

**Re-evaluation of an In-situ Burn and Phytoremediation
Studies
for Onshore Oil Spills**

**E.B. Overton, Ph.D. M.S. Miles, M.S.
Department for Environmental Studies
Louisiana State University**

Disclaimer

This report was prepared under a contract between Louisiana State University and the Louisiana Applied and Educational Oil Spill Research and Development Program (OSRADP). The contents of this document do not necessarily reflect the views and policies of the Louisiana Oil Spill Coordinator's Office, Office of the Governor or those of the Louisiana Applied and Educational Oil Spill Research and Development Program, nor does mention of trade names or commercial products constitute endorsement or recommendation for use by the state of Louisiana.

Table of Contents

Abstract

List of Figures

Figure 1. Soil-oil-bermudagrass treatment

Figure 2. Soil-oil-tall fiscue treatment

Figure 3. Soil-oil-lime-Bermudagrass treatment

Figure 4. Soil-oil-burn treatment

Figure 5. Soil-oil-burn-bermudagrass treatment

List of Tables

Table 1. Target compound list

Table 2. Total alkane results

Table 3. Total aromatic hydrocarbon results

1.0 Introduction

2.0 Methods

2.1. Sample Receipt

2.2. Sediment Extraction Method

2.2.1. High-Level Contamination

2.2.2. Low-Level Contamination

2.3. GC/MS: Instrument Configuration and Calibration

2.4. GC/MS: Data Processing and Interpretation

2.4.1 Quantitative Analysis

2.4.2 Source-Fingerprint

2.5. Data Presentation and Quality Assurance/Quality Control

3.0 Results

3.1. Soil-Oil-Bermudagrass Treatment

3.2. Soil-Oil-Tall Fescue Treatment

3.3. Soil-Oil-Lime-Bermudagrass Treatment

3.4. Soil-Oil-Lime-Bermudagrass Treatment

3.5. Soil-Oil-Lime-Bermudagrass Treatment

4.0 Discussions

5.0 References

2.0 Methods

The purpose of this study was to re-examine, quantitatively, the effects of an in-situ burning and phytoremediation study performed on an oil-contaminated North Louisiana upland soil over a 300-day period. This study was conducted under greenhouse conditions using common bermudagrass and tall fescue as phytoremediators. The following six treatments were established in greenhouse pots that contained 1 kg of Sacul loamy fine sand obtained from the A horizon of Paleudult. Each treatment was replicated three times.

- 30 g spilled oil per kg soil (no burning, no plants)
- 30 g kg⁻¹ spilled oil with burning (no plants)
- 30 g kg⁻¹ spilled oil with common bermudagrass (no burning)
- 30 g kg⁻¹ spilled oil plus 1.5 g kg⁻¹ lime with common bermudagrass (no burning)
- 30 g kg⁻¹ spilled oil with burning and common bermudagrass
- 30 g kg⁻¹ spilled oil with burning and common bermudagrass
- 30 g kg⁻¹ spilled oil with tall fescue

Soil samples from these pots were obtained 0, 50, 100, 200, and 300 days after the treatments were established. A total of 75 soil samples were generated during this original study; six soil samples (rather than 18) were generated for Day 0, as only oiled soil and oiled + burned soil were sampled. The original soil samples were stored at approximately -50 C over the past year. Due to the limited quantity of archived samples, a single soil extraction of the 75 original soil samples was performed. The extracts were then re-analyzed for residual hydrocarbon content by GC/FID and residual hydrocarbon compounds by GC/MS analysis (Roques et al. 1994) in order to assess the impact of the treatments on the restoration of oil-contaminated soil.

2.1 Sample Receipt

Samples, packed in “blue ice” were sent to the Department of Environmental Studies via an overnight carrier service. Upon arrival, each sample was logged into the laboratory, assigned a unique laboratory identification number, and placed in refrigerator/freezer storage. The sediment samples were then stored at ultrafreezer temperatures (<-45°C) to prevent any decomposition. Prior to analysis, the samples were transferred from the freezer storage to refrigeration storage (2-5°C) and allowed to defrost. Immediately prior to extraction, the samples were inspected and qualitative remarks about the sample matrix and appearance recorded in the laboratory notebook. The purpose of this inspection was to determine the level of contamination. This would insure that proper protocol would be followed. The inspection also provided a vehicle for recording information about the sample to aid in qualitative interpretation and QA/QC spot checking.

