Bioremediation of Hydrocarbon Contaminated Arctic Soils

by

Matthew R. Allen

A Thesis submitted
to the Department of Chemistry and Chemical Engineering
In partial fulfillment of the requirements for
the degree of
Master of Engineering

Royal Military College of Canada
Kingston, Ontario
May, 1999

ACKNOWLEDGEMENTS

I would first like to thank my thesis supervisors; Dr. Ken Reimer, the Director of the Environmental Sciences Group and Dr. Bill Mohn, professor of microbiology at the University of British Columbia. They taught me how to integrate the elements of science and project management and introduced me to the field of environmental engineering. Without their guidance and the foundation laid by their research, this thesis would not have been possible.

For her guidance, devotion and patience, I would like to thank Dr. Joan Torrie who helped me with the microbial aspects of the laboratory work and tolerated my incessant questioning. Thanks also go to Dr. Barb Zeeb who taught me about the disciplines of research and technical writing. For assistance in the analysis of the experimental radiolabel results, I would like to acknowledge the entire Analytical Services Group for their rapid turn around times and willingness to explain the science behind the numbers. Finally, a great thank you goes out to all the members of the Environmental Sciences Group who always took the time to offer advice, professional and academic, and forced me to see the humour amidst the chaos.

The experimental work in the field would not have been possible without the help of the ESG Bioremediation Field Team, along with North Warning System, Frontec, and the Department of National Defence site-support staff at FOX-M and CFS Alert. They were always willing to work until sunset and their technical expertise often saved the day. Thanks also go out to Jim Boissonneault, North Warning Systems Office, whose knowledge of the workings of northern logistics and environmental science was invaluable.

For assistance in the production of this thesis, I would like to thank Dr. Ken Reimer, Dr. Barb Zeeb, and Dr. Joan Torrie. I am indebted for their help in "wordsmithing" and data interpretation.

Finally, I would like to acknowledge the continuing financial support of the North Warning System Office and the Director General Environment of the Department of National Defence. I am also grateful to the Association of Canadian Universities in Northern Studies for the gift of the Northern Studentship Trust. To my friends, family, and my girlfriend Clare, thank you for letting me vent and forcing me to keep at it.

EXECUTIVE SUMMARY

Accidental fuel discharges leading to hydrocarbon contamination in soil are a serious environmental concern in northern industrial, commercial, and military operations. Developing dynamic technologies to remediate these soils in the Canadian Arctic poses a significant engineering challenge. The difficulty of transporting heavy, specialized treatment equipment and short treatment seasons require that remediation solutions be efficient, low maintenance and on-site. Physical and biological methods currently available are either logistically or economically not feasible for use in the sensitive ecosystems and extreme climate of the Arctic. Biopiles and landfarms overcome design obstacles for the bioremediation of hydrocarbon contaminated soils.

The objective of this thesis was to examine bioremediation parameters and identify areas for future research needed to make aboveground engineered soil treatment systems an effective means of cleaning up fuel spills in the Arctic.

Laboratory microcosm and field experiments investigated the effects of soil additives including: 1) commercial and bioremediation-specific fertilizers, 2) microbial inoculation with indigenous microbes, 3) surfactants, and 4) the evaluation of several aeration, heating, and insulative options. Experimental and statistical design considerations for enhancing future research were also identified. Experiments were carried out using contaminated soil from two military sites in the Canadian Arctic: Hall Beach and CFS Alert, Nunavut. These experiments tracked hydrocarbon removal through the monitoring of total petroleum hydrocarbon (TPH) changes and ^{14}C-labelled n-dodecane mineralization.

Laboratory microcosm experimental results identified the optimal concentration and type of amendment for each soil type. They also highlighted the need for customized amendment strategies that account for the specific physico-chemical soil characteristics and nutrient profiles at different sites. The addition of a synthetic surfactant and a

commercial fertilizer to Alert soil produced a 16-fold increase in the extent of mineralization of a ^{14}C-labelled hydrocarbon over the response in unfertilized soil. Commercial fertilizer amendment of FOX-M soil produced the greatest increase in the mineralization of a ^{14}C-labelled hydrocarbon while reducing lag periods and increasing initial rates. Mineralization studies also gave evidence of nutrient inhibition from both limited and excess levels of fertilizer and demonstrated the ability of synthetic surfactants to reduce such inhibition in silt soil.

Field experimental results revealed significant changes in TPH levels over time in all biopile and landfarm experiments producing TPH decreases as high as 2757 +/- 743 ppm in Alert medium-scale biopiles over 42 days of treatment. Temperature data gathered from medium-scale biopiles at both sites revealed no clear benefits from aeration or heating systems. Observation of an additional treatment effect over time highlighted the strong effect of volatilization on TPH degradation in uncovered treatments producing TPH decreases as high as 1575 +/- 355 ppm in FOX-M small-scale biopiles over 53 days of treatment.

Analysis of field-scale findings demonstrated that biopiles and landfarms successfully reduced hydrocarbon contamination in soil at two Arctic sites over a short summer treatment season. Laboratory findings provided optimized amendment application ratios and underlined the complexities of modeling TPH degradation with radiolabelled-hydrocarbons in silt soil. An evaluation of the potential impacts of volatilization suggested that remediation with biopile and/or landfarming technologies is an effective, safe treatment option provided that leachate containment and air quality standards are controlled. Furthermore, field data was used to develop statistically valid proposals for data interpretation including increased sample and treatment replication.

TABLE OF CONTENTS

ACKNOWLEDGEMENTS..... ii

EXECUTIVE SUMMARY iii

TABLE OF CONTENTS..... v

LIST OF FIGURES..... ix

LIST OF TABLES..... xi

LIST OF PHOTOGRAPHS..... xii

I. INTRODUCTION..... 1

A. Nature of fuel spills and Arctic hydrocarbon contamination 1

B. Impacts of fuel spills in Arctic ecosystems..... 2

1. Environmental considerations 2
2. Toxicological and human health considerations..... 3

C. Existing technologies for the remediation of hydrocarbon contaminated soil.....4

1. Physical methods 5
i. Soil washing 5
ii. Excavation and Landfilling..... 5
iii. Incineration and Thermal Desorption..... 5
iv. Vacuum Extraction..... 6

2. Biological methods.....6
i. Infiltration Galleries..... 6
ii. Biopiles and Landfarming..... 6

D. Arctic design considerations for the selection of remediation technologies. 7

E. Bioremediation with biopile and landfarming approaches..... 8

1. Bioremediation principles.....8
i. Microbes..... 9
ii. Nutrients 11
iii. Surfactants 12

2. *Biopiles* 13

F. Past research into biopile bioremediation 15

1. Findings to date 15
2. Foundation for further study 16

II. MATERIALS 17

A. Soil Sources 17

1. FOX-M soil 17
2. CFS Alert soil 18

B. Chemicals and soil additives 19

III. METHODS 20

A. Laboratory experiments 20

1. Preparation of soils 21
2. Microcosm design 21
3. Mineralization monitoring 22
4. Liquid scintillation counting procedure for mineralization monitoring 22
5. Experimental Variables 23
6. TPH versus ^{14}C removal 25

B. Field-scale research 26

1. Soil preparation 27
2. Construction of FOX-M small-scale biopiles 28
3. Construction of medium-scale biopiles 30
4. Construction of FOX-M landfarm 34
5. Field-scale soil sampling procedure 35
6. TPH Analysis 37

C. Data presentation & Analysis 40

1. Laboratory-scale data 40
2. Field-scale data 42

IV. RESULTS 42

A. Microcosm studies 42

1. Physico-chemical soil characteristics 42
2. Commercial fertilizer microcosm experiments 43
3. Bioremediation specific fertilizer microcosm experiments 45
4. Surfactant microcosm experiments 45
5. Inoculum microcosm experiments 48
6. TPH versus ^{14}C removal experiments 50

B. Field studies 52

1. Medium-scale experiments 52
2. FOX-M small-scale experiments 55
3. FOX-M landfarm experiments 57

V. DISCUSSION 59

A. Discussion of non-controllable experimental parameters 60

1. Physico-chemical soil characteristics 60
2. Temperature effects 61

B. Discussion of supporting laboratory results 62

1. Commercial fertilizer microcosm experiments 63
2. Bioremediation specific fertilizer microcosm experiments 64
3. Surfactant microcosm experiments 65
4. Microcosm inoculation experiments 66
5. Comparison of FOX-M and Alert microcosm soil additive experiments 67
6. TPH versus ^{14}C removal experiments 69

C. Discussion of field results 71

1. Medium-scale experiments 72
2. FOX-M small-scale experiments 72
3. FOX-M landfarm experiments 73
4. Statistical and experimental design considerations 74
5. Volatilization considerations for landfarming approaches 75

VI. CONCLUSIONS 77

VII. REFERENCES 80

VIII. APPENDICES

Appendix A: UBC experimental results 84
Appendix B: Grain size analysis results 92
Appendix C: Kinetic parameters from Boltzman model 95
Appendix D: Anova results 97
Appendix E: Thermal data 99
Appendix F: Field-scale TPH results 102
Appendix G: Anova sample size calculation 107

LIST OF FIGURES

Figure I-1: Treatment costs for remediation technologies 4

Figure I-2: Schematic of an engineered biopile..... 14

Figure II-1: Map showing the location of FOX-M and CFS Alert..... 18

Figure III-1: Schematic diagram of FOX-M and Alert medium-scale biopiles..... 31

Figure III-2: Cross-section of convex treatment surface for FOX-M landfarm..... 35

Figure IV-1: Impact of commercial fertilizer on ^{14}C-labelled HC mineralization in Alert soil. 44

Figure IV-2: Impact of commercial fertilizer on ^{14}C-labelled HC mineralization in FOX-M soil. 44

Figure IV-3: Impact of bioremediation specific fertilizer on ^{14}C-labelled HC mineralization in Alert soil. 46

Figure IV-4: Impact of bioremediation specific fertilizer on ^{14}C-labelled HC mineralization in FOX-M soil. 46

Figure IV-5: Impact of surfactant on ^{14}C-labelled HC mineralization in Alert soil. 47

Figure IV-6: Impact of surfactant on ^{14}C-labelled HC mineralization in FOX-M soil 47

Figure IV-7: Impact of inoculum and cryopreservative addition on ^{14}C-labelled HC mineralization in Alert soil. 49

Figure IV-8: Impact of inoculum on ^{14}C-labelled HC mineralization in Alert soil. 49

Figure IV-9: Comparison of ^{14}C-labelled HC mineralization and TPH degradation in FOX-M soil. 51

Figure IV-10: Comparison of ^{14}C -labelled HC mineralization and TPH degradation in Alert soil. 51

Figure IV-11: Impact of aeration systems on TPH degradation in FOX-M medium-scale biopiles after 54 days of treatment. 53

Figure IV-12: Impact of aeration systems on TPH degradation in Alert medium-scale biopiles after 42 days of treatment..... 55

Figure IV-13: Impact of soil additives on TPH degradation in FOX-M small-scale biopiles after 53 days of treatment..... 57

Figure IV-14: Impact of soil additives on TPH degradation in FOX-M landfarm treatments after 49 days of treatment..... 59

Figure V-1: Comparison of the impacts of optimal soil additives on ^{14}C mineralization in Alert soil..... 68

Figure V-2: Comparison of the impacts of optimal soil additives on ^{14}C mineralization in FOX-M soil..... 68

LIST OF TABLES

Table I-1: Biodegradability of organic hazardous wastes 1

Table I-2: Microorganisms involved in the bioremediation of organic wastes..... 9

Table III-1: Commercial fertilizer experimental variables..... 23

Table III-2: Bioremediation specific fertilizer experimental variables..... 24

Table III-3: Surfactant experimental variables..... 24

Table III-4: Inoculum dilution experiments..... 25

Table III-5: Field-scale experimental treatments..... 26

Table IV-1: Grain size analysis results..... 43

Table IV-2: TPH levels detected in FOX-M microcosm experiments..... 50

Table IV-3: TPH levels detected in Alert microcosm experiments..... 52

Table IV-4: Mean TPH changes with standard deviations for FOX-M medium-scale biopiles over 54 days of monitoring..... 53

Table IV-5: Mean TPH changes with standard deviations for CFS Alert medium-scale biopiles over 42 days of monitoring..... 54

Table IV-6: Mean TPH changes with standard deviations for FOX-M small-scale biopile treatments over 53 days of monitoring..... 56

Table IV-7: Mean TPH changes with standard deviations for FOX-M landfarm treatments over 49 days of monitoring..... 58

LIST OF PHOTOGRAPHS

Photograph III-1: Soil stockpile constructed by site contractor staff at FOX-M..... 28

Photograph III-2: Leachate collection system built into FOX-M small-scale control pile..... 29

Photograph III-3: Building and fertilizing a small-scale biopile at FOX-M..... 29

Photograph III-4: Completed small-scale biopiles with plastic covers at FOX-M.... 30

Photograph III-5: Building a medium-scale biopile at FOX-M..... 32

Photograph III-6: Partially built medium-scale biopiles at FOX-M. Aeration systems within wooden frames are visible.....33

Photograph III-7: Completed FOX-M medium-scale biopiles..... 33

Photograph III-8: Completed FOX-M landfarm with flagging tape marking individual plots..... 35

Photograph III-9: Sampling a FOX-M small-scale biopile at each corner.....36

Photograph III-10: Sampling a FOX-M medium-scale biopile with a hand auger.....37

Photograph III-11: Excavating soil from the zone of high TPH contamination at Alert..... 38

Photograph III-12: Homogenizing fertilizer and bulking agent in the Alert experimental soil stockpile using a roto-tiller..... 38

Photograph III-13: Medium-scale control biopile constructed outside metal enclosure at Alert..... 39

Photograph III-14: Passively aerated medium-scale biopile at Alert. ABS passive aeration pipes can be seen protruding through impermeable barrier..... 39

Photograph III-15: Greenhouse structure within metal enclosure at Alert. Active and passive aeration medium-scale biopiles are visible inside..... 40

I. INTRODUCTION

The danger of hydrocarbon (HC) contamination is omnipresent in today's mechanized world. This danger is no less pertinent in the Arctic environment. Developing practical, cost-effective ways to deal with HC contamination in soils is an issue that must be addressed by all military, industrial and commercial operations in Canada's North.

A. Nature of fuel spills and Arctic HC contamination

Hydrocarbons belong to the greater family of organic contaminants which also include detergents, PCBs and pesticides and which form the most prevalent group of soil and groundwater contaminants. Organic contaminants have a wide range of degradability in soil with fuels generally being the most readily degradable (Table I-1).

Table I-1: Biodegradability of organic hazardous wastes (adapted from Leahy & Brown, 1994).

READILY DEGRADABLE	MODERATELY DEGRADABLE	HARD TO DEGRADE
Gasoline	#6 Oil	
Jet Fuel	Crude Oil	TCE
Diesel Fuel	Lubricating Oils	PCE
Toluene	Coal Tars	Vinyl Chloride
Benzene	Creosotes	PCBs
Isopropyl Alcohol	Pentachlorophenol	DDT
Methanol	Nitrobenzene	

Arctic HC contamination most commonly stems from fuel spills caused by the transport, storage and transfer of fuels such as diesel, turbo, jet fuel and gasoline. Fuel spills

generally result from mechanical failures at bulk storage (tanks and barrels) and pipeline areas and may go undiscovered for several years in remote Arctic locations.

Fuels differ in their volatility, persistence and concentration and include volatile organic compounds (VOC), semi-volatile organic compounds (SVOC), and other petroleum derivatives (Davis and Russell, 1993). The predominant Arctic HCs are the middle fuel distillate compounds. These contain primarily alkane, cycloalkane, aromatic and olefin components. They come in various straight chain, branched and ring structures containing between nine and twenty carbon atoms each. This group of HCs is typified by No.1 Arctic Diesel fuel, commonly used for variable load and cold temperature applications such as powering generators at remote sites (Emond, 1962). Fuel spills are generally monitored through total petroleum hydrocarbon (TPH) and BTEX (benzene, toluene, ethylene, and xylene) concentration measurements.

B. Impacts of fuel spills in Arctic ecosystems

1. Environmental considerations

In general, Arctic ecosystems are more sensitive and vulnerable to environmental change than southern counterparts. Arctic food chains are relatively simple and involve only a few organisms at each trophic level (Jensen *et al.* 1997). This creates less of a buffer in the event of food chain disruption from environmental contamination.

The soil in the Arctic contains permafrost. Permafrost is permanently frozen soil existing in continuous or broken layers within the soil matrix. It makes soils highly vulnerable to physical changes (e.g. erosion) caused by disturbance to surficial soils. Limited solar energy and water also affect Arctic soil. These combine to slow the release of minerals and organic matter resulting in reduced plant growth and increased recolonization times (Burt, 1991; Freedman and Hutchinson, 1975).

2. Toxicological and human health considerations

Toxicity to terrestrial and aquatic organisms is the primary environmental health issue concerning HC contamination in Arctic soil. The main toxic compounds involved in Arctic soil fuel spills are the aromatics: benzene, toluene, ethylbenzene, xylene, along with naphthalene and other polycyclic aromatic hydrocarbons (PAHs). BTEX compounds have been shown to be carcinogenic in certain animals and epidemiological studies have revealed hematological impacts on humans (CEPA, 1993). Several PAH compounds have been identified as having probable carcinogenic effects on humans and were shown to have neoplastic effects in aquatic organisms in laboratory tests (CEPA, 1994).

To fully assess toxicological impacts, the migration, evaporation and degradation characteristics of HC compounds in Arctic soil must be well-understood (Geotechnical Science Laboratories, 1994; Kostecki and Calabrese, 1990). An important factor is their limited solubility in water, which determines their persistence in sediments and soil matrices. For heavier HCs, this can involve decades (Kershaw and Kershaw, 1986). Specific toxicological guidelines for HC contamination in soil are not well defined and focus mainly on BTEX components. Soil cleanup criteria do exist for some HC compounds, but are currently under review for northern applications (CCME, 1991, 1997; EWG, 1997; GNWT, 1994).

The Arctic middle fuel distillates have common routes of entry into the human body: absorption, ingestion, and inhalation. High vapour concentrations can irritate the mucous membranes, lung tissue, and eyes and are therefore of greatest concern to human receptors in the proximity of fuel spills.

Once HC contaminants enter the sensitive Arctic ecosystem, they can impact environmental and human receptors alike. The aquatic system is not immune as many Arctic operations make use of, and are commonly located near, large bodies of water. These areas are host to a wide range of sea and shore life that could be impacted by HC

contamination. Arctic operations are also frequently located in close proximity to northern communities, magnifying the potential risk to human health.

C. Existing technologies for the remediation of HC contaminated soil

Numerous HC remediation technologies have been developed in recent years, however most of these are only applicable to southern climates. Remediation technologies include both physical (mechanical) and biological methods. Physical methods include i) soil washing, ii) excavation and landfilling, iii) incineration and thermal desorption, and iv) vacuum extraction, while biological methods include i) infiltration galleries and ii) biopiles and landfarming. Generally, biological processes are one half to one third the cost of physical methods (Figure I-1) (Torma, 1994). A brief overview of popular physical and biological methods are outlined with reference to their particular strengths and weaknesses.

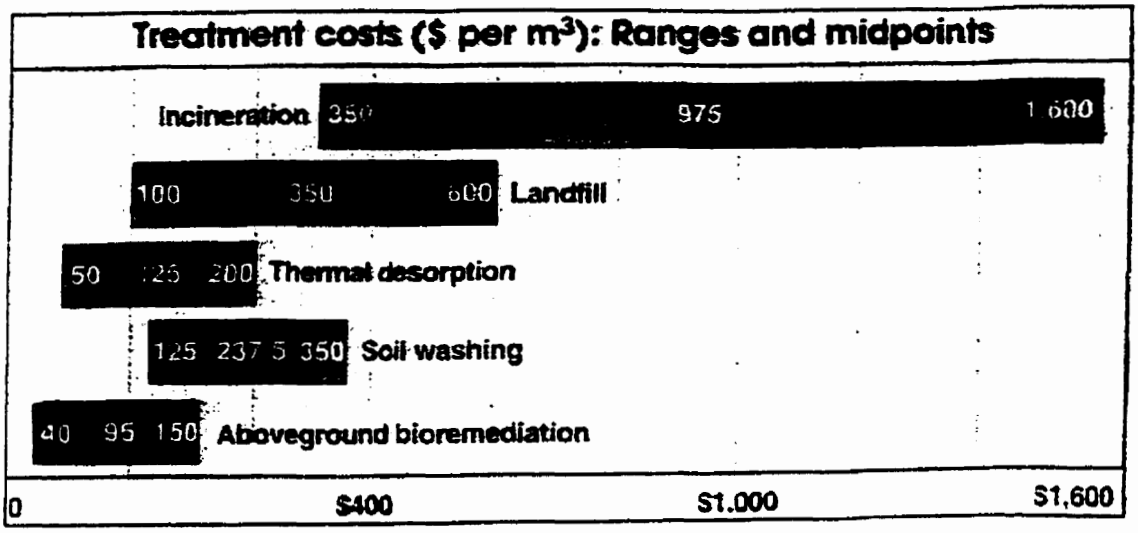


Figure I-1: Treatment costs for remediation technologies (adapted from Leahy & Brown, 1994).

1. Physical methods

i. Soil Washing

Soil washing involves an on-site set-up to scrub soil and remove HCs, which are then treated separately. Soil washing can be carried out with the aid of surfactants, emulsifiers and other additives to increase HC solubility (Kosaric, 1993). The major drawback with this technology is that abrasive additives can harm the natural microbial flora and damage the soil environment (i.e. loss of mineral cycling capacities) (Atlas and Bartha, 1993). Additional steps to remove soil additives after cleanup, non-specificity of cleaning agents, high labour requirements, and low treatment volumes may also serve to reduce efficiency and increase costs of soil washing.

ii. Excavation and Landfilling

This option involves excavating HC contaminated soil with heavy earth-moving equipment and placing it in a regulated landfill. When on-site landfilling is not feasible, soil must be containerized and shipped to a licensed landfill facility most commonly located in the southern regions of Canada. The movement of HC contaminated soil from the north to the south may require a series of air, sea and land routes. These factors plus the need for ongoing monitoring to control fugitive leachate emissions make excavating and landfilling costly and logistically difficult to implement.

iii. Incineration and Thermal Desorption

Thermal desorption and incineration use heat to volatilize and/or destroy HC contaminants. Incineration uses a closed-vessel combustion unit to completely destroy HC components at high temperature, whereas thermal desorption can be carried out *in* or *ex situ* and uses lower temperature ranges to volatilize HC components from the soil. Volatilized components are then captured and/or treated. Influent/effluent streams for both processes face varying regulatory restrictions and monitoring requirements (Kostecki and Calabrese, 1990). These factors, combined with low treatment volumes, reduce efficiency and increase costs for large-scale treatment, making incineration and/or thermal desorption inappropriate in the north.

iv. Vacuum Extraction

In vacuum extraction, a pump draws air through wells constructed above the water table within the contaminated soil. Contaminants volatilize into the vapour phase where they are then captured, treated or exhausted. This *in situ* treatment method removes the need for excavation and *ex situ* remediation. It is not feasible, however for treatment of soils with tight formations (clay), thin unsaturated zones, permafrost or the presence of oils and non-volatile components (Kostecki and Calabrese, 1990). These limitations, especially that of permafrost, make it highly difficult to implement in most northern soils.

2. Biological methods

i. Infiltration Galleries

An infiltration gallery is an *in situ* soil treatment technology that seeks to maximize microbial activity for HC degradation. It is accomplished with down-gradient groundwater pumping wells that recirculate nutrient and oxygen-amended water through the petroleum-impacted soils (Reynolds *et al.* 1998). Equipment requirements are extensive and include pumping wells, recirculating pumps and holding/mixing tanks. In addition, monitoring the extent of HC degradation within the impacted zone may prove difficult (Reynolds *et al.* 1998) as there is the need for free-flowing sub-surface water. The lack of this component makes it logistically impossible to implement in permafrost-impacted Arctic soils. Finally, the need for complex amendment mixing and circulation systems can make this technology economically infeasible for treating large volumes of soil.

ii. Biopiles and Landfarming

Biopiles and landfarms are aboveground treatment cells for the bioremediation of contaminated soil. They can be coupled with biostimulation (addition of nutrients) and/or bioaugmentation (inoculation with microbes). Biopiles involve placing soil in mounds or windrows to promote higher temperatures. For landfarming, soil is excavated, spread thinly (15-30 cm) over a large area to ensure adequate aeration and periodically tilled.

The amount of equipment required depends on the degree of process control required. Regulatory guidelines for VOC emissions may require that off-gases from the treatment cells be captured and treated. Biopiles and/or landfarms can be used for all soil types and can treat large volumes of soil efficiently and economically.

D. Arctic design considerations for the selection of remediation technologies

Arctic conditions present many engineering and design challenges. The fragile Arctic soil environment with permafrost and limited vegetation dictates that mechanical remediation technologies are unfavourable relative to technologies that enhance natural remediation processes (Mackay *et al.* 1980). In addition, the nature of the rugged Arctic landscape poses several complicating factors for the implementation of remediation technologies. For example, transport to most sites is limited to air or sea, and many sea approaches are hindered by pack ice for much of the year, limiting access to heavy equipment and personnel. Technologies requiring large amounts of heavy equipment and specialized treatment apparatus therefore raise treatment costs due to the high cost of shipping. Similarly, shipping contaminated soil or secondary contaminated waste streams off-site incurs high costs. Limited seasonal availability of transport for equipment and personnel underlines the need for technologies that can provide: 1) high degradation rates, and 2) short treatment seasons. Technologies that can be left in place during the winter season with minimal maintenance and supervision are also desirable.

The soil remediation technology selected therefore had to be cost effective, adaptable to harsh and remote conditions, meet regulatory standards, and require minimal supervision and maintenance. Applying these criteria, it became clear that many of the available technologies were not technically or economically feasible. The single technology that satisfied all the requirements was bioremediation with the use of biopiles and landfarming.

E. Bioremediation with biopile and landfarming approaches

1. Bioremediation principles

Both biopiles and landfarming aim to promote the degradation of HC contaminants through bioremediation. Bioremediation is also referred to as biodegradation and can be defined as the use of microorganisms to degrade organic contaminants (Torma, 1994). Bioremediation was first observed in 1895 by Miyoshi (Kosaric, 1993). It occurs naturally in soil ecosystems but generally at rates too low for industrial applications (Torma, 1994). Key bioremediation parameters include temperature, moisture, and nutrient and oxygen supply. Bioremediation technologies seek to maximize microbial metabolism through the optimization of these parameters.

In bioremediation, microorganisms (bacteria, fungi, yeast or microbial extracts) combined with electron acceptors, break down complex chains of HC into smaller chains. For the biodegradation of simple, linear alkanes, HC chains are broken down and converted into alcohol, aldehyde and carboxylic acid fractions through a series of β-oxidations cleaving two carbon atoms from the end of the HC chain at each step. Metabolites are then biochemically transformed and recycled through the environment. The ultimate goal of bioremediation is the complete transformation of toxic contaminants into harmless metabolite by-products such as CO_2, biomass and H_2O (Lei *et al.* 1994). This process is referred to as mineralization.

The effective bioremediation of HC contaminated Arctic soils depends on various physico-chemical characteristics of the soil such as permeability, porosity, grain size and clay/gravel content, pH, and moisture content. Optimum values for these and other characteristics are a pH of 6 to 8, a sand-based/granular soil matrix (more easily remediated than clay-based soil), the absence of competitive carbon sources and oxygen depleting elements, compatible redox and cation exchange potentials, presence of required trace metals, and adequate organic content for proper nutrient cycling (Young

and Cerniglia, 1995). These criteria are likewise essential to the operation of a successful biopile or landfarm system.

i. Microbes

A wide variety of naturally occurring bacteria and fungi contribute to bioremediation processes (Table I-2). Low microbial counts for specific contaminant-degrading consortia can be augmented with selectively enriched inocula. Additional microbes can be inoculated into the soil to supplement indigenous populations. This is especially effective when certain highly toxic and persistent compounds are being targeted (Crawford and Mohn, 1985). Bioaugmentation also serves to significantly decrease the lag phase of the degradation process (ESG, 1999). Inoculating with a known microbial mixture can help to avoid undesirable reaction pathways such as the creation of harmful metabolites (Young and Cerniglia, 1995).

Table I-2: Microorganisms involved in the bioremediation of organic wastes (adapted from Savage *et al.* 1995).

Waste Description	Microorganisms
Crude Oils	*Brevibacterium sp., Flavobacterium sp., Norcadia, Pseudomonas, Flavobacter, Vibrio, Achromobacter*
Hexadecane	*Acinobacter sp., Candida petrophilium, Pseudomonas aeruginosa, Trichosporon pullulans*
Paraffins	*Trichosporon pullulans*
Jet Fuels	*Cladosporium, Hormodendrum*
Napthalene	*Pseudomonas sp.*
Benzene	*Pseudomonas putida*
Kerosene	*Torulopsis, Candida tropicalis, Corynebacterium hydrocarbonclastus*

The effectiveness of inoculating soil with selected microbes is still unproven as many of the biotic and abiotic parameters affecting mineralization are still not known. Furthermore, there is very little information available regarding the 'mixture of microbes' (consortia) required to optimize the mineralization of HCs, especially in Arctic soil. Due to a lack of characterization of the soil environment and the indigenous microbial

communities present, it is also unclear to what extent Arctic microbial consortia resemble those of temperate regions.

Research results for southern soils indicate that, as long as total aerobic heterotrophic and hydrocarbon degrading microorganisms are greater than 10^6 and 10^4 colony forming units per gram, respectively, indigenous populations should be capable of degrading the organic contaminants present (Lei *et al.* 1994). Southern surficial soils with adequate oxygen and nutrient supply typically contain 10^7 to 10^9 microorganisms per gram with approximately 0.1 to 1% of those having petroleum-degrading capabilities (Kostecki and Calabrese, 1990). Arctic soils have much lower organic contents and, as such, it is expected that they also have lower microbial populations and activities.

