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Hydrocarbon Degradation in Louisiana's Salt and Fresh Water Marshes: Inherent Potential, Enhancement, And In-Situ Monitoring Techniques

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**HYDROCARBON DEGRADATION IN LOUISIANA'S SALT AND FRESH
WATER MARSHES: INHERENT POTENTIAL, ENHANCEMENT, AND IN-SITU
MONITORING TECHNIQUES**

A Dissertation

**Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy**

in

The Department of Engineering

by

William A. Jackson

B.S., Rhodes College, 1990

M.S., Louisiana State University, 1993

August 1996

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TABLE OF CONTENTS

ACKNOWLEDGMENTS	ii
LIST OF TABLES	v
LIST OF FIGURES	vi
ABSTRACT	viii
CHAPTER 1. INTRODUCTION	1
CHAPTER 2. INTRINSIC CRUDE OIL DEGRADATION IN WETLANDS AND POTENTIAL FOR ENGINEERED ENHANCED BIODEGRADATION: A REVIEW	6
PHYSICAL AND CHEMICAL FACTORS OF CRUDE OIL AFFECTING ITS BIODEGRADATION	7
Crude Oil Chemical Composition	7
Physical State and Availability	8
ENVIRONMENTAL CONDITIONS CONTROLLING DEGRADATION	9
Temperature	9
Hydrogen Activity	10
Salinity	11
Microbial Populations	11
Nutrient Availability	12
Oxygen Availability	15
MONITORING DEGRADATION OF CRUDE OIL	16
CONCLUSIONS AND IMPLICATIONS	18
CHAPTER 3. THE ROLE OF NUTRIENT ADDITIONS ON CRUDE OIL DEGRADATION IN LOUISIANA SALT MARSHES	20
INTRODUCTION	20
EXPERIMENTAL	22
Laboratory Microcosm Studies	23
Mesocosm Study.	24
Radiorespirometry Studies	25
RESULTS	26
Nutrient Affects on Alkane and PAH Degradation.	26
Radiorespirometry Study.	30
Mesocosm Study.	34
CONCLUSIONS	36

CHAPTER 4. SEASONAL VARIABILITY OF CRUDE OIL RESPIRATION POTENTIAL IN SALT AND FRESH MARSHES	39
INTRODUCTION	39
EXPERIMENTAL	41
Crude Oil Respiration.	41
Site description.	41
Laboratory study.	42
Monitoring Environmental Parameters.	43
RESULTS	43
Fresh Marsh.	43
Salt Marsh	48
DISCUSSION	56
CHAPTER 5. MONITORING CRUDE OIL MINERALIZATION IN SALT MARSHES: USE OF STABLE CARBON ISOTOPE RATIOS. . .	61
INTRODUCTION	61
EXPERIMENTAL	63
Laboratory Microcosm Studies.	63
Field Studies.	64
Analyses.	65
Theoretical.	65
RESULTS AND DISCUSSION	67
Kinetic Experiments.	67
Field Experiment.	72
CONCLUSIONS	79
CHAPTER 6. CONCLUSIONS	81
LITERATURE CITED	85
APPENDEX A	91
APPENDEX B	93
VITA	95

LIST OF TABLES

3.1	Pseudo first-order rate constants of alkane and PAH degradation.	29
3.2	Rates of crude oil mineralization in control, NH_4^+ and NO_3^- amended treatments.. . . .	33
4.1	Seasonal changes in selected environmental conditions in a fresh marsh..	50
4.2	Seasonal changes in selected environmental conditions in a salt marsh... .	57
4.3	Change in mineralization rate under fertilized conditions (R_f) compared to inherent nutrient conditions (R_i). (R_f/R_i)...	59
5.1	Maximum $\delta^{13}\text{C}$ signatures and corresponding total CO_2 production rates measured during the 7 week field study...	73
A.1	Compounds and associated monitored ion retention time.	92

LIST OF FIGURES

1.1	Extent of fresh and salt water coastal marshes in Louisiana	2
3.1	Alkane degradation (C15-C44) as indicated by reduction in hopane ratios: (A) Inhibited Control; (B) Control; (C) Phosphate enriched; (D) Nitrogen enriched; and (E) nitrogen and Phosphate enriched	27
3.2	PAH degradation (phenanthrene,C1-C3; naphthalene, C1-C3) as indicated by reduction in hopane ratios: (A) Inhibited Control; (B) Control; (C) Phosphate enriched; (D) Nitrogen enriched; and (E) nitrogen and Phosphate enriched	28
3.3	Mineralization of crude oil in salt marsh slurries, (A) with NH_4 and (B) NO_3 enhancements	31
3.4	Percent reductions of hopane ratios in reference to a weathered control in large cores after 4 weeks: (A) alkane fraction, (B) PAH fraction	35
4.1	Mineralization curves of hexadecane and phenanthrene in fresh marsh slurries under unamended conditions for May 1995 through March 1996	45
4.2	Mineralization curves of hexadecane and phenanthrene in fresh marsh slurries under fertilized conditions for May 1995 through March 1996	46
4.3	(A) Fresh marsh mineralization rates of phenanthrene and hexadecane. (B) Lag time for phenanthrene and hexadecane	47
4.4	Nutrient enhanced (A) Fresh marsh mineralization rates of phenanthrene and hexadecane. (B) Lag times for phenanthrene and hexadecane	49
4.5	Mineralization curves of hexadecane and phenanthrene in salt marsh slurries under unamended conditions for May 1995 through March 1996	52
4.6	Mineralization curves of hexadecane and phenanthrene in salt marsh slurries under fertilized conditions for May 1995 through March 1996	53

4.7	Salt marsh nutrient unenhanced (A) mineralization rates for hexadecane and phenanthrene (B) Lag time for phenanthrene and hexadecane	54
4.8	Salt marsh nutrient enhanced (A) mineralization rates for hexadecane and phenanthrene (B) Lag time for phenanthrene and hexadecane	55
5.1	(A) Total (C15-C44) alkane hopane ratio versus time in aerated microcosms. (B) Total PAH (phenanthrene, C1-C3; naphthalene, C1-C3) hopane ratio versus time	68
5.2	δ - ¹³ C signatures (0/00) of CO ₂ versus time in aerated microcosms	70
5.3	Cumulative CO ₂ -C production (mg/g soil) from crude oil versus time in aerated microcosms (calculated from isotope dilution equations)	71
5.4	Comparison of final hopane ratios of alkanes and pristane in fertilized and unfertilized salt marsh soils after 7 weeks	74
5.5	Comparison of final hopane ratios of PAHs in fertilized and unfertilized salt marsh soils after 7 weeks	75
5.6	Rates of CO ₂ -C (mg/hour-m ²) mineralized from crude oil in fertilized and unfertilized salt marsh soils (calculated from isotope dilution equations)	77

ABSTRACT

The ability to effectively remediate crude oil from marsh systems is important due to the coexistence of the economically important hydrocarbon industry and ecologically and economically valuable marshes. Laboratory and field studies were initiated to examine the intrinsic ability of coastal marshes to biodegrade crude oil, to determine the ability to enhance degradation using nutrient additions, and develop a superior monitoring technique. The alkane and poly aromatic hydrocarbon (PAH) fractions seem to be independently degraded and these systems appear to have much greater capacity to degrade PAHs than alkanes. Nitrogen was found to be a limiting factor for both alkane and PAH fractions, although the PAH fraction in this crude oil was completely degradable with or without nitrogen enhancements. Phosphate had only a minimal beneficial effect on alkane transformation rates and none for PAH transformation. Seasonal variations were found in both marsh systems although they were greater in the fresh marsh. Seasonal trends in mineralization rates were different for phenanthrene and hexadecane as well as for each marsh. Removing nutrient limitations greatly increased the rate of hexadecane mineralization for most months in both marshes and significantly reduced the lag time to mineralization. Phenanthrene mineralization increased for specific months in the salt marsh and decreased or remained the same in the fresh marsh. Limited correlation was found between environmental conditions and crude oil respiration potential. In laboratory microcosms using salt marsh soils and in field trials it was possible to monitor and quantify crude

oil mineralization by measuring changes in CO₂ δ¹³C signatures and the rate of CO₂ production. These values are easy to obtain and can be combined with simple isotope mass balance equations to determine the rate of mineralization from both the crude oil and indigenous carbon pool. Hydrocarbon degradation was confirmed by simultaneous decreases in alkane-, isoprenoid-, and PAH-hopane ratios. This procedure appears to offer a means of definitively quantifying crude oil mineralization in a sensitive, inexpensive and simple manner in environments with appropriate background δ¹³C signatures.

CHAPTER 1. INTRODUCTION

Petroleum hydrocarbons are an integral and substantial part of our society and the world wide economy. Hydrocarbons are used in almost every aspect of our lives. However, the mix of chemicals which compose "crude oil" and make it suitable as both an energy source and production material can also have serious ecological and health effects if mishandled. Louisiana enjoys approximately 15% of US crude oil production. Numerous major oil refineries and other petrochemical industries are located along both the state's longitudinal (Mississippi river) and latitudinal axis (Inter Coastal Waterway). This necessitates shipping of millions of barrels of crude oil from numerous sources including: 1000's of miles of pipeline (Loop pipeline), barges, and rail cars. Much of this production, shipping, and processing is located in or adjacent to Louisiana's coastal marsh ecosystems. All known means of transport are susceptible to large discharges and there are numerous past discharge events from these activities. Superimposed amongst this oil rich area are 40% of the United States coastal wetlands. The value of these wetlands are immense both ecologically and economically.

Louisiana's coastal marshes can be classified into three main types (Figure 1.1): freshwater, brackish, and salt marshes which are situated on a salinity gradient toward the coast (Mitsch and Gosselink, 1986). All three share the common factor of nearly year round surface saturation; however, they differ greatly in their vegetation, hydrology, and basic controlling biogeochemical processes. Therefore, the response of each of these systems to crude oil discharge may vary greatly. Recovery of oil-impacted

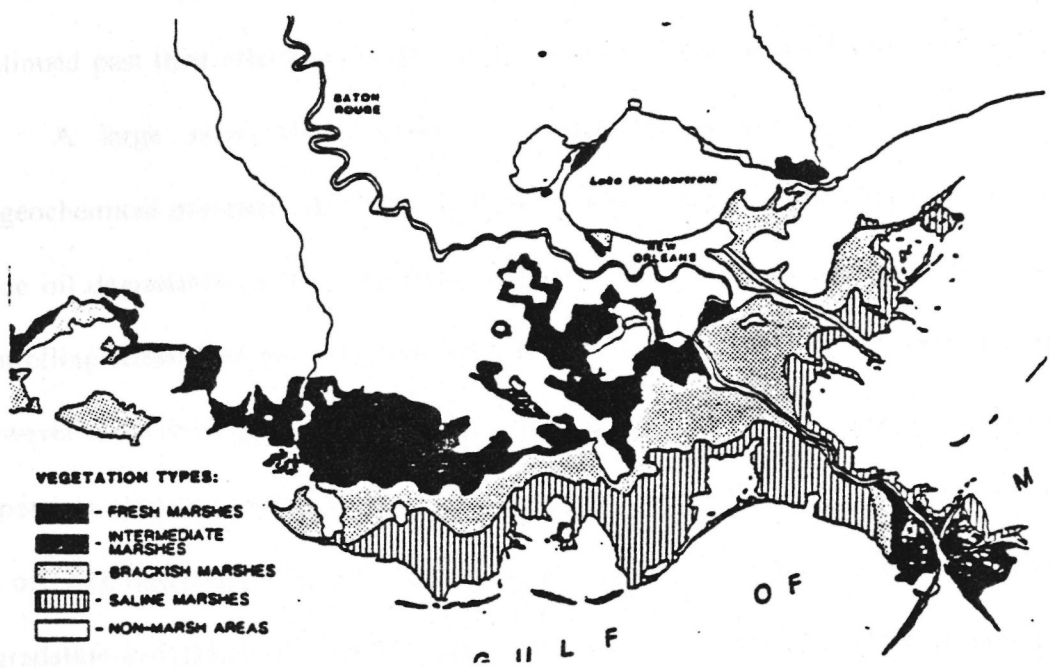


Figure 1.1 Extent of freshwater and saltwater marshes in lower Louisiana.

marshes is a complex series of processes including volatilization, dispersion and biodegradation. Microbial degradation of crude oil is particularly important, since these systems are not amenable to most traditional forms of physical remediation (e.g. sediment stripping) due to their sensitive nature.

The ability to monitor actual mineralization of hydrocarbons is of prime importance whether in a laboratory, field study or an actual remediation project. Hydrocarbons can be sorbed, buried, transformed, or otherwise be undetectable but still pose threats to the system ecology. In addition, it is vital to be able to manage remediation strategies in order to insure that remediation efforts are not wasted nor continued past their effective period, which in themselves can harm the ecosystem.

A large store of information is available on the basic ecology, and biogeochemical processes occurring in these marsh systems. In addition the study of crude oil degradation is well advanced and there are numerous studies on the basic controlling features of both physical and biological aspects of crude oil remediation.

However there is very little information available on the ability of coastal marshes to respond to crude oil releases nor on the controlling factors limiting biodegradation of the oil. The objective of this dissertation is to quantify the potential for hydrocarbon degradation in Louisiana's coastal marshes. The dissertation is divided into 4 sections: A) a review of the current understanding of crude oil biodegradation and current monitoring techniques (Chapter 2), B) the inherent and engineered potential for crude oil biodegradation in Louisiana's coastal marshes (Chapter 3 and 4), C) the use of stable

carbon isotopes to monitor crude oil mineralization (Chapters 5), and D) the conclusions and recommendations for future research (Chapter 6).

Studies were conducted to compare and contrast two types of marshes, freshwater and salt marsh. These two marshes are representative of the two extreme ends of the salinity gradient. These marshes differ in soil type and properties, vegetation and hydrology.

The freshwater marsh site is located in St John the Baptist Parish, Louisiana on the fringe of Lake Des Allemands. The water depth in the marsh ranges from 0-10 cm and is mainly controlled by rainfall events, no tidal fluctuations occur. The soil is highly organic (>90%) and contains large quantities of detrital material from the dominant plant species *Panicum hemitomon* (maiden cane). Numerous other studies in the same area have been performed and most basic biogeochemical information is available including but not inclusive of carbon budgets (Feijtel et al., 1985), sedimentation and accretion (Hatton et al., 1983), nitrogen cycling (Delaune et al., 1986), and carbon dioxide emission (Smith et al. 1983).

The salt marsh site is located at the southern end of the Barataria basin in Louisiana. The water depth is ranges from 2-30 cm under the influence of a small tidal cycle and can be affected by synoptic weather events pushing water in or out of the marsh from or to the gulf respectively. The marsh is characterized by almost homogenous stands of *Spartina alterniflora*. This type marsh is also well characterized and a summary of the biogeochemical processes which occur has been written by Delaune and Patrick, (1979).

Crude oil is a complex mixture of chemicals representing hundreds of classes of chemicals. Crude oils are highly variable depending on their source although they all contain some basic compounds in varying concentrations (e.g. normal alkanes (C1-C40), branched and cyclo-alkanes, isoprenoids, aromatics, and polars. Physical properties (e.g. solubility) of these compounds vary over several orders of magnitude. Biodegradation rates vary as well from the facile alkanes to the recalcitrant isoprenoids and higher molecular weight PAHs. The oil used in this study was a Louisiana "Sweet" crude. This oil is a relatively non-toxic oil with high alkane, moderate PAH and minimal polar concentrations.

Currently little is known concerning the biodegradation of crude oil in coastal marshes and the effects of their unique properties on intrinsic and engineered crude oil biodegradation. These studies will emphasize the intrinsic biodegradation potential, the effectiveness of engineered enhancement, and the ability to accurately monitor degradation in coastal marshes.

The following dissertation is arranged in journal format. Chapter 2 will be submitted to *Wetlands*. Chapter 3 is currently submitted to *Journal of Environmental Quality* and chapter 4 will be submitted soon. Chapter 5 is currently published in *Environmental Science and Technology*.

CHAPTER 2. INTRINSIC CRUDE OIL DEGRADATION IN WETLANDS AND POTENTIAL FOR ENGINEERED ENHANCED BIODEGRADATION: A REVIEW

Recent catastrophic oil spills (e.g. Valdez) have served to increase both public awareness and research into the problems and solutions to crude oil contamination of the environment. Crude oil releases occur daily, albeit on a much smaller scale than the Exxon Valdez, but still pose acute affects to impacted ecosystems. The number of types of ecosystems that can be affected are immense but in general most research has centered on open water, unsaturated soil, or beaches (Leahy and Colwell, 1990). Coastal wetlands/marshes, comprise an important albeit small ecosystem nationwide and are extremely exposed to the risk of crude oil releases. This is due to both their proximity to ocean going oil transportation; and also in areas such as Louisiana, enormous production and processing operations are located literally within these ecosystems. Numerous methods both physical, chemical and biological are simultaneously employed to remediate most other contaminated systems. However, due to the sensitivity, inaccessibility, and intrinsic nature of these systems, biodegradation is the prime method for maximum benefit with a minimum of harm to the ecosystem. Unfortunately, very little work has been conducted on these systems ability to degrade crude oil and even less on possible controlling features which might be relieved in order to enhance degradation. Enormous amounts of research have been conducted on both the intrinsic ability and the ability to stimulate other systems to biodegrade oil. However, adapting this work directly to coastal marshes is highly dubious given the completely different biogeochemical process controlling each system.

The intent of this review is to try to integrate the large amount of information available concerning crude oil degradation with the known biogeochemical processes dominating and defining coastal marsh ecosystems. Specifically, this review hopes to examine: the information now available on intrinsic degradation of hydrocarbons in coastal marshes and the possible effectiveness of current strategies used elsewhere to promote degradation. Applications of recent advances in monitoring techniques will also be addressed.

PHYSICAL AND CHEMICAL FACTORS OF CRUDE OIL AFFECTING ITS BIODEGRADATION

Crude Oil Chemical Composition

One of the most important determinants of crude oil degradation potential is the composition of crude oil. Crude oils are composed of tens of thousands of different compounds. They are generally grouped into four main categories saturates, aromatics, asphaltenes, and resins (Leahy and Colwell, 1990). The order of recalcitrance seems to be dependent on the ecosystem in question. Most researchers have found that the n-alkanes are most easily degraded followed by either branched alkanes or low molecular weight aromatics (Perry, 1984). However, depending on the specific ecosystem in question these three categories can be interchangeable (Cooney et al., 1985, and Fedorak et al., 1981). Both high molecular weight aromatics (PAHs) and polar compounds have extremely low degradation rates in most ecosystems studied. The degradation potential depends on the distribution of the components within the classes, not only due to rates of degradation but also toxicity. Crude oils with high

concentrations of polars can have an inhibitory effect on the degradation of even labile compounds (Leahy and Colwell, 1990).

Physical State and Availability

The physical state in which oil exists in the environment will also affect the degradation potential. The physical distribution of crude oil is important since it can serve as a barrier to oxygen and nutrient diffusion as well as trapping toxic intermediates. Given the low solubilities of almost all crude oil components, surface area becomes a critical factor in degradation potential. Some lower molecular weight alkanes and aromatics may approximate typical substrate dependent degradation rates and other sparingly soluble compounds are dependent more on their water solubility. However, the vast majority of compounds are extremely hydrophobic and available surface area is the controlling parameter (Thomas et al., 1986).

Crude oil availability in coastal marshes will likely be high as compared to other coastal environments (i.e. beaches) due to the large amounts of surface area available due to by litter and live plant coverage. In addition more of the oil will likely be in the sorbed state than other coastal systems due to the high concentrations of organic matter of the marshes (Pardue et al., 1992). This sorption will decrease the concentrations of toxic components but will also reduce the soluble concentration available for degradation. Moreover, desorption rates may be slower and thus increase the persistence of compounds in the environment. Competing with the sorptive process of particulate matter is partitioning of the crude oil components into the dissolved organic pool (DOC) pool. Coastal marshes have one of the highest concentrations of DOC of any

ecosystem (Thurman, 1986). These high concentrations of DOC could decrease the sorption of compounds onto particulate organic matter and increase the water soluble portion. The exact relationship will depend on both the concentration of DOC and the nature of the particulate organic matter. The effect of DOC on biodegradation is unknown. While increased soluble concentrations of hydrocarbons should increase degradation rates, the degradation potential will be less in the water column than the sediment. It is also not known how available complexed DOC-oil components are for microbial uptake and degradation.

Other factors which can serve to increase crude oil availability are dispersants and naturally produced biosurfactants. Bacteria and fungi are able to produce biosurfactants or emulsifying factors which effectively increase the concentrations of compounds available for degradation (Broderick and Cooney, 1982; and Reddy et al., 1983). However, artificial dispersants have been less successful in aiding biodegradation. Dispersants are effective means of dilution but many formulations have been found to inhibit microbial activity and although greater success has been recently achieved the role of the compounds is as yet uncertain in biodegradation (Foght and Westlake, 1982 and Atlas 199).

ENVIRONMENTAL CONDITIONS CONTROLLING DEGRADATION

Other major environmental conditions which have been shown to have significant influences on oil degradation are temperature, salinity, pH, microbial activity, Eh, and nutrient availability.