If a sample contained visible oil, it was labeled highly contaminated and extracted with cyclohexane by the high contamination procedure (i.e. “High”). If no visible oil was detected the samples were extracted by the low contamination or trace method (i.e.

“Low”). Environmental variability or patchiness in the original source soils were only assessed by internal replicate sampling.

2.2 Sediment Extraction Methods

The two extraction methods are outlined below. During sample preparation, an aliquot (1-3 g) of the sample was weighed out. Dry weight determination was then performed by the oven drying method (90°C for a 24-hour period). All solvents used in the analytical analysis were of the highest purity available.

2.2.1 High Level Contamination

For rye, shoot height and biomass showed similar trends, but root length did not. Mean shoot height and biomass was greatest in the Vaminoc inoculated seedlings and were significantly different than control (height, ANOVA $P < 0.001$; biomass ANOVA $P = 0.001$ Dunnet's method, $\alpha = 0.05$). Rye shoot height was lowest in the Smackover 1 inoculated soil (Fig. 3.3, 3.4). Shoot height ranged from 74cm (Vaminoc) to 50.6cm (Smackover 1). Shoot biomass ranged from 60.9g (Vaminoc) to 23.8g in Ranger 4 inoculum. Control seedlings had the lowest mean root length. Root length ranged from 41.2cm with Smackover 5 inoculum to 26.3cm in controls. Rye seedlings inoculated with Smackover 5 inoculum had significantly greater root length than control (Dunnet's method, $\alpha = 0.05$) (Fig. 3.5). Root biomass showed a similar trend to shoot biomass (ANOVA $P < 0.001$). Root biomass ranged from 6.2g (Smackover 2) to 32.5 (Ranger 4). Only Ranger 4 was significantly different from control (Dunnet's method, $\alpha = 0.05$) (Fig 3.6).

2.2.2 Low Level Contamination

Samples containing no visible oil were prepared in a method similar to the “High” samples but were extracted with surrogate and matrix spike standards using a more intense extraction process as typical of trace analyses. The samples were mixed thoroughly and subsampled into clean, solvent rinsed beakers. The amount of material weighed into the beakers were either 25 or 100g (wet weight), depending on sediment type. The larger sample amounts were used for coarse sand and pebble samples. Fine sediments generally only required the smaller sample size. Anhydrous sodium sulfate was added to the sediment samples and mixed until the sample matrixes had the consistency of dry sand. The surrogate standard mix composed of acenaphthene-10, phenanthrene-d10, and terphenyl-d14 was then added (typically, 500uL at 5ng/uL). DCM was added and the samples were extracted by a combination of sonication and tumbling. Initially, the sample was sonicated for 12 minutes in a sonic bath system. The first solvent extract was decanted through a funnel of anhydrous sodium sulfate into a rotary-evaporation flask. Additional solvent was added and the sonication step repeated. The second extract was combined with the first in the same rotary-evaporation flask. The sample was then transferred to a clean, solvent rinsed 500mL amber bottle. Additional solvent was added and the sample tumbled on a Lortone, Inc. rotary tumbler for 24 hours for a thorough and

complete extraction of any remaining oil related constituents. When complete, the extract was combined with the extract from the two sonication steps.

The combined solvent extracts were reduced in volume to 1mL by a combination of rotary evaporation and nitrogen “blow down” (i.e. placing the vial containing the extract under a gentle stream of nitrogen gas). The 1mL sample extracts were then split for analysis: 0.5mL archived for GC/FID analyses and 0.5mL for GC/MS analysis. To the GC/MS sample extract, 0.5mL of the internal standard mix was added. The sample extracts were now ready for GC/MS analysis.