Low average temperatures in the Arctic have serious ramifications for microbial biodegradation and its rates (Atlas, 1986); the colder the temperature, the lower the activity of microorganisms. Only microbial communities able to remain biochemically active over a wide temperature range will be effective in the bioremediation of contaminated soils in the Arctic. There is conclusive evidence of increased microbial growth in the presence of HC substrates and proof that Arctic microbial populations can bioremediate HC fuel components at low temperatures (0° to 10° C). This bioremediation is dominated by indigenous, psychrophilic (i.e. able to thrive in cold temperatures) species (ESG, 1998; Sextone *et al.* 1978). Similar investigations showed that indigenous flora were capable of degrading oil under both psychrophilic and mesophilic conditions (Cook and Westlake, 1974; Hutchinson *et al.* 1994). Cook and Westlake (1974) developed the concept that mixed versus pure microbial populations were more effective for use in fuel biodegradation applications.

The efficiency of microbial processes also depends on the type of substrate being degraded. The n-alkane (Kershaw and Kershaw, 1986) and aromatic mono-, di-, tri-ring compounds common in Arctic fuels are all considered to be degradable by microbes (Cook and Westlake, 1974). Significantly lower rates of biodegradation can be expected in the longer and more complex heavy-end compounds prevalent in lube oils and heavier

fuel blends. This is supported by the findings of Cook & Westlake (1974) demonstrating decreased microbial utilization of the isoprenoid saturate fraction (e.g. phytane, pristane) under psychrophilic conditions at Norman Wells, N.W.T. Light-end HC compounds (e.g. alkanes) are more soluble and can therefore sustain higher cell yields (Cook and Westlake, 1974). They are also biodegraded to lower residual levels over a fixed period of time when compared with heavier compounds (Kostecki and Calabrese, 1990).

ii. Nutrients

Carbon, nitrogen and phosphorus (C,N,P) are the primary nutrients required for biosynthesis and cell growth of microbes involved in bioremediation processes. Studies of contaminant degradation in the Arctic have shown that normal indigenous flora have the capability to degrade HC contaminants more rapidly when supplemented with nutrients in the form of fertilizers (Cook and Westlake, 1973, 1974; Hutchinson *et al.* 1994; Mohn, 1998; Sextone *et al.* 1978). Research into crude oil degradation in the Arctic environment supports the hypothesis that nutrient addition is the most effective way to stimulate HC breakdown (Hutchinson *et al.* 1994). Similar investigations demonstrated that urea-phosphate fertilizer application, with or without inoculum, resulted in statistically significant increases in microbial populations and augmented rates of substrate utilization (Cook and Westlake, 1974). Another field-scale investigation supporting these hypotheses took place during the remediation of the 1989 Exxon Valdez oil spill (Flathman *et al.* 1994). This was also the first successful demonstration of oleophilic fertilizers specifically tailored to the cleanup of HC contaminants.

To fully understand biostimulation effects, particular nutrient interactions within the soil environment must be considered. Accidental discharges of fuel HC may upset the C:N:P nutrient balance in the soil. For efficient growth, most bacteria require 100 parts carbon to 10-13 parts nitrogen and 3 parts phosphorous (von Fahnestock *et al.* 1998). A C:N:P ratio of 100:10:3 is generally considered as the basic nutrient profile required for biopile systems (von Fahnestock *et al.* 1998). At higher ratios, microbes are unable to effectively reproduce. Excessive ratios can also hinder microbial metabolic mechanisms for the

consumption of HC substrates resulting in longer degradation cycles. If the carbon to nutrient ratio is low, excess nitrogen is converted into ammonia (NH_3), upsetting the pH balance and the microbial community. Ammonia is known to be toxic to microorganisms at 300 ppm (Kostecki and Calabrese, 1990). Slow-release agricultural fertilizers have been used to circumvent such toxicity effects by controlling nutrient release over the treatment period (Flathman *et al.* 1994). For phosphorous, a particular concern is its low solubility (and therefore low bioavailability), especially below pH 5.5 and above pH 7.0 (Kostecki and Calabrese, 1990). Phosphorous can also become fixed via precipitation in calcarious soils (Cookson, 1995). Precipitation of nutrients and the resulting low bioavailability are common problems in the bioremediation of HC contaminated soils.

Unfortunately, due to the complexity and heterogeneity of the soil environment, it is difficult to make universal statements regarding the effectiveness of fertilizers on HC degradation rates. Bioremediation with fertilizers can be rendered ineffective by inadequate delivery and distribution of nutrients. Indiscriminate use of inorganic nutrients to stimulate biodegradation may also serve to increase costs and decrease removal rates through the inhibition of microbial processes. Nutrient applications must therefore be matched to contaminant, microbial, and site characteristics through biotreatability assessments of the physico-chemical soil environment (Flathman *et al.* 1994).

iii. Surfactants

The addition of surfactant soil amendments plays a key role in the bioavailability of substrate and nutrient components and optimizes aqueous phase interactions (Finnerty, 1994). By definition, surfactants are any usable and isolatable compound that has some influence on interfaces (Kosaric, 1993) and include compounds that act as emulsifiers and dispersing agents. The ability to overcome physical forces present at air-water, oil-water, and solid-liquid interfaces are the primary qualities of surfactants (Kosaric, 1993).

Many organic compounds are highly insoluble and HC-degrading microorganisms are forced to develop cellular mechanisms to increase their solubility and promote their bioavailability and uptake. As a result, surfactants are commonly found in biological systems where microorganisms are grown on insoluble substrates (Thangamani and Shreve, 1994). Using this knowledge, naturally occurring agents (biosurfactants) and their synthetic counterparts (synthetic surfactants) can be isolated and applied to bioremediation processes.

In soil remediation applications, synthetic and natural surfactants are both commonly used for the cleanup of oil spills, soil contamination and for *in situ* "pump and treat" processes (Kosaric, 1993). Surfactants help to displace pollutants which are adsorbed to the soil (or aquifer) matrix or formed into discrete organic phase mixtures (Non Aqueous Phase Liquids) (Thangamani and Shreve, 1994). The synergistic interactions of surfactants with nutrients and other soil additives in soil are not well documented.

2. Biopiles

The use of biopiles for the bioremediation of HC contaminated soil provides the ideal platform for the optimization of the parameters described above. Biopiles promote greater concentrations of bacteria capable of increasing the rate of bioremediation. They also increase the absorption and retention of radiative solar heat and soil moisture.

The use of engineered biopiles is a modification of standard composting (soil piling) and soil spreading practices (von Fahnestock *et al.* 1998). An engineered aboveground biopile features augmented systems for the control of soil remediation parameters to maximize aerobic microbial metabolism (Davis and Russell, 1993). These systems include aeration, moisture and nutrient addition and leachate collection systems. Leachate collection and recirculation are carried out in lined treatment cells and may make use of graded surfaces to direct leachate flow (Reynolds *et al.* 1998; Flathman *et al.* 1994). Bulking agents can also be added to increase soil porosity and promote better oxygen flow within the pile (Davis and Russell, 1993; Savage *et al.* 1985). Engineered biopiles make maximum use

of limited space and provide the highest degree of control over emissions. A cross-sectional view of a fully engineered biopile is shown in Figure I-2. This set-up may appear elaborate, but costs are comparable to other common solid-phase treatments (Figure I-1).

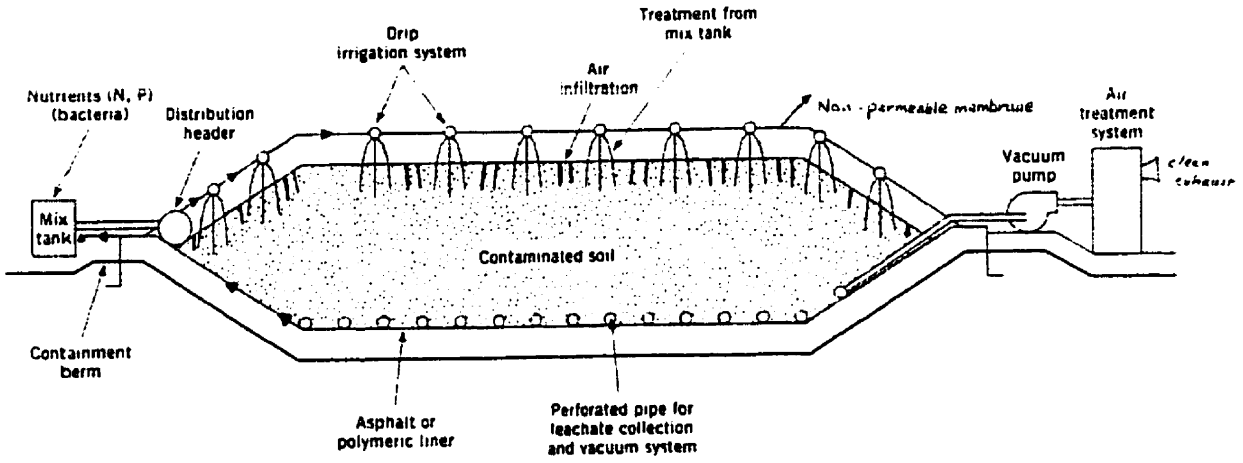


Figure I-2: Schematic of an engineered biopile (adapted from Stoner, 1995).

In biopile construction, the contaminated soil is excavated and heaped within a lined treatment area. The pile is covered with a suitable non-permeable barrier. Water, air, heat and nutrients can be introduced through perforated piping systems in the piles. Biopiles can be coupled with soil vapour extraction and/or biofiltration systems for the capture and/or treatment of VOCs.

Several strategies can be used to monitor the effectiveness of biopiles in HC bioremediation. TPH is the most common measurement of contaminant levels in soil remediation processes as it measures a wide range of compounds present in common industrial HCs. In this thesis, two methods were used to monitor and model the degradation of HCs in Arctic soils. At the field-scale, degradation was monitored using TPH analysis with Gas Chromatography (GC). For the purposes of the microcosm

laboratory experiments and the experiment investigating the comparative degradation of ^{14}C-labelled HC and TPH components, radiolabel (^{14}C) monitoring was employed.

F. Past research into biopile bioremediation

Research has been ongoing at the Environmental Sciences Group (ESG) since 1996 into the effectiveness of aboveground soil treatment systems involving biopiles.

1. Findings to date

Preliminary laboratory experiments, using Arctic soils from former Distant Early Warning Line sites, (Allen and Riddell, 1997) were used to look at small-scale biostimulation and bulking effects. Results of this research suggested that supplementing soils with ammonium phosphate fertilizers and bulking agents, as well as increasing soil temperature, could increase rates of TPH degradation. It also revealed difficulties with monitoring TPH degradation in highly weathered soils.

Past fieldwork (ESG, 1998) demonstrated that biopiles can successfully overcome Arctic bioremediation limitations and effectively remove TPH. This research was carried out in small-scale biopiles (0.25 m^3) on Arctic soil at a former DEW Line site. The same study showed that low N and P levels in the soil limited TPH biodegradation. When supplemented with ammonium-phosphate fertilizer and inoculum (mixed culture of Arctic soil microbes approx. 100 cfu/g), TPH degradation was significantly increased. In less than two months, 80% of the TPH in supplemented soils was degraded compared with less than 10% in unsupplemented biopiles over the same time period. Bulking biopiles with peat appeared to have no effect on degradation rates (ESG, 1998).

In laboratory scale microcosm experiments using Arctic soil from other Distant Early Warning Line sites, investigators examined the effects of: (1) TPH concentration, (2) fertilizers and surfactants (type and concentration), (3) inoculum levels and (4) incubation temperatures on the degradation of several radiolabelled hydrocarbons (ESG, 1999). The

results of these experiments are presented graphically in Appendix A. Briefly, initial TPH levels (100 000 ppm) in the soil had significantly different impacts on the final rate and extent of radiolabelled-HC removal in the various soils tested. This indicated that varying toxicity tolerances existed between the microbial populations at different sites. The impact of surfactant and fertilizer supplements varied depending on soil type and target HC. It was suggested that high fertilizer concentrations (urea + diammonium phosphate) applied to soils with high TPH concentrations were responsible for poor fertilizer performance and that further experiments should be undertaken to optimize application ratios.

Laboratory inoculation results were similar to field results, demonstrating that supplementing the soil with concentrates of indigenous microbes significantly increased the rate and efficiency of HC degradation. Cryoprotectant is a growth substrate of skim milk and honey used as an easily metabolized nutrient source for microbial inoculation. The addition of a cryoprotectant decreased degradative efficiency. It was therefore suggested that optimization of inoculum concentration and the impacts of cryoprotectant on HC degradation be further investigated. Increasing the temperature from 7° to 15° C significantly increased the rate and extent of radiolabelled-HC mineralization in the microcosms (ESG, 1999).

2. Foundation for further study

In summary, past research at the field and laboratory scale and a review of relevant literature indicated that the following parameters required further study: 1) the optimization of soil additives by type and concentration in different soils, 2) inoculation effects, 3) a laboratory microcosm comparison of TPH degradation versus radiolabelled-HC mineralization to allow for the extrapolation of radiolabelled HC data to field conditions, 4) the evaluation of physical and biological degradation mechanisms in field-scale treatments, 5) an investigation of the impacts of aeration and heating on TPH degradation in field-scale, 6) an evaluation of engineering and experimental design

elements and finally, 7) statistical considerations for meaningful and effective data collection.

This thesis was designed to address many of these questions. Its prime objective is to employ a holistic research approach to identify and optimize key bioremediation parameters and experimental design elements for the development of full-scale Arctic soil treatment technologies. Through the identification and optimization of these key parameters prior to large-scale treatment, costly process design flaws should be avoidable.

II. MATERIALS

A. Soil Sources

Soil used in field and laboratory-scale experiments was collected from two sources, FOX-M and CFS Alert. These sites are located in distinctly different Arctic ecosystems and, as such, possess different soil characteristics. The experimental sites both represent typical large-scale fuel spill scenarios and are slated for cleanup as legitimate contaminated sites.

1. FOX-M soil

FOX-M is a North Warning System (NWS) Logistics Support Site and is one of eleven long range radar installations defending Canada's Arctic Coast along the former Distant Early Warning (DEW) Line. It is situated near the community of Hall Beach, on the eastern side of the Melville Peninsula in the Foxe Basin, Nunavut (68°45'34''N, 81°11'41''W) (Map II-1). The site is operational year round with ample support personnel. A pipeline rupture in 1997 at FOX-M released 30 000 L of diesel fuel into the surrounding soil which is predominantly gravel. After the spill was discovered, the contaminated soil was excavated and divided into six stockpiles (approximately 40 m

long x 5 m wide x 2 m high) by site contractor staff. At the request of the NWS Environmental Office, ESG agreed to investigate the effectiveness of biopiles in treating the contaminated soil.

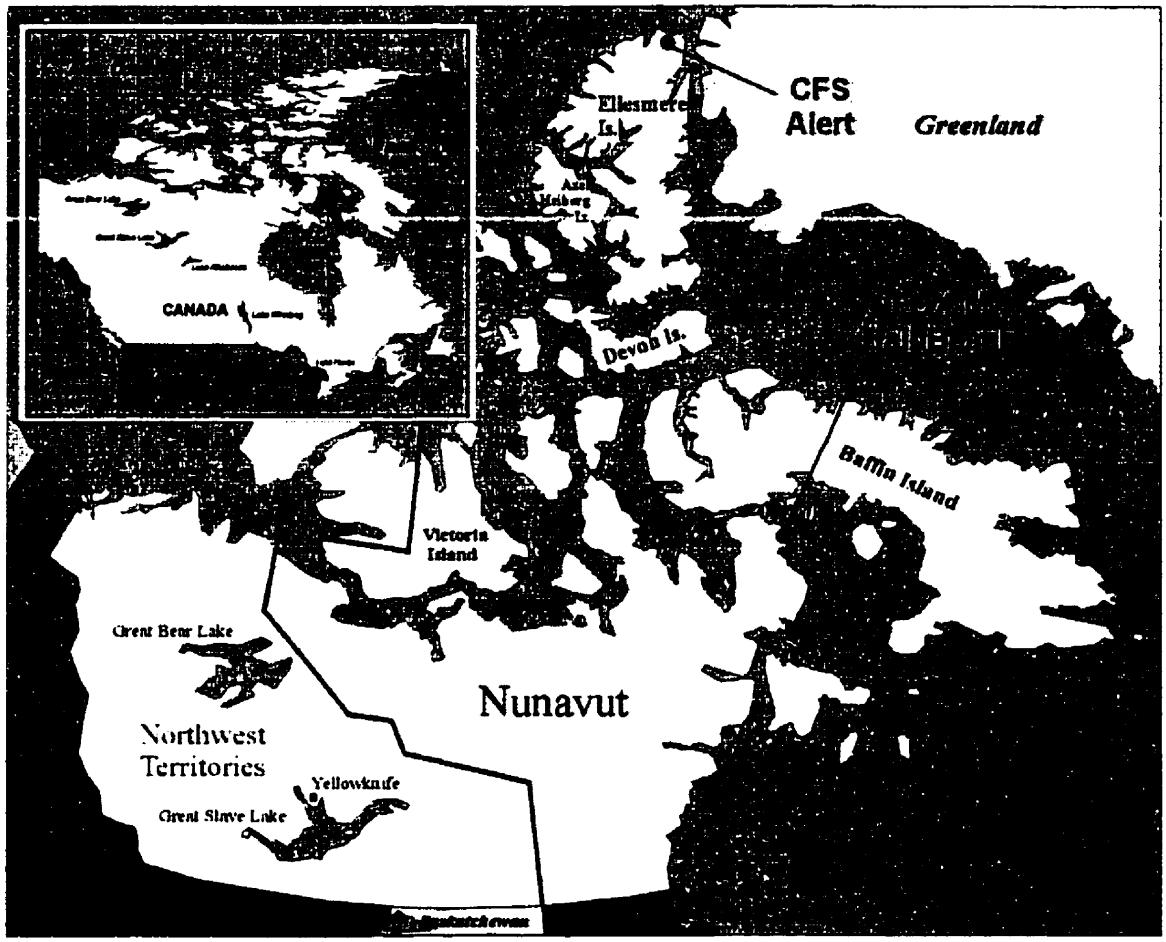


Figure II-1: Map showing the location of FOX-M and CFS Alert.

2. CFS Alert soil

Established in 1950 as a Department of National Defence (DND) communications base, CFS Alert lies at the northeastern tip of Ellesmere Island at 82°30'N, 62°19'W (Map II-1). Due to its extreme northerly location, the summer season is drastically shorter than that encountered at Hall Beach. The HC contamination is in soil with a high clay content near the former power house site. This site lies within a drainage pathway from the station and, as a result, the soil is highly saturated with water.

B. Chemicals and soil additives

Both commercial and bioremediation specific fertilizers and a synthetic surfactant were evaluated in field and laboratory-scale experiments. The commercial fertilizers used were urea (46% N - granular) and diammonium phosphate (18% N, 46% P_2O_5) obtained from Agrico Fertilizer Co. Canada. The bioremediation-specific fertilizer used was Inipol EA 22™ (Elf Aquitaine Inc. Atrix, France), selected after a literature search and technical evaluation of field trial performances in the Exxon Valdez shoreline cleanup (Flathman *et al.* 1994).

Surfactant treatments used Biosolve™, supplied by Biosolve Inc. Canada. Biosolve™, a synthetic surfactant and emulsifying agent, has the ability to improve the bioavailability of TPH to the relevant microorganisms. It was chosen as a representative surfactant agent based on its advertised suitability to soil remediation.

Gro Brix™ (Gro Brix Dist. Co., Mississauga, ON), a cocoa fibre bulking agent, was used to increase airflow and help separate clay particles in Alert soil. This was done to overcome the disparity in soil porosity between the clay soil at Alert and the gravel soil at FOX-M.

The radiolabelled compound used in laboratory microcosm experiments was ^{14}C-labelled n-dodecane (100 μCi) obtained from Sigma-Aldrich Ltd., Canada while a Jet A-1 fuel solvent was procured from Esso Fuel Test Laboratories (Burlington, ON) for the radiolabel addition.

The inoculum for the Alert microcosm bioaugmentation experiments was enriched from indigenous soil microbes collected from the CFS Alert petroleum, oil and lubricant (POL) site by members of ESG and prepared by the University of British Columbia Biotechnology Lab Fermentation Pilot Plant. Cell suspensions were flash frozen in dry ice and ethanol, dried under vacuum, and introduced as rehydrated, lyophilized cultures.

III. METHODS

A. Laboratory experiments

Soil microcosms were used to study HC bioremediation under controlled conditions in a laboratory. Bulk soil samples were collected from the zone of high TPH contamination at FOX-M and Alert, frozen and shipped south. The initial microcosm experiments were initiated within four months of soil collection.

The reproducibility of the experimental protocol was evaluated by comparison with data from a previous experiment using soil from a different source amended with 0.417 g NH_4Cl, 0.044 g NaH_2PO_4 and 0.070 g Na_2HPO_4 in a buffer solution (ESG, 1998). For comparison, an identical experiment was incubated in the laboratory at RMC at 7° C for 35 days producing the anticipated sigmoidal growth response. Once the reproducibility of the protocol was confirmed, microcosm experiments were initiated and monitored in parallel with abiotic and biotic controls.

Radiolabelled-HC mineralization experiments were carried out using 3 g dry weight of soil (equivalent to 3.22 g and 3.59 g for FOX-M and Alert soil, respectively) in each microcosm at 7° C to monitor the impact of; 1) soil additives (commercial fertilizer, bioremediation specific fertilizer and synthetic surfactant), and 2) inocula (enriched indigenous microbes) under varying concentrations. Experiments examined mineralization responses at varying concentrations. Concentrations were selected based on industry recommended levels for each additive. Past research indicated that these industry recommended, or 'standard', levels were inhibitory to mineralization in Arctic soil. As a result, half and quarter concentrations were examined to evaluate mineralization benefits at lower levels. A comparison of TPH degradation vs. ^{14}C-labelled HC mineralization was also carried out.

1. Preparation of soils

Soils were sieved using a 4.75 mm sieve (US #4), and then manually homogenized in a bucket prior to being placed in the microcosms. The water holding capacity (WHC) of the soil was determined gravimetrically by comparing natural, saturated, and dry soil weights. Soil water content was adjusted to 60% moisture (ESG, 1998) according to the individual WHC of each soil, with the addition of 162 μL and 64 μL of water for FOX-M and Alert soils, respectively.

Grain size analysis, in the form of grain size curves (Appendix B), were used to identify the percentage of silt, sand and gravel in each soil. Grain size analysis was carried out by the Department of Civil Engineering at Queen's University (Kingston, ON).

Nutrient analysis (TKN and extractable P) was used to identify background nutrient levels present in both soil environments and was carried out at the Analytical Services Unit (ASU), Queen's University. The significance of the background soil nutrient levels was evaluated by calculating the related C:N:P ratios. These ratios were then compared to the optimal HC bioremediation ratio of 100:13:3 (von Fahnestock *et al.* 1998). The C component for these ratios was determined by taking 80% of the highest average TPH level at each site. These levels were taken from the time zero TPH extraction for the microcosm TPH vs. ^{14}C removal experiment. The resulting ratios do not account for HC components in the radiolabel or diesel solvent and are therefore only useful for quantifying nutrient levels in unlabelled soil.

2. Microcosm design

Triplicate microcosms for each treatment were prepared in 120 mL Qorpak™ glass vials with Teflon™ lined screw caps.

Experiments were carried out using ^{14}C-labelled n-dodecane as a marker. Past research indicated that n-dodecane was the best model HC for comparative studies of TPH

degradation in soil (ESG, 1998). Prior to the addition of the ^{14}C-labelled n-dodecane, selected controls were autoclaved in order to demonstrate that the mineralization of the ^{14}C-labelled n-dodecane was a biological activity. This was done in Qorpak vials with the screw caps slightly open. Where specified, ^{14}C-labelled n-dodecane was added directly to the Qorpak vials. Specifically, 0.45 μCi label was added to each microcosm in 0.89 mL of jet fuel to give 1000 ppm in 3.0 g dry weight of soil. Soil additives for each treatment were injected directly into the vials. The vials were then rolled and gently shaken to distribute the label and additives over the soil. All microcosm vials were sealed and incubated at 7° C. Every 2-5 days, vials were opened to allow for adequate air exchange. All microcosm manipulations were carried out within an ice bath.

3. Mineralization monitoring

Bioremediation was monitored through the evolution of radiolabelled-CO_2 resulting from the microbial mineralization of radiolabelled n-dodecane in the soil. In order to capture the radiolabelled-CO_2 evolved, small tubes containing 0.5 ml of 0.5M NaOH were placed in the microcosm vials. The NaOH solution acted as a CO_2 trap and allowed for the measurement of CO_2 evolved by the following reaction pathway:

$$NaOH + \tfrac{1}{2} CO_2 \rightarrow \tfrac{1}{2} Na_2CO_3 + \tfrac{1}{2} H_2O$$

The trapped, radiolabelled-CO_2 contained in the NaOH solution was pipetted out for mineralization monitoring. The solution was then placed in a scintillation vial and measured by liquid scintillation count (LSC) every two to five days according to a set sampling schedule.

4. Liquid scintillation counting procedure for mineralization monitoring

The scintillation vials, containing approximately 0.5 mL of NaOH plus radiolabelled-CO_2, were sent to the RMC Analytical Services Group (ASG) Laboratory (Kingston, ON) for LSC where 16 mL of Packard Ultima Gold™ Scintillation Cocktail (Fisher Scientific, Canada) was added to each vial. Samples were counted for 15 minutes each on a Packard

Tri-Carb™ 2500TR Liquid Scintillation Analyzer over the 0-2000 keV energy range. A background sample was prepared using 20 mL of the cocktail. The background count was then subtracted from each sample reading. A printed report of the Becquerels (Bq) counted and 2 Sigma percent error was produced for each sample.

5. Experimental Variables

Commercial fertilizer experiments were carried out using urea and diammonium phosphate (DAP) amendments according to recommended concentrations calculated from literature ratio equations (von Fahnestock *et al.* 1998). The fertilizer additions were carried out at standard (i.e. recommended level), half and quarter concentration levels according to the specific nutrient requirements for each soil (Table III-1).

Table III-1: Commercial fertilizer experimental variables.

Soil	Treatment	Amendment
FOX-M	Std. conc.	1.95 mg urea and 0.91 mg DAP
FOX-M	½ conc.	0.97 mg urea and 0.46 mg DAP
FOX-M	¼ conc.	0.49 mg urea and 0.23 mg DAP
Alert	Std. conc.	7.36 mg urea and 3.46 mg DAP
Alert	½ conc.	3.68 mg urea and 1.73 mg DAP
Alert	¼ conc.	1.84 mg urea and 0.86 mg DAP

Bioremediation-specific fertilizer experiments were undertaken using Inipol EA 22™ at varying concentrations. Microcosm additions were based on the recommended 10% wt. application ratio (Lynn, 1998) and average initial TPH concentrations of 3050 ppm and 11 520 ppm for FOX-M and Alert soils, respectively. The fertilizer additions were carried out at standard, half and quarter concentration levels based on the initial TPH levels in each soil (Table III-2).

Table III-2: Bioremediation specific fertilizer experimental variables.

Soil	Treatment	Amendment
FOX-M	Std. conc.	0.915 mg Inipol™
FOX-M	½ conc.	0.457 mg Inipol™
FOX-M	¼ conc.	0.228 mg Inipol™
Alert	Std. conc.	3.456 mg Inipol™
Alert	½ conc.	1.728 mg Inipol™
Alert	¼ conc.	0.864 mg Inipol™

Surfactant experiments were undertaken using Biosolve™ at varying concentrations based on the recommended application ratio of 27 ft^2 contaminated soil per gallon of Biosolve™ concentrate (applied in 3% solution) (Yick, 1998). Surfactant additions were carried out at standard, half and quarter concentration levels (Table III-3). All surfactant experiments were amended with the appropriate amount of commercial fertilizer to ensure adequate nutrient supply.

Table III-3: Surfactant experimental variables.

Soil	Treatment	Amendment
FOX-M	Std. conc.	18 µL Biosolve™, 1.95 mg urea and 0.91 mg DAP
FOX-M	½ conc.	9 µL Biosolve™, 1.95 mg urea and 0.91 mg DAP
FOX-M	¼ conc.	4.5 µL Biosolve™, 1.95 mg urea and 0.91 mg DAP
Alert	Std. conc.	18 µL Biosolve™, 7.36 mg urea and 3.46 mg DAP
Alert	½ conc.	9 µL Biosolve™, 7.36 mg urea and 3.46 mg DAP
Alert	¼ conc.	4.5 µL Biosolve™, 7.36 mg urea and 3.46 mg DAP

Experiments were carried out on Alert soils to complement previously obtained microcosm inoculation data for FOX-M soil (ESG, 1998) by inoculating them with enrichment cultures of indigenous microbes. Cultures used in the experiments were rehydrated with 0.75 mL of cold distilled water and mixed by vortexing. Three serial dilutions of rehydrated lyophilized cultures in cryoprotectant growth medium were used

(Table III-4). The approximate microbial concentration in the undiluted treatment was 10^9 cells per gram of dry weight soil measured by protein assay. The various dilutions were carried out with cold water, mixed and used immediately to inoculate the soil. A separate experiment examined the impact of growth medium alone and competitive substrate effects. All treatments were amended with the standard concentration of commercial fertilizer to ensure adequate nutrient supply.

Table III-4: Inoculum dilution experiments.

Soil	Treatment	Amendment
Alert	Std. inoc. conc.	56 µL undiluted culture, 7.36 mg urea and 3.46 mg DAP
Alert	1/100 inoc. dilution.	56 µL of 1/100 diluted culture, 7.36 mg urea and 3.46 mg DAP
Alert	1/10 000 inoc. dilution	56 µL of 1/10 000 diluted culture, 7.36 mg urea and 3.46 mg DAP

6. TPH versus ^{14}C removal

Microcosm experiments were carried out to compare TPH degradation profiles to the radiolabelled-HC mineralization data. TPH degradation was monitored in both soil types in parallel with radiolabelled-CO_2 evolution for an 8-week period. Triplicate TPH microcosms (with no radiolabel added) were extracted and analyzed at 1, 2, 4, and 8 weeks and compared with mineralization data over the same period. All treatments included the addition of standard concentrations of commercial fertilizer to ensure adequate nutrient supply.

