Winter 2002

Oxygen and nitrate enhanced in situ bioremediation of an oil -contaminated salt marsh

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OXYGEN AND NITRATE ENHANCED *IN SITU* BIOREMEDIATION OF AN OIL-CONTAMINATED SALT MARSH

BY

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DISSERTATION

Submitted to the University of New Hampshire
In Partial Fulfillment of the Requirements for the Degree of

Doctor of Philosophy
in
Engineering: Civil

December 2002

ProQuest Information and Learning Company
300 North Zeeb Road
P.O. Box 1346
Ann Arbor, MI 48106-1346

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ACKNOWLEDGEMENTS

Funds for this research were provided by the Cooperative Institute for Coastal and Estuarine Environmental Technology (CICEET), in cooperation with the National Oceanic and Atmospheric Administration (NOAA) and the New Hampshire Department of Environmental Services (NHDES).

I would like to thank my advisor Dr. Nancy Kinner for her invitation to come to UNH, her teaching and her comments. She has been a star who always points north.

Additionally, I would like to thank Dr. Thomas Ballestero for his help and comments during the field study and for his sense of humor. I would like to thank Dr. Phil Ramsey for his time and willingness to discuss the results and the statistical approach to understand them.

I want to thank to the Colombian Government and especially COLCIENCIAS for the scholarship that allowed me to concentrate on my studies and finish my doctorate. I also thank the Pontificia Universidad Javeriana, especially Dr. Elizabeth Hudson, for giving me the time to finish my writing.

I would like to thank my mother, sisters and brothers for their moral support and love that gave me the strength to finish. I would like to thank to Paula, Maria Isabel Gonzalo for giving me a home and support here at UNH.

Finally I would like to extend my thanks to Marylin my love and life, for her support, sense of humor and final push to finish what I started.

DEDICATION

I would like to dedicate this dissertation to my mother Cecilia and Marylin, my true love. To the memory of Fabio and Elisa, they will always be in my heart.

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ABSTRACT

OXYGEN AND NITRATE ENHANCED *IN SITU* BIOREMEDIATION OF AN OIL-CONTAMINATED SALT MARSH

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Salt marshes are among the most ecologically-sensitive areas to oil spills and remediation activities. Contaminated marshes may take years or decades to recover. Bioremediation is the process of enhancing naturally-occurring biodegradation by supplying limiting nutrients and terminal electron acceptors (TEAs). During this study, two TEAs (O_2 and NO_3^-) were evaluated for their ability to enhance natural *in situ* biodegradation of total petroleum hydrocarbons (TPH) in an oil-contaminated marsh. EPA (9071A) and ASTM (D5831) methods were evaluated for screening TPH in the contaminated marsh sediments. The ASTM Method was selected to evaluate TPH levels in candidate sites at the Fore River Creek salt marsh, Portland, ME impacted by the *Julie N* oil spill in 1996. Two plots in the marsh received air and NO_3^-, two served as controls. Subsurface horizontal wells were used to inject the amendments into the sediments. During 1998-1999, degradation of short chain (SC) and long chain (LC) aliphatics and aromatics, abundance of oil-degrading bacteria, nutrients and *Spartina alterniflora*

growth were monitored.

Results indicated that natural attenuation (control) significantly reduced the TPH. The overall (1998-1999) degradation rates in the controls were 7.8±2.1 and 3.0±1.0 $mg/kg_{dw}/d$ for SC and LC aliphatics, respectively; and 6.9±4.8 $mg/kg_{dw}/d$ for aromatics. The NO_3^- amendment degradation rates for SC aliphatics and aromatics were 4.7±2.4 $mg/kg_{dw}/d$ and 4.5±3.3 $mg/kg_{dw}/d$, respectively. These degradation rates were not significantly different than control rates. During the first season (Summer and Fall 1998), the air and NO_3^- amendments significantly degraded more SC aliphatics than the control, while NO_3^- significantly degraded LC aliphatics. Porewater monitoring indicated more NO_3^- amendment was needed to promote denitrification. In addition, low degradation rates in the amendments plots may have been caused by problems with the well (distribution) system and mass transfer limitations. There was no significant change in the abundance of oil-degraders, probably because they were already established when the study began two years after the spill. Subsurface addition of air and NO_3^- has the potential to accelerate *in situ* biodegradation of Nos. 2 and 4 fuel oils in marsh sediments if problems with the supply of the amendments can be overcome.

CHAPTER I

INTRODUCTION

One of the most important environmental problems, not only in the United States (U.S.) but worldwide, is oil spills onto soil and water. The U.S. uses over 945 million m^3 of oil and petroleum products per year and to satisfy this it produces 473 million m^3 of crude oil and imports 472 million m^3 of crude oil (EPA, 1998). Most soil and water contamination is caused during the loading and unloading of oil in ports. In addition, a significant amount of oil is spilled during the extraction, transport, storage and refining processes.

Of all oil-impacted estuarine and coastal environments, salt marshes are one of the most sensitive and fragile ecosystems (Wright *et al.*, 1997). Salt marshes are highly productive and serve as nursery grounds for fish, shellfish and other marine organisms, providing a place where the young can grow rapidly (Carman *et al.*, 1996). Salt marshes can be impacted by petroleum spills from marine vessels and oil pipelines. Oil may coat plants, remain on the water surface or become associated with the sediments (Wright *et al.*, 1997). Oiled wetlands and salt marshes may take years or decades to recover (Fingas, 2000).

Oil spill remediation in salt marshes differs from remediation of contaminated beaches because mechanical cleaning is not desirable as it causes more damage than the oil (Adrian *et al.*, 1998). Methods used as remediation technologies include cutting,

washing or burning of oiled vegetation, but these may also cause more damage (Wright *et al.*, 1997). In addition, salt marshes are very sensitive to physical damage caused by crews and equipment used during remediation.

The oil released into the environment is affected by natural attenuation processes (biodegradation, dispersion, sorption, volatilization and photodegradation). Natural attenuation modifies the fate and transport of hydrocarbons (HCs) and under favorable conditions, it can act without human intervention to reduce the mass, toxicity, mobility, volume or concentration of the contaminants.

Many naturally-occurring microorganisms can degrade HCs and use the energy they derive from this to create cell biomass and less complex organic compounds or, upon complete degradation, water and carbon dioxide. The ability of microorganisms to degrade a great number of HCs in a wide range of environments has been described in the literature (Alexander, 1994; Atlas, 1978, 1981; Bartha, 1986; Margesin and Schinner, 2001a; McKee and Mendelssohn, 1994).

Bioremediation is the process of enhancing naturally-occurring biodegradation. It consists of a suite of cost-effective and non-invasive cleanup technologies that have gained wide acceptance to restore HC-contaminated environments.

Several oil spill studies have shown that *in situ* biodegradation is a major reason for decreases in HC concentrations. For example, the success of HC degradation was scientifically demonstrated during studies of the *Exxon Valdez* oil spill in Alaska (Lindstrom *et al.*, 1991; Pritchard, 1993; Wolfe *et al.*, 1994). These studies demonstrated that the correct nutrient mass balance (nitrogen and phosphorus) and the presence of the appropriate terminal electron acceptor (TEA) were the most important factors to achieve

higher biodegradation rates in the oil-contaminated environments (rocky intertidal shorelines, primary low energy beaches) (Braddock *et al.*, 1995; Pritchard, 1993; Wolfe *et al.*, 1994).

Coastal salt marsh sediments are saturated, limiting oxygen (O_2) transfer from the atmosphere. Any oxygen dissolving into the porewater is rapidly consumed with depth because of the abundance of organic matter. This results in a thin (few mm) aerobic layer (Shin *et al.*, 2000). The low oxygen concentrations may limit the presence of aerobic microorganisms able to degrade the HCs present in oil-contaminated sediments. As oxygen is depleted, the activity of obligate aerobic microorganisms ceases and facultative and obligate anaerobes use alternate TEAs such as nitrate (NO_3^-), manganese, iron, sulfate (SO_4^{-2}) and carbon dioxide in a sequential fashion (Hurst *et al.*, 1997). In the absence of oxygen, microorganisms can use NO_3^- as an alternative TEA. However, NO_3^- is not usually available in marshes because its concentration is low in marine environments (McKee and Mendelssohn, 1994).

The predominant TEA in salt marsh porewater is SO_4^{-2}, even though the energy yield is less than other TEAs (e.g., NO_3^-). Sulfate reduction is usually the dominant metabolism for oxidation of organic carbon in coastal marine sediments because of the great availability of SO_4^{-2} in marine environments (Rooney-Varga *et al.*, 1997). Sulfate reducing bacteria (SRB) can degrade a variety of HCs (Coates *et al.*, 1997; 1998; Madsen *et al.*, 1995). The HCs usually metabolized by SRBs include small aliphatic alkanes and single aromatics (Heider *et al.*, 1999).

One of the main ways to enhance the *in situ* biodegradation of organic contaminants is to add more efficient TEAs than SO_4^{-2} into salt marsh sediments.

Usually, research projects have focused on adding nutrients on the surface of the beaches and salt marshes. However, studies have shown that tidal and dilution factors remove most of the treatments and they did not go into the pore spaces where the contamination was present (Levin and Gealt, 1997; Venosa *et al.*, 1996).

Availability of nutrients is a major limitation in bioremediation of HC-contaminated soils (Atlas and Bartha, 1973a; Margesin and Schinner, 2001b; Olivieri *et al.*, 1976). Nitrogen and phosphorus are the most important nutrients necessary to achieve biodegradation.

The Cooperative Institute for Coastal and Estuarine Environmental Technology (CICEET) is a national center for the development and application of innovative environmental technologies for monitoring, management, and prevention of contamination in estuarine and coastal waters. The institute is associated with the University of New Hampshire (UNH) and the National Oceanic and Atmospheric Administration (NOAA). This project was funded by CICEET and the New Hampshire Department of Environmental Services (NHDES) to study bioremediation of oil-contaminated salt marshes. The main goal was to conduct a field study to evaluated four methods of bioremediation (natural attenuation, nutrient addition, bioventing and NO_3^- addition) to cleanup a salt marsh contaminated by petroleum HCs.

The specific objectives of the project were to:

- determine the zone(s) within the marsh that were most affected by petroleum contamination, so bioremediation methods would be targeted effectively;

- develop air (TEA), NO_3^- (TEA) and nutrient (nitrogen and phosphorus) delivery and monitoring systems that minimize the impact and invasion of the marsh;
- determine the petroleum attenuation in the marsh for each bioremediation method; and
- recommend the types of salt marsh applications for which each bioremediation method is suitable.

1.1 Objectives

This dissertation research evaluated the ability of two TEAs (oxygen and NO_3^-) to enhance the *in situ* biodegradation of total petroleum hydrocarbons (TPH) in an oil-contaminated salt marsh over a one year period. Evaluations were performed in subsequent years by other graduate students.

The objectives of this dissertation research were to:

- determine the zones within an oil contaminated salt marsh that were most affected by the oil spill;
- evaluate and select an analytical method for the evaluation TPH present in the contaminated salt marsh sediments; and
- determine if addition of oxygen (added as air) and NO_3^- could enhance the *in situ* biodegradation of HCs compared to natural attenuation processes (e.g., volatilization, dilution, dispersion).

1.2 Study Site – Fore River Creek Salt Marsh

The oil-contaminated salt marsh selected for the study was located at Thompson Point in Portland, ME (Figure 1). The salt marsh was contaminated in September 1996, when the Tanker Vessel (T/V) *Julie N* hit the Route 77 Bridge. The *Julie N* lost 353 m^3 and 327 m^3 of Nos. 2 and 4 fuel oils, respectively. After an immediate response, more than 530 m^3 of the fuel was recovered (~80%), and only 150 m^3 was lost to the environment.

The most affected environments were the marshes along the Fore River (Lelyveld, 1996; Porter, 1997). The selection of a study site for the CICEET salt marsh remediation project was coordinated with officials from NHDES, NOAA, and the Maine Department of Environmental Protection (MEDEP). Specific sites were chosen within the Fore River salt marshes in areas where oil droplets were visible in the sediments. The marshes were covered by *Spartina alterniflora* and few invertebrates were present.

1.3 Background Conditions and Experimental Design

In May 1998, four experimental plots (10 m long by 3 m wide) were selected in the Fore River Creek salt marsh and designated for the addition of air (oxygen as TEA) and NO_3^-. Two control plots were also established to evaluate the extend of natural attenuation.

The plots were initially selected based on the visual presence of oil droplets in the sediments and subsequent TPH analysis. Horizontal wells installed in the sediments were used to deliver the amendments (TEAs).

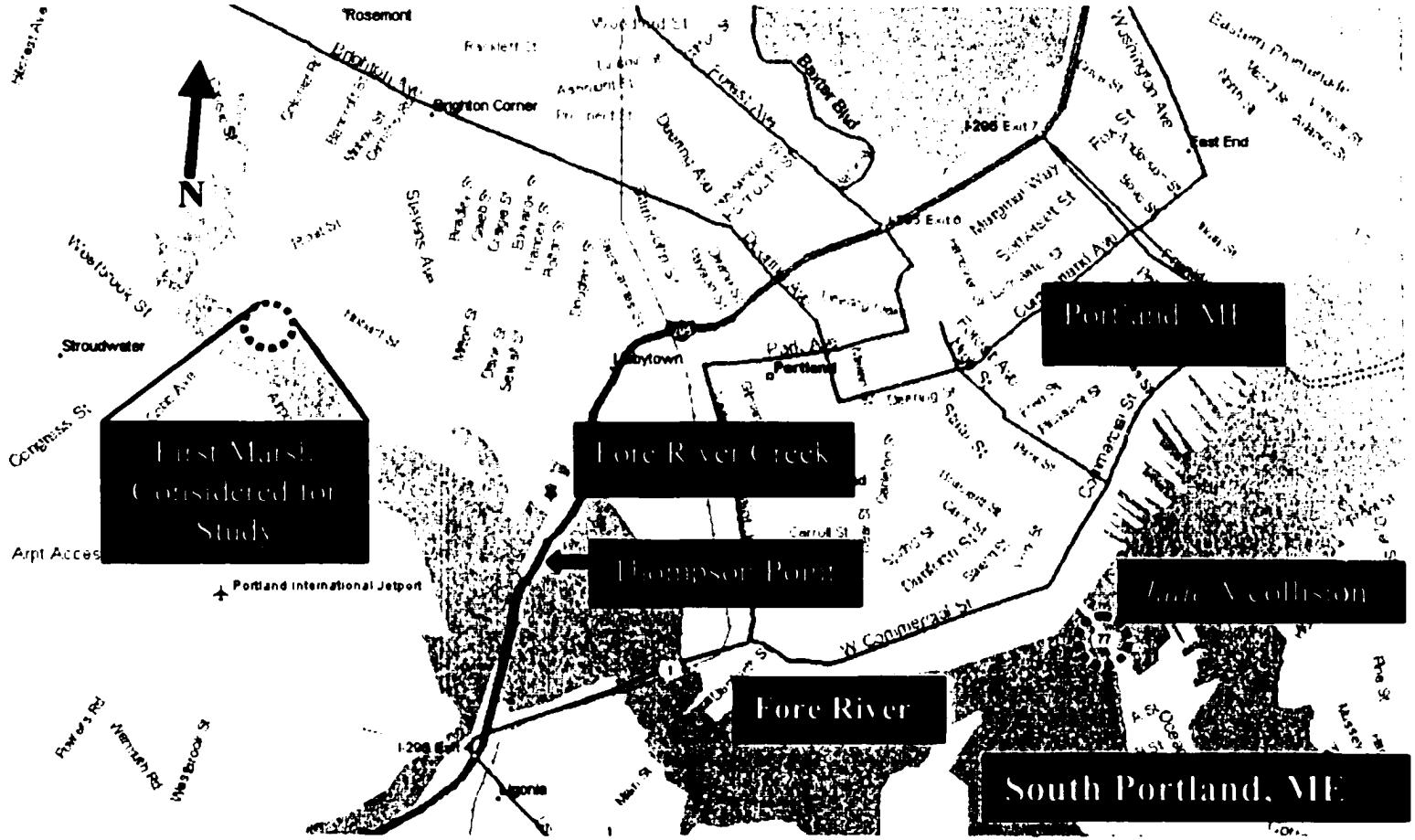


Figure 1. Site selected for the study at the Fore River Creek salt marsh. (taken from http://maps.expedia.com)

While horizontal wells are used for the treatment of terrestrial subsurface contaminated soils, this research was the first time that they were used in a contaminated salt marsh. The wells were buried ~16-20 cm to be below the root zone. The wells consisted of 3.7 m long plastic perforated tubes with a 2.5 cm external diameter and a 1.9 cm internal diameter. The perforations consisted of 1.6 mm holes spaced every 5 cm. The wells were set 60 cm on center and connected to a distribution header made from the same tubing. The wells were installed by first driving a 2.5 cm diameter stainless steel pipe (with a removable drive tip) into the sediments. After the pipe was driven below the marsh surface, the plastic tube was placed inside and the driven pipe was removed, leaving the well behind. Each plot was divided into grids (0.61 m x 0.31 m subplots) for sampling.

1.4 Field Study

The parameters used to monitor and assess the *in situ* HC degradation were: TPH concentration, abundance of oil-degrading bacteria, nutrient concentrations (NO_3^-, ammonia and phosphorus), and plant height and density. Prior to starting the study, two screening methods were evaluated for their ability to detect and quantify TPH in the salt marsh sediments: the U.S. Environmental Protection Agency (EPA) Soxhlet Extraction with Gravimetric Determination of Grease and Oils (EPA, 1994) and the American Society for Testing Materials (ASTM) Standard Test Method for Screening Fuels in Soils (ASTM, 1995). Because of the large number of samples anticipated, the analytical method needed to be low cost, rapid and relatively easy to use. However, the EPA and ASTM methods both experienced significant interferences from the organic matter and

fines in the salt marsh sediments. For this reason, samples were analyzed using the Massachusetts Department of Environmental Protection (MADEP) (MADEP, 1998a) Method for the Determination of Extractable Petroleum Hydrocarbons. This method is designed to measure the concentration of extractable aliphatic and aromatic HCs in water and soil. The aliphatic HCs were quantified as: C_9 through C_{18} (short chain-SC) and C_{19} through C_{36} (long chain-LC). The aromatic HCs were quantified as C_{11} through C_{22}. The aliphatic and aromatic HCs ranges corresponded to compounds with boiling points between 150°C and 500°C. This method uses a solvent extraction, silica gel solid-phase extraction/fractionation process (SPE), and gas chromatography (GC)/flame ionization detection (FID). While more expensive, time consuming and complex, the MADEP method had less interferences than the EPA and ASTM methods.

1.5 Duration of the Study

Field sampling was conducted from June 1998 through October 2001. This dissertation covers the first year from June 1998 to June 1999. During this period, nine sampling events occurred spaced ~3 to 4 weeks apart during the warmer months in New England. Background conditions were evaluated on June 11 and 12, 1998.

Each sampling event took two days to complete. Four subplots within each treatment and control plot were randomly selected for sampling. Sampling was conducted during the 3-4 hr surrounding low tide. Portable catwalks were used as working platforms to minimize the impact on the experimental plots. Two sediment samples were collected from each subplot to evaluate TPH concentration. A sediment

sample was also collected to evaluate the abundance of oil-degrading microorganisms. A porewater sample was used to quantify NO_3^-, ammonia (NH_4^+), and phosphate (PO_4^{-3}) from each subplot. A 30 cm x 30 cm frame was used in each subplot to delineate clusters of plants whose density and height were measured.

CHAPTER II

LITERATURE REVIEW

2.1 Petroleum Contamination

2.1.1 Light Non Aqueous Phase Liquids (LNAPLs)

Many pollutants exist at contaminated sites that are not in the aqueous phase or sorbed to soils, but rather are liquids immiscible with water. The availability of these compounds to biodegradation is drastically reduced (Atlas, 1978). Typically, LNAPLs are composed of molecules that have low aqueous solubility (i.e., the concentration in the water phase is quite low) and high solubility in organic solvents.

LNAPLs are often released by spills or leaks from transporting petroleum tankers. Crude oil LNAPLs have contaminated surface waters, marine sediments, and coastal beaches of the Caribbean Sea, and Atlantic, Pacific and Indian Oceans. Gasoline, petroleum products and industrial solvents have contaminated aquifers and groundwater at a large number of sites because they are often contained in underground storage tanks that after many years of burial corrode and release their contents (EPA, 1995).

If an LNAPL contains two or more compounds (common in oil spills, leakage of underground storage tanks containing gasoline, or at hazardous waste sites), the amount

of each compound present in the aqueous phase that is in equilibrium with the LNAPL phase is a critical factor that affects its transport and fate. This is well known from measurements of the disappearance of different compounds following crude oil spills or under experimental conditions (Atlas, 1981). Compounds present in a LNAPL may be degraded simultaneously or destroyed only after the more susceptible molecules are transformed.

2.1.2 Petroleum HCs

Petroleum is a mix of different HCs containing mainly carbon and hydrogen in their molecular structure. Crude oil has a highly variable composition that depends on the site where it is extracted (Atlas, 1981). However, it contains on average 83 to 86% by weight carbon and 11 to 13% hydrogen. In addition, it has small quantities of sulfur, oxygen and some metals (e.g., iron, chromium, nickel and vanadium). These atoms are arranged in different structural configurations. In general, HCs are divided in two main groups: aliphatics and aromatics. Aliphatics are further divided into alkanes, alkenes and cycloalkanes (Potter and Simmons, 1998).

The alkanes contain only single carbon-carbon bonds. The simplest alkane is methane (CH_4). The general formula for an alkane is C_nH_{2n+2}. Alkenes contain less hydrogen because they have one or more double bonds between their carbons (C=C). The alkene general formula is C_nH_{2n}.

Aliphatic and aromatic compounds differ by the pattern of bonding between adjacent carbons atoms. Aromatics have ring structures and are basically flat and symmetric with clouds of electrons above and below the plane of the molecule. Aromatic carbon-carbon double bonds are called resonance bonds because electrons are shared

between multiple carbon atoms. This imparts chemical stability to the molecule. Aromatic HCs have one or more benzene (C_6) aromatic rings as structural component (Harayama *et al.*, 1999; Potter and Simmons, 1998).

2.1.3 Petroleum Refining

Petroleum fuel mixtures are produced from crude oil through a variety of refining and blending processes. After treatment to remove dissolved gas, dirt and water, crude oil is distilled, producing different petroleum fractions. The fractions can be used directly or can be modified through cracking or reforming. During cracking, LC alkanes are converted into smaller alkanes and alkenes. This is the origin of most of the alkenes because they are not abundant in the crude oil. Reforming converts aliphatics into aromatics. The composition of a distillation fraction can be altered through treatment to add, remove or convert a specific compound. The products from the different refining processes are blended to produce petroleum fuel mixtures with characteristics required for specific uses (Table 1) (Potter and Simmons, 1998).

2.1.4 Impact of Weathering on Petroleum Composition

Petroleum products suffer changes when they are released to the environment in the process called weathering (Potter and Simmons, 1998). The main types of weathering are dissolution of HCs into water, volatilization, photodegradation and biodegradation.

Table 1. General characteristics of individual petroleum fuel mixtures. (Taken from Potter and Simmons, 1998)

Petroleum Fuel Mixture	Density g/mL (20°C)	Alkane Carbon Number Range	Distillate Characteristics	Compound Classes	End Use
Gasoline	~0.73	n-C4 - n-C12	Low-end distillate, Boiling point range of 40-200°C	High concentration of BTEX, Monoaromatics and branched alkanes, Lower concentration of n-alkanes, alkenes cycloalkanes, and naphthalenes, Very low concentration of PAHs	Automotive spark-ignition engines
Kerosene	~0.80	n-C6 - n-C16	Middle distillate, Boiling point range of 150-300°C	High concentration of cycloalkanes and n-alkanes, Lower concentrations of monoaromatics and branched alkanes, Very low concentration of PAHs	Critical kerosene burners
JP-4 fuel	~0.75	n-C5 – n-C14	Middle distillate, Mixture of gasoline (65%) and petroleum distillated (35%), Boiling point range of 150-275°C	High concentration of cycloalkanes and n-alkanes, Lower concentrations of mono aromatics, BTEX and alkenes, Very low concentration of PAHs	Aviation turbine engine
JP-5 fuel	~0.82	n-C8 – n-C17	Middle distillate, Specially blended kerosene Boiling point range of 150-275°C	High concentration of cycloalkanes and n-alkanes, Lower concentration of monoaromatics and branched alkanes, Very low concentration of BTEX and PAHs	Aviation turbine engines, Shipboard engines
JP-7 fuel	~0.80	n-C10 – n-C17	Middle distillate, High flash point kerosene, Boiling point range of 150-275°C	High concentration of cycloalkanes and n-alkanes. Lower concentration of monoaromatics and branched alkanes	Aviation turbine engines

BTEX = Benzene, Toluene, Ethylbenzene and Xylene PAH = Polycyclic Aromatic Hydrocarbons

Table 1. (Continued) General characteristics of individual petroleum fuel mixture (Taken from Potter and Simmons, 1998)

Petroleum Fuel Mixture	Density g/mL (20°C)	Alkane Carbon Number Range	Distillate Characteristics	Compound Classes	End Use
Diesel (#2)	~0.83	n-C8 – n-C21	Middle distillate, Boiling point range of 200-325°C	High concentration of n-alkanes, Lower concentration of branched alkanes, cycloalkanes, monoaromatics, naphthalenes and PAHs, Very low concentrations of BTEXs	High-speed engines
No. 2 fuel oil	~0.90	n-C8 – n-C21	Middle distillate, Boiling point range of 200-325°C	High concentration of n-alkanes, Lower concentration of branched alkanes, cycloalkanes, monoaromatics, naphthalenes and PAHs, Very low concentrations of BTEXs	Domestic burners, Medium capacity commercial, industrial burners
No. 6 fuel oil	~0.95	n-C12 beyond n-C34	Residual oil, Boiling point range of 350-700°C	High concentration of n-alkanes and cycloalkanes, Lower concentration of naphthalene and PAHs, Very low concentration of BTEXs	Commercial burners Industrial burners
Lubricating and motor oil		n-C18 beyond n-C34	Heavy end distillated, Boiling point range of 325-600°C	Lower concentration of barium, High concentration of n-alkanes and cycloalkanes, Very low concentrations of BTEXs and PAHs	Internal combustion engines
Crude oil	~0.94	n-C1 beyond n-C34	Feedstock	High concentration of n-alkanes branched alkanes and cycloalkanes, Lower concentration of BTEX, PAH, and naphthalenes, Variable concentrations of sulfur heterocyclics	

Each HC group is weathered uniquely. For example, aromatics tend to be more soluble in water than aliphatic HCs, whereas aliphatics tend to be more volatile, contaminating the air. Solubility and volatility of all compounds generally decreases with an increase in molecular weight. In general, the more water soluble and volatile compounds are, the more rapidly they are weathered in contaminated soils. Individual rates of dissolution and volatilization are retarded when compounds are present as fuel mixtures. If volatilization rather than dissolution is the dominant weathering process, lower molecular HCs within each series are depleted first. The greater a compound's volatility, the more rapid its loss from an HC mixture. Considering volatilization and dissolution trends together, it is possible to predict the composition of a fuel mixture after its release into the environment (Gustafson *et al.*, 1997). Where volatilization is the predominant process, the loss of lower molecular weight alkanes will be the most significant change in the product. In situations where dissolution is the dominant weathering process (e.g., sediment porewater), the aromatics will be depleted first with benzene removed most rapidly. Photooxidation mainly affects the aromatic compounds in crude oil and converts them to polar species (Dutta and Harayama, 2000).

2.1.5 TPH

TPH analysis is used as a general measure of the presence of crude oil or petroleum products in soil, water or sediments (MADEP, 1998a; Rooney-Varga *et al.*, 1997). TPH is the measurable amount of petroleum based HCs present in the environment. However, TPH is not a direct indicator of the risk (i.e., mobility, toxicity, and exposure to human and environmental receptors) posed by petroleum contamination.

Other analysis or information in addition to a single TPH concentration must be used to evaluated risk (Gustafson, 1998).

EPA Method 418.1 (1994) is the TPH analytical method required by some regulatory agencies. It provides a value of TPH in an environmental medium (e.g., contaminated sand), but does not yield information on the composition of the HC mixture. The amount of TPH measured by EPA 418.1 depends on the ability of the solvent to extract the HCs from the environmental medium and the absorption of infrared (IR) light by the HC in the extract. This method will also detect other organics such as humic acids that act as interferences.

EPA Method 8015 (Modified) (1994) reports the concentration of purgeable and extractable HCs referred to as gasoline (C_6 to C_{10-12}) and diesel fuel (C_{8-12} to C_{24-26}). Purgeable HCs are measured by purge-and-trap GC using FID, while extractable HCs are analyzed by GC following extraction with a solvent and subsequent concentration by evaporation (Gustafson, 1998).

In practice, TPH is defined by the method of analysis used. No single method gives a precise and accurate measurement of TPH for all types of HC contamination. The four most commonly used TPH testing methods include GC, IR spectrometry, gravimetric analysis, and immunoassays (Weisman, 1998).

2.1.6 TPH Determination

The chemical composition of petroleum products is complex and may change over time because of weathering. These factors make it very difficult to select the most appropriate analytical methods for evaluation of environmental samples (Weisman, 1998). Measuring the extent of crude oil degradation is challenging, because crude oil is

a complex mixture of many compounds. Oil degradation is usually based on changes in TPH concentrations (Huesemann, 1995). TPH may be measured by extracting total oil and grease and adsorbing the polar (grease) component onto silica gel and measuring the IR absorption (EPA, 1994). Although this method is subject to interferences, it is satisfactory for monitoring gross changes in TPH (Huesemann, 1995).

More traditional analytical approaches have focused on the identification and evaluation of specific indicator components (e.g., PAH and BTEX). However, because of the inability to analyze for the large numbers of constituents in TPH and the lack of toxicological and other relevant data for many of those constituents that could be individually identified (Edwards *et al.*, 1998), an indicator approach is generally accepted and used by state regulatory agencies for carcinogenic risks posed by TPH.

MADEP has developed a TPH analytical method based on the EPA Modified Method 8015 (EPA, 1994). This method reports the concentration of purgeable and extractable HCs, which are also referred to as gasoline and diesel range organics. The MADEP method is designed to measure the collective concentration of extractable aliphatic and aromatic petroleum HCs in water and soil. The extractable aliphatic HCs are collectively quantified within two ranges; C_9 through C_{18} (SC) and C_{19} through C_{36} (LC) The extractable aromatic HCs are collectively quantitated within the C_{11} through C_{22} range. These aliphatic and aromatic HCs ranges correspond to boiling points between ~ 150°C and 500°C. In this method, a sample is extracted by sonication with methylene chloride, dried over sodium sulfate, solvent exchanged into hexane, and concentrated. Sample cleanup and separation into aromatic and aliphatic fractions is

conducted using commercial silica gel cartridges. The two extracts are re-concentrated and separately analyzed by capillary GC/FID.

Surrogate standards are used to monitor the efficacy of the sample extraction, chromatographic and calibrations systems. The recommended surrogate standards are chloro-octadecane (COD) and ortho-terphenyl (OTP). Using these two compounds, a surrogate spiking solution is prepared. Each sample and blank is fortified with a known concentration of the surrogates.

2.1.7 Estimates of Risk from TPH

There are three approaches that are used to estimate potential human health risks posed by TPH contamination (Gustafson, 1998). The most used is the evaluation of the carcinogenic risk from TPH as an "indicator" approach assuming that the risk is characterized by a small number of compounds (i.e., BTEX, PAHs). The "surrogate" approach assumes that TPH is not included as an indicator and can be characterized by a single compound. This approach can overestimate toxicity and mobility because of the compounds used as surrogates. In the "whole product" approach the toxicity and mobility of the TPH are based on a whole product of similar character (e.g., LC aliphatics). All approaches are similar because they use specific knowledge of a single or few constituents to characterize the many constituents in a HC mixture (Gustafson, 1998).

The TPH Criteria Working Group (TPHCWG), which is a national *ad hoc* committee that was formed to develop a technically risk-based approach to TPH, has developed a combined indicator of the grouping or fraction approach (EPA, 1998; Gustafson *et al.*, 1997; Gustafson, 1998). The basic approach is similar to that developed

by MADEP where the TPH is split into a small number of groups or fractions that have similar properties. The main difference between the MADEP and TPHCWG approaches is that the latter defines groups or fractions of TPH based on the potential mobility of the HC within each group. In the MADEP approach, fractions are based on the available toxicity data (MADEP, 1998a).

2.1.8 Effect of Oil in Marshes

Petroleum HCs can have a profound effect on marshes. Salt marshes are occasionally impacted by crude oil spills from marine vessels and oil pipelines. Oil may cover plants, remain on the water surface or become associated with sediments (Wright *et al.*, 1997). Oil may have physical or toxic effects on marsh plants, animals and microorganisms. Physical effects result from the viscous and adhesive properties of oil. Stomata (gas-exchange pores) of plants may be blocked. The gills and mouthparts of invertebrates may be clogged and the fine structure of birds' feathers may be disrupted. Toxic effects result from the oil's interaction with the biochemical functioning of the contaminated organisms (i.e., the replacement of fatty molecules in cellular membranes resulting in membrane disruption, increased permeability and leakage of cellular contents). In addition, an increase in respiration is often observed, possibly because mitochondria are damaged so that the rate of oxygen use is no longer coupled to other biochemical processes (Dibner, 1978; Walker *et al.*, 1975; Wharfe, 1975).

When large amounts of oil are dispersed into the water column, heterotrophic bacterial growth may show a transient inhibition within hours. The acute toxicity of the most volatile petroleum HCs could also explain the lag phase (Siron *et al.*, 1996). Significant increases in bacterial productivity coupled with decreased primary

productivity, suggest a shift toward heterotrophy following exposure to diesel fuel that could cause serious trophic perturbations. The effects of petroleum pollution may have complex ecological, trophic and biochemical implications in areas like marshes where much of the ecosystem's production and nutrient cycling is microbially mediated (Piehler *et al.*, 1997a).

A compound's toxicity is a major factor determining its biodegradation in a NAPL. Toxicity results from the major solvent or one or more minor components of a heterogeneous NAPL. Many organic solvents suppress microbial proliferation and metabolism (Diaz and Roldan, 1996). Experiments show that differences in petroleum toxicity are attributable to individual fuel composition rather than characteristics of the individual alone (London and Robinson, 1984). As a rule, organic solvents with high values for log K_{OW} ($\geq$ 4.0) do not suppress microbial activity, whereas those with low values for log K_{OW} ($\leq$ 2.0) are highly toxic. (Note: K_{OW} is the octanol-water partition coefficient)

2.2 HC Biodegradation

2.2.1 Biodegradation Process

Problems associated with pollution can be reduced by conventional technologies that remove, alter or isolate the contaminants. These technologies are expensive, and in many cases they do not destroy the contaminant compounds, but transfer them from one environment to another (Hickey, 1995; Hurst *et al.*, 1997; Ritter and Scarborough, 1995). Bioremediation exploits the biological breakdown or biodegradation of organic

contaminants. It is not a new technology. The Romans used it to treat human-generated wastes. They were the first to discharge sewage effluents onto soils, allowing the naturally-occurring microorganisms to degrade them (Chapelle, 1996).

During biodegradation of HCs, microorganisms use the energy they derive from the organic compounds to create cell biomass and less complex organic compounds or, upon complete degradation, water and carbon dioxide (mineralization) (Atlas, 1992). Bioremediation addresses the limitation of more conventional technologies because it can completely degrade the pollutants into inert byproducts, in many cases at reduced cost. As a result, over the past 20 years, bioremediation has grown from a relatively unknown technology to one that is considered for the cleanup of a wide range of contaminants.

Bioremediation has also become an intensive area for research and development in academia, government and industry. Because environmental regulations are becoming more strict regarding the cleanup of contaminated sites, funding for basic and applied research on bioremediation by government agencies and private industry has increased over the past decade (Alexander, 1994; Crawford and Crawford, 1996).

The extent of biodegradation and the rate at which it occurs depend on environmental conditions, the type and number of microorganisms present, and the chemical structure of the contaminants being degraded (Hurst *et al.*, 1997). Bioremediation can be affected by other factors such as temperature, pH, nutrient status, bioavailability and solubility of the organic compounds, biotic interactions, electron donor (ED) and TEA availability, and the composition of the microbial community (Atlas, 1981; McKee and Mendelssohn, 1994). The presence of contaminants in the environment can alter the microbial community structure through selection of species

capable of biodegradation or by causing acute toxicity effects (Long *et al.*, 1995; Macnaughton *et al.*, 1999).

2.2.2 Thermodynamics of Microbial Metabolic Processes

Bioremediation is based mainly on microbial metabolism (Atlas, 1978). Most microbes obtain energy by transferring electrons from EDs to TEAs. Contaminants can act as TEAs or EDs and the rate of the metabolic processes is related to their availability (Chapelle, 1996).

The relationship between reduced (TEA) and oxidized (ED) substrates can be shown as a hierarchy of oxidation-reduction half reactions (Figure 2) (Hurst *et al.*, 1997). In this figure, the vertical axes are oxidation-reduction potential, Eh or pE values. Compounds on the left side of the hierarchy are oxidized, while those on the right are reduced. The transition from oxidized to reduced forms is governed by the redox status of the system and by catalytic mechanisms of microbially-produced enzymes.

Highly oxidizing conditions appear in the upper part of the figure, while highly reducing conditions are in the lower portion. Under environmental conditions, the lower reaction proceeds to the left (electron producing or donating) and the upper reaction proceeds to the right (electron accepting). In nature, a variety of TEAs may be present: oxygen (O_2), nitrate (NO_3^-), manganese (Mn_4^+), iron III (Fe_3^+), sulfate (SO_4^{-2}), and carbon dioxide (CO_2). Each of these coupled half reactions is mediated by microorganisms. When diagonal arrows directing carbohydrate oxidation to these TEAs are drawn, the length of each arrow is proportional to the free energy gained by the microorganisms (Hurst *et al.*, 1997).

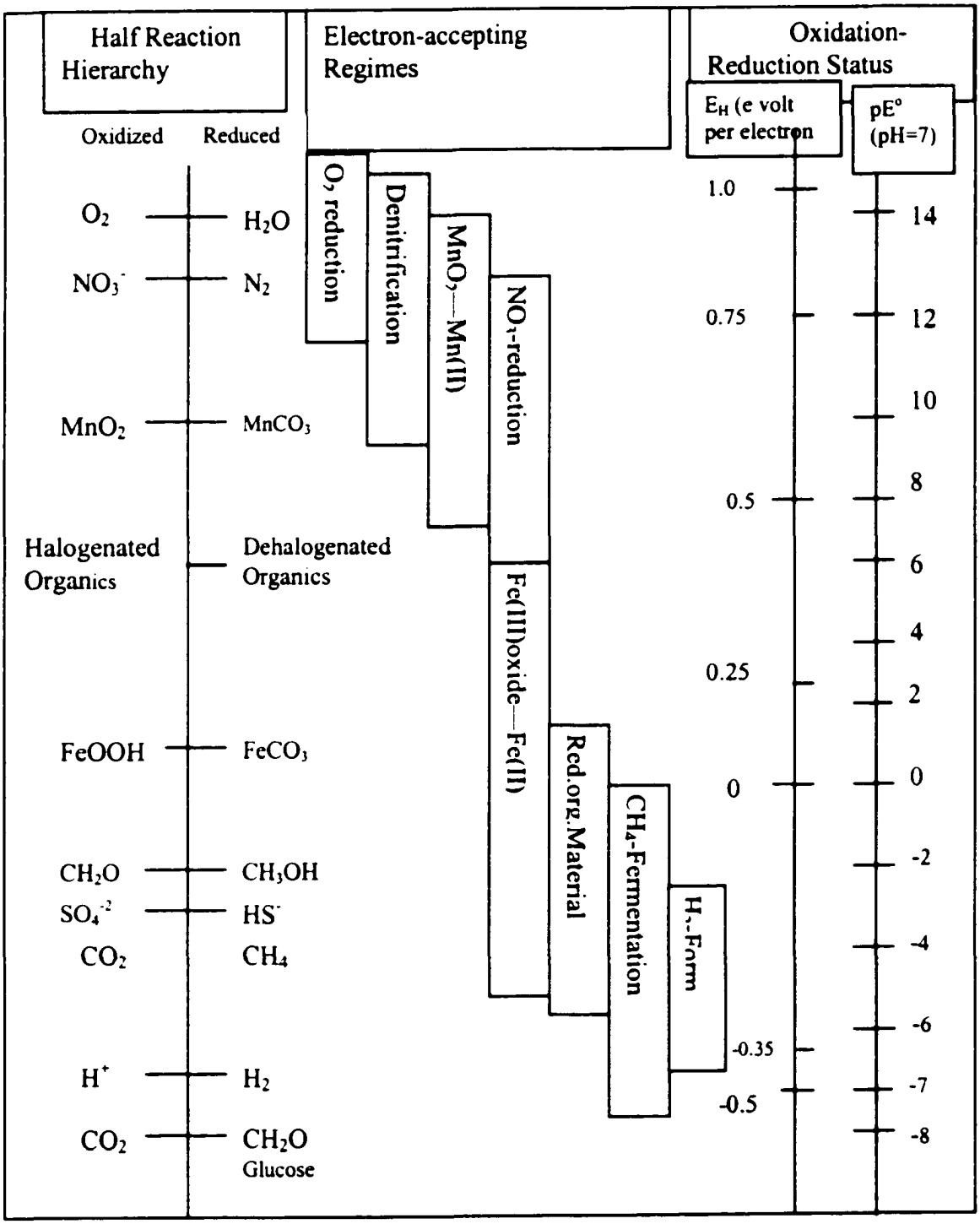


Figure 2. Relationship between reduced and oxidized substrates as a vertically-arranged hierarchy of oxidation-reduction half reactions. (Taken from Hurst *et al.*, 1997)

Microorganisms metabolizing carbohydrates using oxygen as a TEA generate more energy in the form of ATP than those carrying out NO_3^- respiration. These microorganisms, in turn, gain more energy than those using Mn^{+4} and Fe^{+3}.

In a salt marsh, the sediment microflora require an organic substrate as a source of carbon for biosynthesis and to serve as an ED. They also need an organic or inorganic compound to serve as a TEA (McKee and Mendelssohn, 1994). Aerobes and facultative anaerobes use oxygen as long as it is available because the energy yield of this pathway is the highest (Table 2). As oxygen is depleted, the activity of obligate aerobic microorganisms ceases and facultative and obligate anaerobes use the oxidants NO_3^-, Mn^{+4}, Fe^{+3}, SO_4^{-2} and CO_2 in a sequential fashion (Ponnamperua, 1972).

The sequences of the reductions are determined by the energy yield of each reaction leading to the production of reduced compounds such as ammonium (NH_4^+), manganous manganese (Mn^{+2}), ferrous iron (Fe^{+2}), sulfides (S^{-2}, HS^-, H_2S), and methane (CH_4). Due to incomplete decomposition of organic matter under anoxic conditions, organic acids and alcohols are also produced (Table 2). With a sufficiently long adaptation period and an appropriate TEA, most petroleum-HCs can be degraded by anaerobic microbial communities (Coates *et al.*, 1996).

Although reducing conditions may prevail in the waterlogged sediments of a marsh, oxidized zones exist at the soil-water interface and in the rhizosphere of plants growing there. These zones result from the diffusion of oxygen from the overlying water or atmosphere into the soil, but may only be a few millimeters thick (McKee and Mendelssohn, 1994). Oxygen is also transported through the air space in tissues (i.e., aerenchyma) of wetland plants and diffuses out of the roots, forming an oxidized

rhizosphere (Armstron, 1964). The width of the oxic zones depends on the rate of oxygen diffusion to these interfaces and the oxygen demand of the bulk sediment. Oxidized and reduced zones may occur very close together in marsh sediments.

Table 2. Selected reactions illustrating carbon transformation pathways and their energy yields. (Taken from McKee and Mendelssohn, 1994)

Pathway	Reaction	Energy yield (kcal/mol)
Aerobic Respiration	$C_6H_{12}O_6$ (glucose) + $6O_2 = 6CO_2 + 6H_2O$	686.0
Nitrate reduction and Denitrification	$C_6H_{12}O_6$ + 24/6 NO_3^- +24/5 $H^+ = 6CO_2$ + 12/5 N_2 +42/5 H_2O	649.0
Manganese Reduction	$C_6H_{12}O_6$ + 12 MnO_2 + 24 $H^+ = 6CO_2$ + 12 Mn_2^+ +18H_2O	457.8
Iron Reduction	$C_6H_{12}O_6$ + 24$Fe(OH)_3$ + 48$H^+ = 6CO_2$ + 24Fe_2^+ + 66H_2O $C_6H_{12}O_6$ + 12Fe_2O_3 + 48$H^+ = 6CO_2$ + 24Fe_2^+ + 30H_2O	100.4 86.9
Fermentation	$C_6H_{12}O_6 = 2CH_3CHOCOOH$ (lactic acid) $C_6H_{12}O_6 = 2CH_2CH_2OH$ (ethanol) + $2CO_2$	58.0 57.0
Methanogenesis	H_2 + 1/4 $CO_2 = 2CH_4$ + 1/2 H_2O CH_3COO^- + $4H_2 = 2CH_4 + 2H_2O$ $CH_3COO^- = CH_4 + CO_2$	8.3 39.0 6.6
Sulfate Reduction	$CH_3CHOHCOO^-$(lactate) + 1/2$SO_4^=$ + 3/2$H^+ = CH_3COO^-$ (acetate) + CO_2 + 1/2HS^- CH_3COO^- + $SO_4^= = 2CO_2 + 2H_2O + HS^-$	8.9 9.7

2.2.3 Aerobic Respiration

Aerobic respiration is rare in marshes and the rate of oxygen movement from the atmosphere is low, due to diffusion limitations in aqueous solutions. However, microbially-mediated oxidation (e.g., nitrification, methane oxidation, sulfide oxidation) occurs in reduced zones in saturated sediments (McKee and Mendelssohn, 1994). Oxygen is the preferred TEA when its concentration exceeds 0.8 mg/L. Below ~ 0.5 mg/L, there is a potential for NO_3^- competing with oxygen for available electrons. If there is no NO_3^- present, the threshold oxygen concentration may be $\leq$ 0.16 mg/L (Hurst

et al., 1997). If dissolved oxygen is present, it is assumed that aerobic respiration is the predominant process (Lovley *et al.*, 1994). For *in situ* bioremediation of simple HCs, oxygen transport is almost always the rate limiting step in biodegradation (Ritter and Scarborough, 1995). The results of aerobically incubated total heterotrophic plate counts on subsurface soil samples indicate that organisms capable of aerobic growth are present in all sediments tested, even those from anoxic zones (Long *et al.*, 1995). Studies have confirmed that anthracene and naphthalene mineralization are strongly dependent on the availability of oxygen in the marsh sediments (Bauer and Capone, 1985).

In passive bioremediation of petroleum HC contaminated soils and sediments, diffusion is the primary mechanism for supplying oxygen to maximize the effectiveness of treatment (Huesemann and Truex, 1996).

The enhancement of the biodegradation of HCs in soil by forced aeration has been confirmed under field conditions (Yeung *et al.*, 1997). Innovative bioremediation approaches using air injection to stimulate biodegradation *in situ* (e.g., biosparging and bioventing) are now popular. Unfortunately, the efficiency of oxygen transfer *in situ* can vary widely. Because it is difficult to predict or adequately measure this transfer *in situ*, there is a concern that techniques such air sparging or bioventing are often used where they are not appropriate (Stroo, 1996).

2.2.4 Nitrate Reduction and Denitrification

NO_3^- is reduced to nitrogen gas (N_2) by denitrification or NH_4^+ by dissimilatory NO_3^- reduction. Denitrification is often the main process removing dissolved nitrogen in wetlands (Stepanauskas *et al.*, 1996). NO_3^- reduction and denitrification are the major anaerobic processes responsible for HC degradation in microaerophilic-flooded soils

when NO_3^- is present in cold environments (McKee and Mendelssohn, 1994). In the absence of oxygen, microorganisms use NO_3^- as an alternative oxidant. NO_3^- reduction appears to be the preferred pathway when the electron supply (organic carbon) exceeds the amount of available NO_3^- (Hurst *et al.*, 1997; Smith *et al.*, 1991).

Dissimilatory NO_3^- reduction can also contribute to the production of NH_4^+ in reduced environments. Denitrification and nitrification are reported to be the main processes involved in N_2O production in soils (Stepanauskas *et al.*, 1996).

Bench studies have shown that NO_3^- can be used as a TEA for bioremediation of soils contaminated with low molecular weight PAHs (Al-Bashir, 1990; Mester and Kosson, 1996; Mihelcic and Luthy, 1988a, 1988b). A study demonstrated that NO_3^--dependent anaerobic degradation and mineralization of naphthalene by pure cultures is possible (Rockne *et al.*, 2000). The main reasons for using NO_3^- as a TEA are its high solubility in water, very low volatilization rate, and high biodegradation rates that approach those achievable under aerobic conditions. Denitrification represents the optimal redox environment when a tradeoff between volatilization and biodegradation of low molecular weight PAHs is achieved (Burland and Edwards, 1999; Hess *et al.*, 1996; Leduc *et al.*, 1992).

2.2.5 Manganese and Iron Reduction

Mn^{+4} and Fe^{+3} are reduced in anoxic environments (McKee and Mendelssohn, 1994). The energy yield of manganese reduction is slightly lower than NO_3^-, and the energy yield for iron reduction is less than NO_3^- and manganese reduction (Table 2). Iron is one of the most abundant elements in the earth's crust, but is a minor component in aquatic systems because of its relative insolubility in water. The form in which iron that

is found *in situ* is greatly influenced by the *in situ* pH and oxygen concentration (Brock and Madigan, 1997). *Geobacter spp.* have been associated with the oxidation of monoaromatic compounds (e.g., benzene) using Fe^{+3} as a TEA in petroleum-contaminated sediments and aquifers (Lovley *et al.*, 1996; Rooney-Varga *et al.*, 1999). Sulfate reduction is generally inhibited in the presence of Fe^{+3} because iron reducers out compete SRBs for EDs (Weiner and Lovley, 1998b).

2.2.7 Sulfate Reduction

After chloride, the anion most abundant in the marine environment is SO_4^{-2}. In organically-rich marine environments, the microbial reduction of SO_4^{-2} to S^{-2} initiates the cycling of sulfur compounds producing H_2S, the precipitation of FeS and the formation of a disulfide and the deposition of elemental sulfur.

SRB use low molecular weight organic compounds such as lactate ($CH_3CHOHCOO^-$) or acetate (CH_3COO^-) as energy sources and EDs, and use SO_4^{-2} as their TEA. Even though the energy yield is less than for other anaerobic pathways, sulfate reduction often dominates the organic carbon oxidation in coastal marine sediments because of the great availability of SO_4^{-2} in seawater. Sulfate's oxidizing capacity (640-960 mg/L) is 200 times greater than oxygen's (3.2-4.8 mg/L) in marine sediments (McKee and Mendelssohn, 1994). Sulfate reduction is the dominant TEA process and accounts for more than half of the total decomposition of organic matter in salt marshes (Rooney-Varga *et al.*, 1997).

Much of the mineralization of organic matter in coastal marine sediments occurs via anaerobic processes. Dissimilatory reduction of SO_4^{-2} results in the formation of extracellular H_2S. Sulfide oxidation by oxygen occurs at the interface between reduced

sediments and the oxidized zone, typically in a suboxic zone immediately below the aerobic layer. The oxidation of reduced S^{-2} and aerobic respiration of natural organic matter (NOM) are the major oxygen sinks in a salt marsh (Shin *et al.*, 2000). The addition of crude oil represents an additional demand and HC-degrading bacteria may compete for oxygen with the demand from aerobic respiration of organic matter and oxidation of S^{-2} produced at depth.

A wide variety of HC contaminants can be degraded under sulfate reducing conditions in contaminated sediments, suggesting the use of sulfate reduction rather than aerobic respiration as a treatment strategy in natural attenuation of HCs in these sediments (Coates *et al.*, 1996; 1997; Madsen *et al.*, 1995). *S. alterniflora* roots produce low molecular weight organic compounds such as ethanol and malate, and SRBs can use these compounds directly (Haines *et al.*, 1996).

Sulfate reducing activity has been shown to be closely tied to plant growth stages, suggesting that plant-SRB interactions in the *S. alterniflora* rhizosphere play an important role in salt marsh biogeochemical cycles (Rooney-Varga *et al.*, 1997).

The fact that HC metabolism can be linked to sulfate reduction is beneficial because SO_4^{-2} is one of the most abundant electron acceptors in marine environments (Coates *et al.*, 1997). In BTEX-contaminated aquifers, the presence of sulfate reducing conditions and SRBs is coupled with a decrease in HC concentrations in the contaminated sediments (Weiner and Lovley, 1998b; Gieg *et al.*, 1999).

2.2.8 Methanogenesis

When other TEAs are depleted, carbon dioxide can be used as a TEA, being reduced to methane during methanogenesis (Hurst *et al.*, 1997). Methanogens are strict

anaerobes, and may alternatively use low molecular weight organic compounds produced by other bacteria as their electron acceptors (e.g., CH_3COO^-) (McKee and Mendelssohn, 1994). Degradation of toluene under methanogenic conditions has been demonstrated in laboratory studies (Gieg *et al.*, 1999). Degradation of benzene, with the production of methane, is possible after a long period (~30 d) in sediments contaminated with HCs, probably after a microbial consortium is established (Weiner and Lovley, 1998a).

2.2.6 Fermentation

Fermentation is an important process mediated by microorganisms in anoxic conditions. During the fermentation process, when an organic compound is oxidized another organic compound is reduced (McKee and Mendelssohn, 1994). The energy yield is less than a tenth that of aerobic respiration. Hence, the major limitation to fermentative HC degradation is the low energy yield (Atlas, 1975).

2.3 Bioremediation of Petroleum HCs

The microbial degradation of HCs is a long recognized process that has been measured by a variety of strategies (Atlas and Bartha, 1973a; Atlas, 1978; Chapelle, 1996; Leahy and Colwell, 1990; Ritter and Scarborough, 1995).

Biodegradation of the aliphatic HCs has been extensively studied (Watkinson and Morgan, 1990). The metabolic capability to degrade aliphatic pollutants is usually related to the size of the metabolically-active microbial community.

Aromatic HCs, with low solubility in water, are decomposed microbiologically. The metabolic capability to degrade mono-aromatic compounds is usually related to the

level of pollutant exposure (Long *et al.*, 1995). In soil, multi-ranged compounds like anthracene, phenanthrene, pyrene, 1,2-benzopyrene, and chrysene can be biodegraded (Bossert and Bartha, 1986). The microorganisms in soil sediments can also mineralize anthracene and naphthalene (Bauer and Capone, 1985). In pure culture, PAHs such as naphthalene, anthracene, and phenanthrene can serve as carbon sources for bacteria (Bastiaens *et al.*, 2000). Recent observations of HC under anaerobic conditions also indicate the possibility of other pathways for aromatic HC biodegradation (Hughes *et al.*, 1997; Watkinson and Morgan, 1990).

Two-and three-ring PAH compounds are readily degraded, while the four-ring chrysenes are more resistant. While no microorganisms have been isolated that are capable of using four- and five-ring PAHs as sole carbon sources, studies of naturally-occurring microorganisms have demonstrated co-metabolism of pyrene, benz(a)pyrene, benz(a)anthracene, and dibenz(a,h)anthracene in the presence of naphthalene or anthracene (Herbes and Schwall, 1978; Walker *et al.*, 1976; Watkinson and Morgan, 1990; Wrenn and Venosa, 1996). However, co-metabolism may be less important in sediments than in liquid media, possibly because sorptive immobilization of the larger PAHs to sediment particles limits their availability to microorganisms (Herbes and Schwall, 1978). Different isolates have the ability to metabolize naphthalene or phenanthrene as sole carbon sources. Representatives of the genera *Pseudomonas* and *Sphingomonas* form a remarkably high fraction of these isolates (Meyer *et al.*, 1999).

The most stable of the PAHs, hopane (17α(H),21β(H)-hopane) is often used as a control in bioremediation studies (Atlas, 1981; Douglas *et al.*, 1996). In addition, much of the variability inherent in crude oil bioremediation during field studies can be

eliminated when TPH concentrations are normalized to the hopane concentration (Venosa *et al.*, 1997).

The extent of TPH biodegradation is affected by the type of HCs in the contaminant matrix, while the influence of variables such as soil type, fertilizer concentration, microbial abundance and type of treatment (slurry vs. land-treatment) appear to be less significant (Huesemann, 1995; Ramirez *et al.*, 1996).

Molecular studies have shown that the *alk* (C_5 to C_{12} n-alkanes), *nah* (naphthalene), and *xyl* (toluene) genes are responsible for the HC degradation. They are usually located on large catabolic plasmids (Whyte *et al.*, 1997). Significant differences in the frequency and distribution of plasmids among bacterial isolates have been observed (Leahy *et al.*, 1990).

2.4 Biodegradation Mechanisms

The rate of degradation of oil depends on its rate constant and the concentration of the oil (Uraizee *et al.*, 1998). Different mechanisms are postulated to explain how microorganisms use compounds in LNAPLs or metabolize organic solvents with low aqueous solubility. These mechanisms focus on how the compounds are transported from the environment to the cell surface and from the outside of the membrane to the interior to be processed by enzymes. Only the chemicals dissolved in the water are used, but microorganisms can use molecules that enter the aqueous phase by spontaneous partitioning. Microorganisms also excrete some products (e.g., surfactants or emulsifiers) that convert the oil compounds into <1 μm droplets that can be used. The cells must

come in direct contact with the LNAPL, and the solubilized compounds pass through the cell surface into the cytoplasm.

Substrates that have low log K_{OW} values will typically dissolve rapidly in water. The organisms will multiply as they use them as ED. Hence, biodegradation will eventually be limited by the rate of dissolution if the process is ED-limited and will depend on the initial concentration in the water and the spontaneous dissolution rate. Other constituents of the LNAPL can affect the partitioning from the mixture to water. Competition among the active microbial species for oxygen, nitrogen, phosphorus and other inorganic nutrients may also occur (McKee and Mendelssohn, 1994).

The production and use of emulsifiers and surfactants in remediation has received considerable attention in the case of environmental contamination by LNAPLs, oils and oil products (Uraizee *et al.*, 1998). Emulsifiers and surfactants facilitate the partitioning of the molecules from the NAPL in the aqueous phase, thus resulting in enhanced biodegradation. In contrast with a true solution, in which two or more substances are mixed homogeneously at the molecular level, an emulsion is an immiscible liquid that is dispersed intimately in another immiscible liquid as >0.1 μm-1.0 μm droplets (Watkinson and Morgan, 1990). The role of emulsifiers or surfactants in degradation of LNAPLs other than pure aliphatic HCs remains uncertain (Alexander, 1994; Banat *et al.*, 2000). Because of their suspected toxicity, man-made surfactants are usually tested in lower concentrations first in laboratory studies (Madsen *et al.*, 1995). In addition some studies have shown high toxicity of some of these emulsifiers or surfactants during field experiments (Perkins, 1970)

2.5 Aerobic and Anaerobic HC Metabolism

Catabolism of HCs has long been considered as a strictly oxygen-dependent process. For aerobic organisms (e.g., fungi and bacteria), the initial attack of HCs always requires molecular oxygen as TEA. The first enzymes in the metabolic pathways of alkanes are monooxygenases, while aromatic HCs are attacked by either monooxygenases or dioxygenases. These enzymes incorporate hydroxyl groups derived from molecular oxygen, into the aliphatic chain or aromatic ring. The alcohols formed are then oxidized to the corresponding acids; the phenolic compounds generated by ring hydroxylation of the aromatic HCs are direct precursors for oxidative ring cleavage (Harayama *et al.*, 1999). Some microorganisms are also able to catabolize HC compounds under anaerobic conditions. The microorganisms capable of this have developed alternative, oxygen- independent reactions for the initial attack. All anaerobic HC-degrading strains, available as pure cultures, are either denitrifying, Fe^{+3}-reducing bacteria or SRB (Heider *et al.*, 1999; MacRae and Hall, 1998).

The denitrifying species mineralize a variety of alkylbenzenes, including toluene, *m*-xylene, ethylbenzene, propylbenzene, *p*-ethyl toluene and *p*-cymene. These bacteria are typically members of the genera *Pseudomonas*, *Thauera* and *Azoarcus*. Other known species can degrade a wide spectrum of aliphatic HCs, but are restricted to few aromatic HCs. For example, only one species of an Fe^{+3}-reducing bacterium that degrades aromatic HCs (*Geobacter metallireducens*), has been reported (Lovley and Lonergan, 1995). The growth of these bacteria in the environment is probably limited by the availability of the insoluble $Fe(OH)_3$. Several SRBs have been reported to use aromatics,

even though this process yields only small amounts of free energy (-205 kJ/mol of toluene) (Table 3).

Benzoate (or its CoA-thioester) is a central intermediate in the anaerobic mineralization of aromatic HCs (Harayama *et al.*, 1999). Thus, the initial series of reactions in anaerobic toluene degradation apparently involves the conversion of toluene to benzoate (or benzoyl-CoA) (Heider *et al.*, 1999).

Table 3. Stoichiometric equations of anaerobic bacterial toluene oxidation coupled to the reduction of different TEAs. (Taken from Heider *et al.*, 1999).

Denitrifying bacteria $C_7H_8 + 7.2\ NO_3^- + 2OH^-$	$7\ HCO_3^- + 3.6\ N_2 + 0.6\ H_2O$	G^o = -3554 kJ (per mol toluene)
Iron(III)reducing bacteria $C_7H_8 + 94\ Fe(OH)_3$	$7\ Fe\ CO_3 + 29\ Fe_3O_4 + 145\ H_2O$	G^o = -3398 kJ (per mol toluene)
SRB $C_7H_8 + 4.5\ SO4^{-2} + 3\ H20$	$7\ HCO_3^- + 2.5\ H^+ + 4.5\ HS^-$	G^o = -205 kJ (per mol toluene)
Methanogenic		
a) $C_7H_8 + 9\ H_2O$	$7\ HCO_3^- + 3\ H_3C\text{-}COO^- + 4\ H^+ + 6\ H_2$	G^o = +166 kJ (per mol toluene)
b) $6\ H_2 + 1.5\ HCO_3^- + 1.5\ H^+$	$1.5\ CH_4 + 4.5\ H_2O$	G^o = -303 kJ (per 6 mol H_2)
c) $3\ H_3C\text{-}COO^- + 3H_2O$	$3\ CH_4 + 3\ HCO_3^-$	G^o = -93 kJ (per 3 mol acetate)
Sum: $C_7H_8 + 7.5\ H20$	$4.5\ H_2O + 2.5\ HCO_3^- + 2.5\ H^+$	G^o = - 131 kJ (per mol toluene)

a. Proton reducing bacteria; b and c. Methanogens

2.6 Bioremediation Kinetics

Once degradation of a chemical commences, the amount disappearing with time and the shape of disappearance curve will be a function of the specific compound (Alexander, 1994). The study of kinetics of biodegradation in natural environments is often empirical, a reflection of the rudimentary level of knowledge about microbial

populations and activities in these environments. An example of an empirical approach is the power rate model:

$$-dC/dt = kC^n \qquad \text{(Eq. 2.1)}$$

where C is the substrate concentration (M/L^3), t is the time (T), k is the rate constant for chemical disappearance (T^{-1}), and n is a kinetic order (e.g., 1^{st}, 2^{nd}, 3^{rd}). The model can be used to create substrate disappearance curves by varying n and k until a good fit of the data is achieved. The power rate law provides a basic comparison of the goodness of fit of different curves, but it gives no insight into the reason for the shapes and may not have predictive ability (Alexander, 1994). Most biodegradation kinetics is first order:

$$C_t = C_o e^{-kt} \qquad \text{(Eq. 2.2)}$$

where C_t is the substrate concentration at any time (t) and Co is the substrate concentration at t = 0.

2.7 Factors that Affect Biodegradation

2.7.1 Temperature

Temperature has an important role in controlling the nature and extent of microbial metabolism. Metabolism controls *in situ* bioremediation (Alexander, 1994; Atlas, 1975; Walker and Colwell, 1974). Temperature affects the growth rate of HC-degrading microorganisms and the activity of the enzymes involved in the oxidation of HCs (McKee and Mendelssohn, 1994). A general rule is that for every 10°C increase biodegradation rates increase about twofold. However, above a critical temperature biodegradation decreases because of enzyme and protein denaturation (Eweis *et al.*,

1998). In addition, bioavailability and solubility of less soluble hydrophobic substances, such as aliphatic and polyaromatics HCs, are temperature dependent. When the temperature increases, the viscosity decreases affecting diffusion rate. Therefore, higher reaction rates due to smaller boundary layers are expected at elevated temperatures. The increased volatilization and solubility of some HCs at elevated temperature affects toxicity and allows biotransformations with high substrate concentration (Margesin and Schinner, 2001a). However, bioremediation processes can be achieved in environments with low temperature under the right conditions (Margesin and Schinner, 1997; Margesin and Schinner, 2001b). Like total numbers of culturable microorganisms, the numbers of HC-degraders show seasonal fluctuations. Rates of disappearance of HCs are correlated with the mean monthly environmental temperature (Jackson and Pardue, 1997; Rooney-Varga *et al.*, 1997). Biodegradation of petroleum at low temperatures (0°C, 5°C and 10°C) had been observed for the marine environment (Walker and Colwell, 1974).

2.7.2 pH

Neutral or slightly alkaline pHs (6-8) promote optimum microbial activity and oil biodegradation (Dibble and Bartha, 1979). Changes in the pH can have indirect impact on enzymes and nutrient availability (McKee and Mendelssohn, 1994). Wetland soils are typically saturated, a condition that gives rise to a near neutral pH (Breitenbeck and Bryan, 1998). pH can be increased by application of lime, however, the requirements cannot be determined directly by the soil pH since many other characteristics such as soil texture, soil type, conductivity and clay content influence lime requirements. Care must be taken when pH is going to be modified because some elements may become more toxic at different pH ranges (Breitenbeck and Bryan, 1998) or the native microorganisms

can be adapted to site conditions (Kastner *et al.*, 1998). Oil biodegradation can be severely inhibited by dramatic reduction in pH when ammonia is provided as the nitrogen source (Wrenn *et al.*, 1994). However, this is primarily true for closed environments such as laboratory studies (e.g., microcosms) where no dilution from changing aqueous conditions is present (e.g., tides) (Zhu *et al.*, 2001).

2.8 Bacterial Enumeration

While no technique to enumerate specific metabolic types of microorganisms in marine systems is absolute, the Most Probable Number (MPN) technique can give consistent results that are appropriate for relative comparisons among sampling sites (Brown and Braddock, 1990). The MPN method is based on the hypothesis that inoculation of a single cell into a tube of media will produce an observable change in the substrate or culture medium (e.g., a color or turbidity change), the microorganisms are distributed randomly throughout the sample, and growth can be described by a Poisson distribution (Long *et al.*, 1995).

Microorganisms with specific metabolic capabilities, such as oil biodegradation, can be enumerated based on their ability to grow on selective media. Tetrazolium dyes can be used as colorimetric indicators of the biochemical oxidation of any organic substrate. Bacterial oxidation of a substrate generates reduced nicotinamide adenine dinucleotide (NAD), so if the electrons are donated to an electron transport system, a tetrazolium dye may function as an artificial TEA. The reduced product is a colored, insoluble formazan and the density of microorganisms can be calculated based on the number of colored tubes (positives) (Konopka *et al.*, 1998).

The MPN procedure is suitable for organisms that grow on insoluble substrates, because solid media containing a homogeneous distribution of the appropriate carbon source is difficult to prepare (Haines *et al.*, 1996). The low percentage of living bacteria obtained when comparing viable counts (such as MPN) with total direct counts in seawater could be due more to inappropriate technique for detecting the growth ability of living cells than to unacceptable culture conditions (Hurst *et al.*, 1997). MPN calculations can also be enumerated by using the visual observation of pH indicator or turbidity and gas production to detect positive tubes. Changing the detection method (i.e., from MPN to direct counts) could provoke an overestimation of the microbial density (Bianchi and Giuliano, 1996).

MPN procedures have been used to estimate the population density of oil degrading microorganisms in aqueous solutions and soil samples (Long *et al.*, 1995; Piehler *et al.*, 1996; Uraizee *et al.*, 1998). Enumeration of HC-degraders is accomplished most reliably by MPN procedures, because non HC-degrading bacteria can grow on impurities present even in highly purified agar (Wrenn and Venosa, 1996). After the three year period following the *Exxon Valdez* oil spill in Prince William Sound, AK, significantly higher numbers of HC-degrading microorganisms were found at sites within the path of the oil slick than at control sites, indicating rapid acclimation of the resident microbial populations (Braddock *et al.*, 1995; Piehler *et al.*, 1996; Piehler *et al.*, 1997a). The number of HC-degraders varied with depth and location. The ratio of HC-degraders to total number of cells also changed with depth (lower with depth) and site of study (higher close to the site) (Braddock *et al.*, 1995; Venosa *et al.*, 1996).

2.9 Petroleum-Contaminated Salt Marshes and Remediation Techniques

Salt marsh environments are highly sensitive to oiling and high priority is given for their protection during oil spills. Inappropriate response activities can cause harm to oil impacted marshes. Different parameters such as substrate type, plant species, season of impact, oil type, and climate have to be considered and may all affect the eventual recovery of an oil-impacted salt marsh (Table 4) (Hoff, 1995).

Table 4. Cleanup techniques used in marshes. (Taken from Hoff, 1995)

Advantages	Disadvantages
No response Minimal impact (if oil degrades quickly) No physical impact	Potential oiling of birds or wildlife Oil may impact adjacent areas Heavy oil may degrade slowly or form asphalt
Vacuum/pumping Can remove large quantities of oil	Access/deployment of equipment Physical impact
Low pressure flushing Assists in removal by confining oil Lifts oil off sediment surface	Requires careful monitoring Pressure must be controlled Physical impact
Burning Potential to remove oil quickly Can minimize impacts from trampling	Potential damage to plant roots and rhizomes Little known about impacts due to season, destruction of marsh, species composition, air pollution Regulatory concerns
Sediment removal May be only remediation possible for heavily-oiled sediments	Destroys marsh Increased erosion potential Elevation changes could affect regrowth of plants Replanting necessary
Vegetation cutting Leaves most of future plants intact Prevents oiling of birds	May kill plants Potential for increased erosion Must be carefully monitored
Bioremediation Great theoretical potential Low impact	Few case studies available Potential for nutrient enrichment Oxygen may be limiting

2.9.1 Bioremediation in Salt Marshes

A salt marsh is a wetland. Three major features of wetlands are generally recognized: 1) the presence of water at the soil surface or in the root zone, 2) soil conditions that are unique and distinctly different from uplands, and 3) the occurrence of vegetation adapted for growth in saturated or flood soils (McKee and Mendelssohn, 1994). Because of variation in tidal regime, temperature, sediment transport, topography, and hydrology, salt marsh characteristics vary with location and even within individual marshes (Hines *et al.*, 1989). Although marshes are present all along the coast, those in New England tend to be small and characterized by a heavy peat substrate. The hydrology of marshes determines their unique physiochemical qualities, influences the occurrence and distribution of organisms within them, and controls the movements of water, sediments, salts, nutrients, and toxins.

The intermittent or permanent presence of water in wetlands leads to the development of hydric soils, which are characterized by anaerobic conditions in the bulk soil (McKee and Mendelssohn, 1994). The high oxygen demand of marsh soils creates a very small aerobic layer (only few millimeters thick) at the surface of the soil. Most of the marsh soils are dominated by anaerobic processes (Hines *et al.*, 1989; Jackson and Pardue, 1997; McKee and Mendelssohn, 1994; Nixon and Oviatt, 1973; Wright *et al.*, 1997).

Microbial degradation appears to be the major process through which petroleum HCs and other toxic organics are removed from bottom sediments in wetlands (DeLuane *et al.*, 1990). For example, after an experimental oil spill, where a community shift from primary eukaryotic biomass to Gram negative bacterial biomass was observed, the

structure and diversity of the dominant bacterial community changed substantially (Macnaughton *et al.*, 1999). Strains of the same species often have different degradation abilities or HC substrate preferences. However, the taxonomic status of the microorganisms seems highly dependent on the physicochemical factors at a site (e.g., soil structure, water capacity) (Becker and Dott, 1995). The physiological condition of microbial communities represents a definite sum of abilities, within which different contributions of the individual organisms allow a certain variability of the species spectrum. This sum of abilities seems to depend on the carbon source of the salt marsh. Generally, the petroleum contamination is located in the surface layer (0-4 cm) and infrequently reaches the deeper zones because of the fine-textured nature of the sediments (Hershner and Lake, 1980; Mille *et al.*, 1998). The effect of crude oil on microbial processes may be affected by the type of organic material present in the impacted marine sediment (Griffiths *et al.*, 1982).

Redox potential and pH have been shown to control the degradation of many classes of organics in coastal regions in Louisiana bottom sediment. Most of the HCs degrade more rapidly under aerobic conditions. Aerobic microorganisms in oxidized sediment are more capable of degrading HCs than anaerobic microorganisms in reduced sediment at the same pH (DeLaune *et al.*, 1980; DeLuane *et al.*, 1990). Both oxidized and reduced zones may occur in proximity to a wetland soil (McKee and Mendelssohn, 1994).

Results indicate that marsh grasses can survive one, light to moderate dose of oil (Wright *et al.*, 1997). Multiple doses, however, are frequently very deleterious. *S. alterniflora* was the only grass to exhibit sublethal effects of oil dosing on growth

(Hershner and Lake, 1980). Cleanup methods for spilled oil, such as tilling and burning of oiled vegetation, may cause more damage to salt marshes than the spilled oil (Wright *et al.*, 1997). Tilling can move HCs deeper in the sediments where biodegradation is generally slower.

2.9.2 Phytoremediation

In recent years, the use of plants to remediate soils contaminated with organics has become a promising new area of research, particularly for *in situ* cleanup of large volumes of slightly contaminated soils (Adler, 1996; Chang and Corapcioglu, 1998; Cunningham and Berti, 1993; Cunningham *et al.*, 1995; Cunningham *et al.*, 1996; Cunningham *et al.*, 1997; Rogers *et al.*, 1996; Sergeant, 1995). Using different plants, phytoremediation can contain, destroy, or extract organic contaminants from soil (Rock, 1997). Several studies have shown the effective use of plants to remediate soils contaminated with PAHs (Aprill and Sims, 1990; Chang and Corapcioglu, 1998; Kling, 1997; Schwab and Banks, 1994; Simonich and Hites, 1994). Rhizosphere microbial populations may increase bioremediation if the soil is contaminated with organic chemicals (Gatliff, 1994; Nichols *et al.*, 1997). Microbial populations have been reported to be at least two orders of magnitude higher in the soil of the root zone than in an adjacent unplanted soil (Rock, 1997). The limiting factors for the growth of most microbial consortia include oxygen, nutrients and water. As roots penetrate the soil, there is a passive aeration as they release oxygen as part of the normal plant respiration.

Phytodegradation is the process of the plant degrading the contaminant. This may occur because of the plant's transformation or mineralization of the contaminant by

exudates to a less toxic form. For example, there are plants that bring pollutants (e.g., heavy metals) into their plant tissue and store quantities of them. Plants can also influence the movement of contaminants by creating new path of movement with their roots (Jordahl *et al.*, 1997; Rock, 1997). *S. alterniflora* roots can produce low molecular weight organic compounds (e.g., ethanol and malate) when the roots metabolize anaerobically. SRB can use these compounds directly (Hines *et al.*, 1989).

In plant-inhabited soils and sediments, the rhizosphere is an important microhabitat. Biochemical processes (e.g., organic matter decomposition, pollutant degradation, and nonsymbiotic nitrogen fixation) occur at accelerated rates in the rhizosphere and greatly influence ecosystem functions. Despite its importance, very little is known about microbial activity in the rhizosphere or about how it differs from its non-rhizosphere counterpart (Rooney-Varga *et al.*, 1997).

Several factors such as temperature (Howes *et al.*, 1985; McKee and Mendelssohn, 1994), salinity (Buresh *et al.*, 1980), tidal energy (Buresh *et al.*, 1980), oxygen (Dibner, 1978), and NO_3^- (Buresh *et al.*, 1980; Stepanauskas *et al.*, 1996) concentrations, and nutrients (Jackson and Pardue, 1997) are responsible for the variation in *S. alterniflora* growth in marshes. Although the leafy tissues and aerial stems of *S. alterniflora* die back each year, the underground stems (i.e., rhizomes) and roots are perennial structures. These organs, which are essential to the plant's vegetative survival, require oxygen to survive, but cannot obtain it from the anaerobic sediments in which the plant commonly grows. They obtain the necessary oxygen and release carbon dioxide through a system of open spaces and hollow, air-filled tubes that are in contact with the atmosphere via pores in the leaves (i.e., stomata) (Dibner, 1978).

Coastal microbial communities play key roles in nutrient cycling and are a primary food source for many high level organisms. *Spartina sp.* stem microalgal epiphytes have been identified as a source of new nitrogen for salt marshes and they also serve as prey for marsh microfauna. Petroleum has the potential to be toxic to stem epiphytes as well as to be a source of labile organic carbon (Piehler *et al.*, 1997b).

2.9.3 Nutrient Amendment

Availability of nutrients is a major limitation in bioremediation of HC-contaminated soil (Atlas and Bartha, 1973b; Margesin and Schinner, 2001b; Olivieri *et al.*, 1976). Nutrients can be added in the form of inorganic or organic fertilizers (Levin and Gealt, 1997). C/N/P ratios of 100:10:1 (Dibble and Bartha, 1979), 100:10:2, or 120:10:1 (Margesin and Schinner, 1997), 300:15:1 (Ritter and Scarborough, 1995) are recommended. Optimal C/N ratios between 9:1 and 200:1 have been reported for waste oils and sludge (Margesin and Schinner, 1997) and C:N and C:P ratios of 60:1 and 800:1, respectively, for an oil sludge (Dibble and Bartha, 1979). However, in reality, a complete assimilation of petroleum HCs into biomass is not achievable under natural conditions and most of these ratios are based on theoretical and laboratory studies. Some of the compounds are recalcitrant or are metabolized slowly over long periods, so the ratios cannot be predicted accurately and require experimental determination (Dibble and Bartha, 1979).

The rate of biodegradation can be a function of the nitrogen concentration maintained in the porewater of the intertidal sediments. This suggests that the effectiveness of bioremediation can be improved by making real time measurements of nutrient levels in sediments to ensure that adequate, but non-toxic levels of nutrients are

maintained during the treatment (Bragg *et al.*, 1994). The excess of nutrients can cause eutrophication in aquatic environments.

After the *Exxon Valdez* spill, areas treated with water soluble and oleophilic fertilizers were compared to untreated control areas (Levin and Gealt, 1997). Nutrients in the oleophilic fertilizers adhered to the oil covering the rocks and grasses. After approximately two weeks, the areas receiving the oleophilic fertilizer were visibly cleaner at and below the beach surface than other sections of the shoreline (Frederick and Egan, 1994). Studies have shown that adding fertilizers can accelerate the rate of oil removal by a factor of >5 (Bragg *et al.*, 1994).

Bioremediation of petroleum has been correlated with soil nitrogen as a function of soil water (porewater), not soil dry matter (associated with soil) (Walworth *et al.*, 1997). The addition of nitrogen can increase the biomass of *S. alterniflora* up to 28% over controls (Buresh *et al.*, 1980).

Studies have shown that the minimum NO_3^- concentration needed to support the maximum growth rate of alkane-degraders under continuous flow conditions ranges between 0.5 and 2.5 mg NO_3^--N/L. Results have demonstrated that maintenance of a threshold concentration of about 1-2 mg NO_3^--N/L in interstitial porewater in sandy beaches permits HC bioremediation. Since NO_3^- in interstitial porewater is quickly diluted to background levels whenever the incoming tide completely submerges the beach, it has to be applied every day (Venosa *et al.*, 1996).

The addition of nitrogen and phosphorus alone does not result in stimulation of biodegradation (Walworth and Reynolds, 1995), but nitrogen although not P and *Spartina* does result in stimulation of biodegradation. Maximum biodegradation rates were

obtained by the addition of the *Spartina* particulate organic carbon (POC), NH_4^+, and PO_4^{-3} (Piehler and Paerl, 1996). The presence of small organic compounds enhances microbial growth and HCs can be biodegraded by co-metabolism. Nutrient addition is a plausible strategy for improving HC degradation, although its overall effectiveness may be limited by oxygen availability, the type of crude to be degraded, and the form and concentration of the amendments (Adrian *et al.*, 1998; Swannell *et al.*, 1996).

2.9.4 Bioaugmentation

Bioaugmentation requires the addition (seeding) of non-indigenous, or native petroleum-degrading microorganisms into contaminated sites (Atlas, 1981; Lee and Merlyn, 1999; Mishra *et al.*, 2001). In many cases the addition of microorganisms does little to recover contaminated sites under changing environmental conditions (e.g., temperature, pH) (Atlas, 1992; Lee and Merlyn, 1999; Levin and Gealt, 1997). However, under controlled conditions, adapted or modified microorganisms may efficiently degrade target HCs compounds over wide ranges of temperatures and nutrient level (Kastner *et al.*, 1998). The *in situ* competitive interaction between novel and introduced species generally seems, however, to favor the indigenous species. The added microorganisms generally are not adapted to the specific environmental conditions of the contaminated site and their survival is often very limited. Conversely, the reintroduction after enrichment of indigenous microorganisms isolated from contaminated sites with nutrients can help overcome a problem with low microbial abundance in a contaminated site (Korda *et al.*, 1997; Kujat, 1999; Mishra *et al.*, 2001).

2.9.5 Use of Surfactants

Surfactants are compounds capable of reducing surface and interfacial tension at the interfaces between liquids, solids and gases, allowing emulsion mixing or dispersion of sorbed HCs (Banat *et al.*, 2000). Chakrabarty (1985) reported that an emulsifier produced by *Pseudomonas aeruginosa* was able to quickly disperse oil into fine droplets, and inferred that it may be useful in removing oil from contaminated sites. However, some studies have shown the effects of adding surfactants on oil bioremediation are unpredictable (Banat *et al.*, 2000; Bruheim *et al.*, 1997; Bruheim and Eimhjellen, 1998).

2.9.6 Addition of TEAs

Microbial degradation rates of oil within sediments are very low under anoxic conditions (Atlas, 1992; McKee and Mendelssohn, 1994). Recent studies in marshes have demonstrated mixed results for biodegradation rate enhancement with the addition of fertilizers and suggest that oxygen limitation may be the main limiting parameter (Hin *et al.*, 2000; Jackson and Pardue, 1997; Lee and Merlyn, 1999; Swannell and Head, 1994). As indicated by the sediment oxygen demand (SOD) experiments, adding oil and nutrients can increase the SOD *in situ* (Adrian *et al.*, 1998).

Oxygen supplements in the form of Oxygen Release Compounds (ORC) (Regenesis Corporation; San Juan Capistrano, CA) are usually patented formulations consisting of magnesium peroxide and other chemicals. When wetted, the magnesium peroxides react to form oxygen and magnesium hydroxide. According to the manufacturer's literature, ORC is 25-35% magnesium peroxide.

Microbiological investigations focusing on laboratory microcosms and selective enrichment experiments have isolated and characterized different microorganisms and

demonstrated anaerobic degradation of aromatic HCs under redox conditions ranging from denitrification to methanogenesis (Mahne and Tiedje, 1995; Shi *et al.*, 1999; Song *et al.*, 2000; Straub and Buchholz-Cleven, 1998). Biodegradation of three-and four-ringed PAHs by pure cultures has been possible under denitrifying conditions to non detectable levels ($\leq$0.001 mg/L) (McNally *et al.*, 1998, 1999).

In a controlled oil spill (Arabian light crude oil) in a salt marsh (San Jacinto River, TX), KNO_3 was selected as source of NO_3^- to act as TEA. During this study, the addition of NO_3^- did not significantly increase the numbers of aliphatic-degrading, PAH-degrading or total heterotrophic microorganisms compared to the populations in the control plots (Townsend *et al.*, 2000).

A study by Cervantes *et al.* (2001) indicated that humic acid substances might significantly contribute to the intrinsic bioremediation of anaerobic sites contaminated with HC pollutants by serving as TEAs. The dismutation of chlorite by perchlorate-reducing bacteria in anaerobic environments can produce extracellular oxygen. This oxygen can be used by HC-degraders for compounds such as benzene (Coates *et al.*, 1998).

Oxygen is directly used in HC metabolism (e.g., initial conversion to alcohol). Although other oxidants such as NO_3^-, Fe^{+3}, Mn^{+4}, SO_4^{-2} and CO_2 can be used as TEAs, they cannot replace oxygen as a direct reactant (Chayabutra and Ju, 2000). For example, addition of NO_3^- resulted in reduced concentrations of toluene, ethylbenzene, and m,p-xylenes (TEX) in contaminated groundwater in Monroe County, WI. During the study, excess NO_3^- loss was attributed to oxidation of other organics in addition to TEX (Schreiber and Bahr, 2002).

2.10 Oil Spill Incidents

There have been several oil spills where different products have been used in an attempt to enhance biodegradation. In some cases, the authorities have allowed products to enhance remediation to be used for experimental purposes. However, in general, it is difficult to draw valid conclusions from many of these efforts because of the time constraints in planning experiments with appropriate controls after a major spill (Swannell *et al.*, 1996). One notable exception is the work conducted in response to the *Exxon Valdez* spill. The assessment of bioremediation products and techniques for this spill were based on experiments carried out with considerable scientific rigor (Braddock *et al.*, 1995; Bragg *et al.*, 1994; Lindstrom *et al.*, 1991).

Several oil spills have impacted a large number of salt marshes around the world. The best evidence of the effects of an oil spill on a shore community is usually obtained from investigations following the coastal wreck of a tanker or a serious accident during its unloading. Several such spill have occurred, in 1957 the wreck of the *Tampico Maru* in Baja CA and in 1967 the stranding and break-up of the *Torrey Canyon* off Cornwall, England (Nelson-Smith, 1970). In addition, the oil barge *Florida* ran aground in Buzzards Bay on the coast MA in September 1969. An estimated 700 m^3 of No. 2 fuel oil leaked from the barge and was driven on-shore by heavy winds. In the oil-contaminated coastal habitats and subtidal bays many marine and salt marsh organisms died (Burns and Teal, 1979). On the night of May 25-26, 1969, at the site of the Wellen Oil Company storage facility in Jersey City, NJ, an oil tank ruptured releasing a considerable amount of crude oil (Dibner, 1978).