2.3 GC/MS Instrument Configuration and Collaboration

Samples containing no visible oil were prepared in a method similar to the “High” samples but were extracted with surrogate and matrix spike standards using a more intense extraction process as typical of trace analyses. The samples were mixed thoroughly and subsampled into clean, solvent rinsed beakers. The amount of material weighed into the beakers were either 25 or 100g (wet weight), depending on sediment type. The larger sample amounts were used for coarse sand and pebble samples. Fine sediments generally only required the smaller sample size. Anhydrous sodium sulfate was added to the sediment samples and mixed until the sample matrixes had the consistency of dry sand. The surrogate standard mix composed of acenaphthene-10, phenanthrene-d10, and terphenyl-d14 was then added (typically, 500uL at 5ng/uL). DCM was added and the samples were extracted by a combination of sonication and tumbling. Initially, the sample was sonicated for 12 minutes in a sonic bath system. The first solvent extract was decanted through a funnel of anhydrous sodium sulfate into a rotary-evaporation flask. Additional solvent was added and the sonication step repeated. The second extract was combined with the first in the same rotary-evaporation flask. The sample was then transferred to a clean, solvent rinsed 500mL amber bottle. Additional solvent was added and the sample tumbled on a Lortone, Inc. rotary tumbler for 24 hours for a thorough and complete extraction of any remaining oil related constituents. When complete, the extract was combined with the extract from the two sonication steps.

The combined solvent extracts were reduced in volume to 1mL by a combination of rotary evaporation and nitrogen “blow down” (i.e. placing the vial containing the extract under a gentle stream of nitrogen gas). The 1mL sample extracts were then split for analysis: 0.5mL archived for GC/FID analyses and 0.5mL for GC/MS analysis. To the GC/MS sample extract, 0.5mL of the internal standard mix was added. The sample extracts were now ready for GC/MS analysis.

2.4 GC/MS: Data Processing and Interpretation

All collected spectral data were initially processed by HP Chemstation software using a specially written macro developed by LSU. Each macro printout contained the extracted ion chromatography data in addition to raw integration data. These data were also exported to an Excel spreadsheet for quantitative analysis and processing of specific

indexes to highlight source and weathering characteristics. The macro printouts were carefully reviewed and reintegrated as required before source fingerprinting and quantitative analysis.

2.4.1 Quantitative Analysis

The concentration of specific target aliphatic hydrocarbons was determined by an internal standard method compared to a 5-point calibration curve using authentic standards. The analytical method was amendable to the quantitative analysis of total aliphatic hydrocarbons. However, this was not proposed since aliphatic hydrocarbons easily degrade in the marine environment and are not identified as persistent or toxic constituents within petroleum. If required, total aliphatic hydrocarbons were estimated by integrating the entire m/e 85 extracted ion chromatogram. This chromatogram was then compared to an average response factor derived from a standard composed of the normal aliphatic hydrocarbons between nC-10 and nC-33 and the isoprenoids pristane and phytane. The quantitative data were also processed to estimate the percent loss of individual constituents due to weathering independent of oil or sample weight using a hopane normalization technique. In this case, each analyte was normalized relative to the concentration of hopane. The ratio of resolved vs. unresolved aliphatic hydrocarbons was also determined using the same m/e plot, but integrating only the resolved peaks and comparing the summation of the resolved peaks to the integration value of the unresolved peaks, minus the resolved value. Several selected indexes were used to aid in characterization of oil degradation, such as methyl-phenanthrene indexes.

2.4.2 Source-Fingerprinting

The data were processed and interpreted at several levels. First, a comparison was made of the extracted ion chromatographic profiles to determine if any of the samples containing oil appeared to be related. This comparison concentrated on relative composition and weathering. It included a detailed interpretation of the alkylated series's (PNAs, steranes, and triterpanes) distribution pattern. The second level of interpretation was a comparison of source- fingerprint indexes, or ratios: C-2 phenanthrene/C-3 phenanthrenes, C-2 phenanthrenes/C-2 dibenzothiophenes, C-2 dibenzothiophenes/C-3 dibenzothiophenes, and C-3 phenanthrenes/C-3 dibenzothiophene. Additional qualifiers unique to the weathered oil were incorporated into the method. In addition, indexes helpful in determining compositional changes due to degradation may be calculated (e.g. n-C17/pristane, n-C18/phytane, n-C17/n-C18, pristane/phytane).