The soil was analyzed by the ASG using gas chromatography (GC) after *in vitro* extraction. For extraction in the Qorpak vials, 3 g dry weight of soil and 3.5 g anhydrous

Na_2SO_4 were shaken until the mixture flowed freely. Hexane (7.5 mL) was added to each tube and the tubes were shaken vigorously for five minutes at room temperature, left to settle and shaken once again. Portions of the supernatant were then pipetted, passed through a silica column and placed in GC vials for analysis. TPH analysis by GC was carried out at ASG RMC using a HP6890 GC system equipped with a Supelco capillary column (No. 2-4028 SPB-1), airflow of 400 mL/min, carrier flow of 2 mL/min and no internal standard.

B. Field-scale research

Small and medium-scale biopiles and landfarms were constructed to evaluate degradation mechanisms, soil additives and aeration/heating systems (Table III-5). Experiments also aimed to identify the challenges of field-scale experimental design and statistical considerations for data collection. These experiments examined the change in TPH concentration over the 1998 summer treatment season.

Table III-5: Field-scale experimental treatments.

Scale	Site	Pile	Treatment
Small	FOX-M	A & B	Covered control
Small	FOX-M	C & D	Uncovered control
Small	FOX-M	E & F	Covered. 4 kg urea and 2 kg DAP
Small	FOX-M	G & H	Covered. 4 kg urea, 2 kg DAP and 400 mL Biosolve™ concentrate (in 3% solution)
Medium	FOX-M	I	Control (no aeration). 32 kg urea, 16 kg of DAP
Medium	FOX-M	J	Passive Aeration. 32 kg urea, 16 kg of DAP
Medium	FOX-M	K	Active Aeration. 32 kg of urea and 16 kg of DAP
Medium	Alert	AA	Control (no aeration). 100 kg urea, 50 kg of DAP, GroBrix™ bulking agent (@ 10% vol. addition)
Medium	Alert	BB	Passive Aeration. 100 kg urea, 50 kg of DAP, GroBrix™ bulking agent (@ 10% vol. addition)

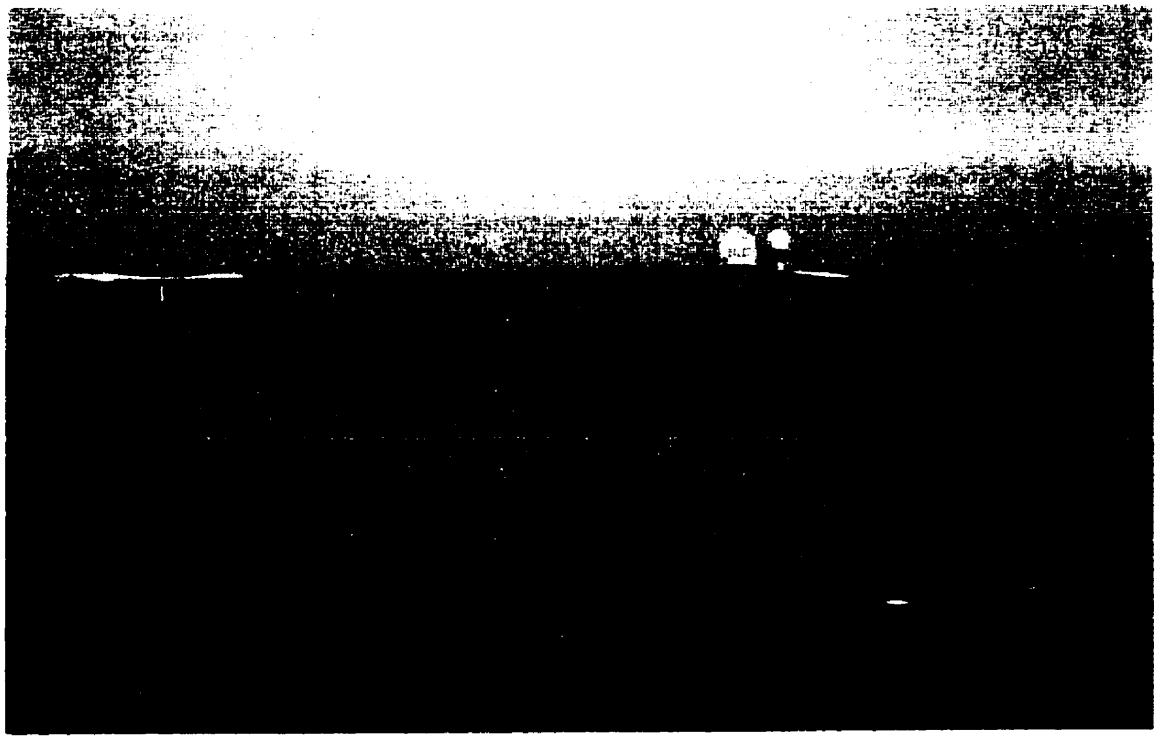
Medium	Alert	CC	Active Aeration. 100 kg of urea and 50 kg of DAP. GroBrix™ bulking agent (@ 10% vol. addition)
Landfarm	FOX-M	L & M	Uncovered Control
Landfarm	FOX-M	N & O	Uncovered. 4kg urea and 2kg DAP
Landfarm	FOX-M	P	Uncovered. 4kg urea, 2kg DAP and 400 mL Biosolve™ concentrate (in 3% solution)
Landfarm	FOX-M	Q	Covered. 4kg urea, and 2kg DAP
Landfarm	FOX-M	R	Uncovered. 400 mL Biosolve™ concentrate (in 3% solution)
Landfarm	FOX-M	S	Covered Control

1. Soil preparation

Soil for the field experiments was chosen based on TPH levels. Average TPH concentrations were determined by GC analysis at ASG to identify the zone of highest contamination at each site.

At FOX-M, the zone of highest TPH concentration within the six stockpiles (Photograph III-1) was determined. The average TPH concentration of the soil selected was 3600 ppm. Soil from the selected zone was removed and placed in a secondary pile which was homogenized with a front-end loader and used as the soil source for the field experiments.

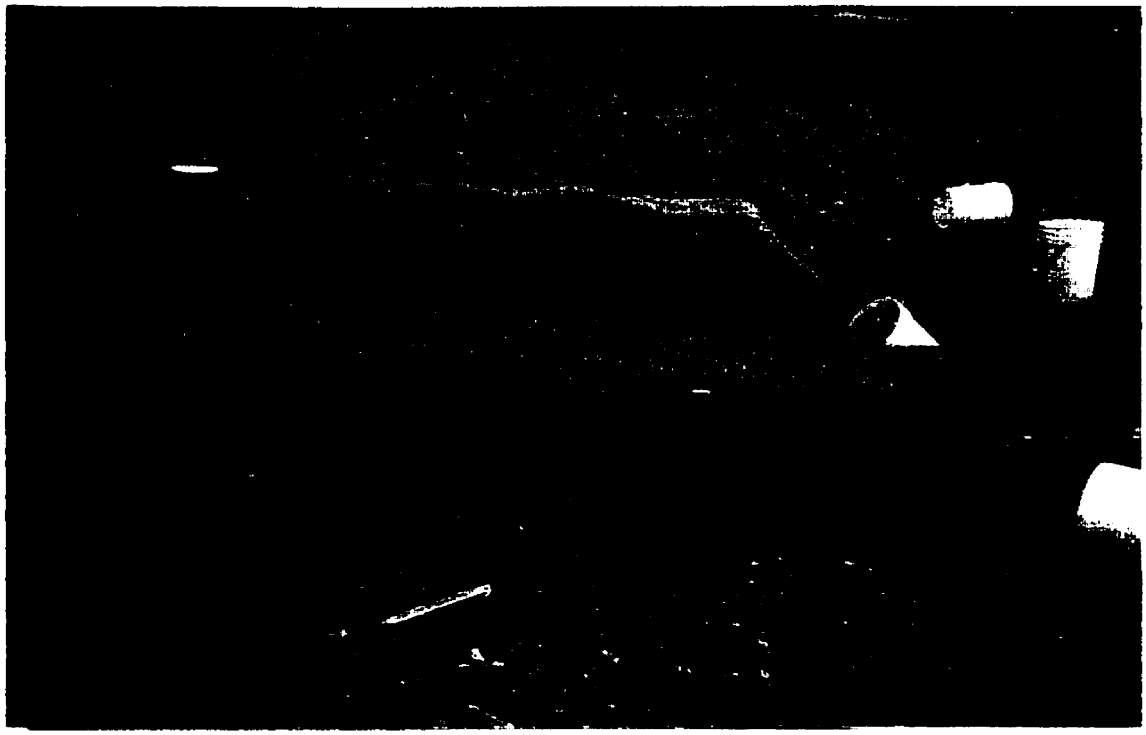
The zone of highest TPH concentration was identified for the power house site soil at Alert. The average TPH concentration of the soil selected was 11,500 ppm. Approximately 45 m^3 of soil was excavated from the site and stockpiled for experimental work. The excavated stockpile was spread out in a 25 cm layer and amended with 300 kg urea and 150 kg of DAP along with 4.5 m^3 (10% vol. addition) of cocoa fibre bulking agent. To ensure homogeneity of initial soil TPH concentrations and even distribution of amendments, the soil was mixed with a gas-powered roto-tiller and then turned over mechanically and collected into a final stockpile with the use of a front-end loader.



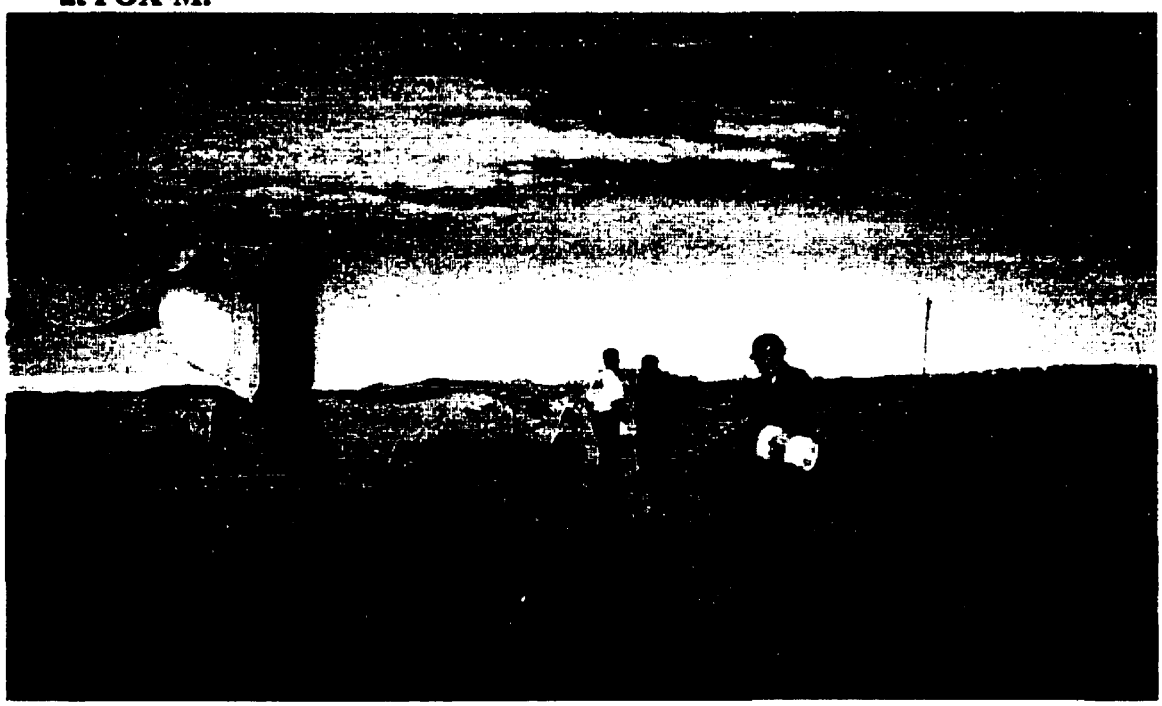
Photograph III-1: Soil stockpile constructed by site contractor staff at FOX-M.

2. Construction of FOX-M small-scale biopiles

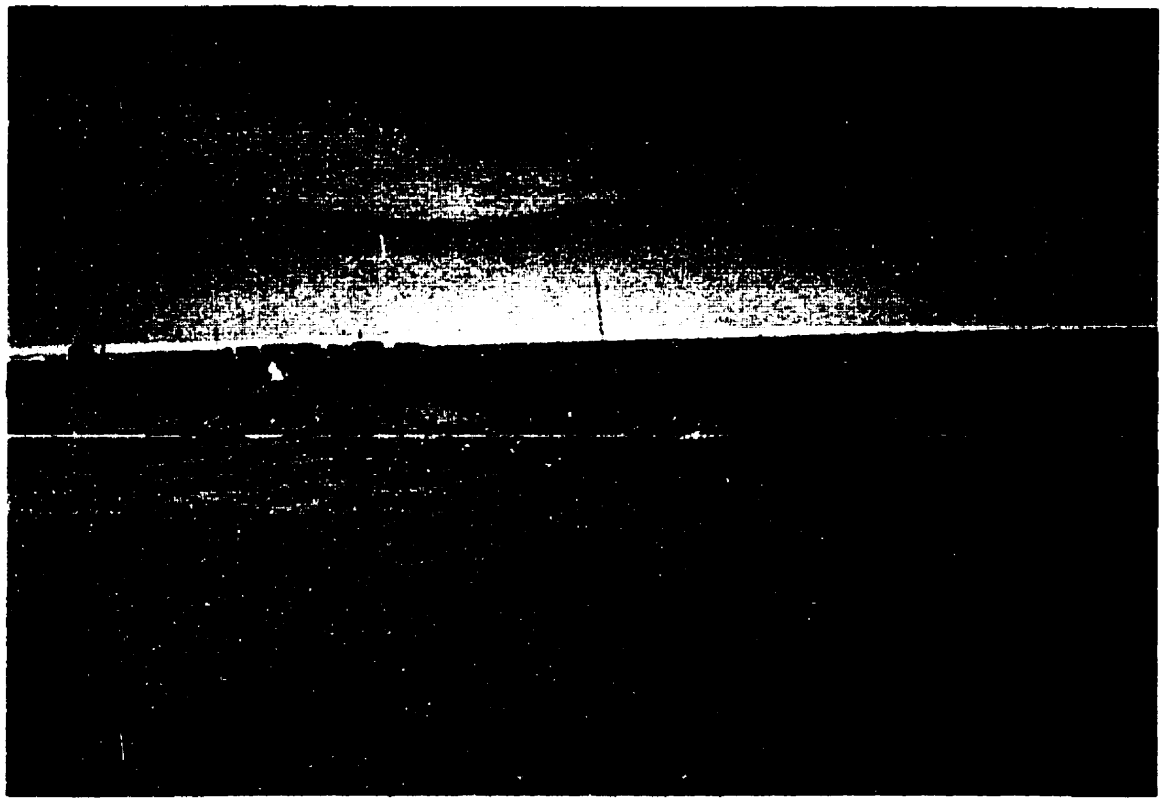
Eight 1.75 m^3 small-scale biopiles (i.e. four treatments with duplicates) were constructed at FOX-M to investigate the effects of; 1) plastic pile covers, 2) commercial fertilizer, and 3) surfactant and commercial fertilizer on TPH degradation (Table III-5). To prevent leaching of TPH from the bottom of the biopiles, they were constructed on high-density polyethylene (HDPE) liners. To evaluate the volume and TPH concentration of the leachate generated, one of the control pile liners was placed on an angled gravel base to channel leachate into a collection bucket (Photograph III-2). During construction, soil was applied to the liners in layers, with commercial fertilizer (urea and DAP) and commercial fertilizer plus Biosolve™ surfactant applied between each layer where specified (Photograph III-3). For the commercial fertilizer additions (piles E-H, Table III-5), twice the industry recommended concentration was used to ensure adequate nutrient supply and distribution. Biopiles were covered with thin sheets of polyethylene to act as vapour/weather barriers except in the case of the uncovered control piles. Covers were secured in place with gravel (Photograph III-4).



Photograph III-2: Leachate collection system built into small-scale control pile "A" at FOX-M.



Photograph III-3: Building and fertilizing a small-scale biopile at FOX-M.



Photograph III-4: Completed small-scale biopiles with plastic covers at FOX-M.

3. Construction of medium-scale biopiles

Three 15 m^3 medium-scale biopiles were constructed at both FOX-M and Alert to investigate the effects of biopiling and aeration systems at this scale (Table III-5). Biopiles were constructed on non-permeable liners to prevent leachate emissions. Duplicate thermocouples were placed at four evenly spaced depths in each pile to monitor soil temperature gradients within the biopiles and thermal flux over the summer treatment season (Figure III-1). These thermocouples were also used to examine thermal increases resulting from aeration, heating and insulative modifications where applicable.

A control pile, an active aeration pile and a passive aeration pile (Table III-5) were constructed at each site. The control piles (piles I & AA; Table III-5) examined the impact of biopiling without aeration. For the aeration treatment biopiles, two different

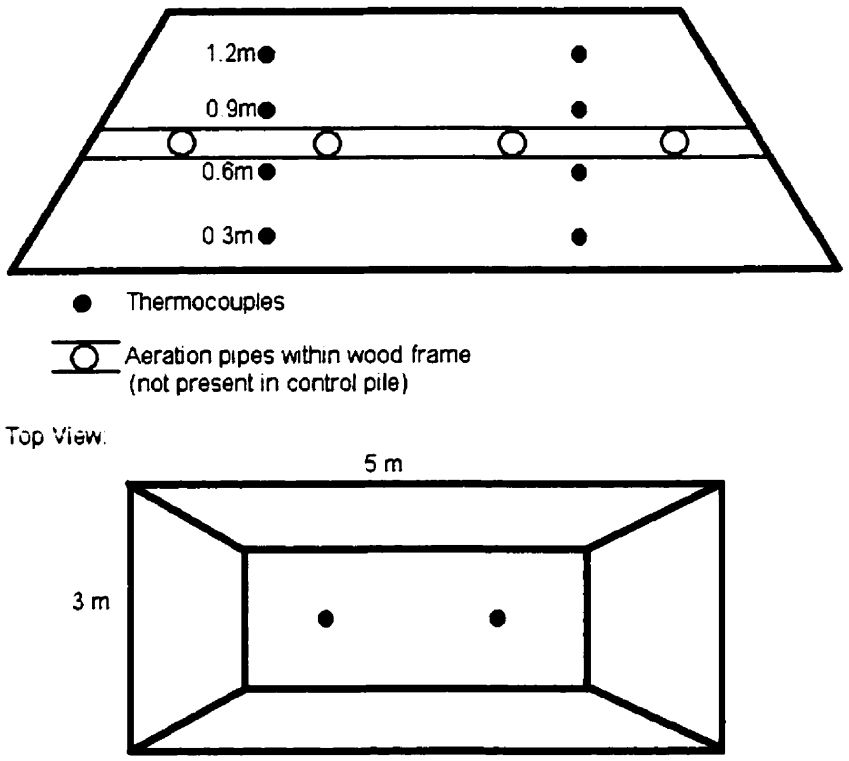


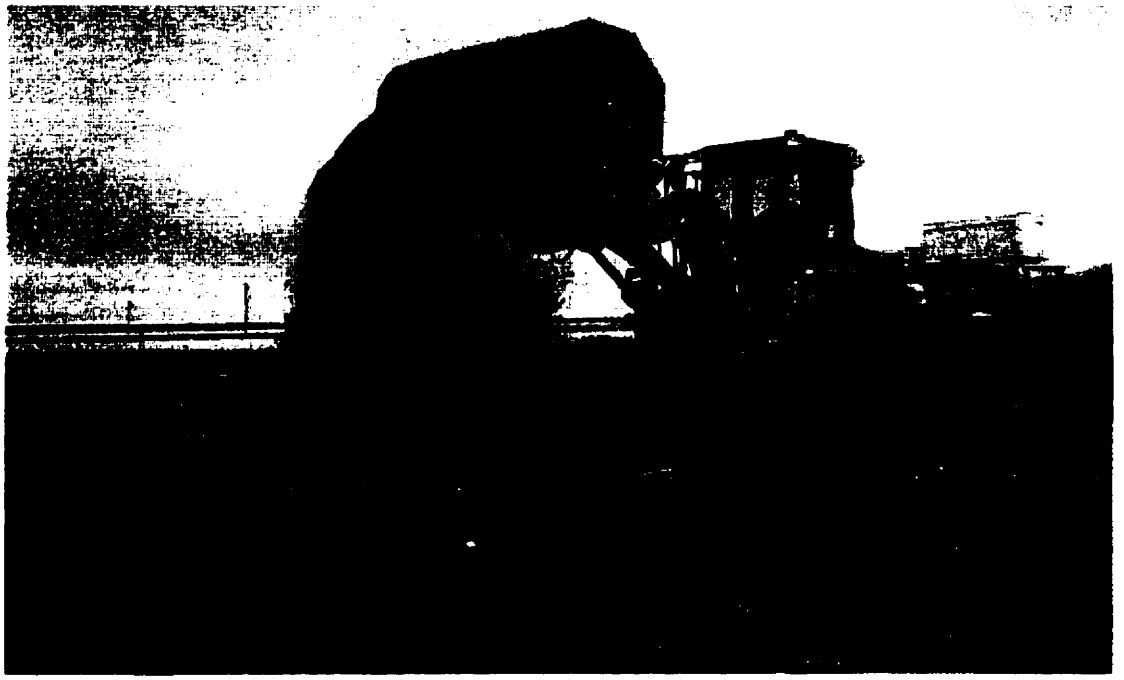
Figure III-1: Schematic diagram of FOX-M and Alert medium-scale biopiles showing the thermocouples (●) and aeration network.

systems were employed; one passive and one active. All biopiles were amended with the appropriate amount of commercial fertilizer for each site.

The aeration system in the passive aeration biopiles (piles J & BB; Table III-5) consisted of 1.5" diameter, perforated (manually drilled, 1 cm diameter holes) ABS plastic pipes to promote passive air diffusion into the biopile. The pipes were evenly spaced along the width of the pile to allow for increased air diffusion into the soil.

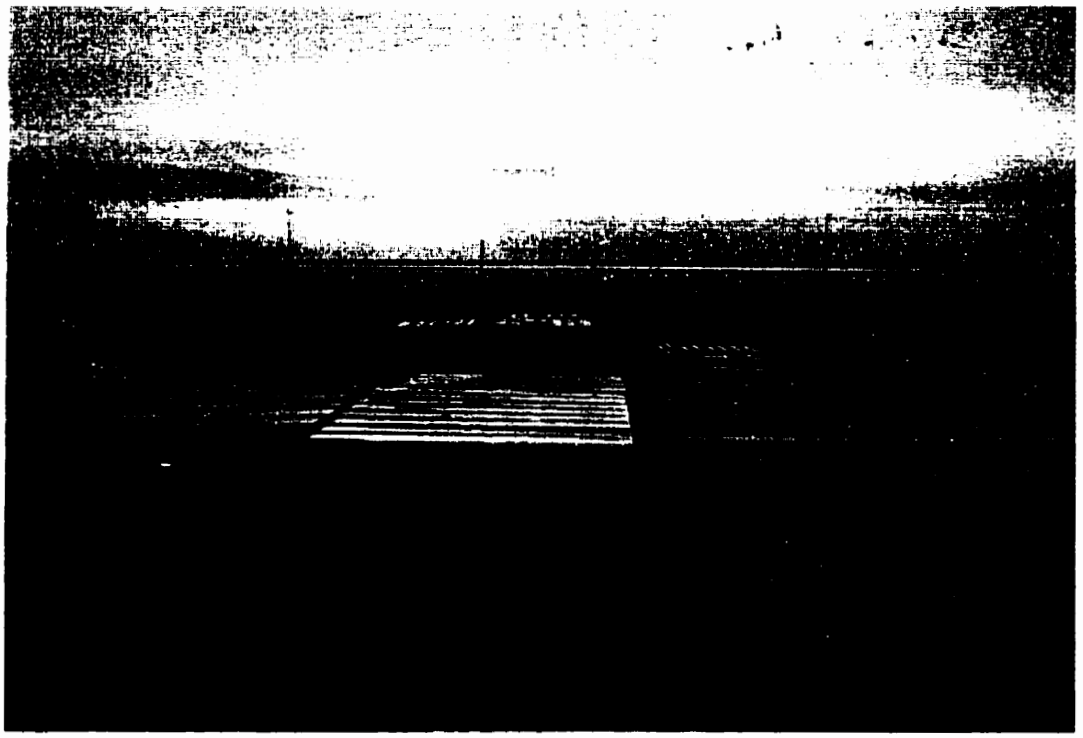
The aeration system in the active aeration biopiles (piles K & CC; Table III-5) consisted of a network of the same pipes connected to an aeration source. The type and source of aeration differed at each site. A Hoover canister central vacuum unit was used for induced aeration at FOX-M. The vacuum was controlled by a timer that activated the

vacuum for half an hour every two hours, 24 hours a day from June 30 , 1998 to August 23, 1998. The Alert forced aeration system (pile CC; Table III-5) was connected to an electric air compressor which operated from 8 August, 1998 through 19 September, 1998. Aeration systems were installed at approximately 1 m from the base (Photograph III-5 & Figure III-1). To prevent compaction of soil and maintain greater void space around the pipes, the aeration systems were housed within a framework of wooden pallets (Photograph III-6).



Photograph III-5: Building a medium-scale biopile at FOX-M.

The voids created by the wooden pallets were filled with soil and piles were shaped to give uniform pile dimensions (Photograph III-7). Completed biopiles were covered with a polyethylene moisture barrier. Vents were cut into the aerated pile covers to allow the passive aeration pipes to protrude and avoid asphyxiation of the vacuum aerated pile.



Photograph III-6: Partially built medium-scale biopiles at FOX-M. Aeration systems within wooden frames are visible (active aeration system in foreground and passive in background).

Photograph III-7: Completed FOX-M medium-scale biopiles. Plastic covers were added after sampling.

Due to colder average temperatures and shorter treatment season, Alert experiments were designed to investigate ways to increase biopile temperatures with the use of; 1) forced air heating, and 2) additional insulative structures. An in-line heater system (designed by the Mech. Eng. Dept., RMC for the Alert team) on the air compressor unit for the active aeration biopile (pile CC; Table III-5) supplied forced air heating. The heater was set to keep a constant inlet air temperature of 18° C. The passive and active aeration biopiles (piles BB & CC; Table III-5) were constructed inside a metal enclosure to minimize heat loss due to wind effects at extremely cold temperatures. The enclosure was used in an attempt to maintain higher ambient air temperatures and extend the treatment season. A greenhouse-style structure was erected within the metal enclosure and on top of the aerated piles for additional insulation. The control biopile (pile AA; Table III-5) was constructed outdoors. Biopiles were built on a foundation of wooden palettes, a layer of 2" Styrofoam SM™ insulation and a polyethylene vapour barrier. A blanket of R-20 fiberglass insulation was placed on top of the vapour barrier for additional insulation.

4. Construction of FOX-M landfarm

Landfarm experiments were carried out to investigate the effects of; 1) plastic covers, 2) commercial fertilizer, 3) commercial fertilizer and surfactant, and 4) surfactant alone on TPH degradation in 15-cm deep treatment plots (Table III-5). Prior to construction, a 7 m x 14 m area was graded to create a convex treatment surface to promote landfarm drainage (Figure III-2). A HDPE liner was laid down over the graded area and covered with the experimental soil. Stakes and flagging tape were used to divide the area into eight 3 m x 3 m treatment plots (Photograph III-8). Commercial fertilizer (urea and DAP) and Biosolve™ were added to the plots as required (Table III-5). Each plot was mixed, aerated (by turning the plot over with shovels), and raked to a uniform depth. The control and commercial fertilizer treatments were duplicated.

