

IN SITU BURNING AND PHYTOREMEDIATION STUDIES ONSHORE OIL SPILLS: AN INTERIM REPORT

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***In Situ* Burning and Phytoremediation Studies for Onshore Oil Spills: An Interim Report**

Abstract

Phytoremediation and *in situ* burning have potential application for the remediation of crude oil contaminated upland sites. The development of phytoremediation for upland oil spills must include the identification of plants that are capable of adapting to and thriving in oiled soil. Furthermore, the effectiveness of *in situ* burning and phytoremediation in restoring oil contaminated soil needs to be evaluated under controlled conditions. This study had several objectives. First, we were interested in identifying potential phytoremediators that are adapted to the soils and climate found within inland Louisiana oil spill sites. This objective was addressed by: (1) observing vegetation growing in existing upland oil spill sites, and (2) screening plants for oil tolerance in the greenhouse. Second, we wanted to evaluate nutrient uptake for plants grown in oiled soil relative to plants grown in uncontaminated soil to see whether crude oil produced any treatable nutritional disorders in plants. Third, we wished to assess the effectiveness of *in situ* burning and phytoremediation under controlled conditions. This was evaluated by measuring the number of hydrocarbon utilizing bacteria and residual hydrocarbon content in oiled soil subjected to *in situ* burning, phytoremediation, or both at five intervals over a 300 day period.

Over 40 different species of native plants were observed growing in oil contaminated soil at existing upland oil spill sites in northern Louisiana. These observations indicated that a variety of plants might be able to persist in crude oil contaminated soil under real world conditions. These plants would, therefore, have the potential to phytoremediate. In greenhouse studies, 46 different types of plants were screened for oil tolerance by growing transplants in soil containing 0, 30, or 60 grams of freshly spilled North Louisiana Sweet Crude oil per kg of typical north Louisiana upland soil (0, 30, or 60 g oil kg⁻¹ soil). Plant height, dry matter, and mortality were determined after a minimum growth period of 28 days. These screening studies indicated that dry matter yield and plant height were reduced gradually in response to increasing rates of crude oil for all plants tested, although some plants appeared to tolerate oiled soil better than others. Several plants were judged to be the most tolerant of crude oil contamination based on their overall appearance and because their growth and development were least affected by crude oil. These apparently oil tolerant plants were: gazania, a drought tolerant ground cover; yellow nutsedge, a tenacious southern weed; johnson grass, a common southern weed; and sorghum sudan grass, a forage crop species. Plant mortality was zero for most plants tested. Based on these studies, it appears feasible to establish vegetation in soil containing up to approximately 5% crude oil or 50 g oil kg⁻¹. Germination studies were conducted in soil containing either 0 or 30 g oil kg⁻¹ soil. Germination rates were very

low in oiled soil, indicating that transplanting may be the best option for establishing vegetation in oiled soil.

Over the course of a 300 day greenhouse study, element uptake by both common bermudagrass and tall fescue was affected by the presence of freshly spilled crude oil (30 g kg⁻¹) in soil. Nitrogen, phosphorus, potassium, calcium, sulfur, and boron concentrations were initially higher in plants that were cultivated in oiled soil during the earliest stage of this study. The levels of these elements increased in the control pots in the following samples so that by the end of the test period their concentrations became similar to or higher than those observed in the oiled plants. This trend in nutrient uptake suggested that either higher biomass production in unoiled soil diluted these elements or that crude oil may have affected the ability of plants to regulate the flow of nutrient ions into and out of their roots. The second possibility may have been due to the oil's effect on root membranes.

In another greenhouse study, we evaluated the effectiveness of burning, phytoremediation, or both in attenuating freshly oiled upland soil. Six treatments were prepared: oiled soil (30 g kg⁻¹), oiled soil with common bermudagrass, oiled soil with tall fescue, oiled-burned soil, oiled-burned soil with common bermudagrass, and oiled-limed soil with common bermudagrass. We found that residual hydrocarbons disappeared rapidly from freshly oiled soil—whether the oil was burned, kept vegetated, subjected to both treatments, or left untreated. The application of lime did not stimulate petroleum degradation despite the low pH of the soil. By Day 300, less than 90% of the originally spilled crude oil remained in the soil of all six oiled treatments. Moreover, there was no difference in residual oil concentration in pots that received burning or phytoremediation treatments relative to concentrations observed in the controls. We need to interject a note of caution: the GC/FID analysis of residual hydrocarbons in our soil samples upon which the following conclusions are based is considered preliminary and needs further verification before it can be accepted. Additional analytical work is planned for the next funding cycle. Until this additional analysis can be performed, we have decided to defer reporting GC/MS analytical results for residual hydrocarbons extracted from these soil samples. In the same study, hydrocarbon utilizing bacteria were found to be orders of magnitude higher in oiled soil than in unoiled soil, indicating that spilled crude oil stimulated soil microbes that are capable of metabolizing hydrocarbons. The implication of these preliminary results is that the application of nutrients (NPK fertilizer) may be an effective means of *in situ* remediation of crude oil affected upland sites (our untreated controls received NPK fertilizer and exhibited high levels of attenuation). Despite these results, revegetation of oiled sites should be considered an important remediation strategy. We believe that the re-introduction of vegetation into oiled upland sites is aesthetically desirable, prevents the soil from being eroded, provides habitat and forage for wildlife, and may accelerate the natural attenuation of crude oil.

1.0 Introduction

Numerous, low volume crude oil spills occur on upland sites in Louisiana. These spills can damage the environment through their toxic effect on soil, water, and native vegetation. Phytoremediation and *in situ* burning are technologies that have the potential to restore crude oil contaminated upland sites. Phytoremediation involves the use of plants to reduce the level of either inorganic or organic contaminants in soil and groundwater (Salt *et al.* 1998; Schnoor *et al.* 1995). This emerging technology has shown promise in remediating crude oil contaminated upland environments (Banks and Schwab 1998; Wiltse *et al.* 1998). *In situ* burning involves the combustion of flammable components in crude oil, thus lowering the hydrocarbon content of oiled soil. Burning has been investigated extensively for marine oil spills, but has not been studied in conjunction with small, upland sites (Allen and Ferek 1993). Although *in situ* burning appears promising, its effectiveness alone and in combination with phytoremediation has not been studied under controlled conditions.