Temperature

Degradation rates generally increase with increasing temperature as with most biological activity. In the laboratory, the maximum is between 30 and 40 °C although in regard to *in situ* studies the optimal temperature may be the temperature at which the specific ecosystem is adapted (Cooney et al., 1985). Researchers have found both thermophilic and mesophilic populations for which maximum rates are higher at temperatures much higher and lower than the normal maximum activity range (Leahy and Cowell, 1990). In coastal marshes, which are primarily along the Gulf coast, temperature effects will be muted and may play only a minor role due to the subtropical climate and warm temperatures of the gulf water.

Hydrogen Activity

Conflicting information has been reported on pH effects. Soils or sediments with extreme pHs have been found to have lower degradation rates but systems with intermediate pH (5-9) have all shown different optimal pHs (Dibble and Bartha, 1979; and Verstraete et al., 1976). In addition, pH effects can be class specific with rate increases in some classes followed by decreases in other. Hambrick et al. (1980) found that salt marshes, where the pH range is normally 7-7.8 in surface sediments, exhibited class specific effects to pH. Octadecane mineralization rates were highest at pH 8 while naphthalene mineralization rates were highest at pH 6.5. Subsurface pH in these sediments can be as low as 5 due to the production of organic acids and dissolution of pyrite. Mineralization was reduced at this pH for both naphthalene and octadecane.

Salinity

Salinity is an important parameter since most coastal marshes contain a gradient of salinity. The salinity will be determined by their proximity to a saline source (gulf or ocean), and the rate of fresh water intrusion. While most studies find increasing salinity decreasing degradation rates, it is unclear whether systems which are normally saline are not as active as fresh systems. Studies involving salinity gradients have generally found that rates are higher in less saline waters. (Spain et al., 1983; Ward and Brock, 1978; Bourquin and Przbyszewski, 1977) However, this trend may be class specific and dependent on the extent of the salinity flux. In two separate studies Shiaris found that some PAHs had higher mineralization rates in more saline waters (Shiaris, 1989 a,b). It is clear is that the environmental conditions which produce optimum degradation rates are dependent on the environmental conditions of the specific ecosystem in question. Changing parameters will cause shifts in population dominance which may or may not be followed by changes in degradation rates.

Microbial Populations

A large portion of the microbial community is able to degrade some oil constituents including yeasts, fungi, algae, and protozoa. (Leahy and Cowell, 1990) However, by far the most important classes of microorganisms are bacteria and fungi. Numerous genera of bacteria are able to degrade oil. However, mixed populations are seen as essential to effective oil degradation (Bossert and Bartha, 1984; and Cooney et al., 1984; and Atlas, 1981). The enormous number of compounds and degradation products which coexist demand a varied population if simultaneous degradation of

multi-class compounds are to degrade effectively. Prior exposure to oil has been seen as an important determinant of initial degradation rates (Bartha and Bossert, 1984). Most ecosystems tested, however, contain indigenous populations of oil degraders that can adapt fairly rapidly to match rates of preexposed populations (Leahy and Cowell, 1990). This may be one of several reasons why field applications of enhanced or acclimated cultures have not been successful in increasing oil degradation (Venosa et al., 1991; and Berkley et al., 1991). Naturally occurring populations seem to be better adapted for their specific environment and able to out compete or compete as efficiently as introduced organisms. Other field studies have also failed to show a correlation between hydrocarbon degradation potential and population of hydrocarbon utilizers (Roubal and Atlas, 1978 and Molina et al., 1995).

Nutrient Availability

Temperature, pH and salinity are important controlling features of crude oil degradation. However, these parameters are difficult to engineer *in situ*. The relationship of crude oil degradation rates to the oxygen and nutrient status of coastal marshes is perhaps of more practical interest from a remediation standpoint. Not only are oxygen and nutrients the controlling features of degradation in most environments, but also they are the two most likely candidates for engineering.

Nutrient (N and P) concentrations are critical for degradation of organic carbon sources. Coastal marshes have high concentrations of both of these components, although most of it is unavailable. Coastal marshes are extremely efficient at internal cycling of nutrients and have large pools of these nutrients trapped in unmineralized

organic forms (Smith et al., 1983). Nitrogen fixation will supply additional nitrogen to these systems and is considered a primary nitrogen source for both fresh and salt marshes (Delaune et al., 1986). However, in general these systems are still nitrogen limited and any externally provided N will quickly be taken up by plants. Natural nitrogen cycling (nitrification-denitrification) increases the competition for available nitrogen. The demand for nutrients, especially nitrogen, will exhibit seasonal fluctuations complicating the understanding of nitrogen availability. No information is available concerning the ability of coastal marshes to supply adequate nutrients to support crude oil degradation. Oil degrading consortia will compete for available nutrients with both plant life and other microbial populations, but no information is currently available on the dynamics of these processes.

Nutrient addition has been a prime candidate for increasing hydrocarbon degradation. Numerous studies have shown that readily available nitrogen and phosphorous sources are necessary for degradation to be effective (Atlas and Bartha, 1973; Bragg et al., 1993; Tabak et al., 1991). The Valdez oil spill spurred an enormous research effort in oil remediation (e.g. Prince, 1992). Many of these studies investigated nutrient addition as a means of accelerating oil degradation. It was found that nutrient addition could increase rates of biodegradation; although, there was much controversy over the form in which nutrients should be applied. Recently, it has been convincingly shown that a critical level of nitrogen was needed in the pore water to detect changes in biodegradation rates. Both Bragg et al., (1994) and Venosa et al., (1995) statistically showed an increase in *in situ* rates of oil degradation in which the pore water

concentration of N was significantly increased. However, these studies as well as the vast majority of research conducted on oil biodegradation were conducted on ecosystems with little or no similarity to coastal marshes. In general, studies at the Valdez and other "beach" sites have found that oleophilic nutrient additions were the most successful in maintaining nutrient concentrations since washout is the primary removal mechanism of nutrients (Atlas and Bartha, 1973; Bragg et al., 1993; and Halmo, 1985). A few studies found that there was a compound specific response with certain classes of compounds being more affected by inorganic than oleophilic additions (Tabak et al., 1991). Much work was also conducted on the release rates of multiple types of nutrient enhancing materials (Glaser et al., 1991 and Safferman, 1991). They found that inorganic additions were best able to raise nutrient levels but least able to maintain them in these systems.

Unfortunately, this work is difficult to apply to marshes. Most work to date has been conducted on cold water, high physical energy, nutrient poor beaches as opposed to highly organic, temperate, oxygen starved, biologically rich sediments. The little work that has been conducted on similar marsh systems (mainly Canada) found very conflicting results. Inorganic not oleophilic nutrient products were found to be more effective in low physical energy systems (marshes) (Lee and Levy, 1989 and 1991). These systems are better able to maintain nutrient concentrations in pore water, due to the limited exchange rate of pore water as well as the high adsorption capacity of the organic soils. Further, there was some evidence of microbial preference to the oleophilic fertilizer over oil as an energy source (Lee and Levy, 1989). Other

researchers have indicated that in these systems it is probable that oxygen, not nutrient conditions, limits degradation of oil (Swannell and Head, 1994; and Lee and Levy, 1991). Addition of nutrients to systems which are oxygen limited will exacerbate the problem due to increased heterotrophic activity or even by the consumption of the nutrient addition as an energy source.

Oxygen Availability

In general, coastal marshes are dominated by anaerobic processes; methanogenesis in freshwater wetlands and sulfate reduction in brackish and saline marshes. Aerobic heterotrophic activity is concentrated at the sediment-water interface in a small (several mm) aerobic layer. The high oxygen demand exerted by decaying vegetation in wetland soils can be met only at a relatively slow rate due to the slow diffusion of O_2 through the water. This results in the formation of a thin aerobic layer at the surface. In oil-impacted wetlands, petroleum components provide an additional overwhelming oxygen demand, in addition to serving as an added barrier to oxygen diffusion. The maintenance of the aerobic layer is critical to microbial degradation of petroleum hydrocarbons since most constituents of crude oil degrade much faster in the presence of molecular oxygen. Due to the high subsidence and burial rates of many coastal marshes there will be a small time period compared to other systems in which crude oil is naturally exposed to molecular oxygen. Accretion rates in this marsh are as high as $0.76 \text{ cm year}^{-1}$ (Smith et al). Oil components which are not degraded before significant burial occurs may become effectively nondegradable unless timely remediation efforts are conducted.

Most components of crude oil have been found to degrade under oxidized conditions and certain components under reduced conditions (Milhelic and Luthy, 1988 a,b; and Leahy and Colwell, 1990). However in general crude oil degradation under reduced (anaerobic) conditions is considered minor and normally not significant to overall reduction of crude oil concentration (Cooney, 1984). Hambrick et al. (1980) found that neither octadecane nor naphthalene were significantly mineralized in highly reduced salt marsh sediments. However, it is probable that many components do transform at environmentally significant rates under anaerobic conditions. While these rates may be too slow to significantly affect free phase oil concentrations, it may be a significant process for low level contamination surrounding the primary spill site.

MONITORING DEGRADATION OF CRUDE OIL

Monitoring crude oil degradation is a complicated process whether in tightly controlled laboratory studies or *in situ*. However, the ability to accurately and, with a high degree of sensitivity, monitor degradation is critical. The difficulty in monitoring crude oil comes from its heterogeneous composition. A large component of the crude oil is volatile or semi-volatile, other components have very low solubilities and thus sorb to other matter. Crude oil can act as a solvent for various individual components, limiting water phase concentrations, and homogeneous mixtures are difficult to achieve since any study mimicking a crude oil release loading rate would have to consist of a free phase and soluble layer. Numerous direct and indirect techniques have been used to follow crude oil degradation for laboratory studies. Indirect techniques include mass residue, chemical oxygen demand (COD), CO₂ production, and oxygen uptake. Direct

techniques include radiorespirometry, and extraction of oil residue with some type of component analyses (e.g. GC-FID or MS). Indirect techniques suffer from the inability to assure that changes in a parameter are attributable to crude oil degradation. Direct methods are superior in that in closed systems for nonvolatile compounds direct losses can be monitored. However, these techniques do not supply information on the extent of component degradation, only transformation of parent compounds to an intermediate, thus no overall mineralization rate can be obtained. Radiorespirometry can supply mineralization and transformation data, however only a few compounds at a time can be monitored and thus large inferences are necessary from a model compound to a class of compounds. All of these techniques are dubiously applied *in situ*.

In situ studies are complicated by physical losses or gains of crude oil from outside the study area as well as extensive volatilization. Relative changes in specific components can not be independently determined to be from degradation. Thus, statistical differences in areas, treatments or other variables are difficult at best. In addition, due to the low degradation rate *in situ* and general noise prevalent in any field data, large time scales must be chosen to see significant differences in studies. Recently, a new technique has solved many of these problems. Degradable oil components are monitored in relation to an undegradable component with similar characteristics. Physical gains or losses of crude oil will not affect this relative ratio while degradation will. Bragg et al. (1994) successfully used this technique *in situ* to concisely show degradation of crude oil following the Exxon Valdez spill. This technique has solved many of the deficiencies in monitoring field studies, however a

number of inadequacies still exist such as: the inability to monitor mineralization not transformation, the realization that only resolved compounds are monitored, the large differences in time needed to see differences in hopane ratios, and the high cost of extraction and GC-MS analyses.

CONCLUSIONS AND IMPLICATIONS

Coastal marshes are at high risk of being exposed to crude oil releases resulting in potential ecological as well as economic damage. It is essential that the intrinsic ability as well as potential for engineered enhancement be characterized in order to mitigate the effects of a crude oil release. Coastal marshes possess unique characteristics which could potentially both enhance or inhibit crude oil degradation. Enhancing properties include the high rate of biological activity, high internal cycling of nutrients, and large surface areas available for degradation to take place. Other features, such as high accretion rates, low oxygen availability, and high sorption rates may inhibit crude oil degradation. Little information is available on the response of marshes to crude oil releases or what features are critical determinants of degradation and which features may be amenable to engineering.

Nutrient availability is a prime candidate for limiting degradation potential. Studies conducted on dissimilar systems have shown this to be a major predictor of degradation potential. However, coastal marshes have such unique properties, in relation to other systems studied, that inferences from these systems are not possible. At present the inherent potential of marshes to degrade crude oil is relatively unknown.

More studies are needed using these systems are needed in order to elucidate the role of marshes in responding to crude oil releases. Cycling of crude oil degradation in response to seasonal changes will be important as both an indicator of controlling features as well as a predictor of engineering strategies to relieve limitations imposed on crude oil degradation. Studies to determine the applicability of engineering strategies used in other systems are beneficial and contribute to the ability of agencies to respond efficiently to releases. Understanding the ability of coastal marshes to degrade crude oil will not only help to recover contaminated marshes more rapidly but may also increase our understanding of how or why other systems respond to crude oil contamination.

CHAPTER 3. THE ROLE OF NUTRIENT ADDITIONS ON CRUDE OIL DEGRADATION IN LOUISIANA SALT MARSHES

INTRODUCTION

Sensitive salt marsh ecosystems are susceptible to impact from spilled and discharged oils. Understanding the response and tolerance of these systems is critical for the design of remediation strategies. Salt marshes differ from other coastal environments (e.g., beaches) since mechanical cleaning is usually not possible due to the sensitivity of these systems. Natural recovery of oil impacted marshes is a complex series of processes including volatilization, dispersion, and biodegradation. Microbial degradation of oil components is particularly important for the recovery of these sensitive systems since it can be engineered and actually removes the compounds from the ecosystem instead of transferring them to another phase with no reduction in toxicity or mass.

The rate and extent of microbial degradation of petroleum hydrocarbons is largely determined by environmental conditions. These conditions include temperature (Bartholomew and Pfaender, 1983), salinity (Bourquin and Pryzybyszewski, 1977), Eh (Hambrick et al., 1980; Pardue et al., 1988), pH (Delaune et al., 1981), and the oxygen and nutrient status of the environment (Cooney, 1984). Biodegradation of petroleum hydrocarbons is primarily an aerobic process, requiring the presence of molecular oxygen (high Eh).

Nutrient addition has been a prime candidate for increasing hydrocarbon degradation. Numerous studies have shown that readily available nitrogen and

phosphorous sources are necessary for degradation to be effective (Atlas and Bartha 1973, Bragg et al. 1993, and Tabak et al. 1991). The Valdez oil spill in Alaska spurred enormous research efforts in oil remediation (e.g. Prince, 1992). Many of these studies investigated nutrient addition as a means of accelerating oil degradation. It was found that nutrient addition could increase rates of biodegradation; although, there was much controversy over the form in which nutrients should be applied. Recently, it has been convincingly shown that a critical level of nitrogen was needed in the pore water to enhance changes in biodegradation rates of crude oil (Bragg et al., 1994 and Venosa et al., 1995). Both, statistically showed an increase in *in situ* rates of oil degradation where the pore water concentration of nitrogen was significantly increased. However, these studies, as well as, the vast majority of research conducted on oil biodegradation, were conducted on ecosystems with little or no similarity to Louisiana's coastal marshes. In general, studies at the Valdez (Alaska) and other "beach" sites have found that oleophilic nutrient additions were the most successful in maintaining nutrient concentrations, since washout is the primary removal mechanism of nutrients (Atlas and Bartha, 1973; Bragg et al., 1993; and Halmo, 1985). A few studies found that there was a compound specific response with certain classes of compounds being more affected by inorganic than oleophilic additions of nutrients (Tabak et al., 1991).

Unfortunately, this work is difficult to apply to Louisiana's and other coastal marshes. Most work to date has been conducted on cold water, high physical energy, nutrient poor beaches as opposed to highly organic, temperate, oxygen starved, biologically rich sediments. The little work that has been conducted on similar marsh

systems found very conflicting results. Inorganic not oleophilic nutrient products were found to be more effective in low physical energy systems (Canadian marshes) (Lee and Levy, 1989 and 1991). These systems are better able to maintain nutrient concentrations in pore water, due to the limited exchange rate of pore water as well as the high adsorption capacity of the organic soils. Further, there was some evidence of microbial preference of the oleophilic fertilizer over oil as an energy source (Lee and Levy, 1989). Other researchers have indicated that oxygen, not nutrient conditions, primarily limits degradation of oil (Swannell and Head, 1994, and Lee and Levy, 1991).

This study investigated the effect of inorganic nutrients on crude oil degradation in salt marsh soils. The critical nutrient components were identified, as well as, the effect of using a reduced or oxidized form of nitrogen on crude oil mineralization. Information produced by this study will be useful in developing remediation strategies in these unique environments as well as highlighting other limiting factors.

EXPERIMENTAL

Crude oil degradation was investigated both in controlled laboratory microcosms, intact core studies, and radiorespirometers. Studies used a "sweet" Louisiana crude oil (SLCO), which is a relatively non-toxic oil with high alkane, low polar, and moderate PAH concentration. The site location was near Port Fourchon at the southwestern end of the Barataria Basin in Louisiana. This site is located in the Leeville oil field. This marsh is continuously flooded with a tidal flux of approximately 0-40 cm and is dominated by the marsh grass, Spartina alterniflora.

Laboratory Microcosm Studies.

Biodegradation experiments were conducted in completely mixed microcosms in which the redox potential (Eh) and pH were continually monitored (Masscheleyn et al., 1992). Microcosms were operated in a completely aerated mode with air flow greater than 15 ml/min. The microcosms contained 1.8 liters of a 60:1 (w/w) slurry produced from surface soils (0-5 cm) obtained from the study site. Microcosms were contaminated with unweathered SLCO at a concentration of 0.7 g oil/g soil. This concentration is the equivalent of a moderate to low oil spill if a contamination depth of 1 cm is assumed. Five treatments were monitored: microbially-inhibited (2 g NaN_3 /flask), control (no fertilizer amendment), phosphate amended (0.05 g-P as K_2HPO_4 / g soil) nitrogen amended (0.05 g-N as NH_4Cl / g soil), and phosphate and nitrogen amended (0.05 g-N and 0.05 g-P/ g soil). Fertilizer amendment concentrations were chosen based on both concentrations from other oil studies and from studies conducted on the site concerning plant growth. The microcosms were sampled for oil components by removing 20 ml of slurry and extracted using liquid extraction with 1:1 (v/v) hexane:acetone. The extracts were passed through MgSO_4 to remove water and concentrated under a stream of dry nitrogen. The oil extracts were analyzed by GC-MS using $17\alpha,21\beta$ -hopane as a normalizing compound (Prince et al., 1994). Using hopane as a normalizing compound (i.e. ratio of compound to hopane concentration) allows only biodegradation to be monitored. Alkanes C12-C44, pristane, and parent, C1, C2, naphthalenes and phenanthrenes were monitored. Populations of oil degraders was

monitored over time by using the "Sheen Screen" method of Brown and Braddock (1990).

Degradation curves were fitted using non-linear regression to a simple first order kinetic equation given below:

$$C/C_0 = Ae^{-BX}$$

where C= substrate's hopane ratio, C_0 = initial hopane ratio, t= time, and A and B are adjustable constants.

Mesocosm Study.

Mesocosm studies used large box cores removed intact from the field and transferred to glass aquariums. Cores were 900 cm² and 20 cm deep. The cores were contaminated with 123 mg oil/cm². This concentration is indicative of a light to moderate oil spill. Five cores were used as controls. Four cores received phosphate (0.5 mg P-K₂HPO₄/ cm²) and a range of nitrogen (0.1, 1.0, 5.0, and 10.0 mg NH₄-N/cm²). At the end of 4 weeks, the mesocosms were cored using 15.3 cm diameter thin-walled aluminum cores. Approximately 20% of the surface area of each treatment was sampled. The top 5 cm of soil was removed and homogenized. Subsamples were taken and mixed with MgSO₄ to reduce the water content. The oil was extracted using supercritical fluid extraction with unmodified CO₂ (flow rate = 11 ml/min; extraction time = thirty minutes; oven temperature = 100°C; restrictor temperature = 175°C; and collection solvent (hexane/acetone 1:1) at 4°C). This extraction procedure had a

recovery of 85-95% depending on the compound. Oil analysis on the extracts were conducted as in the microcosm study.

Radiorespirometry Studies.

Soil samples were obtained using large diameter cores (15 cm diameter). The cores were removed to the lab and a 20:1 m/m soil slurry produced by removing the top 5 cm of the core, mixing with sterile water, and homogenizing with a low speed blender. Twenty ml of the slurry was placed in 125 ml Erlenmeyer flasks containing a glass center well and fitted with Teflon septums. The sediment was contaminated with 0.52 g of oil per g soil the maximum concentration determined without inhibiting degradation. Flasks received $[1-^{14}\text{C}]n$ -hexadecane, (0.1 μCi) soil in a minimum of solvent (10 μl) and comprised less than 0.5% of the compounds total concentration. The $[1-^{14}\text{C}]n$ -hexadecane had a specific activity of 8.7 $\mu\text{Ci}/\text{mg}$. Respirometer flasks were kept in the dark at room temperature ($25^\circ\text{C} \pm 2^\circ\text{C}$) during incubation. Three treatments were examined. Flasks either received no nitrogen, nitrogen as NH_4^+ , or as NO_3^- . Four concentrations of each nitrogen source were examined (10.0, 1.0, 0.1, and 0.01 mg-N/g soil). Four replicates of each treatment were run.

Respiration rates were determined by trapping CO_2 in 1N NaOH in the center well of each flask. Every three or four days the NaOH was removed and replaced, and the headspace of the flasks were injected with 10 ml of pure O_2 . The treatments were sampled for 30-60 days. The spent NaOH was placed in 10 ml of Hionic-fluor (Packard Co. Meriden, CT.) scintillation cocktail. Scintillation counting was performed on Beckman LS 6000SC. Rates of degradation were fitted using linear regression to a

simple zero order kinetic equation given below:

$$C/Co = At + B$$

where C= mineralized hexadecane (cpm), Co= total hexadecane (cpm), t= time and A and B are adjustable constants.

