THE EFFECTS OF A CRUDE OIL SPILL ON THE CROP PRODUCTIVITY AND BIOLOGICAL QUALITY OF AN AGRICULTURAL SOIL, AND THE POTENTIAL FOR PHYTOREMEDIATION OF CRUDE OIL CONTAMINATED LAND

BY

Mitchell D. Timmerman

A Thesis
Submitted to the Faculty of Graduate Studies
in Partial Fulfillment of the Requirements
for the Degree of

MASTER OF SCIENCE

Department of Soil Science
University of Manitoba
Winnipeg, Manitoba

THE UNIVERSITY OF MANITOBA

FACULTY OF GRADUATE STUDIES

*****

COPYRIGHT PERMISSION PAGE

The Effects of a Crude Oil Spill on the Crop Productivity and Biological Quality of an Agricultural Soil, and the Potential for Phytoremediation of Crude Oil Contaminated Land

BY

Mitchell D. Timmerman

A Practicum submitted to the Faculty of Graduate Studies of The University of Manitoba in partial fulfillment of the requirements of the degree of

MASTER OF SCIENCE

ACKNOWLEDGEMENTS

To begin, I would like to acknowledge the primary funding support of Enbridge Pipelines, Inc. Additional funding was provided by the University of Manitoba and Mrs. S. Wright (Douglas L. Campbell Graduate Fellowship).

Special thanks go to the project cooperators, Mr. B. Mason and Mrs. J. Mason. That swather and mower sure came in handy.

Thanks to my committee members, Dr. David Burton and Dr. Isabel Waters for their input into the research activities and the preparation of this document. Good news, Dr. B., no more tantalizing toast smell to torment you every morning. Thanks also to Dr. G. Racz for his advice and guidance as Head of the Department of Soil Science.

I also gratefully acknowledge the technical assistance of the full-time and summer staff at the Department of Soil Science who contributed to the laboratory, field and administrative components of my master's research.

To my fellow graduate students, who toiled long and hard alongside me, I offer thanks for making my time at Soils the best of my life to date (how sad is that?). Your antics and support made me glad that I hit the snooze button on the alarm clock of life.

To my parents, I can't say enough about what you've done for me. You are the reason I've gotten to where I am, and you'll be the reason I get where I'm going.

To my love and nemesis, Claire, thanks for hanging in there.

Finally, to my advisor, Dr. Les Fuller, I deeply appreciate the opportunity you gave me. Thanks to the Master, the young Grasshopper has moved on to the next level.

TABLE OF CONTENTS

Page

LIST OF TABLES

LIST OF FIGURES

Figure **Page**

ABSTRACT

Timmerman, M.D. M.Sc., The University of Manitoba, October, 1999. The Effects of a Crude Oil Spill on the Crop Productivity and Biological Quality of an Agricultural Soil, and the Potential for Phytoremediation of Crude Oil Contaminated Land. Major Professor; Dr. L.G. Fuller.

The release of crude oil onto agricultural land has the potential of taking that land out of production. Its return to production is often the principal goal of site remediation. A two-year study was conducted to examine the effects of a crude oil pipeline spill on the productivity of agricultural land. Experimental plots of wheat (*Triticum aestivum* c.v. Pasqua), canola (*Brassica rapa* c.v. Argentine), bromegrass (*Bromus biebersteinii* Rohman and Schult, c.v. meadow) and alfalfa (*Medicago sativa L. c.v. algonquin*) were established in 1996 within the area of a spill which occurred in October, 1994, as well as on adjacent uncontaminated land. The study soil was a clay loam Gleyed Rego Black Chernozem developed from predominantly moderately fine lacustrine materials. Surface expression at the site was gently undulating. The annual crop plots were sampled for total oil and grease analysis in the spring of each year while sampling in the forage plots occurred only in the first spring.

Over the course of the study, canola demonstrated greater sensitivity than wheat to the presence of crude oil in soil, particularly at higher concentrations. This sensitivity was expressed differently at different points in the growing season. Harvest canola yields in 1996 were only lower than control values in the most highly contaminated plots, a

result also observed in wheat. Nevertheless, the yield reduction was far greater in canola than in wheat. In 1997, wheat growth and yields were variable in the spill plots while canola growth and yields were lower than control values in almost all spill plots. Seed yields in 1996 of both crops were related to total oil and grease levels in 0-30 cm of soil, but the relationship was stronger for canola. The critical soil oil concentration at which canola oilseed yield declined below the mean control value was in the 1000-2000 mg kg^{-1} range. The critical threshold for wheat grain production in 1996 was approximately 2000 mg kg^{-1} oil in soil. No threshold could be established for wheat grain yield in 1997. Although the result was not as apparent as in the previous year, the critical oil concentration for oilseed yield seemed to be similar in 1997 to that found in 1996. Multiple regression analysis confirmed that the presence of oil in soil accounted for more of the variation in oilseed yield than grain yield in 1996. It also indicated that soil salinity was not a major factor influencing the yields of either crop in that year. This agreed with the electrical conductivity data collected in which EC values were above crop tolerance levels in only two experimental sites where yields were evidently not affected. By contrast, soil salinity accounted for more of the yield variation of both crops than did crude oil levels in soil in 1997 based on multiple regression. However, neither factor explained more than a small portion of the yield variation. This occurred despite the fact that EC levels were found to be nearly the same in both years across the experimental sites. Dry soil conditions in the spring of 1997 were likely responsible for the greater influence of salinity on crop yield.

Mid-season aboveground biomass sampling of the forage crops in 1997 revealed that alfalfa was more sensitive to hydrocarbons in soil than was grass. Both forages

performed better in some of the spill sites than in the controls, indicating that small amounts of oil in soil may have stimulated forage growth.

The presence of BTEX and PAHs in crop tissues was negligible.

The spill-affected land examined appears to be approaching its former levels of wheat productivity and forage grass growth but has not been remediated sufficiently to allow normal canola or alfalfa production.

A second objective of the study was to examine the effects of crude oil contamination and a range of remedial treatments on the biological quality of an agricultural soil based on three microbiological indices: microbial biomass carbon (MBC), dehydrogenase activity (DHA) and microbial metabolic diversity (MMD). The four remedial treatments consisted of: meadow bromegrass, alfalfa, fallow with wheat straw incorporation (SF) and unamended fallow (UF_{SP}). An unamended fallow on adjacent uncontaminated land served as a control (UF_{CON}).

The effect of the spill on each of MBC, MMD and DHA was significant at the 0.01, 0.05 and 0.11 probability levels, respectively. Of the four remedial treatments tested, grass had the greatest effect on soil biological quality relative to the UF_{SP}. Alfalfa exhibited a slightly reduced effect compared to grass while the effect of straw incorporation was still more reduced than grass.

A third objective of the research was to compare the efficacies of forage and fallow treatments in reducing hydrocarbon concentrations in an agricultural soil. The treatments were the same as those in the plot trials examining soil biological quality. Composite samples were taken from each of four replicates in each treatment in August, 1996 (t=0) and October, 1997 (t=1). Samples were analyzed for total oil and grease

(CH_2Cl_2 extraction) and total extractable hydrocarbons (total extractable volatile/non-volatile hydrocarbons determined by GC/FID).

Based on general trends, meadow bromegrass enhanced the degradation of crude oil constituents in soil relative to tillage alone more than any other treatment. This trend was most evident in the 30-60 cm depth of soil. The release of organic substrates from grass roots and improved aeration are possible mechanisms for the stimulation of soil microbial activity, resulting in elevated hydrocarbon degradation. The general trend of declining concentrations of total oil and grease indicate degradation of crude oil constituents as an entire group, since the analytical procedure captures the entire spectrum of compounds. The elevated TEH levels are likely the result of longer chain compounds breaking down into shorter chains which fall within the extraction range of the TEH analysis (C_{10}-C_{30}).

FORWARD

This thesis has been prepared in the manuscript format in adherence with the guidelines established by the Department of Soil Science. The referencing style employed throughout this document is that of the Canadian Journal of Soil Science. Three manuscripts will be submitted to the Canadian Journal of Soil Science for publishing. Chapters 3 and 5 will be co-authored by Dr. L.G. Fuller. Chapter 4 will be co-authored by both Dr. Fuller and Dr. D.L. Burton.

1. INTRODUCTION

The introduction of crude oil to agricultural land represents the potentially serious contamination of an important natural resource. Crude oil contains a wide array of potentially hazardous substances, from short-chain, light-weight to long-chain, heavy-weight compounds. Such compounds include volatile BTEX (benzene, toluene, ethylbenzene and xylenes) and relatively non-volatile PAHs (polyaromatic hydrocarbons), all of which can pose a risk to biological health (McGill et al. 1981). Exposure of terrestrial environments to crude oil can come from a number of sources, but pipeline leaks are a major contributor (Rowell 1977a; Smith 1998). Pipelines stretch across much of the agricultural region of the Canadian Prairies, providing ample opportunity for oil contamination of land in the event of pipeline failure (Bank of Montreal 1998; Environment Canada 1974; Environment Canada 1976). The presence of crude oil in soil alters the physical, chemical and biological characteristics of that soil, affecting its ability to support vegetation (Rowell 1977a). Although there have been instances of enhanced plant growth on oil polluted farmland (Carr 1919; Toogood and McGill 1977), such occurrences have generally been restricted to cases of low-level contamination and/or non-agricultural soils (Stebbings 1970; Baker 1970; Baker 1971). For the most part, the introduction of hydrocarbons to soil has either no effect or a deleterious impact on site productivity. Restoring crop production on a crude oil affected soil is a primary aim of site remediation.

Wheat, canola, grass and alfalfa were selected as representative annual and perennial crops to assess the impact of crude oil contamination of soil on agricultural land productivity. The principal hypothesis to be tested was that the presence of crude oil in soil would affect the productivity of agricultural crops commonly grown in the area. It was also hypothesized that crop growth would be related to soil hydrocarbon levels and that plant response would be a function of crop type.

A variety of soil microorganisms are capable of attacking constituents of crude oil in order to generate energy or increase biomass (McGill et al. 1981). Hydrocarbons may also be broken down indirectly via cometabolism as more readily metabolizable substrates are utilized (Hornick et al. 1983). The introduction of crude oil to soil tends to elevate the size and activity of the microbial community (Baldwin 1922; Plice 1948; Dobson and Wilson 1964; Jobson et al. 1972; Biederbeck 1990; Joergensen et al. 1995). After an initial lag phase characterized by stagnation or decline, microbial numbers and activity rebound and rise above those observed in soil without oil. This response pattern is typical of both recently oil-treated and long-term contaminated soils. However, microbial populations and their activities eventually return to control levels as resources become limiting (McGill et al. 1981). Three microbiological indices were used to determine if the biological quality of soil was adversely affected by the presence of crude oil. Forage growth and straw incorporation were tested for their individual effectiveness in improving soil biological quality in a crude oil contaminated soil.

The growth of vegetation has been shown to enhance the dissipation of soil contaminants such as PAHs, which are constituents of crude oil (Aprill and Sims 1990; Reilley et al. 1996; Qiu et al. 1997), as well as crude oil itself (Wiltse et al. 1998). This

enhancement appears to be the result of the effect of plant roots on the soil microbial community. Microbial numbers and activity have been documented to be higher in the vicinity of growing roots than in unvegetated soil (Curl and Truelove 1986). Higher microbial biomass and activity may lead to greater biodegradation of soil pollutants (Reilley et al. 1996). The likely mechanism behind this phenomenon is the release of readily metabolizable organic substrates by roots into the root zone which stimulate the growth and activity of soil microorganisms (Elliot et al. 1984). Forage crops such as grass and alfalfa have demonstrated an ability to enhance the disappearance of organic xenobiotics in soil and offer other attributes including soil stability and wide genetic diversity (April and Sims 1990; Wiltse et al. 1998). Grass and alfalfa plots were established to determine the potential for a phytoremediation approach to the reclamation of a crude oil contaminated soil. Direct carbon amendment in the form of straw incorporation was also tested for its ability to stimulate the breakdown of crude oil in soil.

2. LITERATURE REVIEW

2.1 Introduction

Fossil fuels remain an important energy source for most segments of society in Canada. Crude oil is one type of fossil fuel commonly removed from the earth. Transport of crude oil from extraction sites to refining locations is achieved primarily via pipeline systems. Since much of the extraction occurs in Western Canada, and the crude oil is transported east, pipeline systems run extensively across agricultural land in British Columbia, Alberta, Saskatchewan and Manitoba (Bank of Montreal 1998; Environment Canada 1974; Environment Canada 1976). Agricultural land is susceptible to crude oil exposure in cases of pipeline repair or actual failure. Pipeline releases were cited as the major source of crude oil contamination of farmland by Rowell (1977a) who reported that crude oil spills in Canada occurred at a rate of 356 to 694 per year from 1971-1974. Enbridge Pipelines, Inc. (formerly known as Interprovincial Pipe Line, Inc.) had three pipeline releases of crude oil between June, 1995 and February 1996 onto agricultural land in Saskatchewan and one major spill in 1994 in Manitoba (Smith 1998). A larger number of small spills generally occur from small lines running from extraction sites. Such small spills are typical while major spills are rare (Smith 1998). Leaks from pipelines are responsible for most oil contamination of land. A considerable portion of crude oil affected land is agricultural.

The introduction of crude oil to a terrestrial environment has implications for environmental quality. The effect on soil quality can lead to reduced agricultural productivity. An oil affected site must typically be taken out of production for an extended period of time if no steps are taken to remediate the land (Rowell 1977). Depending on the extent of contamination and the intended utilization of the land, this time period can be reduced.

The biological quality of a soil is also typically affected when hydrocarbons (HCs) are introduced. The microbiology of the soil is of particular importance because it is mainly responsible for the degradation of introduced HCs (McGill et al. 1981). Such microbial parameters as biomass, respiration and enzyme activity may be altered as soil microorganisms respond to the presence of crude oil (Baldwin 1922; Plice 1948; Dobson and Wilson 1964; Jobson et al. 1972; Biederbeck 1990; Joergensen et al. 1995).

In situ biodegradation is a common decontamination process encouraged in the remediation of hydrocarbon (HC) affected lands. Landfarming is a widely accepted approach consisting of fertilizer application and tillage. The addition of carbon to soil to enhance the degradation of HCs is an appealing new strategy because of its potential for cost savings and minimal disturbance to the soil. By adding readily metabolizable sources of carbon to soil, microbial activity is increased and HCs are broken down incidentally while the primary substrate is attacked. Carbon amendments can be made directly through incorporation by cultivation or indirectly through plant root exudation and death. The use of these amendments is less intrusive and can result in the reaching of an endpoint to remediation in comparable or less time than via other methods, such as landfarming.

2.2 Properties of Crude Oil

2.2.1 General Composition

Crude oil is composed of a wide array and large number of HCs as well as other organic compounds containing sulfur, nitrogen and oxygen (Rowell 1977). The composition of oils varies to some degree among all crude petroleums resulting in differences in chemical and physical characteristics (McGill et al. 1981). Whitehead and Breger (1963) identified more than two hundred compounds in crude oil, a number representing only a portion of the entire suite of HCs present.

2.2.1.1 Saturated Compounds. HCs typically constitute a minimum 75% by weight of crude petroleums (McGill et al. 1981), of which alkanes (also called paraffins) are the dominant group (Rowell 1977). Straight chains are the most degradable group of HCs (Evans et al. 1980). The n-alkanes (C_1-C_{33} in length) are a prevalent form of paraffins, constituting as much as 25% of the total weight of crude oil (McGill et al. 1981). Among the n-alkanes, the C_5-C_{10} compounds appear in the greatest numbers (excluding the most volatile) and have been identified as the most toxic fraction to organisms due to greater water solubility (Foster 1962). while heavier components up to C_{35} occur in smaller but still significant amounts. Much longer chain (up to C_{78}) waxes may also be present (Rowell 1977). Among the iso-alkanes (branched alkanes), a less abundant HC group found in crude oil, the 2-, 3- and 4- substituted methyl compounds are the most common. Complex and terminal branching make breakdown of iso-alkanes more difficult, while simple side-chaining has little effect on degradability (Hornick et al. 1983). There are

also mono-, bi- and polycyclic alkanes (naphthenes). Included in the naphthenes are the more volatile C_4 to C_{11} compounds such as cyclopentane, cyclohexane and alkyl forms of each. Cycloalkanes lack terminal methyl groups, the site of initial oxidation, and are thus less susceptible to breakdown than straight chain alkanes (Hornick et al. 1983).

2.2.1.2 Aromatic Compounds. Aromatic substances are the most resistant of the HCs in crude oil to biological attack (Evans et al. 1980). Aromatics constitute a wide range of both volatile and non-volatile compounds. Concentrations of benzene, toluene, 1,2,4-trimethylbenzene and xylene can be as high as 1-2% by weight (McGill et al. 1981). Other common monoaromatics include alkylbenzenes (primarily ones with long side chains), indanes, di- and trinaphthenobenzenes as well as more complex forms with more naphthalene rings attached to a single aromatic ring (Rowell 1977). Bicyclic compounds consist of simple naphthalenes as well as di-, tri- and tetramethyl forms thereof, and C_{11}-C_{14} derivatives (McGill et al. 1981). Less abundant are the tri- and polyaromatics which are highly condensed, short-chained and possess higher sulfur contents (Rowell 1977). Examples include anthracenes, phenanthracenes, pyrene, benzopyrene, acenaphthylenes and several other types. Derivatives of these types contain up to six aromatic rings in their structures. The greater the number of rings in the structures of polyaromatic HCs, the lower the water solubility of these compounds and the greater their resistance to degradation (Hornick et al. 1983). Certain more complex HCs in crude oil show structural similarity to natural substances found in vegetation like carotenes, phenols and chlorophylls.

2.2.1.3 Nitrogen, Sulphur, Oxygen Compounds. Nitrogen occurs in very small amounts in heavy asphaltic compounds and the resin fraction which tend to be of very low volatility. Lighter compounds which contain nitrogen tend to be methylated C_6-C_{10} pyridines and pyrolles or bicyclic structures like indoles and quinolines (Speers and Whitehead 1969).

Most sulphur compounds in crude oil are di- or polyaromatics of low volatility such as thiophenes; the less prevalent volatile fraction is dominated by thiols. Other common S compounds include mono-, bi- and tricyclic sulphides, disulphides, hydrogen sulphide and elemental S (Constantinides and Arich 1967). The typical range for sulphur concentration in petroleum crude is 0.3-3.0% by weight.

Oxygen levels in crude oil tend to be <3% by weight (McGill et al. 1981). A variety of oxygen containing compounds occur in crude oil ranging from the abundant naphthenic or mixed naphthenoaromatic acids and fatty acids to the less prevalent ethers, esters, ketones and phenols (Rowell 1977). Falling mainly in the C_6-C_{12} and C_{14}-C_{19} ranges, the group of naphthenic acids is mostly made up of cyclopentyl and cyclohexyl carboxylic acids. These acids are known for their phytotoxicity and thus their contents in crude oil could influence the severity of the effects of a spill on site plant productivity.

2.2.1.4 Asphaltenes. Polycyclic aromatic and alicyclic compounds with alkyl side chains make up this poorly defined group of crude oil constituents (McGill et al. 1981). These compounds exhibit high molecular weights, ranging from 500 to several thousand, colloidal properties and the ability to carry a charge. The presence of N, S and O in asphaltene structures is not uncommon.

2.2.1.5 Metals. Metal contents of crude oil tend to vary from 0.1-100 ppm (McGill et al. 1981), although concentrations as high as 1000 ppm are possible (Rowell 1977). Several elements were detected by Hitchon et al. (1975) in Alberta crude oils at the ppm level including sodium, chlorine, nickel and iron. A variety of trace elements were found at the ppb level such as bromine, manganese, mercury, chromium, cesium, selenium and zinc. These substances occur as complexes either with alkyl components, porphyrins or N containing molecules.

Metal concentrations in soil have been shown to increase with the addition of crude oil relative to uncontaminated soil. Carls et al. (1995) observed elevated levels of Cr, Ba, Pb and Zn in drilling pad soil compared to control samples with the greatest absolute and proportional differences occurring for Pb. Mean concentrations were <1 ppm for Cr and Ba and <10 ppm for Pb and Zn. Muzaini and Jacob (1996) detected V, Ni and Cr at concentrations as high as >100 ppm each on industrial land onto which oil was spilled. Mean soil contents of these three latter elements were <100 ppm. Pb was found at concentrations <50 ppm while Cd levels were <1 ppm in the contaminated soils. No control values for the surveyed sites were provided.

2.2.1.6 Brine. The potential exists for the salts released during an oil spill to have a greater impact than the oil itself on the quality of soil and therefore plant productivity (McGill et al. 1981). NaCl is the most prevalent form of salt occurring in crude mixtures. Also present are considerable quantities of chlorides, carbonates, bicarbonates and sulphates of K, Ca and Mg. Mean total chloride contents of crude extracts are typically $4.0\text{-}4.5\text{x}10^{4}$ $\mu g\ mL^{-1}$. This value is substantially larger than that of seawater

($2.0\text{-}3.5 \times 10^4$ $\mu g\ mL^{-1}$). The amount of saline water in crude oil is highly variable. The ratio of brine to oil extracted from a deposit is related to the age of a recovery site. The ratio rose from 0.034-0.376 in the period 1951-1974 for oilfields in Alberta (Rowell and Crepin 1977). By contrast, values up to 17 were found for salt water to oil ratios at extraction sites in Kansas (Enright 1963).

2.2.2 General Physical Properties

Crude oil generally exists in the liquid state at room temperature (McGill et al. 1981). Densities of conventional oils fall within the 780-1000 kg m^{-3} range, most being between 800 and 900 kg m^{-3}. The range of typical viscosity values is between 1.6×10^{-4} and 2.3 kg m^{-1} s^{-1} (39°C) (Cuddington and Lowther 1977).

2.2.3 Composition and Characteristics of the St. Leon Spill Oil

Data on 1994 shipments of crude oil by Enbridge Pipelines, Inc. were gathered based on samples collected over a short time frame. Thus, the representativeness of these samples is uncertain (IPL Crude Characteristics Booklet 1994). The particular product that was released onto the St. Leon site was a Husky Synthetic crude oil. Its general composition and some of its physical properties are listed in Tables 2.1 and 2.2. Although no content data was given, it was noted that benzene, toluene, xylene and polycyclic aromatic HCs were all present in the product (Husky Synthetic MSDS 1993). Each of these compounds or group thereof were linked to certain health risks, ranging from carcinogenic, embryotoxic, immunological and teratogenic effects to chromosomal damage and blood diseases.

Table 2.1. Concentrations of constituents in Husky Synthetic product spilled at St. Leon site.

Hydrotreated Gas Oil [+]	Hydrotreated Heavy Naphtha [+]	Hydrotreated Light Naphtha [+]	Butane (Gaseous) [+]	Sulphur *
60-90%	10-30%	3-7%	0.0001-4.0%	0.03%

* IPL Crude Characteristics Booklet 1994.
[+] Material Safety Data Sheet Husky Oil Ltd. 1993.

Table 2.2. Physical properties of Husky Synthetic product spilled at St. Leon site.

Physical State [+]	Vapour Pressure • (kPa)	Density * (kg m^{-3})	Solubility in Water [+]	Pour Point * (°C)	Chemical Stability [+]
Liquid	31.0	850.2	0	<-27	Stable

* IPL Crude Characteristics Booklet 1994.
[+] Material Safety Data Sheet Husky Oil Ltd. 1993.

2.3 Effects of Crude Oil in Soil on Plant Growth

2.3.1 Direct Effect on Seed, Germination and Subsequent Growth

Direct acute toxicity can impede or prevent seed germination (Rowell 1977a). The most toxic components of petroleum are the lightweight volatile fraction. Thus, this effect is most prevalent in the early stages following a spill. Low-boiling compounds are most able to penetrate the seed coat and interfere or halt embryonic development (Udo and Fayemi 1975). The ability of seeds to withstand the phytotoxicity of HCs is strongly related to cell wall structure (Terje 1984). Thus, a structure better equipped to impede the diffusion of volatile oil constituents into cells will limit the impact on the seed. Later plant growth may also be hampered by direct toxic effects, leading to lower biomass yields (Bossert and Bartha 1985; Chaineau et al. 1996). The elimination of the toxic fraction of oil by biodegradation may be responsible for the normal plant growth occurring in soil in which only non-biodegradable compounds persist (Hund and

Traunspurger 1994; Chaineau et al. 1996). In the event of a large release of crude oil, the outer layers of the seed may be penetrated by oil, physically preventing germination.

In unsaturated conditions, the process of germination is impeded by the physical presence of oil. The hydrophobicity of the oil can impede gas exchange and create dry conditions next to the seed, thus inhibiting imbibition (uptake of water by the seed) (Udo and Fayemi 1975; Rowell and Toogood 1977; Amakiri and Onofeghara 1984). Water absorption by plant roots can also be hampered by the hydrophobicity of oil in soil at later growing stages (Swader 1975). Reilley et al. (1996) suggested this reason for reduced forage productivity in HC contaminated soil. This can disrupt root development (Udo and Fayemi 1975; Amakiri and Onofeghara 1984). Ilangovan and Vivekanandan (1990) observed that the presence of oil in soil can alter the geotropic orientation of rice seedlings, thereby reducing their emergence. Bossert and Bartha (1985) reported similar disruption of geotropism in roots and shoots of soybean. Root tips grew horizontally or curled toward the surface while shoot tips and cotyledons curved to the side or downward in crude oil contaminated soil. The suspected cause of these altered growth patterns was the hormonal effect of certain constituents of the petroleum. These compounds structurally resemble plant growth hormones and act in similar fashion to the real hormones. This growth disruption contributed to lower emergence of germinated seedlings and ultimately reduced yields.

2.3.2 Indirect Effect on Crop Growth through Changes in Soil Properties

Changes in soil properties with oil addition can indirectly influence plant growth. Aeration may be reduced physically due to impeded gas exchange at the surface caused

by crusting or the displacement of air and water from soil pores by oil. Oxygen depletion also results from the elevated microbial activity stimulated by the addition of a large carbon source to soil (Rowell 1977a). The decomposition of HCs is predominantly an aerobic process, thus placing a heavy demanding on oxygen supplies. Deficiencies of available forms of nitrogen, phosphorous and other macro and micronutrients can occur due either to inhibited formation of these available forms or competitive immobilization by the stimulated microbial community (Gudin and Syratt 1975; Currier 1951; Rowell 1977a). Nutrients may also become less available because of soil dessication caused by the hydrophobicity of oil (Terje 1984). A lowering of redox potential of soil may result in Fe and Mn toxicity which are more soluble in the reduced form (Rowell 1977a). This latter change is also a result of augmented microbial activity as the dependence of microorganisms on nitrate and sulfate as alternate electron acceptors increases. In poorly drained oil contaminated areas, vegetation can suffer from H_2S toxicity brought on by enhanced activity of sulphide-producers in the soil microbial community (Cook and Westlake 1975).

Water retention is diminished because of the hydrophobicity of oil (Udo and Fayemi 1975; Amakiri and Onofeghara 1984). When soil pores and aggregates become coated with oil, water is repelled away from surfaces. Evaporative losses from the surface may increase due to the darkening effect that oil has on the colour of the soil which leads to greater solar warming (McGill 1978). When oil exposure eliminates or prevents surface cover by vegetation, warming and evaporation can further rise. The moisture status of soil at the time of a spill is therefore critical to the extent of oil infiltration into soil, and subsequently the remediation and recropping of a site (Plice

1948; McGill 1977). Plice (1948) also cited the importance of soil moisture when explaining the reduced growth of several crops in soils to which he had applied crude oil. He downplayed the toxicity factor because of presumed volatilization, and instead emphasized the adverse effect of a disturbed soil hydrology on developing roots in the presence of HCs. He also noted the growth inhibition caused by oxygen deficiencies in a root zone containing HCs. Soil structure can also be adversely affected, such as through destruction of soil aggregates and resulting dispersion (Ellis and Adams 1961). Rowell (1977a) hypothesized that deaggregation results from the loss of natural binding agents such as waxes caused by the solvent action of lighter weight crude oil constituents.

However, long-term positive effects on soil properties are possible as HCs are attacked by soil microorganisms (Ellis and Adams 1961). Soil nitrogen may be increased as a result of elevated microbial activity. The formation of organic matter from oil-derived compounds occurs during the degradation process (Rowell 1977a; Bossert et al. 1984). Physical properties such as aggregation, porosity, water and nutrient retention, erodibility and drainage may all be improved over time as soil organic matter is formed from breakdown products (Rowell 1977; Hornick et al. 1983). In the case of marginally productive soils, such as those high in sand content, the time frame for improvements in physical properties can be relatively shorter in length. Biederbeck (1990) observed over three years that aggregation increased in amount and stability with the incorporation of oily waste sludge into a loamy sand soil. These increases were greater for the higher rate of sludge application. These beneficial changes could promote plant growth as they take effect over time.

2.3.3 Documented Positive Effects on Crop Growth

Instances of enhanced crop growth in response to the addition of crude oil and other types of HCs to soil have been observed. Carr (1919) documented improved soybean growth in soil containing 0.75% crude oil. Stimulation of growth has been generally observed only in cases of very low soil oil levels (<1.0%) and mostly for non-agricultural plants (Stebbings 1970; Baker 1970; Baker 1971). Compounds such as phenylacetic acid and various polynuclear aromatics (PNAs) found in crude oil may be plant growth-promoting substances or precursors to such growth-promoters (Gudin and Syratt 1975; Sims and Overcash 1983). Graf and Nowak (1966) reported that four of six PNAs tested caused increases in yields of summer rye kernels relative to controls. The other two PNAs substantially reduced grain yields. Naphthenic acids, cited for their phytotoxicity by Rowell (1977a), have also been suggested as plant growth-promoters both in the plant itself (Fattah and Wort 1970) and in soil (Severson 1974). However, McGill (1977) stated that direct and immediate enhancement of crop growth only occurs with small additions of certain constituents of oil. Generally, benefits to plant productivity are not to be expected.

2.3.4 Documented Adverse Effects on Crop Growth

Considerable evidence indicates that the presence of HCs in soil has either no effect on plant growth or an increasingly adverse impact with rising levels. The magnitude of a negative effect has been shown to depend on the size of the oil addition and the crop being grown.

Cereal, oilseed, leguminous and other perennial crops have all been studied for their sensitivity to HC contamination of soil. In early research, Murphy (1929) observed that mixing 0.3% by weight crude oil into the upper 10 cm of soil reduced wheat germination by approximately one quarter relative to a control. Treatments of 3 and 9% oil in soil prevented germination entirely. The 0.3% loading rate caused a 27% reduction in the number of growing plants per plot relative to a control. A concentration of approximately 1.5% reduced the number of plants per plot to near zero. Plice (1948) incorporated crude oil into the top 15 cm of soil at rates of 0.1, 0.5 and 1.0% by weight. Stands resulting from spring seeding of cotton, field peas, sorghum and soybeans all declined with increasing soil oil concentration. Subsequent autumn planting of wheat, barley and rye produced nearly complete stands and unaffected yields in all cases.

Chaineau et al. (1997) determined an LC_{50} (50% failure rate) value of 4% by weight fuel oil in a sandy soil for germination of wheat. Clover and barley showed greater sensitivity to the fuel oil with LC_{50} values of 3 and 0.6%, respectively. A dearomatized form of the fuel oil produced a higher LC_{50} value for wheat (18%) and clover (4%) but not for barley (0.4%). Sunflower showed the least susceptibility, based on germination results, to the adverse effects of the fuel oil in both forms. Wheat emergence was not reduced at soil oil levels <1%. However, subsequent growth was hampered at all concentrations. The fuel oil caused significant declines in dry aboveground biomass of wheat measured 45 days after the start of the experiment. Mean decreases in leaf, stem and fruit biomass were >80% for the 0.3% fuel oil treatment. The declines were greater at 0.6 and 1% fuel oil in soil but the magnitude of the declines did not increase linearly. Wheat leaves were chlorotic during the experiment. Trends in

bean growth followed those of wheat, while maize was more resistant to the influence of soil oil on growth. Germination results were reverse with bean and wheat more affected than maize. These results indicate that the effects of HCs in soil can vary among crops and among the growth stages of a particular crop.

Oat germination has been shown to be less susceptible to the adverse effects of crude oil in soil compared to other crops. Schwendinger (1968) recorded only a 2% decline in germinated oat seeds in soil containing 1% crude oil. Even at 3% oil, only 20% less oat seeds germinated relative to an unamended control. Similar lack of sensitivity of oats was found by Rowell and Toogood (1977) who observed no germination losses in soil containing as much as 4% crude oil. However, all successful germinations led to delayed shoot emergence, reduced maximum root and shoot lengths, lower dry weights and higher root:shoot ratios.