Phytoremediation has been used successfully to facilitate *in situ* bioremediation of soil contaminated with compounds such as heavy metals and nonhydrophobic organic contaminants such as pesticides (Schnoor *et al.* 1995; Rock 1996). The primary function of vegetation in the restoration of oil contaminated soil is to increase the biodegradation rate of residual hydrocarbons within the rhizosphere by stimulating microbiological metabolism of these compounds. An added benefit is that plant roots create a porous soil matrix that encourages air and water to move into and through the soil, thereby enhancing aeration and water availability. Several advantages of phytoremediation are that it is *in situ*, passive, and solar driven. Its cost is about 10 to 20% less than the cost associated with mechanical or chemical treatments. Moreover, phytoremediation is faster than natural (unassisted) remediation, aesthetically pleasing, and has high public acceptance.

The presence of oil in soil is known to be toxic to plants (Baker 1970), and establishing vegetation in crude oil contaminated soils can be difficult (Amakiri and Onoreghara 1983 and 1984; Udo and Fayemi 1975). Several plant species show the potential to remediate oil contaminated soil (Aprill and Sims 1990; Gunther *et al.* 1996; Klock 1984; Lee and Banks 1993). For example, Aprill and Sims (1990) reported an increase in the disappearance of polycyclic aromatic hydrocarbons (PAHs) in soil columns planted with prairie grasses. The first successful demonstration of phytoremediation of petroleum contaminated soil on a Gulf Coast agricultural site occurred in 1993 (Betts 1997). Over a 21 month period, 41 and 50% of petroleum compounds were removed from Saint Augustine and rye grass vegetated plots, respectively. Only 21% of petroleum compounds were removed from nonvegetated plots. In another study, Banks and Schwab (1998) reported that the levels of residual hydrocarbons were statistically lower in oiled plots having white clover, tall fescue, or bermudagrass vegetation compared to control plots. After three growing seasons, approximately 50% of the residual hydrocarbons had been removed from the vegetated plots, while only 33% had been removed from the control plots.

Although phytoremediation has been demonstrated, its effectiveness may be affected by both plant species and cultivars within a species (Wiltze *et al.* 1998). The success of some studies was achieved using plant species that are not well suited to conditions found

in northern Louisiana. In order for phytoremediation to be successful, it is necessary to identify plants that will tolerate oil and thrive in the infertile, acidic soils typical of the region.

In situ burning has been used as a treatment technology for marine oil spills for many years, and a thorough analysis has been made on the advantages of this remediation technique (Allen and Ferek 1993). Some of the advantages of *in situ* burning include: (1) high elimination rate, (2) reduction of petroleum compounds to primary combustion products of carbon dioxide and water, (3) minimal environmental impact, and (4) minimal cleanup. Although *in situ* burning has been investigated as a method of removing oil from wetland environments (Baker *et al.* 1987; Bruney and Trimm 1993), there is little information on its applicability for small, upland oil spills. May and Wolfe (1997) presented a synopsis of field experiences (not formal research studies) using controlled burning on inland oil spill sites in Illinois. Only one case involved a small oil spill on a site free from standing water prior to the spill. A fallow cornfield having a small oil spill was burned the same day of the oil spill and tilled prior to establishing normal farming activity. Two years later, representative soil samples met the Illinois Tier I Cleanup Objectives.

The overall goal of this project was to evaluate the potential for using *in situ* burning and phytoremediation to restore oil contaminated upland sites in northern Louisiana. The objectives of this project were to:

1. Observe and identify native vegetation in existing upland oil spill sites in Louisiana. This may provide clues about the types of plants that can tolerate oil in soil and that could, therefore, be effective phytoremediators.
2. Screen a variety of plants for oil tolerance under greenhouse conditions. Plant screening will improve understanding of how plants respond to oil in soil and provide additional insights into the types of plants that may persist in oiled soil in the field.
3. Evaluate the effectiveness of *in situ* burning and phytoremediation in the greenhouse under controlled conditions.
4. Compare the uptake of numerous elements by plants grown in either oiled or unoiled soil to determine whether vegetation established in crude oil contaminated soil may have special nutritional needs.

2.0 Materials and Methods

2.1 Observations of Vegetation at Existing Oil Spill Sites

Five visits to the oil producing region of northern Louisiana were made during the summer of 1998. These observation sites were located near Oil City in Caddo Parish. The sites consisted of areas associated with some aspect of crude oil production (storage tanks, pipelines, wells, etc.) where crude oil had been spilled (Figure 1). Plants found growing in oiled soil were photographed for future reference.

Figure 1 Typical observation sites for plants growing in crude oil contaminated soil.

2.2 Greenhouse Screening for Oil Tolerance

2.2.1 Preparation of Oiled Soil

Soil for the plant screening, germination, *in situ* burning-phytoremediation studies was obtained from the Louisiana Tech University Arboretum located in Ruston. Soil was collected from the A horizon of a Sacul fine sandy loam (clayey, mixed, thermic Aquic Hapludult). A representative soil sample was analyzed by the Louisiana State University Soil Testing Laboratory. Results of these analyses are shown in Table 1. Soil used in all experiments was air dried and passed through a 2 mm mesh screen before crude oil was applied.

To prepare oiled soil for the plant screening studies, 1500 g of sieved, air dried soil was added to a stainless steel mixing bowl and treated with 0, 45, or 90 g of North Louisiana Sweet Crude oil (Calumet Lubricants Company, API gravity of 38.3). Oil spilled at these rates produced soil containing 0, 30, or 60 g crude oil per kg soil. The oil and soil were mixed thoroughly using a hand held electric mixer. The oiled soil was divided equally into three round (10 cm diameter) plastic pots such that each pot contained approximately 500 grams of oiled soil. This produced enough pots to allow the oil spill rate for each plant to be replicated three times. The bottom of each pot was lined with a sheet of Weed-X fabric. The pots were aged for seven to 10 days in a greenhouse to allow time for volatile hydrocarbons to evaporate.

Table 1 Soil test results for Sacul fine sandy loam.