RESULTS

Nutrient Effects on Alkane and PAH Degradation.

There was no detectable decrease in hopane ratios indicating that no significant degradation of crude oil components occurred in either the inhibited, control, or phosphate amended treatment (Figure 3.1A,B,C). Significant degradation (as indicated by decreases in the hopane ratio) occurred in both the nitrogen amended and nitrogen-phosphate amended treatments as indicated by the decreases in hopane ratios (Figure 3.1D and E). Volatilization is not considered a significant factor since only alkanes greater than C=14 were monitored and no decreases were observed in the microbially inhibited control. Although nitrogen is clearly the critical nutrient which limits alkane degradation, the role of phosphate is important. The addition of phosphate had two noticeable effects: an increase in the rate of degradation (Table 3.1) by a factor of three and a decrease in the lag time. PAH degradation showed a noticeably different response to nutrient addition than alkane degradation in this study.

PAH degradation was detectable in all treatments except the microbially inhibited microcosm (Figure 3.2A-E). Some decrease in hopane ratio did occur in the inhibited control but this is probably due to volatilization of naphthalene. Naphthalene

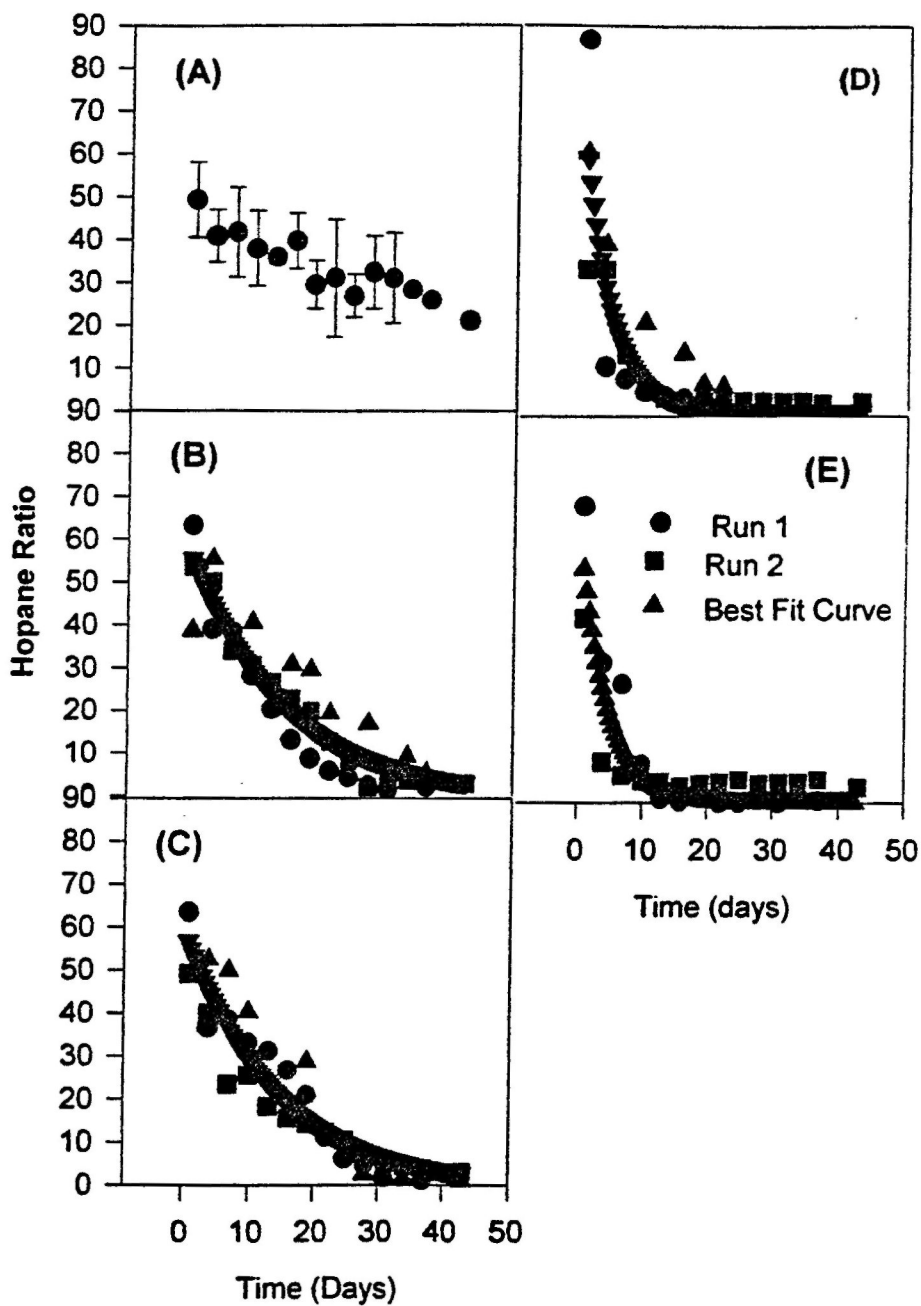


Figure 3.1 Alkane degradation (C15-C44) as indicated by reduction in hopane ratios: (A) Inhibited Control; (B) Control; (C) Phosphate; (D) Nitrogen; and (E) Nitrogen and Phosphate amended.

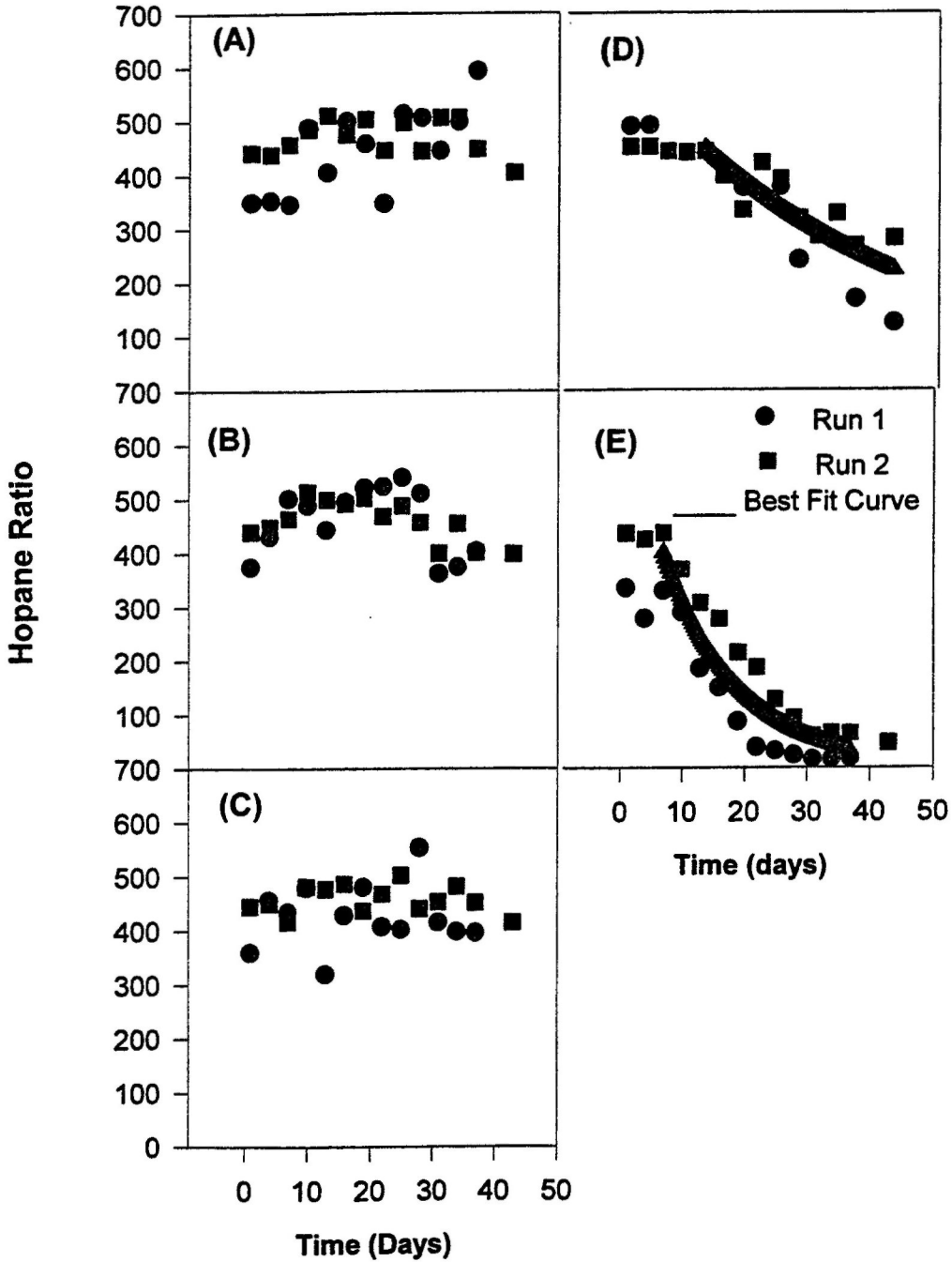


Figure 3.2 PAH degradation (phenanthrene, C1-C3; naphthalene, C1-C3) as indicated by reduction in hopane ratios: (A) Inhibited Control; (B) Control; (C) Phosphate; (D) Nitrogen; and (E) Nitrogen and Phosphate amended.

Table 3.1. Pseudo first-order rate constants of alkane and PAH degradation.

Treatment	ALKANE			PAH		
	Rate constant (t ⁻¹)	std error	R ²	Rate constant (t ⁻¹)	std error	R ²
Inhibited	****	****	****	0.016	.002	.86
Control	****	****	****	0.069	.005	.973
Phosphate Amended	****	****	****	0.072	.004	.977
Nitrogen Amended	0.021	.003	.74	0.24	.021	.973
Nitrogen and Phosphate Amended	0.067	.007	.855	0.239	.026	.956

had the largest Henry's Law constant of all compounds monitored. Nitrogen once again had a significant affect on the rate of degradation. Rates increased by a factor of 3 over treatments not receiving nitrogen (Table 3.1). Interestingly, phosphate appears to have no effect on PAH degradation. Rates of degradation were identical between nitrogen amended and nitrogen and phosphate amended; as well as, between phosphate amended and the control (Table 3.1). Despite significant increases in degradation after amendment with N, PAH degradation proceeded at a rate of $0.069 \pm 0.005 \text{ day}^{-1}$ in the microcosms. Significant PAH degradation can be supported by concentrations of N and P naturally present.

The high rate of PAH degradation in comparison to alkane degradation is interesting. Both naphthalene and phenanthrene have higher solubilities than alkanes (C=15) and greater which could account differences in bioavailability, although it would not explain the lack of effect of phosphate or the ability of the PAH to degrade with no additional nitrogen as opposed to alkanes degradation. Another possibility is that there are distinct populations of bacteria responsible for alkane and PAH degradation. There is no direct evidence of this but we have found in other studies that PAH and alkane degradation are seasonally uncoupled (Jackson and Pardue, IN REVIEW).

Radiorespirometry Study.

Extensive mineralization of crude oil was observed, as indicated by production of $^{14}\text{CO}_2$ from labeled hexadecane, for all treatments as well as the control indicating the high potential for crude oil degradation in these marsh soils (Figure 3.3A and B). Mineralization curves for NH_4^+ and NO_3^{-2} amended treatments as well as the control

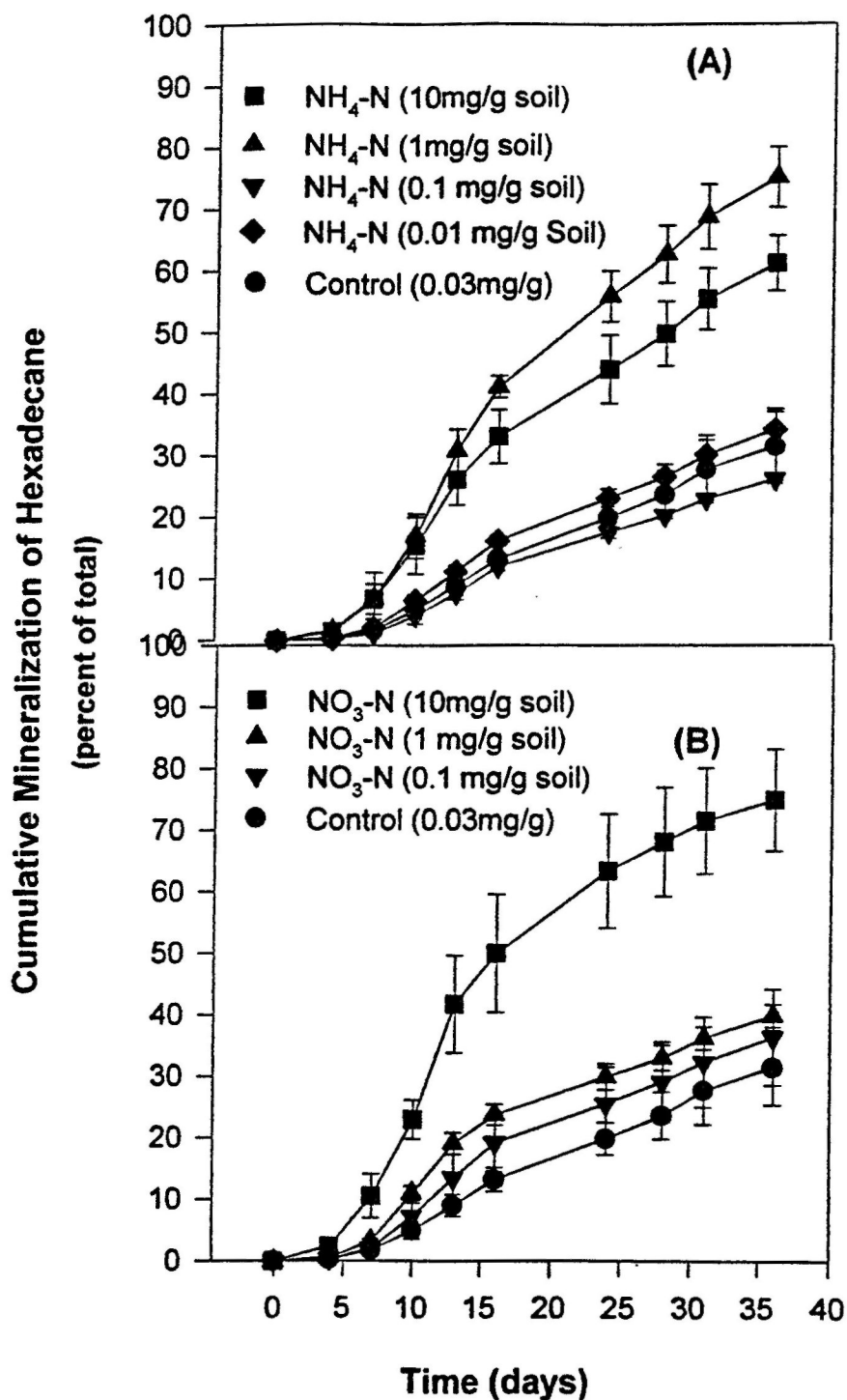


Figure 3.3 Mineralization of crude oil in salt marsh slurries, (A) with NH₄⁺ and (B) NO₃⁻² enhancements.

all seem to have relatively similar shapes. Regardless of nitrogen amendment, mineralization of crude oil was greater than 25% over 32 days. Mineralization was observed after 3-6 days in all treatments. Mineralization rates were quite rapid (0.998-4.22 %/day) in the first 12-15 days and then decreased to a second slower rate (0.721-1.73 %/day).

Table 3.1 presents the two observed rates of mineralization with their respective errors and correlation coefficients. Additions of NH_4^+ increased the initial rapid rate but only when the concentration of NH_4^+ -N added was at least 1 mg/g soil. Additions of N below this did not increase the rate of mineralization significantly as compared to the control. Additions as great as 10 mg/g soil increased the rate of mineralization over the control but inhibited the mineralization in comparison to the 1 mg/g soil treatments. The second slower rate exhibited the same relative order of mineralization as the first initial rapid rate ($1 > 10 > 0.1 = 0.01 = \text{control}$ (0.03 mg/g)). It appears that NH_4^+ has a critical concentration which must be reached before stimulation can occur. This may be due to availability since NH_4^+ has a high sorption rate onto organic soils.

Additions of NO_3^- increased the initial rapid mineralization rate with increasing NO_3^- concentrations. However, only the highest NO_3^- amendment dramatically increased the mineralization rate significantly over the control (by a factor of 4) while the lower amendments (1.0 and 0.1 mg/g) increased the rate only by factors of 1.8 and 1.5 respectively (Table 3.2). The second rate followed a similar pattern with only the highest NO_3^- enhancement having a higher rate than the other NO_3^- enhancements or the control. Ammonium appears to be a more efficient means of stimulating

Table 3.2 Rates of crude oil mineralization in control, NH_4^+ and NO_3^{-2} amended treatments.

Treatment	Initial	std	R ²	Second	std	R ²
		error			error	
	%/day	+ -		%/day	+ -	
Control (0.03mg/g)	1.103	.107	.972	.936	.042	.994
NH_4^+ (10mg/g)	2.756	.165	.989	1.44	.044	.997
NH_4^+ (1mg/g)	3.43	.285	.979	1.73	.065	.996
NH_4^+ (.1mg/g)	.998	.123	.955	.721	.014	.998
NH_4^+ (.01mg/g)	1.362	.117	.978	.917	.025	.987
NO_3^{-2} (10mg/g)	4.22	.335	.981	1.26	.131	.978
NO_3^{-2} (1mg/g)	2.07	.182	.977	.821	.022	.997
NO_3^{-2} (.1mg/g)	1.64	.167	.969	.876	.023	.997

mineralization of crude oil in salt marsh soils. Only one tenth of the concentration of NH_4^+ was needed to stimulate degradation approximately three times the rate of the control. However, NO_3^{-2} does have advantages over NH_4^+ in regards to managing application. Ammonium seems to have a smaller range of concentrations which will increase degradation while not causing inhibition. Nitrate appears to increase, albeit slightly, mineralization with increasing concentrations with no critical level needed before stimulation occurs. Further, any inhibition which may occur would be at much higher concentrations than NH_4^+ , effectively increasing the concentration range which will stimulate not inhibit degradation. This is a critical point since managing pore water concentrations of nutrients is difficult.

Mesocosm Study.

Salt marshes are extremely active biological systems and have an unknown capacity to support crude oil degradation. Large cores were contaminated with crude and oil treated with increasing concentrations of nutrients in order to examine a more realistic field condition on the effects of nutrient enhancements on crude oil degradation in salt marshes. All treatments, both unamended controls and nutrient amended, exhibited significant degradation (Figure 3.4). The PAH degradation was greater than the alkane as in the microcosm study. Alkane degradation of control cores demonstrated a very consistent (15-20%) reduction in hopane ratios as compared to a weathered control, while PAH degradation was more variable (48-65%). Nutrient additions had an unclear effect. The nutrient amended treatments exhibited varying amounts of degradation with the 0.1 and 10 mg/cm^2 nitrogen enhancements having higher

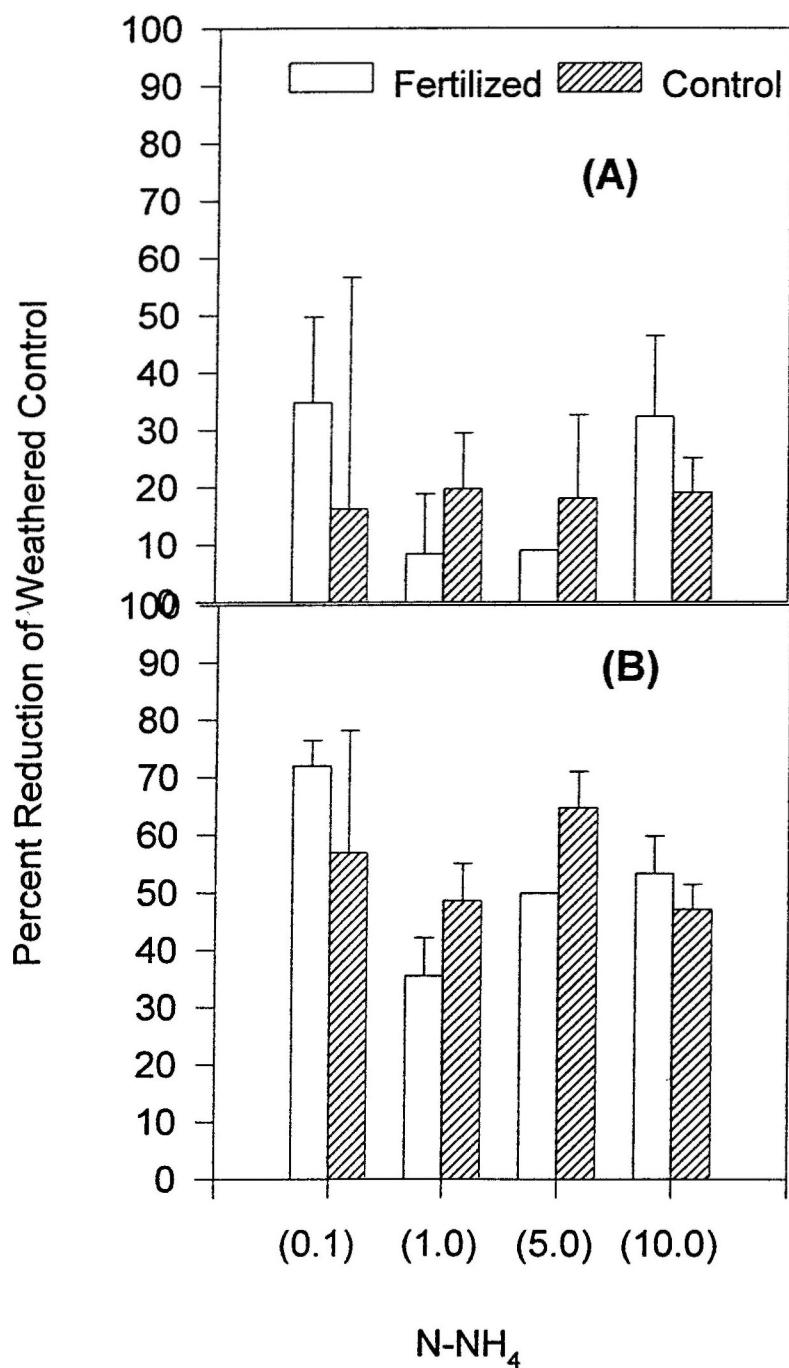


Figure 3.4 Percent reductions of hopane ratios in reference to a weathered control in large cores after 4 weeks: (A) alkane fraction, (B) PAH fraction.

reductions in hopane ratios of both the alkane and PAH fractions, while the intermediate concentrations produced smaller reductions in hopane ratios than the controls. This inconsistent effect was also found in a field study located at this site (Jackson and Pardue, 1996). The limited degradation of the alkane fraction, the largest single component of this crude oil, may be due to oxygen limitations. Salt marshes, due to both their high organic content and sulfide flux, are oxygen depleted with only the top few millimeters oxidized. This may explain why degradation of the alkane fraction was much smaller than in the lab studies in which sediments were not limited by oxygen. Intermediate concentrations of NH_4^+ may have been insufficient to increase crude oil degradation but may have caused an increase in oxygen demand, either by stimulating mineralization of indigenous organic matter or by nitrification of NH_4^+ . PAH degradation may not have been as affected by this limitation since it is only a tenth of the size of the alkane pool. In addition, these sediments seem to have a higher capacity for PAH than alkane degradation. Nutrient enhancement of the alkane fraction would be less pronounced if oxygen was limiting, while given the high rate of PAH degradation with or without nutrient enhancement it is not unexpected that total degradation was not greatly enhanced.

