

## THE PHYTOREMEDIATION OF CREOSOTE CONTAMINATED SOIL

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### INTRODUCTION

Creosote is a common organic wood preservatives used in the wood treating industry for over 100 years. Soil contaminated with creosote has been found in many former and active wood treating plants. Soil contamination at these sites has resulted from past practices and accidental spillage. Physically removing these contaminated soils is not a cost effective method of cleanup. An option in certain situations is to use a form of bioremediation called phytoremediation as a means of degrading these pollutants. Phytoremediation is the use of plants to enhance degradation or sequester harmful contaminants found in soil and water. Phytoremediation is gaining popularity for its low cost in-situ treatment of contaminants. Compared to other remediation technologies, phytoremediation appears to produce results comparable to landfarming and offers protection against erosion, maintains proper soil conditions, and is less laborious than landfarming (Andreotti, et al 2001). Phytoremediation provides four avenues of approach for contaminated areas: 1.) microbial degradation of contaminants within the rhizosphere. 2.) hyperaccumulators where plants uptake and store harmful contaminants, commonly heavy metals, in their roots and shoots. These plants are later harvested and disposed of in a proper manner. 3.) rhizofiltration where plant roots absorb, concentrate or, precipitate heavy metal ions from water. 4.) phytovolatilization where plants uptake volatile organic compounds (VOCs) in groundwater allowing them to be released into the atmosphere via the stomata openings. Surface waters and shallow aquifers were

the first sites where plants were applied as a method of cleanup (Cunningham, 1997; Carman, 1997; Ensley, 2000). Many different plants and trees have been used in the removal or degradation of toxic pollutants. Trees like poplar, willows, and cottonwoods removed contaminants from ground water (Glass, 1998). The biggest disadvantage in using trees for environmental cleanup is the time needed for tree growth. Faster growing flora like grasses may be more suited for some situations.

Although the roots of grasses do not penetrate the soil at depths associated with tree roots, they can proliferate within the topsoil. Most herbaceous plants only produce roots within the first three feet of soil. Alfalfa, in contrast, can produce roots that will extend to a depth of six feet (Stern, 1991).

Without healthy growing plants, phytoremediation would not be successful, thus the ideal environmental conditions for the plants should be provided. For alfalfa, an N-P-K ratio of 0-24-24, respectively, is generally recommended. Boron is often added in trace amounts to promote growth (Kimbrough, 1999). Ryegrass is generally fertilized with (N-P-K), 13-13-13 and a soil pH of 6.5 is needed. Often, ryegrass and alfalfa rotated on a site will make ideal counterparts. The soil pH needed to grow alfalfa is analogous with the soil pH of ryegrass. Ryegrass requires high levels of nitrogen for growth, where as for alfalfa no nitrogen needs to be added because rhizomes fix nitrogen in the soil. Rotating both species will reduce the fertilizer requirements for each growing season (Kimbrough, 1999a).

The objectives of this study were to evaluate the effectiveness of ryegrass for the remediation of creosote during the winter months, and to evaluate the effectiveness of alfalfa for the remediation of creosote during the summer months.

## **MATERIALS AND METHODS**

### Soil Preparation

Soil containing creosote was collected from an old abandoned wood treatment facility located in south MS. The clean soil used in the planted control for creosote was also collected from the same site (a location not contaminated with creosote). After the soils were collected they were air-dried, screened, and debris 3 mm and larger was removed. After the screening process the soils were homogenized by placing them in a soil mixer. Background analysis on the homogenized soil was performed to determine polycyclic aromatic hydrocarbons (PAHs) levels, and nutrient content. Nutrient testing consisted of nitrogen, phosphorus, potassium, and total organic carbon (TOC). Based on these results, Miracle Grow 15-30-15 was added to each of the treatments. A mixture of one teaspoon of Miracle Grow 15-30-15 per gallon of water was used to fertilize all the treatments in the ryegrass experiment. Each pot was fertilized with 20 ml of the Miracle Grow solution. The ryegrass treatments were fertilized twice with a separation of two weeks within the 90 day period. The pots in the alfalfa experiment were fertilized with 25 ml of 0.5 teaspoon per gallon of Miracle Grow solution. They were also fertilized twice with a two week separation.