Properties	Value	Soil Test Interpretation
pH	5.5	moderately acidic
Phosphorus, mg kg ⁻¹	11	low
Potassium, mg kg ⁻¹	3	very low
Calcium, mg kg ⁻¹	176	very low
Magnesium, mg kg ⁻¹	25	very low
Exchangeable bases, cmole kg ⁻¹	1.1	

Texture	fine sandy loam
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2.2.2 Plant Screening

Seeds were germinated in Pro-Mix potting media in plug trays [1.5 cm (w) x 4 cm (h)]. Each pot received five transplants (Figure 2) and was watered with 100 ml of tap water. The pots were placed randomly on an ebb and flow watering table where they were automatically watered twice per day. On warm, clear days when evapotranspiration rates were high, the pots were top misted. The pots were housed within a heated and cooled greenhouse where the air temperature was maintained between 15°C to 35°C. Each pot was fertilized by adding four g of Osmocote Extended Time Release Fertilizer (18-6-12). Insects were controlled with insecticide sprays when necessary. Plants that died from transplant shock during the first week were replaced with fresh transplants. After a minimum 28 day growing period, plant heights, mortality, and dry weights were measured.

Figure 2 *Greenhouse pot containing oiled soil and a wild oats transplant.*

2.2.3 Germination Study

Seventy grams of soil containing either 0 or 30 g crude oil kg⁻¹ soil (prepared as described in the previous section) were added to plastic bathroom cups (5 cm diameter) with holes punched in the bottom. Twenty seeds were placed on the top of the soil and pressed into the soil with another cup. Each treatment was replicated twice. The cups were maintained on an ebb and flow watering table in the greenhouse (as described above) and misted occasionally. Seedlings were removed as they germinated, and the number of germinated seeds was recorded over a 28 day period.

2.2.4 Experimental Design and Statistical Analysis

The plant screening and germination studies were set up as completely randomized designs with three or two replications, respectively. Dry matter yield (grams) and plant height (cm) data were transformed to relative yield and height by dividing yield or height values for 30 or 60 g kg⁻¹ plants by the means for the controls and multiplying by 100. Relative yield and height data for all plants screened were analyzed by PROC REG using the Statistical Analysis System (SAS). Numerous regression models and transformations, such as log, exponential, and inverse functions, were evaluated in order to fit the data to a regression function. A second order polynomial was chosen because it provided the best

fit for the most species.

2.3 *In Situ* Burning-Phytoremediation Greenhouse Study

2.3.1 Preparation of Oiled, Oiled-Limed, and Oiled-Burned Soil

The following soil treatments were prepared using air dried, sieved Sacul topsoil and North Louisiana Sweet Crude oil: (1) oiled soil, (2) oiled-burned soil, and (3) oiled-limed soil. Oiled soil was prepared in small batches by applying 60 g of crude oil to two kg of soil (30 g oil kg⁻¹) in a stainless steel mixing bowl. The soil and oil were mixed thoroughly using a hand held electric mixer and transferred to a 40 l galvanized steel tub. Twenty-five 2 kg batches of oiled soil were prepared in this manner. After all of the small batches had been prepared and transferred to the tub, they were mixed thoroughly using an electric mixer. The oiled-burned soil was prepared in one batch by applying 750 g of crude oil to 25 kg of soil in a 25 l galvanized steel pail. The oil was allowed to seep into the soil for about 15 minutes until approximately one cm of oil remained above the soil surface. The oil was ignited with a propane torch and allowed to burn itself out over approximately 30 minutes (Figure 3). The batches of oiled and oiled-burned soil were stored uncovered in a greenhouse for seven days. Both batches of soil were mixed thoroughly after this aging period and prior to adding transplants. The oiled-limed soil was prepared in four separate batches by mixing 4 kg of aged, oiled soil with 6 g of hydrated lime [Ca(OH)₂] using an electric, hand held mixer.

Figure 3 *In situ* burning of crude oil contaminated soil.

2.3.2 Establishing Treatments

One kg of untreated soil, oiled soil, oiled-limed soil, or oiled-burned soil was added to a square plastic pot [11 cm (w) x 12.5 cm (h)]. The bottom of each pot was lined with a sheet of Weed-X fabric. The pots that received phytoremediation treatments had four transplants added per pot. These transplants were established in plug trays (1.5 cm x 4 cm) using untreated soil as the growing medium. To summarize, the following treatments were established in the pots along with their abbreviations (in parenthesis):

1. soil + 30 g crude oil kg⁻¹ (Oil)
2. soil + 30 g crude oil kg⁻¹ + common bermudagrass (Oil + CB)
3. soil + 30 g crude oil kg⁻¹ + common bermudagrass + 1.5 g lime kg⁻¹ (Oil + lime + CB)
4. soil + 30 g crude oil kg⁻¹ + tall fescue (Oil + TF)
5. soil + 30 g crude oil kg⁻¹ + burning (Oil + burn)
6. soil + 30 g crude oil kg⁻¹ + burning + common bermudagrass (Oil + burn + CB)

7. soil + common bermudagrass (CB)
8. soil + tall fescue (TF)

Sufficient pots were prepared to allow for three replications of each treatment over four sampling periods (total of 12 pots per treatment). These sampling periods were set at 50, 100, 200, and 300 days after vegetation was established in the pots (April 6, 1999). One hundred gram portions of oiled and oil-burned soil (with three replicates) used to generate these treatments were saved as Day 0 samples.

The pots were kept in a climate controlled greenhouse in which the temperature was maintained between 15°C and 35°C. The pots were placed on an ebb and flow watering table that was flooded twice a day and top misted as needed. Five grams of Osmocote (18-6-12) fertilizer granules were added to each pot, and the plants were treated with insecticides as needed. The plants were harvested periodically by clipping the grasses at 10 cm above the soil surface. The clippings were dried in an oven (105°C) and stored for future element analyses.

2.3.3 Soil Sampling Technique

Prior to soil sampling, the grasses were clipped (10 cm height above the soil surface), and the clippings were dried and retained. Next, the soil was removed from each plastic pot as a large plug and placed at the bottom of a paper bag. The paper bags containing the soil plugs were left open, stored in the greenhouse, and allowed to dry for two to four days. Drying the soil plugs was necessary in order to separate the soil from the plant roots; the soil could not be separated from the plant roots while the soil plugs were moist. For those treatments containing plants, soil was separated from the root mass by placing the soil plug in a one quart plastic bag with the stems protruding out of the bag. The soil was kneaded carefully away from the roots, sieved through a 2 mm screen, thoroughly mixed, and retained for microbiological and chemical analysis.