CONCLUSIONS

Salt marshes appear to have relatively high inherent degradation potential for crude oil components. Nutrient additions are plausible strategies for stimulating crude oil degradation although their overall effectiveness may be limited by oxygen availability. The effectiveness of nutrient amendments appears to be dependent on not

only which fraction of crude oil is examined but also on the form and concentration of amendments. Salt marsh systems appear to have a higher inherent potential to degrade the PAHs examined than alkanes. Although it is not possible to tell from this study, likely reasons include greater bioavailability or separate populations of degraders. In either case, this may have important ramifications on remediation efforts. PAHs are normally considered more toxic than alkanes but are a smaller fraction of the crude oil. They appear to be readily degradable with or without nutrient amendments, although amendments can increase the rate of degradation if no other limitations are present and may increase the rate of degradation *in situ*.

Phosphate does not appear to be limiting in these systems and its addition may well be unnecessary at least in the initial stages of remediation efforts. Nitrogen amendments appear to be effective in increasing the degradation rates of alkanes and PAHs in lab studies. The form of nitrogen amendments appears to be fairly important. While both NH_4^+ and NO_3^- did increase degradation at appropriate concentrations, each has different benefits and disadvantages. Nitrate promotes degradation significantly at only very high concentrations but appears to be more available than NH_4^+ at lower concentrations with no inhibiting effects. Ammonium substantially increases degradation at a lower concentration but does appear to have a rather small concentration range for maximum degradation. However, NH_4^+ would be expected to have a higher retention time in these systems than NO_3^- which would have low sorption rates. In addition, NH_4^+ may add an additional oxygen demand which would be detrimental in these oxygen limited systems. Nitrogen amendments in the form of NH_4^+ were also found to

increase degradation, although to a lesser extent, in core studies at similar concentrations to those in the lab studies. The small increase in degradation as compared to the lab study may be due to oxygen limitations. Core studies would be expected to have similar oxygen limitations as field conditions. It may then be necessary to remove this limitation before nutrient applications if maximum benefit is to be achieved.

Successful strategies for large scale in situ bioremediation efforts of crude oil contamination in salt marshes will need to consider the different fractions of oil and either treat for the most recalcitrant or if economically unfeasible then the most damaging. This in itself will need to be determined. Ammonium and NO_3^{-2} can increase degradation of crude oil components, and as mentioned each will have its benefits and disadvantages. Strategies may need to incorporate a cost to benefit index for each type of nutrient amendment incorporating reapplication schedules and increases in degradation rates.

CHAPTER 4. SEASONAL VARIABILITY OF CRUDE OIL RESPIRATION POTENTIAL IN SALT AND FRESH MARSHES

INTRODUCTION

Crude oil releases into the environment are a common and often large scale event. Crude oil is not only physically harmful to the environment but contains many toxic compounds in relatively high concentrations (e.g. PAH's). Louisiana's wetlands are especially at risk. Approximately 40% of U.S. coastal wetlands are located in Louisiana which also supports 15% of U.S. crude oil production. However, while much research has been conducted on crude oil degradation, little information is available on the kinetics of oil degradation in wetland systems. This information is critical since most physical and chemical remediation methods are not possible in wetlands (e.g. mechanical cleaning).

Marsh soils are fundamentally different from typical sediments and unsaturated soils. Both fresh and salt marsh systems are characterized by near constant flooded conditions with periods of high inundation and less frequent periods of partial drying. The marsh soils are highly organic and contain large amounts of nutrients, although most is in an unavailable form. (Delaune et al., 1989, Delaune and Smith, 1985, Feijtel et al., 1985; Delaune et al., 1990) Marshes are in general low energy physical systems but high energy biological systems. The high oxygen demand of marsh soils creates a very small aerobic layer. Most of the marsh soil is dominated by anaerobic processes with the aerobic layer only a few millimeters thick.

Degradation rates of oil components in wetlands are strongly dependent on environmental conditions. These conditions include temperature (Bartholomew and Pfaender, 1983), salinity (Bourquin and Przybyszewski, 1977), Eh (Hambrick et al. 1980; Pardue et al. 1988), pH (Delaune et al. 1981) and the oxygen and nutrient status of the soil (Cooney, 1984). Complicating the microbial response to oil is the natural seasonality of these environmental conditions. In studies of other dissimilar systems it was found that degradation rates of PAH's and alkanes were highly seasonal; although, only limited correlations could be found between specific environmental parameters and degradation rates. (Roubal and Atlas, 1977, and Shiaris, 1989)

The pool of information concerning the effectiveness of nutrient additions for enhancing crude oil remediation has increased greatly in the last decade. Nitrogen and phosphorous and possibly iron have all been indicated repeatedly as critical for successful mineralization. (Leahy and Colwell, 1990 and Dibble and Bartha, 1976) Recently, studies have even conclusively shown the success of nutrient additions *in situ* (Bragg et al., 1994). Others have investigated the type and release rates of the nutrient additions in order to maximize their effectiveness (Glasser et al., 1991 and Safferman, 1991). Other studies have found that the ideal composition of nutrient is dependent on the environment for which it is intended (Lee and Levy, 1987 and 1991). Few researchers have investigated marshes and even fewer, subtropical marshes as in Louisiana, which are not inhibited to any significant effect by temperature decreases in the winter. No information is available concerning the ability of these systems to provide nutrients for hydrocarbon mineralization through internal cycling without

relying on engineered addition. Nutrient concentrations fluctuate greatly in wetlands throughout the year due to the demand and release caused by plant growth and senescence, respectively. It is possible that the timing of the spill could have an effect on the passive ability of the system to degrade oil and determine the need for nutrient addition in wetlands.

These experiments investigated seasonal changes in microbial respiration potential of crude oil under nutrient limited and unlimited conditions. Bimonthly measurements of the mineralization potential of crude oil were conducted in respirometers using south Louisiana crude spiked with ^{14}C -analogues of phenanthrene and hexadecane. These measurements were coupled with seasonal measurements of oil degrading microbial populations, background nutrient concentrations and other pore water parameters.

EXPERIMENTAL

Crude Oil Respiration.

Site description. The salt marsh site was located near Port Fourchon at the southwestern end of the Barataria basin in Louisiana. This site is continuously flooded with a tidal flux of approximately 0-40 cm and is dominated by Spartina alterniflora. The freshwater marsh is located near Lake Des Allemands Louisiana. This site is dominated by Panicum hemitomon. The marsh is continuously flooded but water levels vary dramatically in relation to local rain events or seasonal flooding.

Laboratory Study. Soil samples were obtained using large diameter cores (15 cm). Five cores were obtained approximately every other month from both sites. The cores were

removed to the lab and a 30:1 M/M soil slurry produced by removing the top 5 cm of the core, mixing with sterile water, and homogenizing with a low speed blender. Twenty mls of the slurry was placed in 125 ml Erlenmeyer flasks containing a glass center well and fitted with teflon septums. The sediment was contaminated with 0.52 g of oil per g soil. The crude oil used was a "Sweet Louisiana Crude". This oil is a relatively non-toxic oil with high alkane, low polar, and moderate PAH concentrations. Flasks from each marsh soil received either [1- ^{14}C]*n*-hexadecane, 11.5 $\mu\text{g/g}$ soil or [9- ^{14}C]phenanthrene, 1.94 $\mu\text{g/g}$ soil. The labels had specific activities of 8.7 and 61.0 $\mu\text{Ci/mg}$ respectively. Both labels were added with a minimum of solvent (10 μl) and comprised less than 0.5% of the compounds total concentration in each flask. This produced four treatments: salt marsh soil spiked with ^{14}C -hexadecane, and ^{14}C -phenanthrene; and fresh marsh soil spiked with ^{14}C -hexadecane, and ^{14}C -phenanthrene. Each treatment was conducted under naturally existing nutrient conditions and fertilized conditions. Fertilized conditions were achieved by adding 10,1,0.1 mg/kg soil of N,P, and Fe. Each treatment was replicated 5 times. Respirometer flasks were kept in the dark at room temperature ($25^{\circ}\text{C} \pm 2^{\circ}\text{C}$) dry incubation.

Respiration rates were determined by trapping CO_2 in 1N NaOH in the center well of each flask. Every three or four days the NaOH was removed and replaced, and the flasks were injected with 10 mls of O_2 . The treatments were sampled for 30-60 days. The spent NaOH was placed in 10 ml of Hionic-fluor (Packard Co. Meriden, CT.) scintillation cocktail. Scintillation counting was performed on Beckman LS 6000SC.

Monitoring Environmental Parameters.

A number of environmental conditions, chosen for their likely influence on mineralization rates, were monitored throughout the study. "Peepers" (plexiglass diffusion chambers) were used to collect water samples for testing of NH_4^+ , NO_3^- , DOC, and various cations. These chambers were 45 cm long and capable of holding 8 ml of water every cm. The water wells were separated from the soil matrix by two membranes one 8 and the other 0.45 μm . NH_4^+ was measured by direct Nesslerization, NO_3^- by cadmium reduction and DOC by the method of Moore (1985). Metals were measured on an ICP (Jarrell-Ash). Populations of oil transforming bacteria were measured for each slurry by the method of Brown and Braddock (1990). Mineralization rates were determined by curve fitting the linear portion of the respiration curve. Lag time was defined as the sampling point that greater than 1% cumulative of the label was recovered. This value is greater than the total percent recovery from killed controls.

RESULTS

Fresh Marsh.

Several studies have investigated the ability of fresh water marsh soils to degrade specific contaminants (e.g. Delaune et al. 1990), however no information is available on the ability of these soils to mineralize crude oil at concentrations similar to expected concentrations after a spill. Crude oil mineralization was monitored from May 1994 through March of 1995. Our results indicate that the fresh marsh soil has a high inherent potential to mineralize crude oil. In all samples tested 20-50% and 50-

70% of hexadecane and phenanthrene were mineralized, respectively, with (Figure 4.1) or without (Figure 4.2) any nutrient additions. As can be seen, there were large seasonal variations in both mineralization of hexadecane and phenanthrene using fresh marsh slurries.

In order to quantitatively examine seasonal differences, a mineralization rate was calculated and lag times determined for each mineralization curve and plotted against time (Figure 4.3 and 4.4A-B). The seasonal trends are quite distinct for both hexadecane and phenanthrene. Hexadecane mineralization rates varied from a high of 1.5 day^{-1} in May to a low of 0.5 day^{-1} in November (Figure 4.3A). The mineralization rates generally declined from late spring to late fall. Lag times (elapsed time from contamination to start of mineralization) were fairly consistent and ranged from 1-6 days (Figure 4.3B). Phenanthrene degradation followed a somewhat opposite trend increasing from May to a peak in September (Figure 4.3A). Mineralization rates were slightly more variable and exhibited approximately the same magnitude of change as hexadecane. Phenanthrene appears to follow a pattern reported by Shiaris (1989) in his seasonal study of PAH degradation in estuary sediments. Lag times were generally constant throughout the year and comparable to hexadecane. The mineralization rates of phenanthrene were significantly higher than hexadecane for all dates.

Removing nutrient limitations had a marked effect on both the rates of mineralization as well as seasonal patterns and lag times. Fertilized conditions produced higher rate of mineralization of hexadecane for all months but January and March (Figure 4.4A), and no lag times were observed under fertilized conditions (Figure 4.4B).

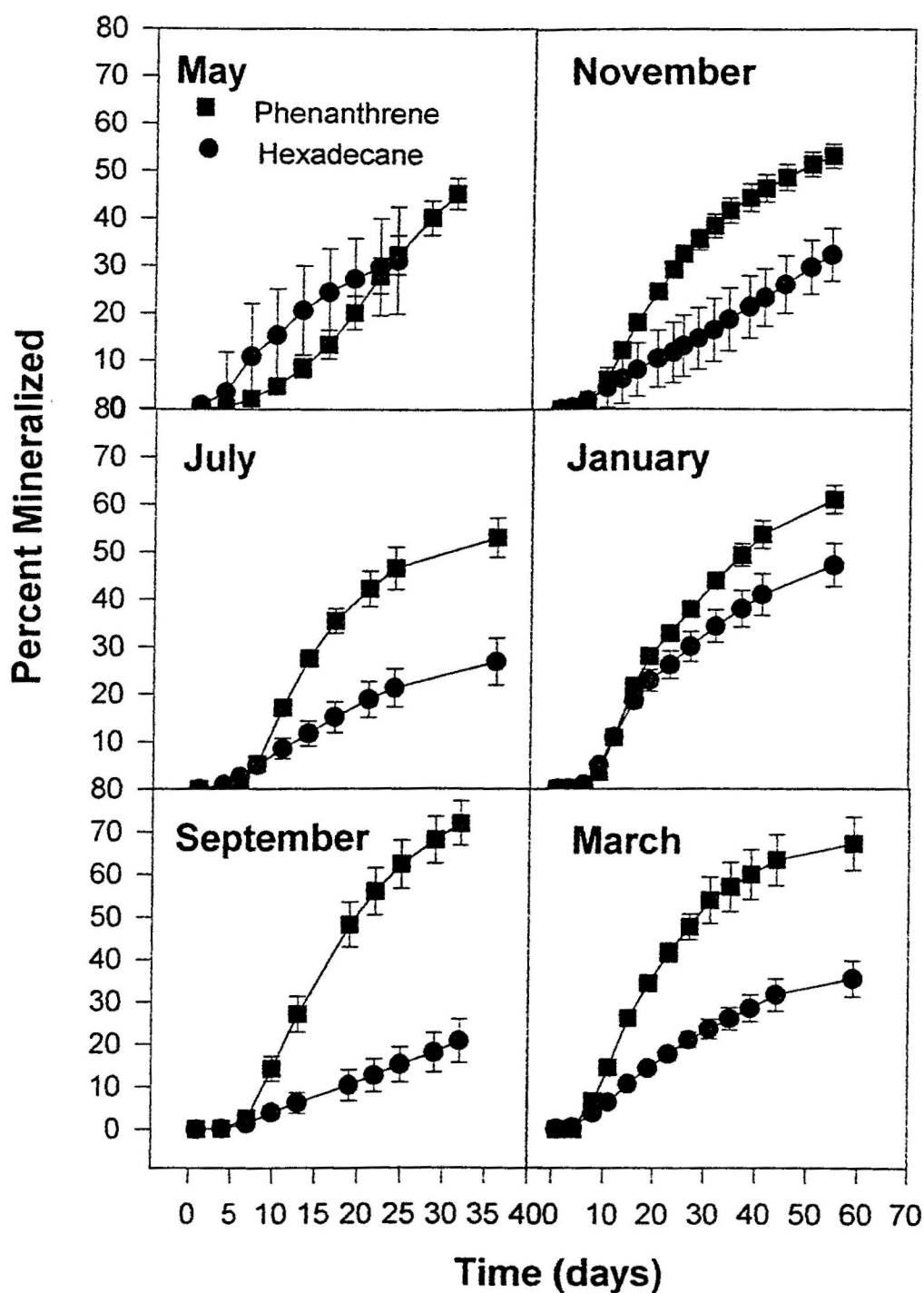


Figure 4.1 Mineralization curves of hexadecane and phenanthrene in fresh marsh slurries under unamended conditions for May 1995 through March 1996.

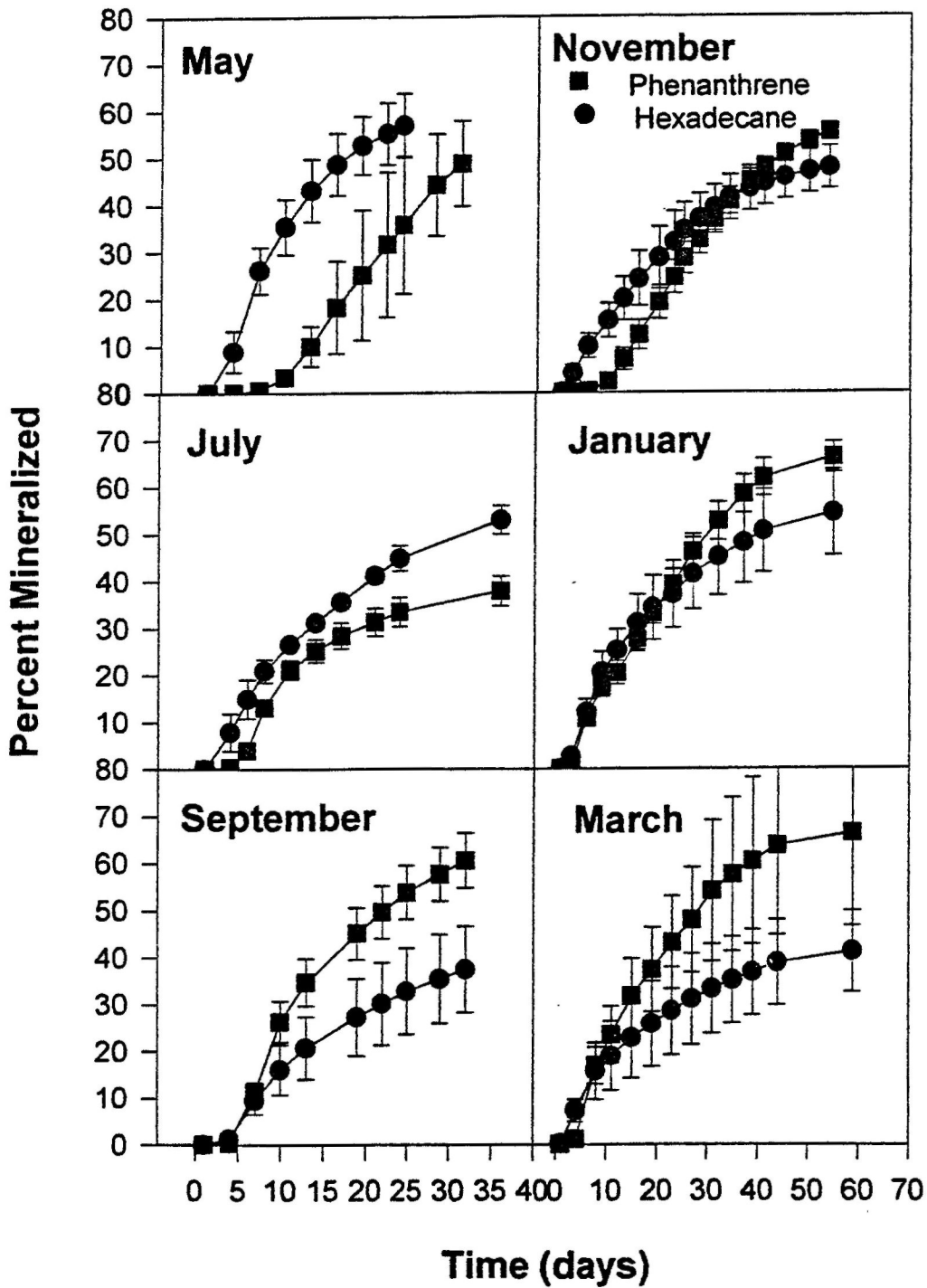


Figure 4.2 Mineralization curves of hexadecane and phenanthrene in fresh marsh slurries under fertilized conditions for May 1995 through March 1996.

