

# **USE OF AMMONIATED BAGASSE TO REMEDiate PETROLEUM CONTAMINATED WETLAND AND FOREST SOILS**

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## **ABSTRACT**

Remediating oil contaminated wetland and forest soils offer a unique challenge, as traditional cleanup techniques may do more damage to the environment than the oil itself. An oil well blowout at Cravens, LA severely impacted a freshwater wetland and surrounding loblolly pine stand. Ammoniated bagasse was applied in-situ to the wetland soil to determine its effectiveness to reduce total petroleum hydrocarbon concentration. Wetland soils treated with rates of 200 kg/ha ammoniated bagasse had a 400-fold decrease in hydrocarbon concentration in the top 10 cm of the soil. A greenhouse study investigated the effectiveness of the bagasse to remediate oil contaminated forest soils. Oil was applied directly to the soil of potted two-year old loblolly pines. Ammoniated bagasse was applied 14 days after the oil. After six months, soil samples were collected and analyzed for total petroleum hydrocarbons. Soils treated with 1700 kg/ha ammoniated bagasse had a 3200-fold decrease in hydrocarbon concentration.

# INTRODUCTION

Oil spills are common occurrences in the oil exploration and production industry. Brown (1) estimated that 6,000,000 tons per year of oily wastes enter the environment uncontrolled. In 1997, 3780 oil spills were reported to the Louisiana Oil Spill Coordinator's Office (2). Most of these are marine spills that usually amount to less than seven tons of oil per incident. Nevertheless, there is an increase in the frequency of oil spills in terrestrial environments. With this increase of terrestrial spill incidents, it has become necessary to develop remediation strategies that will effectively remove the oil with the least amount of harm to the environment. Cleanup methods that are often employed in terrestrial spills include landfarming the contaminated soil, burial, containment in surface impounds, or deep well injection (3). These methods are not always a viable option when remediating oil spills in ecologically sensitive environments, such as wetlands. Traditional remediation procedures employed in these environments may result in more damage to the area than from the oil itself.

Bioremediation is a practice that is commonly employed to cleanup oil spills in terrestrial environments. Microbial degradation of petroleum products in soil, either via naturally occurring or facilitated methods, is a process that is successfully used to reduce soil concentrations of the contaminant to acceptable levels (4). During this process, the microbes attack only specific types of hydrocarbons rather than the entire oily waste. As a result, hydrocarbons are converted to carbon dioxide, water, biomass, or humic materials (5). This is a preferred method to use in oil contaminated wetlands because it results in less harm to the environment than traditional techniques.

Enhancing the bioremediation process can help speed the recovery of an oil contaminated wetland area. This can be accomplished using fertilizers or ammoniated organic wastes, such as bagasse. Bagasse is the organic material remaining after the juice has been squeezed from sugarcane stalks. Cellulose is a primary component of bagasse and it is these cellulose fibers that are capable of absorbing large amounts of oil and water. When bagasse is applied to the surface of contaminated soils it wicks oil from the subsurface to the upper soil depths. Increasing the nitrogen content of bagasse promotes the microbial decomposition of oil by providing an environment that contains oxygen, water, and nutrients, all essential elements needed for successful microbial remediation. Previous studies using ammoniated bagasse have resulted in successful removal of hydrocarbons in coastal marsh soils (6,7).

The objective of this study was to assess the effectiveness of ammoniated bagasse to remediate oil contaminated wetland and forest soils following an oil well blowout. This blowout took place in Cravens, Louisiana in August of 1997. Approximately 24-30 hectares (60-75 acres) of the Kisatchie National Forest were affected by this oil spill, including a 1.7 hectare (4.3 acre) freshwater depressional wetland adjacent to the oil well. All of the wetland vegetation was killed and the soil was severely contaminated by oil and brine. Since a spill of this nature and magnitude has never been recorded in the literature as to date, it offered the opportunity to study and develop a remediation plan that would lead to the restoration of the wetland.

# WETLAND SOIL STUDY

## Materials and Methods

### Description of study area

The study area is located in Cravens, LA (30°59' N, 93°01' W) within the Kisatchie National Forest (Figure 1). The impacted freshwater depression wetland lies approximately 0.2 kilometers (1/8 mile) south of the oil well. After the oil well blowout, an earthen berm was constructed in order to protect the wetland from any overland oil runoff. The berm failed after a heavy rain washed it away, and as a result oil and brine flowed unimpeded into the wetland (Figure 2). Overland flow of oil also occurred west of the well into Little Sixmile Creek, which is located less than 1/8 mile from the well.

The soils of the upland area have been mapped as a Ruston fine sandy loam (Fine-loamy, siliceous, subactive, thermic Typic Paleudult). The soil of the wetland is a Guyton silt loam (Fine-silty, siliceous, active, thermic Typic Glossaqualf), a typical wetland soil. Loblolly pine (*Pinus taeda*) and longleaf pine (*Pinus palustris*) dominate the vegetation of the upland area. Dominant vegetation within the wetland includes sweetgum (*Liquidambar styraciflua*), black gum (*Nyssa sylvatica*), and tupelo gum (*Nyssa aquatica*).

### C-K Associates, Inc. preliminary site data

After the oil well blowout in August 1997, C-K Associates, Inc. was hired by the owners of the well to conduct an ecological and environmental assessment of the site. A sample of oil from the wellhead was characterized for petroleum hydrocarbons (Table 1). The total petroleum hydrocarbon (TPH) concentration was 910,000 parts per million (ppm) and the concentrations of 2-methylnaphthalene and naphthalene were the highest of the semi-volatile organic compounds. In June of 1998, C-K Associates, Inc. collected wetland soil samples from three random locations within the wetland and analyzed these samples for TPH, electrical conductivity, pH, and priority pollutant metals (Table 2). The elevated concentrations of TPH revealed the need for remediation of the oil contaminated wetland soil.

### Wetland remediation

The oil well blowout occurred during August 1997. During July 1998, soil samples from four randomly selected locations within the wetland were taken at depths of 0-10, 10-20, and 20-30 centimeters (cm). The electrical conductivity (EC) and pH for the soil samples were determined using a saturated soil paste extract according to the methods of Page *et al.* (8). The samples were analyzed for EC in order to determine the extent of brine contamination within the wetland. After much discussion, the United States Forest Service decided the most economic and efficient method for removing the surface oil from the wetland was incineration.

The wetland was burned in December 1998 (Figure 3). The burn resulted in volatilization of nearly all of the surface oil, from 69,000 ppm pre-burn to 2000 ppm post-burn. Three days after the burn, three transects were established within the wetland and five locations, at 10 meter (m) intervals, along each transect were selected for soil sampling. Sampling depths were 0-10, 10-20, and 20-30 cm. These samples were analyzed for pH, EC, and sodium according to the methods of Page *et al.* (8). Soil samples from four selected upland sites were also taken at the ends of two of the transects. These samples were taken at depths of 0-10, 10-20, and 20-30 cm and analyzed for pH, EC, and sodium (Na) concentration according to the methods of Page *et al.* (8).