2.3.4 Microbial Analysis

Soil samples were analyzed for hydrocarbon utilizing bacteria (HUB) by the most probable number (NPN) technique using North Louisiana Sweet Crude oil as the carbon source (Chaîneau *et al.* 1996). Eleven grams of soil were added to sterilized dilution jars containing 99 ml of de-ionized water. Ten-fold serial dilutions were prepared beginning with 10^{-1} and ending with 10^{-6} . Dilutions were adjusted for microbial growth as needed. Tubes contained a mineral salt medium consisting of 0.68 g L⁻¹ KH₂PO₄, 1.79 g L⁻¹ NaHPO₄, 0.35 g L⁻¹ MgSO₄, 1 g L⁻¹ NO₃NH₄, 1 g L⁻¹ CaCl₂, 0.4 mg L⁻¹ FeSO₄, and 0.1 mL L⁻¹ of a solution that contained 100 mg L⁻¹ of H₃BO₄, MnSO₄, ZnSO₄, CuSO₄, and CoCl₂. Each tube contained 5.0 ml of sterilized mineral salt solution, 1.0 ml of inoculum from the dilutions, and 0.1 ml of crude oil. A series of five tubes per dilution was

established and incubated for 28 days at $24 \pm 1^\circ \text{C}$. After examination for positive tubes, standard McCrady tables were used to determine the number of viable organisms.

2.3.5 Chemical Analysis

Oil residues were extracted from the soil and analyzed at the Institute for Environmental Studies' (Louisiana State University) analytical laboratory using procedures developed by Henry and Overton (1993). Gas chromatography/mass spectroscopy (GC/MS) analysis was used to identify individual compounds (Roques *et al* 1994); gas chromatography/flame ionization detection (GC/FID) analysis was used to measure the total hydrocarbon residues. Soil samples were extracted by weighing 30 g samples into 150 ml beakers. Each sample was extracted twice—one sample for GC/FID analysis and the other for GC/MS analysis. Anhydrous sodium sulfate (Na_2SO_4) was added to absorb moisture until samples had the consistency of dry sand. After the soil and Na_2SO_4 were mixed thoroughly, 1 ml of a surrogate was added. Surrogates were used to trace the recovery of hydrocarbon residues during the extraction process. The GC/MS surrogate standard mix consisted of 2-fluorophenol, phenol- d_6 , nitrobenzene- d_5 , 2-fluorobiphenyl, 2,4,6-tribromophenol and 4-terphenyl- d_{14} . This mix was added at a concentration of 12mg/ml in 1 ml of dichloromethane (DCM). The GC/FID surrogate, o-terphenyl, was added at a concentration of 200m/ml in 1 ml of DCM. Next, 50 ml of DCM were added to the beakers and sonicated for 15 minutes. After being allowed to settle, the DCM solution was decanted and filtered by gravity through Na_2SO_4 . The DCM extraction was repeated two additional times. The funnel was washed with additional portions of DCM. Solvent extracts were concentrated to 5 ml by rotary evaporation and nitrogen blow down and stored at 4°C in sealed vials for analysis. Extracts were analyzed using a Hewlett-Packard 5890A gas chromatograph equipped with a capillary column and flame ionization detector.

2.3.6 Plant Tissue Analysis

Common bermudagrass and tall fescue clippings obtained during the course of the *in situ* burning/phytoremediation study described in this section were obtained over four periods. The only plant tissue samples that were analyzed were from the CB, TF, Oil + CB, and Oil + TF treatments. The first set of clippings was obtained for the growth period between Day 0 and Day 50. Clippings from these samples were comprised of leaves or stems obtained from plants that were to be sacrificed to obtain Day 50 soil samples. The plants were clipped irregularly to maintain the length of tillers at approximately 10 to 50 cm. Thus, the clippings that comprised Day 50 tissue samples were a combination of any plant material harvested prior to Day 50 plus the entire above-ground portion of the plant obtained at Day 50 when the plants were sacrificed. Grass clippings for the periods of Day 50 to 100, Days 100 to 200, and Days 200 to 300 were obtained in a similar fashion. After the grass clippings were dried in an oven (three days

at 105°C), they were ground in a Wiley mill (to less than 2 mm diameter) and stored for future analysis. The grass tissue samples were analyzed at the Plant Tissue Analysis Laboratory (Agronomy Department) at Louisiana State University. Nitrogen content was measured using the Dumas method (Simonne *et al.* 1995). The Dumas method is a dry combustion analysis for total N in solid-phase samples and is based on extremely rapid volatilization by the complete flash combustion of the sample. For the analysis of the other elements (Ca, Mg, S, P, K, Al, B, Cd, Cu, Fe, Mn, Na, Ni, Pb, and Zn), plant material was digested, and the digests analyzed by ICP (Inductively Coupled Plasma) spectroscopy (Huang and Schulte 1985; Jones *et al.* 1991).

2.3.7 Experimental Design and Statistical Analysis

This study was established as a repeated measures experimental design and a modified split plot design. For the analysis of the *in situ* burning/phytoremediation study, the six oiled soil treatments were considered the whole plots, and the five time periods were split plots. For the plant tissue analysis data, the whole plots were the soil and plant treatments with four time periods serving as the split plots. The HUB, GC/FID, and plant tissue analysis data were analyzed using PROC GLM of the Statistical Analysis System (SAS). The HUB count data (CFU g⁻¹) were transformed to their natural logarithms in order to normalize the data. Microbial analysis data for Day 200 were not obtained due to problems with sample contamination.

2.3.8 Evaporation Study

One hundred grams of crude oil were spilled on top of 500 grams of air dried soil contained in a round plastic pot (10 cm diameter). These treatments were replicated three times. The pots were maintained on a greenhouse bench between 2 February and 28 September (1999). The pots were weighed periodically over the 221 day test period to determine evaporation losses. The greenhouse did not have a cooling system, and daytime temperatures exceeded 50°C on many days during the study.

3.0 Results and Discussion

3.1 Observations at Existing Spill Sites

Over 40 different plant species were observed to be growing within existing oil spill sites in northern Louisiana. Many of these plants were identified as native grasses, sedges, or other herbaceous plants (Figure 4). Unfortunately, taxonomic identification was not achieved for any of these plant species. We observed plants thriving in oil spills within a variety of different upland environments, such as sunny meadows, shaded

woodlands, and wet, low spots. In several instances, plants were found growing in soil that appeared to contain significant amounts of crude oil, although highly polluted locations were usually devoid of plant life. These field observations indicate that it may be possible to re-vegetate crude oil affected upland sites, because native vegetation appears to tolerate oiled soil under actual field conditions.