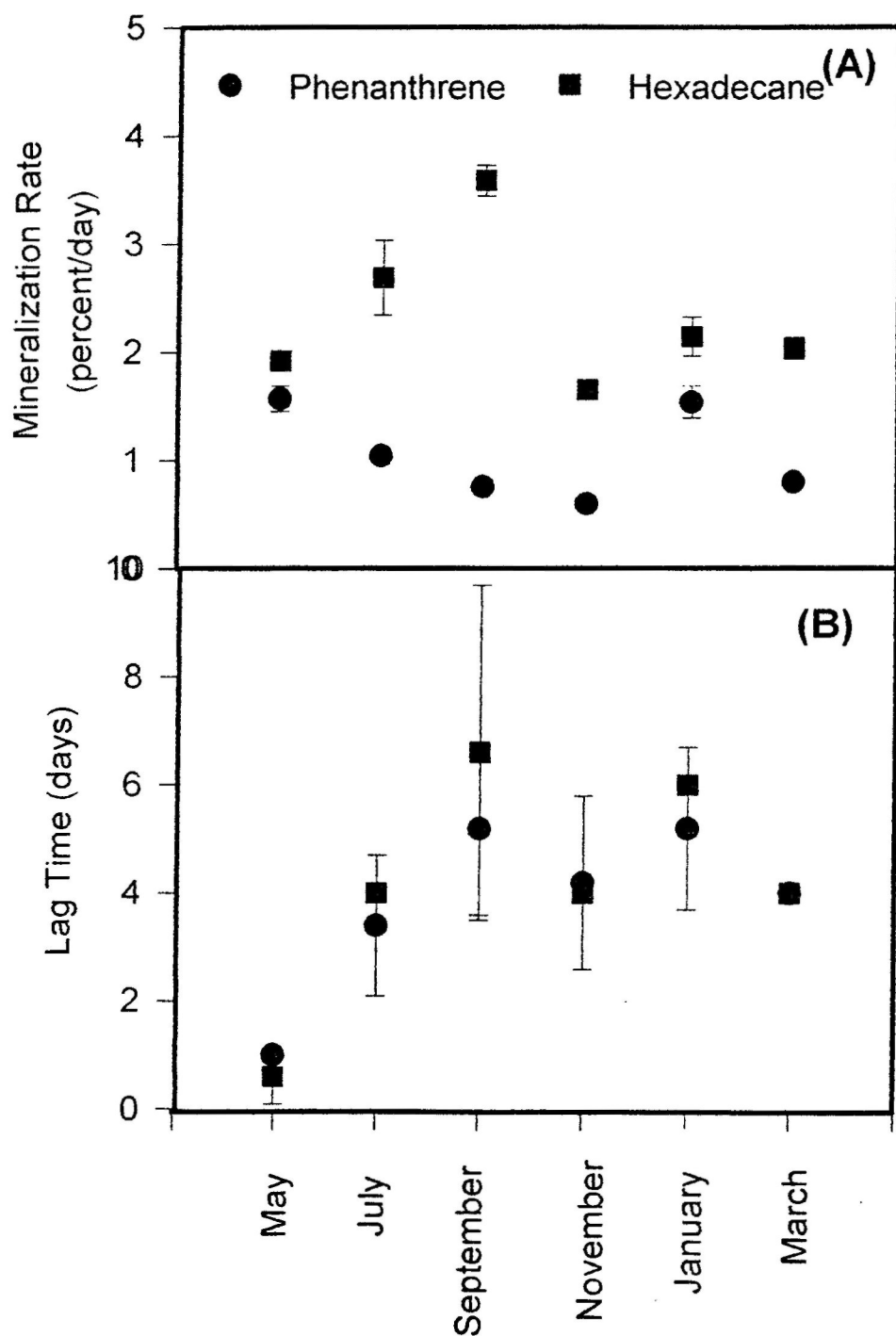


Figure 4.3 (A) Fresh marsh mineralization rates of phenanthrene and hexadecane. (B) Lag time for phenanthrene and hexadecane.

Seasonal trends of hexadecane mineralization did not change from nutrient limited studies with rates decreasing from May to March with the exception of a slight increase in January. This suggests mineralization rates of hexadecane are limited by other factors during these two months.

Phenanthrene rates were more consistent under fertilized conditions (Figure 4.3A and 4.4A). Rates either decreased or remained constant compared to nutrient limited rates. Seasonal variation was markedly reduced under fertilized conditions. There was no consistent effect of nutrients on phenanthrene lag times which increased in some months and decreased in others (Figure 4.3D and 4.4D).

A number of environmental conditions were monitored throughout the year and are listed in Table 4.1. An attempt was made to correlate environmental conditions with mineralization rates in fresh marshes. In general, no correlations were found with one exception. Phenanthrene mineralization under natural nutrient conditions was found to be significantly correlated ($R^2=.951$) to populations of oil degraders at the 0.1 level of significance ($df=4$). The lack of correlation is in agreement with other seasonal studies which have found limited and sporadic associations between degradation rates and environmental parameters even though the sediments exhibit seasonal differences in rates (Shiaris, 1989).

Salt Marsh

Crude oil mineralization potential in the salt marsh was determined 6 times from May 1994 through March of 1995. Mineralization of crude oil components was markedly different in salt marsh studies than in fresh marsh studies. The inherent

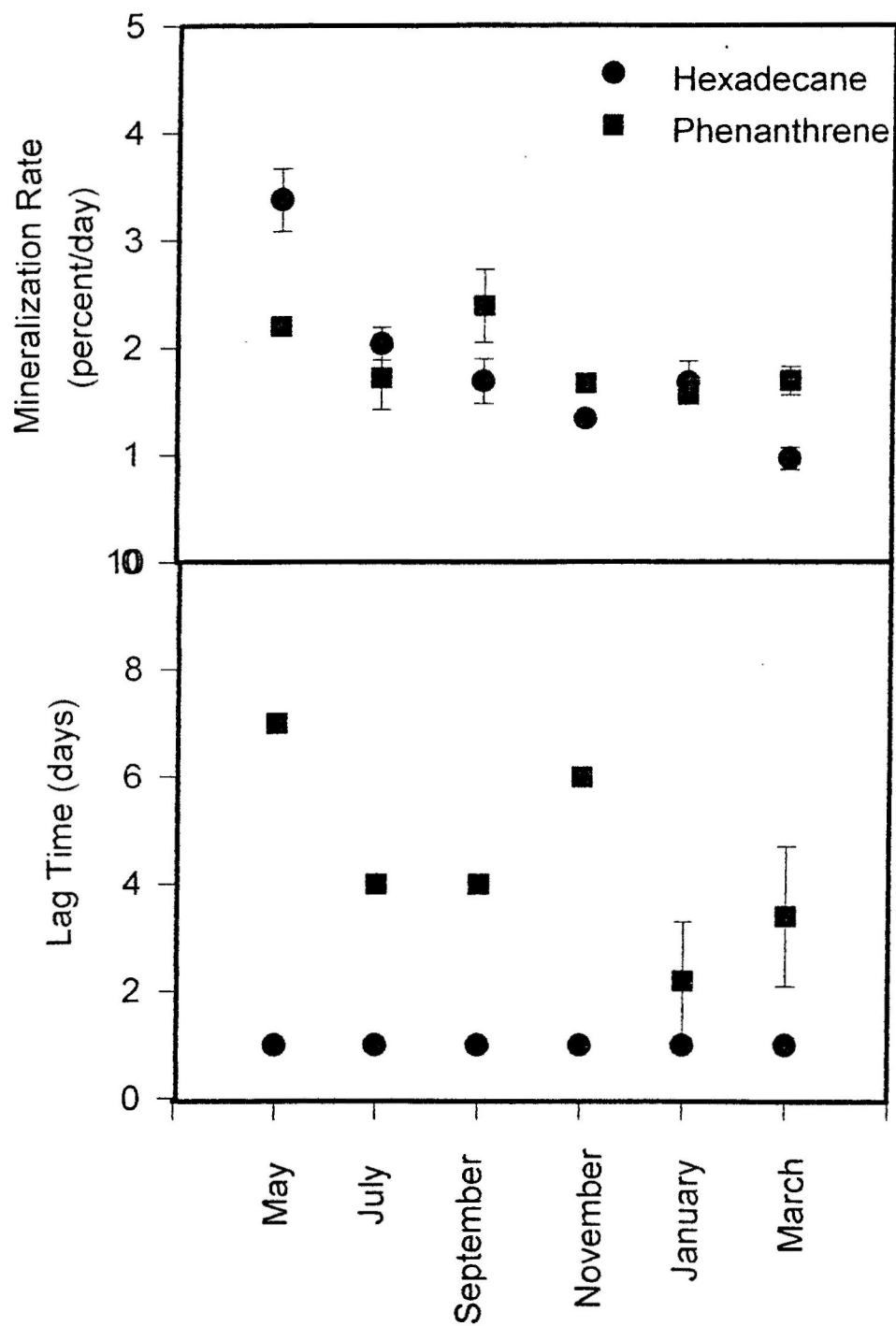


Figure 4.4 Nutrient enhanced (A) Fresh marsh mineralization rates of phenanthrene and hexadecane. (B) Lag times for phenanthrene and hexadecane.

Table 4.1 Seasonal changes in selected environmental conditions in a fresh marsh.

Month	Log bacterial population (cfu/g soil)	Ammonia (mg/l)	DOC (mg/l)	Soluble Iron (mg/l)
May	4.6	1.2	13.4	5.4
July	6.1	.08	17.6	2.8
September	6.4	1.4	*	13.1
November	4.7	7.8	3.3	2.7
January	5.0	8.1	3.9	9.6
March	5.0	12.2	13.8	3.5

potential of the salt marsh to mineralize oil without nutrient additions is much lower than the fresh marsh (Figure 4.5). Phenanthrene and hexadecane were degraded for all months tested, albeit slightly for some months. However, if nutrients are added, the mineralization potential increases dramatically (Figure 4.6). As can be easily seen from Figures 4.5 and 4.6, there were large seasonal variations in both the rate and lag time of the nutrient limited and unlimited studies.

Mineralization rates of hexadecane ranged by over an order of magnitude and peaked in September and January with very low rates in spring and summer months (Figure 4.7A). Phenanthrene mineralization was much more consistent throughout the year. As in the fresh marsh studies, phenanthrene mineralization seems to be independent of hexadecane. Lag times for biodegradation of both compounds were quite long (7-21 days) and were highly variable for a given month and between months (Figure 4.7B). Seasonal trends in lag times were similar for both compounds although differences in overall magnitude varied. Removing nutrient limitations had very unique effects on the alkane and PAH fractions of the crude oil. Hexadecane degradation increased enormously and seasonal variations were markedly reduced with only the July and March dates significantly different than other months (Figure 4.7 and 4.8A). Mineralization rates of phenanthrene were enhanced by the addition of fertilizer for only the July, January and March dates. The low rates of mineralization for May, September, November seem to be limited by some other factor. Seasonal variation was much greater under nutrient unlimited conditions and independent of hexadecane variation. Hexadecane mineralization was much more rapid with little seasonal variation

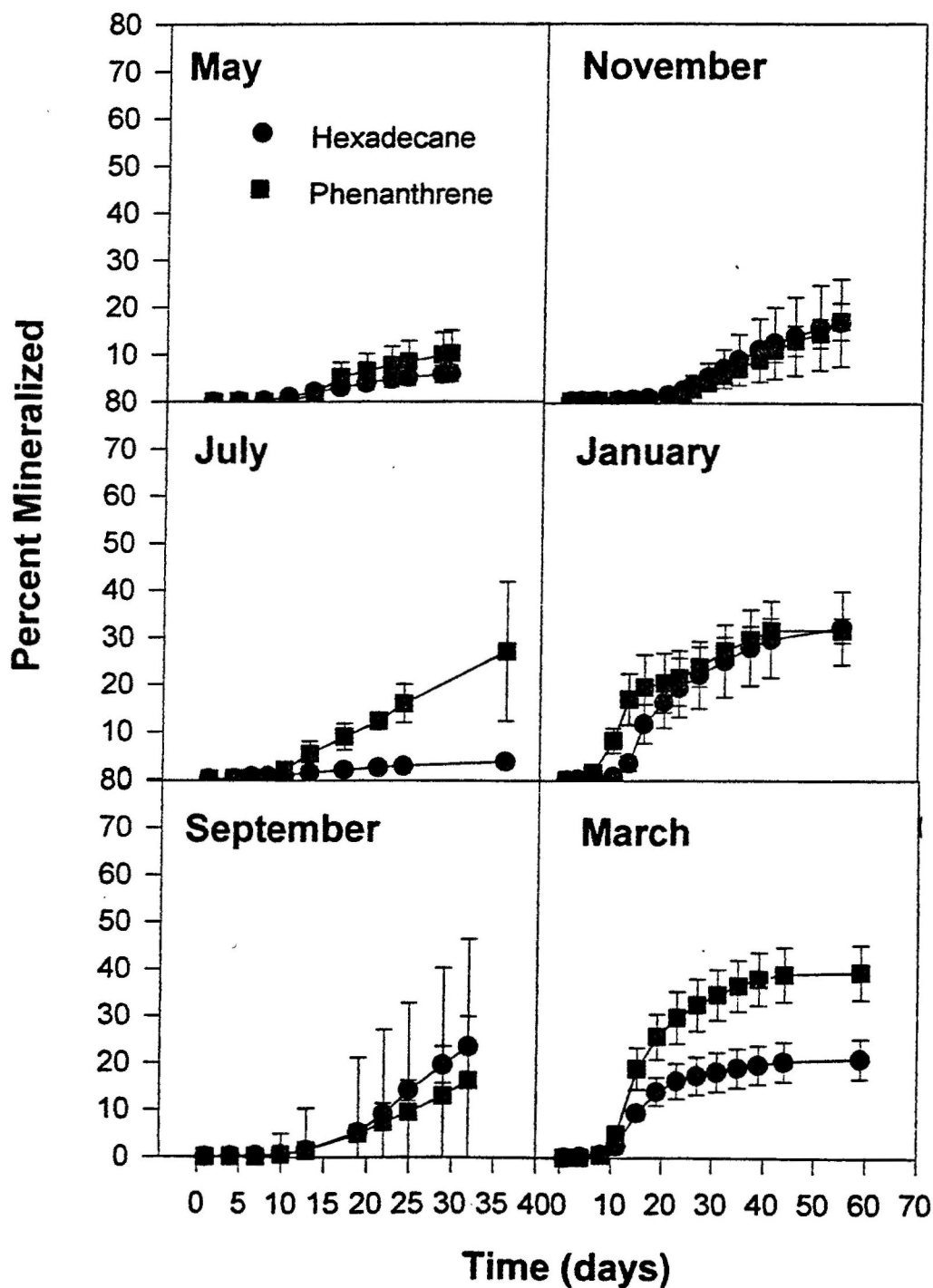


Figure 4.5 Mineralization curves of hexadecane and phenanthrene in salt marsh slurries under unamended conditions for May 1995 through March 1996.

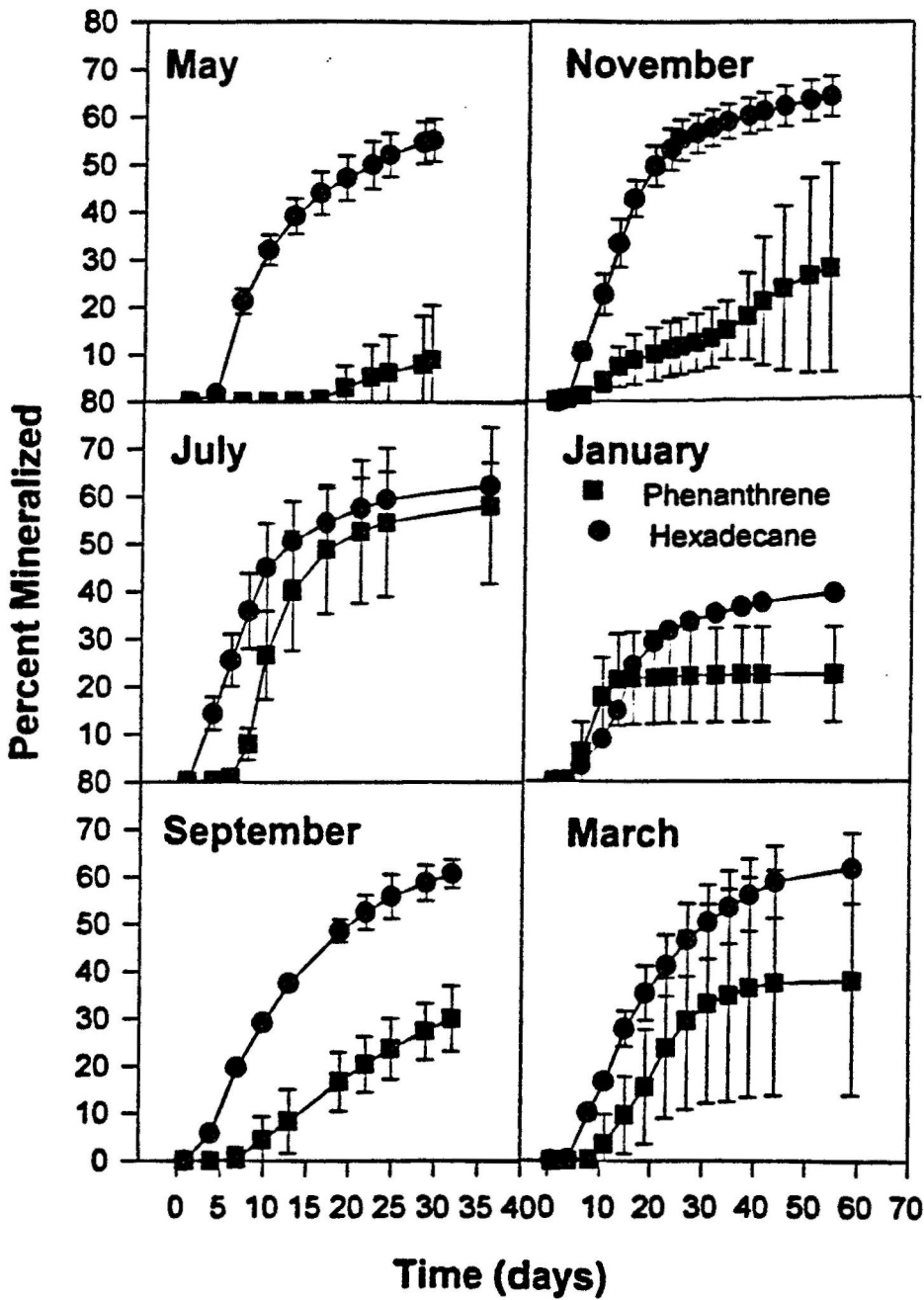


Figure 4.6 Mineralization curves of hexadecane and phenanthrene in salt marsh slurries under fertilized conditions for May 1995 through March 1996.

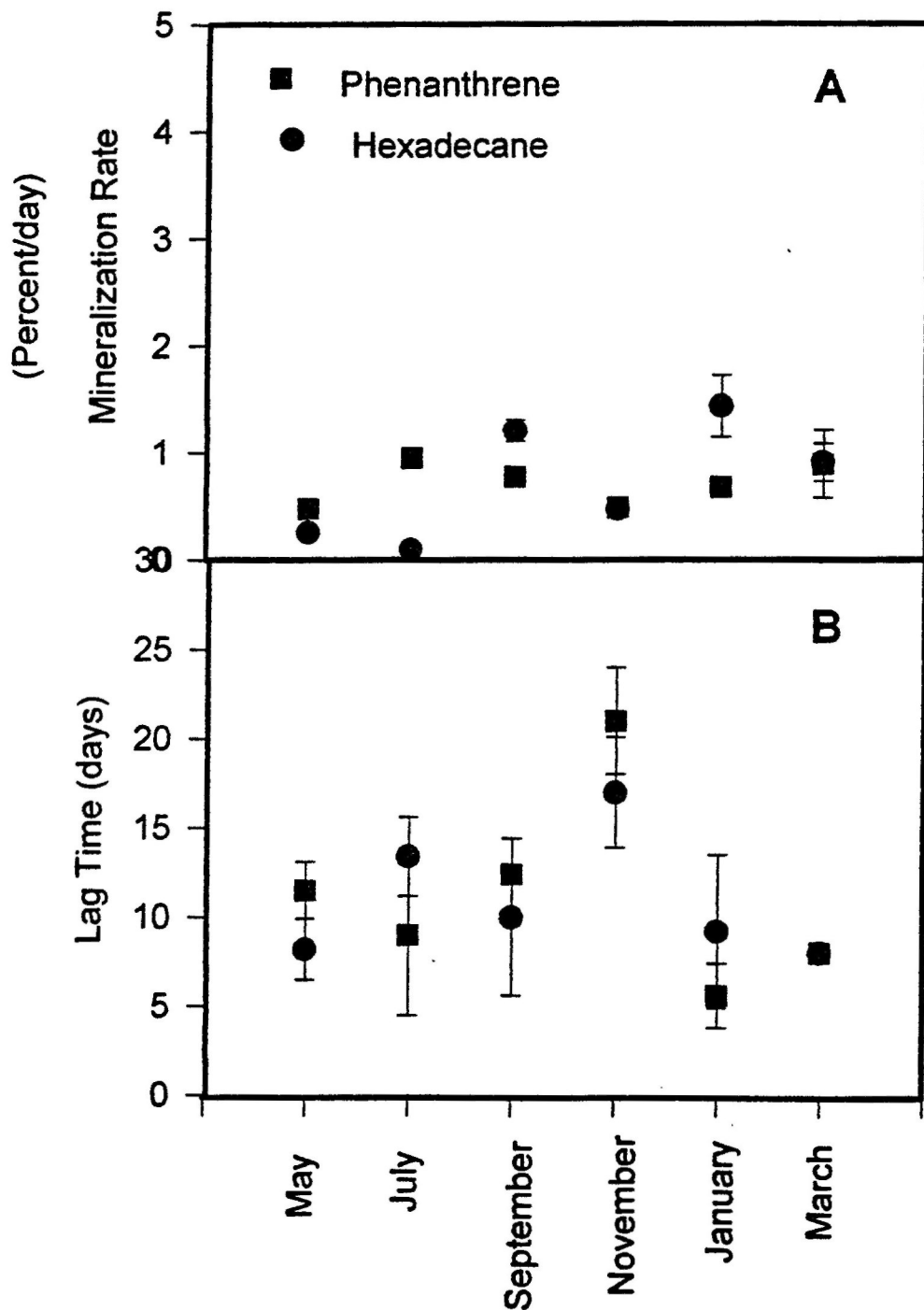


Figure 4.7 Salt marsh nutrient unenhanced (A) mineralization rates for hexadecane and phenanthrene (B) Lag time for phenanthrene and hexadecane.