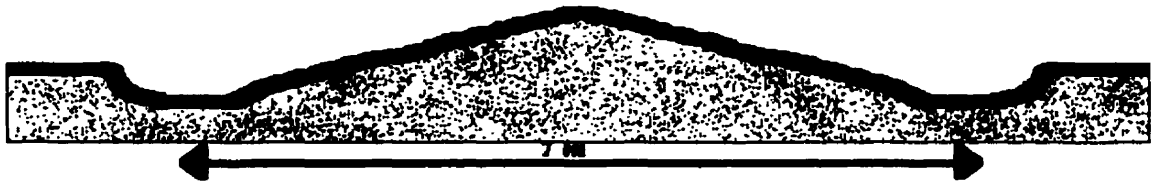
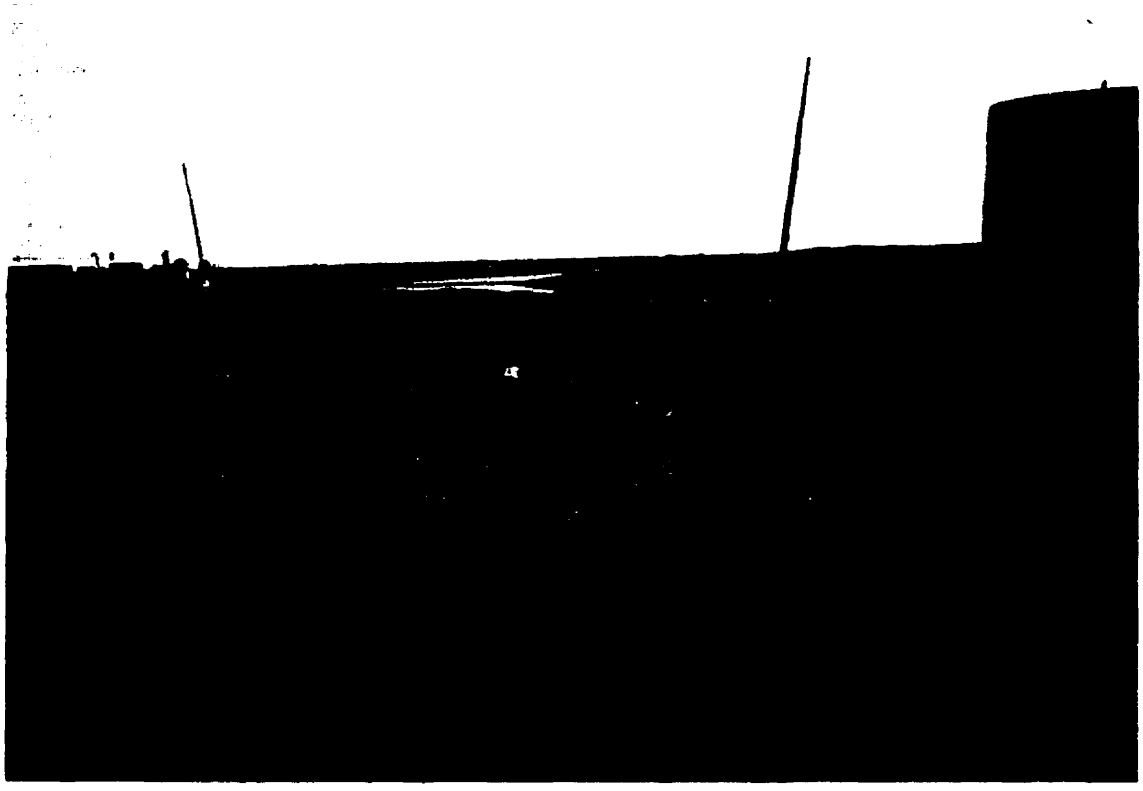


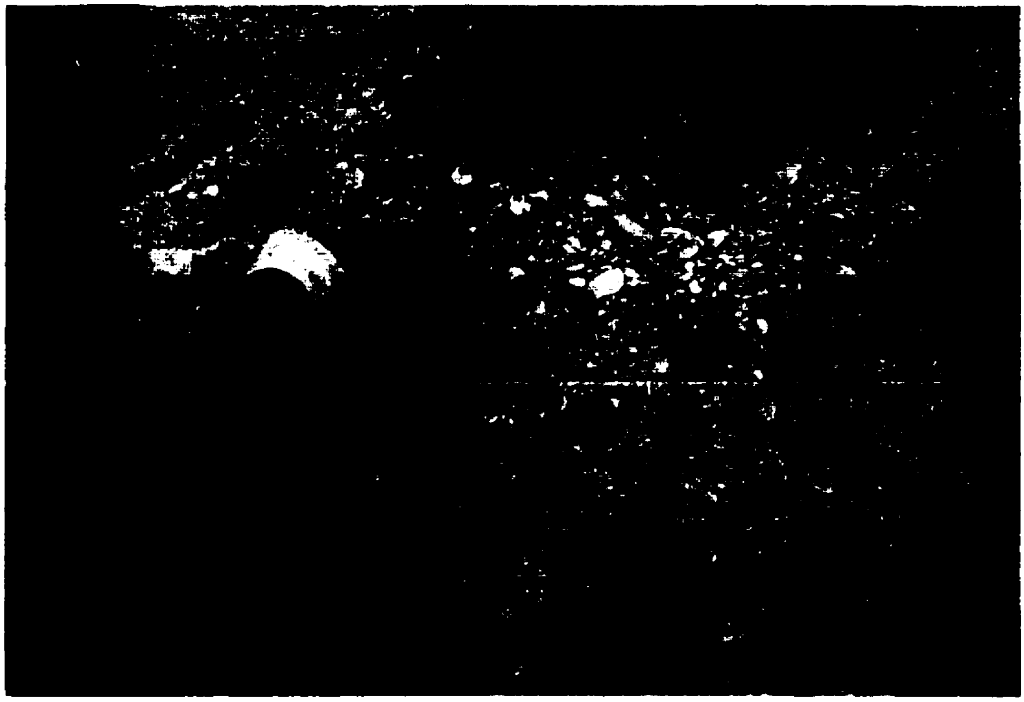
Figure III-2: Cross-section of convex treatment surface for FOX-M landfarm.



Photograph III-8: Completed FOX-M landfarm (background) with flagging tape marking individual plots.

5. Field-scale soil sampling procedure

All soils were sampled at the beginning and end of the treatment season to monitor TPH levels. For the FOX-M small-scale biopiles, 50 cm deep pits were dug at each corner and sampled (Photograph III-9). Four samples were taken from each pile during each sampling event.



Photograph III-9: Sampling a FOX-M small-scale biopile at each corner.

For the medium-scale biopiles, samples were collected from each corner of the pile at two depths (0.5 m and 1.0 m from the base). The samples were collected at 50-60 cm depth in the pile using a hand auger (Photograph III-10). Eight samples were collected from each pile during each sampling event.

The landfarm plots were sampled 1 m in from each corner at a depth of approximately 5 cm. Four samples were collected from each plot during each sampling event.

Each soil sample was placed into a 125 mL amber glass jar using a clean metal scoop and leaving no headspace. The samples were frozen and shipped south for analysis. Duplicate samples were collected every tenth sample for quality assurance/quality control (QA/QC) purposes. After sampling, pits were backfilled and covers replaced, where appropriate.

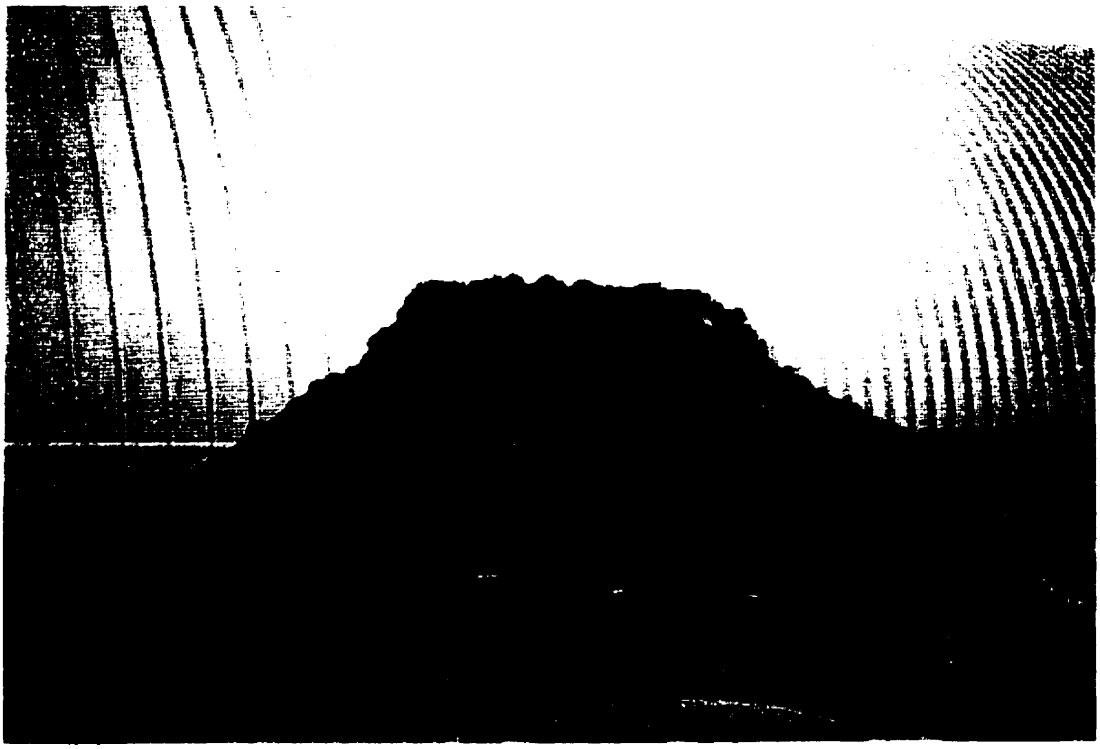
Photograph III-10: Sampling a FOX-M medium-scale biopile with a hand auger (indicated by the arrow).

6. TPH Analysis

For the purpose of monitoring TPH levels in field experiments, soil samples were analyzed by GC at the ASG Laboratory. Soil samples were homogenized and sub-samples dried for moisture determination. A wet sample (10 g dry wt. equivalent) was ground with anhydrous sodium sulfate and Ottawa sand to a free-flowing powder, and loaded into a round-bottomed flask. Pesticide grade hexane (20 mL) was added, and the flask ultrasonically agitated. A 1 mL aliquot of the hexane extract was pipetted from the flask in a manner ensuring no transfer of solid material, and sealed in a GC vial. GC analysis was carried out using a HP6890 GC system equipped with a Supelco capillary column (No. 2-4028 SPB-1), airflow of 400 mL/min, carrier flow of 2 mL/min and no internal standard.

Photograph III-11: Excavating soil from the zone of high TPH contamination at Alert.

Photograph III-12: Homogenizing fertilizer and bulking agent in the Alert experimental soil stockpile using a roto-tiller.



Photograph III-13: Medium-scale control biopile constructed outside metal enclosure at Alert.

Photograph III-14: Passively aerated medium-scale biopile at Alert. ABS passive aeration pipes can be seen protruding through impermeable barrier.

Photograph III-15: Greenhouse structure within metal enclosure at Alert. Active and passive aeration medium-scale biopiles are visible inside.

C. Data presentation & Analysis

1. Laboratory-scale data

Data from the ^{14}C-labelled HC mineralization experiments was obtained from liquid scintillation count analysis in the form of a Becquerel (Bq) count and 2 sigma (i.e. percent error) value. The Bq value corresponded to the activity of the radiolabelled-CO_2 collected in the NaOH solution over each sampling interval. The cumulative sum was compared to the total activity (Bq) of the radiolabel added to each microcosm. The total amount was measured by LSC analysis of the full radiolabel dose (0.45 μCi) dissolved in the jet fuel and NaOH solution. For graphical analysis, time accumulated counts (as a cumulative percentage of total label added) were plotted against time (days). Triplicate microcosms were sampled according to a rotating sampling schedule (i.e. AB, BC, AC, ABC, etc...). Counts were measured for either two or three of each triplicate set of

microcosms and averaged. The resulting curve represents the mineralization response curve or $\%^{14}C$ mineralized (Figures IV-1 to IV-10).

This type of curve ideally fits the microbial growth curve with lag, exponential growth, stationary and decay components. Hydrocarbon mineralization experiments adequately demonstrating this type of response were expected to fit a logistic growth model: a type of exponential growth limited by the carrying capacity of the environment. Previous research showed that the Boltzman fit closely matched experimental data (ESG, 1998). For sigmoidal curve fitting purposes, an integrated and parameterized form of the Boltzman equation is most appropriate. Applicable portions of the experimental data were analyzed and interpreted at the Dept. of Microbiology, University of British Columbia using the Boltzman equation described below.

$$Y = Ymax - [(Ymax - Yinit) / (1 + \exp (k (T-1)))]$$

Where Y is the final extent of mineralization, k the rate constant, T is time and T_{mid} the time required for half of the maximum mineralization to occur.

A curve-fitting module in Microcal Origin™ (Microcal Software, Inc., Northampton, Mass.) used this equation to fit experimental data and determine the value of the defining kinetic parameters. These parameters were then used for quantitative comparison of the mineralization curves and relative biodegradation performance in the different soils and treatments (e.g. lag times, rate constants, maximum amount of mineralization). Parameters could only be calculated and compared for treatments that produced the required sigmoidal mineralization pattern. The majority of microcosm mineralization responses did not exhibit a pronounced stationary phase and as such, only lag phase parameters could be calculated using the Boltzman method. A summary of the kinetic data obtained is presented in Appendix C.

2. Field-scale data

Small, medium-scale and landfarm data were analyzed using Excel™ for Windows and JMP™ for Apple™. Statistical analysis (nested and two way Anova analysis) was employed to examine the change in TPH concentration over the summer treatment season according to 1) time, 2) treatment, 3) time by treatment and/or 4) time by replicate within treatment interactions. A summary of the statistical results generated by the model is presented in Appendix D.

IV. RESULTS

A. Microcosm studies

Microcosm studies detected no mineralization of ^{14}C-labelled HC in Alert or FOX-M sterile soil treatments (Figure IV-6). Alert soil treatment blanks (unfertilized, non-sterile soil) showed that approximately 3.5% of the ^{14}C-labelled HC was mineralized to CO_2 after 37 days incubation at 7° C (Figure IV-1). In contrast, FOX-M treatment blanks produced no detectable mineralization over the same time period (Figure IV-2).

1. Physico-chemical soil characteristics

Nutrient analysis revealed that Alert soil had higher concentrations of both N and P. TKN nitrogen concentrations were 700 ppm for Alert and 300 ppm for FOX-M soil samples. Extractable (bioavailable) phosphorous concentrations were 6.3 and 4.4 ppm for Alert and FOX-M soil samples, respectively.

Grain size compositions for both soils indicate the percentages of silt, sand and gravel before and after the soil was sieved for use in the microcosm experiments (Table IV-1).

Table IV-1: Grain size analysis results.

Soil	Condition	% Silt	% Sand	% Gravel
Alert	Unsieved	39	23	38
Alert	Sieved	55	1	14
FOX-M	Unsieved	0	28	65
FOX-M	Sieved	0	56	30

2. Commercial fertilizer microcosm experiments

^{14}C-labelled HC mineralization results, under various commercial fertilizer application ratios for each soil type, revealed that the quarter concentration of commercial fertilizer performed best in Alert soil while the half concentration was superior in FOX-M soil (Figures IV-1 & IV-2). Furthermore, shorter lag periods, higher rates and greater extents of mineralization were observed in FOX-M soil compared to Alert soil.

In Alert soil microcosms, the quarter concentration of commercial fertilizer produced the highest rate of mineralization between approximately 21 and 29 days (Figure IV-5). The quarter concentration also produced the greatest average final extent of mineralization (33% ^{14}C-labelled HC mineralization within 37 days). However, upon closer examination, standard error measures overlap with those of the half standard concentration indicating that there was no significant difference between the two final extents. Mineralization rates for both the half and quarter concentration treatments did not appear to be decreasing by the 37 day point. The three fertilizer concentrations tested in Alert soil produced similar lag periods ranging from 7.1 to 8.1 days (Appendix C).

FOX-M mineralization results showed that treatment with the half concentration exhibited the highest average rate and extent of mineralization of 45%. This was supported by Boltzman kinetic parameters indicating that the half concentration level produced the shortest lag phase (4.6 d) and reached half of its maximum mineralization level (T_{mid}) in the shortest time of 12.8 days (Appendix C).

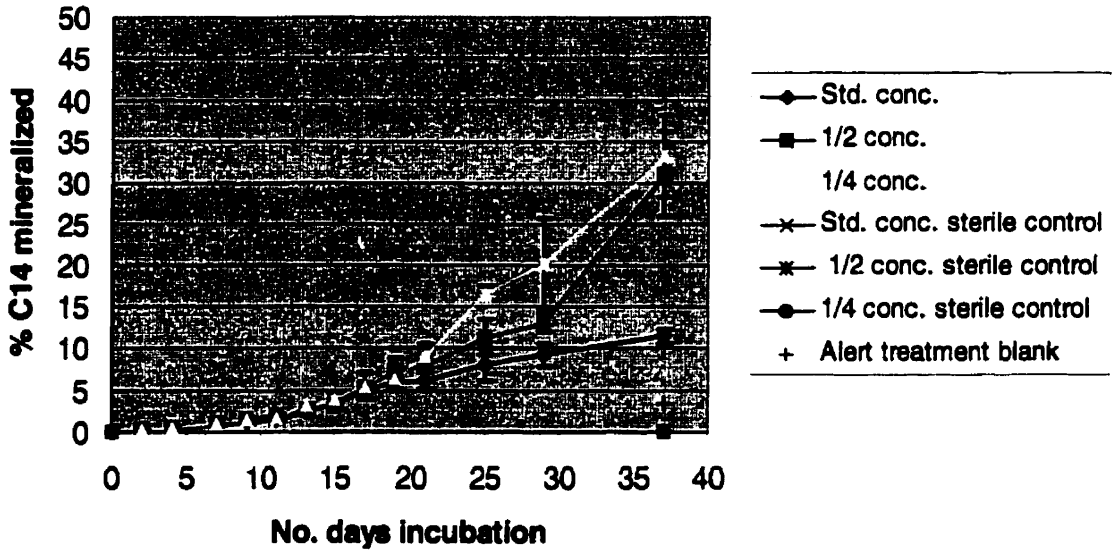


Figure IV-1: Impact of commercial fertilizer on ^{14}C-labelled HC mineralization in Alert soil: std. conc., ½ conc., ¼ conc., sterile blank (unfertilized sterile soil), treatment blank (unfertilized soil). Error bars represent +/- standard error of the mean. Error measures for controls and blanks are less than reportable increment.

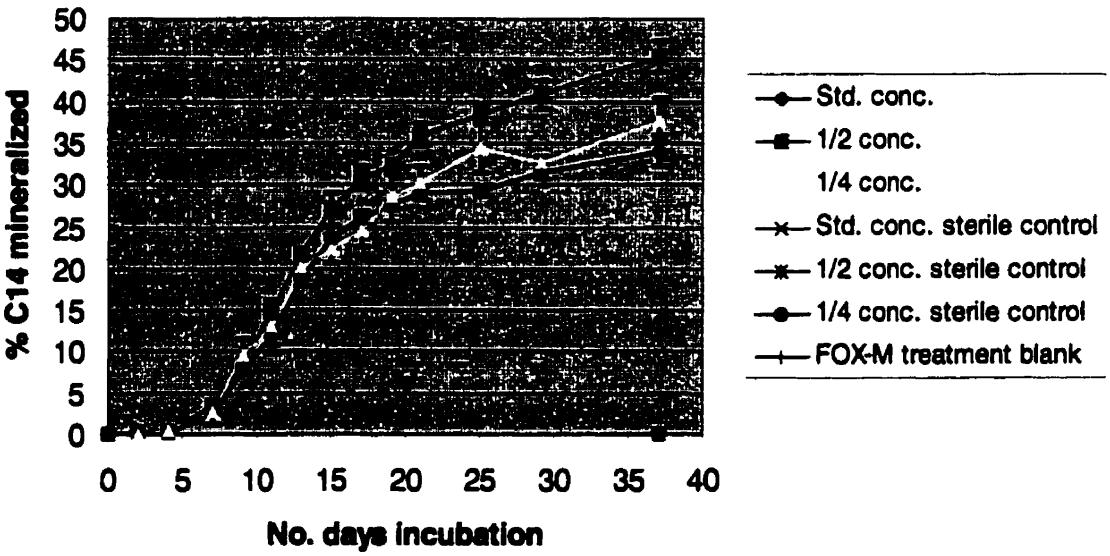


Figure IV-2: Impact of commercial fertilizer on ^{14}C-labelled HC mineralization in FOX-M soil: std. conc., ½ conc., ¼ conc., sterile blank (unfertilized sterile soil), treatment blank (unfertilized soil). Error bars represent +/- standard error of the mean. Error measures for controls and blanks are less than reportable increment.

3. Bioremediation specific fertilizer microcosm experiments

^{14}C-labelled HC mineralization results, under various bioremediation specific fertilizer application ratios for each soil type, revealed that the standard concentration of bioremediation specific fertilizer produced optimal rates and extents of mineralization in both Alert and FOX-M soils (Figures IV-3 & IV-4). Mineralization curves for Alert and FOX-M treatments did not appear to reach the stationary phase within the monitoring period. Boltzman data revealed slightly shorter lag phases in Alert soil compared to FOX-M soil (5.8 d vs. 7.6 d) (Appendix C).

Mineralization of ^{14}C-labelled HC within Alert soil microcosms for the standard level of bioremediation specific fertilizer reached an average maximum of 14% in 37 days of monitoring. In FOX-M soil, mineralization in the standard concentration reached a very similar average maximum (of 17%) ^{14}C-labelled HC mineralization over the 37-day treatment period.

4. Surfactant microcosm experiments

^{14}C-labelled HC mineralization results, under various surfactant application ratios for each soil type, revealed that the quarter surfactant concentration performed best in Alert soil while the standard concentration was superior in FOX-M soil (Figures IV-5 & IV-6). Boltzman data revealed shorter lag periods in Alert soil than in FOX-M soil under optimal surfactant conditions (7.05 d vs. 11.2 d) (Appendix C).

Treatment of Alert soil with surfactant in the absence of fertilizer (Figure IV-5 – "Alert std. conc. (no fert.)"), did not increase the rate or extent of mineralization of ^{14}C-labelled HC over the treatment blank (unfertilized, non-sterile soil). Mineralization results in the Alert half concentration (9 μL/microcosm) surfactant treatment produced an average maximum of 44%. However, when standard error is factored in, results show that halving the concentration of surfactant, although causing higher replicate variability, did not significantly improve the rate or extent of mineralization compared to the standard

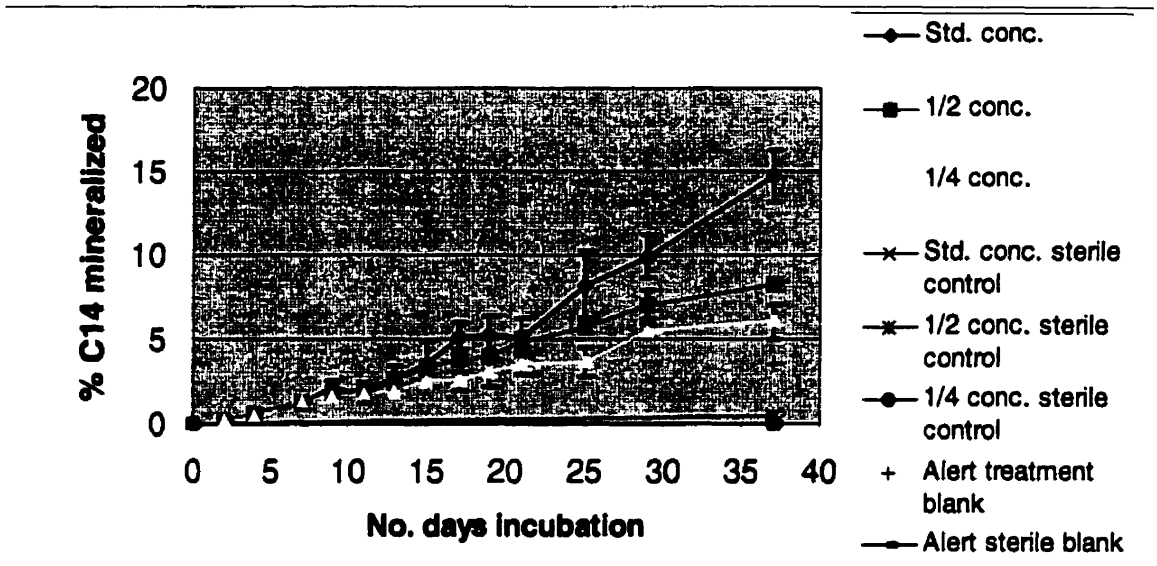


Figure IV-3: Impact of bioremediation specific fertilizer on ^{14}C-labelled HC mineralization in Alert soil: std. conc., ½ conc., ¼ conc., sterile blank (unfertilized sterile soil), treatment blank (unfertilized soil). Error bars represent +/- standard error of the mean. Error measures for controls and blanks are less than reportable increment.

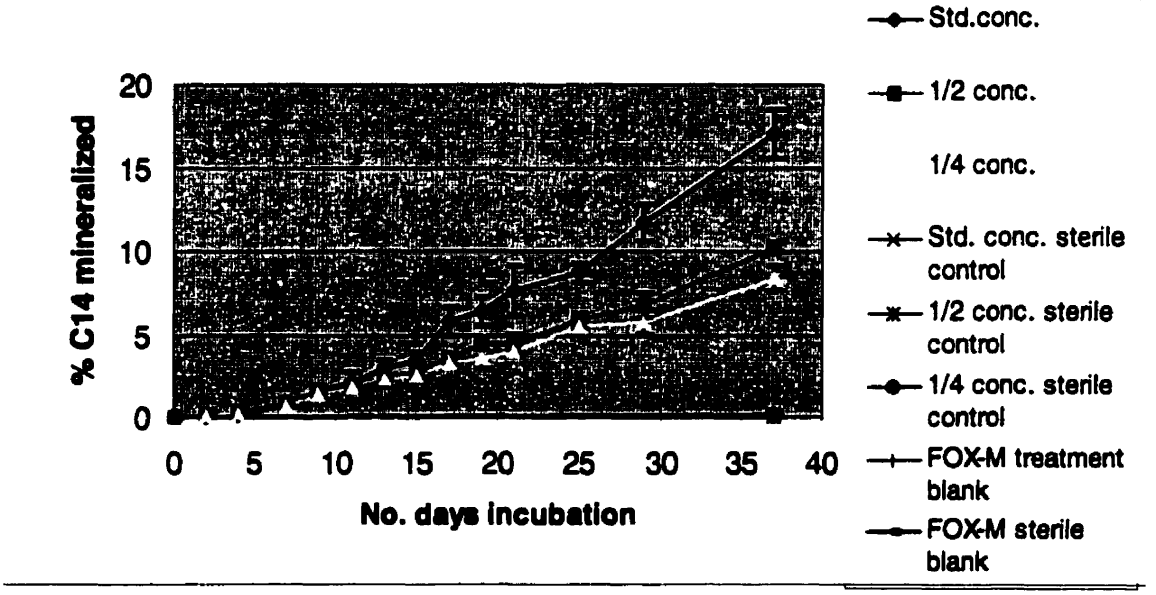


Figure IV-4: Impact of bioremediation specific fertilizer on ^{14}C-labelled HC mineralization in FOX-M soil: std. conc., ½ conc., ¼ conc., sterile blank (unfertilized sterile soil), treatment blank (unfertilized soil). Error bars represent +/- standard error of the mean. Error measures for controls and blanks are less than reportable increment.

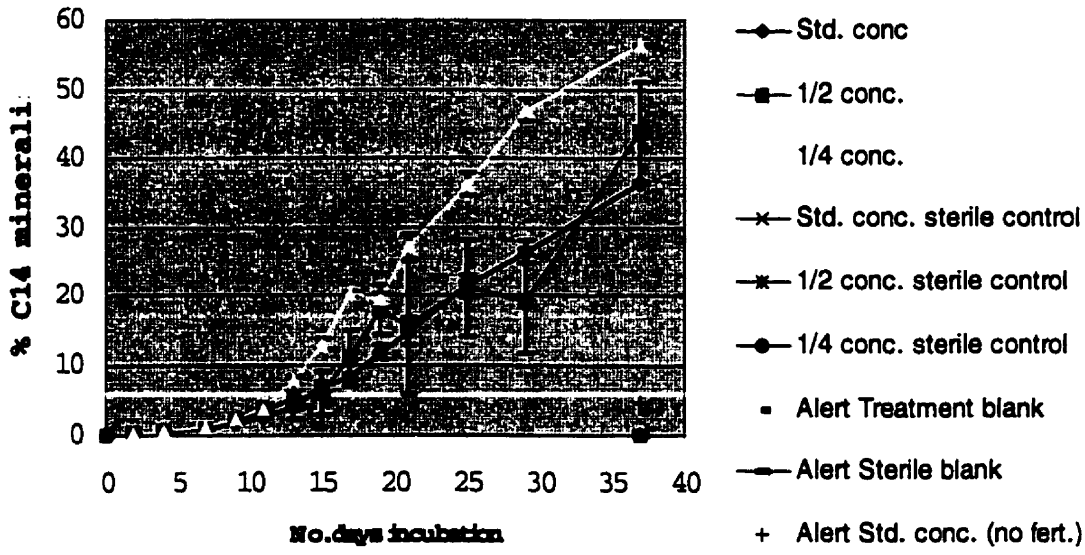


Figure IV-5: Impact of surfactant on ^{14}C-labelled HC mineralization in Alert soil: std. conc., ½ conc., ¼ conc., sterile blank (unfertilized sterile soil), treatment blank (unfertilized soil). All surfactant treatments amended with std. conc. of commercial fertilizer. Error bars represent +/- standard error of the mean. Error measures for controls and blanks are less than reportable increment.

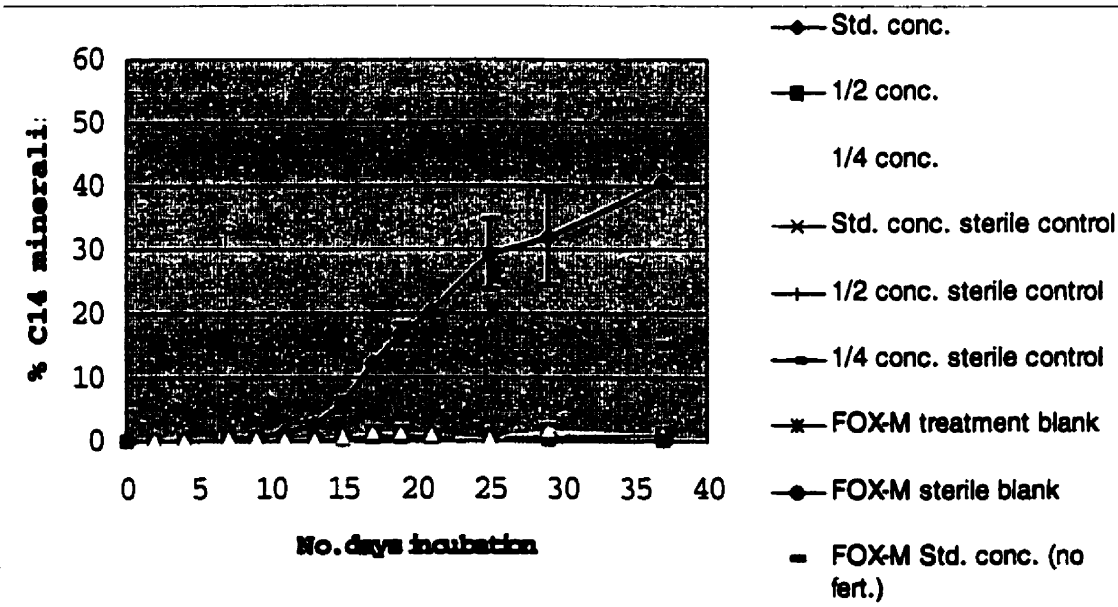


Figure IV-6: Impact of surfactant on ^{14}C-labelled HC mineralization in FOX-M soil: std. conc., ½ conc., ¼ conc., sterile blank (unfertilized sterile soil), treatment blank (unfertilized soil). All surfactant treatments amended with std. conc. of commercial fertilizer. Error bars represent +/- standard error of the mean. Error measures for controls and blanks are less than reportable increment.

concentration treatment. The strongest interaction of fertilizer and surfactant was observed in the quarter concentration (4.5 μL/microcosm) treatment producing the highest average maximum rate and extent of mineralization at 56% over 37 days.