Ammoniated bagasse (ABG) was used in combination with lime ( $\text{CaCO}_3$ ) and topsoil to test the effectiveness of the bagasse to bioremediate the wetland. Test plots were established using open-ended cylinders (30 cm diameter / 105 cm height) constructed from plastic corrugated drainage pipe driven 15-20 cm into the soil (Figure 4). In May 1999, the bagasse, lime, and topsoil were added to each cylinder. One week before the bagasse was added,  $\text{CaCO}_3$  was incorporated into the top 5 cm of the soil at a rate of 1500 kg/ha in order to raise the pH of the soil to approximately 6.5. The rates of bagasse added were 0, 50, 100, and 200 kg/ha, with five replications of each treatment randomly placed within the wetland. These rates were based upon the post-burn total petroleum hydrocarbon (TPH) analyses performed by C-K Associates, Inc. (9). Topsoil collected from the non-contaminated upland area was added to the wetland soil at a rate of 0.015 kg/ha in order to ensure an adequate microbial population within each cylinder. The bagasse and topsoil were mixed together and incorporated into the top 2 cm of the soil.

Soil samples from each cylinder were collected at depths of 0-5, 5-10, and 10-20 cm at 21 day intervals beginning in June 1999 and analyzed for TPH, EC, and pH. After a 90 day study period the test cylinders were removed and soil samples were collected at depths of 0-10 and 10-20 cm.

Total petroleum hydrocarbon concentration for all samples was determined using a Buck Scientific Total Hydrocarbon FT-IR Analyzer using a known concentration of weathered crude oil obtained from the research site. Residual oil concentration for all soil samples was determined in aliquots of mixed soil extractions using carbon disulfide ( $\text{CS}_2$ ) as the extractant.  $\text{CS}_2$  was used because hydrocarbon compounds are readily miscible in it and it does not contain any C-H bonds that would interfere with FT-IR analysis (10). A standard curve was created using weathered crude oil taken from the contaminated wetland. Standard solutions of 10, 25, 50, 75, and 100 ppm were used to calibrate the hydrocarbon analyzer each day before analysis.

Fifteen grams of contaminated wetland soil was placed into a glass jar. Twenty milliliters of  $\text{CS}_2$  were added to the soil and the jar loosely sealed. The soil and  $\text{CS}_2$  were sonified for 30 minutes. One milliliter of the  $\text{CS}_2$  extractant was collected after sonification using a glass syringe and diluted with non-contaminated  $\text{CS}_2$ . This extractant sample was then analyzed using the Buck analyzer and TPH concentration determined from the standard curves created earlier.

Electrical conductivity and pH of the soils were analyzed according to the methods of Page *et al.* (8). A Perkin-Elmer ICP-AES was used to analyze the soil samples collected in September 1999 for water-soluble cations (Al, Ca, Cd, Cu, Fe, K, Mg, Mn, Na, Ni, Pb, Se, and Zn). Statistical analysis of the wetland soil hydrocarbon data was performed using the SAS GLM procedure. An  $\alpha$ -value of 0.05 was used to determine the significance of interactions of treatment, date, depth, and replications.

## **Results**

### **Pre- and post-burn soil pH and electrical conductivity analyses**

Pre-burn wetland soil analysis from samples collected in July 1998 (Table 3) indicated that the 0-10 cm soil layer had a low concentration of brine with an average EC of 434  $\mu\text{S}/\text{m}$  (microSiemens per meter). The average EC readings for the 10-20 cm and 20-30 cm layers were 637  $\mu\text{S}/\text{m}$  and 611  $\mu\text{S}/\text{m}$ , respectively. pH readings for the soil ranged from 4.7 to 5.5.

Post-burn analysis of the wetland soils sampled in December 1998 showed a decrease in EC (Table 4). The average electrical conductivity of the 0-10 cm soil layer was 236.7  $\mu\text{S/m}$ , average pH was 5.2, and the average Na concentration was 26.6 ppm. The average EC for the 10-20 cm soil layer was 259.7  $\mu\text{S/m}$ , average pH was 4.8, and average Na concentration was 29.7 ppm. The average EC for the 20-30 cm soil layer was 374.9  $\mu\text{S/m}$ , average pH was 4.6, and average Na concentration was 31.5 ppm.

Comparing EC results for the pre- and post-burn soil analysis indicated a decrease in salinity after the burn. In the 0-10 cm soil layer the EC decreased from 434  $\mu\text{S/m}$  to 236  $\mu\text{S/m}$ , in the 10-20 cm layer from 637  $\mu\text{S/m}$  to 259  $\mu\text{S/m}$ , and in the 20-30 cm layer from 611  $\mu\text{S/m}$  to 374  $\mu\text{S/m}$ . As a result of these analyses, the U.S. Forest Service concluded that the brine was no longer a deterrent to wetland remediation and restoration because the soil salinities were well below the critical value (400,000  $\mu\text{S/m}$ ) necessary for vegetative survival (11). Efforts then focused on removal of the residual oil in the soil.

Post-burn analysis of the upland soil samples is also presented in Table 4. As expected, these soils had lower electrical conductivities, due to less brine contamination, and higher pH values when compared to the wetland soils. As with the wetland soils, brine will not be an obstacle to overcome when restoring and managing vegetation on these upland environments.

### **Soil hydrocarbon analysis**

The 90-day period for the wetland study ended in September 1999. Soil samples collected in June, July, August, and September were analyzed for TPH. Results of the analyses suggest that the ABG decreased the overall hydrocarbon concentration of the contaminated wetland soils. The mean hydrocarbon concentration for all soil samples collected during June, July, August, and September was 626 ppm, with a maximum concentration of 9643 ppm and a minimum concentration of 5 ppm. This is decrease in the post-burn TPH concentration of 2000 ppm measured by C-K Associates, Inc. (9). The standard deviation of 1347 ppm illustrates the high variability of oil concentration within the wetland.

The results of the TPH soil analyses are presented in Tables 5 and 6 and Figures 5 and 6. Replications of each treatment have been averaged. Since there was no significant difference in the TPH values ( $p=0.8592$ ) of the 0-5 cm and 5-10 cm layers in June, July, and August, these values were averaged into one composite 0-10 cm layer in order to compare results with the last sampling period.

In the 0-10 cm layer (Figure 5), the wicking action of ABG, which has been proven in previous studies with ABG (6,7), may also be occurring in the 100 and 200 kg/ha treatments in this study. In June, 21 days after the bagasse was added, the 200 kg/ha treatment had the highest mean concentration of oil (904 ppm) compared to the other treatments, as the bagasse may have drawn oil from the subsurface to the soil surface. From June to July, the 200 kg/ha treatment may have continued to wick oil to the surface, which would be represented by the increase in mean TPH concentration (904 ppm to 942 ppm). From July to August there is a decrease (942 ppm to 199 ppm) in mean TPH in the 200 kg/ha treatment. Previous studies (6,7) have shown that as oil is absorbed by the bagasse, accelerated microbial degradation decreases TPH concentration. Similar accelerated degradation may have occurred in the Cravens wetland soils and would account for the decrease in the 200 kg/ha treatment from July to August. The increase in mean TPH from August to September in the 200 kg/ha treatment (199 ppm to 1336 ppm) may be as result of

the microbes exhausting their nitrogen source (the ABG) or the occurrence of a substantial rain event in August 1999. As a result of this rain event, residual oil that remained in the 10-20 cm layer may have been translocated to the 0-10 cm layer, therefore increasing the TPH concentration in the surface soil.

The 100 kg/ha treatment shows a similar trend as the 200 kg/ha treatment during the study period. The 100 kg/ha treatment seems to be wicking oil from the subsurface to the soil surface. There is a decrease in mean TPH concentration from June to July in the 100 kg/ha treatment (747 ppm to 242 ppm). The increase in mean TPH concentration from August to September (246 ppm to 2071 ppm) may be as a result of the rain event that occurred in August. Oil may have been moved from the subsurface to the surface as water moved through the wetland. The statistical difference between the August and September data would help to support this conclusion.