During the years 1970-1972, the Nipisi, Rainbow, and Old Peace River pipeline spills occurred in the lesser Slave Lake area of northern Alberta, Canada. After 25 years, results on these wetlands showed that: 15-43% of the residual oil in surface samples (0-4 cm) has been removed by weathering. Subsurface samples (10-40 cm) had the greatest quantities of oil even 25 years after the spill, indicating that the recovery rates were slow. The extent of contamination and degree of degradation correlated strongly with sample depth (Wang *et al.*, 1998).

On the night of March 16, 1978, the supertanker *Amoco Cadiz* drifted onto the North Brittany coast of France, releasing 223,000 m^3 of crude oil (123,000 m^3 of the Iranian light and 100,000 m^3 of Arabian light). Up to 64,000 m^3 of this was estimated to have contaminated the Ile Grande salt marshes during the four weeks following the grounding (Mille *et al.*, 1998). Most of the beach cleanup efforts focused on pumping and mechanical recovery. These operations caused some of the oil to penetrate the sand. Four different products were tested to assess the possibility of promoting the biodegradation of the trapped oil (Swannell *et al.*, 1996): 1) a commercial cleaning compound containing nutrients especially adapted to restore oiled soils; 2) a mixture of lyophilized, bacteria adapted to HC, a chemical dispersant, and a nutrient; 3) a chemical fertilizer used in agricultural applications; and 4) a powder treated with 0.1 % surfactant. Some changes in oil concentrations were found after these experiments, but the limited results were inconclusive. It was not clear if the disappearance of the oil was physically or biologically mediated (Swannell *et al.*, 1996).

On July 28, 1983, the Greek tanker *Shinoussa* collided with two tank barges in the Houston Ship Channel, Galveston Bay, TX, releasing approximately 3,000 m^3 of partially

refined catalytic feedstock oil over 2 days, which spread onto the surrounding coastline. Alpha Biosea (Alpha Environmental, Houston, TX) a product composed of a lyophilized bacterial mixture and inorganic nitrogen and phosphorus was applied eight days after the spill in selected areas of Pelican Island and Marrow Marsh. Two plots on the beach were treated, and two were left as controls. The 15 m diameter experimental plots were sampled on a routine basis. The results showed that there were no significant differences between pre-and post-treatment samples after 96 hr of treatment with any of the selected methods (Swannell *et al.*, 1996).

A pipeline broke on April 1985 near Nairn, LA, resulting in the release of 120 m^3 crude oil into a brackish salt marsh dominated by a vegetative mixture of *S. patens*, *S. alterniflora* and *Distichlis spicata.* Studies after the spill showed that small oil concentrations (0.28 Lt/m^2) had toxic effects on the marsh plants. Prolonged contact of the oil with the photosynthetic leaf issue and the subsequent movement of the oil into the marsh substrate may have been the primary causes for the plant death and low live percentage of cover exhibited after the spill (Mendelssohn *et al.*, 1990).

On June 8, 1990, the Norwegian tanker *Mega Borg* was off-loading crude oil from the Italian tanker *Fraqmura* about 90 km off the Texas coast. Following an explosion and fire, there was a release of 45 m^3 of crude oil. Two portions of the slick were treated with a product containing Alpha Biosea. Little change was observed in the control areas and conclusive evidence of the enhancement's bioremediation effectivenes was not obtained because of limitations in the sampling strategy (Aldhous, 1991; Swannell *et al.*, 1996).

In January 1990, fuel oil from a pipeline failure was spilled into the Arthur Kill, NJ waterway contaminating a gravel beach on the Prall's Island bird sanctuary. Mechanical methods were used to remove the bulk of the oil. A slow-release fertilizer (Customblen; Sierra Chemicals, Mustang, NV) was placed in trenches to encourage biodegradation. Over a 93 d period, subsamples were taken, together with beach and water samples. No clear trends of increased bioremediation from the fertilized plots could be identified during the experiment. However, there was high variability in the levels of TPH, which may have masked any effects (Swannell *et al.*, 1996).

On October 31, 1990, a well blowout off in Seal Beach, CA, resulted in the release of approximately 20 m^3 of crude oil that contaminated 8,000 to 12,000 m^2 of marsh grassland in the Seal Beach National Wildlife Refuge. One week after the incident, a combination of a microbial product used in sewage treatment plants (INOC 8162) and a commercial fertilizer (Miracle Gro 30-6-6) was hand sprayed onto the marsh. After 35 days of monitoring no differences between the treated and untreated plots were observed. The microbial products were also not effective in accelerating biodegradation of oil under controlled laboratory conditions (Swannell *et al.*, 1996).

The tanker *Exxon Valdez* ran aground on Bligh Reef in the Gulf of Alaska on March 24, 1989, spilling approximately 41,000 m^3 of Alaska North Slope crude oil. A major response effort was mounted at sea to recover the oil, but the weather and tidal conditions resulted in the contamination of about 2,090 km of coastline. Many cleanup techniques of shorelines were tried, these included cold and warm water washing, steam cleaning, and manual oil recovery techniques. Initially, the main aim was to remove the heaviest concentrations of oil to minimize the impact on wildlife and fisheries (Bragg *et*

al., 1994; Swannell *et al.*, 1996). Shortly after the spill, it was suggested that bioremediation could enhance the rates of oil removal from the contaminated beaches (Lindstrom *et al.*, 1991). As a preliminary step, the number of oil-degrading microorganisms on oiled beaches was determined as compared to controls. The number of microorganisms increased by as much as 10,000 times to an average level of 10^6 cell/g of beach material (Swannell *et al.*, 1996). Then, it was necessary to establish which factors were likely to limit biodegradation and which specific hydrocarbon components were biodegradable. The experiments demonstrated unequivocally that the microbial population in Prince William Sound could rapidly biodegrade the aliphatic and aromatic fractions of the Prudhoe Bay crude oil in the presence of suitable nitrogen and phosphorus sources. The highest mineralization rates were noted in the test systems treated with the highest concentration of nitrogen (Swannell *et al.*, 1996). Hence, the addition of nutrients, and not seeding with microbes, was thought to be the most appropriate bioremediation strategy (Bragg *et al.*, 1994).

In Snug Harbor located on the southeastern side of Knight Island in Prince William Sound, three oil-contaminated cobble plots were evaluated. They were treated with an oleophilic nutrient amendment, Inipol EAP 22 (Elf Aquataine, France), Woodace briquettes, or left untreated (control). Microbial numbers increased in all plots during the experiment. No significant differences in microbial numbers or TPH concentration were noted between the treatments and the controls. The amounts of oil extracted from beach sediment were highly variable, making the results difficult to interpret. Even though the chemical data were not conclusive, there was at least some evidence that the treatment with Inipol EAP 22 did encourage microbial biodegradation of the oil (Frederick and

Egan, 1994; Stone, 1992; Swannell and Head, 1994; Swannell *et al.*, 1996). Elevated mineralization potentials, coupled with increased numbers of HC degraders, indicated that natural HC biodegradation was enhanced. However, the microbial counts alone were not sufficient to determine *in situ* rates of crude oil biodegradation (Lindstrom *et al.*, 1991). The high energy environmental conditions (e.g., wave action) at the spill site and cleanup efforts resulted in extensive dispersion of the oil. About 50% of the oil was biodegraded *in situ* on beaches and in the water columns, 14% was recovered or disposed, and about 20% was subject to evaporation and photolysis (Wolfe *et al.*, 1994).

2.11 *Julie* N Spill

In September 27, 1996, the T/V *Julie N*, inbound with a cargo of 34,000 m^3 of Nos. 2 and 4 fuel oils struck the Rte 77 Bridge, spanning Portland, ME, as it went through the draw span. In the collision, four holds were damaged: the fore peak tank, forward bunker tank, a void tank/space, and the No.1 port cargo tank. The forward bunker tank lost 353 m^3 of No. 4 fuel oil and the No.1 port cargo tank lost 327 m^3 of No. 2 diesel, totaling 680 m^3 of oil spilled (US Coast Guard, USCG) (Lelyveld, 1996; Porter, 1997). The National Transportation Safety Board said that incorrect instructions from a docking pilot to the helmsman of the tanker caused the vessel to sideswipe a concrete bridge pillar (Kent, 1996). Although much of the oil volatilized over a period of days, the spill fouled beaches and marshes and killed a large amount of marine life in a natural sanctuary (Lelyveld, 1996). The terminal to which the *Julie N* was heading had booms in place by the time the ship arrived. Although good weather helped the subsequent cleanup, a strong wind moved the oil to the Fore River salt marshes. Within minutes,

emergency services had swung into action, with the Coast Guard warning returning lobster fishermen to stay out of the harbor, and lobster dealers shutting off water circulation machinery to prevent oil from reaching nearby fish farms (Porter, 1997; Rice, 1998). Nearly all of the salt marsh vegetation from the I-295 Bridge north to the within 300 m of the Congress Street Bridge near Stroudwater, then south to and including Long Creek was oiled (Reilly, 1998) (Figure 1).

High-volume pressure washing flow, and hot water flushing were conducted along some of the contaminated areas, and were effective in removing some pooled oil, however, a heavy surface coating remained. A test application of Corexit® 9850 (Nalco/Exxon Energy Chemicals; Sugar Land, TX), a biodegradable surfactant, was conducted on marsh vegetation at Thompson Point and was confined to exposed surfaces. The areas that were exposed to oil experienced good removal but the underlying materials remained heavily oiled. The different agencies (MEDEP, the U.S. Fish and Wildlife Service (USFWS), NOAA and EPA) recommended that use of Corexit 9850 should not continue because: 1) the vegetation was not visibly cleaned enough to warrant the risk of introducing more oil into the water column and sediments; 2) it appeared that a significant amount of the released oil was not immediately recoverable; and 3) the test site represented the best operational considerations in terms of good access and even then the effectiveness was limited (Reilly, 1998). Another two options were evaluated: cutting the oiled vegetation and "no action". Consensus among the regulatory group was for the "no-action" alternative.

Teachers and students from the Waynflete School (Portland, ME) conducted one of the first studies in the area. They established two study sites on the East side of

Thompson Point near where the School's outdoors classrooms, nature trails and marsh and river shorelines are located. During the study, they measured stem density of the marsh grass in the plots, to determine whether the oil, which coated the *Spartina sp*, affected the rate of growth of new grass the following spring (Millard, 1997).

Brown (1997) detected total PAH concentrations in Fore River sediments ranging from 2.0 to 62.0 mg/kg (dry weight basis). Only four of the 25 sediments samples analyzed contained petrogenic PAHs attributable to the *Julie N* oil (Brown *et al.*, 1997), however no sampling locations were given. Of the 34 lobster samples analyzed, 22 indicated a contribution from *Julie N* oil. Of the 47 water samples analyzed, 31 samples were identified as contained *Julie N* oil residues, with most samples having from 70% to 95% PAHs. Six of the samples contained PAHs signatures from other (non-*Julie N*) petroleum sources (Brown *et al.*, 1997).

2.12 Research Justification

One of the most important environmental problems is the spill of crude oil and refined products (e.g., Nos. 2 and 4 fuel oils) onto soil and water environments occurred during the extraction, transport, storage and refining processes. Of all oil-impacted estuarine and coastal environments, salt marshes are generally the most sensitive and fragile ecological systems (Wright *et al.*, 1997). The remediation of an oil-contaminated salt marsh is very difficult. Follow up studies from past spills have documented that inappropriate response activities can cause more harm than good (Hoff, 1995). In addition, the ability of salt marshes to recover after an oil spill is very slow because the anaerobic environment (sulfate-reducing) conditions predominant in the sediments. For

this reason the remediation of oil-contaminated salt marshes throught natural attenuation could take years or decades (Bachoon *et al.*, 2001; Burns and Teal, 1979; Mille *et al.*, 1998). Bioremediation, which has emerged as one of the most effective and inexpensive methods to recover oil-contaminated groundwater and soil (Kinner, 1996), could be used for the remediation of oil-contaminated salt marshes.

Some studies have demonstrated the advantages of adding nutrients (mainly nitrogen) to contaminated marine shorelines (Lindstrom *et al.*, 1991; Piehler and Paerl, 1996; Pritchard and Costa, 1991; Venosa *et al.*, 1996). However, this is the first research to evaluate the amendment of oxygen and NO_3^- using a buried horizontal well system meant to deliver amendments to the rhizosphere of an oil-contaminated salt marsh.

CHAPTER III

METHODS AND MATERIALS

This study evaluated the ability of two TEAs (oxygen and NO_3^-) to enhance *in situ* bioremediation in an oil-contaminated salt marsh located along the Fore River Creek in Portland, ME. Two plots were selected for the amendment additions and two plots served as controls to evaluate the natural attenuation process. Background conditions were assessed in all four plots prior to adding amendments. The amendment of the TEAs and sampling events were conducted during Summer and Fall 1998 and Spring 1999. The parameters used to monitor and assess biodegradation were: TPH concentration, abundance of oil-degraders, porewater nutrient concentrations, and plant height and density. Prior to the field evaluation, three methods of TPH analysis were compared: the ASTM (1995) Standard Test Method for Screening Fuels in Soils, the EPA (1994) Oil and Grease Extraction Method for Sludge and Sediment Samples, and the MADEP (1998) Method for the Determination of Extractable Petroleum Hydrocarbons (EPH).

3.1 TPH Methods

TPH is sometimes referred to as hydrocarbon oil, extractable hydrocarbon, or oil and grease. There are many analytical techniques available that measure TPH concentrations in the environment. No single method measures the entire range of

petroleum-derived HCs. The methods vary in the way HCs are extracted, concentrated, and detected. In addition, they measure slightly different groups of petroleum HCs. Therefore, the definition of TPH depends on the analytical method used (Weisman, 1998).

3.1.1 EPA Oil and Grease Gravimetric Method

This method is used to quantify low concentrations of oil and grease by chemically drying the sample and performing a Soxhlet extraction (EPA, 1994; Standard-Methods, 2000). It was evaluated because of its high percent recovery in fuel oil-contaminated soils and the availability of the equipment needed in the laboratory (Simonton, 1998).

A 10.00 g sediment sample was weighed (P1200N, Balance, Mettler; Hightown, NJ) and mixed thoroughly with 10.00 g of granular anhydrous sodium sulfate (ACS grade) (EM Scientific; Gibbstown, NJ) previously pre-dried at 400°C for 4 h in a shallow tray. The sodium sulfate was used to absorb the water present in the sample. The mixture was placed in a 20 mL glass extraction thimble (VWR; Boston, MA) and covered using glass fibers to avoid spilling. The thimble was placed in a Soxhlet apparatus and 150 mL of hexane (ACS grade) (Burdick and Jackson; Muskegon, MI) were circulated at a rate of 20 cycles/h for 4 h to extract the TPH. Boiling chips were used during the extraction to minimize superheating. The extract was filtered into a pre-weighed boiling flask using Whatman #40 filter paper (Maidstone, UK) and 4.5 g silica gel (100-200 mesh) (Fisher Scientific; Fair Lawn, NJ) were added to remove the grease. The hexane was evaporated from the boiling flask. The flask was then allowed to cool in

a ventilation hood for 3 h and was weighed on an analytical balance (Ohaus AS200; Florham Park, NJ). The flask's net weight represented the TPH in the sample:

$$mgTPH/kg_{dw}\ soil = \frac{\text{gain in weight of flask (g)} \times \left(\frac{1000\ mg\ TPH}{g\ TPH} \times \frac{1000\ g\ soil}{kg\ soil}\right)}{\text{weight of the solids (g)} \times \text{dry weight fraction}} \quad \text{(Eq.3.1)}$$

To calculate the dry weight fraction, another 10.00 g of the sample was weighed and dried overnight at 100°C. The sample was cooled to room temperature and weighed to determine the dry weight fraction:

$$\text{Dry weight fraction} = \frac{\text{weight dry sample (g)}}{\text{weight wet sample (g)}} \quad \text{(Eq. 3.2)}$$

For quality control, three types of blanks were used: clean sand (CS) (Ossipee Aggregate; Ossipee, NH) combusted at 550°C for 90 min to eliminate any organic matter present, an organic soil (OS) (Hoffman; Lancaster, NY), and sand previously contaminated (PCS) with No. 2 fuel oil used in another study (Simonton, 1998). The blanks were spiked with 0.5 g of No. 2 fuel oil (Proulx Oil; Newmarket, NH). Percent recoveries were obtained by dividing the weight increase of the spiked samples by the weight change of a spiked blank. Percent recoveries were considered acceptable if they were within 75% and 115% of the theoretical value (EPA, 2000).

3.1.2 ASTM Method for Screening Fuels in Soils

This method is a screening procedure to determine the presence of fuel compounds in soil. It can be used to identify the presence of contamination or to estimate the concentration of a fuel in a soil using average response factors (ASTM, 1995; Sorini *et al.*, 1997).

A 5.00 g sediment sample was placed in a preweighed 125 mL wide mouth, glass sample bottle with a Teflon®-lid cap (VWR). 5.00 g of calcium oxide (Certified Grade) (Fisher Scientific) was mixed with the sample to remove water and humic acids. The calcium oxide was pre-dried at 900°C for 12 h and stored in a desiccator prior to use. 50.0 mL of isopropyl alcohol (Reagent Grade) (Fisher Scientific) were poured into the mixture. A Teflon® magnetic stir bar (VWR) was placed in the bottle prior to capping. The slurry was stirred for 3 min. After a ~5 min settling period, 10 mL of the supernatant were collected with a syringe and filtered using a disposable 0.45 μm polytetrafluoroethylene cartridge (Osmotics; Livermore, CA). The filtrate was poured in a 1 cm pathlength quartz cuvette and the sample's absorbance was read at 254 nm in a spectrometer (Hitachi U-2000; New York, NY).

A calibration curve was obtained using No. 2 fuel oil (Proulx Oil) at four different concentrations: 40.0, 80.0, 120.0 and 160.0 mg/L (n=2). These standards were selected because they were inside of the 0.000 to 1.000 absorbance unit (AU) range. Isopropyl alcohol was used to zero the instrument. Any sample above 1 AU was diluted into the range using isopropyl alcohol. A calibration curve was created from the standards (mg/L vs. absorbance) and used to calculate the TPH concentration associated with the samples (mg/kg). The initial concentration in mg/L was multiplied by a factor of 10 to find the concentration in mg/kg. The factor of 10 was used for a solvent volume-to-soil mass ratio of 50.0 mL of isopropyl alcohol: 5.00 g of soil. If the extract was diluted, an appropriate correction was made.

For quality control, a sample was monitored that only contained isopropyl alcohol and calcium oxide. Two types of blanks were used: CS and OS. One set of blanks was

spiked with 20 µL of No. 2 fuel oil, and the extractions conducted. Recoveries were calculated by comparing the absorbance of the extract from the spiked sample with the absorbance of a solution of 20 µL of No. 2 fuel oil in 50 mL of isopropyl alcohol. After correction for any material appearing in the unspiked sample, the recovery had to be within ± 20% of the true value (ASTM, 1995).

3.1.3 MADEP Method for the Determination of Extractable Petroleum HCs

This method is designed to measure the concentration of extractable aliphatic and aromatic petroleum HCs in water and soil (MADEP, 1998a). The aliphatic HCs are quantified as: C_9 through C_{18} (SC), and C_{19} through C_{36} (LC). The aromatic HCs are quantified as C_{11} and C_{22}. The aliphatic and aromatic HC ranges correspond to compounds with boiling points between 150°C and 500°C. This method uses an SPE process, and GC/FID.

Approximately 5 g of the sample (weighed to the nearest 0.01 g) were placed into a 60 mL amber glass bottle with a Teflon® lid (VWR). 5.00 g of anhydrous sodium sulfate (Na_2SO_4) (ACS grade) (Fisher Scientific) was blended with the sample. A disposable tongue depressor was used to transfer and mix the sample. The sodium sulfate, pre-dried at 400°C for 4 h, removed water from the sample. 200 µL of a surrogate, with a final concentration of 40.0 mg/mL, was added to the sample. The surrogates used for the aliphatic and aromatic fractions were COD (AccuStandard, Inc; New Haven, CT) and OTP (AccuStandard), respectively. Using a pipette dispenser (Brinkmann Instrument, Inc.), 40.0 mL of methylene chloride, (CH_2Cl_2) (GC grade) (Fisher Scientific) were added to the bottle for the solvent extraction phase.

3.1.3.1 Sample Extraction

The extraction was conducted using an ultrasonic and liquid processer sonicator, (Misonix Inc; Farmingdale, NY) for 5 min in a pulse mode (1 sec). The 5 min sonication cycle was performed three times. The sonicator was tuned before use, following the manufacturer's specifications. The instrument was located in a soundproof box inside a fume hood. Between samples, the horns were cleaned with 70% isopropyl alcohol (Fisher Scientific) (commercial grade). After the extraction, the samples were filtered into a concentrator tube using a glass funnel and Whatman #40 filter paper. The extraction bottle was rinsed twice with 5 mL of methylene chloride to ensure that all of the TPH was collected. The methylene chloride was concentrated to 1.0 mL using a bench top concentrator (TurboVap II® Concentration Workstation; Zymark Corp; Hopkington, MA). The concentration consisted of a water bath at 40°C with a constant nitrogen flow (15 psi; Ultra High Purity grade) (Northeast Air Gas; Dover, NH). For the solvent exchange, 50 mL of hexane (GC grade) (Fisher Scientific) were added. The sample was concentrated to 1 mL in the Turbo Vap as described previously.

3.1.3.2 Silica Gel Cleanup and Fractionation

Using a Pasteur pipette, the extract was transferred to a 5 g/20 mL commercially-prepared SPE silica gel cartridge (Waters Corporation; Milford, MA). The cartridge was pre-cleaned by passing 30.0 mL of methylene chloride through it, prior to being pre-dried in a convection oven at 100°C for 3 h, and stored in a desiccator until use. The cartridges were rinsed with 60.0 mL of hexane prior to being loaded with sample. The concentrator tube was rinsed with 1.0 mL of hexane, and the rinsate transferred into the silica gel cartridge. Silica gel separates petroleum distillates into aliphatic and aromatic fractions.

In order to elute the aliphatic fraction, 20.0 mL of hexane were passed through the column and collected in the concentrator tube. 20.0 mL of methylene chloride were subsequently passed through the cartridge to elute the aromatic fraction. This extract was collected in a second concentrator tube. The samples were concentrated to 1.0 mL in the Turbo Vap and transferred into labeled 15 mL glass vials with Teflon®-lined screw caps (VWR). Each concentrator tube was rinsed with 1.0 mL of hexane for the aliphatic fraction or 1.0 mL of methylene chloride for the aromatic fraction to obtain a final volume of 2 mL. 200 μL of the internal standard (IS) 5-alpha androstane (AccuStandard Inc) (200 ng/μL) were added to the final extract. Samples were extracted within 7 d after collection and the extracts analyzed within 40 d (MADEP, 1998a).

3.1.3.3 GC Analysis

A Perkin-Elmer (Norwalk, CT) 1020 GC Plus with a Nelson Model 1020 personal integrator and an Autosystem GC equipped with an FID was used for the TPH analysis. An RTX®-5 (Crossbond 5% diphenyl-95% dimethyl polysiloxane; Restek Corporation; Bellafonte, PA) capillary column performed the separation (30 m long, 0.32 mm internal diameter, 0.25 μm film thickness). The total run time was 44.5 min with an initial lockout time of 2.9 min to avoid the integration of the solvent peak. The area sensitivity and base sensitivity were 1350 and 65, respectively. The skim sensitivity was 10% and the minimal peak area was 1.00000×10^5 area counts.

The initial oven temperature of 60°C was maintained for 1 min and then increased at rate of 8.0°C/min to 290°C. The injector and detector temperatures were 285°C and 315°C, respectively. The column pressure was 15 psi. A 0.5 min equilibration time at 60°C was required prior to initiation of a run. The split valve was open for the first 0.35

min of the run to eliminate excess of solvent (Rice, 1998). The analog output settings for the integrator were 10 mV at an attenuation of 4. The injection of the sample from the autosampler was in a fast speed mode. The injection volume was 2.0 μL.

3.1.3.4 GC Methods

In order to analyze the samples and standards, four different methods were created in the GC integrator (EPH 1, EPH 2, EPH 3, and EPH 4; EPH= Extractable Petroleum Hydrocarbons). EPH 1 was used to analyze the aliphatic and first part of the aromatic standards. This method used valley-to-valley baseline integration. EPH 3 was used for the second part of the aromatic standard (after 21.00 min). This method forced a horizontal projection of the baseline and was used to separate aromatic peaks that were very close together (i.e., indenol [1,2,3-cd] pyrene and dibenzo [a,h] anthracene with retention times of 33.227 min and 33.332 min, respectively). EPH 2 and EPH 4 were used for samples. EPH 2 identified and quantified the surrogates and internal standards. This method used valley-to-valley baseline integration. EPH 4 was used to quantify the aromatic samples and the C_9-C_{18} and C_{18}-C_{36} ranges for the aliphatic samples with base-to-base baseline integration.

3.1.3.5 IS Calibration Procedure

The internal standard calibration procedure for aromatic and aliphatic HCs used five concentrations (4.55, 9.10, 45.46, 72.73 and 81.82 ng/μL). The aromatic HC calibration set (DRH-006-CAL SET) (AccuStandard) was composed of 17 aromatic compounds and an aromatic surrogate (Table 5). The aliphatic HC calibration set (DRH-007-CAL SET) (AccuStandard) was composed of 14 aliphatic compounds and an aliphatic surrogate (Table 6)

2 μL of each calibration standard were injected into the GC. The peak area responses were tabulated using the concentration of each compound and the internal standard. Then, the response factors (RF) were calculated for each individual compound:

$$RF = \frac{(A_s)(C_{is})}{(A_{is})(C_s)} \qquad \text{(Eq. 3.3)}$$

where: A_s is the response for the analyte to be measured (area counts), C_{is} is the concentration of the internal standard (ng/μL), A_{is} is the response for the internal standard (area counts), and C_s is the concentration of the analyte to be measured (ng/μL).

If the RF value over the working range was <25% relative standard deviation (RSD), it was assumed to be invariant, and the range RF (RRF) was used for calculations (MADEP, 1998a). An RRF was established for each HC range. The summation of the peak areas of all components in a specific range were tabulated against the total mass injected (Table 7) to calculate the RRFs for the C_9-C_{18} aliphatic HCs, C_{19}-C_{36} aliphatic HCs, and C_{11}-C_{22} aromatic HCs.

$$\text{Range RF} = \frac{(A_s)(C_{is})}{(A_{is})(C_s)} \qquad \text{(Eq. 3.4)}$$

where: A_s is the summation of peak areas of component standards (e.g., C_9-C_{18}, 6 components), (area counts), C_{is} is the concentration of internal standard (ng/μL), A_{is} is the response for the internal standard (area counts), and C_s is the total mass concentration of injected standards (ng/μL).Each RRF was verified every sampling event, and after every 20 samples, by injecting a mid-level calibration standard to verify instrument performance and linearity.

Table 5. Aromatic HC calibration standards with the calculated average RTs and their respective windows (± 0.000 min).

Compound	Mean RT (min)	RT Window (min)
Naphthalene	7.647	7.613-7.682
2-Methylnaphthalene	9.615	9.569-9.663
Acenaphthylene	12.289	12.230-12.350
Acenaphthene	12.868	12.804-12.934
Fluorene	14.441	14.370-14.513
Phenanthrene	17.385	17.310-17.461
Antracene	17.527	17.440-17.616
Ortho-Terphenyl (surrogate)	18.886	18.817-18.955
5-alpha Androstane (IS)	20.388	20.350-20.426
Fluoranthene	21.126	21.048-21.205
Pyrene	21.792	21.704-21.880
Benzo(a)Anthracene	25.630	25.549-25.712
Chrysene	25.761	25.660-25.862
Benzo(b)Fluoranthene	28.839	28.754-28.925
Benzo(k)Fluoranthene	28.917	28.804-29.031
Benzo(a)Pyrene	29.701	29.614-29.789
Indeno(1,2,3-cd)Pyrene	33.227	33.087-33.367
Dibezo(a,h)Anthracene	33.331	33.187-33.476
Benzo(g,h,i)Perylene	34.185	34.023-34.349

Table 6. Aliphatic hydrocarbon calibration standards with the calculated average RTs and their respective windows (± 0.000 min).

Compound	Carbon Number	Mean RT (min)	RT Window (min)
n-Nonane	9	3.004	2.986-3.022
n-Decane	10	4.351	4.334-4.367
n-Dodecane	12	7.786	7.748-7.824
n-Tetradecane	14	11.286	11.230-11.342
n-Hexadecane	16	14.416	13.539-15.293
n-Octadecane	18	17.407	17.339-17.475
n-Nonadecane	19	18.758	18.687-18.828
n-Eicosane	20	20.044	19.971-20.117
5-alpha Androstane (IS)		20.382	20.350-20.414
1-Chloro-octadecane (surrogate)		21.152	21.083-21.220
n-Docosane	22	22.451	22.371-22.530
n-Tetracosane	24	24.660	24.582-24.739
n-Hexacosane	26	26.704	26.625-26.783
n-Octacosane	28	28.599	28.522-28.676
n-Triacontane	30	30.407	30.326-30.488
n-Hexatriacotane	36	39.901	39.724-40.079

If the relative percent difference (RPD) for any analyte varied from the predicted response (Eq. 3.4) by more than ±25%, a new calibration curve was prepared for that analyte or range (QC analysis are presented in Chapter IV).

Table 7. Calibration standard concentrations and masses used for MADEP Method.

Components	Concentration of Standard Analytes (ng/μL)*				
	4.55	9.10	45.46	72.73	81.82
Total Mass C_9-C_{18} (SC) Aliphatic HCs, ng (6 components)	54.6	109.2	545.5	872.7	981.8
Total Mass C_{19}-C_{36} (LC) Aliphatic CHs, ng (8 components)	120.8	145.6	727.3	1,163.6	1,309.1
Total Mass C_{11}-C_{22} Aromatic HCs/PAHs, ng (17 components)	154.7	309.4	1,545.6	2,472.8	2,781.8

*2 μL injection

3.1.3.6 RT Windows

Three injections of the aromatic and aliphatic standards were made in the course of a 72 h period using EPH 1 and EPH 3. The mean RTs were calculated as the average of 15 values for each surrogate (OTP and COD), the IS, and each aromatic and aliphatic compound (Tables 5 and 6). The RT windows for each compound were calculated as ±3 times the standard deviation of the mean RT.

The range RT windows were defined as beginning 0.1 min before the RT of the beginning marker compound and ending 0.1 min after the RT of the ending marker compound. C_{19} was the beginning and ending marker compound for the two different aliphatic ranges. The C_9-C_{18} aliphatic HC range ended 0.01 min before the elution of the C_{19} peak. The C_{19}-C_{36} aliphatic HC range began 0.01 min before the elution of the C_{19} peak.

3.1.3.7 Sample Analysis

The GC sequence began with a blank (hexane for aliphatic HC samples and methylene chloride for aromatic HC samples). The blank identified whether contamination was present in the liner or GC column. If any peak was detected, a second blank was injected to recheck the column. If this was clean (no peaks), a calibration standard was run to verify instrument performance and linearity. Then, the sample extracts were analyzed. Every 20 samples, a blank and a calibration standard were analyzed. The output from the GC reported the RT and the area counts for each peak.

Identification of an analyte occurred when a peak from an extract fell within the daily RT window. Aliphatic and aromatic ranges were determined by the collective integration of all peak elutions between specified compounds (e.g., n-nonane and n-octadecane for the C_9-C_{18} aliphatic range). For the quantification on a peak area basis by IS, collective peak area integration for the fractional ranges, was from the baseline (including the unresolved complex mixture areas). For the integration of individual surrogate compounds and IS, a valley-to-valley approach was used.

3.1.3.8 TPH Calculations

The concentration of a specific HC range in a sample was determined by calculating the amount of analyte or HC injected, from the peak response, based upon the analyte/IS response ratio (MADEP, 1998a):

$$\text{Concentration (ug/kg)} = \frac{(A_x)(C_{is})(D)}{(A_{is})(RF)(W_d)} \qquad \text{(Eq. 3.5)}$$

where: A_x is the response of the analyte, HC range/being measured (area counts), C_{is} is the mass of IS added to extract (ng), D is the dilution factor (1) (dimensionless), A_{is} is the

response of the IS (area counts), RF is the RF for analyte or HC range/TPH (dimensionless), and W_d is the dry weight of sample extracted (g), (eq 3.6 – 3.8)

3.1.3.9 Calculation of Dry Weight of Sample

Dry weight were calculated as follows:

$$\% \text{ Moisture} = \frac{\text{g wet sample} - \text{g dry sample}}{\text{g wet sample}} \times 100 \quad \text{(Eq. 3.6)}$$

$$\% \text{ Dry Solids} = (100\ \%) - (\% \text{ Moisture}) \quad \text{(Eq. 3.7)}$$

$$W_d\ (g) = (\% \text{ Dry solids}/100)(\text{g of extracted sample}) \quad \text{(Eq. 3.8)}$$

3.2 Porewater Analysis

3.2.1 Phosphate Analysis

Orthophosphate (PO_4^{-3}) was analyzed by the Environmental Coastal Chemistry Laboratory (ECCL) at UNH using a Lachat Continuous Flow Analyzer (Milwaukee, WI) with the QuikChem Method 31-115-01-3-A. In this method, the PO_4^{-3} reacts with ammonium molybdate and antimony potassium tartrate under acidic conditions to form a complex. The complex is reduced with ascorbic acid to form a blue color, which absorbs light at 880 nm. The absorbance is proportional to the concentration for PO_4^{-3} in the sample (Lachat-Instruments, 1994).

During the analysis, a calibration curve was created using three standards (0.50, 1.00 and 2.00 μM). All standards were prepared using MilliQ® (Millipore; Bedford, MA) water. As part of the QA/QC criteria, an initial calibration blank (ICB) was run before the samples and continuing calibration blanks (CCB) and continuing calibration verification standards (CCV) were run between the samples to detect any carryover. In addition, a duplicate was run after 15 samples. If the response for a sample was out of range of the calibration curve, a dilution was conducted and a dilution factor was used to calculate the final concentration. No silicate or arsenate interferences were found. Precision was judged by percent coefficient of variation (%CV) using 1.0 and 5.0 μM standards. Percent recovery (%P) was evaluated using artificial seawater spiked with PO_4^{-3}.

3.2.2 Nitrate and Nitrite Analysis

The NO_3^-, and nitrite (NO_2^-) were analyzed by the Jackson Estuarine Laboratory (JEL) at UNH. The samples were run through a cadmium column (where all NO_3^- was reduced to NO_2^-). Then, NO_2^- was determined alone. In this method, NO_2^- is diazotizated by sulfanilamide and N-(1-naphthyl) ethylendiamine dihydrocloride to create a pink color measured at 520 nm.

The NO_3^- and NO_2^- were analyzed using a Lachat Continuous Flow Analyzer with JEL Standard Operating Procedure (SOP) 1.12 derived from EPA (1992). During the analysis, a calibration curve was created from standards (1.00 and 2.50 and 5.00 μM). All standards were prepared using Milli Q® water. No interferences were present during the analysis of the samples. QA/QC and precision were analyzed as described for the PO_4^{-3} analysis.

3.2.3 Ammonia Analysis

NH_4^+ was analyzed by JEL using a Continuous Flow Analyzer with the QuikChem Method 31-107-06-1-C. This method was based on the Berthelot reaction where NH_4^+ reacts with alkaline phenol and then with sodium hypochlorite to form indophenol blue. Sodium nitroprusside (nitroferricyanide) is added to enhance sensitivity. The absorbance of the reaction product is measured at 630 nm and is directly proportional to the original NH_4^+ concentration (Lachat-Instruments, 1994).

During the analysis, a calibration curve was created using three standards (10.0, 20.0 and 50.0 μg N/L). All standards were prepared using Milli Q® water. No interferences from color, turbidity, calcium, magnesium, sulfite or organic matter were present in the samples analyzed. QA/QC and precision were analyzed as described for PO_4^{-3}.

3.3 Enumeration of Oil-Degrading Bacteria

In this study, the MPN technique was used for quantifying oil-degrading bacteria present in the salt marsh sediments. This method determines the abundance of microorganisms, present in a sample with the metabolic capacity to degrade or use different HCs. Microbial growth occurs in a liquid mineral medium (Bushnell Haas (BH); Difco Products; Detroit, MI), with an HC as a carbon and energy source. The presence of active microorganisms is confirmed by the reduction of INT. When the INT is reduced, it is possible to observe a precipitate and red/pink color in the tubes that contain metabolically-active microorganisms with the capacity to degrade HCs. These

tubes are considered positives (Brown and Braddock, 1990; Haines *et al.*, 1996; Konopka *et al.*, 1998; Wrenn and Venosa, 1996).

1.0 g of sediment was used to inoculate a dilution tube containing 9.0 mL sterile buffered peptone water (BPW). This tube was vigorously mixed for 30 s using a vortex. A 1.0 mL aliquot was transferred to a new BPW dilution tube to provide a 1:100 dilution of the sediment. This tube was mixed and the procedure continued to provide a decimal dilution series adequate for enumerating the oil-degrading bacteria. Four dilutions sets of five MPN replicates were used for each sediment sample. Typical dilution series ranged from 10^{-1} to 10^{-6}.

MPN tubes contained 9 mL of BH broth supplemented with 2% sodium chloride, inoculated with 1.0 mL of the sediment dilution and 0.1 mL of No.2 fuel as a carbon and energy source. Blank tubes were included that contained No. 2 oil fuel, but no inoculum. Controls had no fuel.

The MPN tubes were incubated at 20 ± 2°C for 28 d. After the incubation, the tubes were inspected for evidence of emulsification (breakup/droplet formation) of the fuel pellicle in the top of the BH broth according to Brown and Braddock (1990), turbidity, and reduction of INT (Haines *et al.*, 1996).

To determine INT reduction, 1.5 mL of 0.3% INT (Research Organics, Inc.; Cleveland, OH) were added to each tube, and after incubating for 20 min at 20°C, red or pink tubes were counted as positive. A second reading was conducted after 16 h to check for any new positive tubes. A combination of positive/negative tubes for the most representative three dilutions was recorded and the score compared to published 5-tube MPN reference tables (Haines *et al.*, 1996).

3.4 Site Selection

The salt marsh chosen for the field studies had to meet the following criteria: a) be impacted by a recent oil spill with a relatively uniform contaminant distribution (TPH concentrations higher than 5,000 mg/kg_{dw}), b) be close to UNH where the microbial and chemical analysis were conducted, c) have easy access for installing the horizontal wells and sampling, and d) have access to power and water needed for the distribution system.

Site selection was coordinated with officials from NHDES, MEDEP and NOAA. A salt marsh located along the main channel of the Fore River was initially considered for the study (Figure 1). However, the results indicated that the TPH concentrations were lower than the expected to conduct this research (See Chapter IV). For this reason a second marsh was evaluated and selected for the study. The selected marsh was located at Thompson Point in Portland, ME (Figure 1). The salt marsh was contaminated in September 1996, when the T/V *Julie N* collided with the Rte. 77 bridge, spilling 353 m^3 of No. 4 and 327 m^3 of No. 2 fuel oils. After an immediate response, more than 530 m^3 were recovered (80%), but approximately 150 m^3 of oil were lost to the environment. The most affected environments were the marshes along the Fore River (Lelyveld, 1996; Porter, 1997).

Reilly (1998) reported that the vegetation in the marshes between Thompson Point and the Congress Street Bridge still showed evidence of stress in 1997. "In this area, there were scattered open patches in the marsh where much of the *S. alterniflora* vegetation had died and not regrown from the roots, and the broken-off dead stems from the previous year growth were still visible"(Reilly, 1998).

The selected Fore River Creek salt marsh lies between the property owned by Peter Van Wyck (Boston, MA) and the Waynflete School (Figure 3). The Fore River Creek salt marsh has a 2 m mean diurnal tidal range and is mainly dominated by short *S. alterniflora* (Figure 4). The salt marsh conditions are similar to those thought New England as it is small, low energy and characterized by the presence of heavy peat substrate (Howes *et al.*, 1985; Nixon and Oviatt, 1973).

3.5 Experimental Plots

Four plots were selected for the evaluation and installation of the amendments and controls (Figure 5). The plots were selected initially based on visual observation of the oil in the sediments and the TPH concentration was later confirmed by sediment analysis. Two plots were used to evaluate the effectiveness of the air and NO_3^- amendments. In addition, two plots were used as controls to evaluate the natural attenuation process.

3.6 Design of Experimental Plots

Each plot was 10 m long by 3 m wide. The corners of each plot were marked using wooden poles (5.1 cm x 7.6 cm x 1.8 m) that were hammered 1.0 m into the marsh sediment. The amendment plots were divided into 0.6 m intervals with two sections of 0.3 m on the left and right ends of the plots. At each 0.6 m interval, a 3.7 m plastic pipe (2.5 cm external diameter, 1.9 cm internal diameter) was buried approximately 20 cm below the marsh surface.

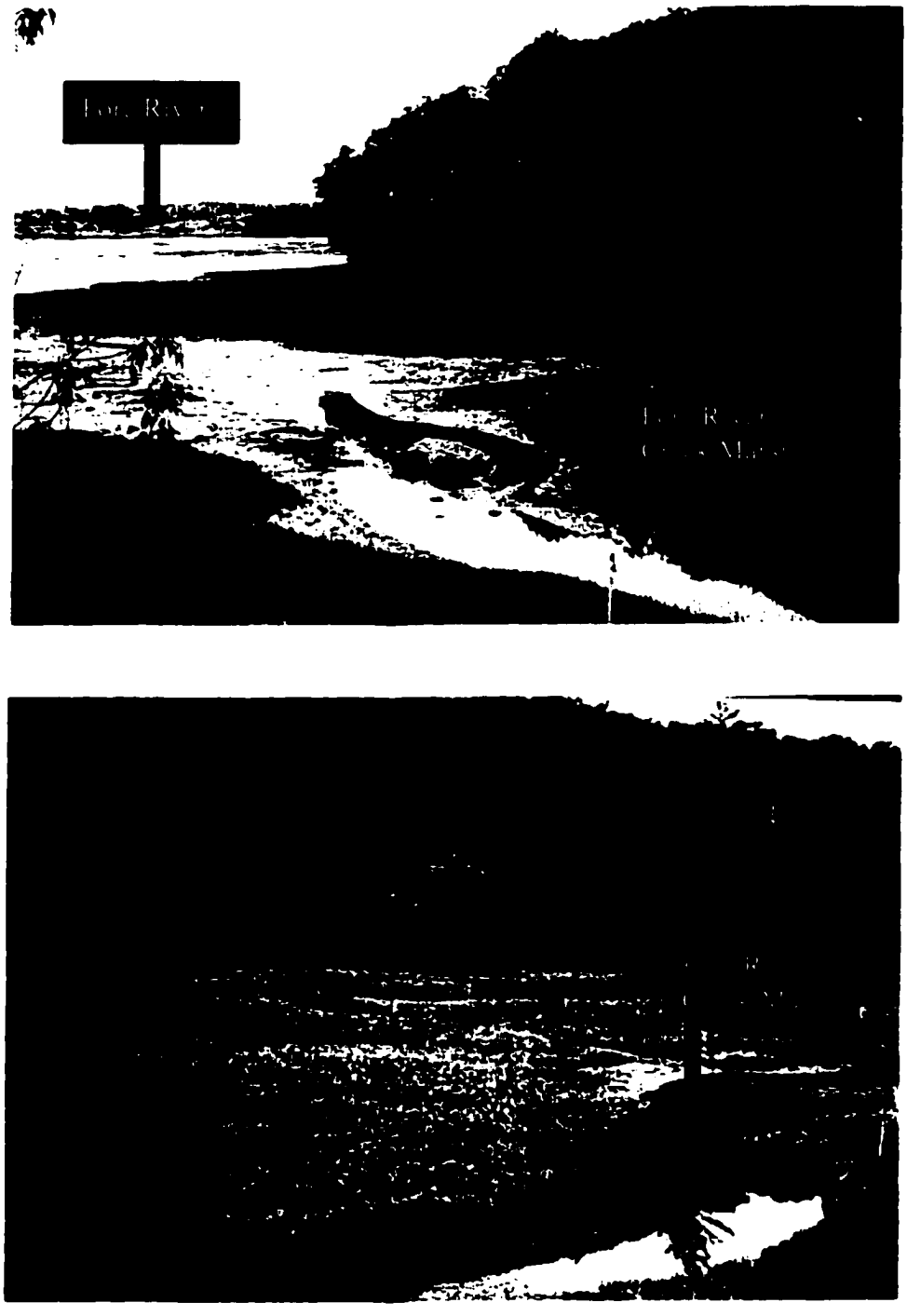
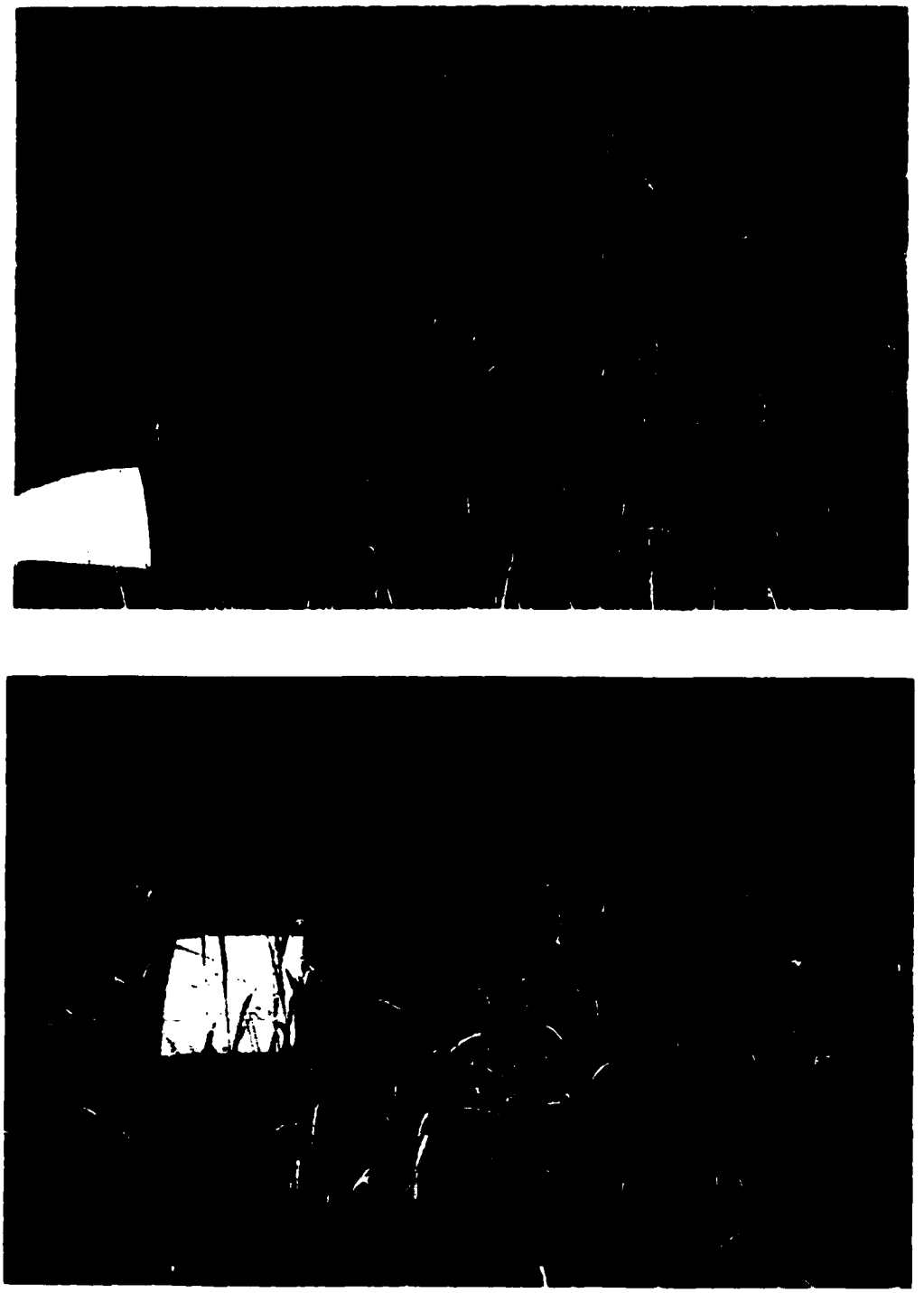


Figure 3. View of the Fore River Creek salt marsh selected for the study. Top: Fore River and Fore River Creek salt marsh (view looking south). Bottom: Fore River Creek salt marsh (view looking north).



B

Figure 4. *S. alterniflora* in the Fore River Creek salt marsh. Top: June , 1998.

Bottom: August 1998.

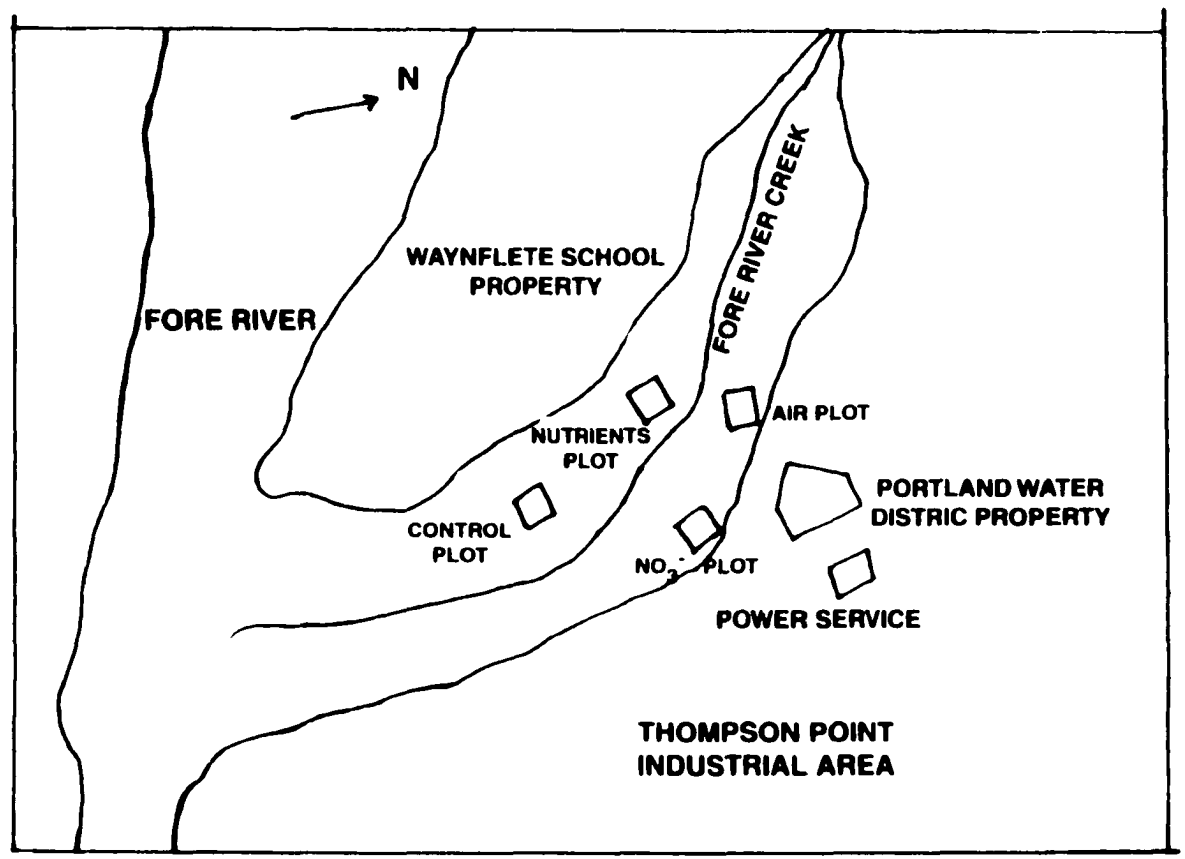


Figure 5. Relative location of the plots in the Fore River Creek salt marsh. (Not to scale)

This depth was chosen based on the estimated contaminant depth of ~4 cm and the depth of the rhizosphere. The final volume per plot surrounding the wells was 2.27 m^3 (Figures 6 and 7).

The plastic pipe was perforated to facilitate the proper distribution of air or NO_3^-. To place the plastic pipes into the salt marsh, a hollow metal pipe, with a bolt inserted into its leading end, was manually driven into the sediments (Figure 8). The perforated plastic pipe was placed inside of the metal pipe once it was driven fully into the sediments. The metal pipe was then pulled out of the sediments, leaving the plastic pipe and the bolt behind. The 15 plastic pipes (horizontal wells) were connected to a distribution header using T connectors. The plastic distribution header and tube connectors were buried into the sediments and partially into the bank of the marsh. The header was connected to the air or NO_3^- supply (Figure 9). The installation of the distribution lines inside of amendment plots was done in May 1998, when the plant growth was just starting, so fewer roots and plants were affected. However, the sides of the plots where the distribution headers were buried were heavily impacted.

Each plot was subdivided to obtain sampling grids of 0.61 m x 0.31 m and six sections 0.23 m wide where the sampling platforms (catwalks) were placed (Figure 10). 120 sampling grids were available within each plot. Each grid was assigned a number from 1 to 120. A random number generator was used to select the subplots to be sampled during each sampling event.

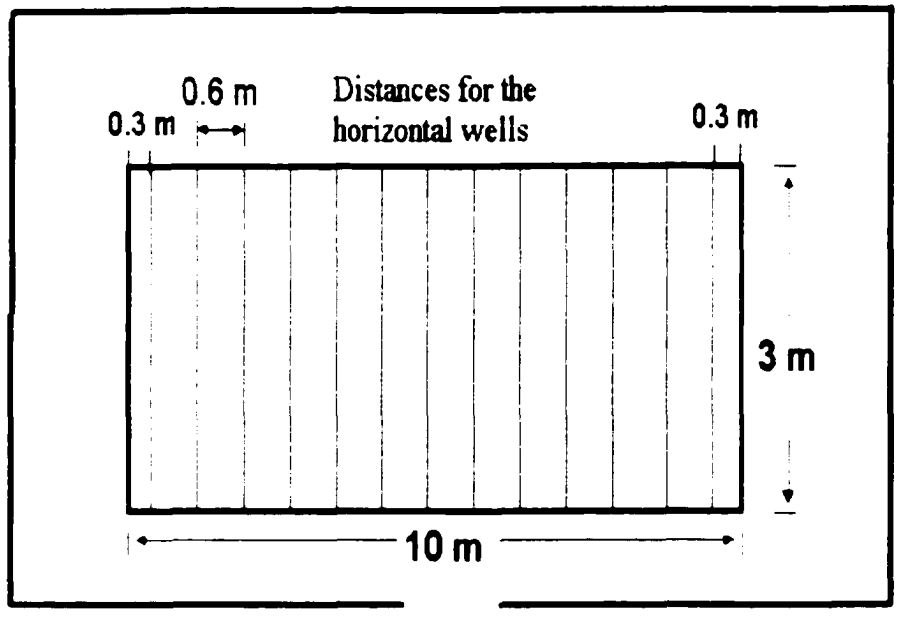


Figure 6. Experimental plot design for oxygen and NO_3^-.

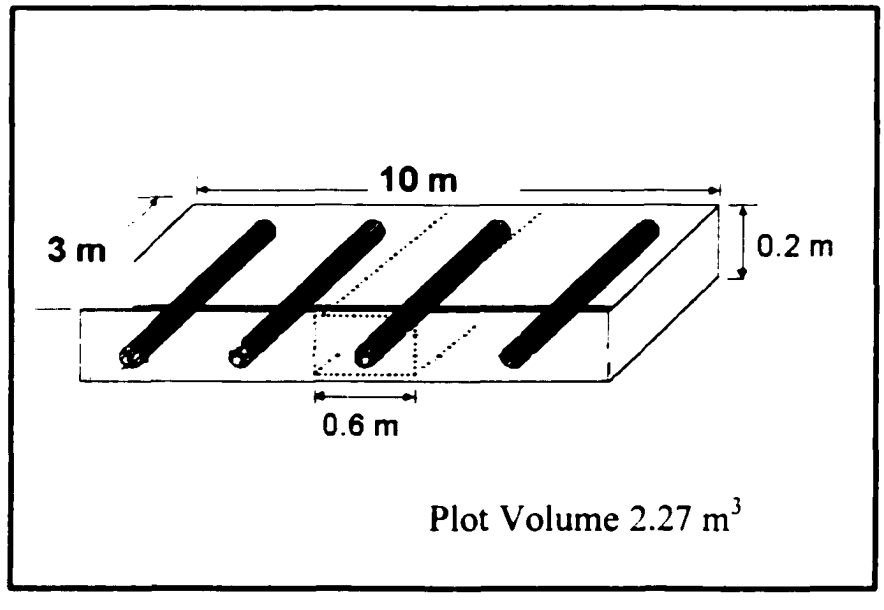


Figure 7. Experimental plot volume.

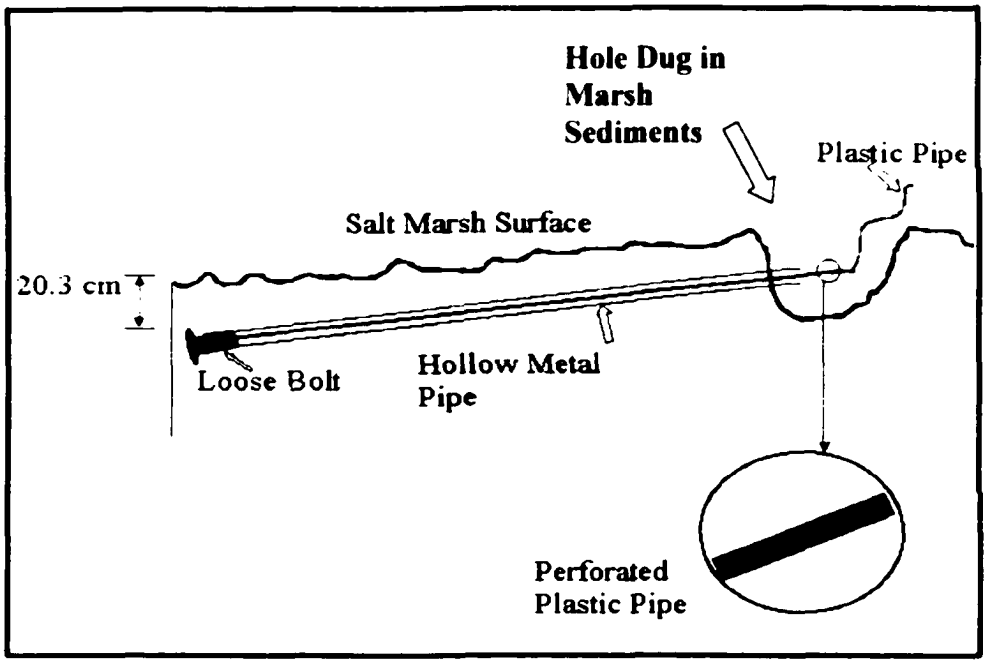


Figure 8. Installation of the horizontal wells used for the distribution of the TEAs.

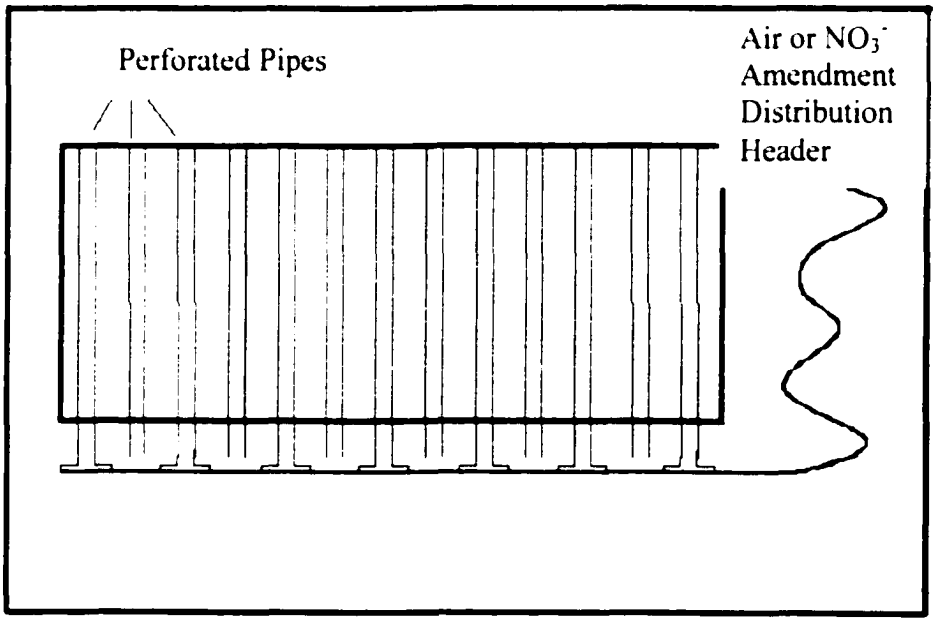


Figure 9. Distribution system for the experimental plots. Plastic pipes were connected to a distribution header for the addition of the Amendments.

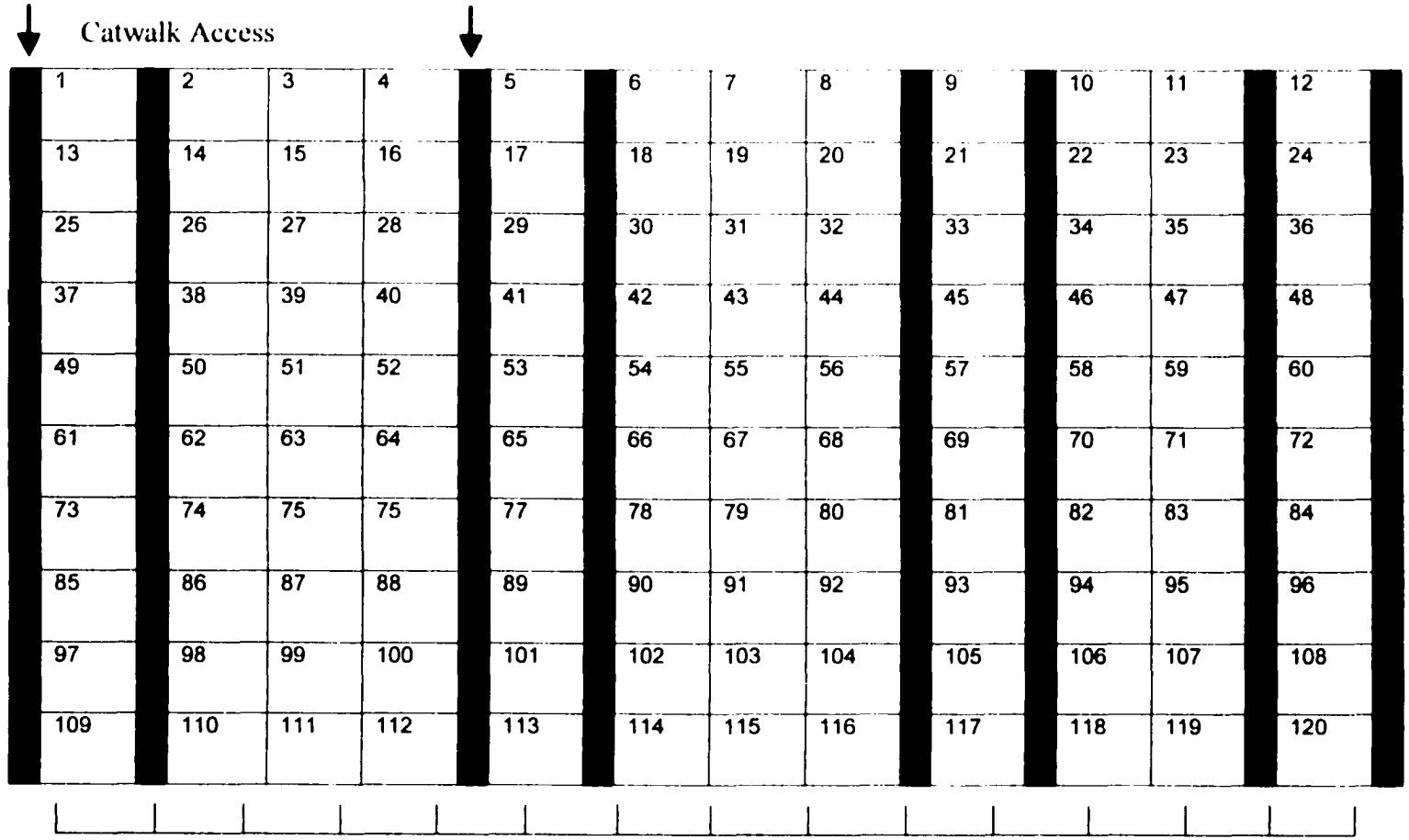


Figure 10. Experimental plot grids. Each plot was divided into 120 subplots (0.3 x 0.6 m) and spaces were left where the catwalks legs could rest (0.23 m).

3.7 Sampling Events

The field sampling of this dissertation research was conducted from June 1998 through June 1999. During this period, there were nine sampling events (Table 8). Background conditions were evaluated on June 11 and 12, 1998. Samples for TPH, salinity, plant density, plant height, abundance of oil-degrading microorganisms, NO_3^-, NH_4^+, and PO_4^{2-} were collected.

Table 8. Sampling events from June 1998 through Spring 1999.

Sampling Event	Date	Year
1	June 11,12	1998
2	July 8,9	1998
3	July 27,28	1998
4	August 18,19	1998
5	September 19,20	1998
6	October 17,18	1998
7	April 10,11	1999
8	May 12,13	1999
9	June 10,11	1999

Sampling was a two day process. Four different subplots (0.6 x 0.3 m) were randomly selected for each sampling event. The sampling was conducted during low tide (~4h). Portable catwalks were used to avoid damaging the marsh grass inside of the plots (Figure 11). The catwalks, used to access inside of the plots, were placed in corridors with in the plots previously defined for this purpose. Sampling was not conducted in the winter assuming that microbial activity was minimal.

3.8 Sampling Methods

During each sampling event a marked cord was used as a template to frame the plot and color cords were used side to side to identify the selected subplot (Figure 12).

The portable catwalks were made of a panel of fiberglass isophthalic-polyester grating (IKG Fiberglass Systems; Nashville, TN). This material was selected because it is light and resistant to saline and cold environments. The catwalk panels were 1.0 m x 1.5 m, and they were mounted on aluminum legs and supported by two wood poles 2.0 m x 0.8 m long and 0.2 m thick.

Two sediment samples were collected from each subplot to evaluate TPH concentration. A sediment sample was also collected to evaluate the abundance of oil-degrading microorganisms. A porewater sample was collected to quantify NO_3^-, NH_4^+, and PO_4^{3-}. In addition, a frame 30 cm x 30 cm was used inside of each subplot to evaluated plant density and plant height (Figure 13).

All samples were taken using plastic spoons to avoid contamination between samples. During the sampling event, all person collecting samples wore neoprene gloves (18 mm thickness and 33 cm length) (VWR) to avoid any contact with oil-contaminated sediments and disposable latex gloves (VWR) to avoid cross-contamination between subplots (Figure 14).

3.8.1 TPH

TPH samples were collected from the top sediments (~ 4.0 cm) in the selected subplots. The samples were placed in 120 mL amber wide-mouth glass bottles with Teflon®-lined screw caps (VWR).

Figure 11. Catwalks used during the study to avoid damaging the salt marsh.

Figure 12. Selected subplot during a sampling event. Note colored cords delineating subplots and quadrant used sampling.

Figure 13. Plant stem height and plant density measurements.

Figure 14. Double gloves used to avoid cross-contamination during sampling.

The bottles were previously decontaminated by washing them with Extran® laboratory detergent (VWR). They were dried in a Thelco® oven (Precision Scientific) at 100°C and combusted at 500°C for 90 min. The caps were acid washed (chromic acid) and rinsed 3 times with MilliQ® water. Samples were cooled to 4°C on blue ice in coolers immediately after collection and transported to the UNH laboratory with in ~5 h. TPH samples were stored at 4°C in the laboratory for a maximum of 7 d prior to extraction. Extract were held at 0°C for a maximum of 40 d prior GC analysis.

3.8.2 Microbial Abundance

The sediments for microbial studies were collected from the top ~ 4.0 cm in the selected subplots. The samples were placed in plastic Ziploc® bags (SC Johnson; Racine, WI). Samples were cooled to 4°C on blue ice immediately after collection and transported to the JEL at UNH to be processed during the next 3-4 d.

3.8.3 Porewater

Porewater samples were collected using a porous ceramic cup (lysimeter) that was inserted horizontally (top 4 cm) into the salt marsh sediments. The ceramic cup (10.0 cm long x 1.0 cm diameter) (Soil Moisture Equipment Corp; Goleta, CA) was attached to a plastic tube (50 cm length x 0.32 cm diameter). The cup was attached to the tubing using epoxy resin (Soil Mixture Equipment Corp) and wrapped with Teflon® tape (VWR). The other side of the tubing was attached to a 125 mL side arm flask. The flask was sealed with a rubber stopper. A hand vacuum pump (Soil Mixture Equipment Corp) attached to the flask was used to create a 350 to 370 mm Hg vacuum. Approximately 60 to 70 mL of porewater was obtained after a vacuum was applied for 45 min . The filtrate

was poured into a 125 mL high-density polyethylene bottle (VWR). Samples were cooled to 4°C on blue ice immediately after collection and transported to the UNH laboratory where they were frozen until analysis at the end of the research (~6 months).

The lysimeters were decontaminated for 2 h in a 50% HCl solution and subsequently soaked in MilliQ® water for 2 d, rinsed for 2 h in methanol, and finally soaked 1 d in MilliQ® water. They were air dried. The flasks and polyethylene bottles were washed with 50% HCl and rinsed with MilliQ® 3 times.

3.9 Amendment Addition

A low flow pump (DOA, Gast Manufacturing, Inc.; Lombard, IL) (3.2 m^3/h) was used to add air. The pump worked 24h during the entire study. Sodium nitrate ($NaNO_3$) was used as the NO_3^- amendment. To prepare the solution, 430 g of $NaNO_3$ were mixed with 0.028 m^3 of water for a final concentration of 11.04 g NO_3^-/L. Mixing occurred in a Rubbermaid® (Wooster, OH) (~0.26 m^3) tank. The solution was delivered by gravity using the distribution network (Figure 15). The solution was made at the site weekly and delivered immediately after preparation. The volume of injected water was calculated based on the volume of the sediments to treat (2.27 m^3). 0.028 m^3 of water flushed the distribution lines after the injection assuring to release all of the NO_3^- solution into the sediment (Ballestero, 1999). The amendment was added at the beginning of a low tide to avoid loss of the solution, this was assumed to be the best case so the solution would not be diluted by the tide and could stay longer in the sediments. The estimate final concentration of N-NO_3^- in the porewater was ~31.2 mg N/L. A porosity of 0.4 pore size value was assumed to calculate the total volume of the experimental plots (2.27 m^3).

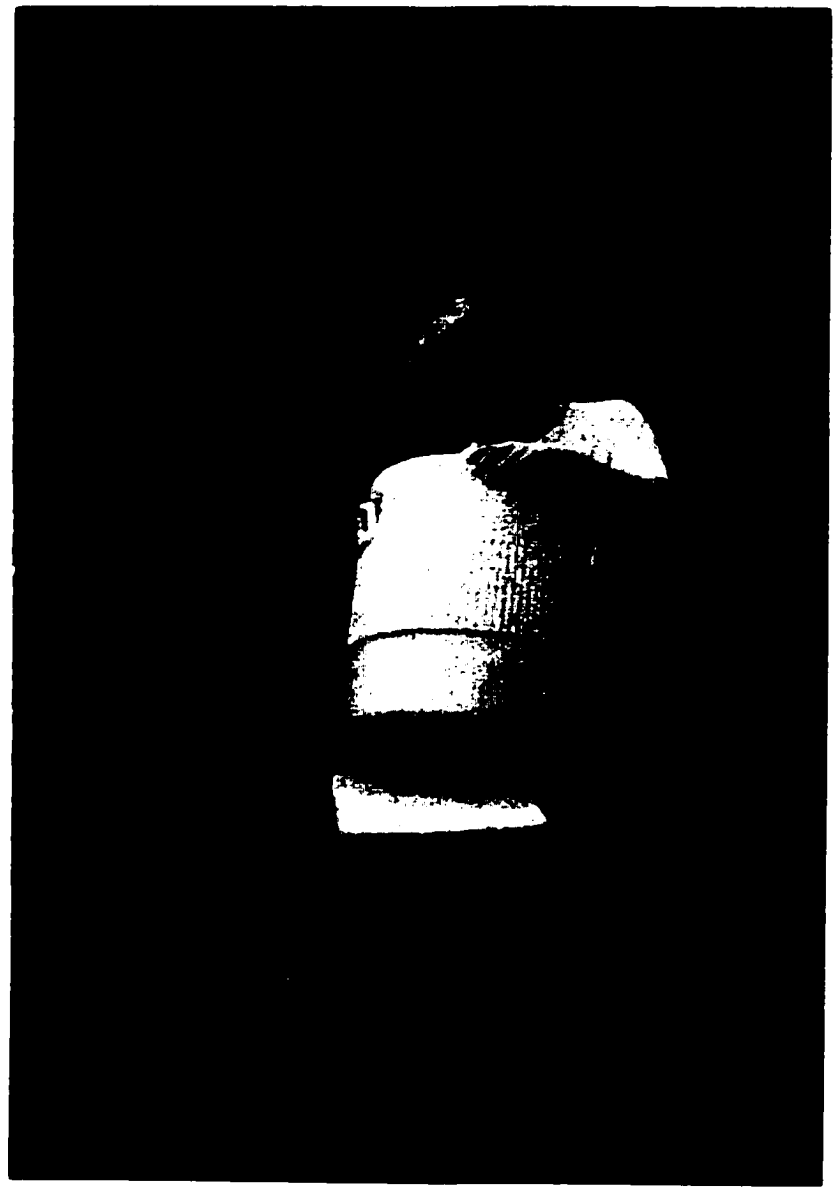


Figure 15. Addition of NO_3^- solution by gravity during the Fore River Creek salt marsh study..

CHAPTER IV

RESULTS AND DISCUSSION

Two TEAs (NO_3^-, and oxygen) were evaluated for their ability to enhance *in situ* biodegradation of TPH in the Fore River Creek salt marsh contaminated by the *Julie N* oil spill in September 1996. Horizontal wells, placed in the sediments of the contaminated salt marsh in June 1998, were used to distribute the TEAs. The effects of the amendments were evaluated from June 11, 1998 until June 10, 1999. During this period, nine sampling events were conducted. Sampling was not conducted during colder months (November-March) because of the difficulty in accessing the site and the assumption that microbial metabolism and rhizosphere activity would be limited due to the low temperatures.

The objectives of this dissertation research were to:

- determine the zones within an oil contaminated salt marsh that were most affected by the oil spill;
- evaluate and select an analytical method for the evaluation TPH present in the contaminated salt marsh sediments; and
- determine if addition of oxygen (added as air) and NO_3^- could enhance the *in situ* biodegradation of HCs compared to the natural attenuation process.

Prior to beginning the study, two screening methods were evaluated for their ability to detect and quantify TPH in the salt marsh sediments: the EPA (1994) Method

and the ASTM (1995) Method. These methods were compared to the MADEP (1998) Method for Extractable Petroleum Hydrocarbons. During the field study, TPH concentrations in the sediments were analyzed using the MADEP Method. The abundance of oil-degrading bacteria; porewater NO_3^-, NH_4^+, and PO_4^{-3} concentrations; and plant growth and density were also monitored during each event.

4.1 Evaluation of Screening Methods for TPH

Many non-halogenated HCs can be used as an energy and carbon source by naturally-occurring microorganisms and be completely biodegraded to water and carbon dioxide (complete mineralization). One important indicator of the effectiveness of a bioremediation process is the decrease in the *in situ* concentration of the contaminants. Hence, it was crucial that the analytical method chosen for this research project be able to detect differences in TPH concentration on the order of 500 mg/kg_{dw}. Ideally, the selected screening methods would also be low cost, relatively easy to perform and yield rapid results. For example, the EPA Method is simple, quick and inexpensive. During a subsurface bioventing study at UNH, it had achieved good recoveries for TPH in No. 2 fuel oil-contaminated soils (Simonton, 1998). In addition, the equipment needed to conduct the EPA Method was also available in the laboratory. The ASTM Method was also evaluated because it is a standard test method for screening fuels in soil (ASTM, 1995), has a high sample throughput, and is inexpensive and simple to perform.

Three types of soil were analyzed to compare the two screening methods: a commercial organic soil (OS), sand contaminated with No. 2 fuel oil (PCS), and clean sand (CS). Samples from the Fore River oil-contaminated salt marshes were also used to

compare the efficiency of the extraction and analysis of the two analytical methods. The methods were compared based on their Method Detection Limit (MDL), Limit of Quantification (LOQ), precision (expressed as %CV) and ability to detect No. 2 fuel oil spiked into the samples (expressed as %P). Statistical analyses were generally conducted using a probability (p) of 0.05 (p=0.05).

4.1.1 EPA Method

The EPA Method was evaluated because it may be used to quantify low concentrations of TPH in sediments and sludges (EPA, 1999) and it was used in a previous study at the UNH laboratory (Simonton, 1998). This method is not applicable to measurements of materials that volatilize at temperatures below ~85°C. The EPA Method uses a Soxhlet extraction (with hexane as the solvent) and subsequent gravimetric analysis to determine the TPH present in the sample. The sediment TPH concentrations were expressed as dry weight (dw) (mg TPH/kg_{dw}) in order to compare samples on an equal basis (Appendix A).

To calculate the MDL and LOQ, seven CS replicates were analyzed under laboratory conditions (Table 9). The MDL was calculated as 3.75 (one tail *t* statistic, p=0.01) times the standard deviation of the CS replicates (Standard Methods, 2000). The LOQ was calculated as 10 times the standard deviation of the CS replicates. The calculated MDL and LOQ were 773 mg TPH/kg_{dw} and 2,060 mg TPH/kg_{dw}, respectively (Appendix D).

Although the MDL and LOQ were calculated based on CS samples, some of the concentrations found were relatively high (300 and 503 mg/kg_{dw}). This indicated that some contamination was present in the CS or more likely that small particles were being

transported from the extraction thimble into the flask during the Soxhlet extraction affecting the flask weight and, therefore, the TPH concentration.

Table 9. CS replicates used to calculate the MDL and LOQ of the EPA Method.

Sample	TPH mg/kg_{dw}
1	30
2	10
3	140
4	ND
5	503
6	ND
7	300
Mean	197
Standard Deviation	206

ND = Not detected

The Ohaus analytical balance used to make the gravimetric measurements had a manufacturer reported detectable difference of ±0.5 mg, which represented approximately ± 50 mg TPH/kg_{dw} (0.5 mg TPH/10 g_{dw} sample x 1000 g_{dw}/kg_{dw}), so differences in TPH concentration of $\leq$ 50 mg/kg_{dw} could not be distinguished.

The TPH concentration of the OS was significantly greater than the CS and significantly larger than the MDL (one tail, p=0.05), but not significantly different (NSD) from the LOQ (two tail *t*-test, p=0.05) (Table 10). The data suggested that some compounds present in the OS caused a positive interference during the analysis. Some of the NOM associated with the OS was probably extracted by the hexane and detected as TPH.

The precision for all of the sample matrices whose concentrations were above the MDL and LOQ (i.e., the spiked CS and OS) were acceptable compared to the published %CV of $\leq$ 25% (Table 10) (EPA, 2000).

To be acceptable, the average %P for the EPA Method must be between 75% and 115% (EPA, 2000). %Ps obtained during the analysis of the spiked CS and pre-contaminated sand (PCS) were within this range (Table 10). The %P for the OS was lower, but NSD (one tail *t*-test, p=0.05) than the recommended range.

Table 10. TPH concentrations obtained from the EPA Method analysis of laboratory-spiked known samples.

Sample	N	TPH[b] mg/kg_{dw}	%CV	% P
CS	3	(140±194)BDL*	-	-
Spiked CS[a]	4	38,000±2,540	±7 %	76.0±5.1
PSC	3	12,000±2,142	±18 %	-
Spiked PCS[a]	4	55,000±6,653	±12 %	84.0±4.5
OS	4	2,000±1,828	[±91 %]	-
Spiked OS[a]	5	100,000±12,370	±12 %	70.0±8.7

[a]500 mg of No. 2 fuel oil spiked into 10 g of sample (50,000 mg/kg).
b (means ± 1 standard deviation)
*BDL = below MDL of 773 mg/kg_{dw} BQL= below LOQ of 2,060 mg/kg_{dw}
{} %P significantly lower than published range (75-115%) p=0.05
[ ] %CV exceeds acceptable published value of ≤ 25 %
The TPH concentrations in the spiked samples are modified by the dry weight .

The spiked CS %P was NSD from the results previously obtained for a bioventing study (72.0±2.5%) (two tail *t*-test, p=0.05) (Simonton, 1998) indicating a consistent recovery of No. 2 fuel oil within the laboratory. Initially, the concentration of the spiked samples was based on a study that had been conducted at the UNH laboratory with the PCS. This spike resulted in concentrations 2 or 3 times higher than the CS and OS, the desired spiked range recommended by EPA. The lower %CV and higher %P of the spiked CS, PCS and OS samples could been caused by the high concentration (50,000 mg/kg) used.

Overall, the tests with the EPA Method on known soil matrices yielded acceptable precision and accuracy compared with published values. The organic compounds (co-extracted by the hexane) present in the unspiked OS matrix affected the precision of the method at low TPH concentrations, while at higher concentrations precision and accuracy were NSD from published values.

Several problems occurred during the first attempt to analyze the actual marsh samples with the EPA Method. Sediments in New England salt marshes contain very fine (silt and clay size) particles in addition to roots and rhizomes (Hines *et al.*, 1989). Although big roots were avoided during sample collection, the Soxhlet extraction was very slow because the fine particles clogged the extraction thimble, reducing solvent flow through the sample. In addition, some of the sediment and sodium sulfate (used to absorb the water from the sample) flooded the thimble and fell into the extraction flask. This problem was solved by placing a small piece of glass wool on top of the sample inside the thimble (Figure 16). After resolving these problems, marsh samples were analyzed with this modified Soxhlet method (Table 11). Sites 1-4, from which the sediments were collected, were located in the salt marsh along the Fore River main channel.

The %CVs for the salt marsh samples analyzed using the modified Soxhlet extraction were very large (Table 11) compared to the published criterion (%CV $\leq$ 25%). The poor precision was probably a function of the variability (uneven distribution) of TPH in the contaminated marsh sediments and within sample heterogeneity (Pritchard, 1993; Reynolds *et al.*, 1997). High *in situ* variability in sediments can be overcome if the samples are thoroughly mixed (manually or mechanically) to homogenize them.

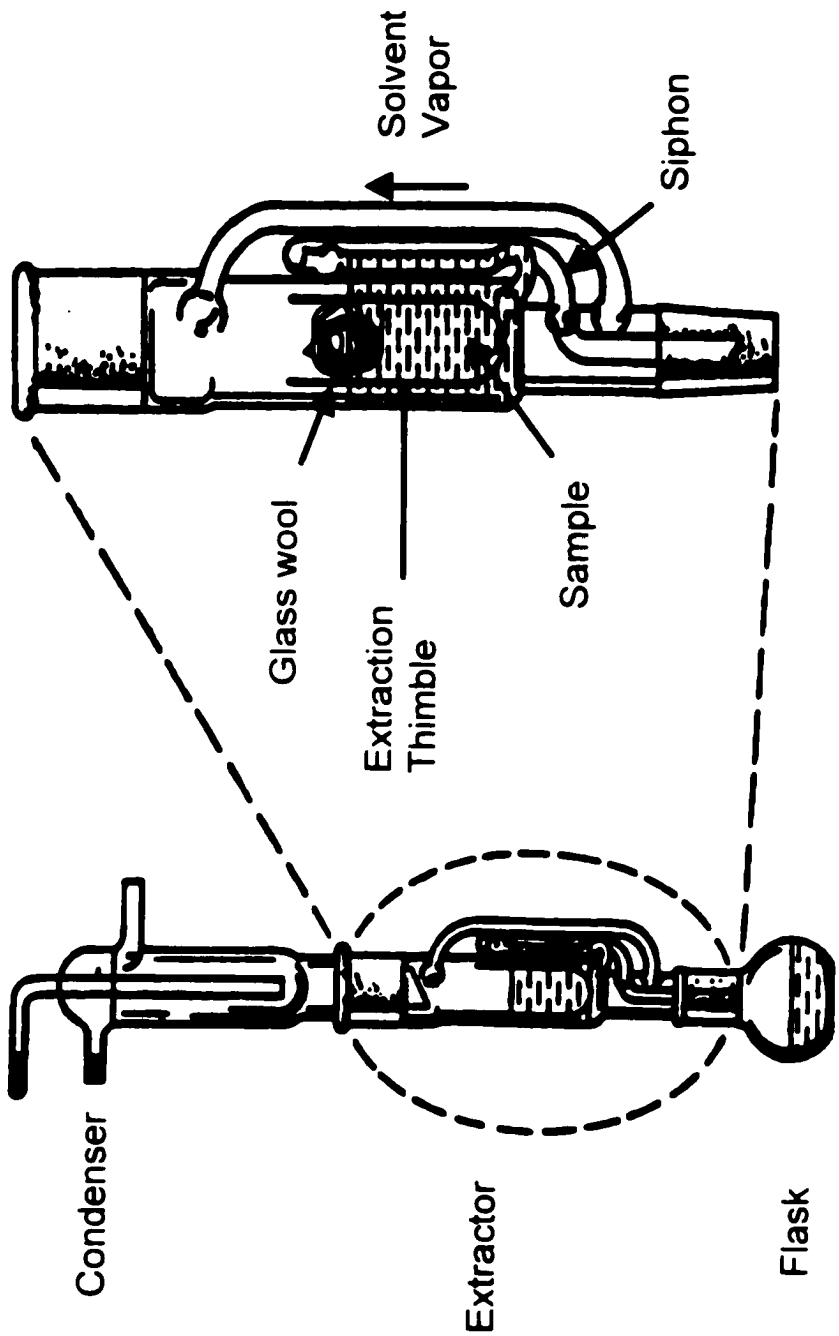


Figure 16. Schematic diagram of the Soxhlet extraction set-up used in the EPA Method.