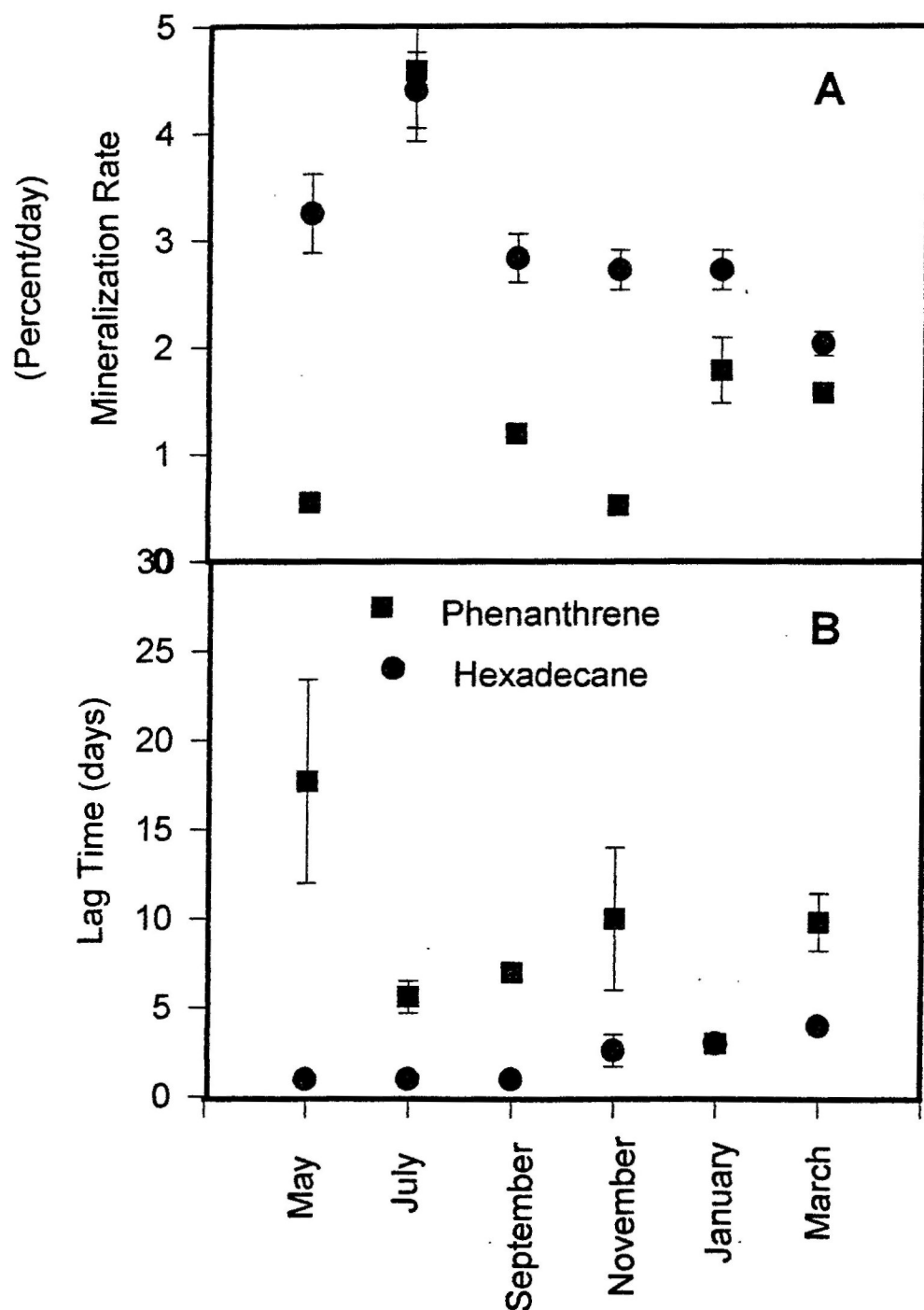


Figure 4.8 Salt marsh nutrient enhanced (A) mineralization rates for hexadecane and phenanthrene (B) Lag time for phenanthrene and hexadecane.

in lag times (Figure 4.7 and 4.8B). The results are very similar to the results observed in the fresh marsh, suggesting the decrease in lag time is related to the substrate, not specific bacteria population. The general seasonal variation in lag time for PAH mineralization followed the same trend as in nutrient limited conditions with only minor changes.

Environmental conditions listed in Table 4.2 were checked for correlations with mineralization rates of hexadecane and phenanthrene. No correlations were found under fertilized conditions. However, under natural nutrient conditions hexadecane and phenanthrene mineralization rates positively correlated with populations of oil degraders (5% level of significance with $df=4$ and a $R^2=0.857$) and (10% level of significance with $df=4$ and $R^2=0.756$) respectively.

DISCUSSION

The fresh marsh and salt marsh soils have different inherent mineralization potentials in response to crude oil contamination. While mineralization rates of hexadecane encompass the same range of rates between the two systems, the seasonal cycling is unique to each system. Phenanthrene mineralization under nutrient limited conditions is quite high in both systems, although the fresh marsh rates are significantly higher than the salt marsh for all dates. In both cases it would appear that crude oil degradation in the marsh under ambient nutrient conditions are potentially substantial but vary greatly seasonally and between classes of compounds. The addition of nutrients to increase degradation is one of the most likely and well studied ideas to increase degradation. However, few studies have examined the effectiveness of fertilizer

Table 4.2 Seasonal changes in selected environmental conditions in a salt marsh.

Month	Log bacterial population (cfu/g soil)	Ammonia (mg/l)	DOC (mg/l)	Soluble Iron (mg/l)
May	4.1	1.9	21.8	1.5
July	4.8	15.3	34.2	0.2
September	3.5	21.4	27.9	0.4
November	3.7	10.2	3.5	0.6
January	1.8	6.5	*	4.0
March	1.9	7.0	10.3	0.6

additions seasonally to examine if there are times when it is inefficient or harmful to increase nutrient conditions. Table 4.3 presents the percent increase in mineralization rates for both compounds in both marshes. The salt marsh responded positively to nutrient additions. Both compounds were mineralized at a higher rate throughout the year. The salt marsh appears to be nutrient limited throughout the year and there does not appear to be any correlation between PAH and alkane rates. It is still clear that certain seasons will experience a much greater benefit from nutrient addition than others.

The response of the fresh marsh to nutrient additions was not uniform. It is clear that hexadecane and, by extension, alkane mineralization was greatly enhanced by nutrient addition for most months. However the increased nutrient conditions decreased PAH mineralization in the fresh marsh for all but 2 months tested and in only one case was an increase observed. Therefore, it might be counterproductive to fertilize the fresh marsh in winter (January and March) since there is only a slight benefit to alkane mineralization and a decrease in PAH. While this study does not assume to predict if these seasonal patterns are constant from year to year, it is clear that the effectiveness that nutrient additions may have will depend on the season in which they are applied. Further, the crude oil components will have to be ranked in order of risk. In many increase in months the hexadecane rates was almost equalled by a reduction in PAH rates. While alkane concentrations are usually higher than PAHs, the PAH component is generally the more toxic and a strategy may have to be devised to treat each problem in order of risk. Another factor which will need to be weighed is the effect of fertilizer

Table 4.3 Change in mineralization rate under fertilized conditions (R_f) compared to inherent nutrient conditions (R_i). (R_f/R_i)

Month	Fresh		Salt	
	Hexadecane	Phenanthrene	Hexadecane	Phenanthrene
May	2.15	1.14	12.5	1.16
July	1.96	0.64	43.7	4.8
September	2.23	0.66	2.34	1.54
November	2.24	1.01	5.7	1.07
January	1.09	0.72	1.89	2.62
March	1.2	0.82	1.81	1.76

on lag time. Both marshes have high rates of accretion and limited depths to which oxygen is available. Long lag times increase the amount of oil which will be unavailable for mineralization. Nutrient additions were very effective in decreasing the time to alkane degradation and less so for PAH. It is not known if the lag times will apply to field conditions, but the results certainly indicate a possible benefit to nutrient additions rarely addressed and of extreme importance to these systems.

Seasonal changes in mineralization potential of crude oil will play a major role in the effectiveness of any response to a contamination event. Environmental conditions clearly influence mineralization rates even though poor correlations were found with many measured field parameters with the exception of microbial populations. However, the effects of specific environmental conditions are probably dependent on a number of inter-related influences and coupled with competition between different populations of bacteria and thus, difficult to determine.

CHAPTER 5. MONITORING CRUDE OIL MINERALIZATION IN SALT MARSHES: USE OF STABLE CARBON ISOTOPE RATIOS.*

INTRODUCTION

Sensitive salt marsh ecosystems are susceptible to impact from spilled and discharged oils. Major oil recovery and processing operations are located in these ecosystems. Louisiana is especially impacted since it contains approximately 40% of the U.S. coastal wetlands and 15% of U.S. crude oil production. Passive and engineered bioremediation are two approaches that may provide a solution to oil spills in marshes. The ability to monitor mineralization of hydrocarbons is of prime importance in a successful remediation strategy. It is vital to be able to insure hydrocarbon mineralization since hydrocarbons can be sorbed, transformed, buried or otherwise be undetectable but still pose threats to the existing system ecology. Further, monitoring hydrocarbon mineralization would allow effective management of remediation strategies. This would assure that remediation efforts are not wasted nor continued past their effective period, since remediation efforts in themselves can harm the ecosystem (Owens et al., 1993). Previously, absolute amounts of hydrocarbons were monitored, but the ability to successfully correlate loss of detectable hydrocarbons to biodegradation has been difficult. One successful technique has been monitoring changes in oil composition in relation to a stable non-degradable compound (Bragg et al., 1993 and 1995). Hopanes have been successfully used as a conservative marker

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allowing confirmation of *in situ* biodegradation. This method also allows more accurate determination of relative rates and lag periods since only biological degradation should change these ratios after easily weathered components are lost from the mixture. However, two disadvantages to this method exist, one; its lack of ability to demonstrate mineralization instead of transformation, and two; its inability to measure absolute oil degradation, since only "resolved" compounds are quantified. Further, sampling large areas of contaminated sediment requires extensive labor, high cost for GC-MS analysis and large numbers of samples for statistical verification of results.

A promising new technique for the detection and quantification of hydrocarbon mineralization is the use of stable carbon isotope ratios (Aggarwal and Hinchey, 1991). Carbon dioxide has different $^{13}\text{C}/^{12}\text{C}$ ratios depending on the source of the gas. Crude oils are more depleted in ^{13}C and thus the mineralization of oil produces CO_2 with lower $\delta^{13}\text{C}$ values (Stahl, 1980; Suchomel et al., 1990; McMahon et al., 1990). Highly organic marsh soils have distinctly different $\delta^{13}\text{C}$ signatures. In marshes, the $^{13}\text{C}/^{12}\text{C}$ ratios of soil organic matter is primarily controlled by the type of vegetation inhabiting the surface. Plants utilize two different fixation pathways. C_3 plants utilize the Calvin cycle and their $\delta^{13}\text{C}$ signature is lower than plants which utilize the C_4 pathway. C_4 plants have an additional pathway, "the Hatch-Slack pathway", which discriminates less against $^{13}\text{CO}_2$ (Smith and Epstein, 1971; Vogel, 1980). Salt marshes are predominantly colonized by C_4 plants and have $\delta^{13}\text{C}$ signatures of -14.4 to -17.7 ‰ (Chumura et al., 1987). It is likely that CO_2 evolved from salt marshes will also reflect this signature. Oil has a $\delta^{13}\text{C}$ value of -29 to -32 ‰ depending on the source of the oil. Therefore, if crude

oil biodegradation is occurring in a contaminated salt marsh, the $\delta^{13}\text{C}$ value of the evolved CO_2 should decrease. If this occurs, it would be possible to qualify and quantify hydrocarbon degradation by measuring total CO_2 production and changes in the ^{13}C signature of CO_2 produced from the marsh. Trapping and quantifying CO_2 is a simple process and numerous labs provide relatively inexpensive stable isotope analysis.

Here we demonstrate a simple method for monitoring and quantifying hydrocarbon mineralization using $\delta^{13}\text{C}$ values, both in controlled laboratory studies and field trials.

EXPERIMENTAL

Crude oil degradation was investigated both in controlled laboratory microcosms and in a small *in situ* study. Both studies used a "sweet" Louisiana crude oil (SLCO), which is a relatively non-toxic oil with high alkane, low polar, and moderate PAH concentrations. The site location was near Port Fourchon at the southwestern end of the Barataria basin in Louisiana. This site is located in the Leeville oil field. This marsh is continuously flooded with a tidal flux of approximately 0-40 cm and dominated by *Spartina alterniflora*.

Laboratory Microcosm Studies.

Biodegradation experiments were conducted in completely mixed microcosms in which the redox potential (Eh) and pH were continually monitored (Masscheleyn et al. 1992). Microcosms were operated in a completely aerated mode with air flow greater than 15 ml/min. The microcosms contained 1.8 liters of a 60:1 (w/w) slurry produced from surface soils (0-5 cm) obtained from the study site. Microcosms were contaminated

with unweathered SLCO at a concentration of 0.7 g oil/g soil. Three treatments were monitored: microbially-inhibited (2 g NaN_3 /flask), control (no fertilizer amendment), and fertilized (0.05 g-N and 0.05 g-P/ g soil). In addition, a treatment containing no oil was used to monitor background CO_2 production and natural $\delta^{13}\text{C}$ values. The flasks were aerated continuously with CO_2 -free air. The CO_2 was stripped from the incoming air using two in-line NaOH (3N) traps.

The microcosms were sampled for oil components by removing 20 ml of slurry and extracted using liquid extraction with 1:1 (V/V) hexane:acetone. The extracts were passed through MgSO_4 to remove water and concentrated under a stream of dry nitrogen. The CO_2 produced from the microcosms was trapped in 1N NaOH.

Field Studies.

An *in situ* field experiment was conducted in replicated 900 cm^2 plots. Marsh plots were isolated using plexiglass diffusion chambers consisting of a permanently installed base and a removable cover. These chambers allow for tidal fluctuations but retard sediment loss (Mathais et al., 1980). Two treatments were investigated: oil and oil plus fertilizer. The soil was contaminated (1.16 kg oil/ m^2) during low tide and allowed to sorb for 24 hours before the tidal cycle was allowed to reestablish. Every two weeks nitrogen (55 g-N/ m^2) and phosphate (55 g-P/ m^2) were added to the fertilized treatments. Once a week the chambers were equipped with NaOH (1N) traps, closed to the atmosphere, and purged with CO_2 free air. The CO_2 produced was collected for approximately one tidal cycle or 20 hours. Total CO_2 production ($\text{g}/\text{m}^2\text{-day}$) and the $\delta^{13}\text{C}$

value of the collected CO₂ were determined. The $\delta^{13}\text{C}$ signature of CO₂ produced from uncontaminated marsh soils was determined by using the same method.

After seven weeks, the soil within the chambers was cored using 15.3 cm diameter thin-walled aluminum cores. Approximately 20% of the surface area of each treatment was sampled. The top 3 cm of soil was removed and homogenized. Subsamples were taken and mixed with MgSO₄ to reduce the water content. The oil was extracted using supercritical fluid extraction with unmodified CO₂ (flow rate = 11 ml/min; extraction time = thirty minutes; oven temperature = 100°C; restrictor temperature = 175°C; and collection solvent (hexane/acetone 1:1) at 4°C). This extraction procedure had a recovery of 85-95% depending on the compound. Hopane ratios of oil extracted from marsh soils were compared to a weathered sample. The weathered sample was produced by exposing a sample of oil to atmospheric conditions for a similar period of time and then extracting the oil using the same procedure as used for the field samples.

Analyses.

The amount of CO₂ produced was determined by titration with HCl using a mixed indicator and the $\delta^{13}\text{C}$ signature determined by Geochron Laboratories (Cambridge, MA). The oil extracts were analyzed by GC-MS using 17 α ,21 β -hopane as a normalizing compound (Prince et al., 1994). Alkanes C12-C44, pristane, and parent, C1, C2, naphthalenes and phenanthrenes were monitored.

Theoretical.

The rate of CO₂ produced from each carbon source can be easily computed using three equations relating the CO₂ production and $\delta^{13}\text{C}$ signature.

$$R_o + R_i = R_t \quad (1)$$

$$\frac{R_o}{R_t} (S_o) + \frac{R_i}{R_t} (S_i) = S_t \quad (2)$$

$$\frac{R_o}{R_t} + \frac{R_i}{R_t} = 1 \quad (3)$$

where R_o and R_i are the rates of CO_2 production from the crude oil and indigenous carbon sources, respectively, and R_t is the total rate of CO_2 production. S_o and S_i are the $\delta^{13}\text{C}$ signatures of the crude oil and indigenous organic matter and S_t is the measured $\delta^{13}\text{C}$ signature of the produced CO_2 . S_o, S_i, S_t and R_t are experimentally determined. R_o and R_i can then be determined from equations 2 and 3. This assumes that CO_2 is generated from only these two carbon pools and that there is no addition of atmospheric CO_2 .

The method also assumes that the process of "microbial fractionation" of ^{13}C will have only a minor effect on the $\delta^{13}\text{C}$ signature of CO_2 evolved from contaminated marsh soils. Previous studies have demonstrated only small differences in the $\delta^{13}\text{C}$ signature of microbially-produced CO_2 and the parent crude oil. Additionally, differences between the $\delta^{13}\text{C}$ signature of various important components of crude oil have been shown to be minimal (Stahl, 1980; Kenicutt, 1988). These studies indicate that fractionation of different components of crude oils is a minor process and would have a negligible effect on the calculations performed using these equations in salt marshes.

RESULTS AND DISCUSSION

Kinetic Experiments.

The microcosm studies were conducted in triplicate and a representative run is presented for both alkane and PAH degradation. The microcosm studies showed rapid and nearly complete (greater than 90% reduction in the hopane ratio) degradation of parent alkanes in the fertilized treatments. In the unfertilized treatment, there was less than a 10% reduction in the hopane ratio of the alkanes (Figure 5.1A). PAH degradation was evident in both unfertilized and fertilized treatments (Figure 5.1B). The rate of PAH degradation differed greatly between treatments. There was no detectable lag time in the fertilized treatments while the unfertilized treatments had a brief lag period and degraded at a slower rate. Both treatments had equivalent ultimate reduction of the parent PAH hopane ratio (~85%). Significant PAH volatilization losses were noted in the inhibited control (reductions in hopane ratios from ~70 to ~35). Losses are largely thought to be due to naphthalene present. This suggests that these salt marsh soils are capable of hydrocarbon degradation and that fertilizers will affect specific classes of compounds differently. Extensive degradation occurred for all monitored compounds in the fertilized treatments, while only the PAH (10% of the alkane concentration) degraded extensively in the unfertilized microcosm.