Rowell and Toogood (1977) reported good barley germination at one test site in lightly oiled plots (2.5%) and poor germination in more heavily oiled plots (6.1% and 11.1%) one year after application of crude oil to soil. The plots were remediated using fertilizer and tillage. Harvest yields were slightly lower than control values in the light treatment plots while yields in the two heavier treatment plots were zero. Germination was restored to control values in the high-oil plots two years after oil application to soil, but yields only improved slowly over several years of remediation. In the same study by Rowell and Toogood (1977) rapeseed was a secondary test crop, seeded 3 and 4 years after crude oil application at the two study sites. During that time, plots received various cultivation and/or fertilization treatments. Initial crude oil levels were 2.5% by weight in the light treatment plots. Analysis done the year before rapeseed was first seeded

showed that the soil at the two study sites had crude oil contents between 0.4 and 0.7%. By the second year of seeding, soil oil levels were roughly the same as the previous year, being in the range of 0.4 to 0.6%. These concentrations are within the range of those present in the soil in this study. At both study locations, rapeseed yields in most of the remediated plots were comparable to those in control plots in both years of crop trials.

Rapeseed has exhibited considerable sensitivity to the presence of oil in soil. A fuel oil concentration of 0.8-1.0% by weight, depending on the density of the oil used, reduced yields by 65% in a loamy sand (Kloke and Sahm 1961). Declines in rapeseed yield of 66-94% occurred in the first season following applications of approximately 0.8-2.0% fuel oil by weight to soil (Kloke and Leh 1963). An oil treatment in the range of 3.2-4.0% resulted in an absence of growth in the first year following application. Yield declines were only 18% in the third year of production due to a waning in the harmful effects of the oil.

Toogood (1977) conducted crop field trials in which plots received 1/3% to 2% crude oil by weight. Other than tillage, no steps were taken to remediate the soil. He reported no adverse effects of petroleum presence in soil on wheat or oat germination in a Luvisolic soil. Barley and flax demonstrated comparatively greater sensitivity to oil, while canola was the most susceptible to adverse effects. Four years of canola crops failed to germinate when grown in soil which received a 2% initial loading rate. Grasses and legumes had to be reseeded in order to become established. Similar though less striking trends were detected for the same crops on a Chernozemic soil.

Toogood (1977) recorded progressively greater declines in regrowth of hay stands with increasing oil levels three years after oil incorporation into field plots of Luvisolic

soils. No remediation techniques had been applied to the plots. Compared to the control (taken as 100% cover), the 1/3 and 2/3% oil plots showed relatively minor decreases in cover of 2 and 10%, respectively. Serious declines were observed in the 1, 1 1/3 and 2% oil treatments in which the stand estimates were 73, 65 and 52% compared to the control. Less marked impacts were reported for a Chernozemic soil. Four years after oil application, spring-seeded plots of fescue, brome and alfalfa exhibited oil-induced stress by autumn.

Klokk (1992) measured, in general, increasingly major declines in germination and vegetative growth of perennial vegetation with increasing rates of oil applied to soil. There were several instances of enhanced growth by the addition of low doses of oil to soil relative to a control. The plants tested included meadow grass (*Poa pratensis*), red fescue (*Festuca rubra*) and clover (*Trifolium repens*).

Wiltse et al. (1998) reported that the agronomic performance of alfalfa one year after establishment was hampered by the presence of 20 000 mg kg^{-1} crude oil in soil. Total yield and root mass declined 32 and 47%, respectively, in soil to which oil was applied compared to untreated soil. Compared to untreated soil, the soil receiving oil produced shorter plants which took longer to mature. Towards the end of the experiment, however, all agronomic variables except for root mass increased in value. This change in performance as the trial wore on was believed to be a result of a diminishing contaminant effect on plant growth as crude oil constituents were volatilized or degraded.

Toogood and McGill (1977) made a tentative classification of a number of crops based on general categories for resistance to growth impediment by oil introduced to soil.

Oats, wheat, sunflower and several grasses including brome were listed as having good tolerance. Rowell (1977b) also described brome grass as having low sensitivity to the presence of oil in soil. Barley, white clover, timothy and creeping red fescue were considered as moderately tolerant. Canola and alfalfa were characterized as having poor tolerance. This classification generally agrees with other crop ratings for sensitivity to oil in soil, with some exceptions. For example, Rowell (1977b) placed wheat in the medium tolerance class. Flax has also been described as being somewhat to highly susceptible to the adverse effects caused by oil in soil (Toogood 1977; Rowell 1977b).

The type of oil influences the effect of HC exposure on plant growth. Toxicity tends to be greater for lighter weight and less viscous liquids like fuel oil than for crude oils and sludges. 0.6% by weight fuel oil in 0-60 cm of soil was shown by Swader (1975) to cause yield declines for deep-rooting crops. Crude oil levels must typically be higher in soil than this latter concentration in order for serious yield reductions to occur (McGill et al. 1981).

2.3.5 Critical Oil Concentrations for Crop Growth

Attempts have been made to identify critical thresholds for successful plant growth in soils containing HCs. Carr (1919) observed that soybean growth was unaffected in soils possessing less than 4.0% crude oil. Biederbeck et al. (1997) suggested an optimal initial soil concentration of 0.7-1.0% waste HCs in attempting to improve the soil quality of marginal lands. Racz and Cansfield (1977) recommended an application rate of 1.0% for refinery HC wastes added to a high clay soil. This loading rate can be sustained provided that adequate tillage and fertilization is conducted and that

the soil is fallowed for one year before continuing crop production. This recommendation was made specifically for barley, the crop under investigation in their study. Toogood et al. (1977) found that 0.3-0.95% crude oil in soil was acceptable for unaffected barley growth, provided that sufficient soil nutrient levels were maintained. After several years of remediation, canola yields in plots containing 0.4-0.6% crude oil by weight were comparable to those in check plots. Overcash in Dueul (1990) determined tolerance levels for crops grown in soil which had just been treated with oil. Canola could tolerate a single oil application which was <0.5% by weight, while wheat could withstand a <1.5% loading rate. Perennial grasses demonstrated a tolerance for oil concentrations which were >3%. Alfalfa seeds were capable of germinating when exposed to crude oil levels in soil as high as 50 000 mg kg^{-1} (Wiltse et al. 1998).

Rowell and Toogood (1977) noted that texture and soil organic matter levels will influence critical oil concentrations for seed germination as a result of the balance between water and oil holding capacities of soil. Texture may also help determine such thresholds for crop production through its influence on HC degradation. Rhykerd et al. (1995) demonstrated that mixing an amount of a clay-loam soil into a sandy clay-loam soil increased the rate and extent of oil breakdown in the latter soil, possibly by elevating its pH and microbial degrader populations. Texture may thus influence the degree of competitive nutrient consumption by soil microorganisms but also the length of time required for site productivity to be restored to pre-spill levels.

2.3.6 Plant Uptake of Polyaromatic Hydrocarbons (PAHs)

PAHs are important constituents of crude oil because of their prevalence and their potential health risk to organisms which absorb them from the environment. The behaviour of certain PAHs under similar soil and crop conditions has been found to vary. Shabad and Cohan (1972) found the natural background levels of one PAH, benzo(a)pyrene (B(a)P), to be 0.29 and 27.0 $\mu g\ kg^{-1}$ in spring wheat seed and stem, respectively. These authors also determined that B(a)P contents of wheat plant parts were not related to soil levels. The highest B(a)P amounts were found in spring wheat straw (27.0 and 26.7 $\mu g\ kg^{-1}$ from crops grown in soil containing 1.6 and 170 μg B(a)P kg^{-1}, respectively). Wagner and Siddiqi (1970) found similar results for B(a)P contents in summer wheat, except that the highest concentrations were in the stem. By contrast, 3,4-benzfluoranthene presence in wheat plant parts was substantially greater and was a function of soil levels, with smaller differences between the various plant components assayed (Wagner and Siddiqi 1970). Despite these differing fates in the wheat plant, both PAHs exhibited biomagnification based on their concentrations in the seed, stem and straw.

A number of researchers have detected PAHs only in root parts of plants grown in soil treated with sewage sludge and none in aboveground parts (Hulster et al. 1994; Wild et al. 1992; Wild and Jones 1992). Wild et al. (1992) measured PAH concentrations which were consistently $<1\ mg\ kg^{-1}$ in the tissues of grass, clover and barley crops grown in sewage amended soils. PAH levels in aboveground plant parts were not related to soil levels; absorption from the atmosphere was proposed to be the pathway of uptake. Plant uptake of organic substances from soil has been correlated with

octanol/water partition coefficients (K_{OW}) (Trapp et al. 1990). Chaineau et al. (1996) detected no petroleum HCs in wheat, pea and maize seeds produced on mildly polluted land, demonstrating that uptake did not occur from soil containing plant-tolerable oil levels. Chaineau et al. (1997) found that, even at soil HC levels of 1%, absorption of low K_{OW} compounds by maize was extremely minimal. However, the authors pointed out that the potential phytoavailability of polar by-products of HC biodegradation may exist.

2.4 Effects of Crude Oil on Soil Biological Quality

2.4.1 Soil Quality and Soil Health

The concept of soil quality is becoming increasingly important as land is assessed for its "health" in both ecosystem and human-use contexts. Doran and Safley (1997) defined soil health as "the continued capacity of soil to function as a vital living system, within ecosystem and land-use boundaries, to sustain biological productivity, promote the quality of air and water environments, and maintain plant, animal and human health." The authors also noted the ability of soil to continue to function under changes induced by human or natural causes over time. The concept of soil quality is preferred by some as it is considered a more quantifiable entity based on physical, chemical and biological properties. The biology of soils plays a vital role in a number of soil processes including nutrient cycling, soil organic matter decomposition, soil development and pollutant stabilization and degradation (Turco et al. 1994). Soil biological phenomena also contribute heavily to the resiliency of the soil system when subjected to a disturbance (Karlen et al. 1992). Soil quality reflects system productivity and should therefore reflect

potential agronomic productivity (Yakovchenko et al. 1996). Since the biology of soil is an essential part of soil quality, soil microbiological properties should then serve as useful indicators of the health of an agricultural system. The dynamics of the soil microbial community, in particular, are a dominant force at the ecosystem level in both pristine and disturbed environments (Turco et al. 1992). In the latter systems, soil microbes regulate restoration processes which make it possible for floral and faunal life to recolonize a perturbed site. Thus, soil microflora, being a major fraction of soil biology, are considered to be an essential component of soil quality (Howard 1947; Higa 1991).

2.4.2 Biological Indicators of Soil Quality

Soil microbial parameters have been proposed as biological indicators in order to determine the effects of disturbance on soil quality (Turco et al. 1992). The utility of soil microorganisms in this manner is made possible by their high sensitivity to changes in their habitat. The objective is to measure a particular parameter(s) which can describe the biological state of the soil system. This information can then reveal the general condition of soil, including the physical and chemical aspects, because of the interrelatedness of these three categories of soil properties.

Holloway and Stork (1991) identified a number of criteria which should be met by an effective bioquality indicator. The indicator should: 1) react promptly and accurately to a disturbance; 2) be related to ecosystem function in some capacity; 3) be ubiquitous but also be specific in it its response to an introduced change and 4) enable easy, economical and reliable measurement. Visser and Parkinson (1992) recognized

three levels of organization for soil biological indices: population, community and ecosystem. These authors recommended ecosystem (i.e. process) level examinations of soil biology in order to quickly establish the state and on-going change in soil biological quality. At this level are such microbial parameters as microbial biomass carbon and soil enzymes. Zak et al. (1994) argued the importance of functional diversity of the soil microbial community in the overall biological quality of the soil environment.

Sparling (1997) recognized the importance of establishing target values for microbiological indices which are used to assess soil quality. In order to make accurate statements about the state of a soil's biological quality or health, minimum, maximum or optimal levels of microbial community size and activity must be defined. 'Baseline' or threshold values below or above which biological quality is deemed to be affected by a disturbance would indicate when present conditions or trends in the soil environment are no longer acceptable. In such instances, land use or management changes or remedial actions would then be warranted to arrest declines in soil quality.

The impacts of changes in the status of soil biological indicators must be identified. This understanding will provide context for the process of setting threshold values and justification for any subsequent changes in human activity which utilizes and affects the soil resource.

2.4.2.1 Soil Microbial Biomass. Rasmussen and Collins (1991) recognized soil organic matter (SOM) as the most all-encompassing measure of soil quality. Within SOM is the carbon associated with microbial biomass (MB). Fluxes in MB may follow changes in SOM but within a much more practical time frame than for SOM itself (Powlson et al.

1987; Sparling 1992). Methods of quantifying microbial biomass offer an estimate of the portion of SOM which is alive at a given moment in time, i.e., the "standing crop" (Voroney et al. 1993). Microbial biomass carbon (MBC) represents stored energy for fueling microbial reactions, thus making it a measure of potential activity of soil microflora (Rice et al. 1996). Because of the influence of MB on soil physical and chemical properties, it is an integrative indicator of soil quality. This characteristic of MB may eliminate the need for multiple analyses (Rice et al. 1996). The rapid turnover of MB, <1 yr according to Paul (1984), enables early detection of effects from land contamination by such contaminants as metals (Chander and Brookes 1991 a,b, 1993). However, this sensitivity to introduced substances can lead to the masking of long-term changes in soil quality by recent amendments (Fauci and Dick 1992).

2.4.2.2 Soil Enzymes – Dehydrogenase. Soil enzymes fulfill a critical role in many biochemical processes and are an important aspect of soil quality. They may also serve as process level indicators of soil quality. Measurement of soil enzyme activities has been suggested as a technique for assessing the impact of crude oil introduction on soil microbiology (Rowell 1977). The extraction of soil enzymes has proven to be inefficient due to enzyme breakdown during the procedure (Tabatabai 1982). Consequently, the preferred alternative to direct extraction has been the measurement of enzyme activities (Dick 1994). This approach is considered acceptable because activities are (a) strongly associated with other aspects of soil quality including SOM, soil structure and MB or general activity; (b) show a rapid response to introduced conditions (within 1-2 yr) and (c) serve as a historical marker of previous soil conditions affected by either management

or disturbance. Biological activity should be higher in soils of higher overall quality, presumably documented in part by elevated enzyme production, stabilization and activity.

Dehydrogenase is a widely examined oxidoreductase. The pathways of this enzyme contribute heavily to the oxidation of SOM through the transfer of hydrogen from substrates to acceptors (Dick 1994). Assays in general encompass the activities of enzymes associated with both living and non-living soil components. Therefore, it is not always possible to confirm that the source of activity is a viable microbial population. This situation may produce a poor correlation between enzyme activity and MB. Dehydrogenase is exempt from this difficulty. It is an intracellular enzyme and therefore associated with only living cells. Thus, it is related to total metabolic activity. Despite this, there have been several instances of poor correlation between dehydrogenase and microbial activity measurements such as viable microbial counts, oxygen uptake and carbon dioxide production (Frankenberger and Dick 1983; Ross 1973; Howard 1972).

Howard (1972) put forth an hypothesis to explain these failed relationships. Extracellular phenol oxidases are capable of initiating the dehydrogenase reaction, thus competing for substrate. This competition could diminish the utility of dehydrogenase activity as an indicator of viable microbial activity. Nevertheless, its close association with living microorganisms in soil make dehydrogenase a sensitive responder to disturbance or amendments in the soil system, and therefore a potential indicator of soil biological quality. Dehydrogenase activity has also been directly related to agronomic parameters including crop yield, compost amendment and rates of straw application (v. Boguslawski et al. 1976; Cole et al. 1994).

2.4.2.3 Metabolic Diversity. Biodiversity is a highly regarded though unclearly defined element of nature (Lubchenco et al. 1991). Solbrig (1991) delineated three components of biodiversity: taxonomy, genetics and functionality. Methodological constraints and information gaps have limited the study of the first two components in the field of microbiology (Klopatek et al. 1993). Consequently, a shift in focus to functional diversity may yield an improved understanding of the ecosystem level contribution of soil microorganisms. Zak et al. (1994) suggested that functionality may prove to be more readily measurable and is certainly an equally crucial element of biodiversity. Garland and Mills (1991) developed the application of the BIOLOG redox procedure to the determination of functional diversity of microbial communities. It is based on the measurement of sole-carbon-source utilization by heterotrophic organisms which can be sampled from a variety of environments. The microplate identification system can be used to assess the functional or metabolic diversity of the microbial community of a soil (Zak et al. 1994). Within the confines of this procedure, microbial metabolic diversity is operationally defined as the proportion of substrates utilized by bacteria existing in soil extracts. This approach provides an opportunity to detect biologically meaningful trends in substrate utilization.

2.4.3 The Effects of Crude Oil Introduction on Soil Microbiological Variables

The nature of the soil environment is a function of the physical and chemical characteristics of the soil, and the soil environment determines both the quantitative and qualitative nature of the soil microbial community (Alexander 1961). Any introduced disturbance to the soil environment can thus potentially alter the state of the soil

microbial community. The introduction of crude oil is one such disturbance which affects the biological quality of soil.

The results of investigations into the effects of crude oil on soil microbiology have typically shown that microbial activity is elevated by the presence of petroleum HCs. The evidence has been documented for microbial numbers, O_2 consumption and CO_2 evolution (Baldwin 1922; Plice 1948; Dobson and Wilson 1964; Jobson et al. 1972; Biederbeck 1990; Joergensen et al. 1995). The response patterns of microbial populations to oil addition to soil have tended to consist of initial small declines in total numbers followed by rapid growth. The initial declines may be due either to toxicity of certain volatile crude oil constituents (Buddin 1914; Rowell 1977) or the reduced availability of mineral nitrogen caused by HC-utilizing populations which immobilize N (Hornick et al. 1983). Eventually, total microbial numbers rebound as shifts in the composition of the soil microbial community occurs (Overcash and Pal 1979). Larger populations following addition of HCs to soil are primarily the result of adaptation and growth of oil degraders (McGill et al. 1981). While an increase in the total microbial population in soil is usually observed, species diversity tends to decline (Gossen and Parkinson 1974; Jensen 1975; Llanos and Kjoller 1976). Ultimately, population levels drop as available substrate is consumed and essential nutrients, such as N and P, become limiting (McGill et al. 1981). Microbial respiration follows the trends of population numbers.

Considering the contribution of microorganisms to the soil ecosystem (Turco et al. 1994), the potential effects of crude oil contamination on soil microbiology are of importance.

2.4.4 The Effect of Plant Growth or Carbon Amendment on Soil Microbiology

Both the establishment of vegetation (Ross and Cairns 1982; Curl and Truelove 1986; Drury et al. 1991) and addition of wheat straw (Powlson et al. 1987; Ocio and Brookes 1990) have been shown to affect aspects of the soil microbial community. In each case, the treatment applies carbon to soil which is expected to influence microbial indices based on the strong association of microbial biomass (MB) and activity with SOM (Collins et al. 1992). Plant growth can also influence microbial activity by modifying soil structure, aerating soil and distributing root-associated microorganisms throughout soil as roots proliferate.

The establishment of forage crops has been used to increase soil microbial biomass relative to uncropped soil (Drury et al. 1991). Peaks in microbial biomass carbon (MBC) were associated with periods of vigorous growth. Ryegrass growth increased soil MBC based on the measurement of glucose-induced maximal initial respiration rate (Ross and Cairns 1982). Microbial numbers have been shown to be greater in alfalfa planted soils than in bare soils in both contaminated and uncontaminated soils (Lee and Banks 1993; Schwab and Banks 1994). The size of the microbial community in the root zone has been demonstrated to be a function of plant type and species (Perfect et al. 1990; Drury et al. 1991).

Ocio and Brookes (1990) observed increases in MB that were nearly 100 and 50% in a sandy loam and clay soil, respectively, following the recent amendment of wheat straw. Measurements were taken 13 and 35 d after amendment. Powlson et al. (1987) found significant increases (45 and 37%) in MB after 18 years of straw application to two sandy soils. Schnurer et al. (1985) obtained similar results for 27 year

plots which had received yearly straw inputs. By contrast, Ritz et al. (1992) reported no prolonged beneficial effect of a single straw amendment on MB and suggested multiple yearly applications as a means to this end. Scow et al. (1994) supported this belief with their observation that 3 years of cover crop incorporations were required for elevated MBC in soils under organic versus conventional soil management practices. The combined presence of petroleum HCs and either a rhizosphere or incorporated straw may produce unique results for various measures of soil microbiological activity.

Certain grasses, such as ryegrass, have demonstrated an ability to enhance respiratory and enzyme activities in soil (Ross and Cairns 1982). The amendment of soil with plant roots significantly increased soil dehydrogenase enzyme activity relative to that of unamended soil (Christensen et al. 1992). Stevenson (1959) made preliminary observations of responses in soil dehydrogenase activity to the addition of crop residues to soil. He found that the relationship between the activity of this enzyme and oxygen uptake held when he used a number of different crop residues. In a follow-up investigation, Stevenson (1962) reported that the amendment of chopped biomass from 8 week old wheat to the same soil in which it had been grown elevated dehydrogenase activity above that of an unamended soil. The effect peaked after 10 d and then activity diminished towards the control value at 66 d. Straw incorporation rate has been shown to affect DHA (v. Boguslawski et al. 1976). Higher rates of addition generated higher DHA in soil.

Straw incorporation has been shown to elevate levels of substrate utilization in different soil types and for different frequencies of application (Bossio and Scow 1995). The enhancement occurred in both a near-neutral clay which received a single

application of mature, dry rice straw and in an acid loam which received successive straw applications over 6 years. The comparison treatment in both cases was land on which straw was burned. This effect was attributed to altered microbial community composition due to the organic amendment in a carbon limited soil environment.

2.5 Methods of Hydrocarbon Analysis

2.5.1 Analysis for Total Oil and Grease

Total oil and grease has been a commonly measured variable in assessing HC presence in soil. The procedure for quantifying total oil and grease in soil is a gravimetric determination based on a Soxhlet extraction with methylene chloride (CH_2Cl_2) (McGill and Rowell 1977). This solvent was preferred by the authors for its efficiency of extraction, low boiling point, low hazard level and relatively low cost compared to other extractants. Brown and Deuel (1983) also favoured methylene chloride because of it does not tend to withdraw constituents of native organic matter in any significant amounts. The results can be expressed on a per cent by weight basis or in kg oil m^{-2}. When comparing results from different soils, the latter units are more suitable because it includes soil bulk density which varies among soils.

2.5.2 Analysis for Total Extractable Hydrocarbons

Another measure of HC content in soil is the quantity of total extractable HCs (TEH). This category of HCs represents the C_{10}-C_{30} fraction which is extractable by GC/FID (gas chromatography using a flame ionization detector). The method for TEH

determination thus examines a specific portion of crude oil in soil in contrast to the more general HC analysis for total oil and grease.

2.5.3 Native Soil Hydrocarbons and Degradation Products

HCs such as fats and waxes occur naturally in soils. McGill et al. (1981) estimated that 1-5% and 5-20% of organic matter in mineral soils and organic soils, respectively, is extractable by lipid solvents. Other researchers have found complex polycyclic aromatic HCs which are naturally present in soils at levels of 50-500 $\mu g\ kg^{-1}$. The amount and type of HCs in soils depends for the most part on pH and vegetation factors. The effect of pH may be the most important, determining either the type of vegetation that can grow or the hydrocarbon decomposition rate. Higher acidity in soil has been associated with high levels of HCs and reduced rates of microbially-mediated degradation. The presence of native HCs in soils must be accounted for in quantifying the content of crude oil or other fossil fuels in soil.

Some products of the degradation process are able to combine with constituents of either soil organic matter or the HC mixture itself (Rowell 1977a). The result are new humic substances which may exhibit properties different from those of the original HCs, preventing the detection of these new compounds by extraction procedures. This represents a possible source of error in the determination of HC content of a soil.

2.6 Biological Degradation of Hydrocarbons

A wide range of microorganisms, including bacteria, actinomycetes, yeasts and filamentous fungi are capable of breaking down crude oil, often generating energy or

allocating carbon to biomass in the process (Hornick et al. 1983; McGill et al. 1983). Besides these two forms of carbon utilization, soil microbes also break down HCs in soil through *cometabolism* (Hornick et al. 1983). This metabolic process was first described by Leadbetter and Foster (1959). It involves the concurrent oxidation of a molecule, which can not serve as a sole carbon source, with that of a metabolizable substrate. The versatility of the starting enzyme in metabolizing different substrates is the likely factor which enables cometabolic decomposition of HCs. The cometabolically altered compound remains unmetabolizable for the co-oxidizing microorganisms, but is likely to become susceptible to decomposition by other microbes over time.

Soil salinity has been found to affect the degradation of crude oil in soils and marine sediments. The metabolic activity of microorganisms is generally inhibited by salinity (Walker and Calwell 1975). Thus, salinity may affect oil dissipation in soil through its influence on microbial activity. Rhykerd et al. (1995) observed less CO_2 production and more residual oil in soil to which motor-oil and NaCl were applied than in soil treated with only motor-oil. These trends were magnified as soil EC levels increased from 40 to 120 to 200 dS m^{-1}, values which represent extremely high soil salinity. The highest NaCl treatment represented a typical salinity level for oil field brine. By contrast, numbers of HC-degrading microbes did not fluctuate appreciably with changing EC levels. The authors hypothesized that the osmotic effect of the NaCl on soil microorganisms was limited to their activity and not their viability. Oil degradation based on rates of O_2 uptake and CO_2 production was shown to decrease with increasing concentrations of NaCl in cultures of crude oil degraders taken from marine

sediments (Haines et al. 1994). Cumulative oxygen consumption and CO_2 evolution were not affected by NaCl levels.

A portion of crude oil remains at the end of the degradation process due to its resistance to microbial attack. This recalcitrance is caused by the low degradability of the remaining compounds and their low availability to soil microorganisms (Harmsen 1991). During the degradation process, the composition of crude oil changes, with less reactive compounds persisting. These compounds also tend to be of low volatility and only slightly water soluble.

2.7 Reclamation of Crude Oil Contaminated Land

2.7.1 *In Situ* Bioremediation

The basis of *in situ* bioremediation is the enhancement of microbial activity in contaminated soil by optimizing soil conditions which enable this activity and, therefore, the degradation of introduced compounds. Optimization is typically achieved by supplying the essential requirements for the soil microbial community to grow and function, such as nutrients, oxygen (in the case of aerobic microorganisms), water and electron acceptors.

2.7.2 Landfarming

Landfarming, also known as land treatment, has become a widely used approach to the remediation of soils contaminated with organic pollutants. It is commonly applied to HC contaminated land with the intent being to encourage volatile escape to the atmosphere and biodegradation of soil contaminants as environmental fates (Reilley et al.

1996). The former fate has become a less desirable means of disposal because of concerns over air quality. Landfarming is a relatively simple and inexpensive technique based on natural biological processes (Harmsen 1991). Oil-containing soil can be treated on-site in either a constructed unit or in an unaltered contaminated site provided that the area is confined and manageable.

The procedure consists of optimizing the conditions necessary for HC degradation by resident soil microorganisms: adequate levels of nutrients, oxygen and contaminant bioavailability (Bollag et al. 1994). Dilution of the contaminant is also often done by mixing the affected soil with unpolluted soil (Pierzynski et al. 1994). Nutrients, primarily inorganic nitrogen and phosphorous, are applied in the form of fertilizers. The application of fertilizer can be effective in countering the phytotoxicity of HCs in soil, even in years of drought (Biederbeck 1990). Of particular importance is the soil C:N ratio which is increased by the addition of HCs to soil which are carbon rich and nitrogen poor. The application of inorganic N restores the C:N balance in soil to a level which promotes microbial activity. The target soil C:N ratio is typically reported as being 10:1 (Jobson et al. 1974) or 20:1 (Cook 1977).

Cultivation improves aeration and microbial-contaminant contact in HC-affected soil by redistributing oil, nutrients and microbes. Replenishing the oxygen supply in soil is essential for remediation of HC contamination because the biodegradation of HC contaminants is largely an aerobic process. In the case of PAHs and other crude oil constituents, breakdown can only occur along an aerobic metabolic pathway (Harmsen et al. 1997). However, landfarming has a depth limitation of 0.5 m or less based on feasible tillage depth (Bollag et al. 1994). Toogood et al. (1977) found no major differences in

crop growth when comparing the relative efficacies of intensive cultivation (deep and frequent rototilling) versus normal tillage practices (shallow rototilling carried out only for weed control). These results led the researchers to believe that there were also no major differences in oil dissipation and that standard cultivation was sufficient to stimulate the activity of soil microorganisms.

The separate effects of nutrient amendment (laboratory experiments) and tillage (laboratory and field experiments) were demonstrated by Hoeks et al. (1988). Both the addition of inorganic N and P and regular mixing of contaminated soil accelerated HC breakdown and increased total degradation based on declines in O_2 concentration. Toogood et al. (1977) brought about successful remediation of oil amended field plots using a variety of treatment combinations. Oudot et al. (1989) demonstrated the effectiveness of landfarming when 94% reductions in free fossil HCs were achieved in a field study. However, the authors warned that not all the carbon associated with these degraded compounds could be expected to be removed from the soil. Metabolic by-products were likely to persist due to incomplete mineralization, particularly in deeper soil.

Harmsen et al. (1994) suggested that landfarming has two phases. The first phase is short-term intensive landfarming in which conditions for the degradation process are optimized. In this first step, the bioavailable fraction of an organic contaminant is metabolized by soil microbial degraders. When this bioavailable fraction is eliminated, only the recalcitrant fraction remains. The amount of this latter portion of the contaminant is unaffected by continued intensive landfarming. At this point, the proposed second phase, extensive landfarming, should begin in which alternative means

to cultivation are used to aerate the soil. This second phase is intended to address the residual nonbioavailable fraction which could be metabolized by soil microbes if it could be made accessible. Thus, extensive landfarming is a long-term process of removing adsorbed molecules which must slowly detach from soil particles and enter the soil solution. The greater the exposure time of soil to pollutant and the greater the affinity of the compound to soil particles, the longer the time period that will be required for the nonbioavailable fraction to become available. Measures proposed by the authors to economically optimize conditions for contaminant degradation included nutrient and compost amendments to improve soil structure and the establishment of suitable vegetation to fix N and aerate the soil.

2.7.3 Phytoremediation

The use of vegetation has been proposed as an innovative approach to remediation of land contaminated with HCs (Reilley et al. 1996). This approach is less site-invasive, simpler and cheaper than other remediation technologies. It can serve as alternative or supplemental measures to other accepted practices such as landfarming.

Soil microorganisms contribute heavily to HC degradation in soil (McGill et al. 1981). The presence of a rhizosphere can augment microbial numbers and stimulate microbial activity (Curl and Truelove 1986). A proposed mechanism of this phenomenon is *rhizodeposition* of readily metabolized organic substrates (Elliot et al. 1984). Plants can release from their growing roots as much as 15-40% of the carbon derived from photosynthesis (Cunningham et al. 1997). Several kinds of substances are deposited into the root zone or rhizosphere, including root exudates (passive release),

secretions (active release), plant mucilages (substances from root cap cells, cell walls and epidermal cells), mucigel (a complex mixture of plant materials) and lysates (from the lysis of old epidermal cells) (Rovira et al. 1979). These substances are generally made up of readily metabolizable organic compounds (Rovira and Davey 1974; Reilley et al. 1996). These substrates range from light-weight compounds including amino acids, organic and fatty acids and simple sugars to heavier polymers such as polysaccharides (Curl and Truelove 1986). Depending on their nature, rhizodeposits can also increase the availability of soil nutrients, serve as chemoattractants or exert an antiseptic effect on certain soil microbes (Shann and Boyle 1994). Direct carbon amendments into soil, predominantly through tillage, may have similar effects. This elevated microbial activity in the root zone or depth of carbon incorporation may enhance the degradation of HCs in soil.

Research has found that the effect of plant growth on the soil environment varies among plant species and types (Curl and Truelove 1986; Bachmann and Kinzel 1992). Plant rooting patterns and root morphology may contribute to this variation as well as determine the volume of soil influenced by root growth (Walton et al. 1994; Shann and Boyle 1994). Root surface area, diameter, surface:volume ratio and total biomass as well as rooting depth influence the effect of plant roots on the soil environment. Forage stands exhibit many attributes in their use in revegetating oil contaminated land. These crops provide a surface cover and extensive root system, together improving soil stability. The deep-rooting pattern of forages enables access to water, nutrients and contaminants at greater soil depths. Perennial plant roots also influence the soil environment for a greater portion of the growing season than do those of annual plants

(Walton et al. 1994). Microbial biomass carbon (MBC) has been shown to be relatively stable throughout a growing season under perennial crops (Chantigny et al. 1996). This stability is explained by the fact that the root systems of perennials continuously grow and release rhizodeposits from spring to autumn following the establishment year. Chantigny et al. (1996) also found a significant correlation between MBC and plant-derived water-soluble organic C. This result indicated that the size of the microbial community associated with a given rhizosphere was a function of the rhizodeposition patterns of the particular type of vegetation.

