Biodiesel Waste Products as Soil Amendments - Field Study and Runoff Impacts

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The objectives of this research project were to evaluate the effects of crude glycerol on plant and microbial systems in relation to soils and the potential for runoff contamination. Three tests were used: Concentration comparison in soil with respirometry, plant germination and growth, and runoff testing by test plot application. This project is a continuation of project MBTC 3025 and some of the results included in this report were previously reported in the previous report.

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INTRODUCTION

Biodiesel is a carbon neutral biofuel often made from waste vegetable oil sourced from the restaurant industry or increasingly from other wastes or crops rich in lipids. Biodiesel is created through the transesterification of lipids such as vegetable oil or animal fat with an alcohol such as methanol and in the presence of a base such as potassium hydroxide. Transesterification involves reacting an alcohol with an ester resulting in a new alcohol and a new ester. The organic group of the alcohol is exchanged with the organic group of the ester. In the case of biodiesel, the biodiesel is the new ester (linoleic acid methyl ester) and glycerol is the new alcohol (glycyl alcohol).

Glycerol is also known as glycerin, glycerine (UK), glycyl alcohol, and trihydroxypropane . Pure, it is a clear odorless colorless viscous liquid, but as a product of biodiesel production, it is a dark brown viscous liquid with an odor quite similar to the vegetable oil from which it comes. It has the molecular formula $C_3H_8O_3$ and is very soluble in water as well as being hygroscopic which means it attracts and absorbs water from the air. Figure 1 shows a space-filling model of what a glycerol molecule looks like. Its basic form is that of a propane molecule with three hydrogen atoms replaced with hydroxyl groups.

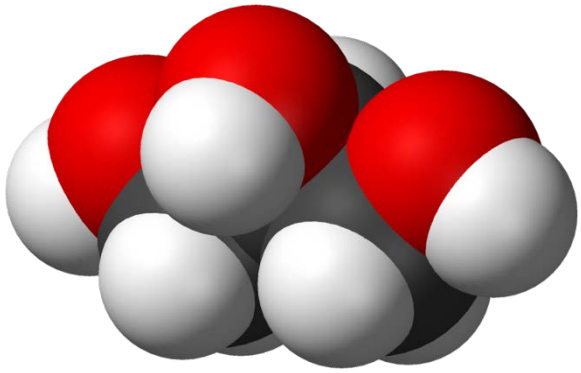


Figure 1. Space Filling Model of Glycerol Molecule.

The mixture that is the byproduct of biodiesel is primarily glycerol but it contains measurable amounts of the alcohol (usually methanol) used in transesterification and the base (usually potassium hydroxide or sodium hydroxide) used as a catalyst. It also typically contains remnants of vegetable oil and water.

Much glycerol created during biodiesel production is incinerated. This releases carbon into the atmosphere and mitigates some of the carbon dioxide reduction biodiesel offers as a biofuel. Glycerol does replace other fuels used for incineration, but its use as such is contrary to the spirit of biofuels utilization. Glycerol has many other uses such as animal food supplement, and its aforementioned utilization in makeup, foods, and hygiene products. The

demand for these uses is small compared to the increasing volume being produced in the burgeoning biofuels industry. Biodiesel production has so modified the marketplace that there is only one major producer of synthetic glycerol remaining, Dow Chemical Company.

It is in this environment that the concept of using glycerol as a soil amendment finds its genesis. To that end, the idea has not received much study. Cayuela et al (2010) showed that glycerol as a soil amendment has implications for carbon sequestration and greenhouse gas emissions. Glycerol may actually show benefits for soils because it can raise the organic content of soil. However, little is yet known about the toxicity according to Biodiesel Magazine (2008). It is generally assumed to be nontoxic due to its source in natural oils and its use in foods, but there are few studies on microbial, biological, plant and soil systems.

Schoenau et al. (2009) found that glycerol was effective in increasing soil organic content but it required supplemental fertilizer due to nitrogen and phosphorus tied up by microorganisms during decomposition in the soil. The immobilization of phosphorus may be of benefit in watersheds suffering from excessive phosphorus runoff due to land application of poultry litter.

Our research examined the effects of the application of glycerol on microbial oxygen utilization, plant germination and growth, and the Total Organic Carbon (TOC) found in runoff water. A microbial respiration test was used to evaluate toxicity to microorganisms at various concentrations from 0.01 percent to 10 percent by weight. A plant germination and growth test were conducted to evaluate the effects of germination and growth at concentrations between 0.01 and 10 percent. Finally, runoff tests were performed to compare the TOC concentrations in runoff from control plots and plots treated with fertilizer, glycerol, and fertilized glycerol applications.

If waste glycerol from the production of biodiesel is found not to be toxic when used as a soil amendment, it should also be able to be used in dust control, replanting, and landscaping rather than incinerated. As well as disposing of glycerol in a more ecologically sound and climate change responsible way, it could also demonstrate beneficial effects in soils and limit the need for other soil amendments. If glycerol is shown to be benign or even advantageous, guidelines for application rates will be created based on the outcomes of this research.

PROJECT OBJECTIVES

The objectives of this research were to evaluate the toxicity of methanol-stripped glycerol sourced from biodiesel production on microbial, biological, and plant systems in soils. Three tests were performed: 1. Repirometry; 2. Plant germination and growth; and 3. Test plot application with runoff analysis.

The specific objectives were:

1. Measure microbial respiration in soil samples with varying concentrations of glycerol present in the samples.

2. Qualitatively evaluate the germination and growth rates of grass seed in soils dosed with varying concentrations of glycerol.

3. Measure Total Organic Carbon (TOC) in samples of runoff water from test plots having had glycerol and/or fertilizer applied to them.

RELATED WORK

Schoenau et al. (2009) studied several biofuel, crop and animal processing byproducts including dry and wet distillers grains, dehydrated alfalfa, thin stillage, and glycerol as soil amendments. They established that glycerol effectively increased soil organic content but required supplemental fertilizer to compensate for the tie up of nutrients by microorganisms in the process of respiration and decomposition in the soil. Neither glycerol nor any of the other byproducts tested had significant biological effects. At the application rates in the study, glycerol did not affect chemical soil parameters measured including pH, salinity, or soluble metals. The authors of the study noted that "glycerin addition may be of greatest benefit in increasing soil organic carbon content and carbon sequestration, compared to the alternative of incinerating the glycerin." They found beneficial effects of applications as high as 10,000 kg per hectare (1% by weight, assuming a soil depth of 10 cm), however that rate would require excessive amounts of fertilizer to compensate for nutrient tie-up. Shoenau suggested an application rate of approximately 1000 kg per hectare (0.1% by weight) would be appropriate. Because of the tendency of glycerol to tie up nutrients in the soil, it may be beneficial in certain watersheds (such as the one this research has taken place in) which are sensitive to runoff with large amounts of phosphorus.

Qian et al. (2011) in a study closely related to the Shoenau study tested the effects of soil amendment with thin stillage (a byproduct of ethanol production) and glycerol in Saskatchewan. As a soil amendment, glycerol only contains carbon, hydrogen, and oxygen and was effective in increasing the organic carbon content of the soil. Neither amendment tested showed biologically significant effects in other aspects tested including pH, salinity, or soluble metals.

Cayuela et al. (2010) added ten different amendments to soils (manure digestates, rapeseed meal, distilled dried grains with solubles, nonfermentables from hydrolysis of different lignocellulosic materials, and biochars) and investigated soil carbon and nitrogen cycling. It was found that biofuel byproducts as soil amendments contain large amounts of readily degradable carbon leading to short term nitrogen immobilization limiting their prospective use as fertilizers. The authors also suggested that these products should be utilized in a way that allows them to degrade somewhat to maintain biological activity and nutrient cycling but still maintain persistence in the soil.

Hall (2010) studied soy based foam insulation as a soil amendment. He examined toxicity in activated sludge systems, earthworm populations, and plant environments. Hall found that it was difficult to work with the foam due to its very low density causing it not to maintain homogenous mixtures in stirred soil, activated sludge for respirometry and so he proposed an alternate method. The plant studies in this research will be performed similarly to Hall's

methods, but glycerol does not suffer from the same difficulties as foam due to the fact that it can be diluted in water and mixed easily with soil. Hall's control results were highly precise, but the other studies suffered from poor repeatability due to the physical effects of foam chunks.

Dror et al. (2000) examined the effects of soil amendments including sewage sludge on the dynamics of kerosene attenuation on field plots. The plots were then leached using sprinkler irrigation. The tests lasted 100 days. They discovered that soil amendments may enhance the rate of kerosene degradation and reduce the residual amount as compared to untreated soil.

In a study by Chung et al. (2005) glycerol was used as an ingredient for a granulated biofungicide to control *Rhizoctonia solani* colonization in soil to prevent damping-off disease of Chinese cabbage. Germination of the cabbage was not negatively affected by the presence of glycerol (Chung, Huangb, and Huang, 2005.

Siddiqui and Shaukat (2002) examined the effects of zinc and glycerol individually or in concert to improve biocontrolling activity of indigenous and non-native bacteria, namely *Pseudomonas aeruginosa* and *Pseudomonas fluorescens*. They came to the conclusion that both zinc and glycerol together and separately increased efficacy against root knot nematodes as well as improved tomato plant growth and bacterial rhizosphere colonization. Glucose alone was found to inhibit nematicidal activity of the bacteria.

Rod Rodriguez-Kabana of Auburn University has developed and patented a glycerol based product that is injected into soil to control weeds and crop destroying nematodes (AAES, 2008). He suggests that this product could be utilized in organic farming and expects it to be widely available in a few years.