For example, the PCS contaminated with a No. 2 fuel oil had a relatively low % CV because it was slowly mixed (Table 10) on a plastic tarp with shovels and rakes (Simonton, 1998). However, the PCS was fairly uniform sand and tended to have relatively little heterogeneity. The salt marsh sediments were much more heterogeneous because of the presence of *S. alterniflora* roots and rhizomes interspersed among the fine sediments and their different redox conditions and sorptive capacities for TPH. In addition, TPH is an LNAPL that does not dissolve readily in water and forms droplets at high concentrations, resulting in phase separation.

Table 11. TPH in the Fore River Creek salt marsh samples using the EPA Method.

Sample	N	TPH mg/kg_{dw}	%CV	% P
Site 1	4	4,000±2,776	[± 69 %]	-
Spiked Site 1[a]	1	6,099		{71.0}
Site 2	4	3,000±2,460	[± 82 %]	-
Spiked Site 2[a]	1	3,877		{54.7}
Site 3	4	4,000±2,908	[± 73 %]	-
Spiked Site 3[a]	1	4,901		{64.2}
Site 4	4	3,000±3,018	[± 101 %]	-
Spiked Site 4[a]	1	8,327		{61.6}
Blank (CS)	4	(200±246)BDL*	-	-
Spiked Blank (CS)[a]	1	(1,310)BQL*		

[a]19 mg of No. 2 fuel oil into 10 g of sample (1,900 mg/kg) *BDL = below MDL of 773 mg/kg_{dw}
BQL= below LOQ of 2,060 mg/kg_{dw} [ ] %CV exceeds acceptable published value of ≤ 25 %
{} %P lower than published range (75-115%)

A CS sample was spiked and used as a control to evaluate laboratory performance during the analysis of the contaminated salt marsh sediments. During this analysis, the CS was spiked with a lower concentration (1,900 mg/kg) than the one used with the

laboratory-spiked known samples (50,000 mg/kg). Unfortunately, this 1,900 mg/kg spike was only slightly higher than the method's LOQ, which was a poor choice. Only 1,310 mg/kg_{dw} of the spike CS were recovered, which is below the LOQ, so the %P could not be calculated for the CS for this sample run.

The salt marsh samples were also spiked with 1,900 mg/kg of TPH. The dry weight of the marsh sediments was 0.3, so the spike was equivalent to ~6, 500 mg TPH/kg_{dw} which was barely or not within the 2-3x spiking window required by EPA. Again, this confounded any determination of %P for the EPA Method with respect to the marsh samples. The mean recovery for the salt marsh samples (63.0±6.7%) was significantly lower (SL) (one tail *t*-test, p=0.05) than the %P initially obtained for the CS (Table 10) and the lowest acceptable %P (75%) reported by the EPA, but NSD (two tail *t*-test, p=0.01) (Table 11). The mean %P for the salt marsh sediments was also SL than the recovery obtained for the spiked PCS (one tail *t*-test, p=0.05), but not SD than the recovery obtained for the spiked OS (two tail *t*-test, p=0.05) (Table 10). The low recoveries indicated than some interference was limiting TPH recovery in the salt marsh sediments. Sulfur compounds, organic compounds and chlorophyll that may be present in marsh sediments, are all soluble in hexane and have been reported to lower efficiency and interfere with the EPA method (EPA, 1999). The presence and concentration of these potential interferences are a function of the season, microbial activity and plant growth. Therefore, %Ps will likely vary spatially and temporally within the marsh. The use of higher spikes concentrations for the salt marsh samples could also have improved the %P making it easier to detect the spike.

The mean TPH concentration found in the four sites within the contaminated salt marsh sediments of the Fore River was 3,000±2,584 mg/kg_{dw}. This concentration was lower than the 10,000 mg/kg_{dw} desired to conduct the *in situ* bioremediation study. The lower concentrations could have been the result of the low %P of EPA Method, the marsh areas tested being less heavily impacted by the *Julie N* spill or natural attenuation of the TPH between the September 1996 spill and February 1997 when sampling occurred.

Using the EPA method on Fore River Creek marsh samples with concentrations of 3,000±2,584 mg/kg_{dw}, ~100 replicate samples (p=0.05) from each plot would be needed to observe a change in concentration of 500 mg/kg_{dw} (See Equation 4.1 below) (Appendix D). The standard deviations associated with the TPH concentrations observed between the different replicates for all sites were very large necessitating analysis of a very high number of samples to achieve the small detectable differences (500 mg TPH/kg_{dw}) between samples. The large number of replicates made it impractical to use the EPA Method as a screening tool during this research. Generally, the more heterogeneous the system, the more sample replication is needed to achieve a low %CV and high %P (Zhu *et al.*, 2001).

Number of replicates of a sample required:

$$N \geq \left[\frac{t\,s}{U}\right]^2 \qquad \text{(Eq. 4.1)}$$

where: N is the number of samples, t is the Student's *t*-statistic for a given confidence level (one tail, p=0.05), s is the overall standard deviation of marsh samples, and U is the

acceptable level of uncertainty or detectable difference desired between samples (i.e., 500 mg TPH/kg_{dw}).

4.1.2 ASTM Method

The ASTM Method was evaluated because of its high sample throughput (~30 samples per day; 10 min/sample) (ASTM, 1995), low cost (< $10 per sample), and simplicity (Sorini *et al.*, 1997). This method uses an isopropyl alcohol extraction that is fast (3 min/sample). It has been used for monitoring organic-rich soils, which often cause interferences for other screening methods (Sorini *et al.*, 1997, 2001).

Three calibration curves were prepared for the ASTM Method to calculate the TPH concentrations in the marsh sediments (one for each batch of samples processed) (Figure 17). The slopes and y intercepts of the calibration curves were NSD (two tail *t*-test, p=0.05) (Table 12). The readbacks were within of the 95% C.I. of the calibration curves and the ± 20% range recommended by ASTM (1995). The correlation coefficients (r^2) for the calibration curves (Table 12) were higher than the minimum accepted value (0.995) for absorbance calibration curves (Standard Methods, 2000) Hence, all calibrations curves were acceptable and consistent between runs.

Table 12. Calibration curves used for the TPH determination for the ASTM Method.

Calibration Curve	Slope (absorbance/mg/L)	y intercept (absorbance)	r^2	n
1	$4.41x10^{-3}±6.3x10^{-5}$	$8.9x10^{-3}±4.5x10^{-2}$	0.9990	4
2	$4.22x10^{-3}±4.5x10^{-5}$	$7.0x10^{-3}±7.0x10^{-3}$	0.9997	4
3	$4.36x10^{-3}±9.0x10^{-6}$	$2.5x10^{-3}±1.4x10^{-4}$	0.9999	4

[ ] exceeds the minimum accepted value of 0.995

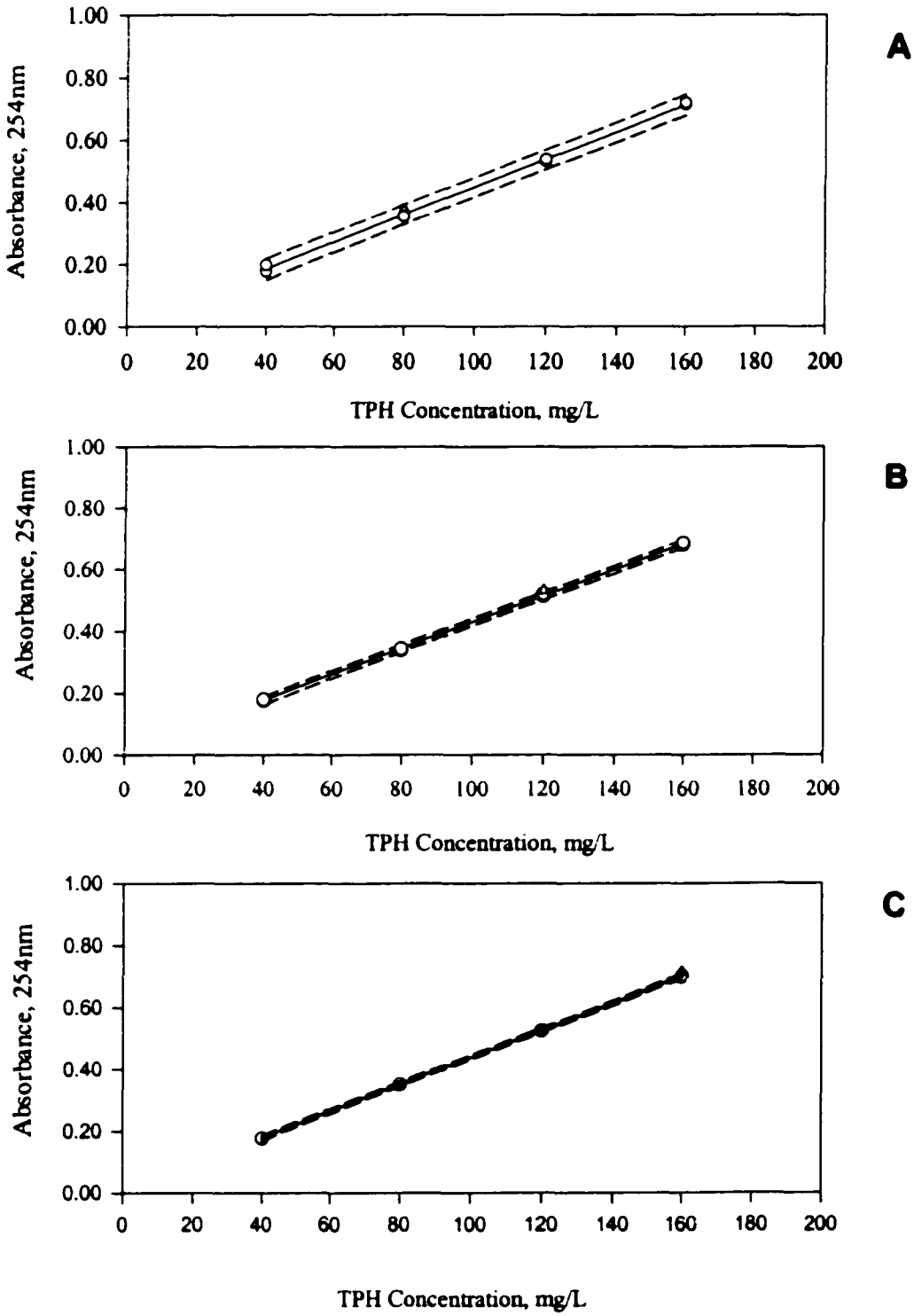


Figure 17. Calibration curves for the ASTM Method. Four different standards (o) were used (40.0, 80.0, 120.0 and 160.0 mg/L). A). Curve 1 Readback (Δ)(80 mg/L). B) Curve 2 Readback(Δ)(120 mg/L). C) Curve 3 Readback (Δ)(160 mg/L). Slopes and y-intercepts for calibration curves shown in Table 12.

The absorbance of the lowest calibration standard (i.e., 40 mg/L) was used to calculate the MDL (52 mg/kg_{dw}) (one tail *t*-test, p=0.01) (Appendix D) (N.B., n=6 because the 40 mg/L standard was analyzed twice for each of the calibration runs. The 5.2 mg/L MDL for the liquid concentration corresponds to a MDL of 52 mg/kg_{dw} in the sediment samples (ASTM, 1995) (Table 13)). The LOQ of 180 mg/kg_{dw} (equivalent to 18 mg/L in liquid) was calculated as 10 times the standard deviation of the absorbance of the 40 mg/L standard (ASTM, 1995) (Appendix A). This LOQ was higher than that reported by ASTM for sand contaminated with diesel fuel (75 mg/kg), possibly because different conditions were used during the analysis. Unfortunately, ASTM did not report the calibration standard used to calculate its LOQ.

The ASTM Method was initially evaluated by analyzing CS and OS (Table 13). Not surprisingly, the TPH concentration for CS was lower than the MDL. As with the EPA Method, the unspiked OS had a significantly higher concentration than the LOQ. It was assumed that there was little to no TPH in the commercial OS used. Sorini (1997) reported that some humic materials present in OS can interfere at 254 nm, the wavelength recommended in the ASTM Method. It is likely that humic materials were present in the OS.

The %CV for the CS was higher than for the spiked samples probably because the TPH concentration was less than the calculated MDL (52 mg/kg_{dw}). There is usually a large amount of variation in data near detection limit. ASTM reported %CVs of 7% for diesel-contaminated sand and OS (802±54 mg/kg and 618±42 mg/kg, respectively). The precision obtained in this study for the spiked CS and OS samples was NSD from the published precision (β test, p=0.05), and was therefore considered acceptable. The spike

concentration was higher than 2 to 3 times the TPH concentration found in the samples. This high concentration of the spike helped reduce variation and improved the %P.

Table 13. TPH concentrations obtained from the ASTM Method analysis of laboratory-spiked known samples.

Sample	n	TPH mg/kg_{dw}	%CV	% Recovery of Spike, %P
Clean sand (CS)	2	(50±19)BDL*	[± 38 %]	-
Spiked CS[a]	2	1,800±77	± 4 %	94.3±4.0
Organic soil (OS)	2	270±18	± 7 %	-
Spiked OS[a]	2	2,900±199	± 7 %	78.0±5.7

[a]9.7 mg of No. 2 fuel oil to 5 g sample (1,940 mg/kg)). *BDL = below MDL of 52 mg/kg_{dw}
[ ] %CV exceeds acceptable published value of ≤ 7 % BQL= below LOQ of 180 mg/kg_{dw}
{} %P significantly lower than published range (80%-120%) at p=0.05
The TPH concentrations in the spiked samples are modified by the dry weight.

The %Ps for the spiked CS and OS were NSD the ASTM recommended range (80%-120%) (Table 13) (two tail *t*-test, p=0.05). The %Ps for the spiked CS and OS were also NSD from each other (two tail *t*-test, p=0.05), but were SD at an 80% CI. Not surprisingly, the OS %P was lower than the CS, most likely due to the negative interference from humic substances retaining the TPH in the OS. In spite of this, the rapid isopropyl extraction and spectrophotometric analysis were able to recover an acceptable amount the No. 2 fuel oil from the OS.

The samples collected in the Fore River Creek salt marsh and previously analyzed by the EPA Method (Table 11) were also evaluated by the ASTM Method (Table 14). The marsh samples had higher %CVs than the spiked CS and OS analyzed previously (Table 13) and than published for the method. This was expected because of the highly variable composition of the sediments and the uneven distribution of TPH within them (Pritchard, 1993; Reynolds *et al.*, 1997).

Table 14. TPH in the Fore River Creek salt marsh samples analyzed using the ASTM Method.

Sample	n	TPH mg/kg_{dw}	%CV	%P
Site 1	4	3,000±2,359	[± 79 %]	-
Spiked Site 1[a]	1	6,327		112.2
Site 2	4	1,400±791	[± 56 %]	-
Spiked Site 2[a]	1	2,284		116.6
Site 3	4	6,800±3,445	[± 51 %]	-
Spiked Site 3[a]	1	4,499		98.6
Site 4	4	6,500±5,296	[± 82 %]	-
Spiked Site 4[a]	1	8,245		108.3
Blank (CS)	2	(110±16)BQL[*]	[± 15 %]	-
Spiked Blank (CS)[a]	1	1,946		102.0

[a]9.7 mg of No. 2 fuel oil to 5 g sample (1,940 mg/kg)). [*]BDL = below MDL of 52 mg/kg_{dw}
[ ] %CV exceeds acceptable published value of ≤ 7 % BQL= below LOQ of 180 mg/kg_{dw}
{} %P significantly lower than published range (80%-120%) at p=0.05

Although, the spike concentration (1,940 mg/kg) used during the marsh sediments analysis was lower than the recommended 2-3x concentration (~6,000 mg/kg_{dw}), all %Ps for the salt marsh sediments were in the 80%-120% range recommended by the ASTM Method. Most recoveries were higher than 100%. Sorini *et al.* (2001) reported that ASTM Method could yield higher concentrations than other methods because of its sensitivity to aromatic compounds present in the samples. Overall, the ASTM Method had better %Ps when spiked samples were analyzed, perhaps because the isopropyl alcohol extraction is more efficient. Unfortunately, only small numbers of replicates were analyzed during this part of the experiment. In future work, the number of replicates should be greater than one or two.

The TPH concentrations in the contaminated salt marsh sediments measured by the ASTM Method were lower than the desired concentration (~10,000 mg/kg_{dw}) for the bioremediation field study. Using the ASTM Method on Fore River Creek salt marsh with a TPH concentration of 4,500±3,790 mg/kg_{dw}, >100 replicates ($p=0.05$) of each sample would be needed to observe a change in concentration of 500 mg/kg_{dw} (Equation 4.1) (Appendix D). The large number of replicates was driven by the large standard deviations associated with the TPH concentrations in the marsh sediments relative to the desired detectable difference of 500 mg/kg_{dw}. This high number of replicates made it impractical to use the ASTM Method to evaluate the disappearance of TPH during the *in situ* bioremediation study.

4.1.3 Comparison of the EPA and ASTM Methods

The calculated MDL and LOQ were one order of magnitude lower for the ASTM Method than the EPA Method (Table 15). Hence, small TPH concentrations could be better detected and quantified using the ASTM Method. This occurred because the detection limits for the EPA Method were calculated by analysis of the standard deviation of the CS in contrast to the ASTM Method where the detection limits were calculated with the lowest calibration standard (40 mg/L). Both methods exhibited possible negative analytical interferences (%P < published criterion) from organic compounds in the unspiked OS samples (Table 15). This effect was much greater in the EPA Method when the marsh samples were analyzed indicating the EPA Method would be more problematic with the actual sediments.

Table 15. Summary of the detection limits, %CVs and %Ps for the EPA and ASTM Methods.

	EPA Method							ASTM Method						
	MDL= 773 mg/kg_{dw} LOQ= 2,060 mg/kg_{dw}							MDL= 52 mg/kg_{dw} LOQ= 180 mg/kg_{dw}						
Sample	TPH		± % CV		%P			TPH		± % CV		%P		
	n	Concentration mg/kg_{dw}	Value	Spec.* Met	n	Value	Spec.* Met	n	Concentration mg/kg_{dw}	Value	Spec.* Met	n	Value	Spec.* Met
CS	3	(140±194)BDL						2	(50±19)BDL					
SCS	4	38,000±2,540	7%	Yes	4	76±5	Yes		1,800±77	4%	Yes	2	94±4	Yes
OS	4	(2,000±1,828)BLQ						2	270±18	7%	Yes			
SOS	5	100,000±12,370	12%	Yes	5	70±8	Yes		2,900±199	7%	Yes	2	78±6	Yes
PCS	3	12,000±2,142	18%	Yes										
SPCS	4	55,000±6,653	12%	Yes	4	84±4	Yes							
Site 1	4	4,000±2,776	69%	No	1	71	No	4	3,000±2,359	79%	No	1	112	Yes
Site 2	4	3,000±2,460	82%	No	1	55	No	4	1,400±791	56%	No	1	117	Yes
Site 3	4	4,000±2,908	73%	No	1	64	No	4	6,800±3,445	51%	No	1	99	Yes
Site 4	4	3,000±3,018	101%	No	1	62	No	4	6,500±5,296	82%	No	1	108	Yes
Mean						63±7							109±8	

*Meets published method criterion.

Precision for the EPA and ASTM Methods were within their recommended ranges when unspiked and spiked laboratory samples were analyzed. However, the ASTM Method had significantly better precision for spiked and unspiked OS than the EPA Method (F-test, p=0.05) (Table 16). Comparing the precision for samples from each site within the contaminated salt marsh indicated the two methods were NSD (F-test, p=0.05). However, all %CVs for the marsh sediments were outside the range specified by both methods because of the high *in situ* spatial variability in TPH and the highly heterogeneous conditions in the salt marsh sediments (e.g., fine sediment particles and *S. alterniflora* roots).

The %Ps of the two methods were within the published criteria for each method for spiked CS and OS. The %Ps for the two methods for the spiked OS samples were NSD (two tail *t*-test, p=0.05). However, the %P was significantly higher for the ASTM Method for the spiked CS (one tail *t*-test, p=0.05). With the contaminated salt marsh sediments, the two methods had SD %Ps (two tail *t*-test, p=0.05). The ASTM Method met the published criterion (±20%), but the EPA Method did not. These results indicated that the ASTM Method was preferred with respect to %P.

Table 16. Statistical comparison of the %CVs and %Ps for the EPA and ASTM Methods.

Sample	Concentration mg/kg_{dw}	%CV	%P
Spiked CS	-	NSD	SD
OS	SD	SD	NSD
Salt marsh sediments	NSD	NSD	SD

The extraction process of the samples was faster for the ASTM Method (0.05h) (EPA Method = 4h) because the samples could be weighted in the same bottles in which

the extraction was conducted and shaking was faster than performing a Soxhlet extraction. The very fine nature of the marsh sediments slowed the Soxhlet extraction step (4 samples/d). The ASTM Method was less expensive (~$10 vs. $30 per sample) because the EPA Method requires a large number of Soxhlet setups in the laboratory, whereas the ASTM Method uses disposable syringes and polytetrafluoroethylene filters.

Overall, the ASTM Method was judged to be the better method of the two for analyzing of the TPH of the salt marsh sediments because it had a lower calculated MDL and better precision for spiked and unspiked OS, and a higher and acceptable %P during the analysis of the spiked salt marsh sediments. The only problem with the ASTM Method was the high %CV for the marsh sediments which required a large number of replicates to be analyzed to obtain a detectable differences of 500 mg/kg_{dw}. In fact, the large number of replicates (> ~50) required for both methods resulted in the decision to seek another analytical method to evaluate the TPH degradation in the marsh sediments for use in the bioremediation study.

4.2 TPH Concentrations in the Fore River Creek Salt Marsh

The Fore River Creek salt marsh at Thompson Point (Portland, ME) was selected as the study site because oil droplets were observed in the sediments (Figure 18) and it was easy to get onto the marsh for the installation of the distribution wells and sampling. Power and water needed for the distribution systems were also readily available at the site. Once the Fore River Creek salt marsh was selected for the study, the location of individual test plots within the site had to be determined.

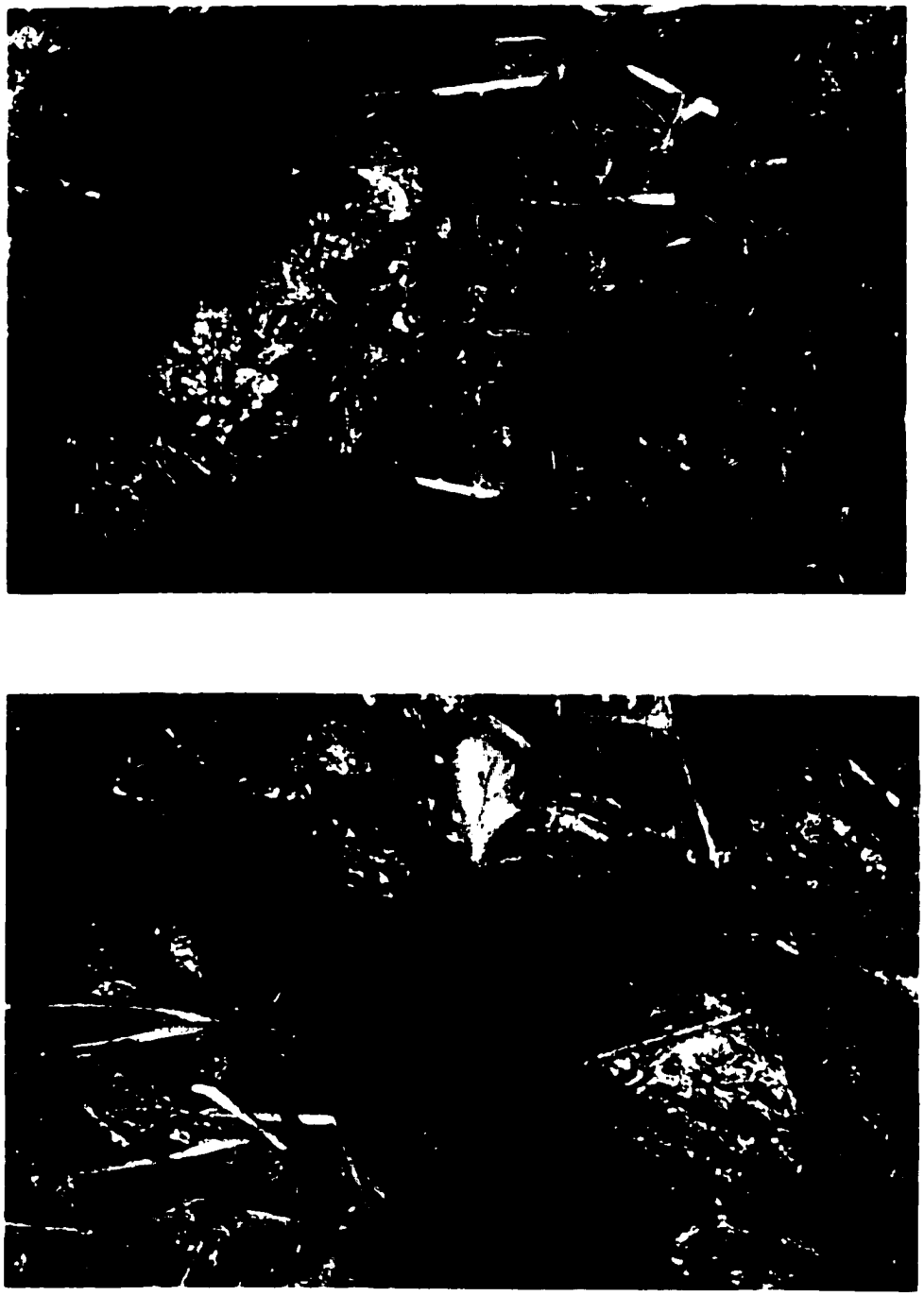


Figure 18. Droplets of oil in the sediments in the Fore River Creek salt marsh.

During a visit to the site in February 1998, four potential test plots locations (A,B,C,D) were identified at the entrance of the marsh, where most of the contamination from the *Julie N* spill occurred (Reilly, 1998). Two sets of sediment samples from each location were analyzed using the ASTM Method (February 17 and March 6, 1998) (Table 17). Subsequently, Dr. Lehmann (NOAA) realized that Site A was part of a long-term monitoring program on the recovery of *S. alterniflora* being conducted by his agency and Research Planning International (Columbia, SC). Therefore, this plot could not be used for the bioremediation study. In addition, Plot B had low sediment TPH concentrations (<5,000 mg/kg_{dw}) (Table 17), so two more locations (E and F) were selected to determine their efficacy as test plots (Figure 19).

Table 17. TPH for the initial candidate test plots in the Fore River Creek salt marsh (ASTM Method).

		Set of Samples			
		First (February 17,1998)		Second (March 6, 1998)	
Sample	n	TPH mg/kg_{dw}	%CV	TPH mg/kg_{dw}	%CV
Plot A	4	10,000±6,325	[± 63 %]	13,000±7,830	[± 60 %]
Plot B	4	4,000±2,377	[± 59 %]	2,900±710	[± 25 %]
Plot C	4	18,000±8,748	[± 49 %]	19,000±13,194	[± 69 %]
Plot D	4	17,000±15,962	[± 93 %]	23,000±9,192	[± 40 %]
Plot E	4	7,000±2,582	[± 36 %]	7,000±2,348	[± 35 %]
Plot F	4	9,000±3,635	[± 42 %]	9,000±3,650	[± 41 %]
Blank (CS)	2	(85±15)BQL*	[± 18 %]	(130±32)BQL*	[± 25 %]

*BQL=below LOQ of 180 mg/kg_{dw} [ ] %CV exceeds acceptable published value of ≤ 7 %

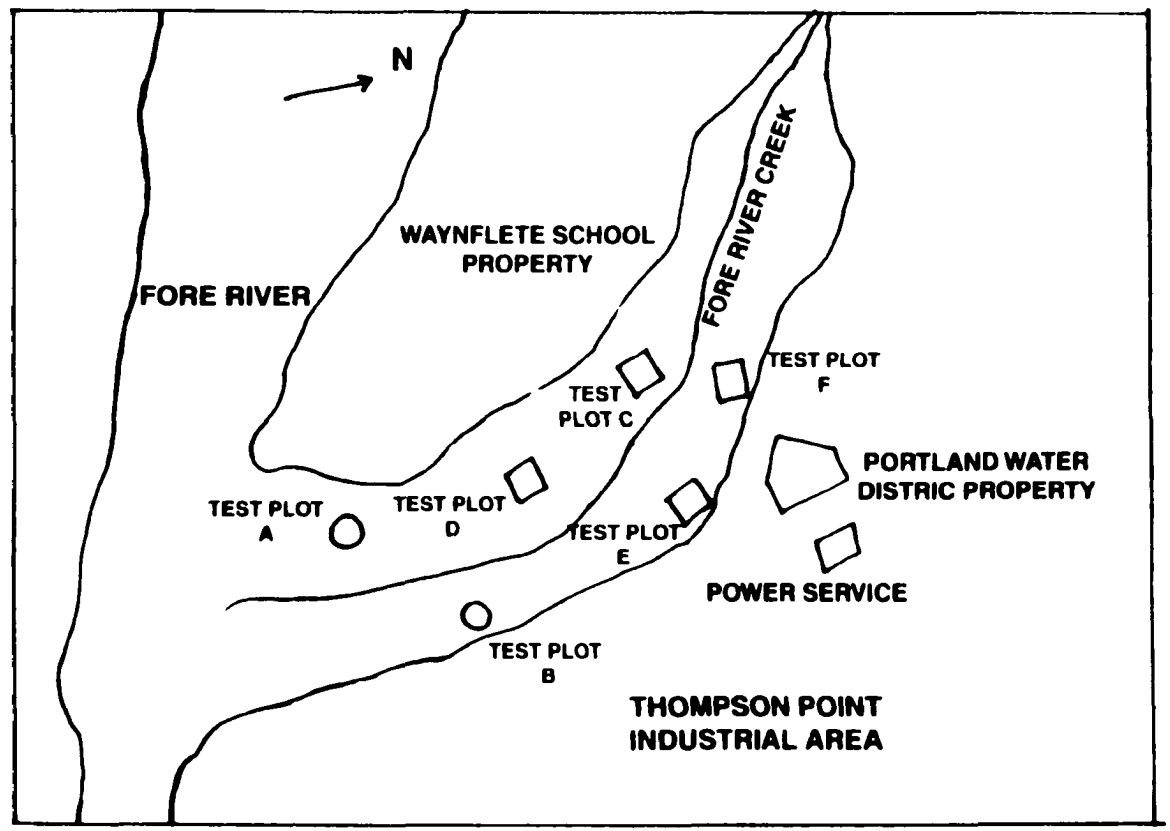


Figure 19. Relative location of the candidate test plots in the Fore River Creek salt marsh. (Not to scale)

The TPH concentration in the blank (CS) was again lower that the LOQ as expected, and for both events the %CVs for the CS were lower than previously obtained (±38%) (Table 13). This increase in precision was probably the result of the increased operator experience with the method. During this part of the study, Calibration Curve 3 was prepared and used (Figure 17C). The concentrations obtained in each of the plots on the two days (e.g., A vs. A) were NSD (two tail *t*-test, p=0.05) indicating that there was no significant short term change in TPH during this period of cold weather. Plots C, D, E, F were selected for use in the bioremediation study because their TPH concentrations were closer or greater than the desired 10,000 mg/kg_{dw} and they were not being used by NOAA.

The variability in the TPH concentration for the salt marsh sediments was worse than that of the OS (F test, p=0.05) (Table 13). However, the %CVs for the February and March samples were NSD (Bartlett test, p=0.05) from the %CVs found previously with the ASTM Method for the contaminated salt marsh sediments (Table 14). The relative highly %CVs obtained did not meet the ASTM Method criteria ($\leq$ 7%) probably because of the high *in situ* variability of the TPH. For some plots, one of the replicates had a markedly different TPH concentration than the others (e.g., Site D, sampled on February 2, 1998) increasing the %CVs (Figures 20 and 21). Again, this was attributed to pockets of TPH causing high *in situ* variability in TPH.

A third sampling round was conducted on March 25, 1998 to compare the results obtained by UNH using the ASTM Method with those of Aquarian Analytical, Inc. (Canterbury, NH) using the MADEP Method (Table 18).

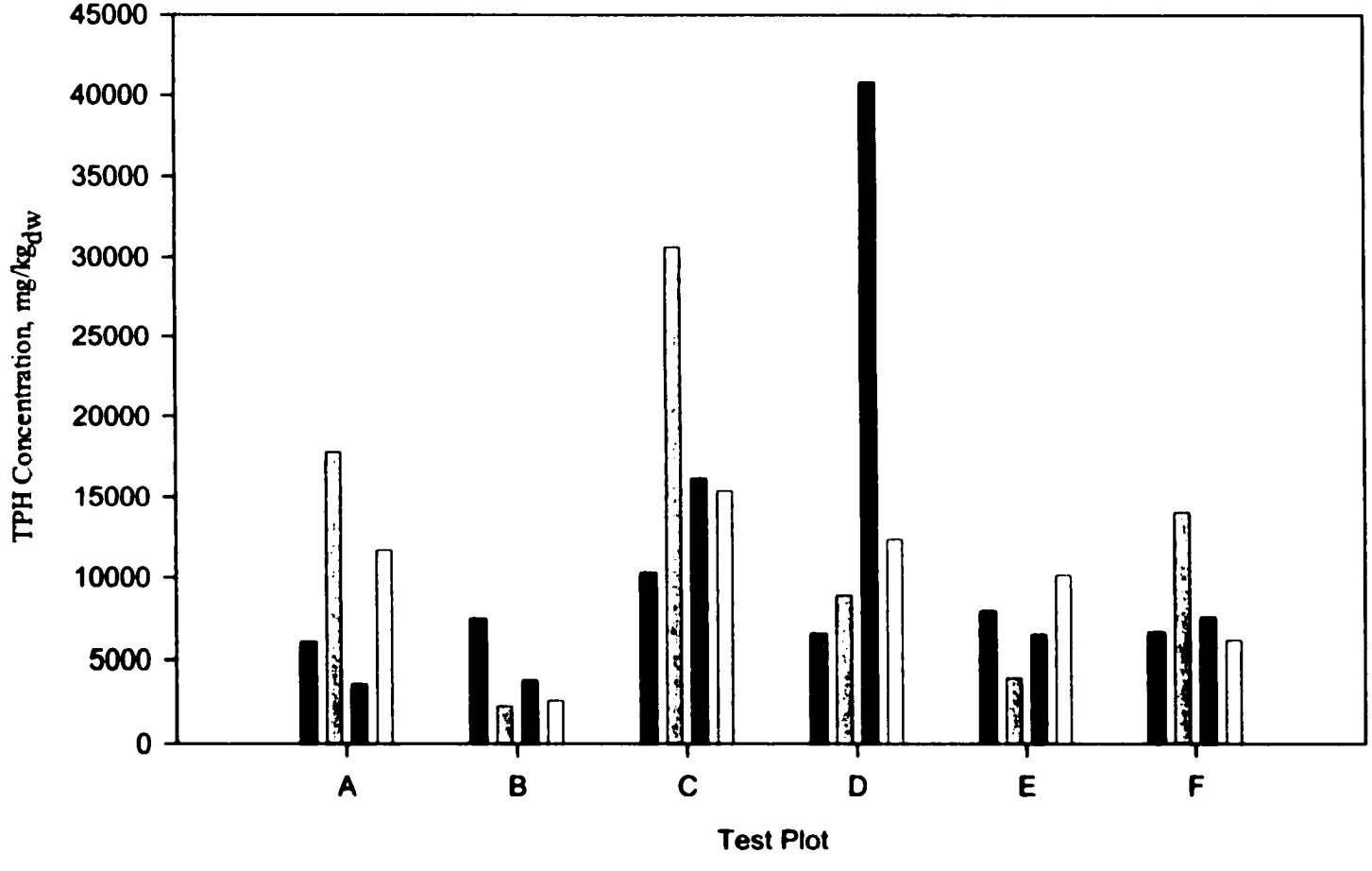


Figure 20. TPH concentrations using the ASTM Method for the candidate test plots from the Fore River Creek salt marsh on February 17, 1998. (4 replicates for each plot)

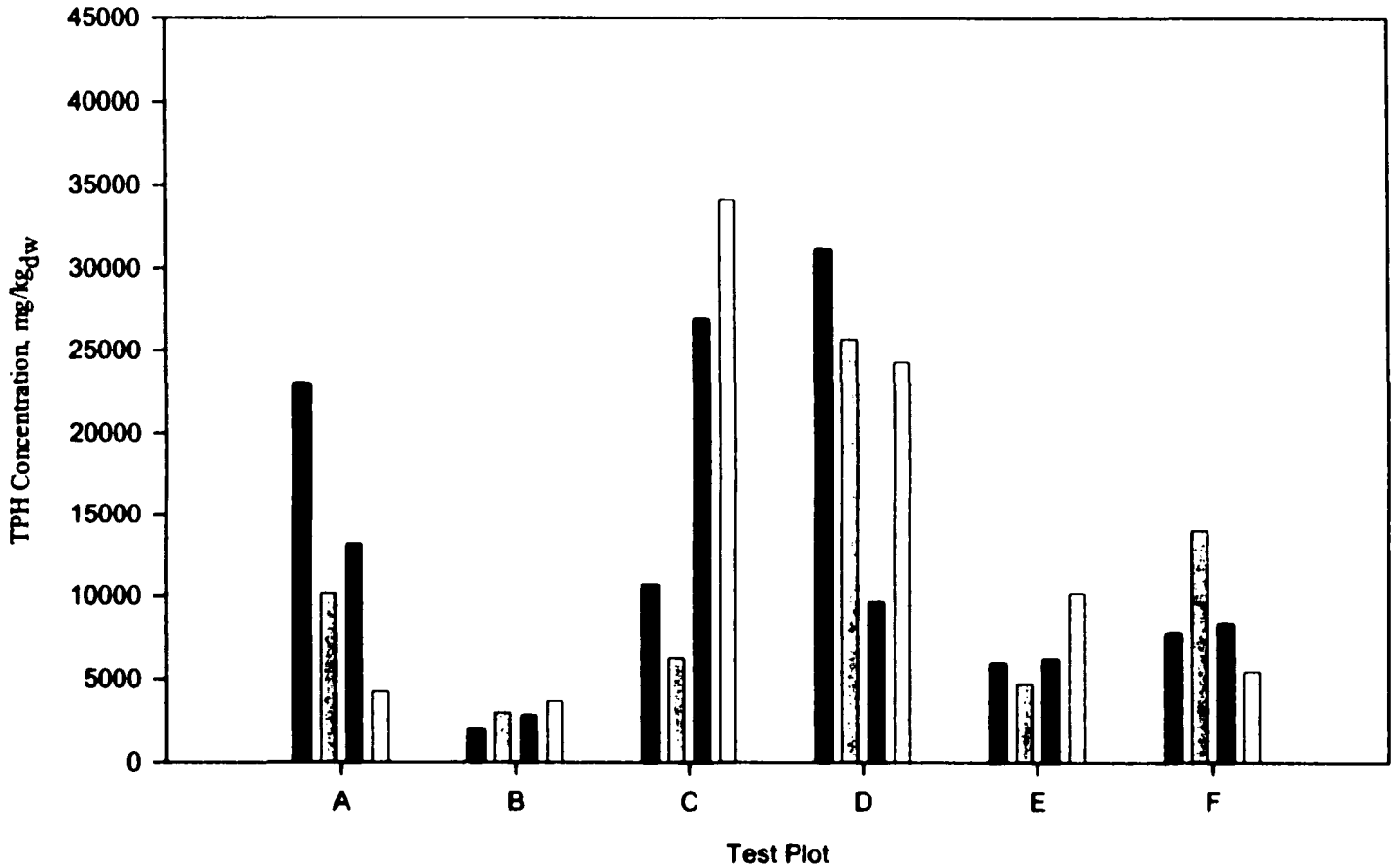


Figure 21. TPH concentrations using the ASTM Method for the candidate test plots from the Fore River Creek salt marsh on March 6, 1998. (n=4 replicates)

Table 18. TPH concentrations in split salt marsh sediments samples (ASTM and MADEP Methods).

	ASTM Method (n=2)		MADEP Method (n=1)
Sample	TPH mg/kg_{dw}	%CV	TPH mg/kg_{dw}
Plot C	12,500±175	± 1.4 %	5,700
Plot D	10,500±437	± 4.1 %	7,300
Plot E	17,000±4,881	[± 29 %]	2,900
Plot F	7,000±190	± 2.7 %	3,700
Blank (CS)	(23±9.7)BDL*	[± 42 %]	

*BDL=below MDL of 52 mg/kg_{dw} for the ASTM Method
[ ] %CV exceeds acceptable published value of ≤ 7 %

The concentration obtained for the CS was BDL, as expected indicating that the operator was getting better at analyzing the blanks. The high %CV of the CS was expected in this concentration range. The %CVs from the split samples (Plots C,D,E,F) analyzed at the UNH laboratory were significantly lower than the values previously obtained from the marsh samples analyzed with the ASTM Method (e.g., E vs. E) (F test, p=0.05) (Table 17) and most had better precision than recommended by ASTM (1995). This could be explained in part because of the special attention given to the homogenization of the samples before taking the three subsamples: one to send to Aquarian Analytical and two analyzed at UNH.

The TPH concentrations for each plot on March 25, 1998 were NSD (two tail *t*-test, p=0.05) from those found in the first and second sampling rounds (except for Site E that was SH) (Table 18). It is possible that during this event one of the sample locations in Plot E may have had some oil droplets.

TPH concentrations found in the split marsh samples analyzed with the ASTM Method were significantly higher (one tail *t*-test, p=0.05) by as much as ~5,000 mg/kg_{dw}. than the concentrations reported by the Aquarian Analytical using the MADEP Method. For Plots C, D and F differences were > 50%, while for Plot E differences were > 200%.

Previously, the ASTM Method had high %Ps when marsh sediments were spiked with No. 2 fuel oil (Table 15), which could have been the result of NOM that absorbed 254 nm light (Sorini *et al.*, 2001). Aquarian Analytical reported a %P of 80±14% for the marsh samples analyzed by the MADEP Method (based on surrogate recovery).

The MADEP Method was used for all subsequent analysis because it was reported to yield reliable results that could be used to assess and evaluate the extent of bioremediation occurring during the study (Rice, 1998; Weisman, 1998). The MADEP method increased the time required to analyze the samples (12 samples/d) and the cost per sample was higher (~$50/sample). However, it allowed TPH characterization (SC and LC aliphatics and aromatics HCs) that is important when monitoring the effectiveness of *in situ* biodegradation. The MADEP Method is reported (Weisman, 1998) to have less interferences with NOM because the identification and quantification of TPH are conducted using gas chromatography.

4.2.1 Depth of Contamination in the Marsh Sediments

Sediment samples were taken at three depths in Plot D of the Fore River Creek salt marsh (n=1): 0 to 4 cm, 4 to 6 cm and 9 to 11 cm. The samples were sent to Aquarian Analytical and analyzed using the MADEP Method (Table 19). The highest TPH concentration was present in the top interval. This amount of TPH penetration in the sediments ~19 months after the *Julie N* spill was consistent with what has been observed in other contaminated marshes. Burns and Teal (1979) found ~4,300 mg TPH/kg from 0 to 5 cm and 15 mg TPH/kg from 45 to 50 cm below the surface during studies two years after a West Falmouth, MA spill of No. 2 oil. They suggested that the oil concentration decreased exponentially with depth because of diffusion or small scale

mixing. In addition, they found that five years after the spill, while the overall TPH concentration had decreased, the concentration was still highest between 0 and 5 cm (~2,100 mg TPH/kg).

Table 19. TPH concentrations at depth in the Fore River Creek salt marsh sediments of Plot D (n=1).

Depth, cm	TPH Concentration, mg/kg_{dw}
0-4	6,700
4-6	24
9-11	640

The rate and depth of oil penetration also depends on the porosity of the substrate (Zhu *et al.*, 2001). For coarse-grained shorelines (e.g., cobble and sandy beaches), oil can penetrate deeper and stay longer, compared to fine-grained sediments (e.g., silts and clay) such as found in salt marshes. Generally, in salt marshes if no action is taken (e.g., tilling), the spilled oil will not travel deeper into the sediments. Under a controlled No. 2 fuel oil spill, Hershner and Lake (1980) found that the maximum oil penetration in a Chesapeake Bay salt marsh was < 20 cm. Five and half months after dosing ended, the highest concentration was present in the 0 to 10 cm region. They suggested that because the marsh studied was relatively young (in geological time), the depth of marsh peat and nature of the underlying sediment contributed to the minimal penetration. Mille *et al.* (1998) studied the Ile Grande salt marshes (Brittany, France) that were contaminated by the *Amoco Cadiz* spill in 1978. After the spill, they found a penetration similar to that in the Fore River Creek salt marsh. They found no contamination in the lower layers (12 to 20 cm) in any of the locations they studied. As a result of this evaluation, sediment

samples were collected from 0-4 cm during the *in situ* bioremediation study in the Fore River Creek marsh.

4.3 QC for MADEP Method

This section presents the QC results for the MADEP Method during this dissertation research.

4.3.1 Instrument QC

The surrogates (COD and OTP) and IS (5-alpha-androstane) were adequately resolved in the GC chromatograms having RTs separate from the individual components in the aliphatic and aromatic calibration standards and eluting at or near the representative RTs specified in the MADEP Method. Using the split injection, the first aliphatic peak (n-nonane; n-C_9) peak was resolved from the solvent (hexane or methylene chloride) peak. The RT windows were calculated and adjusted three times during the study (Tables 20 and 21). They were slightly different than those cited in the MADEP Method. because the column and chromatographic conditions used were specific for the UNH laboratory (See Chapter III-Materials and Methods). The calibration standard checks run between samples were used to identify and verify the IS and surrogate RTs in each event. Initially, there was a problem for the aromatic peaks that were very close (i.e., Indenol [1,2,3-cd] pyrene and dibenzo [a,h] anthracene). This was resolved using a suggestion by Dr. Rice (Aquarian Analytical) to force a horizontal projection during the GC integration after the pyrene. Overall, there were no problems with the RT windows during the bioremediation study.

Table 20. RT windows for the aliphatic standards in the MADEP Method during the study.

	MADEP	RT Calibration 1 (min)		RT Calibration 2 (min)		RT Calibration 3 (min)	
Compound	RT(min)	Mean	Window	Mean	Window	Mean	Window
n-Nonane (C_9)	3.14	3.088	3.017 -3.159	3.117	3.028 -3.205	3.004	2.986 -3.022
n-Decane (C_{10})	4.55	4.441	4.401 -4.481	4.542	4.432 -4.652	4.351	4.334 -4.367
n-Dodecane (C_{12})	7.86	7.882	7.836 -7.928	8.065	7.922 -8.207	7.786	7.748 -7.824
n-Tetradecane (C_{14})	11.10	11.390	11.334 -11.445	11.605	11.442 -11.767	11.286	11.230 -11.342
n-Hexadecane (C_{16})	14.05	14.608	14.545 -14.672	14.847	14.676 -15.018	14.416	13.539 -15.293
n-Octadecane (C_{18})	16.71	17.520	17.450 -17.589	17.781	17.604 -17.958	17.407	17.339 -17.475
n-Nonadecane (C_{19})	17.95	18.873	18.801 -18.945	19.148	18.969 -19.327	18.758	18.687 -18.828
n-Eicosane (C_{20})	19.14	20.162	20.092 -20.232	20.449	20.267 -20.631	20.044	19.971 -20.117
5-alpha Androstane (IS)	N/A	20.502	20.424 -20.579	20.805	20.610 -21.001	20.382	20.350 -20.414
COD (surrogate)	20.13	21.277	21.206 -21.348	21.568	21.382 -21.755	21.152	21.083 -21.220
n-Docosane (C_{22})	21.35	22.574	22.498 -22.650	22.884	22.697 -23.071	22.451	22.371 -22.530
n-Tetracosane (C_{24})	23.40	24.789	24.709 -24.868	25.124	24.939 -25.310	24.660	24.582 -24.739
n-Hexacosane (C_{26})	25.29	26.834	26.753 -26.915	27.195	27.010 -27.381	26.704	26.625 -26.783
n-Octacosane (C_{28})	27.04	28.732	28.645 -28.818	29.116	28.929 -29.303	28.599	28.522 -28.676
n-Triacontane (C_{30})	28.69	30.561	30.458 -30.663	31.050	30.819 -31.282	30.407	30.326 -30.488
n-Hexatriacotane (C_{36})	34.82	40.393	40.119 -40.666	42.145	41.453 -42.838	39.901	39.724 -40.079

RT Calibration 1 used for June and July 1998.
RT Calibration 2 used for August, September and October 1998.
RT Calibration 3 used for April, May and June 1999.
N/A Not available.

Table 21. RT windows for the aromatic standards in the MADEP Method during the study.

	MADEP	RT Calibration 1 (min)		RT Calibration 2 (min)		RT Calibration 3 (min)	
Compound	RT(min)	Mean	Window	Mean	Rt Window	Mean	Window
Naphthalene	7.660	7.756	7.718-7.793	7.903	7.744-8.063	7.648	7.613-7.682
2-Methylnaphthalene	9.490	9.723	9.683-9.763	9.896	9.716-10.076	9.616	9.569-9.663
Acenaphthylene	11.930	12.402	12.359-12.445	12.600	12.403-12.796	12.290	12.230-12.350
Acenaphthene	12.460	12.983	12.939-13.026	13.189	12.990-13.388	12.869	12.804-12.934
Fluorene	13.890	14.556	14.513-14.599	14.771	14.564-14.977	14.442	14.370-14.513
Phenanthrene	16.540	17.503	17.460-17.546	17.740	17.522-17.957	17.386	17.310-17.461
Anthracene	16.660	17.642	17.593-17.692	17.882	17.658-18.106	17.528	17.440-17.616
OTP (surrogate)	17.950	19.013	18.975-19.051	19.251	19.043-19.460	18.886	18.817-18.955
5-alpha Androstane (IS)	N/A	20.512	20.497-20.526	20.807	20.594-21.020	20.388	20.350-20.426
Fluoranthene	19.920	21.248	21.200-21.296	21.519	21.288-21.750	21.127	21.048-21.205
Pyrene	20.510	21.913	21.859-21.967	22.192	21.958-22.427	21.792	21.704-21.880
Benzo(a)Anthracene	24.080	25.750	25.692-25.808	26.065	25.825-26.305	25.630	25.549-25.712
Chrysene	24.210	26.026	24.911-27.141	26.199	25.954-26.444	25.761	25.660-25.862
Benzo(b)Fluoranthene	26.940	28.961	28.893-29.028	29.306	29.055-29.556	28.839	28.754-28.925
Benzo(k)Fluoranthene	27.020	29.039	28.952-29.126	29.386	29.129-29.643	28.917	28.804-29.031
Benzo(a)Pyrene	27.660	29.703	28.787-30.619	30.189	29.915-30.463	29.701	29.614-29.789
Indeno(1,2,3-cd)Pyrene	30.250	33.401	33.261-33.542	34.031	33.606-34.456	33.227	33.087-33.367
Dibenzo(a,h)Anthracene	30.360	33.535	33.390-33.680	34.183	33.764-34.602	33.332	33.187-33.476
Benzo(g,h,i)Perylene	30.760	34.380	34.198-34.562	35.096	34.619-35.572	34.186	34.023-34.349

RT Calibration 1 used for June and July1998.
RT Calibration 2 used for August, September and October1998.
RT Calibration 3 used for April, May and June1999.
N/A Not available.

4.3.2 Calibration

An internal calibration method was used. The calibration was based on the five calibration standards and an IS. During the study, three calibrations were prepared: June 9, 1998 [(n=1) (used for June10-11, July 7-8 and July 26-27, 1998)], August 13, 1998 [(n=3) (used for August 17-18, September 18-19 and October 16-17, 1998)] and April 27, 1999 [(n=3) (used for April 9-10, May 11-12 and June 9-10, 1999)] (Appendix B). The calibration data were used to generate RFs in each standard concentration for each compound. RRFs as the arithmetic average of the individual RFs for the SC, and LC aliphatics and aromatics were also calculated.

All calibrations, based on the RFs, were generally acceptable because the %CVs were $\leq$ 25% over the working range (Tables 22 and 23) (MADEP, 1998a). The mean RRF for each calibration was used to calculate SC and LC aliphatic and aromatic concentrations for each sampling event (Table 24).

For the first calibration (June 9, 1998), some of the %CVs for the aromatic compounds were $\geq$ 25%, especially for those compounds that were resolving close together (e.g., benzo (k) fluoranthene and benzo (a) pyrene). After discussing this problem with Dr. Rice (Aquarian Analytical), the integration method was adjusted to baseline to separate these peaks. The calibration was reintegrated and the %CVs were reduced to $\leq$ 25%. The corrected RRFs were used to calculate the TPH concentrations for the June 10-11, July 8-7 and July 26-27, 1998 data (Table 24). For the second calibration (August 13, 1998), some of the C_{12}-C_{18} data had %CVs > 25%.

Table 22. %CVs for the aliphatic standards in the MADEP Method used in the calibrations.

Compound	% CVs of Calibrations						
Calibration Curve #	1	2			3		
n-Nonane (C_9)	7.1	10.4	19.8	23.7	13.5	5.9	15.3
n-Decane (C_{10})	9.3	10.9	21.7	27.6	14.6	1.7	4.0
n-Dodecane (C_{12})	7.8	12.3	22.0	30.0*	6.1	10.3	4.6
n-Tetradecane (C_{14})	6.1	12.9	20.7	30.0*	3.3	9.9	5.1
n-Hexadecane (C_{16})	5.3	12.7	19.7	28.0*	5.6	8.1	4.4
n-Octadecane (C_{18})	15.6	12.2	19.2	25.8*	5.6	6.3	4.5
n-Nonadecane (C_{19})	7.5	12.0	18.7	25.0	5.4	5.5	4.0
n-Eicosane (C_{20})	6.1	11.2	21.4	24.3	5.0	4.8	4.0
COD (surrogate)	9.2	7.4	18.6	24.8	6.5	5.7	5.3
n-Docosane (C_{22})	8.9	11.0	18.7	23.9	5.1	3.9	3.6
n-Tetracosane (C_{24})	6.5	11.2	19.2	23.9	5.4	3.6	3.6
n-Hexacosane (C_{26})	6.9	11.7	19.7	24.5	6.2	4.7	4.9
n-Octacosane (C_{28})	7.5	10.7	19.7	24.6	5.0	4.5	5.4
n-Triacontane (C_{30})	6.9	12.4	24.5	24.9	8.6	6.9	8.3
n-Hexatriacotane (C_{36})	9.1	19.0	19.8	27.8*	18.4	19.7	20.4

* Exceeds the 25% maximum %CV cited by the MADEP Method.

For Calibration Curve 1, only one replicate is shown because the data file containing the others was lost.

Table 23 %CVs for the aromatic standards in the MADEP Method used in the calibrations.

Compound	% CV of Calibrations						
	1	2			3		
Naphthalene	4.7	5.5	3.0	1.8	10.6	9.8	7.2
2-Methylnaphthalene	4.6	5.2	3.1	2.1	10.2	9.5	7.5
Acenaphthylene	3.7	5.3	3.2	1.7	8.6	8.2	6.1
Acenaphthene	4.0	4.8	3.3	2.2	6.7	6.3	5.2
Fluorene	4.4	4.5	3.5	2.5	5.7	5.5	5.0
Phenanthrene	5.4	5.9	4.2	3.8	11.2	13.0	10.3
Anthracene	6.5	6.3	3.9	4.3	12.1	12.3	11.3
OTP (surrogate)	5.0	5.4	4.4	4.0	5.7	5.4	4.7
Fluoranthene	4.8	6.8	5.5	5.9	6.8	5.0	5.0
Pyrene	4.4	7.4	5.6	6.0	6.0	9.7	5.4
Benzo(a)Anthracene	5.4	10.8	8.3	12.1	11.9	11.5	11.7
Chrysene	5.4	10.6	8.6	11.5	2.0	3.3	2.5
Benzo(b)Fluoranthene	4.2	11.0	9.7	12.7	18.1	18.0	18.0
Benzo(k)Fluoranthene	10.0	5.9	5.4	7.5	19.7	17.7	14.8
Benzo(a)Pyrene	13.3	7.7	14.2	13.6	3.7	2.4	3.8
Indeno(1,2,3-cd)Pyrene	4.6	8.6	14.0	11.0	11.6	13.9	22.9
Dibenzo(a,h)Anthracene	6.5	12.8	25.2*	21.6	11.1	10.4	5.9
Benzo(g,h,i)Perylene	6.6	10.4	23.6	20.3	16.6	8.6	17.4

* Exceeds the 25% maximum %CV cited by the MADEP Method.

For Calibration Curve 1, only one replicate is shown because the data file containing the others was lost.

However, because these %CVs were close to 25% and the %CVs of the other data generated for the calibration were ≤ 25%, all of the data were used for the August 17-18, September 18-19 and October 16-17, 1998 events.

Table 24. Mean RRFs used for calculation of SC and LC aliphatic and aromatic concentrations.

Fraction	RRFs		
	Calibration 1 (June 9,1998)	Calibration 2 (August 13, 1998)	Calibration 3 (April 27,1999)
SC Aliphatics	0.93	0.77	0.98
LC Aliphatics	0.98	0.82	0.91
Aromatics	1.00	0.79	0.84

MADEP specifies that the RRFs should be ~1.00.

In order to demonstrate the absence of mass discrimination during the study, the ratio of n-octadecane (C_{28}) to n-Eicosane (C_{20}) area counts was evaluated for the standards used during the calibrations (Figure 22). This ratio is used because both C_{28} and C_{20} belong to the LC aliphatic fraction. The MADEP Method stipulates that the ratio must be ≥ 0.85 (MADEP, 1998a).

Initially, the C_{28}/C_{20} ratio was not monitored during the study. After realizing this omission, the ratio was calculated based on the aliphatic check standards (Figure 23). Generally the ratios were all ≥ 0.85. Although September 18-19 and October 16-17, 1998 ratios were ≤ 0.85, the data were used because they were close to the stated value. A new column was purchased, a new calibration prepared on April 27, 1999 and, and all samples (Figure 22) from April 9-10, 1999 were reanalyzed. With the new column and calibration, all of the ratios were ≥ 0.85. [N.B., All of the data shown in this dissertation for April 9-10, May 11-12 and June 9-10, 1999 were generated using this column.]

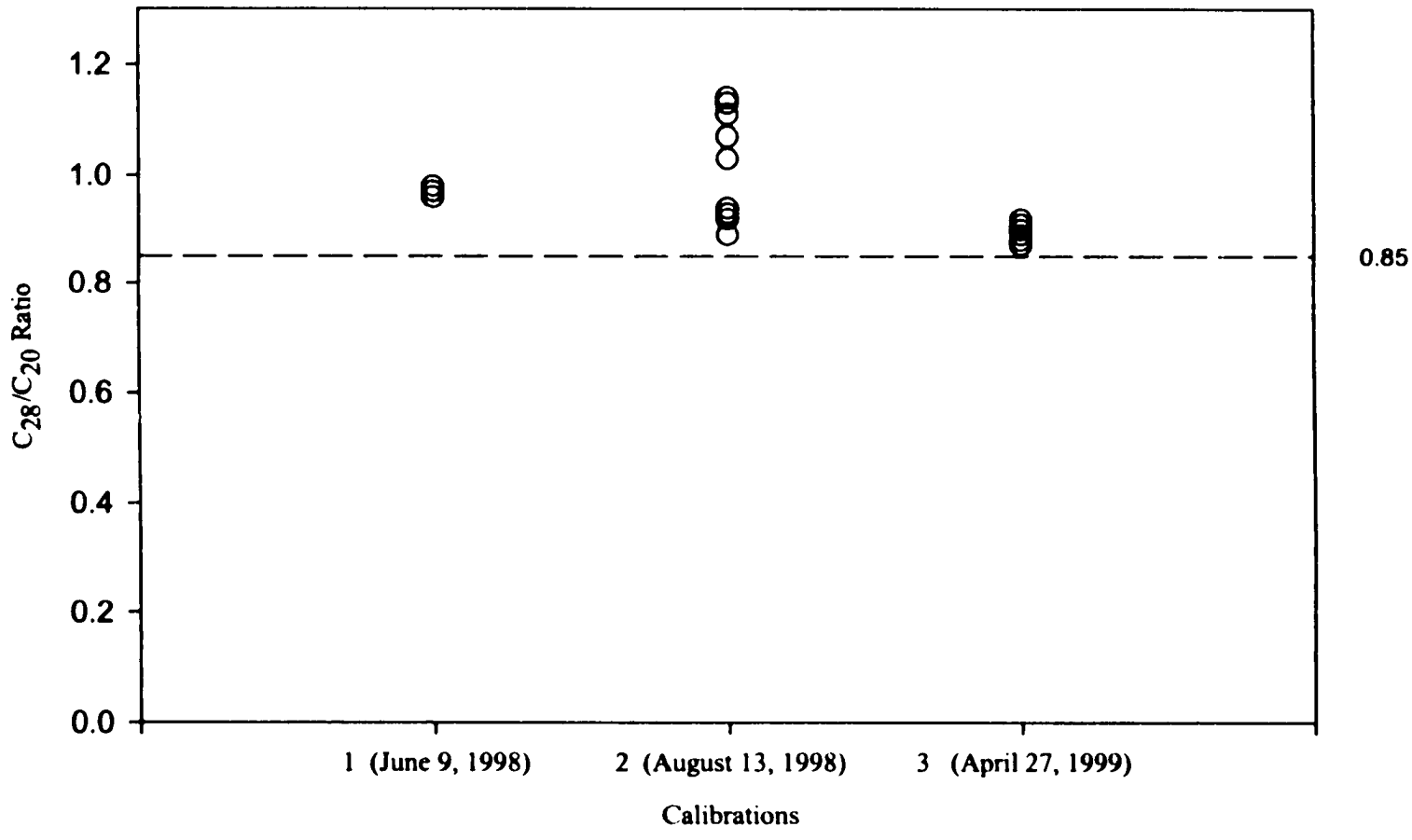


Figure 22. C_{28}/C_{20} ratios for the aliphatic calibration standards evaluated for the different calibrations. 0.85 is the C_{28}/C_{20} ratio stipulated by the MADEP Method.

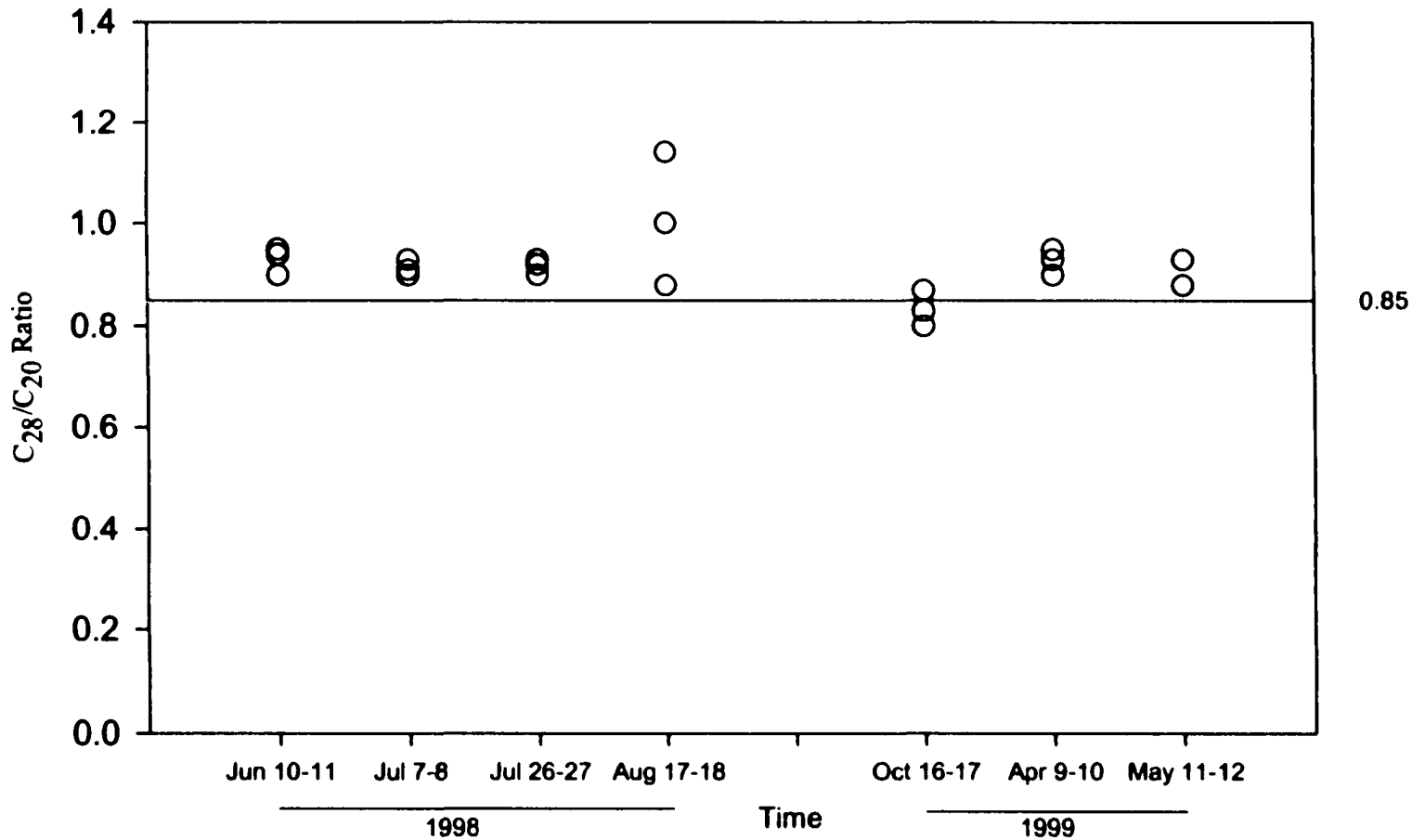


Figure 23. C_{28}/C_{20} ratios for the aliphatic calibration standards checks during the sampling events. 0.85 is the C_{28}/C_{20} ratio stipulated by the MADEP Method.

4.3.3 QC Monitoring

After every 20 samples analyzed during each sampling event, calibration check standards were run to monitor QC. These standards were prepared from the same stock solution as the calibration standards for Calibrations 1, 2 and 3, and were generally mid-level concentrations.

The calibration checks for each sampling event were also used to calculate RFs and those were compared, as RPDs with the RFs from the calibrations curves (1, 2 or 3) for the specific concentration of the standard (Figure 24). The MADEP Method specifies that the RPDs must be $\leq$ 25%. This criterion was meet throughout the study for all compounds.

4.3.4 Solvent, Laboratory and Trip Blanks.

Solvent blanks (hexane or methylene chloride) were analyzed at the beginning of each batch of samples to evaluate if there was any contamination in the solvent or GC (e.g., column, liner or detector). If any peaks were detected, then several solvent blanks were run to clean the GC and determine if the contamination persisted that it was in the solvent. Fine aggregate sand (Ossipee Aggregate) was used for laboratory blanks (LBs) and trip blanks (TBs) to evaluate the sampling performance and analyze recovery in a blank matrix. The sand was muffled at 550°C for 2 h to eliminate organic contamination. For TBs, the muffled sand was placed in pre-cleaned glass bottles and transported to the field to monitor potential contamination during sampling, transportation and storage. The LBs were kept in the laboratory in a close jar until processing. The TBs and LBs were spiked with the IS and aliphatic and aromatic surrogates just before extraction.

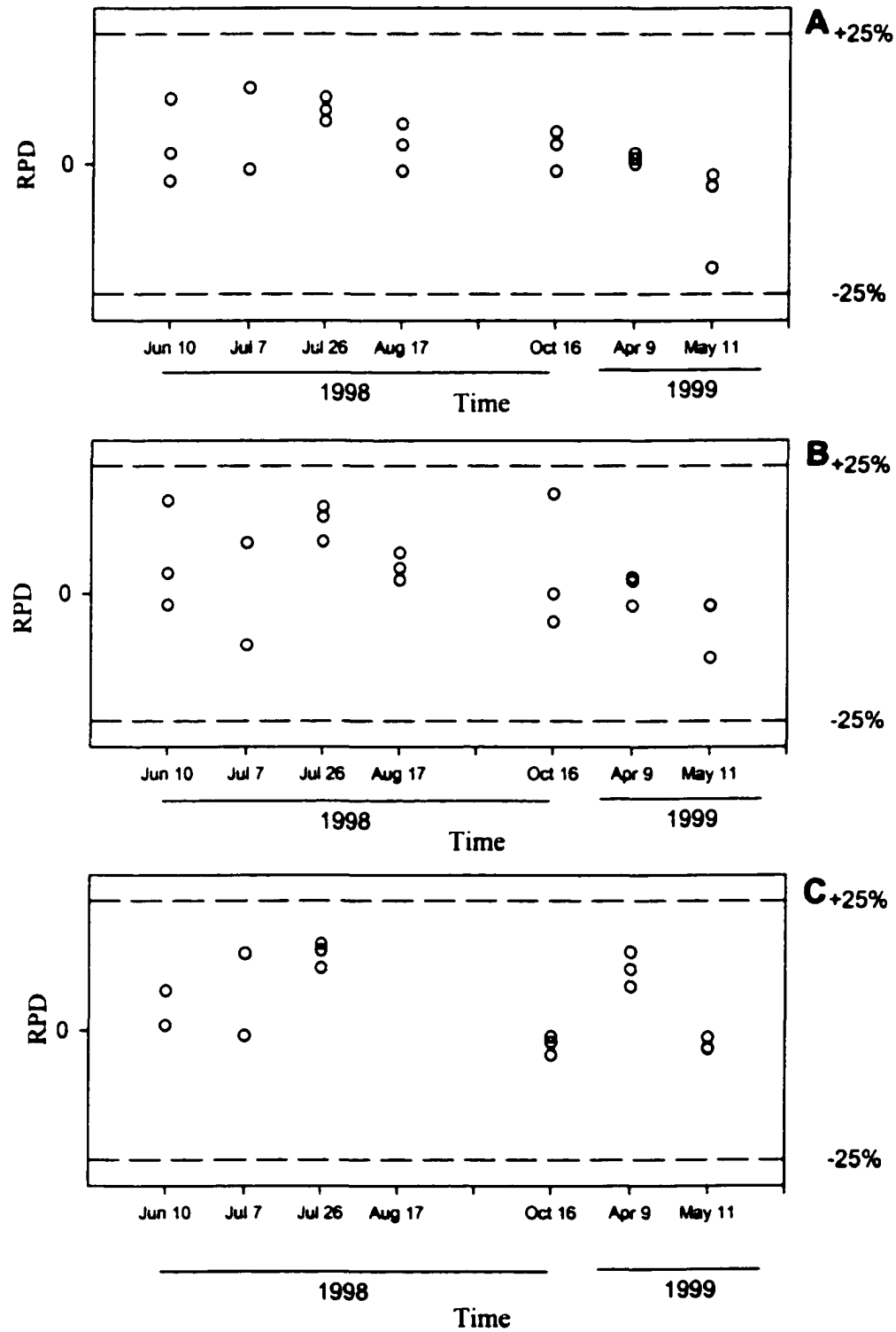


Figure 24. RPDs during the sampling events. A) SC aliphatics, B) LC aliphatics, and C) aromatics. ±25% is the RPD stipulated by the MADEP Method.

The TBs and LBs were exposed to all glassware, solvents, reagents, and equipment used during the analysis. In addition, their area counts were used for baseline correction of peaks attributable to the leaching of plasticizers or other contamination from the silica gel SPE columns (MADEP, 1998a).

The LBs and TBs for July 7-8 and July 26-27, 1998 were contaminated with SC and LC aliphatics and aromatics (Figure 25). After checking all procedures (by running methylene chloride through the extraction and GC process), it was determined that the sand used for the LBs was contaminated with TPH. During the study, large (kg) batches of the sand were muffled and placed in a single wide-cap mouth plastic container and stored for long periods of time (~6 weeks). Because, the storage container was plastic, it could not be muffled. It is likely that the sand was contaminated by mistake. After the problem was discovered, only small amounts of sand (g) were muffled and these were kept in low volume pre-muffled, sealed glass bottles for short periods of time (~3 d) prior to use. All SC and LC aliphatic and aromatic concentrations were corrected by subtracting the mean area counts for the blanks from the sample area counts for each event as recommended by the MADEP Method. To be able to use the sample data from July 7-8 and July 26-27, 1998, the area counts for the LBs and TBs for August 1998 through June 1999 were averaged and those values were used to correct the sample TPH concentrations for July 1998 (Figure 25).

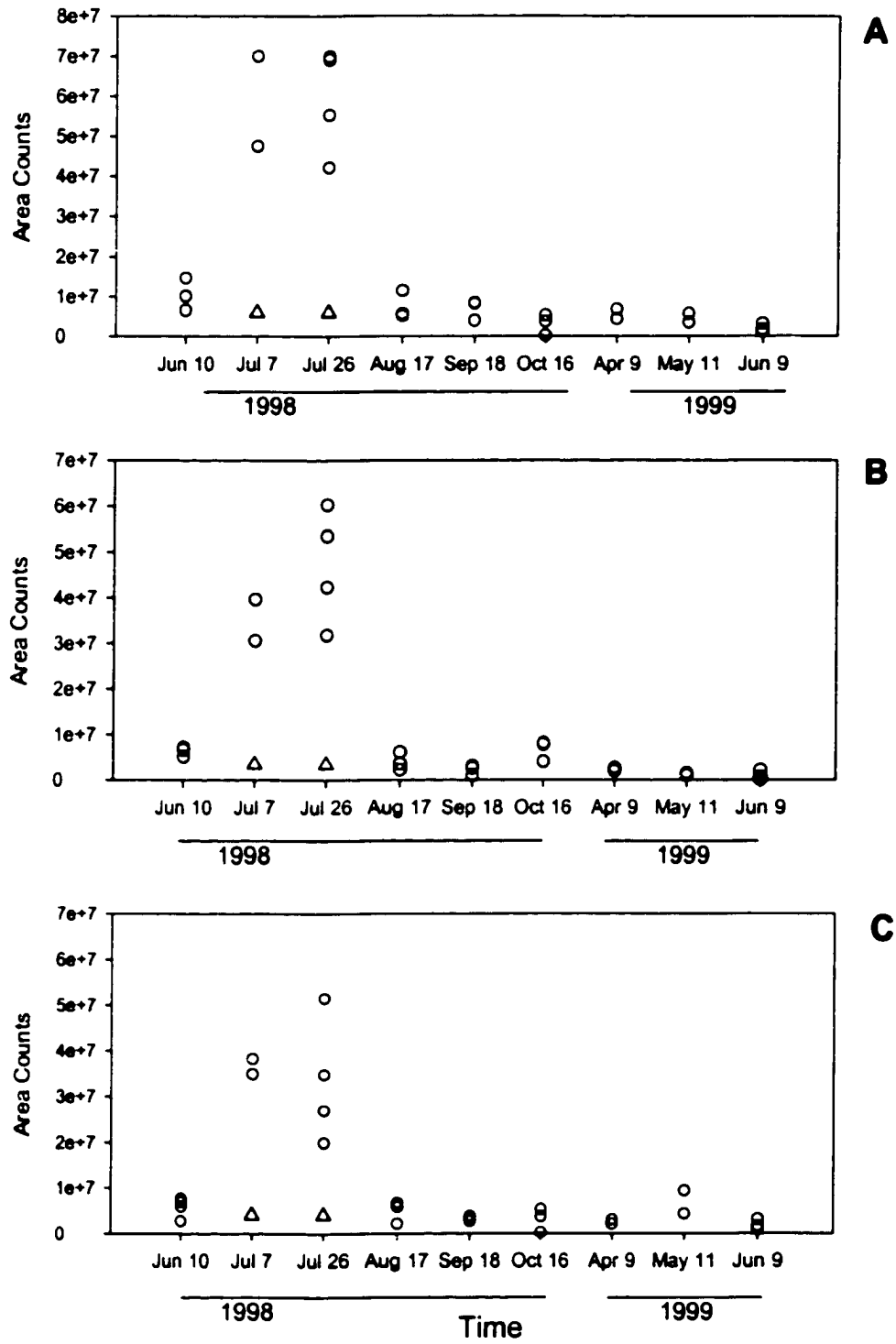


Figure 25. Area counts for LBs and TBs. A) SC aliphatics, B) LC aliphatics and C) aromatics. Δ is the mean blank used value for August 1998-June 1999 instead of the contaminated blanks originally associated with the July 7 and 26, 1998 sampling dates.

4.3.5 Surrogate Recovery

Two surrogates were used (OTP and COD for the aromatic and aliphatic fractions, respectively). They were added to the samples in the laboratory just before extraction began, so that the overall efficiency of the method could be determined.

A surrogate's behavior mimics that of the analytes of interest, but surrogate compounds are not typically found in natural environments. Hence, they can be used to correct extraction efficiency and %P in each sample. The surrogate recoveries were also used to compensate the SC and LC aliphatics and aromatic concentrations for any loss or gain in the %P during the extraction and analysis.

4.3.5.1 Blank Surrogate Recovery

The acceptable surrogate range used in this study (40 to 140 %P) is specified by the MADEP Method. Some of the initial surrogate recoveries for TBs and LBs (June 10-11, 1998) were low and out of range (Figure 26).

The main reason for this was the inexperience of the analyst with the MADEP Method. The extraction process must be done carefully to avoid loss of the surrogate (e.g., via sonication, and filtration and concentration of the extract). One problem noted in August 1998 was that the extraction solvent (methylene chloride) was being shaken out of the vial during sonication. To avoid this problem, the power of the sonicator was decreased until loss of solvent was not evident. In addition, Dr. Rice (Aquarian Analytical Laboratory) recommended that the surrogates and IS be added to blanks and samples using GC glass micro-syringes (VWR) instead of a micropipette. Extraction vials were also placed into a beaker containing cold water to minimize solvent heating and TPH volatilization during sonication.

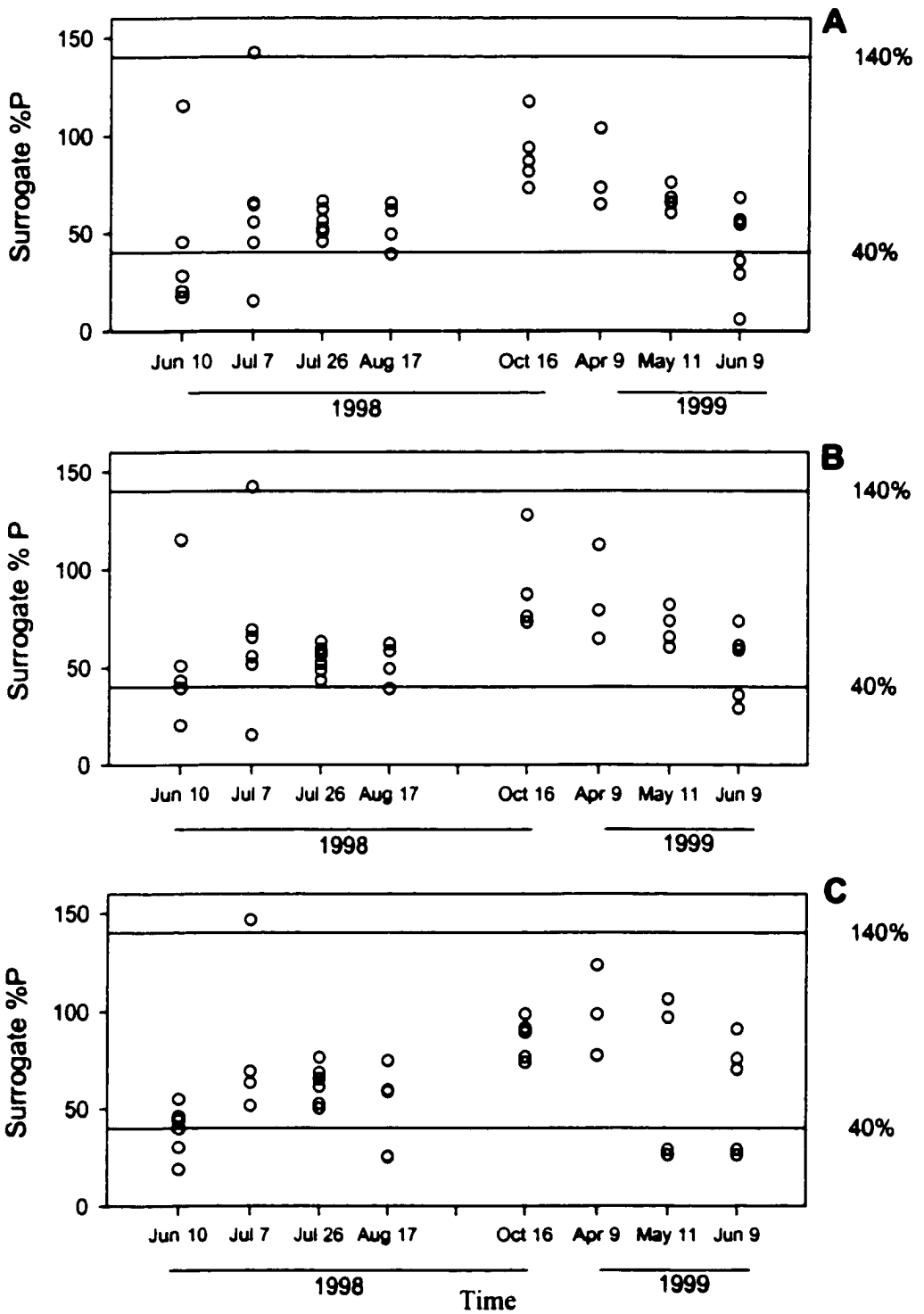


Figure 26. Control chart for LB and TB surrogate %Ps .The surrogates used were COD and OTP for the aliphatic and aromatic fractions, respectively. A) SC aliphatics, B) LC aliphatics and C) aromatics. 40-140 is the %P range stipulated by the MADEP Method.

During rinsing of the sonicator horns, special care was also taken to avoid TPH losses. Because of these changes, most of the blanks recoveries after August 1998 were higher and within the 40-140% range recommended by the MADEP (Figure 26). Lower recoveries were observed for June 9-10, 1999 because a new group of graduate students had taken over the analyses.

Samples from September 18-19, 1998 were discarded because the SPE fractionation was conducted using Supelco (Bellefonte, PA) silica gel columns instead of those manufactured by Waters Corporation (Milford, MA) that were out of stock in September 1998. The extraction performed with the Supelco columns did not meet QC criteria. By the time, the Waters columns became available the maximum holding time (14 days) for the September 18 and 19 sediment samples had been exceeded, so they could not be reanalyzed. Therefore, no TPH data is shown for September 18-19, 1998.

4.3.5.2 Sample Surrogate Recovery

Because of the high water and NOM content of the salt marsh sediments, as well as the small particle size, the surrogate recoveries in the samples were lower than for the LBs and TBs (Figure 27). The original draft of the MADEP method specified a Soxhlet extraction. However, after the second interlaboratory "Round Robin" evaluation was conducted by MADEP, a sonication extraction procedure was found to yield good %Ps for sand samples (MADEP, 1998b). The sonication extraction is not as rigorous as other extraction methods and is not recommended for applications where high extraction efficiencies of analytes at very low concentrations are necessary (e.g., demonstration of effectiveness of corrective action) (EPA, 1994). However, the TPH concentrations in this study in the salt marsh sediments were high (4,000 to 5000 mg TPH/kg_{dw}).

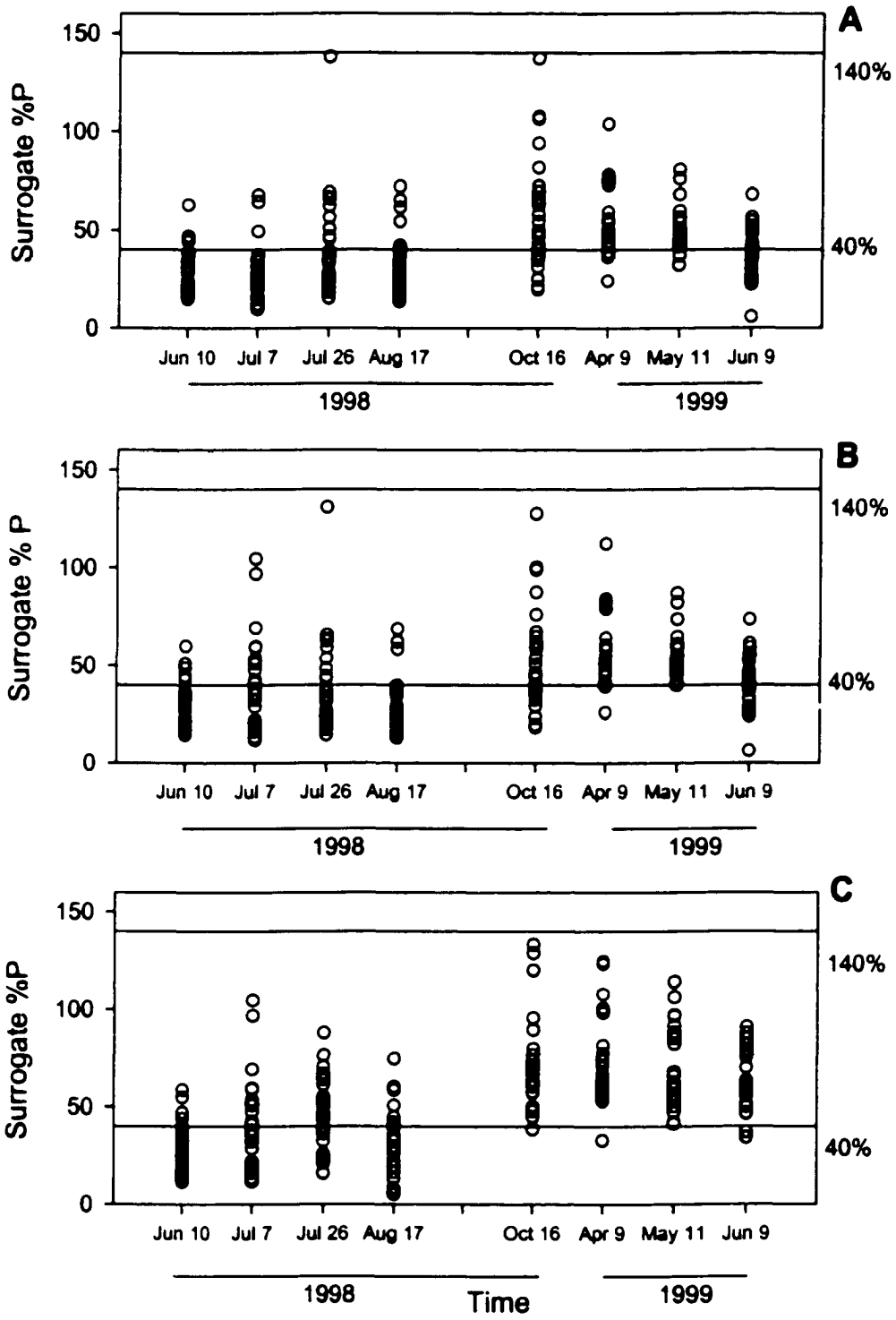


Figure 27. Control chart for the samples surrogate %Ps. The surrogates used were COD and OTP for the aliphatics and aromatic fractions, respectively. A) SC aliphatics, B) LC aliphatics and C) aromatics. 40-140 is the %P range stipulated by the MADEP Method.