Figure 4 *Native plants found growing in existing oil spill sites.*

3.2 Greenhouse Screening Studies

3.2.1 Oil Tolerance Screening Study

Increasing levels of crude oil in soil gradually reduced the plant height and dry matter yield of wild oats (Figure 5), the first plant screened for oil tolerance. Wild oats was stressed significantly, but appeared to tolerate up to 60 g oil per kg soil with only 20% mortality. At 90 g oil kg⁻¹, the stress was acute and resulted in 80% mortality. Based on the response of wild oats, the highest concentration of crude oil selected for the screening study was set at 60 g kg⁻¹ in order to place the plants under considerable stress, but not enough to be lethal. Thus, 0, 30, and 60 g oil per kg soil were chosen because these levels of crude oil were expected to produce zero, moderate, and significant stress in the test plants. The effect of crude oil on each of the 46 plants tested is summarized in Table 2, where the relative dry matter yields and percent mortality are reported. Increasing levels of crude oil in soil produced a gradual decline in plant growth and a gradual increase in mortality. Of all plant parameters measured, dry matter yield appeared to be the most reliable indicator of plant growth and vitality in response to crude oil. The relative plant height was affected less by the presence of crude oil in soil than the relative yield. Moreover, in many instances, plants growing in oiled soil would appear to be under significant stress despite having only marginal reductions in their heights. The visual symptoms of crude oil stress were: stunted growth leaf chlorosis and necrosis, stunted and discolored root systems, and plant death. Sometimes plants died quickly soon after being transplanted into oiled soil; this did not occur to any significant degree with transplants in control pots. The harmful effect of crude oil on some transplants may not have been due to oil's phytotoxicity, but rather to dehydration resulting from poor soil to root contact. The root bound plug containing potting medium that was transplanted always made firm contact with soil in the control pots. In oiled soil, however, the transplant plugs did not bind well with the soil. As a result of this poor soil to root contact, many fresh transplants toppled over, with their leaves lying on the oiled soil, and their roots out of contact with the soil. This condition lead to transplant death within a couple of days following transplanting for some of the plants. A majority of the plants

screened were easy to establish and had minimal transplant deaths, while a few plants required additional transplants to become fully established in oiled soil. Once established, crude oil slowly reduced the vigor of all plants over the screening period. Affected plants slowly took on the symptoms of crude oil stress described above. The minimal amount of mortality that did occur was the result of long-term stress that appeared to kill the affected plants slowly.

The breadth of plant response to crude oil observed in this study can be seen in three different plants. Crimson clover (Figure 6) was one of the plants most severely affected by the level of oil in soil; barnyardgrass's (Figure 7) intermediate response was typical of most plants tested; gazania (Figure 8) was one of the least affected plants and showed some degree of oil tolerance. The response of the various plants to crude oil in soil suggests several important possibilities. First, there were noticeable differences in the overall growth and vigor of plants cultivated in oiled soil. These differences indicate that screening additional plants for oil tolerance may lead to the identification of plants that have an even higher probability of persisting in crude oil contaminated soils. Based on this study, gazania, johnson grass, sorghum sudan grass, and yellow nutsedge would be suitable phytoremediation candidates for field trials because of their relative tolerance of oil in soil and their adaptation to the soil and climate found in Louisiana upland locations. Second, plant response data suggest that the upper limit of crude oil tolerance may be on the order of approximately 5% crude oil in soil (50 g kg^{-1}), or possibly even higher if growth conditions are ideal. Earlier we described how the relative dry matter yield and plant height for wild oats were essentially zero at 90 g oil kg^{-1} soil, while its response to crude oil between 0 and 60 g oil kg^{-1} soil was somewhat typical for most plants screened (Figure 5). This would indicate that crude oil would be quite lethal to vegetation when it approached 8 to 10% of the soil mass. Studies conducted in the field, however, would be needed to determine the upper limits of plant tolerance to oil in soil. In addition, a plant's ability to tolerate crude oil in soil under greenhouse conditions might not translate into long-term persistence under actual field conditions where other factors such as inadequate moisture, insect damage, and disease can produce additional stress upon the vegetation.

All plants exhibited a good fit of their relative dry matter yield and height to the rate of spilled oil by second order, polynomial regression equations. While these regression equations may not represent the ideal biological model of plant response to toxicity, they did the best job of fitting the data. The coefficient of determinations (R^2) was usually greater than 0.90. The investigators had expected to use confidence intervals to separate the plants into groups of similarly responding plants. Unfortunately, the confidence intervals for relative dry matter yields and plant heights were too wide to group the plants definitively.

For several reasons, the results generated by this screening study should be viewed with caution with regard to predicting the usefulness of individual plants as phytoremediators of crude oil spills. First, the plants were screened in freshly spilled crude oil. The composition of aged crude oil would be quite different than fresh oil, and plant response to crude oil may change as the oil weathers. Weathered crude oil would be

expected to exhibit a lower degree of phytotoxicity. Second, greenhouse studies have always been conducted to provide a crude approximation for how a soil-plant system might behave under real world conditions. Many environmental factors that plants might encounter under field conditions, such as drought or insect stress, do not impact plant growth in the greenhouse. This screening study was conducted: (1) to determine the range of oil concentration in soil that could promote the use of plants to remediate crude oil spills and, (2) to identify plants that may possess some degree of oil tolerance.

Figure 5 *Relative dry matter yield and plant height of wild oats cultivated in soil containing 0, 30, 60, or 90 g crude oil kg⁻¹ soil.*

Table 2 Mean relative dry matter yield (and percent mortality) in soil containing 30 and 60 g oil kg⁻¹ soil

Plant	% Relative Yield (% Mortality)	
	30 g oil kg ⁻¹	60 g oil kg ⁻¹
Alfalfa	58†	23 (20)
Alkali sacaton	56	34
Annual blue grass	34	24
Annual rye grass	40	22
Anza Wheat	64	38
Austrian winter pea	58	41
Barley	62	35
Barnyardgrass	52	29
Bent grass	52	19 (13)
Big bluestem	52	24
Chicory	49	14
Common bermudagrass	40	41
Cosmos	49	32
Cowpea	68 (7)	49 (7)
Crimson clover	27 (7)	15 (13)
Dallis grass	67	44
Elbon rye	52	17 (7)
Gazania	96	50
Hairy vetch	43	25 (40)

Johnson grass	78	61
Klein grass	56	22
Love grass	46	13
Matua prairie grass	32	19
Maximillian sunflower	38	29 (7)
Millet	74	52 (7)
Moss verbena	26	13 (13)
Mt. Barker clover	34 (20)	9 (40)
Novella English pea	57	32 (7)
Oats	52 (7)	25 (13)
Piper sudan grass	55	27
Plains coreopsis	69	37
Rape	52	21 (27)
Red top	27	18 (7)
Reed canary grass	28	17
Rescuegrass	78	44
Rough blue grass	44 (13)	20 (7)
Sorghum sudan grass	78	54
Smooth brome	28	13
Sweet pea	50	37 (7)
Tall fescue	36	22
Texas bluebonnet	25 (53)	12 (67)
Timothy	30	15 (7)
Western wheat grass	35	17 (27)
Wild oats	47	18 (20)
Winter wheat	68	43
Yellow nutsedge	60	58

† Zero percent mortality values have been omitted.