The toxicity of glycerol resulting from the transesterification of vegetable oil that produces biodiesel is based on the concentration of methanol in the mixture. Methanol will evaporate into the atmosphere in approximately a week if the container is left open. Heating the product will increase the evaporation rate as well. After the methanol has been stripped, the resulting crude glycerol is generally considered non-toxic and biodegradable (Tickell, 2003). According to the glycerol Material Safety Data Sheet (MSDS) it is a skin and eye irritant with no known carcinogenic effects on animals or humans and is non-hazardous if ingested (EMD Chemicals Inc., 2004).

In the previous MBTC study (Soerens, 2011), results of the respirometry studies suggested that there is no microbial inhibition due to the glycerol. In plant studies, there was inhibition of growth and germination with glycerol quantities above 1% by weight. Lower levels of glycerol did not appear to inhibit plant germination or growth and in fact appeared to be

beneficial to growth. In worm assays, glycerol concentrations above 1% were fatal on contact with earthworms due the glycerol absorbing water and desiccating the worms. Worms survived when exposed to glycerol in lower concentrations.

METHODS AND MATERIALS

Glycerol

Glycerol used in all tests was obtained from the University of Arkansas Facilities Management department. To strip methanol from the product, it was stored in an open container in a fume hood to allow the methanol to evaporate. A titration was performed with the glycerol to ascertain the amount of acid it would take to neutralize the high pH to a manageable level. The acid used was 1 Normal H_2SO_4.

Microbial Toxicity Test

The microbial toxicity test was carried out using a soil mixture consisting of five parts commercially available topsoil, five parts commercially available composted cow manure, and one part uncomposted chicken litter. Each test batch was made from one common quantity of soil. Soil portions were separated from the common quantity and weighed and then mixed with the corresponding amount of glycerol. Soil mixtures were tested containing 0%, 0.01%, 0.03%, 0.1%, 0.3%, 1%, 3%, and 10% glycerol by weight.

The apparatus used was an AER-200 Respirometer system provided by Challenge Technology of Springdale Arkansas. The apparatus consists of eight identical cells each containing a perforated plastic tube lined with screen that allows gas transfer from the environment of the cell to the soil sample held within. In the bottom of the cell is a magnetic stirring apparatus that homogenizes the atmosphere in the cell. The magnetic stirrers are operated by the apparatus in the platform upon which all the cells sit. At the top of each tube is a small container into which is placed 3 mL of potassium hydroxide (KOH) which absorbs carbon dioxide (CO_2) produced by the sample in a reaction that produces potassium bicarbonate ($KOH + CO_2 \rightarrow KHCO_3$). The cells are sealed with a rubber O-ring and a threaded cap. Attached to each cell is a thin tube which has an inline 0.004 inch orifice and leads to a bubble counter. The bubble counter consists of eight clear plexi-glass containers with hold mineral oil. Each container houses a backlighting light emitting diode (LED) and a light receiving sensor. When a bubble is drawn through the oil by the negative pressure in the cell, it causes the light produced by the LED to bend around the bubble in the oil which causes it not to strike the sensor. The respirometer registers this as a count and sends a signal to an attached computer which notes a volume of oxygen based on an assumed bubble size. Bubbles are drawn from a common manifold that is supplied with pure oxygen by an attached compressed oxygen cylinder with a regulator and a 0.004 inch orifice. The pressure is set by allowing the oxygen to bubble through a pipe in a jar with approximately 1 mm of water above the orifice.

Respirometer data measured in milligrams of oxygen uptake were recorded every minute. The length of a cycle was approximately one week or less. The slope of the plot of uptake versus time during the main period of activity is the Oxygen Uptake Rate (OUR) which is expressed in units of mg/hr. The main period of activity is defined as the time from shortly after the beginning of the test to the point where the ability of the vial of KOH to absorb CO_2 is exhausted. At that point, bacteria in the sample, no longer able to draw oxygen from the system, switch to anaerobic respiration and begin net production of gases which the respirometer cannot measure. This results in a leveling off and/or jumps in a graph of oxygen consumption.

Plant Test

The plant test was begun on September 6, 2011 using a soil mixture consisting of five parts commercially available topsoil, five parts commercially available composted cow manure, and one part uncomposted chicken litter. The containers used were commercially available plant starter kits. Each kit contained eight flats of nine pods each. The total of eight pairs of flats represented the eight concentrations of glycerol tested, 0%, 0.01%, 0.03%, 0.1%, 0.3%, 1%, 3%, and 10% glycerol by weight. The soil was prepared from a common quantity mixed in a food grade plastic bucket. Batches were separated from the common quantity, weighed and mixed with the corresponding volume of glycerol. In each pod was placed approximately 12 tall fescue (*Festuca arundinacea*) seeds and all were covered with a thin layer of soil unmixed with glycerol. Due to supply shortages, one flat of nine pods representing 10% glycerol by weight was replaced by a 3 inch square pot which was seeded with approximately 108 tall fescue seeds. Figure 2 shows the method for planting seeds in the pods.

Figure 2. Seeds Sown in Soil Pods.

Both kits were watered with deionized water, an equal amount from below to prevent cross-contamination twice a week. The plants were kept under fluorescent lighting containing two

30 daylight (6500K color temperature) bulbs and two 30 watt soft white (3000K color temperature) bulbs. The bulbs were located approximately 18 inches above the plants. At four times during the course of the experiment (October 11, 2011, October 18, 2011, October 25, 2011, and November 8, 2011), the grass was trimmed using the height of a pestle as a measurement to assure even height. The same pestle was used to weigh the clippings. Figure 3 demonstrates the method. The clippings were weighed using an analytical balance and the values recorded to three decimal places. Throughout the experiment, pictures were taken to discern qualitatively the differences in results.

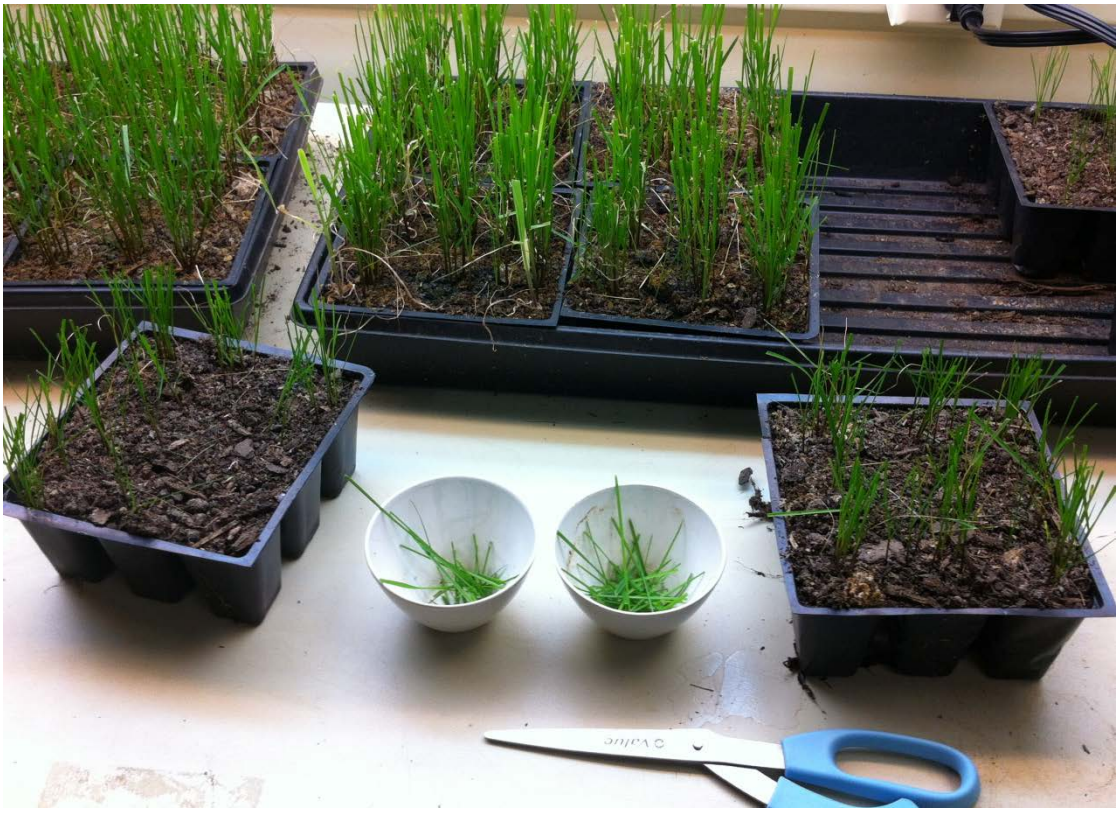


Figure 3. Trimming Procedure

Runoff Test

The runoff test was carried out using test plots created in 2007 and located at the University of Arkansas Parasitology Farm (36°04’43.88” N, 94°17’04.87” W, Elev. 1240 feet). In the recent years of no usage, the plots had become overgrown with grass and weeds and some of the plots had been damaged by cows stepping on them and gophers digging under them. The grass was trimmed and the catchment gutters were cleaned in preparation for the tests. On May 3, 2012, the plots were dosed in four groups of four with the following applications: Four plots with 1000 kg/hectare glycerol, four plots with 300 kg/hectare sodium nitrate

(nitrate of soda, a nitrogenous fertilizer) as nitrogen, four plots with the same amounts of both glycerol and sodium nitrate, and four plots as controls with no application.

One week later was the first runoff test. To simulate rainfall, an apparatus, shown in Figure 4, was built to span the plots and provide a spray of tap water. The rainfall simulator was intended to simulate rainfall at 50 mm/hr however due to fluctuating water pressure in the available supply, rainfall application rates varied somewhat. The actual amount of precipitation applied to the plots was controlled and recorded by a water meter and hose-end ball valves.