Prairie grasses, in particular, have been proposed as suitable candidates for phytoremediation for several reasons. The fibrous root systems exhibited by grasses, especially sod-forming species, maximize surface area over which the rhizosphere effect can occur. The zone of particularly enhanced microbial activity is along the interface between the root surface and the soil matrix known as the rhizoplane (Foster and Bowen 1982). The genetic diversity of prairie grasses may offer considerable flexibility in degrading capabilities. Finally, the perennial life cycle of grasses requires a single establishment year, minimizing input costs, and provides continuous weed competition and soil stability (Aprill and Sims 1990).

Recent research has shown that the growth of grass can accelerate the degradation of certain polycyclic aromatic HCs (PAHs) which are constituents of crude oil. Aprill and Sims (1990) monitored the change in concentration of four PAHs under eight prairie grass species with manure amendments. PAH declines were consistently greater in rhizosphere treatments than unvegetated soils after 59 days. The greater decreases in rhizosphere soils became statistically significant after 151 days.

Reilley et al. (1996) studied anthracene and pyrene dissipation under four grass treatments in two soils, one which was already contaminated and another which had not been previously exposed to PAHs. Except for the 'uncontaminated + anthracene' set, in which all treatments had undetectable concentrations after 16 weeks, PAH levels were lower in all vegetated soils than unplanted soils after 24 weeks. PAH mineralization, based on $^{14}CO_2$ evolution from ^{14}C-labeled compounds, was greatest in planted systems with organic acid amendments.

Prairie Buffalograss (*Buchloe dactyloides* var. Prairie) has demonstrated an ability to significantly enhance naphthalene disappearance in 0-30 cm of a clay soil but not below 30 cm (Qiu et al. 1997). The absence of an effect in subsurface soil was attributed to a lack of root growth in very high moisture conditions. Other test low molecular weight (LMW) PAHs were also lower in concentration in the surface soil under the grass than in unvegetated soil, although the results were not significant. High molecular weight (HMW) PAHs were higher in concentration in seeded than in unseeded plots throughout the soil profile. In another part of the same study, Qiu et al. (1997) found Kleingrass (*Panicum coloratum* var. Verde) to be the most effective among several grasses tested in enhancing both LMW and HMW PAH dissipation from soil. The superiority of this particular grass species was possibly attributable to its more extensive root system than those of other grasses. Other grass species had success in promoting reductions in LMW PAH concentrations but, unlike Kleingrass, failed to augment losses of HMW PAHs from soil relative to an unplanted control.

Alfalfa has also demonstrated the potential to enhance the degradation of individual HCs (Reilley et al. 1996) as well as crude oil in soil (Wiltse et al. 1998).

Reilley et al. (1996) observed anthracene concentrations in alfalfa pots were significantly less than those in unplanted pots after 24 weeks; the soil in both sets of pots was previously contaminated with petroleum HCs and spiked with a mixture of anthracene and pyrene. In the same period of time, pyrene degradation was significantly greater under alfalfa than in the absence of vegetation in uncontaminated soil spiked with the PAH mixture. The difference between pyrene levels in alfalfa planted and unplanted pots with contaminated soil + PAHs was not statistically significant but still considerable. Wiltse et al. (1998) reported significantly enhanced HC dissipation in alfalfa-planted soil containing 2% (w/w) crude oil relative to unvegetated soil. However, this result was only obtained with two of twenty genotypes tested. Thus, variability for phytoremediation capabilities among alfalfa genotypes was detected, indicating the possibility for breeding manipulation in order to maximize HC-degrading potential (Wiltse et al. 1998).

Crop residues with a narrow range of C/N ratios promote microbial activity in soil (Drury et al. 1990). Certain rhizosphere microbial populations may be enhanced in their activity by the narrow C/N ratio of legumes (Rothrock and Hargrove 1988). Leguminous vegetation has successfully colonized oil-polluted sites, perhaps because their N-fixing abilities give them a competitive advantage over other plants in N-limited soils (Gudin and Syratt 1975). Consequently, legumes were suggested as good candidates for revegetating HC-contaminated sites and to improve the rhizosphere.

The effect of vegetation on the fate of HCs in soil has not been consistent for all soil-plant-contaminant systems. Watkins et al. (1994) observed less naphthalene mineralization in soil microcosms with Bell Rhodesgrass (*Chloris gayana*) than in

unplanted soil microcosms. It was speculated that the reduced mineralization in the vegetated microcosms was possibly due to plant uptake and subsequent volatilization of naphthalene. Alternatively, the introduction of organic compounds from grass roots may have elevated the competition for resources between naphthalene degrading and non-degrading microorganisms. It was also suggested that enzyme induction for naphthalene metabolism may have been reduced by the stimulation of enzymatic pathways directed at other readily available substrates.

A proposed explanation for enhanced contaminant degradation in the root zone is the effect of root presence on the soil microbial community. Plant roots provide physical habitat for a microbial community to occupy, a structure upon which colonies can form (Walton et al. 1994). Plant roots also release, both passively and actively, organic compounds which are readily metabolized by soil microorganisms in the vicinity of roots (Rovira and Davey 1974). As a result, the size and activity of microbial populations are augmented, a phenomenon known as the rhizosphere effect. Curl and Truelove (1986) reported that typical rhizosphere populations are 2-20 times larger than in non-rhizosphere soils, though 100 fold differences have been reported. This enhancement of the microbial community, and some selected organisms in particular, may be responsible for the accelerated degradation of HCs in rhizosphere soil. In attacking organic root exudates (primary substrate), soil microorganisms may also break down HCs (secondary substrate) which are susceptible to the primary metabolic pathway. This process of cometabolism may also be stimulated by applying readily decomposable carbon amendments to contaminated soil.

Plant root growth may enhance the decomposition of pollutants in soil by other means. Soil aeration is augmented in the rhizosphere of prairie grasses through the proliferation of their fibrous roots which are able to influence a considerable volume of soil as deep as the roots can reach (Qiu et al. 1994). Growing roots extract water from soil pores which can then be filled with air (Qiu et al. 1997). Root growth may also improve soil structure by encouraging aggregation. The death and subsequent decay of roots leave behind vacant channels through which oxygen and water may pass. In a grass rhizosphere, from one quarter to one half of roots in the upper portion of the soil profile are dead or dying (Newman 1985). Improved aeration and moisture content contribute to greater microbial activity and degradation of organic contaminants. Alternatively, some plants can take up contaminants *(phytoextraction)* which can then be removed from the soil when the plants are harvested (Cunningham et al. 1997). Such pollutants include metals which are present in varying amounts in crude oil (Bollag et al. 1994). A concurrent approach to phytoextraction is *phytostabilization* in which contaminants are held in place by vegetation which limits contaminant escape from the site by erosion or leaching (Cunningham et al. 1997). Pollutants may be absorbed by or adsorbed to plant roots or be immobilized in the soil due to water uptake by plants, preventing vertical migration in the soil profile. By elevating soil organic matter levels in the root zone, plants may also affect the fate of a contaminant in terms of its sorption, bioavailability and vertical transport in the profile (Qiu et al. 1997).

2.8 Regulatory Control of Crude Oil Levels in Soil

No guidelines exist for crude oil specifically. However, there are recommended values for mineral oil and grease. In addition, the Canadian Council of Ministers of the Environment (CCME) developed soil quality guidelines for twenty known pollutants which serve as surrogates for the various boiling ranges (CCME 1997). Several of the compounds addressed in the CCME document are constituents of crude oil. These guidelines are of a general nature and are not legally binding. The CCME document provides only guidance for the determination of acceptable soil levels for the specific chemicals examined. Two types of guidelines are given. Environmental soil quality guidelines are developed from toxicological data to establish threshold values for critical receptors. Human health soil quality guidelines are derived through a process similar to that of a site-specific risk assessment. Toxicity thresholds are established for non-carcinogens while guidelines for carcinogens are derived in the context of incremental risk over a lifetime of exposure to soil.

When using the guidelines, site-specific conditions and the standards of the appropriate jurisdiction should be taken into account in all cases.

Table 2.3 lists the CCME 1997 Recommended Guidelines for several compounds which are constituents of crude oil. In instances in which the 1997 values are greater than those of the previously applied 1991 Interim soil Quality Criteria, the latter values were chosen. This step is to account for environmental receptors and/or pathways which were not included in the determination of the guidelines.

Table 2.3. CCME 1997 recommended soil quality guidelines for mineral oil and grease and five individual compounds which are constituents of crude oil (mg kg^{-1}).

	Land Use			
Compound	**Agricultural**	**Residential/ Parkland**	**Commercial**	**Industrial**
Mineral oil and grease	1000	5000	5000	5000
Benzene	0.05	0.5	5	5
Ethylbenzene	0.1	1.2	20	20
Toluene	0.1	0.8	0.8	0.8
Benzo(a)pyrene	0.1	0.7	0.7	0.7
Naphthalene	0.1	0.6	22	22

2.9 Summary

Crude oil is a complex mixture of predominantly hydrocarbons as well as nitrogen, sulfur and oxygen-containing compounds. The presence of crude oil in soil typically has an adverse effect on plant growth which has been observed in both early (Schwendinger 1968; Rowell and Toogood 1977; Chaineau et al. 1997) and advanced (Rowell and Toogood 1977; Toogood 1977; Klokk 1992; Wiltse et al. 1998) stages of growth. Depressed plant growth may be attributable to either direct effects (toxicity, physical obstruction and development impedance) (Rowell 1977a; Bossert and Bartha 1985; Chaineau et al. 1996) or indirect effects through changes in soil properties (reduced aeration, water content or nutrient levels) (Gudin and Syratt 1975; Rowell 1977a; Terje 1984). Addition of crude oil to soil has also been shown to affect soil microbiology (Gossen and Parkinson 1974; Biederbeck 1990; Jorgensen et al. 1995) due in part to conditions similar to those influencing plant growth (Rowell 1977a; Hornick et al. 1983). Plant growth also influences the growth and activity of soil microorganisms (Ross and Cairns 1982; Curl and Truelove 1986; Drury et al. 1991), which may explain enhanced hydrogen dissipation in vegetated soil (Reilley et al. 1996).

3. THE EFFECTS OF A CRUDE OIL SPILL AND SUBSEQUENT REMEDIATION ON AGRICULTURAL LAND PRODUCTIVITY

3.1 Abstract

A two-year study was conducted to examine the effects of a crude oil pipeline spill on the productivity of agricultural land. The study was also intended to ascertain the degree to which a particular spill site had been rehabilitated with respect to the production of crops commonly grown in the local area.

Based on trends in the agronomic variables examined, canola was more susceptible to the adverse effects of the spill than was wheat, particularly at higher concentrations. Crop sensitivity to oil contamination varied over the course of the growing season. Harvest canola yields in 1996 were only lower than control values in the most highly contaminated plots, a result also observed in wheat. Nevertheless, the yield reduction was far greater in canola than in wheat. In 1997, wheat growth and yields were variable in the spill plots while canola growth and yields were lower than control values in almost all spill plots. Seed yields in 1996 of both crops were related to total oil and grease levels in 0-30 cm of soil, but the relationship was stronger for canola. The critical soil oil concentration at which canola oilseed yield declined below the mean control value was in the 1000-2000 mg kg^{-1} range. The critical threshold for wheat grain production in 1996 was approximately 2000 mg kg^{-1} oil in soil. No threshold could be

established for wheat grain yield in 1997. Although the result was not as apparent as in the previous year, the critical oil concentration for oilseed yield seemed to be similar in 1997 to that found in 1996.

Multiple regression analysis confirmed that the presence of oil in soil accounted for more of the variation in oilseed yield than grain yield in 1996. It also indicated that soil salinity was not a major factor influencing the yields of either crop in that year. This agreed with the electrical conductivity data collected in which EC values were above crop tolerance levels in only two experimental sites where yields were evidently not affected. Neither factor explained more than a small portion of the yield variation in 1997.

Mid-season aboveground biomass sampling of the forage crops in 1997 revealed that alfalfa was more sensitive to hydrocarbons in soil than was grass. Both forages performed better in some of the spill sites than in the controls, indicating that small amounts of oil in soil may have stimulated forage growth.

Transfer of BTEX and PAHs from soil to crop tissues was negligible.

The spill-affected land examined appears to be approaching its former level of productivity for wheat and forage grass but has not been remediated sufficiently to allow normal canola or alfalfa production.

3.2 Introduction

That agricultural land is commonly exposed to crude oil in extraction regions and along pipelines has been well recognized for decades (Carr 1919; Murphy 1929; Plice

1948). Rowell (1977a) stated that pipeline ruptures were the primary source of petroleum pollution in the terrestrial environment. The potential for adverse effects of petroleum hydrocarbon contamination on the agronomy of a spill site has been a major concern. Murphy (1929) identified the restoration of site productivity as the top priority in rehabilitating agricultural land. In addition to crop production concerns, the concept of soil quality, in all its aspects, should be addressed when assessing the impact of a disturbance on the soil environment (Yakovchenko et al. 1996; Sims et al. 1997). Crop yield can nevertheless serve as an indicator of soil quality because it is a product of all interacting components of the soil system (Granatstein and Bezdicek 1992) and a measure of system productivity (Yakovchenko et al. 1996). However, rather than being based on absolute productivity, a yield based assessment of soil quality should focus on more informative measures such as stability over time and resiliency following disturbance (Conway 1985). In the absence of remedial action, a substantial portion of oil affected land tends to be unproductive for considerable time (Rowell 1977a). Landfarming has become an accepted approach in the remediation of hydrocarbon contaminated soils (Reilley et al. 1996). This technique consists of tillage to improve soil aeration and fertilizer application, predominantly N based, to lower the C:N ratio of the soil. These actions are designed to enhance the activity of soil microorganisms responsible for hydrocarbon degradation.

Crude oil in soil can affect plant growth directly through toxicity, physical obstruction and interference with normal physiological functions (Rowell 1977a; Amakiri and Onofeghara 1984; Bossert and Bartha 1985; Reilley et al. 1996).

Crude oil in soil can also interfere with plant growth by altering the conditions of the soil environment. Changes in soil properties with oil addition include reduced aeration (Rowell 1977a), reduced water retention (Schwendinger 1968), nutrient deficiencies (Rowell 1977a) and altered soil structure (Ellis and Adams 1961).

Instances of enhanced crop growth in response to the addition of crude oil and other types of hydrocarbons to soil have been observed (Carr 1919; Toogood and McGill 1977). However, McGill (1977) stated that direct and short-term enhancement of crop growth only occurs with small additions of certain constituents of oil. Generally, benefits to plant productivity are not to be expected.

Considerable evidence indicates that the presence of hydrocarbons in soil has either no effect on plant growth or an increasingly adverse impact with rising levels. The magnitude of a negative effect has been shown to depend on the size of the oil addition and the crop being grown. The effects of hydrocarbon amendment of soil on a number of crop types has been investigated, including cereals, oilseeds, legumes and other perennial crops. In early research, Murphy (1929) observed that mixing 0.3% by weight crude oil into the upper 10 cm of soil reduced wheat germination by approximately one quarter relative to a control. Treatments of 3 and 9% oil in soil prevented germination entirely. The 0.3% loading rate caused a 27% reduction in the number of growing plants per plot relative to a control. A concentration of approximately 1.5% reduced the number of plants per plot to near zero. The germination and yields of other cereal crops have also demonstrated varied sensitivities to crude oil in soil (Schwendinger 1968; Rowell and Toogood (1977). Oilseed crops such as rapeseed has shown a greater susceptibility to the

adverse effects of soil contamination by crude oil (Kloke and Sahm 1961; Kloke and Leh 1963; Rowell and Toogood 1977).

Estimates of critical crude oil concentrations in soil for successful crop growth have been made for barley of 1.0% HCs in soil by weight (Racz and Cansfield 1977) and for wheat of <1.5% (Overcash in Dueul 1990). A critical oil concentration for canola was approximated to be <0.5% (Overcash in Dueul 1990). Biederbeck et al. (1997) suggested a loading rate of 0.7-1.0% hydrocarbons in soil for normal crop production in general. A threshold of >3.0% oil concentration in soil was estimated for perennial grasses (Overcash in Dueul 1990).

With the knowledge that the presence of crude oil in soil can affect plant growth, a study was undertaken with the objective being to evaluate the degree to which an area of farmland exposed to crude oil had been remediated. The assessment was to be made based on several crop growth parameters for selected annual and perennial crops. The hypothesis under consideration was that crop growth and yield would be related to hydrocarbon levels in soil, thereby indicating the degree to which the site had been remediated and the threshold for successful crop production. A second objective was to determine if certain hydrocarbons, which may be constituents of crude oil, are present in plant tissue sampled on a contaminated site. Such hydrocarbon components may pose a health risk to consumers of crop residues.

3.3 Materials and Methods

The study was conducted at the site of a pipeline rupture which released crude oil onto agricultural land near St. Leon in southcentral Manitoba in October, 1994. The soil

at this location is an imperfectly drained Gleyed Rego Black Chernozem (Joyale Series) with a clay loam surface texture. The soil developed from moderately fine lacustrine material, for the most part overlying till. Surface expression at the site varies somewhat because of a natural swale running from north to south, but generally the surface is gently undulating. This changes at the south end of the spill area where it is slightly concave. A slight elevational gradient (1-2%) exists from the north to the south end of the affected field. The released petroleum flowed along this gradient and eventually pooled in a slight depressional zone at the southernmost extent of the spill. As a result, this latter zone showed initially high soil hydrocarbon levels. Initial cleanup measures consisted of berm construction to contain the spill, pumping pooled petroleum off the surface and then burning the remainder of the oil still exposed. An additional step was taken to effectively dilute the amount of crude oil present in the "hot spot" at the southern tip of the spill area. Contaminated soil was excavated from the depressional zone and deposited at a nearby location in the spill area which exhibited much lower crude oil concentrations. The mildly contaminated soil at this latter position was then placed in the depressional zone which had been the original high oil zone.

In 1995, a method of soil treatment known as landfarming, consisting of fertilizer application and regular tillage, was employed as part of the long-term remediation strategy of the owner of the pipeline. Landfarming continued during the subsequent two years in the spill area except where experimental sites were established for University of Manitoba research.

Following an initial site visit by Department of Soil Science researchers in 1996, locations were selected for experimental sites in the spill area. The selection process was

based on two criteria: soil hydrocarbon data collected by Clifton Associates Ltd. and the growth of fall rye planted in late 1995. The intention was to position the research sites so as to reflect the substantial variation in hydrocarbon concentration across the spill area. The rationale behind site selection in the spill area is provided in Table 3.1. Three control sites were located west of the spill area on nearby uncontaminated land such that variation in productivity from the north to the south end of the field could be represented (Figure 3.1). In the first year of the study, five experimental sites in the spill area (SP1-SP5) and three sites on the control land (CON1-CON3) were established. In the second year, an additional spill site (SP1-B) was positioned adjacent to SP1 to evaluate the benefits, if any, that an extra year of tillage had on productivity in the spill area.

The annual crops to be tested were wheat (*Triticum aestivum* L. c.v. Pasqua) and canola (*Brassica rapa* c.v. Argentine), chosen because they are commonly grown in the St. Leon area. These two crops also represent two major crop types: cereals and oilseeds. The wheat variety planted was Pasqua. The canola type was Argentine and the variety was Crusher. Grass and alfalfa were also assayed to discover the effect of the spill on the productivity of common forage crops. The effect of these types of vegetation on hydrocarbon levels in soil was also investigated in a separate study to determine their potential application as a phytoremediation strategy. The forage crops grown were meadow bromegrass (*Bromus biebersteinii*. Rohman and Schult) and alfalfa (*Medicago sativa* L. c.v. algonquin).

The design of each experimental site is illustrated in Figure 3.2. The fallow plots (T7 and T8) were included with the forage plots as part of a separate study on phytoremedial and amendment techniques in land reclamation. Each research site was

Table 3.1 Rationale used to select locations within the spill area for research sites.

Site ID	Rationale
SP1	This experimental site was situated at the northern tip of the spill area near the original release point where hydrocarbon concentrations were high. Growth of the fall rye was poor. This location represented the high end of the contamination range and provided a means to assess the effects of significant concentrations of crude oil on crop growth and soil quality.
SP1-B	This site, added in 1997, was positioned directly over a sampling point (S16) used by Clifton Associates, providing two years of hydrocarbon levels for that location. TPH values were relatively moderate and had declined from 5370 ppm to 3800 ppm after one season of landfarming in 1996. The location was also adjacent to SP1, such that conditions at the two sites would be comparable during the growing season.
SP2	This site was placed in an area between two sampling bore holes (Clifton Associates Ltd. 1995) from which low concentrations of hydrocarbons were measured. The good growth of fall rye concurred with the reported hydrocarbon levels. It was believed that this location represented minimal contamination.
SP3	According to the Clifton Associates Ltd. (1995) report, it was expected that this site represented an area with intermediate concentrations of crude oil. The growth of fall rye was patchy.
SP4	This site was selected because it was the area that received excavated soil obtained from the most southern extent of the spill area. The growth of fall rye in this area was virtually absent, implying high crude oil content presumably transferred from the excavated land. SP4 represents high hydrocarbon concentrations.
SP5	The southern tip of the spill site included an area that had previously been excavated due to the initial accumulation of oil and resulting high hydrocarbon concentrations in the surface soil. The majority of the contaminated soil at this site was removed by reclamation personnel in 1995 and spread at the location where the SP4 research site was placed. The fall rye at SP5 appeared to be healthy and exhibited good growth in the spring of 1996, implying low levels of oil at that time.

30 m by 30 m in dimensions. Hydrocarbon levels in soil were a function of crude oil distribution in soil at the time of the spill. Thus, pre-determined oil treatments could not be assigned to the crop plots, rather the treatments were approximated through the selection of research site locations across the spill area. Treatments were, therefore, not truly replicated. Nevertheless, each crop plot was divided into four replicates in order to

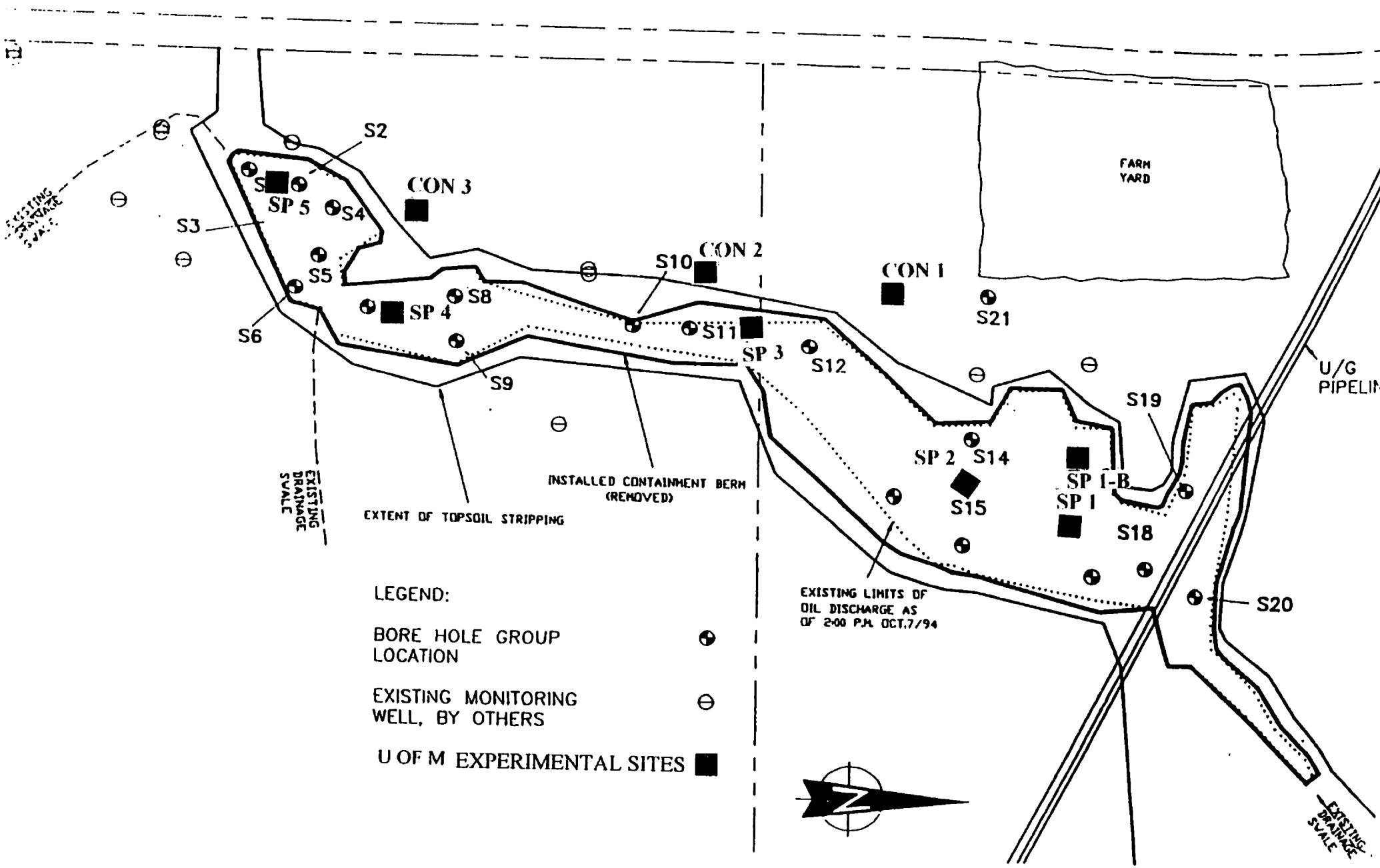

Figure 3.1 Map of the study area (adapted from Clifton Associates 1995) indicating bore hole group locations and existing monitoring wells. Approximate positions of experimental sites established for the study (■) have been added.

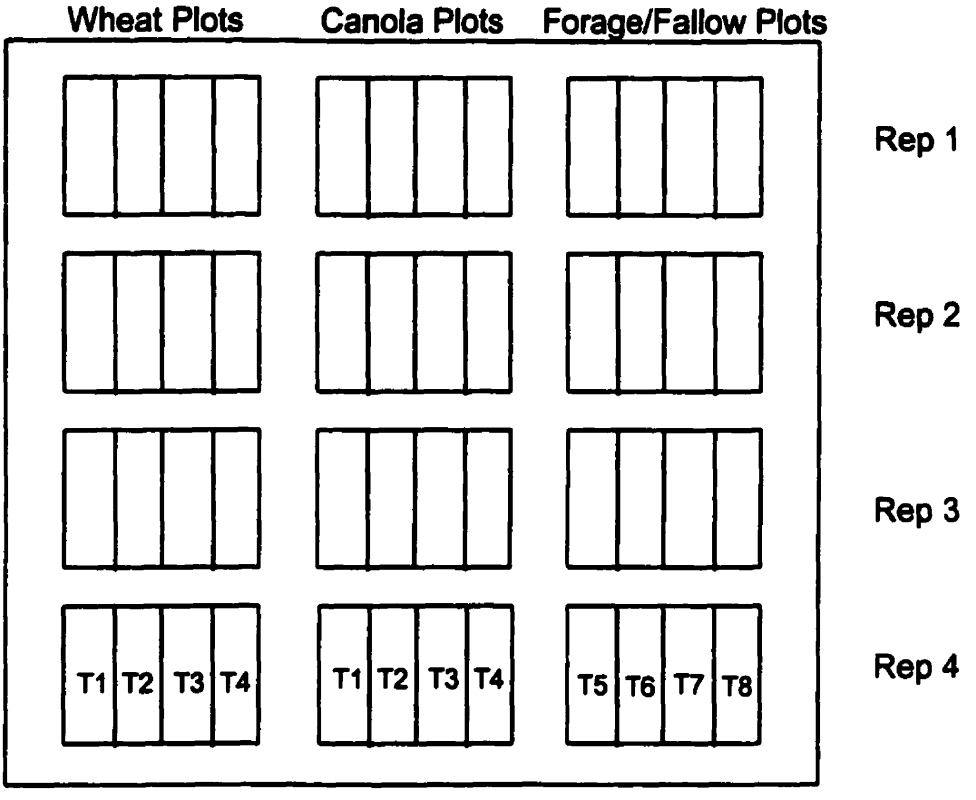

Figure 3.2 Schematic digram illustrating plot layout in each 30 m x 30 m experimental site. Control annual crop plots consisted of T1, T2, T3 and T4 subplots corresponding to N fertilizer rates of 0, 60, 120 and 180 kg N ha^{-1}. All control annual crop plots also received 40 kg P_2O_5 ha^{-1} with the seed each year. Spill annual crop plots consisted of T1, T2, T3 and T4 subplots which were designated only for sampling purposes. These subplots received no extra fertilizer N as soil N levels were high in the spill area (Figures 3.3 and 3.4). All spill annual crop plots did, however, receive 40 kg P_2O_5 ha^{-1} with the seed each year. Treatments T5, T6, T7 and T8 correspond to bromegrass, alfalfa, straw incorporated fallow and unamended fallow, respectively. No fertilizer was added to these plots.

assess variability in productivity across each plot. Each of the wheat and canola blocks was further subdivided into four sub-plots in order to account for within-block variability in crop productivity. Due to the uncontrolled nature of the experiment, regular statistical analysis of results was not appropriate. Instead, only means and standard errors were determined and displayed in the figures. Regression analysis was performed to relate harvest seed yields to total oil and grease in soil and electrical conductivity.

In the control sites, fertilizer trials were conducted both study years in the wheat and canola plots. The treatments consisted of four N rates: 0, 60, 120 and 180 kg N ha^{-1} (T1, T2, T3 and T4 respectively) applied as 34-0-0. The trials were run as a randomized complete block design. Soil analysis on grab samples taken in the spring of 1996 showed that N levels in the control research sites were similar (Table 3.2). Calculations of yield response to soil N were then based on the fertilizer treatments only.

Table 3.2 Total inorganic N (kg ha^{-1}) in control experimental sites in the spring of 1996.

	Soil Depth	
Experimental Site	**0-30 cm**	**30-60 cm**
CON 1	65.2	28.4
CON 2	63.2	31.2
CON 3	62.4	29.6

In 1997, residual soil nitrate concentrations were determined using the method described by Maynard and Kalra (1993) based on KCl extraction and analysis with a Technicon Autoanalyzer. Analysis was done on composite samples taken from three equally spaced points within each fertilizer trial sub-plot. All sub-plots received the same fertilizer treatments as in the first year. By combining the treatment levels with residual

values, N supply was calculated for determining crop responses to fertilizer. No trials were conducted in the spill sites in either year due to the high N levels already present in the soil as part of the landfarming activities (Figures 3.3 and 3.4). All wheat and canola plots in both control and spill sites received 40 kg P_2O_5 ha^{-1}.

Seeding of the annual and perennial crops was carried out in the spring of 1996. Seeding rates for wheat, canola, brome grass and alfalfa were approximately 75, 6, 7 and 7 kg ha^{-1} respectively. Soil samples were then taken for total oil and grease analysis from all replicates in all spill sites to ascertain the variability in hydrocarbon concentration. Thus, sampling was done prior to the establishment of any cropping or fallow treatments. The analytical method was developed by McGill and Rowell (1977). Five randomly selected replicates in each control site were also sampled to determine "background" levels, i.e. amounts of substances from organic matter detected as crude oil compounds. The analysis is a gravimetric determination of the total amount of oil and grease present in a sample on a percentage basis. It consists of a Soxhlet extraction using methylene chloride (CH_2Cl_2) as the solvent. The method does not extract a particular fraction of oil compounds but instead attempts to quantify the amount of all oil in soil. Composite samples were taken using a Dutch auger from four points 2.5 to 3.5 m apart within each replicate block. The four points were equidistant from each other in a rectangular configuration approximately 1 m from each of two sides of each 5 m by 6 m block. Samples were stored in sealed plastic bags and kept in a field cooler until being placed into 4°C storage. The soil was later air-dried, ground and then submitted to a commercial lab for determination of total oil and grease based on the method described by McGill and Rowell (1977).

