 (cc)	
		Yeast	**Mold**
Chemical (+)	Black	----------	33333
	Green	----------	5000
	Blue/Green	20889	----------
Non- Chemical (-)	Black	----------	55333
	Green	----------	12556
	Blue/Green	25667	----------

	Colors	Corrected Average Total Fungi/cm^3 (cc)			
		Yeast	**% change**	**Mold**	**% change**
Chemical (+)	Black	----------	----------	25100	75.3%
	Green	----------	----------	5973	119.469%
	Blue/Green	8952	42.857%	----------	----------
Non- Chemical (-)	Black	----------	----------	41667	75.3%
	Green	----------	----------	15000	119.469%
	Blue/Green	11000	42.857%	----------	----------

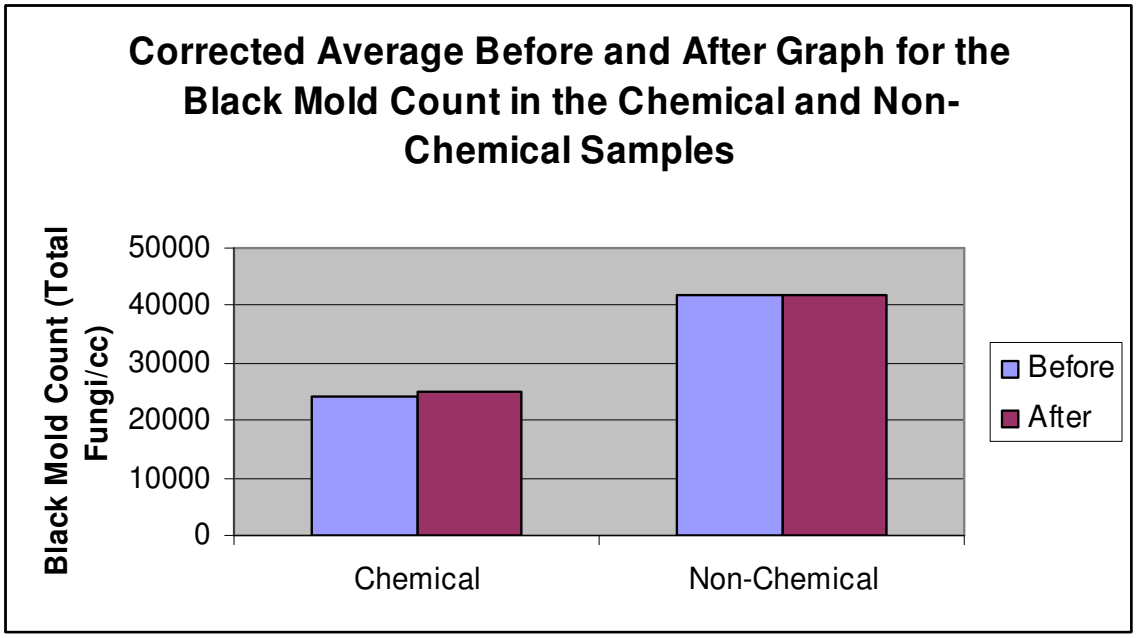
Data Graphs

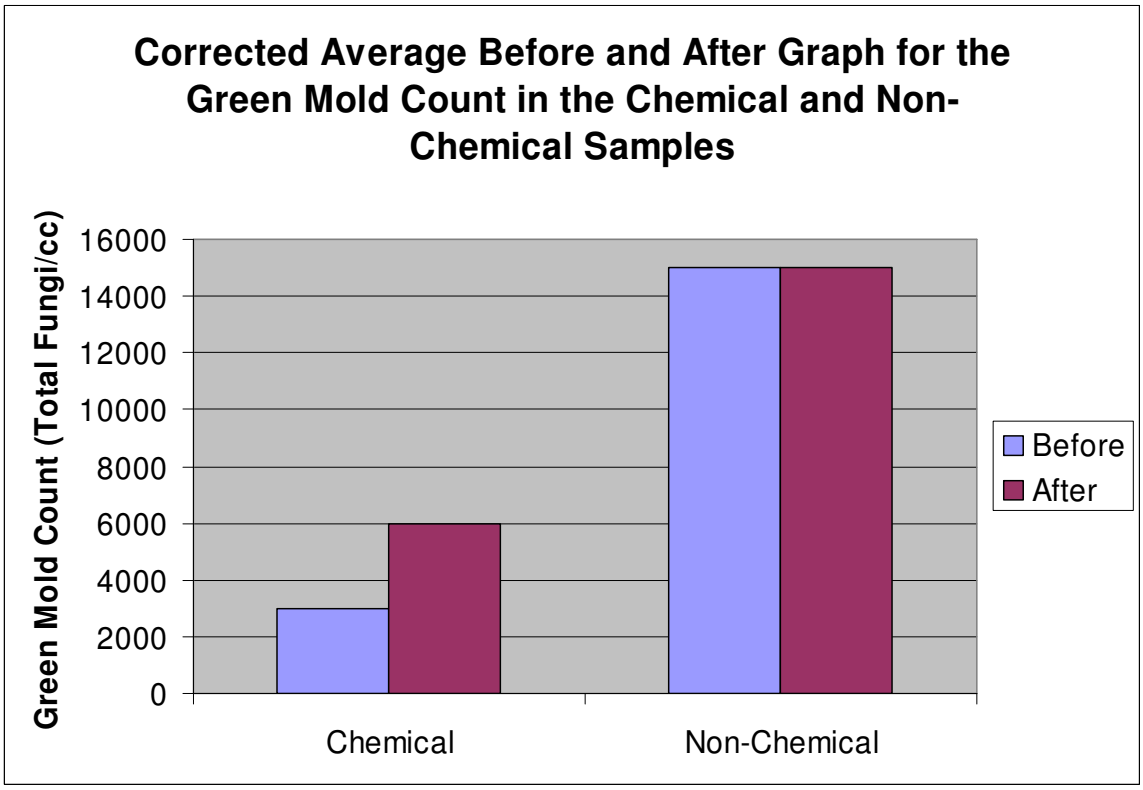


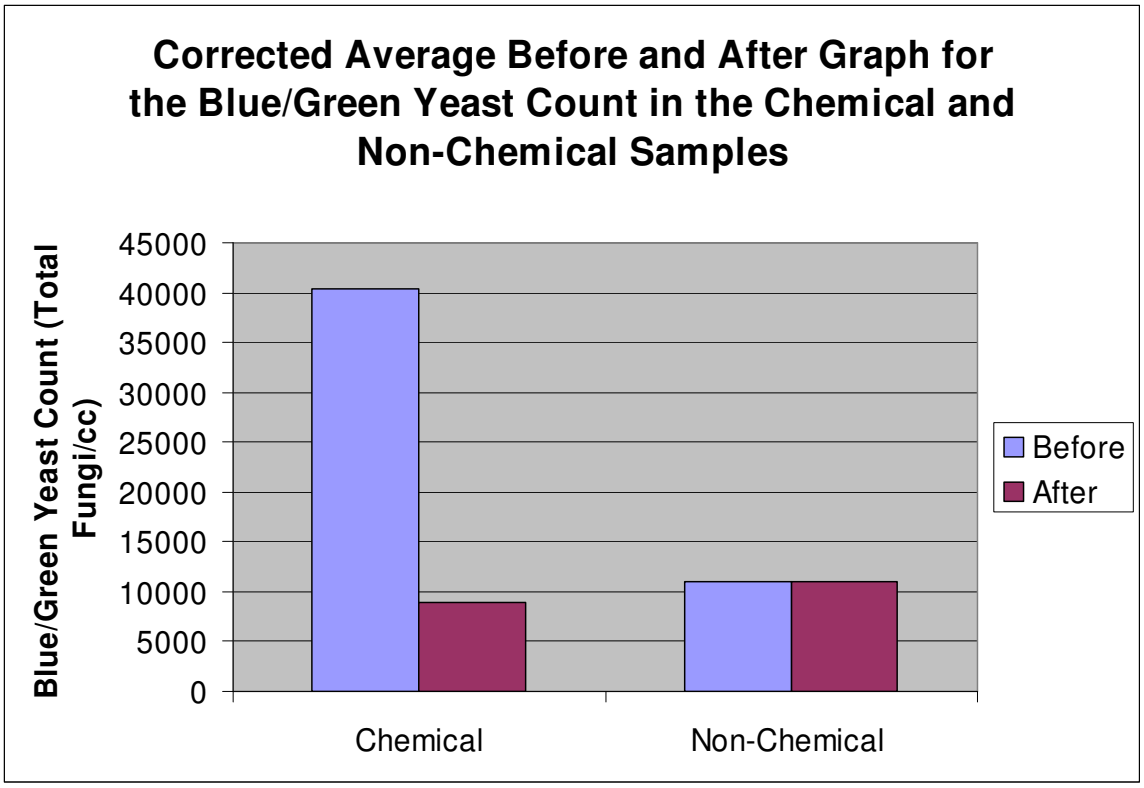




Corrected Differences


Corrected Average Before and After Graph for the Black Mold Count in the Chemical and Non-Chemical Samples
Black Mold Count (Total Fungi/cc)
50000
40000
30000
20000
10000
0
Chemical
Non-Chemical
Before
After