The 50 kg/ha treatment had little or no effect on the degradation of oil in the wetland soils. The TPH values for the 50 kg/ha treatment ranged from 164 ppm to 422 ppm. There is no statistical difference in TPH concentration between months. The temporal variability questions the effectiveness of the 50 kg/ha treatment to absorb and bioremediate oil contaminated wetland soils. In the 0 kg/ha controls, hydrocarbon concentrations ranged from 215 ppm to 2960 ppm. The significant increase in TPH concentration from August to September in the controls (402 ppm to 2960 ppm) helps to support the conclusion that the rain event in August resulted in the translocation of residual oil in the soil subsurface to be translocated to the soil surface.

In the 10-20 cm layer (Table 6 and Figure 6), all of the treatments remained below a mean TPH concentration of 300 ppm. The lowest TPH concentration was 40 ppm and the highest concentration was 241 ppm. There was no statistical difference between treatments for the 10-20 cm soil layer. The high spatial and temporal variability of oil within the wetland makes it difficult to prove that oil was being wicked by the ABG to the soil surface.

### **Statistical analysis of wetland soil hydrocarbon data**

Statistical analysis of the wetland soil hydrocarbon data was performed using the PROC GLM procedure. An  $\alpha$ -value of 0.05 was used to determine significance of interactions of treatment, date, depth, and replications (Table 7). The effect of adding different amounts of bagasse (treatment) to reduce soil hydrocarbon concentrations was not found to be statistically significant ( $p=0.1756$ ). Date and depth were found to be significant with  $p$ -values of 0.0001 and 0.0001, respectively. Date by depth was also found to be significant ( $p=0.0001$ ). Other interactions between treatment, date, depth, and replication were not found to be significant.

Time was found to be significant in the removal of hydrocarbons from the soil. This is probably due to the increase in TPH from August to September in some of the treatments. Increases in TPH concentration in the 0 kg/ha, 100 kg/ha and 200 kg/ha treatments from August to September are probably due to the rain event that occurred at the end of August.

Hydrocarbons were greater in the 0-10 cm soil layer than in the 10-20 cm soil layer. The average hydrocarbon concentration of the 0-10 cm soil layer, pooling all treatments, was 595 ppm and the average concentration for the 10-20 cm soil layer was 175 ppm. This may be due to the absorbing ability of the bagasse to translocate oil from the 10-20 cm depth to the soil surface where microbial degradation can take place or the oil did not penetrate the soil deep enough to cause significant contamination in the 10-20 cm layer. The effect of the

rain event in August may also be the cause of the highly significant differences in TPH between the two soil layers.

### **Electrical conductivity and pH analysis**

All of the soil samples taken in June, July, August, and September were analyzed for electrical conductivity (Figures 7 and 8) and pH (Figures 9 and 10) using a saturated soil paste according to the methods of Page *et al.* (1982). The pH and EC data were pooled for all treatments for each month, as ABG does not affect these soil properties. For the 0-10 cm and 10-20 cm soil layers the electrical conductivity remained below 500  $\mu\text{S/m}$  for the entire study period. There is a decrease from August to September in both soil layers. This is due to the rain event that occurred at the end of August. This rain caused the residual brine in the soil to be diluted, thereby reducing EC values. During this period the EC dropped below 300  $\mu\text{S/m}$ . These results further illustrate that brine is no longer an obstacle to overcome to restore the site.

Foght and Westlake (12) reported that the optimal pH range for microbial degradation of hydrocarbons in soil is 6 to 8. For the 0-10 cm and 10-20 cm soil layers, the pH during June remained below 6. During July and August, the pH for both soil layers was at or near 6, optimizing soil conditions needed for accelerated degradation rates. From August to September there is a decrease in pH to below 6. This is probably due to the rain event that occurred in August.

The EC and pH data help to support the conclusion that the rain event in August may have caused oil to be moved to the soil surface. The water entering the wetland from the rain caused a decrease in the EC and pH of both the soil layers sampled. The drop in EC in the 10-20 cm layer proves that water from the rain event did infiltrate the subsurface and had the potential to translocate oil to the surface. This would help to explain the increase in TPH in the 0-10 cm soil layer from August to September.

### **Cation analysis**

Results of the cation analysis indicated that there was no element present that would pose an environmental hazard or inhibit remediation of the wetland (Table 8). Iron concentrations were equal to or less than 1 ppm, with an average concentration of 0.152 ppm. These concentrations do not pose an environmental threat. Sodium concentrations ranged from 83 to 112 ppm, with an average concentration of 95 ppm. Even though chlorides were not determined, its concentration is assumed to be approximately equal to Na concentration. The average concentrations of Ca, K, and Mg are 2.5, 0.72, and 0.65 ppm, respectively. These concentrations would add only approximately 4 ppm to the chloride concentration.

### **Discussion and Conclusions**

The wetland vegetation at the Cravens blowout site was completely destroyed as a result of the oil and brine contamination. The hydrology, which is the most important component of wetland restoration, was minimally affected (9). There have been substantial reductions in brine contamination since the blowout occurred and it is no longer a deterrent to wetland remediation. As a result, restoration efforts should focus on the removal of residual oil from the soil and revegetation of the site by selected wetland species.

The use of ammoniated organic wastes, specifically bagasse, has been found to be a reliable application to enhance the remediation of ecologically sensitive environments that have been subjected to oil contamination (6,7). It was expected that ABG would have a similar effect in the wetland impacted by the Cravens oilwell blowout if rapidly applied after the spill. Results of the study, though, do not statistically prove that ABG promoted

biodegradation of the oil contaminated soil. The spatial variation of the oil within the wetland made it difficult to prove that successful remediation was achieved.

There was no statistical difference among ABG treatments during individual monthly sampling periods (Tables 5 and 6). September is the only month when there is any statistical difference between treatments. All of the treatments, except the 50 kg/ha treatment, had increases in TPH concentration in the 0-10 cm soil layer. This was most likely due to the rain event in August that resulted in residual oil in the subsurface to be translocated to the soil surface. This was supported by the data that shows EC's decreasing in the 0-10 cm layer from August to September as a result of dilution from the rain.

Even though this study did not statistically prove the effectiveness of ammoniated bagasse to bioremediate the wetland, it still may be a useful tool at this and other similar sites. The high spatial variability of oil within the Cravens wetland makes it difficult to determine the rates of ABG that would need to be applied in order to achieve successful remediation. Results of this study indicate that rates of ABG greater than 200 kg/ha would need to be applied in order to account for the spatial variation of the oil within the wetland. Larger treatment blocks would need to be established within the wetland using ABG rates greater than 200 kg/ha to determine the correct amount of ABG to be added to effectively remediate the wetland soils. Rates of ABG applied could be 0, 500, 750, 1000, 1500 kg/ha. Similar sampling procedures and laboratory techniques used in this study would be adequate for this type of investigation.

In oil spills such as these, the difficulty lies not only with removing the oil from the environment, but also restoring the area to its natural conditions. To restore the contaminated area, it must be decided if revegetation will occur by natural succession or if human intervention will be employed to accelerate the restoration process. This should be decided on a site-by-site basis as all spills and environmental conditions are not the same.