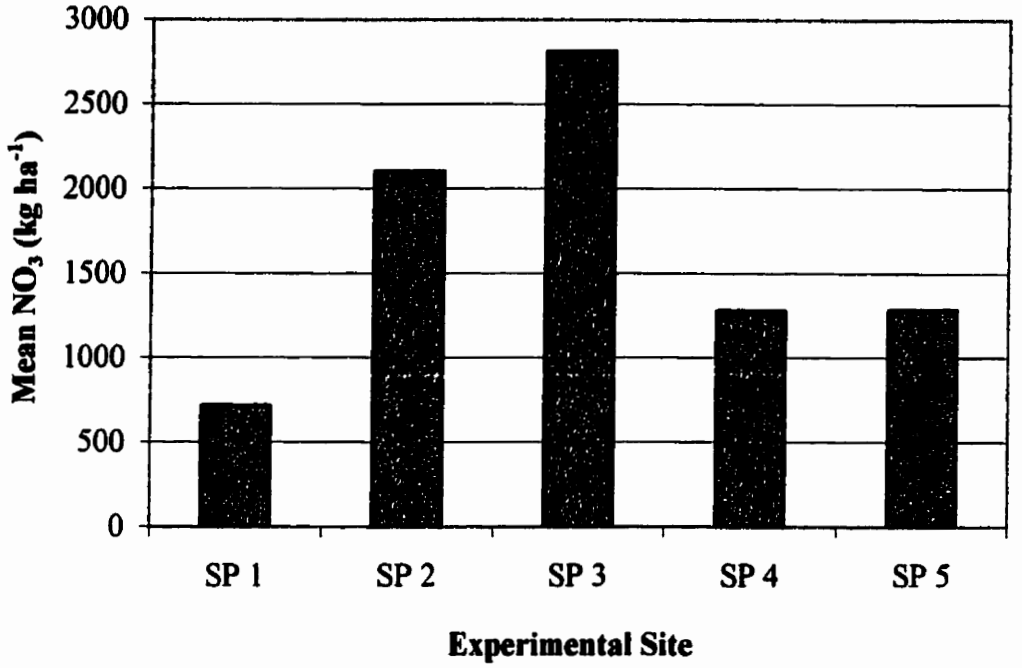

Figure 3.3 Nitrate levels in the top 60 cm of soil in the spill sites in spring, 1996. Each bar represents the mean of values for the 0-30 and 30-60 cm depths based on one sample per site.

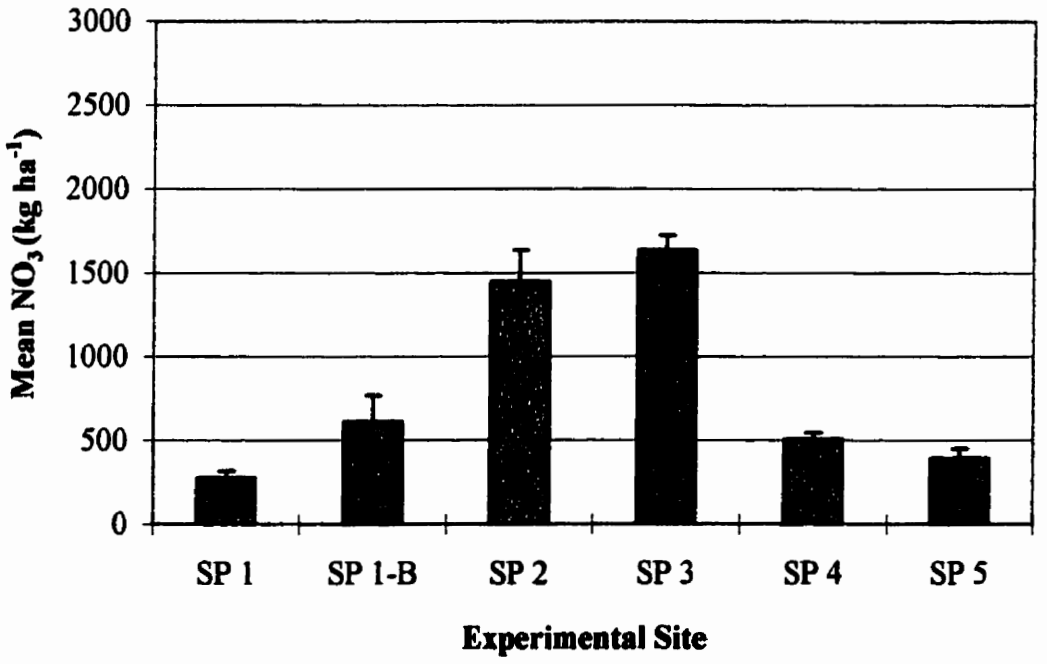

Figure 3.4 Nitrate levels in the top 60 cm of soil in the spill sites in spring, 1997. Each bar represents the mean of values for the 0-30 and 30-60 cm depths across four replicates.

In the spring of 1997, total oil and grease levels were determined for samples taken from the spill wheat and canola plots only because the forage and fallow treatments were well established. Composite samples were again taken from four equidistant points in a rectangular configuration within a given block. For background values, subsamples of those obtained for the control site fertilizer trials were composited within each of four randomly selected replicates in all three control sites.

Soil electrical conductivity (EC) was determined on the same samples as for the hydrocarbon analysis, following the saturation extract method of Janzen (1993). Values obtained with a conductivity meter were based on a temperature of 25°C. This analysis for soil salinity was performed on both 1996 and 1997 samples and identified plots in which salinity could confound the effect of the crude oil. Crop growth and yield could then be related to both of these factors.

Data on three stages of growth were collected for wheat and canola in 1996: emergence counts, mid-season above-ground biomass and harvest yields of grain or oilseed and straw. Emergence counts were taken to assess the impact of the spill on early crop growth. Data was collected for rows 17, 18 and 19 of each replicate. Counting began at a point 2 m into each block from its northern edge. Emerged plants were counted over a distance of 0.5 m.

Mid-season biomass was evaluated to ascertain the progress of crop growth during the growing season. In 1996, above-ground biomass was sampled from a single row from a randomly selected sub-plot of each block in the spill sites and from all sub-plots in each block of the control sites. The same row was sampled in each block unless the row happened to fall within the tractor tire tracks in which compaction of the seed bed

had occurred, hampering plant growth. A 4 m distance of crop was cut, leaving a 50 cm buffer from both the north and south edges of the block.

At harvest, two rows were sampled from all sub-plots in spill and control sites. Two 3.5 m lengths of crop were harvested, leaving 0.75 m buffer lengths at each end of the rows. Sampling greater lengths from a minimal number of rows enabled greater inclusion of variability across a given block. The same two rows were sampled in each block wherever possible. Exceptions arose where the sampling rows fell within the tractor tire tracks. This situation arose in both spill and control plots and so there were no implications for sampling bias.

Composite samples of wheat grain, canola oilseed and straw from both crops from spill sites showing the highest crude oil concentrations in soil (SP1 and SP4) and from a control were submitted to Norwest Labs for hydrocarbon analysis. These plant tissue samples were analyzed for BTEX (benzene, toluene, ethylbenzene and xylenes) by EPA Method 8020 and for PAHs (polyaromatic hydrocarbons) by EPA Method 8270 for solid matrix.

In 1997, data was again collected on wheat and canola growth in all experimental sites. However, extremely wet site conditions prevented the recording of emergence counts before plants had grown too large to be differentiated from each other. At mid-season, above-ground biomass was sampled from 1 m lengths in each of four adjacent rows in each sub-plot to account for the large lateral variability in growth that was evident across a given replicate in 1997. Exceptions to this rule were made to avoid rows that fell in tire tracks. In such instances, two rows on either side of the tire tracks were sampled. Sampling began 0.5 m in from the northern edge of each block.

At harvest, plant tissue was sampled in the same way from the same rows as at mid-season. The starting point for cutting was 0.5 m beyond the end point of sampling at mid-season.

Mid-season above-ground biomass was also sampled in the forage plots of all experimental sites in 1997. Three equally spaced 0.25 m^2 quadrats were sampled in each block of meadow brome grass and alfalfa.

Composite samples of wheat grain and canola oilseed from the two most highly contaminated sites and a control were again submitted for hydrocarbon analysis. The same was done for the two forage crops in which only above-ground biomass was analyzed.

Throughout each growing season, various tasks were performed to maintain the research plots. In 1996, herbicide application was completed early in the summer in the control sites where weed populations were significant. No spraying was required in the spill sites due to the minimal weed growth. Further weed control was achieved by mowing in the buffer strips around plots and by hand weeding in the forage plots. In 1997, herbicides were applied early in the summer in all the annual crop plots due to weed resurgence at the spill sites. In addition, irrigation of all canola plots was conducted in late spring due to poor emergence. A volume equivalent to one half inch precipitation was applied in an effort to promote germination and emergence.

3.4 Results and Discussion

3.4.1 Variability of Oil Content in Soil

The five research sites in the spill area represented a wide range of crude oil concentrations in soil. In 1996, the lowest mean value for total oil and grease was below detection (0.05%) for both depths of soil in all crop plots containing oil residues. The highest mean levels for the 0-30 cm depth in the wheat and canola plots were 0.71 and 1.55%, respectively (Figures 3.5 and 3.6). The highest mean levels for the 30-60 cm depth in the wheat and canola plots were 0.67 and 0.85%, respectively. Three of the spill sites, SP2, SP3 and SP5, possessed low to very low crude oil concentrations in both soil depths in both wheat and canola plots with the exception of the canola plot in SP2. This latter plot contained a wider range of hydrocarbon levels with a mean greater than 0.2% in both soil depths. The remaining sites, SP1 and SP4, exhibited moderate to high crude oil contamination within the context of this particular spill area. The wheat plots in these two research sites had nearly identical mean total oil and grease concentrations in the 0-30 cm depth. These oil levels were lower than those found in the canola plots, with the SP4 mean being the highest across the entire spill area. Total oil contents of soil for the 30-60 cm depth of both annual crop plots in SP1 and SP4 were more comparable.

In 1997, mean crude oil concentrations in the original five spill sites were similar to those of the previous year. Individual soil samples measured from below detection (0.05%) to 1.85% total oil and grease by weight. Crop growth in SP4 in 1996 appeared to be partitioned along an east-west line through the site, with noticeably better growth in the north half than the south half of the wheat and canola plots. Due to this observed growth pattern of the annual crops in the first season, SP4 was broken up into separate

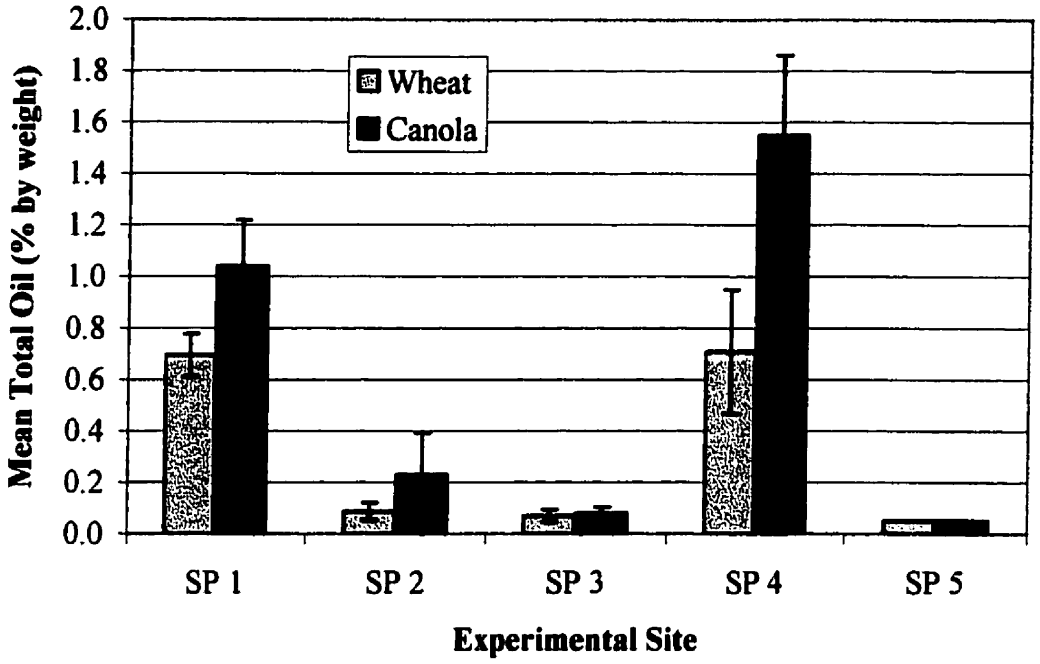

Figure 3.5 Total oil and grease in 0-30 cm depth of wheat and canola plots in 1996. Each bar represents the mean of four field replicates each comprised of four composited subsamples.

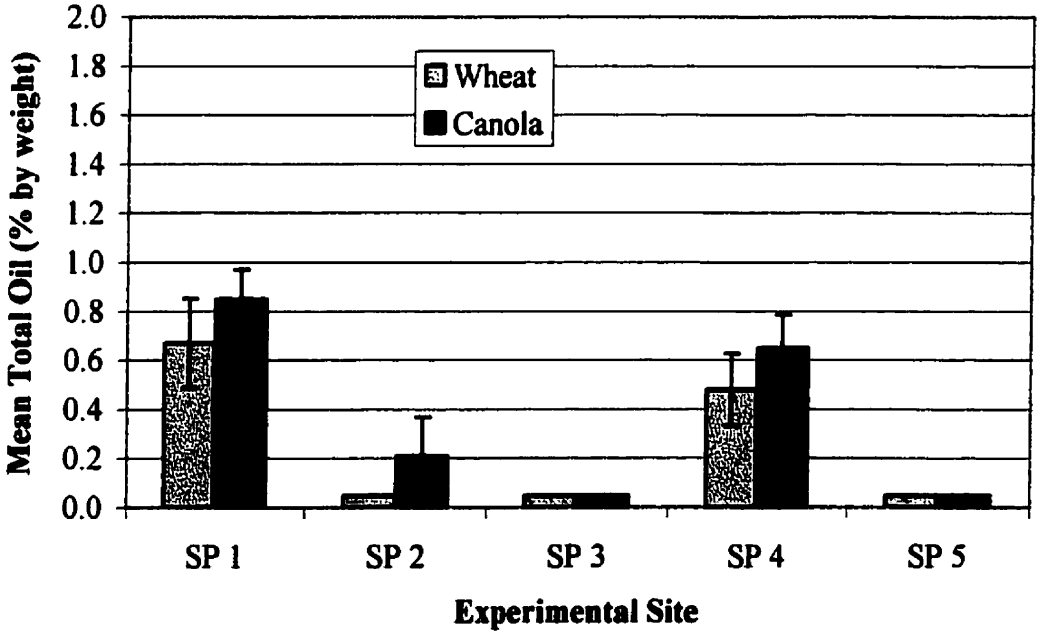

Figure 3.6 Total oil and grease in 30-60 cm depth of wheat and canola plots in 1996. Each bar represents the mean of four field replicates each comprised of five composited subsamples.

subplots, the northern two blocks (SP4-a) and the southern two blocks (SP4-b), for all year two data analyses. Total oil and grease contents in the 0-30 cm depth were 0.34% and 0.80% in SP4-a and SP4-b, respectively, when replicates from both crop plots were averaged. Thus, the delineation of two subplots in SP4 was warranted. The total oil levels in SP2, SP3 and SP5 were again at the low end of the concentration range (Figures 3.7 and 3.8). In all three of these sites, one crop plot had more oil in it on average than the other in both soil depths, though the differences were generally small. A second tier of research sites, consisting of SP1, SP4 and SP1-B (the new site) had moderate to high crude oil levels in the 0-30 cm depth. These groupings were less apparent for the 30-60 cm depth. In the upper depth the highest hydrocarbon levels were found at SP1 while SP1-B and SP4 exhibited slightly lower hydrocarbon levels. The greatest difference in mean total oil and grease in the 0-30 cm depth between two crop plots occurred in SP1. Differences between crop plots in other sites were more minor. In the 30-60 cm depth, SP1 also exhibited a sizable difference in total oil between crop plots, though the largest difference in concentration was found in SP4-b.

Data on total oil and grease in the forage and fallow plots were collected only in 1996. The forage and fallow treatments were well established by the following year, making the same sampling scheme impractical and not useful for interpretation. A more intensive study of soil oil levels under forage and fallow treatments was undertaken concurrently but only in one experimental site in order to limit costs. The intent was to relate forage growth, measured the year following establishment, to the general extent of soil contamination in each experimental site. For the 0-30 cm depth, the highest concentrations were found in SP4-a, followed by SP4-b and SP1 (Figure 3.9). For the

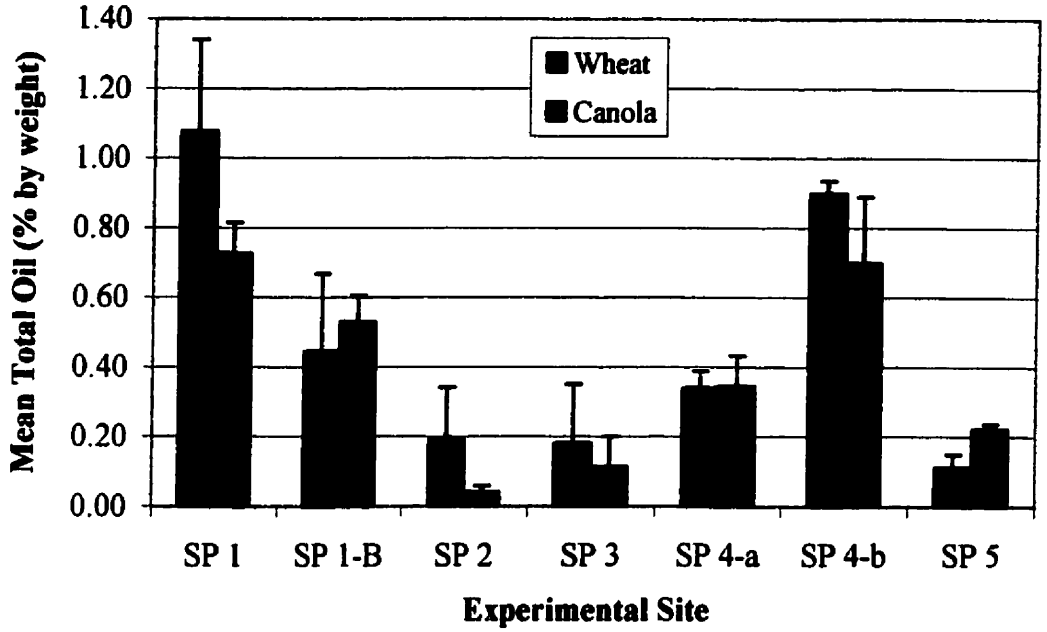

Figure 3.7 Total oil and grease in 0-30 cm depth of wheat and canola plots in 1997. Each bar represents the mean of four replicates, except for SP4-a and which are derived from two replicates. The composite sample in each replic was derived from four subsamples.

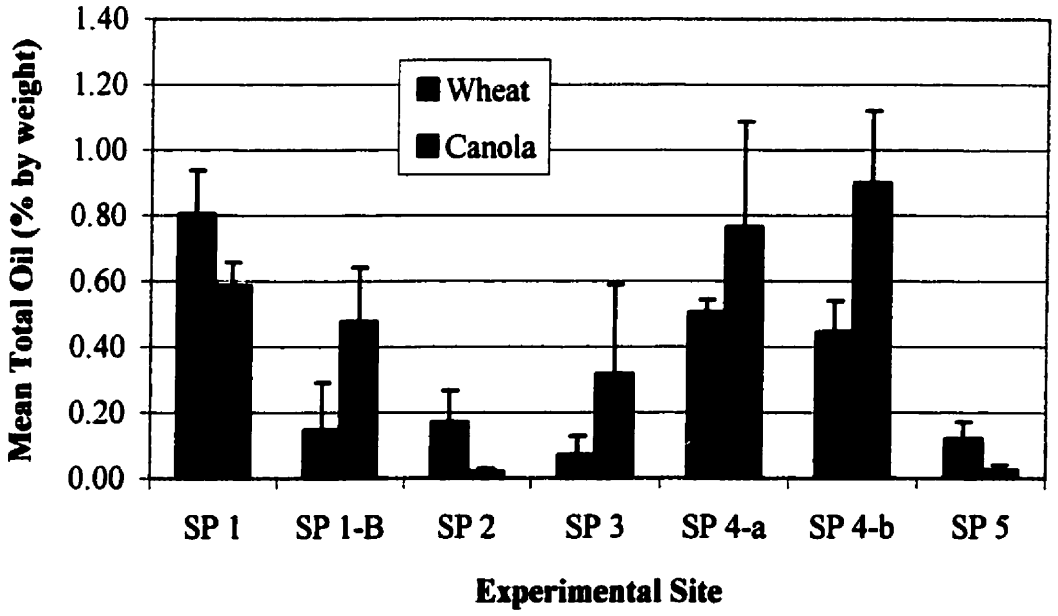

Figure 3.8 Total oil and grease in 30-60 cm depth of wheat and canola plots in 1997. Each bar represents the mean of four replicates, except for SP4-a and which are derived from two replicates. The composite sample in each replic was derived from four subsamples.

30-60 cm depth, these three research sites exhibited similar lower hydrocarbon levels than in the upper depth. Total oil contents of soil were appreciably lower in the other three sites, with the lowest occurring in SP5.

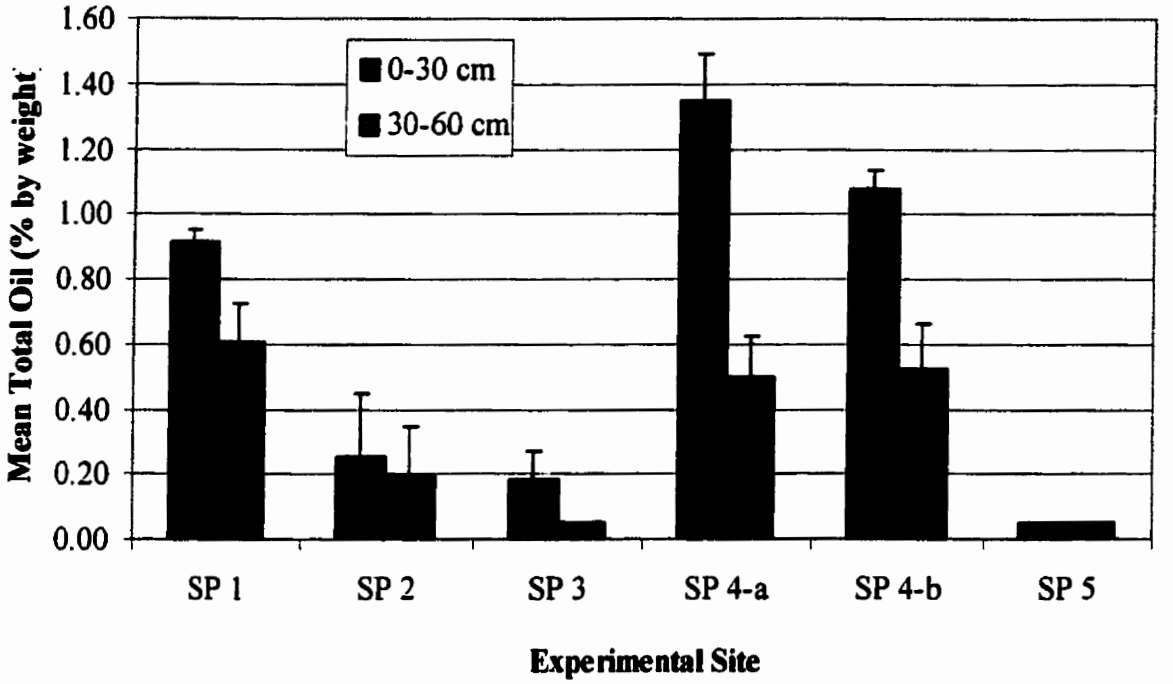

Figure 3.9 Total oil and grease in forage and fallow plots in 1996. Each bar represents a mean of 4 replicates, except for SP4-a and -b which are means of 2 replicates, each comprised of 4 composited subsamples.

3.4.2 Soil Electrical Conductivity (EC) Results

Growth of sensitive crops such as canola is inhibited when grown in a soil exhibiting an electrical conductivity value between 4 and 8 mS cm^{-1} (Table 3.3). Differences in EC between crop plots within each research site were minor and so means were calculated for individual research sites. Results were consistent over the two field seasons. All control sites exhibited mean EC values less than 4 mS cm^{-1} in the 0-30 cm depth and near 4 or 5 mS cm^{-1} in the 30-60 cm depth (Figures 3.10 and 3.11). SP2

and SP3 had mean ECs in the 6-8 mS cm^{-1} range, indicating that salinity could have affected crop growth in these two sites. All other experimental sites in the spill area exhibited mean EC levels which were very near or within the tolerance range for crop growth.

Table 3.3. Salinity classes with corresponding degrees of crop effects.*

Salinity Class	Electrical Conductivity (dS m^{-1})	Crop Effect
Non-saline	< 4	No effect
Weakly saline	4 – 8	Sensitive crops affected
Moderately saline	8 – 15	Substantial growth reduction in most crops
Strongly saline	> 15	Few plants survive

*Expert Committee on Soil Survey. 1982. The Canada Soil Information System (CanSIS) Manual for describing soils in the field. Research Branch, Agriculture Canada, Ottawa

3.4.3 Emergence of Wheat and Canola

Although little research has focused specifically on wheat or canola emergence in oil contaminated soils, germination in the presence of oil has been extensively examined for a number of crops. Since it is the precursor to emergence, research done on germination in oiled soil can be applied to the results of this study.

Emergence of wheat differed markedly from canola in the first year of the study. In all spill sites, wheat emergence was as good as emergence in the control plots located outside the spill area (Figure 3.12). It appears that even in the sites with greatest crude oil concentrations (SP1 and SP4), wheat was able to emerge as effectively as in uncontaminated sites.

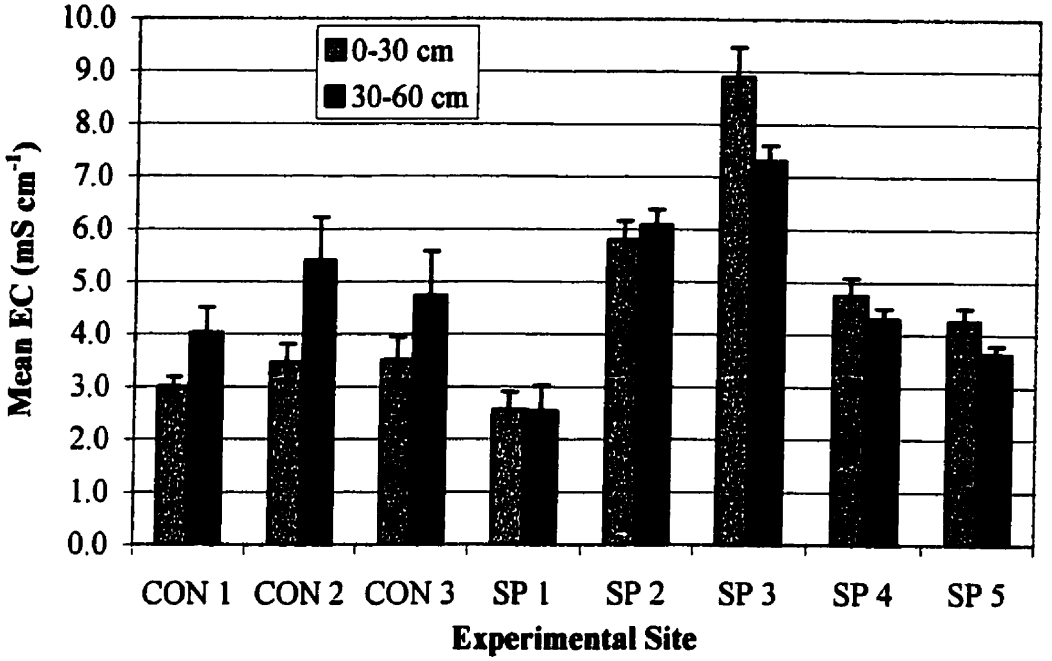

Figure 3.10 Electrical conductivities for soil at each experimental site in 1996. Each bar represents the mean of eight field replicates each comprised of four composited subsamples.

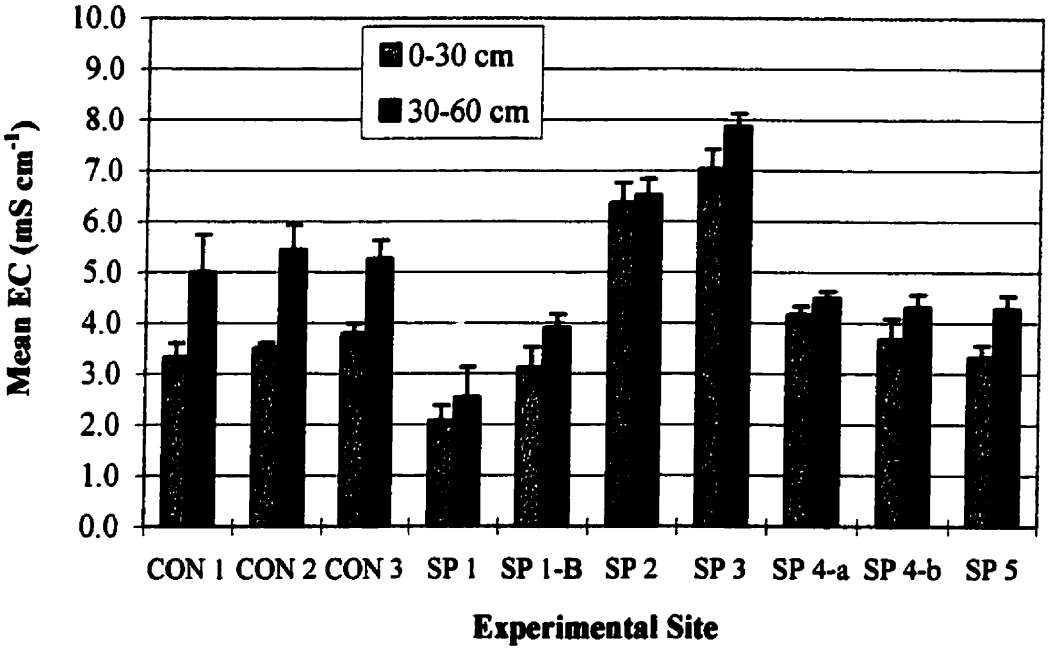

Figure 3.11 Electrical conductivities for soil at each experimental site in 1997. Each bar represents a mean of four replicates, except for SP4-a and -b which are means of two replicates, each comprised of four composited subsamples.

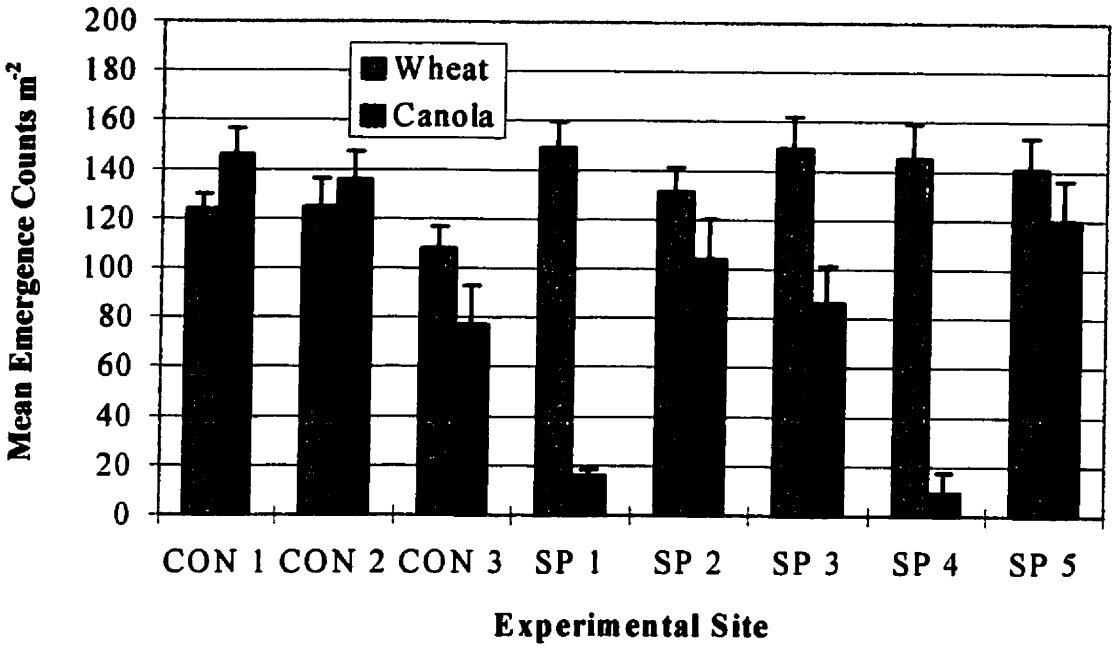

Figure 3.12 Emergence counts for wheat and canola in 1996. Each bar represents the mean of twelve replicates.