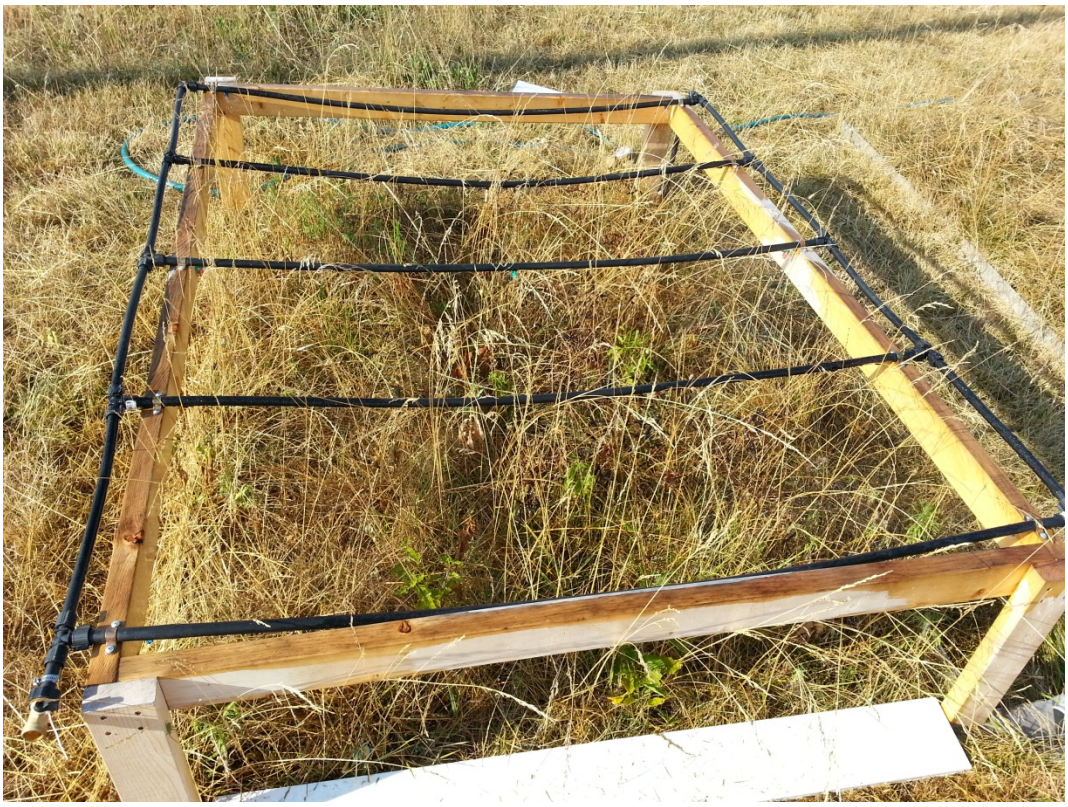


Figure 4 Runoff Rainfall Apparatus

The runoff was initially collected in one gallon containers. When approximately one gallon was collected, two 200 mL samples were collected from it. This was done because it was found that if only 200 mL were collected initially, a substantial proportion of it was water accidentally collected from the rainfall simulator without having contacted the soil. Therefore, a large initial water sample was collected to provide a more characteristic and uniform sample. One of the 200 mL sampling bottles of each plot was delivered to the University of Arkansas Water Laboratory for TOC analysis and the other was frozen to be utilized for possible future analysis.

RESULTS

Titration

A titration of a sample of glycerol was performed using 1N H_2SO_4. The resulting graph is found in Figure 5.

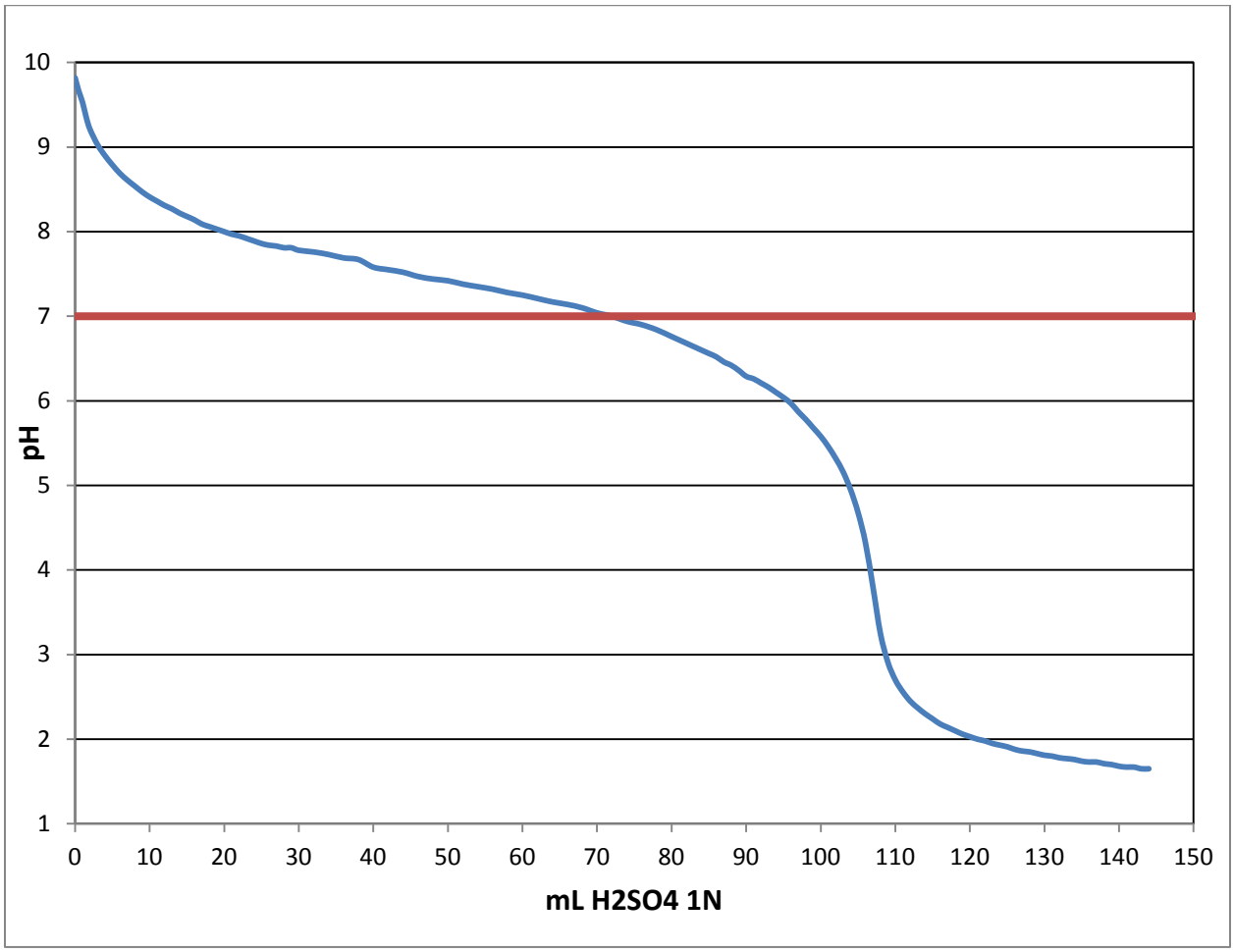


Figure 5. Titration of Glycerol with 1N H_2SO_4.

The tritration shows that the glycerol is highly basic due to the strong base catalysts used.

Microbial Toxicity Test Results

Figure 6 shows a representative respirometer cycle. The respirometer has 8 cells. In the cycle shown in Figure 6, two cells had no glycerol (control), four cells had 0.03% glycerol, and two cells had 10% glycerol. Two of the 0.03% replicates tracked very closely together. A third 0.03% replicate started with the other two but then hit a constant, that is, there was no further

uptake. A fourth 0.03% replicate had no uptake at all during the experiment. These errors were likely due to a failure in the cell or tubing that didn't allow more oxygen in or a different failure of the system for that particular cell. The cell labeled number three did not function effectively in any test throughout the entire testing period. This is understood to be caused by a flaw in the gasket mechanism that seals the cell and it was unable to be repaired during the testing period.

Similar errors were observed in a number of the respirometer runs. Another commonly seen error was a nearly vertical rise in the uptake curve due to temporary back pressure in the respirometer cells or tubing. In the lower of the two 10% runs in this experiment, there was an initial jump in the apparent oxygen uptake to 974 mg within the first 30 minutes and then no additional uptake for 3 hours, which is similar to the lag period in the other cells. When the initial jump was subtracted off, the uptake curve was similar to that of the other 10% cell. The adjusted data are what is shown in Figure 6. Note that this adjustment does not affect the oxygen uptake rate (OUR), which is the slope of the uptake versus time plot during the growth period (after the lag).