Sonication was preferred for the marsh samples because of its short extraction process (~0.25 h) compared to the Soxhlet extraction (4 h). In addition, the dual horn sonicator allowed extraction of two samples concurrently.

Several critical steps responsible for the low recoveries with the sediment samples were identified. During the initial sampling events, the sediments were placed in extraction vials and the surrogates and sodium sulfate were added. After removing the water from the sample, the sodium sulfate formed a compact layer preventing complete mixing of the sample and solvent contact with the sediments. This problem was solved by rigorously mixing the samples and minimizing holding time before adding the solvent (methylene chloride) and starting the sonication. Filtration of some samples was very slow (>45 min) because the fine sediments clogged the filter paper and the only way to continue was to transfer the material to a new filter. During the transfer, some surrogate may have been lost. In addition, clogging of the silica gel column made the solvent elute very slowly from some samples.

After solving these issues and the problems described for the blanks (Section 4.3.5.1), most of the %Ps for the aromatic surrogate from October 1998 to June 1999 were within the recommended range (40-140%) (MADEP, 1998a). For the same period, the aliphatic recoveries were lower probably because some aliphatics were retained in the silica gel columns and eluting with the aromatic fraction.

4.3.6 IS Monitoring

The IS was added to the LBs and TBs, as well as the samples immediately after the extraction process was completed. The IS was used to monitor the GC's performance during the analysis of each event and between events.

4.3.6.1 Blank IS

By mistake, the IS concentration used for June 10-11, 1998 (4 ng/μL) was an order of magnitude lower than the concentration used for the rest of the events (Figure 28). The IS concentration was increased because it was too close to the low end of the calibration standards. Hence, TPH data from June 10-11, 1998 were not considered during the statistical analysis of the data. All of the IS area counts for October 16-17, 1998 were also somewhat low. Based on this and the low C_{28}/C_{20} ratios (< 0.85) observed for October 16-17, 1998 and April 9-10, 1999, a new column was installed in the GC and this corrected the problem. The April 1999 samples were reanalyzed with the new column. The October 1998 samples could not be reanalyzed because their hold time exceeded the published value when the problem was finally solved.

4.3.6.2 Sample IS

The IS area counts for the samples in June 10-11 and October 16-17, 1998 were low (Figures 29 and 30) as were the IS area counts blank (See Section 4.3.6.1). The June 1998 data were not used (See Section 4.3.6.1). Although the IS area counts for the October 16-17, 1998 samples were somewhat lower than for the other sampling events, they were used during the statistical analysis. No correction was made to these data. During some of the events (July 26-27 and August 17-18, 1998), some of the samples had high IS area counts. This may have occurred because higher volumes of the IS solution were added to the extract by mistake. These data were also used. These decisions were made because there were not criteria in the MADEP Method requiring their exclusion and some variation in IS area counts is expected.

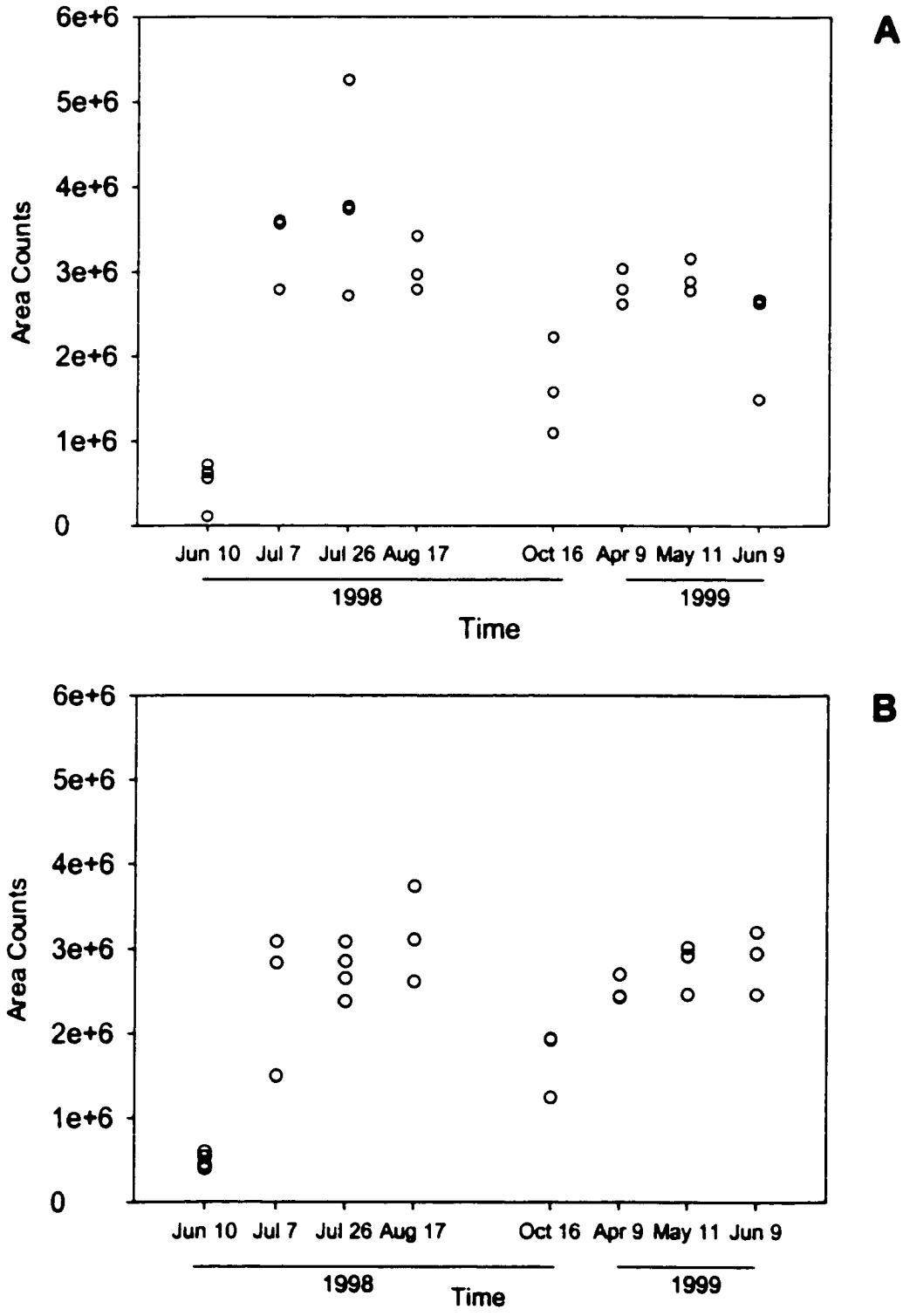


Figure 28. Control chart of the IS area counts for the LBs and TBs. A) aliphatics and B) aromatics.

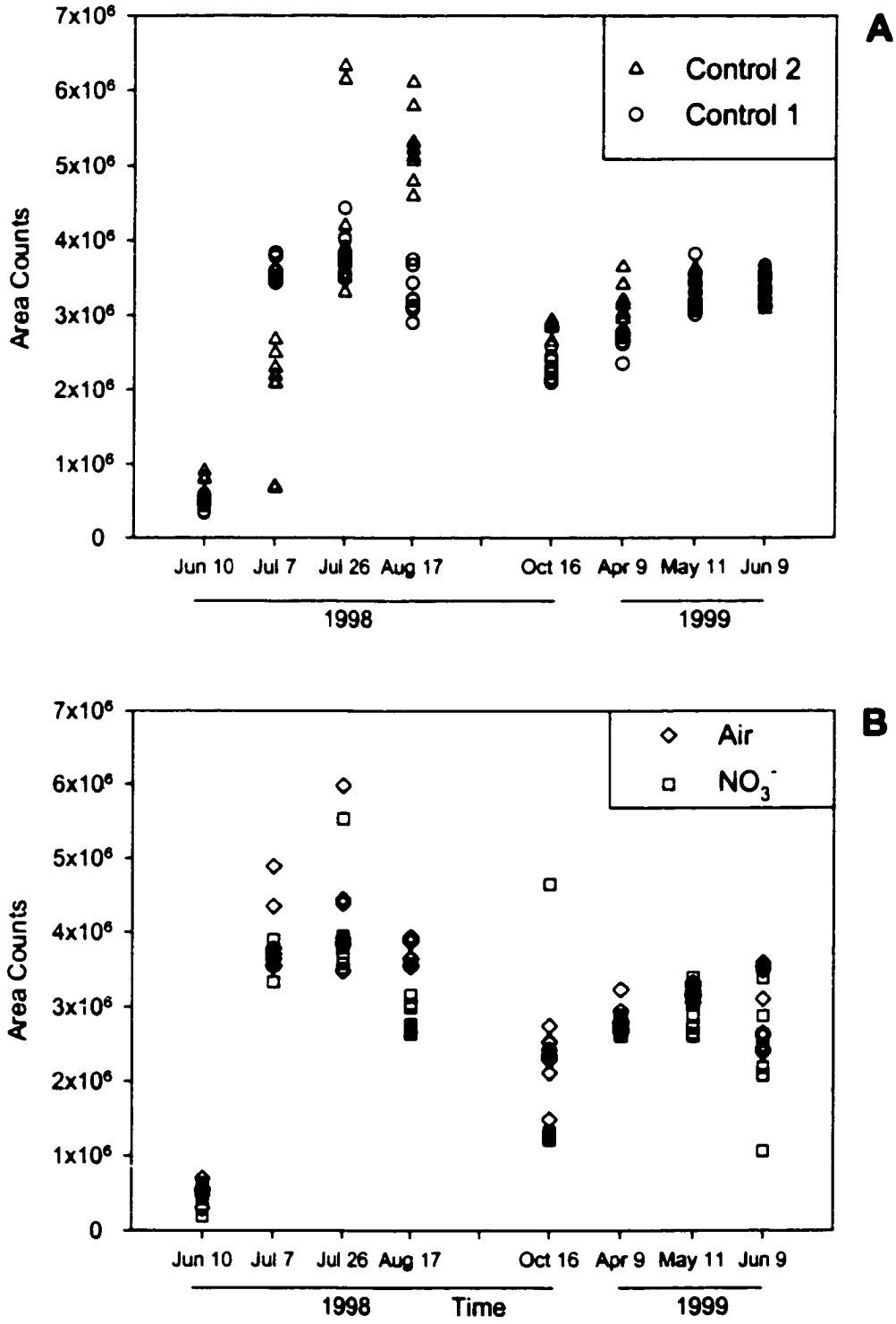


Figure 29. Control chart for the IS area counts for aliphatic samples. A) controls and B) treatments.

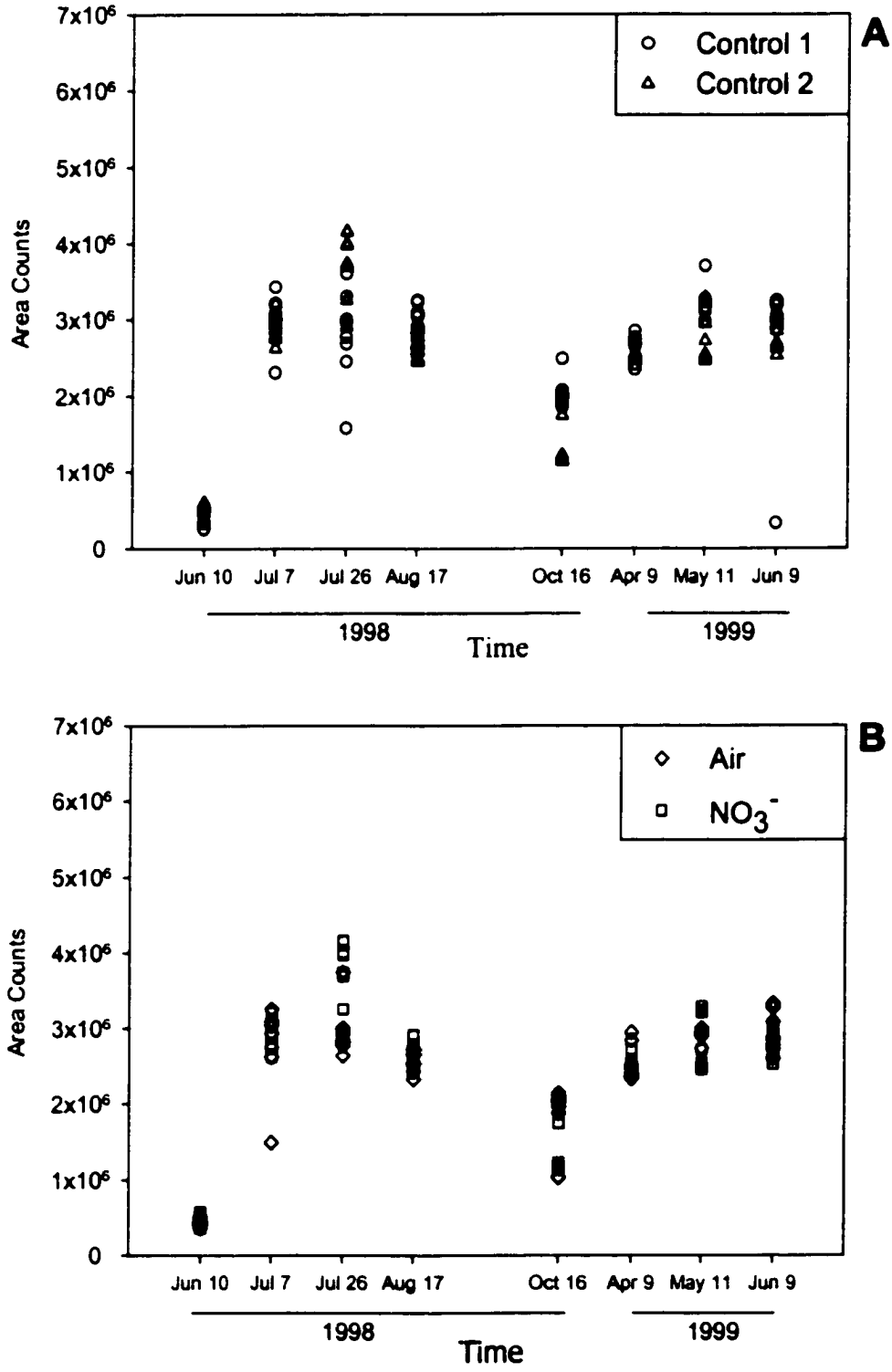


Figure 30. Control chart for the IS area counts for aromatic samples. A) controls and B) treatments.

4.3.7 Distribution of the TPH Data

The distribution of the data was analyzed by plotting the residuals for each TPH fractions: SC aliphatics (Figure 31), LC aliphatics (Figure 32), and aromatics (Figure 33). The residuals were plotted to observe if the log transformation had a normal distribution. Normal distribution was required for the analysis of covariance linear model (ACLM) and also stabilizes variance (Ramsey, 2002). The residual plots represent the observed minus the predicted concentration. The transformed TPH concentrations were randomly distributed around zero and there were no trend or pattern observed. Therefore, all of the TPH data were log transformed. The TPH concentrations were calculated by summing the SC and LC aliphatic and aromatic concentrations. As a result, the TPH data were redundant and more variable, so they were not used in any statistical analyses.

4.4 Analysis of Covariance

ACLM was developed to determine if the amendments (air and NO_3^-) enhanced biodegradation of the TPH fractions compared to the control plots. The ACLM was used to test the hypothesis that the amendments had a significant effect on the degradation of the TPH fractions (SC and LC aliphatics and aromatics) over time compared to the control (amendments vs. control).

The ACLM was performed using the statistical software JMP IN, Version 3.2.6 (SAS Institute INC; Cary, NC).

The general ACLM is:

$$\text{Log } Y = \mu + Ai + Bj(i) + Dl + ADil + E(i,j,l)$$

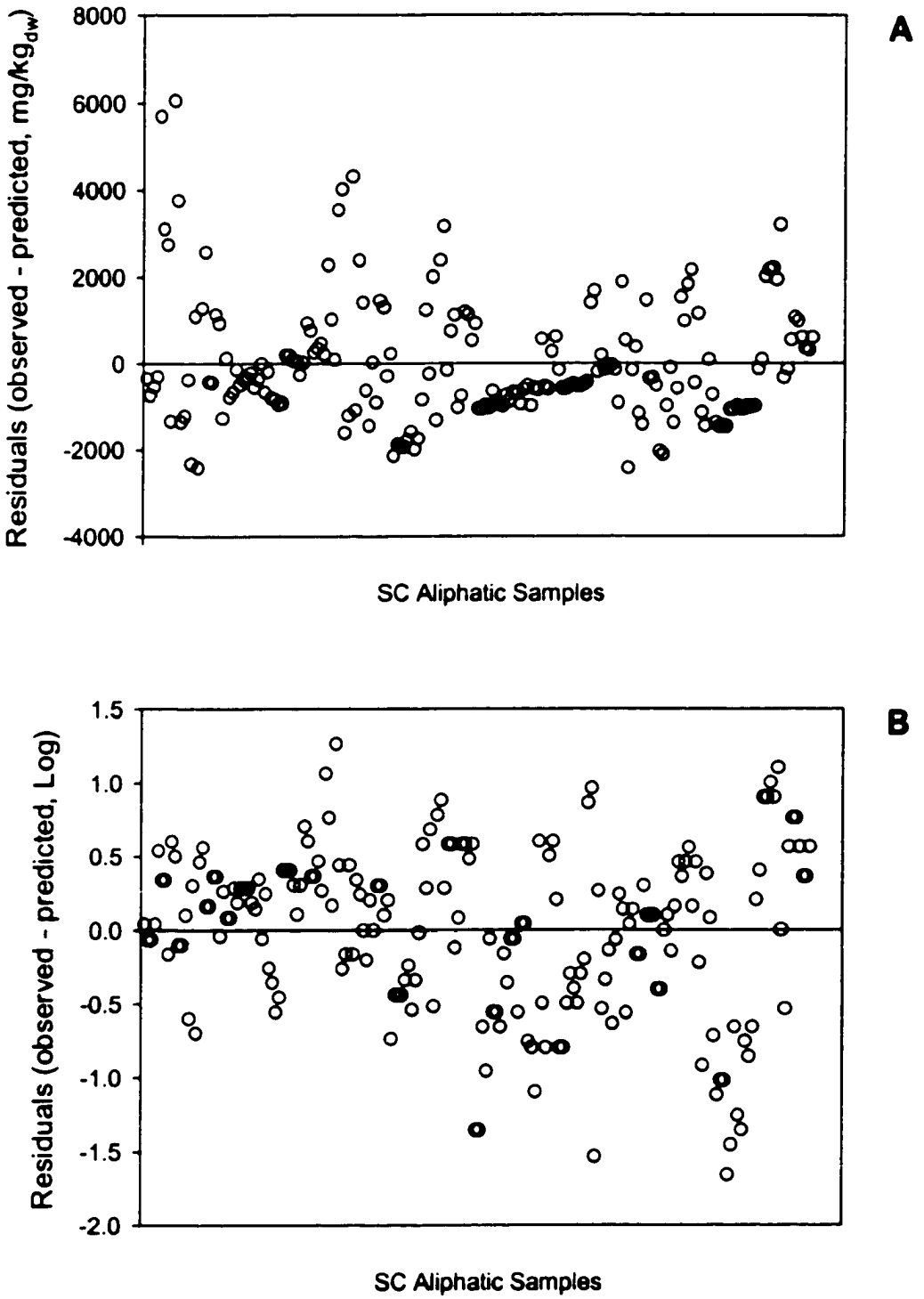


Figure 31. Residuals plots for the SC aliphatic fraction. A) arithmetic and B) log transformation.

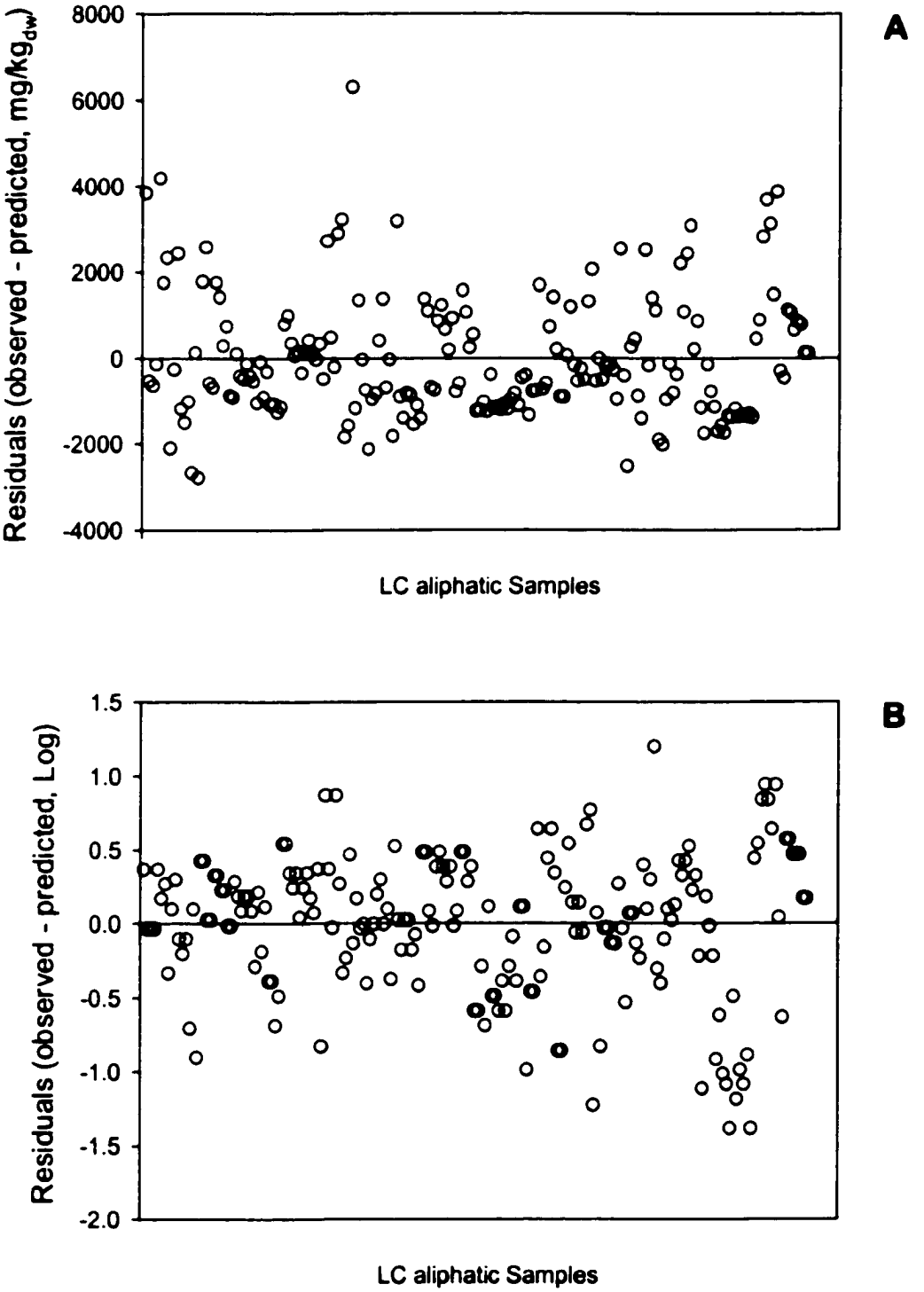


Figure 32. Residuals plots for the LC aliphatic. A) arithmetic and B) log transformation.

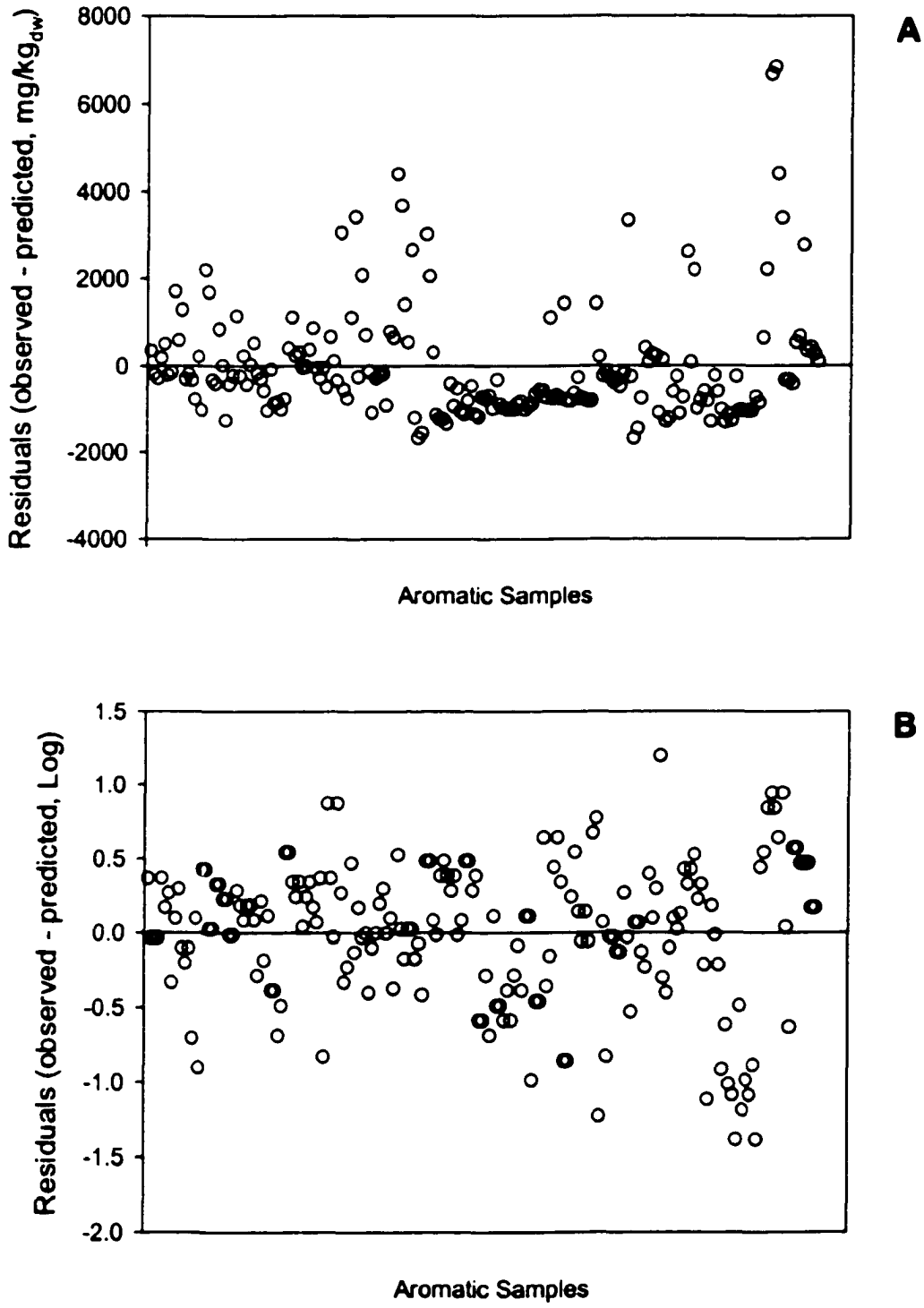


Figure 33. Residuals plots for the aromatic fraction. A) arithmetic and B) log transformation.

where: Y is the response variable (for the SC aliphatic, LC aliphatic and aromatic concentrations); μ is the theoretical overall mean of the response; Ai is the individual treatment (amendments or control); Bj(i) is the within treatment plot sample location (Subplots: a, b, c and d for this study); Dl is time (days); ADil is the interaction of the treatment effects with time; and E(i,j,l) is a random error associated with the i^{th} treatment, j^{th} sample location within the i^{th} treatment plot, and l^{th} time period.

In the analysis of the ACLM, the amendment (Ai), is the nominal factor and time is the continuous factor (covariate). The interaction of the two factors (ADil), is used to determine if the effect of time and degradation depend upon the specific amendment (air or NO_3^-). The within plot factor (Bj(i)) is used to test for the significance of the treatment effect (Ai). The JMP IN form of the ACLM is: treatment, time, treatment * time, and Plot[treatment]{random}.

To facilitate the interpretation of the analysis, the ACLM was reparameterized as an indicator variable regression model (IVRM). The ACLM and IVRM are statistically equivalent and either form can be used to perform the data analysis (Myers, 1990). The IVRM is developed by representing the levels of the treatments as a set of indicator variables (Z_i), which can only have values of 0 or 1. In general, the number of indicator variables required for the regression model is one less than the number of treatment levels. For the Fore River Creek salt marsh study, there were three treatments levels requiring two indicator variables (Z_1 and Z_2). If the treatment was air, then $Z_1 = 1$ and $Z_2 = 0$. If the treatment was NO_3^-, then $Z_1 = 0$ and $Z_2 = 1$. If the treatment was the control, then $Z_1 = 0$ and $Z_2 = 0$. The IVRM produced a linear regression model describing the

relationship of each treatment (amendment) level to the responses of the SC and LC aliphatics and aromatics.

The IVRM, containing interaction terms between time and the amendment, was:

$$\text{Log } Y = \mu + \beta_1 Z_1 + \beta_2 Z_2 + \beta_3 \text{time}^* Z_1 + \beta_4 \text{time}^* Z_2 + \beta_5 \text{time}$$

where: Y is the response variable (SC aliphatics, LC aliphatics or aromatics); μ is the intercept of the regression model when $Z_1 = 0$ and $Z_2 = 0$, which is the control treatment level; Z_1 and Z_2 are the indicator variables (1 for the amendments and 0 for the control) (air = 1,0) (nitrate = 0,1) (control = 0,0); $\beta_{1,2}$ shift the intercepts of the simple linear regression models for air and NO_3^-, respectively; β_5 is the slope of time for the control; and $\beta_{3,4,}$ are the adjustment for the slope of each specific treatment . The time factor was the main parameter that considered the natural attenuation of the TPH in the Fore River Creek salt marsh.

After replacing the indicator variables (Z values) with their settings for each treatment, the specific models were:

Air Amendment model:

$$\text{Log } Y = (\mu+\beta_1) + (\beta_3+\beta_5)\text{time},$$

NO_3^- Amendment model:

$$\text{Log } Y = (\mu+\beta_2) + (\beta_4+\beta_5)\text{time, and}$$

Control model:

$$\text{Log } Y = \mu + \beta_5\text{time}.$$

The regression model provided an easy form of direct comparison of the air and NO_3^- amendments to the control. The IVRM provided a statistically-based test of significance for each of the terms in the model.

The hypothesis during the study was that the amendments (NO_3^-, air) would enhance the *in situ* bioremediation of the TPH fractions in the oil-contaminated Fore River Creek salt marsh sediments during the period of the study relative to the natural attenuation of the fractions occurring in the control (no amendment). A more negative slope for the model relative to the control indicated greater degradation of the TPH fractions during the study.

The output displayed the probability that one of the variables (air, NO_3^- or control) used in the model could have a significant effect over the TPH degradation. For this study, it was assumed that a $p \leq 0.2$ meant that the variables (treatments) were significant. A p of this size is often used during biological studies (Simonton, 1998) because of the high variability observed in biological systems and in the distribution of the TPH fractions in sediments.

The subplot term (B) was not considered in the IVRM because it was negligible indicating that the subplot sampled (a, b, c, or d) did not have a significant effect during the study. Interestingly, the replicates taken in each subplot (aa, ab) generally had a greater variability that the subplot sampled. This could indicate that the *in situ* variability (e.g., caused by presence of oil droplets) was greater within subplots than between subplots.

4.5 TPH Results

4.5.1 Seasonal Variability

This study was conducted over one year to observe the effect of the seasons on the biodegradation of TPH in the Fore River Creek salt marsh sediments. The horizontal well installation and first sampling events were conducted during Summer and Fall 1998 (June-October) and the second sampling period covered the Spring 1999 (April-early June). The TPH results are presented in Section 4.5 and discussed in Section 4.6. The data are contained in Appendix C.

4.5.2 Summer and Fall 1998

For visual purposes, the TPH fractions were plotted as simple regressions without considering the amendment-time effect (Figures 34-37).

During Summer and Fall 1998, the IVRM showed that there was significant biodegradation of all three TPH fractions in the control plot (Table 25). The negative slopes indicated that there was a reduction in the concentrations during this season.

Table 25. Treatment slopes and probabilities obtained using the IVRM during Summer and Fall 1998.

Treatment	Treatment Slopes ($1x10^{-3}$)			p value		
	Log SC	Log LC	Log Aromatics	Log SC	Log LC	Log Aromatics
Control	-2.190	-1.886	-7.245	**0.1097**	**0.1137**	**< .0001**
Air	-4.338	-0.608	-2.268	**0.1979**	0.5805	**0.1257**
NO_3^-	-10.422	-5.274	-7.647	**0.0189**	**0.0117**	0.5356

Shading indicates a significant $p \leq 0.20$

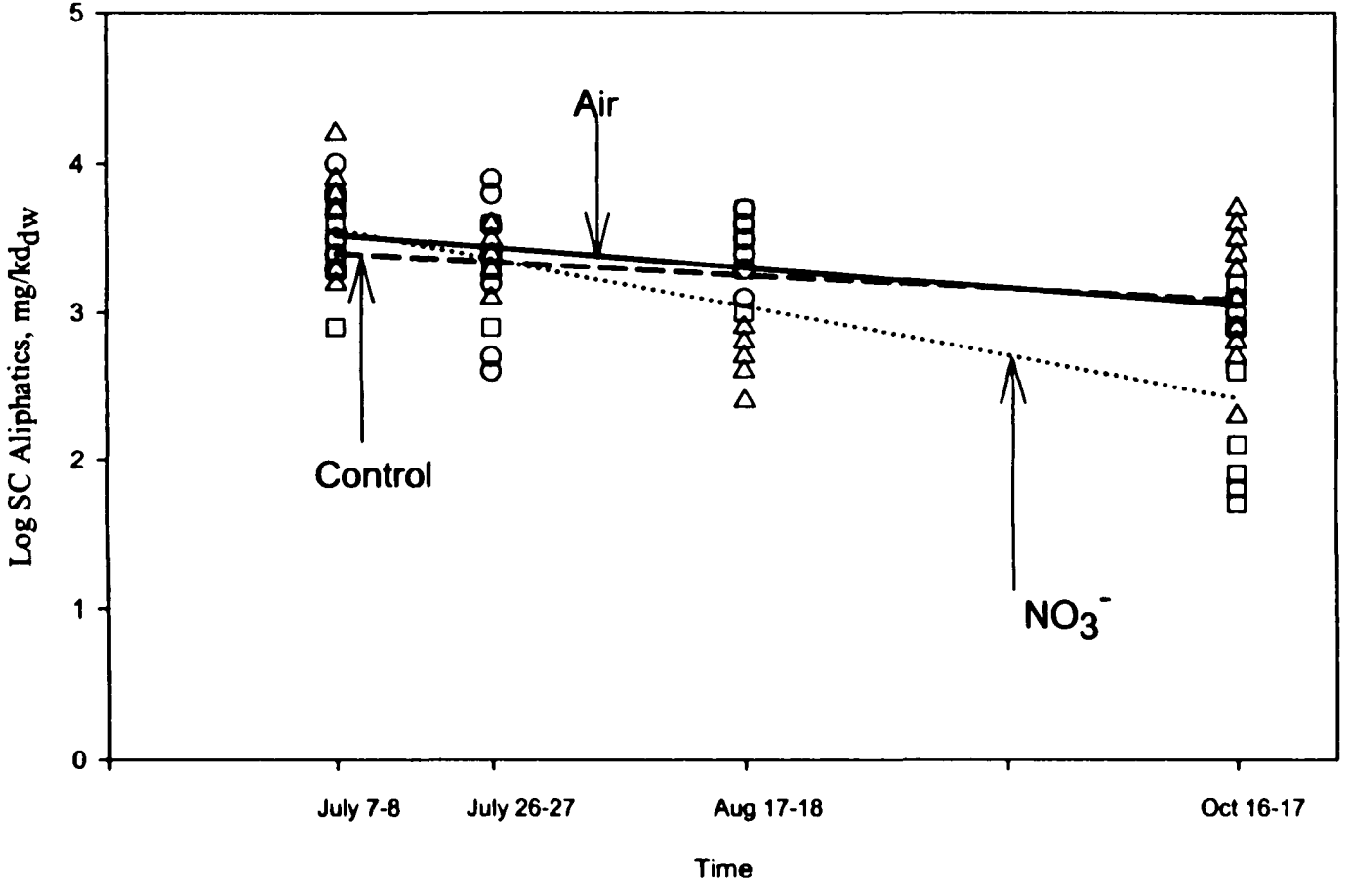


Figure 34. SC aliphatic concentrations during the Summer and Fall 1998. For visual comparison only. Does not include amendment-time interaction effects. Δ=Control, O=Air, 0=NO_3^-

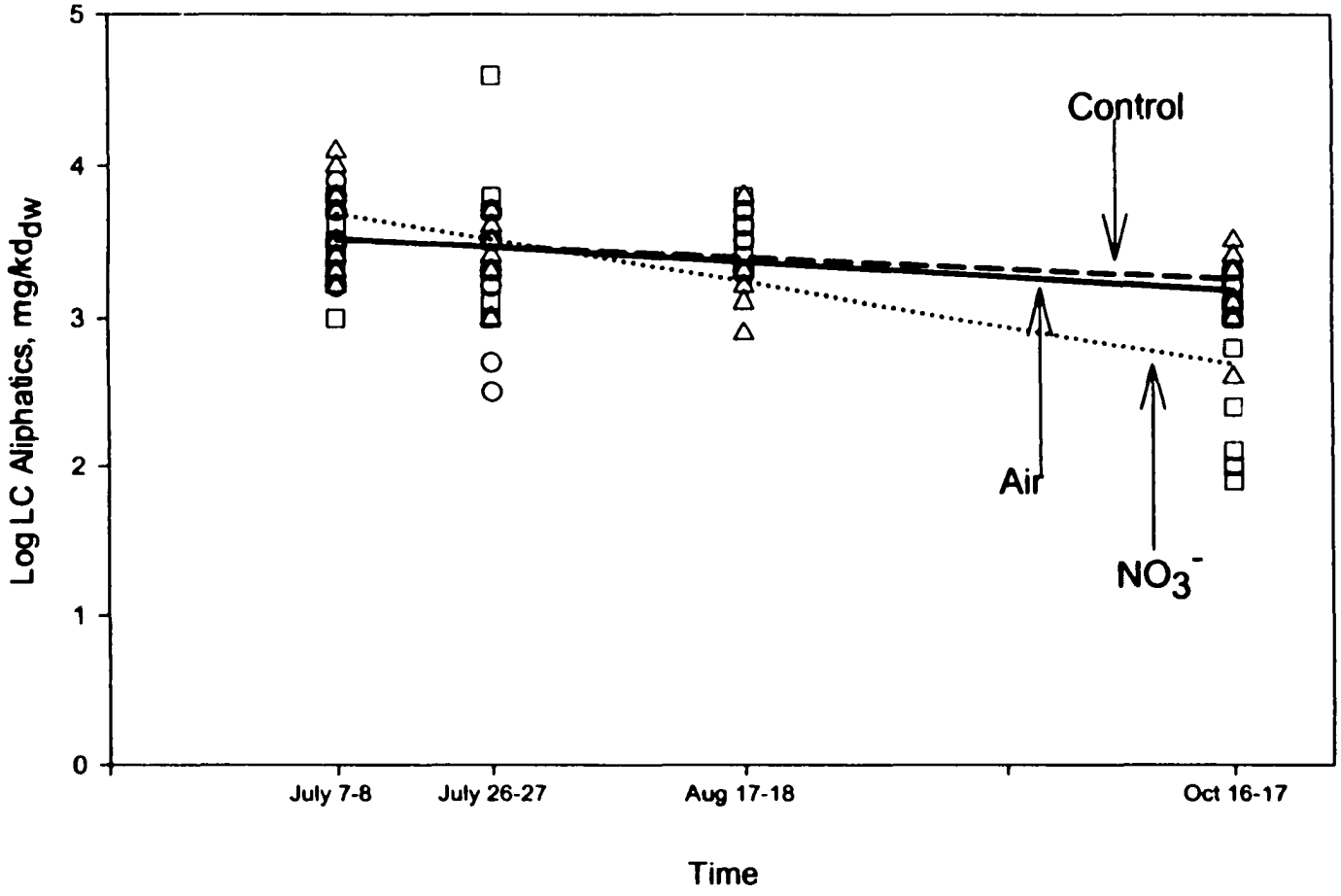


Figure 35. LC aliphatic concentrations during the Summer and Fall 1998. For visual comparison only. Does not include amendment-time interaction effects. Δ=Control, O=Air, □=NO_3^-

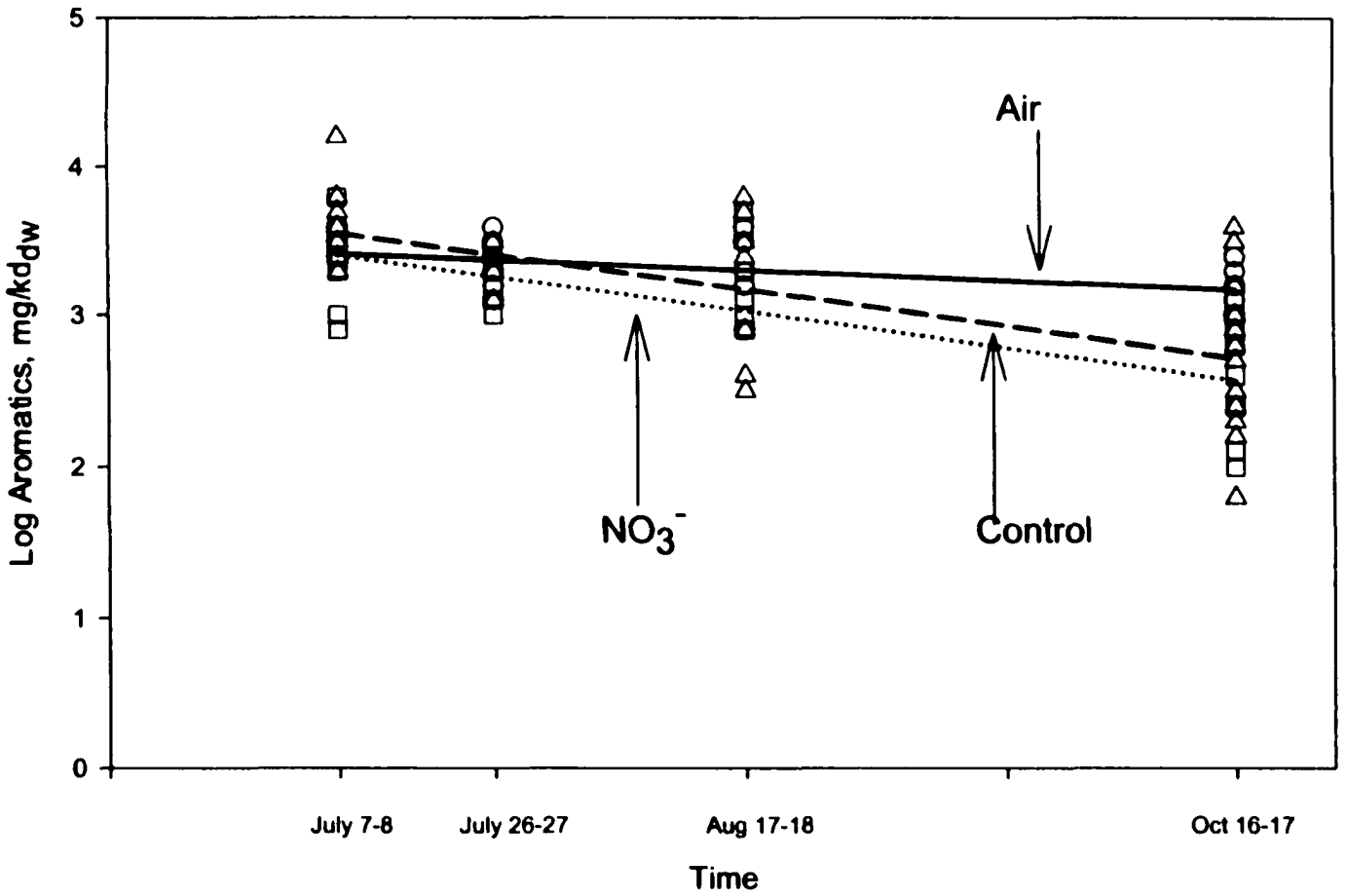


Figure 36. Aromatics concentrations during the Summer and Fall 1998. For visual comparison only. Does not include amendment-time interaction effects. Δ=Control, O=Air, □=NO_3^-

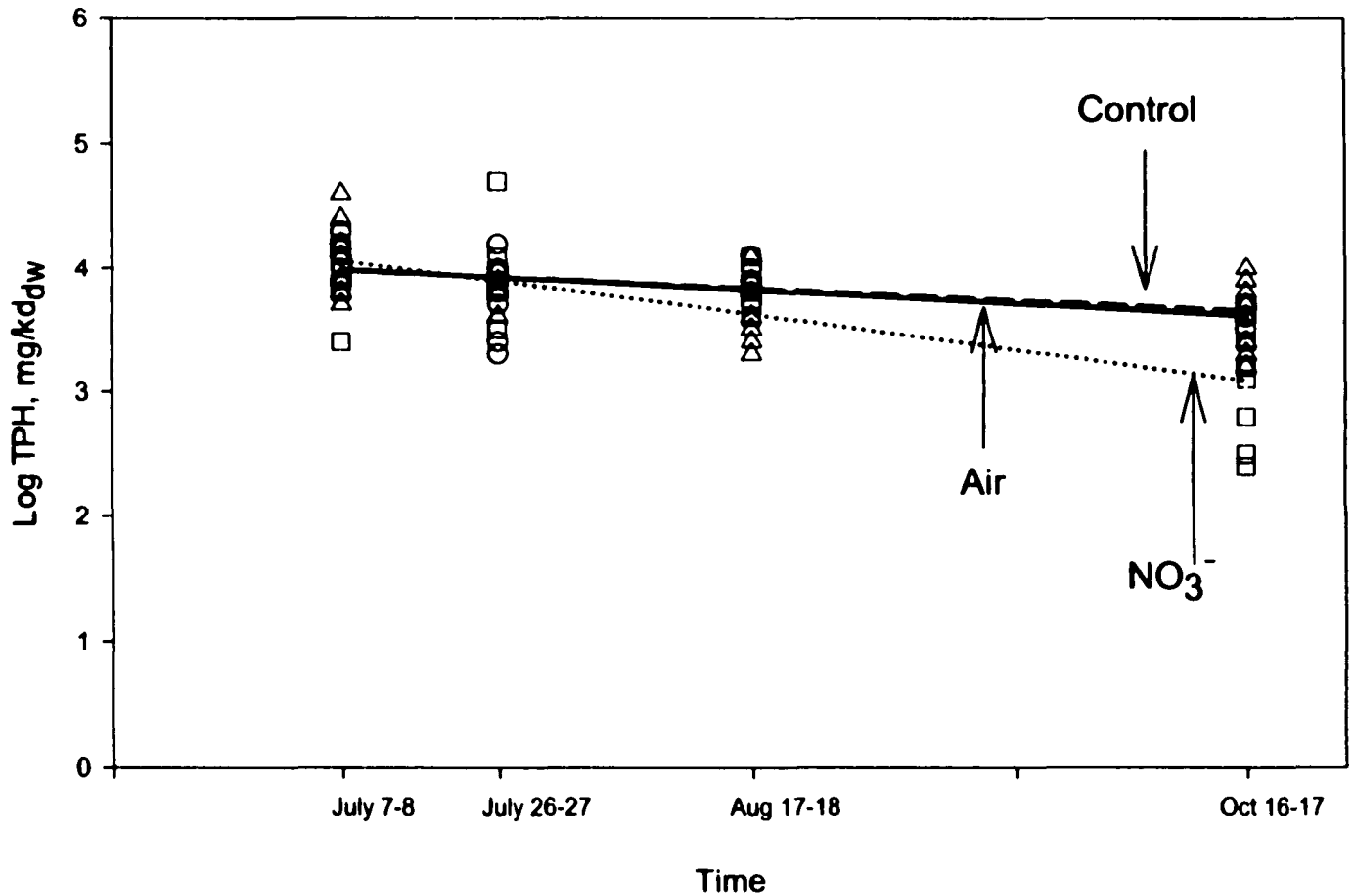


Figure 37. TPH concentrations during the Summer and Fall 1998. For visual comparison only. Does not include amendment-time interaction effects. Δ=Control, O=Air, □=NO_3^-.

The main remediation process causing the natural attenuation of the TPH in the control plot was probably biodegradation (microbial metabolism of the TPH fractions) because ~19 months after of the *Julie N* spill, most of the volatile TPH compounds were likely gone and weathering (e.g., volatilization) had already occurred. It is possible that some dilution and losses by tidal and wave action were occurring. The extend of this could be ascertained by comparing losses over the summer season vs. the winter months when TPH biodegradation should have been limited by the cold temperatures.

In the air plot, there was significant biodegradation of the SC aliphatics and aromatic fractions. The slope for the SC aliphatics was more negative than the control slope for this fraction indicating that the addition of air enhanced removal of these aliphatics compared to natural attenuation alone (i.e., the control). It was unlikely that the TPH removal was due to air stripping because the air flow used was low (3.2 m^3/h) and intended to achieve biodegradation not volatilization. The horizontal well network was designed to promote bioventing in contrast to a soil vapor extraction (SVE) system where the flow rates are optimized for volatilization (Lesson and Hinchee, 1996). The NO_3^- plot showed a significant degradation of the aliphatic fractions (SC and LC) compared to the control (i.e., $p < 0.20$ and slope more negative than control). The IVRM also showed that the control (natural attenuation) was more effective at reducing the aromatic concentrations than either of the amendments.

4.5.3 April-June 1999

Again, for visual purposes the TPH fractions were plotted as simple regressions without considering the amendment-time effect (Figures 38-41). During this season, the slopes for all of the treatments were positive (Table 26). However, only the p values for

the NO_3^- plot were significant ($p< 0.20$). The data indicated that no TPH degradation was occurring during this season in any of the treatment plots. To observe if any sampling event was driving these observations, the NO_3^- data (SC and LC aliphatics and aromatics) for the 1999 season were compared using a Tukey-Kramer test ($p<0.005$). The analysis indicated that the mean concentrations for the SC and LC aliphatics were significantly lower for the April 1999 event. During this event, the TPH concentrations in the control and air plots for all fractions were somewhat lower than those in May and June 1999 (Figures 42-44). However, in the NO_3^- plot, the April concentrations were very low and the May concentrations were high. This accentuated the trend of increasing TPH and could have been caused by sampling subplots that had very low concentrations of TPH during April and higher TPH during the May and June events (e.g., contamination pockets).

Table 26. Treatment slopes and probabilities obtained using the IVRM during Spring 1999.

Treatment	Treatment Slopes ($1x10^{-3}$)			p value		
	Log SC	Log LC	Log Aromatics	Log SC	Log LC	Log Aromatics
Control	+0.718	+1.644	+0.843	0.8454	0.6364	0.8127
Air	+6.306	+3.949	+3.199	0.4947	0.7601	0.8306
NO_3^-	+19.496	+17.896	+12.388	**0.0024**	**0.0038**	**0.0695**

Shading indicates a significant $p \leq 0.20$

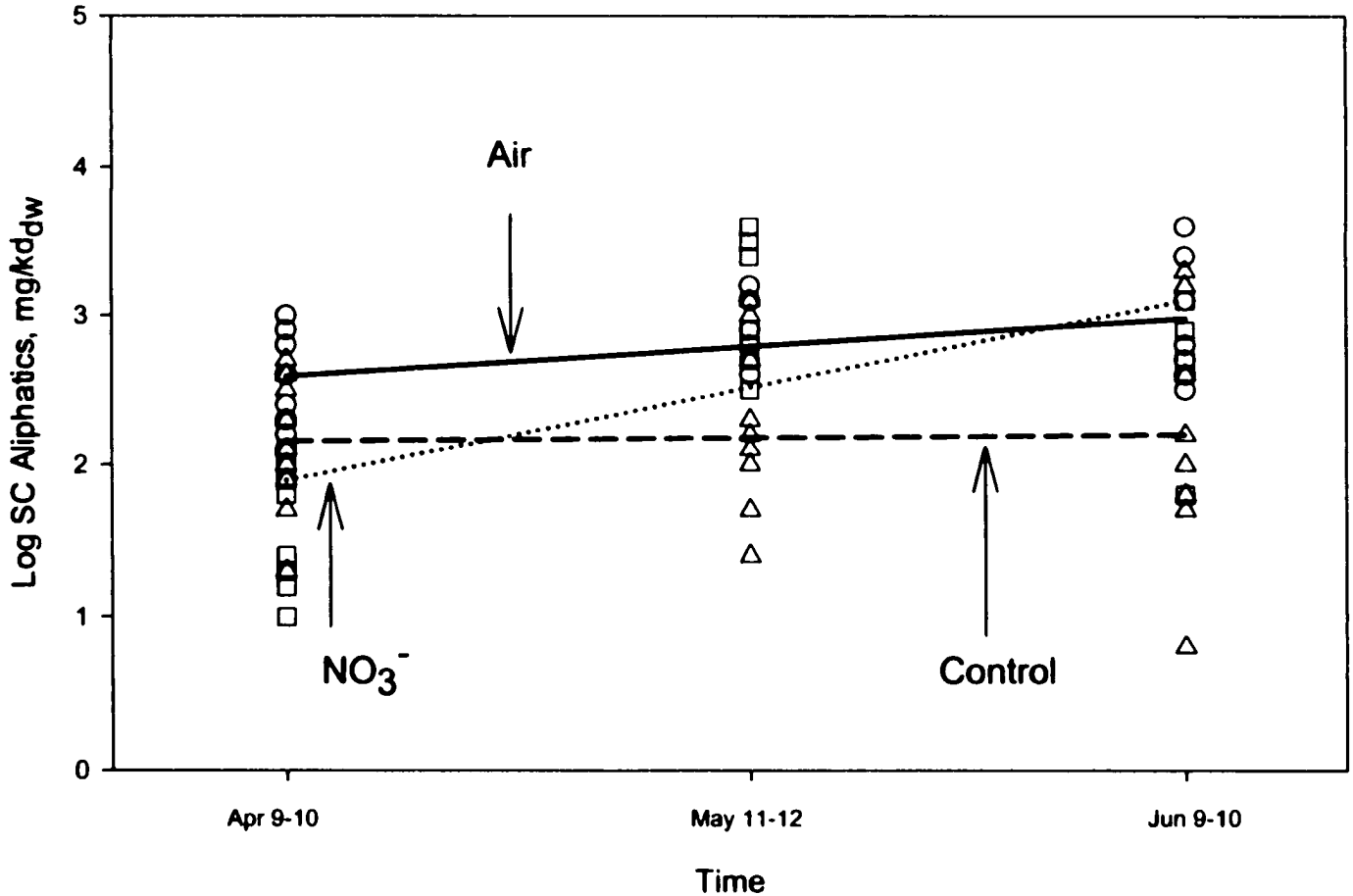


Figure 38. SC aliphatic concentrations during the Spring 1999. For visual comparison only. Does not include amendment-time interaction effects. Δ=Control, O=Air, □=NO_3^-

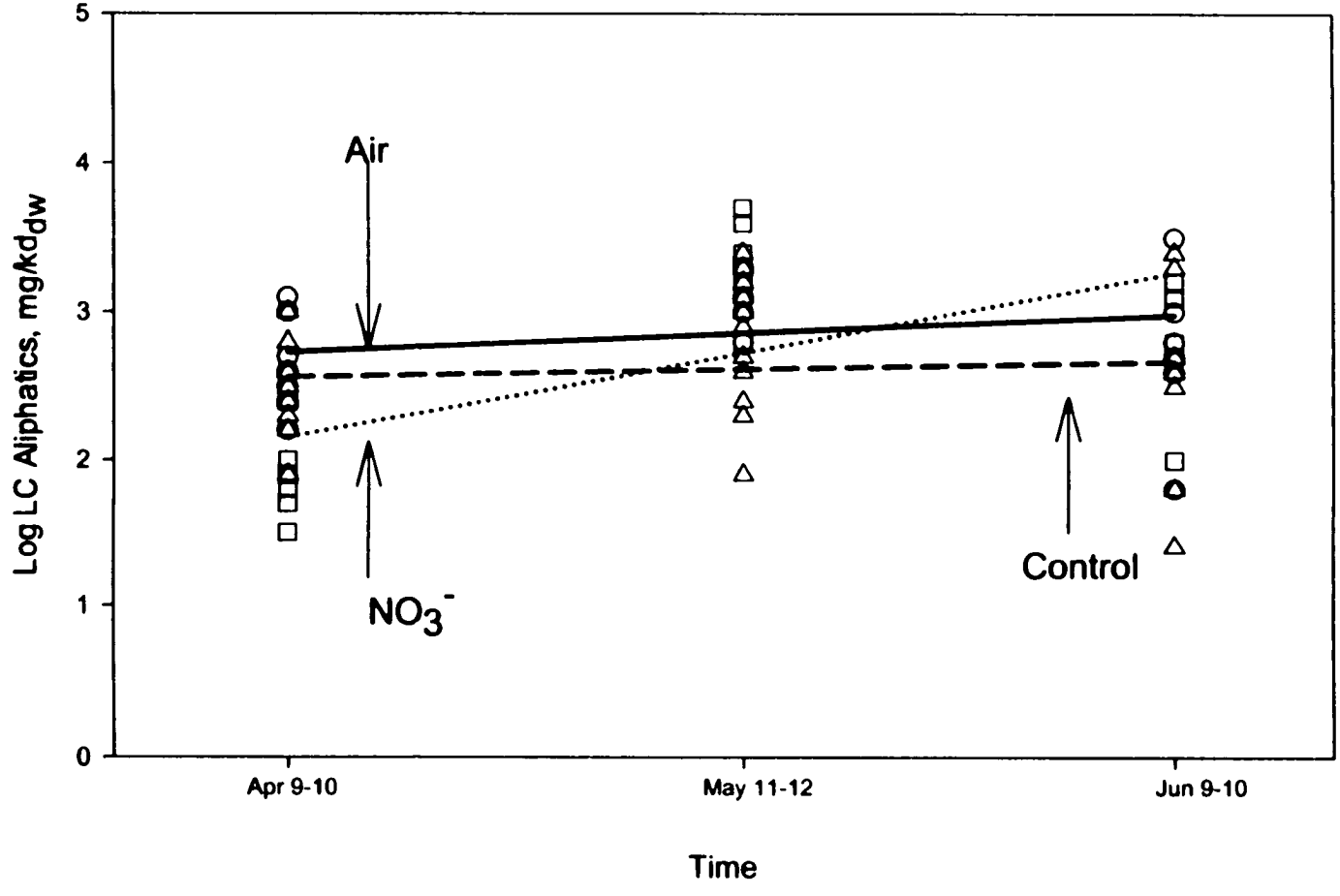


Figure 39. LC aliphatic concentrations during the Spring 1999. For visual comparison only. Does not include amendment-time interaction effects. Δ=Control, O=Air, □=NO_3^-

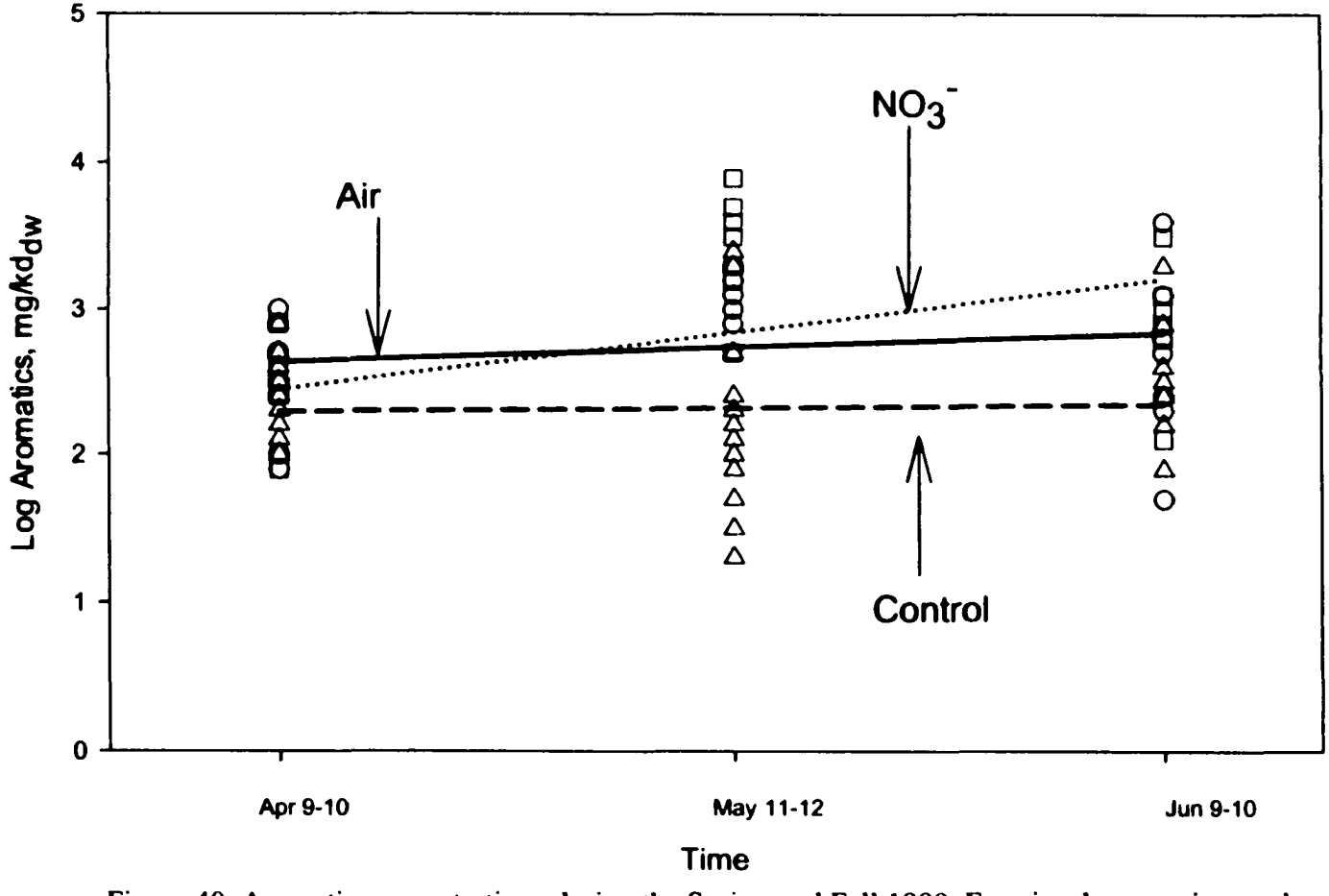


Figure 40. Aromatic concentrations during the Spring and Fall 1999. For visual comparison only. Does not include amendment-time interaction effects. Δ=Control, O=Air, □=NO_3^-

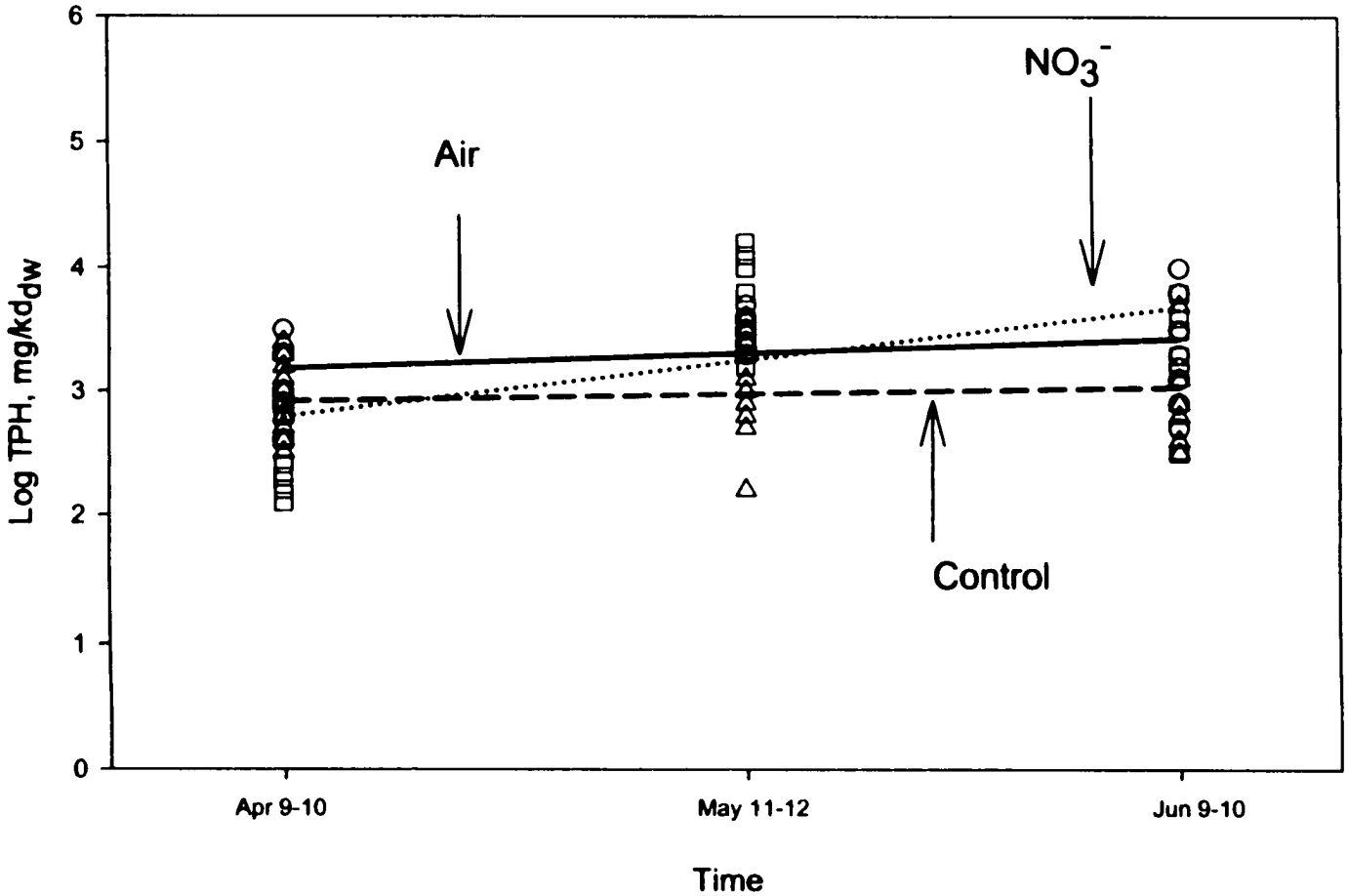


Figure 41. TPH concentrations during the Spring 1999. For visual comparison only.
Does not include amendment-time interaction effects. Δ=Control, O=Air, □=NO_3^-

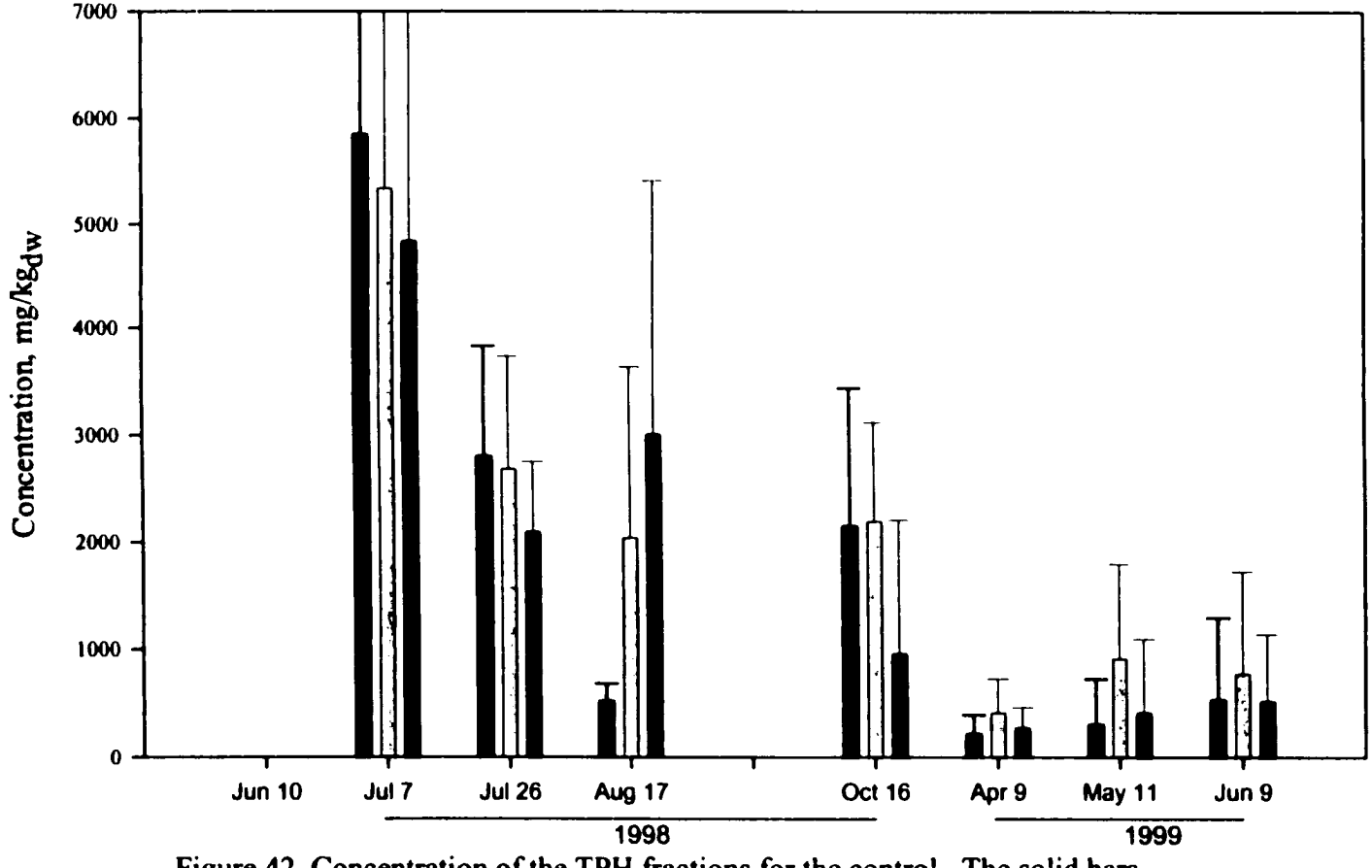


Figure 42. Concentration of the TPH fractions for the control. The solid bars are the mean of eight samples and the error bars represent one standard deviation.

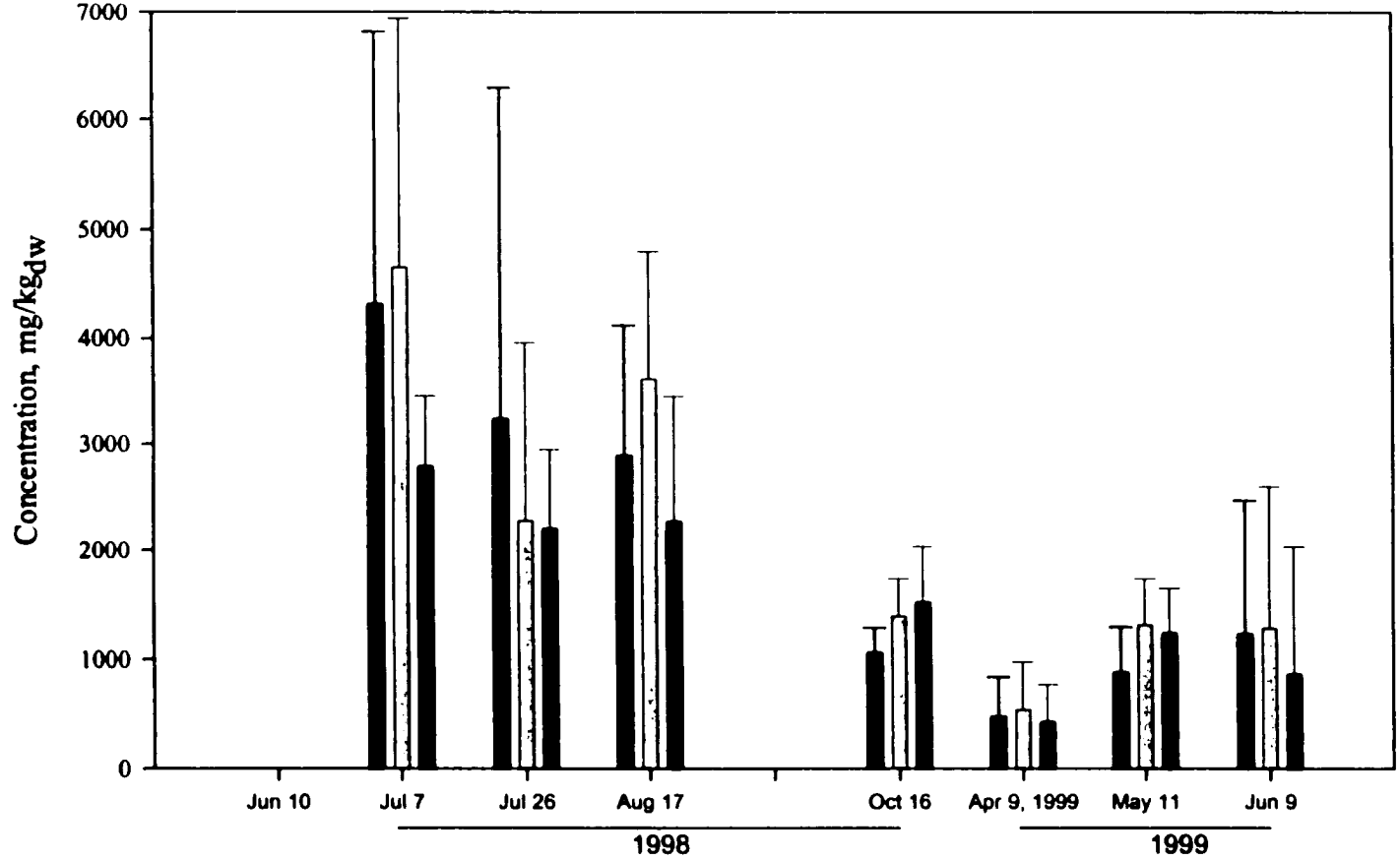


Figure 43. Concentration of the TPH fractions for the air treatment. The solid bars are the mean of eight samples and the error bars represent one standard deviation.

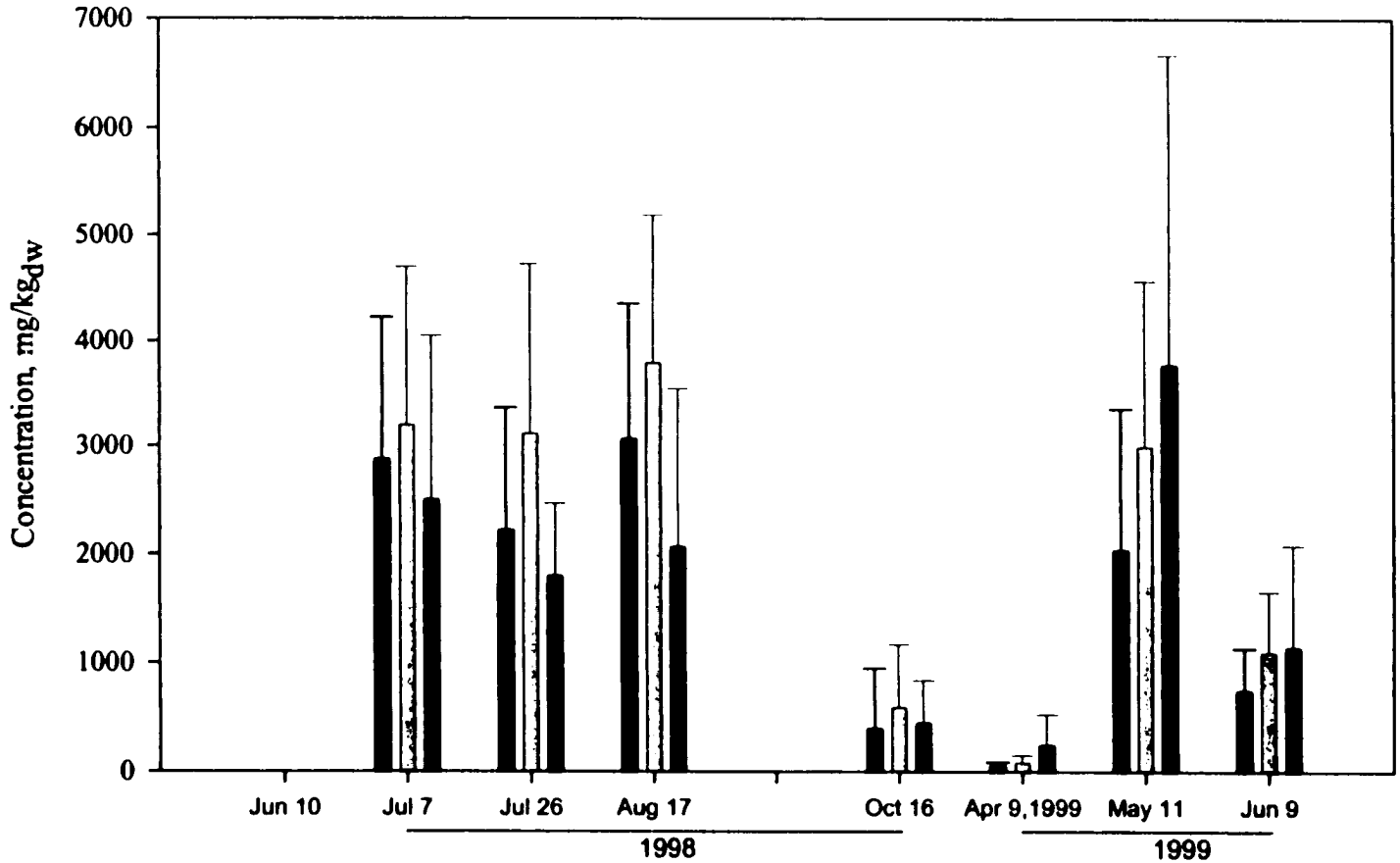


Figure 44. Concentration of the TPH fractions for the NO_3^- treatment. The solid bars are the mean of eight samples and the error bars represent one standard deviation.

4.5.4 Overall Results (June 1998-June 1999)

Again for visual purposes, the TPH fractions were plotted as simple regressions without considering the amendment-time effect (Figures 45-48). Over the one year period, natural attenuation in the control plot significantly reduced all TPH fractions (Table 27). There was no significant decrease in the air plot. The SC aliphatic and aromatic concentrations significantly decreased in the NO_3^- plot, but the removals were not greater than the control. The Spring 1999 data for the air and NO_3^- plot, where the concentrations in May were high, greatly influenced the finding that there was no decrease in TPH for the amendments during the one year period.

Table 27. Treatment slopes and probabilities obtained using the IVRM during 1998-1999.

Treatment	Treatment Slopes ($1x10^{-3}$)			p value		
	Log SC	Log LC	Log Aromatics	Log SC	Log LC	Log Aromatics
Control	-3.763	-2.683	-3.122	**<0.0001**	**<0.0001**	**<0.0001**
Air	-1.922	-2.103	-1.979	0.7769	0.6520	0.6215
NO_3^-	-2.197	-2.388	-0.973	**0.1280**	0.3557	**0.1055**

Shading indicates a significant $p \leq 0.20$

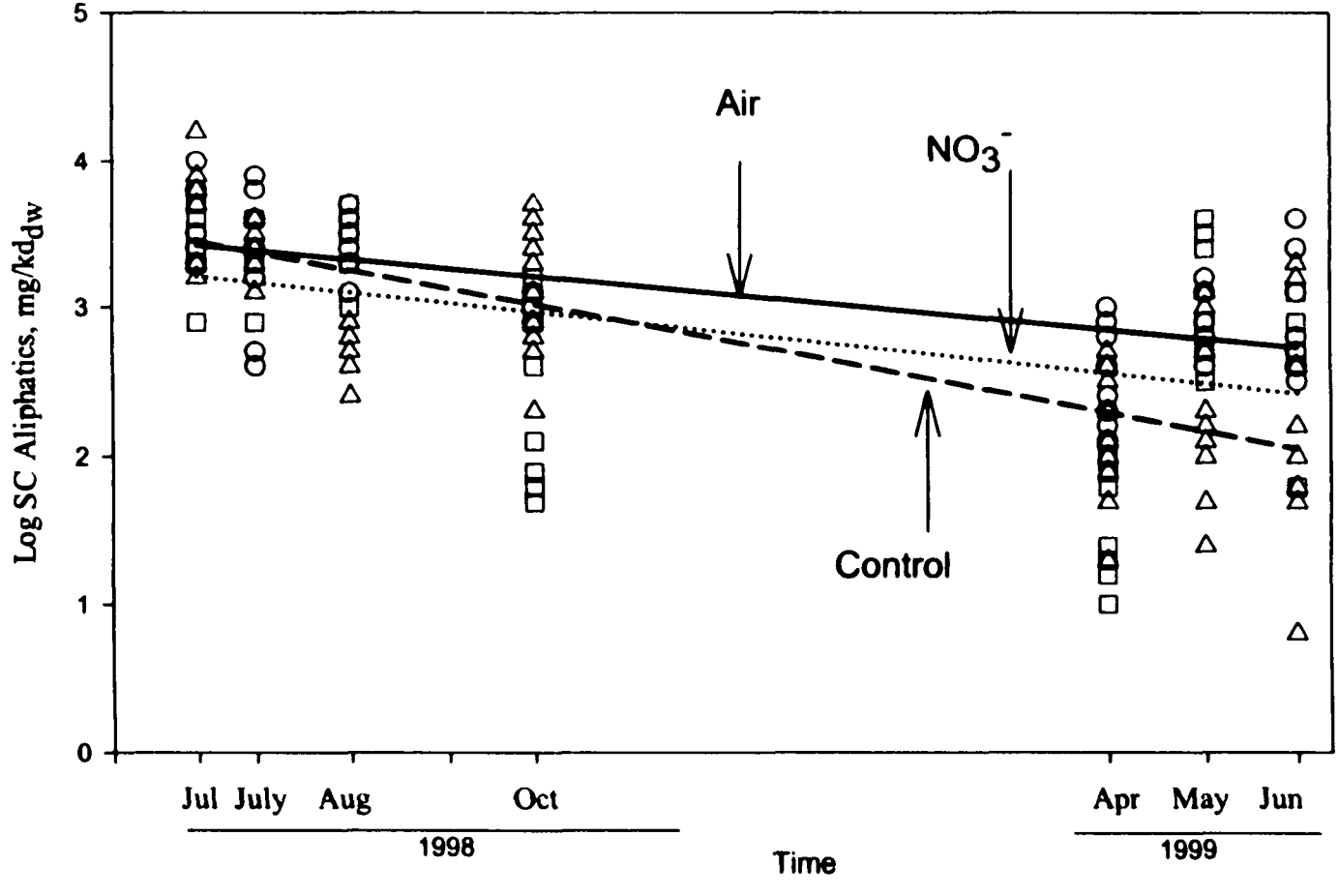


Figure 45. SC aliphatics concentrations during the study. For visual comparisons only.

Does not include amendment-time interaction effects. Δ=Control, O=Air, □=NO_3^-

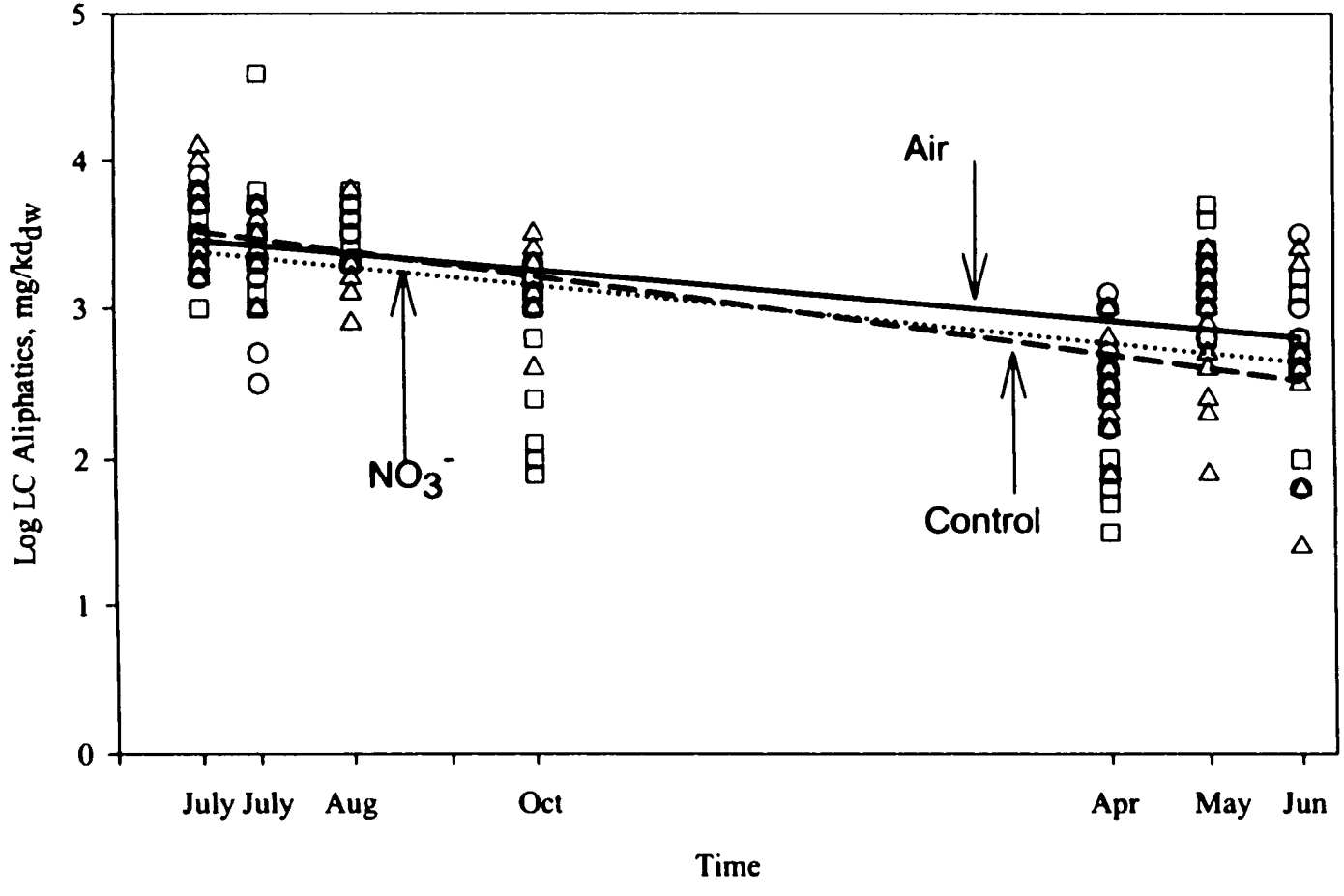


Figure 46. LC aliphatic concentrations during the study. For visual comparisons only.