One important aspect that is often overlooked at restoration sites is monitoring and observation. In many cases, after restoration practices have been implemented the site is ignored and assumed to be a success. Most times this is not the case. Establishing vegetation at a site does not define success. The functional activity of the wetland within the surrounding landscape must also be assessed. This is especially true for oil contaminated wetlands which are often completely destroyed. At the Cravens blowout site, soil samples should be taken monthly after the bagasse has been added in order to determine its success at removing the residual oil from the soil. After oil concentrations have decreased to acceptable levels, a seed bank with selected species can be introduced into the wetland. Monitoring of vegetation should continue until successful establishment is certain.

Species to be introduced into the overstory of Cravens site may include black gum (*Nyssa sylvatica*), sweet gum (*Liquidambar styraciflua*), tupelo gum (*Nyssa aquatica*), sweet bay (*Laurus nobilis*), water oak (*Quercus nigra*), and bald cypress (*Taxodium distichum*). Understory vegetation may include American beautyberry (*Callicarpa americana*), muscadine (*Vitis rotundifolia*), and sassafras (*Sassafras albidum*). These species can be established by planting seeds, seedlings, or stem cuttings, plant-soil transfers, use of a donor seed bank, or natural succession. A key to successful revegetation is to reduce the amount of noxious, invasive plants that may colonize the site. Examples of some invasive species are Chinese tallow (*Sapium sebiferum*), hybrid cattail (*Typha glauca*), and purple loosestrife

(*Lythrum salicaria*). With continued monitoring and observation, the odds for successful restoration of the oil contaminated wetland will increase.

As of this writing, the Forest Service has rejected the plan submitted to them to incorporate ABG into the remediation of the wetland. Instead, they used heavy equipment to excavate the top 0-15 cm of the oil contaminated wetland soil and will attempt to begin revegetation of the site in the next 3 to 6 months.

## FOREST SOIL STUDY

### Introduction

A greenhouse oil study was performed to evaluate the efficiency of ammoniated bagasse to remediate oil contaminated forest soils. The United States Forest Service donated approximately 1000 one-year old loblolly pine (*Pinus taeda*) seedlings for this study. These seedlings were planted in March 1999 in tree pots (15 cm width / 41 cm height) filled with the A and B horizons of a Malbis silt loam (Fine-loamy, siliceous, subactive, thermic Plinthic Paleudult) in order to imitate forest soil conditions. The seedlings were watered with de-ionized water every other watering in order to reduce salt buildup in the soil. The seedlings grew for one year under monitored greenhouse conditions before any treatments were applied.

### Materials and Methods

A 4x4 factorial design with four rates of oil and four rates of ABG with five replications of each treatment was employed. The seedlings used in this study were randomly selected from the greenhouse population and assigned a treatment. Eighty total seedlings were used in this study. The rates of oil added to the soil were 0, 431, 862, and 1724 L/ha. These rates of oil are equivalent to 0, 100, 200, and 400 mL/seedling. The oil was applied directly to the soil surface on May 8, 2000. Height and diameter measurements for each seedling were taken before the oil was applied. The ABG was added to the soil 14 days after the oil treatments at rates of 0, 431, 862, and 1724 kg/ha and mixed into the upper 2 cm of the soil. These rates of ABG are equivalent to 0, 100, 200, and 400 g/seedling. The ABG was added 14 days after the oil applications in order to imitate response times in typical oil spill situations.

The seedlings were monitored and watered with de-ionized water every other watering to reduce salt buildup in the soil. The seedlings were harvested in October 2000. A visual health rating system was used in this study to assess the effects of the soil applied oil on the pine seedlings. Soil samples for each treatment were taken approximately six months after ABG application and analyzed for TPH concentration employing the same methods used to analyze the TPH of the wetland soils. Statistical analysis of the hydrocarbon soil data was performed using the SAS GLM procedure.

### Results

The TPH analysis of the soils showed that the ABG decreased the hydrocarbon concentrations when added at sufficient rates (Figure 11). In soils treated with 862 L/ha oil, ABG reduced TPH concentrations when added at rates of 1724 kg/ha (18201 ppm to 1438 ppm) and 862 kg/ha (18201 ppm to 3585 ppm). In soils treated with 1724 L/ha oil, ABG reduced concentrations when added at rates of 1724 kg/ha (18571 ppm to 574 ppm) and 862 kg/ha (18571 ppm to 915 ppm). A less dramatic decline in oil concentration occurred with the 431 kg/ha ABG treatments. As with the wetland soil study, high variability exists within treatments. Results of this study show that ABG has the ability to enhance the remediation of oil contaminated forest soils.

Adding crude oil directly to the soil resulted in rapid negative reactions from the seedlings. The seedlings treated with the higher rates of soil applied oil (862 L/ha and 1724 L/ha) showed signs of severe stress and as a result most the seedlings died (Table 9). The 1724 L/ha oil treated seedlings showed severe signs of stress within one week after application and all of the seedlings were dead three weeks after application. Signs of stress included needle wilting, chlorosis, necrosis, and premature needle loss. The 862 L/ha oil treated seedlings showed similar signs of stress, but took longer to die. Only four of the twenty 862 L/ha oil treated seedlings did not die by the October harvest date. The 0 L/ha and 431 L/ha oil treated seedlings showed little or no signs of stress. Wilting of some needles was the only visual stress seen as a result of the oil application. Changes in height and diameter were statistically significant, with both measurements having p-values of <0.0001.

## **Conclusions**

The application of the ABG did not seem to increase the odds of survival of the seedlings when exposed to high concentrations of oil in the soil. The health of the seedling and rate of bagasse applied to the soil was not significant ( $p=0.3702$ ). By the time the ABG was added, the high concentrations of oil had already injured the seedling beyond recovery. If the ABG was applied sooner than 14 days after the oil was applied, results may have been different. If applied one to three days after oil spills and at appropriate rates equal to TPH soil concentrations, ABG may be able to prevent the death of some forest seedlings and other vegetation by preventing oil interference with root functions.

The number of fine roots per tree is a function of the ecological and environmental conditions of the site. Most fine roots die in unfavorable conditions, but reform rapidly when conditions become more favorable. Obviously, oil spills on a site will degrade the ecological and environmental conditions. If oil contaminated forest soils are remediated as soon as possible after the spill, then the negative effects on vegetation may be reduced.

Oil added directly to the soil had detrimental effects on the pine seedlings. Some type of root interaction with the oil probably caused these effects. Oil which coats absorbing roots may potentially inhibit water and nutrient uptake resulting in drought-like reactions from the vegetation (i.e. needle wilting and chlorosis). The oil may also bind soil particles together impeding water and oxygen, which may affect nutrient availability to the plants. These conditions may mimic soil water deficits, which in turn may cause leaf water deficits. When leaf water deficits occur, reduction of leaf area and closing of stomata inhibits photosynthesis. This type of interference would directly affect growth and survival of the plant. Udo and Fayemi (1975) found that plants growing in oil contaminated soil had reduced growth rates and chlorotic leaves and were dehydrated, indicating soil water deficiencies. This type interaction of oil with absorbing roots and/or the soil caused of death of these seedlings.

The ammoniated bagasse was efficient in removing oil from the forest soil and reducing TPH concentration. If sufficient amounts of ABG are applied immediately after an oil spill, the negative effects on vegetation may have the potential to be reduced. The ABG would be a beneficial tool to use at oil contaminated forest sites. Further field studies with ABG and oil contaminated forest sites should continue. A study repeating the greenhouse study in a field situation would help to determine the effectiveness of ABG under natural environmental conditions. Also, testing ABG at smaller oil spill sites may help to assess its effectiveness at remediating forest soils during larger spill events.