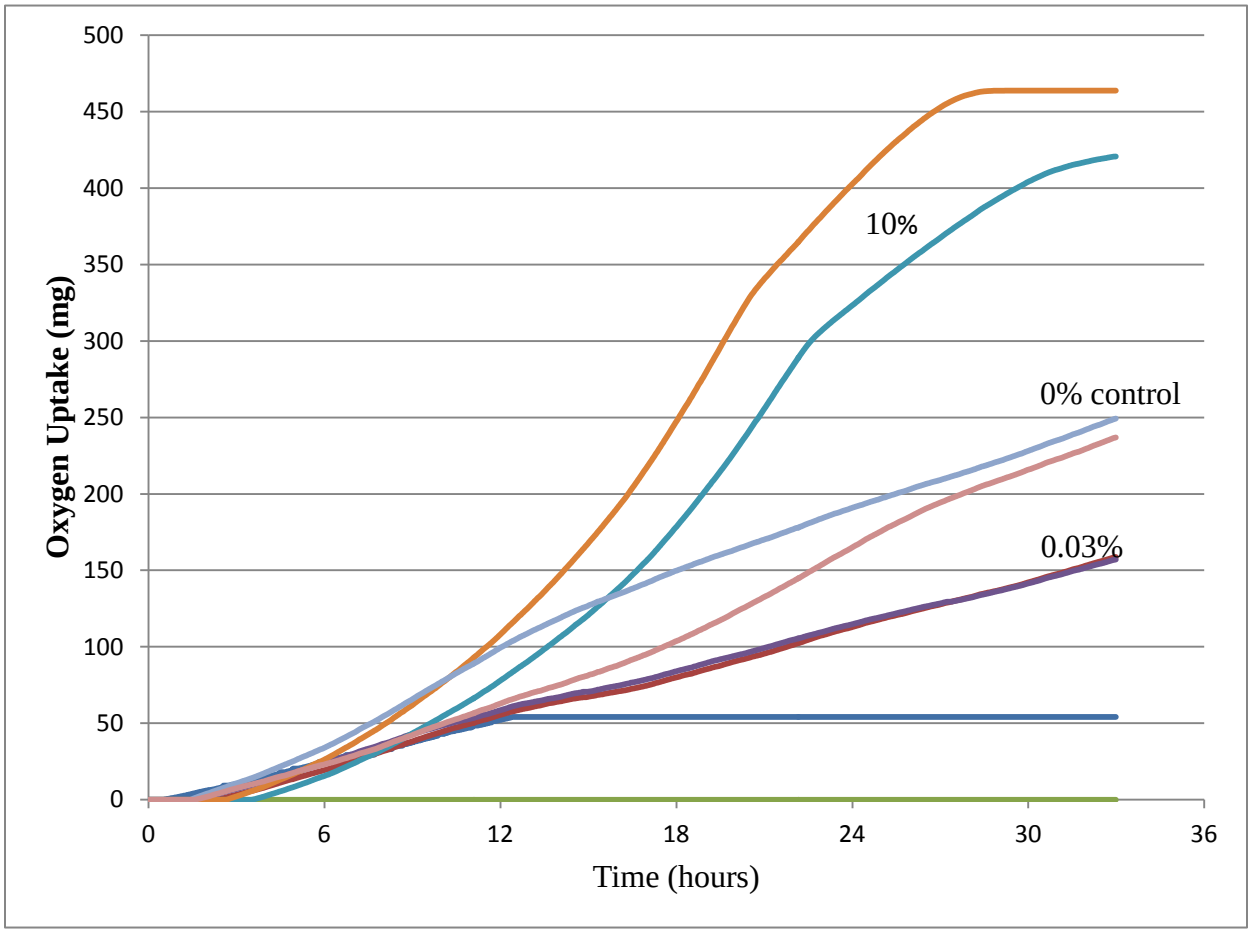


Figure 6. Oxygen Uptake During Respirometer Cycle

From this cycle, the OUR was calculated using the slope beginning a 8 hours and ending at 25 hours, which is the primary period of microbial activity. The 10% curves plateaued after this point and became anaerobic. For the 0.03% cell that stopped at 54 mg uptake at 12.4 hours, the rate was the slope of the graph from 8 to 12 hours. The uptake rates are shown in Table 6.

Table 1. OUR and Glycerol Content ([1]rate 8-12 hours; [2]no uptake data).

Glycerol %	OUR (mg/hr)
0	8.095
0	8.068
0.03	[1]4.872
0.03	4.787
0.03	------[2]
0.03	4.697
10	19.327
10	23.963

There is noteworthy agreement between replicates for the same treatment with the exception of the failed cells. However, it is not clear why the 0.03% glycerol would be less than the control and the 10% more. Each treatment was prepared in one batch and then separated into replicates. Perhaps there is a physical factor that is consistent among the replicates but is different between the preparations. A similar result was seen in the 0.01% set of plant growth tests. Although it cannot concluded whether the glycerol increases or decreases microbiological activity, from this run it can be concluded that there is microbial activity in the presence of glycerol and that glycerol does not severely inhibit microbial activity.

Figure 7 shows a respirometer cycle with all 8 cells tested as controls without glycerol. Two of the cells had an apparent uptake at the beginning of the test and two of the cells had a longer lag than the other cells. This can be explained by the nature of the apparatus in that the oil in the bubble counters takes time to be drawn into the correct position to count bubbles and during that time, the meniscus of the oil in the bubble counters may cross the sight line of the sensor and cause erroneous jumps in the data. In spite of these anomalies, the slope of the curve can still be used to calculate an OUR. One of the cells had zero uptake and cannot be used as with other tests. There is good precision among the values and a tight confidence interval has been calculated. Minor variations seen in all curves at the same time are likely due to temperature changes in the lab caused by cycling of air conditioning units or opening and closing of doors. Table 2 is a summary of the OUR values for this test.

Table 2. OUR values of cells without glycerol.

OUR values (mg/hr)	4.006	3.593	3.405	3.473	4.177	4.113	4.536
mean	3.900						
95% confidence interval	+/-	0.389	=	(3.512, 4.289)			

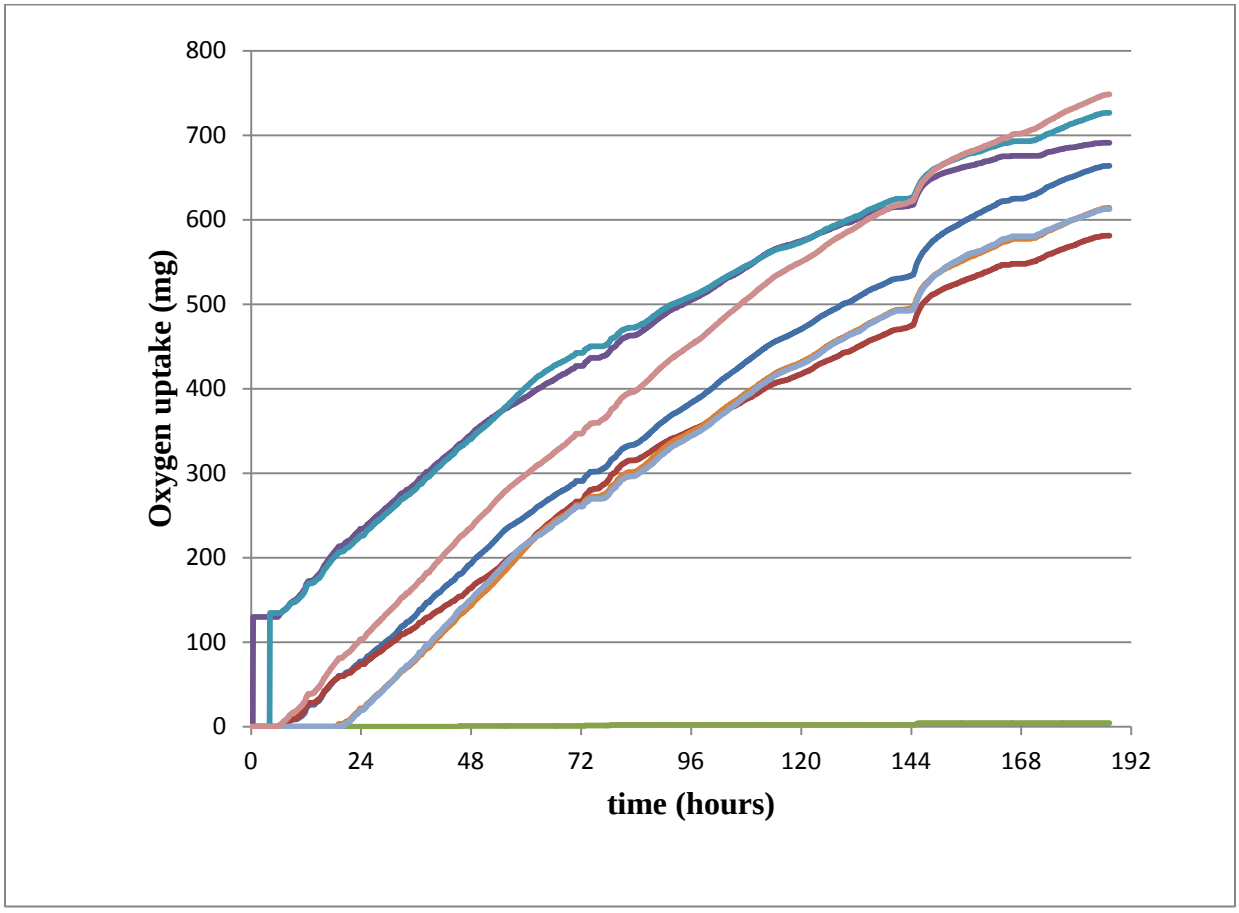


Figure 7. Respirometer Cycle with 8 Control Replicates

Figure 8 shows a cycle with four replicates each of two glycerol concentrations – 0.1% and 3%. It was observed that the 3% cells had higher oxygen uptake than the 0.1%. One of the 0.1% cells failed. Table 3 is a summary of the data.