Does not include amendment-time interaction effects. Δ=Control, O=Air, □=NO_3^-

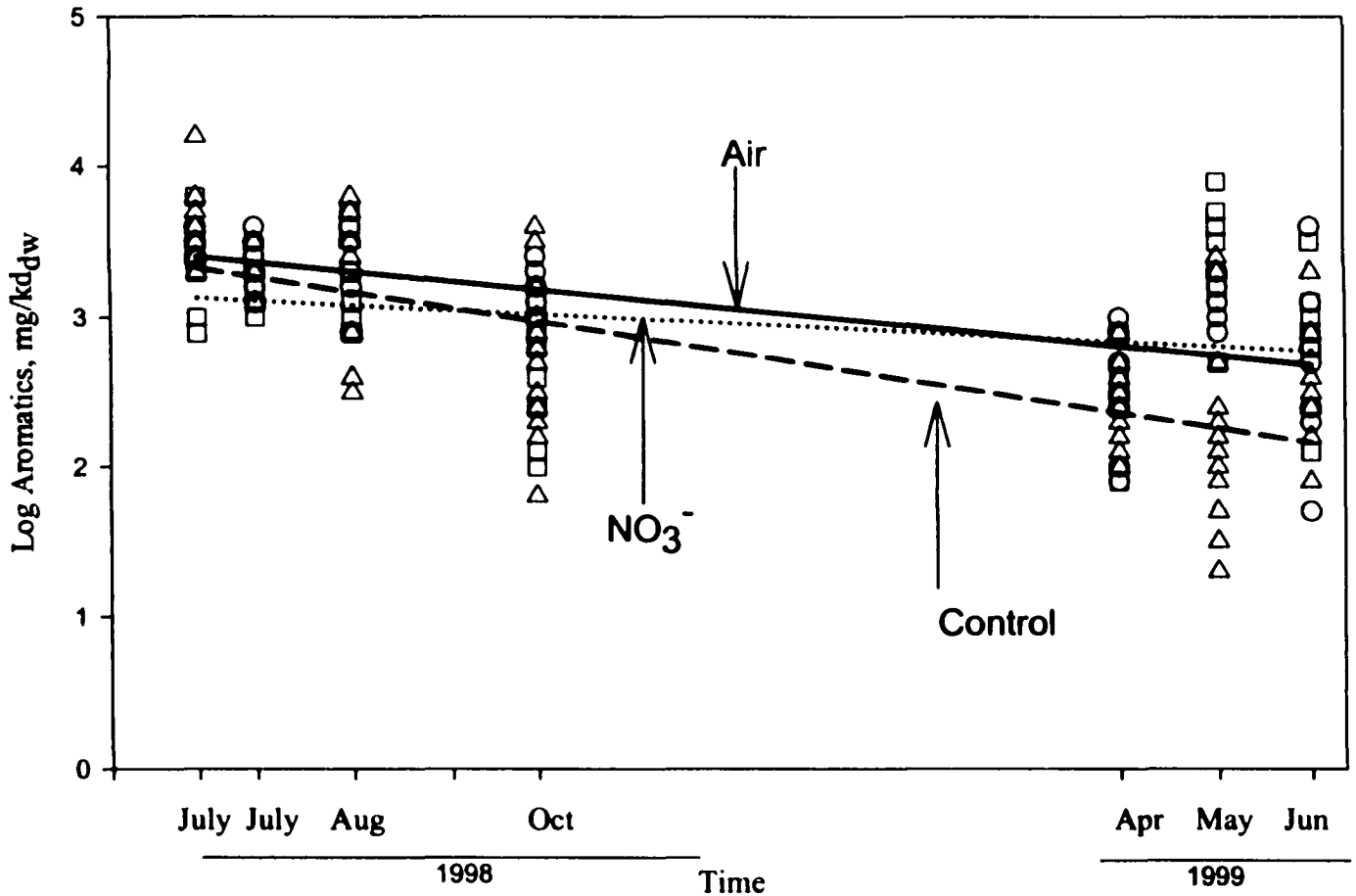


Figure 47. Aromatic concentrations during the study. For visual comparisons only.

Does not include amendment-time interaction effects. Δ=Control, O=Air, □=NO_3^-

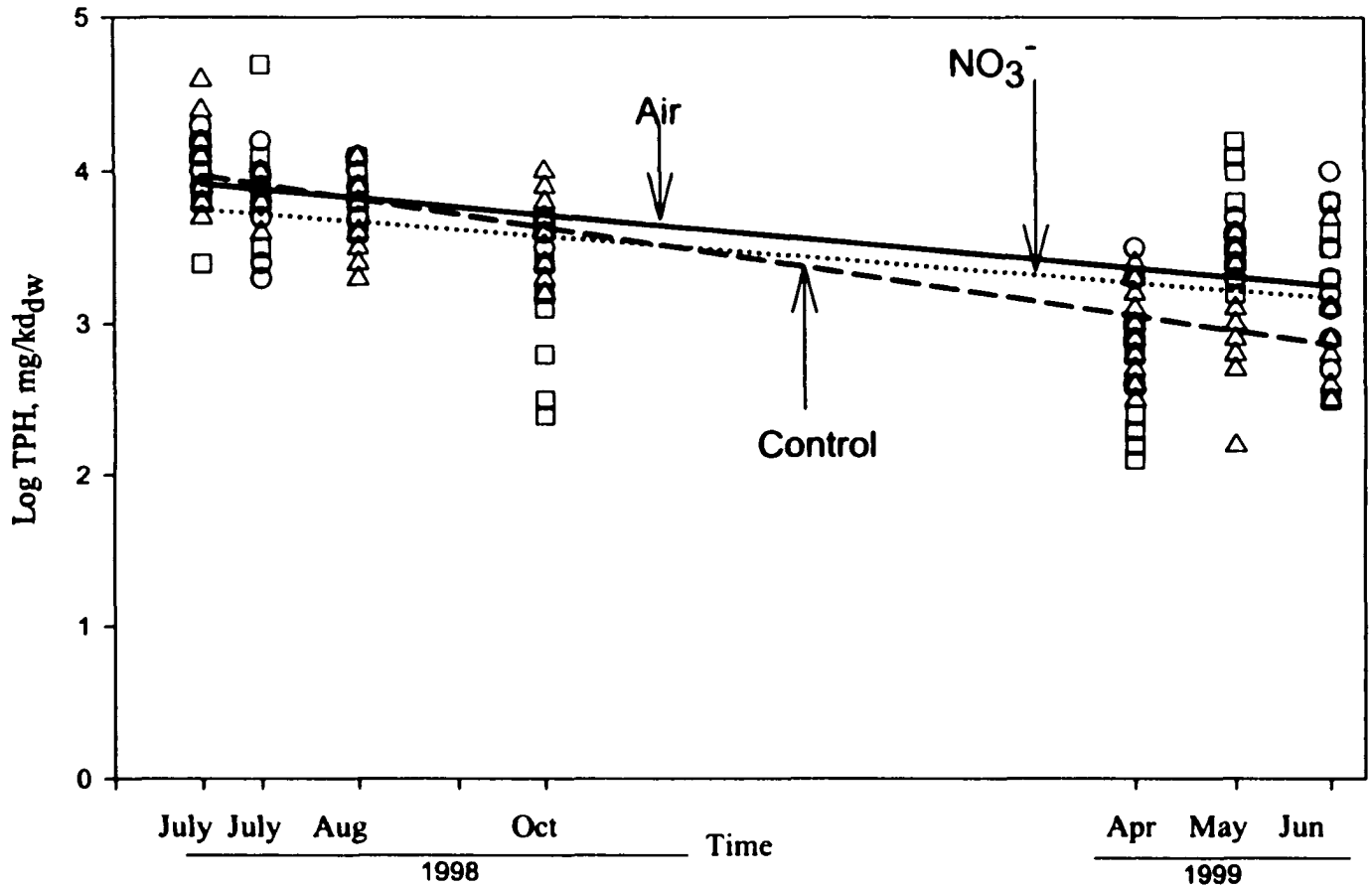


Figure 48. TPH concentrations during the study. For visual comparisons only.

Does not include amendment-time interaction effects. Δ=Control, O=Air, □=NO_3^-

4.6 TPH Biodegradation

4.6.1 Aliphatic Biodegradation

The SC and LC aliphatics are C_9 to C_{18} and C_{19} to C_{36} HCs, respectively. They may be saturated (alkanes) or unsaturated (alkenes or alkynes) and straight chain, simply branched or highly branched molecules. They have limited solubility (i.e., they are hydrophobic) because they are composed entirely of carbon-carbon and carbon-hydrogen linkages (Watkinson and Morgan, 1990). The solubility of aliphatics rapidly decreases with increasing molecular weight. Hence, SC aliphatics are more water soluble than LC aliphatics. A wide variety of bacteria, filamentous fungi and yeast can metabolize aliphatics, but biodegradation is hampered by their bioavailability (i.e., their limited solubility in water and high sorptive capacity) (Atlas, 1975).

During 1998 and the overall study, the control plot had a significant decrease of all TPH fractions (Table 28) indicating that natural attenuation was occurring. Natural attenuation is the combination of the physical, chemical and biological processes that reduce TPH concentrations in the environment. The main natural attenuation processes in a salt marsh are biodegradation, volatilization, dissolution/dilution by wave and tidal action, sediment removal by ice, and tidal forces. Volatilization generally occurs when the oil spreads on the water during the first hours of a spill. The spreading process increases the surface area of the spill enhancing mass transfer via evaporation (Zhu *et al.*, 2001). Because the *Julie N* spill occurred ~19 months before the start of this study and evaporation removes the majority of alkanes smaller than C_{15} within one to ten days after a spill, volatilization was probably not responsible for the decrease in TPH observed in

the control plot during the study. However, some volatile components may have persisted longer because the oil had permeated into the sediments.

Because the Fore River Creek salt marsh is a low energy system, dissolution of the TPH by waves was probably not a significant factor in TPH removal. Dissolution into the tidal waters could have affected the TPH concentration and would have been more significant during the warmer months of June to September than October, April and May.

Table 28. Treatments and their significant reduction in TPH fractions.

Treatment	June-October 1998	April-June 1999	June 1998-June 1999
Control	SC, LC, aromatics	-	SC, LC, aromatics
Air	SC, aromatics	-	-
NO_3^-	SC, LC	-	SC, aromatics

Some TPH loss in the Fore River Creek marsh could have been caused by the redistribution of the sediments. However, it was difficult to assess this impact because at the same time that some TPH was removed from the control plot other TPH could have entered. A detailed hydraulic and sediment analysis would need to be conducted to determine the extend of sediment redistribution in the marsh. Although it was in a different environment, Venosa *et al.* (1996) reported that sand exchange had no effect on the observed biodegradation rates in the control plot under a controlled oil (Bonny Light) spill on the shoreline of Delaware Bay. However, sand has low sorptive capacity compared to the salt marsh sediments. During the Fore River Creek study, ice movement during the winter may have resulted in losses of TPH because there was a decrease in the

TPH concentration during the winter in the control plot, and during this period, cold temperatures probably reduced microbial metabolism.

It is likely that biodegradation accounted for some, if not all, of the TPH removal observed in the control plot. Biodegradation of No. 2 fuel oil has been observed in natural systems by many researchers (Carman *et al.*, 1996; Hess *et al.*, 1996; London and Robinson, 1984; Piehler and Paerl, 1996; Reynolds *et al.*, 1997; Simonton, 1998; Sorini *et al.*, 1997; Suarez and Rifai, 1999; Walker *et al.*, 1975; Yang *et al.*, 2000). Generally, the SC aliphatics are the first TPH fraction to be biodegraded because they are the most bioavailable. Until recently, it was thought that biodegradation of aliphatics and aromatics could only be initiated under aerobic conditions since oxygenase reactions appear to be necessary for the initial metabolic activation of the molecule to be degraded (Watkinson and Morgan, 1990). However, studies have now demonstrated the degradation of TPH under microaerophilic (NO_3^-) and sulfate-reducing conditions (Coates *et al.*, 1997; Rueter *et al.*, 1994).

Since the *Julie N* spill occurred almost ~19 months before the bioremediation study began, there is a high probability that microorganisms capable of degrading TPH and related compounds were present in the sediments because they are ubiquitous in marine and soil environments (Zhu *et al.*, 2001). The most readily available TEA in the marsh sediments of the control was SO_4^{-2}. The highest sulfate reduction rates have been observed in the top ~3 cm of salt marsh environments where often the concentration is ~ 2,000 mg SO_4^{-2}/L (Hines *et al.*, 1989; Shin *et al.*, 2000). Even if it were diluted 90% by freshwater coming into the marsh (e.g., Fore River Creek or runoff), the sulfate concentration would be more than adequate for reduction of the existing TPH. In

addition, the highest TPH concentrations were found in the top ~4 cm in the Fore River Creek salt marsh where sulfate reduction rates are typically the greatest (Hin *et al.*, 2000).

The other possible TEA for aliphatic biodegradation in the marsh sediment was oxygen. In the Fore River Creek marsh, *S. alterniflora* could have aerated the contaminated sediments through its roots (Rock, 1997). The extension of plant roots into the sediment also augments soil-surface contact serving as a natural ventilation system (Lin and Mendelssohn, 1998). Because aliphatics are more biodegradable than aromatics, the former would probably have been used first.

An important factor that probably was limiting the biodegradation of the aromatics and aliphatics in the marsh was the presence of clay or humic fractions in the sediment matrix. These materials make TPH compounds less bioavailable (Zhu *et al.*, 2001). Because TPH compounds are hydrophobic, they are often sorbed or trapped in the sediment pores by capillary action (Chayabutra and Ju, 2000). In addition, the low aqueous solubility of some of the compounds (e.g., naphthalene and phenanthrene) makes them less available in the water phase where microorganisms can access them (Carman *et al.*, 1996; Huesemann, 1995).

The addition of NO_3^- enhanced the biodegradation of the aliphatic fraction (SC and LC) during the 1998 season (vs. the control) (Table 28). Similar results have been described by Bachoon *et al.* (2001) in a Georgia salt marsh microcosms study where the addition of NO_3^- (as an inorganic nutrient) was the most effective treatment for the enhancement of TPH degradation (mainly alkanes and a lesser extend the aromatic fraction). The concentration of residual alkanes ($>C_{19}$) was reduced to less than the 10% of the residual values in the control treatment (Bachoon *et al.*, 2001). Other researchers

have also observed enhanced biodegradation of SC and LC aliphatics upon the addition of NO_3^- (Adrian *et al.*, 1998; Coates *et al.*, 1997; 1998). It is difficult to assess whether the NO_3^- was used as a TEA (dissimilatory pathway) and/or as a nutrient (nitrogen source; assimilatory pathway). The nitrogen concentration in the porewater of the Fore River Creek sediments (~ 2.0 mg N/L) (as NO_3^- and NH_4^+) could have served as a nutrient to support the TPH biodegradation based on stoichiometry (See Section 4.8). Porewater nitrogen concentrations of 1-2 mg N/L and 1.5 mg/L have been successful in maintaining TPH biodegradation on sandy beaches (Bragg *et al.*, 1994; Venosa *et al.*, 1996). However, these nitrogen concentrations would probably be too low for degrading all of the TPH in the Fore River Creek marsh because of the high sediment NOM content.

Air only enhanced the biodegradation of the SC aliphatics during the first season (Table 28). Oxygen is the TEA that provides the highest energy yield per molecule of TPH and aerobic conditions generally provide the fastest means of biodegradation. However, oxygen is a limiting factor for TPH biodegradation in most salt marshes (Zhu *et al.*, 2001). Few studies have been conducted adding oxygen as a TEA in salt marshes because of the technical difficulties and high cost of delivering it into the sediment. Adrian *et al.* (1998) studied aerated Louisiana marsh cores (15 cm diameter and 30 cm long) artificially-contaminated with weathered Louisiana crude oil and ^{14}C hexadecane. $^{14}CO_2$ production was monitored to assess biodegradation. ORC (Regenesis Corp), a patented material consisting mainly of magnesium peroxide and other chemicals, was used to deliver oxygen into the marsh sediments. ORC addition did not increase available oxygen in the porewater and hence did not increase TPH biodegradation.

A theoretical oxygen supply rate of 110 g/cm^2 was reported (Adrian *et al.*, 1998) to be necessary in the marsh, which exceeds any reasonable application rate.

One of the possible reasons why the air treatment did not have a more positive effect during this study may be because in a salt marsh, sulfate reduction is the dominant metabolic process and accounts for >50% the total decomposition of NOM (Rooney-Varga *et al.*, 1997). This sulfate-reducing activity is closely related to plant growth and microbial metabolism suggesting that plant-SRB interaction in the *S. alterniflora* rhizosphere plays an important role in salt marsh biogeochemical cycles (Coates *et al.*, 1996; Hin *et al.*, 2000; Hines *et al.*, 1989; Rooney-Varga *et al.*, 1997). The oxidation of reduced sulfides present in this environment and the aerobic respiration of NOM are the major oxygen sinks in a salt marsh (Shin *et al.*, 2000). This could be a potential problem when adding oxygen as TEA in a marsh. Probably the air injected did not provide enough oxygen to sustain aerobic TPH biodegradation as well as meet the demand resulting from the oxidation of natural compounds (e.g., sulfides and NOM).

During the 1999 season, the TPH concentrations appeared to decrease and then increase in all plots. This could have occurred because of losses (e.g., ice or storms) over the winter followed by deposition of sediments. In addition, warmer temperatures may have released some of the TPH sorbed in the sediments by increasing the solubility of the HCs immediately after the winter.

There are several engineering-based reasons why the NO_3^- and air amendments may not have been totally effective in the biodegradation of all TPH fractions compared to the natural attenuation. The amendments may not have been delivered in a homogeneous way to the total volume of the treatment plot. The NO_3^- solution and the

air may have followed the path of less resistance, going immediately to the surface of the sediments where they were removed by the tidal water. The NO_3^- was dissolved in freshwater which was less dense than the saline porewater. Hence, it may have risen to the marsh surface due to density differences. Another possibility could be that the NO_3^- solution and air were following the path made by the metallic pipe that was used to install the horizontal wells. If the sediments did not consolidate around the horizontal wells, as expected, the NO_3^- solution and air could have exited ground via these channels. No liquid was observed coming out of the ground after injection. In the air plot bubbles were observed where the head of the distribution system pipes was buried indicating incomplete consolidation was at least initially (~2 months) a problem.

It is also possible that the concentrations of NO_3^- (71.0 g NO_3^--N/L) and air (3.2 m^3/h) were lower than the amount needed for biodegradation of TPH. Although the NO_3^- amendment was injected weekly, it could have been used rapidly. One of the main challenges associated with biostimulation in oil-contaminated coastal areas is maintaining optimum nitrogen concentrations in contact with the oil and the microorganisms (Zhu *et al*, 2001). Salt marsh sediments also have higher nitrogen demands due to the size of microbial population within an organic carbon-rich environment (Lee and Merlyn, 1999).

Mass transfer limitations could have been an important factor during the Fore River Creek salt marsh study. Because most of the TPH compounds have limited solubility in water, they may have been unavailable to the microbes. Diffusion of the HCs and TEAs in the marsh sediments follows Fick's law (Equation 4.2), which can describe the molecular movement of these biologically-important parameters.

$$J = D\left(\frac{\Delta C}{\Delta X}\right) \qquad \text{(Eq. 4.2)}$$

where: J is the mass flux (M/T^1L^2), D is the diffusivity coefficient (L^2/T), C is the concentration (M/L^3) and X is the distance (L). Factors such as low temperature, laminar flow conditions, limited mixing in the marsh sediments may have limited the mass transfer of relatively low soluble TPH to the active microorganisms. Varying ionic strength (salinity) may have change the availability and conformation of the HCs. Lower TPH concentrations could also have limited the flux of HCs to the microbes. In addition, over time, the composition of the TPH mixture probably changed because more biodegradable compounds were being degraded, leaving a preponderance of compounds more resistant to biodegradation.

During the first part of the study (Summer and Fall 1998), because of the higher *in situ* TPH concentrations present in the Fore River Creek marsh, biodegradation was probably limited by the flux of TEAs (oxygen and NO_3^-). Subsequently, during the second season (Spring 1999) when the HC concentrations were lower, *in situ* biodegradation was probably limited by the flux of biodegradable TPH to the microorganisms in the marsh sediment. Probably the main factors controlling this were the concentration and sorption/desorption of TPH molecules in the marsh sediments.

An important parameter that could have affected the aerobic biodegradation in the salt marsh sediment was the limited gas permeability through the sediments. This movement is a function of the sediment structure, grain size and moisture content. The fine nature of the salt marsh sediments makes it difficult for gases to move through them. This helps create anaerobic environments in most marshes. In addition, because the

organic-rich nature of the marsh exerts a large TEA demand, microorganisms immediately scavenge any oxygen diffusing into the sediments.

Some differences were observed in the plant growth between the plots and within them indicating that the distribution of *S. alterniflora* could have impacted in the biodegradation of aliphatics during the study. Abundance of active metabolic microorganisms in the rhizosphere and the presence of plant roots and their importance for the production of exudates and passive oxygenation could have had a significant effect during the study (Heider *et al.*, 1999) (See Section 4.9).

4.6.2 Aromatic Biodegradation

The aromatics include monocyclic compounds (e.g., benzene, toluene and xylene) and PAHs (e.g., naphthalene, anthracene and phenanthrene), which have two or more aromatic rings. In general, aromatics are more resistant to biodegradation than SC and some LC aliphatics. Monoaromatics are toxic to some microorganisms because of their solvent action on cell membranes, but in low concentrations they are degraded under aerobic and anaerobic conditions (Zhu *et al.*, 2001). It is unlikely that many monoaromatics were present in the Fore River Creek sediments because they are not common in No. 2 or No. 4 fuels and when present, they volatilize rapidly (within hours or days of a spill). PAH biodegradation is limited because these compounds are hydrophobic, have low aqueous solubility and a strong sorptive capacity to sediments reducing their bioavailability (Banat *et al.*, 2000). Usually, they must be degraded into smaller ring compounds before they can be degraded (Atlas, 1981).

During 1998 and the overall study, the aromatic HCs in the control plot decreased ($p<0.20$) (Table 28) probably as a result of natural attenuation. The natural attenuation of aromatic HCs is similar to the natural attenuation described for aliphatic HCs. However, although most of the aromatic compounds have low solubility in water, the smaller compounds (<2 rings) are more soluble and can be reduced by dilution processes (tidal action). Large aromatic compound (>3 rings) tend to sorb to the sediment organic particles and therefore are retained much longer in marine sediments (Atlas, 1981). Because the *Julie N* spill occurred ~19 months before this study and PAH are generally less volatile than aliphatic compounds, volatilization was probably not responsible for the decrease in aromatic HCs in the control plot.

Generally, most aromatics can be degraded under aerobic conditions (Bauer and Capone, 1985; Bossert and Bartha, 1986; Wrenn and Venosa, 1996). Some low molecular aromatics have been degraded under denitrifying conditions (Al-Bashir, 1990; Burland and Edwards, 1999; Leduc *et al.*, 1992; MacRae and Hall, 1998; Mihelcic and Luthy, 1988a, 1988b). Some aromatics have been degraded under sulfate-reducing (Coates *et al.*, 1996; 1997; Coates *et al.*, 1998; Rueter *et al.*, 1994) and methanogenic conditions (Heider *et al.*, 1999). It is most likely that any biodegradation of the aromatics that occurred in the control plot was a function of aerobic respiration (diffusion of oxygen into the top layers of sediments) or sulfate-reduction (because of the predominance of sulfate in seawater).

Although the IVRM model indicated that the air treatment had a significant effect ($p<0.20$) during 1998, the degradation rates for this plot and the control were not significantly different. The Fore River Creek research is the first study to evaluate the

addition of air (bioventing) by a horizontal well system into the salt marsh sediments. Aerobic conditions are generally considered necessary for extensive degradation of oil since major degradative pathways for aliphatics and aromatics involve enzymes that required oxygen (oxygenases) (Atlas, 1991; Zhu *et al.*, 2001). Oxygen is the TEA that yields more energy (ATP) per molecule of TPH during the microbial metabolism. Probably one of the main reasons why the air treatment did not achieve higher degradation rates during this study was because of mass transfer limitations. The efficiency of oxygen mass transfer in a marsh is known to be restricted by the low gas permeability of the very fine and organic-rich sediments and limitations between phases including liquid/gas (water/oxygen), and liquid/microbes (dissolved oxygen/microorganisms) (EPA, 1995).

Although the biodegradation of aromatics under denitrifying conditions is not as extensive as under aerobic conditions, it has been reported in several studies under laboratory and environmental conditions (MacRae and Hall, 1998; McNally *et al.*, 1998, 1999). However, comparison with these studies is difficult because either pure cultures or laboratory conditions were used and there was little correlation between NO_3^- use and PAH degradation. Typically, smaller and more soluble PAHs were degraded faster than larger, less soluble compounds.

During the Fore River Creek study, the NO_3^- did not enhance the degradation of aromatics. The IVRM model indicated that NO_3^- had a significant effect during the overall study however, the biodegradation rates for the NO_3^- and the control were not significantly different. As noted for aliphatic HCs (Section 4.6.1), problems with the NO_3^- delivering and availability could have limited its effectiveness and availability.

4.6.3 Degradation Rates

Degradation rates were calculated (Appendix D) (Table 29) for the treatments that the IVRM indicated had a significant effect on the removal of TPH during the study (Table 28). During the 1998 season, all plots exhibited significant linear degradation of SC aliphatics. The air and NO_3^- amendment degradation rates (as judged by the slope of best fit line in the mg TPH/kg_{dw} vs. time plot) were significantly greater than the biodegradation rate of the control (one tail *t*-test, $p<0.05$.). This corroborated the results of the IVRM (Table 28).

For the LC aliphatics, the NO_3^- treatment had a significantly higher degradation rate than the control, while for the aromatic fraction the air and control rates were NSD (one tail *t*-test, $p<0.05$). During the 1999 season, none of the treatments had a significant effect on the degradation of any TPH fraction during the study. Again, these results corroborated the results of the IVRM (Table 28).

Table 29. Treatments achieving significant reduction in TPH concentration ($p<0.2$) and their biodegradation rates (mg/kg_{dw}/d ±2s).

	1998+	1999	Overall (1998-1999)++
SC aliphatics	Control (13.1±12.8) **Air (27.6±17.5)*** **NO_3^- (20.6±10.9)***	-	Control (8.7±3.9) NO_3^- (5.0±4.9)
LC aliphatics	Control (13.6±12.2) **NO_3^- (23.7±13.0)***	-	Control (6.2±3.5)
Aromatics	Control (19.2±11.2) Air (16.9±9.9)	-	Control (7.2±3.8) NO_3^- (2.2±4.3)

Shaded = IVRM indicated degradation significantly better TPH removal than control (Table 28).
*indicates degradation rate significantly greater than control (one tail *t*-test, $p=0.05$).
+ Assumes linear degradation model ++ Assumes first order degradation model.

During the overall study (1998-1999), it was assumed that first-order degradation occurred (See Figures 42-44). The first order degradation rate constants (k) (T^{-1}) were estimated from the slope of the best-fit line on a plot of Ln concentration (mg/kg_{dw}) vs. time. During the entire study, the control and NO_3^- exhibited significant degradation of the SC aliphatics, but their rates were NSD from each other (one tail *t*-test, $p<0.05$). Neither of the amendments had a significant effect on the degradation of the LC aliphatics. For the aromatic fraction, the control and NO_3^- exhibited significant degradation, but their rates were NSD from each other (one tail *t*-test, $p<0.05$). These results also corroborated those of the IVRM (Table 28).

Suarez and Rifai (1999) summarized data from approximately 280 biodegradation studies for BTEX and chlorinated solvents. They reported that the specific conditions used in the experiments greatly affected the degradation rates observed. Some of the factors that caused this variation included: the nature of the microorganisms present (consortium or pure culture), competition among different cultures for the substrate, the medium or environment in which the experiment was conducted (aqueous phase, soil or sediments), the temperature, the size of the microbial community, and the availability of TEAs, nutrients and substrate (ED). A comparison between laboratory and field degradation rates indicated that those obtained in the laboratory were generally higher than field rates, because the laboratory studies maintained more favorable conditions (Suarez and Rifai, 1999).

In a laboratory study, Al-Bashir *et al.* (1990) studied the biodegradation of ^{14}C-labelled naphthalene in pristine and oil-contaminated soil slurries under denitrifying

conditions. When the initial naphthalene concentration was 50 mg/L, a biodegradation rate of 1.3 mg/kg/d was observed over 50 days incubation (T= 35°C). At higher naphthalene concentrations (500 mg/kg), they observed a rate of 1.8 mg/kg/d. The rates obtained in the Fore River Creek salt marsh and by Al-Bashir *et al.* (1990) were NSD (two tail *t*-test, $p<0.05$), corroborating our results.

During bioventing studies of Florida soils contaminated with JP-4 jet fuel, the degradation rates ranged from approximately 2 to 20 mg/kg/d, with average values of 5 mg/kg/d (Lesson and Hinchee, 1996). Although, the air treatment had no significant effect during the overall study in the Fore River Creek salt marsh, it had a significant effect during the 1998 season. During that season, the air showed a significant biodegradation of SC aliphatics and aromatics with removal rates of 27.6±17.5 and 16.9±9.9 $mg/kg_{dw}/d$, respectively. These biodegradation rates were NSD from those reported in the Florida study.

Venosa et al. (1996), under a controlled crude oil spill (artificially weathered) (2,040L) in Delaware beach, found higher first-order degradation rates in the control ($-26.0 \times 10^{-3} d^{-1}$ and $-21.0 \times 10^{-3} d^{-1}$) for the alkanes and aromatics, respectively. The biodegradation rates obtained during Venosa's study were normalized using hopane. The main reason to observe lower degradation rates during the Fore River Creek marsh study are that the study was conducted ~19 months after the No. 2 fuel oil spill.

4.6.4 Subplot Distribution

It was possible that the location of the subplots may have affected the TPH results (Figures 49-51). The proximity of a subplot to: the borders where catwalks were placed (e.g., Subplots 1, 13, 25, 12, 24); the distribution header (e.g., 1, 2, 3...12); the entrance of the distribution system to the plot (e.g., 109, 110, 111...120), the center or the border of the plot (e.g., 67 vs.115) and the underground horizontal wells (e.g., 8,43 vs. 21,48) could have been a factor in the distribution of the amendments.

The mass (concentration x volume) of amendment (e.g., NO_3^-) added was calculated assuming equal distribution radially in all directions from the well over a 0.6 m x 0.3 m x 18 cm volume. However, if the solution was not evenly distributed, some part of the subplot could have been devoid of amendment because the amendment may have followed the path of less resistance to the surface of the sediment. Also, some subplots could have received more amendment than planned.

In order to check for patterns of removal as a function of subplot position the means for SC and LC aliphatics and aromatics (for each sampling event) were compared statistically by using a Tukey-Kramer test ($p<0.05$.). This test was used because it allowed graphical and numerical observation of the data (mean $\pm$ 2s.) for each subplot compared to the other subplots (a vs. b vs. c vs. d). During each sampling event four subplots were sampled (e.g., 3c meaning third sampling event subplot c). Only 4 of the 63 evaluated (7 sampling events x 3 fractions x 3 plots) were significantly different. The results corroborated those of the IVRM (See Section 4.4) that indicated that the subplot sampled did not have a significant effect on the degradation.

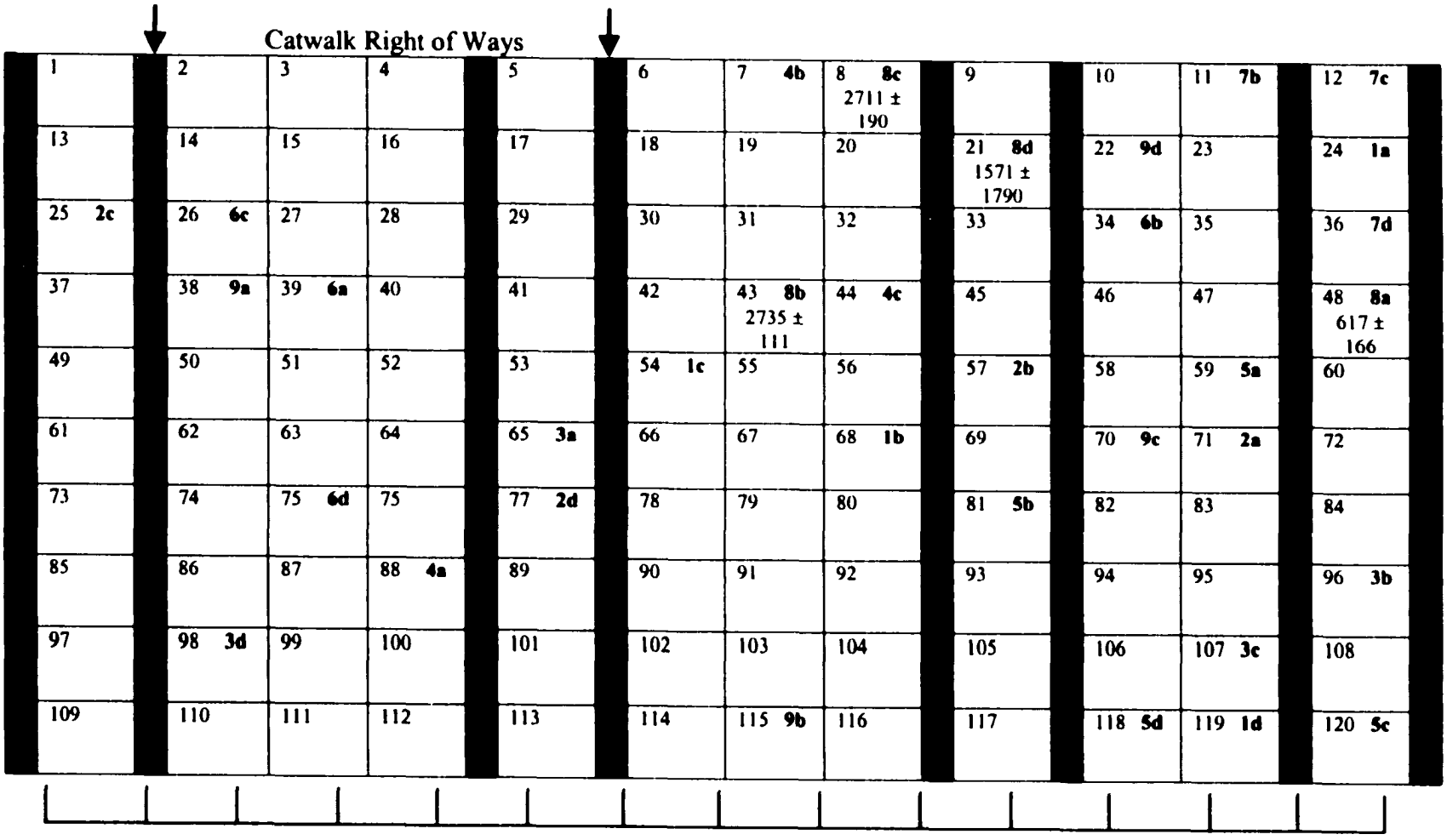


Figure 49. Spatial diagram for the NO_3^- plot and the sampled subplots (a,b,c,d) for each event. The SC concentrations for May 1999 (Event 8) are shown as an example.

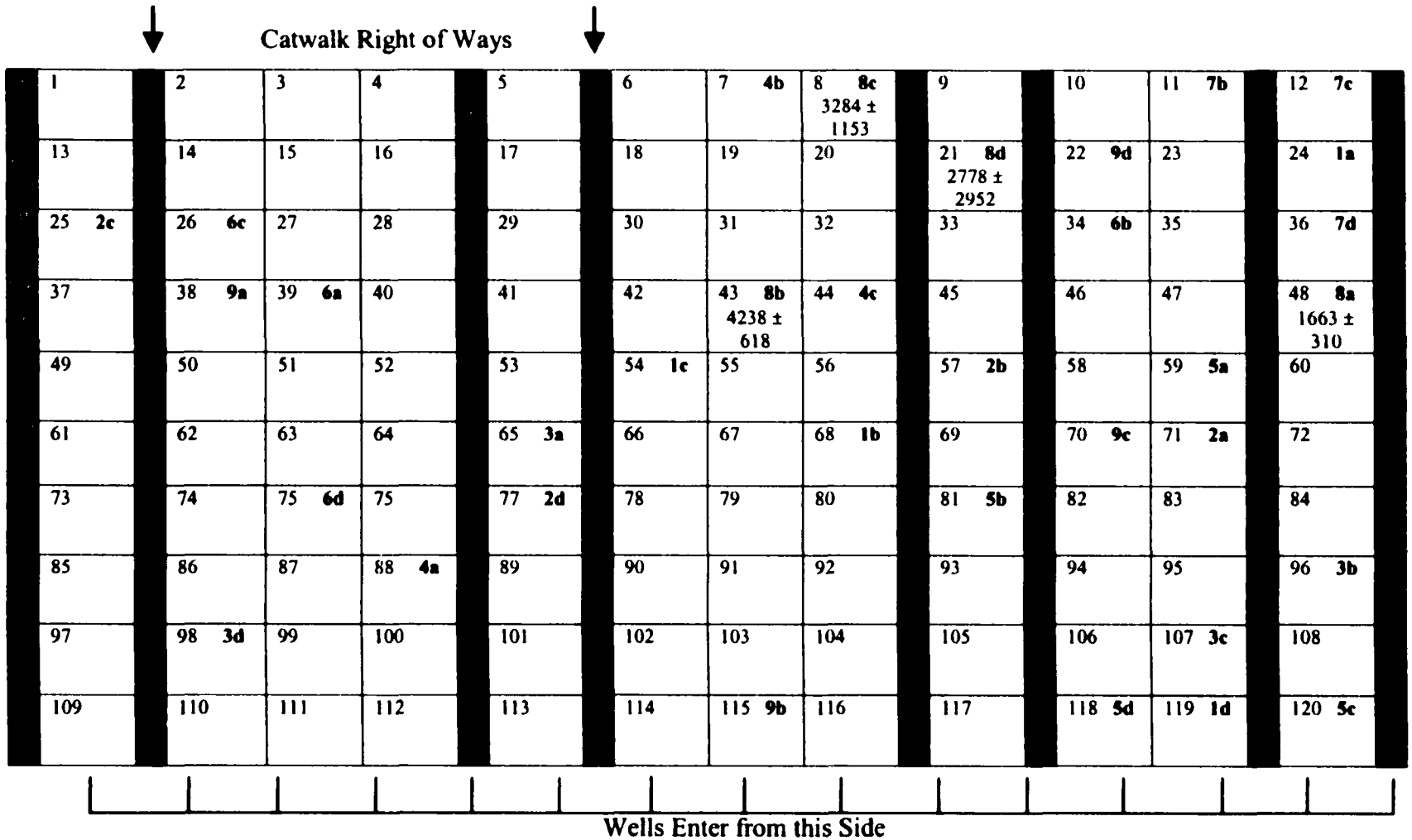
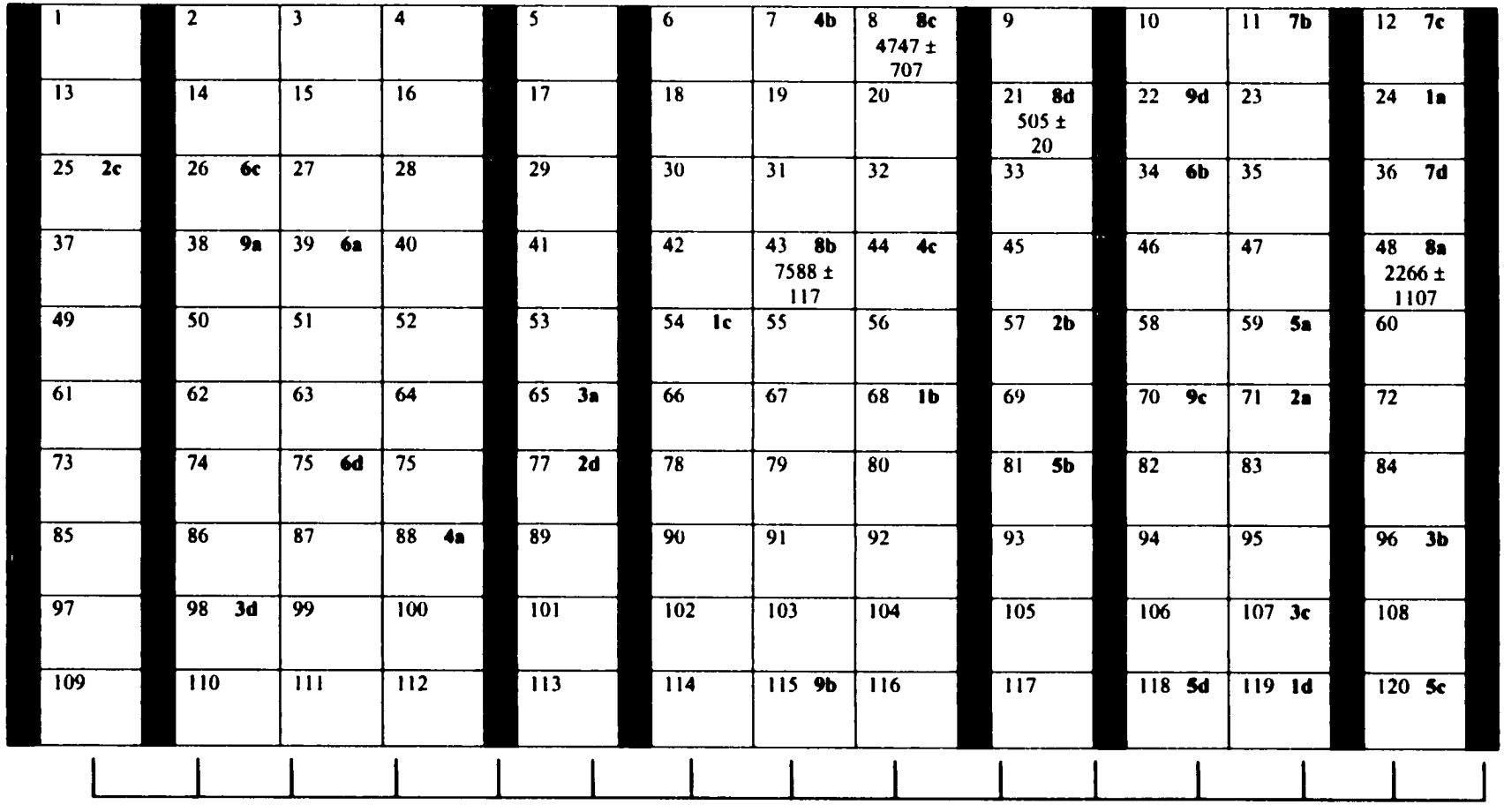


Figure 49. Spatial diagram for the NO_3^- plot and the sampled subplots (a,b,c,d) for each event. The LC concentrations for May 1999 (Event 8) are shown as an example.



Wells Enter from this Side

Figure 49. Spatial diagram for the NO_3^- plot and the sampled subplots (a,b,c,d) for each event. The aromatics concentrations for May 1999 (Event 8) are shown as an example.

In the control plot, all subplots were NSD, while in the air plot (SC aliphatics), Subplot 8C was significantly lower than 8D. In the NO_3^- plot (SC aliphatics), Subplot 3C was SL than Subplots 3A, 3B and 3 D. For the LC aliphatics, 4B and 4C were SH than 4A. In the aromatic fraction, 8D was SL than 8A, 8B and 8C. No trends with respect to proximity to the catwalks platforms, distribution system or horizontal wells were evident in any of the plots for any of the events for the SC, and LC aliphatics or aromatics.

4.7 Abundance of TPH-Degraders

The abundance of TPH-degrading microorganisms was evaluated by colorimetric INT reduction coupled with an MPN method. When INT is reduced, it is possible to observe a precipitate and color change in the oil-containing MPN tubes that contain active microorganisms with the capacity to degrade TPH. In the marine environment, bacteria are the predominant TPH-degraders, along with a few fungi. The role of algal and protistan communities in TPH biodegradation is unknown (Leahy and Colwell, 1990). The abundance of TPH degraders for the first two sampling events was high and variable. During this period, turbidity was used as an indicator of growth (Brown and Braddock, 1990). However, a precipitate that formed in the liquid media interfered with the readings creating false positives (Jones, 1999). Starting on July 26-27, 1998, INT was added to evaluate the presence of TPH-degrading microorganisms (Haines *et al.*, 1996; Konopka *et al.*, 1998). Hence, only MPN data from July 26-27, 1998 thought June 9-10, 1999 were used in this study (Figure 52) (Appendix C).

While researchers have shown that the number of TPH-degrading microorganisms

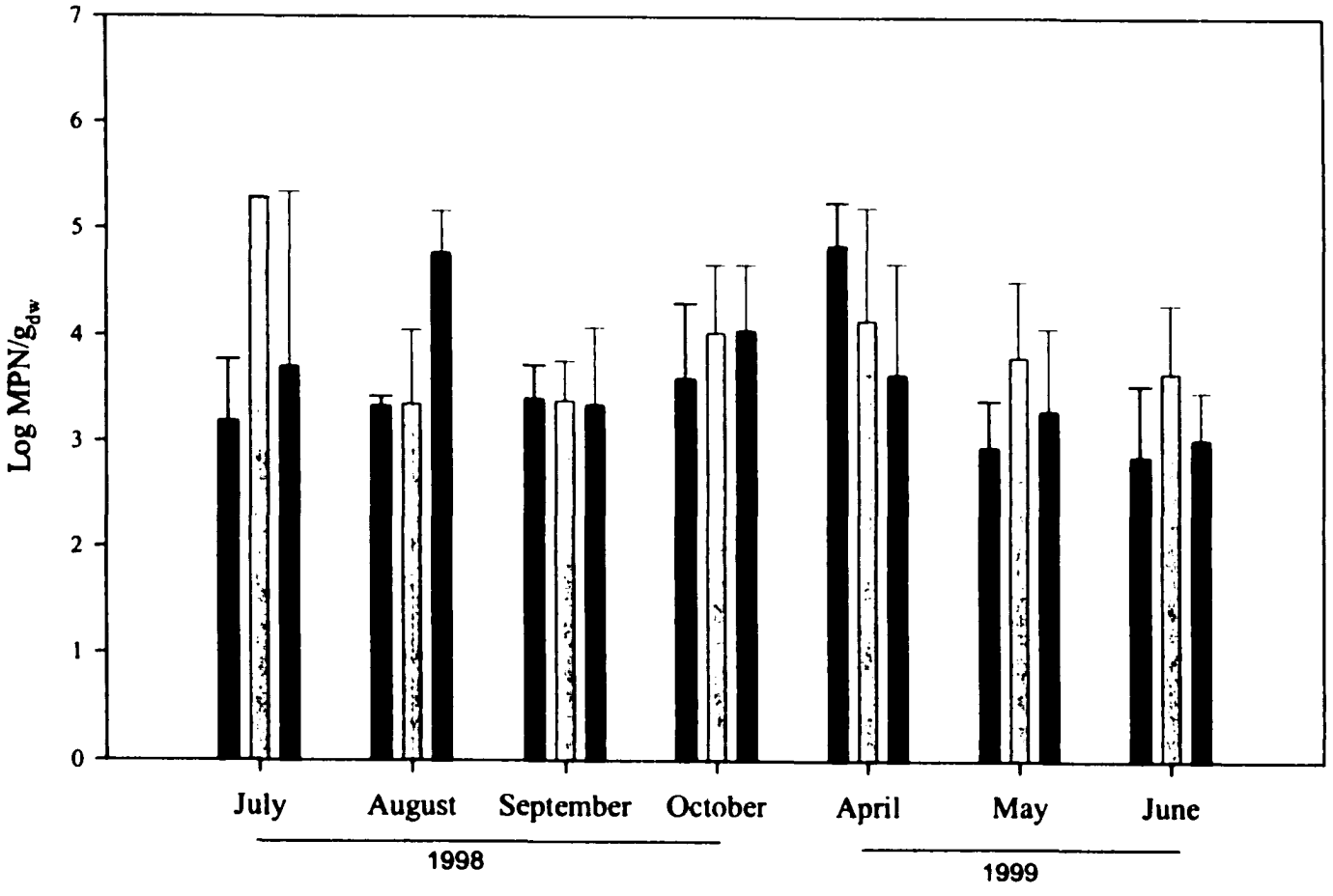


Figure 52. Log of the MPN for the TPH-degrading bacteria.

and their proportion in the heterotrophic community increase upon exposure to petroleum (Leahy and Colwell, 1990; Zhu *et al.*, 2001), especially in the first two weeks (Braddock *et al.*, 1995; Lindstrom *et al.*, 1991), such a change was not expected in the Fore River Creek salt marsh study because the spill had occurred ~19 months earlier.

One of the four MPN replicates in the NO_3^- plot for July 26-27, 1998 and one for August 17-18, 1998 were one order of magnitude higher than the rest of the MPN data during the study (Figure 53), so these results were assumed to be outliers, they were not considered during the statistical analysis. In July 1998, the air MPN data were significantly higher (ANOVA, $p<0.05$) than those from the control and NO_3^- treatment. In August 1998, the NO_3^- plot had significantly higher MPN counts than the control and air plots. In general, these occurrences were isolated and there were not consistent trends in the abundance of TPH degraders over time.

The mean MPN abundances (July 26-27, 1998 to June 1999) for the treatments (NO_3^- and air amendment) were also compared to the control data using a Mann-Whitney U test ($p<0.05$) (Table 30). This non-parametric method requires a minimum sample size of three for each sample class and is used in remediation field studies (Braddock *et al.*, 1995; Lindstrom *et al.*, 1991). The TPH-degrading community was significantly higher ($p<0.05$) in the air treatment when compared with NO_3^- and control treatments. There was not a significant difference ($p<0.05$) between the microbial counts for the NO_3^- treatment and the control over time (Figure 54). Interestingly, the air treatment only had the highest degradation rate during the 1998 season for the SC aliphatic fraction (Table 29). Possibly, the high degradation rates were related to a greater abundance of TPH-degraders during the 1998 season.

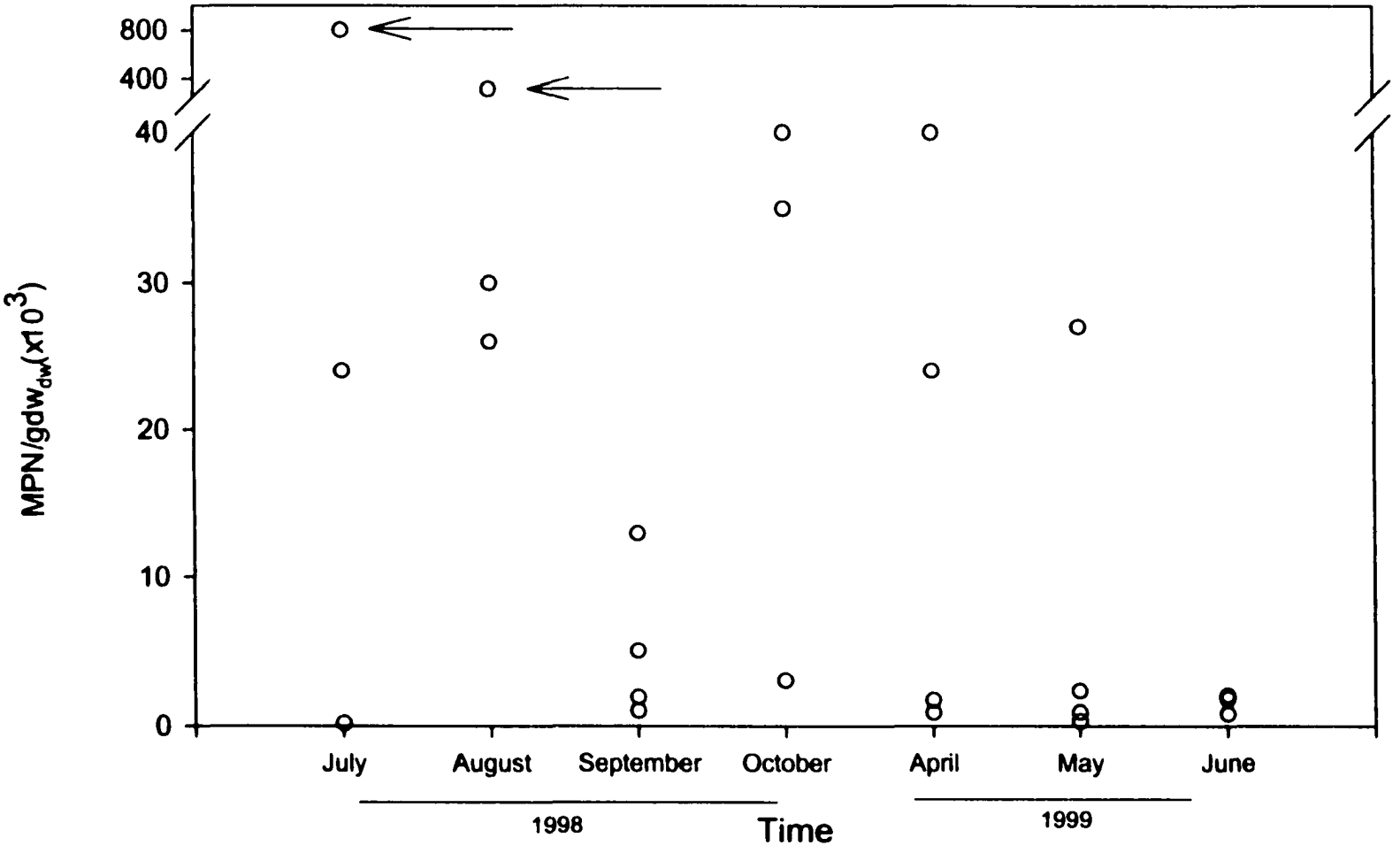


Figure 53. MPN of the TPH-degrading bacteria for the NO_3^- plot. Note line break on y axis. Arrows indicate data that was considered out of range and not used in statistical analysis.

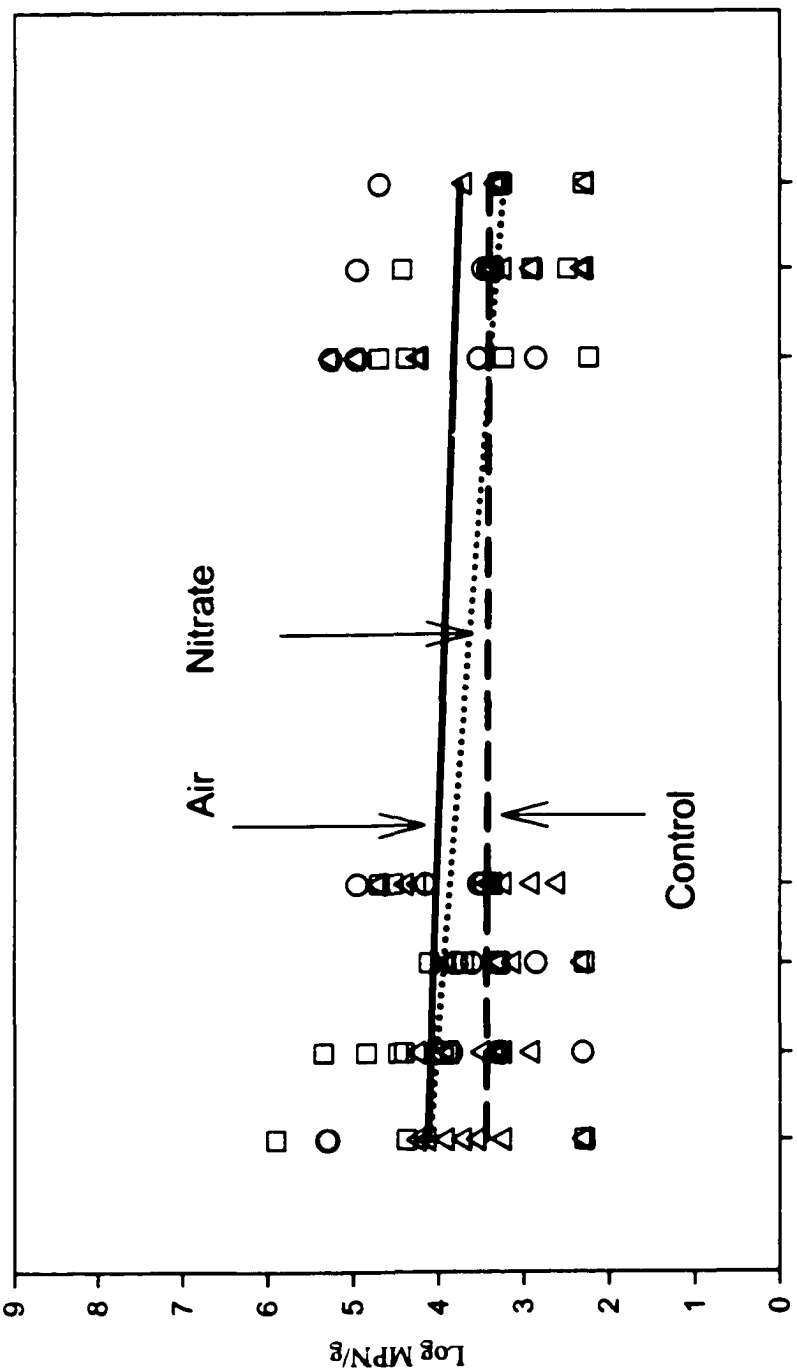


Figure 54. Log of the MPN for the TPH degrading-bacteria. Δ=Control, O=Air, □=NO_3^-

Table 30. Mean TPH-degrading bacteria MPN for July 26-27, 1998 through June 1999.

Treatment	Mean MPN/g_{dw}	Standard Deviation MPN/g_{dw}	Lower 95% MPN/g_{dw}	Upper 95% MPN/g_{dw}
Control	16,900	40,000	6,500	27,000
Air	47,700	72,000	28,500	67,000
NO_3^-	15,000	19,000	9,500	20,500

The data were plotted for the different treatments to visualize the relationship between the MPN counts and HCs concentrations during the study (Figures 55-57). The control plot showed higher slopes and correlation coefficients (r^2) between the MPN data and the TPH fraction concentrations than the air and NO_3^- plots (Table 31). The trend observed in the control plot with the slopes and correlation coefficients (Figures 55-57) indicated that higher MPN data were observed at higher TPH concentrations. This was expected because the higher organic carbon (electron donor) concentrations could support higher numbers of TPH degraders. The correlation coefficients for the SC and LC aliphatics, as well as, the TPH, were significant in the control plot (two tail *t*-test, $p<0.2$) supporting the trend of higher MPN data with higher TPH concentrations.

Table 31. Treatment slopes for the relationships between MPN counts and TPH concentrations for July 26-27, 1998 through June 1999.

	Treatment Slopes (Log MPN/TPH fraction) ($MPN/kg/mg/kg_{dw}$)			Correlation Coefficient (r^2)		
	Control	Air	NO_3^-	Control	Air	NO_3^-
SC Aliphatics	0.5099	0.0327	0.0755	**0.2023**	0.0024	0.0029
LC Aliphatics	0.4828	-0.2738	0.6513	**0.1048**	0.0167	0.0020
Aromatics	0.1937	0.1759	0.0957	0.0235	0.0080	0.0026
TPH	0.5286	-0.0466	0.0754	**0.1193**	0.0040	0.0021

Shading indicates a significant $p \leq 0.20$

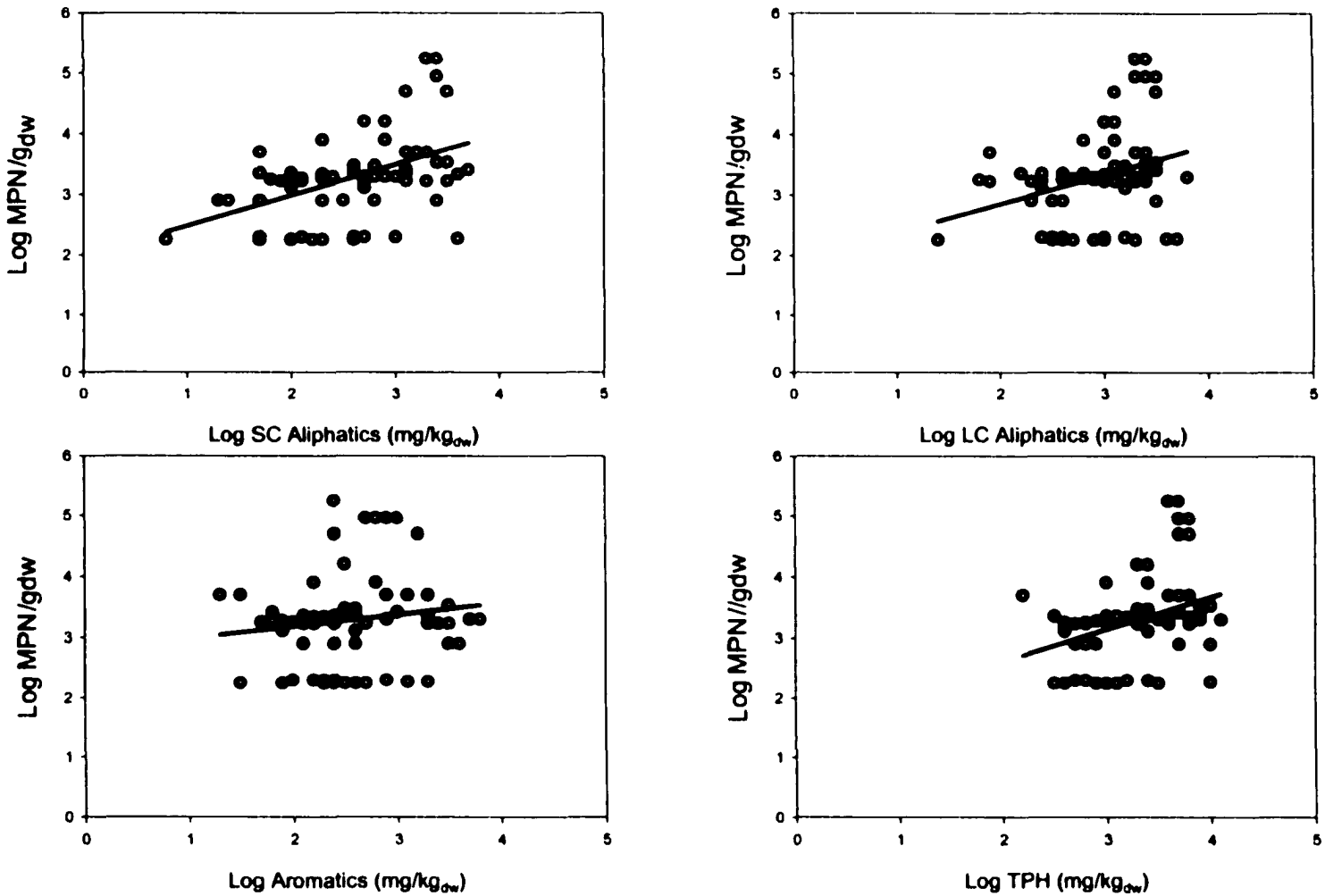


Figure 55. Relationship between log TPH concentrations and log MPN data for the control. Data on regression in Table 32.

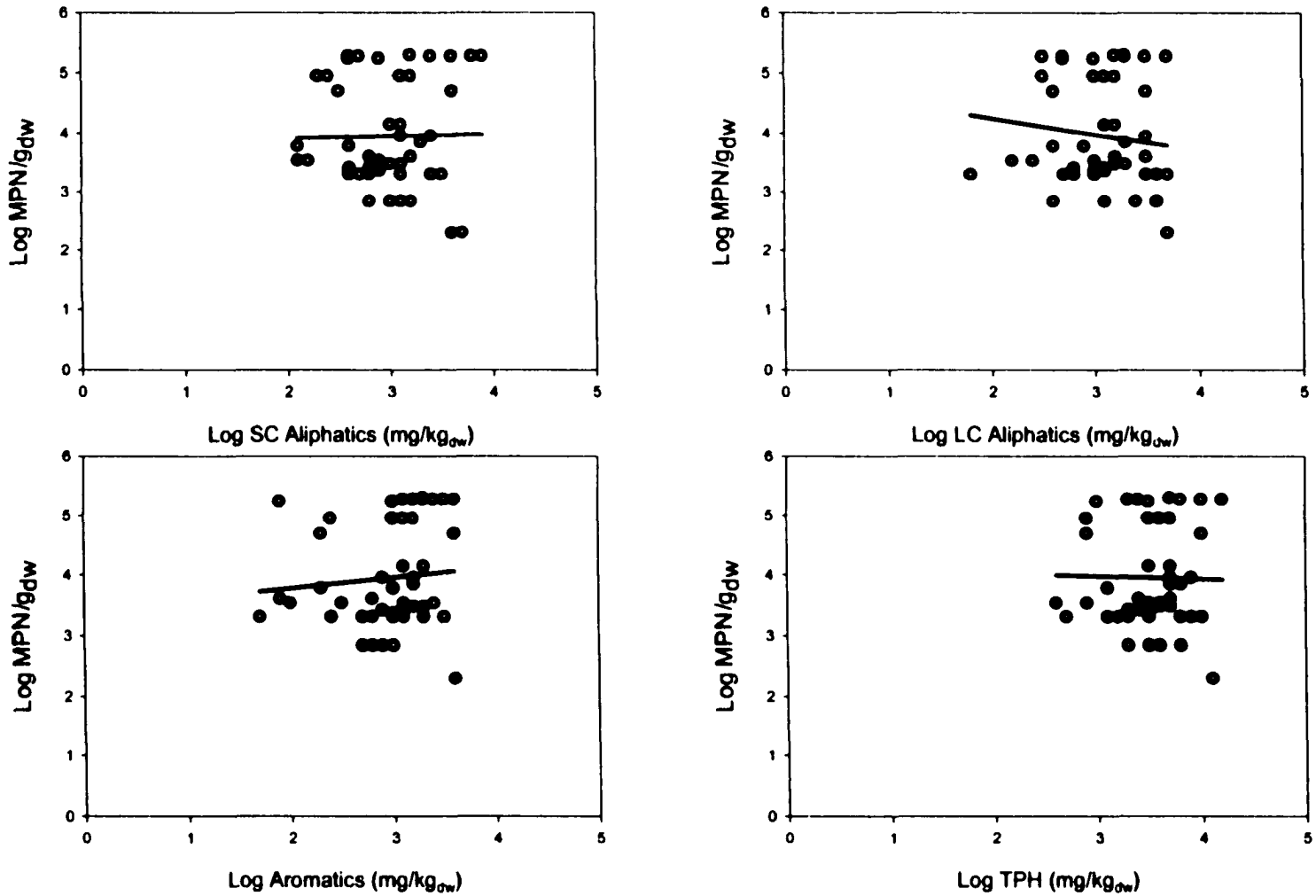


Figure 56. Relationship between log TPH and log MPN data for the air amendment. Data on regression in Table 32.

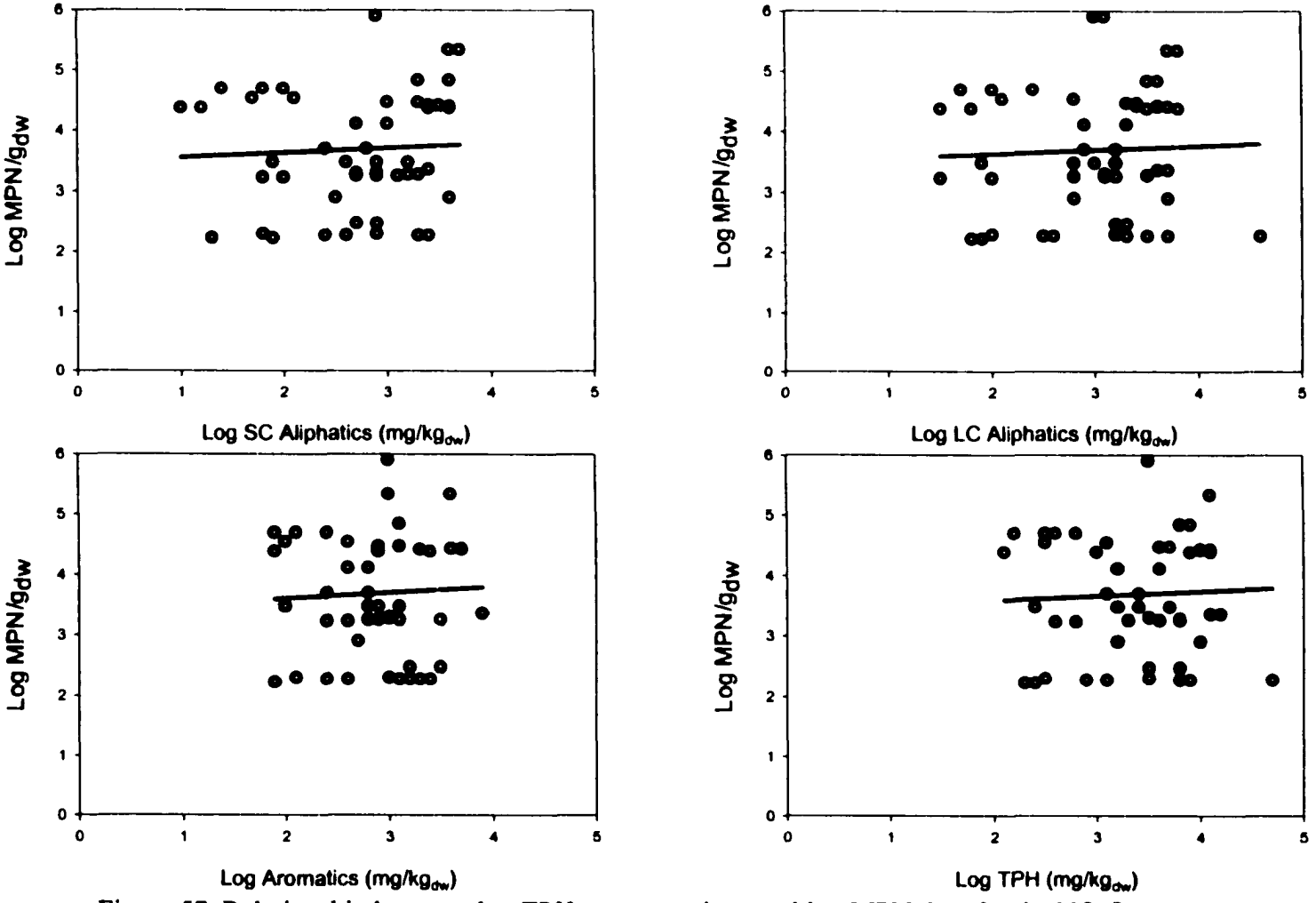


Figure 57. Relationship between log TPH concentrations and log MPN data for the NO_3^- amendment. Data on regression in Table 32.

Braddock *et al.* (1995) reported that in intertidal (shoreline) environments MPN abundance of 20,000-50,000/g_{dw} almost a year after the Exxon Valdez spill, which were similar to the range found in the control at the Fore River marsh (Table 30) ~ 19 months after the *Julie N* spill. It is possible that the structure and diversity of the dominant microbial community in the Fore River Creek marsh changed with the addition of NO_3^- and air, but not the abundances as observed by Macnaughton *et al.* (1999). It is also possible that the abundance of the target population (e.g., denitrifiers, aerobes) was so small that it was masked by other populations of degraders (e.g., SRB). Such small changes may not have been observed due the variability in the MPN (Uraizee *et al.*, 1998). There is the possibility that the TEAs stimulated the microbial community to degrade NOM, especially if the TPH remaining was more difficult to degrade or the concentration too low to be used efficiently. If sediment samples were not properly shaken or if clumping of bacterial cells occurred, the MPN value could also have underestimated the real bacterial density, but a trend should still have been present (Standard Methods, 2000). In addition, large numbers of microorganisms may have been needed to observe a color change of the INT (Haines *et al.*, 1996).

In results similar to these in the Fore River Creek study, many researchers have observed only small changes in microbial abundance in response to a spike of TPH contamination after the first period of exposure. For example, Carman *et al.* (1996) found in a 28 d microcosm study (with Louisiana marsh sediments) that the bacterial abundance had not significantly increased after the addition of diesel-contaminated sediments. However, they found an increase in the PAH degradation suggesting that an adequate microbial community was present. McCurdy *et al.* (1997) were also unable to

observe a stimulation of the TPH degraders over a 650 d period in soil cores studies (Louisiana marshes). They suggested that previous oil spills in the salt marsh could have increased the microbial abundance before the study or the large variance in the counts from soil samples could have masked changes. Townsend *et al.* (1996) evaluated the effect of two biostimulation treatments ($(NH_4)PO_4$ and KNO_3) on a controlled oil spill (Arabian light crude oil) in a marsh (San Jacinto River, TX). Again, there was an initial (30 d) increase in the number of aliphatic- and PAH degraders, but the amendments did not significantly increase microbial abundance compared to control plots. By Day 140, all populations were back to baseline. Venosa *et al.* (1999) evaluated four different treatments in response to a controlled oil spill: no oil (control), oil alone (Bonny Light crude oil), oil and nutrients; and oil, nutrients and an indigenous bacterial inoculum. The MPN data indicated that the alkane degraders did not increase with time, while the aromatic degraders increased during the first 15 d and then both populations decreased steadily. With an average TPH degradation rate of ~5mg/kg_{dw}/d in the Fore River Creek salt marsh study, it was unlikely there would be a marked increase in the abundance of TPH degraders, especially because the community was already acclimated 19 months after the oil spill.

4.8 Nutrient Analysis

Nutrient availability (mainly nitrogen) can be one of the most common limiting factors of oil biodegradation along marine shorelines (Atlas, 1981; Bartha, 1986; Venosa *et al.*, 1996). Therefore, monitoring nutrients, particularly concentrations in porewater, is critical to understanding the potential of oil bioremediation (Lee and Merlyn, 1999; Zhu

et al., 2001). An appropriate C:N:P ratio is necessary to ensure that oil biodegradation is not limited by nutrients (Reynolds *et al.*, 1997). Different ratios have been recommended ranging from 60:1 and 800:1 for C:N and C:P, respectively (Dibble and Bartha, 1979), 160:1 C:N (American Petroleum Institute (API), 1985), 100:10:2 C:N:P (Margesin and Schinner, 1997), and 20:20:1 C:N:P (Belloso *et al.*, 1998). However, maintaining a specific nutrient ratio *in situ* is nearly impossible, especially in marine environments, because of the dynamic washout and dilution caused by tides and waves (Zhu *et al.*, 2001).

4.8.1 Porewater NO_3^-

Because oil degradation takes place mainly in the interface between the TPH molecules and the water or sediment particles where the microorganisms reside, the effectiveness of biostimulation depends on the nitrogen concentration in the interstitial porewater of contaminated sediments (Bragg *et al.*, 1994; Venosa *et al.*, 1996).

The porewater NO_3^- concentration was highly variable in all of the plots during the Fore River Creek study (Figure 58) (Appendix C). Only samples from June 1998 through April 1999 were analyzed for NO_3^- because of some analytical problems (e.g., salinity interferences). In the control plot, the porewater NO_3^- concentration significantly decreased from ~0.5 mg to ~0.01 mg NO_3^--N/L (Tukey-Kramer, $p<0.05$) possibly because of the NO_3^- demand exerted by the oil biodegradation, *S. alterniflora* and the lack of adequate supply. Generally, NO_3^- concentrations in salt marshes are low in comparison to other nitrogen sources such as NH_4^+ because of the reducing conditions (McKee and Mendelssohn, 1994).

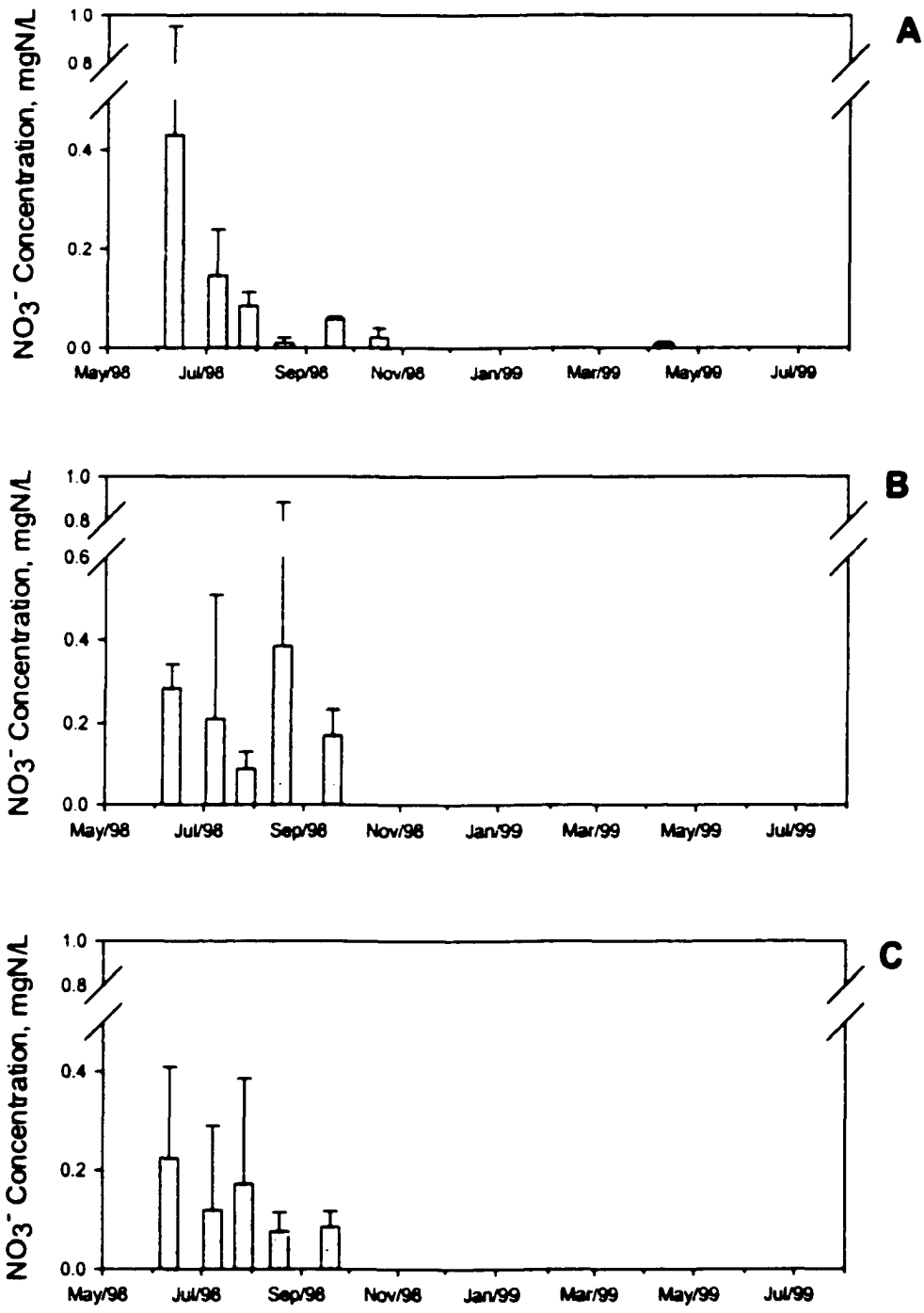


Figure 58. NO_3^- concentration in the porewater samples of the Fore River Creek plots. A) control, B) air and C) NO_3^-. Note line breaks on y axis

It is very difficult to assess if the decrease in the NO_3^- concentration in the control plot during the Fore River Creek study was a result of it being used as a TEA (dissimilatory pathway) or as a source of nitrogen (nutrient) (assimilatory pathway).

Jones (2000) reported in a technical characterization of the estuarine and coastal waters in New Hampshire, that although different concentrations of nitrogen are observed within locations, there is a seasonal pattern. Late fall through early spring, high inorganic nitrogen concentrations are observed, while by late spring through early fall low inorganic nitrogen concentrations are present. This pattern was observed at the Fore River Creek salt marsh except for April 1999. During that month, the inorganic nitrogen concentrations were low corresponding to lower TPH, especially in the NO_3^- plot.

In the air plot, the NO_3^- concentration (0.3 ± 0.2 mg N/L) was somewhat variable, and did not change significantly during the study (Tukey-Kramer, $p<0.05$). Similar results were also observed in the NO_3^- plot (0.2± 0.1 mg N/L) (Tukey-Kramer, $p<0.05$).

The NO_3^- concentration in the porewater of the NO_3^- plot (plot volume = 2.27 m^3) was supposed to be 32 mg N/L. The *in situ* NO_3^- porewater concentrations were ~ two order of magnitude below this desired value. During the Fore River Creek study, the NO_3^- solution was added after the sampling events to avoid bias when collecting porewater. The NO_3^- concentrations found in the porewater samples suggested that either microorganisms were using NO_3^- very quickly because of the high demand (i.e., NO_3^- was very limiting in the environment) or it was washed out by tidal action. NO_3^- can also serve as TEA for the reoxidation of sulfides present in a salt marsh (Davidova *et al.*, 2001).

Field tests conducted after the *Exxon Valdez* oil spill in Prince William Sound showed that the rate of oil biodegradation in three sandy beaches was accelerated by maintaining average interstitial nitrogen concentrations (as NO_3^- or NH_4^+) of about 1.5 mg N/L (Bragg *et al.*, 1994). A microcosm study with sandy sediments suggested that optimum rates of biodegradation could be sustained by maintaining *in situ* concentrations approaching 1-2 mg N/L in interstitial porewater (as NO_3^- or NH_4^+) (Venosa *et al.*, 1996). Zhu *et al.* (2001) noted that bioestimulation of oil-impacted beaches should occur when nitrogen concentrations (as NO_3^- or NH_4^+) in the porewater are at least 2 to 5-10 mg N/L,. The higher concentrations should only be used after a broad analysis of cost, environmental impact and practicality. However, these concentrations may be lower than the nitrogen demand in the Fore River Creek marsh, which has a much higher organic content and thus greater nutrient demand (i.e., growing plants) than a beach.

Based on the stoichiometry of the degradation of SC and LC aliphatics and aromatics under denitrifying conditions (Table 32), ~ 158 mg N (NO_3^- as TEA) would be needed to completely degrade ~0.1 g SC fraction (Appendix D). Considering that the Fore River Creek marsh had ~4,600 g SC/plot, it would theoretically require ~7,268 g N (NO_3^- as TEA) for the complete degradation. Based on the theoretical (Appendix D) for the LC aliphatics 4,908 g/plot and aromatics 3,834 g/plot degradation: 7,803 g N and 6,594 g N, respectively, would be needed. The total of N (NO_3^- as TEA) theoretically needed to degrade the initial TPH in the Fore River Creek salt marsh would be approximately 19,979 g N. During the study (1998-1999), 2,154 g N (as NO_3^-) were added to the NO_3^- plot. This indicates that only 10.6% of the nitrogen required as NO_3^- (to be used as TEA) was added (Appendix D). In order to supply the needed NO3$^-$-N, the

porewater should be monitored frequently to maintain the optimum concentration of the TEA *in situ* (Bragg *et al.*, 1994; Venosa *et al.*, 1996; Zhu *et al.*, 2001).

During the Fore River Creek marsh study, 430 g of $NaNO_3$ were added weekly for an expected porewater final concentration of 32 mg N/L (Appendix D). This concentration is lower than that reported by Townsend *et al.* (1996) in an oil-contaminated salt marsh (San Jacinto River, TX) (40 mg available N/kg_{dw} for the nutrient treatment, and 40 mg available N/kg_{dw} plus 100 mg NO_3^--N/kg_{dw} for the TEA treatment). In addition, the Fore River Creek salt marsh had a higher TPH concentration: SC (2,900±1,400 mg/kg_{dw}), LC (3,200±1,500 mg/kg_{dw}) and aromatics (2,500±1,500 mg/kg_{dw}) than Townsend et al. (aliphatics (~869 mg/kg_{dw}) and aromatics (~271 mg/kg_{dw})).

Table 32. Theoretical stoichiometry for TPH fraction degradation by using NO_3^- as TEA.

Compound	Theoretical Stoichiometry
SC Aliphatic $C_{14}H_{30}$	$C_{14}H_{30} + 22.4\ NO_3^- + 48.4\ H^+ \rightarrow 14\ CO_2 + 11.2\ N_2 + 39.2\ H_20$
LC Aliphatic $C_{28}H_{58}$	$C_{28}H_{58} + 44.8\ NO_3^- + 99.8\ H^+ \rightarrow 28\ CO_2 + 22.4\ N_2 + 78.4\ H_20$
Aromatic $C_{17}H_{17}$	$C_{17}H_{17} + 27.2\ NO_3^- + 78.2\ H^+ \rightarrow 17\ CO_2 + 13.6\ N_2 + 47.6\ H_20$

Based on typical C_nH_n compound

In salt marsh sediments Adrian *et al.* (1998) observed little to no enhancement of degradation for the lowest chain length alkanes (C_{11} and C_{12}) with the exception of a plot where NO_3^- was added at 500 mg NO_3^--N/kg. In addition, the NO_3^- at this concentration stimulated biodegradation of almost all other alkanes at two to three times the rate of the control. Their porewater concentrations were ~90-292 mg N/L. However, they found that NH_4^+ was a better amendment because it required only 20% of the concentration that NO_3^- did to achieve the same degradation rates. Not only were the porewater NO_3^-

concentrations much higher in the Adrian *et al.* (1998) study, but also the concentration of the crude oil was higher too (700,000 mg oil/kg soil). In addition, during the Adrian *et al.* (1998) study, they did not experience nitrogen losses by tidal action because they used microcosms.