CO₂ production was correspondingly greater in the fertilized treatment. The average rate of total CO₂ production for the non-oiled fertilized (NOF), oiled no fertilizer (O), and oiled fertilized (OF) microcosms was 0.174, 0.396, 1.86 mg CO₂-C/day-gram soil respectively. The large increase in CO₂ production between the OF and the NOF

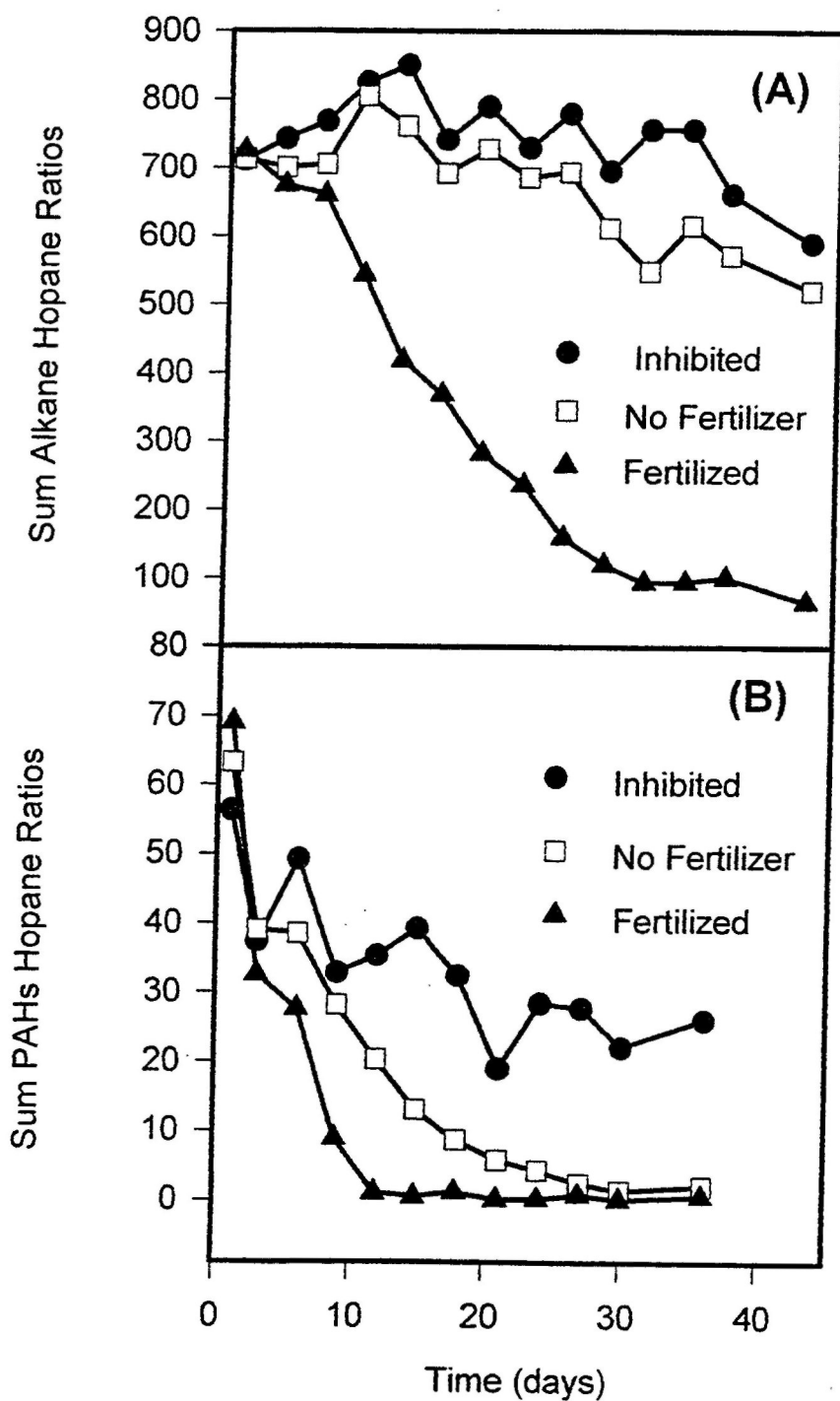


Figure 5.1 (A) Total (C15-C44) alkane hopane ratio versus time in aerated microcosms. (B) Total PAH (phenanthrene, C1-C3; naphthalene, C1-C3) hopane ratio versus time.

treatments suggests that the increase in CO₂ production is from the mineralization of the crude oil and not indigenous carbon sources.

There was a definite decrease in the $\delta^{13}\text{C}$ signature of evolved CO₂ in both treatments contaminated with oil (Figure 5.2). The measured $\delta^{13}\text{C}$ signature of CO₂ evolved from noncontaminated soil is $-17.1 \pm 0.50/00$. The fertilized treatment approaches the $\delta^{13}\text{C}$ ratio of pure oil (-29.1) and varies between -27 and -29 0/00. The nonfertilized treatment varies between -25 and -27 0/00. While the $\delta^{13}\text{C}$ signatures of CO₂ evolved from the two treatments are within several 0/00, total CO₂ evolution is dramatically different. The $\delta^{13}\text{C}$ signature is dependent on both the signatures of the carbon sources and the amount each source contributes to the total CO₂ evolved (Aggarwal and Hinchee, 1991). The small difference in the ratios between the two treatments is due to the small amount of CO₂ produced from the soil carbon. This causes the S_i term in equation 3 to become very small and thus the $\delta^{13}\text{C}$ signature is controlled by the mineralization of oil.

This technique also can be used to quantify the amount of crude oil mineralized. By using the $\delta^{13}\text{C}$ signatures, CO₂ production data and the isotope dilution equations, the amount of CO₂-C produced from crude oil can be computed (Figure 5.3). The predictive ability of these equations is supported by the similarity between the first order rate constants in the reduction of hopane ratios (Figure 5.1A) and the calculated CO₂ production from the crude oil carbon pool (Figure 5.3), 0.082 and 0.087 day⁻¹, respectively. In addition, the average total CO₂-C from non hydrocarbon sources in the OF and O microcosms predicted by equations 1-3 (6.6 and 4.8 mg/g soil) is similar to the total CO₂ directly measured from the NOF microcosm (9.6 mg/g soil). While there are

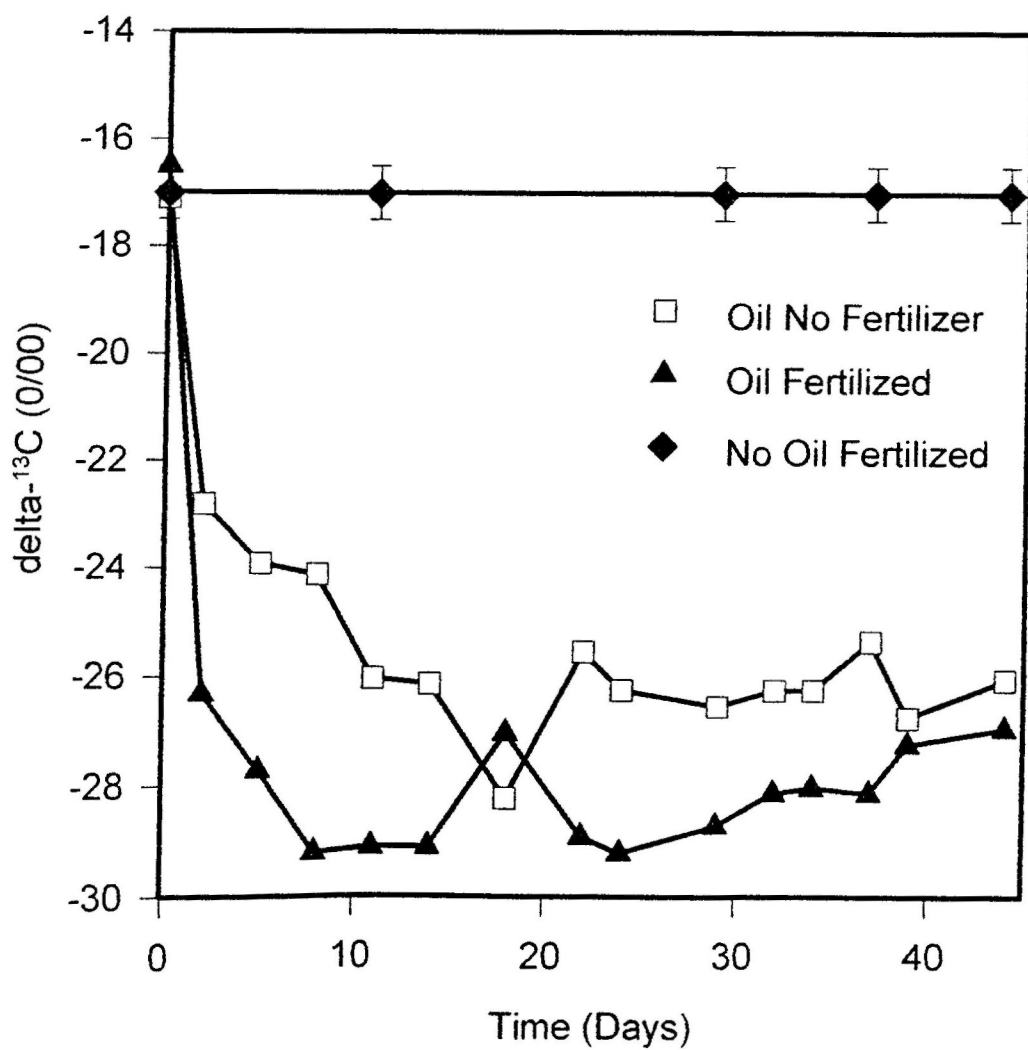


Figure 5. 2 $\delta^{13}\text{C}$ signatures (‰) of CO_2 versus time in aerated microcosms.

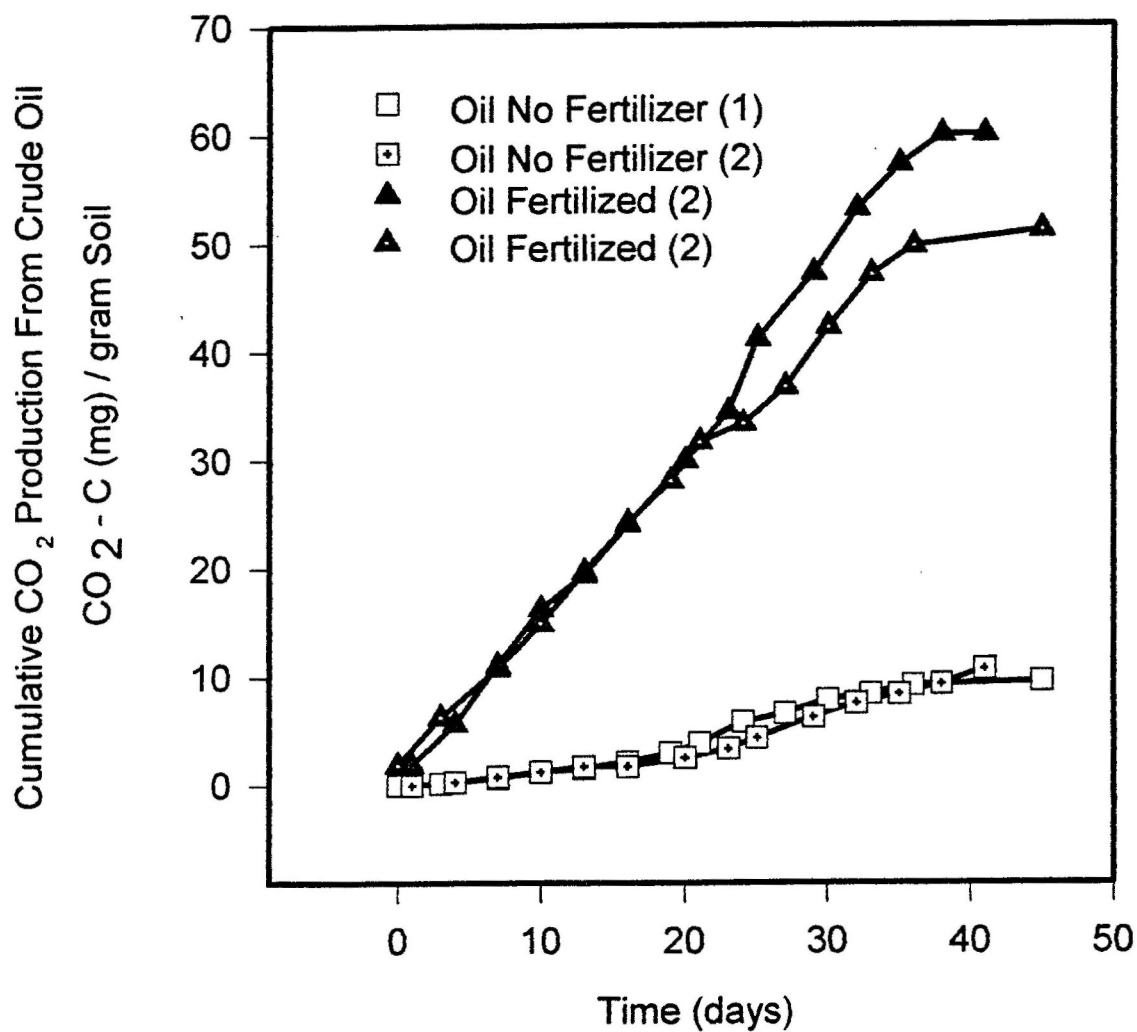


Figure 5.3 Cumulative CO₂-C production (mg/g soil) from crude oil versus time in aerated microcosms (calculated from isotope dilution equations).

large differences in the conditions of the treatments, the similarity of these values support the ability of these equations to accurately predict CO₂ production of each source. The importance of the $\delta^{13}\text{C}$ data is its ability to calculate mineralization rates directly. It measures the final product while monitoring hopane ratios only measures the disappearance of the parent compound, not mineralization. The microcosm experiments support the ability to use $\delta^{13}\text{C}$ signatures in conjunction with CO₂ production to qualitatively and quantitatively monitor crude oil degradation.

Field experiment.

A small-scale field experiment was conducted to verify the ability to detect $\delta^{13}\text{C}$ signature changes in CO₂ evolved *in situ* from oil-contaminated soils. Significant decreases in the $\delta^{13}\text{C}$ signature of evolved CO₂ were detected five weeks after oiling in fertilized (F1 and F2) and unfertilized (C1 and C2) treatments (Table 5.1). As expected, changes in the $\delta^{13}\text{C}$ signature of CO₂ evolved from contaminated field plots were not as extreme as in the dilute microcosm slurries. This is due to the large amount of background CO₂ production from indigenous organic matter in the plots. Changes in the $\delta^{13}\text{C}$ signature of evolved CO₂ were easily detectable, however, at these oil concentrations. Decreases in the alkane- and PAH-hopane ratios accompanied these changes in $\delta^{13}\text{C}$ signature of evolved CO₂ for all treatments (Figure 5.4 and 5.5). However, the C1 and C2-phenanthrene appear to be stable over the time period of this experiment.

The CO₂ production rates and the $\delta^{13}\text{C}$ signatures measured were used to calculate the CO₂ produced from crude oil (Figure 5.6). No mineralization of crude oil was

Table 5.1 Maximum $\delta^{13}\text{C}$ signatures and corresponding total CO_2 production rates measured during the 7 week field study.

Treatment	Week	$\delta^{13}\text{C}$ signatures (‰)	Total $\text{CO}_2\text{-C}$ (mg/hour- m^2)
Fertilized 1 (F1)	5	17.9	14.22
Fertilized 2 (F2)	5	18.5	8.11
Unfertilized 1 (C1)	6	19.1	17.1
Unfertilized 2 (C2)	6	18.9	12.6

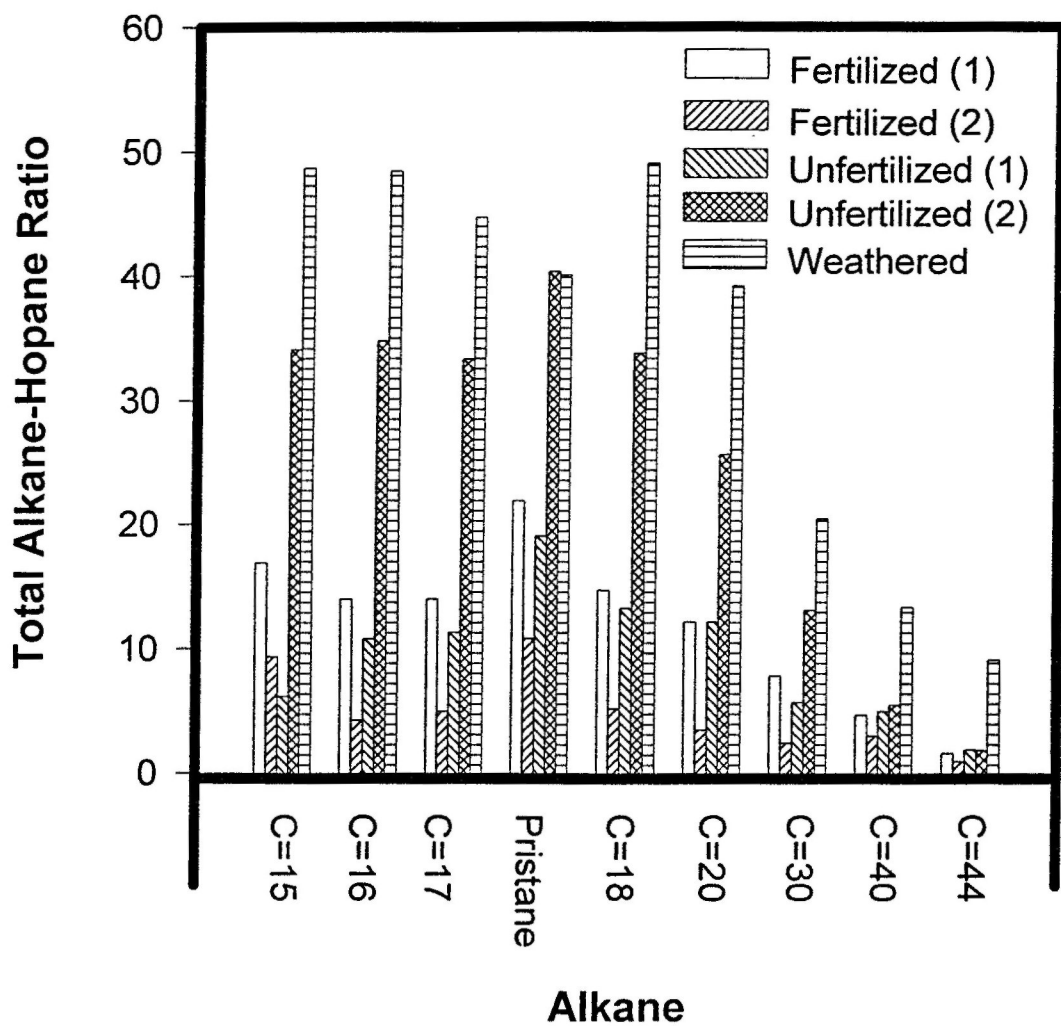


Figure 5.4 Comparison of final hopane ratios of alkanes and pristane in fertilized and unfertilized salt marsh soils after 7 weeks.

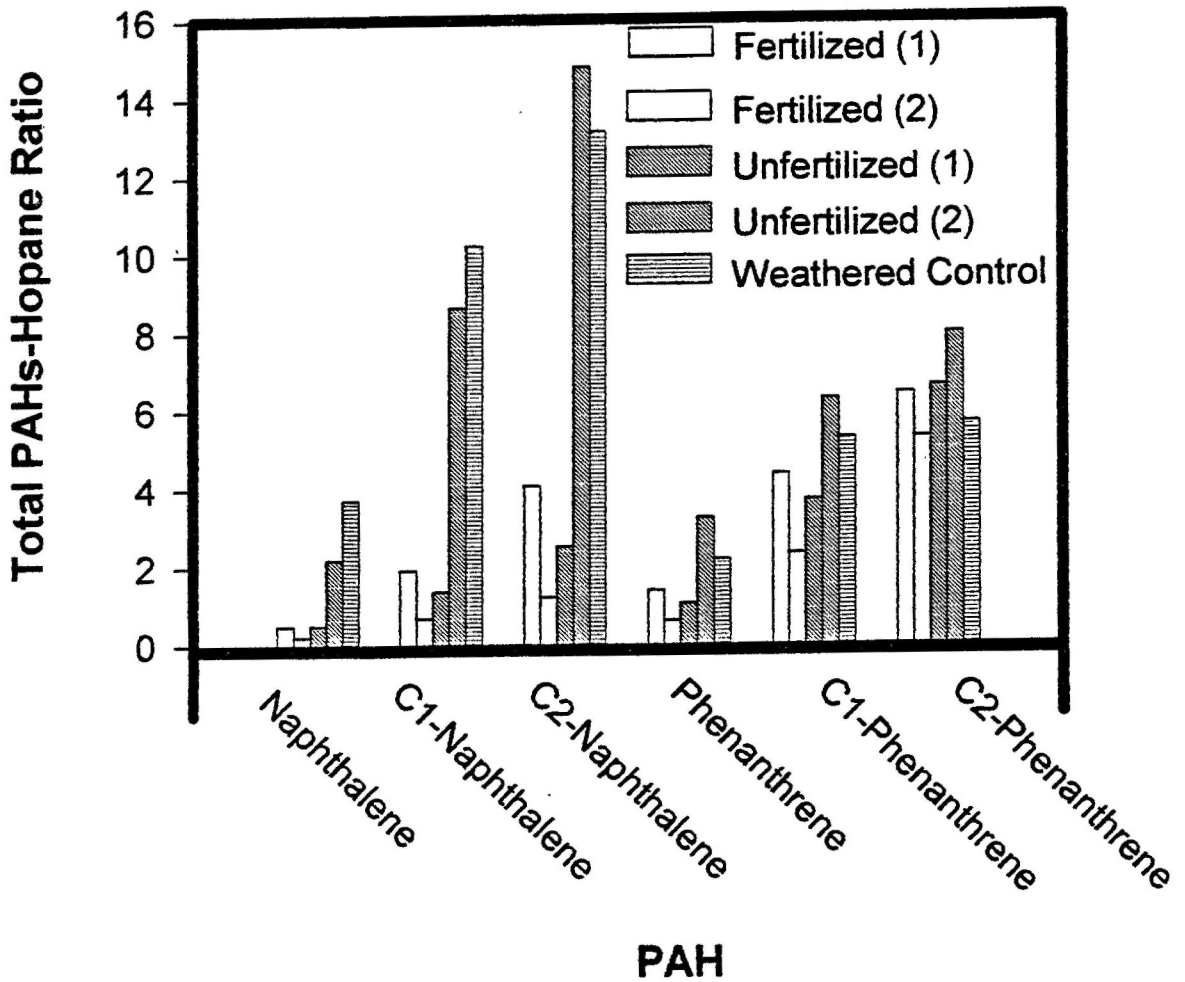


Figure 5.5 Comparison of final hopane ratios of PAHs in fertilized and unfertilized salt marsh soils after 7 weeks.

detected until week 2 and the majority of mineralization appears to begin at week 5. The fertilized treatments appeared to show higher mineralization rates before the unfertilized and to mineralize at a more even prolonged rate. The unfertilized treatments have a more intense rate of mineralization but for only one sampling date.