Table 3. Results of respirometer run with 0.10% and 3% glycerol

0.10%				
OUR values	6.720	6.647	5.827	
mean	6.398			
95% confidence interval	+/-	1.232	= (5.166, 7.631)	
3%				
OUR values	23.187	23.663	25.112	23.349
mean	23.828			
95% confidence interval	+/-	1.399	= (22.429, 25.226)	

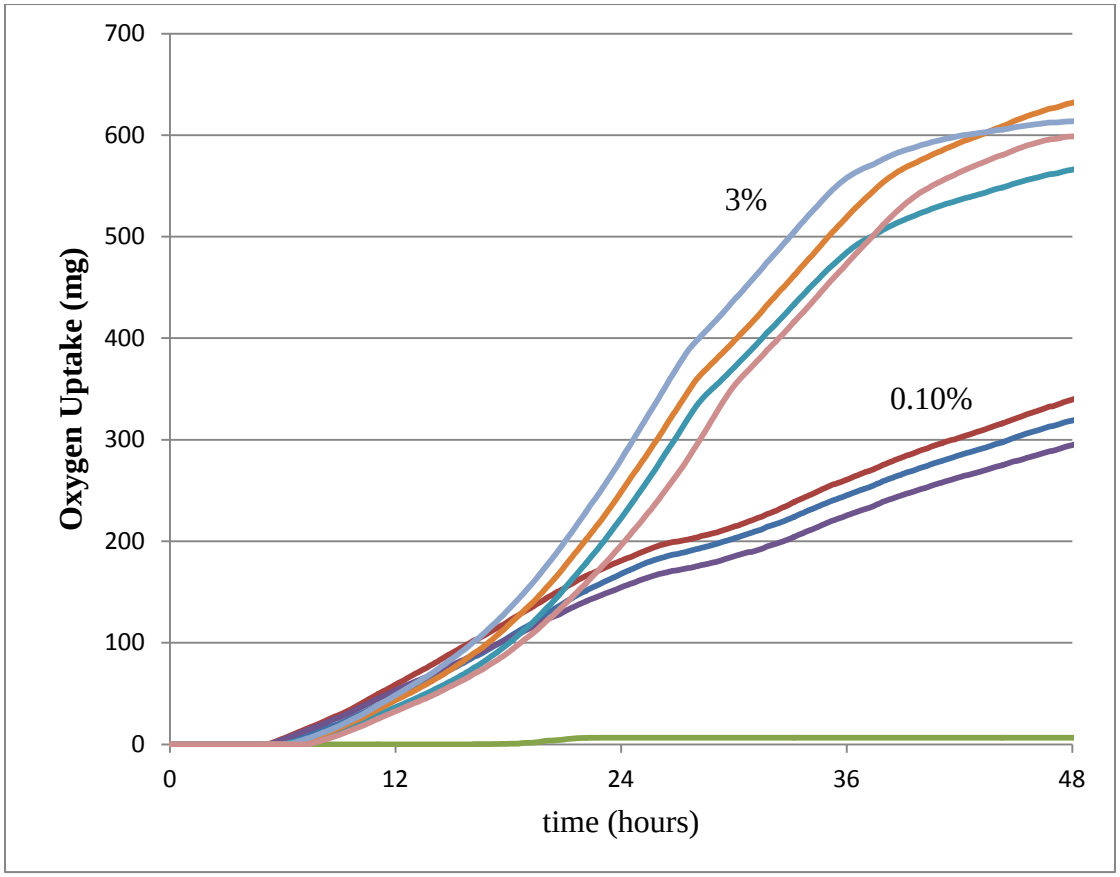


Figure 8. Respirometer run with 0.10% and 3% glycerol

From these results it is clear that the oxygen uptake for 3% is significantly more than that for 0.10%. The oxygen uptake rates for the 3% glycerol cells are similar to that for the 10% cells in the other cycle. It appears that glycerol as an organic substrate is easily utilized by soil microbes. It is clear that glycerol does not prevent microbial activity. Table 4 summarizes the data for the three respirometer runs summarized.

Table 4. Summary of Respirometer Cycles

Glycerol concentration	OUR: Mean +/- 95% CI
0%	8.095, 8.068 (two values)
0%	3.900 +/- 0.389
0.03%	4.785 +/- 0.217
0.10%	6.398 +/- 1.232
3%	23.828 +/- 1.399
10%	19.327, 23.963 (two values)

Plant Test Results

There was a significant correlation between the concentration of the glycerol in the soil and the resulting growth of the grass. As seen in Figure 9, growth generally increased with increasing concentration until the threshold of approximately 1% where growth began to decline in comparison to the results of lower concentrations. The soil in the pods containing 3% and 10% glycerol was dry and crunchy. This is possibly due to the fact that glycerol is hygroscopic which means it attracts and holds water molecules from the surrounding environment. This would have made the water unavailable to the plants.

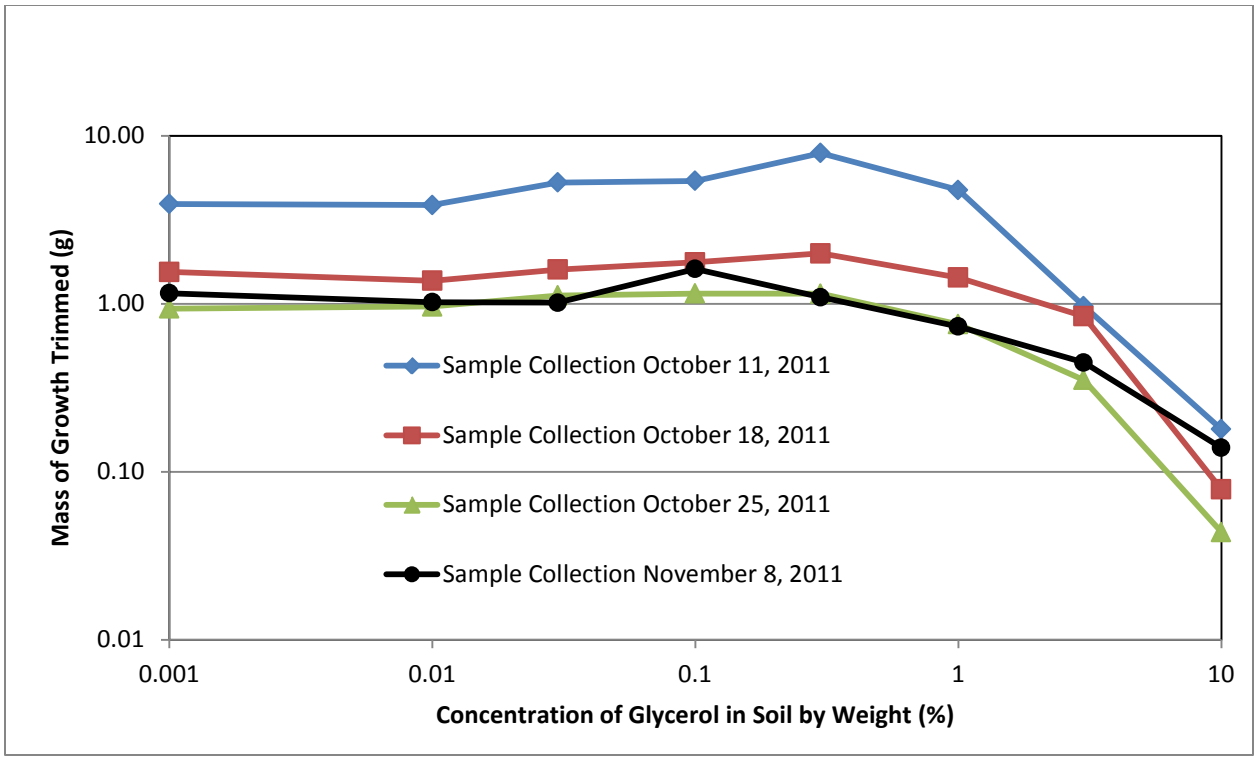
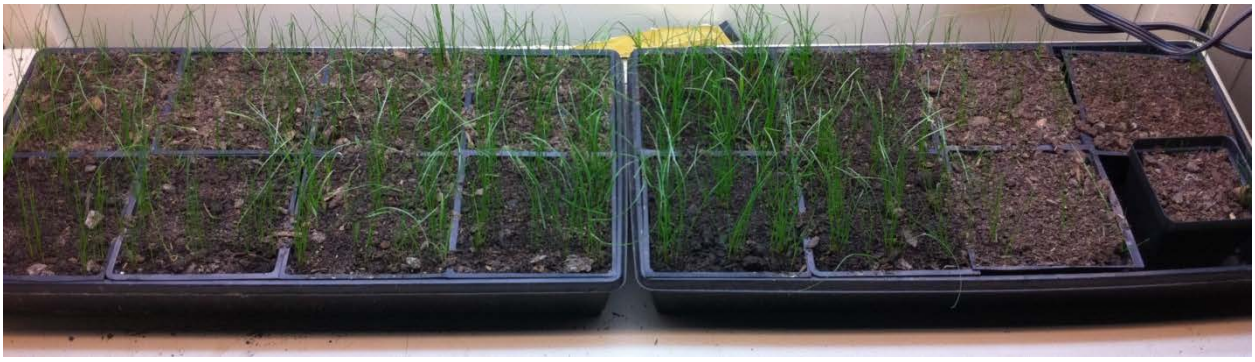


Figure 9. Grass Clippings Mass versus Glycerol Concentration in Soil (log-log scale)

Figure 10 demonstrates the differences between the different soil concentrations visually. From the control to higher concentrations, the soil becomes darker as water is absorbed from the air and made available to the plants. At the threshold of 3%, the glycerol sequesters water from the soil and makes it dry and coarse.



0% 0.01% 0.03% 0.1% 0.3% 1.0% 3.0% 10%

Figure 10. Photograph of Early Growth Showing Soil Effects.

The mass of trimmings are listed in Table 5 and the growth rate in grams per day is shown in Table 6.

Table 5. Harvest (g) versus Glycerol Concentration (% by weight)

	Harvest			
Conc. (%)	1	2	3	4
10	0.18	0.08	0.04	0.14
3	0.97	0.84	0.35	0.45
1	4.76	1.44	0.76	0.73
0.3	7.90	2.00	1.15	1.09
0.1	5.39	1.76	1.15	1.61
0.03	5.27	1.60	1.12	1.02
0.01	3.87	1.37	0.96	1.02
0.001	3.93	1.55	0.94	1.16

Table 6. Growth (g/d) by Harvest

	Harvest				
Conc. (%)	1	2	3	4	Average
0	0.127	0.310	0.134	0.083	0.164
0.01	0.125	0.274	0.138	0.073	0.153
0.03	0.170	0.320	0.160	0.073	0.181
0.1	0.174	0.353	0.164	0.115	0.202
0.3	0.250	0.400	0.164	0.078	0.223
1	0.154	0.287	0.108	0.052	0.150
3	0.031	0.169	0.050	0.032	0.071
10	0.006	0.016	0.006	0.010	0.010

Table 7 shows the effect of time on growth. This is likely due to the natural process of the plant, but it also shows some of the effect of glycerol. Figure 11 is a graphical representation of this data.