One of the main reasons to use horizontal wells during the Fore River Creek salt marsh study was to minimize the potential for washout of the NO_3^- by the tide. This approach assumed that nutrients dissolved in freshwater would be brought into contact with the oiled sediments assuming equal movement in all directions. However, the delivery system may not have worked properly as discussed in Section 4.6. The horizontal well delivery system in the Fore River Creek marsh should be assessed using a tracer to monitor the amendment pathways *in situ* after injection.

4.8.2 Porewater NH_4^+

The NH_4^+-N concentration during the Fore River Creek study was highly variable in all plots (Figure 59) (Appendix C) and was not significantly different between the different treatments (ANOVA, $p<0.05$). The NH_4^+-N was higher than the NO_3^--N concentration in all cases as expected because the majority of the nitrogen in salt marsh sediments is in the form of NH_4^+ because of the reducing conditions (McKee and Mendelssohn, 1994). The concentration of the NH_4^+ did not change significantly in any of the plots during the study (Tukey-Kramer, $p<0.05$).

The inorganic nitrogen concentrations in the porewater (NO_3^--N- and NH_4^+-N) in the air, NO_3^-, and control plots were ~3.0 mg N/L, ~1.8 mg N/L and ~2.6 mg N/L, respectively. These nitrogen concentrations are similar to levels that supported biodegradation of oil on contaminated beaches, however, the concentration of available

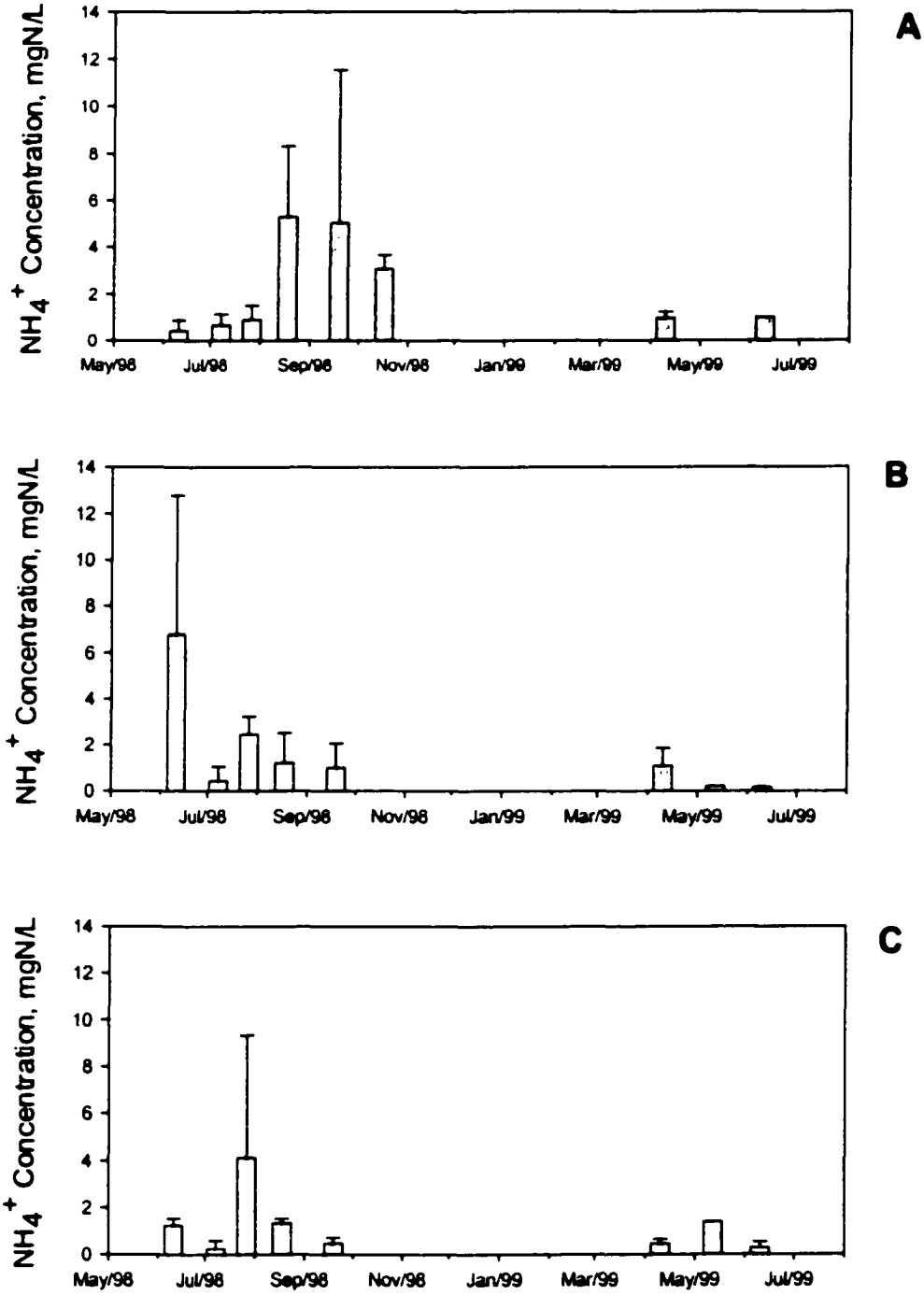


Figure 59. NH_4^+ concentration in the porewater samples of the Fore River Creek plots. A) control, B) air and C) NO_3^-

NOM is much higher in salt marsh environments (See Section 4.8.1) (Bragg *et al.*, 1994; Venosa *et al.*, 1996; Zhu *et al.*, 2001). In fact, Townsend *et al.* (1996) and Adrian *et al.* (1998) found concentrations 1-2 orders of magnitude higher were needed in salt marshes. Therefore, it is likely that nitrogen concentrations in the Fore River Creek salt marsh may not have been adequate to stimulate TPH degradation. NH_4^+ is a source of inorganic nitrogen for microorganisms and plants (*S. alterniflora*) and is less likely to be lost from a marsh by washout because it is able to sorb to organic matter more efficiently than NO_3^- (Adrian *et al.*, 1998). For future amendments, NH_4^+ should be considered as the nitrogen source if TPH biodegradation is nutrient limited. However, unlike NO_3^- NH_4^+ cannot serve as a TEA because the nitrogen is already reduced (-3 valence).

4.8.3 Porewater PO_4^{-3}

Phosphorus and other minerals (e.g., potassium, magnesium) must be incorporated into biomass. Phosphorus is generally used as a structural element and is present in DNA and RNA molecules and as co-factor in some enzymes. Low phosphorus concentrations available at a TPH contaminated site could limit the rate of the microbial degradation of TPH.

The PO_4^{-3} concentrations present in the porewater during the Fore River Creek study were highly variable (Appendix C). The control plot had high PO_4^{-3} concentrations at the end of the Summer and early Fall 1998 season (Figure 60), followed by a drastic decrease during October 1998 and April 1999. This pattern was similar in the NO_3^- plot where higher PO_4^{-3} concentrations in the porewater were present during the June-August 1998 compared to September-October 1998 and April-June 1999. The air plot had lower porewater PO_4^{-3} concentrations compared to the control and NO_3^- plots.

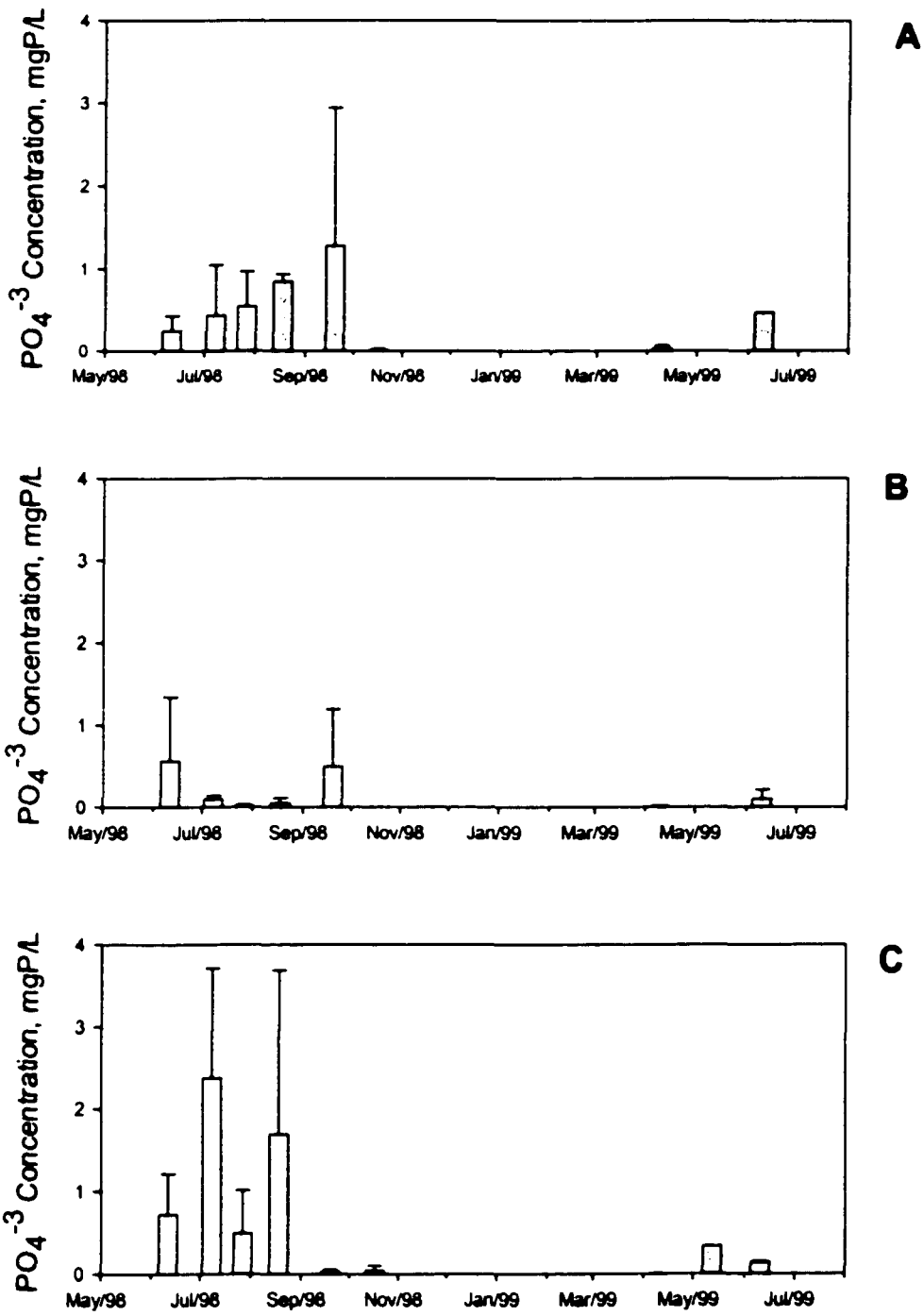


Figure 60. PO_4^{-3} concentration in the porewater samples of the Fore River Creek plots. A) control, B) air and C) NO_3^-

These lower concentrations could have been indicative of a higher uptake of PO_4^{-3} by the active microbial population. For example, the air plot also had a significantly higher MPN counts for TPH degraders and a significantly higher degradation rate for the SC aliphatics during the Summer and Fall 1998.

Wright *et al.* (1995), in a mesocosm study using salt marsh sediments contaminated with light crude oil, found that the application of phosphorus significantly enhanced TPH degradation during the winter, but not during summer. They concluded that warmer temperatures influenced phosphorus diffusion from sediments. This type of trend was not observed in the Fore River Creek marsh. Lower PO_4^{-3} concentrations occurred during the Fall (October 1998) and early Spring (April 1999).

The PO_4^{-3} porewater concentrations in the Summer and Fall 1998 were enough to support the biodegradation of the TPH present in the Fore River Creek marsh sediments compared to Wright *et al.*'s levels (40 kg PO_4^{-3}/ha). The low PO_4^{-3} concentrations observed in the Fore River Creek marsh during the 1999 season could have limited biodegradation, however, the lower TPH during that period may also have exerted a lower demand for PO_4^{-3}.

4.9 Plant Growth and Density

The stem height and density of *S. alterniflora* was measured in each subplot during the sampling events (Appendix C). *S. alterniflora*, the predominant plant in the Fore River Creek marsh, is usually found in environments where it can be flooded. It is an important primary producer that acts as the initial source of detrital food on which all of salt marsh organisms depend (DeLaune *et al.*, 1979). *S. alterniflora* spreads asexually

by means of a subterranean rhizome system. The plant forms a dense net that encourages the deposition of fine sediment particles including organic matter (salt marsh peat) (Lin and Mendelssohn, 1998).

4.9.1 Seasonal Variation

In the Fore River Creek marsh, the *Spartina* started to grow during the spring and reached its highest stem height during August and September 1998 (Figure 61). In late summer, *S. alterniflora* flowered and by late fall (October, 1998), the grasses died back and showed no growth until the following spring (May 1999).

A similar growth pattern was described by Hines (1989) during a study of sulfate reduction in a northern New England salt marsh (upper region of Great Bay, NH). Although leafs and aerial stems of *S. alterniflora* die back each year, the underground rhizomes are perennial structures (Dibner, 1978). Plants can transport oxygen to their roots, which contain some air-filled tissue. Hence, they are not asphyxiated when they are inundated by the tide (Dibner, 1978).

The 1998 and 1999 data were used to evaluate and compare the stem height growth and density of *S. alterniflora* between the different amendments. The blade height and density data were not normally distributed, typical of plant population growth, so they were log transformed for statistical analysis. The tallest stems were present in September 1998, July-August 1998 and August 1998 for the NO_3^-, control and air plots, respectively (Table 33). The *S. alterniflora* stem density in the control plot showed a decreasing trend during the 1998 season compared to the treatment plots (Figure 62).

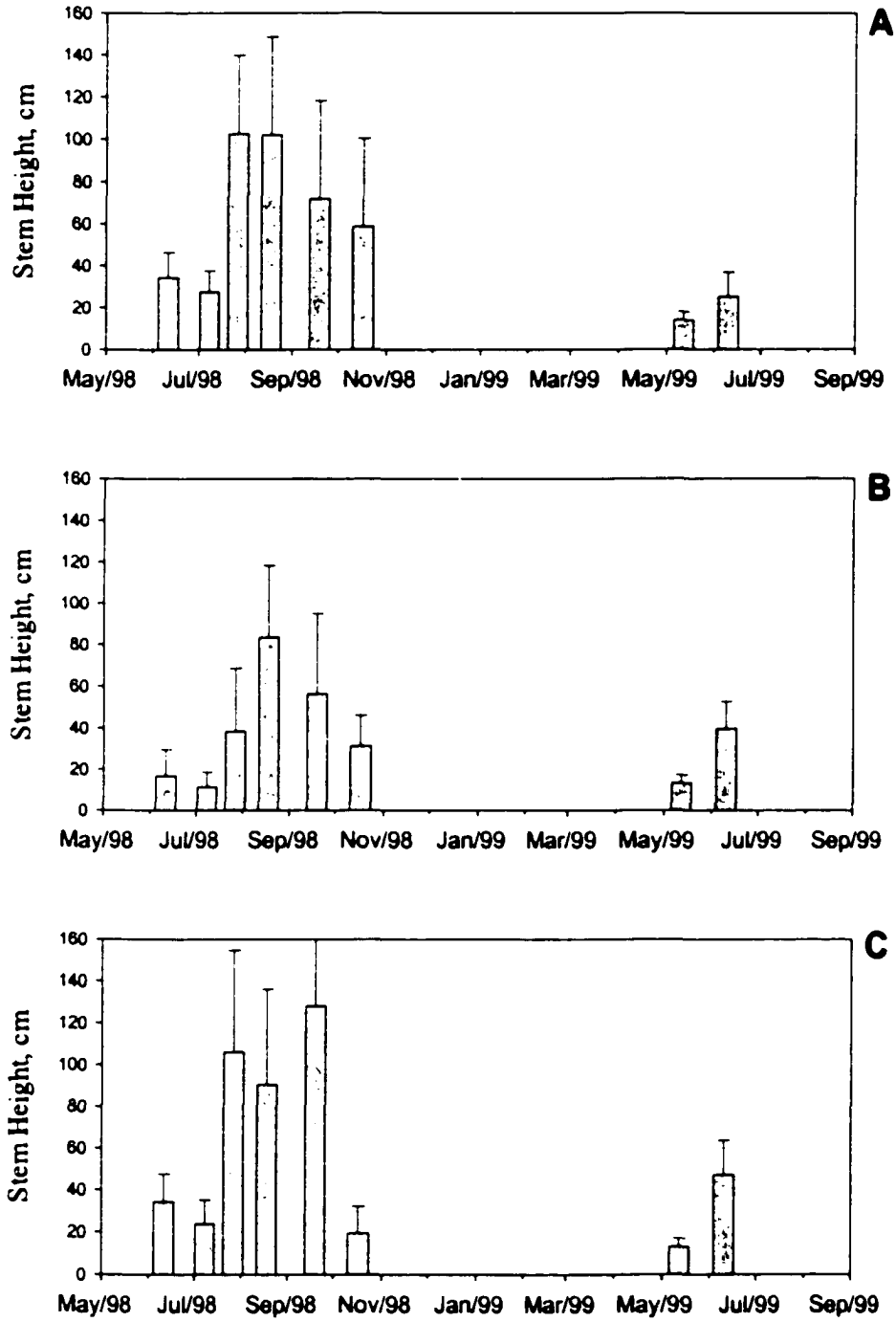


Figure 61. *S. alterniflora* stem heights. A) control, B) air and C) NO_3^-

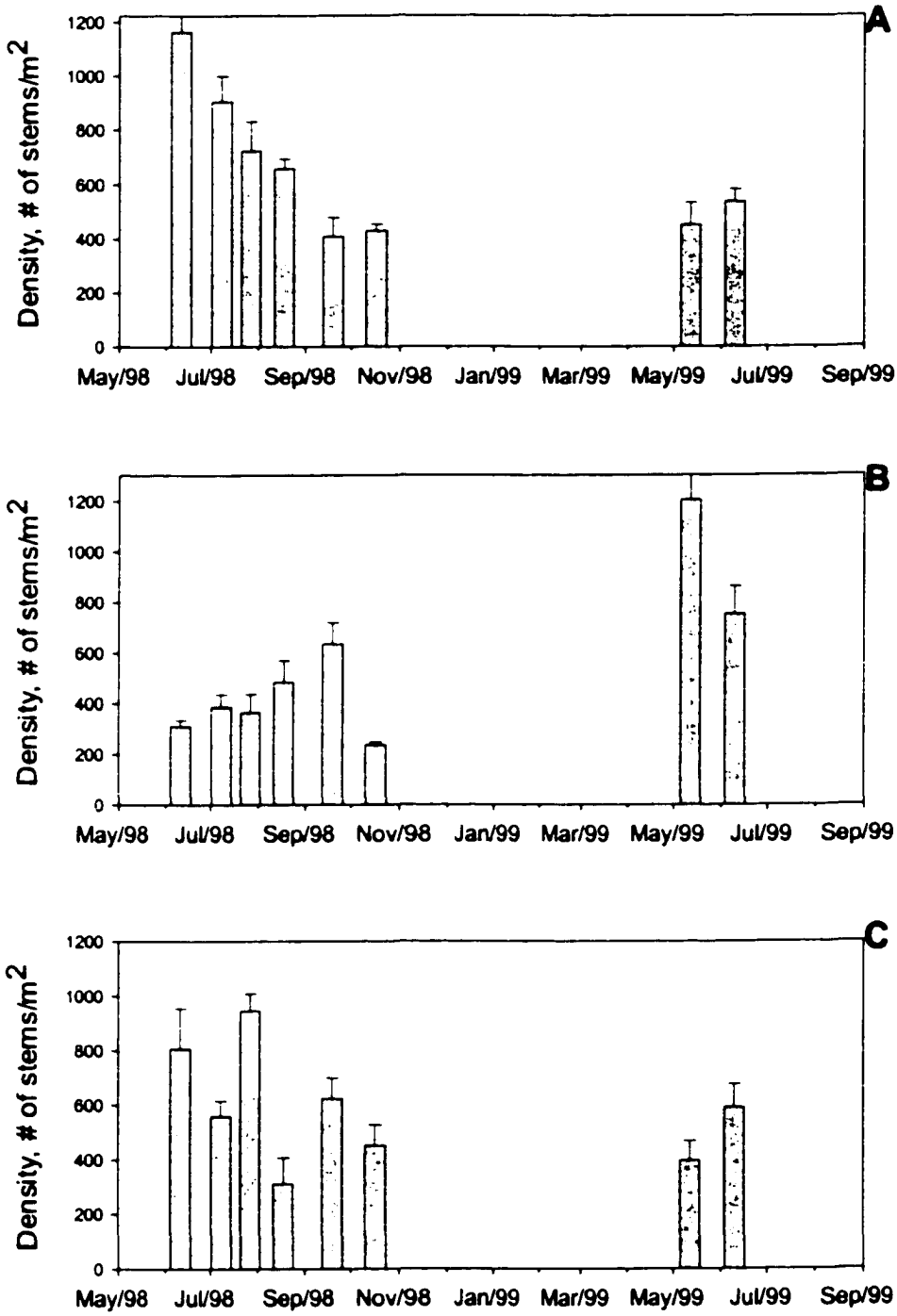


Figure 62. *S. alterniflora* densities. A) control, B) air and C) NO_3^-

Table 33. *S. alterniflora* stem height during the months with the tallest plants.

cm	Control		Air			NO_3^-		
	July, August 1998	**September October 1998**	**August 1998**	**July, September October 1998**	**June 1999**	**September 1999**	**July, August 1999**	**June 1999**
Mean	88.5	46.9	73.6	33.1	37.4	117.8	83.0	43.8
Std. Dev.	1.9	2.5	1.8	2.5	1.47	1.8	2.0	1.6
Max.	171.0	164.1	134.0	146.9	68.1	170.8	169.1	74.0
Min.	9.0	6.0	12.0	3.0	10.0	12.0	12.0	6.0

The *S. alterniflora* mean densities were not significant different between the different treatments (Tukey-Kramer, $p<0.05$) (Table 34). The densities in the air plot on May and June 1999 were significantly higher than the other events for this treatment (Tukey-Kramer, $p<0.05$.). It is possible that the subplots sampled had a high number of small plants in the quadrant counted compared to the other treatments.

Table 34. *S alterniflora* during the months with the highest densities.

# of stems/m^2	Control		Air		NO_3^-	
	June-July 1998	May-June 1999	June-July 1998	May-June 1999	June-July 1998	May-June 1999
Mean	21.6	11.5	8.3	22.8	13.4	11.5
Std. Dev.	8.8	5.7	6.1	10.5	9.3	7.1
Max.	27.2	16.3	12.1	31.5	19.3	17.4
Min.	16.0	6.7	4.4	14.0	7.5	5.6

4.9.2 Treatment Effect

The plant stems in the control and NO_3^- treatments were significantly higher than those in the air plot when they were compared using one-way ANOVA ($p<0.05$). However, this was true even at the beginning of the study before the amendments were added when the air plot had the lowest stem height (Figure 61). This indicated that the amendments did not have a significant effect in the *S. alterniflora* height over the course of the study.

4.9.2.1 IVRM Analysis

The IVRM was used to determine if the NO_3^- and air amendments were related to the *S. alterniflora* stem length. The IVRM was basically the same used initially to observe the effect of the treatments on the degradation of TPH (See Section 4.4), except that the log of *S. alterniflora* stem height was used as a variable. The July, August and September 1998 stem height data were used because these were the months with the tallest plants and most stable growth (Table 33). The model indicated that none of the plots had a higher effect in the *S. alterniflora* stems (Table 35). The control and NO_3^- had higher *S. alterniflora* stems compared to the air (Tukey-Kramer test, $p<0.05$). This agreed with the earlier findings indicating that the treatments (control, air or NO_3^-) did not have a significant effect on the plants' growth.

Table 35. Treatment slopes and their probabilities using the IVRM.

Treatment	Treatment Slopes ($1x10^{-4}$)	p value
	Log stem height	Log stem height
Control	0.81	**0.002**
Air	1.76	**<0.0001**
NO_3^-	1.81	**0.0142**

Shading indicates a significant $p \leq 0.20$

4.9.3 *S. alterniflora* and HC Biodegradation

S. alterniflora stem height data was plotted against the degradation of the TPH (SC and LC aliphatics and aromatics) to observe possible relationships during the study (Figures 63-65). None of the slopes (Table 36) were significantly different from zero (two tailed *t*-test, $p<0.05$) indicating that there was no apparent relationship between the *S. alterniflora* stem height and the degradation of the SC and LC aliphatics or aromatic. Similar results to the obtained in this study at the Fore River Creek salt marsh were observed by Wright *et al.* (1996). They found that *S. alterniflora* did not significantly influence the degradation of artificially weathered Arabian crude oil in a salt marsh (Galveston, TX) mesocosms.

Table 36. Slopes of the best-fit linear relationship between *S. alterniflora* growth and TPH

	Control		Air		NO_3^-	
	Slope	r^2	Slope	r^2	Slope	r^2
SC Aliphatics	-0.191±1.132	0.0282	-0.083±0.735	0.0130	+0.089±0.449	0.0388
LC Aliphatics	-0.177±1.468	0.0130	+0.034±0.736	0.0022	+0.075±0.456	0.0267
Aromatics	-0.277±1.236	0.0485	-0.280±0.639	0.1645	-0.025±0.601	0.0017
TPH	-0.218±1.512	0.0207	-0.125±0.804	0.0239	+0.045±0.546	0.0069

Buresh *et al.* (1980) found that nitrogen availability is an important factor influencing *S. alterniflora* productivity in salt marshes (Louisiana). Although, it is likely that in the Fore River Creek salt marsh, NO_3^- did not enhance S. *alterniflora* growth vs. the control because this was used rapidly by *in situ* microorganisms.

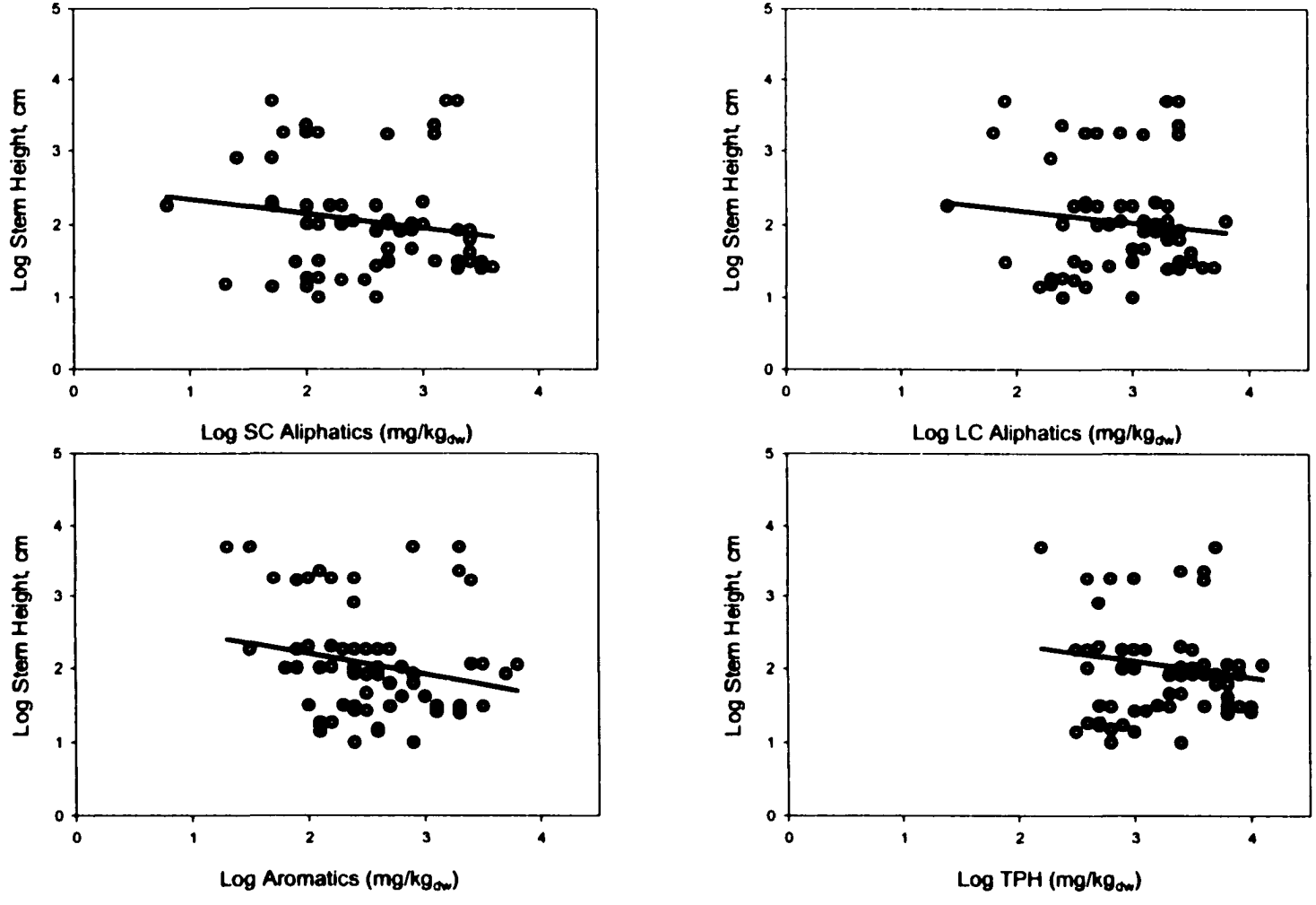


Figure 63. Relationship between the log TPH concentration and log *S. alterniflora* stem height for the control. Data on regression is presented in Table 38.

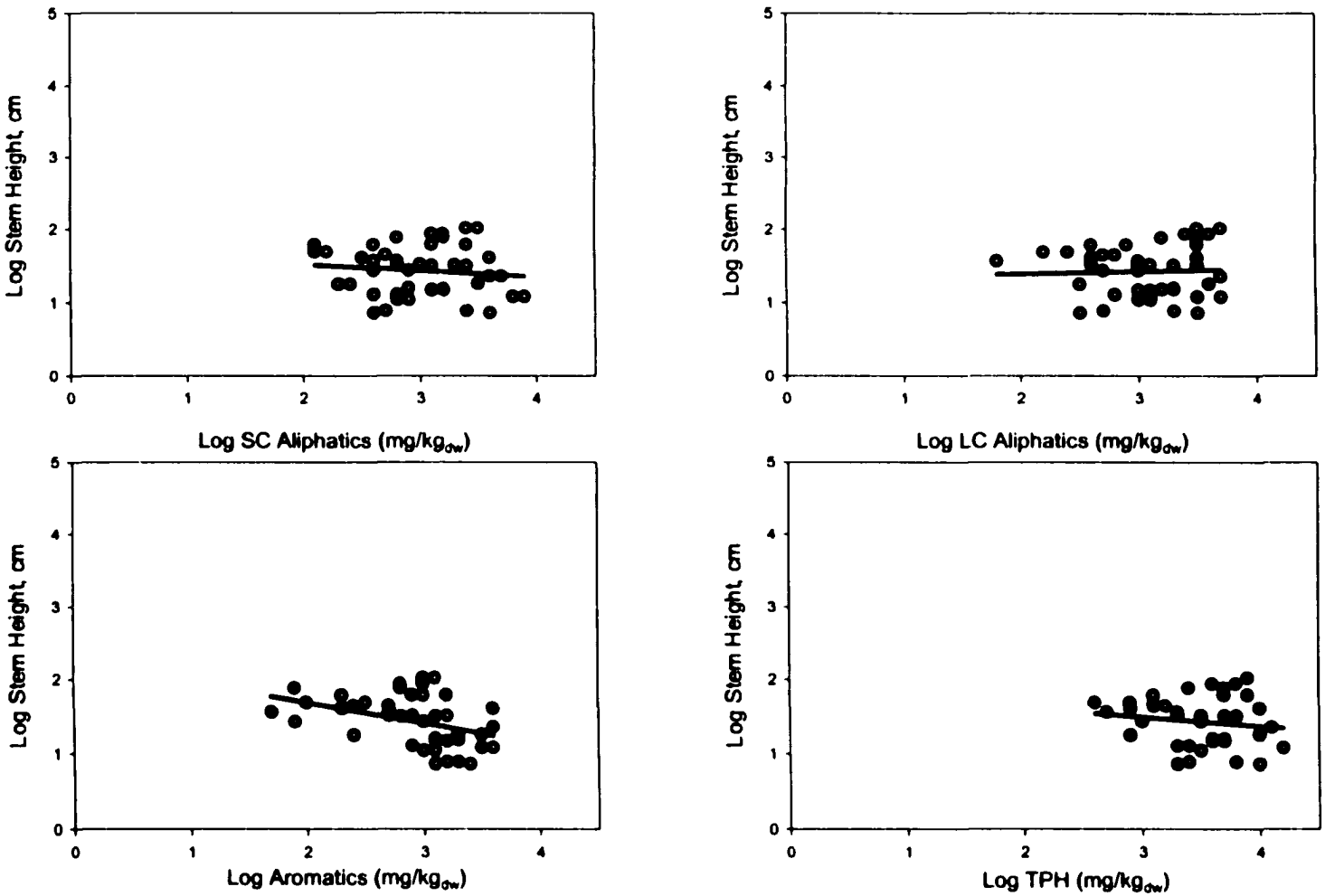


Figure 64. Relationship between the log TPH concentration and log *S. alterniflora* stem height for the air amendment. Data on regression is presented in Table 38.

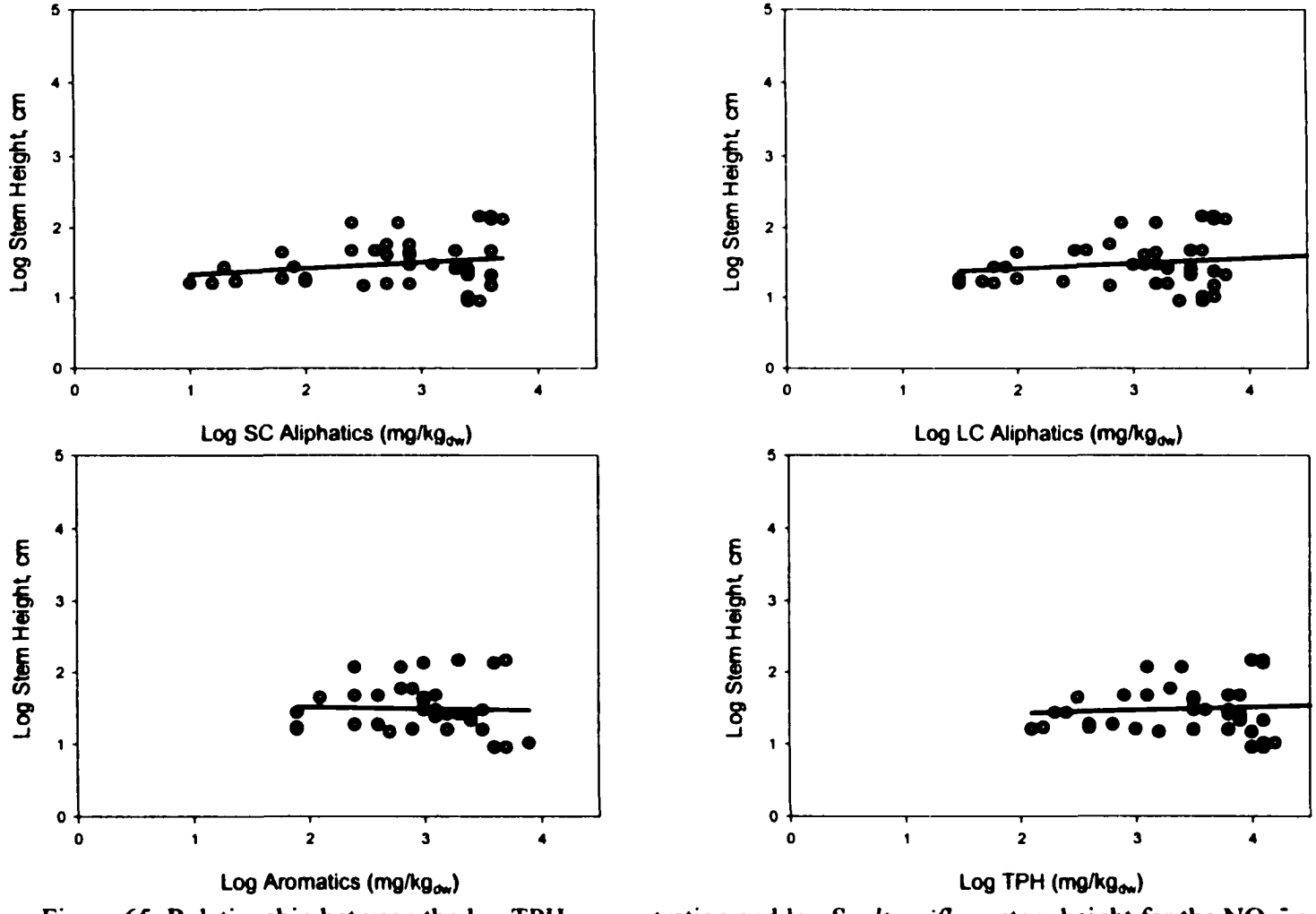


Figure 65. Relationship between the log TPH concentration and log *S. alterniflora* stem height for the NO_3^- amendment. Data on regression is presented in Table 38.

Burns and Teal (1980) reported that after 16 months of an oil spill, there was an inverse correlation between the amount of oil in surface sediments and plant growth (*S. alterniflora*). However, they found that areas showing concentrations greater than 1,000 to 2,000 mg No. 2 fuel oil/kg in the sediments contained non-living plants. The results obtained during the Fore River Creek salt marsh disagreed with Burns and Teal's study because *S. alterniflora* was growing in sediments contaminated with concentrations ~ 5,000 mg/kg_{dw} after a similar period of time. There was no correlation between the TPH and the *S. alterniflora* growth. DeLaune *et al.* (1979) reported that *S. alterniflora* can apparently tolerate large amounts of crude oil without any direct short-term harmful effect on standing crop biomass.

During the sampling process in the Fore River Creek salt marsh, catwalks were used to avoid damaging the plants and sediments in the treatment plots. However, because the limited number of platforms some damage was caused outside and on the periphery of the plots. Foot and mechanical traffic is known to damage vegetation and drive the HCs into the anaerobic layer of the sediments where they may persist for decades (Hoff, 1995). The foot traffic was clearly detrimental as the track remained visible for months after sampling was done for the season (Figure 66). However, there was no relationship between the plots located in the border of the plots and low biodegradation of TPH (See Section 4.9.4).

4.9.4 *S. alterniflora* Coverage

The marsh is a habitat with high rates of microbial activity that are strongly affected by plant growth and activity (Hines *et al.*, 1999). Therefore, it was important to assess the potential impact of *S. alterniflora* cover on TPH concentration.

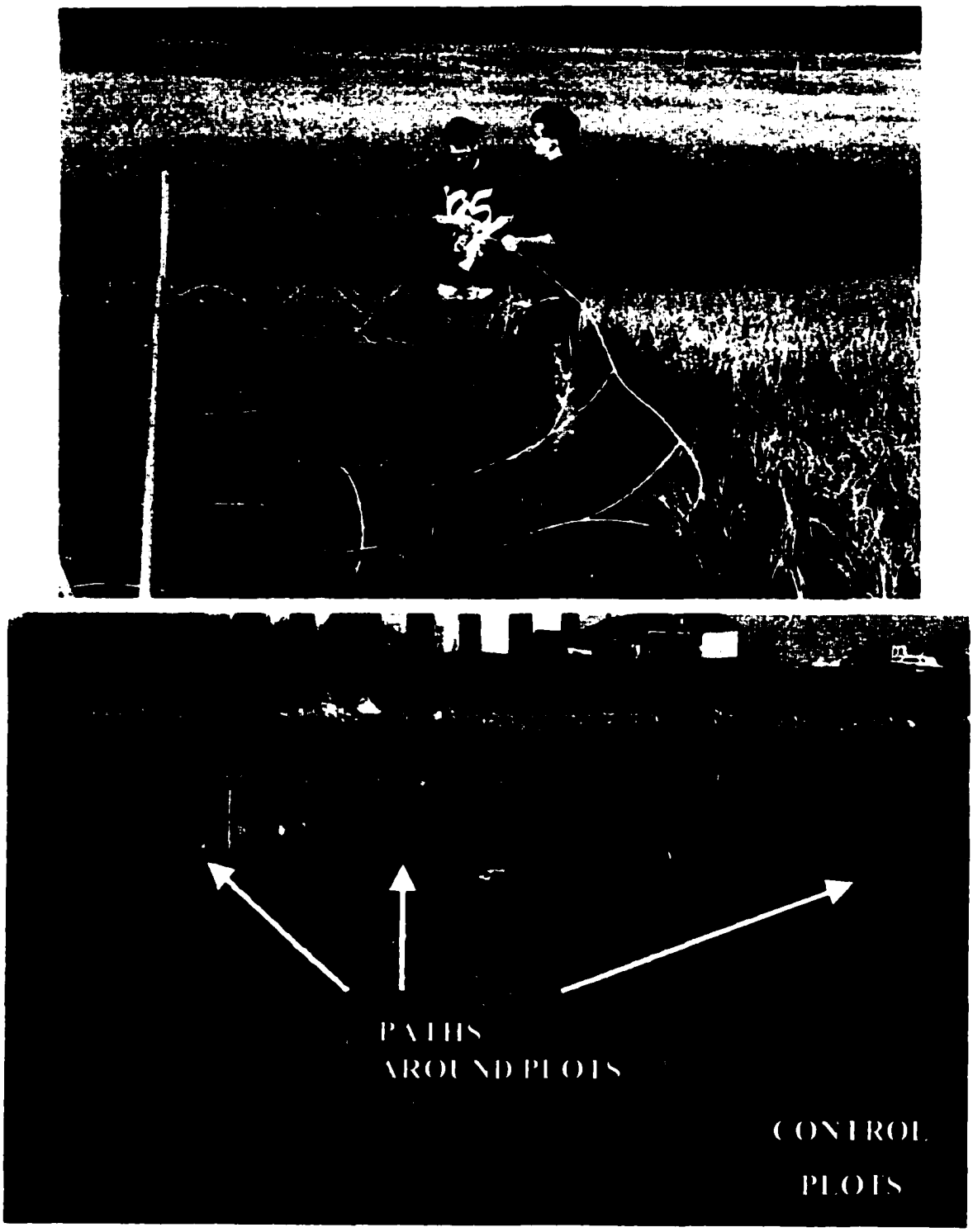


Figure 66. Damage caused outside of the plots and around the treatments by pedestrian traffic.

Because the plots were initially selected during the Spring 1998, when no plants were no growing, it was difficult to observed whether the plant coverage in each plot was homogeneous (Figure 67). Data from the NO_3^- plot in August 1998 were analyzed because it was the only time when two of the subplots sampled (c and d) did not have plants, while the other two subplots (a and d) did. The stem height and density data from the NO_3^- subplots (a, b, c, d) were compared (Figure 68) (Table 37) using a Tukey-Kramer test ($p<0.05$). The SC aliphatic and the aromatic concentrations were not significantly different between the barren and *S. alterniflora* subplots, but the LC aliphatic concentrations were. In addition, the IVRM indicated that NO_3^- had a significant effect on the biodegradation of LC aliphatics during the 1998 season. There is not enough statistical evidence during this study to assess if the *S. alterniflora* cover affected TPH degradation at the Fore River Creek salt marsh.

Little research has been conducted to assess the capacity of vegetation to enhance the degradation of oil in marshes. Lin and Mendelssohn (1998) evaluated two salt marsh plants, *Juncus roemerianus and S. alterniflora*, in the biodegradation of artificially weathered crude oil (45,000 mg/kg_{dw}). They also found that *S. alterniflora* did not enhance degradation compared to the control while *J. roemerianus* did most likely due to its higher biomass and transpiration rates.

4.10 Summary

One of the major objectives of this dissertation was to determine if the addition of oxygen (added as air) and NO_3^- could enhance the *in situ* biodegradation of HCs

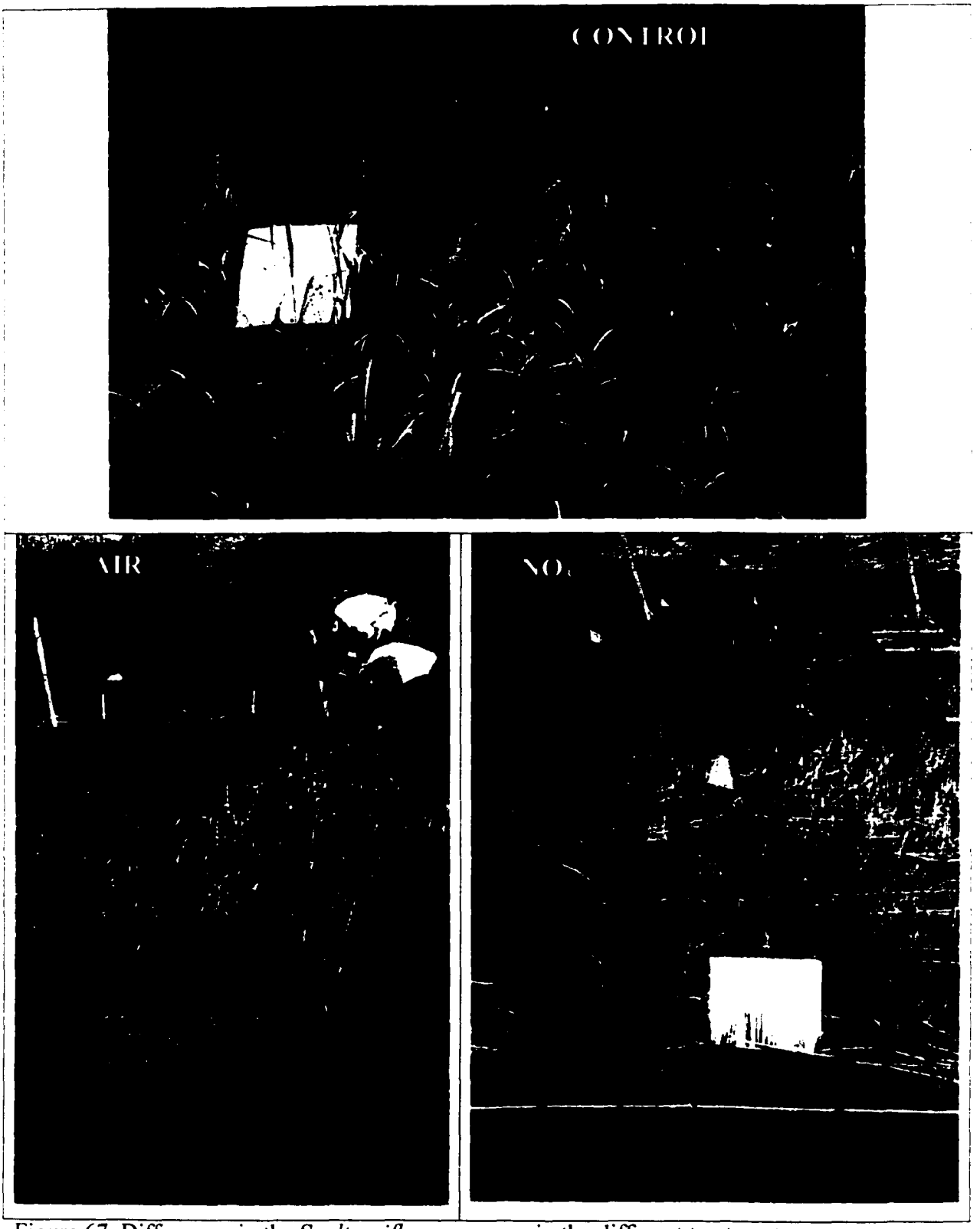


Figure 67. Differences in the *S. alterniflora* coverage in the different treatments.

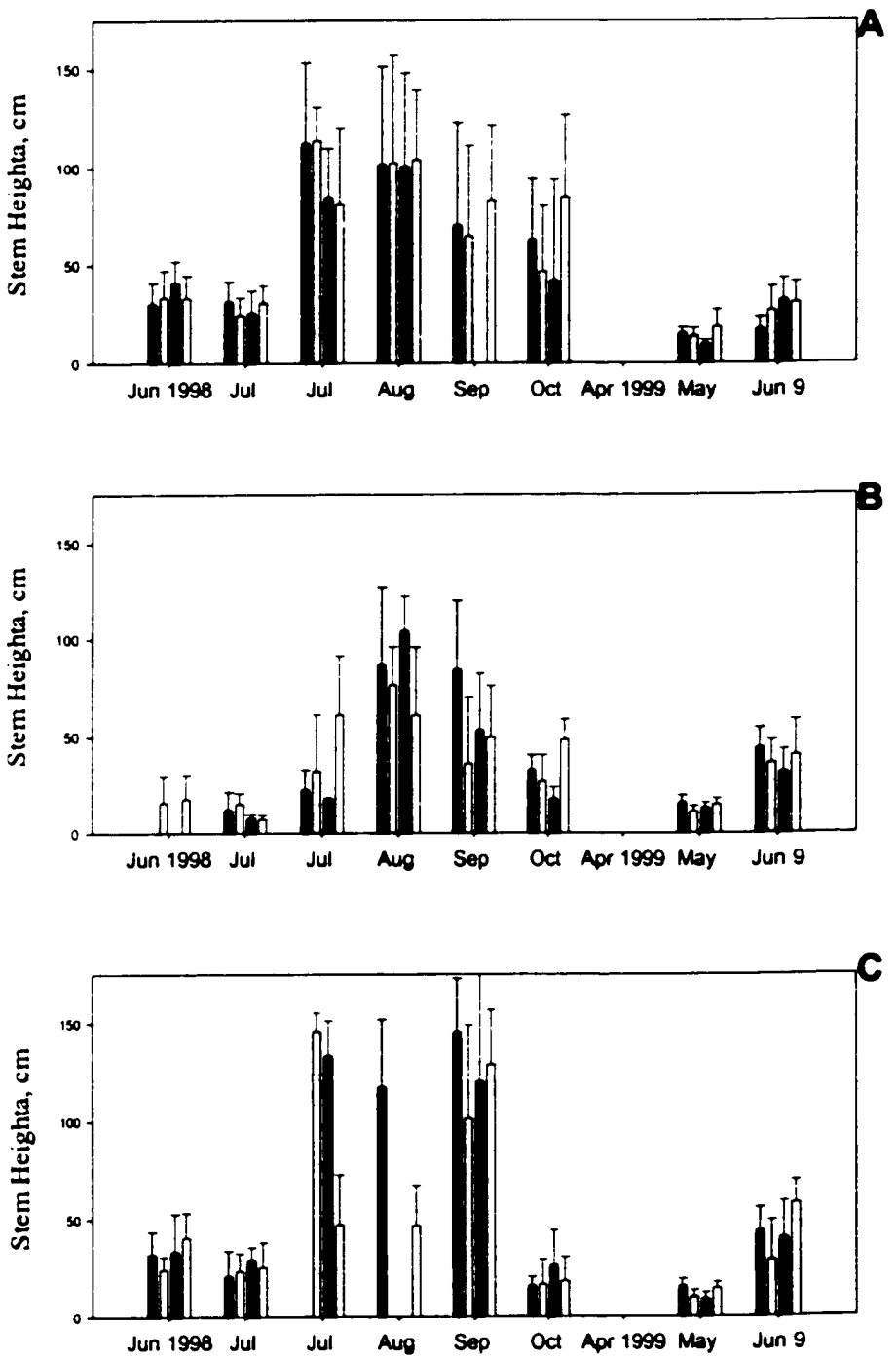


Figure 68. *S. alterniflora* stem height in the subplots (a,b,c,d). The solid bars are the mean of stem height measurements per subplot, and the error bars represent one standard deviation. A) control, B) air and C) NO_3^-

Table 37. *S. alterniflora* growth and SC and LC aliphatic and aromatic concentrations for the NO_3^- plot during August 1998.

Subplot	*S. alterniflora* growth		Concentration (mg/kg_{dw}) (n=2)		
	# Plants	Stem height (cm)	SC aliphatics	LC aliphatics	Aromatics
a	18	117.4±34.3	1,417±553	2,106±311	1,065±272
b	-	-	3,665±394	4,350±787	3,330±1,789
c	-	-	4,395±235	5,457±462	2,581±1,257
d	11	46.8±20.5	2,754±1,146	3,254±467	1,296±141

compared to the natural attenuation process in an oil-contaminated salt marsh. During the overall study (1998-1999), the addition of oxygen and NO_3^- as TEAs did not enhance the *in situ* degradation of TPH compared to natural attenuation. Because the study was conducted ~19 months after the *Julie N* spill and considering that the Fore River salt marsh is a low energy system, biodegradation was considered to be the main natural attenuation process acting on the TPH removal in all of the plots. Over the one year period, none of the treatments had significantly higher biodegradation rates than observed in the control plot for the different TPH fractions (SC and LC aliphatics, and aromatics). However, during the 1998 season (Summer and Fall), the addition of oxygen and NO_3^- yielded significantly higher degradation rates for the SC aliphatics and NO_3^- also yielded significantly higher degradation of the LC aliphatics.

The low nitrogen concentrations observed in the porewater samples and mass transfer limitations could be the main factors that limited the *in situ* TPH biodegradation during the Fore River Creek study. Based on porewater analysis, the *in situ* NO_3^- concentrations in the NO_3^- plot were low (<0.15 mg N/L). Also, stoichiometry indicated

that the amount of NO_3^- added was ~11% of that needed to degrade all the TPH using NO_3^- as a TEA. The degradation obtained in the air plot also indicated that the oxygen delivered into the sediments was not enough to enhance aerobic TPH biodegradation over that observed in the control. The low biodegradation rates in the treatment plots could also indicate that the treatments were not properly delivered into the marsh sediments by the horizontal well system.

Based on the results obtained during this dissertation research it will be necessary to conduct more studies to understand better the fate of the amendments and the hydrologic conditions present in the marsh sediments to optimize the biodegradation rates. Although, subsurface horizontal wells (inserted 0.6 m apart) could be an acceptable system to deliver the amendments directly into the sediments of a small oil-contaminated salt marsh, this system is not practical for larger marshes. A more efficient subsurface injection system and longer residence times of the amendments will be necessary.

CHAPTER V

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

5.1.1 Evaluation of Screening Methods for TPH and Site Selection

During the first part of the study, the EPA and ASTM Methods were evaluated as screening tools for TPH determination. The MDL and LOQ for the ASTM Method (52 mg/kg_{dw} and 180 mg/kg_{dw}, respectively) were lower than for the EPA Method (773 mg/kg_{dw} and 2,060 mg/kg_{dw}, respectively) indicating that small TPH concentrations would be better quantified using the ASTM Method. The precision and accuracy were within the published ranges for each method when they were evaluated with unspiked and spiked laboratory samples (CS and OS). The precision of the two methods was NSD, but was out of the published ranges when TPH-contaminated sediments from the Fore River Creek salt marsh were evaluated. The percent recovery (109±8%) for the ASTM Method was within the recommended ranges and SH than for the EPA Method (63±7%). The ASTM Method also had a higher sample throughput (~30 samples per day; 10 min/sample) and was less expensive (~$15) compared to the EPA Method. Overall, the ASTM Method was better for screening TPH in the Fore River Creek salt marsh

sediments. However, the presence of NOM in the marsh caused a significant overestimation of TPH in the sediments by the ASTM Method and the variability was so high that >50 replicates would have needed to obtain a detectable difference of 500 mg TPH/kg_{dw}. Using the ASTM Method, it was possible to determine the plots with higher TPH concentrations in the Fore River Creek salt marsh.

During the study, the MADEP Method was used to analyze the TPH in the sediments. This method could detect specific fractions of TPH (SC and LC aliphatics, and aromatics) and was recommended by NHDES for the TPH analysis. Although some initial problems occurred during the first sampling event, the MADEP Method exhibited acceptable QC, except that the recovery of the surrogates (COD and OTP) was lower than that recommended by MADEP. This effect was the result of the NOM in the sediments and was consistent throughout the study. Therefore, the data could be used for comparative purposes as long as the QC criteria established were consistently achieved.

5.1.2 TPH Biodegradation

During the first season of the study (Summer and Fall 1998), there was a significant degradation of the SC and LC aliphatics and aromatics, indicating that natural attenuation was occurring. In the air plot, removal of the SC aliphatics and aromatics was significantly greater than in the control. The NO_3^- plot had a significantly greater reduction of the SC and LC aliphatics compared to the control. Because the *Julie N* spill occurred ~19 months before the study began and the Fore River Creek salt marsh is a low energy system, it is likely that biodegradation accounted for most, if not all, of the TPH removal observed in the plots. The biodegradation rates observed during 1998 for the SC aliphatics were significantly higher in the treatments (27.6±17.5 mg/kg_{dw}/d and

20.6±10.9 mg/kg_{dw}/d for the air and NO_3^- plots, respectively) than in the control (13.1±12.8 mg/kg_{dw}/d). For the LC aliphatics, the biodegradation rate for the NO_3^- plot (23.7±13.0 mg/kg_{dw}/d) was significantly higher than the control plot (13.6±12.2 mg/kg_{dw}/d). The biodegradation rate for the aromatics was NSD than in the control. During the 1999 season, none of the plots (control, air and NO_3^-) exhibited a significant reduction in the TPH fractions. This may have been because less TPH was present in the marsh sediments limiting the degradation rates and because there were problems with the amendment delivery systems.

The overall results after a year of study for the Fore River Creek salt marsh indicated that significant degradation of SC and LC aliphatics and aromatics occurred in the control as a result of natural attenuation (as indicated by the IVRM model and degradation rates) and neither the air nor NO_3^- amendments enhanced degradation over this natural process.

Natural biodegradation rates could have been limited during the study because of the low porewater nitrogen concentrations, mass transfer limitations and the presence of less biodegradable HCs remaining at the end of the study. The ability of the amendments to enhance biodegradation could have been limited because there was not a complete distribution of the amendments into the sediments, mass transfer limitations of TEAs to the microorganisms, and the low mass application of NO_3^-/air used during the study. During the 1998 season (Fall and Summer), the flux of TEAs was probably the main factor limiting the TPH degradation. However, during the 1999 season (Spring), the flux of biodegradable TPHs was most likely the limiting factor because the HC concentrations were much lower limiting mass transfer.

5.1.3 Bacterial Abundance

The use of INT as an indicator for the presence of active TPH-degrading microorganisms during the MPN procedure was more reliable than the observation of turbidity to assess presence/absence. This was evident during the first sampling events in June 1998 when a precipitate present in liquid media interfered with the readings creating false positives. There was no significant change in the abundance of TPH-degrading bacteria during the study. However, a significant increase was not expected because the microbial population was probably already adapted almost 19 months after the spill. The control was the only plot that had higher MPN counts associated with higher TPH concentrations. The air plot exhibited significantly higher MPN data, but this could not be correlated to higher biodegradation rates during the study.

5.1.4 Nutrient Analysis

The porewater NO_3^- concentration in the control plot significantly decreased from ~0.5 mg to ~0.01 mg NO_3^--N/L. This could have been related to seasonal patterns observed in marsh environments. The amendment concentration in the porewater of the NO_3^- plot was NSD than the porewater NO_3^- in the air treatment indicating that the NO_3^- injected weekly was either rapidly used supporting TPH or NOM biodegradation or removed by dilution (e.g., tidal action). The *in situ* concentrations were two orders of magnitude below the desired goal of 32 mg NO_3^--N/L used during the study. Based on stoichiometry, the 32 mg/L concentration desired in the porewater for use as a TEA supplied only 11% of the mass of NO3$^-$-N needed to maintain and degrade the TPH. Previous studies reported that to avoid nitrogen limitation during TPH biodegradation, it is necessary to maintain a concentration in the range of 0.8 to 10 mg N-NO_3^-/L in the

porewater. However, most of these studies have been conducted in (non organic-rich) sandy beaches (Bragg *et al.*, 1994; Venosa *et al.*, 1996; Zhu *et al.*, 2001). The NO_3^- mass application rate required for denitrification of the TPH within the Fore River Creek salt marsh environment was much higher than for beaches because of the TEA demand exerted by the NOM (available as a carbon source) and the demand for NO_3^- by growing plants (i.e., *S. alterniflora*).

The porewater ammonia concentration found during the Fore River Creek study ranged from 1.8 to 3.0 mg $N\text{-}NH_4^+/L$ indicating that nitrogen, as a nutrient (not as TEA), was probably also limiting the TPH biodegradation. The lower PO_4^{-3} concentrations observed during the second season (1999) (~0.005 mg $PO_4^{-3}\text{-}P/L$) could also have contributed to the lower TPH biodegradation observed during this period.

5.1.5 Plant Growth

There was no relationship between *S. alterniflora* heights and densities and the decrease of TPH during the study. Some areas of the air and NO_3^- plots were not completely covered by *S. alterniflora*. Although there was not enough data for statistical analysis, it seems that the absence of *S. alterniflora* affected the TPH biodegradation. This could indicate that the location of the plots in the Fore River Creek salt marsh could have affected the study.

During the overall study, the addition of TEAs (oxygen and NO_3^-) did not enhance the biodegradation over the natural attenuation monitored in the control plots. However, during the 1998 season, the air and NO_3^- plots showed a significantly higher degradation of SC aliphatics. The NO_3^- plot also had significantly higher degradation of LC aliphatics. Problems with the delivery system, the low *in situ* nitrogen

concentrations, and mass transfer could have slowed subsequent biodegradation of TPH in the Fore River Creek marsh.

More studies are necessary to better understand the fate of the amendments and the hydrologic conditions present in the marsh sediments. The optimization of these parameters could increase biodegradation rates. Closely-spaced subsurface horizontal wells (0.6 m) used in the Fore River Creek salt marsh could be an acceptable system to deliver the amendments directly into the sediments of a small oil-contaminated marsh. However, this system is not practical for larger marshes. A more efficient subsurface injection system that achieves longer residence times of the amendments will allow longer contact time between the microorganisms, TPH and TEAs.

5.2 Recommendations for Future Research

Based on the results obtained during the Fore River Creek study, the concentration of NO_3^- injected could be increased (~10x) to obtain the necessary *in situ* porewater concentration. In addition, NH_4^+ should also be added because of its low *in situ* concentration in the marsh sediments. The frequency of the NO_3^- injection should be evaluated with the help of tracer studies because this study did not maintain a sufficient NO_3^- concentration *in situ*. The fate of the amendments should be assessed using hydrological and chemical studies. In addition, NO_3^- and nutrients have to be monitored more frequently.

The use of biomarkers would help to differentiate between the biodegradation of TPH and the physical-chemical losses. An ideal biomarker is not formed during the weathering or biological process and is non-biodegradable or relatively resistant to

biodegradation (Zhu *et al.*, 2001). Although hopane (17α(H),21β(H)-hopane) has been recognized as an ideal biomarker for oil biodegradation, its concentration in refined product such as No. 2 fuel oil (present at the Fore River Creek salt marsh) is very reduced. Therefore, phenanthrenes, anthracenes and chrysenes could be used as biomarkers to assess biodegradation.

Toxicological analysis could be conducted to evaluate the effect of the treatments. These analyses could be conducted in parallel during the study to evaluate the presence of toxic compounds. A series of bioassay tests with organisms from different trophic levels could be used (e.g., bacteria, algae and microinvertebrates).

MPN counts for aliphatics and aromatics degraders (Wrenn and Venosa, 1996) could be evaluated to assess community changes and their relationship during the TPH fraction degradation (SC and LC aliphatics and aromatics). In addition, it would be useful to determine the diversity of bacterial communities during the biodegradation process. Molecular methods, such as the polymerase chain reaction (PCR) and denaturing gradient gel electrophoresis (DGGE), could be useful tools to understand the microbial ecology during bioremediation. These techniques monitor microorganisms that are difficult to growth under laboratory conditions.

Finally, alternative subsurface delivery systems could be implemented and optimized associated with hydrological and fate and transport studies to maximize the distribution of the amendments into the marsh sediments to more effectively enhance *in situ* bioremediation.

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APPENDIX A.1 SCREENING METHODS (EPA AND ASTM METHODS)

Table A1.1 TPH using EPA Method in laboratory samples.

Sample	sample wt [g]	Flask weight [g]	dry fraction [g]	TPH [mg/Kgdw]	mean [mg/Kgdw]	std.dev. [mg/Kgdw]	% Recovery %P	std. dev s(%P)
CS	10.00	0.0003	1.00	30.0				
CS	10.03	0.0001	1.00	10.0				
CS	10.01	0.0014	1.00	139.9				
					60	**70**		
CSS	10.00	0.3556	1.00	35563.8			0.71	
CSS	10.00	0.3704	1.00	37046.1			0.74	
CSS	10.00	0.3721	1.00	37217.9			0.75	
CSS	10.00	0.4146	1.00	41468.8			0.83	
					37824	**2541**	**0.76**	**0.05**
OS	10.00	0.0000	0.35					
OS	10.01	0.0000	0.35					
OS	10.00	0.0140	0.38	3678.8				
OS	10.02	0.0039	0.36	1094.1				
					2386	**1828**		
OSS	10.00	0.3474	0.38	91287.1			0.68	
OSS	10.02	0.3738	0.35	105676.4			0.73	
OSS	10.00	0.3153	0.37	86360.3			0.61	
OSS	10.00	0.4163	0.35	117549.6			0.81	
OSS	10.01	0.3427	0.35	96670.7			0.67	
					99509	**12370**	**0.71**	**0.09**
PCS	10.00	0.1202	1.07	11277.7				
PCS	10.01	0.1563	1.07	14650.1				
PCS	10.00	0.0965	0.90	10676.8				
					12202	**2142**		
PCSS	10.00	0.5746	0.90	63573.8			0.90	
PCSS	10.00	0.5363	0.96	56025.1			0.82	
PCSS	10.00	0.5289	1.07	49623.7			0.81	
PCSS	10.00	0.5281	1.07	49548.7			0.81	
					54693	**6653**	**0.84**	**0.04**

CS=clean sand OS=organic soil PCS=pre-contaminated sand S=spiked (50,000 mg/kg)

Table A1.2 TPH using EPA Method in salt marsh samples.

Sample	sample wt [g]	Flask weight [g]	dry fraction [g]	TPH [mg/Kgdw]	mean [mg/Kgdw]	std.dev [mg/Kgdw]	% Recovery %P
Site 1-1	10.00	0.0060	0.33	1836.5			
Site 1-2	10.00	0.0019	0.28	675.0			
Site 1-3	10.00	0.0162	0.29	5620.6			
Site 1-4	10.00	0.0165	0.26	6313.7			
					3611	**2776**	
SSite 1-1	10.00	0.0154	0.25	6099.4			0.71
Site 2-1	10.00	0.0098	0.33	3004.4			
Site 2-2	10.00	0.0018	0.33	540.1			
Site 2-3	10.00	0.0126	0.20	6277.5			
Site 2-4	10.01	0.0056	0.31	1821.9			
					2911	**2460**	
SSite 2-2	10.00	0.0122	0.31	3876.6			0.55
Site 3-1	10.01	0.0071	0.24	2984.8			
Site 3-2	10.02		0.26				
Site 3-3	10.01	0.0048	0.25	1925.1			
Site 3-4	10.02	0.0254	0.34	7407.1			
					4106	**2908**	
SSite 3-3	10.01	0.0170	0.35	4900.8			0.64
Site 4-1	10.00	0.0001	0.21	47.5			
Site 4-2	10.00	0.0003	0.22	137.0			
Site 4-3	10.02	0.0178	0.28	6261.3			
Site 4-4	10.01	0.0106	0.28	3762.7			
					2552	**3018**	
SSite 4-4	10.01	0.0223	0.27	8327.4			0.62
CS	10.00	0.0000	0.79	0.0			
CS	10.01	0.0039	0.77	503.3			
CS	10.01	0.0000	1.00	0.0			
CS	10.00	0.0030	1.00	300.0			
					201	**246**	
CSS	10.00	0.0131	1.00	1310.2			0.69

S=spiked (1,900 mg/kg)

Table A1.3 TPH using ASTM Method in laboratory samples.

sample	Absorb 254 nm	dilution	Aprox [mg/L]	Aprox [mg/Kg]	dry fraction [g]	TPH [mg/Kgdw]	mean [mg/Kgdw]	std dev [mg/Kgdw]	%Recovery %P	Mean X%P	Std dev s%P
CS	0.037	1	6.4	63.9	1.00	63.9					
CS	0.025	1	3.7	36.6	1.00	36.7	**50**	**19**			
OS	0.680	2	10.0	100.2	0.35	282.8					
OS	0.749	2	9.1	91.1	0.35	257.2	**270**	**18**			
CSS	0.791	2	177.5	1774.8	1.00	1774.8			91.5		
CSS	0.839	2	188.4	1883.8	1.00	1884.1	**1829**	**77**	97.1	**94.3**	**4.0**
OSS	0.680	2	152.3	1523.0	0.55	2748.0			73.6		
OSS	0.749	2	168.0	1679.5	0.55	3030.5	**2889**	**200**	81.6	**77.6**	**5.7**

CS=clean sand OS=organic soil S=spiked

Table A1.4 TPH using ASTM Method in salt marsh samples.

sample	Absorb 254 nm	dilution	new absorb 254 nm	Aprox [mg/L]	dry fraction [g]	TPH [mg/Kgdw]	mean [mg/Kgdw]	std dev [mg/Kgdw]
Site 1-1	2.714	6	0.454	101.0	0.33	1855.0		
Site 1-2	3.673	8	0.77	222.6	0.28	6327.9		
Site 1-3	1.233	7	0.236	51.5	0.29	1251.8		
Site 1-4	3.687	8	0.652	145.9	0.26	4467.5	**3476**	**2359**
Site 2-1	3.404	7	0.478	106.5	0.33	2284.6		
Site 2-2	0.907	2	0.505	112.6	0.33	675.6		
Site 2-3	1.435	7	0.251	54.9	0.20	1916.3		
Site 2-4	1.084	8	0.152	32.5	0.31	846.3	**1431**	**791**
Site 3-1	3.670	7	0.655	146.6	0.24	4319.0		
Site 3-2	3.673	9	0.491	109.4	0.26	11704.5		
Site 3-3	3.671	9	0.699	202.0	0.25	6487.3		
Site 3-4	3.669	10	0.757	192.5	0.34	4498.9	**6752**	**3444**
Site 4-1	3.009	7	0.422	93.8	0.21	3117.2		
Site 4-2	3.665	8	0.498	111.0	0.22	4054.9		
Site 4-3	3.673	10	0.29	63.8	0.28	14391.1		
Site 4-4	3.674	8	0.689	154.3	0.28	4387.2	**6488**	**5296**
CS	0.061	1	0.061	11.8	1.00	118.4		
CS	0.051	1	0.051	9.6	1.00	95.7	**107**	**16**

CS=clean sand

A1.5 TPH using ASTM Method in the candidates sites at the Fore River Creek salt marsh

sample	dilution	new absorb 254 nm	Aprox [mg/L]	corrected [mg/L]	Aprox [mg/Kgdw]	Mean [mg/Kgdw]	std dev [mg/Kgdw]
Plot A-1	15	0.654	153.3	2299.8	22997.6		
Plot A-2	8	0.542	126.8	1014.2	10142.2		
Plot A-3	8	0.702	164.7	1317.5	13175.4		
Plot A-4	8	0.232	53.3	426.5	4265.4		
						12645	**7830**
Plot B-1	8	0.111	24.6	197.2	1971.6		
Plot B-2	8	0.166	37.7	301.4	3014.2		
Plot B-3	8	0.158	35.8	286.3	2862.6		
Plot B-4	8	0.202	46.2	369.7	3696.7		
						2886	**710**
Plot C-1	8	0.571	133.6	1069.2	10691.9		
Plot C-2	8	0.337	78.2	625.6	6255.9		
Plot C-3	15	0.705	165.4	2481.0	24810.4		
Plot C-4	15	0.707	165.9	2488.2	24881.5		
						16660	**9624**
Plot D-1	15	0.715	167.8	2516.6	25165.9		
Plot D-2	15	0.716	168.0	2520.1	25201.4		
Plot D-3	8	0.517	120.9	966.8	9668.2		
Plot D-4	15	0.69	161.8	2427.7	24277.3		
						21078	**7619**
CS	1	0.051	10.4	10.4	104.3		
CS	1	0.07	14.9	14.9	149.3		
						127	**32**

A1.6 TPH using ASTM Method in the candidates sites at the Fore River Creek salt marsh

sample	dilution	new absorb 254 nm	Aprox [mg/L]	corrected [mg/L]	Aprox [mg/Kgdw]	Mean [mg/Kgdw]	std dev [mg/Kgdw]
Plot E-1	8	0.598	99.1	792.4	7924.3		
Plot E-2	8	0.389	49.6	397.2	3971.6		
Plot E-3	8	0.525	81.8	654.4	6543.7		
Plot E-4	8	0.715	126.7	1013.7	10137.1		
						7144	**2581**
Plot E-5	8	0.493	74.2	593.9	5938.5		
Plot E-6	8	0.43	59.3	474.7	4747.0		
Plot E-7	8	0.508	77.8	622.2	6222.2		
Plot E-8	8	0.716	127.0	1015.6	10156.0		
						6766	**2349**
Plot F-1	8	0.588	96.7	773.5	7735.2		
Plot F-2	15	0.574	93.4	1400.7	14007.1		
Plot F-3	8	0.621	104.5	835.9	8359.3		
Plot F-4	8	0.467	68.1	544.7	5446.8		
						8887	**3636**
Plot F-5	8	0.531	83.2	665.7	6657.2		
Plot F-6	15	0.574	93.4	1400.7	14007.1		
Plot F-7	8	0.58	94.8	758.4	7583.9		
Plot F-8	8	0.505	77.1	616.5	6165.5		
						8603	**3650**
CS	1	0.012	2.3	2.3	22.9		
CS	1	0.008	1.4	1.4	13.7	**18**	**6**

A1.7 TPH concentration in the split samples (ASTM and MADEP Methods).

sample	dilution	new absorb 254 nm	Aprox [mg/L]	corrected [mg/L]	Aprox [mg/Kg]	Mean [mg/Kgdw]	std dev [mg/Kgdw]
Plot C-1	9	0.602	137.4	1236.8	12368.3		
Plot C-2	9	0.614	140.2	1261.6	12615.7		
						12492	**175**
Plot D-1	9	0.514	117.3	1055.4	10554.3		
Plot D-2	9	0.511	116.6	1049.2	10492.4		
						10523	**44**
Plot E-1	13	0.679	155.1	2015.8	20158.0		
Plot E-2	9	0.645	147.3	1325.5	13254.7		
						16706	**4881**
Plot F-1	9	0.352	80.2	721.5	7214.8		
Plot F-2	9	0.339	77.2	694.7	6946.9		
						7081	**189**
CS	1	0.009	1.6	1.6	16.0		
CS	1	0.015	3.0	3.0	29.8	**23**	**10**

APPENDIX B.1 CALIBRATION CURVES FOR THE MADEP METHOD

B.1 Calibration for the MADEP Method

B.1.1 Aliphatic Calibration

	Area accounts					Response factor								Range response factor						
	1 ng/uL	5 ng/uL	20 ng/uL	40 ng/uL	50 ng/uL	1 ng/mL	5 ng/mL	20 ng/mL	40 ng/mL	50 ng/mL	mean	std dev	%CV	1 ng/mL	5 ng/mL	20 ng/mL	40 ng/mL	50 ng/mL	mean	std dev
C9	118476	599653	2449545	4976625	6186261	0 77	0 91	0 89	0 88	0 80	0 85	0 06	7 1							
C10	161818	644101	2529017	5126004	6412359	1 06	0 98	0 92	0 90	0 83	0 94	0 09	9 3							
C12	116489	563948	2538641	5271744	6598236	0 76	0 86	0 92	0 93	0 85	0 86	0 07	7 8							
C14	130671	603355	2693409	5580552	6863595	0 85	0 92	0 97	0 98	0 89	0 92	0 06	6 1	0 95	0 93	0 96	0 96	0 86	0 93	0 04
C16	142865	614373	2773332	5758166	6929799	0 93	0 94	1 00	1 02	0 89	0 96	0 05	5 3							
C18	202270	623924	2849950	5865039	6985032	1 32	0 95	1 03	1 03	0 90	1 05	0 16	15 6							
C19	171791	649504	2915720	6049368	7140614	1 12	0 99	1 06	1 07	0 92	1 03	0 08	7 5							
C20	180855	650015	2897249	5974376	7062310	1 05	0 99	1 05	1 05	0 91	1 01	0 06	6 1							
INT STD	611986	524713	552531	567232	619714															
SURR	150853	540310	2435395	5040355	5973615	0 99	0 82	0 88	0 89	0 77	0 87	0 08	9 2							
C22	178453	663458	2892770	5987458	7039289	1 17	1 01	1 05	1 05	0 91	1 04	0 09	8 9							
C24	162914	660060	2885095	5950582	6986912	1 06	1 01	1 04	1 05	0 90	1 01	0 07	6 5	1 05	0 96	1 01	1 01	0 87	0 98	0 07
C26	163470	646857	2870363	5921409	6926426	1 07	0 99	1 04	1 04	0 89	1 01	0 07	6 9							
C28	164793	637327	2821527	5814705	6809588	1 08	0 97	1 02	1 03	0 88	0 99	0 07	7 5							
C30	155692	614110	2720063	5591945	6567638	1 02	0 94	0 98	0 99	0 85	0 95	0 07	6 9							
C36	128611	499643	2213020	4609902	5109878	0 84	0 76	0 80	0 81	0 66	0 78	0 07	9 1							
Ratio C28/C20 =	1 02	0 98	0 97	0 97	0 96															

B.1.2 Aliphatic Calibration (A)

	Area accounts					Response factor								Range response factor						
	4 6 ng/uL	9 1 ng/uL	45 5 ng/uL	72 7 ng/uL	81 8 ng/uL	4 6 ng/mL	9 1 ng/mL	45 5 ng/mL	72 7 ng/mL	81 8 ng/mL	mean	std dev	%CV	4 6 ng/mL	9 1 ng/mL	45 5 ng/mL	72 7 ng/mL	81 8 ng/mL	mean	std dev
C9	410770	848080	4093944	4245258	6099396	0 72	0 82	0 75	0 61	0 77	0 73	0 08	10 4							
C10	352667	776893	4042582	4262680	6092593	0 61	0 75	0 74	0 62	0 77	0 70	0 08	10 9							
C12	347267	772596	4268184	4528889	6458144	0 60	0 74	0 78	0 66	0 82	0 72	0 09	12 3	0 65	0 79	0 81	0 68	0 85	0 76	0 09
C14	358459	799609	4509306	4816473	6845091	0 62	0 77	0 83	0 70	0 87	0 76	0 10	12 9							
C16	380858	845721	4759502	5079310	7203683	0 66	0 81	0 87	0 73	0 91	0 80	0 10	12 7							
C18	398589	873867	4906962	5264711	7430557	0 69	0 84	0 90	0 76	0 94	0 83	0 10	12 2							
C19	417328	909238	5130278	5509761	7756295	0 73	0 87	0 94	0 80	0 98	0 86	0 10	12 0							
C20	418963	896162	5008487	5385900	7574359	0 73	0 86	0 92	0 78	0 96	0 85	0 10	11 2							
INT STD	2294056	2078024	2183598	1727424	1752502															
SURR	438987	799836	4393898	5662894	7126492	0 76	0 77	0 80	0 87	0 90	0 82	0 06	7 4							
C22	424930	900645	5030560	5440954	7640183	0 74	0 87	0 92	0 79	0 97	0 86	0 09	11 0							
C24	418823	897360	5006764	5430055	7608861	0 73	0 86	0 92	0 79	0 96	0 85	0 10	11 2							
C26	409519	874530	4985098	5406015	7563432	0 71	0 84	0 91	0 78	0 96	0 84	0 10	11 7	0 68	0 80	0 87	0 75	0 92	0 80	0 10
C28	414237	856997	4872406	5311006	7421071	0 72	0 82	0 89	0 77	0 94	0 83	0 09	10 7							
C30	386551	830746	4797642	5204202	7300519	0 67	0 80	0 88	0 75	0 93	0 81	0 10	12 4							
C36	230796	491050	3295710	3692328	5178200	0 40	0 47	0 60	0 53	0 66	0 53	0 10	19 0							
Ratio C28/C20 =	0 94	1 07	1 11	0 89																

B1.2 Aliphatic Calibration (B)

	Area accounts					Response factor								Range response factor						
	4 6 ng/uL	9 1 ng/uL	45 5 ng/uL	72 7 ng/uL	81 8 ng/uL	4 6 ng/mL	9 1 ng/mL	45 5 ng/mL	72 7 ng/mL	81 8 ng/mL	mean	std dev	%CV	4 6 ng/mL	9 1 ng/mL	45 5 ng/mL	72 7 ng/mL	81 8 ng/mL	mean	std dev
C10	348659	759026	3949927	6865715	5300250	0 61	0 73	0 72	0 99	0 67	0 75	0 15	19 8							
C12	338265	759847	4228004	7210104	5624972	0 59	0 73	0 77	1 04	0 71	0 77	0 17	21 7	0 65	0 77	0 75	0 78	0 84	0 76	0 07
C14	348657	788717	4625927	7549147	5974301	0 61	0 76	0 85	1 09	0 76	0 81	0 18	22 0							
C16	374812	842669	5054217	7826764	6292227	0 65	0 81	0 93	1 13	0 80	0 86	0 18	20 7							
C18	394985	883944	5367859	7985793	6509653	0 69	0 85	0 98	1 16	0 83	0 90	0 18	19 7							
C19	416926	925414	5649977	8294032	6805242	0 73	0 89	1 03	1 20	0 86	0 94	0 18	19 2							
C20	412696	913495	5561869	8090807	6663256	0 72	0 88	1 02	1 17	0 84	0 93	0 17	18 7							
INT STD	2245399	2091157	2406510	2382244	1543531															
SURR	425663	813233	4881030	8343705	6262650	0 74	0 78	0 89	1 21	0 79	0 88	0 19	21 4							
C22	417006	921043	5658869	8130630	6746573	0 73	0 89	1 04	1 18	0 86	0 94	0 17	18 6							
C24	413172	912230	5657979	8092415	6744237	0 72	0 88	1 04	1 17	0 86	0 93	0 17	18 7							
C26	407157	892167	5646387	8034269	6702538	0 71	0 86	1 03	1 16	0 85	0 92	0 18	19 2	0 68	0 81	0 89	0 81	0 92	0 82	0 09
C28	392013	866731	5524927	7863664	6540062	0 68	0 83	1 01	1 14	0 83	0 90	0 18	19 7							
C30	384355	849628	5454432	7707646	6458965	0 67	0 82	1 00	1 12	0 82	0 88	0 17	19 7							
C36	229926	527157	3660630	5211357	4567411	0 40	0 51	0 71	0 75	0 58	0 59	0 14	24 5							

Ratio C28/C20 = 0 92 1 07 1 13 0 94

B.1.2 Aliphatic Calibration (C)

	Area accounts					Response factor								Range response factor						
	4.6 ng/uL	9 1 ng/uL	45 5 ng/uL	72 7 ng/uL	81 8 ng/uL	4 6 ng/mL	9 1 ng/mL	45 5 ng/mL	72 7 ng/mL	81 8 ng/mL	mean	std dev	%CV	4 6 ng/mL	9 1 ng/mL	45 5 ng/mL	72 7 ng/mL	81 8 ng/mL	mean	std dev
C9	402160	646297	4060068	7090556	8279894	0 70	0 62	0 74	1 03	1 05	0 83	0 20	23 7							
C10	338766	631521	3960010	7042159	8215751	0 59	0 61	0 73	1 02	1 04	0 80	0 22	27 6							
C12	327155	642881	4112518	7399565	8577186	0 57	0 62	0 75	1 07	1 09	0 82	0 25	30 0	0 67	0 69	0 83	0 83	0 89	0 78	0 10
C14	336037	684761	4327523	7775601	8944336	0 59	0 66	0 79	1 13	1 13	0 86	0 26	30 0							
C16	357064	760240	4557594	8098460	9251350	0 62	0 73	0 83	1 17	1 17	0 91	0 25	28 0							
C18	376895	832777	4706839	8261469	9437619	0 66	0 80	0 86	1 20	1 20	0 94	0 24	25 8							
C19	397625	891930	4912753	8622234	9800836	0 69	0 86	0 90	1 25	1 24	0 99	0 25	25 0							
C20	393479	869159	4800965	8419479	9561722	0 69	0 85	0 88	1 22	1 21	0 97	0 24	24 3							
INT STD	2137433	2026536	2054241	2286474	2204413															
SURR	404627	785606	4187886	7994005	9006159	0 70	0 76	0 77	1 16	1 14	0 91	0 22	24 8							
C22	397149	915987	4833366	8479460	9618074	0 69	0 88	0 89	1 23	1 22	0 98	0 23	23 9							
C24	395733	920647	4806830	8462578	9599602	0 69	0 89	0 88	1 22	1 22	0 98	0 23	23 9							
C26	386290	914729	4762026	8420733	9540302	0 67	0 88	0 87	1 22	1 21	0 97	0 24	24 5	0 69	0 85	0 88	0 88	0 92	0 84	0 09
C28	376848	894965	4632448	8260443	9322764	0 66	0 86	0 85	1 20	1 18	0 95	0 23	24 6							
C30	367386	878796	4554396	8128532	9161010	0 64	0 84	0 83	1 18	1 16	0 93	0 23	24 9							
C36	220192	585866	3046716	5503805	6071492	0 38	0 56	0 56	0 80	0 77	0 61	0 17	27 8							

Ratio C28/C20 = 0.93 1.14 1 11 1.03

B.1.3 Aliphatic Calibration (A)