**Table 1.** Petroleum Hydrocarbon Characterization for Oil Wellhead Sample from Cravens, Louisiana Oilwell Blowout Site.\*

Semi-volatile Organic Compound	Concentration (ppm)
2-methylnaphthalene	2100
Acenaphthalene	<198
Acenaphthylene	<198
Anthracene	<198
Benzo(a)anthracene	<198
Benzo(a)pyrene	<198
Benzo(b)fluorathene	<198
Benzo(h,h,l)perylene	<198
Benzo(k)fluorathene	<198
Chrysene	<198
Dibenz(a,h)anthracene	<198

Fluorathene	<198
Fluorene	<198
Ideno(1,2,3-c,d)pyrene	<198
Naphthalene	200
Phenanthrene	<198
Pyrene	<198

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\*From C-K Associates, Inc. (9)

**Table 2.** Soil Data from Oil Contaminated Wetland at Cravens, Louisiana Oilwell Blowout Site (June 1998).\*

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TPH – 69,000 ppm

Electrical Conductivity – 837  $\mu$ S/m

pH – 5.05

Priority Pollutant Metals

<u>Metal</u>	<u>Concentration (ppm)</u>
Antimony	<1.0
Arsenic	<1.0
Beryllium	<0.2
Cadmium	<0.5
Chromium	2.1
Copper	1.3
Lead	6.7
Mercury	<0.1

Nickel	<1.0
Selenium	<0.5
Silver	<1.0
Thallium	<1.0
Zinc	1.6

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\*From C-K Associates, Inc. (9)

**Table 3.** Pre-Burn Wetland Soil Analysis from the Cravens, LA Oilwell Blowout Site (July 1998).

Sample Depth	Electrical Conductivity (s.d.) ( $\mu\text{S}/\text{m}$ )	pH (s.d.)
0-10 cm	434 (130)	5.5 (0.3)
10-20 cm	637 (273)	4.9 (0.8)
20-30 cm	611 (281)	4.7 (0.6)

**Table 4.** Post-Burn Wetland and Upland Soil Analysis from the Cravens, LA Oilwell Blowout Site (December 1998).

Sample Location And Depth	Electrical Conductivity (s.d.) ( $\mu\text{S}/\text{m}$ )	pH (s.d.) (ppm)	Sodium (s.d.)
Wetland 0-10 cm	236.7 (115.9)	5.2 (0.9)	26.6 (13.9)
Wetland 10-20 cm	259.7 (102.9)	4.8 (0.6)	29.7 (15.6)
Wetland 20-30 cm	374.9 (145.2)	4.6 (0.5)	31.5 (21.6)
Upland 0-10 cm	262.8 (37.5)	5.4 (0.9)	20.3 (6.5)
Upland 10-20 cm	175.3 (55.0)	5.0 (0.7)	16.5 (8.0)

Upland 20-30 cm	167.0 (47.5)	5.0 (0.6)	14.5 (10.4)
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**Table 5.** Total Petroleum Hydrocarbon Means ( $\mu$ ) and Standard Deviations (s) for 0-10 cm Soil Layer in the Oil Contaminated Wetland at Cravens, LA as a Result of Different Ammoniated Bagasse (ABG) Treatments.

ABG Treatment Applications	June		July		August		September	
	$\mu$	s	$\mu$	s	$\mu$	s	$\mu$	s
0 kg/ha	215 <sup>Aa</sup>	99	335 <sup>Aa</sup>	280	402 <sup>Aa</sup>	425	2960 <sup>Bb</sup>	2775
50 kg/ha	164 <sup>Aa</sup>	89	290 <sup>Aa</sup>	296	422 <sup>Aa</sup>	603	371 <sup>Aa</sup>	155
100 kg/ha	747 <sup>Aab</sup>	715	242 <sup>Ab</sup>	143	246 <sup>Ab</sup>	194	2071 <sup>Ba</sup>	1598
200 kg/ha	904 <sup>Aa</sup>	932	942 <sup>Aa</sup>	811	199 <sup>Aa</sup>	237	1336 <sup>Ba</sup>	1080

Means with the same lower case letters within rows are not significantly different.

Means with the same upper case letters within columns are not significantly different.

**Table 6.** Total Petroleum Hydrocarbon Means ( $\mu$ ) and Standard Deviations (s) for 10-20 cm Soil Layer in Oil Contaminated Wetland at Cravens, LA as a Result of Different Ammoniated Bagasse (ABG) Treatments.

ABG Treatment Applications	June		July		August		September	
	$\mu$	s	$\mu$	s	$\mu$	s	$\mu$	s

0 kg/ha	129 <sup>Aa</sup>	77	103 <sup>Aa</sup>	66	193 <sup>Aa</sup>	92	111 <sup>Aa</sup>	30
50 kg/ha	115 <sup>Aa</sup>	57	65 <sup>Aa</sup>	11	40 <sup>Aa</sup>	9	126 <sup>Aa</sup>	28
100 kg/ha	241 <sup>Aa</sup>	145	240 <sup>Aa</sup>	165	106 <sup>Aa</sup>	41	192 <sup>Aa</sup>	142
200 kg/ha	136 <sup>Aa</sup>	109	115 <sup>Aa</sup>	54	114 <sup>Aa</sup>	139	121 <sup>Aa</sup>	59

Means with the same lower case letters within rows are not significantly different.

Means with the same upper case letters within columns are not significantly different.

**Table 7.** ANOVA Table of F and p Values for Wetland Soil Hydrocarbon Data Collected from the Oil Contaminated Wetland at Cravens, LA.

Source	F Value	Pr > F
Treatment	1.70	0.1756
Date	8.15	0.0001
Depth	29.94	0.0001
Replication(Treatment)	1.30	0.2290
Date*Treatment	1.50	0.1679
Date*Replication(Treatment)	0.97	0.5393
Date*Depth	7.99	0.0001
Treatment*Depth	1.13	0.3440
Date*Treatment*Depth	1.63	0.1268

**Table 8.** Mean Cation Concentrations for Wetland Soil Samples.<sup>a</sup>

Cation	0-10 cm Soil Depth	10-20 cm Soil Depth	Average	U.S. EPA Drinking Water Standards
Al	0.068	0.133	0.100	0.200
Ca	4.633	0.371	2.502	NA
Cd	0.000	0.001	0.000	0.005
Cu	0.001	0.001	0.001	1.300
Fe	0.105	0.199	0.152	0.300
K	0.852	0.598	0.725	NA
Mg	0.858	0.440	0.649	NA
Mn	0.020	0.006	0.013	0.050
Na	94.964	95.921	95.442	50.000
Ni	0.002	0.001	0.002	0.100
Pb	0.006	0.004	0.005	0.015
Se	0.005	0.006	0.006	0.050
Zn	0.001	0.001	0.001	5.000

<sup>a</sup>Concentrations are expressed in parts per million (ppm).  
NA – Not available

**Table 9.** Mean Health Ratings, Height Change, and Diameter Change of Greenhouse Grown Two-year Old Loblolly Pine in Response to Soil Applied Oil.