In FOX-M soils (Figure IV-6), surfactant without fertilizer performed better than the unfertilized, non-sterile treatment blank (treatment blank not visible but along baseline). Contrary to what was seen in Alert soil, the presence of surfactant alone increased the ability of indigenous populations to mineralize ^{14}C-labelled HC in the absence of nutrient amendments. Surfactant in the presence of commercial fertilizer performed better than surfactant alone. An average maximum level of 40% ^{14}C-labelled HC mineralization was achieved using the standard concentration of surfactant (18 μL) and commercial fertilizer (1.95 mg urea, 0.91 mg DAP) in less than 30 days. At this optimal level, there was a significant improvement over the 34% mineralization observed in FOX-M standard commercial fertilizer experiments (without surfactant). Mineralization detected for the half and quarter concentration treatments was negligible over the treatment period.

5. Inoculum microcosm experiments

^{14}C-labelled HC mineralization results, under various inoculum and cryoprotectant applications, revealed that the standard concentration of inoculum produced the highest mineralization response in both sterile and non-sterile soil (Figures IV-7 & IV-8).

The lack of detectable mineralization in the sterile, fertilized blank indicated that, even in the presence of standard levels of commercial fertilizer, no detectable mineralization resulted in sterile soil over the 35-day treatment period (Figure IV-7). The cryopreservative blank (non-sterile, unfertilized soil) produced an average maximum of 1.5% ^{14}C-labelled HC mineralization over the 35-day treatment period.

The treatment effect control (fertilized soil with cryoprotectant) produced an average maximum of 4.7% mineralization (Figure IV-7). Similar lag periods were observed in both cases (Appendix C).

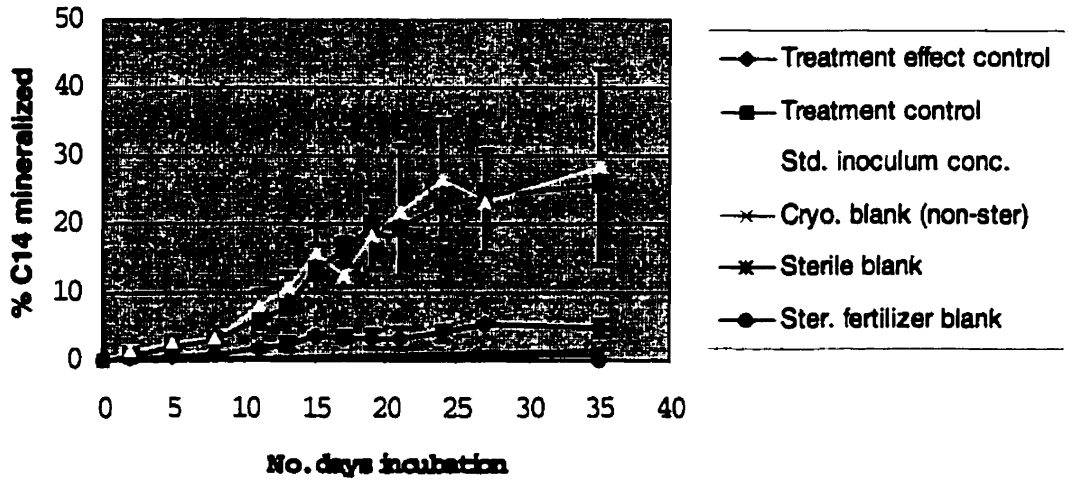


Figure IV-7: Impact of inoculum and cryopreservative addition on ^{14}C-labelled HC mineralization in Alert soil: treatment effect control (fertilized soil with cryopreservative), treatment control (sterile, fertilized soil with standard inoculum concentration), cryo. blank (non-sterile unfertilized soil), sterile blank (sterile soil with cryopreservative), ster. fertilizer blank (sterile, fertilized soil with cryopreservative). Applicable treatments amended with std. conc. of commercial fertilizer. Error bars represent +/- standard error of the mean. Error measures for controls and blanks are less than reportable increment.

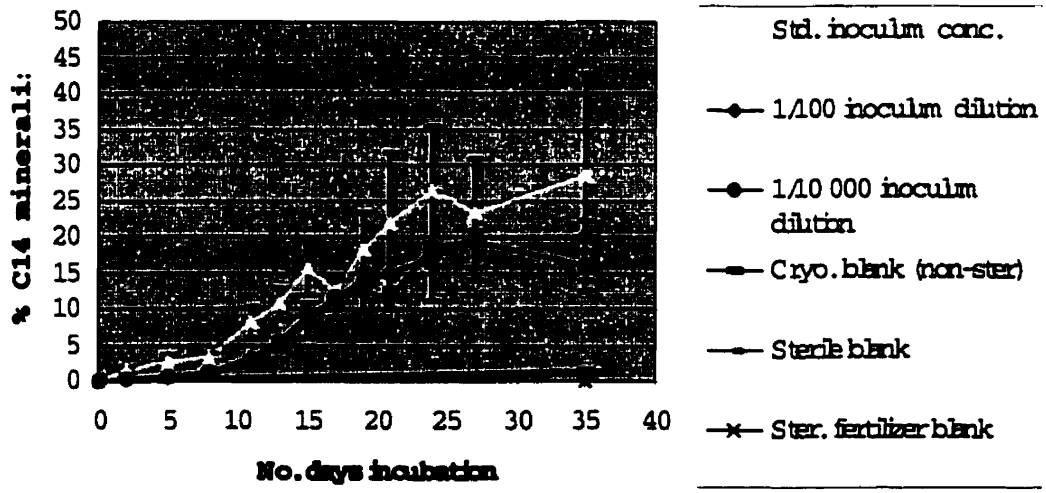


Figure IV-8: Impact of inoculum on ^{14}C-labelled HC mineralization in Alert soil: std. conc. (undiluted inoculum), 1/100 inoculum dilution, 1/10 000 dilution, cryo. blank (fertilized soil), sterile blank (sterile soil with cryopreservative), ster. fertilizer blank (sterile, fertilized soil with cryopreservative). Inoculum includes cryopreservative. Applicable treatments amended with std. conc. of commercial fertilizer. Error bars represent +/- standard error of the mean. Error measures for controls and blanks are less than reportable increment.

The standard inoculum concentration outperformed the 1/100 and 1/10 000 dilutions resulting in an average maximum ^{14}C-labelled HC mineralization level of 28% over the treatment period (Figure IV-8). The treatment control (standard inoculum concentration applied to sterile soil) produced a parallel mineralization response (within standard error measures) to the standard inoculum treatment (Figure IV-7). The standard inoculum concentration treatment had a slightly shorter lag period at 1.4 days compared to 2.7 days for the treatment control with similar Tmid periods of 15.1 and 15.8 days, respectively (Appendix C).

6. TPH versus ^{14}C removal experiments

A comparison of ^{14}C-labelled HC mineralization and TPH degradation in each soil revealed distinctly different patterns. Results revealed a good correlation of the two measures in FOX-M soil and an apparent TPH increase in Alert soil (Figures IV-9 and IV-10).

The mineralization of ^{14}C-radiolabelled n-dodecane curve revealed a similar pattern to that of TPH degradation in FOX-M soil (Figure IV-9). Although the two curves followed the same form, they were separated by a gap in terms of extent of overall removal. TPH degradation reached an average maximum of approximately 80% within 20 days (Table IV-2). N-dodecane mineralization was approximately 37% completed over the 45-day monitoring period.

Table IV-2: TPH levels detected in FOX-M microcosm experiments.

Time (days)	Mean TPH (ppm)	Std. dev.
0	1647	54
9	583	78
19	292	14
29	323	135
47	387	35

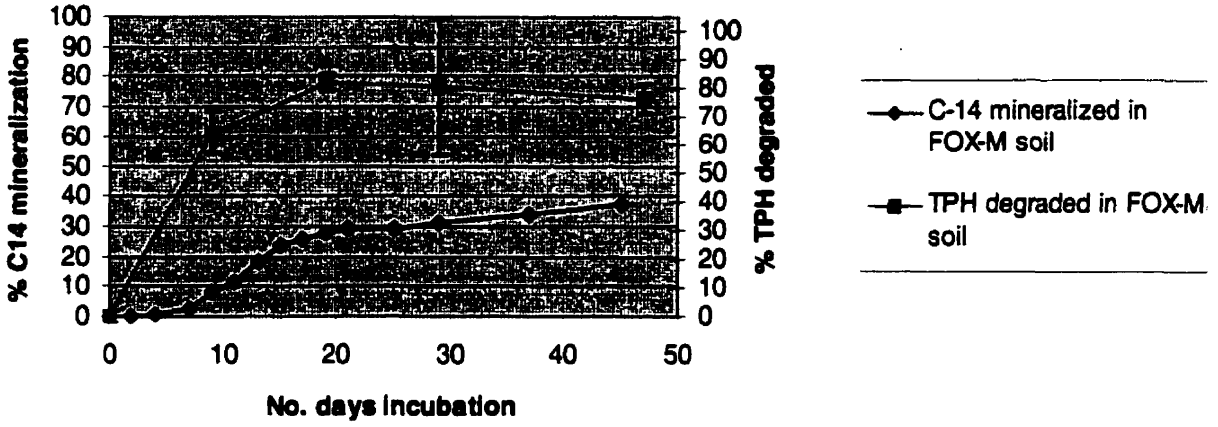


Figure IV-9: Comparison of ^{14}C-labelled HC mineralization and TPH degradation in FOX-M soil. Soil was fertilized with standard commercial fertilizer concentration. Radiolabel introduced in 1000 ppm Arctic diesel solvent. No radiolabel was added to TPH microcosms. Error bars represent +/- standard error of the mean. Error measures on ^{14}C mineralization curve are less than reportable increment.

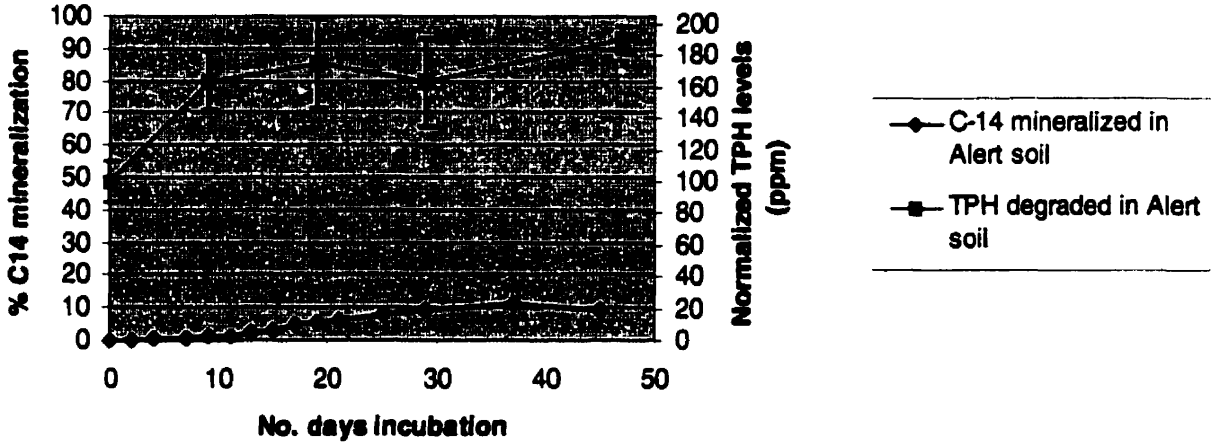


Figure IV-10: Comparison of ^{14}C -labelled HC mineralization and TPH degradation in Alert soil. Soil was fertilized with standard commercial fertilizer concentration. Radiolabel introduced in 1000 ppm Arctic diesel solvent. No radiolabel was added to TPH microcosms. Error bars represent +/- standard error of the mean. Error measures on individual ^{14}C mineralization curves are less than reportable increment.

A distinctly different TPH degradation pattern emerged for Alert soil (Figure IV-10 & Table IV-3) showing increasing TPH levels over time. For graphical analysis, it was necessary to present the data in a different manner. TPH values were expressed in terms of relative amount, whereby the TPH value at day 0 was given the value of 100%. Mineralization followed the expected curve for ^{14}C-labelled HC reaching an average maximum of over 10% in 45 days. This result correlated with the average 11.5% mineralization recorded in other Alert soil microcosms amended with the standard level of commercial fertilizer. TPH levels detected in Alert microcosms appeared to follow an increasing trend over the monitoring period. The final TPH level detected revealed that almost twice the amount of TPH was detected after 45 days than at time zero (an approximate 90% increase).

Table IV-3: TPH levels detected in Alert microcosm experiments.

Time (days)	Mean TPH (ppm)	Std. dev.
0	5071	1165
9	8303	1644
19	8874	2507
29	8260	2504
47	9643	708

B. Field (*in vivo*) studies

Field-scale TPH results for the small, medium-scale and landfarm experiments are presented graphically along with statistical Anova findings. Results are integrated with thermal data (Appendix E) gathered from thermocouples in the medium-scale biopiles at each site.

1. Medium-scale experiments

Medium-scale biopile TPH degradation results for FOX-M soil show a decrease in TPH levels after 54 days of monitoring in all treatments (Table IV-4, Figure IV-11 &

Appendix F). The mean decrease in the active aeration biopile was highest. All medium-scale treatments had large standard deviations. A two-way Anova confirmed that data and residuals were normally distributed and independent of predicted values. The variation in initial TPH levels among medium-scale biopiles was not significant ($\alpha = 0.05$) (Appendix D). However, there was a statistically significant decrease in TPH levels detected within all treatments during the monitoring period attributed to the effect of biopiling over time alone (Appendix D). This indicated that the highest mean decrease detected in the actively aerated pile was not due to a treatment by time interaction. Additional data collected from the medium-scale control pile revealed that TPH levels in leachate samples collected with the graded liner and bucket were below detection (< 5 ppm).

Table IV-4: Mean TPH changes with standard deviations for FOX-M medium-scale biopiles over 54 days of monitoring.

Treatment	Mean TPH decrease (ppm)	Std. dev. of the mean
Control	631	621
Active aeration	707	643
Passive aeration	486	890

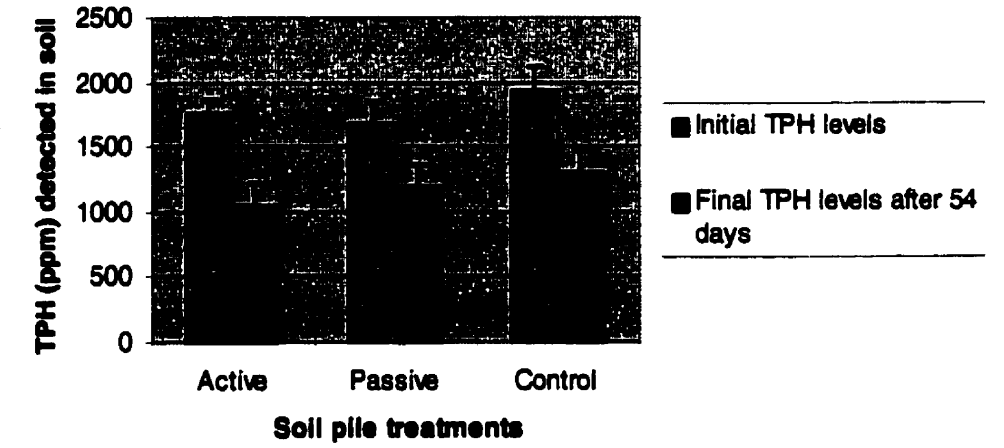


Figure IV-11: Impact of aeration systems on TPH degradation in FOX-M medium-scale biopiles after 54 days of treatment. All treatments were amended with standard concentration of commercial fertilizer. Error bars represent +/- standard error of the mean.

Thermal data indicated that average temperatures in FOX-M biopiles were between 10 – 15° C (Figure E-1: Appendix E). Sub-zero conditions were not recorded within the piles over the monitoring period. No obvious thermal increases from increased microbial activity were recorded in the actively aerated pile indicating that the active aeration system was not effective at raising pile temperature over the passive and control biopile treatments.

Medium-scale biopile TPH degradation results for Alert soil show decreases in TPH levels for the active and passive aeration biopile treatments (Table IV-5 & Figure IV-12). Only the active and passive aeration treatments were sampled at the end of the summer period and as such, the control pile was only used for collection of thermal data. The mean decrease in TPH levels detected in the passively aerated pile was greater than that of the more variable, actively aerated biopile (Appendix F). A two-way Anova revealed a leptokurtic distribution and a logarithmic function was applied to reduce residual dependence. Within both the actively and passively aerated treatments, there was a statistically significant decrease in TPH levels over the monitoring period attributed to a time interaction only. This suggests that the greater decrease in the passively aerated pile was not due to a treatment effect (Figure IV-12) (Appendix D).

Table IV-5: Mean TPH changes with standard deviations for CFS Alert medium-scale biopiles over 42 days of monitoring.

Treatment	Mean TPH decrease (ppm)	Std. dev. of the mean
Control	N/A	N/A
Active aeration	2100	1577
Passive aeration	2757	744

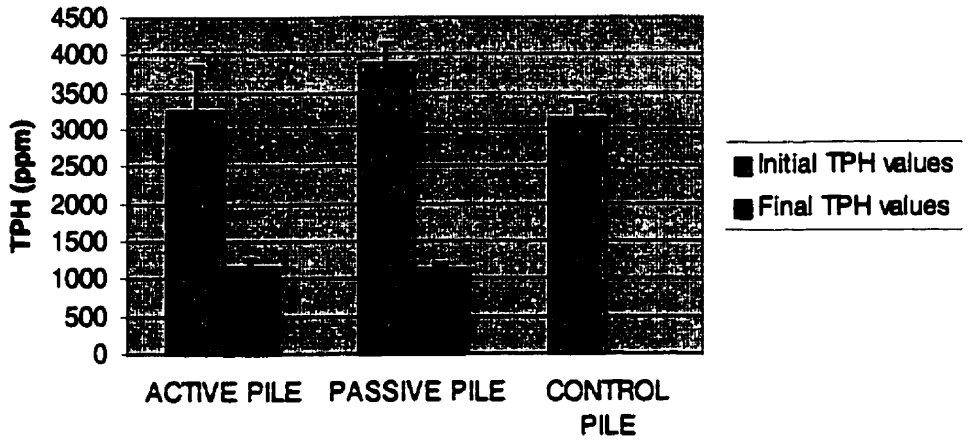


Figure IV-12: Impact of aeration systems on TPH degradation in Alert medium-scale biopiles after 42 days of treatment. All treatments were amended with standard concentration of commercial fertilizer and cocoa fibre bulking agent. Error bars represent +/- standard error of the mean. Note: control pile was only sampled at time zero.

For Alert experiments, thermal data indicated that all piles had approximately the same initial temperatures (Figure E-2:Appendix E). As the season progressed, the control pile (located outdoors) quickly fell below zero (which precluded the final sampling) while the aerated piles (housed in the metal enclosure) fell below 0° C 15-18 days later. On average, higher temperatures were recorded in the active and passive aeration piles compared to the outdoor control. The average temperature over the season was between 2 - 5° C for the aerated piles and 0 - 2° C for the control. Larger thermal gradients were recorded in the outdoor control pile than in the indoor piles (Figure E-3: Appendix E).

2. FOX-M small-scale experiments

FOX-M TPH degradation results from small-scale experiments did not reveal a decrease in mean TPH levels in all treatments (Figure IV-13 & Table IV-6). The largest mean TPH decrease was detected in the duplicate uncovered control piles. Two-way Anova analysis demonstrated normal distribution of data and residuals. Residuals were also

independent of predicted values confirming that the parametric assumptions made by the Anova model were appropriate.

Table IV-6: Mean TPH changes with standard deviations for FOX-M small-scale biopile treatments over 53 days of monitoring.

Treatment	Mean TPH decrease (ppm)	Std. dev. of the mean
Control #1	-25	814
Control #2	-737	407
Uncovered Control #1	1577	355
Uncovered Control #2	1000	216
Commercial Fertilizer #1	-1082	374
Commercial Fertilizer #2	-1066	829
Surfactant + Commercial Fertilizer #1	-1412	530
Surfactant + Commercial Fertilizer #2	545	599

A significant decrease in TPH levels was detected in the uncovered control piles. Anova analysis showed that this decrease was a result of a time by treatment interaction (α= 0.05) (Appendix D). When the Anova was repeated excluding the uncovered control pile data, the apparent TPH level increases (Figure IV-13) in the remaining treatments were no longer significant due to a time by treatment interaction but by a time interaction. This time interaction was not previously observed and confirmed the significance of the uncovered pile treatment by time interaction. In both cases, an additional pile by time

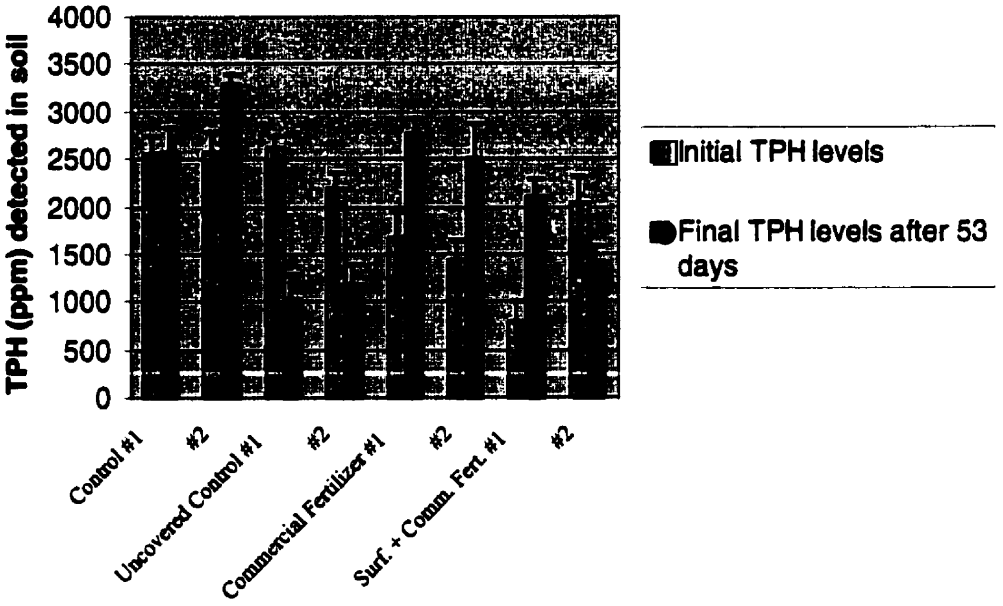


Figure IV-13: Impact of soil additives on TPH degradation in FOX-M small-scale biopiles after 53 days of treatment. Additives were introduced at standard concentration. Error bars represent +/- standard error of the mean.

within treatment effect was observed revealing significant heterogeneity between piles within treatments over time (Appendix D).

3. FOX-M landfarm experiments

TPH degradation results for FOX-M landfarm experiments indicated that the highest mean change in TPH levels detected over the treatment period was recorded in the unamended control treatment plots (Figure IV-14 & Table IV-7). For the purpose of the Anova analysis, the duplicate control and commercial fertilizer plots were combined and treated as single non-duplicated treatments. This was possible because there was no significant difference within these treatments for initial and final TPH data sets. A two-way Anova analysis was performed and revealed a leptokurtic distribution of data and significant dependence of residuals. A logarithmic function was applied to better distribute and lower the dependence of residuals. The final Anova revealed significant effects from treatment, time and treatment by time interactions (Appendix D). This

indicated that the mean decrease in TPH levels detected in the unamended, uncovered control plots was due to a treatment effect. This significant loss of TPH in the uncovered controls suggested that the increased loss of TPH components was due to the effects of volatilization and not bioremediation.

Table IV-7: Mean TPH changes with standard deviations for FOX-M landfarm treatments over 49 days of monitoring.

Treatment	Mean TPH decrease (ppm)	Std. dev. of the mean
Control plot #1	1415	1584
Control plot #2	1015	697
Commercial Fertilizer plot #1	604	499
Commercial Fertilizer plot #2	80	150
Surfactant + Commercial Fertilizer plot	-71	144
Commercial Fertilizer + covered plot	22	105
Surfactant only	392	166
Unfertilized covered plot	384	318

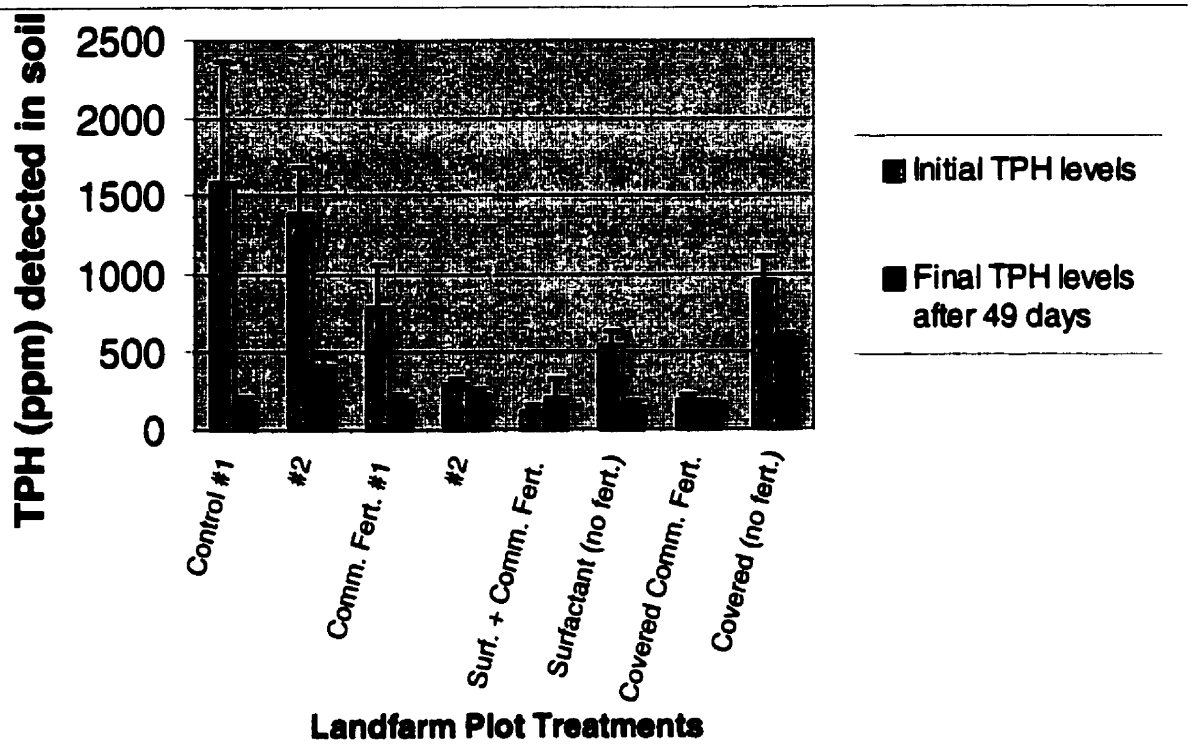


Figure IV-14: Impact of soil additives on TPH degradation in FOX-M landfarm treatments after 49 days of treatment. Error bars represent +/- standard error of the mean.

V. DISCUSSION

The laboratory and field-scale experimental results provided many interesting findings at the micro and macro-scale. Laboratory data demonstrated the positive impacts of biostimulation and bioaugmentation, revealed specific inhibitory effects, and allowed for the identification of the optimal type and concentration of amendment for each soil. These findings are complementary to the field-scale results which highlighted the effectiveness of biopiling and landfarming, the strong role of volatilization in uncovered treatments and a high degree of TPH variance. This chapter provides further interpretation of these results and translates the key findings into design changes that can be used in future research.

A. Discussion of non-controllable experimental parameters

1. Physico-chemical soil characteristics

The C:N:P ratios of 100:9:0.1 for Alert soil and 100:22.8:0.3 for FOX-M soil, calculated from background nutrient levels at each site, indicated that both soils lacked the P levels required to deal with the amount of HCs present at each site. They also indicated that N levels were excessively high for FOX-M and low for Alert. The low P levels support the initial hypothesis that biostimulation was required for HC bioremediation at each site. It is difficult to comment on the significance of the excess N levels in FOX-M soil without a formal review of Arctic microbial nutrient requirements and resulting optimal C:N:P ratios.

Higher nutrient levels in Alert compared to FOX-M soil were initially predicted by the increased mineralization response in Alert treatment blanks (Figure IV-1). Although higher than those in FOX-M soil, Alert background nutrient levels were still too low in P to account for the mineralization recorded. It is therefore suspected that some other mechanism (e.g. increased nutrient bioavailability) is responsible for the background mineralization observed. The discrepancy also suggested that the ratios recommended in the literature may not accurately reflect nutrient requirements and delivery mechanisms in Arctic soil environments.

The fertilizer microcosm experimental findings indicated that optimum levels for the commercial fertilizer examined (Figures IV-1 & IV-2) were below those recommended in literature; one half and one quarter of the recommended concentration for FOX-M and Alert soils, respectively. Lower fertilizer requirements would translate directly into lower nutrient requirements and therefore lower N:P ratios and suggest that the ratio could be as low as one quarter of the recommended level in certain soils (i.e. 100:3.25:0.75). Nevertheless, even with this lower ratio, background P levels would still have been inadequate.

The results of the grain size analysis confirmed initial qualitative assumptions regarding the clay and gravel nature of Alert and FOX-M soils, respectively. This relationship held true for soil compositions even after they were sieved for use in the microcosm experiments.

The different compositions of the two soils (e.g. high silt content in Alert soil) may have contributed to the unique mineralization responses observed in the microcosm experiments. Soil with high silt and clay levels have low porosity and permeability, which can affect the retention and attenuation of organic contaminants (Kostecki and Calabrese, 1990). These soil properties may have played a role in the unique biostimulant interactions observed in Alert soil (e.g. the ability of surfactants to overcome inhibitory fertilizer effects). Soil characteristics may also be linked to the apparent increase in TPH levels observed in Alert microcosms suggesting that silty soil types interfere with the degradation and/or monitoring of TPH.