	Area accounts				Response factor							Range response factor					
	4.6 ng/uL	18.2 ng/uL	45.5 ng/uL	90.9 ng/uL	4.6 ng/uL	18.2 ng/uL	45.5 ng/uL	90.9 ng/uL	mean	std dev	%CV	4.6 ng/uL	18.2 ng/uL	45.5 ng/uL	90.9 ng/uL	mean	std dev
C9	669639	2100313	5564879	1.1E+07	1.19	0.92	0.96	0.91	0.99	0.13	13.5						
C10	689179	2096743	5617657	1.1E+07	1.23	0.92	0.96	0.93	1.01	0.15	14.6						
C12	487890	2126510	5848929	1.1E+07	0.87	0.93	1.00	0.96	0.94	0.06	6.1						
C14	569696	2226506	6136983	1.2E+07	1.01	0.97	1.05	1.00	1.01	0.03	3.3	1.03	0.96	1.02	0.98	1.00	0.04
C16	527926	2292392	6267324	1.2E+07	0.94	1.00	1.08	1.02	1.01	0.06	5.6						
C18	533676	2326768	6329494	1.2E+07	0.95	1.02	1.09	1.03	1.02	0.06	5.6						
C19	549010	2387096	6492964	1.2E+07	0.98	1.04	1.11	1.05	1.05	0.06	5.4						
C20	544144	2353556	6375879	1.2E+07	0.97	1.03	1.09	1.04	1.03	0.05	5.0						
INT STD	2246643	2290947	2329095	2359507													
SURR	439250	1942951	5327237	1E+07	0.78	0.85	0.91	0.87	0.85	0.06	6.5						
C22	535983	2320912	6290095	1.2E+07	0.95	1.01	1.08	1.02	1.02	0.05	5.1						
C24	519954	2260665	6149849	1.2E+07	0.92	0.99	1.06	1.00	0.99	0.05	5.4	0.79	0.87	0.94	0.90	0.91	0.03
C26	496831	2196209	5975544	1.2E+07	0.88	0.96	1.03	0.98	0.96	0.06	6.2						
C28	486050	2083583	5676913	1.1E+07	0.86	0.91	0.97	0.93	0.92	0.05	5.0						
C30	403599	1849126	5109782	1E+07	0.72	0.81	0.88	0.85	0.81	0.07	8.6						
C36		550043	1776995	4121966	0.00	0.24	0.31	0.35	0.30	0.05	18.4						
Ratio C28/C20 =		0.89	0.89	0.89	0.90												

B.1.3 Aliphatic Calibration (B)

	Area accounts				Response factor							Range response factor					
	4 6 ng/uL	18 2 ng/uL	45 5 ng/uL	90 9 ng/uL	4 6 ng/uL	18 2 ng/uL	45 5 ng/uL	90 9 ng/uL	mean	std dev	%CV	4 6 ng/uL	18 2 ng/uL	45 5 ng/uL	90 9 ng/uL	mean	std dev
C9	604209	2199159	5631646	1 1E+07	1 03	0 92	0 92	0 92	0 95	0 06	5 9						
C10	557606	2195396	5700628	1 1E+07	0 95	0 92	0 93	0 95	0 94	0 02	1 7						
C12	455364	2220693	5945948	1.2E+07	0 78	0 93	0 97	0 98	0 91	0 09	10 3						
C14	482060	2328690	6280210	1 2E+07	0 82	0 98	1 03	1 02	0 96	0 10	9 9	0 90	0 96	1 00	0 99	0 96	0 05
C16	513929	2391953	6456720	1 2E+07	0 88	1 00	1 05	1 03	0 99	0 08	8 1						
C18	540821	2435799	6565223	1 3E+07	0 92	1 02	1 07	1 04	1 01	0 06	6 3						
C19	566235	2491067	6754346	1 3E+07	0 97	1 04	1 10	1 06	1 04	0 06	5 5						
C20	567202	2454340	6655627	1 3E+07	0 97	1 03	1 09	1 04	1 03	0 05	4 8						
INT STD	2342004	2385618	2447677	2411471													
SURR	465678	2027442	5578912	1E+07	0 79	0 85	0 91	0 87	0 86	0 05	5 7						
C22	576444	2432030	6612500	1 2E+07	0 98	1 02	1 08	1 02	1 03	0 04	3 9						
C24	572813	2370837	6487292	1.2E+07	0 98	0 99	1 06	1 00	1 01	0 04	3 6	0 81	0 87	0 95	0 90	0 91	0 04
C26	541527	2290985	6326573	1 2E+07	0 92	0 96	1 03	0 97	0 97	0 05	4 7						
C28	524362	2166535	6047080	1 1E+07	0 89	0 91	0 99	0 92	0 93	0 04	4 5						
C30	448524	1933951	5522643	1E+07	0 77	0 81	0 90	0 84	0 83	0 06	6 9						
C36		556772	2078581	4014141	0 00	0 23	0 34	0 33	0 30	0 06	19 7						

Ratio C28/C20 = 0 92 0 86 0 91 0 89

B.1.3 Aliphatic Calibration (C)

	Area accounts				Response factor							Range response factor					
	4 6 ng/uL	18 2 ng/uL	45 5 ng/uL	90 9 ng/uL	4 6 ng/uL	18 2 ng/uL	45 5 ng/uL	90 9 ng/uL	mean	std dev	%CV	4 6 ng/uL	18 2 ng/uL	45 5 ng/uL	90 9 ng/uL	mean	std dev
C9	771609	2384650	6278992	1 2E+07	1 21	0 91	0 94	0 89	0 99	0 15	15 3						
C10	636711	2377460	6385197	1 2E+07	1 00	0 92	0 98	0 92	0 95	0 04	4 0						
C12	567651	2405486	6635634	1 2E+07	0 89	0 93	1 00	0 95	0 94	0 04	4 6						
C14	588930	2521728	6953772	1 3E+07	0 92	0 97	1 04	1 00	0 98	0 05	5 1	0 99	0 96	1 01	0 97	0 98	0 03
C16	607091	2586946	7085912	1.3E+07	0 96	1 00	1 06	1 01	1 01	0 04	4 4						
C18	611838	2623116	7164724	1.3E+07	0 96	1 01	1 08	1 02	1 02	0 05	4 5						
C19	632226	2686718	7308551	1 4E+07	1 00	1 04	1 10	1 04	1 04	0 04	4 0						
C20	625606	2658690	7231662	1 3E+07	0 98	1 03	1 09	1 03	1 03	0 04	4 0						
INT STD	2538196	2590056	2664032	2620242													
SURR	507466	2201279	6052312	1 1E+07	0 80	0 85	0 91	0 86	0 85	0 04	5 3						
C22	627251	2629880	7167274	1 3E+07	0 99	1 02	1 08	1 02	1 02	0 04	3 6						
C24	615554	2568170	7021002	1 3E+07	0 97	0 99	1 05	0 99	1 00	0 04	3 6	0 80	0 88	0 94	0 90	0 91	0 03
C26	578064	2496179	6839391	1 3E+07	0 91	0 96	1 03	0 97	0 97	0 05	4 9						
C28	544736	2368579	6516913	1 2E+07	0 86	0 91	0 98	0 92	0 92	0 05	5 4						
C30	465805	2130838	5957164	1 1E+07	0 73	0 82	0 89	0 86	0 83	0 07	8 3						
C36		618111	2222406	4697013	0 00	0 24	0 33	0 36	0 31	0 06	20 4						

Ratio C28/C20 = 0 87 0 89 0 90 0 90

B.1.4 Aromatic Calibration (A)

	Area accounts				Response factor							Range response factor					
	5 ng/uL	20 ng/uL	40 ng/uL	50 ng/uL	5 ng/mL	20 ng/mL	40 ng/mL	50 ng/mL	mean	std dev	%CV	5 ng/mL	20 ng/mL	40 ng/mL	50 ng/mL	mean	std dev
Napthalene	678737	2735704	5726871	6739010	1 15	1 22	1 28	1 17	1 20	0 06	4 7						
2-MethylNapthalene	690715	2760696	5748475	6706185	1 17	1 23	1 28	1 16	1 21	0 06	4 6						
Acenapthylene	671905	2618391	5393774	6360135	1 14	1 16	1 20	1 10	1 15	0 04	3 7						
Acenapthene	692419	2680591	5602902	6550676	1 17	1 19	1 25	1 13	1 19	0 05	4 0	1 03	1 04	1 08	0 99	1 03	0 04
Fluorene	675759	2656307	5536359	6441035	1 14	1 18	1 23	1 11	1 17	0 05	4 4						
Phenanthrene	632466	2558462	5340887	6140225	1 07	1 14	1 19	1 06	1 12	0 06	5 4						
Anthracene	524451	2144505	4506584	5953252	0 89	0 95	1 00	1 03	0 97	0 06	6 5						
OTP (surrogate)	709633	2818846	5828921	6680056	1 20	1 25	1 30	1 16	1 23	0 06	5 0						
5-Alpha-Androstane	472626	449899	449016	462146													
Fluoranthrene	657239	2533826	5160150	5943564	1 11	1 13	1 15	1 03	1 10	0 05	4 8						
Pyrene	634857	2460674	5095870	5899887	1 07	1 09	1 13	1 02	1 08	0 05	4 4						
Benz(a)Anthracene	548486	2211881	4606016	5282340	0 93	0 98	1 03	0 91	0 96	0 05	5 4						
Chrysene	558652	2322030	4772545	5595257	0 95	1 03	1 06	0 97	1 00	0 05	5 4						
Benzo(b)Fluoranthe	558743	2179901	4472824	5209383	0 95	0 97	1 00	0 90	0 95	0 04	4 2						
Benzo(k)Fluoranten	709140	2457226	4867907	5413090	1 20	1 09	1 08	0 94	1 08	0 11	10 0						
Benzo(a)Pyrene	655512	1942217	4027118	4861128	1 11	0 86	0 90	0 84	0 93	0 12	13 3						
Indeno[123cd]pyren	474325	1833168	3915870	4526217	0 80	0 81	0 87	0 78	0 82	0 04	4 6						
Dibenz[a,h]anthrace	485934	1917548	4074642	4494688	0 82	0 85	0 91	0 78	0 84	0 05	6 5						
Benzo(ghi)perylene	456383	1703051	3550011	5057082	0 77	0 76	0 79	0 88	0 80	0 05	6 6						

B.1.5 Aromatic Calibration (A)

	Area accounts				Response factor							Range response factor					
	4 6 ng/uL	18 2 ng/uL	45 5 ng/uL	90 9 ng/uL	4 6 ng/uL	18 2 ng/uL	45 5 ng/uL	90 9 ng/uL	mean	std dev	%CV	4 6 ng/uL	18 2 ng/uL	45 5 ng/uL	90 9 ng/uL	mean	std dev
Napthalene	698662	3251331	6921916	16434632	0 96	1 04	0 96	1 06	1 00	0 06	5 5						
2-MethylNapthalene	696237	3223766	6840494	16206473	0 95	1 03	0 95	1 05	0 99	0 05	5 2						
Acenapthylene	640169	2902178	6039230	14965570	0 88	0 93	0 86	0 97	0 91	0 05	5 3						
Acenapthene	686418	3144076	6666159	15638780	0 94	1 01	0 93	1 02	0 97	0 05	4 8	0 75	0 83	0 78	0 87	0 81	0 06
Fluorene	693629	3124664	6642290	15819510	0 95	1 00	0 93	1 02	0 97	0 04	4 5						
Phenanthrene	656925	3099020	6648796	15630448	0 90	0 99	0 93	1 02	0 96	0 06	5 9						
Anthracene	603475	2819261	7714904	14491424	0 83	0 90	0 83	0 93	0 87	0 05	6 3						
OTP (surrogate)	686895	3231686	6952637	16307468	0 94	1 04	0 96	1 05	1 00	0 05	5 4						
5-Alpha-Androstane	2922263	3121634	3727417	3100056													
Fluoranthrene	622963	3006802	6344619	15324528	0 85	0 96	0 90	0 99	0 92	0 06	6 8						
Pyrene	619456	2997290	6395590	15565628	0 85	0 96	0 90	1 00	0 93	0 07	7 4						
Benz(a)Anthracene	501214	2548338	7327942	13822733	0 69	0 82	0 79	0 89	0 79	0 09	10 8						
Chrysene	509309	2580831	7414937	13999771	0 70	0 83	0 80	0 90	0 81	0 09	10 6						
Benzo(b)Fluoranthe	453237	2286011	6622134	12690130	0 62	0 73	0 71	0 81	0 72	0 08	11 0						
Benzo(k)Fluoranten	546751	2497994	6695241	12656637	0 75	0 80	0 72	0 82	0 77	0 05	5 9						
Benzo(a)Pyrene	442932	1973998	5626871	10975972	0 61	0 63	0 60	0 71	0 64	0 05	7 7						
Indeno[123cd]pyren	308747	1582099	4790904	9149985	0 42	0 51	0 51	0 59	0 54	0 05	8 6						
Dibenz[a,h]anthrace	288978	1547148	4532385	8402430	0 40	0 50	0 49	0 54	0 48	0 06	12 8						
Benzo(ghi)perylene	296576	1497601	4386064	8122438	0 41	0 48	0 47	0 52	0 47	0 05	10 4						

B.1.5 Aromatic Calibration (B)

	Area accounts				Response factor							Range response factor					
	4 6 ng/uL	18 2 ng/uL	45 5 ng/uL	90 9 ng/uL	4 6 ng/uL	18 2 ng/uL	45 5 ng/uL	90 9 ng/uL	mean	std dev	%CV	4 6 ng/uL	18 2 ng/uL	45 5 ng/uL	90 9 ng/uL	mean	std dev
Napthalene	632249	2716196	6873263	13847410	1 10	1 15	1 09	1 14	1 12	0 03	3 0						
2-MethylNapthalene	624065	2677765	6762263	13691174	1 08	1 14	1 07	1 13	1 10	0 03	3 1						
Acenapthylene	605789	2517527	6337471	13026820	1 05	1 07	1 00	1 08	1 05	0 03	3 2						
Acenapthene	614879	2637884	6585198	13333910	1 07	1 12	1 04	1 10	1 08	0 04	3 3	0 73	0 80	0 76	0 83	0 78	0 04
Fluorene	607650	2571948	6381957	13070349	1 05	1 09	1 01	1 08	1 06	0 04	3 5						
Phenanthrene	551712	2399984	5980763	12398578	0 96	1 02	0 94	1 02	0 99	0 04	4 2						
Anthracene	527907	2292575	5805272	11958662	0 92	0 97	0 92	0 99	0 95	0 04	3 9						
OTP (surrogate)	565365	2469662	6159326	12824246	0 98	1 05	0 97	1 06	1 02	0 04	4 4						
5-Alpha-Androstane	2302138	2357109	2531672	2419837													
Fluoranthrene	478987	2152268	5378785	11276316	0 83	0 91	0 85	0 93	0 88	0 05	5 5						
Pyrene	484573	2152642	5368706	11405200	0 84	0 91	0 85	0 94	0 89	0 05	5 6						
Benz(a)Anthracene	366065	1661824	4292364	9348957	0 64	0 71	0 68	0 77	0 70	0 06	8 3						
Chrysene	366596	1694768	4311770	9429678	0 64	0 72	0 68	0 78	0 70	0 06	8 6						
Benzo(b)Fluoranthe	298059	1406874	3617818	7910832	0 52	0 60	0 57	0 65	0 58	0 06	9 7						
Benzo(k)Fluoranten	377007	1672377	3949533	7889006	0 66	0 71	0 62	0 65	0 66	0 04	5 4						
Benzo(a)Pyrene	235167	1320885	3229279	6832299	0 41	0 56	0 51	0 56	0 51	0 07	14 2						
Indeno[123cd]pyren	124717	790171	2328291	5315317	0 22	0 34	0 37	0 44	0 38	0 05	14 0						
Dibenz[a,h]anthrace	125568	755747	2329294	4977532	0 22	0 32	0 37	0 41	0 33	0 08	25 2						
Benzo(ghi)perylene	125363	782747	2219896	4816965	0 22	0 33	0 35	0 40	0 32	0 08	23 6						

B.1.5 Aromatic Calibration (C)

	Area accounts				Response factor							Range response factor					
	4 6 ng/uL	18 2 ng/uL	45 5 ng/uL	90 9 ng/uL	4 6 ng/uL	18 2 ng/uL	45 5 ng/uL	90 9 ng/uL	mean	std dev	%CV	4 6 ng/uL	18 2 ng/uL	45 5 ng/uL	90 9 ng/uL	mean	std dev
Napthalene	652240	2825821	7309786	14019276	1 07	1 10	1 09	1 06	1 08	0 02	1 8						
2-MethylNapthalene	648059	2849438	7183448	14034643	1 07	1 11	1 07	1 06	1 08	0 02	2 1						
Acenapthylene	615987	2636160	6620224	13644306	1 01	1 03	0 99	1 03	1 01	0 02	1 7						
Acenapthene	635140	2796356	6958772	13966073	1 06	1 09	1 04	1 06	1 06	0 02	2 2	0 72	0 81	0 76	0 82	0 78	0 05
Fluorene	623545	2732897	6744275	13891200	1 03	1 07	1 01	1 05	1 04	0 03	2 5						
Phenanthrene	581178	2598608	6333147	13454586	0 96	1 01	0 95	1 02	0 98	0 04	3 8						
Anthracene	549463	2457309	5968955	12866450	0 91	0 96	0 89	0 97	0 93	0 04	4 3						
OTP (surrogate)	595913	2682770	6536476	13866092	0 98	1 05	0 98	1 05	1 01	0 04	4 0						
5-Alpha-Androstane	2425527	2560591	2674090	2642107													
Fluoranthrene	511676	2369066	5675256	12484956	0 84	0 93	0 85	0 94	0 89	0 05	5 9						
Pyrene	515904	2369775	5674406	12622657	0 85	0 93	0 85	0 96	0 89	0 05	6 0						
Benz(a)Anthracene	359038	1656850	4515076	10449773	0 59	0 73	0 68	0 79	0 70	0 08	12 1						
Chrysene	369523	1683258	4557942	10560212	0 61	0 74	0 68	0 80	0 71	0 08	11 5						
Benzo(b)Fluoranthe	299561	1582455	3816508	8845339	0 49	0 62	0 57	0 67	0 59	0 07	12 7						
Benzo(k)Fluoranten	380897	1843356	4085239	8974793	0 63	0 72	0 61	0 68	0 66	0 05	7 5						
Benzo(a)Pyrene	254650	1434591	3449437	7612622	0 42	0 56	0 52	0 58	0 52	0 07	13 6						
Indeno[123cd]pyren	147442	942919	2529090	5929217	0 24	0 37	0 38	0 45	0 40	0 04	11 0						
Dibenz[a,h]anthrace	149434	936292	2430157	5640586	0 25	0 37	0 36	0 43	0 35	0 08	21 6						
Benzo(ghi)perylene	148286	929048	2373766	5381438	0 24	0 36	0 35	0 41	0 34	0 07	20 3						

B.1.6 Aromatic Calibration (A)

	Area accounts				Response factor							Range response factor					
	4 6 ng/uL	18 2 ng/uL	45 5 ng/uL	90 9 ng/uL	4 6 ng/uL	18 2 ng/uL	45 5 ng/uL	90 9 ng/uL	mean	std dev	%CV	4 6 ng/uL	18 2 ng/uL	45 5 ng/uL	90 9 ng/uL	mean	std dev
Napthalene	527416	2344010	6179166	12407517	1 01	1 11	1 20	1 29	1 15	0 12	10 6						
2-MethylNapthalene	521194	2356845	6125660	12183682	1 00	1 11	1 19	1 27	1 14	0 12	10 2						
Acenapthylene	517955	2280224	5877738	11646398	0 99	1 08	1 14	1 21	1 10	0 09	8 6						
Acenapthene	539663	2367962	5971862	11638430	1 03	1 12	1 16	1 21	1 13	0 08	6 7	0 75	0 86	0 87	0 90	0 84	0 07
Fluorene	536032	2370868	5834523	11295394	1 02	1 12	1 13	1 17	1 11	0 06	5 7						
Phenanthrene	437235	2165678	5411790	10426030	0 83	1 02	1 05	1 08	1 00	0 11	11 2						
Anthracene	427102	2121438	5364223	10460354	0 82	1 00	1 04	1 09	0 99	0 12	12 1						
OTP (surrogate)	519430	2316020	5680356	10899444	0 99	1 09	1 10	1 13	1 08	0 06	5 7						
5-Alpha-Androstane	2092440	2120960	2056150	1924647													
Fluoranthrene	438621	2050200	4927184	9284522	0 84	0 97	0 96	0 95	0 93	0 06	6 8						
Pyrene	445482	2043408	4892271	9331166	0 85	0 96	0 95	0 97	0 93	0 06	6 0						
Benz(a)Anthracene	321869	1678414	4051152	7692512	0 61	0 79	0 79	0 80	0 75	0 09	11 9						
Chrysene	435076	1807549	4192491	7893933	0 83	0 85	0 82	0 82	0 83	0 02	2 0						
Benzo(b)Fluroanthe	239879	1466057	3502633	6595900	0 46	0 69	0 68	0 69	0 63	0 11	18 1						
Benzo(k)Fluoranten	560350	1755866	3819088	6771922	1 07	0 83	0 74	0 70	0 84	0 16	19 7						
Benzo(a)Pyrene	335910	1372832	3134976	5772420	0 64	0 65	0 61	0 60	0 62	0 02	3 7						
Indeno(123cd)pyrene		793630	2218345	4545330	0 00	0 37	0 43	0 47	0 43	0 05	11 6						
Dibenz(a,h)anthrace	198456	974562	2545090	4522208	0 38	0 46	0 50	0 47	0 45	0 05	11 1						
Benzo(ghi)perylene	156547	901681	2249988	4121924	0 30	0 43	0 44	0 43	0 40	0 07	16 6						

B.1.6 Aromatic Calibration (B)

	Area accounts				Response factor							Range response factor					
	4 6 ng/uL	18 2 ng/uL	45 5 ng/uL	90 9 ng/uL	4 6 ng/uL	18 2 ng/uL	45 5 ng/uL	90 9 ng/uL	mean	std dev	%CV	4 6 ng/uL	18 2 ng/uL	45 5 ng/uL	90 9 ng/uL	mean	std dev
Napthalene	551206	2441378	6346035	12986410	1 00	1 12	1 20	1 27	1 15	0 11	9 8						
2-MethylNapthalene	544634	2446304	6280927	12762705	0 99	1 12	1 19	1 24	1 14	0 11	9 5						
Acenapthylene	531881	2317613	5923497	12072234	0 97	1 06	1 12	1 18	1 08	0 09	8 2						
Acenapthene	562170	2440847	6112716	12193288	1 02	1 12	1 15	1 19	1 12	0 07	6 3	0 75	0 86	0 87	0 89	0 84	0 06
Fluorene	557857	2446305	5972879	11854680	1 02	1 12	1 13	1 16	1 10	0 06	5 5						
Phenanthrene	436531	2227035	5649323	11000868	0 80	1 02	1 05	1 07	0 98	0 13	13 0						
Anthracene	439286	2192290	5486396	10940287	0 80	1 00	1 04	1 07	0 98	0 12	12 3						
OTP (surrogate)	546385	2378018	5827158	11578648	1 00	1 09	1 10	1 13	1 08	0 06	5 4						
5-Alpha-Androstane	2193869	2183893	2117953	2050989													
Fluoranthrene	473580	2099431	5007737	9835828	0 86	0 96	0 95	0 96	0 93	0 05	5 0						
Pyrene	427987	2090327	5016974	9901644	0 78	0 96	0 95	0 97	0 91	0 09	9 7						
Benz(a)Anthracene	340105	1716881	4151252	8221853	0 62	0 79	0 78	0 80	0 75	0 09	11 5						
Chrysene	430389	1856673	4303420	8385109	0 78	0 85	0 81	0 82	0 82	0 03	3 3						
Benzo(b)Fluroanthe	254062	1498183	3624160	7165501	0 46	0 69	0 68	0 70	0 63	0 11	18 0						
Benzo(k)Fluoranten	561005	1781500	3861334	7184841	1 02	0 82	0 73	0 70	0 82	0 14	17 7						
Benzo(a)Pyrene	342434	1379301	3193278	6186235	0 62	0 63	0 60	0 60	0 62	0 01	2 4						
Indeno(123cd)pyrene		812813	2336764	5052762	0 00	0 37	0 44	0 49	0 44	0 06	13 9						
Dibenz(a,h)anthrace	316175	1181928	2583731	4882318	0 58	0 54	0 49	0 46	0 52	0 05	10 4						
Benzo(ghi)perylene	205268	1001697	2336765	4387263	0 37	0 46	0 44	0 43	0 43	0 04	8 6						

B.1.6 Aromatic Calibration (C)

	Area accounts				Response factor							Range response factor					
	4 6 ng/uL	18 2 ng/uL	45 5 ng/uL	90 9 ng/uL	4 6 ng/uL	18 2 ng/uL	45 5 ng/uL	90 9 ng/uL	mean	std dev	%CV	4 6 ng/uL	18 2 ng/uL	45 5 ng/uL	90 9 ng/uL	mean	std dev
Napthalene	591862	2559876	6698196	13585640	0 96	1 15	1 15	1 11	1 10	0 08	7 2						
2-MethylNapthalene	585581	2557802	6645798	13620456	0 97	1 14	1 14	1 11	1 09	0 08	7 5						
Acenapthylene	568770	2363804	6231384	13198106	0 94	1 06	1 07	1 08	1 04	0 06	6 1						
Acenapthene	609075	2534813	6528200	13532902	1 01	1 13	1 12	1 10	1 09	0 06	5 2	0 75	0 85	0 87	0 86	0 84	0 06
Fluorene	607196	2524560	6421579	13460711	1 01	1 13	1 10	1 10	1 08	0 05	5 0						
Phenanthrene	506113	2287142	6058778	12957336	0 84	1 02	1 04	1 06	0 99	0 10	10 3						
Anthracene	477409	2167722	5805839	12467528	0 79	0 97	1 01	1 02	0 95	0 11	11 3						
OTP (surrogate)	602816	2440276	6362526	13580946	1 00	1 09	1 09	1 11	1 07	0 05	4 7						
5-Alpha-Androstane	2411486	2234446	2329604	2449657													
Fluoranthrene	526138	2136904	5609687	12023434	0 88	0 96	0 96	0 98	0 94	0 05	5 0						
Pyrene	526804	2124542	5578596	12176361	0 87	0 95	0 96	0 99	0 94	0 05	5 4						
Benz(a)Anthracene	386053	1721317	4703600	10357226	0 64	0 77	0 81	0 85	0 77	0 09	11 7						
Chrysene	531099	1865036	4876765	10576167	0 88	0 83	0 84	0 86	0 85	0 02	2 5						
Benzo(b)Fluroanthe	292547	1506386	4143137	9209166	0 48	0 67	0 71	0 75	0 66	0 12	18 0						
Benzo(k)Fluoranten	612787	1770259	4488060	9206375	1 02	0 79	0 77	0 75	0 83	0 12	14 8						
Benzo(a)Pyrene	359938	1358281	3658400	7962217	0 60	0 61	0 63	0 65	0 62	0 02	3 8						
Indeno(123cd)pyrene		784078	2839566	6881010	0 00	0 35	0 49	0 56	0 47	0 11	22 9						
Dibenz[a,h]anthrace	265669	1064789	3050701	6459672	0 47	0 48	0 52	0 53	0 50	0 03	5 9						
Benzo(ghi)perylene	197877	989290	2784389	6103586	0 33	0 44	0 48	0 50	0 44	0 06	17 4						

APPENDIX C.1 FORE RIVER CREEK SALT MARSH DATA

TPH Concentration using MADEP Method

C.1.1 Short Chain Aliphatics

Amendment		Ax	Cis	D	Ais	RF	Wd	concentration mg/kg dw	concentration mg/kg dw	surrogate area account	surrogate recove mg/kg dw	% recovery surrogate	concentration %P
Nitrate	1Aa	3112431	16000	5 0	533646	0 9	1 7	296837	297	256369	4 9	20 7	1437
Nitrate	Ab	1605577	16000	5 0	438837	0 9	1 5	212998	213	189808	5 0	18 6	1146
Nitrate	Ba	5904229	16000	5 0	469670	0 9	1 5	728912	729	402645	9 9	36 9	1977
Nitrate	Bb	3693991	16000	5 0	469579	0 9	1 4	524275	524	367376	9 9	33 6	1558
Nitrate	Ca	1110865	16000	5 0	336170	0 9	1 9	151233	151	244560	6 7	31 3	483
Nitrate	Cb	16667670	16000	5 0	450876	0 9	1 8	1780851	1781	244508	5 2	23 3	7636
Nitrate	Da	3507547	16000	5 0	510633	0 9	1 1	514166	514	258867	7 6	21 8	2358
Nitrate	Db	466800	16000	5 0	656208	0 9	1 0	58532	59	238716	6 0	15 6	374
Air	Aa	15465214	16000	5 0	451180	0 9	1 5	1933399	1933	315707	7 9	30 1	6425
Air	Ab	16392686	16000	5 0	349211	0 9	1 6	2554120	2554	311678	9 7	38 4	6654
Air	Ba	6464869	16000	5 0	584715	0 9	1 5	623385	623	207417	4 0	15 3	4086
Air	Bb	1467862	16000	5 0	511956	0 9	1 5	159971	160	243535	5 3	20 5	782
Air	Ca	15465214	16000	5 0	451180	0 9	1 5	1952111	1952	315707	6 0	30 1	6487
Air	Cb	7290307	16000	5 0	526876	0 9	1 5	775341	775	373546	7 9	30 5	2543
Air	Da	23131689	16000	5 0	563523	0 9	1 6	2272836	2273	309086	6 1	24 0	9464
Air	Db	10501336	16000	5 0	300130	0 9	1 7	1763706	1764	247507	8 3	36 5	4973
Control	Aa	7897987	16000	5 0	548026	0 9	1 0	1250793	1251	245886	7 8	19 3	6482
Control	Ab	476097	16000	5 0	141008	0 9	1 1	266371	266	206250	23 1	62 9	423
Control	Ba	1567796	16000	5 0	225330	0 9	1 1	548547	549	244469	17 1	46 7	1176
Control	Bb	1668233	16000	5 0	428982	0 9	1 2	269222	269	280649	9 7	28 1	1028
Control	Ca	399054	16000	5 0	626344	0 9	1 2	46259	46	256730	6 0	17 6	263
Control	Cb	1293367	16000	5 0	657986	0 9	1 2	138846	139	240107	5 2	15 7	885
Control	Da	2377206	16000	5 0	618859	0 9	1 2	266178	266	217836	4 9	15 1	1758
Control	Db	1291356	16000	5 0	611789	0 9	1 1	164119	164	217991	5 5	15 3	1071
field trip		6545278	16000	5 0	505051	0 9	5 6	198857	199	202387	1 2	17 2	1154
field trip		10117536	16000	5 0	554040	0 9	5 1	310403	310	487988	3 0	37 9	819
control		10048615	16000	5 0	717818	0 9	5 8	205899	206	756712	3 1	45 3	454
control		14684240	16000	5 0	622812	0 9	4 5	455005	455	639361	4 0	44 1	1031

Amendment		Ax	Cis	D	Ais	RF	Wd	concentration mg/kg dw	concentration mg/kg dw	surrogate area account	surrogate recove mg/kg dw	% recovery surrogate	concentration %P
Nitrate	2Aa	15089244	40000	1 0	3435402	0 9	1 6	118126	118	721802	5 7	22 6	523
Nitrate	Ab	45451913	40000	1 0	3829323	0 9	1 1	485776	486	724452	7 7	20 3	2388
Nitrate	Ba	88554519	40000	1 0	3584401	0 9	1 9	422511	423	662079	4 0	19 6	2160
Nitrate	Bb	18339327	40000	1 0	3675591	0 9	1 7	132006	132	1639011	11 8	49 3	268
Nitrate	Ca	107547184	40000	1 0	3462048	0 9	1 9	718030	718	1103788	7 4	34 3	2095
Nitrate	Cb	158856883	40000	1 0	3779108	0 9	1 9	945045	945	1209198	7 2	34 4	2747
Nitrate	Da	10322042	40000	1 0	3463220	0 9	1 2	104099	104	989399	9 8	30 1	346
Nitrate	Db	7463026	40000	1 0	3510875	0 9	1 1	81626	82	1154711	12 6	36 4	231
Air	Aa	26866894	40000	1 0	4350334	0 9	1 2	218354	218	897536	7 3	22 2	984
Air	Ab	42157857	40000	1 0	3775884	0 9	1 6	308882	309	984446	7 1	27 5	1124
Air	Ba	95944026	40000	1 0	3710557	0 9	1 7	855898	856	1284321	8 8	37 2	1782
Air	Bb	87073649	40000	1 0	3648170	0 9	1 5	664132	664	1220731	9 3	36 0	1846
Air	Ca	110213932	40000	1 0	3562541	0 9	1 4	938667	939	826983	7 0	25 0	3761
Air	Cb	199487250	40000	1 0	3885848	0 9	1 6	569936	570	1016844	2 9	11 3	5049
Air	Da	202447302	40000	1 0	4889888	0 9	1 4	1287950	1288	1226922	7 8	27 0	4774
Air	Db	8414115	40000	1 0	3548447	0 9	1 4	73766	74	918229	8 1	27 8	266
Control	Aa	47105595	40000	1 0	3897509	0 9	1 4	370723	371	429771	3 4	11 9	3127
Control	Ab	15434991	40000	1 0	3337343	0 9	1 4	144052	144	979462	9 1	31 6	457
Control	Ba	127827649	40000	1 0	3896821	0 9	1 1	1264822	1266	575823	5 7	15 9	7964
Control	Bb	126411774	40000	1 0	3896821	0 9	1 4	984138	984	2453799	19 1	67 7	1454
Control	Ca	54389062	40000	1 0	3830817	0 9	1 1	577593	578	506505	5 4	13 0	3851
Control	Cb	22416414	40000	1 0	3753033	0 9	1 6	156887	157	841736	5 9	24 1	650
Control	Da	40680578	40000	1 0	3640235	0 9	1 1	444455	444	669167	7 3	19 8	2249
Control	Db	14367603	40000	1 0	3588598	0 9	1 1	161591	162	532816	6 0	16 1	1007
field trip		47498238	40000	1 0	3580461	0 9	5 0	113912	114	1894527	4 5	56 9	200
control		60923444	40000	1 0	3580461	0 9	5 0	167034	167	2141063	5 1	64 3	260

Amendment	As	Cs	D	As	RF	Wd	concentration mg/kg dw	concentration mg/kg dw	surrogate area account	surrogate recove mg/kg dw	% recovery surrogate	concentration %/t
Nitrate	52236520	40000	10	4015356	0.9	14	388309	368	677775	5.0	18.1	2140
Nitrate	24832504	40000	10	3554159	0.9	13	222625	223	926867	8.3	26.1	791
Nitrate	21527270	40000	10	3742106	0.9	13	187945	186	907663	7.9	26.1	721
Nitrate	25011526	40000	10	3818585	0.9	11	250447	250	1195630	12.0	33.7	744
Nitrate	3037979	40000	10	3466580	0.9	19	20030	20	1505650	9.9	46.4	43
Nitrate	22891419	40000	10	3662458	0.9	19	142631	143	2257715	14.1	65.9	217
Nitrate	83637485	40000	10	4031078	0.9	16	577410	577	1906300	13.1	50.9	1134
Nitrate	110119232	40000	10	4426566	0.9	16	662736	663	1478359	8.9	38.9	1846
Nitrate	170152319	40000	10	4390253	0.9	14	1222147	1222	1066040	7.7	26.1	4681
Air	303566563	40000	10	4442003	0.9	13	2185065	2185	1606739	11.6	36.9	5612
Air	30566562	40000	10	3915719	0.9	19	178355	178	1202981	7.0	33.0	540
Air	74367388	40000	10	3624348	0.9	16	529428	529	2002645	14.3	56.3	940
Air	51632066	40000	10	3467300	0.9	14	398017	398	1070177	8.2	33.0	1206
Air	14176762	40000	10	5974049	0.9	16	73462	73	3833834	19.9	69.0	106
Air	91512003	40000	10	3844296	0.9	16	620544	621	891234	6.0	24.9	2490
Air	29106807	40000	10	3863283	0.9	16	202850	203	4033820	34.4	137.7	147
Air	1502801	40000	10	3803923	0.9	11	15301	15	892654	9.1	28.2	81
Air	9006737	40000	10	3690085	0.9	13	77473	77	1391806	12.0	38.4	202
Air	17033799	40000	10	3727033	0.9	11	185631	186	920091	10.1	26.7	696
Air	8039945	40000	10	3831220	0.9	13	69172	69	1002543	8.6	27.4	252
Air	67750807	40000	10	5529364	0.9	17	316446	316	785192	3.7	15.3	2073
Air	68719300	40000	10	3842610	0.9	16	410414	410	748565	4.5	20.4	2010
Air	13824884	40000	10	3694665	0.9	12	143906	144	914630	9.5	27.4	524
Air	15740662	40000	10	3617913	0.9	12	162547	163	760564	7.9	23.2	699
Air	89780372	40000	10	3799152	0.9	56	142569	143	2175440	4.4	62.1	230
Air	89143065	40000	10	5250747	0.9	56	101280	101	2225424	3.3	48.6	223
Air	55247561	40000	10	3736513	0.9	53	119125	119	1763007	3.8	50.7	236
Air	42081873	40000	10	3668876	0.9	50	101081	101	2203510	8.3	66.4	152
Amendment	As	Cs	D	As	RF	Wd	concentration mg/kg dw	concentration mg/kg dw	surrogate area account	surrogate recove mg/kg dw	% recovery surrogate	concentration %/t
Nitrate	44222621	40000	10	3201156	0.8	20	302670	303	876551	6.0	29.5	1026
Nitrate	12906373	40000	10	3666466	0.8	21	724877	725	1367035	7.7	40.1	1808
Nitrate	28804430	40000	10	3115937	0.8	14	2838212	2838	2085694	19.9	72.0	3843
Nitrate	12843718	40000	10	3741741	0.8	17	880326	880	904077	8.2	26.0	3385
Nitrate	8856414	40000	10	3426000	0.8	14	777327	777	565665	5.1	18.4	4229
Nitrate	20047800	40000	10	3211119	0.8	16	1635121	1635	1070546	9.7	35.8	4562
Nitrate	37217180	40000	10	3080742	0.8	17	307320	307	453088	3.7	15.8	1944
Nitrate	56222109	40000	10	2891176	0.8	14	616719	617	488275	4.9	17.3	3564
Air	94218868	40000	10	3666915	0.8	15	769785	770	662513	5.7	20.9	3687
Air	92671466	40000	10	3630238	0.8	14	716976	717	526139	4.0	14.4	4986
Air	94110097	40000	10	3644521	0.8	20	562404	562	974042	5.8	28.7	1958
Air	94512314	40000	10	3648717	0.8	19	601092	601	1017614	6.5	30.8	1950
Air	110886836	40000	10	3918469	0.8	17	736607	736	756870	5.0	20.8	3542
Air	88243742	40000	10	3666977	0.8	20	466178	466	506145	2.7	14.0	3337
Air	38664026	40000	10	3666986	0.8	15	298056	298	907155	7.3	26.6	1122
Air	84535174	40000	10	3671731	0.8	18	507037	508	725129	4.4	20.1	2522
Control	2639663	40000	10	3496581	0.8	11	34581	35	338397	5.2	13.7	252
Control	2286861	40000	10	2884689	0.8	10	121419	121	658078	9.8	23.7	513
Control	2783944	40000	10	2783944	0.8	15	110849	111	561537	6.2	23.7	468
Control	2736001	40000	10	2736001	0.8	16	140016	140	781063	7.9	30.4	461
Control	3576173	40000	10	3162561	0.8	14	237844	238	994195	10.0	39.1	606
Control	1147224	40000	10	3038962	0.8	11	233731	233	956632	8.0	28.8	807
field trip	2719869	40000	10	2982518	0.8	12	272147	272	1074356	13.6	38.5	403
control	13237	40000	10	2791312	0.8	5.6	13347	13	1183131	13.9	41.9	650
control	15347	40000	10	5662657	0.8	5.6	19863	19	1999827	4.4	61.3	22
control	19863	40000	10	3421514	0.8	5.3	27	27	2074149	4.9	66.2	41

Amendment	Rep	As	Cs	D	Au	RF	Wd	concentration mg/kg dw	concentration mg/kg dw	surrogate area account	surrogate recove mg/kg dw	% recovery surrogate	concentration %P
Nitrate	A	24516618	40000	10	2668802	0.6	17	233757	234	911156	87	364	842
Nitrate	A	8025145	40000	10	4631063	0.6	16	46359	46	725610	42	168	275
Nitrate	A	28099916	40000	10	2931361	0.6	11	361490	361	486820	63	179	2024
Nitrate	A	44227089	40000	10	2688774	0.6	15	477506	478	713212	76	285	1874
Nitrate	A	14003257	40000	10	2682852	0.6	09	236980	237	664394	111	266	888
Nitrate	A	6117934	40000	10	2624712	0.6	10	130324	130	697087	112	280	456
Nitrate	A	5920803	40000	10	2785438	0.6	13	70447	70	709225	84	274	257
Nitrate	A	9132947	40000	10	3124770	0.6	12	104071	104	696058	80	241	433
Air	A	85429854	40000	10	3191696	0.6	17	505600	506	647442	73	319	1584
Air	A	56023662	40000	10	3088745	0.6	19	408334	408	1026537	76	306	1141
Air	A	40163662	40000	10	2976393	0.6	19	386707	386	1513625	113	546	989
Air	A	85088712	40000	10	3186603	0.6	18	630974	631	1054975	78	368	1783
Air	A	127337791	40000	10	3784842	0.6	17	844188	844	1298637	86	369	2288
Air	A	152560947	40000	10	2718031	0.6	14	1779117	1779	1462905	171	578	3076
Air	A	231025457	40000	10	4520664	0.6	18	124070	125	1293400	70	308	408
Air	A	6338623	40000	10	2733090	0.6	17	59744	60	1291304	122	508	118
Control	A	10666874	40000	10	2748264	0.6	13	131014	131	347349	43	136	984
Control	A	6329329	40000	10	2705886	0.6	12	87162	87	986070	136	392	222
Control	A	3813211	40000	10	2836870	0.6	12	48759	47	1256780	163	476	88
Control	A	10018897	40000	10	2797231	0.6	11	143564	144	675327	97	280	553
Control	A	4638643	40000	10	2863095	0.6	13	53463	53	687533	79	256	208
Control	A	663834	40000	10	2814606	0.6	12	83382	83	1561215	202	604	130
Control	A	21282014	40000	10	2916161	0.6	11	287133	287	1007534	136	371	773
Control	A	4947271	40000	10	2894645	0.6	10	82566	83	1005430	166	401	208
Bad trip	A	2546357	40000	10	2716339	0.6	5.3	7028	7	2497974	69	920	6
control	A	4088662	40000	10	2480017	0.6	5.3	13325	13	1791046	59	783	17
control	A	1734633	40000	10	2636184	0.6	5.6	5099	5	2065624	61	851	6
Amendment	Rep	As	Cs	D	As	RF	Wd	concentration mg/kg dw	concentration mg/kg dw	surrogate area account	surrogate recove mg/kg dw	% recovery surrogate	concentration %P
Nitrate	A	6343231	40000	10	2233466	0.6	10	151112	151	665662	166	401	377
Nitrate	A	15804207	40000	10	2584148	0.6	10	31123	31	840367	176	429	73
Nitrate	A	16229477	40000	10	2154028	0.6	07	563283	563	578042	201	360	1608
Nitrate	A	13220035	40000	10	2441551	0.6	07	385680	386	910751	266	487	702
Nitrate	A	1902313	40000	10	2068126	0.6	09	51839	52	603766	185	376	138
Nitrate	A	982464	40000	10	2298916	0.6	10	23067	23	826253	192	469	49
Nitrate	A	1543202	40000	10	2402081	0.6	10	34705	35	909462	205	494	70
Air	A	1826676	40000	10	2126880	0.6	11	40833	41	1174540	261	721	58
Air	A	13752335	40000	10	2423557	0.6	08	360795	361	1005443	286	842	721
Air	A	15302273	40000	10	2111800	0.6	08	487024	487	938276	299	861	830
Air	A	21089781	40000	10	2745621	0.6	08	500569	501	786562	177	384	1373
Air	A	42497753	40000	10	2510371	0.6	08	1081023	1081	2052784	522	1084	1016
Air	A	32740289	40000	10	2535246	0.6	09	720992	721	1242714	274	640	1126
Air	A	28406899	40000	10	1463801	0.6	08	1254110	1254	1220723	539	1074	1168
Air	A	33966405	40000	10	2346780	0.6	08	886956	886	1246206	328	663	1292
Air	A	21721739	40000	10	2280420	0.6	08	603239	603	1104888	307	630	956
Control	A	3801422	40000	10	2373759	0.6	06	134157	134	364456	136	200	660
Control	A	98427561	40000	10	4649443	0.6	06	1633497	1633	2356885	439	882	2771
Control	A	5776340	40000	10	1276417	0.6	07	319239	319	245456	136	251	1272
Control	A	11176282	40000	10	1254657	0.6	07	732965	733	199881	131	208	3022
Control	A	2476315	40000	10	1265437	0.6	07	141502	142	667911	369	720	197
Control	A	17841708	40000	10	1265388	0.6	07	987536	988	245458	136	253	3900
Control	A	18603881	40000	10	1312980	0.6	07	831181	831	199881	112	199	4882
Bad trip	A	9079736	40000	10	1217948	0.6	09	422361	422	290034	135	312	1354
control	A	3666663	40000	10	2224695	0.6	5.7	16006	16	1300003	6.6	616	20
control	A	5308143	40000	10	1573516	0.6	5.7	48979	49	1130575	6.6	608	52
control	A	8169348	40000	10	1099894	0.6	5.7	60045	60	1144561	9.7	1371	50

Amendment	Rep	Au	Cs	D	As	RF	Wd	concentration mg/kg dw	concentration mg/kg dw	surrogate area account	surrogate recove mg/kg dw	% recovery surrogate	concentration %dr
Nitrate	7A	2991153	40000	10	26171155	10	10	4976	5	1213613	19.8	47.3	10
Nitrate	A	408637	40000	10	2303530	10	09	8173	8	1073790	21.4	46.5	18
Nitrate	A	3041180	40000	10	2641397	10	09	51003	51	1426544	23.9	55.1	93
Nitrate	B	926905	40000	10	26819233	10	09	15656	16	1426544	24.1	55.6	28
Nitrate	B	1054408	40000	10	27731569	10	10	16085	16	2043140	31.3	76.3	21
Nitrate	C	2119426	40000	10	27356838	10	10	30130	30	976212	13.9	36.4	83
Nitrate	D	1564222	40000	10	27126967	10	09	25665	26	982315	19.1	39.9	80
Nitrate	D	2356321	40000	10	27169752	10	10	34586	35	1043915	15.3	38.5	90
Air	A	23205664	40000	10	29433065	11	11	302983	303	1236646	19.2	43.0	704
Air	A	27281962	40000	10	26569787	10	11	388057	388	980174	13.9	36.8	1075
Air	B	8169639	40000	10	32329871	10	11	96350	96	782524	9.0	24.1	400
Air	B	27668664	40000	10	27123990	10	11	386266	386	1162756	10.2	43.7	883
Air	C	10624434	40000	10	26716112	10	09	193633	194	1961832	34.8	74.9	256
Air	C	8630974	40000	10	28020056	10	12	109422	109	1300369	10.7	49.5	221
Air	D	8242527	40000	10	27936753	10	10	68705	67	1475124	11.9	53.9	124
Air	D	5658163	40000	10	27780412	10	09	90131	90	1613337	24.8	59.2	152
Control	A	536373	40000	10	27500070	10	09	9099	9	1129667	19.2	41.9	22
Control	A	448606	40000	10	26113372	10	07	10296	10	1253023	28.7	49.0	21
Control	B	2681474	40000	10	26477722	10	09	42719	43	1198260	19.1	43.0	98
Control	B	1438852	40000	10	27243381	10	09	24486	24	1256982	21.4	47.1	52
Control	C	12788304	40000	10	26896875	10	09	200197	200	1322970	20.7	49.7	420
Control	C	3247200	40000	10	27062208	10	08	65194	65	1257080	25.2	47.4	138
Control	D	3627863	40000	10	26636875	10	09	64061	64	1406809	24.9	50.2	126
Control	D	3675252	40000	10	26411176	10	09	69491	69	2011644	36.1	77.7	88
field trip	A	4211436	40000	10	26113379	10	06	11743	12	2660179	7.4	103.8	11
control	A	6716338	40000	10	27169047	10	06	17543	18	1966302	6.2	73.0	24
Amendment	Rep	Au	Cs	D	As	RF	Wd	concentration mg/kg dw	concentration mg/kg dw	surrogate area account	surrogate recove mg/kg dw	% recovery surrogate	concentration %dr
Nitrate	8A	15664473	40000	10	30730614	10	10	221182	221	1269926	18.3	44.4	498
Nitrate	A	26478257	40000	10	31119817	10	10	372442	372	1497744	21.1	50.7	734
Nitrate	B	10671316?	40000	10	34339826	10	10	1265647	1266	1548244	18.4	47.7	2666
Nitrate	B	6970036?	40000	10	33633324	10	09	904135	904	1074826	13.9	32.1	2813
Nitrate	C	10546620?	40000	10	30073?5	10	10	1542320	1542	1542298	22.5	54.2	2645
Nitrate	C	7749860?	40000	10	31454?0	10	10	1075431	1075	1241806	17.1	41.7	2577
Nitrate	D	11184956?	40000	10	30922?6?	10	09	1615291	1615	1231801	17.6	42.1	3837
Nitrate	D	11324293	40000	10	36103?8	10	10	127872	128	1511886	17.1	41.9	305
Air	A	27936389	40000	10	32803?8	12	09	306727	307	1146383	12.6	37.0	820
Air	A	26709835	40000	10	33244?0	10	09	360324	360	1373212	18.5	43.7	825
Air	B	26980111	40000	10	30609?5	10	10	350626	351	1422470	19.2	49.1	714
Air	B	26811669	40000	10	31349?2	10	10	346576	347	1494230	19.3	50.4	688
Air	C	17883315	40000	10	31436?5	12	10	207339	207	1675446	19.4	58.3	368
Air	C	32665651	40000	10	31466?0	11	10	362631	363	1778343	21.4	59.7	668
Air	D	98516575	40000	10	32063?1	10	10	722986	723	1388717	10.2	49.7	1581
Air	D	40713179	40000	10	31670?7	11	10	620970	621	1329672	18.6	44.4	1380
Control	A	1381747	40000	10	27243?1	08	08	25631	26	1256982	23.3	48.6	83
Control	A	582703	40000	10	26113?2	07	07	13025	13	1253023	27.5	50.7	26
Control	B	32211579	40000	10	31160?4	08	08	525077	525	1260705	20.9	43.4	1206
Control	B	3819147	40000	10	26411?6	08	08	77116	77	2011644	40.6	80.5	86
Control	C	1237043	40000	10	33926?4	08	08	19985	20	1430886	23.1	44.6	45
Control	C	25980111	40000	10	30609?5	08	08	450802	451	1422470	24.7	49.1	918
Control	D	32211579	40000	10	31160?4	08	08	547772	548	1260705	21.8	43.4	1261
Control	D	12711199	40000	10	26896?5	08	08	231453	231	1322970	24.1	48.4	478
field trip	A	5679736	40000	10	26896?7	10	08	27421	27	2070827	10.0	75.9	36
control	A	3362251	40000	10	27740?1	10	31	16991	17	1786074	8.9	68.2	24

Amendment	Rep	As	Cs	D	As	RF	Wd	concentration mg/kg	concentration mg/kg dw	surrogate area account	surrogate recove mg/kg dw	% recovery surrogate	concentration %/t
Air	2	24888931	40000	10	3671770	10	13	219526	220	1630674	14.5	40.3	455
Air	2	26747327	40000	10	3211072	10	16	237109	237	1315445	10.8	43.3	548
Air	2	26764129	40000	10	3288512	10	14	242679	243	1129803	10.2	38.3	666
Air	3	5110	40000	10	3310336	10	17	39	0	1236268	9.4	39.5	0
Air	3	93045413	40000	10	3655308	10	15	737563	738	1030200	8.2	29.8	2476
Air	3	47707875	40000	10	3396304	10	14	429870	430	1111368	10.0	34.9	1233
Air	3	23622175	40000	10	3514687	10	20	140935	141	1611517	9.5	48.5	291
Air	3	201462870	40000	10	3466171	10	17	1412961	1413	1241212	8.7	37.9	3733
Nitrate	1	4708867	40000	10	3495065	10	20	28744	29	1586458	9.7	48.0	60
Nitrate	1	29974597	40000	10	3564006	10	20	167816	168	760674	4.4	22.4	750
Nitrate	1	44466035	40000	10	2607161	10	16	455076	455	882349	9.0	36.8	1272
Nitrate	1	25140831	40000	10	2388617	10	15	295506	296	560681	6.6	24.8	1191
Nitrate	1	19466615	40000	10	2647716	10	15	214346	214	665812	7.3	26.6	806
Nitrate	1	15864403	40000	10	2430227	10	15	180113	180	787093	9.4	34.2	552
Nitrate	1	19471017	40000	10	3108786	10	16	156089	156	900568	7.6	30.6	509
Nitrate	1	40874365	40000	10	3536313	10	17	288013	288	1196731	8.5	35.8	805
Control	1	57133083	40000	10	2878026	10	13	667830	668	1124407	13.1	41.3	1617
Control	1	50627568	40000	10	3386405	10	14	447466	447	782801	6.7	23.6	1879
Control	1	93909	40000	10	1058146	10	13	2954	3	518240	16.5	51.8	6
Control	1	13038231	40000	10	2078377	10	14	196152	196	992715	14.0	60.5	367
Control	1	20841131	40000	10	2188110	10	12	32739	33	1057530	10.6	51.1	84
Control	1	3677880	40000	10	2529887	10	14	42071	42	1057530	12.1	42.5	99
Control	1	51468686	40000	10	2624119	10	12	68871	69	1052821	14.0	42.4	162
Control	1	1751552	40000	10	2465762	10	15	20073	20	942246	10.6	40.4	50
Red Inp	1	3471446	40000	10	2670211	10	5.1	10799	11	1427834	4.4	56.5	19
Red Inp	1	2003876	40000	10	1490876	10	5.0	11830	12	767674	4.3	54.4	22
control	1	4356944	40000	10	2527574	10	5.0	13897	14	1060117	5.4	68.0	20
control	1	3201941	40000	10	2656812	10	5.8	8064	8	1554566	0.4	6.7	141

C.1.2 Long Chain Aliphatics

Amendment		Ax	Cis	D	Ais	RF	Wd	concentration mg/kg dw	concentration mg/kg dw	surrogate area account	surrogate recove mg/kg dw	% recovery surrogate	concentration %P
Nitrate	1Aa	2892972	16000	5 0	533646	1 0	1 7	262393	262	256389	4 7	19 7	1335
Nitrate	Ab	2256597	16000	5 0	436837	1 0	1 5	284700	285	189606	4 8	17 7	1611
Nitrate	Ba	12136786	16000	5 0	469670	1 0	1 5	1425207	1425	402645	9 5	35 1	4065
Nitrate	Bb	9884486	16000	5 0	469579	1 0	1 4	1266637	1266	367376	9 4	32 0	3955
Nitrate	Ca	4423954	16000	5 0	336170	1 0	1 9	572779	573	244560	6 3	29 8	1925
Nitrate	Cb	16176579	16000	5 0	450876	1 0	1 8	1643726	1644	244506	5 0	22 2	7411
Nitrate	Da	3837255	16000	5 0	510633	1 0	1 1	534948	535	256867	7 2	20 7	2580
Nitrate	Db	3180799	16000	5 0	656208	1 0	1 0	377685	378	238716	5 7	14 9	2536
Air	Aa	19808412	16000	5 0	451180	1 0	1 5	2356080	2355	315707	7 5	28 6	8229
Air	Ab	10392128	16000	5 0	349211	1 0	1 6	1539859	1540	311678	9 2	36 5	4218
Air	Ba	11344573	16000	5 0	664715	1 0	1 5	1040337	1040	207417	3 8	14 5	7170
Air	Bb	3664863	16000	5 0	511956	1 0	1 5	381915	382	243535	5 0	19 5	1963
Air	Ca	19808412	16000	5 0	451180	1 0	1 5	2377873	2378	315707	7 6	28 6	8309
Air	Cb	13630261	16000	5 0	526876	1 0	1 5	1378610	1379	373546	7 6	29 0	4754
Air	Da	19414984	16000	5 0	563523	1 0	1 6	1814215	1814	309086	5 8	22 8	7944
Air	Db	8798482	16000	5 0	300130	1 0	1 7	1405335	1405	247507	7 9	33 7	4167
Control	Aa	636999	16000	5 0	548026	1 0	1 0	95789	96	245886	7 4	18 4	522
Control	Ab	248121	16000	5 0	141008	1 0	1 1	132022	132	206250	21 9	59 8	221
Control	Ba	4754752	16000	5 0	225330	1 0	1 1	1582132	1582	244469	16 3	44 4	3566
Control	Bb	2629109	16000	5 0	426982	1 0	1 2	433484	433	280649	9 3	26 6	1620
Control	Ca	1880613	16000	5 0	628344	1 0	1 2	205120	205	256730	5 7	16 7	1227
Control	Cb	7323980	16000	5 0	667966	1 0	1 2	747733	748	240107	4 9	14 9	5010
Control	Da	474415	16000	5 0	618659	1 0	1 2	50519	51	217836	4 6	14 4	351
Control	Db	529043	16000	5 0	611789	1 0	1 1	63943	64	217991	5 3	14 6	439
field trip		5080880	16000	5 0	505051	1 0	5 6	146226	146	202387	1 2	16 4	892
control		6668317	16000	5 0	554040	1 0	5 1	194270	194	687988	4 0	50 8	383
control		7143661	16000	5 0	717818	1 0	5 8	139207	139	756712	2 9	43 1	323
control		7128539	16000	5 0	622612	1 0	4 5	210353	210	739351	4 4	48 6	433
Amendment		Ax	Cis	D	Ais	RF	Wd	concentration mg/kg dw	concentration mg/kg dw	surrogate area account	surrogate recove mg/kg dw	% recovery surrogate	concentration %P
Nitrate	2Aa	43309030	40000	1 0	3436402	1 0	1 60	322873	323	721802	5 4	21 5	1503
Nitrate	Ab	84448787	40000	1 0	3829323	1 0	1 05	858358	858	724452	7 4	19 3	4437
Nitrate	Ba	68438026	40000	1 0	3584401	1 0	1 95	401135	401	662079	3 8	18 6	2156
Nitrate	Bb	39797488	40000	1 0	3575591	1 0	1 67	272434	272	1639011	11 2	46 9	581
Nitrate	Ca	165152798	40000	1 0	3462048	1 0	1 86	1048625	1049	1103788	7 0	32 6	3217
Nitrate	Cb	200992600	40000	1 0	3779108	1 0	1 91	1137126	1137	1209198	6 8	32 7	3478
Nitrate	Da	48173559	40000	1 0	3463220	1 0	1 23	462039	462	980399	9 3	26 6	1614
Nitrate	Db	38040380	40000	1 0	3510875	1 0	1 12	395695	396	1154711	12 0	33 6	1177
Air	Aa	57976636	40000	1 0	4350334	1 0	1 22	446297	446	397536	3 1	9 3	4798
Air	Ab	82110544	40000	1 0	3775884	1 0	1 56	571770	572	984446	6 7	26 1	2189
Air	Ba	127336140	40000	1 0	3710557	1 0	1 70	827614	828	1264321	8 3	35 4	2338
Air	Bb	130201029	40000	1 0	3848170	1 0	1 55	944436	944	1220731	8 9	34 2	2760
Air	Ca	195615031	40000	1 0	3562541	1 0	1 42	1583802	1584	826983	6 7	23 7	6672
Air	Cb	178483878	40000	1 0	3685848	1 0	1 55	484954	485	1016844	2 8	10 7	4518
Air	Da	218623984	40000	1 0	4889886	1 0	1 38	1322743	1323	1226922	7 4	25 7	5156
Air	Db	24814706	40000	1 0	3548447	1 0	1 36	206894	207	918229	7 7	26 5	782
Control	Aa	146475186	40000	1 0	3897509	1 0	1 40	1098307	1098	829771	4 7	16 5	6635
Control	Ab	26211233	40000	1 0	3337343	1 0	1 38	232643	233	979462	8 7	30 0	775
Control	Ba	117733742	40000	1 0	3896821	1 0	1 12	1107888	1108	775623	7 3	20 4	5444
Control	Bb	140456094	40000	1 0	3896821	1 0	1 42	1039920	1040	2453799	18 2	64 4	1615
Control	Ca	79846672	40000	1 0	3630817	1 0	0 89	1008902	1009	508606	6 4	14 3	7073
Control	Cb	50621922	40000	1 0	3753033	1 0	1 64	336508	337	841736	5 6	22 9	1467
Control	Da	57302472	40000	1 0	3640235	1 0	1 06	595394	595	669167	7 0	18 8	3166
Control	Db	18841930	40000	1 0	3568596	1 0	1 07	201534	202	532616	5 7	15 3	1320
field trip		39617940	40000	1 0	3580461	1 0	5 01	90364	90	1894527	4 3	54 1	167
control		30530008	40000	1 0	3580461	1 0	5 03	69358	69	2141083	4 9	61 1	113

Amendment	Ar	Ca	D	As	Rf	Wd	concentration mg/kg dw	surrogate area account	surrogate recove mg/kg dw	% recovery surrogate	concentration %P
Nitrate	9443000	40000	10	4015350	10	14	667549	677775	4.6	17.3	3658
Nitrate	6853026	40000	10	3554159	10	13	593860	920887	7.9	26.8	2220
Nitrate	919784 53	40000	10	3742108	10	13	763662	907663	7.5	24.8	3079
Nitrate	13824 3807	40000	10	3818585	10	11	1316471	1196530	11.4	32.0	4112
Nitrate	4220806	40000	10	3486680	10	19	289127	1505650	9.4	44.2	610
Nitrate	73454186	40000	10	3662458	10	19	435871	2257715	13.4	62.7	896
Nitrate	1165326922	40000	10	4031078	10	16	782399	1909360	12.5	48.4	1574
Nitrate	13571580 4	40000	10	4428546	10	16	776782	1478359	9.5	34.1	2276
Air	80997087	40000	10	4360253	10	14	416863	1088040	7.3	24.8	1676
Air	257869402	40000	10	4442003	10	13	1765531	1608739	11.0	37.0	4766
Air	86600276	40000	10	3915719	10	19	398781	1202981	8.7	31.4	1174
Air	64804182	40000	10	3824348	10	16	574537	2002845	13.6	53.5	1074
Air	48808627	40000	10	3487300	10	16	343161	1070177	7.6	31.4	1094
Air	17068985	40000	10	5974049	10	14	84077	3633634	18.9	88.6	1267
Air	787291 56	40000	10	3844296	10	16	494813	891234	5.7	23.7	2087
Air	24699457	40000	10	3853283	10	16	163709	4933620	32.7	130.9	125
Control	15621211	40000	10	3803623	10	11	151256	882634	8.6	24.0	630
Control	4427329	40000	10	3898095	10	13	36217	1391808	11.4	38.5	96
Control	9823600	40000	10	3727833	10	11	99740	926091	9.6	25.4	393
Control	29753777	40000	10	3831220	10	13	243543	1002563	8.2	28.1	634
Control	72516494	40000	10	5620354	10	17	322116	785192	3.5	14.5	2216
Control	81123997	40000	10	3942610	10	18	460788	748566	4.3	19.4	2373
Control	20754139	40000	10	3564665	10	12	205452	914830	9.1	26.1	787
Control	2446835	40000	10	3617913	10	12	259852	780564	7.5	22.1	1176
field trip	60254072	40000	10	3789152	10	16	117078	2175460	4.2	58.0	198
field trip	42364861	40000	10	5258747	10	8.6	58050	2225524	3.1	43.3	135
control	31747646	40000	10	3735513	10	5.3	66102	1763007	3.6	48.3	135
control	5347803 8	40000	10	3566876	10	5.0	122104	2203510	5.0	63.2	193

Amendment	Ar	Ca	D	As	Rf	Wd	concentration mg/kg dw	surrogate area account	surrogate recove mg/kg dw	% recovery surrogate	concentration %P
Nitrate	81296569	40000	10	3201156	08	1.86	529175	878557	5.7	28.1	1880
Nitrate	16800174 6	40000	10	3868466	08	2.08	880741	1387035	7.3	38.1	2326
Nitrate	3709196 37	40000	10	3115637	08	1.45	3359186	2068994	10.9	88.5	4907
Nitrate	1439122 62	40000	10	3741741	08	1.86	838085	904677	5.9	24.7	3794
Nitrate	8960847 6	40000	10	3426000	08	1.44	743826	465685	4.0	14.5	5131
Nitrate	2542111 79	40000	10	3211119	08	1.64	1971824	1070546	8.3	34.1	5784
Nitrate	5688847 6	40000	10	3080742	08	1.66	439742	453086	3.6	15.0	2624
Nitrate	6865784 5	40000	10	2897176	08	1.40	688886	466275	4.7	16.5	3585
Air	11470388 0	40000	10	3555915	08	1.46	891242	682513	6.4	19.9	4488
Air	894072 11	40000	10	3835236	08	1.43	724385	526139	3.6	13.7	5279
Air	1018091 45	40000	10	3646521	08	1.97	678814	974042	6.5	27.3	2118
Air	9746853 3	40000	10	3548717	08	1.91	589486	1017614	6.2	29.3	2010
Air	1360138 43	40000	10	3618499	08	1.86	880736	759870	4.6	19.8	4459
Air	1086 3074	40000	10	3985077	08	2.04	844239	506145	2.6	13.3	4135
Air	9881687 6	40000	10	3569966	08	1.45	780322	907155	7.0	25.3	3000
Air	1156542 60	40000	10	3871731	08	1.86	660884	725129	4.1	19.2	3451
Control	7798717	40000	10	2838983	08	1.66	1146900	336397	4.9	13.0	880
Control	9345532 5	40000	10	2966861	08	0.96	1323947	656078	9.3	22.5	5886
Control	4088668	40000	10	2804668	08	1.52	406587	591537	5.9	22.5	1805
Control	3865154 7	40000	10	2763944	08	1.53	374946	781053	7.5	28.9	1297
Control	7312885 5	40000	10	2738001	08	1.56	700317	984186	9.6	37.2	1886
Control	8308562 7	40000	10	3678173	08	1.43	503020	956632	7.7	27.4	1833
Control	3320108 8	40000	10	3162561	08	1.08	389295	1074365	12.9	34.7	1150
field trip	9475296 1	40000	10	3039662	08	1.20	635500	1183131	13.2	39.8	1596
control	2202344	40000	10	2962518	08	5.58	5444	1089627	4.2	58.3	9
control	3672734	40000	10	2791312	08	5.58	8374	1068324	4.5	62.2	15
control	8052204	40000	10	3421514	08	5.34	13560	2074140	4.6	62.0	22

Amendment		Ax	Cis	D	Ais	RF	Wd	concentration mg/kg dw	concentration mg/kg dw	surrogate area account	surrogate recove mg/kg dw	% recovery surrogate	concentration %P
Nitrate	5Aa	54338904	40000	1 0	2668602	0 8	1 66	492726	493	911156	8 3	34 6	1422
Nitrate	Ab	20773283	40000	1 0	4631063	0 8	1 61	114124	114	725610	4 0	16 0	712
Nitrate	Ba	42162933	40000	1 0	2931361	0 8	1 14	515837	516	486920	6 0	17 0	3037
Nitrate	Bb	86215612	40000	1 0	2668774	0 8	1 50	891675	892	713212	7 2	27 1	3288
Nitrate	Ca	34636332	40000	1 0	2663852	0 8	0 95	557482	557	656394	10 6	25 1	2222
Nitrate	Cb	14905173	40000	1 0	2624712	0 8	1 02	227565	228	697067	10 6	27 2	838
Nitrate	Da	7921677	40000	1 0	2785438	0 8	1 30	89636	90	709225	8 0	26 0	344
Nitrate	Db	7592510	40000	1 0	3124770	0 8	1 21	82280	82	699058	7 6	22 9	360
Air	Aa	155242248	40000	1 0	3191696	0 8	1 74	1140879	1141	947462	7 0	30 4	3759
Air	Ab	114329820	40000	1 0	3088745	0 8	1 90	798185	798	1028537	7 2	34 0	2344
Air	Ba	106011576	40000	1 0	2979393	0 8	1 94	749641	750	1513925	10 7	52 0	1443
Air	Bb	163967744	40000	1 0	3169503	0 8	1 83	1156550	1157	1054975	7 4	34 0	3398
Air	Ca	268301238	40000	1 0	3784842	0 8	1 71	1817643	1818	1298837	8 2	35 1	5181
Air	Cb	147508910	40000	1 0	2719031	0 8	1 36	1635949	1636	1462905	16 2	55 0	2974
Air	Da	41331452	40000	1 0	4520954	0 8	1 76	212076	212	1293400	8 6	29 3	725
Air	Db	19553441	40000	1 0	2733099	0 8	1 67	175321	175	1291304	11 6	46 3	383
Control	Aa	19033467	40000	1 0	2746264	0 8	1 26	224664	225	347349	4 1	12 9	1738
Control	Ab	17612678	40000	1 0	2705866	0 8	1 15	230667	231	988070	12 9	37 3	619
Control	Ba	9845681	40000	1 0	2835670	0 8	1 17	118711	119	1255789	15 5	45 3	262
Control	Bb	27645154	40000	1 0	2797231	0 8	1 07	376731	377	675327	9 2	24 7	1526
Control	Ca	14464299	40000	1 0	2893096	0 8	1 29	158600	159	687533	7 5	24 3	653
Control	Cb	24172111	40000	1 0	2814906	0 8	1 20	293231	293	1561215	19 2	57 4	511
Control	Da	38613171	40000	1 0	2916161	0 8	1 09	469785	470	1007534	12 9	36 3	1330
Control	Db	14950821	40000	1 0	2694645	0 8	0 98	237382	237	1005430	16 0	38 2	622
field trip		3063811	40000	1 0	2716339	0 8	5 34	8640	9	2497974	7 0	94 0	9
control		2615418	40000	1 0	2460017	0 8	5 34	8144	8	1791046	5 6	74 4	11
control		948043	40000	1 0	2636184	0 8	5 34	2755	3	2085624	6 1	80 9	3
Amendment		Ax	Cis	D	Ais	RF	Wd	concentration mg/kg dw	concentration mg/kg dw	surrogate area account	surrogate recove mg/kg dw	% recovery surrogate	concentration %P
Nitrate	6Aa	11395658	40000	1 0	2233466	0 8	1 0	256724	257	686892	15 5	37 3	686
Nitrate	Ab	1847422	40000	1 0	2584148	0 8	1 0	36577	36	849367	16 4	39 9	89
Nitrate	Ba	17170840	40000	1 0	2154026	0 8	0 7	554680	555	578042	18 7	32 6	1701
Nitrate	Bb	17720872	40000	1 0	2441561	0 8	0 7	481063	481	910751	24 7	45 3	1061
Nitrate	Ca	9829672	40000	1 0	2098126	0 8	0 9	244240	244	603766	15 3	36 0	698
Nitrate	Cb	2649596	40000	1 0	2299916	0 8	1 0	57294	57	826253	17 9	43 7	131
Nitrate	Da	5766489	40000	1 0	2402061	0 8	1 0	120701	121	909482	19 0	46 0	262
Nitrate	Db	3260151	40000	1 0	2126880	0 8	1 1	67915	68	1174540	24 3	67 1	101
Air	Aa	18338914	40000	1 0	2423557	0 8	0 8	484985	485	1005943	26 6	80 4	982
Air	Ab	16794864	40000	1 0	2111900	0 8	0 8	497507	498	939276	27 8	84 0	921
Air	Ba	30879893	40000	1 0	2745621	0 8	0 8	663900	664	766562	16 5	33 9	1957
Air	Bb	59863569	40000	1 0	2519371	0 8	0 8	1412580	1413	2052784	48 8	99 0	1427
Air	Ca	39701274	40000	1 0	2535248	0 8	0 9	813393	813	1242714	25 5	59 6	1366
Air	Cb	41698840	40000	1 0	1483801	0 8	0 8	1713154	1713	1220723	50 2	100 0	1714
Air	Da	37564731	40000	1 0	2346780	0 8	0 8	921458	921	1246205	30 6	64 5	1428
Air	Db	29490472	40000	1 0	2290420	0 8	0 8	762266	762	1104888	28 6	58 6	1300
Control	Aa	2340269	40000	1 0	2373759	0 8	0 6	81140	81	364456	12 6	18 7	435
Control	Ab	114759788	40000	1 0	4649443	0 8	0 6	1989675	1990	2368685	40 9	61 6	3231
Control	Ba	13428886	40000	1 0	1276417	0 8	0 7	690765	691	245458	12 6	23 4	2966
Control	Bb	3663460	40000	1 0	1254657	0 8	0 6	223618	224	199981	12 2	19 4	1155
Control	Ca	13920972	40000	1 0	1265537	0 8	0 7	740892	741	697911	37 1	67 0	1106
Control	Cb	12431625	40000	1 0	1266388	0 8	0 7	640432	640	245458	12 6	23 6	2717
Control	Da	10923607	40000	1 0	1312680	0 8	0 7	570192	570	199981	10 4	18 5	3080
Control	Db	16939992	40000	1 0	1217988	0 8	0 9	733277	733	290934	12 6	29 0	2526
field trip		3998080	40000	1 0	2224886	0 8	5 7	15397	15	1390003	5 4	75 9	20
control		7761879	40000	1 0	1573518	0 8	5 7	42256	42	1130575	6 2	87 3	48
control		8055627	40000	1 0	1089694	0 8	5 7	63327	63	1144561	9 0	127 6	50

Amendment	Rep	Ca	D	As	RF	Wd	concentration mg/kg dw	concentration mg/kg	surrogate area account	surrogate recove mg/kg dw	% recovery surrogate	concentration %P
Nitrate	7	40000	10	2617156	10	10	33673	34	1213613	21.4	61.2	66
Nitrate	Aa	40000	10	2353530	10	09	14222	14	1073799	23.2	60.3	28
Nitrate	Aa	40000	10	2641397	10	09	137763	136	1425644	25.9	59.6	231
Nitrate	Ba	40000	10	2619233	10	09	30744	31	1426544	26.1	60.1	51
Nitrate	Ba	40000	10	2731569	10	10	63285	63	2043148	33.8	62.5	77
Nitrate	Ca	40000	10	2736539	10	09	25990	26	978212	15.0	39.4	66
Nitrate	Ca	40000	10	2712697	10	09	42250	42	982315	17.4	40.0	106
Nitrate	Ca	40000	10	2708752	10	10	13446	13	1043815	16.6	41.6	32
Air	Aa	40000	10	2943095	10	11	168238	168	1236846	17.5	46.5	382
Air	Aa	40000	10	2656787	10	11	531927	532	980174	15.1	39.6	1336
Air	Ba	40000	10	3239987	10	11	128992	129	782324	9.7	26.0	498
Air	Ba	40000	10	2712390	10	11	522518	523	1182756	17.5	47.3	1105
Air	Ca	40000	10	2671612	10	09	264317	264	1961832	37.7	81.0	326
Air	Ca	40000	10	2802056	10	12	177779	178	1360389	18.0	53.6	332
Air	Ca	40000	10	2703683	10	10	84981	85	1475124	12.9	56.3	146
Control	Aa	40000	10	2780412	10	10	167019	167	1613337	20.8	64.0	261
Control	Aa	40000	10	2750070	10	09	81445	81	1126597	20.8	45.3	180
Control	Ba	40000	10	2611372	10	07	100832	101	1253023	31.0	62.9	180
Control	Ba	40000	10	2647222	10	09	177220	177	1198260	20.7	49.5	361
Control	Ca	40000	10	2724361	10	09	82168	82	1256882	23.1	50.9	161
Control	Ca	40000	10	2686875	10	09	521418	521	1322970	22.4	50.5	1032
Control	Ca	40000	10	2706208	10	08	127631	128	1257080	27.3	51.3	249
Control	Ba	40000	10	2663875	10	09	141289	141	1409809	26.9	54.3	280
Control	Ba	40000	10	2641176	10	09	175472	175	2011044	39.0	84.0	209
field trip	Ba	40000	10	2613379	10	30	11183	11	2680179	14.8	112.3	10
control	Ca	40000	10	2786947	10	30	13486	13	1966302	10.4	79.0	17
Amendment	Rep	Ca	D	As	RF	Wd	concentration mg/kg dw	concentration mg/kg	surrogate area account	surrogate recove mg/kg dw	% recovery surrogate	concentration %P
Nitrate	8	40000	10	3073614	10	10	691825	692	1268926	19.5	47.9	1444
Nitrate	Aa	40000	10	3119817	10	10	1031139	1031	1497744	22.8	54.8	1862
Nitrate	Aa	40000	10	3433926	10	10	1966255	1966	1548244	19.8	61.5	3801
Nitrate	Ba	40000	10	3535324	10	09	1924363	1924	1274826	17.9	41.2	4675
Nitrate	Ba	40000	10	3007375	10	10	2400163	2400	1542298	24.3	58.5	4100
Nitrate	Ca	40000	10	3145620	10	10	1112509	1113	1241808	18.5	45.1	2466
Nitrate	Ca	40000	10	3092265	10	09	2211943	2212	1231801	19.2	45.5	4665
Nitrate	Ca	40000	10	3610336	10	10	312336	312	1511886	18.4	45.3	680
Air	Aa	40000	10	3260388	10	12	710724	711	1148353	13.6	40.0	1776
Air	Aa	40000	10	3324630	10	09	935565	936	1373212	20.0	47.2	1984
Air	Ba	40000	10	3060975	10	10	710831	711	1422470	20.7	53.0	1340
Air	Ba	40000	10	3134872	10	10	572850	573	1494230	20.9	54.4	1063
Air	Ca	40000	10	3143825	10	12	702091	702	1675448	21.0	60.8	1154
Air	Ca	40000	10	3146080	10	11	416480	416	1776343	23.1	64.5	646
Air	Ca	40000	10	3208361	10	10	548411	548	1388717	11.0	49.4	1110
Control	Aa	40000	10	3167087	10	11	672413	672	1329672	17.9	47.9	1403
Control	Aa	40000	10	2724381	10	08	110202	110	1256982	26.2	52.7	209
Control	Ba	40000	10	2611372	10	07	121274	121	1253023	29.7	54.8	221
Control	Ba	40000	10	3116024	10	08	1294453	1294	1280765	22.5	49.9	2684
Control	Ca	40000	10	2641176	10	08	219863	220	2011044	43.9	89.9	253
Control	Ca	40000	10	3382874	10	08	186870	190	1430888	25.0	49.1	394
Control	Ca	40000	10	3060975	10	08	913914	914	1422470	26.7	53.0	1723
Control	Ba	40000	10	3116024	10	08	1130248	1130	1280765	23.5	46.9	2400
field trip	Ba	40000	10	2686875	10	08	624885	625	1322970	26.0	52.3	1196
control	Ca	40000	10	2686077	10	30	5600	6	2070827	10.8	81.9	7
				2774071	10	31	7769	8	1786074	9.6	73.6	11

Amendment	Rep	As	Cs	D	As	Rf	Wd	concentration mg/kg dw	concentration mg/kg dw	surrogate area account	surrogate recove mg/kg dw	% recovery surrogate	concentration %P
Air	1	34892843	40000	10	3571779	10	13	335075	335	1630874	15.7	82.1	643
Air	2	27372241	40000	10	3211072	10	16	243806	244	1315445	11.7	46.8	521
Air	3	36554633	40000	10	3288512	10	14	351086	351	1129803	11.1	38.2	896
Air	4	3831275	40000	10	3310336	10	17	29879	30	1230266	10.1	42.6	70
Air	5	122665728	40000	10	3655306	10	15	1052626	1053	1030200	8.8	32.2	3272
Air	6	40366082	40000	10	3396304	10	14	362772	363	1111388	10.8	37.7	1043
Air	7	29106412	40000	10	3514687	10	20	186335	186	1811517	10.3	82.3	356
Air	8	185809434	40000	10	3466171	10	17	1407916	1408	1241212	9.4	40.9	3444
Nitrate	1	7367147	40000	10	3495065	10	20	48565	49	1588458	10.5	51.9	94
Nitrate	2	64882176	40000	10	3584006	10	20	406440	406	760674	4.6	24.2	1982
Nitrate	3	56925675	40000	10	2607161	10	16	629115	629	882349	9.6	38.6	1026
Nitrate	4	23984254	40000	10	2398617	10	15	326020	326	560681	7.1	26.6	1217
Nitrate	5	34033780	40000	10	2647718	10	15	404711	405	885612	7.9	28.7	1410
Nitrate	6	38818928	40000	10	2430227	10	15	500038	500	787063	10.1	37.0	1362
Nitrate	7	24886302	40000	10	3106766	10	16	227134	227	900568	8.2	33.1	686
Nitrate	8	34277236	40000	10	3336313	10	17	262110	262	1198731	9.2	38.6	678
Control	1	66661430	40000	10	2878026	10	13	840462	840	1124407	14.2	44.6	1884
Control	2	7096811	40000	10	3386405	10	14	673361	673	762801	7.2	25.7	2619
Control	3	4726519	40000	10	1056146	10	13	14538	15	518240	17.8	58.9	26
Control	4	19897946	40000	10	2078377	10	14	303076	303	992715	15.1	84.5	556
Control	5	1923366	40000	10	2186110	10	12	32928	33	1057530	17.9	88.2	59
Control	6	13660269	40000	10	2029887	10	14	186991	186	1057530	13.1	45.9	388
Control	7	13125940	40000	10	2624119	10	12	189119	189	1052921	15.2	45.8	413
Control	8	10354018	40000	10	2465782	10	15	129142	129	942246	11.7	43.6	294
field trip	1	5733239	40000	10	2670211	10	51	1926	2	1427834	4.6	61.0	3
field trip	2	122186	40000	10	1400876	10	50	746	1	767674	4.7	58.8	1
control	1	461364	40000	10	2627574	10	50	1560	2	1660117	5.6	73.4	2
control	2	2103063	40000	10	2656612	10	50	5741	6	155456	0.4	6.2	93

C.1.3 Aromatics

Amendment		Ax	Cis	D	Ais	RF	Wd	concentration mg/kg dw	concentration mg/kg dw	surrogate area account	surrogate recove mg/kg dw	% recovery surrogate	concentration %P
Nitrate	1Aa	416806	16000	5 0	452410	1 0	1 7	42200	42	158699	3 2	13 6	311
Nitrate	Ab	223323	16000	5 0	258675	1 0	1 5	45199	45	160307	6 5	24 0	189
Nitrate	Ba	9234581	16000	5 0	314664	1 0	1 5	1531491	1531	270993	9 0	33 3	4594
Nitrate	Bb	4643560	16000	5 0	470044	1 0	1 4	562115	562	442269	10 7	38 4	1543
Nitrate	Ca	5340060	16000	5 0	500252	1 0	1 9	439684	440	218910	3 6	16 9	2596
Nitrate	Cb	14721278	16000	5 0	442123	1 0	1 8	1443616	1444	359463	7 1	31 5	4587
Nitrate	Da	1703763	16000	5 0	607952	1 0	1 1	188794	189	305873	6 8	19 5	969
Nitrate	Db	4325522	16000	5 0	577799	1 0	1 0	552006	552	333176	8 5	22 3	2473
Air	Aa	16323767	16000	5 0	341634	1 0	1 5	2425671	2426	322842	9 6	38 6	6631
Air	Ab	1005273	16000	5 0	515696	1 0	1 6	95456	95	338597	6 4	25 4	376
Air	Ba	1620271	16000	5 0	337805	1 0	1 5	243389	243	293197	8 8	33 6	724
Air	Bb	4937826	16000	5 0	602996	1 0	1 5	411197	411	400759	6 7	25 7	1598
Air	Ca	12462616	16000	5 0	455353	1 0	1 5	1402809	1403	301065	6 8	25 6	5462
Air	Cb	12333275	16000	5 0	566791	1 0	1 5	1093603	1094	478118	8 5	32 5	3361
Air	Da	16988279	16000	5 0	525012	1 0	1 6	1583951	1584	390837	7 3	28 8	5497
Air	Db	10214186	16000	5 0	555065	1 0	1 7	834815	835	675821	11 0	47 1	1771
Control	Aa	992381	16000	5 0	458932	1 0	1 0	168904	169	182342	6 2	15 4	1096
Control	Ab	2699483	16000	5 0	575059	1 0	1 1	333305	333	173092	4 3	11 7	2861
Control	Ba	2321942	16000	5 0	575059	1 0	1 1	286498	286	242377	6 0	16 3	1756
Control	Bb	2765070	16000	5 0	509472	1 0	1 2	363260	363	173092	4 5	13 2	2762
Control	Ca	1225918	16000	5 0	412426	1 0	1 2	194856	195	368095	11 7	34 5	564
Control	Cb	1936807	16000	5 0	509364	1 0	1 2	242089	242	368095	9 2	28 0	865
Control	Da	1243203	16000	5 0	414143	1 0	1 2	187210	187	376486	11 3	35 2	532
Control	Db	1266721	16000	5 0	368654	1 0	1 1	240446	240	376486	14 3	39 5	608
field trip		7646486	16000	5 0	346901	1 0	5 6	304400	304	244978	2 0	27 3	1114
control		6913093	16000	5 0	534618	1 0	5 1	197816	198	756241	4 3	54 8	361
control		5606981	16000	5 0	361814	1 0	5 8	216114	216	411032	3 0	44 0	491
control		2704607	16000	5 0	400698	1 0	4 5	117392	117	606360	5 3	58 6	200