Soil Applied Oil Treatment	Mean Health Rating*	Mean Height Change (cm)	Mean Diameter Change (mm)
0 L/ha	1.2 <sup>c</sup>	3.2 <sup>a</sup>	1.48 <sup>a</sup>
431 L/ha	1.2 <sup>c</sup>	1.8 <sup>b</sup>	1.35 <sup>a</sup>
862 L/ha	4.3 <sup>b</sup>	1.1 <sup>bc</sup>	0.77 <sup>b</sup>
1724 L/ha	5.0 <sup>a</sup>	0.0 <sup>c</sup>	0.41 <sup>b</sup>

\*Ratings: 1 (healthy, no visual signs of stress); 2 (slight visual signs of stress, few chlorotic and wilting needles); 3 (moderate signs of stress, chlorotic needles, wilting needles, some premature needle loss); 4 (severe signs of stress, chlorotic and necrotic needles, extreme wilting, premature needle loss); 5 (seedling is dead, all needles brown).

Means with the same letter within columns are not significantly different at  $\alpha=0.05$ .



**Figure 1.** Location of Cravens, LA Research Site.



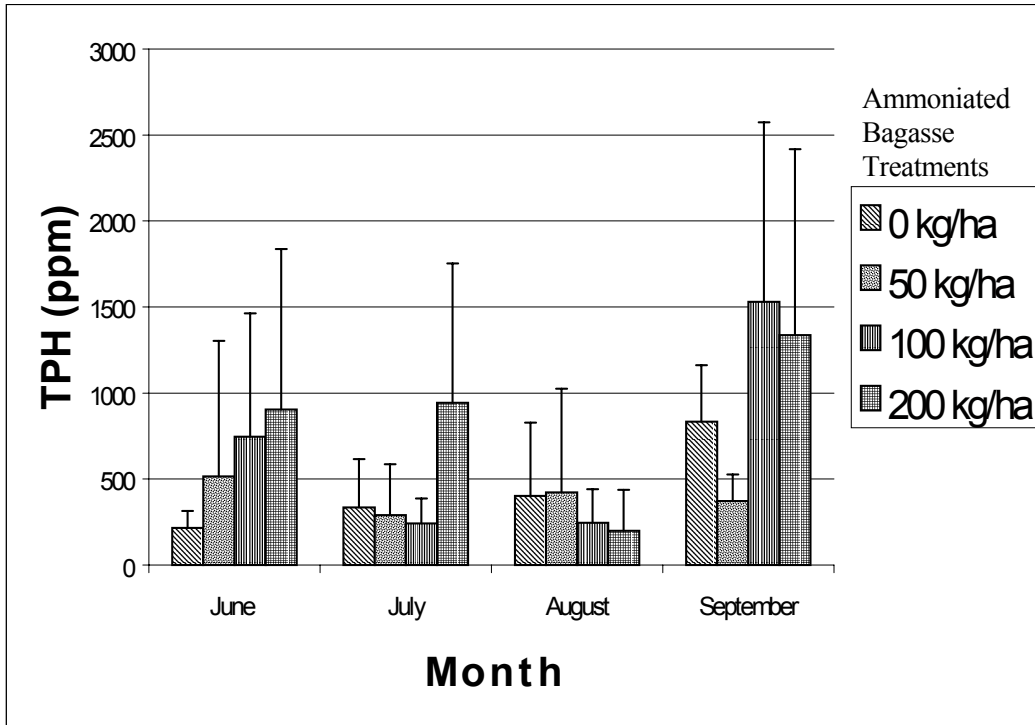
**Figure 2.** Cravens, LA Oil Contaminated Wetland, July 1998 (pre-burn).



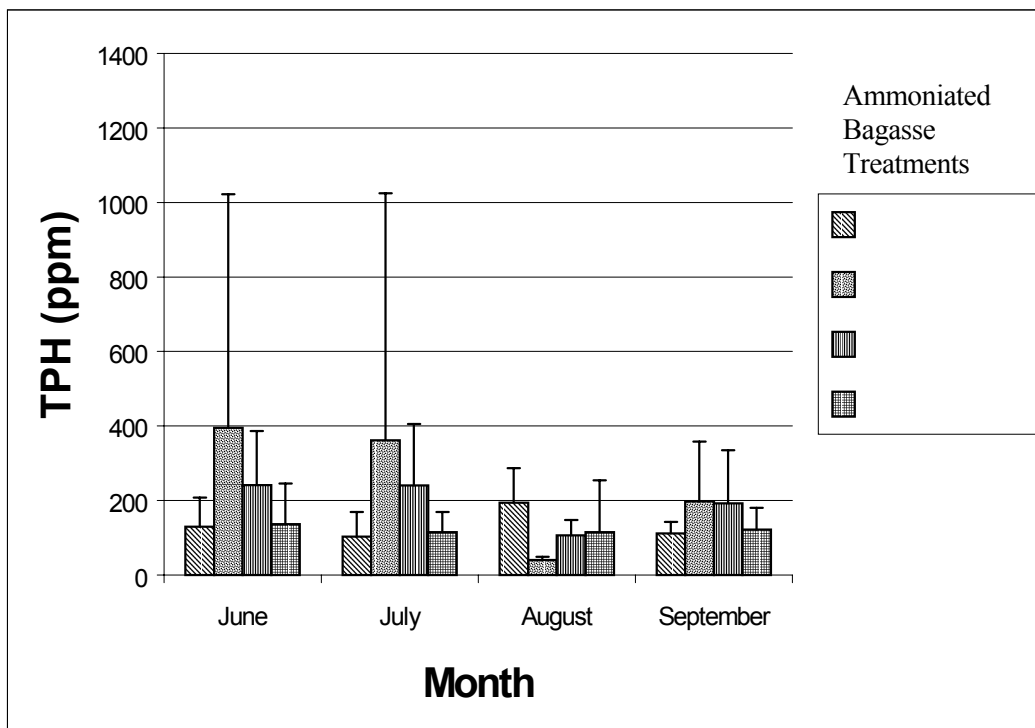
**Figure 3.** Cravens, LA Oil Contaminated Wetland, December 1998 (post-burn).



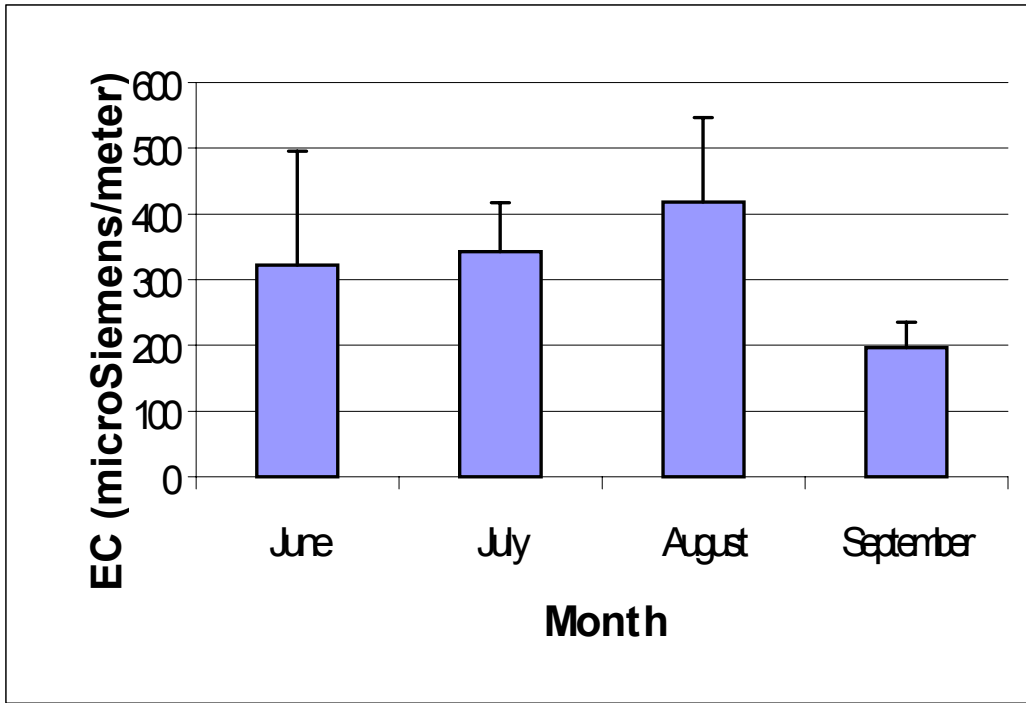
**Figure 4.** Test Cylinder Located in Cravens, LA Oil Contaminated Wetland.