2.5 Data Presentation and Quality Assurance/Quality Control

The data were processed and interpreted at several levels. First, a comparison was made of the extracted ion chromatographic profiles to determine if any of the samples containing oil appeared to be related. This comparison concentrated on relative composition and weathering. It included a detailed interpretation of the alkylated series's (PNAs, steranes, and triterpanes) distribution pattern. The second level of interpretation was a comparison of source- fingerprint indexes, or ratios: C-2 phenanthrene/C-3

phenanthrenes, C-2 phenanthrenes/C-2 dibenzothiophenes, C-2 dibenzothiophenes/C-3 dibenzothiophenes, and C-3 phenanthrenes/C-3 dibenzothiophene. Additional qualifiers unique to the weathered oil were incorporated into the method. In addition, indexes helpful in determining compositional changes due to degradation may be calculated (e.g. n-C17/pristane, n-C18/phytane, n-C17/n-C18, pristane/phytane).

3.0 Results

Decreasing levels of alkanes and aromatic hydrocarbons were exhibited by all treatments over the 300 day test period. Figures 1-5 graphically show that all treatments possess similar degradation patterns. Individual total alkane and aromatic hydrocarbon reduction rates are summarized in Tables 2 and 3, respectively.

3.1 Soil-Oil-Bermudagrass Treatment

The total alkane pattern for the oil-soil-bermudagrass treatment exhibited a sharp decrease in alkane levels from Day 0 to Day 50, followed by a slight decrease in alkane levels from Day 50 to Day 200. After Day 200 the alkane levels again dropped sharply to produce a 98% total alkane reduction. The total aromatic hydrocarbon pattern, like the alkane pattern, exhibited a steady decline in aromatic hydrocarbon concentration and a 99% reduction over the 300 day test period.

3.2 Soil-Oil-Tall Fescue Treatment

The total alkane pattern for the oil-soil-tall fescue treatment exhibited a sharp decrease in alkane levels from Day 0 to Day 50, followed by a steady decline in alkane levels from Day 50 to Day 300. The total aromatic hydrocarbon pattern exhibited a steady decline in aromatic hydrocarbon concentration over the 300 day test period. Both the alkane and aromatic hydrocarbon levels achieved 99% reduction rates by the conclusion of the experiment.

3.3 Soil-Oil-Lime-Bermudagrass Treatment

The total alkane pattern for the oil-soil-lime-bermudagrass treatment exhibited a 35% increase in alkane levels from Day 50 to Day 100, followed by a steady decline in alkane levels from Day 100 to Day 300. The total aromatic hydrocarbon pattern exhibited a steady decline in aromatic hydrocarbon concentration over the 300 day test period. The alkane and aromatic hydrocarbon levels were reduced by 98% and 99%, respectively. The increases in alkane levels at Day 50 suggest possible sampling error, but fluctuations in aromatic hydrocarbon levels at that time do not reinforce this hypothesis.

3.4 Soil-Oil-Burn Treatment

The total alkane pattern for the oil-soil-burn treatment exhibited a 300% increase in alkane levels from Day 50 to Day 100, followed by a steady decline in alkane levels from

Day 100 to Day 300. The total aromatic hydrocarbon pattern, like the alkane pattern, exhibited a 90% increase in aromatic hydrocarbon concentration from Day 50 to Day 100. The alkane and aromatic hydrocarbon levels were reduced by 98% and 97%, respectively. Increases in alkane and aromatic hydrocarbon levels at Day 50 suggest possible sampling error.

3.5 Soil-Oil-Burn-Bermudagrass Treatment

Both the total alkane and aromatic hydrocarbon patterns for the oil-soil-burn-bermudagrass treatment exhibited a steady decrease in levels from Day 0 to Day 300. The alkane and aromatic hydrocarbon levels were both reduced by 99%.