The soils (250 g) were placed in 500 cm<sup>3</sup> plastic pots with holes drilled in the bottom for water absorption. The pots were then placed into aluminum pans lined with paper towels creating a small gap between the pans and the pots. Twenty pots were created in this fashion for each treatment (clean soil + plants, creosote + plants, and creosote control). Fifteen seeds were added to each pot designated as plant treatments. After the seeds germinated and became established the number of plants in each pot was adjusted to be consistent for all treatments. Each pot analyzed in the ryegrass study contained eleven plants, and pots used in the alfalfa study contained thirteen plants. Water was added to the pans allowing the soils to absorb water from the bottom. During the winter (Ryegrass study) each pan was filled to total capacity on a schedule of every other day. For the alfalfa study during the summer water was added daily. Pans used for both the winter and summer studies had a maximum capacity of one gallon of water. Samples were collected every 45 days for analysis.

#### Experimental Controls

Two types of controls were used in this experiment. One consisted of contaminated soil with no plants. The second control was clean soil containing plants to establish the fact that all of the necessary requirements were met to promote the growth of alfalfa and ryegrass. The plants from these controls were also tested for selected PAHs compounds found in creosote, in order to establish background levels of PAHs. All controls for this experiment were subjected to the same conditions and received the same amount of sunlight, fertilizer, and water throughout the experiment as the test subjects.

### Seed Variety

Marshall ryegrass (Supreme Brand Wild Game Mix) was used in the cool season treatments. Alfagraze alfalfa inoculated with Rhizobium meliloti and R. leguminosarum biovar trifolii was used in the summer treatments.

### Soil Moisture Content

The soil moisture content was measured by placing soil samples in an oven for a minimum of four hours and recording the weight loss. The moisture content was recorded as a percentage based on the initial wet soil weight (EPA, 1986).

### Soxhlet Extraction for Soil & Plant Biomass

EPA method 3540 (EPA, 1986) was used to extract the selected PAHs from the soil and plant tissue. One milliliter of each extracted sample was further processed using a silica gel cleanup. The samples were analyzed by gas chromatography using EPA method 8100 for the following PAHs: naphthalene, 2-methylnaphthalene, 1-methylnaphthalene, biphenyl, acenaphthylene, acenaphthene, dibenzofuran, fluorene, phenanthrene, anthracene, carbazole, fluoranthracene, pyrene, 1,2-benzanthracene, chrysene, benzo (a) pyrene, and benzo (ghi) pyrene.

### Leachate Analysis

At the conclusion of the experiments one sample of water (500 ml) contained in one of the pans was used for leachate analysis. The sample was extracted by using EPA method 3520. The extracts were analyzed for PAHs using gas chromatography method 8100.

### Oil & Grease Analysis

Method 5520B (Clesceri, 1989) was also performed to determine total oil and grease content on all the extracted soil samples.

### Microbial Counts

Microbial counts were performed by conducting serial dilutions on the soil samples. Four media were selected for each of the three soil treatments. Two media for each treatment were selective for bacteria. The other two media were selective for fungi.

Nutrient agar (NA) was produced using the manufacturer's specifications for the product. Creosote / nutrient agar (C) was prepared by adding 1 ml of creosote standard consisting of 20 mg/ml (creosote in methanol) to each liter of media. Potato dextrose agar with antibiotics (PDAA) was prepared using the manufacturer's specifications plus chlorotetracycline (30 mg/L) and streptomycin sulfate (120 mg/L) was added. The antibiotic solution was then sterile filtered into the potato dextrose agar. Potato dextrose agar with antibiotics and creosote (PDAAC) was prepared in the same manner as the PDAA. Creosote standard (1 ml) listed above was added to the PDAA solution.

NA was used to recover total bacteria populations. C was used to recover the viable bacteria acclimated to creosote. PDAA enumerated all the fungi present in the soil sample. PDAAC was used to obtain the fungi populations tolerant to creosote.

### Statistical Analysis

The concentration of creosote was analyzed using a completely randomized design. Replicates from each treatment were compared using a two-way analysis of variance with replicates to test for significance between treatments. Testing was executed using Microsoft Excel version 2001 with an  $\alpha = 0.05$ .

## **RESULTS AND DISCUSSION**

### **Ryegrass Experiment**

#### Moisture Content for Ryegrass Study

It was expected that the creosote, due to its hydrophobic properties, would reduce the moisture content of soil. However by day 90 the moisture content for clean soil and creosote soil planted with ryegrass were almost identical 28% (Table 1). The creosote + plant treatment moisture content was 8.6% greater than the unplanted creosote control which indicates that the presence of ryegrass in creosote contaminated soil increased moisture content. An increase in soil moisture content by the growth of ryegrass is important because at higher levels it should increase the availability of nutrients to support the growth of the bacteria and fungi that can degrade creosote.