These results are not consistent with Murphy (1929) who observed 24% of wheat kernels failed to germinate after being planted in soil containing 0.3% crude oil by weight in the 0-10 cm depth. This concentration falls within the range of hydrocarbon levels among spill plots in the St. Leon study. In the study by Murphy (1929), treatments of 3% and 9% oil content of soil were necessary to prevent any wheat germination. Chaineau et al. (1997) reported no reduced emergence of wheat seedlings in a fertilized sandy soil having <1% fuel oil by weight. A value of 4% fuel oil prevented one half of wheat kernels from germinating. Previous work documented normal germination in soil with a maximum oil concentration of 0.2% (Chaineau et al. 1996). Other cereal crops have demonstrated considerable tolerance in early growth stages to oil contamination of soil. Oat seeds have germinated normally in soil containing 1% (Schwendinger 1968) and 4% crude oil (Rowell and Toogood 1977). Rowell and Toogood (1977) also found that germination of barley could tolerate 2.5% crude oil by weight without any reduction.

With respect to canola, however, emergence was substantially reduced in SP1 and SP4 relative to control plots in 1996. Emergence of canola in SP2, SP3 and SP5 was slightly less than that encountered in control plots CON1 and CON2, but not CON3 (Figure 3.12). The poor results in the heavily contaminated soil agree with the findings of Toogood (1977) who observed no canola germination in four consecutive plantings in soil having 2% crude oil by weight. However, in the latter study, basic tillage was the only remedial measure undertaken in the plots.

When interpreting emergence counts and other crop production results, the differences in total oil and grease concentrations between wheat and canola plots of both SP1 and SP4 should be considered. For the 0-30 cm depth of soil, mean hydrocarbon levels were substantially greater in the canola plots than in the wheat plots (Figure 3.5). This could have implications for determining the sensitivity of crop types to crude oil presence in soil.

In mid to late spring of 1997, it became clear that canola emergence was quite poor in several of the spill and control plots. The evidently dry field conditions were likely responsible for failed germination of canola seeds and therefore the emergence problems. Emergence in the wheat plots appeared to be somewhat affected in only two of the experimental sites, SP2 and SP3. In an effort to stimulate canola germination and consequently emergence, the canola plots were irrigated in late spring. Five days later successful emergence was observed in the irrigated plots, although it was very patchy.

However, by the time of mid-season biomass sampling, it was evident that much of the canola crop which did emerge had ultimately failed to survive. The failure was most severe in SP1 and SP1-B where there was no crop to sample. Patches of entirely

absent growth were also observed in SP2, SP3, SP4 and in all three control canola plots. Canola growth in SP1 and SP4 was expected to be poor based on the low productivity at those locations last year. The severe decline in productivity in the canola plots at SP2 and SP3 from the first season was presumably due to site conditions inhibitory to crop growth that were not present in 1996.

3.4.4 Mid-season Biomass

3.4.4.1 Annual Crops. In 1996, the growth of wheat in spill sites SP2, SP3 and SP5 as indicated by total above-ground biomass was as good if not better than that in the control plots (Figure 3.13). However, in SP1 and SP4, the wheat crop began to show evidence of stress as indicated by the lower biomass yields relative to the control plots. Even though the emergence of wheat in these plots was similar between spill and control plots, it appears that by mid-season the vigor of the crop had been reduced relative to the controls. Canola growth by mid-season was reduced in all spill plots relative to control plots CON1 and CON2. The canola plots in SP1 and SP4 showed poor canola biomass production which is consistent with the poor emergence observed in the spring for these spill plots. Toogood (1974) measured vegetative growth for a wide spectrum of crops including cereals, oilseeds and perennial forages. However, the effect of light spills of crude oil on rapeseed growth could not be ascertained as the crops consistently failed to germinate over four consecutive years. Reported results centered entirely on the germination trials also carried out and so no indication of the sensitivity of wheat to oil in soil based on vegetative growth was given. No other research has focused on above-ground biomass in oil contaminated soils during the growing season.

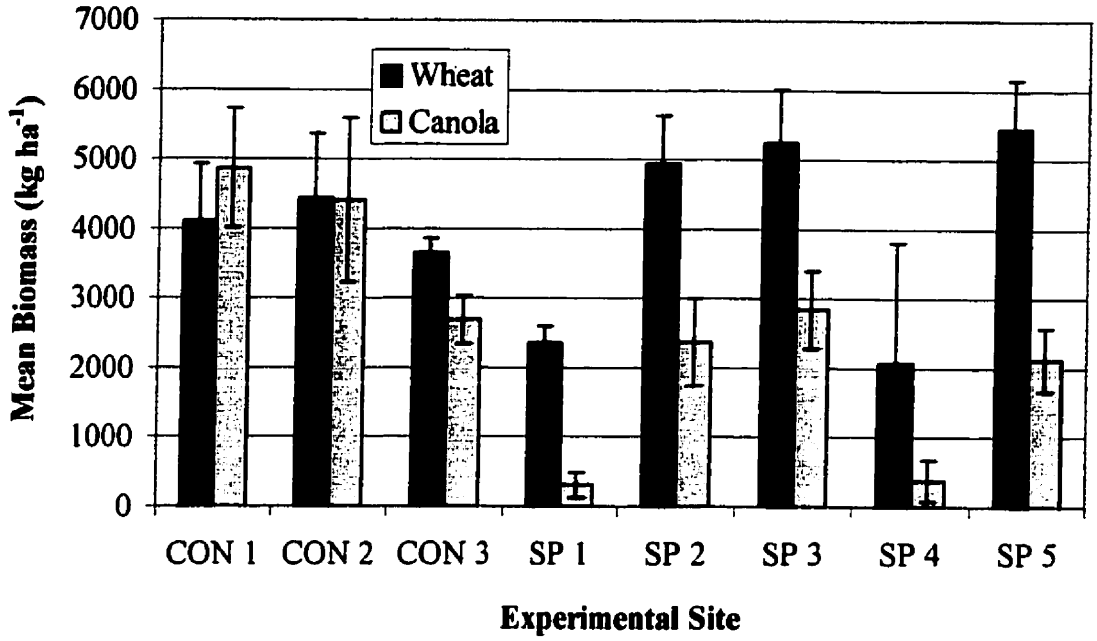

Figure 3.13 Mid-season above-ground biomass of annual crops in 1996. Each control (CON) and spill (SP) bar represents the mean of twelve and four replicates, respectively.

The presence of crude oil appeared to have an increasing effect on emerged plants as they passed the earliest stages of growth and began to demand more growth requirements from the soil. This applies primarily to the wheat plots which exhibited no emergence problems earlier in the season. It may also apply to those canola plots which had only somewhat reduced emergence (SP2, SP3 and SP5).

In 1997, wheat growth in spill plots SP1, SP2, SP3, SP4-b and SP5 based on total aboveground biomass was less vigorous than that in the control plots (Figure 3.14). By contrast SP1-B and SP4-a wheat performance was comparable to that of wheat in the control plots. In 1997, the presence of crude oil and the amount of oil in soil did not have a consistent effect on mid-season biomass. Canola biomass in SP5 was greater than that measured for all three controls. All other spill canola plots showed lower productivity at

mid-season than control plots. Mid-season productivity of canola in 1996 was affected by hydrocarbon contamination, regardless of salinity levels.

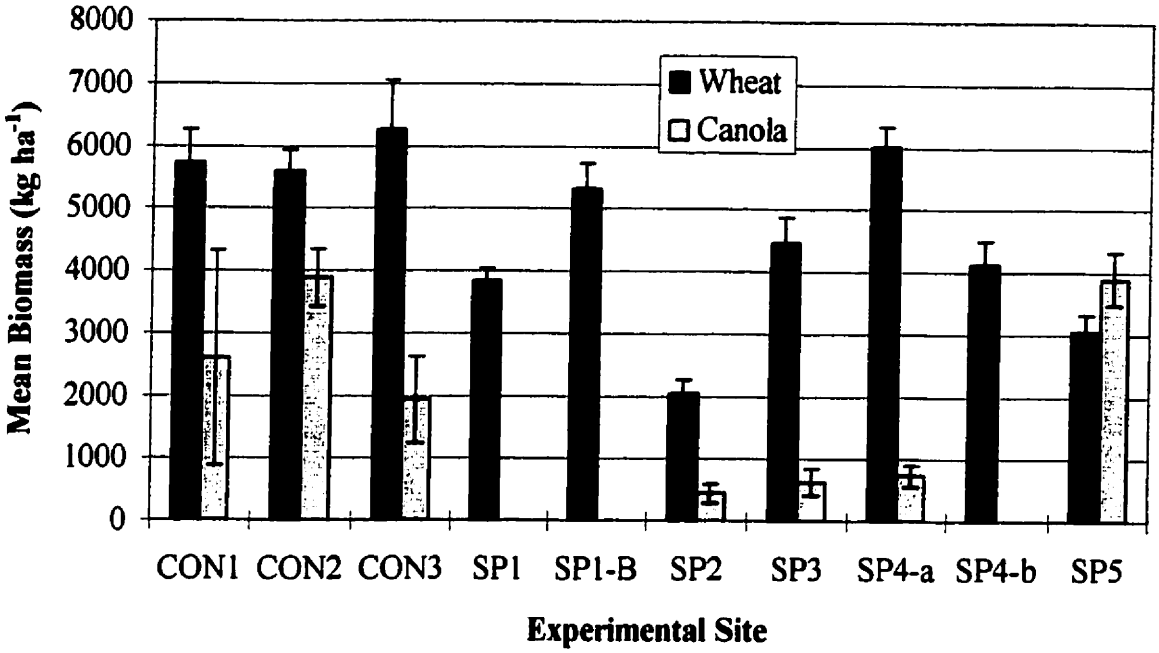

Fig. 3.14 Midseason aboveground biomass of wheat and canola in 1997. Each bar represents the mean of sixteen replicates.

Considering the heavy rate of fertilizer application as part of the landfarming activities, nitrogen should not have been limiting in the spill area. However, differences in nitrate levels in the 0-60 cm depth of soil among the spill sites may account in part for the differences in crop biomass at mid-season. The higher nitrate concentrations in the upper 60 cm of soil in SP2 and SP3 than in the other spill sites may indicate that there was less competition between plants and soil microorganisms for nitrogen in those sites. The crude oil levels in SP2 and SP3 were relatively low, resulting in lower activity by oil degrading microbes and therefore a lower microbial demand for soil nitrogen. Thus, the effect of sufficiently high amounts of crude oil in soil on nitrogen availability to plants

may be at least partly responsible for the crop growth reductions recorded for the spill area research sites.

It should be noted that due to heavy rains in early summer of 1997, SP5 was flooded for a considerable length of time (as long as two weeks). The excessive moisture in both wheat and canola plots possibly led to elevated incidence of disease, relative to other experimental sites, which could have reduced midseason yields.

3.4.4.2 Forage Crops. Aboveground biomass was sampled in the forage plots mid-way through the 1997 season. Grass productivity as indicated by mid-season biomass was greater in SP2, SP3 and SP5 than in all controls (Figure 3.15). Growth in SP1 and SP4-a reached higher levels than in CON1 and CON2 but was below that in CON3. Grass biomass in SP4-b was lower than in all three controls.

Alfalfa biomass in SP2, SP3 and SP5 also measured higher than in the controls. Productivity in SP1 and both SP4 subplots was below that in the controls. SP4-b had no observed alfalfa growth. Mid-season biomass values for grass were greater than alfalfa in SP1, SP4-b and SP5. Alfalfa showed greater productivity in SP3 and in all three controls. Forage yields were comparable to each other in SP2 and SP4-a.

The observed tolerance for hydrocarbons in some of the forage plots, particularly with respect to grass, is consistent with other research findings. Overcash in Dueul (1990) found that perennial grasses could withstand >3% oil content of soil. Toogood (1974) reported relatively unaffected hay stands (the constituents of which were not clearly indicated) grown in soil containing 1/3 and 2/3% oil. Oil treatments of 1% and greater caused considerable losses in surface coverage by the hay stands. The results

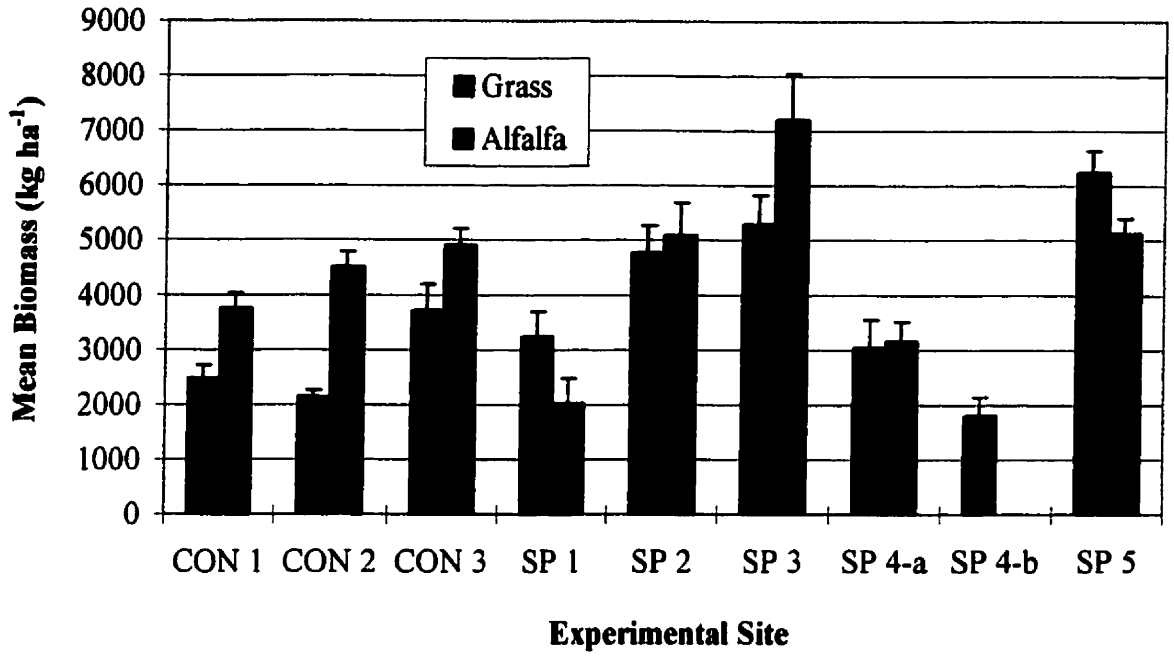

Figure 3.15 Mid-season aboveground biomass in forage plots in 1997. Each bar represents the mean of twelve replicates.

were recorded several years after the oil was applied to soil which never received any remedial measures. Four years after oil application, crops of fescue, brome and alfalfa were seeded. These plots showed no symptoms of damage by hydrocarbons in soil in the year following establishment. Rowell (1977) characterized brome grass as having a low sensitivity to hydrocarbons in soil.

Klokk (1992) measured, in general, increasingly major declines in germination and vegetative growth of perennial vegetation with increasing rates of oil applied to soil. There were several instances of enhanced growth by the addition of low doses of oil to soil relative to a control. The plants tested included meadow grass, red fescue and clover.

Reilley et al. (1996) observed varied results for forage productivity after 24 weeks in a clay soil receiving several hydrocarbon treatments: uncontaminated (UN), uncontaminated + PAH (UN + PAH) and contaminated + PAH (CN +PAH). The PAH

spike consisted of anthracene and pyrene added at a concentration of 100 mg kg^{-1}. The contaminated soil contained petroleum compounds including anthracene and pyrene which were at concentrations of 0.6 and 1.4 mg kg^{-1}. These hydrocarbon levels are below the detection limit for total oil and grease in soil in this study. Fescue, described as a cool-season grass, was not significantly affected in its growth by the addition of anthracene and pyrene to soil compared to untreated soil. This result was observed both in the UN + PAH and the CN + PAH treatments. Two warm-season grasses, sudangrass and switchgrass, produced significantly less shoot and root biomass in the CN + PAH soil than in uncontaminated soils with and without the PAH spike. Alfalfa root biomass was significantly reduced in the contaminated + PAH treatment relative to the other two treatments. Alfalfa shoot biomass was significantly lower in the CN +PAH soil than in the UN + PAH soil and substantially lower than in the UN soil. Disturbance of soil-plant water relations by hydrocarbon presence was suggested as the cause of reduced forage biomass. These forage crops showed considerably greater sensitivity to hydrocarbons in soil than the grass and alfalfa grown in this study.

Wiltse et al. (1998) measured an effect of 20 000 mg kg^{-1} crude oil in soil on the agronomic performance of alfalfa one year after establishment. Total yield was down 32% and root mass was lower by 47% in oiled soil compared to untreated soil. Mean plant height was lower and maturation times were longer in soil with oil versus soil without oil. However, all agronomic variables except for root mass improved towards the end of the experiment. This was supposedly because of a diminishing contaminant effect on plant growth as the crude oil constituents were modified or eliminated.

Based on mid-season above-ground biomass, alfalfa demonstrated a greater sensitivity than grass to the presence of crude oil in soil. The response of grass to hydrocarbons in soil was much more variable. Both forage crops may have been stimulated in their growth by low levels of crude oil in several of the spill plots, indicated by the better productivity in these plots than in the control plots.

3.4.5 Harvest Results

3.4.5.1 N Response Curves. In 1996, the wheat grain and canola oilseed yields in control plots were dependent on the rate of fertilizer N added (Figures 3.16 and 3.17). The yield of wheat and canola in the control plots increased with added nitrogen fertilizer. The yields obtained in the control plots reached a plateau indicating that the yields from these plots can be used to estimate the yield potential of these soils under the conditions within the control plots during the 1996 growing season.

In 1997, annual wheat grain and canola oilseed yields in control plots were plotted against nitrogen supply. N supply was calculated using residual soil N levels from year one and applied N amounts in year two (Figures 3.18 and 3.19). Wheat grain yields in control pots did not vary greatly with N supply. CON1 and CON2 wheat yields generally reached a plateau indicating that yield potential at these two sites was adequately estimated for the 1997 growing season.

Canola oilseed yields demonstrated a greater response to varying soil N levels. CON3 canola yield reached a plateau while CON1 oilseed production rose gradually with increasing N supply. The CON2 curve was strikingly different from the other two curves in showing a stronger relationship between canola yield and N supply. The varied

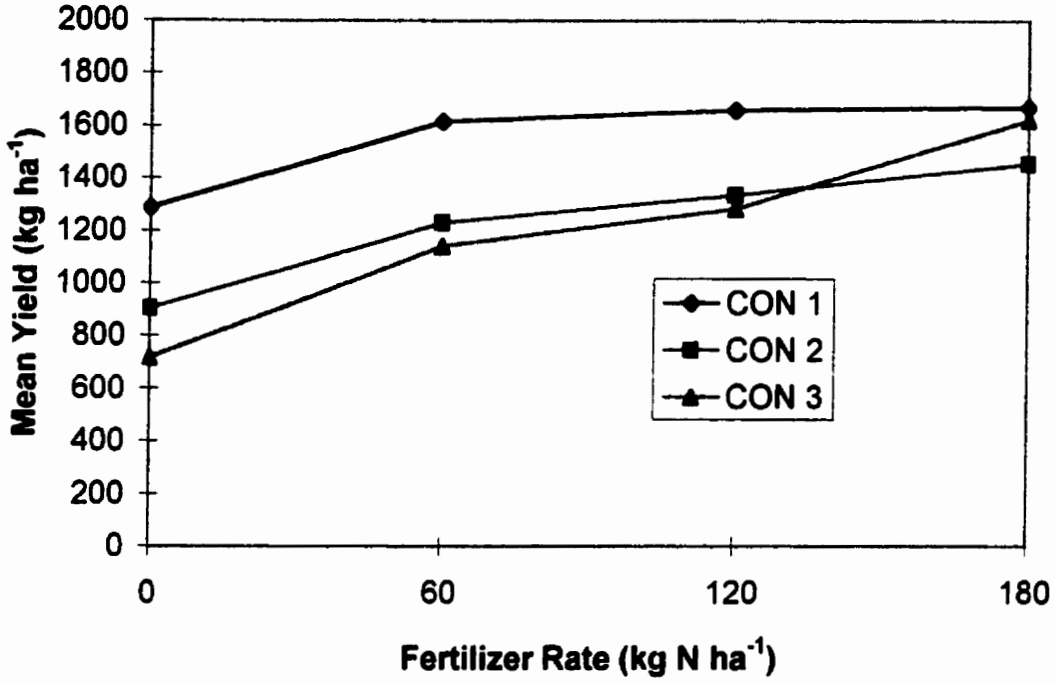

Figure 3.16 Wheat grain yield in control plots as a function of added fertilizer nitrogen. Each point on the curves represents a mean of four replicates.

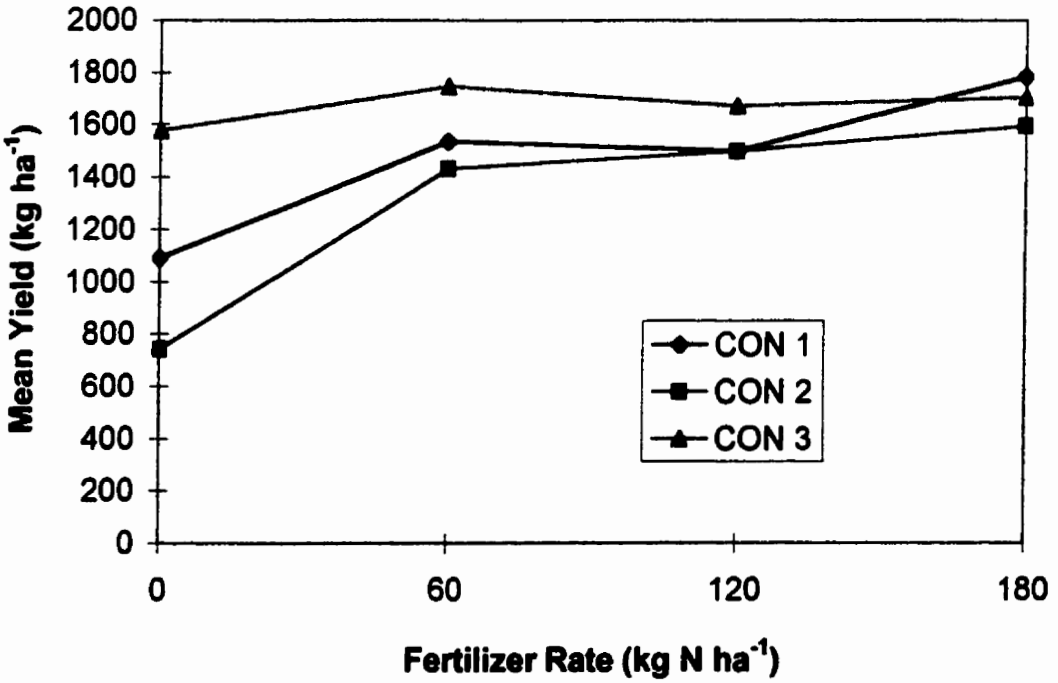

Figure 3.17 Canola oilseed yield in control plots as a function of added fertilizer nitrogen. Each point on the curves represents a mean of four replicates.

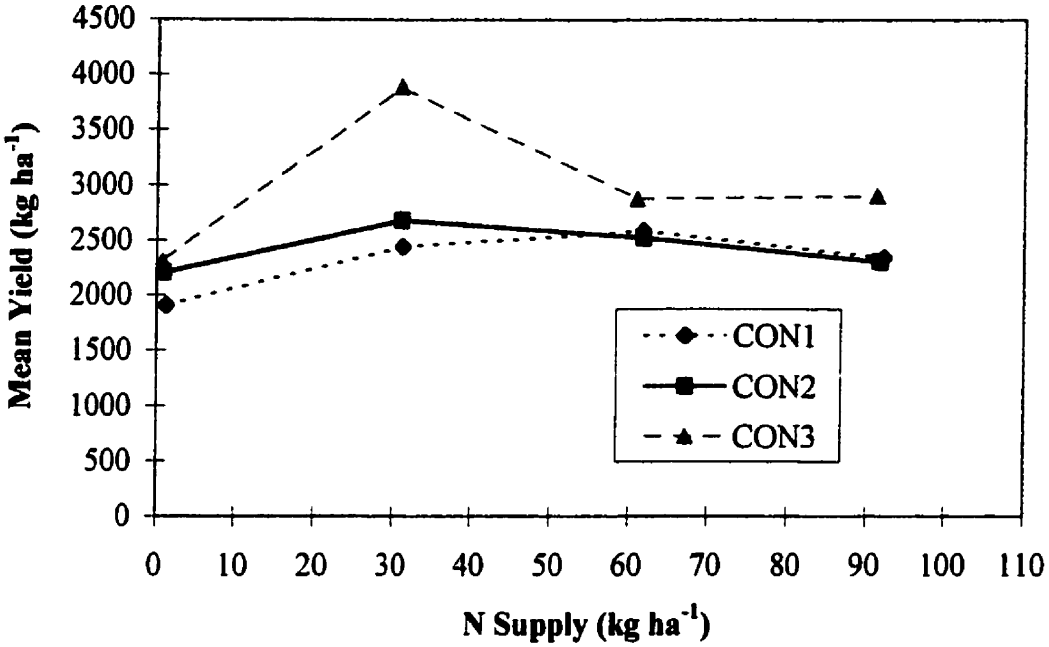

Figure 3.18 Wheat yield response curves based on 0-60 cm soil N supply (residual + 1/2 applied NO_3) for the three control sites in 1997. Each point on the curves represents the mean of four replictes.

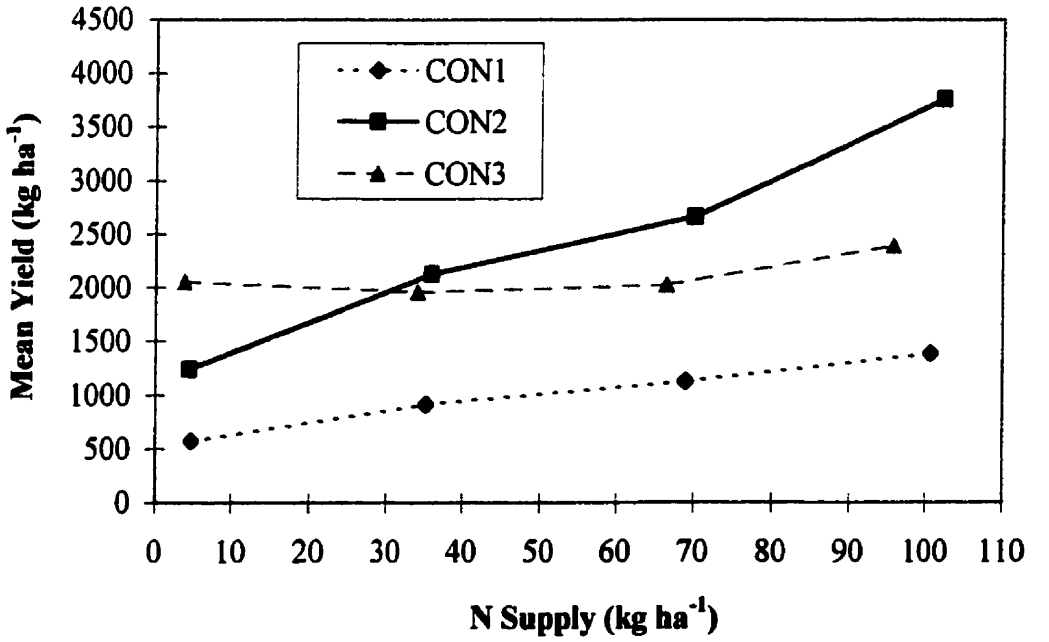

Figure 3.19 Canola yield response curves based on 0-60 cm soil N supply (residual + 1/2 applied NO_3) for the three control sites in 1997. Each point on the curves represents the mean of four replictes.

response of canola yield to N supply in the control plots, particularly in CON2, does not ensure that yield potential has been properly represented. Despite the lack of uniform response, mean control yields based on the highest N supply only were used to compare maximum yield potential in uncontaminated land with crop productivity in the spill area. Yields at the highest N supply were either higher than or comparable to average yields for all N levels within a given plot. Thus, the yields with highest N supply were deemed suitable for comparisons of productivity.

There was a vast difference in N supply between the control and spill research sites. Mean nitrate concentrations in the spill sites (Figure 3.4) were from approximately three to sixteen times the highest mean N supply in the control plots (Figures 3.18 and 3.19).

3.4.5.2 Crop Yields. The grain, oilseed and straw yields of the control plots displayed in Figures 3.20 and 3.21 are means for the highest (180 kg N ha^{-1}) fertilizer treatment only. This enables a comparison between productivity in the spill plots to the maximum yield potential for uncontaminated farmland adjacent to the spill area.

In 1996, the final wheat grain yields in SP2 and SP3 were similar to the yields obtained in the control plots (Figure 3.20). However, grain yields at SP1 and SP4 appeared to be somewhat depressed relative to the controls. The wheat at SP1 and SP4 had begun to show signs of stress earlier as indicated by the mid-season biomass and this translated into lower yields of both grain and straw at these sites (Figures 3.20 and 3.21). It is noteworthy that the greatest grain yield occurred at SP5 where the excavation had occurred in 1995.

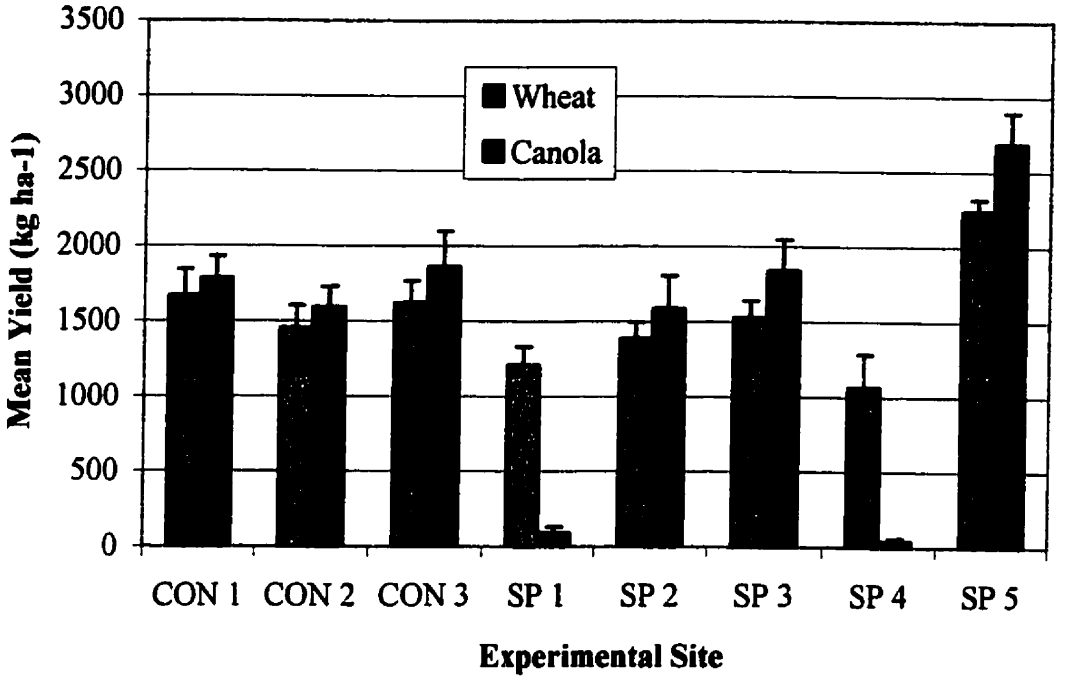

Figure 3.20 Wheat grain and canola oilseed yields in control (CON) and spill (SP) sites in 1996. Each bar represents the mean of sixteen replicates.

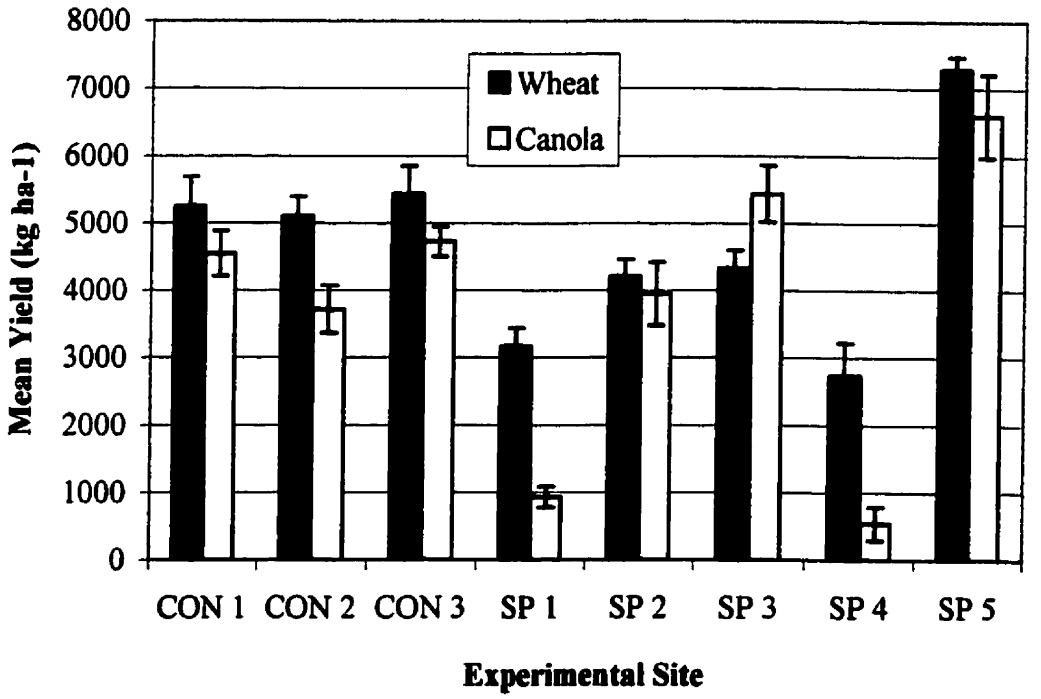

Figure 3.21 Wheat and canola straw yields in control (CON) and spill (SP) sites in 1996. Each bar represents the mean of sixteen replicates.