Figure 6 *Relative dry matter yield, plant height, and photograph of crimson clover cultivated in 0, 30, and 60 g crude oil kg⁻¹ soil.*

Figure 7 *Relative dry matter yield, plant height, and photograph of barnyardgrass cultivated in 0, 30, and 60 g crude oil kg⁻¹ soil.*

Figure 8 *Relative dry matter yield, plant height, and photograph of gazania cultivated in 0, 30, and 60 g crude oil kg⁻¹ soil.*

3.2.2 Germination Study

Crude oil in soil suppressed seed germination in all plants tested (Table 3). Most plants had zero germination in oiled soil, and only one species (cowpea) had a germination rate in oiled soil close to the germination rate in the control. Decreased germination rates of seeds planted in oiled soil have been reported by others (Udo and Fayemi 1975; Amakiri and Onofeghara 1984). These findings imply that establishing vegetation in oiled uplands may require the use of transplants rather than direct seeding, especially as the concentration of oil approaches 30 g kg⁻¹.

Table 3 Percent seed germination in the control and in oiled soil

Plant	% Germination	
	0 g oil kg ⁻¹	30 g oil kg ⁻¹
Annual blue grass	95	0
Annual rye grass	90	0
Austrian winter pea	100	0
Anza Wheat	90	5
Barley	95	0
Coastal bermudagrass	42	0
Cowpea	32	25

Crimson clover	97	12
Elbon rye	80	0
Hairy vetch	75	0
Matua prairie grass	97	0
Millet	50	0
Mt. Barker clover	60	7
Novella English pea	97	0
Oats	92	0
Piper sudan grass	82	0
Rape	97	10
Rough blue grass	77	0
Sorghum sudan grass	87	0
Sweet pea	50	0
Tall fescue	82	0
Texas bluebonnet	47	0
Wild oats	20	0
Winter wheat	92	0
Yellow nutsedge	32	0

3.3 *In Situ* Burning and Phytoremediation Greenhouse Study

3.3.1 Plant Growth

Common bermudagrass and tall fescue plants fared well over the 300 day growth period in both the unoiled and oiled treatments (Figure 9). No transplants died and all appeared to have excellent vigor. The leaves and stems were clipped periodically because they produced large quantities of plant material (e.g. bermudagrass tillers grew to more than 75 cm in length at times). The clippings were dried (105°C), ground up, and stored for elemental analysis. This aspect of the study demonstrates that plants can thrive for extended periods of time in heavily oiled soil when properly watered and fertilized.

Figure 9 *Common bermudagrass and tall fescue plants cultivated in 30 g crude oil treatment at Day 98.*

3.3.2 Microbial Analysis

The number of hydrocarbon utilizing bacteria (HUB) in all treatments containing oil contaminated soil was consistently at least several orders of magnitude higher than in the unoiled, vegetated controls (CB, TF). For example, the mean number of HUB in oiled soil treatments was 8.3×10^6 CFU/g, but only 4.4×10^4 in the unoiled controls over the three sampling periods. Analysis of variance indicated that the main treatment effect and time x treatment interaction were both highly significant (probability < 0.0001). Treatment main effects are shown in Table 4, and the treatment x time interaction is shown in Figure 10. Unexpectedly, the treatment main effects show that the highest levels of HUB were found in the unvegetated, oiled treatments, and that lime application apparently did not stimulate microbial activity. The number of HUB was higher in oiled and unoiled treatments containing tall fescue than in the corresponding treatments planted with common bermudagrass. The time x treatment interaction showed that the number of HUB in unoiled, vegetated treatments (TF and CB) was lower at Day 100 than at the other sampling dates. This decline in HUB did not, however, occur in the oiled treatments.

Table 4 Main effect of oil and burn treatments on hydrocarbon utilizing bacteria (HUB)

Treatment	ln HUB (CFU g ⁻¹) †
Oil	15.6 a
Oil + burn	15.2 ab
Oil + TF	15.1 ab
Oil + lime + CB	14.3 bc
Oil + CB	13.7 c
Oil + burn + CB	13.6 c
TF	9.6 d
CB	8.5 e

†Mean ln HUB followed by a different lower case letter are significantly different based on Fisher's LSD (probability < 0.05).

Figure 10 *Interactive effect of treatment x time on the number of HUB in the various treatments.*

3.3.3 Chemical Analysis

The GC/FID analytical results presented in this section are considered preliminary; the authors believe additional analyses should be undertaken before these results are accepted. The GC/FID analysis performed to date shows that the level of residual hydrocarbons in all six oiled treatments declined substantially between Days 0 and 300 (Figure 11). By Day 300, the mean level of residual hydrocarbons in all oiled treatments was only 8% of that detected on Day 0. In other words, 92% of the original hydrocarbons were absent from the oiled soil. The decline in the residual level of hydrocarbons was likely due to both evaporation and microbial degradation. Metabolism of spilled crude oil by soil microbes has been reported in numerous studies (Atlas 1991; Lee and Banks 1993; Chaîneau 1996). The increase in HUB in oiled soil observed in this study (as described in the previous section) is considered evidence that microbial degradation of oil may have occurred. The rate or degree of biodegradation depends upon many environmental factors, such as temperature, moisture, pH, and soil fertility. Most of the conditions that promote microbial degradation of crude oil were near optimum during the course of our study; the soil was kept moist, warm, and supplied with nutrients for the entire 300 day test period. Based on optimal biological conditions that existed in the oiled pots, significant microbial decomposition of the residual hydrocarbons should have occurred during this study. Oil evaporation rates are dependent upon the composition of the oil and environmental factors such as temperature, soil moisture, and wind speed. Oil evaporation potential was assessed in a separate study. Here we observed rapid and extensive evaporation of hydrocarbons following the spilling of crude oil onto air dried soil. Almost 50% of the spilled crude oil evaporated during the 221 days that the evaporation study was conducted (Figure 12). Oiled soil in the evaporation study was subjected to different conditions than that which occurred in the *in situ* burning/phytoremediation study. We would expect less evaporation over the 300 day *in situ* burning/phytoremediation study than during the 221 day evaporation study for the following reasons. First, oiled soil in the *in situ* burning/phytoremediation study was subjected to much cooler daytime temperatures (30 to 35° C versus 40 to 50°+ C). Second, the daily additions of water to the pots would have reduced evaporation potential relative to that experienced in the evaporation study. However, it is likely that a significant amount of oil may have been lost by evaporation during the course of the *in situ* burning/phytoremediation study, although the specific amount lost by evaporation remains unknown. We propose that the rapid decline in residual hydrocarbons that occurred between Days 0 and 50 was due to evaporation and degradation of the most volatile, readily degradable hydrocarbons. The slower rate of attenuation that occurred between Days 50 and 300 was likely due mainly to microbial degradation although evaporation could have contributed to some decline in the level of residual hydrocarbons.