#### Microbial Counts for Ryegrass

A slight population increase was observed for only the first 45 days, but not throughout the 90 days of the trials for the pots containing ryegrass (Figure 1). The reasons for the decrease in populations from day 45 to day 90 could not be determined. Random fluctuations in the bacteria and fungi counts could have been a factor. Another factor that could have influenced the population numbers was the weather conditions toward the end of the 90 days; the temperature started to increase rapidly in the non-vented greenhouse and this could have induced physiological changes affecting the root exudates produced by the ryegrass impacting the bacteria and fungi populations.

It was expected that the bacteria and fungi populations would be different between the clean and creosote contaminated soils. Especially since two of the four plating mediums used were selective for creosote. No differences between the bacteria and fungi population could be determined for each of the soils. This is probably because the bacteria and fungi populations came from soils from the same site, with less than 1/4 a mile separating the two locations where the soils had been collected. Even though the clean soil did not contain creosote, it did contain bacteria and fungi populations tolerant to creosote.

#### Creosote Concentrations for Ryegrass

The presence of ryegrass did not significantly reduce the creosote content of the contaminated soils compared to the controls after 90 days of growth (Figure 2). The manner in which the soil was prepared provided an oxygen rich environment for existing bacteria and fungi populations. Placing the soil loosely

into each of the pots along with water and nutrients created ideal conditions for bioremediation of the creosote constituents in the unplanted controls.

The amount of creosote lost over time from day 0 (548 ppm) is recorded in Table 2 for the ryegrass study. The difference in the percent creosote lost by day 45 for both plants and controls was only 2.0%. By day 90 the difference between the ryegrass and the controls had increased to 6.0%, suggesting that over a longer period of time the amount of creosote degraded in the presence of plants could become significant. The average concentration of PAHs based on ring size showed only a reduction of 4 ring PAHs and inconsistent results for other groups.

#### Ryegrass Shoot and Root Observations

On the basis of visual observations of the bottom of the pots, the presence of creosote in soil appeared to have a negative effect on the root growth. Fewer roots were visible in the bottom of the pot containing creosote compared to the pot containing clean soil for the first 45 days. Observations of the bottom of the pots on day 79 suggested the same trend of having less roots in the presence of creosote. However, the conclusion that root development by day 79 was hindered in the presence of creosote was retracted after the ryegrass plants were removed from the soil in order to make a full comparison of the root systems.

The development of shoots and leaves was stunted in the presence of creosote at the initial part of the experiment. However, by day 79 no differences could be determined in the shoot and leaves between the two soil treatments, indicating ryegrass is capable of growing without any observable effects in creosote contaminated soil. Plant biomass was extracted to determine the

presence PAHs within shoots and roots. No PAHs could be detected within these tissues.

#### Ryegrass Oil and Grease Analysis

Oil and grease analysis provides an account of all the creosote and other oily organic compounds present in the soil, which is in contrast to the GC analysis where only, selected creosote constituents are identified. After a complete analysis of the oil and grease concentration within the samples, it was determined that the presence of ryegrass did not significantly reduce the concentration (Figure 3). Day 45 results show a difference between ryegrass and control treatments of 12.8%. An analysis of the oil and grease concentration for day 90 showed that the unplanted control was 28.4% higher than the ryegrass treatment. Although this reduction was not statistically significant, it does indicate that a longer exposure period could result in a significantly greater reduction of oil and grease. These reductions were considerably greater than those seen for selected PAHs, which indicates that the microorganisms preferably biodegraded components other than the PAHs analyzed in this study.

## **Alfalfa Experiment**

### Moisture Content for Alfalfa Study

The addition of alfalfa plants did not increase the moisture content enough to be considered substantial after the plants had reached maturity in the creosote contaminated soil (Table 3). This is in contrast to the ryegrass study where the presence of ryegrass increased the moisture content in the planted versus the unplanted controls. Some degradation did occur in the presence of ryegrass; in the case of alfalfa no degradation between the treatments exists. A difference in moisture content was expected between the creosote contaminated soils plus or minus alfalfa because it was assumed there would be an increased water demand from the alfalfa plants. It was observed that the root systems of the alfalfa plants extended out of the pots into the trays giving them direct access to water. This would eliminate the need for the plants to pull water up through the contaminated soils and probably explains the lack of increase in soil moisture content.