The final oilseed yields of canola were severely depressed in SP1 and SP4 relative to controls (Figure 3.20). This is consistent with poor emergence and poor mid-season biomass production at these sites. Oilseed yields in SP2 and SP3 were comparable to those of the control plots. This represents an improvement in the level of productivity at harvest in these two spill sites from that shown at mid-season. The SP5 site once again showed substantially higher yields than all other plots including the controls. The same trends were observed for canola straw yields (Figure 3.21).

Declines in wheat grain yield have been observed in soil containing relatively low levels of hydrocarbons (Chaineau et al. 1996). Grain yields decreased 16% below control values when grown a year after oil application in plots having an initial 0.2% concentration of hydrocarbons from drill cuttings. An 8% decline in grain yields was observed in plots initially containing 0.1% hydrocarbons. The authors attributed the yield losses in treated plots to the inability of the wheat to extend the rhizosphere beyond the upper 25 cm of soil, the soil depth with the greatest amount of hydrocarbons. The crude oil concentrations in both sets of crop plots in SP1 and SP4 were much lower than those first applied in a study of barley and rapeseed production in soil receiving crude oil at two sites (Toogood et al. 1977). In that experiment, barley yields in soil with an original oil content of 2.5% by weight reached control levels within a single year after fertilization and only two months of tillage. In previous tests, for which no data were given, barley had shown considerable sensitivity to the presence of oil, and greater sensitivity than wheat. Rapeseed was a secondary test crop, seeded 3 and 4 years after crude oil application at two sites. During that time, plots received various cultivation and/or fertilization treatments. At both study locations, rapeseed yields in most of the

remediated plots initially having 2.5% crude oil were comparable to those in control plots. Analysis done the year before rapeseed was first seeded showed that the soil at the two study sites had between 0.4 and 0.7% crude oil contents. Soil oil levels in the second year of seeding had remained the same or dropped slightly to a range of 0.4 to 0.6%. These concentrations are within the range of those at the St. Leon site.

The greater production of grain and straw at SP5 for both wheat and canola relative to the control plots may be attributed to excavation of the contaminated soil combined with ample nitrogen levels and soil moisture during the growing season. The excavation has apparently removed the negative effect of the crude oil from SP5. In fact, relatively small amounts of hydrocarbons remaining in the soil deposited at SP5 may have had a positive influence on crop growth at that location. This effect could have resulted indirectly from improved soil properties which influence plant growth, a change which could have only come about if the time between the St. Leon spill and experimental crop growth (2 years) was sufficient. The presence of hydrocarbons in soil is believed to have plant growth-promoting properties at very low levels because of certain constituents of crude petroleum (Gudin and Syratt 1975; McGill 1977). Some research has demonstrated this effect for particular agricultural crops (Carr 1919) but for the most part this enhancement of vegetative growth has occurred with non-agricultural plants (Stebbings 1970; Baker 1970; Baker 1971).

The high productivity of the SP5 site must be weighed against the poor results of SP4 where the contaminated soil was spread. The depressed growth at SP4 can be related to the high crude oil content of the root zone. Soil salinity did not contribute to the poor productivity at SP4 based on EC analysis for both soil depths (Table 3.3). This suggests

that crude oil concentrations in the soil that was spread at that location were sufficiently high to depress crop growth. It is also possible that the part of the field where SP5 was situated is inherently more productive than the control land because of its lower elevation and therefore presumably greater moisture content. Oil levels may have been so extremely low or even zero (unknown since analytical results were below detection), that they had neither a positive nor a negative influence on crop growth in SP5. It should also be noted that differences in the seed bed existed between the spill and control areas. The spill area had received extensive and regular treatment by a tiller over a full season the previous year, resulting in smaller soil aggregates near the surface. By contrast, the control soil showed signs of compaction, with large heavy clods broken up at the surface providing a less amenable seed bed. These conditions may have inhibited the growth of the small sized canola seed and seedling in the controls. Nevertheless, the canola yields were still lower in the high-oil zones of the spill area than on the control land.

In 1997, relatively low variability in final wheat grain yields was observed in the control plots (Figure 3.22). The grain yields in SP1-B, SP3 and SP4-a were all higher than those of the control plots. SP2 and SP4-b wheat yields were comparable to those of CON1 and CON2 but fell considerably below that recorded for CON3. Productivity in SP2 improved markedly from midseason. As at the time of midseason sampling, SP1 and SP5 values were lower than control values at harvest. Neither of the plots were able to make up the shortfall during the latter portion of the growing season. Similar groupings existed for wheat straw yields, with SP1-B, SP3 and SP4-a comparable to the controls while the other spill plots had lower straw yields than the controls (Figure 3.23). The good performance of wheat in most of the plots with higher oil levels is again contrary to

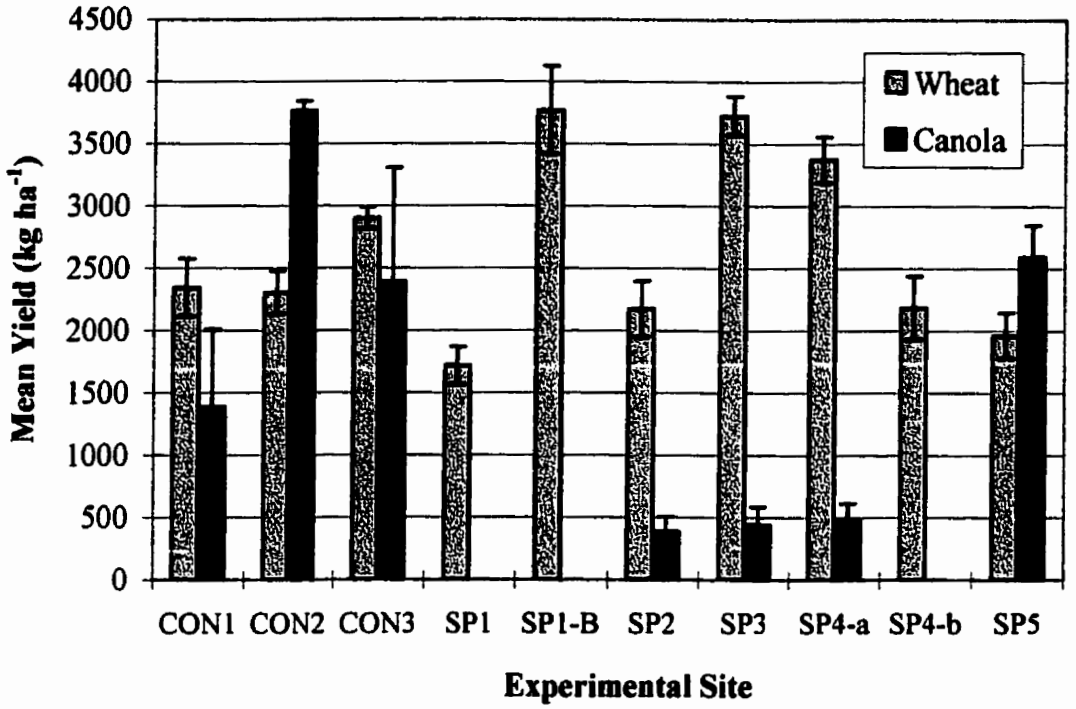

Figure 3.22 Wheat grain and canola oilseed yields in control (CON) and spill (SP) sites in 1997. Each bar represents the mean of sixteen replicates.

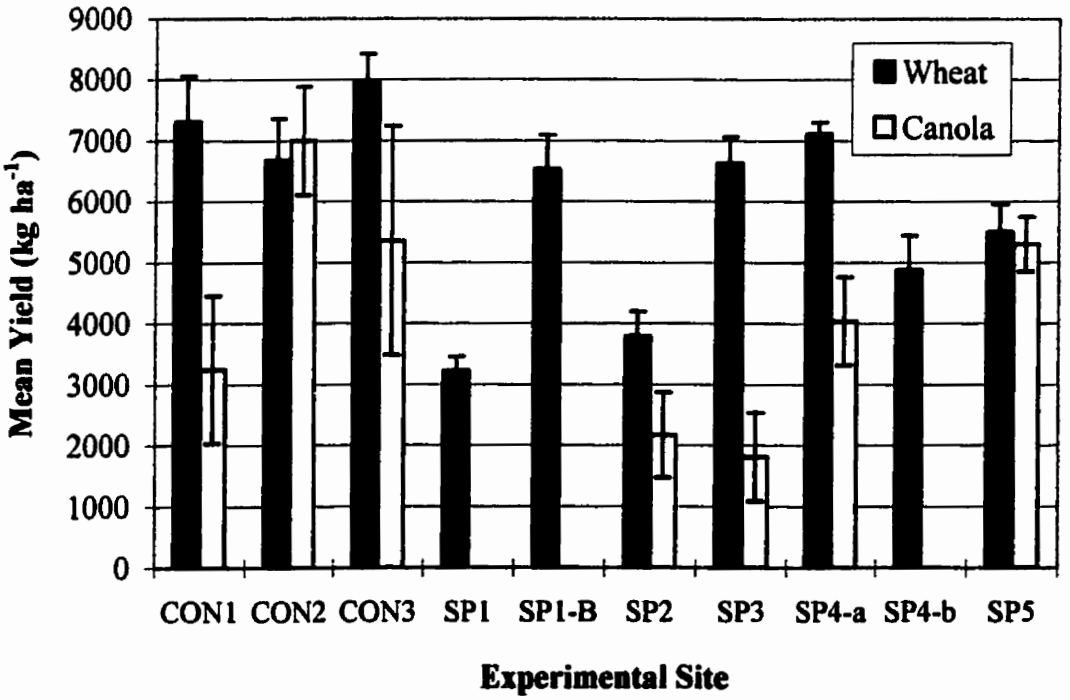

Figure 3.23 Wheat and canola straw yields in control (CON) and spill (SP) sites in 1997. Each bar represents the mean of sixteen replicates.

the findings of Chaineau et al. (1996). In that study, wheat yields were reduced significantly in soil having concentrations of 0.2% or less.

Only the canola yield in SP5 was comparable in any way to control yields (Figure 3.22). The spill plots SP1 to SP4 showed reduced canola growth relative to the control plots. Germination and/or emergence problems were possibly responsible for these results. It is apparent that canola growth in the spill plots (except SP5) remained below that expected for these soils under undisturbed conditions. Whether this was attributable solely to the presence of excessive concentrations of oil in soil is not clear at this point. Dry spring conditions may have contributed to the lower yields in 1997, through a combination of poor emergence and death of the seedlings which did emerge. However, since all canola plots received irrigation water and the control plots outyielded SP1 through SP4, it is likely that the presence of oil residues within these soils has contributed significantly to reduced canola productivity. Canola straw production followed very similar patterns across all sites except SP4-a which ranked closer to the control plots than in the case of oilseed yield (Figure 3.23). These results again conflict with those of Toogood et al. (1977) who observed near normal rapeseed yields in plots containing crude oil levels similar to those found in the spill plots of this study.

The splitting of experimental site SP4 into two sub-sites for the purposes of analysis revealed the variability in crop growth over a very short distance that was possible in the spill area. Differences in crop performance corresponded to differences in hydrocarbon levels between the northern and southern halves of the research site. The greatest contrast in contamination lay in the 0-30 cm depth in which crude oil content in SP4-b (the southern two replicates) was more than twice that of SP4-a (the northern

replicates). It appears that the deposition of soil from the vicinity of SP5 onto the SP4 location is still affecting crop production at this latter research site. Salinity was probably not a limiting factor as EC values were approximately 4 mS cm^{-1} in the SP4 soil in 1997.

The depressed wheat production in SP5 was possibly attributable to fungal disease brought on by prolonged wet soil conditions caused by heavy precipitation in early summer. The adverse effect of disease on wheat yield was exacerbated by the low-lying slope position of this research site which resulted in flooding conditions. The excavation that took place at SP5 does not appear to have been a detrimental factor in determining crop yield. No negative impact on canola yield was observed based on the performance of the oilseed crop at this spill site in comparison with the control canola plots. In fact, canola yields may have been increased by the presence of small residues of crude oil in SP5 as they appeared to have been the previous season.

The new experimental site established in year two, SP1-B, exhibited productivity consistent with that measured at locations in the spill area of similar hydrocarbon levels. Wheat production was high while canola yield was recorded as zero. The substantially lower oil concentrations, which may have been achieved as a result of more extensive landfarming, than at the nearby SP1 site makes a direct comparison difficult (although all other conditions should have been very similar). Consequently, it is difficult to confirm that an additional season of tillage created better growing conditions at SP1-B than at SP1 for the production of wheat and canola. Salinity was not a limiting factor at either of these two sites.

Initial growth of wheat in SP2 and SP3 was less vigorous, possibly due to the dry soil conditions which were worsened by the high salinity at these two sites. The harvest

yields indicate that the impact of these early limitations was minimal on wheat production. The consistently strong performance of wheat in the control plots confirms this latter assertion.

3.4.5.3 Harvest Index Values. Harvest Index (HI) values were calculated for both wheat and canola in each of the research plots (Table 3.4). This ratio (straw yield/grain yield) reveals how plants partitioned carbon between straw and grain. Thus, the HI may serve as a measure of the suitability of the growing conditions under which a crop has developed. Except for SP4 in year one, all wheat spill plots exhibited mean HI values which were either lower than or within the HI mean range of the control plots. Thus, the oil spill had a minimal impact on the ability of a wheat crop to allocate carbon to grain.

By contrast, almost all spill canola plots throughout the study had mean HI values which were greater than the range of HI means in the control plots. Only SP5 possessed mean HI values which fell within the control range. HIs for several of the spill plots could not be calculated in 1997 because of the absence of any oilseed yields. Thus, unlike wheat, canola plants exhibited considerable difficulty in allocating carbon to seed. This further reflects the negative effect of the spill on canola productivity as well as the lower sensitivity of wheat than canola to the presence of oil in soil.

Table 3.4. Harvest Index (HI) values (Straw Yield/Grain Yield) for wheat and canola in control (CON) and spill (SP) experimental sites (means of four replicates; harvest index for each control plot was calculated for the 180 kg N ha^{-1} treatment only in 1996 and for the highest N supply treatment in 1997). S.E. is standard error.

Research Sites	1996				1997			
	Wheat		Canola		Wheat		Canola	
	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.
CON1	3.2	0.1	2.5	0.0	3.1	0.05	2.8	0.33
CON2	3.5	0.2	2.3	0.0	2.9	0.17	1.9	0.25
CON3	2.9	0.1	2.6	0.3	2.8	0.12	2.3	0.28
SP1	2.8	0.1	21.1	0.4	1.9	0.06	0.0	0.00
SP1-B	-	-	-	-	1.8	0.08	0.0	0.00
SP2	3.1	0.1	3.1	0.4	1.9	0.24	6.1	1.54
SP3	2.9	0.1	3.1	0.2	1.8	0.05	4.5	0.47
SP4	8.9	5.6	18.3	3.6	-	-	-	-
SP4-a	-	-	-	-	2.1	0.07	14.3	5.72
SP4-b	-	-	-	-	2.2	0.03	0.0	0.00
SP5	3.3	0.2	2.5	0.1	2.9	0.14	2.1	0.06

3.4.6 Regression Analysis

The regression of crop yield with soil crude oil content may provide an indication of the oil concentration at which crop growth is reduced below that of the control plots. This concentration would be useful for management of spill sites on agricultural land by providing a target concentration for remediation efforts below which no effect on crop growth can be demonstrated. Such a critical concentration would also be useful to decide how much contaminated soil can safely be spread on agricultural soils without affecting crop growth. The poor growth at SP4 is likely due to the excessive addition of contaminated soil from SP5 thereby exceeding the critical oil concentration for growth of canola and wheat.

3.4.6.1 Wheat Regression. In 1996, the regression for wheat was highly significant with a coefficient of determination of 0.49 (Figure 3.24). This suggests that almost half of the variability in wheat yields could be explained by the presence of oil within the 0-30 cm root zone. The scatter of the data around the regression line suggests that factors other than oil content influenced yield variability. However, a significant negative linear relationship existed between wheat yield and oil concentrations. The mean yield of the control plots is plotted as a horizontal line in the figure. The regression line and the control yield line intersect at an oil content of approximately 0.2% (or 2000 mg/kg). This result suggests that oil content must drop to a concentration below this level before there is no evidence of depressed grain yield relative to the control plots. It must be stressed that this number is for the growing conditions of 1996 and thus is an estimate of a critical oil concentration for successful crop production based on limited results. Previously recommended application rates of hydrocarbons for purposes of disposal or land improvement have been substantially higher than the value found for wheat in this study. However, these addition rates were for types of petroleum other than crude oil. Racz and Cansfield (1977) advised a ceiling rate of 1.0% by weight for disposal of refinery hydrocarbon wastes on a high clay soil to maintain unaffected barley growth. Biederbeck (1997) suggested a range of 0.7 to 1.0% as acceptable for waste hydrocarbon concentrations on marginal lands in order to improve soil quality and crop productivity.

Regression analysis conducted for the 1997 wheat crop established no significant relationship between grain yield and total oil and grease in the 0-30 cm depth of soil. The results were not statistically significant with a coefficient of determination of 0.05 based on a linear model (Figure 3.25). The apparently random distribution of the data points

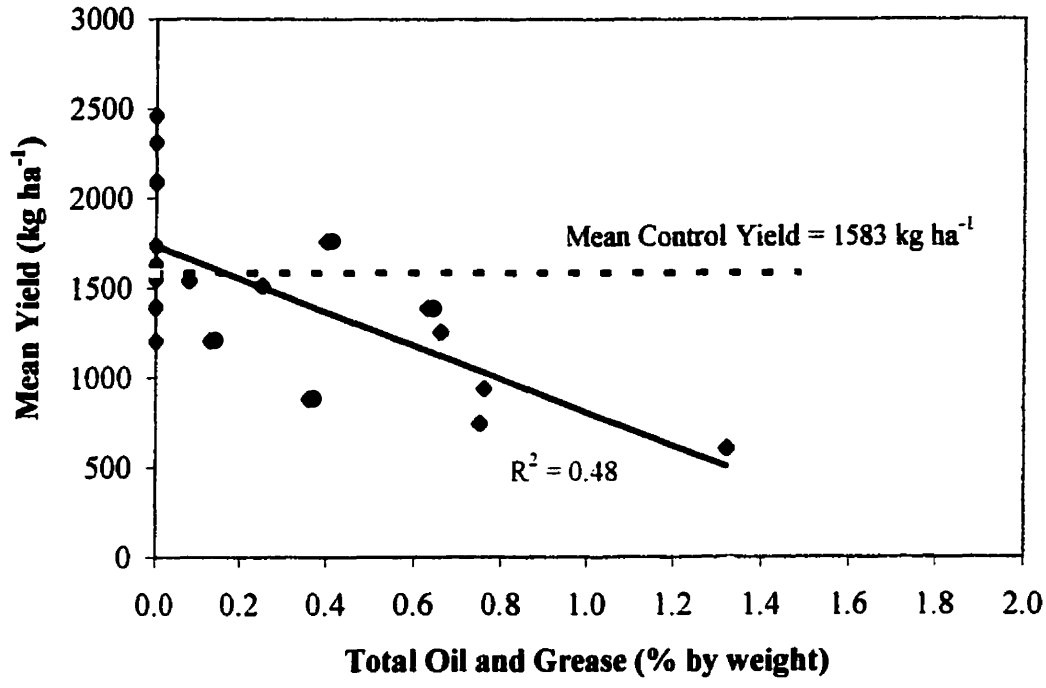

Figure 3.24 Regression of wheat grain yield in the spill plots versus total oil and grease in soil for the 0-30 cm depth in 1996.

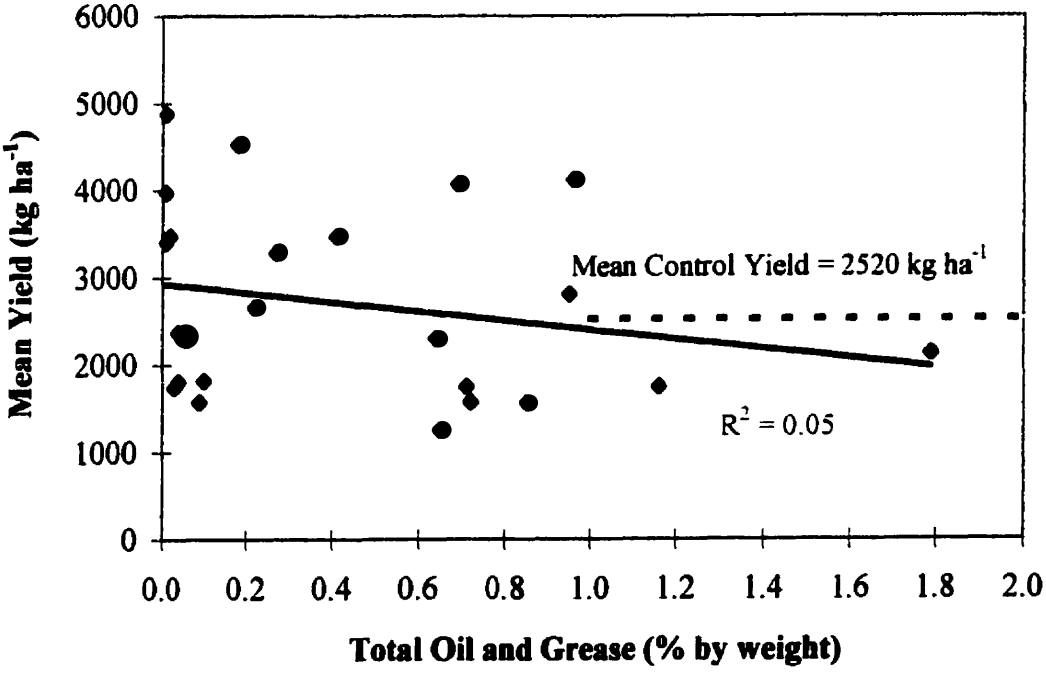

Figure 3.25 Regression of wheat grain yield in the spill plots versus total oil and grease in soil for the 0-30 cm depth in 1997.

around the regression line indicates that the variability in grain yield is largely caused by factors other than soil contamination. These other factors include environmental and agronomic conditions. This suggests that only a small amount of the variation in wheat yield is attributable to the presence of crude oil in the upper root zone. The mean yield of the control plots is represented by the horizontal line in the figure. Due to the poor relationship between yield and crude oil content in the 1997 year, it is not possible to estimate a threshold hydrocarbon concentration above which grain yield is reduced in the spill area.

3.4.6.2 Canola Regression. The lack of data points between the upper and lower clusters of data points in the regression was unavoidable due to the absence of canola subplots with oil concentrations between 0.2 and 0.7% (Figure 3.26). However, based on the distribution of the data points that are there, it was assumed that first-order rate relationship existed between canola oilseed yield and oil concentration in the 0-30 cm depth. The coefficient of determination for the regression was 0.95 indicating good agreement between the data and the equation used to model the yield response to oil concentration. This is supported by the tight fit of the data points about the regression curve. Therefore, concentration of total oil and grease explained 95% of the variability in oilseed yields. The mean control yield for canola is also indicated by a horizontal line. The regression line and control yield lines intersect somewhere between 0.1 and 0.2% oil. Canola growth rapidly declined with increasing oil concentrations above this value. This concentration range is below the critical threshold found by Overcash in Dueul (1990) for successful canola growth (<0.5%). An estimate of a critical oil concentration for canola

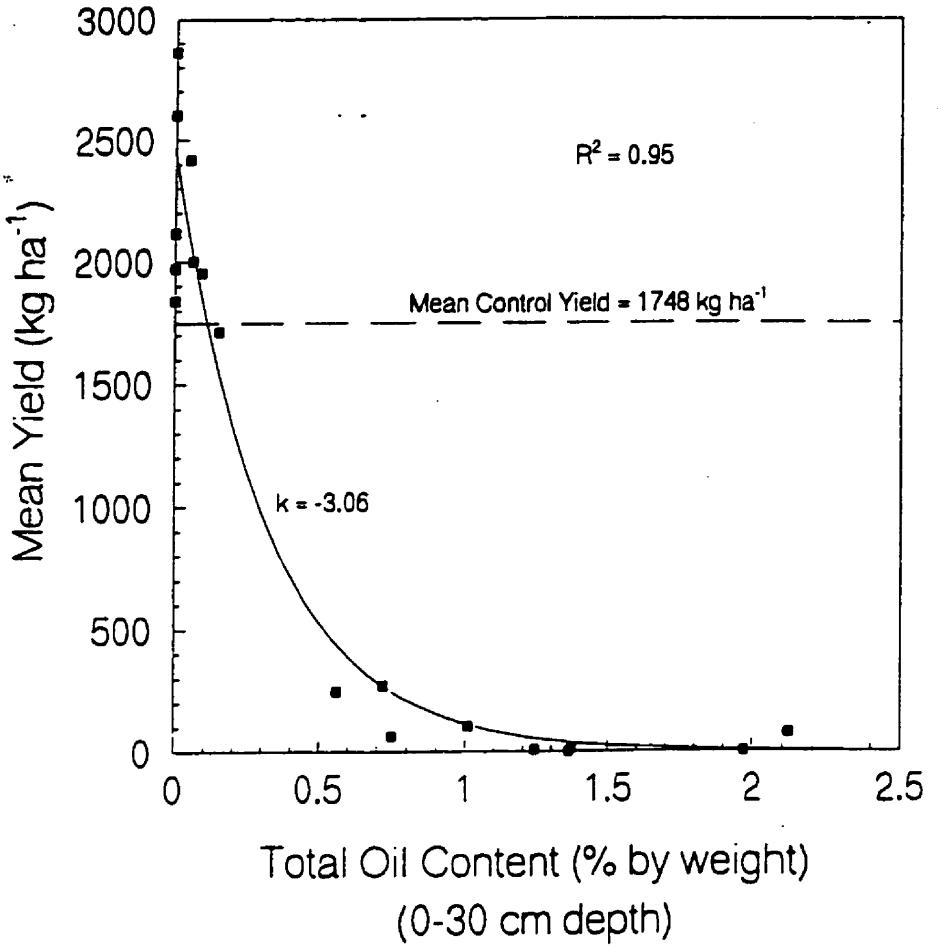

Figure 3.26 Regression of canola oilseed yield in the spill plots versus total oil and grease in soil for the 0-30 cm depth in 1996.

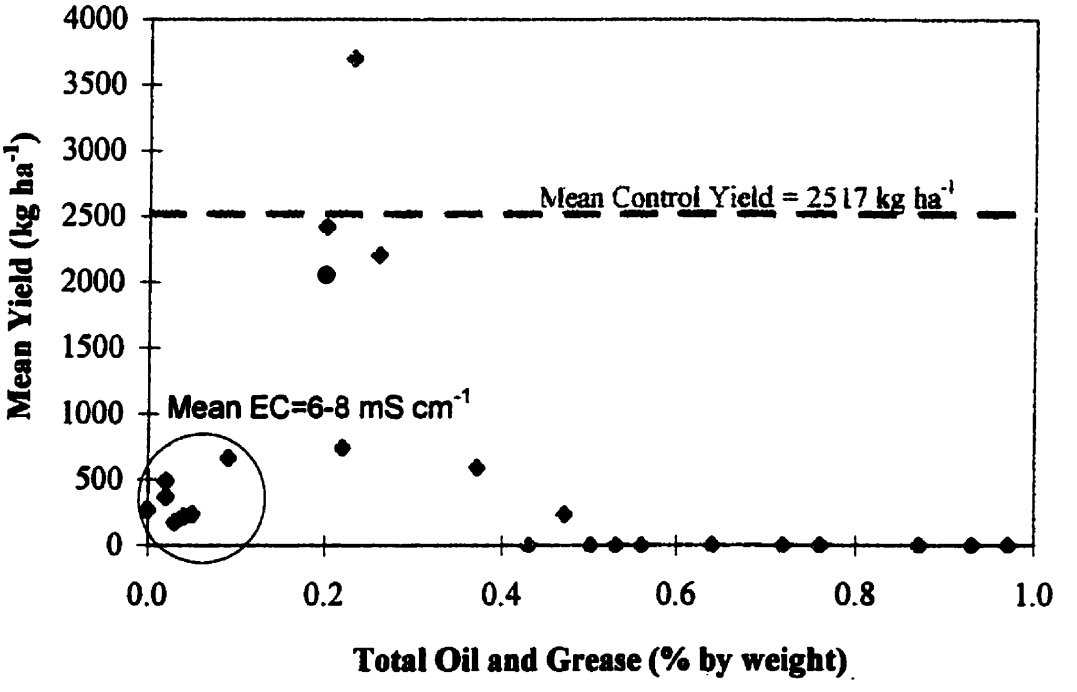

Figure 3.27 Plot of canola oilseed yield in the spill plots versus total oil and grease in soil for the 0-30 cm depth in 1997.

can be obtained from the crop trials of Toogood et al. (1977). Yields of rapeseed in treatment plots were comparable to those in control plots at two sites after several years of remediation. Soil analysis showed oil levels were 0.4-0.7% crude oil by weight in the year before the first seeding and 0.4-0.6% in the second year of seeding. The oil content of these plots was initially established at 2.5%.

In its entirety, the 1997 data set for canola oilseed yield plotted against total oil and grease in the 0-30 cm depth exhibits no evident pattern of distribution (Figure 3.27). Thus, a regression of the canola data fails to reveal any trends. However, this lack of pattern in the distribution of the data is due largely to the collection of points based on low yield and low oil content of soil (enclosed by the circle in the figure). These points represent blocks in SP2 and SP3 which experienced poor emergence and growth. The mean electrical conductivity values for the sites to which these points belonged were between 6 and 8 mS cm^{-1} and thus soil salt content may be at least partly responsible for reduced emergence and growth. When these points are not considered, the data follow a steep decline from high yields at low oil levels to low yields at high oil content. This decline in yield occurs near 0.2% total oil. This threshold for successful canola growth is consistent with the value determined in 1996.

3.4.6.3 Multiple Regression. A stepwise multiple regression analysis of the crop yield data from each year of the study was performed to ascertain the strength of relationship between yield and each of the two independent variables, total oil and grease content of soil and soil electrical conductivity. This type of approach is useful because both factors likely affected crop growth simultaneously but perhaps to different degrees. This

procedure was also intended to determine the general magnitude of each factor's influence on yield.

The 1996 wheat yield data produced an R^2 of 0.402 for the crude oil factor and an R^2 of 0.101 for the EC factor (Table 3.5). These values imply that roughly 40% of the variation in wheat yield in 1996 was attributable to the presence of crude oil in the soil, while only 10% of the variation was attributable to soil salinity. The 'P' values indicate that the fit of each of the factors in the regression model was significant at the 1 and 5% levels, respectively. These results indicate that hydrocarbon levels had a greater effect on final wheat yield than EC levels. Approximately 50% of wheat yield variation was caused by other factors.

Relating canola yield to crude oil concentrations in soil generated an R^2 of 0.733 while the regression of yield against EC values produced an R^2 of 0.004. Thus, canola yield in 1996 was much more profoundly influenced by the presence of crude oil than by soil salinity. Hydrocarbon levels in the canola plots accounted for considerably more of the variation in oilseed yield than they did for grain yield in the wheat plots. The fit of crude oil content in the regression model was significant at the 1% level while the fit of soil EC was not significant.

The presence of crude oil in the upper 30 cm of soil was not a factor in the production of wheat in 1997 based on multiple regression analysis. When wheat grain yield was tested against only the two factors of total oil and grease and electrical conductivity, both in the 0-30 cm depth of soil, soil EC accounted for all the variation in yield (Table 3.5). However, factors other than EC more greatly influenced wheat grain

yield as evidenced by the low R^2 value of 0.19 for EC. The fit of soil EC in the regression model was significant at the 10% level.