The effect of *in situ* burning on residual HC levels could be seen by comparing levels in the oil treatment to levels in the oil + burn at Day 0. Burning reduced the mean hydrocarbon concentration from $36.8 \times 10^3 \text{ mg kg}^{-1}$ to $23.0 \times 10^3 \text{ mg kg}^{-1}$, a 38% reduction. The decline in hydrocarbons observed based on GC/FID analysis in this study was comparable to the loss of oil from burning measured during a small pilot study we performed (not described in the Materials & Methods section). In this test, 150 g of crude oil were added to 1500 g of air dried soil in a metal coffee can. The oil was allowed to seep into the soil before it was ignited, as was done when preparing burned treatments for the *in situ* burning/phytoremediation study. After the burn was complete, the burned oiled soil was allowed to cool before it was weighed to determine oil loss. In this case, 94.3 g of the oil remained, indicating a 37% decline in the level of residual oil. The percent loss of oil from combustion was essentially the same in both studies.

The GC/FID analysis of the soil samples generated during the course of this study suggests several significant results. First, neither common bermudagrass nor tall fescue affected the rate of crude oil attenuation. Second, an over 90% reduction in hydrocarbon content was achieved in unvegetated controls after 300 days. Third, while *in situ* burning initially reduced the level of residual hydrocarbons in soil, the difference in residual oil content between oiled and oil + burned soil was nil by Day 300. A number of studies have reported that phytoremediation led to a greater attenuation of crude oil than occurred in unvegetated soils (Aprill and Sims 1990; Banks and Schwab 1998). There are a few reports of marginal to no effect of vegetation on the remediation of crude oil (Wiltse *et al.* 1998; Chaîneau 2000). In fact, Chaîneau *et al.* (2000) observed crude oil attenuation rates in fertilized (NPK) soil comparable to rates in fertilized plus vegetated soil. They did report, however, that remediation was significantly reduced in unfertilized soil and concluded that the application of essential nutrients enhanced the remediation process. In our study, we did not include an unfertilized control because we felt that there was insufficient native fertility in Sacul soil to sustain common bermudagrass or tall fescue in greenhouse pots for a 300 day growth period. Other studies have shown increased rates of petroleum biodegradation from the application of nutrients (Atlas 1991; Bossert and Bartha 1984). Fertilization may provide the most cost-effective means of achieving rapid attenuation of crude oil spills. We believe, however, that crude oil spills in the uplands should be looked at from a broader perspective given that oil spills involve more than just the addition of toxic, hydrophobic, and undesirable substances to soil. Crude oil spills in the uplands tend to pose additional environmental risks because they can kill the native vegetation and prevent it from becoming re-established. Restoration of crude oil affected upland sites should have both the removal of hydrocarbons and re-establishment of vegetation as primary goals. For this reason, we believe that upland oil spill research should focus on **vegetative restoration**, rather than strictly on phytoremediation. We define vegetative restoration as the permanent re-vegetation of crude oil affected upland sites (primary goal) where phytoremediation would be a desirable, but not a required secondary benefit, of affected areas that have become vegetated.

Figure 11 *Level of residual hydrocarbons in treatments between Days 0 and 300.*

Figure 12 *Residual crude oil remaining over time during the course of the evaporation study.*

3.3.4 Plant Tissue Analysis

The concentrations of all macronutrients and several micronutrients in the grass clippings were found to be affected by the presence of crude oil in soil. The statistical significance of the various main effects and interactions for the 13 elements tested as determined by analysis of variance are summarized in Table 5. Crude oil produced statistically significant ($P=0.05$) main or interactive effects on N, P, K, Ca, Mg, S, B, and Mn tissue concentration in either common bermudagrass, or tall fescue, or both. All significant oil by time and oil by plant by time interactive effects are shown in Figures 13 through 25. Each of the significant main effects of oil on element concentration have not been reported because wherever these were significant, a higher order interaction took precedence. For example, although the main effect of oil on K concentration was significant ($P=0.006$); the oil by time interaction ($P=0.01$) and the oil by plant by time interaction ($P=0.05$) also were significant and thus overshadowed the main effect.

Overall, the concentrations of all oil affected nutrients except magnesium were higher for plants grown in oiled soil than in unoiled soil in the Day 0 to 50 tissue samples (Figures 13-18, 20-25). As the plants matured over the course of the 300 day growing period, their concentrations either decreased in the oiled plants or increased in the unoiled plants or both. Except for manganese, the concentration of these elements in the last tissue samples collected (Day 200 to 300) was either higher or equivalent in unoiled soil compared to concentrations in oiled soil, a reversal of what was detected in the Day 0 to 50 samples. Sometimes these reversals in element concentration were more pronounced for common bermudagrass (phosphorus and potassium; Figures 15 and 17) or tall fescue (sulfur and boron; Figures 21 and 25). Tissue magnesium levels (Figure 19) were higher in oiled plants during Days 50 to 100, but were similar to those in unoiled plants in the other periods. Tissue manganese levels were higher in oiled plants for the entire duration of the study (Figure 22). The difference in manganese concentration between oiled versus unoiled plants declined over time and was greater for common bermudagrass than for tall fescue (Figure 23).