### Microbial Counts for Alfalfa

The basis for phytoremediation is that an increase in bacteria and fungi populations within the rhizosphere will lead to a decrease in creosote concentration over time. Although the bacteria and fungi populations in the ryegrass study did not increase throughout the 90 days some decrease in creosote did occur. In the case of alfalfa, the bacteria and fungi populations had a slight increase throughout the 90 days compared to the appreciable creosote controls (Figure 4).

### Creosote Concentrations for Alfalfa

Neither the presence of alfalfa nor ryegrass significantly reduced the total amount of selected creosote constituents within the test pots during the 90 day studies. Figure 5 compares the mean total PAHs for each treatment for days 45 and 90. The concentration of PAHs for days 45 and 90 are identical between each of the treatments for the given day. The alfalfa results for PAHs gives no indication that an increased study period would create a significant difference between planted and unplanted controls. Table 4 provides the percent creosote loss from day 0 (508 ppm) for the alfalfa study. Only a 3.7% reduction of PAHs had taken place between days 45 and 90 for each treatment. These results indicate that alfalfa did not enhance the degradation of creosote in contaminated soil. The average concentration of PAHs based on ring size showed a reduction of 2, 3, 4, and 5 ring PAHs for day 45 but no reduction of the PAHs occurred for day 90.

### Alfalfa Shoot and Root Observations

No differences in growth characteristics could be determined between the alfalfa plants grown in creosote contaminated soil and the clean soil. The creosote had no effect on the amount of shoot development. No lag phase at the start of the experiment was observed like the one reported earlier for ryegrass. The creosote did seem to improve the drought tolerance of the alfalfa plants. During hot and dry periods when a water deficiency could develop, the leaves of the alfalfa plants growing in clean soil would fold up to reduce water loss. In

contrast, the alfalfa plants grown in creosote contaminated soil remained open during this period.

No observable differences in root growth were apparent for alfalfa grown in creosote contaminated or clean soil. Plant biomass was extracted and no PAHs could be detected within shoots and roots of the alfalfa plants.

#### Alfalfa Oil and Grease Analysis

The average oil and grease content for days 45 and 90 (control minus plants and alfalfa pots) are provided in Figure 6. These results indicated alfalfa did not reduce the oil and grease present within the soil. The day 45 results seemed promising because the alfalfa pots showed less oil and grease, however the differences were not significant. By day 90 of the alfalfa study no reduction of oil and grease content was apparent in the presence of alfalfa. The control pots had a lower mean concentration compared to the alfalfa pots, but were not statistically different.

#### Leachate Analysis

The results of the pot leachate analysis were also analogous to the published literature regarding the leaching potential of organic compounds. None of the creosote constituents were found to be present in the water samples collected from either of the plant studies or creosote controls. Leachate analysis is also important to insure that the reduction of creosote constituents is directly related to microbial and fungi activity within the soil.

## **CONCLUSION**

Growing marshall ryegrass on creosote contaminated soil did not result in any significant reduction in the creosote concentration after 90 days of exposure. However there was a slight trend in creosote reduction, suggesting that growth of ryegrass over a longer time period may possibly result in a significant reduction in creosote concentration. Furthermore, there are many different ryegrass varieties that might be more efficient at remediating creosote and future research should be directed at evaluating some of these different varieties.

Alfagraze alfalfa showed no sign of reducing the creosote content of contaminated soils. Like ryegrass, there are many different varieties available on the market. Alfagraze does provide two benefits to areas contaminated with creosote: 1) alfagraze could produce excellent ground cover reducing the spread of creosote through ground water runoff and dust particles without causing harm to grazing animals in the area. 2) alfagraze could also increase the nitrogen content of soils which is a needed for crops like ryegrass to grow.

TABLE 1. SOIL MOISTURE CONTENT FOR THE RYEGRASS STUDY.