Description	Initial Concentration (mg/Kg)	Final Concentration (mg/Kg)	% Reduction
Soil+Oil+Bermudagrass	1854	21	99
Soil+Oil+Tall Fescue	1854	10	99
Soil+Oil+Lime+Bermudagrass	1854	40	98
Soil+Oil+Burn	1765	30	98
Soil+Oil+Burn+Bermudagrass	1765	19	99

Table 2. Total Alkane Results

Description	Initial Concentration (mg/Kg)	Final Concentration (mg/Kg)	% Reduction
Soil+Oil+Bermudagrass	78	1.0	99
Soil+Oil+Tall Fescue	78	1.0	99
Soil+Oil+Lime+Bermudagrass	78	1.0	97
Soil+Oil+Burn	75	2.0	98
Soil+Oil+Burn+Bermudagrass	75	0.6	99

Table 3. Total Aromatic Hydrocarbon Results

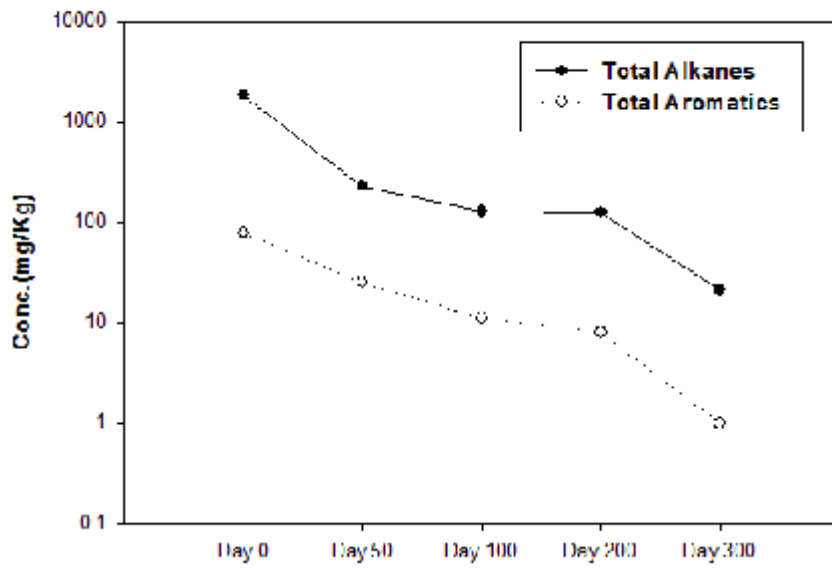


Figure 1. Soil-Oil-Bermudagrass Treatment

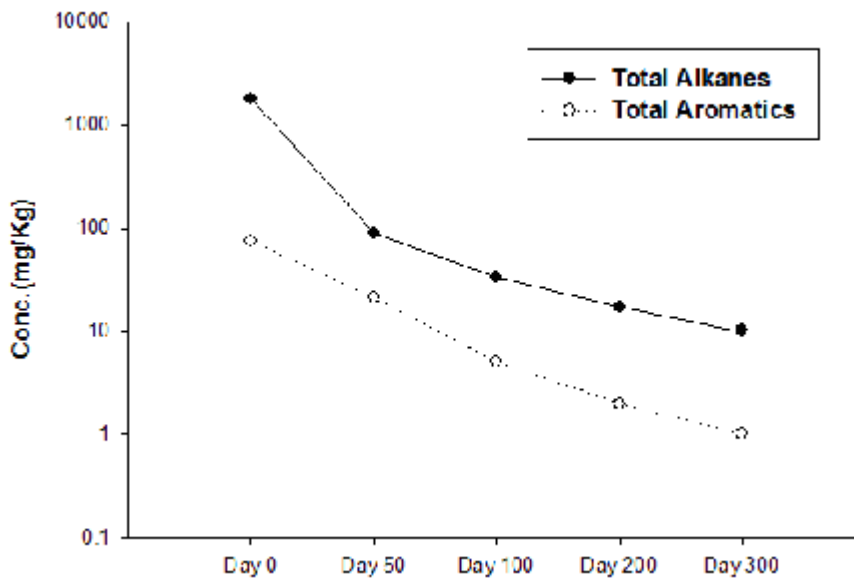


Figure 2. Soil-Oil-Tall Fescue Treatment

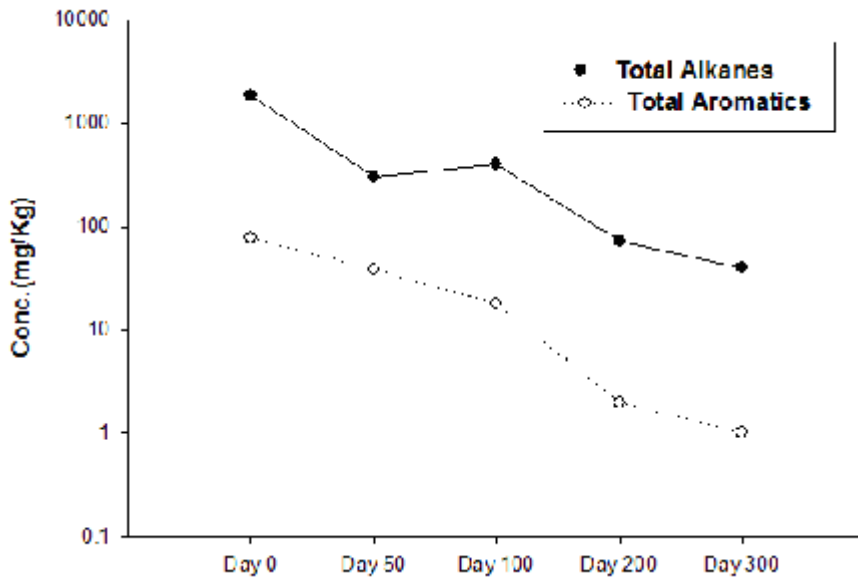


Figure 3. Soil-Oil-Lime-Bermudagrass Treatment

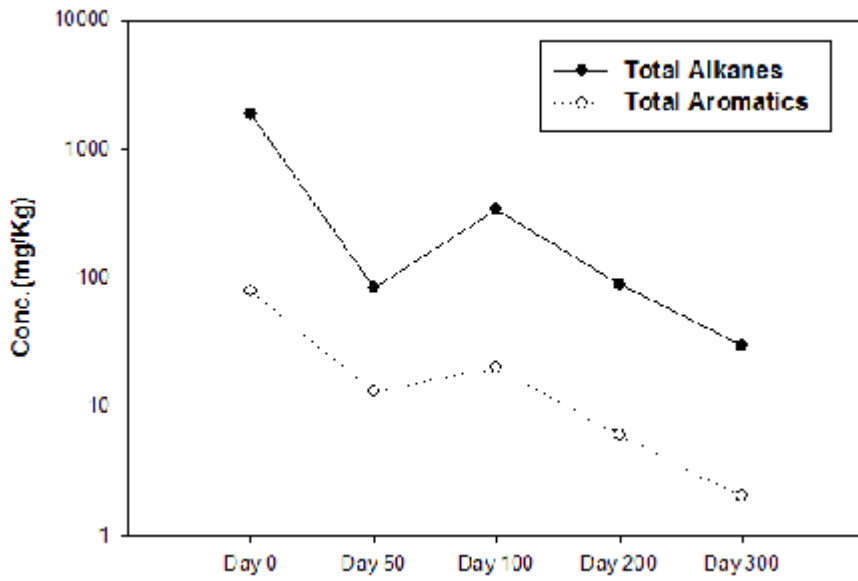


Figure 4. Soil-Oil-Burn Treatment

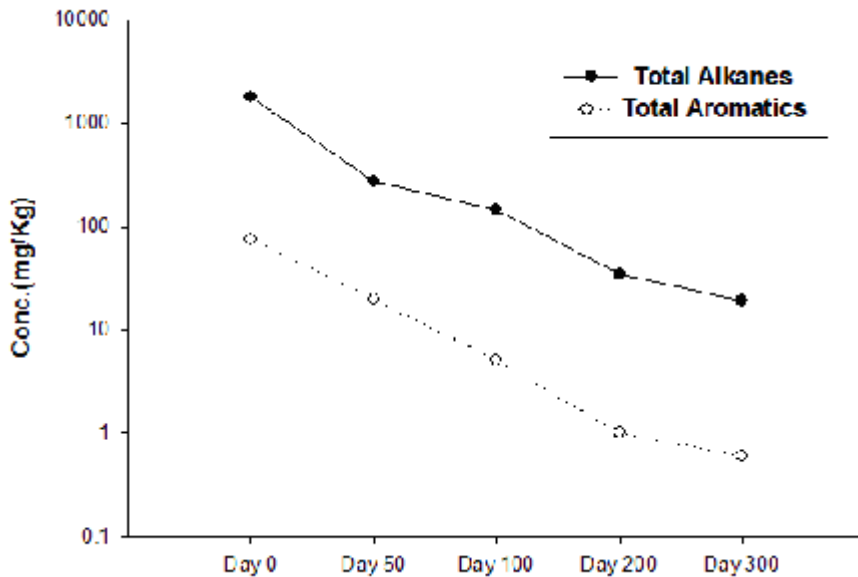


Figure 5. Soil-Oil-Burn-Bermudagrass Treatment

4.0 Conclusion

Data from this project suggest that there is no significant difference in alkane and aromatic hydrocarbon reduction between the oil burning, nonburning, and lime addition treatments. This is important information relevant to assessing the effectiveness of in-situ burning and phytoremediation for restoring upland agricultural and forested land in Louisiana contaminated by petroleum spills.