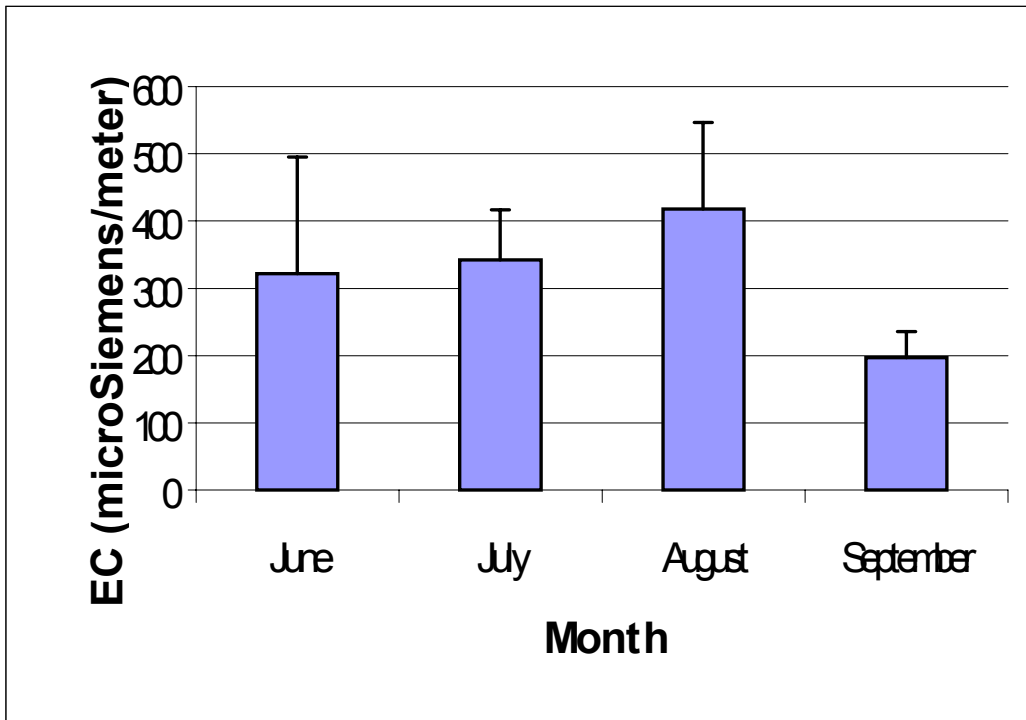
**Figure 5.** Total Petroleum Hydrocarbons in the 0-10 cm Soil Layer Resulting from Different Ammoniated Bagasse Treatments (error bars are standard deviation).



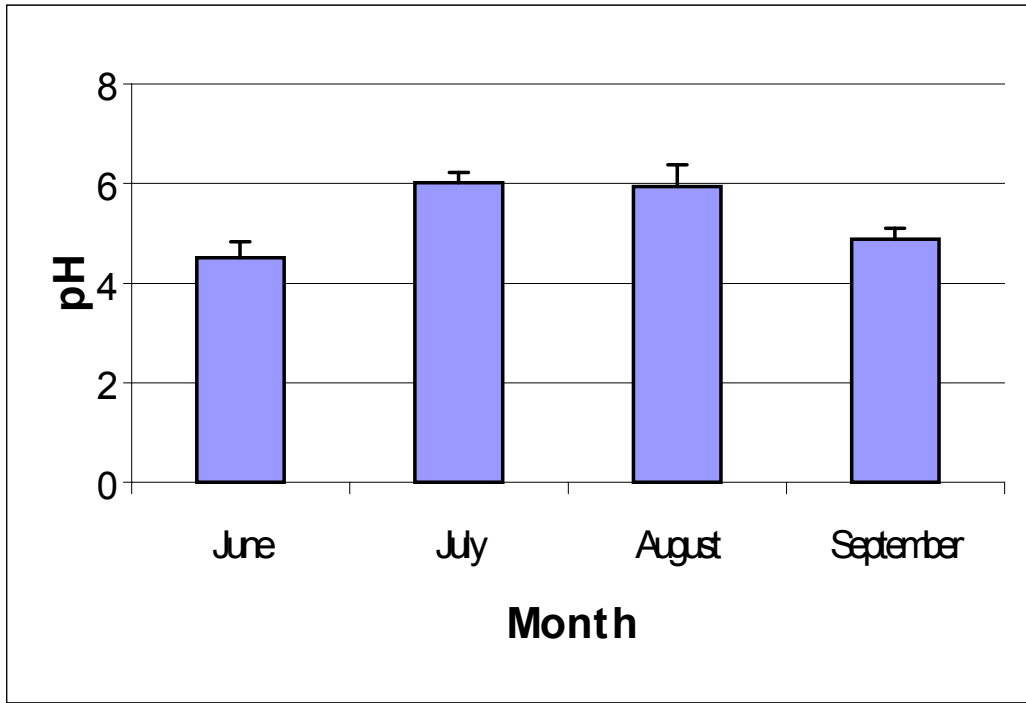
**Figure 6.** Total Petroleum Hydrocarbons in the 10-20 cm Soil Layer Resulting from Different Ammoniated Bagasse Treatments (error bars are standard deviation).



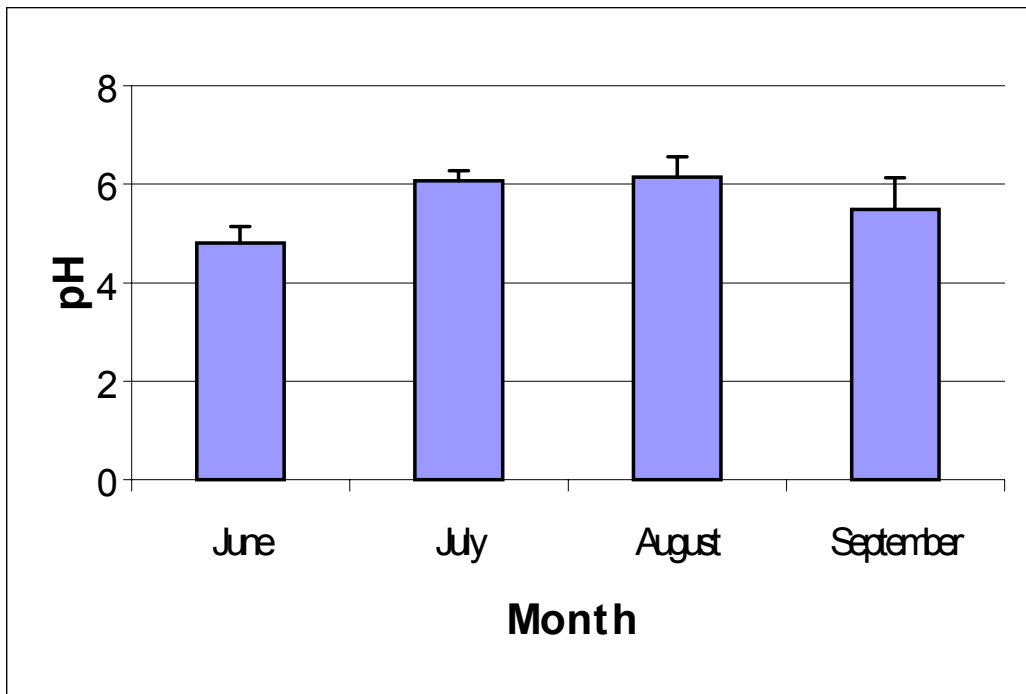
**Figure 7.** Electrical Conductivity in the 0-10 cm Soil Layer at the Oil Contaminated Wetland in Cravens, LA (error bars are standard deviation).