2. Temperature effects

Temperature data gathered from FOX-M and Alert medium-scale biopiles revealed important thermal trends (Appendix E) including; 1) average temperatures at FOX-M were higher than average temperatures at Alert, 2) temperatures in Alert indoor treatments were higher than those in Alert outdoor treatments, and 3) distinct temperature gradients were observed within the Alert outdoor control pile towards the end of the monitoring period, several of which are discussed below. The absence of a significant correlation between elapsed time and reduction in TPH concentration at the medium scale for either site, however, makes it difficult to attribute thermal trends to aeration and/or heating treatment effects.

Alert thermal data did not offer conclusive evidence that the forced air heating system offered any pronounced temperature benefits (in its current design) compared to the passively aerated biopile (Figure E-2: Appendix E). Marginal temperature differences between outdoor and indoor biopiles suggest that non-insulated metal and greenhouse

enclosures do not drastically increase pile temperatures. However, the 15-18 day delay of sub-zero temperature onset does demonstrate the ability of such structures to extend treatment seasons by temporarily preventing the freezing of piles.

FOX-M thermal data revealed substantially higher average outdoor seasonal temperatures than Alert. This was anticipated given the given the more southerly location of FOX-M. All FOX-M biopiles displayed similar thermal profiles, with no clear benefits from either aeration system.

B. Discussion of supporting laboratory results

Biostimulation, the addition of emulsifying agents and bioaugmentation all positively stimulated mineralization of n-dodecane. The degree of stimulation was different for each type and concentration of soil additive, as well as soil type tested. Other biostimulation findings showed that both nutrient limitation and excess nutrient additions inhibited microbial degradation processes. Furthermore, experimental results proved that biological processes exclusively controlled and dominated the mineralization of ^{14}C-labelled HC in the microcosms.

The addition of fertilizer to FOX-M soils appeared to be slightly more beneficial to the mineralization process than in Alert soils. This could be due to the fact that the high silt content of Alert soil adversely affected fertilizer bioavailability: ionic fertilizer components are commonly bound by charged components within silt soil matrices impacting nutrient availability and transport (Kostecki and Calabrese, 1990).

Substantial mineralization was observed in the microcosm experiments incubated at 7° C. This demonstrated the relative benefits of degradation at temperatures similar to those encountered in the field. Past research (ESG, 1999) showed that microcosm incubation at 15° C further improved mineralization responses. Incubation at this higher temperature decreased lag periods and increased rates of mineralization of n-dodecane but had no impact on the final extent of mineralization. This suggests that incubation of future

microcosm experiments at 15° C vice the 7° C used in this study will afford a more complete view of the mineralization response. This is important for those microcosms that did not reach the stationary phase during the monitoring period, thereby restricting the calculation of Boltzman kinetic constants for all treatments and complicating the comparison process. It is still not clear whether optimal microcosm temperatures can be extrapolated to other HC in the TPH range or to field-scale treatments. These findings suggest that maintaining higher temperatures for future field experiments would increase TPH degradation over the initial phases, thereby shortening treatment times.

1. Commercial fertilizer microcosm experiments

The commercial fertilizer microcosm experiments revealed a distinct difference between the two soil types in their response to biostimulation. Inhibitory effects at high fertilizer concentrations were more dramatic in Alert soil, whereas for FOX-M soil, biostimulation produced a more pronounced mineralization response regardless of concentration. However, a reduction in the commercial fertilizer concentration (i.e. from the industry recommended standard level) improved the rate and extent of mineralization in both soils.

The lack of mineralization in sterile controls demonstrated that the addition of fertilizer itself was not responsible for the mineralization of ^{14}C-labelled HC and did not introduce significant numbers of foreign microbes. It also indicates that the mineralization detected in non-sterile experiments was due to microbial activity.

The Alert treatment blank showed that, even in the absence of biostimulation, indigenous microbes were still capable of mineralizing n-dodecane at background nutrient levels. Additional Alert mineralization results indicated that the standard concentration was not only excessive but appeared to inhibit the bioprocess that it was meant to stimulate. Reducing the concentration suggested that the optimal level was somewhere between one half and one quarter of the standard concentration given that the quarter level provided

short-term rate increases but the same final extent of mineralization within standard error measures.

The absence of mineralization in the FOX-M soil treatment blanks suggested that nutrient levels were too low for indigenous microbial populations to stimulate biodegradation of the ^{14}C-labelled HC. The different treatment blank responses in the two soils are difficult to explain if the microbial consortia are assumed to be the same in each. If two distinct populations were present, as is reasonable, the different results may be due to unique nutrient requirements, bioavailability and/or delivery mechanisms.

For FOX-M soil, mineralization results indicated that the standard concentration was excessive and possibly inhibitory. Reduction of fertilizer concentrations provided clear benefits for both the rates and final extents of mineralization. Up to the 25 day point, the half and quarter concentration treatments exhibited similar rates after which point the rate for the quarter concentration treatment began to decline. This suggested that fertilizer levels in the quarter concentration treatment were nearing exhaustion. It would appear that the half concentration significantly improved mineralization performance and that this treatment provided nutrient levels that were neither excessive nor limiting.

The performance increase with reduced commercial fertilizer concentrations is useful *a posteriori* in re-evaluating the use of twice the standard level of commercial fertilizer in the field-scale experiments. Although it is unclear whether the inhibitory effects of increased fertilizer concentration seen in microcosms can be extrapolated to the field scale, it is assumed that the use of this newly optimized level in future FOX-M treatments will improve bioremediation responses at all scales.

2. Bioremediation specific fertilizer microcosm experiments

The addition of bioremediation specific fertilizer produced moderate mineralization increases in both soils. Overall, mineralization rates and final extents were lower than those recorded in commercial fertilizer experiments.

The mineralization response for the optimal standard concentration of bioremediation specific fertilizer in FOX-M and Alert soil did not reach a maximum within the monitoring period. Given that mineralization was still occurring without any signs of inhibition, the final extent of mineralization had likely not yet been reached. This result could be linked to low metabolic rates for the uptake of nutrient formulation provided in this fertilizer. Further microcosm experiments should be undertaken to evaluate potential mineralization improvements over longer periods and/or at higher concentrations.

3. Surfactant microcosm experiments

Experimental results suggested that surfactants affect nutrient delivery in both Alert and FOX-M soils. This was demonstrated by the increased mineralization response in microcosms amended with surfactant plus the standard concentration of commercial fertilizer compared to those amended with the standard concentration of commercial fertilizer alone. The ability of surfactants to overcome commercial fertilizer inhibitory effects may be explained by the following mechanisms: 1) that surfactant binds excess fertilizer (thereby temporarily reducing bioavailability) and masks its inhibitory effects, or that 2) surfactant improves fertilizer distribution within the soil matrix. The latter may help prevent microbial burnout in hyper-accumulated zones and a lack of growth in underfertilized zones.

The ability of surfactant to reduce inhibitory effects suggests that higher levels of commercial fertilizer could be used in field applications. This would allow for a greater extent of bioremediation with commercial fertilizer levels that, in the absence of surfactant, would be inhibitory. This would translate into longer treatment periods with a single fertilizer dosage, reducing the need for multiple applications and eliminating the danger of nutrient limitation over long treatment periods.

Due to the inexplicable lack of mineralization response in the half and quarter concentration surfactant treatments for FOX-M soil, it is recommended that these experiments be repeated. However, from data collected, it appears that either 1)

inadequate fertilizer was added to the microcosms, 2) the microbes within the microcosm were dead, or that 3) viable microbes were present within the microcosm but were inactive.

Surfactant experiments were amended with the standard concentration of commercial fertilizer (except those testing the effect of surfactant alone). It is unclear what the optimum level of surfactant and consequent mineralization response would be if the newly optimized levels of commercial fertilizer were used. It remains to be seen whether the surfactant would interact with these optimal levels in an additive or synergistic manner. Surfactant experiments should therefore be repeated using optimal levels of commercial fertilizer in order to evaluate performance increases.

4. Microcosm inoculation experiments

The experiments that examined the effect of varying concentrations of inoculum on HC biodegradation in Alert soils suggested that the standard level of inoculum was most effective at promoting ^{14}C mineralization. When standard error measures were included, the standard level was only advantageous over the first 15 days after which point the error bars overlapped with the two diluted treatments. This was consistent with FOX-M data which exhibited shorter lag phases and increased mineralization rates for the higher inoculum concentration over the initial stages with similar final extents of mineralization for all concentration levels (ESG, 1999). This suggests that lower inoculum concentrations would be more cost effective given long enough treatment periods.

When the results of the treatment control (undiluted inoculum in sterile soil) and the standard inoculum concentration (in unsterile soil) were compared, equal mineralization responses (within standard error measures) were found. This suggested that indigenous populations contributed little to the mineralization of n-dodecane and that the inoculated consortium dominated the mineralization process. The most obvious explanation for this is the fact that inoculated populations had been selectively enriched for HC degraders making them genetically predisposed to mineralize the n-dodecane more efficiently.

Other potential explanations for the similarity between the mineralization responses of the standard inoculum in sterile and non-sterile soil include; 1) inoculated consortia were better adapted to standard commercial fertilizer and cryoprotectant levels which proved inhibitory to indigenous populations, 2) indigenous populations may not have been able to acclimate and reproduce effectively in the presence of the high microbial populations of the inoculated consortia (i.e. unable to compete for nutrient and substrate supplies). All of these potential factors give evidence that inoculated microbes differed from indigenous populations with respect to metabolic ability.

The addition of just cryoprotectant to sterile soil did not promote the mineralization of n-dodecane and thus, did not introduce significant microbial contaminants. In non-sterile soil, cryoprotectant appeared to interfere with metabolic pathways in indigenous populations. This is supported by the results of the treatment effect control (fertilized, non-sterile soil with cryoprotectant) which produced an average maximum of 4.7% ^{14}C-labelled HC mineralization compared to an average of 11.5% in comparable treatments amended with equal levels of commercial fertilizer without cryoprotectant (Figure IV-1). The negative impact of cryoprotectant on mineralization of n-dodecane may be attributable to: 1) competitive metabolism (i.e. microbes preferentially degrading the cryoprotectant over n-dodecane components) or 2) toxic inhibition from high cryoprotectant concentrations that indigenous populations had not previously been exposed to, or 3) selective enrichment for non-HC degrading populations that used the cryoprotectant as a growth substrate.

5. Comparison of FOX-M and Alert microcosm soil additive experiments

A comparison of FOX-M and Alert microcosm soil additive experiments was conducted by selecting the most effective amendment concentration from each. The highest ^{14}C mineralization responses from the various experiments in each soil are presented graphically as Figures V-1 & V-2.

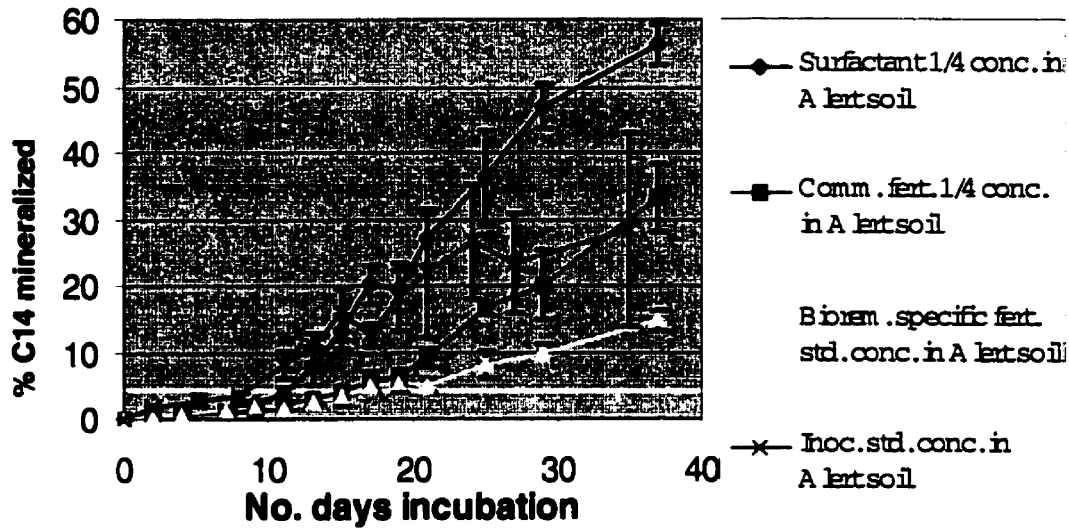


Figure V-1: Comparison of the impacts of optimal soil additives on ^{14}C mineralization in Alert soil. Error bars represent +/- standard error of the mean. Error measures on individual ^{14}C mineralization curves are less than reportable increment.

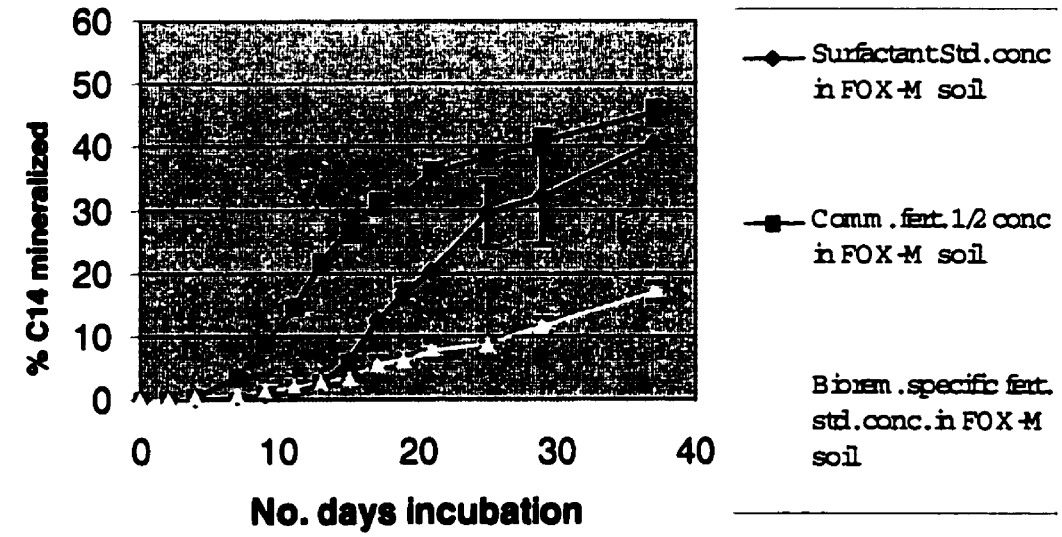


Figure V-2: Comparison of the impacts of optimal soil additives on ^{14}C mineralization in FOX-M soil. Error bars represent +/- standard error of the mean. Error measures on individual ^{14}C mineralization curves are less than reportable increment.

The Alert soil microcosm experiments that displayed the largest effects on mineralization (Figure V-1) were surfactant at one quarter of the standard concentration amended with the standard concentration of commercial fertilizer, commercial fertilizer at one quarter of the standard concentration, bioremediation specific fertilizer at the standard concentration, and inoculum at the standard (undiluted) concentration.

Of these amendments, the addition of one quarter of the standard level of synthetic surfactant combined with the standard level of commercial fertilizer produced the greatest increase in ^{14}C-labelled HC mineralization in Alert soil over the monitoring period. This is a four-fold increase over standard commercial fertilizer alone and a 16-fold increase over the mineralization response in unfertilized soil. When standard error is included, the standard inoculum concentration performed the same as the surfactant treatment for the first 27 days after which point the surfactant treatment exhibited a superior mineralization response.

For FOX-M experiments, the optimal additive concentrations that were selected from each experiment were surfactant at standard concentration amended with the standard concentration of commercial fertilizer, commercial fertilizer at one half of the standard concentration, and bioremediation specific fertilizer at the standard concentration.

FOX-M microcosm results revealed the strongest mineralization response with one half of the standard level of commercial fertilizer over all other additives and concentrations tested. These experiments also exhibited a shorter lag period, greater initial rates and a greater final extent of mineralization.

6. TPH versus ^{14}C removal experiments

TPH versus ^{14}C removal experiments demonstrated that the use of radiolabelled compounds was a useful tool for modeling TPH degradation in soil. Experimental results revealed a gap between ^{14}C and TPH removal curves and an apparent increase in TPH levels over time in silty soil.

The most intriguing result from the FOX-M comparison experiments was the gap between the TPH degradation and ^{14}C mineralization curves. It can be attributed to several factors, including; 1) TPH is a measure of a broad range of HC, whereas the ^{14}C method measures only a small component of the HC range (C_{12}; dodecane) and, therefore, only a small range of metabolic activity, 2) the accepted approach to the biochemistry of aerobic HC biodegradation suggests that if one C atom is mineralized then complete mineralization of the entire HC will follow (Mohn, 1999). Since only one C atom in the dodecane compound is labelled, radiolabelled CO_2 evolution only gives evidence of partial HC mineralization. For every labelled C atom mineralized, the subsequent mineralization of the rest of the HC would go unrecorded, 3) the use of the diesel fuel solvent to dilute the n-dodecane introduced an alternate C source that may have been preferentially degraded over the radiolabel, 4) the addition of the ^{14}C compound and diesel fuel increased the metabolic burden within the soil over and above initial TPH levels present. Microbes resident in the soil would already have undergone selective enrichment for the original TPH compounds thereby preferentially degrading it over the newly introduced HC components in the radiolabelled mixture, 5) mineralization of a radiolabelled HC is a much more complex process to track than the degradation of TPH. Some of the ^{14}C-labelled HC gets incorporated into biomass during mineralization. This portion of the HC does not produce radiolabelled-CO_2 and, as such, does not get recorded as having been degraded. Conversely, all of the TPH change would be recorded as a loss whether the HC is partially degraded, mineralized or assimilated into biomass.

Alert soil microcosm TPH degradation results were surprising, giving an overall increase in TPH levels over the monitoring period. The overall TPH increase is believed to reflect difficulties in the extraction process and/or the desorption of HC products from interstitial soil pores as degradation proceeded. It is assumed that desorption released TPH components from the silty soil that were previously 'hidden' to the analytical extraction process thereby creating a false TPH profile where HC components appeared to be created and not destroyed. This process could be assisted by the release of microbially produced surfactant compounds (Kosaric, 1993). Another feasible explanation is that the

clay components within the soil caused partitioning of TPH between the interstitial and surficial areas of the soil. The equilibrium between absorbed/adsorbed and available fuels must be kept in mind when measuring and monitoring the degree of available TPH within the complex matrix of clay and silt soils. Once the available surface TPH is degraded, equilibrium is reestablished by the release of more TPH from interstitial spaces where the hydrophobic HC compounds reside. In the assay, this appears as an increase, whereas the initial degradation responsible for the desorption is not recorded.

In summary, the results for FOX-M soil demonstrated that both radiolabelled HCs and TPH measurements can be used to monitor microbial degradation. The accuracy of these measures as a comparative tool is demonstrated by the similar shape of the two curves. The techniques differ in sensitivity (as demonstrated by the gap between the two curves) and the differences suggest that there may be difficulties in extrapolating ^{14}C microcosm results to TPH data from field work. Additional findings from the Alert comparison experiments underlined the difficulty in monitoring TPH degradation in silty soil. This finding reinforced the need to quantify the physico-chemical parameters of all soils prior to laboratory or field investigations to predict these types of monitoring anomalies.

C. Discussion of field results

Field experimental results revealed that all biopile and landfarm experiments underwent significant changes in TPH levels over time. Only in the case of the FOX-M small-scale uncovered control piles and uncovered landfarm control treatments was the decrease in TPH attributable to a treatment effect over time. This interaction highlighted the strong effect of volatilization on TPH degradation in uncovered treatments. The small-scale biopile experiments exhibited an additional time by pile within treatment interaction (i.e. significant heterogeneity between piles within treatments over time).

In general, field experiments showed no significant HC bioremediation benefits from any of the soil aeration, heating or amendment treatments. Nonetheless, the lessons learned

regarding the effects of volatilization and the experimental design and the associated statistical implications discovered are invaluable.

1. Medium-scale experiments

The presence of a time interaction in the medium-scale experiments proved that piling of soil alone served to significantly reduce TPH over a short summer treatment season. Anova analysis indicated that the aeration systems used at Alert and FOX-M had no significant impact on TPH removal. It was originally assumed that active aeration would reduce TPH levels by increased vapour stripping as suggested by field observation of VOCs in the FOX-M vacuum exhaust stream.

There are several reasons why the aeration systems may have been ineffective, including: 1) there was no way of evaluating whether volumetric air flows in the active aeration systems and/or the air manifold setup were capable of providing increased O_2 supply over passive O_2 penetration, 2) the use of the wooden pallets may have created preferential flow pathways through the sides of the pile that short-circuited airflow from the main body of the pile, and finally 3) there was no way of measuring airflow dynamics or flow pathways within the pile.

2. FOX-M small-scale experiments

Small-scale experiments also demonstrated that volatilization plays an important role in TPH loss. This was evident in the significant decrease in the uncovered controls. Anova analysis without the treatment effect of the uncovered control piles indicated that the balance of the treatments, aimed at promoting bioremediation processes, actually produced significant TPH level increases (α= 0.01) over the monitoring period. It is believed that these increases are due to augmented availability of HC components within the soil, possibly as a consequence of biological activity (e.g. microbial surfactant production) or due to simple partitioning of TPH components following initial TPH removal. This anomaly may also be linked to the same mechanisms responsible for the

TPH increases observed in the Alert microcosm TPH experiments. Consultation with industry suggested that this effect has been seen by others. In these observations, surfactants produced sharp increases in detected TPH levels shortly after application due to increased desorption of HC components from soil matrices (Yick, 1998).

3. FOX-M landfarm experiments

The presence of significant time and time by treatment interactions suggest several factors that may influence landfarm remediation. However, similar final TPH levels for each treatment make it difficult to pinpoint which treatment was responsible for the interactions. The presence of 1) the highest mean TPH decrease in the uncovered control plots and 2) the added evidence from the treatment effect observed in the small-scale uncovered control piles suggested that volatilization in the uncovered landfarm controls played a key role.

Additional landfarm data emphasized the impact of volatilization on TPH loss. This was evident in the mean initial FOX-M landfarm concentrations compared to those of the small and medium-scale FOX-M biopiles. For example, consider the mean initial TPH concentration for all treatments in the FOX-M small-scale biopiles of 1939 ppm +/- 732 ppm (std. dev.) compared with the mean 713 ppm +/- 740.5 ppm TPH concentration for all treatments in the FOX-M landfarm. This suggested that large amounts of TPH were removed from the soil through the additional mechanical manipulation, manual raking and waiting period (5-days during which the soil was left in the uncovered stockpile) associated with landfarm construction.

The difference in initial concentrations after five days of additional physical manipulation underlines the effect of mechanical 'remediation'. In addition, it suggests that different degradative mechanisms and kinetic effects may have been present in the different experiments (i.e. medium and small-scale biopiles and landfarms). This could have affected the extent and mechanism (i.e. physical or biological) of TPH degradation in each experiment. Furthermore, lower initial concentrations in a given treatment would

demand different microbial responses as well as the correspondingly altered nutrient requirements.

4. Statistical and experimental design considerations

Field-scale findings also identified a high degree of variance in TPH concentrations present, even in homogenized soil. There are several possible explanations for the large variances in TPH levels detected in the field experiments. The primary factors, abiotic, biotic and experimental, are presumed to be; 1) differences in initial microbial populations and metabolic activities, which produce degradation gradients within the soil over time, 2) heterogeneity of soil particle composition, and/or 3) contaminant gradients within the soil.

The high variance recorded in the TPH data indicates that soil homogeneity in the treatments could have been improved. Increased homogeneity would lower standard deviations and increase the precision of analytical tools. This would most easily be achieved through more thorough mixing of soil prior to treatment construction with the use of heavy equipment for bulk homogenization. The incorporation of amendments in lifts during construction could be replaced with bulk amendment addition concurrent to the homogenization of soil prior to construction. This would ensure that amendments impact all parts of the treatment cell equally. All of the procedures would, of course, also increase TPH loss due to volatilization.

The variance recorded in the field-scale treatments was used to calculate the sample size (n) required to detect a true difference of a given magnitude between treatments. By translating this finding into a hypothetical field scenario, it was determined that in order to detect a true TPH concentration difference of 20% ($p = 0.8$, $\alpha = 0.05$) between two of three populations (i.e. treatments, piles or plots) with a coefficient of variance of 25%, the minimum required sample size would be 26 samples/treatment (Appendix G). This scenario would be equivalent to wanting to detect a 1000 ppm difference between two of three biopiles with an average initial TPH concentration of 5000 ppm and a standard

deviation of 1250 ppm. By extension, this calculation shows that as the desired resolution level increases (e.g. wanting to detect smaller TPH differences) so to does the required sample size.

The most significant finding highlighted by the Anova analysis was the presence of a time by pile within treatment interaction in the FOX-M small-scale biopiles. This indicated that the response of piles through time was significantly heterogeneous even within treatments. This result stressed the necessity for duplication at the pile level within treatments (i.e. all treatments must be evaluated in duplicate piles/plots). In cases where duplication is not possible, treatment effects from piles could potentially be confounded with those from the treatments themselves. This eliminates the ability to detect whether changes in concentration for a given pile over time are due to the effect of the treatment or merely the idiosyncratic nature of the piles themselves.

Experimental design improvements from the synthesis of the time by pile within treatment interaction in chorus with higher replication within treatments (e.g. n=26), will increase the efficiency and precision with which future field investigations can be executed.

5. Volatilization considerations for landfarming approaches

A powerful finding from the field experiments was the strong role of volatilization in TPH degradation highlighted by the treatment by time interaction for the uncovered small-scale and landfarm control treatments. This was attributed to the effects of mechanical soil mixing/manipulation and treatment in uncovered soil piles/plots.

The loss of volatile TPH components and its role in the apparent degradation of HC contaminants in soil are well documented (Stiver and Mackay, 1984). The findings from the research in this thesis underline the importance of such mechanisms. Volatilization, while resulting in significant HC loss, has important environmental, regulatory and human health considerations. These factors were examined by an evaluation of similar southern environmental scenarios. There appear to be no standards or guidelines that

specifically address this issue in a soil treatment context at either the provincial or federal levels. There are, however, Ontario Ministry of the Environment and Energy (MOEE, 1994) standards regulating emissions of VOCs from a point of impingement (POI) approach. These standards stipulate maximum BTEX emission levels for smokestacks and industrial air effluent streams. These standards can also be applied to volatilization of VOCs from soil. In order to make direct comparisons, treatment cells must be treated as equivalent POI or discrete air effluent sources. Using this approach and the data collected for the TPH decrease that occurred during the construction of the FOX-M landfarm, a quantitative mass/volume value can be calculated and compared to the POI standards. This calculation yields a value of 2000 $\mu g/m^3$ (30 min. avg.) for a 1400 ppm TPH loss over a 72 m^2 landfarm plot during the first five days of operation. Comparing this to the POI standards and noting that BTEX would only form a fraction of the TPH, it is well within the range of the 4000, 2000, 2300 $\mu g/m^3$ (30 min. avg.) limits for ethylbenzene, toluene, and xylene, respectively. HC contaminated sites with higher initial soil contamination levels and/or more volatile fuel components could feasibly produce VOC emissions in excess of these criteria and consequently pose legitimate environmental hazards.

A further comparison can be made by considering how the volatilization issues are dealt with in southern regions. In the case of diesel fuel spill response in Ontario, for example, absorbent materials (sand and soil) used to confine and collect the spills are then used as cover material at regulated landfill sites (MOEE, 1999). This is explicitly done so that the diesel fuel will volatilize from the soil. This could conceivably imply that such practices are deemed to be environmentally safe. It is important to note, however, that key issues of leachate containment and proximity to potential receptors are inherently mitigated with treatment at regulated landfill sites. Arctic treatment scenarios do not often afford these safeguards. If leachate collection and adequate buffer zones (i.e. between the treatment area and local receptors) can be properly incorporated into the remediation design, it is feasible to assume that similar methods could be employed in the Arctic.

Specific guidelines and criteria governing VOC emissions from soil remediation operations are still under development. For the present time, such operations must strive to protect air quality, control leachate emissions and ensure that adequate buffer zones are in place. The following quote from the MOEE - Guideline for Use at Contaminated Sites in Ontario outlines the guiding principles that will help shape future legislation:

"The ongoing, uncontrolled release of volatile compounds to the air as part of a remedial action is not acceptable... every effort should be made to recover volatile contaminants and prevent release to the atmosphere." (CCOHS, 1998).

This type of statement provides a conceptual framework for future designs in the temporary absence of formal guidelines. Given the lack of clear direction at this juncture, it is essential that a cautionary and due diligent approach be taken.

VI. CONCLUSIONS

Results from the laboratory microcosm experiments focussed primarily on micro-scale bioremediation parameters: those dealing with soil additives and microbial degradation kinetics. The optimal concentration and type of amendment (fertilizer, surfactant and inoculum) by soil type were investigated and successfully identified. These results highlighted the need for customized amendment strategies that account for the specific physico-chemical soil characteristics and nutrient profiles at different sites. The radiolabelled-HC mineralization studies also gave evidence of nutrient inhibition from both limited and excess levels and demonstrated the ability of synthetic surfactants to overcome this inhibition in a silt soil. Difficulties in monitoring and modeling TPH degradation in silt-based, weathered soil were also demonstrated through comparisons with ^{14}C-labelled mineralization.

Field-scale experiments (small, medium-scale biopiles and landfarm treatments) focussed on macro-scale bioremediation parameters, investigating elements of experimental and process design. In addition, these experiments confirmed the microcosm findings of

nutrient inhibition and the key role played by specific physico-chemical soil characteristics.

From an experimental and statistical design perspective, field-scale results confirmed the high variance in TPH levels inherent to fuel contaminated Arctic soil. They also suggested that increased homogeneity would increase the power and precision of analytical tools. This knowledge was translated into design improvements involving increased sample and treatment replication.

The power of degradation through volatilization as a result of both mechanical soil mixing/manipulation and treatment in uncovered soil piles/plots was also demonstrated. After a review of related environmental, regulatory and human health aspects, it was determined that treating contaminated soils with combined soil spreading and biopiling approaches is feasible as long as VOC and leachate issues are properly addressed. Guidelines for this type of treatment are currently under review and future designs should incorporate the latest guidelines from a due diligence perspective.