This research project is relevant to the OSRADP's objectives in that it contributes basic knowledge that can be used to develop appropriate response technology to onshore oil spills in North Louisiana. Since this project involves the application of Louisiana oil to Louisiana soil, the results are directly applicable to onshore oil spills in Louisiana. If in-situ burning and phytoremediation are successful, the results of this research will produce an efficient and cost-effective in-situ remediation technique. Research into small, onshore oil spills will have the greatest impact on the small oil producers that comprise a large part of the Louisiana oil industry. If simple and inexpensive in-situ remediation can achieve an acceptable threshold level, a defensible recommendation could be made that more costly mechanical and chemical techniques are not needed. This one recommendation alone may save small oil producers a large portion of their remediation costs.

5.0 References

Aprill, W. and R. C. Sims. 1990. Evaluation of the use of prairie grasses for stimulating polycyclic aromatic hydrocarbon treatment in soil. *Chemosphere*. 20 (1-2)253-265.

- Banks, K. 1996. Phytoremediation work in cooperation with EPA's Region 7/8 Hazardous Waste Substance Center (HSRC) and two industry partners. Workshop on Phytoremediation of Organic Contaminants, Dallas, TX Dec 18-19, 1996.
- Betts, K. 1997. TPH soil cleanup aided by ground cover. *Environ. Sci. Tech. News.* 31 (5), 214A.
- Günther, Thomas, Utz Dornberger, and Wolfgang Fritsche. 1996. Effects of ryegrass on biodegradation of hydrocarbons in soil. *Chemosphere.* 33 (2)203-215.
- Henry, Charles, Edward Overton. 1993. Source-fingerprinting and compound specific quantitative analysis of oil contaminated soils and sediments. Louisiana State University, Institute for Environmental Studies, Report Number: IES93-01
- Klokk, Terje. 1984. Effects of oil pollution on the germination and vegetative growth of five species of vascular plant. *Oil Petrochem. Pollut.* 2(1):25-30.
- Lee, E. and M.K. Banks. 1993. Bioremediation of petroleum contaminated soil using vegetation: microbial study. *J. Environ. Sci. Health. Part A, Environmental Science and Engineering.* 28(10):2187-2198.
- Rock, Steven. 1996. Phytoremediation of organic compounds: Mechanisms of action and target contaminants. Workshop on Phytoremediation of Organic Contaminants, Dallas, TX, December 18-19, 1996.
- Roques, D.E., E.B. Overton, and C.B. Henry. 1994. Using gas chromatography/mass spectroscopy fingerprint analysis to document process and progress of oil degradation. *J. Environ. Qual.* 23:851-855.
- Wiltse, C.C., W.L. Rooney, Z. Chen, A.P. Schwab, and M.K. Banks. 1998. Greenhouse evaluation of agronomic and crude oil-Phytoremediation potential among alfalfa genotypes. *J. Environ. Qual.* 27:169-173.