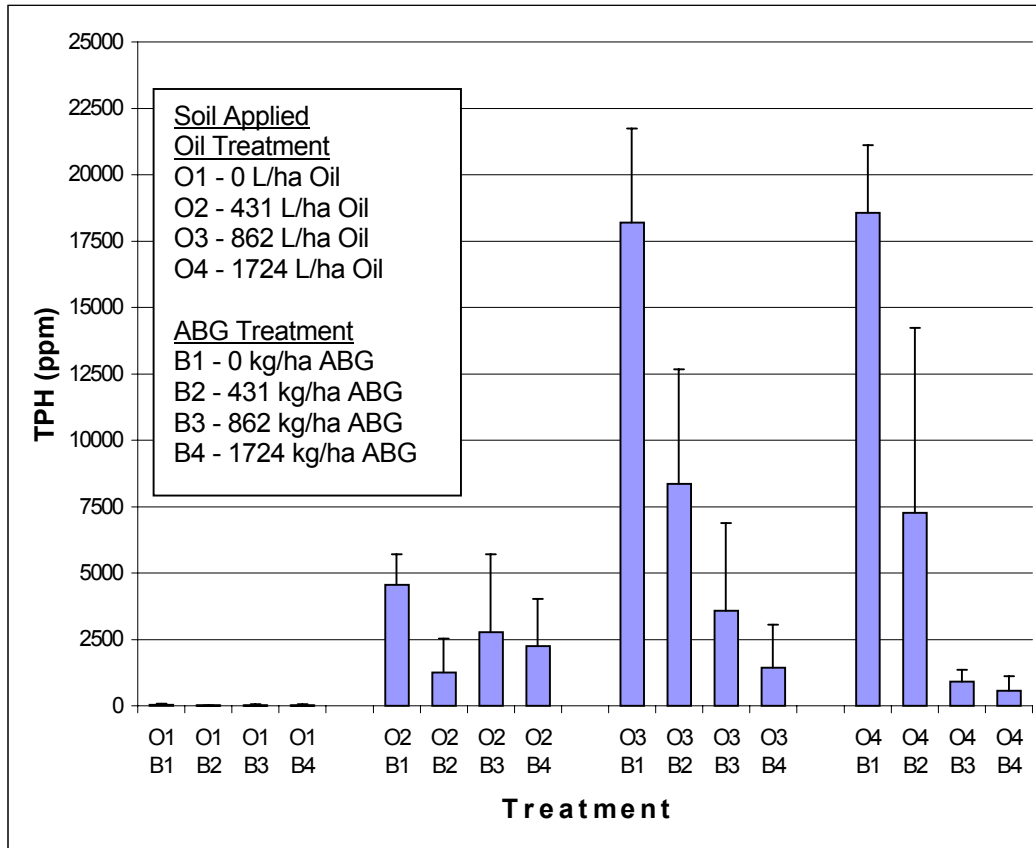
**Figure 8.** Electrical Conductivity in the 10-20 cm Soil Layer at the Oil Contaminated Wetland in Cravens, LA (error bars are standard deviation).



**Figure 9.** Soil pH of the 0-10 cm Soil Layer at the Oil Contaminated Wetland in Cravens, LA (error bars are standard deviation).



**Figure 10.** Soil pH of the 10-20 cm Soil Layer at the Oil Contaminated Wetland in Cravens, LA (error bars are standard deviation).



**Figure 11.** Soil Total Petroleum Hydrocarbon Concentration as a Result of Application of Ammoniated Bagasse (ABG) to Oil Contaminated Forest Soil (error bars are standard deviation).

## REFERENCES CITED

1. Brown, L.R., "Oil-degrading microorganisms," *Chem. Eng. Prog.* October, 35-40 (1987).
2. Davis, D., "Opening remarks," Louisiana Oil Spill Research and Development Program, Oil Spill Research Symposium, Baton Rouge, LA (1998).
3. Breitenbeck, G.A. and Grace, B., "Use of Ammoniated Cellulosic Materials for Remediation of Oil-Contaminated Wetlands," Michael Holiday & Associates. Louisiana Oil Spill Research and Development Program, OSRADP Technical Report Series 96-001, 22 pp. (1999).
4. Englert, C.J., Kenzie, E.J., and Dragun, J., "Bioremediation of petroleum products in soil," In: Principles and Practices for Petroleum Contaminated Soils. E.J. Calabrese and P.T. Kosteci (eds.). Ann Arbor, MI. Lewis Publishers (1993).
5. Huesemann, M.H., "Guidelines for land-treating petroleum hydrocarbon contaminated soils," *Journal of Soil Contamination*. **3**:1-20 (1994).
6. DeSilva, A.P., "Ammoniation of Cellulosic Materials for Use in Fertilization and Oil Spill Remediation," Ph.D. Dissertation. Louisiana State University, Baton Rouge, Louisiana. 157 pp. (1995).
7. Grace, B., "The Use of N-Rich Absorbent Materials for the *In Situ* Remediation of Crude Oil Contaminated Wetlands," Masters Thesis. Louisiana State University, Baton Rouge, Louisiana. 60 pp. (1997).
8. Page, A.L., Miller, R.H., and Keeney, D.R., Methods of Soil Analysis: Chemical and Microbiological Properties (2<sup>nd</sup> Edition), 1159 pp. Madison, WI. American Society of Agronomy (1982).
9. C-K Associates, Inc., "SONAT Exploration Company, Cravens, LA: Results of field activities and laboratory analysis for the delineated study area," (1999).
10. Barre, B., "Composting to Enhance Microbial Degradation of Chlorinated and Non-chlorinated Hydrocarbons in a Contaminated Wetland," Masters Thesis. Louisiana State University, Baton Rouge, Louisiana. 93 pp. (1997).
11. Bohn, H.L., McNeal, B.L., and O'Connor, G.A., Soil Chemistry (2<sup>nd</sup> Edition), 341 pp. New York. John Wiley and Sons (1985).
12. Foght, J.M. and Westlake, D.W.S., "Biodegradation of hydrocarbons in freshwater," In: Oil in Freshwater: Chemistry, Biology, Countermeasure Technology. S.E. Hrudey and J.H. Vandermeulen (ed.). New York. Pergamon Press (1987).