Soil EC in the 0-30 cm depth also had a somewhat greater effect on canola oilseed yield than oil content in that depth. Both factors fit the multiple regression model well based on the 'p' values, but both factors also accounted for only small portions of the variation in yield. The poor regression of yield with oil was probably caused by the dry soil conditions which, exacerbated by high salinity in SP2 and SP3, resulted in reduced canola growth in 1997. These conditions depressed oilseed yields in plots with relatively low hydrocarbon levels, thus weakening the overall relationship between yield and oil content of soil across the spill area.

Table 3.5 Multiple regression results.

	1996				1997			
	Wheat		Canola		Wheat		Canola	
Statistic	Oil	EC	Oil	EC	Oil	EC	Oil	EC
R^2	0.402	0.101	0.733	0.004	0.000	0.190	0.130	0.197
P	0.000	0.018	0.000	0.609	0.939	0.074	0.004	0.022

3.4.7 Presence of BTEX and PAHs in Crop Tissue

Grain, oilseed and straw tissue samples from the annual crops in both years of the study and aboveground plant tissue from the forages were submitted to a commercial lab to be analyzed for BTEX and PAHs. At the end of the first season, composite grain and straw were taken from wheat plots in each of CON1, SP1 and SP4. Composite oilseed and straw samples were taken from canola plots in CON1 and SP1 only, due to the absence of canola growth in SP4 in 1996. The following year, annual crop tissues were

sampled in CON1 and SP4 while forage crop samples were taken from the CON1 and SP1 plots.

For the most part BTEX levels were near or below the detection limit (Tables 3.6 and 3.8). The highest levels detected were still <1 mg kg^{-1}. These amounts are too low to be of any concern with respect to contaminant uptake by vegetation. All PAHs for which samples were analyzed were at levels below the detection limit (Tables 3.7 and 3.9). Thus, BTEX and PAHs were either present at extremely low concentrations just above detection limits or were entirely absent in crop tissue samples.

The results of this study were consistent with those of previous research on cereal, forage and pulse crops. Certain PAHs have been shown to accumulate in the tissues of agricultural crops. Shabad and Cohan (1972) reported natural background levels of benzo(a)pyrene (B(a)P) (0.29 and 27.0 μg kg^{-1} in spring wheat seed and stem, respectively). No relationship was found between the soil and wheat contents of this PAH. The highest B(a)P amounts were detected in spring wheat straw (27.0 and 26.7 μg kg^{-1} from crops grown in soil containing 1.6 and 170 μg B(a)P kg^{-1}, respectively). Concentrations of B(a)P in summer wheat were also found to be unrelated to soil levels, but the greatest amounts accumulated in the stem (Wagner and Siddiqi 1970). 3,4-benzfluoranthene, by contrast, was present in substantially greater quantities in wheat tissue and its presence was a function of soil levels. There were also smaller differences between the various plant components assayed (Wagner and Siddiqi 1970).

In other instances, PAHs have not been found in plants grown on land treated with PAH-contaminated mixtures, such as sewage sludge (Hulster et al. 1994; Wild and Jones 1992). Wild et al. (1992) consistently detected PAH concentrations of <1 mg kg^{-1}

Table 3.6 Concentration of BTEX compounds (mg kg^{-1}) in 1996 crop tissue.

BTEX	Experimental Site and Tissue										
	SP1* Grain	SP4* Grain	CON1 Grain	SP1* Wht Straw	SP4* Wht Straw	CON1* Wht Straw	SP1* Oilseed	CON1* Oilseed	SP1* Can Straw	CON1* Can Straw	Detection Limit
Benzene	<0.07	<0.07	<0.02	<0.15	<0.17	<0.20	<0.05	<0.18	<0.10	<0.04	0.02
Toluene	<0.07	<0.07	0.03	<0.15	<0.17	<0.20	<0.05	<0.18	<0.10	<0.04	0.02
Ethylbenzene	<0.07	<0.07	<0.02	<0.15	<0.17	<0.20	<0.05	<0.18	<0.10	<0.04	0.02
Total Xylenes (o,m & p)	0.07	<0.07	0.04	<0.15	<0.17	<0.20	0.13	<0.18	<0.10	<0.04	0.02

**Note detection limit due to difficult matrix.*

Table 3.7 Concentrations of Polynuclear Aromatic Hydrocarbons (PAHs) (mg kg^{-1}) in 1996 crop tissue.

PAH	Experimental Site and Tissue										
	SP1 Grain	SP4 Grain	CON1 Grain	SP1 Wht Straw	SP4 Wht Straw	CON1 Wht Straw	SP1 Oilseed	CON1 Oilseed	SP1 Can Straw	CON1 Can Straw	Detection Limit
Naphthalene	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	0.1
Acenaphthylene	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	0.1
Acenaphthene	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	0.1
Fluorene	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	0.1
Phenanthrene	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	0.1
Anthracene	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	0.1
Fluoranthene	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	0.1
Pyrene	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	0.1
Benzo(a)anthracene	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	0.1
Chrysene	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	0.1
Benzo-fluoranthenes (b&k)	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	0.1
Benzo(a)pyrene	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	0.1
Indeno(1,2,3-c,d)pyrene	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	0.1
Dibenzo(a,h)anthracene	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	0.1
Benzo(g,h,i)perylene	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	0.1

Table 3.8 Concentration of BTEX compounds (mg kg^{-1}) in 1997 crop tissue.

BTEX	Experimental Site and Tissue								
	SP4 Grain	CON1 Grain	SP4 Oilseed	CON1 Oilseed	SP1 Grass	CON1 Grass	SP1 Alfalfa	CON1 Alfalfa	Detection Limit
Benzene	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	0.02
Toluene	<0.02	0.02	0.09	<0.02	0.34	0.05	0.05	0.08	0.02
Ethylbenzene	<0.02	<0.02	0.03	<0.02	0.03	0.02	0.03	0.05	0.02
Total Xylenes (o,m & p)	<0.02	0.06	0.19	0.05	0.15	0.14	0.18	0.26	0.02

Table 3.9 Concentrations of Polynuclear Aromatic Hydrocarbons (PAHs) (mg kg^{-1}) in 1997 crop tissue.

PAH	Experimental Site and Tissue								
	SP4 Grain	CON1 Grain	SP4 Oilseed	CON1 Oilseed	SP1 Grass	CON1 Grass	SP1 Alfalfa	CON1 Alfalfa	Detection Limit
Naphthalene	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	0.1
Acenaphthylene	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	0.1
Acenaphthene	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	0.1
Fluorene	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	0.1
Phenanthrene	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	0.1
Anthracene	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	0.1
Fluoranthene	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	0.1
Pyrene	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	0.1
Benzo(a)anthracene	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	0.1
Chrysene	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	0.1
Benzo-fluoranthenes (b&k)	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	0.1
Benzo(a)pyrene	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	0.1
Indeno(1,2,3-c,d)pyrene	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	0.1
Dibenzo(a,h)anthracene	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	0.1
Benzo(g,h,i)perylene	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	0.1

in the tissues of grass, clover and barley crops grown in sewage amended soils. PAH levels in aboveground plant parts were not related to soil levels; absorption from the atmosphere was proposed to be the pathway of uptake. Grass has demonstrated no tendency to possess either low or high molecular weight PAHs in tissues in other instances (Qui et al. 1997). This observation was made for several grass species growing in soil containing PAHs in concentrations ranging in the thousands ppm. Chaineau et al. (1996) demonstrated that no hydrocarbons were present in seeds of wheat, maize and pea seeds placed in soil with oil levels compatible with crop growth. Negligible concentrations of fuel oil constituents were found in maize harvested from stands on soil with hydrocarbon levels as high as 1% (Chaineau et al. 1997).

Chaineau et al. (1997) noted that polar by-products of hydrocarbon biodegradation in soil may be available to plants. In addition, the appearance of PAH breakdown products in plant tissue, which would indicate the former presence of the parent compounds, has not been investigated.

3.5 Summary and Conclusions

Results of the study demonstrated that the crude oil spill still affected the agronomy of the exposed land two and three years after the initial release. In 1996, wheat growth was not influenced by the presence of hydrocarbons in the soil until mid-season, when it was somewhat reduced in only the most highly contaminated plots. Harvest wheat yields of grain and straw were not diminished in the spill area compared to the adjacent unaffected land. However, within the spill area, wheat grain yield was somewhat related to the crude oil content of soil according to regression analysis. By

contrast, canola growth was hampered throughout the growing season in soil with the highest hydrocarbon levels. Emergence counts, mid-season aboveground biomass and harvest yields of oilseed and straw were all lower in the most heavily contaminated plots than in the control plots. The canola crop in the high oil plots also had difficulty in allocating its resources to the generation of seed relative to straw production. Oilseed yield exhibited a strong relationship with total oil and grease in 0-30 cm of soil, with yield dropping off drastically when soil oil content was above 0.1-0.2%.

In 1997, wheat production demonstrated much less susceptibility to adverse hydrocarbon effects than in the previous year. At mid-season, wheat growth was lower in several of the spill plots relative to the controls, but salinity and disease factors may have been at work in two of these plots. Few differences in grain and straw harvest yields were observed between wheat plots within and outside the spill area. Wheat grain yield was independent of hydrocarbon concentrations in soil based on regression analysis. Dry soil conditions confounded the results for canola to a certain degree. Despite this circumstance, similar trends were observed as in the previous year for canola with the exception of two experimental sites which exhibited higher salinity than all other sites. At these two sites, canola growth was seriously depressed. This latter result was observed at both mid-season and harvest. No emergence data was collected due to adverse site conditions. In the most heavily contaminated plots, in which salinity was not a factor, the canola crop was virtually absent. The relationship between canola oilseed yield and crude oil concentrations would likely have been strong if not for the influence of salinity in two of the plots. Nevertheless, the decline of yield with rising hydrocarbon levels followed a pattern similar to that of the previous year.

Over the course of the study, canola exhibited a greater sensitivity to the presence of oil within the root zone than did wheat. A critical crude oil concentration above which crop yield was reduced could only be repeatedly estimated for canola, and only with a correction for salinity effects in the second year. This threshold was in the range of 1000 to 2000 mg kg^{-1}. Wheat yield was related to soil hydrocarbon levels only in the first year when the threshold concentration for yield reduction was approximately 2000 mg kg^{-1}. Establishing a critical crude oil concentration at which crop growth and yield begins to be negatively affected may enable the calculation of what amount of contaminated soil can be safely spread without depressing the plant productivity of a site.

The results of the study, from year one in particular, suggest that canola may be a useful crop for bioassay purposes on crude oil affected agricultural lands. The growth and yield of canola may potentially be used as a screening tool to identify portions of a spill area needing special remedial actions. This would provide a means to allocate remediation resources to critical zones within the spill area.

Uptake of BTEX and PAHs from crude oil by wheat, canola, brome grass and alfalfa crops does not appear to be a prevalent fate pathway for hydrocarbons introduced to agricultural soil.

The St. Leon spill site appears to be approaching a level of productivity for wheat which would be expected in that area on land with no crude oil contamination. This assertion is based on the most recent results. The evidence for this statement consists of the comparable performance of research plots within the spill area versus control plots on adjacent land and the lack of relationship between grain yield and total oil and grease in 0-30 cm of soil. However, site productivity with respect to canola remains well below

that typical for the affected field. In both years of the study, canola exhibited a sensitivity to crude oil in soil. Thus, determining the endpoint of remediation for agricultural land depends on the types of crops to be grown and their respective sensitivities to the presence of crude oil or other hydrocarbons in soil.

4. THE EFFECTS OF A CRUDE OIL SPILL ON MICROBIOLOGICAL INDICES OF SOIL BIOLOGICAL QUALITY

4.1 Abstract

The concept of soil quality is becoming increasingly important as land is assessed for its "health" in both ecosystem and human-use contexts. Introducing a contaminant to soil can affect the quality of a soil, including the biological component. A study was conducted to examine the effects of a crude oil spill and a range of remedial treatments on the biological quality of an agricultural soil based on three microbiological indices: microbial biomass carbon (MBC), dehydrogenase activity (DHA) and microbial metabolic diversity (MMD). The study was conducted at the site of a pipeline rupture which released crude oil onto agricultural land near the village of St. Leon in southern Manitoba. Experimental plots were located in the spill area and on adjacent unexposed land. The soils at both locations are clay loam Gleyed Rego Black Chernozems developed from lacustrine materials alone at the spill site and from lacustrine over till at the control site. The four remedial treatments consisted of: meadow bromegrass, (*Bromus biebersteinii.* Rohman and Schult), alfalfa (*Medicago sativa* L. c.v. Algonquin), fallow with wheat straw incorporation (SF) and unamended fallow (UF_{SP}). An unamended fallow on adjacent uncontaminated land served as a control (UF_{CON}). The effect of the spill on each of MBC, MMD and DHA was significant at the 0.01, 0.05

and 0.11 probability levels, respectively. Of the four remedial treatments tested, grass had the greatest effect on soil biological quality relative to the UF_{SP}. Alfalfa exhibited a slightly reduced effect compared to grass. The incorporation of wheat straw did not affect either MBC or DHA, but did significantly ($p=0.02$) enhance MMD in the spill experimental site relative to the UF_{SP}.

4.2 Introduction

The concept of soil quality is becoming increasingly important as land is assessed for its "health" in both ecosystem and human-use contexts. The biology of soils plays a vital role in a number of soil processes including nutrient cycling, soil organic matter decomposition, soil development, maintenance of tilth and structure, and pollutant stabilization and degradation (Weil et al. 1993; Turco et al. 1994). Soil biological phenomena also contribute heavily to the resiliency of the soil system when subjected to a disturbance (Karlen et al. 1992). The dynamics of the soil microbial community, in particular, are a dominant force at the ecosystem level in both pristine and disturbed environments (Turco et al. 1992). In the latter systems, soil microbes regulate restoration processes which make it possible for floral and faunal life to recolonize a perturbed site. Soil quality reflects system productivity and should therefore reflect potential agronomic productivity (Yakovchenko et al. 1996). Since the biology of soil is an essential part of soil quality (Howard 1947; Higa 1991), soil microbiological properties should then serve as useful indicators of the health of an agroecosystem.

Soil microbial parameters have been proposed as biological indicators in order to determine the effects of a disturbance on soil quality (Turco et al. 1992). As a vital element of ecosystem function, the soil microbial community can reflect changes in the ecological functioning of soil. Measuring soil microbiological variables can thus reveal the functional status of the soil at particular point in time.

Rasmussen and Collins (1991) recognized soil organic matter (SOM) as the most all-encompassing measure of soil quality. Within SOM is the carbon associated with microbial biomass (MB). Microbial biomass carbon (MBC) represents stored energy for fueling microbial reactions, thus making it a measure of potential activity of soil microflora (Rice et al. 1996).

Soil enzymes fulfill a critical role in many biochemical processes and are an important aspect of soil quality. They may also serve as process level indicators of soil quality. The magnitude of a given enzyme activity in one soil relative to another may indicate a difference in soil quality, depending on the relative conditions of the soils. Dehydrogenase is a widely examined oxidoreductase. An assay of its activity serves as a measure of general metabolic activity of the soil microbial community (Friedel et al. 1994). The pathways of this enzyme contribute heavily to the oxidation of SOM through the transfer of hydrogen from substrates to acceptors (Dick 1994). Dehydrogenase is an intracellular enzyme and therefore associated with only living cells (Skujins and Burns 1976). Thus, it is related to total metabolic activity. Rossel and Tarradellas (1991) suggested dehydrogenase activity (DHA) as an assessment tool for contaminated soils provided that it is considered a short-term or substrate-induced activity. Its close association with living microorganisms in soil may make dehydrogenase a sensitive

responder to disturbance or amendments in the soil system, and therefore a potential indicator of soil biological quality (Dick et al. 1994; Warman and Cooper 1997).

Biodiversity is a highly regarded though unclearly defined element of nature (Lubchenco et al. 1991). Solbrig (1991) delineated three components of biodiversity: taxonomy, genetics and functionality. Methodological constraints and information gaps have limited the study of the first two components in the field of microbiology (Klopatek et al. 1993). Consequently, a shift in focus to functional diversity may yield an improved understanding of the ecosystem level contribution of soil microorganisms. Zak et al. (1994) suggested that functionality may prove to be more readily measurable and that it is certainly an equally crucial element of biodiversity. A microbial community represents a functional unit defined by the totality of its metabolic capabilities (Wunsche et al. 1995). Therefore, the pattern of substrate utilization of a microbial community should be a product of both its quantitative and qualitative composition. This physiological profile should then reveal its metabolic potential.

Considering the contribution of microorganisms to the soil ecosystem (Turco et al. 1994), the potential effects of crude oil contamination on soil microbiology are of considerable importance. Both the establishment of vegetation (Rouatt et al. 1960; Curl and Truelove 1986) and addition of wheat straw (Powlson et al. 1987; Ocio and Brookes 1990) have been shown to affect aspects of the soil microbial community. In each case, the treatment applies carbon to soil which influences microbial indices based on the strong association of microbial biomass and activity with SOM (Collins et al. 1992). The combined presence of petroleum hydrocarbons and either a rhizosphere or incorporated straw may produce unique results for various measures of soil microbiological activity.

Thus, the objective of the research was to measure the effect of a crude oil spill on soil biological quality via three indicators: (1) the size of the microbial community based on total biomass carbon; (2) general microbial activity based on the dehydrogenase enzyme and (3) microbial metabolic diversity using the Biolog microplate identification system. It was hypothesized that the presence of crude oil would adversely affect the biological quality of an agricultural soil. A further hypothesis was that the establishment of a forage crop, grass or alfalfa, or the incorporation of wheat straw into soil containing crude oil would improve soil biological quality relative to an unamended fallow.

4.3 Materials and Methods

The study was conducted at the site of a pipeline rupture which released crude oil onto agricultural land near the village of St. Leon in southern Manitoba. Experimental plots were located on both the spill and adjacent unaffected land. Soil profile and landscape data for the spill and control experimental sites is presented in Table 4.1. Mean total oil and grease levels in the contaminated plots were not significantly different from each other (Figure 4.1; Tables 5.2 and 5.3 in Chapter 5). The treatments had been established for phytoremediation and carbon amendment trials which examined the effects of permanent forage cover or straw incorporation on hydrocarbon levels in soil. The five treatments consisted of: meadow bromegrass (*Bromus biebersteinii.* Rohman and Schult), alfalfa (*Medicago sativa* L. c.v. Algonquin), fallow with wheat straw incorporation (SF), spill or contaminated unamended fallow (UF_{SP}) and control unamended fallow (UF_{CON}). The two unamended fallows were intended to

simulate landfarming concurrently underway on the remainder of the spill area. All plots had received approximately 3500 kg ha^{-1} fertilizer nitrogen as part of the landfarming activities on the contaminated land prior to the experimental trials. Treatments were arranged roughly north-south side by side in the field in four replicates, each block being 1.5 m by 5 m.

Table 4.1 Properties of typical soil profiles on control and spill land.

Research Site	Horizons	Depth (cm)	Texture	Surface Expression	Comments on Profile
Spill	Apk	0-18	CL	Level	Moderately fine lacustrine parent material throughout Evidence of disturbance Most gypsum found at 70-90 cm
	AC	18-32	CL		
	Ckgjs	32-90	SiC		
	Ckgs	90-120	SiCL		
Control	Ap	0-26	CL	Level	Moderately fine lacustrine overlying till Gypsum found at 40-50 cm
	AC	26-40	CL		
	Ckgj	40-95	SiC		
	IICkgj	90-120	SiCL		

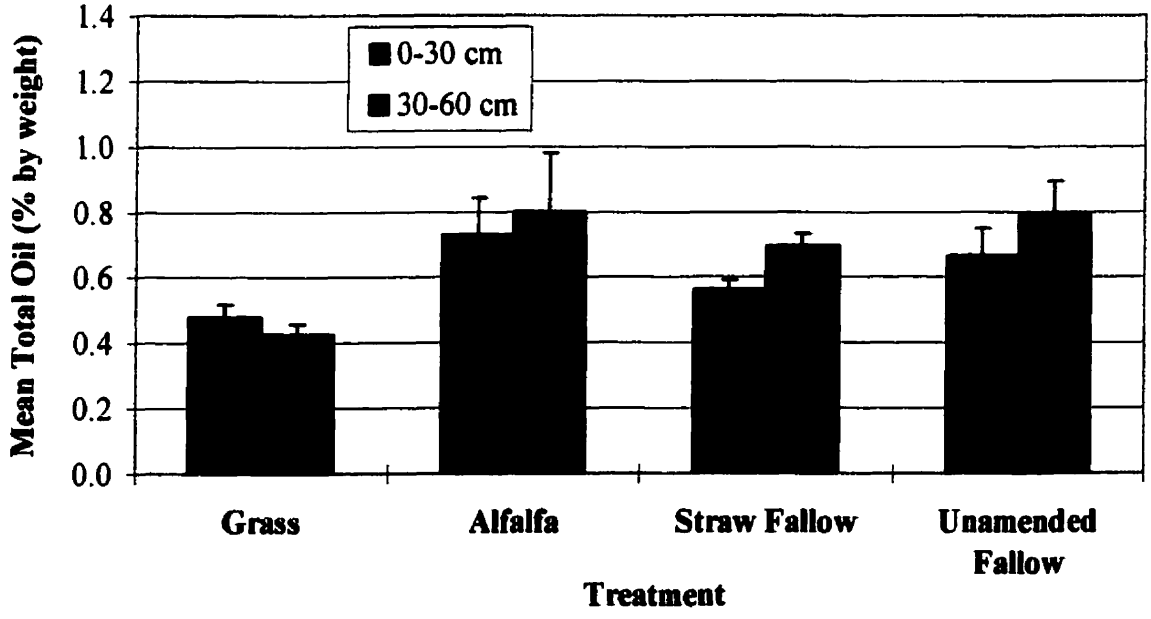

Figure 4.1 Total oil and grease levels in the forage and fallow plots in 1997. Each bar represents the mean of four replicates.

The forages were seeded in June/96 at a rate of 7 kg ha^{-1}. Straw was incorporated in August, 1996 at a rate of 4100 kg ha^{-1}, a rate similar to that typically applied to farm fields at harvest. The straw fallow plot was tilled at the same time as the unamended fallow plot in the second year. Tillage passes in the fallows numbered approximately ten in each year which matched the landfarming tillage regime on the adjacent soil.

Three samples from the 0-15 cm depth were taken 1.25 m apart in the centre of each of four replicates with dutch augers in August, 1997. In order to keep the samples field moist, they were stored first in a portable cooler for transport and then at 4°C until analyses were performed within two days.

MBC was determined using the method described by Voroney et al. (1993). Sieving was not done as the soil, being from the surface, was already dominated by small aggregates and because it was desirable to maintain physical field conditions as much as possible. Twenty-five gram samples were extracted with a 1:2 soil mass to extractant volume ratio. The samples were frozen at -15°C until analysis. Organic C was measured according to Method No. 455-76W/A, Technicon Industrial Systems, using an AutoAnalyzer II system equipped with a No. 116-D660-01 manifold.

DHA was measured based on the procedure outlined by Dick et al. (1996) which was modified from the original assay developed by Lenhard (1956). During 24 h incubation at 37°C, TTC (2,3,5-triphenyltetrazolium chloride) was reduced to TPF (2,3,5-triphenyl formazan), the colour intensity of which was analyzed using a spectrophotometer at a wavelength of 485 nm.

MMD was assessed using the multiple substrate microplate identification system (BIOLOG, Inc.) developed by Garland and Mills (1991). The basic intent of the procedure is to identify the metabolism of a substrate by a community of microorganisms extracted from the soil by observing colour formation from a redox-sensitive dye in the plate wells. In so doing, the microbial community present in the extract can be characterized for its functional diversity based on the utilization of substrates present in the plate wells. A purple colour develops when a tetrazolium dye is reduced at the same time that the substrate in the well is oxidized. Ten gram soil samples (on an oven-dry weight basis) were transferred aseptically to water agar dilution bottles, each containing 90 mL of water agar solution and ten 5 mm glass beads. After the bottles spent 30 min on a lateral shaker, 1 mL of each soil suspension was taken and deposited into a saline dilution bottle containing 99 mL of physiological saline solution (10^{-3} g mL^{-1}). The saline dilutions bottles were shaken by hand. Finally, each of the 96 wells in the Biolog plates were inoculated with 100 μL of the final dilution. The plates were then incubated on an orbital shaker at 25°C. The plates were taken out of incubation for inspection at 24, 48, 72 and 96 hours. At each reading, the wells were inspected for colour appearance, indicating the utilization of the substrate in a particular well. Microbial metabolic diversity, based on per cent substrate utilization, was then determined for each soil sample. These values were then averaged to find a mean MMD for each treatment.

Statistical analysis was performed using Systat 7.0. Results were analyzed using one-way ANOVA and Fisher's Least Significant Difference Test which in Systat

generates a matrix of pairwise comparison probabilities for all treatments. This output reveals at what level of probability a given comparison is significant.

4.4 Results and Discussion

4.4.1 Forage Establishment

In general, establishment and growth in the forage plots was successful but not uniform for both crops. The grass emergence and growth was better than alfalfa throughout the replicates. Mean mid-season aboveground biomass in the spill experimental site was 3240 and 2023 kg ha^{-1} for grass and alfalfa, respectively. Mean biomass in the control experimental site was 2477 and 3760 kg ha^{-1} for grass and alfalfa, respectively. Thus, the potential impact of grass growth on soil biological quality was not limited by its agronomic performance. The same can not be said for alfalfa.

4.4.2 Microbial Biomass Carbon (MBC)

An estimate of the size of the microbial community was based on total biomass carbon (Figure 4.2). Treatment means were significantly different at the 1% probability level (Table 4.2). Significantly larger populations ($p=0.05$) were supported in the grass, alfalfa and control unamended fallow (UF_{CON}) treatments than in the straw fallow (SF). Highly significantly larger populations ($p=0.01$) were present in the grass, alfalfa and the UF_{CON} plots than in the spill unamended fallow (UF_{SP}) (Table 4.3).

Through chemical or physical changes in the soil environment, the presence of crude oil negatively altered the soil microbial habitat. Many of the various species of microorganisms present at this particular site may have been sensitive to toxic crude oil

constituents, initially and over an extended period of time. Crude oil has been described as having antiseptic properties in soil (Buddin 1914). This resulted in the UF_{CON} exhibiting greater MBC than the UF_{SP}. The high levels of MBC in the UF_{CON} seems to indicate that the study soil possessed ample substrate, despite the absence of vegetation, for soil microorganisms but lacked the inhibitory influence of crude oil compounds. Typically, microbial populations susceptible to oil toxicity decline immediately after exposure, causing total microbial numbers to initially decrease or remain stagnant

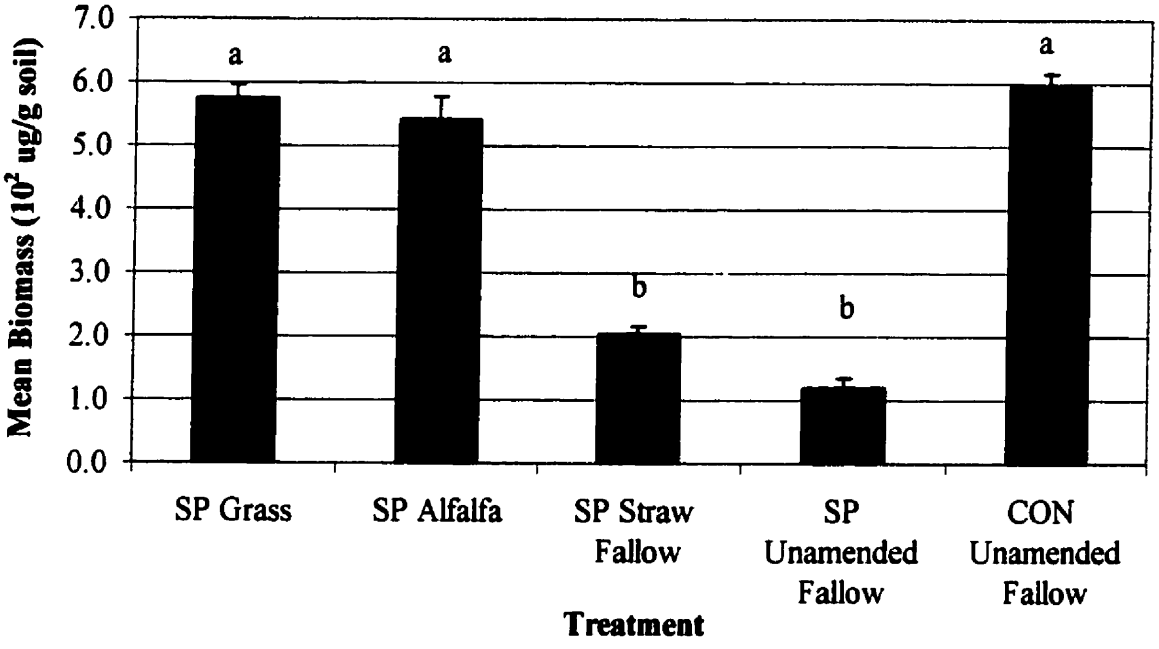

Figure 4.2. Microbial biomass carbon in 0-15 cm depth of spill (SP) and control (CON) forage and fallow plots. Each bar represents the mean of four replicates (p=0.05).

Table 4.2. Analysis of Variance results for soil microbial biomass carbon data.

Source	Sum-of-Squares	df	Mean-Square	F-ratio	P
Treatment	51299.52	4	12824.88	6.28	0.0036
Error	30654.38	15	2043.63		

Table 4.3. Matrix of pairwise comparison probabilities for soil microbial biomass carbon based on Fisher's Least Significant Difference Test.

	Grass	Alfalfa	SF	UF_{SP}	UF_{CON}
Grass	1				
Alfalfa	0.80	1			
SF	0.01	0.02	1		
UF_{SP}	0.00	0.00	0.52	1	
UF_{CON}	0.88	0.68	0.01	0.00	1

(Baldwin 1922; Plice 1948; Odu 1972; Gossen and Parkinson 1974; Biederbeck 1990). As resistant populations and hydrocarbon-degrading strains increase in prominence, total numbers rebound to levels above those found in untreated soils. Microbial numbers can rise for months following oil application to soil (Matthews 1924; Chaineau 1996). Eventually, the size of the soil microbial community returns to control levels. This latter trend may not occur until years after initial soil exposure to oil (Matthews 1924; Biederbeck 1990; Chaineau 1996). In this study, the soil microbial community may have followed this general pattern until equilibrium was reached. At this point, a new equilibrium was established by altered soil conditions which could not sustain a microbial biomass as large as the one supported before exposure to crude oil. Thus, the length of time elapsed between the exposure of soil to hydrocarbons and the collection and analysis of samples is an important consideration in interpreting results.

When a crude oil spill occurs, there is a natural partitioning of hydrocarbon fractions. Low weight, low viscosity compounds migrate downward while a high molecular weight, highly viscous fraction remains at the surface bound to clay and organic matter (Duffy et al. 1977). The heavy fraction contains recalcitrant components, such as complex aromatics and asphaltenes (McGill et al. 1981) which could affect the surface soil over the long term as they are not rapidly biodegraded. Samples for this

study were taken from the 0-15 cm depth. The hydrophobicity of the hydrocarbons may have repelled moisture away from the surface and interior of soil aggregates, preventing colonization by microorganisms.

The presence of forage crops on crude oil affected land restored the size of the biomass to levels similar to those in unvegetated, uncontaminated soil and greater than those in the unvegetated, contaminated soil. This latter result is consistent with the findings of Lee and Banks (1993) in which microbial numbers were substantially greater in alfalfa planted soils than in bare soils. This trend was observed for uncontaminated soil, uncontaminated soil spiked with either PAHs or aliphatics and contaminated soil also spiked with either type of hydrocarbons. Alfalfa growth was said to have shown an ability to augment soil microbial activity in both polluted an unpolluted soils. Similar results were obtained in research conducted by Schwab and Banks (1994) for microbial counts in contaminated and uncontaminated alfalfa rhizospheres which were either spiked with PAHs or left untreated. Establishing forage crops has increased soil microbial biomass relative to uncropped soil within three years (Drury et al. 1991). Peaks in microbial biomass carbon (MBC) were associated with periods of vigorous growth. Similar findings were obtained by Carter (1986) for three year old ryegrass. Ryegrass growth has been proven to increase soil MBC based on the measurement of glucose-induced maximal initial respiration rate (Ross and Cairns 1982).