Table 7. Daily Growth for the Duration of the Test

	Harvest				
Conc. (%)	1	2	3	4	Average
0	0.127	0.310	0.134	0.083	0.164
0.01	0.125	0.274	0.138	0.073	0.153
0.03	0.170	0.320	0.160	0.073	0.181
0.1	0.174	0.353	0.164	0.115	0.202
0.3	0.250	0.400	0.164	0.078	0.223
1	0.154	0.287	0.108	0.052	0.150
3	0.031	0.169	0.050	0.032	0.071
10	0.006	0.016	0.006	0.010	0.010

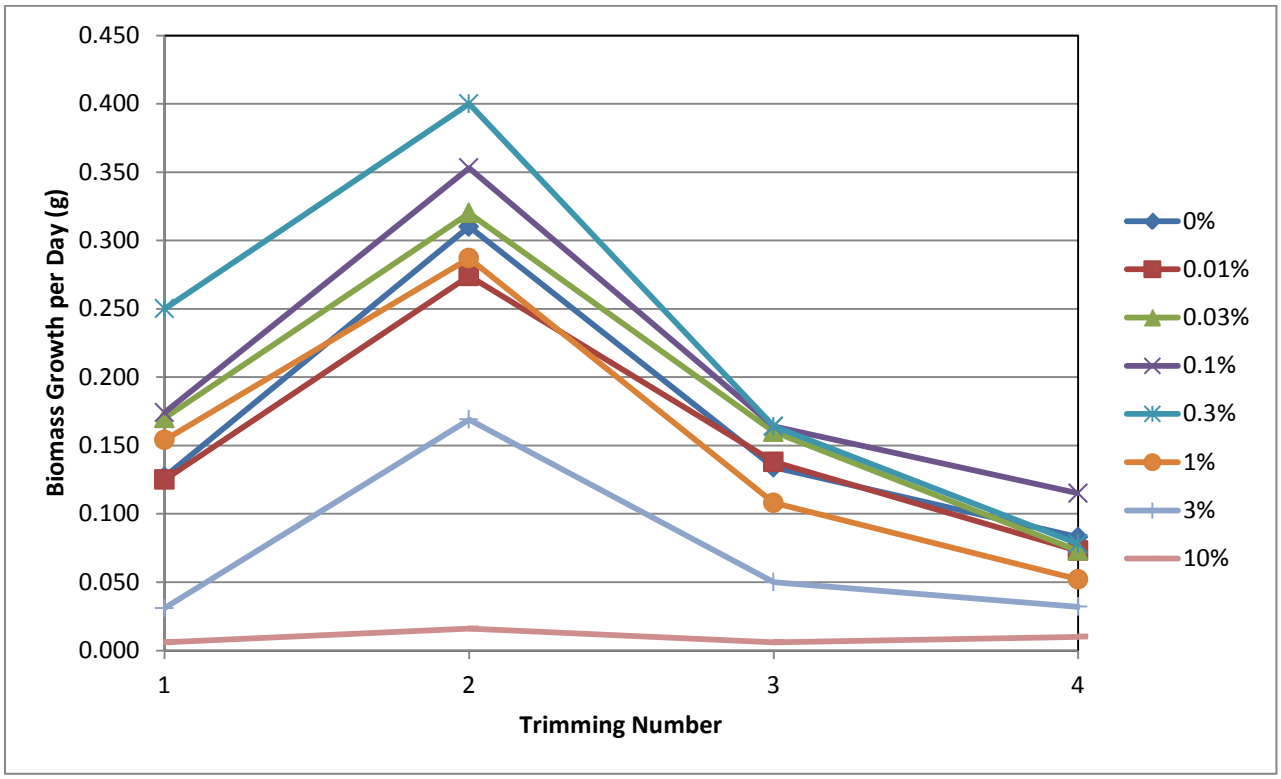


Figure 11. Biomass Growth over Time

To investigate which differences were significant, a series of paired t-Tests (two-tail) were performed to identify significant differences between the growth rate with glycerol and the control. The method compares the growth rate of the glycerol cells at each harvest to the growth rate of the control. These results are listed in Table 8. Three of the treatments showed significant differences ($p < \alpha$) from the control at an $\alpha = 0.05$ level. The 3% and 10% glycerol treatments showed significantly less growth than the control, which can be seen in Figure 8. The 0.1% glycerol application showed significantly more growth than the control.

Table 8. Results of Paired t-test between treatment and control

Glycerol %	p value	conclusion
0.01	0.303	
0.03	0.225	
0.1	**0.002**	**> control**
0.3	0.132	
1	0.400	
3	**0.016**	**< control**
10	**0.050**	**< control**

At the conclusion of the plant germination and growth test, a sample of soil containing each glycerol concentration was removed from the test area, mixed with deionized water and tested for pH level. The results of the pH tests were in the range of 7.9 to 8.1. Thus, it is apparent that the soil buffers the high pH of crude glycerol to levels consistent with common soils.

Runoff Test Results

Runoff tests show correlations between Total Organic Carbon (TOC) in the runoff water and the applied chemicals. Figure 12 shows the results of runoff water testing from plots designated as controls (C), for fertilizer application (F), for glycerol application (G), and for both (B).

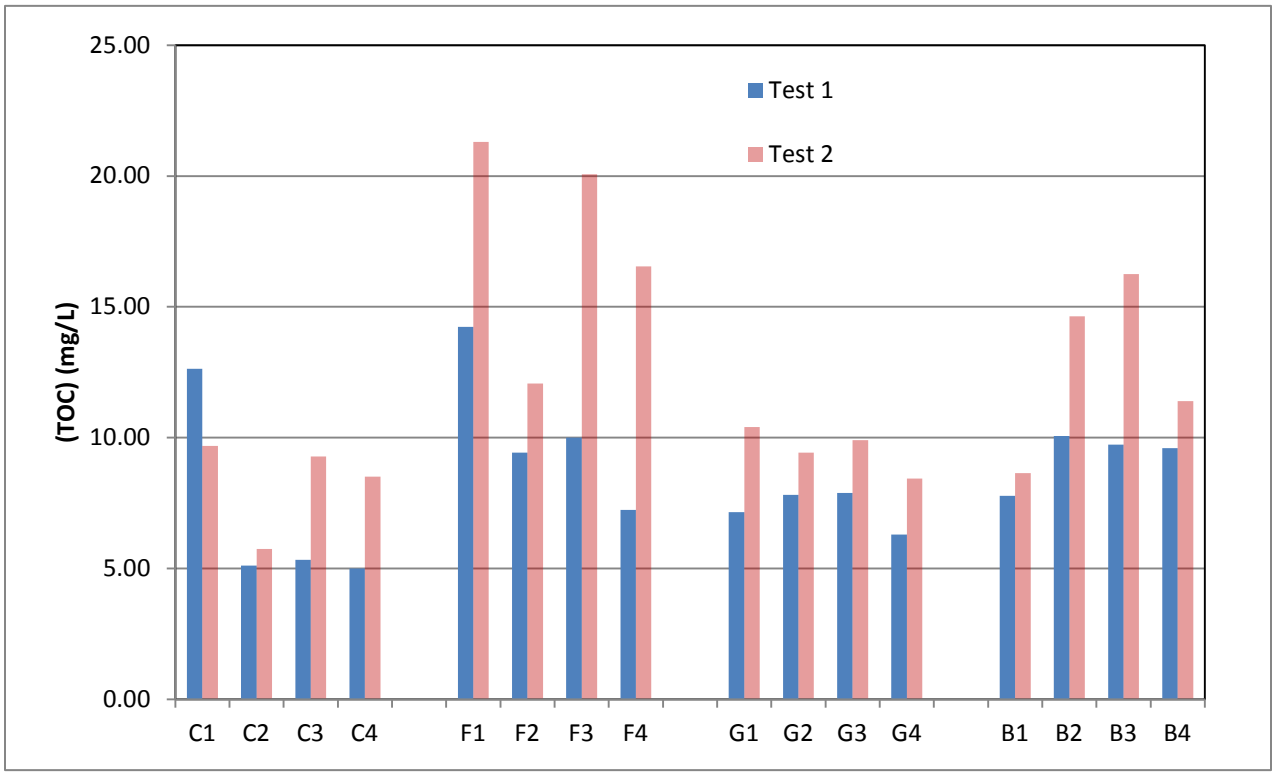


Figure 12. TOC Results for Runoff Replicates.

Between the two sampling dates there is a readily visible correlation between the different applications. The control group shows the lowest TOC runoff and the glycerol group shows a similar reading but slightly higher. The fertilizer only group shows the highest TOC and the group with fertilizer and glycerol shows less, but greater than the glycerol group. An Analysis of Variance (ANOVA) test was performed on sets of data from both runoff tests. The results showed that there was no significant difference between control and glycerol plots and fertilized plots for the test performed after one week. ANOVA showed significant difference between fertilized and non-fertilized plots for the second test after one month but no significant difference between the control and glycerol applied plots.

Figure 13 shows the averages and 90% confidence intervals for the mean TOC concentration in runoff.