The dependence of *in situ* microbial oil degradation on available nutrient concentrations, mainly N and P, has been demonstrated repeatedly. Most field work has centered on cold water, high energy beaches with inherently low nutrient concentrations (Bragg et al., 1994; Lindstrom et al., 1991). In general, oleophilic nutrients seem to be the most successful in these environments (Tabak et al., 1991; Atlas and Bartha, 1973; Glasser et al., 1991). Increases in degradation rates produced from nutrient addition are variable depending on the compound. Limited work has been performed involving low energy wetland systems. These systems seem to be amenable to either inorganic or oleophilic nutrient additions, with some studies suggesting microbial degradation of the oleophilic nutrient over oil (Lee and Levy, 1994 and 1989).

Louisiana's salt marshes are low energy (physical) systems, tidal effects are mainly limited to surface water with little pore water exchange. Inorganic nutrient addition would appear to be a plausible strategy for enhancement of oil degradation. However, Swannell (1994) has suggested that low energy systems may be controlled by oxygen limitations rather than nutrient limitations. Salt marshes, mainly due to their high organic content, are oxygen depleted with only the top few millimeters oxidized. While detected some studies have shown that specific components of oil will degrade

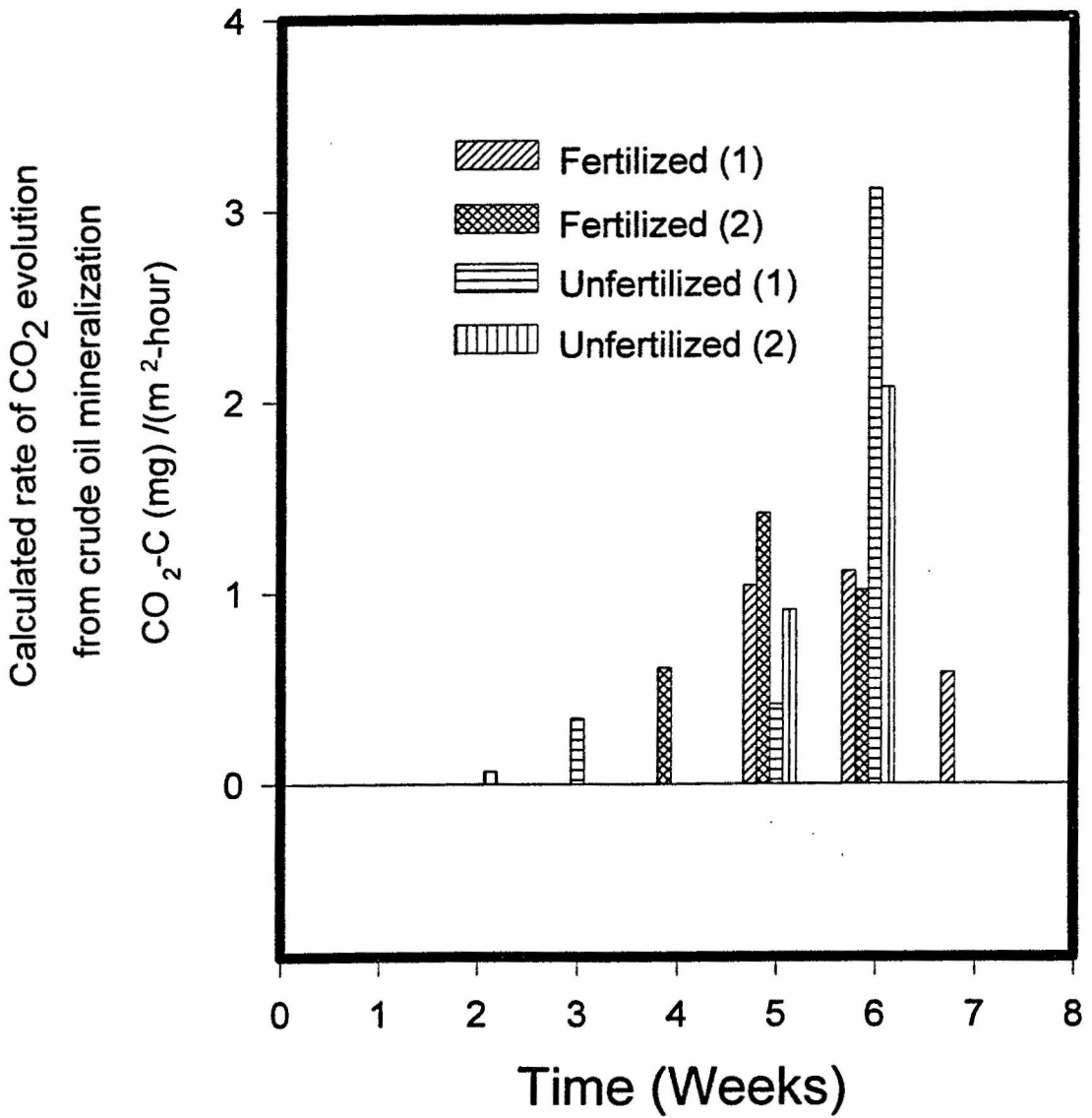


Figure 5.6 Rates of CO₂-C (mg/hour-m²) mineralized from crude oil in fertilized and unfertilized salt marsh soils (calculated from isotope dilution equations).

anaerobically, these rates are much slower than aerobic rates of crude oil degradation (Hambrick et al., 1980).

Amendments of fertilizer inconclusively increased degradation as evidenced by hopane ratios of specific oil components. Reductions in total hopane ratios between treatments varied from little to almost complete with treatment $F2 > F1 \sim C1 > C2$. Reductions in hopane ratios were consistent across compounds although little degradation was found for the C1 and C2 phenanthrenes. The total predicted amount of CO_2 -C mineralized from oil over the seven one-day collections were 42, 46, 58, and 46 mg for treatments F1, F2, C1, C2 respectively. The lack of effect of the fertilizer could be due to oxygen limitations in the soil.

Fertilizer amendments did significantly increase pore water NH_4^+ concentrations. The concentration of NH_4^+ -N (water extractable) in the soils at the conclusion of the experiment was 0.462, 0.78, and 2.458 mg NH_4 -N/g soil for the unoiled, oiled unfertilized, and oiled fertilized respectively. Even though the concentration of NH_4^+ in the fertilized treatment is much higher, the unfertilized soils still contain significant levels of NH_4^+ . This suggests that N was not limiting oil biodegradation in this salt marsh. Surprisingly, no increase in CO_2 evolution was measured between oiled and unoiled treatments. The average rates of total CO_2 production were 314, 346 and 265 mg CO_2 -C/m²-day for the nonoiled, oiled unfertilized, and oiled fertilized soils respectively. These values are in good agreement with previously measured rates (Feijtel et al., 1985). The NH_4^+ concentrations, coupled with the constant CO_2 production, indicate that the rate of

degradation may be controlled by oxygen transfer and not nutrient or carbon availability. If oxygen is limiting, then fertilization would have little effect.

The sensitivity of this method is dependent on the difference in $\delta^{13}\text{C}$ signatures and the rate of CO_2 production. Large amounts of indigenous respiration will increase the rate of oil mineralization necessary to detect changes in $\delta^{13}\text{C}$ signatures of evolved CO_2 . However, our results indicate that it could easily be more sensitive than any other methods used *in situ*. Rates of crude oil mineralization of the treatments ranged from approximately 0.2-3 mg $\text{CO}_2\text{-C}/\text{hour-m}^2$. Perhaps more importantly, it is the only method that directly measures mineralization not transformation.

CONCLUSIONS

Monitoring *in situ* oil biodegradation is possible using a variety of techniques. However, all techniques to date suffer from a variety of deficiencies. The relatively slow rate of oil degradation *in situ* and the large spatial variability present, allow most methods to be useful only over long periods of time, normally months. This complicates, if not makes impossible, the ability to determine which management techniques are stimulating or inhibiting biodegradation. In addition the effects of short-term environmental factors such as tides are impossible to determine. Lastly, due to physical losses, applying biodegradation rates calculated from month-long intervals will have large errors.

Stable carbon isotope signatures of produced CO_2 offer the ability to solve these problems in environments such as salt marshes with appropriate background $\delta^{13}\text{C}$ signatures. The method is sensitive enough to measure mineralization rates in time periods of a few hours, depending on the rate of biodegradation and concentration of oil.

This allows short-term effects of fertilizers, dispersants and environmental factors to be determined. In addition, quantitating oil mineralization is possible. Physical losses are not necessary to determine since the rates of mineralization can be determined for an area directly instead of back calculating to a previous concentration which is dependent on physical losses. This method does have limitations. It can not resolve specific compounds, and it is also not applicable to many areas since it is only useful in areas with appropriate background signatures. However, coupling this method with other methods such as monitoring hopane ratios would greatly enhance the ability to monitor oil degradation. The cost of this method is relatively inexpensive and little sample preparation necessary.

CHAPTER 6. CONCLUSIONS

The incident of a major oil spill in the coastal marshes of the Gulf coast is a near certainty in the future. Recently, spills have occurred in Galveston Bay and historically in Louisiana's southern most parishes. Coastal marshes are unique systems with many characteristics (large surface areas, large stores and high internal cycling of nutrients, high biological activity, temperate climates, low pore water exchange rates, etc..) which promote degradation in relation to other commonly affected systems (e.g. beaches, open water, and subsurface soils). However, some of these same characteristics along with others (high accretion rates and low oxygen availability) could as easily be expected to inhibit or limit degradation. There is an enormous amount of literature on crude oil degradation in dissimilar systems but this information will have little direct application to coastal marshes given their unique properties. A review found little information regarding the controlling aspects and inherent potential for crude oil degradation in coastal marshes, the seasonal influence and variability of crude oil degradation, and a serious deficiency in *in situ* monitoring techniques.

Laboratory studies were initiated to determine the ability of nutrient enhancements to promote degradation. Both the composition and form of nutrient enhancements were examined. The alkane and PAH fractions seem to be independently degraded and these systems appear to have much greater capacity to degrade PAHs. Nitrogen was found to be a limiting factor for both alkane and PAH fractions, although the PAH fraction was completely degradable with or without nitrogen enhancements.

Phosphate had only a minimal beneficial effect on alkane transformation rates and none for PAH transformation. Nitrogen in either a reduced (NH_4) or oxidized form (NO_3) appears to be able to increase degradation in lab studies. Ammonium stimulated mineralization to a greater extent at lower concentrations; however, it seems to have a fairly narrow range of concentrations which will maximize degradation and fairly high concentrations are necessary for any stimulation to occur. Nitrate appears to stimulate mineralization at lower concentrations but significant stimulation required 10 times the concentration of ammonium, no inhibition was found.

In undisturbed core studies PAHs also had higher transformation rates and there was little difference in nitrogen enhanced and control cores. It is likely that PAHs will degrade in these systems without any nutrient enhancements. The alkane fraction was degraded to a lesser extent and only very large concentrations of nitrogen appeared to stimulate degradation even slightly. A small in situ study also showed little benefit to nutrient enhancements for either fraction. Oxygen limitations are the most likely reason. Little increase in CO_2 production was observed between uncoiled and oiled cores suggesting that biological mineralization activity is limited by oxygen availability, although nutrient limitations should still exist once oxygen limitation are removed.

Seasonal variations in crude oil degradation was pronounced in both fresh and salt marsh systems although the pattern was quite distinct. Variations appear to be uncoupled from most single environmental parameters, in addition the PAH and alkane fraction appear to be uncoupled. The PAH fraction of crude oil was mineralized to a greater extent for both systems as in other studies. The fresh marsh appears to have a

greater potential to degrade crude oil than the salt marsh. Nutrient enhancements had distinct affects for each marsh. The salt marsh is nutrient limited throughout the year, while the fresh marsh apparently is nutrient limited for only selected seasons and fractions. PAH mineralization appears to be unaffected by nutrient additions throughout the year and alkane mineralization was only slightly enhanced in winter. Another important effect of nutrient additions is a decrease in lag times. These systems have high accretion rates and oxygen limitations. Decreasing the lag time to degradation will increase the time that the crude oil coexists with oxidized sediments and thus is efficiently degradable and easily amendable to remediation efforts. Nutrient additions significantly decreased lag times in both the fresh and salt marsh systems although the response was more pronounced in the salt marsh. Strategies for remediation will be much more efficient if both the class of compounds, the type of marsh, and the season are incorporated into remediation efforts.

One of the major limitations in studying crude oil biodegradation as well as other pollutants is the lack of a sensitive, and specific (i.e. not affected by physical or chemical factors) monitoring technique, and a complete lack of ability to monitor mineralization *in situ*. Many possible stimulatory or inhibiting influences both natural and introduced could potentially have significant effects but on time scales too small to be monitored by current techniques. In addition most current techniques are technically demanding and expensive; a major drawback when considering monitoring large scale spills covering hundreds of acres. The use of stable carbon isotope ratios to monitor crude oil degradation solves many of these problems.

By monitoring stable carbon isotope signatures of produced CO₂, it is possible to quantify the mineralization of crude oil *in situ* in appropriate environments. In both laboratory experiments and field studies it was possible to quantify crude oil mineralization. The technique is simple and fairly inexpensive in relation to GC-MS analyses which is commonly used. Monitoring mineralization not transformation is important because many compounds can be transformed to more toxic intermediates. In addition, it would be possible to determine the ultimate fate of crude oil between mineralized and residual pools, an important factor in determining the success of remediation efforts and studying the ultimate fate of introduced crude oil. The method is sensitive enough to allow short term effects such as tides, dispersants or fertilizer amendments to be determined.

This study while elucidating many facets of the ability of coastal marshes to degrade crude oil, the role of nutrient enhancements, and seasonal influences has also illustrated many areas in which a fundamental lack of knowledge exists. Future work should further explore the differences in nutrient form with special emphasis on the differences on *in situ* remediation strategies which exist between the ability of increasing degradation and the cost of application. Oxygen limitation will also play a critical role in any remediation strategy involving these systems and a thorough understanding of the relationship between oxygen demand exerted naturally and with additional carbon substrate (crude oil) as well as, the influence of nutrient enhancements on both uncontaminated and contaminated systems.

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APPENDIX A.

Crude oil analyses were performed using a Hewlett-Packard 5890 gas chromatograph coupled with a 5972A mass selective detector (GC-MS) in the selected ion monitoring (SIM) mode. Chromatographic conditions were as follows: A 1 μ L splitless injection onto a 30 m (length), 0.53 μ m (diameter) HP-5 capillary column into an injector held at 300°C; initial oven temperature was at 55°C (3 minutes), programmed to 310°C at 6°C per minute; GC-MS interface held at 280°C. A range of deuterated compounds served as internal standards: 1,4-dichlorobenzene-*d*4, naphthalene-*d*8, acenaphthene-*d*8, phenanthrene-*d*10, and chrysene-*d*12. The following ion groups were monitored: Quantitation was performed by using 5-point calibration curves referenced to the deuterated internal standards. Authentic standards (Supelco, Inc.) were used for each calibration curve. Calibration checks and blanks were performed with each 24 hour sample set.

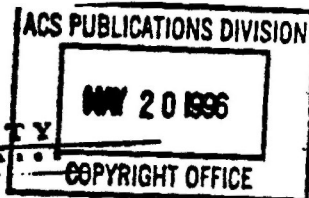
Table A.1 Compounds and associated monitored ion and retention time.

I o n (m/z)	Compound monitored	Structure	Retention time (min.)
85.05	Decane	$C_{10}H_{22}$	7.5
	Undecane	$C_{11}H_{24}$	10.06
	Dodecane	$C_{12}H_{26}$	12.52
	Tridecane	$C_{13}H_{28}$	14.85
	Tetradecane	$C_{14}H_{30}$	17.03
	Pentadecane	$C_{15}H_{32}$	19.10
	Hexadecane	$C_{16}H_{34}$	21.05
	Heptadecane	$C_{17}H_{36}$	22.91
	Pristane	$C_{17}H_{36}$	23.04
	Octadecane	$C_{18}H_{38}$	24.67
	n-eicosane	$C_{20}H_{42}$	27.95
	n-tetracosane	$C_{24}H_{50}$	33.72
	n-octacosane	$C_{28}H_{58}$	38.65
	n-dotriacontane	$C_{32}H_{66}$	42.96
	n-hexatriacontane	$C_{36}H_{74}$	46.92
128.10	Naphthalene	$C_{10}H_8$	12.09
142.00	C-1 naphthalenes	$C_{11}H_{11}$	range of peaks
152.00	Acenaphthylene	$C_{12}H_8$	18.04
153.00	Acenaphthene	$C_{12}H_{10}$	18.78
156.00	C-2 naphthalene	$C_{12}H_{14}$	range of peaks
166.10	Fluorene	$C_{13}H_{10}$	20.72
178.05	Phenanthrene	$C_{14}H_{10}$	24.33
	Anthracene	$C_{14}H_{10}$	24.49
192.00	C-1 phenanthrene	$C_{15}H_{13}$	range of peaks
202.05	Fluoranthene	$C_{15}H_{10}$	28.88
	Pyrene	$C_{16}H_{10}$	29.67
206.00	C-2 phenanthrene	$C_{16}H_{16}$	range of peaks
228.10	Benzo(a)anthracene	$C_{18}H_{12}$	34.38
	Chrysene	$C_{18}H_{12}$	34.55
252.10	Benzo(b)fluoranthene	$C_{19}H_{12}$	38.28
	Benzo(k)fluoranthene	$C_{19}H_{12}$	38.38
	Benzo(a)pyrene	$C_{20}H_{12}$	39.30
276.10	Benzo(g,h,i)-perylene		42.70
	Indeno(1,2,3-cd)-pyrene		43.37
278.10	Dibenzo(a,h)-anthracene	$C_{22}H_{14}$	42.83

APPENDIX B.



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To: Arleen Courtney
American Chemical Society
1155 16th st. North West
Washington D.C. 20036

May 20, 1996

Dear Madame

I am writing to request permission to include in my dissertation a paper recently published in your journal. The title of the paper is "Monitoring Crude Oil Mineralization in Salt Marshes: Use of Stable Carbon Isotope Ratios" in *Envir. Sci. Tech.* Vol 30:4, pp. 1139-1144. I will also agree to print the ACS copyright credit line on the first page of the article.

Thank you very much for your attention to this matter, please refer to the address or fax number in the letter head, or if further information is required my number is 504-388-1153.

Sincerely, Andrew Jackson



American Chemical Society

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WASHINGTON, D.C. 20036
Phone (202) 872-4367 or -4368
Fax (202) 872-6060

FAX NUMBER: 504/388-5990

DATE: May 20, 1996

MEMORANDUM

TO: Andrew Jackson
Louisiana State University
Department of Civil & Environmental Engineering
College of Engineering
Baton Rouge, Louisiana 70803-5401

FROM: C. Arleen Courtney *C. Arleen Courtney*
Copyright Assistant

RE: Your letter dated May 20, 1996

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Thank you for writing. If you have any questions, please call me at 202/872-4368.

4/25/96

VITA

W. Andrew Jackson was born July 12, 1968 in Shreveport, Louisiana. He graduated from Rhodes College in Memphis, Tennessee in 1990 with a bachelor of science in Biology. In August, 1990 he enrolled as an Alumni Fellow in the graduate program in Environmental Engineering at Louisiana State University at Baton Rouge, Louisiana. He received the master of science degree in Engineering Science with a major in Environmental Engineering in 1993 under the direction of Dr. Dipak Roy. In October of 1993 he joined Dr. Pardue's research team and in 1995 was employed as a research associate. He will receive his doctorate in August of 1996.

DOCTORAL EXAMINATION AND DISSERTATION REPORT

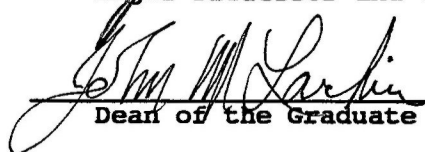
Candidate: William A. Jackson

Major Field: Engineering Science

Title of Dissertation: Hydrocarbon Degradation in Louisiana's
Salt and Fresh Marshes: Inherent Potential, Enhancement, and
In-Situ Monitoring Techniques.

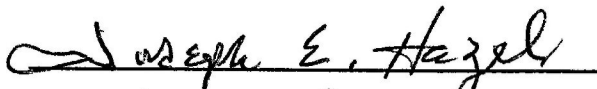
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EXAMINING COMMITTEE:









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