Treatment	Day 0	Day 45	Day 90
Clean + Ryegrass	5.4%	18.1%	27.9%
Creosote + Ryegrass	4.3%	13.0%	28.6%
Creosote Control	4.3%	9.8%	20.7%

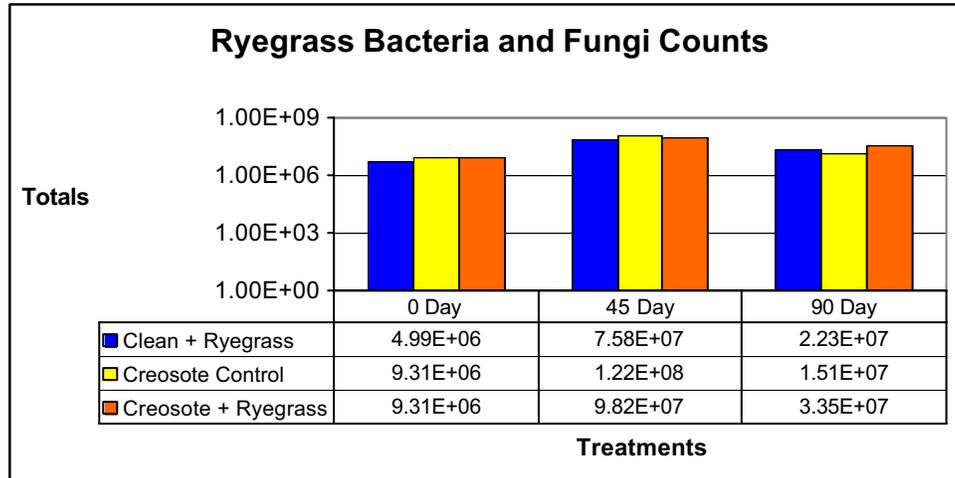


Figure 1. Combined average total of bacteria and fungi populations within each sample of the ryegrass study. Each treatment represents the average of four replicates.

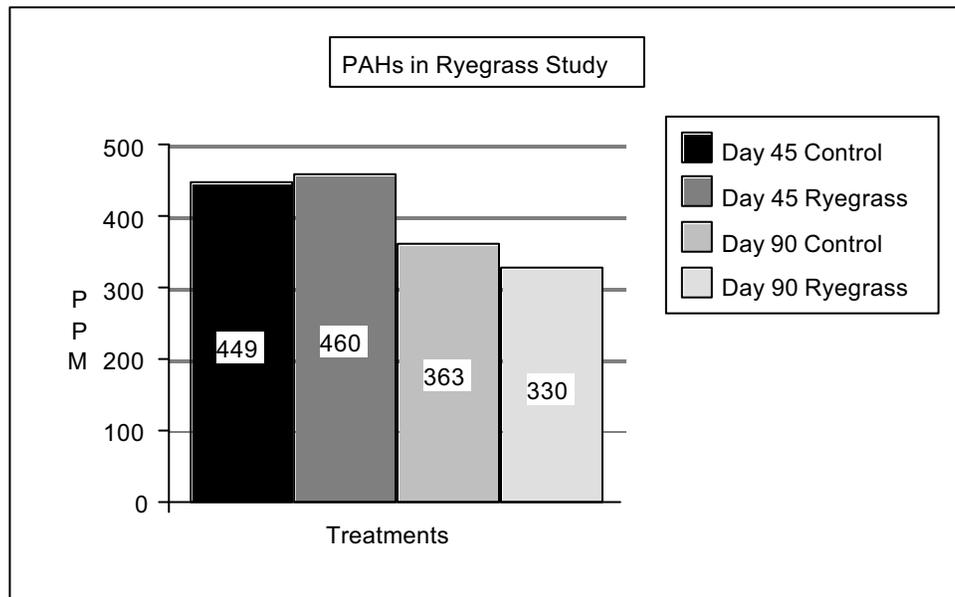


Figure 2. Mean concentration of selected PAHs for ryegrass experiment. Treatments are not significantly different at the  $\alpha = .05$  Level. Each treatment represents the analysis of four replicates.