The incorporation of all the major findings at the micro and macro-scale provides strong direction for future work in this field. In particular, this research demonstrates that, if VOC emissions are shown to be below BTEX emission standards, landfarming in the presence of the recommended environmental safeguards could be used for remediation of volatile HC components in the soil. The heavier, more recalcitrant compounds, more efficiently treated by biological means, could then be treated in a biopile cell. These biopile cells would incorporate the optimized type and concentrations of soil additives (accounting for site-specific physico-chemical characteristics and biostimulation impacts) developed in the microcosm experiments along with the next generation of aeration/heating system. The newly devised experimental design elements of higher sample replication and duplication within treatments would then be incorporated in order to more effectively study the efficiency of these systems and magnify the power of future findings.

The data collected in the various experiments expand our knowledge of bioremediation at all levels. This research accomplished the objectives of this thesis: to investigate and optimize the impact of several bioremediation parameters and identify areas for future research needed to make aboveground engineered soil treatment systems an effective means of cleaning up fuel spills in the Arctic.

In closing, recommendations for future work in this area are to:

1. Incorporate findings for optimal type and concentration of soil amendments into upcoming large-scale treatment designs and future laboratory investigations. It is recommended that Alert experiments make use of one quarter of the standard level of surfactant combined with the standard level of commercial fertilizer. For FOX-M experiments, one half of the standard level of commercial fertilizer is recommended for best results.

2. Further optimize aeration and heating systems for large-scale treatments in order to evaluate effects of augmented oxygen supply and treatment temperatures.

3. Increased homogenization of soil, contaminant, and amendment distribution prior to pile construction is recommended in order to reduce analytical variance and avoid toxic inhibition in super-concentrated zones.

4. Increase the number of samples collected from each treatment pile/plot in order to have the statistical power to detect significant changes between piles. It is therefore recommended that no less than 26 samples per pile/plot be collected at each sampling.

5. Increased replication between piles/plots within treatments is required to overcome the heterogeneous behaviour within treatments over time. It is recommended that treatments at all scales be duplicated.

6. In light of evolving guidelines for landfarming VOC emissions, it is recommended that a cautionary approach be taken until formal guidelines are promulgated. Until that time, combined landfarming and biopiling approaches are recommended if leachate and VOC emission hazards are mitigated through the use of lined treatment facilities, adequate buffer zones and evaluation of airborne BTEX concentrations.

VII. REFERENCES

Allen, M.R. and Riddell, E.G. (1997) Biopile Remediation of Hydrocarbon Contaminated Soils in the Arctic. Royal Military College of Canada Undergraduate Thesis: CME-CCE-CM417-5-97.

Atlas, R.M. (1986) Fate of Petroleum pollutants in Arctic ecosystems. *Water Science and Technology* **18**(2), 59-67.

Atlas, R.M. and Bartha, R. (1993) Microbial Ecology: Fundamentals and Applications, 3rd ed., Benjamin/Cummings Publ. Co., CA.

Burt, P. (1991) Barrenland Beauties: Showy Plants of the Arctic Coast, Outcrop Ltd.

Canadian Centre for Occupational Health and Safety (CCOHS) (1998) Canadian Enviro-OSH Legislation plus Standards - CD-ROM.

Canadian Council of Ministers of the Environment (CCME) (1991) Interim Canadian Environmental Quality Criteria for Contaminated Sites.

Canadian Council of Ministers of the Environment (CCME) (1997) Recommended Canadian Soil Quality Guidelines, ISBN 1-895-925-92-4.

Canadian Environmental Protection Act (CEPA) (1994) Government of Canada, Environment Canada, Health Canada: Priority Substance Lists: PAH.

Canadian Environmental Protection Act (CEPA) (1993) Government of Canada, Environment Canada, Health Canada: Priority Substance Lists: BTEX.

Cook, F.D. and Westlake, D.W.S. (1973) Biodegradability of Northern Crude Oils, Environmental-Social Program Northern Pipelines, Task Force on Northern Oil Development, Report #73-20.

Cook, F.D. and Westlake, D.W.S. (1974) Microbiological Degradation of Northern Crude Oils for the Environmental-Social Program Northern Pipelines, Task Force on Northern Oil Development, Report #74-1.

Cookson, J.T. (1995) Bioremediation Engineering: Design and Application, McGraw-Hill Inc.

Crawford, R.L. and Mohn, W.W. (1985) Microbiological removal of pentachlorophenol from soil using a *Flavobacterium*. *Enzyme Microbial Technology* **7**, 617-620.

Davis, K.J. and Russell, D.J. (1993) Soil treatment technologies combined. *Pollution Engineering* **July**, 54-58.

Emond, J.P. (1962) Performance of Fuels. Course Materials (Reprinted with permission by the Royal Military College of Canada).

Environmental Sciences Group (ESG) (1998) Hydrocarbon Bioremediation of Arctic Sites. RMC-CCE-ES-98-10.

Environmental Sciences Group (ESG) (1999) Experimental Bioremediation Technologies for Hydrocarbon and PCB Contaminated Soils at Arctic Sites. RMC-CCE-ES-99-19.

Environmental Working Group (EWG) (1997) Second Report of the DEW Line Cleanup EWG.

Flathman, P.E., Jerger, D.E. and Exner, J.H. (1994) Bioremediation Field Experience, Lewis Publishers., Boca Raton.

Finnerty, W.R. (1994) Biosurfactants in environmental biotechnology. *Curr. Opion. Biotechnol.* **5**, 291-295.

Freedman, W. and Hutchinson, T.C. (1975) Physical and biological effects of experimental crude oil spills on low Arctic tundra in the vicinity of Tuktoyaktuk, N.W.T., Canada. *Canadian Journal of Botany* **54**, 2219-2230.

Geotechnical Science Laboratories - Carleton University (1994) Report to Department of National Defence: Study of movement of hydrocarbons through freezing and thawing soils – DEW Line Cleanup, Final Report.

Government of the Northwest Territories (GNWT) (1994) Guideline for Contaminated Site Remediation in the N.W.T., Department of Renewable Resources.

Hutchinson, T.C., Hellebust, J. and Telford, M. (1994) Oil Spill effects on vegetation and soil microfauna at Norman Wells and Tuktoyaktuk, N.W.T., Environmental-Social Program Northern Pipelines, Task Force on Northern Oil Development, Report # 74-14.

Jensen, J., Adare, K. and Shearer, R. (Eds) (1997) Ecosystem Uptake and Effects. *Canadian Arctic Contaminants Assessment Report.* Indian and Northern Affairs Canada, Ottawa.

Kershaw, G.P. and Kershaw, L.J. (1986) Ecological characteristics of a 35 year old crude-oil spills in tundra plant communities of the Mackenzie Mountains, N.W.T. *Canadian Journal of Botany* **64**, 2935-2947.

Kosaric, N. (Ed) (1993) Biosurfactants – Production, properties and applications, Marcel Dekker Inc., New York, NY.

Kostecki, P.T. and Calabrese, E.J. (1990) Petroleum Contaminated Soils-Vol. 1 & 3, Lewis Publishers.

Leahy, M.C. and Brown, R.A. (1994) Bioremediation: Optimizing Results. *Chemical Engineering* **May**, 108-115.

Lei, J., Sansregret, J.L. and Cyr, B. (1994) Biopiles and biofilters combined for soil cleanup. *Pollution Engineering* **Jun**, 56-58.

Lynn, J. (1998) Elf Acquitaine – Atochem, U.S.A. Personal Conversation.

Mackay, D., Ng, T.W., Shiu, W.Y. and Reuben, B. (1980) The Degradation of Crude Oil in Northern Soils. Environmental Studies #18, Environment Canada.

Mohn, W.M. (1998 & 1999) University of British Columbia. Personal Conversation.

Ontario Ministry of the Environment and Energy (MOEE) (1994) Summary of Point of Impingement Standards, Ambient Air Quality Criteria and Approvals Screening Levels, Standards Development Branch.

Ontario Ministry of the Environment and Energy (MOEE): Abatement section (1999) Personal Conversation – John Boyle.

Reynolds, C.M., Braley, W.A., Travis, M.D., Perry, L.B., Iskandar, I.K. (1998) Bioremediation of Hydrocarbon-Contaminated Soils and Groundwater in Northern Climates. US Army Corps of Engineers: Cold Regions Research & Engineering Laboratory.

Savage, G.M., Diaz, L.F. and Golueke, C.G. (1985) Disposing of organic hazardous wastes by composting. *Biocycle* **26**, 31-34.

Sextone, A.P., Gustin, T. and Atlas, R. (1978) Long term interactions of microorganisms and Prudhoe Bay crude oil in tundra soils at Barrow, Alaska. *Arctic* **31** (3), 348-354.

Stiver, W. and Mackay, D. (1984) Evaporation rate of spills of hydrocarbon and petroleum mixtures. *Enviro. Sci. and Technol.* **18**, 834-840.

Stoner, DL. (ed.) (1994) Biotechnology for the Treatment of Hazardous Waste, CRC Press Inc., Boca Raton, FLA.

Thangamani, S. and Shreve, G.S. (1994) Effect of anionic biosurfactant on hexadecane partitioning in multi-phase systems. *Environ. Sci. Technol.* **28**(12), 1993-2000.

Torma, A.E. (1994) The basics of bioremediation. *Pollution Engineering* **June**, 46-47.

von Fahnestock, F.M., Kratzke, R.J., Major, W.R. and Wickramanayake, G.B. (1998) Biopile Design, Operation and Maintenance Handbook for Treating Hydrocarbon-Contaminated Soils, Batelle Press.

Yick, B. (1998) Biosolve Canada. Personal Conversation.

Young, L.Y. and Cerniglia, C.E. (eds.) (1995) Microbial Transformation and Degradation of Toxic Organic Chemicals, Wiley-Liss Inc., New York, NY.

APPENDIX A

UBC EXPERIMENTAL RESULTS

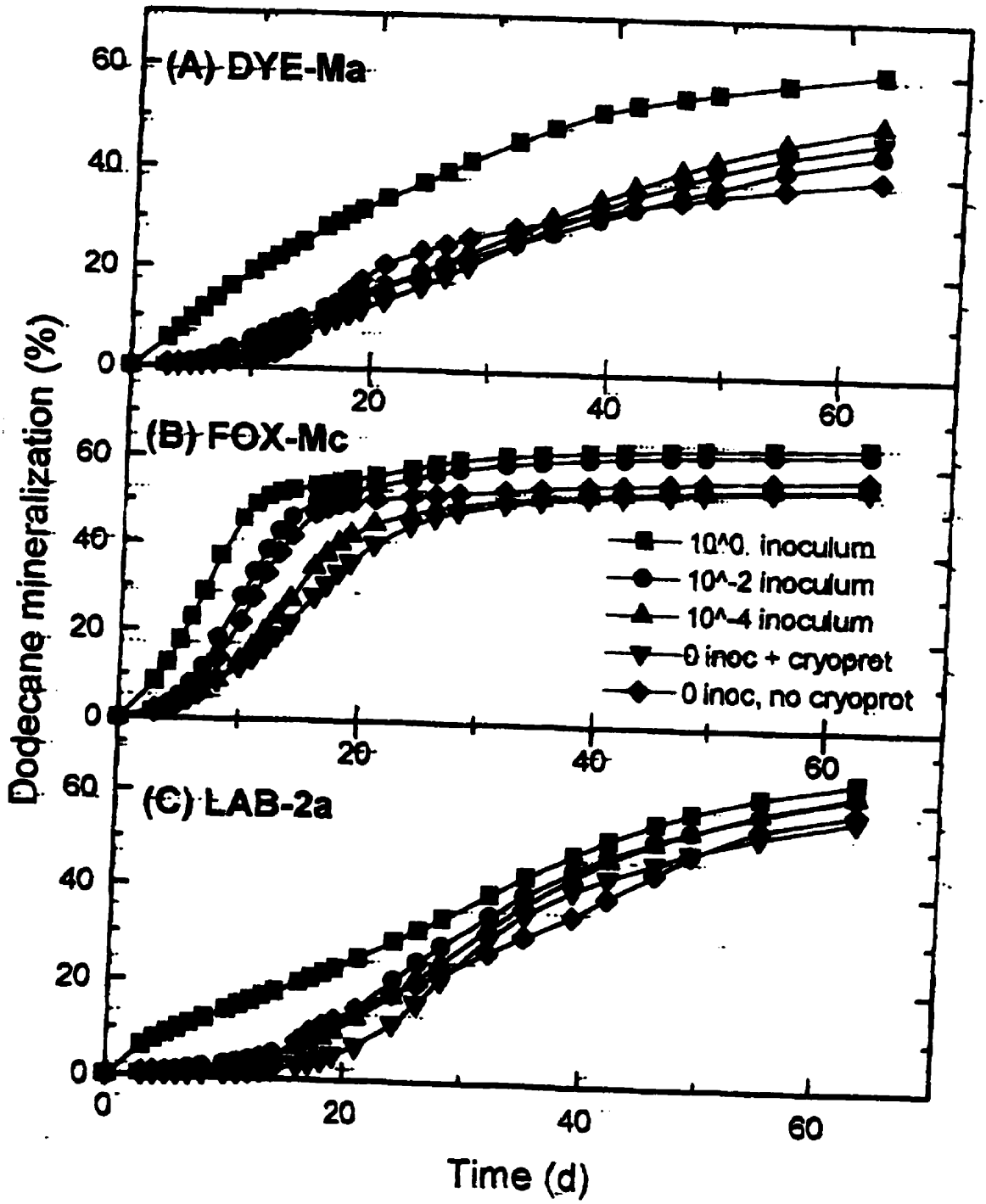
Table 1. Characteristics of soil samples and kinetic constants for dodecane mineralization in each soil sample.

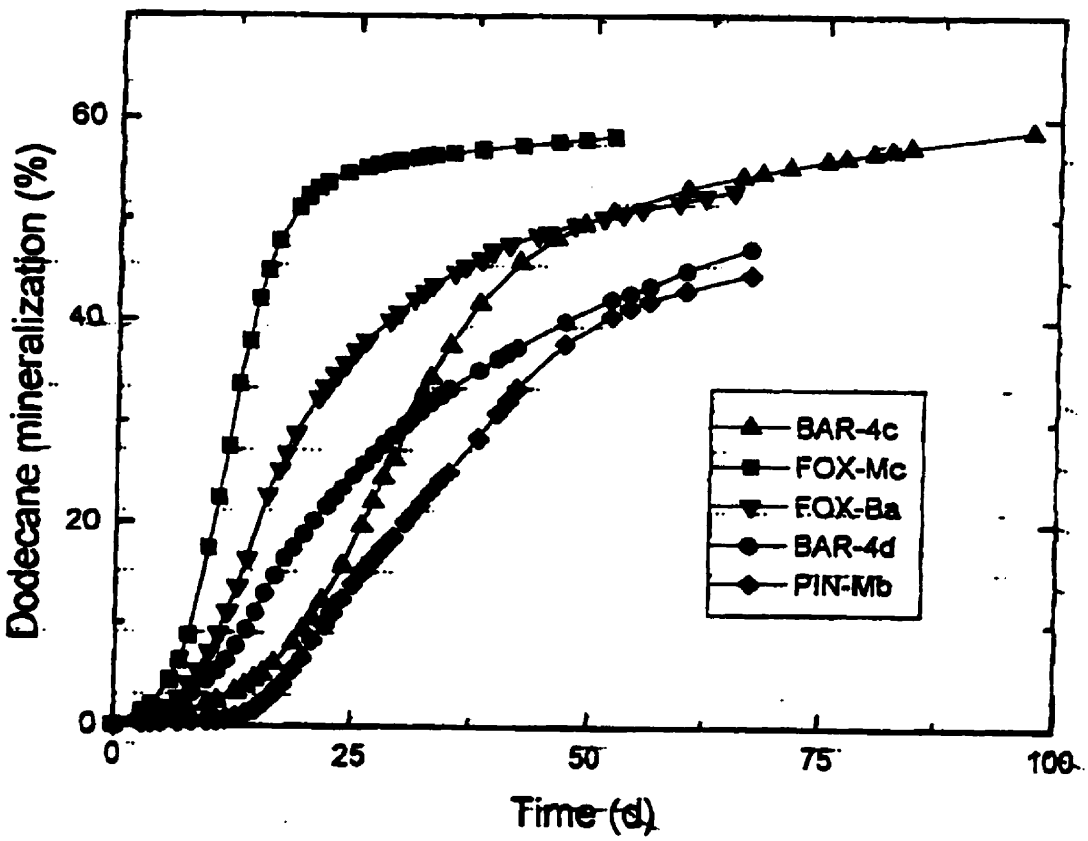
Soil	TPH	WHC	pH	C	OC	N	P	% Composition				Metals (μg/g)							Lag	T_{mid}	k	Y_{max}
	(μg/g)	(g/g)		(%)	(%)	(μg/g)	(μg/g)	Gravel	Sand	Silt	Clay	Cu	Ni	Co	Pb	Zn	Cr	As	(d)	(d)	(d^{-1})	(%)
BAR-1a	11500	0.18	7.3	2.7	0.73	400	1.3	50.0	85.2	11.2	3.6	20.2	21.2	0.0	16	60	27	5.1	5.0	35	0.07	66
BAR-4a	25200	0.33	7.4	7.4	6.83	1000	2.6	15.1	68.7	15.1	16.2	15.9	21.9	8.5	21	108	0	12.9	2.0	11	0.34	43
BAR-4b	8020	0.18	7.7	2.4	2.25	300	4.6	26.0	88.8	6.9	4.3	9.6	14.3	6.4	11	59	24	14.8	7.4	22	0.31	44
BAR-4c	24100	0.24	7.0	4.2	4.25	300	9.5	11.0	89.6	5.3	5.1	11.4	22.0	0.0	1443	514	30	7.5	6.0	29	0.17	51
BAR-4d	1230	0.29	7.8	2.1	1.47	800	5.6	13.8	78.2	12.7	9.1	20.2	22.6	7.8	23	92	0	12.8	4.0	17	0.21	30
BAR-4e	115	0.18	7.8	0.6	0.59	200	12.0	19.5	95.2	4.2	0.6	16.2	13.7	5.7	0	33	0	18.7	11.4	27	0.15	59
CAM-4a	9830	0.20	6.9	1.0	0.95	500	2.0	58.8	93.7	4.7	1.6	5.9	5.9	7.8	135	51	32	0.9	5.6	31	0.12	56
CAM-4b	4400	0.22	6.6	1.0	1.00	600	2.0	40.4	82.4	12.3	5.3	8.0	5.8	8.7	10	60	27	1.0	4.2	28	0.14	60
CAM-4c	2020	0.24	6.8	1.4	1.40	1000	2.3	31.6	81.2	11.2	7.6	11.6	8.7	8.7	0	53	42	0.9	2.6	23	0.15	61
DYE-Ma	250	0.17	6.8	0.2	0.15	200	2.6	17.8	83.8	14.2	3.0	24.8	27.2	7.8	10	49	87	0.4	7.5	12	0.60	40
FOX-Ba	2060	0.46	5.6	1.5	1.50	800	6.1	7.0	68.3	27.2	4.5	34.6	28.5	10.0	10	92	65	18.8	5.0	15	0.27	39
FOX-Bb	9120	0.44	5.3	1.5	1.50	900	6.1	11.8	75.0	20.9	4.1	27.0	27.9	9.2	0	89	64	16.6	4.0	21	0.06	64
FOX-Ma	14400	0.14	7.9	11.7	1.96	300	0.0	45.0	77.0	18.4	4.6	5.1	5.7	0.0	10	22	0	1.7	4.9	16	0.51	37
FOX-Mb	12600	0.12	7.8	12.7	4.30	400	0.0	45.3	68.6	24.4	7.0	5.0	11.9	0.0	40	90	26	2.0	7.7	20	0.37	41
FOX-Mc	46000	0.08	7.2	15.3	6.21	300	0.3	39.5	68.8	22.9	8.3	8.4	6.2	0.0	36	68	0	1.7	2.3	12	0.38	55
LAB-2a	5370	0.17	5.9	0.2	0.15	200	3.8	29.5	89.5	9.2	1.3	18.2	27.2	6.8	20	33	72	0.8	11.0	49	0.08	61
PIN-Mb	195	0.15	8.2	8.9	0.30	200	1.0	31.8	88.2	10.0	1.8	7.1	0.0	0.0	0	35	0	2.2	13.2	25	0.13	34
PIN-Mc	6390	0.19	7.8	8.6	0.81	200	6.9	19.9	89.1	9.5	1.4	14.2	0.0	0.0	103	67	0	1.7	7.0	13	0.47	29

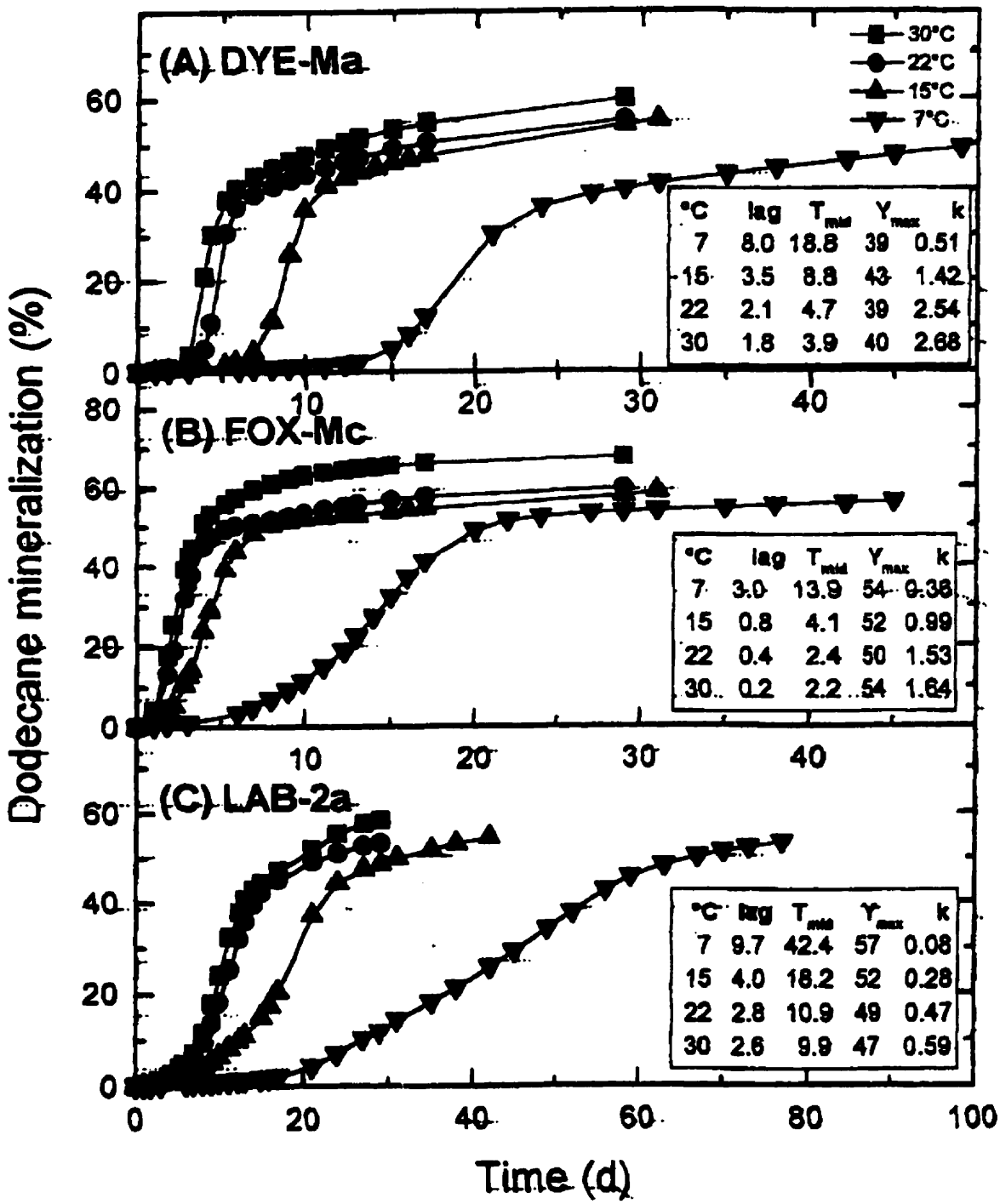
Note: WHC = water holding capacity
OC = organic content

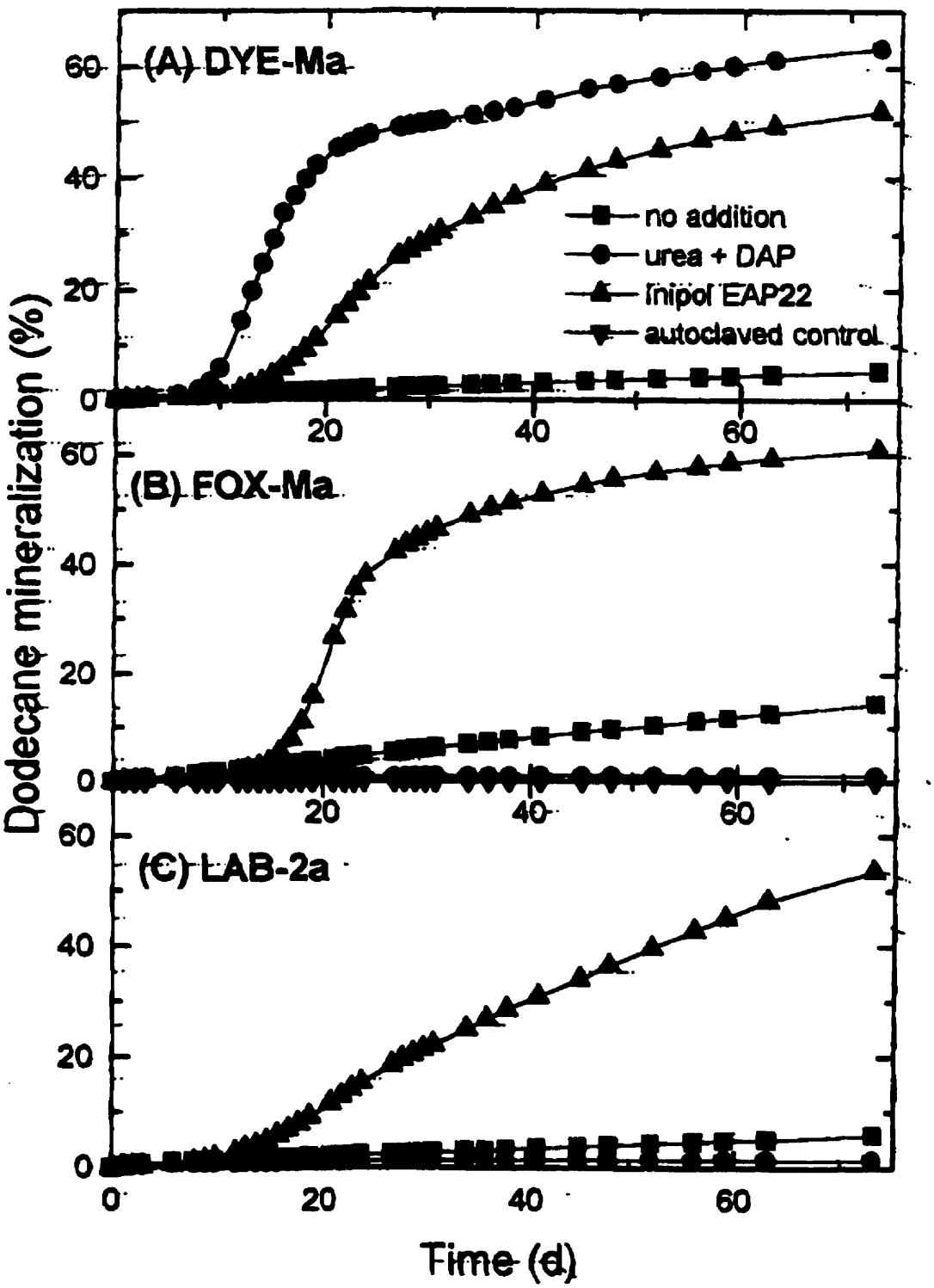
Table 2. Kinetic constants for dodecane mineralization in three soils with different concentrations of added Jet A-1 fuel.