The implications of how crude oil affects element uptake are not known with any certainty and have received only modest study (Udo and Fayemi 1975). Our results

indicate that in the earliest growth stages, plants grown in oiled soil accumulated many of these essential nutrients in higher concentrations than in unoiled soil. There are two plausible explanations for this. First, crude oil suppressed plant growth, and these plants produced less biomass than did plants grown in uncontaminated soil. The adsorbed nutrients may have been diluted to a greater degree in the larger amounts of plant material produced in unoiled soil. This phenomenon is observed routinely in plant nutrition studies. Second, crude oil could have damaged root membranes sufficiently to alter ion uptake. There are complex physiological and biochemical mechanisms that enable plants to regulate the types and amounts of ions that are brought into their roots from the surrounding soil. The regulation of ion import and export into the root occurs within membranes at the root soil interface. During the course of the greenhouse screening studies for oil tolerance (described earlier), we observed that plants cultivated in oiled soil showed significant root discoloration. Plant roots in oiled soil may have become increasingly less affected over time as the level of residual hydrocarbons declined. The gradual dissipation of residual hydrocarbons between Days 0 and 300 meant that plant roots could have recouped their ability to regulate the flow of ions into and out of their roots, thereby leading to levels of elements similar to those observed in the controls.

The practical implication of these results is that insufficient quantities of essential plant nutrients or excess amounts of toxic ions did not appear to reduce plant growth in oiled soil. This suggests that the diminished plant growth and vigor that we observed in oiled soil probably could not be ameliorated by chemically amending the soil with materials such as fertilizer or limestone. Clearly, a sufficient reduction of the level of residual hydrocarbons by microbial decomposition would be the single most important precursor to establishing vegetation in crude oil affected upland soils. Our results imply that nutrient uptake in oiled soil becomes similar to that in unoiled soil as the level of residual hydrocarbons declines.

Table 5 Summary of analysis of variance probabilities (probability >F) testing oil, time, and plant type main and interactive effects on macronutrient content in grass clippings

Effect	Element					
	N	P	K	Ca	Mg	S
	Probability > F					
Oil	NS	0.06	0.006	0.002	0.05	NS
Plant	0.0001	0.0001	0.0001	0.0004	0.0001	0.09
Oil x Plant	NS	0.01	NS	NS	NS	NS
Time	0.006	0.0001	0.0001	0.0001	0.0001	0.09

Oil x Time	0.0001	0.0001	0.01	0.002	0.01	0.0002
Plant x Time	0.03	NS	0.0008	NS	NS	0.0001
Oil x Plant x Time	NS	0.02	0.05	NS	NS	0.002

Table 6 Summary of analysis of variance probabilities (probability >F) testing oil, time, and plant type main and interactive effects on trace element content in grass clippings

Effect	Element						
	Al	B	Cu	Fe	Mn	Na	Zn
	Probability > F						
Oil	NS	NS	NS	0.07	0.0001	NS	NS
Plant	0.0004	0.0001	NS	0.0001	0.004	0.0001	NS
Oil x Plant	NS	NS	NS	0.07	0.06	NS	NS
Time	0.0001	0.0005	0.0001	0.001	0.0001	0.001	NS
Oil x Time	NS	0.009	NS	NS	0.0001	NS	NS
Plant x Time	0.03	NS	NS	0.03	0.06	0.0004	NS
Oil x Plant x Time	NS	0.04	0.08	NS	0.03	NS	NS

Figure 13 *Mean tissue nitrogen concentration in oiled or unoiled soil over the 300 day growth period.*

Figure 14 *Mean tissue phosphorus concentration in oiled or unoiled soil over the 300 day growth period.*

Figure 15 *Mean tissue phosphorus concentration for either common bermudagrass (CB) or tall fescue (TF) in oiled or unoled soil over the 300 day growth period.*

Figure 16 *Mean tissue potassium concentration in oiled or unoled soil over the 300 day growth period.*

Figure 17 *Mean tissue potassium concentration for either common bermudagrass (CB) or tall fescue (TF) in oiled or unoled soil over the 300 day growth period.*

Figure 18 *Mean tissue calcium concentration in oiled or unoled soil over the 300 day growth period.+*

Figure 19 *Mean tissue magnesium concentration in oiled or unoled soil over the 300 day growth period.*

Figure 20 *Mean tissue sulfur concentration in oiled or unoled soil over the 300 day growth period.*

Figure 21 *Mean tissue sulfur concentration for either common bermudagrass (CB) or tall fescue (TF) in oiled or unoled soil over the 300 day growth period.*

Figure 22 *Mean tissue manganese concentration in oiled or unoled soil over the 300 day growth period.*

Figure 23 *Mean tissue manganese concentration for either common bermudagrass (CB) or tall fescue (TF) in oiled or unoled soil over the 300 day growth period.*

Figure 24 *Mean tissue boron concentration in oiled or unoled soil over the 300 day growth period.*

Figure 25 *Mean tissue boron concentration for either common bermudagrass (CB) or tall fescue (TF) in oiled or unoled soil over the 300 day growth period.*

4.0 Conclusions

Observations at existing oil spill sites in northern Louisiana indicated that a variety of native plants can persist in oiled soil under real world conditions. This provides some assurance that we can re-establish and maintain vegetation in crude oil contaminated upland sites. In greenhouse screening studies, response to crude oil in soil (within the range of 0 to 60 g oil kg⁻¹ soil) by 46 different plant types was somewhat variable. All plants showed a gradual reduction in growth and vigor in response to increasing rates of spilled oil, although some plants were less affected by crude oil in soil than others. Of the plants evaluated, gazania, johnson grass, yellow nutsedge, and sorghum sudan grass exhibited the greatest oil tolerance on the basis of their growth in oiled soil. Plant mortality was low for all plants tested. Seed germination was reduced severely in soil containing as little as 30 g oil kg⁻¹ soil, indicating that the establishment of vegetation by seed would be impractical. The presence of crude oil in soil initially increased nutrient element uptake by plants, although this trend was reversed once the level of residual crude oil declined. Under greenhouse conditions, burning, phytoremediation, or both substantially reduced residual hydrocarbon concentrations in soil, but were no more effective than fertilizing with NPK. This conclusion is preliminary, pending additional analytical efforts in the coming year. Despite this finding, crude oil affected upland soils should be restored vegetatively, where permanent vegetation is established in order to return the location to a more natural state, protect the soil from erosion, encourage phytoremediation, and increase wildlife habitat.

5.0 References

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