Amendment		Ax	Cis	D	Ais	RF	Wd	concentration mg/kg dw	concentration mg/kg dw	surrogate area account	surrogate recove mg/kg dw	% recovery surrogate	concentration %P
Nitrate	2Aa	66863465	40000	1 0	3013775	1 0	1 6	537963	538	889593	7 2	28 6	1883
Nitrate	Ab	80727205	40000	1 0	2306114	1 0	1 1	1269391	1269	634847	10 1	26 6	4840
Nitrate	Ba	53331035	40000	1 0	3072197	1 0	1 9	345135	345	631156	4 1	19 9	1736
Nitrate	Bb	39410550	40000	1 0	2936536	1 0	1 7	310977	311	1556577	12 3	51 4	605
Nitrate	Ca	39793899	40000	1 0	3072197	1 0	1 9	269453	269	1120067	7 6	35 3	764
Nitrate	Cb	163114654	40000	1 0	3426013	1 0	1 9	983320	983	2087889	12 3	59 0	1634
Nitrate	Da	89833536	40000	1 0	3206019	1 0	1 2	880788	881	1175433	11 5	36 5	2483
Nitrate	Db	71921587	40000	1 0	2831368	1 0	1 1	877896	878	1207996	14 7	41 3	2126
Air	Aa	42568218	40000	1 0	3038030	1 0	1 2	446253	446	680649	7 1	21 7	2058
Air	Ab	109496091	40000	1 0	3252792	1 0	1 6	837592	838	1381166	10 6	41 1	2039
Air	Ba	137229428	40000	1 0	1489523	1 0	1 7	2102637	2103	1608378	24 6	104 5	2012
Air	Bb	125762624	40000	1 0	3094382	1 0	1 5	1017795	1018	1356295	11 0	42 4	2400
Air	Ca	122121051	40000	1 0	2623761	1 0	1 4	1270986	1271	1288535	13 4	47 5	2675
Air	Cb	114411874	40000	1 0	2749146	1 0	1 6	1036480	1036	1448883	13 1	51 0	2032
Air	Da	227335032	40000	1 0	2934039	1 0	1 4	2169331	2169	2934039	28 0	98 8	2242
Air	Db	227837217	40000	1 0	3038989	1 0	1 4	2099041	2099	1666510	15 4	53 1	3956
Control	Aa	114310974	40000	1 0	3059835	1 0	1 4	1031389	1031	1879483	17 0	59 4	1735
Control	Ab	53641985	40000	1 0	2935536	1 0	1 4	512236	512	1147921	11 0	37 8	1354
Control	Ba	133866776	40000	1 0	3207554	1 0	1 1	1448282	1448	666682	7 2	20 2	7179
Control	Bb	39375801	40000	1 0	3207554	1 0	1 4	335175	335	438216	3 7	13 2	2536
Control	Ca	40766152	40000	1 0	2623761	1 0	0 9	674560	675	438216	7 3	16 2	4174
Control	Cb	69786871	40000	1 0	2786193	1 0	1 6	991349	991	926597	7 9	32 2	1838
Control	Da	26721732	40000	1 0	3035428	1 0	1 1	338688	339	389825	4 4	11 8	2874
Control	Db	26736618	40000	1 0	2749146	1 0	1 1	351287	351	505917	6 6	17 8	1973
field trip		34884610	40000	1 0	2831368	1 0	5 0	95165	95	1504160	4 1	51 4	185
control		36171786	40000	1 0	1489523	1 0	5 0	197267	197	1063600	5 5	69 1	286

Amendment		Ax	Cis	D	Ais	RF	Wd	concentration mg/kg dw	concentration mg/kg dw	surrogate area account	surrogate recove mg/kg dw	% recovery surrogate	concentration %P
Nitrate	3Aa	110055664	40000	1 0	3304504	1 0	1 44	694641	695	1556703	12 7	45 6	1963
Nitrate	Ab	87756226	40000	1 0	3611271	1 0	1 35	606879	607	1427526	11 3	38 3	1822
Nitrate	Ba	52202886	40000	1 0	3000671	1 0	1 32	511532	512	2186864	21 4	70 5	725
Nitrate	Bb	66390783	40000	1 0	2774659	1 0	1 12	823406	823	1456669	18 1	50 9	1619
Nitrate	Ca	33208641	40000	1 0	1577874	1 0	1 87	435440	435	1434067	18 8	87 9	495
Nitrate	Cb	50600770	40000	1 0	2451068	1 0	1 87	426904	427	1693200	14 3	66 8	639
Nitrate	Da	94202689	40000	1 0	2970856	1 0	1 55	791356	791	1980427	16 5	63 9	1239
Nitrate	Db	113113467	40000	1 0	2681368	1 0	1 61	1011904	1012	1784572	16 0	64 4	1571
Air	Aa	132997522	40000	1 0	3743230	1 0	1 36	1008350	1008	1699172	12 9	43 9	2296
Air	Ab	26080614	40000	1 0	3004322	1 0	1 35	249539	250	489792	4 7	15 8	1582
Air	Ba	117005749	40000	1 0	2921719	1 0	1 88	822950	823	1617721	11 4	53 6	1536
Air	Bb	86378422	40000	1 0	2986267	1 0	1 58	708758	709	1440798	11 8	46 7	1518
Air	Ca	54531549	40000	1 0	2776425	1 0	1 60	475196	475	1127159	9 8	39 3	1210
Air	Cb	58038398	40000	1 0	2640829	1 0	1 39	621799	622	1764301	18 6	64 6	962
Air	Da	123659361	40000	1 0	2817251	1 0	1 65	1029802	1030	1526456	12 7	52 5	1962
Air	Db	47636867	40000	1 0	2850996	1 0	1 60	405537	406	1617721	13 7	54 9	739
Control	Aa	27022556	40000	1 0	4156465	1 0	1 11	226665	227	1001365	8 4	23 3	972
Control	Ab	17975688	40000	1 0	4003770	1 0	1 28	135518	136	1330694	10 0	32 2	421
Control	Ba	22690464	40000	1 0	4166471	1 0	1 06	199155	199	1049429	9 2	24 4	817
Control	Bb	28899364	40000	1 0	3980719	1 0	1 27	219313	219	940775	7 2	22 9	959
Control	Ca	61134946	40000	1 0	2911874	1 0	1 67	647640	648	1305794	10 4	43 4	1493
Control	Cb	53891569	40000	1 0	3739501	1 0	1 83	305403	305	1396523	7 9	36 1	846
Control	Da	38071170	40000	1 0	3697383	1 0	1 15	345783	346	800742	7 3	21 0	1650
Control	Db	41964208	40000	1 0	3257269	1 0	1 18	421213	421	860654	8 6	25 6	1647
field trip		26840823	40000	1 0	2852431	1 0	5 58	66216	66	1803892	4 4	61 2	107
control		19972209	40000	1 0	2853229	1 0	5 58	52171	52	1435609	3 6	52 4	100
control		51416533	40000	1 0	3083397	1 0	5 34	120879	121	1597047	3 8	50 1	241
control		34706821	40000	1 0	3083397	1 0	5 34	81595	82	2430407	5 7	76 3	107
Amendment		Ax	Cis	D	Ais	RF	Wd	concentration mg/kg dw	concentration mg/kg dw	surrogate area account	surrogate recove mg/kg dw	% recovery surrogate	concentration %P
Nitrate	4Aa	52007530	40000	1 0	2621443	0 8	2 0	391209	391	1213783	9 1	44 8	873
Nitrate	Ab	118363047	40000	1 0	2903126	0 8	2 1	755610	756	1802753	11 5	60 1	1258
Nitrate	Ba	87317981	40000	1 0	3231068	0 8	1 4	721687	722	524452	4 3	15 7	4596
Nitrate	Bb	108751689	40000	1 0	3083986	0 8	1 7	813938	814	1256468	9 4	39 4	2065
Nitrate	Ca	19191108	40000	1 0	3043873	0 8	1 4	169870	170	127916	1 1	4 1	4177
Nitrate	Cb	268800493	40000	1 0	3250929	0 8	1 6	1948963	1949	6648333	48 2	197 8	986
Nitrate	Da	36465973	40000	1 0	2552344	0 8	1 7	327107	327	721186	6 5	27 3	1198
Nitrate	Db	37773660	40000	1 0	2818350	0 8	1 4	370175	370	772518	7 6	26 5	1396
Air	Aa	54441097	40000	1 0	2646033	0 8	1 5	539472	539	363486	3 5	12 9	4173
Air	Ab	107656613	40000	1 0	2433499	0 8	1 4	1200541	1201	823162	9 2	32 7	3888
Air	Ba	98510040	40000	1 0	2735686	0 8	2 0	691688	692	1184340	8 5	41 9	1662
Air	Bb	87181666	40000	1 0	2711992	0 8	1 9	652981	653	1171036	8 8	41 8	1563
Air	Ca	103636017	40000	1 0	2519346	0 8	1 7	982286	982	886228	8 2	34 0	2830
Air	Cb	68040776	40000	1 0	2329577	0 8	2 0	553570	554	668744	5 4	27 8	1993
Air	Da	26892378	40000	1 0	2662859	0 8	1 5	287153	287	1108632	11 1	40 2	714
Air	Db	66317452	40000	1 0	2546260	0 8	1 8	545309	545	922465	7 6	35 1	1556
Control	Aa	37223320	40000	1 0	2577037	0 8	1 1	530633	531	221400	3 2	8 3	6383
Control	Ab	16182366	40000	1 0	2489105	0 8	1 0	261117	261	118720	1 9	4 6	5668
Control	Ba	27844374	40000	1 0	2699194	0 8	1 5	262015	262	215818	2 0	7 7	3387
Control	Bb	14588636	40000	1 0	2674661	0 8	1 5	137649	138	150779	1 4	5 5	2523
Control	Ca	26625727	40000	1 0	2917466	0 8	1 6	243289	243	158188	1 3	5 2	4637
Control	Cb	17926572	40000	1 0	2765023	0 8	1 4	174972	175	644196	6 3	22 5	776
Control	Da	8561934	40000	1 0	2907567	0 8	1 1	105990	106	1034483	12 8	34 4	308
Control	Db	16371025	40000	1 0	2448844	0 8	1 2	215290	215	1280917	16 8	50 6	425
field trip		5796564	40000	1 0	2613147	0 8	5 6	15371	15	2014245	5 3	74 6	21
control		66478640	40000	1 0	3107773	0 8	5 6	148254	148	1881042	4 2	58 6	253
control		2077549	40000	1 0	3735152	0 8	5 3	4032	4	1749539	3 4	45 3	9

Amendment	Sub	Ax	Cs	D	As	Rf	Wd	concentration mg/kg dw	concentration mg/kg dw	surrogate area account	surrogate recove mg/kg dw	% recovery surrogate	concentration %dl
Nitrate	A	26300240	40000	10	2203752	0.6	1.7	275987	276	1037791	10.9	45.6	605
Nitrate	A	10036743	40000	10	2761458	0.6	1.6	87500	88	1053117	9.2	38.9	237
Nitrate	A	18798480	40000	10	2490721	0.6	1.1	255202	255	820552	8.4	24.0	1062
Nitrate	A	38248518	40000	10	2767883	0.6	1.5	355414	355	1128142	10.5	30.4	901
Nitrate	A	14198685	40000	10	2404462	0.6	0.9	241403	241	1060054	10.1	42.9	583
Nitrate	A	6266886	40000	10	2453994	0.6	1.0	98845	97	675446	10.4	28.6	364
Nitrate	A	7886076	40000	10	2585343	0.6	1.3	90981	91	872299	10.1	32.6	279
Nitrate	A	10454663	40000	10	2180057	0.6	1.2	153685	154	935235	13.7	41.5	370
Ax	A	63213960	40000	10	2517261	0.6	1.7	557585	558	1584418	14.0	60.9	916
Ax	A	38845170	40000	10	2263810	0.6	1.9	332166	332	1291123	11.6	56.2	602
Ax	A	49578627	40000	10	2122618	0.6	1.9	466661	466	1753294	16.5	79.9	583
Ax	A	6653707	40000	10	2371096	0.6	1.6	58460	58	1768481	15.8	72.2	81
Ax	A	102043499	40000	10	2949975	0.6	1.7	781134	781	2581633	19.6	84.0	930
Ax	A	60099949	40000	10	2578254	0.6	1.4	997272	997	2330720	25.8	87.5	1140
Ax	A	16398777	40000	10	2357942	0.6	1.6	171305	171	2339888	21.8	88.0	178
Ax	A	69075866	40000	10	2180286	0.6	1.7	741532	742	1776323	19.1	79.6	932
Control	A	6888686	40000	10	2521864	0.6	1.3	108299	108	2252091	27.4	88.4	125
Control	A	6600686	40000	10	2533451	0.6	1.2	87376	87	909667	12.0	34.7	252
Control	A	2966975	40000	10	2558701	0.6	1.2	38283	38	1176284	15.2	44.5	86
Control	A	7282788	40000	10	2686825	0.6	1.1	98513	99	732174	9.9	28.6	371
Control	A	1887844	40000	10	2198163	0.6	1.3	25782	26	787980	10.8	34.7	74
Control	A	3722451	40000	10	2192016	0.6	1.2	54877	55	1778884	26.2	78.4	70
Control	A	17030206	40000	10	2578868	0.6	1.1	234036	234	1106740	15.2	41.5	564
Control	A	5203238	40000	10	2218742	0.6	1.0	98046	98	1266070	23.1	56.2	174
Control	A	2750278	40000	10	2074825	0.6	0.3	9810	10	2454162	8.6	114.5	6
field trip	A	3741075	40000	10	2724518	0.6	0.3	9864	10	1758424	4.7	62.5	16
control	A	2976026	40000	10	2363864	0.6	0.3	9050	9	1840351	5.0	66.8	14
Amendment	Sub	Ax	Cs	D	As	Rf	Wd	concentration mg/kg dw	concentration mg/kg dw	surrogate area account	surrogate recove mg/kg dw	% recovery surrogate	concentration %dl
Nitrate	A	13660309	40000	10	2070539	0.6	1.0	264532	265	957481	18.5	44.7	591
Nitrate	A	3240834	40000	10	1875118	0.6	1.0	68497	68	1206202	25.6	62.2	110
Nitrate	A	26302247	40000	10	1954919	0.6	0.7	746242	746	1279501	38.3	63.3	1178
Nitrate	A	21758974	40000	10	2032360	0.6	0.7	565073	565	1458660	37.9	69.5	813
Nitrate	A	10686261	40000	10	1880333	0.6	0.9	240521	241	1163457	26.5	60.5	387
Nitrate	A	3606470	40000	10	1995231	0.6	1.0	77524	78	1471837	29.2	71.4	109
Nitrate	A	11091573	40000	10	2011214	0.6	1.0	220820	221	1665372	33.0	79.7	277
Nitrate	A	5741676	40000	10	2482960	0.6	1.1	81097	81	1567420	22.1	61.1	133
Ax	A	28888053	40000	10	2020612	0.6	0.8	674252	674	1188580	30.0	58.9	1186
Ax	A	38468138	40000	10	1961888	0.6	0.8	977390	978	777041	19.7	38.3	2561
Ax	A	30152665	40000	10	2104159	0.6	0.8	673650	674	1247361	27.9	57.4	1174
Ax	A	85103606	40000	10	2056773	0.6	0.8	1986442	1986	2547218	58.9	119.9	1640
Ax	A	41882796	40000	10	1878075	0.6	0.9	925887	926	1851988	40.8	95.5	960
Ax	A	39197001	40000	10	1029761	0.6	0.8	1847752	1848	1366445	64.6	128.7	1436
Ax	A	62262250	40000	10	2136453	0.6	0.8	1334780	1335	1527328	32.7	68.1	1931
Control	A	36348270	40000	10	1035282	0.6	0.8	1665349	1666	1424515	64.9	133.1	1243
Control	A	84738277	40000	10	2030677	0.6	0.6	3057710	3058	1450005	46.6	89.1	4426
Control	A	81754635	40000	10	2052049	0.6	0.6	2567675	2566	1567971	46.1	73.9	3456
Control	A	18561706	40000	10	1151474	0.6	0.7	844239	844	1584801	26.6	49.2	1716
Control	A	2502148	40000	10	1136763	0.6	0.6	134364	134	584018	31.4	49.6	270
Control	A	1863856	40000	10	1143619	0.6	0.7	88803	89	584455	27.4	49.5	180
Control	A	1831740	40000	10	1222377	0.6	0.7	77704	78	567803	24.1	44.9	173
Control	A	1270240	40000	10	1744733	0.6	0.7	39915	40	1200966	37.6	66.6	80
field trip	A	13268223	40000	10	1167253	0.6	0.9	478019	478	578790	20.8	48.0	966
control	A	162087	40000	10	1933129	0.6	0.7	572	1	1471440	5.2	7.7	-1
control	A	5211606	40000	10	1923565	0.6	0.7	18453	18	1522389	6.4	78.6	24
control	A	3656690	40000	10	1234817	0.6	0.7	19860	20	1140905	6.3	89.4	22

Amendment		Ax	Cis	D	Ais	RF	Wd	concentration mg/kg dw	concentration mg/kg dw	surrogate area account	surrogate recove mg/kg dw	% recovery surrogate	concentration %P
Nitrate	7Aa	22090610	40000	1 0	2350476	0 8	1 0	467260	467	1054694	22 3	53 4	876
Nitrate	Ab	2382113	40000	1 0	2709114	0 8	0 9	48129	48	1302721	26 3	57 2	84
Nitrate	Ba	2744706	40000	1 0	2410653	0 8	0 9	56786	59	1545241	33 1	76 2	77
Nitrate	Bb	2598016	40000	1 0	2647116	0 8	0 9	47073	47	1545241	26 0	64 5	73
Nitrate	Ca	4565511	40000	1 0	2754943	0 8	1 0	80616	81	2326729	41 2	100 4	80
Nitrate	Cb	2656085	40000	1 0	2644477	0 8	1 0	48953	49	1235783	21 2	56 6	86
Nitrate	Da	14169006	40000	1 0	2751183	0 8	0 9	267175	267	1534151	26 9	66 3	403
Nitrate	Db	4984683	40000	1 0	2709237	0 8	1 0	87154	87	745474	13 0	32 7	266
Air	Aa	32898012	40000	1 0	2839557	0 8	1 1	518372	518	1518665	23 9	63 6	815
Air	Ab	26192411	40000	1 0	2942562	0 8	1 1	400598	401	1827429	27 9	73 8	542
Air	Ba	2587414	40000	1 0	2346584	0 8	1 1	49000	49	1192698	22 6	60 4	81
Air	Bb	32744899	40000	1 0	2513876	0 8	1 1	574283	574	1175343	20 6	56 6	1033
Air	Ca	13098832	40000	1 0	2362666	0 8	0 9	306466	306	2472086	57 8	124 4	246
Air	Cb	11941160	40000	1 0	2333678	0 8	1 2	204745	205	1449654	24 9	73 9	277
Air	Da	7519766	40000	1 0	2459771	0 8	1 8	80567	81	1592698	17 1	77 0	106
Air	Db	13842972	40000	1 0	2399311	0 8	1 0	287617	288	1644863	34 2	81 5	363
Control	Aa	9999003	40000	1 0	2458936	0 8	0 9	221533	222	1166137	25 8	66 3	393
Control	Ab	8310337	40000	1 0	2717103	0 8	0 7	213077	213	1347637	34 6	59 0	361
Control	Ba	10896729	40000	1 0	2498679	0 8	0 9	230680	231	1117076	23 6	53 2	434
Control	Bb	3968234	40000	1 0	2511401	0 8	0 9	85168	85	1319313	28 4	62 5	136
Control	Ca	22505625	40000	1 0	2567243	0 8	0 9	464750	465	1245696	26 7	57 9	802
Control	Cb	5346040	40000	1 0	2456827	0 8	0 8	137636	138	1226951	31 6	59 3	232
Control	Da	4600092	40000	1 0	2471714	0 8	0 8	107304	107	1480096	36 3	71 2	151
Control	Db	5774529	40000	1 0	2538475	0 8	0 9	125673	126	2300327	50 0	107 8	117
field trip		1998798	40000	1 0	2428492	0 8	3 0	12898	13	2517406	16 2	123 3	10
control		2958030	40000	1 0	2434143	0 8	3 0	19027	19	2015386	13 0	98 5	19

Amendment		Ax	Cis	D	Ais	RF	Wd	concentration mg/kg dw	concentration mg/kg dw	surrogate area account	surrogate recove mg/kg dw	% recovery surrogate	concentration %P
Nitrate	8Aa	37113392	40000	1 0	3107092	0 8	1 0	620438	620	1034661	17 3	41 8	1483
Nitrate	Ab	101033616	40000	1 0	3265059	0 8	1 0	1613806	1614	1375932	22 0	52 9	3048
Nitrate	Ba	228137886	40000	1 0	3106491	0 8	1 0	3556306	3556	1171410	18 3	47 4	7506
Nitrate	Bb	183862030	40000	1 0	3154445	0 8	0 9	3176888	3177	1039981	18 0	41 4	7670
Nitrate	Ca	195081687	40000	1 0	3233276	0 8	1 0	3152276	3152	1546149	25 0	60 1	5247
Nitrate	Cb	133046878	40000	1 0	2975290	0 8	1 0	2306561	2307	1292397	22 4	54 6	4227
Nitrate	Da	12399495	40000	1 0	3107092	0 8	0 9	211798	212	1034661	17 7	41 8	506
Nitrate	Db	24087733	40000	1 0	3701216	0 8	1 0	332779	333	1948880	26 9	66 1	503
Air	Aa	51609828	40000	1 0	2992195	0 8	1 2	738290	738	1413939	20 2	59 4	1244
Air	Ab	91406018	40000	1 0	2942298	0 8	0 9	1656886	1656	1993179	38 1	85 1	1946
Air	Ba	48899996	40000	1 0	2733624	0 8	1 0	678240	678	1787934	32 1	82 2	1089
Air	Bb	60061217	40000	1 0	2695845	0 8	1 0	998828	999	2028518	33 7	88 0	1135
Air	Ca	50276344	40000	1 0	2999860	0 8	1 2	726015	726	2185153	31 6	91 5	793
Air	Cb	52747507	40000	1 0	2990890	0 8	1 1	794544	795	2298791	34 6	96 8	823
Air	Da	121618825	40000	1 0	2902607	0 8	1 8	1172800	1173	2241276	21 6	97 0	1209
Air	Db	94521839	40000	1 0	2998611	0 8	1 1	1482992	1483	2081227	32 7	87 3	1700
Control	Aa	6508861	40000	1 0	2498679	0 8	0 8	156449	156	1117076	26 9	56 2	279
Control	Ab	5672125	40000	1 0	2458936	0 8	0 7	157325	157	1166137	32 3	59 5	264
Control	Ba	5447651	40000	1 0	2538475	0 8	0 8	129547	130	2300327	54 7	113 8	114
Control	Bb	76748871	40000	1 0	2942298	0 8	0 8	1653834	1654	1993179	42 9	85 1	1943
Control	Ca	3064731	40000	1 0	3263209	0 8	0 8	60810	61	1774332	36 2	67 9	90
Control	Cb	3983459	40000	1 0	2717103	0 8	0 8	92541	93	1347637	31 3	62 3	149
Control	Da	2222164	40000	1 0	3208538	0 8	0 8	43615	44	1288258	26 2	50 4	87
Control	Db	70509243	40000	1 0	3233276	0 8	0 8	1363664	1364	1546149	29 9	60 1	2270
field trip		9316005	40000	1 0	2916098	0 8	3 0	52883	53	2464771	14 0	106 2	50
control		4292580	40000	1 0	3011599	0 8	3 1	23326	23	2323294	12 6	98 9	24

Amendment	Au	Ca	D	Au	RF	Wd	concentration mg/kg dw	concentration mg/kg dw	surrogate area account	surrogate recove mg/kg dw	% recovery surrogate	concentration ug/l
Air	30536971	40000	10	3217811	0.6	13	414794	415	2178421	23.5	86.0	486
Air	21788252	40000	10	3210884	0.6	16	213568	214	2060319	20.5	81.8	261
Air	29702522	40000	10	3043482	0.6	14	345831	346	1907139	19.8	70.1	404
Air	4088345	40000	10	2863194	0.6	17	42516	43	1801027	18.7	79.0	54
Air	43563309	40000	10	324815	0.6	15	4620512	4621	981388	104.0	379.6	1217
Air	36500073	40000	10	3178401	0.6	14	402982	403	1565407	17.6	61.9	491
Air	23070570	40000	10	3242434	0.6	20	175829	176	2263768	17.3	67.7	200
Nitrate	18687238	40000	10	2863457	0.6	17	1849005	1849	1210158	11.8	51.3	3804
Nitrate	12911477	40000	10	3087851	0.6	20	103567	104	1921230	15.6	78.2	132
Nitrate	73839171	40000	10	2726896	0.6	20	669843	670	1336056	12.1	61.6	1086
Nitrate	47514167	40000	10	3327916	0.6	16	452723	453	977619	9.3	38.9	1227
Nitrate	15172403	40000	10	3270106	0.6	15	1548217	1548	1214880	12.4	49.7	3317
Nitrate	43900845	40000	10	2876136	0.6	15	529868	530	1344715	16.2	58.7	922
Nitrate	58686618	40000	10	2777963	0.6	15	739990	740	1982622	20.9	97.2	972
Nitrate	42261756	40000	10	2596587	0.6	16	507738	507	1279644	15.4	61.8	820
Control	53142100	40000	10	2851127	0.6	17	554670	555	1929092	20.1	86.0	653
Control	75711043	40000	10	3053665	0.6	13	991159	991	1219985	18.0	90.2	1975
Control	29880060	40000	10	2525707	0.6	14	418318	418	1118185	15.7	58.6	752
Control	12971881	40000	10	2956152	0.6	13	175250	175	1327564	17.9	56.4	311
Control	15916204	40000	10	3022174	0.6	14	183476	183	1117748	12.9	49.5	386
Control	8021385	40000	10	2631486	0.6	12	93472	93	716186	11.1	34.2	273
Control	8374549	40000	10	2847364	0.6	14	105151	105	1387761	17.4	61.2	172
Control	8226560	40000	10	2863773	0.6	12	144503	145	1346311	20.9	63.2	229
Control	3470112	40000	10	2819967	0.6	15	44452	44	1231278	15.8	69.0	75
field trip	1486527	40000	10	3194570	0.6	61	4600	5	1763777	5.5	70.1	7
control	3138560	40000	10	2947613	0.6	60	10650	11	1775205	6.0	75.7	14
	1068260	40000	10	2461616	0.6	50	4326	4	1781942	7.2	80.9	5

C.1.4 TPH and Log TPH concentrations for the treatments

Treatment	Days	Event	Plot	Subplot	SC mg/kgdw	LC mg/kgdw	Aromatics mg/kgdw	TPH mg/kgdw	Log SC mg/kgdw	Log LC mg/kgdw	Log Arom mg/kgdw	Log TPH mg/kgdw
Air	0	1	A	a	6425	8229	6631	21285	38	39	38	43
Air	0	1	A	b	6654	4218	376	11248	38	36	26	41
Air	0	1	B	a	4066	7170	725	11981	36	39	29	41
Air	0	1	B	b	782	1983	1599	4343	29	33	32	36
Air	0	1	C	a	6487	8309	5482	20277	38	39	37	43
Air	0	1	C	b	2543	4754	3361	10658	34	37	35	40
Air	0	1	D	a	9464	7944	5497	22905	40	39	37	44
Air	0	1	D	b	4973	4167	1771	10911	37	36	32	40
Air	28	2	A	a	984	4798	2058	7840	30	37	33	39
Air	28	2	A	b	1124	2189	2039	5352	31	33	33	37
Air	28	2	B	a	1762	2338	2012	6113	32	34	33	38
Air	28	2	B	b	1846	2760	2400	7006	33	34	34	38
Air	28	2	C	a	3761	6672	2675	13107	36	38	34	41
Air	28	2	C	b	5049	4518	2032	11599	37	37	33	41
Air	28	2	D	a	4774	5156	2242	12172	37	37	34	41
Air	28	2	D	b	265	782	3956	5003	24	29	36	37
Air	47	3	A	a	4681	1678	2296	8655	37	32	34	39
Air	47	3	A	b	5612	4768	1582	11961	37	37	32	41
Air	47	3	B	a	540	1174	1536	3250	27	31	32	35
Air	47	3	B	b	940	1074	1518	3532	30	30	32	35
Air	47	3	C	a	1206	1094	1210	3510	31	30	31	35
Air	47	3	C	b	107	128	962	1196	20	21	30	31
Air	47	3	D	a	2490	2087	1962	6539	34	33	33	38
Air	47	3	D	b	147	125	739	1011	22	21	29	30
Air	78	4	A	a	3687	4488	4173	12348	36	37	36	41
Air	78	4	A	b	4969	5279	3666	13915	37	37	36	41
Air	78	4	B	a	1956	2119	1652	5728	33	33	32	38
Air	78	4	B	b	1950	2011	1563	5523	33	33	32	37
Air	78	4	C	a	3542	4459	2830	10832	35	36	35	40
Air	78	4	C	b	3338	4135	1993	9466	35	36	33	40
Air	78	4	D	a	1122	3000	714	4836	31	35	29	37
Air	78	4	D	b	2522	3451	1556	7529	34	35	32	39
Air	110	5	A	a	1584	3759	916	6258	32	36	30	38
Air	110	5	A	b	1141	2344	602	4087	31	34	28	36
Air	110	5	B	a	669	1443	583	2695	28	32	28	34
Air	110	5	B	b	1783	3398	61	5242	32	35	19	37
Air	110	5	C	a	2288	5181	930	8399	34	37	30	39
Air	110	5	C	b	3076	2974	1140	7189	35	35	31	39
Air	110	5	D	a	406	725	178	1310	26	29	23	31
Air	110	5	D	b	118	363	932	1413	21	26	30	31
Air	138	6	A	a	721	982	1185	2887	29	30	31	35
Air	138	6	A	b	839	921	2551	4310	29	30	34	36
Air	138	6	B	a	1373	1957	1174	4505	31	33	31	37
Air	138	6	B	b	1016	1427	1640	4083	30	32	32	36
Air	138	6	C	a	1126	1366	969	3461	31	31	30	35
Air	138	6	C	b	1168	1714	1436	4317	31	32	32	36
Air	138	6	D	a	1292	1428	1931	4652	31	32	33	37
Air	138	6	D	b	958	1301	1243	3502	30	31	31	35
Air	327	7	A	a	704	362	815	1881	28	26	29	33
Air	327	7	A	b	1075	1335	543	2953	30	31	27	35
Air	327	7	B	a	400	496	81	977	26	27	19	30
Air	327	7	B	b	883	1105	1033	3021	29	30	30	35
Air	327	7	C	a	259	326	246	831	24	25	24	29
Air	327	7	C	b	221	332	277	830	23	25	24	29
Air	327	7	D	a	124	146	105	374	21	22	20	26
Air	327	7	D	b	152	261	353	766	22	24	25	29
Air	359	8	A	a	829	1779	1244	3851	29	33	31	36
Air	359	8	A	b	825	1984	1946	4755	29	33	33	37
Air	359	8	B	a	714	1340	1069	3123	29	31	30	35
Air	359	8	B	b	688	1053	1135	2876	28	30	31	35
Air	359	8	C	a	368	1154	793	2316	26	31	29	34
Air	359	8	C	b	658	646	823	2126	28	28	29	33
Air	359	8	D	a	1581	1110	1209	3900	32	30	31	36
Air	359	8	D	b	1399	1403	1700	4502	31	31	32	37
Air	389	9	A	a	455	643	488	1586	27	28	27	32
Air	389	9	A	b	548	521	261	1330	27	27	24	31
Air	389	9	B	a	668	895	494	2057	28	30	27	33
Air	389	9	B	b	567	70	54	691	28	18	17	28
Air	389	9	C	a	2476	3272	1217	6965	34	35	31	38
Air	389	9	C	b	1233	1043	651	2927	31	30	28	35
Air	389	9	D	a	291	356	201	847	25	26	23	29
Air	389	9	D	b	3733	3444	3604	10781	36	35	36	40

Treatment	Days	Event	Plot	Subplot	Short mg/kgdw	Long mg/kgdw	Aromatics mg/kgdw	TPH mg/kgdw	Log Short mg/kgdw	Log Long mg/kgdw	Log Arom mg/kgdw	Log TPH mg/kgdw
Nitrate	0	1	A	a	1437	1335	311	3083	3.2	3.1	2.5	3.5
Nitrate	0	1	A	b	1146	1611	189	2946	3.1	3.2	2.3	3.5
Nitrate	0	1	B	a	1977	4065	4594	10636	3.3	3.6	3.7	4.0
Nitrate	0	1	B	b	1566	3955	1544	7057	3.2	3.6	3.2	3.8
Nitrate	0	1	C	a	483	1925	2596	5004	2.7	3.3	3.4	3.7
Nitrate	0	1	C	b	7636	7411	4587	19634	3.9	3.9	3.7	4.3
Nitrate	0	1	D	a	2358	2580	970	5908	3.4	3.4	3.0	3.8
Nitrate	0	1	D	b	374	2538	2473	5386	2.6	3.4	3.4	3.7
Nitrate	28	2	A	a	523	1503	1884	3909	2.7	3.2	3.3	3.6
Nitrate	28	2	A	b	2388	4437	4840	11666	3.4	3.6	3.7	4.1
Nitrate	28	2	B	a	2160	2156	1736	6053	3.3	3.3	3.2	3.8
Nitrate	28	2	B	b	268	581	606	1454	2.4	2.8	2.8	3.2
Nitrate	28	2	C	a	2095	3217	764	6075	3.3	3.5	2.9	3.8
Nitrate	28	2	C	b	2747	3476	1634	7856	3.4	3.5	3.2	3.9
Nitrate	28	2	D	a	346	1614	2483	4443	2.5	3.2	3.4	3.6
Nitrate	28	2	D	b	231	1177	2127	3534	2.4	3.1	3.3	3.5
Nitrate	47	3	A	a	2140	3868	1963	7970	3.3	3.6	3.3	3.9
Nitrate	47	3	A	b	791	2220	1822	4833	2.9	3.3	3.3	3.7
Nitrate	47	3	B	a	721	3079	725	4525	2.9	3.5	2.9	3.7
Nitrate	47	3	B	b	744	4112	1619	6475	2.9	3.6	3.2	3.8
Nitrate	47	3	C	a	43	610	495	1148	1.6	2.8	2.7	3.1
Nitrate	47	3	C	b	217	695	639	1551	2.3	2.8	2.8	3.2
Nitrate	47	3	D	a	1134	1574	1239	3947	3.1	3.2	3.1	3.6
Nitrate	47	3	D	b	1847	2276	1571	5693	3.3	3.4	3.2	3.8
Nitrate	78	4	A	a	1026	1886	873	3785	3.0	3.3	2.9	3.6
Nitrate	78	4	A	b	1808	2326	1258	5392	3.3	3.4	3.1	3.7
Nitrate	78	4	B	a	3943	4907	4595	13445	3.6	3.7	3.7	4.1
Nitrate	78	4	B	b	3387	3795	2066	9246	3.5	3.6	3.3	4.0
Nitrate	78	4	C	a	4229	5131	4177	13538	3.6	3.7	3.6	4.1
Nitrate	78	4	C	b	4562	5784	985	11331	3.7	3.8	3.0	4.1
Nitrate	78	4	D	a	1944	2924	1196	6064	3.3	3.5	3.1	3.8
Nitrate	78	4	D	b	3564	3585	1396	8544	3.6	3.6	3.1	3.9
Nitrate	110	5	A	a	642	1422	606	2669	2.8	3.2	2.8	3.4
Nitrate	110	5	A	b	275	712	237	1225	2.4	2.9	2.4	3.1
Nitrate	110	5	B	a	2024	3037	1062	6124	3.3	3.5	3.0	3.8
Nitrate	110	5	B	b	1674	3288	901	5863	3.2	3.5	3.0	3.8
Nitrate	110	5	C	a	899	2223	563	3684	3.0	3.3	2.8	3.6
Nitrate	110	5	C	b	456	838	364	1658	2.7	2.9	2.6	3.2
Nitrate	110	5	D	a	257	344	279	880	2.4	2.5	2.4	2.9
Nitrate	110	5	D	b	433	360	370	1163	2.6	2.6	2.6	3.1
Nitrate	138	6	A	a	377	668	591	1656	2.6	2.8	2.8	3.2
Nitrate	138	6	A	b	73	89	110	272	1.9	1.9	2.0	2.4
Nitrate	138	6	B	a	1606	1701	1178	4487	3.2	3.2	3.1	3.7
Nitrate	138	6	B	b	792	1061	813	2666	2.9	3.0	2.9	3.4
Nitrate	138	6	C	a	138	698	397	1233	2.1	2.8	2.6	3.1
Nitrate	138	6	C	b	49	131	109	289	1.7	2.1	2.0	2.5
Nitrate	138	6	D	a	70	262	277	609	1.8	2.4	2.4	2.8
Nitrate	138	6	D	b	56	101	133	290	1.8	2.0	2.1	2.5
Nitrate	327	7	A	a	10	66	876	952	1.0	1.8	2.9	3.0
Nitrate	327	7	A	b	18	28	84	130	1.2	1.5	1.9	2.1
Nitrate	327	7	B	a	93	231	77	401	2.0	2.4	1.9	2.6
Nitrate	327	7	B	b	26	51	73	152	1.5	1.7	1.9	2.2
Nitrate	327	7	C	a	21	77	80	178	1.3	1.9	1.9	2.3
Nitrate	327	7	C	b	63	66	88	237	1.9	1.8	1.9	2.4
Nitrate	327	7	D	a	70	106	403	578	1.8	2.0	2.6	2.8
Nitrate	327	7	D	b	90	32	266	389	2.0	1.5	2.4	2.6
Nitrate	359	8	A	a	499	1444	1483	3425	2.7	3.2	3.2	3.5
Nitrate	359	8	A	b	734	1882	3048	5664	2.9	3.3	3.5	3.8
Nitrate	359	8	B	a	2656	3801	7505	13962	3.4	3.6	3.9	4.1
Nitrate	359	8	B	b	2813	4675	7670	15156	3.4	3.7	3.9	4.2
Nitrate	359	8	C	a	2845	4100	5247	12192	3.5	3.6	3.7	4.1
Nitrate	359	8	C	b	2577	2469	4227	9273	3.4	3.4	3.6	4.0
Nitrate	359	8	D	a	3837	4885	506	9208	3.6	3.7	2.7	4.0
Nitrate	359	8	D	b	305	690	503	1498	2.5	2.8	2.7	3.2
Nitrate	389	9	A	a	60	94	133	286	1.8	2.0	2.1	2.5
Nitrate	389	9	A	b	750	1682	1089	3521	2.9	3.2	3.0	3.5
Nitrate	389	9	B	a	1272	1628	1227	4127	3.1	3.2	3.1	3.6
Nitrate	389	9	B	b	1191	1217	3317	5725	3.1	3.1	3.5	3.8
Nitrate	389	9	C	a	806	1410	902	3119	2.9	3.1	3.0	3.5
Nitrate	389	9	C	b	552	1353	972	2877	2.7	3.1	3.0	3.5
Nitrate	389	9	D	a	509	686	820	2016	2.7	2.8	2.9	3.3
Nitrate	389	9	D	b	805	679	653	2136	2.9	2.8	2.8	3.3

Treatment	Days	Event	Plot	Subplot	Short mg/kgdw	Long mg/kgdw	Aromatics mg/kgdw	TPH mg/kgdw	Log Short mg/kgdw	Log Long mg/kgdw	Log Arom mg/kgdw	Log TPH mg/kgdw
Control	0	1	A	a	6482	522	1098	8102	38	27	30	39
Control	0	1	A	b	423	221	2881	3505	26	23	35	35
Control	0	1	B	a	1176	3565	1756	6497	31	36	32	38
Control	0	1	B	b	1028	1620	2762	5410	30	32	34	37
Control	0	1	C	a	283	1227	564	2056	24	31	28	33
Control	0	1	C	b	885	5010	865	6760	29	37	29	38
Control	0	1	D	a	1758	351	532	2641	32	25	27	34
Control	0	1	D	b	1071	439	608	2118	30	26	28	33
Control	28	2	A	a	3127	8635	1735	11498	35	38	32	41
Control	28	2	A	b	457	775	1354	2586	27	29	31	34
Control	28	2	B	a	7964	5444	7179	20587	39	37	39	43
Control	28	2	B	b	1454	1615	2535	5604	32	32	34	37
Control	28	2	C	a	3851	7073	4174	15098	36	38	36	42
Control	28	2	C	b	650	1467	1838	3955	28	32	33	36
Control	28	2	D	a	2249	3168	2874	8291	34	35	35	39
Control	28	2	D	b	1007	1320	1973	4299	30	31	33	36
Control	47	3	A	a	61	630	972	1663	18	28	30	32
Control	47	3	A	b	202	99	421	723	23	20	26	29
Control	47	3	B	a	695	393	817	1904	28	26	29	33
Control	47	3	B	b	252	934	959	2145	24	30	30	33
Control	47	3	C	a	2073	2218	1493	5784	33	33	32	38
Control	47	3	C	b	2011	2373	846	5230	33	34	29	37
Control	47	3	D	a	525	787	1650	2962	27	29	32	35
Control	47	3	D	b	699	1176	1647	3523	28	31	32	35
Control	78	4	A	a	252	880	6383	7515	24	29	38	39
Control	78	4	A	b	513	5666	5658	12057	27	38	38	41
Control	78	4	B	a	468	1805	3387	5659	27	33	35	38
Control	78	4	B	b	461	1297	2523	4282	27	31	34	36
Control	78	4	C	a	609	1886	4637	7131	28	33	37	39
Control	78	4	C	b	807	1834	776	3416	29	33	29	35
Control	78	4	D	a	403	1150	308	1861	26	31	25	33
Control	78	4	D	b	650	1598	425	2671	28	32	26	34
Control	110	5	A	a	984	1738	125	2828	30	32	21	35
Control	110	5	A	b	223	619	252	1093	23	28	24	30
Control	110	5	B	a	98	262	86	447	20	24	19	26
Control	110	5	B	b	553	1526	371	2450	27	32	26	34
Control	110	5	C	a	209	653	74	936	23	28	19	30
Control	110	5	C	b	138	511	70	719	21	27	18	29
Control	110	5	D	a	773	1330	564	2666	29	31	28	34
Control	110	5	D	b	206	622	174	1002	23	28	22	30
Control	138	6	A	a	669	435	4425	5530	28	26	36	37
Control	138	6	A	b	2771	3231	3459	9461	34	35	35	40
Control	138	6	B	a	1272	2956	1718	5946	31	35	32	38
Control	138	6	B	b	3523	1155	270	4947	35	31	24	37
Control	138	6	C	a	197	1105	180	1482	23	30	23	32
Control	138	6	C	b	3900	2717	173	6790	36	34	22	38
Control	138	6	D	a	4682	3080	60	7822	37	35	18	39
Control	138	6	D	b	1354	2526	998	4877	31	34	30	37
Control	327	7	A	a	22	180	393	595	13	23	26	28
Control	327	7	A	b	21	190	361	573	13	23	26	28
Control	327	7	B	a	99	381	434	915	20	26	26	30
Control	327	7	B	b	52	161	136	350	17	22	21	25
Control	327	7	C	a	429	1032	802	2263	26	30	29	34
Control	327	7	C	b	136	249	232	618	21	24	24	28
Control	327	7	D	a	128	260	151	538	21	24	22	27
Control	327	7	D	b	89	209	117	415	20	23	21	26
Control	359	8	A	a	53	209	279	540	17	23	24	27
Control	359	8	A	b	26	221	264	511	14	23	24	27
Control	359	8	B	a	1209	2684	114	4007	31	34	21	36
Control	359	8	B	b	96	253	1943	2292	20	24	33	34
Control	359	8	C	a	45	394	90	529	17	26	20	27
Control	359	8	C	b	918	1723	149	2789	30	32	22	34
Control	359	8	D	a	1261	2409	87	3756	31	34	19	36
Control	359	8	D	b	478	1196	2270	3944	27	31	34	36
Control	389	9	A	a	1617	1885	1975	5477	32	33	33	37
Control	389	9	A	b	1879	2619	752	5250	33	34	29	37
Control	389	9	B	a	6	26	311	343	08	14	25	25
Control	389	9	B	b	387	556	395	1337	26	27	26	31
Control	389	9	C	a	33	793	273	1099	15	29	24	30
Control	389	9	C	b	99	366	172	639	20	26	22	28
Control	389	9	D	a	162	413	229	804	22	26	24	29
Control	389	9	D	b	50	294	75	419	17	25	19	26

Treatment	Days	Event	Plot	Subplot	Short mg/kgdw	Long mg/kgdw	Aromatics mg/kgdw	TPH mg/kgdw	Log Short mg/kgdw	Log Long mg/kgdw	Log Arom mg/kgdw	Log TPH mg/kgdw
Control	28	2	A	a	1672	479	1556	3706	32	27	32	36
Control	28	2	A	b	519	92	4815	5426	27	20	37	37
Control	28	2	B	a	1265	407	1999	3671	31	26	33	36
Control	28	2	B	b	2567	12	713	3292	34	11	29	35
Control	28	2	C	a	932	326	2822	4080	30	25	35	36
Control	28	2	C	b	831	313	2405	3549	29	25	34	36
Control	28	2	D	a	815	269	1932	3015	29	24	33	35
Control	28	2	D	b	486	127	7923	8536	27	21	39	39
Control	47	3	A	a	3313	5479	20	8811	35	37	13	39
Control	47	3	A	b	1694	1692	118	3504	32	32	21	35
Control	47	3	B	a	5339	9834	107	15079	37	40	20	42
Control	47	3	B	b	1385	2430	200	4015	31	34	23	36
Control	47	3	C	a	3149	3717	741	7606	35	36	29	39
Control	47	3	C	b	3589	4169	605	8343	36	36	28	39
Control	47	3	D	a	2309	3384	207	5900	34	35	23	38
Control	47	3	D	b	1677	2640	621	4938	32	34	28	37
Control	110	5	A	a	803	798	45	1443	28	29	16	32
Control	110	5	A	b	342	506	108	956	25	27	20	30
Control	110	5	B	a	196	244	42	481	23	24	16	27
Control	110	5	B	b	333	653	82	1067	25	28	19	30
Control	110	5	C	a	244	506	75	824	24	27	19	29
Control	110	5	C	b	36	83	34	153	16	19	15	22
Control	110	5	D	a	48	90	31	169	17	20	15	22
Control	110	5	D	b	57	127	58	241	18	21	18	24
Control	138	6	A	a	2276	2041	474	4791	34	33	27	37
Control	138	6	A	b	2652	2782	879	6313	34	34	29	38
Control	138	6	B	a	488	1053	346	1886	27	30	25	33
Control	138	6	B	b	761	1253	295	2309	29	31	25	34
Control	138	6	C	a	2730	3424	802	6756	34	35	28	38
Control	138	6	C	b	2663	2920	939	6521	34	35	30	38
Control	138	6	D	a	2056	2090	281	4427	33	33	24	36
Control	138	6	D	b	2454	2403	229	5086	34	34	24	37
Control	327	7	A	a	335	327	129	790	25	25	21	29
Control	327	7	A	b	214	222	114	550	23	23	21	27
Control	327	7	B	a	399	446	244	1088	26	26	24	30
Control	327	7	B	b	370	586	302	1257	26	28	25	31
Control	327	7	C	a	130	316	103	549	21	25	20	27
Control	327	7	C	b	460	959	193	1612	27	30	23	32
Control	327	7	D	a	556	1017	244	1816	27	30	24	33
Control	327	7	D	b	89	79	479	646	19	19	27	28
Control	359	8	A	a	55	80	33	167	17	19	15	22
Control	359	8	A	b	56	73	19	146	17	19	13	22
Control	359	8	B	a	112	1056	203	1370	20	30	23	31
Control	359	8	B	b	152	2188	557	2897	22	33	27	35
Control	359	8	C	a	117	830	90	1036	21	29	20	30
Control	359	8	C	b	101	452	55	608	20	27	17	28
Control	359	8	D	a	153	748	31	933	22	29	15	30
Control	359	8	D	b	204	491	31	726	23	27	15	29

C.1.5 MPN and Log MPN data

Treatment	days	Event	Plot		MPN/gdw	Log MPN/gdw
Air	0	1	A	a	140000000	8.15
Air	0	1	B	a	17000000	7.23
Air	0	1	C	a	5000000	6.70
Air	0	1	D	a	140000000	8.15
Air	28	2	A	a	1900000	6.28
Air	28	2	B	a	20000000	7.30
Air	28	2	C	a	140000000	8.15
Air	28	2	D	a	140000000	8.15
Air	47	3	A	a	190000	5.28
Air	47	3	B	a	200000	5.30
Air	47	3	C	a	190000	5.28
Air	47	3	D	a	190000	5.28
Air	78	4	A	a	200	2.30
Air	78	4	B	a	7000	3.85
Air	78	4	C	a	2000	3.30
Air	78	4	D	a	9000	3.95
Air	110	5	A	a	700	2.85
Air	110	5	B	a	4000	3.60
Air	110	5	C	a	2000	3.30
Air	110	5	D	a	6000	3.78
Air	138	6	A	a	3400	3.53
Air	138	6	B	a	3000	3.48
Air	138	6	C	a	90000	4.95
Air	138	6	D	a	14000	4.15
Air	327	7	A	a	700	2.85
Air	327	7	B	a	176000	5.25
Air	327	7	C	a	90000	4.95
Air	327	7	D	a	3400	3.53
Air	359	8	A	a	3000	3.48
Air	359	8	B	a	2300	3.36
Air	359	8	C	a	2600	3.41
Air	359	8	D	a	90000	4.95
Air	389	9	A	a	2000	3.30
Air	389	9	B	a	2000	3.30
Air	389	9	C	a	2000	3.30
Air	389	9	D	a	50000	4.70
Nitrate	0	1	A	a	190000	5.28
Nitrate	0	1	B	a	140000000	8.15
Nitrate	0	1	C	a	300000000	8.48
Nitrate	0	1	D	a	300000000	8.48
Nitrate	28	2	A	a	17000	4.23
Nitrate	28	2	B	a	190	2.28
Nitrate	28	2	C	a	190	2.28
Nitrate	28	2	D	a	190	2.28
Nitrate	47	3	A	a	24000	4.38
Nitrate	47	3	B	a	190	2.28
Nitrate	47	3	C	a	800000	5.90
Nitrate	47	3	D	a	190	2.28
Nitrate	78	4	A	a	30000	4.48
Nitrate	78	4	B	a	26000	4.41
Nitrate	78	4	C	a	220000	5.34
Nitrate	78	4	D	a	70000	4.85
Nitrate	110	5	A	a	5000	3.70

Treatment	days	Event	Plot		MPN/gdw	Log MPN/gdw
Nitrate	110	5	B	a	1900	3.28
Nitrate	110	5	C	a	13000	4.11
Nitrate	110	5	D	a	190	2.28
Nitrate	138	6	A	a	3000	3.48
Nitrate	138	6	B	a	3000	3.48
Nitrate	138	6	C	a	35000	4.54
Nitrate	138	6	D	a	50000	4.70
Nitrate	327	7	A	a	24000	4.38
Nitrate	327	7	B	a	50000	4.70
Nitrate	327	7	C	a	170	2.23
Nitrate	327	7	D	a	1700	3.23
Nitrate	359	8	A	a	300	2.48
Nitrate	359	8	B	a	2300	3.36
Nitrate	359	8	C	a	27000	4.43
Nitrate	359	8	D	a	800	2.90
Nitrate	389	9	A	a	200	2.30
Nitrate	389	9	B	a	1800	3.26
Nitrate	389	9	C	a	2000	3.30
Nitrate	389	9	D	a	1800	3.26
Control	0	1	A	a	190000	5.28
Control	0	1	B	a	190000	5.28
Control	0	1	C	a	12000000	7.08
Control	0	1	D	a	190000	5.28
Control	28	2	A	a	17000	4.23
Control	28	2	B	a	90000	4.95
Control	28	2	C	a	200000	5.30
Control	28	2	D	a	13000	4.11
Control	47	3	A	a	5000	3.70
Control	47	3	B	a	1700	3.23
Control	47	3	C	a	190	2.28
Control	47	3	D	a	3400	3.53
Control	78	4	A	a	2000	3.30
Control	78	4	B	a	1700	3.23
Control	78	4	C	a	2000	3.30
Control	78	4	D	a	3000	3.48
Control	110	5	A	a	2000	3.30
Control	110	5	B	a	1300	3.11
Control	110	5	C	a	1900	3.28
Control	110	5	D	a	8000	3.90
Control	138	6	A	a	800	2.90
Control	138	6	B	a	50000	4.70
Control	138	6	C	a	2200	3.34
Control	138	6	D	a	2600	3.41
Control	327	7	A	a	90000	4.95
Control	327	7	B	a	16000	4.20
Control	327	7	C	a	90000	4.95
Control	327	7	D	a	176000	5.25
Control	359	8	A	a	800	2.90
Control	359	8	B	a	2300	3.36
Control	359	8	C	a	200	2.30
Control	359	8	D	a	1700	3.23
Control	389	9	A	a	5000	3.70
Control	389	9	B	a	180	2.26
Control	389	9	C	a	1800	3.26
Control	389	9	D	a	180	2.26

C.1.6 *S. alterniflora* Height and Density

Sampling Event 1

	AIR					NITRATE					CONTROL			
	A	B	C	D	Dens.	A	B	C	D	Dens.	A	B	C	D	Dens.
# stems		13.0	0.0	16.0	29.0	11.0	21.0	6.0	37.0	75.0	29.0	22.0	23.0	34.0	108.0
average (cm)		15.8		17.7	16.8	32.0	24.3	33.6	40.7	34.3	30.7	33.7	41.1	33.4	34.4
std dev (cm)		13.7		12.5	12.8	11.5	6.5	19.1	12.5	13.4	10.4	13.6	10.8	11.5	12.0
heights (cm)		4.0		6.3		20.6	12.2	41.9	48.8		34.8	52.0	47.0	29.0	
		3.9		8.6		37.6	19.1	46.6	42.5		15.2	48.1	48.0	47.0	
		29.0		24.4		36.9	20.1	8.1	22.2		31.0	48.2	49.0	48.0	
		21.5		10.2		41.6	24.3	10.2	36.4		8.7	22.3	27.0	54.0	
		40.6		30.9		41.0	20.2	46.7	52.5		52.0	36.0	35.0	61.0	
		3.2		7.8		52.5	24.9	48.3	61.4		32.1	7.1	41.0	42.0	
		7.6		7.4		37.0	19.3		47.8		29.6	10.8	56.0	32.0	
		5.4		2.6		21.9	24.7		43.4		24.1	41.0	40.0	38.0	
		36.6		38.6		19.9	25.1		53.5		39.6	31.6	23.0	48.0	
		19.2		33.0		28.2	28.2		52.6		42.0	22.7	28.0	43.0	
		26.1		28.5		15.6	32.6		51.8		30.4	41.8	53.0	35.0	
		3.4		26.7			20.8		18.0		31.0	24.0	35.0	29.0	
		5.5		33.3			22.4		46.6		27.0	19.6	38.0	16.0	
				12.1			35.7		27.6		21.0	17.4	45.0	26.0	
				6.5			38.1		54.0		20.7	21.8	67.0	39.0	
				5.5			24.1		59.2		29.6	49.0	42.0	31.0	
							13.0		57.7		17.1	47.6	45.0	27.0	
							20.6		37.8		27.6	44.4	36.0	17.0	
							27.9		34.2		38.1	28.4	49.0	33.0	
							31.2		19.6		31.6	41.9	38.0	38.0	
							25.4		44.8		39.9	43.6	24.0	26.0	
									26.2		40.0	42.9	33.0	34.0	
									44.5		44.6		49.0	33.0	
									27.6		37.0			31.0	
									26.7		29.4			13.0	
									48.6		42.1			17.0	
									32.1		38.0			32.0	
									25.0		29.0			14.0	
									44.6		7.6			45.0	
									14.2					44.0	
									43.7					21.0	
									48.8					23.0	
									44.7					33.0	
									50.6					35.0	
									44.5						
									27.4						
									46.2						

Sampling Event 2

	AIR					NITRATE					CONTROL			
	A	B	C	D	Dens.	A	B	C	D	Dens.	A	B	C	D	Dens.
# stems	14.0	11.0	4.0	7.0	36.0	19.0	15.0	7.0	11.0	52.0	9.0	30.0	21.0	24.0	84.0
average (cm)	12.0	15.1	7.8	7.3	11.6	20.8	23.6	29.3	25.5	23.8	31.6	24.9	28.0	31.1	27.7
std dev (cm)	9.5	5.6	1.7	2.1	7.3	13.2	9.1	6.6	12.6	11.3	10.3	8.9	11.3	8.8	9.9
heights (cm)	13.0	14.0	10.0	4.0		14.0	13.0	38.0	29.0		37.0	34.0	41.0	25.0	
	12.0	14.0	6.0	6.0		14.0	13.0	31.0	35.0		39.0	32.0	41.0	38.0	
	12.0	15.0	8.0	8.0		6.0	26.0	30.0	31.0		24.0	10.0	35.0	27.0	
	2.0	7.0	7.0	11.0		7.0	30.0	16.0	20.0		45.0	34.0	13.0	35.0	
	5.0	8.0		7.0		53.0	21.0	30.0	14.0		38.0	35.0	35.0	43.0	
	5.0	11.0		8.0		28.0	11.0	31.0	47.0		42.0	30.0	12.0	38.0	
	6.0	12.0		7.0		22.0	27.0	29.0	6.0		19.0	32.0	38.0	18.0	
	3.0	19.0				19.0	32.0		26.0		18.0	34.0	18.0	28.0	
	6.0	19.0				7.0	23.0		8.0		24.0	35.0	36.0	45.0	
	12.0	23.0				8.0	32.0		29.0			28.0	10.0	34.0	
	12.0	24.0				31.0	33.0		37.0			21.0	20.0	34.0	
	14.0					6.0	34.0					27.0	7.0	20.0	
	28.0					24.0	5.0					28.0	12.0	48.0	
	36.0					14.0	27.0					34.0	36.0	40.0	
						26.0	27.0					18.0	16.0	31.0	
						36.0						19.0	26.0	19.0	
						6.0						19.0	30.0	23.0	
						35.0						20.0	32.0	13.0	
						36.0						20.0	21.0	35.0	
												42.0	31.0	34.0	
												17.0	36.0	28.0	
												20.0		34.0	
												16.0		28.0	
												17.0		28.0	
												17.0			
												11.0			
												22.0			
												17.0			
												42.0			
												17.0			

Sampling Event 3

	AIR					NITRATE					CONTROL				
	A	B	C	D	Dens.	A	B	C	D	Dens.	A	B	C	D	Dens.
# stems	5.0	17.0	2.0	10.0	34.0	0.0	9.0	13.0	12.0	34.0	32.0	12.0	11.0	12.0	67.0
average (cm)	22.6	32.3	18.0	61.2	38.5		146.1	133.5	47.2	108.4	112.5	113.9	84.5	81.7	102.7
std dev (cm)	10.3	29.0	0.0	30.4	30.2		9.3	17.8	25.7	48.5	40.9	17.0	25.3	38.9	37.2
heights (cm)	30.0	5.0	18.0	85.0			140.0	136.0	31.0		101.0	135.0	95.0	137.0	
	27.0	3.0	18.0	84.0			144.0	137.0	84.0		161.0	146.0	98.0	14.0	
	22.0	3.0		60.0			142.0	136.0	77.0		140.0	134.0	93.0	132.0	
	29.0	36.5		98.0			151.0	97.0	71.0		123.0	113.0	85.0	79.0	
	5.0	8.0		81.0			167.0	134.0	76.0		129.0	98.0	88.0	34.0	
		28.0		45.0			151.0	100.0	38.0		132.0	96.0	74.0	108.0	
		27.5		90.0			138.0	129.0	17.0		136.0	99.0	108.0	114.0	
		10.5		23.0			137.0	136.0	12.0		125.0	104.0	108.0	95.0	
		20.0		19.0			145.0	146.0	61.0		122.0	127.0	110.0	65.0	
		25.0		27.0				159.0	47.0		137.0	105.0	33.0	80.0	
		19.0						154.0	33.0		121.0	108.0	44.0	37.0	
		88.0						128.0	21.0		142.0	104.0		86.0	
		22.5						139.0			138.0				
		48.0									107.0				
		31.0									121.0				
		90.0									147.0				
		84.0									52.0				
											52.0				
											58.0				
											28.0				
											27.0				
											61.0				
											19.0				
											127.0				
											142.0				
											128.0				
											145.0				
											128.0				
											140.0				
											136.0				
											171.0				
											106.0				

Sampling Event 4

	AIR					NITRATE					CONTROL				
	A	B	C	D	Dens.	A	B	C	D	Dens.	A	B	C	D	Dens.
# stems	13.0	5.0	14.0	13.0	45.0	18.0	0.0	0.0	11.0	29.0	19.0	16.0	11.0	15.0	61.0
average (cm)	86.6	78.6	104.4	61.0	83.6	117.4			46.8	90.6	101.4	102.4	100.7	104.1	102.2
std dev (cm)	40.2	19.5	18.1	35.0	34.7	34.3			20.5	45.6	49.6	55.1	47.2	35.5	46.6
heights (cm)	70.0	61.0	131.0	94.0		117.0			63.0		17.0	14.0	9.0	13.0	
	57.0	64.0	133.0	100.0		92.0			44.0		108.1	108.3	109.9	110.6	
	127.0	105.0	101.0	104.0		136.0			37.0		144.0	153.0	63.0	98.0	
	134.0	89.0	100.0	114.0		148.0			46.0		138.0	148.0	102.0	97.0	
	105.0	64.0	76.0	21.0		140.0			37.0		136.0	152.0	134.0	57.0	
	52.0		123.0	23.0		131.0			31.0		127.0	50.0	147.0	91.0	
	122.0		133.0	17.0		143.0			16.0		140.0	80.0	33.0	141.0	
	12.0		98.0	43.0		118.0			69.0		135.0	160.0	133.0	65.0	
	125.0		88.0	56.0		127.0			41.0		127.0	157.0	158.0	134.0	
	118.0		94.0	53.0		125.0			91.0		130.0	154.0	105.0	126.0	
	42.0		100.0	89.0		149.0			40.0		129.0	148.0	114.0	117.0	
	54.0		101.0	26.0		146.0					125.0	145.0		131.0	
	108.0		95.0	53.0		31.0					137.0	39.0		131.0	
			91.0			120.0					133.0	41.0		130.0	
						123.0					110.0	43.0		120.0	
						116.0					16.0	46.0			
						119.0					14.0				
						32.0					30.0				
											33.0				

Sampling Event 5

	AIR					NITRATE					CONTROL				
	A	B	C	D	Dens.	A	B	C	D	Dens.	A	B	C	D	Dens.
# stems	20 0	23 0	8 0	8 0	59 0	18.0	6 0	22 0	12 0	58 0	14 0	13 0	0 0	11 0	38 0
average (cm)	84 3	36 2	53 3	49 9	56 7	145 6	101 3	120 5	129 2	128 1	70 4	64 8		63 4	72 3
std dev (cm)	35 9	34 1	29 1	26 2	38 5	27 2	47 7	55 4	27 6	43 5	52 6	46 3		38 3	46 0
heights (cm)	102 0	71 5	106 0	66 0		156 0	12 0	122 0	149 0		164 0	123 0		95 0	
	44 0	38 5	66 0	62 5		167 0	127 0	159 0	134 0		139 0	38 0		114 0	
	116 0	39 0	64 0	75 0		158 0	132 0	149 0	137 0		154 0	52 0		123 0	
	42 0	33 0	40 5	18 0		160 0	95 0	148 0	134 0		79 0	30 0		110 0	
	39 0	32 0	36 0	18 0		147 0	99 0	143 0	127 0		23 0	124 0		84 0	
	95 0	36 0	29 0	30 0		170 0	143 0	158 0	116 0		32 0	122 0		78 0	
	86 0	4 0	25 0	66 0		140 0		162 0	157 0		56 0	140 0		77 0	
	124 0	6 0	40 0	44 0		148 0		163 0	142 0		106 0	77 0		100 5	
	64 0	5 0				47 0		149 0	136 0		60 0	22 0		113 0	
	108 0	3 5				167 0		0 0	163 0		73 0	17 0		13 0	
	27 0	5 0				133 0		37 0	70 0		21 0	49 0		12 0	
	37 0	51 5				164 0		16 0	63 0		19 0	20 0			
	43 0	43 0				158 0		23 0			20 0	29 0			
	147 0	96 0				132 0		39 0			20 0				
	120 0	14 0				147 0		153 0							
	108 0	6 5				140 0		146 0							
	108 0	13 0				144 0		142 0							
	106 0	108 0				143 0		158 0							
	62 0	107 0						124 0							
	107 0	79 0						154 0							
		13 0						142 0							
		10 0						164 0							
		19 0													

Sampling Event 6

	AIR					NITRATE					CONTROL				
	A	B	C	D	Dens.	A	B	C	D	Dens.	A	B	C	D	Dens.
# stems	4 0	6 0	6 0	6 0	22 0	12 0	10 0	10 0	10 0	42 0	9 0	13 0	8 0	10 0	40 0
average (cm)	32 8	27 0	17 7	48 7	31 4	16 0	16 8	27 0	16 4	19 4	62 8	46 7	42 1	84 7	58 9
std dev (cm)	7 8	13 8	6 2	10 0	15 2	4 5	12 8	17 4	12 5	12 7	31 1	34 2	51 7	42 3	41 6
heights (cm)	40 0	28 0	25 0	58 0		20 0	7 0	10 0	12 0		62 0	73 0	130 0	105 0	
	38 0	38 0	10 0	62 0		23 0	3 0	10 0	13 0		79 0	28 0	20 0	15 0	
	23 0	10 0	19 0	40 0		15 0	5 0	15 0	9 0		79 0	71 0	11 0	112 0	
	30 0	38 0	12 0	40 0		13 0	35 0	6 0	7 0		78 0	120 0	10 0	91 0	
		40 0	16 0	40 0		10 0	30 0	16 0	34 0		100 0	38 0	7 0	33 0	
		10 0	24 0	52 0		15 0	33 0	33 0	16 0		74 0	56 0	11 0	110 0	
						13 0	25 0	50 0	34 0		71 0	70 0	120 0	113 0	
						10 0	3 0	50 0	40 0		16 0	59 0	28 0	28 0	
						15 0	12 0	40 0	9 0		6 0	10 0		130 0	
						15 0	15 0	40 0	10 0			8 0		110 0	
						20 0						7 0			
						23 0						8 0			
												61 0			

Sampling Event 8

	AIR					NITRATE					CONTROL				
	A	B	C	D	Dens.	A	B	C	D	Dens.	A	B	C	D	Dens.
# stems	35.0	36.0	18.0	23.0	112.0	7.0	14.0	1.0	15.0	37.0	19.0	14.0	7.0	2.0	42.0
average (cm)	15.7	11.0	12.8	14.7	12.7	15.7	10.3	9.0	14.6	13.3	15.2	14.0	10.0	18.5	13.6
std dev (cm)	3.7	2.9	3.0	3.3	4.3	3.7	3.4	3.5	3.2	4.0	3.0	4.0	1.9	9.2	4.3
heights (cm)	5.0	8.0	7.0	9.0		21.0	17.0	9.0	8.0		17.0	14.0	10.0	12.0	
	19.0	12.0	12.0	16.0		17.0	17.0	14.0	9.0		18.0	12.0	9.0	25.0	
	11.0	10.0	17.0	21.0		15.0	8.0		18.0		18.0	18.0	11.0		
	17.0	9.0	12.0	13.0		18.0	14.0		13.0		14.0	12.0	13.0		
	15.0	17.0	15.0	15.0		12.0	11.0		18.0		11.0	12.0	9.0		
	16.0	19.0	11.0	8.0		10.0	8.0		15.0		13.0	18.0	11.0		
	15.0	11.0	17.0	9.0		17.0	9.0		15.0		14.0	17.0	7.0		
	12.0	15.0	16.0	16.0			9.0		15.0		9.0	19.0			
	16.0	11.0	12.0	17.0			7.0		17.0		18.0	18.0			
	17.0	10.0	16.0	16.0			11.0		18.0		21.0	17.0			
	15.0	10.0	16.0	13.0			9.0		15.0		14.0	13.0			
	14.0	7.0	11.0	13.0			7.0		16.0		17.0	8.0			
	19.0	10.0	10.0	17.0			9.0		15.0		16.0	6.0			
	23.0	11.0	14.0	20.0			8.0		17.0		13.0	12.0			
	17.0	15.0	8.0	17.0					10.0		19.0				
	16.0	10.0	11.0	15.0							14.0				
	14.0	9.0	12.0	15.0							15.0				
	16.0	9.0	13.0	17.0							16.0				
	24.0	12.0		16.0							12.0				
	19.0	9.0		14.0											
	18.0	12.0		16.0											
	13.0	11.0		14.0											
	11.0	13.0		10.0											
	20.0	5.0													
	17.0	6.0													
	17.0	11.0													
	10.0	10.0													
	11.0	12.0													
	16.0	13.0													
	21.0	15.0													
	12.0	12.0													
	16.0	14.0													
	17.0	10.0													
	15.0	8.0													
	15.0	11.0													
		10.0													

Sampling Event 9

	AIR					NITRATE					CONTROL				
	A	B	C	D	Dens.	A	B	C	D	Dens.	A	B	C	D	Dens.
# stems	29.0	23.0	7.0	11.0	70.0	19.0	3.0	13.0	20.0	55.0	17.0	14.0	7.0	12.0	50.0
average (cm)	44.2	36.5	31.7	40.6	38.6	43.6	29.3	40.5	58.1	44.9	17.2	27.1	32.1	30.8	27.1
std dev (cm)	10.5	11.8	11.8	18.0	14.8	12.1	20.2	18.7	11.9	16.1	6.6	11.9	11.2	10.9	11.1
heights (cm)	37.0	44.0	45.0	57.0		61.0	42.0	28.0	60.0		29.0	19.0	33.0	31.0	
	35.0	10.0	21.0	13.0		19.0	40.0	26.0	50.0		9.0	25.0	31.0	39.0	
	39.0	34.0	22.0	54.0		34.0	8.0	59.0	68.0		10.9	30.0	49.0	15.0	
	32.0	38.0	42.0	61.0		33.0		70.0	51.0		17.0	46.0	19.0	21.0	
	40.0	59.0	44.0	63.0		39.0		70.0	66.0		14.0	37.0	18.0	44.0	
	42.0	46.0	30.0	28.0		50.0		24.0	28.0		24.0	33.0	33.0	33.0	
	37.0	47.0	18.0	53.0		44.0		38.0	67.0		23.0	46.0	42.0	24.0	
	18.0	41.0		32.0		63.0		25.0	51.0		31.0	39.0		30.0	
	47.0	20.0		43.0		54.0		63.0	39.0		15.0	16.0		29.0	
	58.0	19.0		21.0		42.0		34.0	39.0		22.0	24.0		40.0	
	35.0	36.0		22.0		49.0		18.0	68.0		17.0	12.0		15.0	
	57.0	30.0				42.0		27.0	59.0		18.0	13.0		49.0	
	48.0	39.0				43.0		44.0	61.0		13.0	13.0			
	38.0	41.0				53.0			71.0		13.0	26.0			
	33.0	47.0				34.0			57.0		10.0				
	45.0	44.0				53.0			74.0		18.0				
	60.0	56.0				53.0			61.0		10.0				
	37.0	45.0				20.0			67.0						
	42.0	33.0				47.0			63.0						
	68.0	24.0							63.0						
	42.0	32.0													
	42.0	30.0													
	61.0	25.0													
	46.0														
	43.0														
	57.0														
	48.0														
	47.0														
	49.0														

Nitrate-N Concentration

Sampling Event	Treatment								
		air mean (mg/L)	std dev (mg/L)		nitrate mean (mg/L)	std dev (mg/L)		control mean (mg/L)	std dev (mg/L)
1	0.32			0.09			0.80		
1	0.24	0.28	0.06	0.35	0.22	0.18	0.06	0.43	0.52
2	0.42			0.24			0.08		
2	0.00	0.21	0.30	0.00	0.12	0.17	0.21	0.15	0.09
3	0.12			0.32			0.10		
3	0.06	0.09	0.04	0.02	0.17	0.21	0.07	0.09	0.03
4	0.74			0.05			0.02		
4	0.03	0.39	0.50	0.10	0.08	0.04	0.00	0.01	0.01
5	0.13			0.06			0.06		
5	0.21	0.17	0.06	0.11	0.09	0.03	0.06	0.06	0.00
6	ND			ND			0.03		
6	ND			ND			0.01	0.02	0.02
7	ND			ND			0.01		
7	ND			ND			0.01	0.01	0.00
8	ND			ND			ND		
8	ND			ND			ND		
9	ND			ND			ND		
9	ND			ND			ND		

ND=not dedermined

Sampling Event	Phosphate-P Concentration								
	Treatment								
		air mean (mg/L)	std dev (mg/L)		nitrate mean (mg/L)	std dev (mg/L)		control mean (mg/L)	std dev (mg/L)
1	0.02			1.07			0.37		
1	1.11	0.57	0.77	0.37	0.72	0.49	0.12	0.25	0.18
2	0.13			4.73			0.87		
2	0.07	0.10	0.04	0.01	2.37	3.34	0.00	0.43	0.61
3	0.01			0.86			0.85		
3	0.03	0.02	0.01	0.13	0.50	0.52	0.24	0.54	0.43
4	0.01			3.10			0.79		
4	0.09	0.05	0.06	0.28	1.69	1.99	0.91	0.85	0.09
5	0.01			0.01			0.11		
5	0.99	0.50	0.69	0.04	0.03	0.02	2.45	1.28	1.66
6	0.00			0.01			0.01		
6	0.00	0.00	0.00	0.08	0.05	0.06	0.02	0.02	0.01
7	0.00			0.00			0.01		
7	0.01	0.01	0.01	0.01	0.00	0.00	0.05	0.03	0.03
8	0.01			0.34			ND		
8	0.01	0.01	0.00	0.34	0.34	0.00	ND		
9	0.18			0.14			0.47		
9	0.02	0.10	0.11	0.15	0.14	0.01	0.47	0.47	0.00

ND=not dedermined

Sampling Event	Amonia-N Concentration								
	Treatment								
		air mean (mg/L)	std dev (mg/L)		nitrate mean (mg/L)	std dev (mg/L)		control mean (mg/L)	std dev (mg/L)
1	12.46			1.46			0.12		
1	1.14	6.80	8.00	1.06	1.26	0.28	0.75	0.44	0.44
2	0.87			0.49			1.01		
2	0.00	0.44	0.62	0.00	0.24	0.35	0.38	0.69	0.45
3	3.01			0.46			1.33		
3	1.94	2.47	0.76	7.82	4.14	5.20	0.49	0.91	0.60
4	2.14			1.48			7.45		
4	0.32	1.23	1.29	1.22	1.35	0.18	3.18	5.32	3.02
5	0.29			0.33			0.47		
5	1.76	1.03	1.04	0.65	0.49	0.22	9.64	5.05	6.49
6	ND			ND			2.68		
6	ND			ND			3.50	3.09	0.58
7	0.57			0.60			1.16		
7	1.63	1.10	0.75	0.36	0.48	0.17	0.79	0.98	0.26
8	0.22			1.40			ND		
8	0.22	0.22	0.00	1.40	1.40	0.00	ND		
9	0.18			0.13			1.03		
9	0.13	0.15	0.03	0.47	0.30	0.24	1.03	1.03	0.00

ND=not dedermined

APPENDIX D.1 CALCULATIONS DURING THE STUDY

D1.1 Detection Levels for the EPA Method

Table D1.1. TPH using EPA Method. The results are the mean and standard deviation for the CS used to calculate the MDL and LOQ.

Sample	TPH mg/kg_{dw}
1	30
2	10
3	140
4	ND
5	503
6	ND
7	300
Mean	197
Standard Deviation	206

ND = not detected Note: The values were obtained in two different sets of samples (1-3 Table A1.1 and 4-7 Table A1.2).

EPA (1994) specifies that:

MDL = ts

where : t = 3.75 (one tail t test, p=0.01), and s = 206 mg/kg_{dw}

MDL = 206 mg/kg_{dw} x 3.75 = 773 mg/kg_{dw}

Standard Methods specifies that:

LOQ = 10s

Where: s = 206 mg/kg_{dw}

LOQ = 2,060 mg/kg_{dw}

D1.2 Detection Levels for the ASTM Method

Table D1.2. The results are the mean absorbance and standard deviation for the lowest calibration standard (40 mg No. 2 fuel oil/L) used to calculate the MDL and LOQ.

Sample	Absorbance (AU)
1	0.179
2	0.198
3	0.176
4	0.179
5	0.177
6	0.177
Mean	0.181
Standard Deviation	0.0841

AU = absorbance units

The MDL was calculated as 3.37 (one tailed t test, p=0.01) times the standard deviation of the lowest liquid calibration standard used (40 mg/L). Then, the concentration (mg/L) corresponding to the absorbance was determined using the line of best fit for the calibration curve data and multiplied by the solvent/sample ratio to obtain the mg TPH/kg_{dw}. MDL = 52 mg/kg_{dw}. The LOQ was calculated as 10 times the standard deviation and transformed to mg/kg_{dw} following the same procedure for the MDL.

LOQ= 180 mg/kg_{dw}.

Example of MDL calculation:

MDL_{AU}= ts = (3.37) (0.0841 Au) = 0.28 AU

From the calibration curve, 0.28 AU corresponds to a concentration of 5.2 mg TPH/L.

Using the solvent/sample ratio, this is converted to mg TPH/kg_{dw}

MDL = 5.2 mg TPH/L x 50 mL solvent/5 g soil x 1000 g /kg x L/1000mL x 1 (dry weight fraction for CS)

MDL = 52 mg/kg_{dw}

D1.3 Number of Samples for the EPA Method

The number of samples was calculated based on Equation 4.1 (See Results and Discussion) and its related figure from Standard Methods (2000). The number of samples required in estimating a mean concentration is:

$$N \geq \left[\frac{ts}{U}\right]^2 \qquad \text{(Eq. 4.1)}$$

where: N = number of samples, t = Student's t-statistic for a given confidence level (one tail, p=0.05, n = 16 marsh samples from which s was derived), s = overall standard deviation of marsh samples (2,544 mgTPH/kg_{dw}), and U = acceptable level of uncertainty or detectable difference desired between samples (1,000 or 500 mgTPH/kg_{dw}).

For a U of 1,000 mgTPH/kg_{dw}, N = ~ 25 samples

For a U of 500 mgTPH/kg_{dw}, N = ~ 100 samples

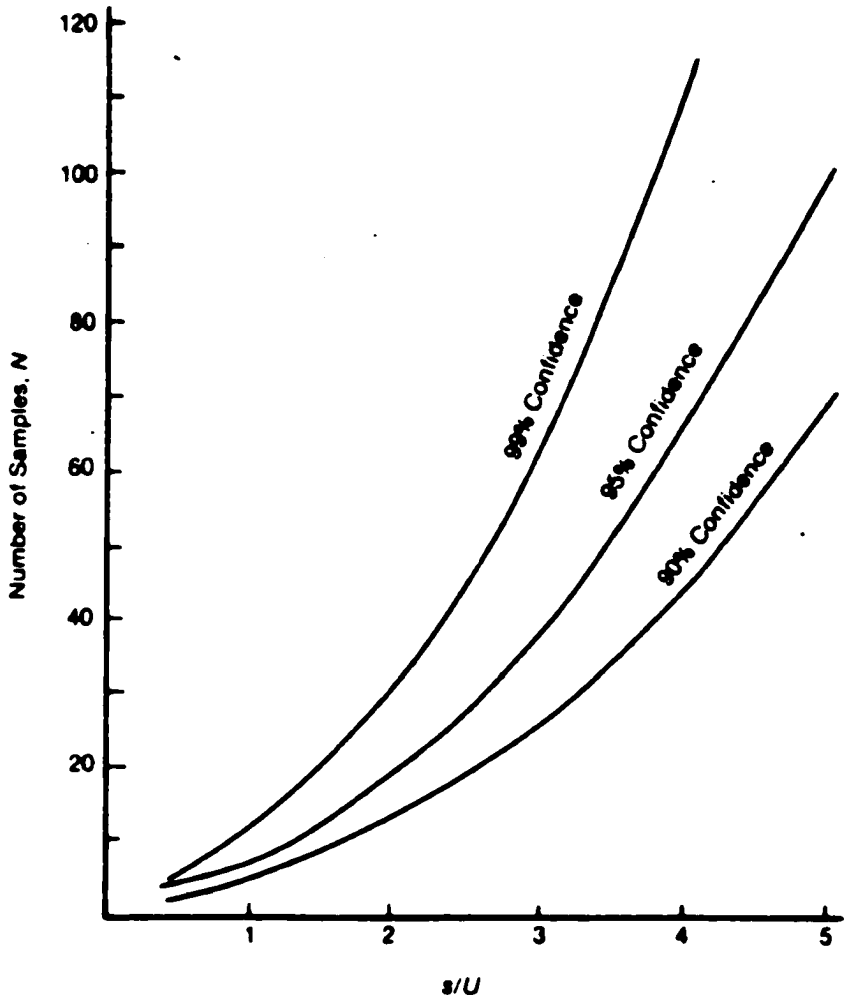

Figure D1.3. Approximate number of samples required in estimating a mean concentration. (Taken from Standard Methods, 2000).

D1.4 Plot Volume

30 ft x 10 ft x 8 in x 1 ft/12 in = 200 ft^3

Assumes the radius of influence of the wells will allow penetration of the amendment to a maximum depth of 8 in. below the sediment surface.

200 ft^3 x 0.0283 m^3/ft^3 = 5.66 m^3

Total plot volume = 5.66 m^3

Liquid volume in sediment pore spaces assuming 0.4 porosity = 2.27 m^3 = (5.66m^3 x 0.4)

Plot volume in the top 2 in. of the sediments where the highest TPH concentrations were found:

30 ft x 10 ft x 2 in x 1 ft/12 in x 0.0283 m^3/ft^3 = 1.42 m^3

Liquid volume in the 2 in. assuming 0.4 porosity = 0.57 m^3

D1.5 Stoichiometry for TPH degradation Using NO_3^- as TEA

Table D1.5. Stoichiometry for the biodegradation of SC and LC aliphatics, and aromatics using NO_3^- as TEA.

Compound	Theoretical Stoichiometry
SC Aliphatic $C_{14}H_{30}$	$C_{14}H_{30} + 22.4\ NO_3^- + 48.4\ H^+ \rightarrow 14\ CO_2 + 11.2\ N_2 + 39.2\ H_2O$
LC Aliphatic $C_{28}H_{58}$	$C_{28}H_{58} + 44.8\ NO_3^- + 99.8\ H^+ \rightarrow 28\ CO_2 + 22.4\ N_2 + 78.4\ H_2O$
Aromatic $C_{17}H_{17}$	$C_{17}H_{17} + 27.2\ NO_3^- + 78.2\ H^+ \rightarrow 17\ CO_2 + 13.6\ N_2 + 47.6\ H_2O$

Calculation for the theoretical NO_3^- demand during the degradation of an SC aliphatic:

For one mole of an SC aliphatic molecule ($C_{14}H_{30}$ M.W= 198 g), 22.4 moles of NO_3^--N (Nitrogen M.W = 14 g) is needed as a TEA for a total of 313.6 g NO_3^--N.

Based on the stoichiometry, to degrade 0.100 g of SC aliphatics, 0.158 g NO_3^--N will be needed:

$$\frac{198\text{g SC aliphatic}}{313.6\text{g } NO_3^- - N} \times \frac{X\text{ g } NO_3^- - N}{0.100\text{g SC aliphatic}} = 0.158\text{g } NO_3^- - N$$

Based on the stoichiometry, 0.159 g and 0.172 g of NO_3^--N will be needed to degrade 0.100 g of LC aliphatics and aromatics, respectively.

D1.6 Concentration of the NO_3^- in the amendment and expected concentration in the porewater

Concentration of the NO_3^- solution added/week during the study:

430 g $NaNO_3$ were added. That is equivalent to 313.7 g as NO_3^- and 70.8 g as N

$NaNO_3$ M.W. = 85 g, NO_3^- M.W. = 62 g and, N M.W. = 14 g.

Concentration in the total plot volume (5.66 m^3 or 5,663 L)(Appendix D1.4):

(313.7g NO_3^- x 1000 mg/g) / 5,663 L = 55.4 mg NO_3^-/L

NO_3^- = 55.4 mg/L, and N= 12.5 mg/L

Concentration in the pore volume (2,270 L) (Appendix D1.4):

NO_3^- = 138.2 mg/L and N= 31.19 mg/L

D1.7 Mass of TPH per plot

Density of sediments ρ_s:

$\rho_s = S_s\rho_w$

where: S_s is the sediment specific gravity. It was assumed S_s= 1.8 because it is a typical value for marsh sediments (Ballestero 2002), and ρ_w is the density of water (1,000 kg/m^3).

ρ_s = 1.8 (1,000 kg/m^3) = 1,800 kg/m^3

Solid fraction, S_f:

$S_f = 1 - \phi$

Where: ϕ = 0.4 = porosity (assumed)

S_f = 0.6

SC aliphatic fraction concentration in the Fore River Creek marsh:

~3,000 mg/kg_{dw} at the start of the study

Mass CS aliphatics in treatment volume = (solid fraction x TPH concentration x ρ_s x vol. plot)

Mass SC aliphatics = 0.6 x 3,000 mg/kg_{dw} x 1800 kg/m^3 x 1.42 m^3 x g/1000mg

Mass SC aliphatics = 4,601 mg SC/plot

Based on the stoichiometry (Appendix D1.5): ~0.158 g of $NO3^-$-N are needed to treat 0.100 g of SC aliphatics, so for the SC (4,600 g/plot) in the Fore River Creek marsh, ~ 7,268 g of N will be needed.

For the LC aliphatics (3,200 mg/kg_{dw}) and aromatics (2,500 mg/kg_{dw})

0.158 NO_3^--N/0.100 g CS = X g NO_3^-N needed/4,600 g SC/plot

g NO_3^-N needed x 1000 mg/g = 7,268 mg NO_3^-N needed to degrade SC aliphatics in the plot

Bases on the stoichiometry (Appendix D1.5): for the LC (4,908 g) and aromatics (3,834 mg), 7,803 g NO_3^--N and 6,594 g NO_3^--N respectively, will be needed.

The total of N (NO_3^- as TEA) theoretically needed to degrade the total TPH in the Fore River Creek salt marsh (SC +LC + aromatics) will be 19,979 g NO_3^--N

During the study, 70.8 g NO_3^--N/week were added during 30 weeks for a total of 2,124 g of NO_3^--N, that is ~10.6 % of the theoretical value needed to degrade the SC and LC aliphatics, and aromatics.

D1.8 Degradation Rates

Table D1.8 TPH fraction degradation rates for the Fore River Creek salt marsh.

	Degradation rates ($mg/kg_{dw}/d \pm 2s$.) during the Summer and Fall, 1998		
	Control	Air	Nitrate
SC Aliphatics	+13.1±12.8	+27.6±17.5	+20.7±10.9
LC Aliphatics	+13.6±12.21	+22.7±14.9	+23.7±12.99
Aromatics	+19.2±11.2	+16.9±9.9	+10.2±9.9
	Degradation rates ($mg/kg_{dw}/d \pm 2s$) during the Spring, 1999		
	Control	Air	Nitrate
SC Aliphatics	-4.7±5.9	-12.1±12.8	-11.7±18.7
LC Aliphatics	-7.6±9.9	-11.9±14.1	-17.1±25.2
Aromatics	-3.9±7.2	-7.3±12.9	-15.5±38.2
	Degradation rates constant ($x10^{-3}$)(d^{-1})(± 2s) during the entire study		
	Control	Air	Nitrate
SC Aliphatics	+8.7±3.9	+4.4±3.5	+5.0±4.9
LC Aliphatics	+6.2±3.5	+4.1±3.5	+4.4±4.6
Aromatics	+7.2±3.8	+4.4±4.4	+2.2±4.3

(+) means that the TPH fraction concentration is decreasing. This corresponds to negative slopes on the plots of TPH concentration vs. time.