TABLE 2. AVERAGE CREOSOTE CONSTITUENT LOSS FROM INITIAL CONCENTRATION FOR THE RYEGRASS STUDY

Ryegrass Day 45	16.10%
Controls Day 45	18.10%
Ryegrass Day 90	39.80%
Controls Day 90	33.8%

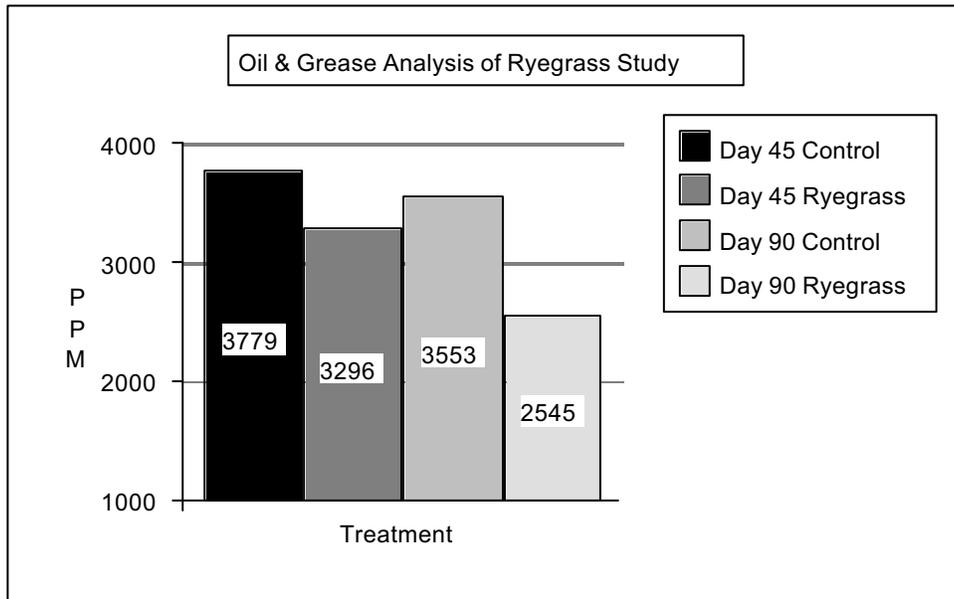


Figure 3. Mean concentration of oil and grease for the ryegrass experiment. Treatments are not significantly different at the  $\alpha = .05$  Level. Each treatment represents the analysis of four replicates.

TABLE 3. SOIL MOISTURE CONTENT FOR THE ALFALFA STUDY.

Treatment	Day 0	Day 45	Day 90
Creosote + Alfalfa	1.7%	19.4%	23.7%
Creosote Control	1.7%	23.0%	23.3%
Clean + Alfalfa	2.2%	24.6%	25.0%

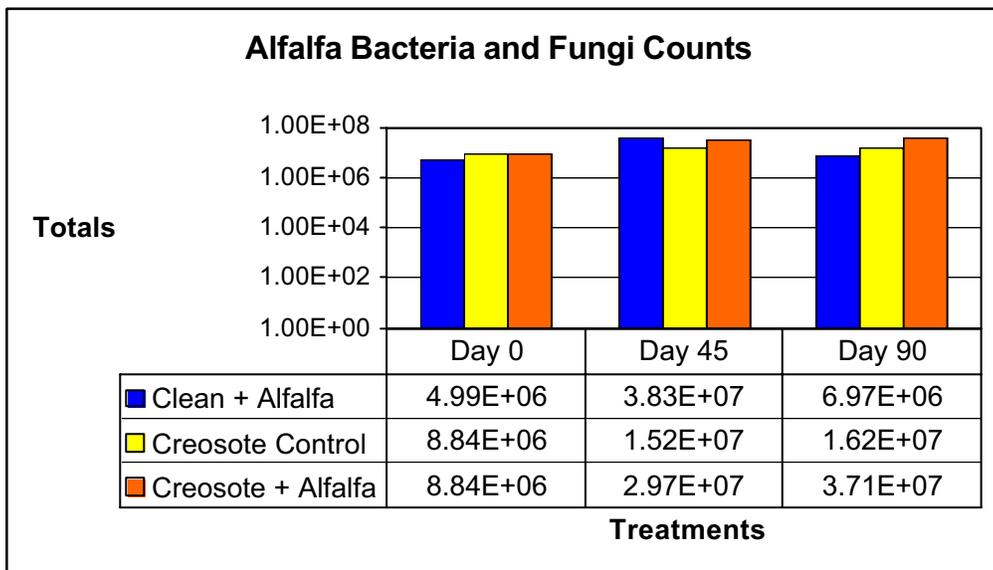


Figure 4. Combined average total of bacteria and fungi populations within each sample of the alfalfa study. Each treatment represents the average of three replicates.