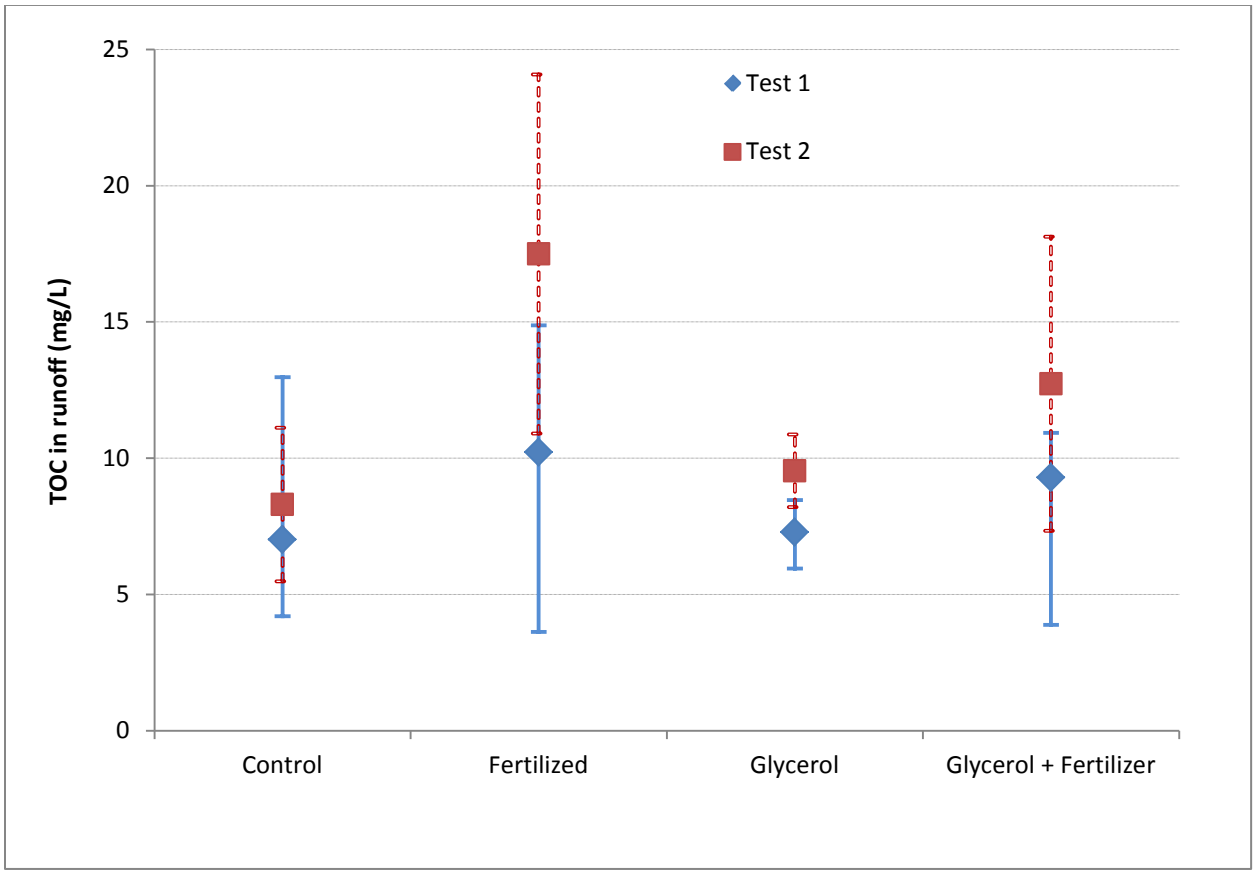


Figure 13. TOC (mg/L) in Runoff: Mean +/- 90% Confidence Intervals

Between the two tests, the average TOC of the runoff water increased by 3.57 mg/L. A t-Test showed that such a difference is statistically significant. However, what is more important are the average differences among the groupings. The control group showed an average increase of 1.29 mg/L. The fertilizer group showed an average increase of 7.28 mg/L. The glycerol group showed an increase of 2.26 mg/L. The group of plots applied with both fertilizer and glycerol showed an average increase of 3.96 mg/L. Table 9 shows the results and differences.

Table 9 Runoff Results – TOC concentrations (mg/L)

TOC concentrations			
Treatment	Test 1	Test 2	dif
C1	12.63	9.68	-2.95
C2	5.11	5.75	0.64
C3	5.33	9.28	3.95
C4	5.00	8.51	3.51
Control avg	7.02 +/- 1.87	8.31 +/- 0.89	1.29
	(mean +/- standard error)		
F1	14.23	21.30	7.07
F2	9.42	12.07	2.65
F3	10.00	20.07	10.07
F4	7.24	16.55	9.31
Fertilizer avg	10.22 +/- 1.46	17.50 +/- 2.07	7.28
G1	7.15	10.41	3.26
G2	7.81	9.42	1.61
G3	7.89	9.90	2.01
G4	6.30	8.44	2.14
Glycerol avg	7.29 +/- 0.37	9.54 +/- 0.42	2.26
B1	7.77	8.64	0.87
B2	10.06	14.64	4.58
B3	9.73	16.25	6.52
B4	9.60	11.40	1.80
Glycerol and Fertilizer avg	9.29 +/- 0.52	12.73 +/- 1.70	3.44
		Average difference =	3.57

CONCLUSIONS AND RECOMMENDATIONS

The results of this research show that a moderate and measured application of glycerol as a soil amendment has no negative effect on soil microbiology, some benefit for plant germination and growth, and no significant increase of Total Organic Carbon from glycerol treated grass plots. High concentrations of glycerol can have a negative effect on plant germination and growth.

The results suggest that glycerol is not toxic to soil microbial communities. In the respirometer testing, the presence of glycerol in a wide range of concentrations in soil showed no inhibition in oxygen uptake. One test with a low concentration of glycerol may have shown a slight inhibition in oxygen uptake relative to the control, but all tests with glycerin showed oxygen uptake.

Plant tests indicated that glycerol concentrations greater than 1% by weight are detrimental to grass germination and growth. Concentrations less than 1% do not inhibit growth and may by beneficial to growth. Results suggest that the best concentration to apply to achieve increase in growth is 0.1% to 0.3% or approximately 1000 to 4000 lb/acre. It is clear that very high concentrations such as 3%, 10%, and above are detrimental to the growth of the plants. The previous MBTC project (Soerens, 2011) showed that high glycerol concentrations are detrimental to animal life (worms) as well. It is important to assure limited or no negative effects to wildlife and thus it is necessary to limit the application of glycerol to acceptable and benign or beneficial levels.

Glycerol waste from biodiesel has a high pH from the base used in the process. The titration of glycerol demonstrated by the steep negative slope at the beginning that the pH of the crude product can be lowered easily. And as was shown at the close of plant growth testing, pH is brought down rapidly and significantly by the natural buffering capability of the soil itself. Even so, it may be of benefit to acidic soils and could lessen the application of lime to such soils.

In field runoff tests measuring total organic carbon in runoff samples, there was not a significant difference between the glycerol treated grass plots and the untreated (control) grass plots. TOC from the fertilized plots was higher than the controls or the glycerol plots. The plots with both glycerol and fertilizer had slightly higher TOC runoff than the controls and the glycerol only, but less than those with fertilizer only. All plots had increased TOC in the second runoff test with the fertilizer-only plots showing the most increase and the glycerol-only plots the least increase.

An interesting not is that, during field testing, cows became very interested in glycerol that had been spilled on the ground. Such was the case that the cows actually ate holes in the turf as they sought the sweet glycerol that had soaked in. Fortunately, the test plots had been fenced off prior to the commencement of the experiments and turf damage was confined to areas outside the confines of the extent of the testing. It may be necessary to separate cattle from areas where glycerol is being land applied temporarily so that they may not cause damage to the turf and consequently cause increased erosion and sediment in runoff.

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Appendix A – Respirometer Data

Data files available upon request.

Appendix B – Growth and Germination Data

Growth Tests – Plant mass

	Raw	Weight	Raw	Weight	Harvest 1	Raw	Weight	Raw	Weight	Harvest 2
10	45.9915	0.0272	46.7222	0.1795	0.1795	45.9815	0.0195	46.6177	0.0787	0.0787
3	46.7351	0.7708	47.708	1.1653	0.96805	47.0945	1.1325	47.0945	0.5555	0.844
1	51.0976	5.1333	50.9356	4.3929	4.7631	47.4022	1.4402	47.9688	1.4298	1.435
0.3	53.116	7.1517	55.1913	8.6486	7.90015	47.9169	1.9549	48.5796	2.0406	1.99775
0.1	50.2064	4.2421	53.075	6.5323	5.3872	47.6514	1.6894	48.3786	1.8396	1.7645
0.03	50.9164	4.9521	52.1324	5.5897	5.2709	47.3826	1.4206	48.3169	1.7779	1.59925
0.01	49.3863	3.422	50.8594	4.3167	3.86935	47.2415	1.2795	48.0014	1.4624	1.37095
0	50.1111	4.1468	50.2654	3.7227	3.93475	47.443	1.481	48.1574	1.6184	1.5497
	45.9643		46.5427			45.962		46.539		

Raw	Weight	Raw	Weight	Harvest 3	Raw	Weight	Raw	Weight	Harvest 4
45.9935	0.032	46.5743	0.0438	0.0438	46.02	0.0548	46.6816	0.1392	0.1392
46.2553	0.2938	46.941	0.4105	0.35215	46.3917	0.4265	47.0117	0.4693	0.4479
46.7143	0.7528	47.2906	0.7601	0.75645	46.7568	0.7916	47.2165	0.6741	0.73285
47.0628	1.1013	47.7254	1.1949	1.1481	47.045	1.0798	47.6503	1.1079	1.09385
47.081	1.1195	47.713	1.1825	1.151	47.9532	1.988	47.7808	1.2384	1.6132
46.9636	1.0021	47.7714	1.2409	1.1215	46.9335	0.9683	47.6055	1.0631	1.0157
46.7751	0.8136	47.6456	1.1151	0.96435	46.925	0.9598	47.6265	1.0841	1.02195
46.8933	0.9318	47.4708	0.9403	0.93605	47.1493	1.1841	47.6719	1.1295	1.1568
45.9615		46.5305			45.9652		46.5424		

9/6/2011	10/13/2011	10/18/2011	10/25/2011	11/8/2011
Concentra	1	2	3	4
10	0.1795	0.0787	0.0438	0.1392
3	0.96805	0.844	0.35215	0.4479
1	4.7631	1.435	0.75645	0.73285
0.3	7.90015	1.99775	1.1481	1.09385
0.1	5.3872	1.7645	1.151	1.6132
0.03	5.2709	1.59925	1.1215	1.0157
0.01	3.86935	1.37095	0.96435	1.02195
0.001	3.93475	1.5497	0.93605	1.1568