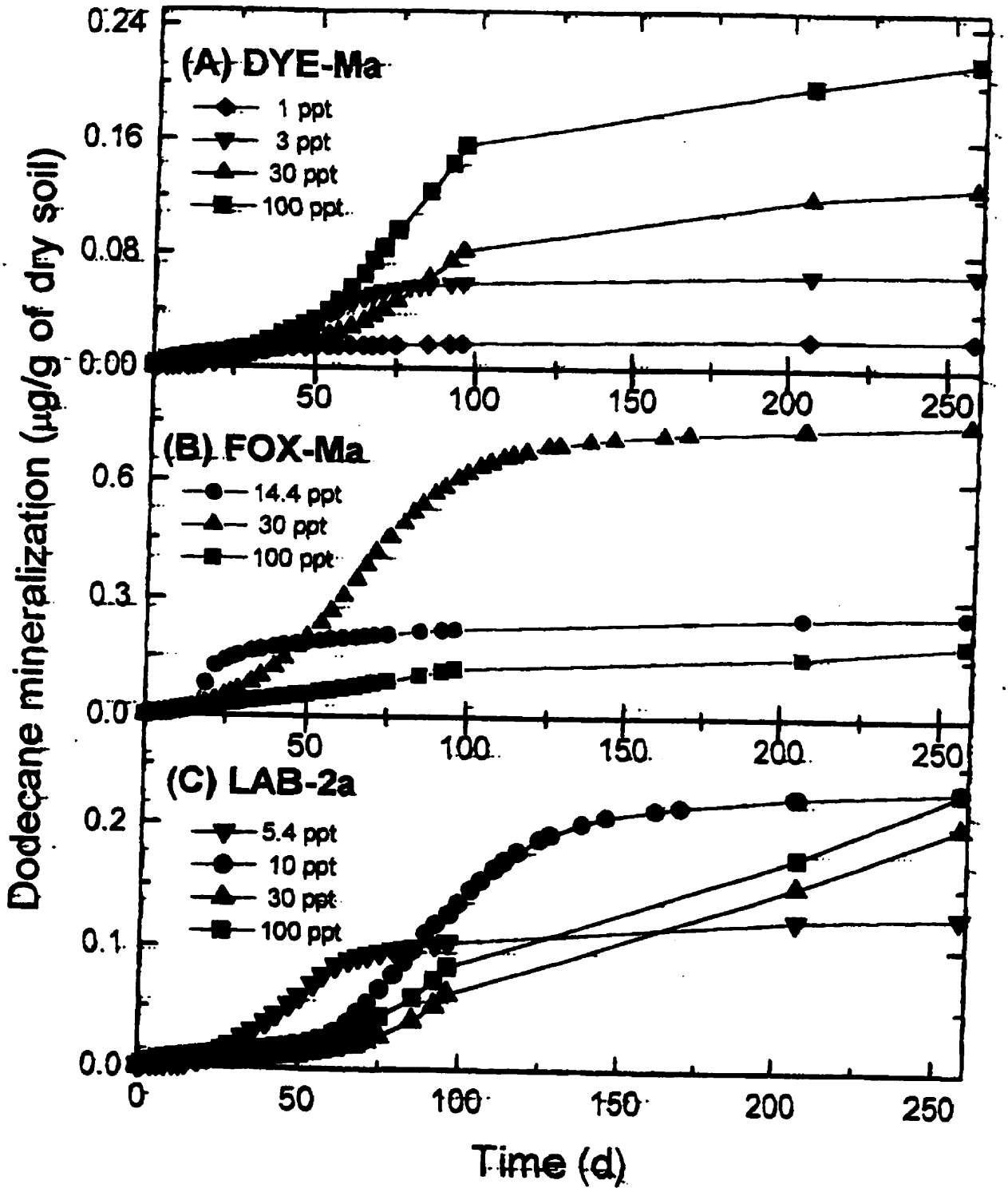
	TPH (ppt)	χ^2	lag (d)	k (d^{-1})	T_{mid} (d)	Y_{max} (ppt)
FOX-MA	14.4	3.70E-04	5.1	0.156	21.4	0.208
	30	3.00E-05	9.4	0.055	65.1	0.724
	100	1.70E-04	26.7	0.015	65.1	0.148
DYE-MA	1	3.00E-06	7.9	0.192	21.3	0.016
	3	2.00E-06	13.6	0.065	45.1	0.064
	30	2.00E-06	32.7	0.051	84.0	0.124
	100	8.00E-06	50.0	0.061	78.1	0.207
LAB2-A	5.4	2.00E-05	9.9	0.063	47.9	0.117
	10	1.00E-05	15.0	0.056	90.3	0.221
	30	4.00E-05	44.4	0.042	114	0.178
	100	5.00E-05	66.8	0.031	115	0.210


(A) DYE-Ma
(B) FOX-Mc
(C) LAB-2a
Dodecane mineralization (%)
Time (d)
10^0. inoculum
10^-2 inoculum
10^-4 inoculum
0 inoc + cryoprot
0 inoc, no cryoprot
0
20
40
60


Dodecane mineralization (%)
60
40
20
0
0
25
50
75
100
Time (d)
BAR-4c
FOX-Mc
FOX-Ba
BAR-4d
PIN-Mb


(A) DYE-Ma
30°C
22°C
15°C
7°C
°C lag T_{mid} Y_{max} k
7 8.0 18.8 39 0.51
15 3.5 8.8 43 1.42
22 2.1 4.7 39 2.54
30 1.8 3.9 40 2.68
(B) FOX-Mc
°C lag T_{mid} Y_{max} k
7 3.0 13.9 54 0.38
15 0.8 4.1 52 0.99
22 0.4 2.4 50 1.53
30 0.2 2.2 54 1.64
(C) LAB-2a
°C lag T_{mid} Y_{max} k
7 9.7 42.4 57 0.08
15 4.0 18.2 52 0.28
22 2.8 10.9 49 0.47
30 2.6 9.9 47 0.59
Dodecane mineralization (%)
Time (d)


(A) DYE-Ma
(B) FOX-Ma
(C) LAB-2a
no addition
urea + DAP
Inipol EAP22
autoclaved control
Dodecane mineralization (%)
Time (d)
0
20
40
60


(A) DYE-Ma
1 ppt
3 ppt
30 ppt
100 ppt
(B) FOX-Ma
14.4 ppt
30 ppt
100 ppt
(C) LAB-2a
5.4 ppt
10 ppt
30 ppt
100 ppt
Dodecane mineralization (µg/g of dry soil)
Time (d)
0.24
0.16
0.08
0.00
0.6
0.3
0.0
0.2
0.1
0.0
0
50
100
150
200
250

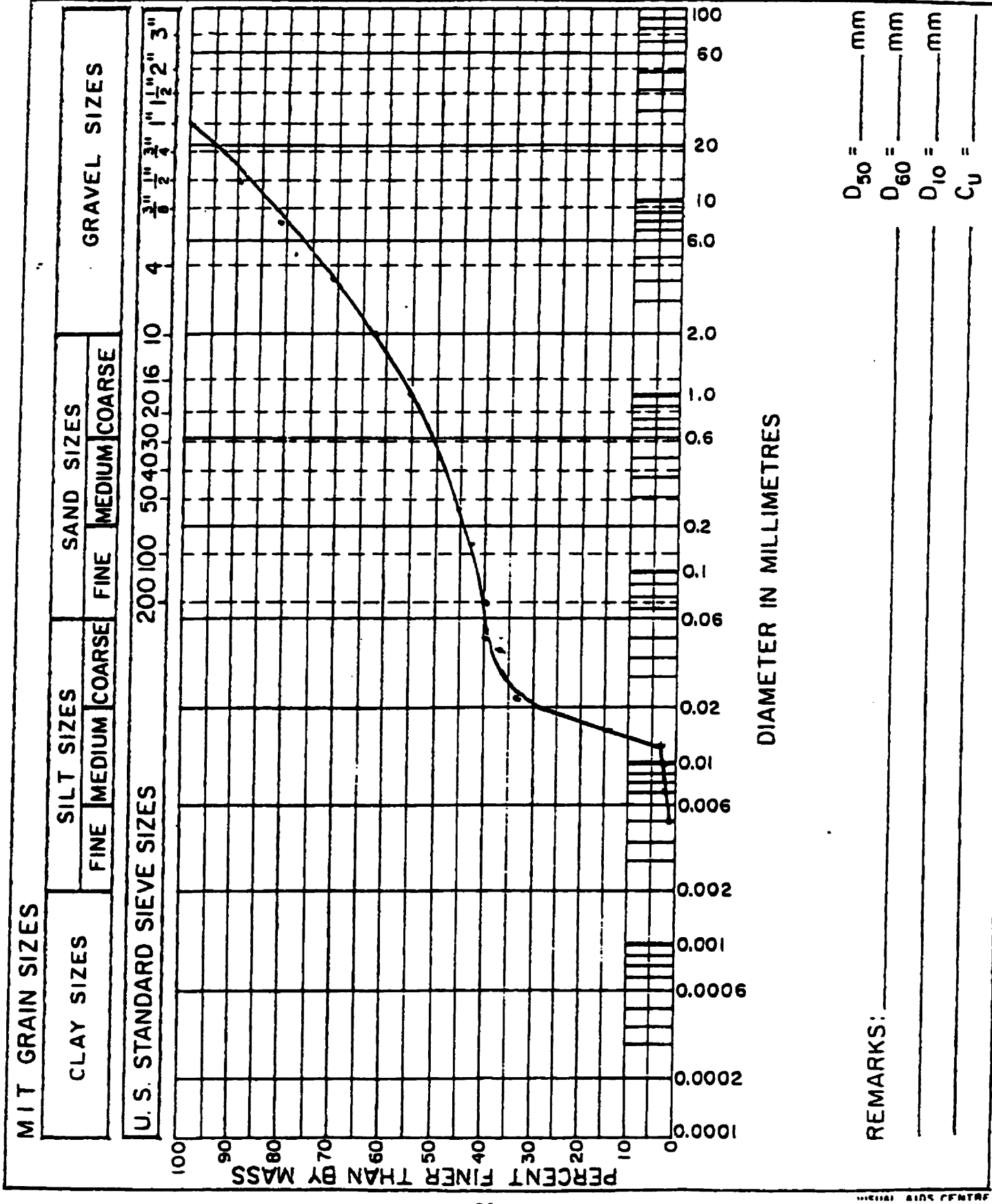
APPENDIX B

GRAIN SIZE ANALYSIS RESULTS

QUEEN'S UNIVERSITY
DEPARTMENT OF CIVIL ENGINEERING
SOIL MECHANICS LABORATORY

GRAIN SIZE CURVE - 1

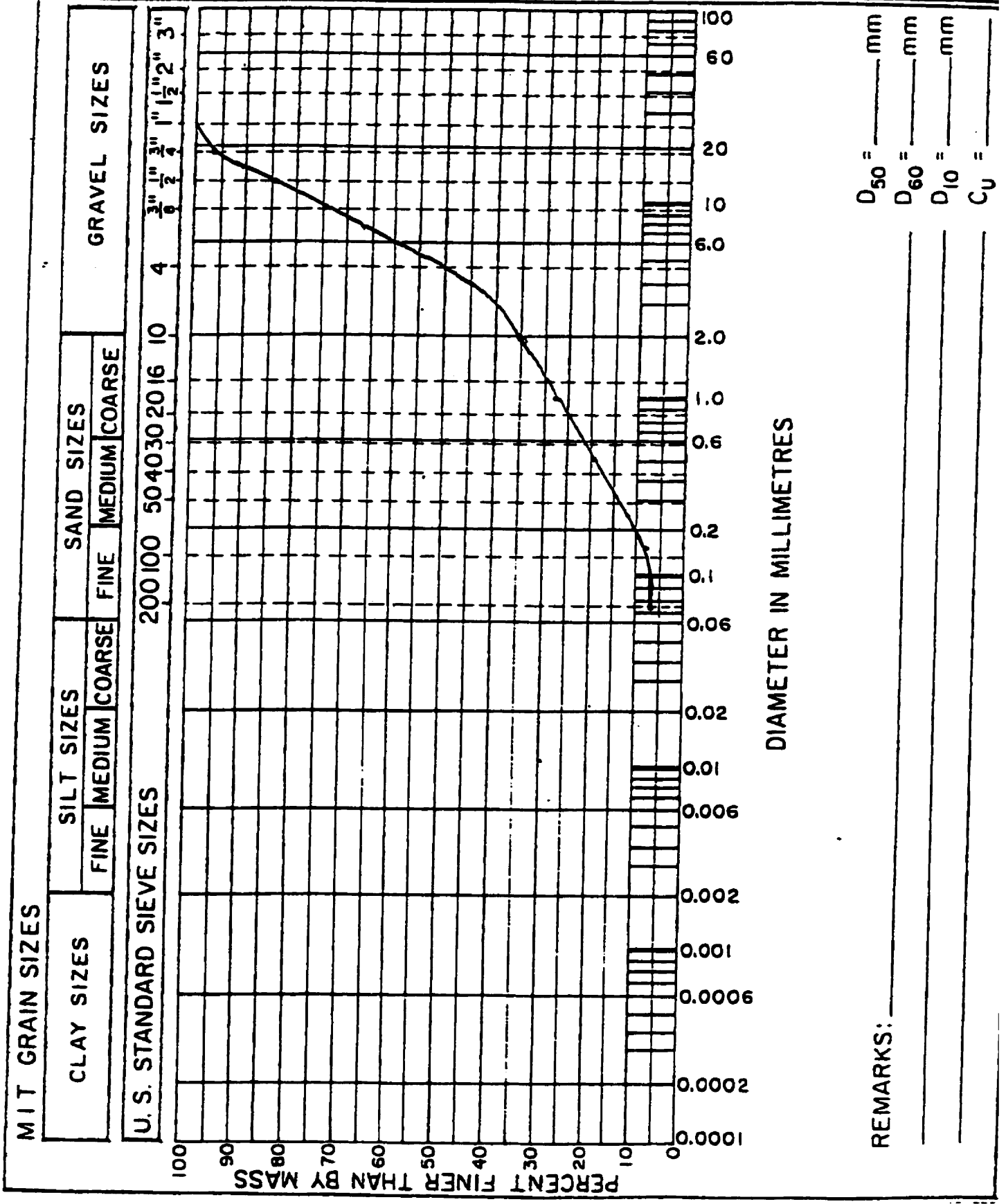
SITE	
SAMPLE	98-12082 A - ALERT
LOCATION	
HOLE	DEPTH
TECHNICIAN PNG	DATE 3/3/99



QUEEN'S UNIVERSITY
DEPARTMENT OF CIVIL ENGINEERING
SOIL MECHANICS LABORATORY

GRAIN SIZE CURVE - 1

PROJECT	
SITE	
SAMPLE	98 - 25148 . FOX-M
LOCATION	
HOLE	DEPTH
TECHNICIAN	PNG DATE 3/3/99



APPENDIX C

KINETIC PARAMETERS FROM BOLTZMAN MODEL

Kinetic Parameters from Boltzman Model

Treatment variable	lag (@ 1%) d	k 1/d	Tmid d	Ymax %
Inoculation Experiments				
Treatment effect control	8.2	0.167	12.1	5
Treatment control	2.7	0.268	15.1	25.3
Std. inoc. conc.	1.4	0.189	15.8	29
1/100 dilution inoc. conc.	7	0.269	16.2	20.2
1/10 000 dilution inoc. conc.	7.5	0.309	16.1	17.9
Bioremediation specific fertilizer experiments				
FOX-M Std. conc.	7.7	N/A	N/A	N/A
FOX-M 1/2 conc.	7.4	N/A	N/A	N/A
FOX-M 1/4 conc.	7.6	N/A	N/A	N/A
Alert Std. conc.	6	N/A	N/A	N/A
Alert 1/2 conc.	5.6	N/A	N/A	N/A
Alert 1/4 conc.	5.6	N/A	N/A	N/A
Commercial fertilizer experiments				
FOX-M Std. conc.	4.9	0.283	12.3	33.2
FOX-M 1/2 conc.	4.6	0.242	12.8	42.5
FOX-M 1/4 conc.	4.7	0.235	12.4	35.7
Alert Std. conc.	8.1	0.2	18.3	10.6
Alert 1/2 conc.	7.1	N/A	N/A	N/A
Alert 1/4 conc.	7.3	N/A	N/A	N/A
Surfactant experiments				
FOX-M Std. conc.	11.2	0.464	19.4	23.3
Alert Std. conc.	7	0.159	24.4	41.1
Alert 1/2 conc.	7	N/A	N/A	N/A
Alert 1/4 conc.	7.1	0.177	21.8	60.2

APPENDIX D

ANOVA RESULTS

Treatment	Effect Test	F Ratio	Prob > F
FOX-M small	treatment	20.09	<.0001
	pile(treatment)	1.43	0.2371
	time	1.01	0.3189
	treatment x time	19.99	<.0001
	pile x time (treatment)	6.16	0.0004
FOX-M landfarm ln (conc)	treatment	11.43	<.0001
	time	20.01	<.0001
	treatment x time	4.26	0.0025
FOX-M medium scale	treatment	1.11	0.3382
	time	20.55	<.0001
	treatment x time	0.42	0.6564
Alert medium scale ln (conc)	treatment	1.25	0.2714
	time	79.23	<.0001
	treatment x time	1.66	0.208

APPENDIX E

THERMAL DATA

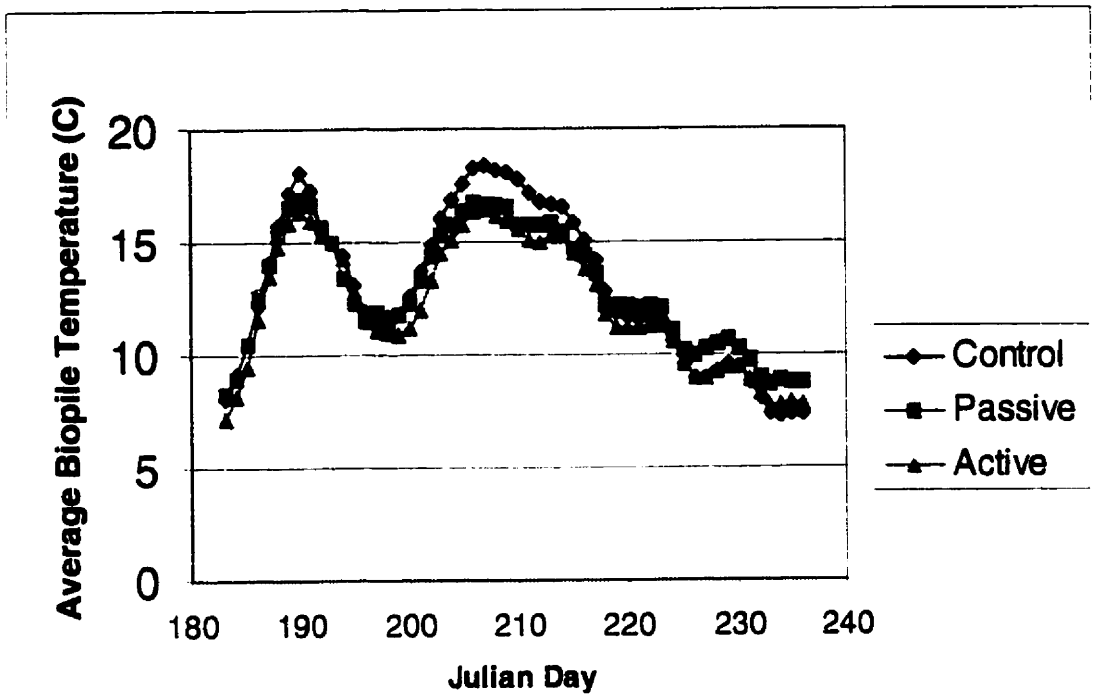


Figure E-1: Temperature profiles for FOX-M medium-scale biopiles from 2 July to 24 August, 1998.

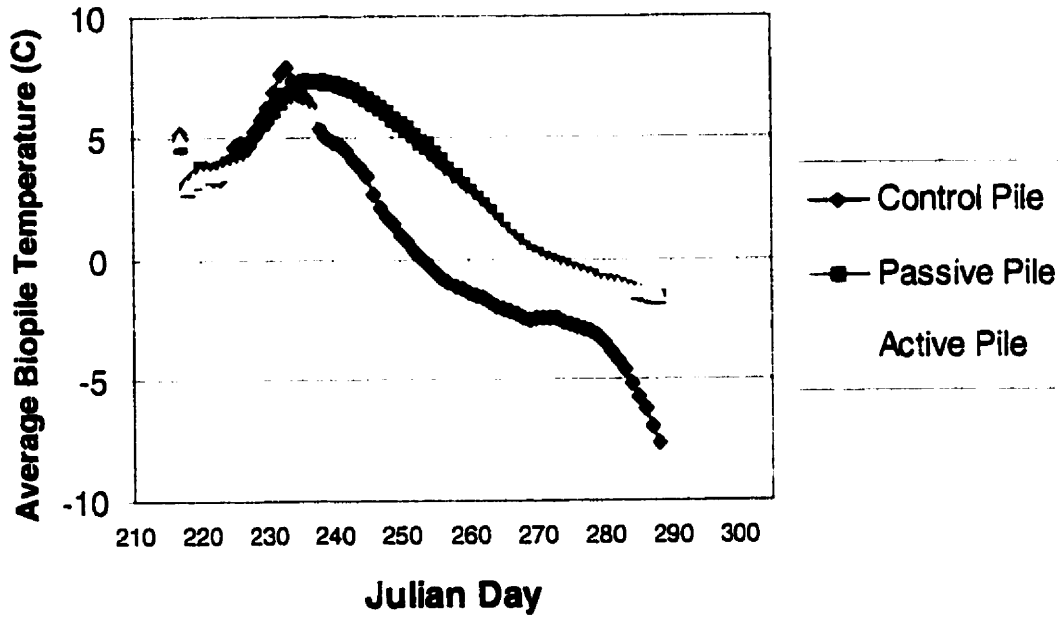


Figure E-2: Temperature profiles for Alert medium-scale biopiles from 5 August to 15 October, 1998.

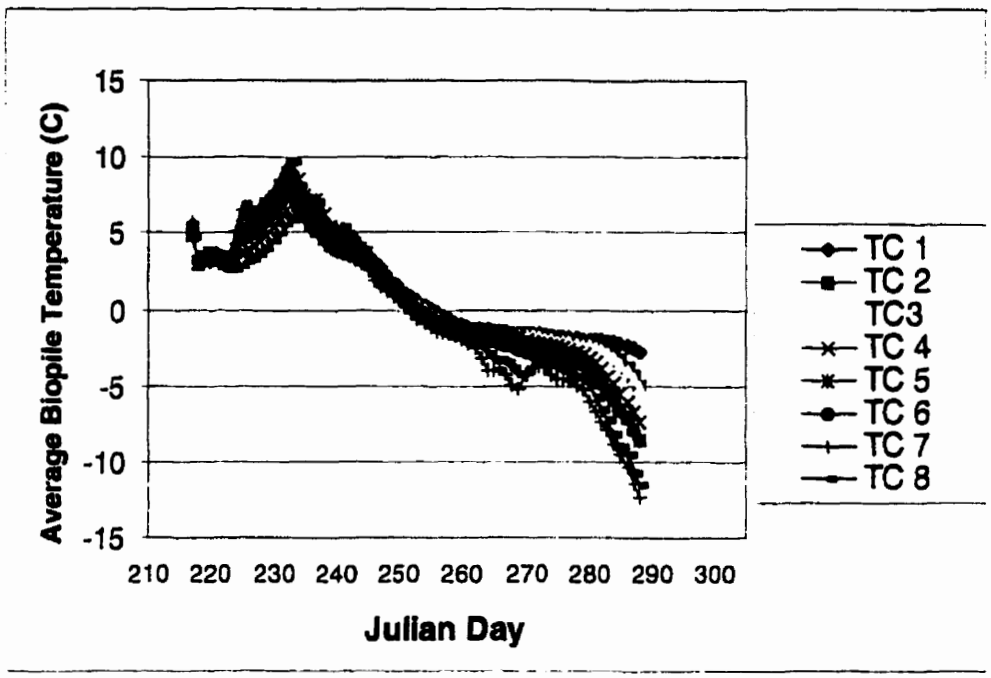


Figure E-3: Temperature profile for Alert outdoor control pile from 5 August to 15 October, 1998. [Note: TC1 and TC2 were duplicate thermocouples placed in the base of the pile - thermocouple numbers increase with height in the pile].

APPENDIX F

FIELD-SCALE TPH RESULTS

FOX-M Medium Scale Biopiles

Initial sampling		Final sampling		Initial [TPH] - Final [TPH]
Active Pile				
98-25082	1900	98-25207	1100	800
98-25083	1500	98-25212	1900	-400
98-25084	1600	98-25208	1400	200
98-25085	2500	98-25213	770	1730
98-25086	1400	98-25209	810	590
98-25087	1500	98-25214	500	1000
98-25088	1800	98-25211	1400	400
98-25089	1650	98-25215	530	1120
Passive Pile				
98-25091	930	98-25201	1900	-970
98-25092	2400	98-25205	610	1790
98-25093	1600	98-25202	1500	100
98-25094	2200	98-25206	1000	1200
98-25095	2000	98-25198	1300	700
98-25097	1100	98-25203	800	300
98-25098	1500	98-25200	1650	-150
98-25099	2000	98-25204	730	1270
Control Pile				
98-25100	1100	98-25191	1300	-200
98-25102	2100	98-25196	1300	800
98-25103	2200	98-25193	650	1550
98-25104	1600	98-25197	740	860
98-25105	2400	98-25189	2000	400
98-25106	2600	98-25194	1300	1300
98-25107	1600	98-25190	1300	300
98-25108	2000	98-24195	1800	200

FOX-M Small Scale Biopiles

Initial sampling		Final sampling		Initial [TPH] - Final [TPH]
Control Pile #1				
98-25048	2200	98-25157	3300	-1100
98-25049	2700	98-25155	2200	500
98-25050	3000	98-25156	2300	700
98-25051	2300	98-25158	2500	-200
Control Pile #2				
98-25056	3000	98-25162	3200	-200
98-25057	2100	98-25159	3100	-1000
98-25058	2850	98-25160	3500	-650
98-25059	2300	98-25161	3400	-1100
Uncovered Control Pile #1				
98-25052	2550	98-25164	440	2110
98-25053	3000	98-25165	1600	1400
98-25054	2500	98-25166	1100	1400
98-25055	2400	98-25163	1000	1400
Uncovered Control Pile #2				
98-25060	1700	98-25170	500	1200
98-25061	2500	98-25167	1400	1100
98-25062	2100	98-25169	1400	700
98-25063	2400	98-25168	1400	1000
Commercial Fertilizer #1				
98-25064	970	98-25178	2600	-1630
98-25065	1800	98-25177	2600	-800
98-25066	2300	98-25175	3300	-1000
98-25067	1600	98-25176	2500	-900
Commercial Fertilizer #2				
98-25068	1900	98-25171	3100, 3200, 3000	-1200
98-25069	1700	98-25172	1800	-100
98-25070	1600	98-25173	1900	-865
98-25071	1100	98-25174	3200	-2100
98-25072	470	98-25173	1900	
Surf + Comm Fert #1				
98-25073	330	98-25180	2300	-1970
98-25074	760	98-25181	2500	-1740
98-25075	810	98-25182	1650	-840
98-25076	1300	98-25179	2400	-1100
Surf + Comm Fert #2				
98-25077	2700	98-25184	1900	800
98-25078	2300	98-25188	1200	1100
98-25079	1600	98-25186	1700	-100
98-25081	1600	98-25185	1300	300

FOX-M Landfarm

Initial sampling		Final sampling		Initial [TPH] - Final [TPH]
Control Plot #1				
98-25109	310	98-25216	290	110
98-25111	840	98-25217	99	741
98-25112	3900	98-25219	190	3710
98-25113	1200	98-25218	100	1100
Control Plot #2				
98-25114	540	98-25220	540	0
98-25115	1400	98-25222	240	1160
98-25116	1600	98-25224	270	1330
98-25117	2000	98-25223	430	1570
Commercial Fertilizer #1				
98-25118	1500	98-25225	290	1210
98-25119	530	98-25226	150	380
98-25120	910	98-25228	140	770
98-25121	225	98-25227	170	55
Commercial Fertilizer #2				
98-25122	310	98-25229	200	110
98-25123	250	98-25231	390	-140
98-25124	240	98-25233	180	60
98-25126	290	98-25232	110	180
Surfactant + Commercial Fertilizer				
98-25127	200	98-25234	280	-80
98-25128	70	98-25235	340	-270
98-25129	70	98-25237	66	4
98-25130	140	98-25236	80	60
Commercial Fertilizer + Covered Plot				
98-25131	300	98-25238	130	170
98-25132	170	98-25239	160	10
98-25133	130	98-25241	140	-10
98-25134	150	98-25240	230	-80
Surfactant Only				
98-25135	810	98-25242	155	655
98-25137	480	98-25244	200	280
98-25138	390	98-25246	<40	350
98-25139	500	98-25245	200	300
Unfertilized Covered Plot				
98-25140	865	98-25247	630	245
98-25141	1100	98-25248	470	630
98-25142	1300	98-25250	640	660
98-25143	550	98-25249	550	0

Alert medium scale Biopiles

Initial sampling		Final sampling		Initial [TPH] - Final [TPH]
ACTIVE PILE				
98-12150	3000	98-12464	1300	1700
98-12417	2600	98-12466	890	1710
98-12416	2200	98-12476	1100	1100
98-12415	5700	98-12478	930	4770
98-12148	5200	98-12470	1800	3400
98-12147	4500	98-12472	1200	3300
98-12420	1650	98-12465	990	660
98-12419	1100	98-12463	940	160
PASSIVE PILE				
98-12146	4100	98-12467	1000	3100
98-12145	4800	98-12468	1200	3600
98-12140	3100	98-12480	790	2310
98-12139	3400	98-12477	880	2520
98-12142	5100	98-12471	1200	3900
98-12141	3600	98-12479	970	2630
98-12144	2900	98-12475	1300	1600
98-12143	4000	98-12469	1600	2400
CONTROL PILE				
98-12430	2200			
98-12429	3000			
98-12422	3800			
98-12421	2600			
98-12424	4200			
98-12423	3400			
98-12428	2600			
98-12425	3265			

APPENDIX G

ANOVA SAMPLE SIZE CALCULATION

Finding sample size required for an Anova

Number of replications needed to detect a given "true" difference between two means

Goal: To determine how many samples (n) need to be taken from each population to be 80% (P= 0.8) certain of detecting a 20% difference between two means in a given number of populations (a) at the 0.5% level of significance (α)?

Assumptions:

1. A coefficient of variation of 25% for TPH levels in soil
2. Three non-duplicated treatments (a = 3) from which we would like to be able to detect a difference between two of the three means

Calculations:

First, the degrees of freedom must be calculated: $v = a(n-1)$

$v = 3(n-1)$

Number of samples (n) can be solved by iteration in the following equation:

$$n \geq 2\,(\sigma/\delta)^2\,\{t_{\alpha\,[v]} + t_{2\,(1-P)[v]}\}^2$$

- where (σ/δ) is the true standard deviation divided by the smallest true difference that you want to detect. If precise values are not known, a ratio of coefficient of variation over desired percent difference detectable can be supplemented.
- in this case, $(\sigma/\delta) = 25\% / 20\% = 1.25$
- this is equivalent to wanting to detect a 1000 ppm difference between 2 of 3 biopile treatments with an average initial TPH concentration of 5000 ppm with a standard deviation of 1250 ppm.
- where t represents the critical value of the t distribution at a given coordinate

First, try an estimate of n = 25, making v = 72, which yields $n \geq 25.8$

Next, we try n = 26, making v = 75, which yields $n \geq 25.8$

Therefore, round up to 26 to be conservative. This is the same as the previous answer indicating that the iteration has reached stability. This implies that 26 samples per population are necessary.