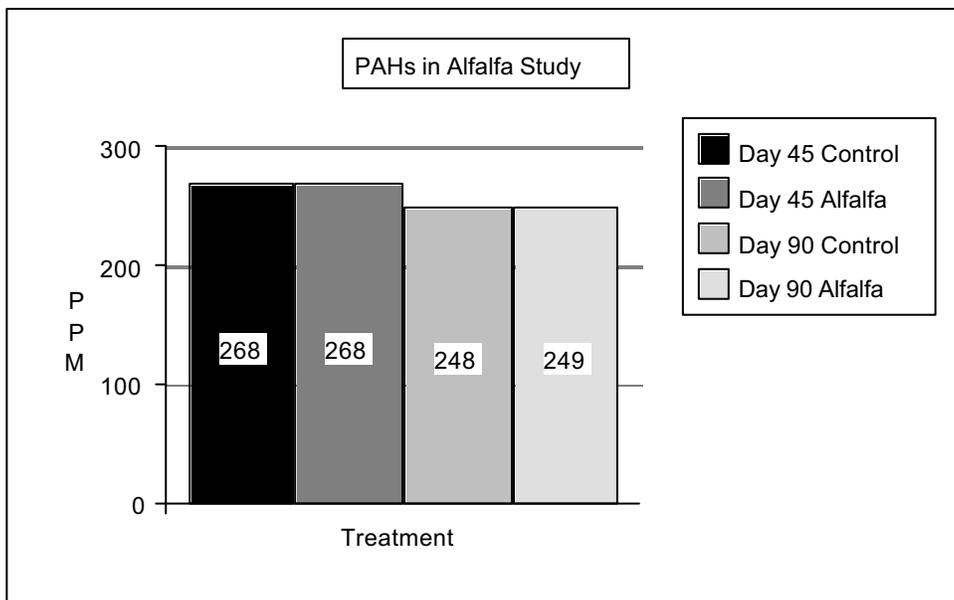


Figure 5. Mean concentration of selected PAHs for alfalfa experiment. Treatments are not significantly different at the  $\alpha = .05$  Level. Each treatment represents the analysis of three replicates.

TABLE 4. AVERAGE CREOSOTE CONSTITUENT LOSS FROM INITIAL CONCENTRATION FOR THE ALFALFA STUDY

Alfalfa Day 45	47.50%
Controls Day 45	47.50%
Alfalfa Day 90	51.00%
Controls Day 90	51.40%

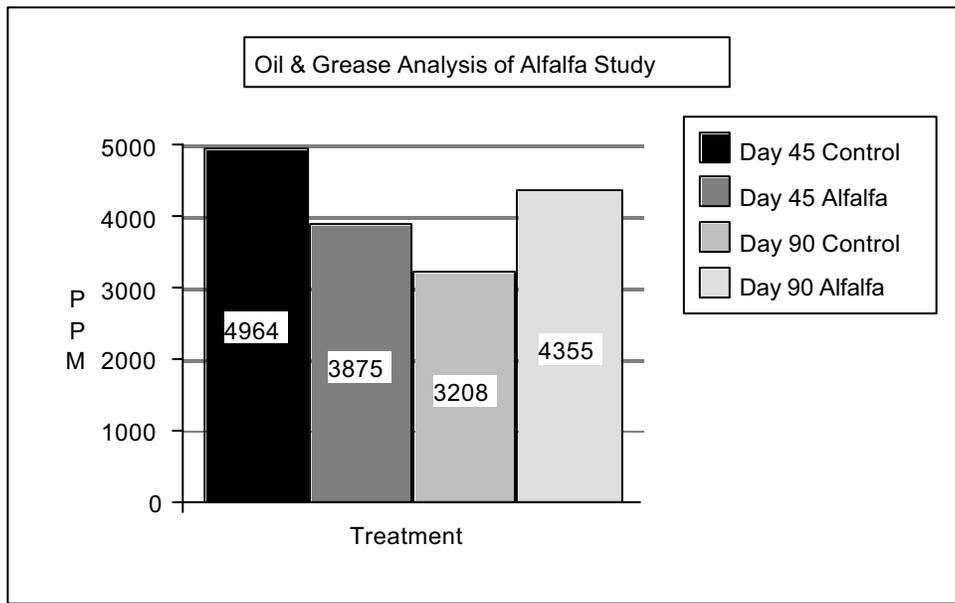


Figure 6. Mean concentration of oil and grease for the alfalfa experiment. Treatments are not significantly different at the  $\alpha = .05$  Level. Each treatment represents the analysis of three replicates.

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