Corrected Average Before and After Graph for the Green Mold Count in the Chemical and Non-Chemical Samples
Green Mold Count (Total Fungi/cc)
16000
14000
12000
10000
8000
6000
4000
2000
0
Chemical
Non-Chemical
Before
After



Conclusion:

During the course of our experiment, we noticed multiple patterns within our data. With the uncorrected average graphs, there were noticeable differences in the average cm^3 amount of yeasts and molds between the 'before' and 'after' samples. According to the original, uncorrected average graphs, black mold, in the chemical and non-chemical plots, increased from 24,000 cc to 33,333 cc (chemical plots) and from 41, 667 cc to 55,333 cc (non-chemical plots). Green molds increased in the chemical plots from 3,000 cc to 5,000 cc, and decreased in the non-chemical plots from 15,000 cc to 12,556 cc. Blue/green yeasts decreased in the chemical plots from 40,333 cc to 20,889cc, and increased in the non-chemical plots from 11,000 cc to 25,667 cc.

However, because the non-chemical plots, in order to act as controls, needed to remain the same in the before and after samples, we needed to correct the data and make

corrected graphs. The numbers in the non-chemical plots, despite our attempts to control the general conditions, increased or decreased due to reasons that were out of our jurisdiction and control. Therefore, what we did was found the difference in percentage between the before and after samples for the non-chemical plots, and applied the same exact change to the after samples for the chemical plots. This made the average graphs for the non-chemical plots for each color of yeast and mold equal in the before and after samples (letting them serve as a control). The change for the chemical plots might have increased or decreased, depending on the corrected percentage. Our data's change could change the conclusions drawn on the experiment as a whole.

According to the corrected average tables and graphs, the after sample data for black mold decreased by 24.7%, making the cc for the chemical plots increase from 24,000 cc to 25,100 cc. The after data for green mold increased by 19.469%, making the cc for the chemical plots increase from 3,000 cc to 5,973 cc. Lastly, the after data for blue/green yeasts decreased by 57.143%, and so the cc for the chemical plots decreased from 40,333 cc to 8,952 cc. This data should be relatively accurate, allowing us to draw a conclusion about the answer to our problem

After performing the experiment, we found out that our hypothesis was proven wrong. The only biodiversity that actually decreased as a result of chemical runoff (fertilizer) was the blue/green yeast, and yet this was the only kind of yeast that we could identify in counting the cc on the plate. The majority of the biodiversity of the species counted, which were the black and green molds, increased by a small, yet substantial amount, proving our logic and hypothesis incorrect. For proof, we referenced the average graphs, which stated that black and green mold increased from 24,000 cc to 25,100 cc

and 3,000 cc to 5,973 cc, respectively. Two thirds of our data contradicted our intentional belief regarding the biodiversity of molds and yeasts in the soil.

In addition to coming up with this solid conclusion, we must mention a small yet significant flaw in our experiment, and therefore in our data. We believe strongly that we proved what was mentioned in the previous paragraph, but, after performing our experiment, realized that we did not have enough samples for each of the initial before trials from the chemical and non-chemical plots. While taking the soil samples for the before trials, we ran out of time, and were only able to collect one sample from each of the six plots, which means only one from the chemical and non-chemical plots for each replication. Because of this, our "averages" for the before samples are the only data we collected for that specific part of the experiment, causing a flaw to occur in our experiment, observations, and analysis. Without at least three samples from the chemical and non-chemical plots in each replication, our averages could never be as accurate as we would have liked. The problem lies within the fact that the data could have changed either in favor of or against our hypothesis, a fact that we can never know.

Thus, even though we have come to a substantial conclusion based on our carefully collected and recorded data, we cannot know, in reality, what the actual outcome of our experiment might have been. However, thinking about what might have been can never get anyone anywhere in biology or any science for that matter. It is for this reason that we must profess our belief in the one obvious conclusion available to draw for our experiment: fertilizer, when applied as shown, causes mold biodiversity to increase, and yeast biodiversity to decrease drastically.