ANOVA on plant mass

Anova: Two-Factor Without Replication

SUMMARY	*Count*	*Sum*	*Average*	*Variance*
10	4	0.4412	0.1103	0.003682
3	4	2.6121	0.653025	0.089438
1	4	7.6874	1.92185	3.693868
0.3	4	12.13985	3.034963	10.69134
0.1	4	9.9159	2.478975	3.82711
0.03	4	9.00735	2.251838	4.115436
0.01	4	7.2266	1.80665	1.923263
0.001	4	7.5773	1.894325	1.914778
1	8	32.273	4.034125	6.161993
2	8	10.63985	1.329981	0.367156
3	8	6.4734	0.809175	0.166698
4	8	7.22145	0.902681	0.207361

ANOVA

ce of Varic	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Rows	25.50812	7	3.644017	3.354222	0.014657	2.487578
Columns	55.96241	3	18.65414	17.17064	7.25E-06	3.072467
Error	22.81434	21	1.086397			
Total	104.2849	31				

Growth Rates

g/day					
Growth Rates					
	31	5	7	15	days
	1	2	3	4	average
0	0.127	0.310	0.134	0.083	0.163
0.01	0.125	0.274	0.138	0.073	0.152
0.03	0.170	0.320	0.160	0.073	0.181
0.1	0.174	0.353	0.164	0.115	0.202
0.3	0.255	0.400	0.164	0.078	0.224
1	0.154	0.287	0.108	0.052	0.150
3	0.031	0.169	0.050	0.032	0.071
10	0.006	0.016	0.006	0.010	0.009

ANOVA on growth rates

Anova: Two-Factor Without Replication

SUMMARY	*Count*	*Sum*	*Average*	*Variance*
0	4	0.653217	0.163304	0.01007
0.01	4	0.609768	0.152442	0.007371
0.03	4	0.722643	0.180661	0.010531
0.1	4	0.806338	0.201584	0.010836
0.3	4	0.89654	0.224135	0.018882
1	4	0.601059	0.150265	0.010026
3	4	0.282327	0.070582	0.004365
10	4	0.03773	0.009433	2.11E-05
1	8	1.041065	0.130133	0.006412
2	8	2.12797	0.265996	0.014686
3	8	0.924771	0.115596	0.003402
4	8	0.515818	0.064477	0.001058

ANOVA

ce of Varic	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Rows	0.140253	7	0.020036	10.88515	9.37E-06	2.487578
Columns	0.177648	3	0.059216	32.17052	4.86E-08	3.072467
Error	0.038655	21	0.001841			
Total	0.356556	31				

Example of paired t-test

t-Test: Paired Two Sample for Means		
	10	*0*
Mean	0.009433	0.163304
Variance	2.11E-05	0.01007
Observations	4	4
Pearson Correlation	0.802222	
Hypothesized Mean Difference	0	
df	3	
t Stat	-3.18249	
P(T<=t) one-tail	0.024999	
t Critical one-tail	2.353363	
P(T<=t) two-tail	0.049998	
t Critical two-tail	3.182446	

Appendix C – Runoff Data

Total Organic Carbon Test			
Plot	Designation	Test 1	Test 2
1	C1	12.63	9.68
2	F1	14.23	21.30
3	G1	7.15	10.41
4	B1	7.77	8.64
5	C2	5.11	5.75
6	F2	9.42	12.07
7	F3	10.00	20.07
8	B2	10.06	14.64
9	C3	5.33	9.28
10	F4	7.24	16.55
11	G2	7.81	9.42
12	B3	9.73	16.25
13	C4	5.00	8.51
14	G3	7.89	9.90
15	G4	6.30	8.44
16	B4	9.60	11.40

TOC Arranged by Group		
Plot	Test 1	Test 2
C1	12.63	9.68
C2	5.11	5.75
C3	5.33	9.28
C4	5.00	8.51
F1	14.23	21.30
F2	9.42	12.07
F3	10.00	20.07
F4	7.24	16.55
G1	7.15	10.41
G2	7.81	9.42
G3	7.89	9.90
G4	6.30	8.44
B1	7.77	8.64
B2	10.06	14.64
B3	9.73	16.25
B4	9.60	11.40

Averages		
	Test 1	Test 2
Control	7.02	8.31
Fertilizer	10.22	17.50
Glycerol	7.29	9.54
Both	9.29	12.73

Averages	
Test 1	Test 2
8.45	12.02

Difference Between Tests				Group Average Difference
C1	12.63	9.68	-2.95	
C2	5.11	5.75	0.64	
C3	5.33	9.28	3.95	1.29
C4	5.00	8.51	3.51	
F1	14.23	21.30	7.07	7.28
F2	9.42	12.07	2.65	
F3	10.00	20.07	10.07	
F4	7.24	16.55	9.31	
G1	7.15	10.41	3.26	2.26
G2	7.81	9.42	1.61	
G3	7.89	9.90	2.01	
G4	6.30	8.44	2.14	
B1	7.77	8.64	0.87	3.96
B2	10.06	14.64	4.58	
B3	9.73	16.25	6.52	
B4	9.60	11.40	1.80	
Average Difference			3.57	

Difference Between Tests			
C1	12.63	9.68	-2.95
C2	5.11	5.75	0.64
C3	5.33	9.28	3.95
C4	5.00	8.51	3.51
F1	14.23	21.30	7.07
F2	9.42	12.07	2.65
F3	10.00	20.07	10.07
F4	7.24	16.55	9.31
G1	7.15	10.41	3.26
G2	7.81	9.42	1.61
G3	7.89	9.90	2.01
G4	6.30	8.44	2.14
B1	7.77	8.64	0.87
B2	10.06	14.64	4.58
B3	9.73	16.25	6.52
B4	9.60	11.40	1.80
Average Difference			3.57

t-Test: Paired Two Sample for Means		
	Variable 1	*Variable 2*
Mean	8.454375	12.01938
Variance	6.768773	19.95263
Observations	16	16
Pearson Correlation	0.667517	
Hypothesized Mean Difference	0	
df	15	
t Stat	-4.25974	
P(T<=t) one-tail	0.000343	
t Critical one-tail	1.75305	
P(T<=t) two-tail	0.000685	
t Critical two-tail	2.13145	

ANOVA Test 1		
	No Fertilizer	Fertilizer
No Glycerol	12.63	14.23
	5.11	9.42
	5.33	10.00
	5.00	7.24
Glycerol	7.15	7.77
	7.81	10.06
	7.89	9.73
	6.30	9.60

Anova: Two-Factor With Replication

SUMMARY	No Fertilizer	Fertilizer	Total
No Glycerol			
Count	4	4	8
Sum	28.07	40.89	68.96
Average	7.0175	10.2225	8.62
Variance	14.01889167	8.549625	12.607
Glycerol			
Count	4	4	8
Sum	29.15	37.16	66.31
Average	7.2875	9.29	8.2888
Variance	0.543358333	1.064333	1.8347
Total			
Count	8	8	
Sum	57.22	78.05	
Average	7.1525	9.75625	
Variance	6.261792857	4.368713	

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	0.43890625	1	0.4389	0.072618	0.79214	4.747225
Columns	27.11805625	1	27.118	4.486734	0.055714	4.747225
Interaction	1.44600625	1	1.446	0.239245	0.63357	4.747225
Within	72.528625	12	6.0441			
Total	101.5315938	15				

ANOVA Test 2		
	No Fertilizer	Fertilizer
No Glycerol	9.68	21.30
	5.75	12.07
	9.28	20.07
	8.51	16.55
Glycerol	10.41	8.64
	9.42	14.64
	9.90	16.25
	8.44	11.40

Anova: Two-Factor With Replication

SUMMARY	No Fertilizer	Fertilizer	Total
No Glycerol			
Count	4	4	8
Sum	33.22	69.99	103.21
Average	8.305	17.4975	12.901
Variance	3.1371	17.14409	32.835
Glycerol			
Count	4	4	8
Sum	38.17	50.93	89.1
Average	9.5425	12.7325	11.138
Variance	0.703625	11.51183	8.1427
Total			
Count	8	8	
Sum	71.39	120.92	
Average	8.92375	15.115	
Variance	2.083569643	18.76831	

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	12.44325625	1	12.443	1.531636	0.239536	4.747225
Columns	153.3263063	1	153.33	18.87288	0.000954	4.747225
Interaction	36.03000625	1	36.03	4.434921	0.056942	4.747225
Within	97.489925	12	8.1242			
Total	299.2894938	15				

ANOVA Average		
	No Fertilizer	Fertilizer
No Glycerol	11.16	17.77
	5.43	10.75
	7.31	15.04
	6.76	11.90
Glycerol	8.78	8.21
	8.62	12.35
	8.90	12.99
	7.37	10.50

Anova: Two-Factor With Replication

SUMMARY	No Fertilizer	Fertilizer	Total
No Glycerol			
Count	4	4	8
Sum	30.645	55.44	86.085
Average	7.66125	13.86	10.761
Variance	6.044322917	10.0647	17.882
Glycerol			
Count	4	4	8
Sum	33.66	44.045	77.705
Average	8.415	11.01125	9.7131
Variance	0.49855	4.614706	4.1173
Total			
Count	8	8	
Sum	64.305	99.485	
Average	8.038125	12.43563	
Variance	2.966413839	8.609853	

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	4.389025	1	4.389	0.827249	0.380975	4.747225
Columns	77.352025	1	77.352	14.5794	0.002447	4.747225
Interaction	12.97800625	1	12.978	2.44611	0.143792	4.747225
Within	63.6668375	12	5.3056			
Total	158.3858938	15				