Drury et al. (1991) measured a larger microbial biomass in soil seeded to reed canarygrass than in soil supporting several other forage and annual crops throughout the summer. The difference between microbial biomass under reed canarygrass and alfalfa was significant only in July. Bromegrass has produced larger MBC amounts in soil than

alfalfa (Perfect et al. 1990). This latter result was possibly attributable to the significant differences in root length and weight between the two forage crops. In this study, no difference in MBC were observed between grass and alfalfa.

Forage growth stimulated an enlargement of the soil microbial community in contaminated soil relative to an unvegetated oil plot, possibly by improving the soil habitat for microorganisms. A likely mechanism for this phenomenon is *rhizodeposition* by growing roots. Several kinds of substances are deposited into the root zone or *rhizosphere,* including root exudates (passive release), secretions (active release), plant mucilages (substances from a number of different sources), mucigel (a complex mixture of plant materials) and lysates (from the lysis of old epidermal cells) (Rovira et al. 1979). These substances are generally made up of readily metabolizable organic compounds (Rovira and Davey 1974; Reilley et al. 1996). They serve as additional substrate for soil microorganisms in the rhizosphere. For instance, alfalfa growth has been shown to significantly increase carbohydrate contents in soil after two years and significantly increase soil organic carbon levels after three years, all relative to fallow (Angers 1992). The well documented observation of elevated microbial numbers and activity in the root zone is known as the *rhizosphere effect.* Curl and Truelove (1986) reported that a 2-20 fold increase in microbial populations is typical over non-rhizosphere soils, though 100 fold increases are possible.

The addition of straw to soil containing crude oil failed to produce a recovery of the biomass. This result does not agree with the findings of other short and long-term studies. Differences in the number and size of straw applications and the length of time between amendment and sampling for analysis could account for the conflicting results.

In a laboratory study, Ocio and Brookes (1990) observed increases that were nearly 100 and 50% in a sandy loam and clay soil, respectively, following the recent amendment of 2% w/w wheat straw. This straw content represents approximately 40 t ha^{-1}, roughly an order of magnitude larger than the amendment made in this study. Sampling was done to a depth of 15 cm and MB measurements were made only 13 and 35 d after amendment. Subsequent research in the field demonstrated similar short-term effects from a single 10 t ha^{-1} wheat straw application to soil (Ocio et al. 1991). MBC doubled immediately following straw addition before slowly dropping over the rest of the trial period. However, straw-amended soils still contained *ca* 20% more MBC almost one year after the straw was incorporated than did unamended soils. Powlson et al. (1987) found significant increases (45 and 37%) in microbial biomass after 18 yr of straw application to two sandy soils. 4 t dry matter ha^{-1} of straw was chopped and incorporated along with 1 t ha^{-1} of stubble into the upper 20 cm of soil. Samples were taken to a 25 cm depth in the spring following the final autumn straw application. Schnurer et al. (1985) obtained similar results for 27 yr plots which had received yearly straw inputs.

In this study, the time between straw amendment and soil sampling, *ca* 14 months, was too long for the persistence of any flush of growth which may have occurred immediately after the carbon addition (Allison and Killham 1988). Also, the single addition of straw could not enable any cumulative effect that might have come with subsequent amendments like the one observed by Powlson et al. (1987). Ritz et al. (1992) reported no prolonged beneficial effect of a single straw amendment on MB in the field and suggested multiple yearly applications as a means to this end. Scow et al. (1994) agreed with this suggestion when they observed that 3 yr of cover crop

incorporations were required to elevate MBC in soils under organic versus conventional soil management practices. Ritz et al. (1992) described cereal straw as "chemically recalcitrant." The authors stated that a single pulse of this type of C source did not provide the kind of readily metabolizable substrate that could stimulate soil microbial growth like other amendments such as sucrose. In this study, the crude oil in the SF soil continued to suppress the MB through toxic effects or damage to microbial habitat. However, since the values for MB in the two forage plots were significantly greater than the MB in the UF_{SP}, the presence of crude oil did not prevent population increases from occurring under all carbon-input treatments.

4.4.3 Dehydrogenase Activity (DHA)

Stevenson (1959) proposed that DHA could be used an index of microbial activity. He based this idea on the correlation he found for DHA with oxygen consumption. Recent support for the use of DHA to this end (Turco et al. 1994; Dick 1994) has been qualified because of inconsistent correlations between DHA and other measures of microbial activity (Ross 1973; Sparling 1981; Frankenberger and Dick 1983; Falih and Wainwright 1996). Nevertheless, the fact that the dehydrogenase enzyme is associated only with living cells makes an assay of DHA an attractive method for assessing general soil microbial activity (v. Boguslawski et al. 1976; Dick et al. 1994; Cooper and Warman 1997). In addition, DHA correlations with other activity variables have demonstrated its utility in measuring microbial activity in other instances (Ladd and Paul 1973; Nannipieri et al. 1990; Serra-Wittling et al. 1995).

In this study, DHA means were significantly different from each other at an alpha level of 0.01 (Table 4.4). Growth of the forage crops enhanced DHA relative to the UF_{SP} and SF (Figure 4.3). The effect of forage growth was significant at the 1 and 5% levels for grass and alfalfa, respectively (Table 4.5). Rhizodeposition is likely responsible for stimulating this enzymatic pathway in the forage plots, resulting in the higher activity levels. Other studies have shown that vegetation affects the activities of enzymes. The amendment of soil with plant roots significantly increased soil dehydrogenase enzyme activity relative to that of unamended soil (Christensen et al. 1992).

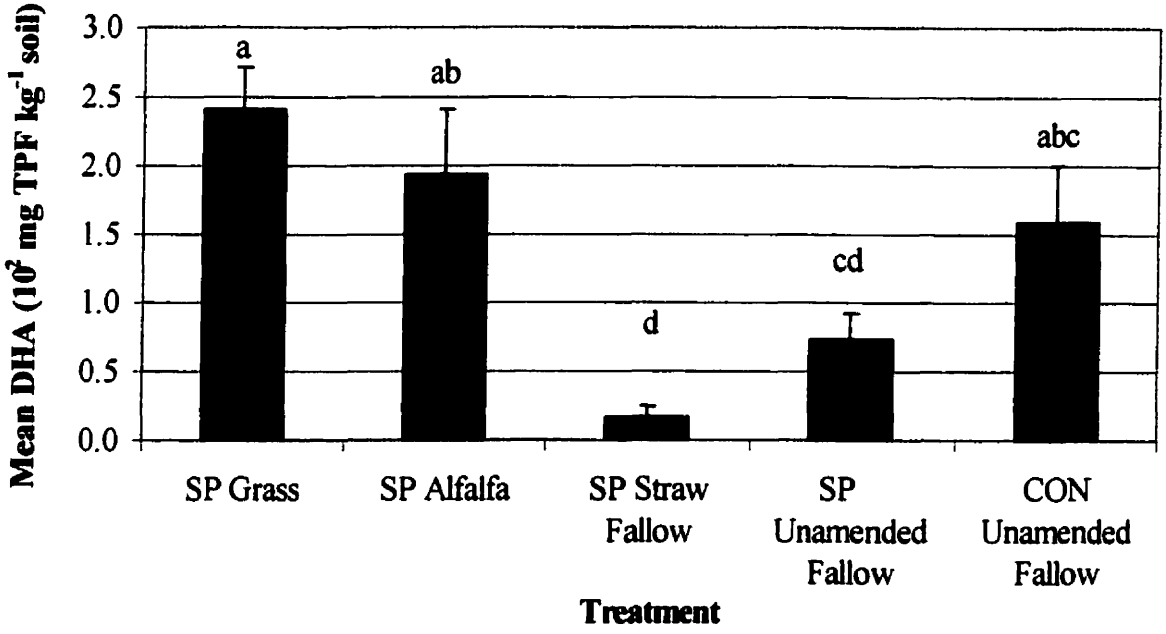

Figure 4.3. Dehydrogenase activity in 0-15 cm depth of spill (SP) and control (CON) plots. Each bar represents the mean of a variable number of replicates due to variable extraction success (p=0.05).

Table 4.4. Analysis of Variance results for DHA data.

Source	Sum-of-Squares	df	Mean-Square	F-ratio	P
Treatment	224906.39	4	56226.60	4.73	0.0035
Error	439625.99	37	11881.78		

Table 4.5. Matrix of pairwise comparison probabilities for DHA based on Fisher's Least Significant Difference Test.

	Grass	Alfalfa	SF	UF_{SP}	UF_{CON}
Grass	1				
Alfalfa	0.33	1			
SF	0.00	0.01	1		
UF_{SP}	0.00	0.03	0.40	1	
UF_{CON}	0.09	0.49	0.03	0.11	1

soil (Christensen et al. 1992). Ryegrass significantly enhanced the activities of a range of soil enzymes relative to a control without ryegrass (Ross and Cairns 1982). Root exudation and the addition of dead root material were suggested as the principal means of enhancing microbial activity.

Stevenson (1959) made preliminary observations of responses in soil DHA to the addition of crop residues to soil. He found that the relationship between the activity of this enzyme and oxygen uptake held when he used a number of different crop residues. In a follow-up investigation, Stevenson (1962) reported that the amendment of chopped plant biomass from 8 week old wheat to the same soil in which it had been grown elevated DHA above that of an unamended soil. The effect peaked after 10 d and then activity diminished towards the control value at 66 d. Some research has found that DHA was stimulated by straw incorporation to soil and increased with a higher rate of addition (v. Boguslawski et al. 1976). By contrast, other inquiries have observed no consistent trends in DHA response to straw amendment to soil (Ritz et al. 1992).

DHA has also been augmented by the addition of other types of high-carbon materials to soil. Amending pesticide-contaminated soil with mature compost derived from yard trimmings significantly increased DHA in soil relative to mixing polluted soil

with unpolluted soil (Cole et al. 1994). Serra-Wittling et al. (1995) measured elevated DHA in soils amended with compost having a C:N ratio near 10. The effect was significantly additive with greater proportions of compost in the compost-soil mixtures. Cooper and Warman (1997) reported elevated DHA in a silty clay soil receiving as much as 8 t C ha^{-1} organic amendment in the form of composted chicken manure compared to unamended soil. Higher DHAs were also observed at lower rates of organic amendment compared to the control. The increase in DHA may have been related to the increase in organic C content in the amended soil following compost addition. DHA has been related to the readily available fraction of soil organic C (Fraser et al. 1988). In the Cooper and Warman (1997) study, DHA was not affected by the organic amendment in a sandy loam soil, possibly because organic C levels were already high and likely at equilibrium. Sugar beets serving as a rich source of readily metabolizable carbon for soil microorganisms increased DHA relative to an unamended control (Falih and Wainwright 1996).

In this study, the dehydrogenase pathway was not stimulated by added straw at the time of sampling (*ca* 14 months after straw incorporation). DHA in the SF was also significantly lower than the UF_{CON}. This demonstrated that a straw amendment did not improve the resilience of the dehydrogenase system of the contaminated soil. As dehydrogenase is closely associated with active MB, similar reasons as for MBC could explain this result.

The statistical difference between the means for UF_{SP} and UF_{CON} had an associated probability of 0.11. Despite this result, there was a generally negative trend in

DHA in the UF_{SP} compared to the UF_{CON}. The adverse effect of crude oil presence on the soil microbial community was less pronounced for dehydrogenase than for MBC.

The effect of soil contamination on DHA has been previously investigated (Rossel and Tarradellas 1991). Over the course of a 203 d experiment, DHA was reduced 45-60% by the application to soil of 100 $\mu g\ g^{-1}$ tributylin (TBT), a persistent biocide. As a result, DHA was considered to be a potential tool in assessing the impact of xenobiotics on the physiologically active soil microbial biomass. However, the authors advised that DHA should be determined as a substrate-induced maximum initial activity because it was not related to long-term microbial respiration. Thus, DHA did not represent the effective total activity of the MB, only its potential total activity. The usefulness of DHA in this regard with respect to crude oil contamination of soil was not clearly demonstrated in this study. However, DHA may have served as a measure of substrate availability in soil with respect to hydrocarbons as well as any organic amendments.

The high levels of nitrate in the spill area soil (>270 kg $NO_3\ ha^{-1}$ in 1997), a result of the landfarming remediation practices undertaken, could undermine the accuracy of the dehydrogenase assay results. Bremner and Tabatabai (1972) found that nitrate or nitrite at a concentration of 10 μmoles g^{-1} of soil caused DHA to be underestimated by a range of 19 to 34% in three different soils. These forms of inorganic nitrogen possibly either directly inhibited the enzyme or diverted electrons away from TTC (2,3,5-triphenyltetrazolium chloride), the compound which is reduced in the assay. Based on 1996 N levels in the spill plots, which were approximately one fifth that of the most

similar soil in the Bremner and Tabatabai (1972) study, DHAs could have been underestimated by 7% or more.

4.4.4 Activity Index

The mean activities illustrated in Figure 4.3 are absolute values for the various treatments. To express the values on a per unit biomass basis, an activity index is calculated (Figure 4.4). This calculation effectively standardizes the measured activity for the size of the community by determining the activity/biomass ratio. AI means were significantly different with a probability of 0.07 (Table 4.6). The mean AI for the UF_{SP} was significantly different from that for all other treatments at the 5% level, except in the case of grass in which the difference was significant at the 7% level (Table 4.7). All other treatments were not significantly different from each other even at the 10% level. The UF_{SP} had a smaller but more active biomass on a per unit basis relative to the UF_{CON}. This difference could be due to the presence of hydrocarbons in the spill fallow which serve as additional substrate for soil microorganisms. Consequently, a greater proportion of the biomass was active along the dehydrogenase enzymatic pathway. The UF_{CON} had a much larger but less active biomass on a per unit basis, possibly due to the stability of the soil system on the uncontaminated land. The microbial communities in the forage, SF and UF_{CON} plots all had similar DHA per unit biomass. The forage plots and the UF_{CON} each supported a relatively large microbial biomass which was also relatively highly active. The SF contained a relatively small biomass of relatively low activity.

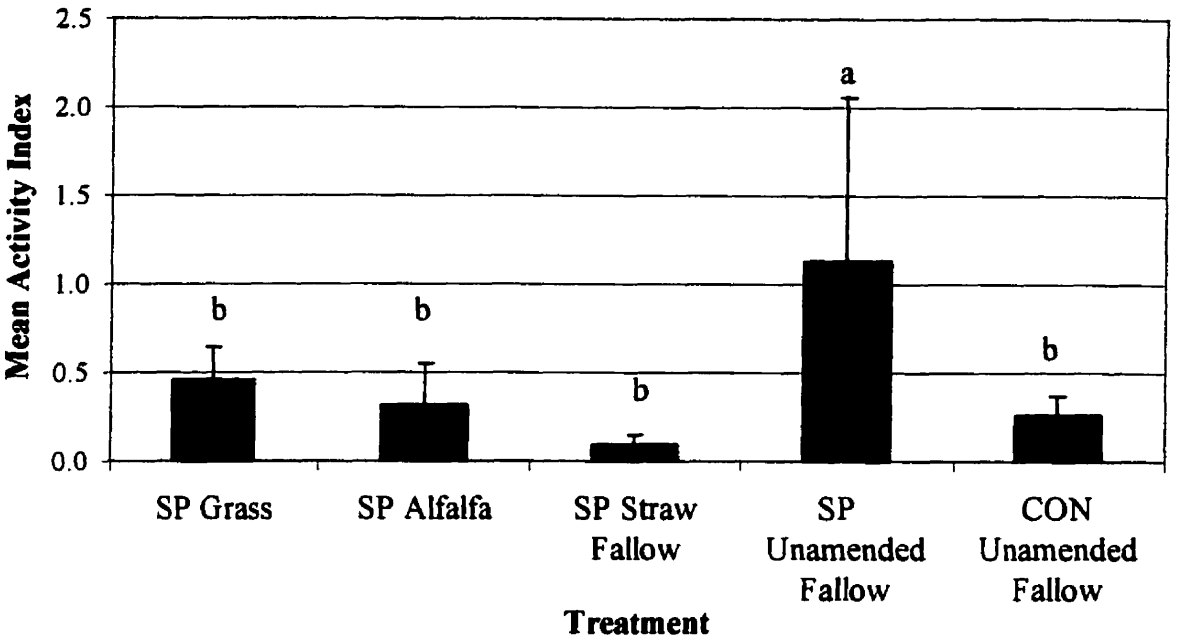

Figure 4.4. Dehydrogenase activity index (activity/biomass) for 0-15 cm of spill (SP) and control (CON) plots. Alfalfa and straw fallow bars represent the means of three replicates while all other bars represent means of four replicates (p=0.1).

Table 4.6. Analysis of Variance results for AI data.

Source	Sum-of-Squares	df	Mean-Square	F-ratio	P
Treatment	2.481	4	0.620	2.855	0.067
Error	2.824	13	0.217		

Table 4.7. Matrix of pairwise comparison probabilities for AI based on Fisher's Least Significant Difference Test.

	Grass	Alfalfa	SF	UF_{SP}	UF_{CON}
Grass	1				
Alfalfa	0.69	1			
SF	0.29	0.53	1		
UF_{SP}	0.06	0.04	0.01	1	
UF_{CON}	0.55	0.88	0.60	0.02	1

Stevenson (1959) found that DHA did not follow changes in bacterial numbers unless the experimental soils were amended with fresh crop residues. Casida et al. (1964) observed relatively constant DHA while microbial populations fluctuated slightly in a soil to which no additional moisture or nutrients were added. By contrast, activity of the enzyme did relate to total bacterial metabolism in a soil receiving weekly additions of water and nutrients. Increases in DHA matched a rapid rise in numbers of gram positive bacteria. When the population rise reached a plateau and the microbes reduced their metabolic activity, a sharp decline in DHA occurred. It may be that DHA serves better as a measure of general microbial activity in terms of respiration and not the size of the microbial community (Stevenson 1959). In this way, DHA may still be a useful measure of substrate availability and/or general microbial activity, while the size of the microbial community may be of less relevance to assessing the overall functioning of the soil MB.

4.4.5 Microbial Metabolic Diversity (MMD)

Garland and Mills (1991) developed the application of the Biolog redox procedure to the determination of functional diversity of microbial communities. It is based on the measurement of sole-carbon-source utilization by heterotrophic organisms which can be sampled from a variety of environments. Differences in breakdown of the 95 substrates in the microplate wells reveal differences in the metabolic capabilities of microbial communities.

Significant differences ($p=0.06$) were found between means of substrate utilization in the various treatments based on the Biolog assay (Table 4.8). Extracted

microbial communities from the UF_{CON} and SF exhibited significantly higher substrate utilization than those from the grass and UF_{SP} treatments based on a LSD test at the 10% level (Table 4.9 and Figure 4.5). The mean utilization under alfalfa was not significantly different from any of the other treatments. Variation was minimal for the UF_{CON} results, suggesting functional stability at this site, while variation was great for UF_{SP}, possibly attributable to disturbance caused by the spill. It appears that root exudation in the forage plots did not expand the metabolic range of soil microorganisms relative to that of the UF_{SP}.

The effect of the oil spill on MMD observed in this study is consistent with other research. Wunsche et al. (1995) observed altered patterns of substrate utilization in a Biolog assay of both recently oil-treated and long-term oil contaminated soils compared to soil without oil. The change in physiological profile was attributed to a shift in relative abundance towards autochthonous microbial populations living on hydrocarbons. This was confirmed by measuring the proportion of hydrocarbon-utilizing bacteria in the

Table 4.8. Analysis of Variance results for MMD data.

Source	Sum-of-Squares	df	Mean-Square	F-ratio	P
Treatment	0.04	4	0.01	2.91	0.06
Error	0.05	15	0.00		

Table 4.9. Matrix of pairwise comparison probabilities for MMD data based on Fisher's Least Significant Difference Test.

	Grass	Alfalfa	SF	UF_{SP}	UF_{CON}
Grass	1				
Alfalfa	0.40	1			
SF	0.07	0.31	1		
UF_{SP}	0.58	0.17	0.02	1	
UF_{CON}	0.04	0.19	0.76	0.01	1

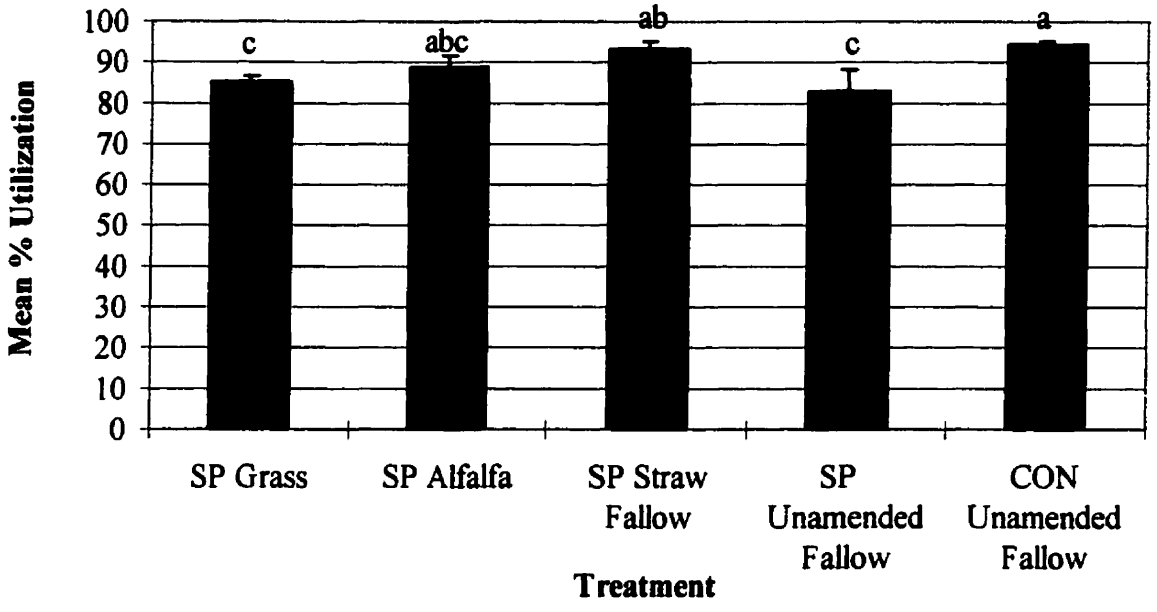

Figure 4.5. Substrate utilization in multiple substrate GN microplate wells (BIOLOG™, Inc.) by soil microorganisms from 0-15 cm depth of spill (SP) and control (CON) plots. Each bar represents the mean of four replicates (p=0.1).

adapted soil microbial communities of the contaminated soils. These findings, and those of this study, indicate that physiological profiling based on the multiple-substrate microplate system may be an effective approach to assessing the impact of oil pollution on the MMD of soil.

The results for MMD in this study parallel those for the DHA index data. Higher specific activity, lower metabolic diversity and high variability, as found in the UF_{SP}, are characteristic of disturbed systems. The return to lower specific activity and greater metabolic diversity, both being less variable, is indicative of a return to a "climax" state. This change was observed in the SF and the UF_{CON} was already in this stable condition. Such stability could arguably be considered of higher quality than an unstable situation.

Straw incorporation has been shown to elevate levels of substrate utilization in different soil types and for different frequencies of application (Bossio and Scow 1995).

The enhancement occurred in both an approximately neutral clay which received a single application of mature, dry rice straw and in an acid loam which received successive straw applications over 6 yr. The comparison treatment in both cases was land on which straw was burned. This effect was attributed to altered microbial community composition following the organic amendment in a carbon limited soil environment. This research agrees with the finding of this study that straw amendment to a fallowed soil increased MMD relative to an unamended fallow. Although the amount of carbon introduced to the soil in this study in the form of petroleum hydrocarbons is large, and the range of compounds in crude oil is also large, other more metabolically important carbon sources were still limiting in the unamended fallow. The lower MMD in the UF_{SP} could also reflect the impact of toxicity caused by certain constituents of the crude oil. The incorporation of straw may have introduced a wider range of substrates, modifying the make-up of the soil microbial community and leading to greater functional diversity.

Wunsche et al. (1995) be noted a number of limitations inherent in the Biolog method. The results are limited to experimental conditions, including the substrates found in the microplates and the efficacy of the extraction of microorganisms from soil for inoculation of the microplates. In addition, this technique is selects for only certain metabolic types of microorganisms (aerobic or facultatively anaerobic, heterotrophic and copiotrophic). These microbes must also metabolize the test substrates within the inspection times prescribed by the procedure. Although the number and range of substrates in the plates are extensive, these substrates represent only a portion of all the substrates present in soil.

4.5 Summary and Conclusions

Several microbiological indices were used to assess the impact of the presence of crude oil on the biological quality of an agricultural soil, as well as the potential for bioquality enhancement through site management treatments. The spill had a significant effect on soil biological quality based on MBC (1% level) and MMD (5% level), and a similar general trend for DHA. Contrary to previous research (Matthews 1924; Biederbeck 1990; Chaineau 1996), the microbial community was depressed in its size and activity by the presence of crude oil relative to uncontaminated soil. It is possible that any initial stimulation of soil microorganisms that might have occurred shortly after the spill had diminished. By the time of this investigation, microbial activity in the UF_{SP} based on the three indices examined had dropped below control levels, possibly because of long-term toxic effects or changes in soil conditions. This pattern is typical with respect to microbial numbers and respiration (Rowell 1977).

Of the five treatments tested, meadow bromegrass had the greatest effect on soil biological quality. Grass growth has been shown to elevate the values of soil microbiological indices including respiration, decomposition, organic carbon and N mineralization relative to cultivated crops (Follet and Schimel 1989; Weil et al. 1993). In this study, the grass treatment was able to restore the levels of MBC and DHA in soil to near or above those found in uncontaminated soil. As a result, the grass treatment exhibited the highest activity index based on dehydrogenase. However, grass did not enhance the MMD of the soil microbial community based on per cent substrate utilization

measured via the Biolog assay. Algonquin alfalfa exhibited a slightly reduced effect compared to grass, an observation likely attributable to differences in rooting pattern and therefore exudation. No statistically significant differences were found, however, between the two forage crops among the examined parameters.

The incorporation of wheat straw did not have a positive effect on either MBC or DHA. The length of time between amendment and analysis was likely too long for any initial positive responses which may have taken place to still be detected and the single application was probably insufficient to adequately stimulate decomposers or their activity. However, MMD was enhanced in the straw fallow. This latter result concurred with findings of a previous study (Bossio and Scow 1995).

The introduction of crude oil to an agricultural soil adversely affected its biological quality based on the microbiological indices studied. The establishment of perennial forage crops, meadow bromegrass in particular, is recommended as a potential means of restoring the soil biological quality of a crude oil contaminated soil.

5. FORAGE CROPS AND FALLOW AFFECT CRUDE OIL CONCENTRATIONS IN AN AGRICULTURAL SOIL

5.1 Abstract

Field trials were conducted on the site of a crude oil spill on agricultural land to compare the efficacies of phytoremedial and fallow approaches in reducing hydrocarbon (HC) concentrations. The cropping treatments consisted of two forages, meadow bromegrass (*Bromus biebersteinii* Rohman and Schult) and alfalfa (*Medicago sativa* L.). Two fallow plots were established, one with straw as a carbon amendment and another with no amendment to simulate landfarming bioremediation.

Levels of total oil and grease decreased in the 0-30 cm depth in all four treatments, but no significant differences existed between treatment means. However, the general trends in the data indicated that greater declines occurred under grass than under the other treatments. The differences in trends were more striking in the 30-60 cm depth in which the only predominantly declining trend occurred in the grass treatment. TEH levels increased in all treatments in both depths, but to a greater degree in the 30-60 cm depth. Means were not significantly different for the TEH results. The most prevalent trend of increasing TEH levels were recorded for grass in both depths. The general trends of declining total oil concentrations indicate degradation of crude oil constituents as an entire group, since the analytical procedure captures the entire

spectrum of compounds. The elevated TEH levels are likely the result of longer chain compounds breaking down into shorter chains which fall within the extraction range of the TEH analysis (C_{10}-C_{30}). These results demonstrate that degradation occurred and that longer carbon chain compounds were the principle target of the breakdown process. Based on general trends, meadow bromegrass enhanced the degradation of crude oil constituents in soil relative to tillage alone more than any other treatment. This trend was most evident in the 30-60 cm depth of soil. The release of organic substrates from grass roots and improved aeration are possible mechanisms for the stimulation of soil microbial activity, resulting in elevated HC degradation.

5.2 Introduction

The establishment of vegetation has become a promising approach to the remediation on land contaminated with xenobiotics (Cunningham et al. 1995). Soil microorganisms contribute heavily to HC degradation in soil (McGill et al. 1981) and soil microbial biomass and activity is enhanced in the rhizosphere relative to bare soil (Walton et al. 1994). Phytoremediation has been proposed as an effective approach to reclaiming HC contaminated land (Reilley et al. 1990). This strategy offers several benefits to a remediation process, including reduced cost, complexity, management and intrusiveness compared to other remediation technologies. The use of plants to promote contaminant dissipation in soil can serve as an alternative or supplemental measure to other accepted practices such as landfarming. The latter technique consists of nutrient addition and tillage on unvegetated land, both intended to optimize conditions for soil

microbial activity and, consequently, biodegradation of hydrocarbons (HCs) in soil (Lee and Banks 1993; Reilley et al. 1996).

A widely accepted explanation for enhanced contaminant degradation in the root zone is the effect of root presence on the soil microbial community. Plant roots provide physical habitat for a microbial community to occupy, a structure upon which colonies can form (Walton et al. 1994). In addition, plant roots release, both passively and actively, organic compounds which are readily metabolized by soil microorganisms in the vicinity of roots (Rovira and Davey 1974). This continuous process can be described by the term *rhizodeposition* (Newman 1985). Several kinds of substances are deposited into the root zone or *rhizosphere,* including root exudates (passive release), secretions (active release), plant mucilages (substances from a number of different sources), mucigel (a complex mixture of plant materials) and lysates (from the lysis of old epidermal cells) (Rovira et al. 1979).. In response to the addition of these compounds to the root zone, the size and activity of microbial populations are augmented, a phenomenon known as the *rhizosphere effect* (Elliot et al. 1984; Curl and Truelove 1986). Typical rhizosphere populations are 2-20 times larger than in non-rhizosphere soils, though 100 fold differences have been reported (Curl and Truelove 1986).

This enhancement of the microbial community, and some selected organisms in particular, may be responsible for the accelerated degradation of HCs in rhizosphere soil. The rhizosphere provides habitat for a diverse microbial community which may work synergistically to break down pollutants (Anderson et al. 1995). In attacking organic root exudates (primary substrate), soil microorganisms may also break down HCs (secondary

substrate) which are susceptible to the primary metabolic pathway, a process called *cometabolism* (Hornick et al. 1983).

The introduction of organic materials to soil in other forms can also elevate soil microbial activity. Straw amendments to soil, for instance, affect aspects of the soil microbial community (v. Boguslawski et al. 1976; Powlson et al. 1987; Ocio and Brookes 1990). The elevated microbial activity in the root zone or depth of carbon incorporation may enhance the degradation of HCs in soil.

Recent research has shown that the presence of vegetation can accelerate the degradation of certain polycyclic aromatic HCs (PAHs) which are constituents of crude oil. Aprill and Sims (1990) monitored the change in concentration of four PAHs under eight prairie grass species with manure amendments. PAH declines were consistently greater in rhizosphere treatments than unvegetated soils after 59 days. The greater decreases in rhizosphere soils became statistically significant after 151 days.

Reilley et al. (1996) studied anthracene and pyrene dissipation under four grass treatments in two soils, one which was already contaminated and another which had not been previously exposed to PAHs. Except for the 'uncontaminated + anthracene' set, in which all treatments had undetectable concentrations after 16 weeks, PAH levels were lower in all vegetated soils than unplanted soils after 24 weeks. PAH mineralization, based on $^{14}CO_2$ evolution from ^{14}C-labeled compounds, was greatest in planted systems with organic acid amendments.

The effect of vegetation on the fate of HCs in soil has not been consistent for all soil-plant-contaminant systems. Watkins et al. (1994) observed less naphthalene

mineralization in soil microcosms with Bell Rhodesgrass than in unplanted soil microcosms.

Forage crops provide a surface cover and extensive root system, together improving soil stability. Prairie grasses, in particular, have been proposed as preferred candidates for phytoremediation (Aprill and Sims 1990). The fibrous root systems exhibited by grasses, especially sod-forming species, maximize the surface area over which the rhizosphere effect can occur. The zone of particularly enhanced microbial activity is along the interface between the root surface and soil matrix known as the *rhizoplane* (Foster and Bowen 1982). The genetic diversity of prairie grasses may offer considerable flexibility in degrading capabilities.

Alfalfa has also demonstrated the potential to enhance the degradation of individual HCs (Reilley et al. 1996) as well as crude oil in soil (Wiltse et al. 1998). Variability for phytoremediation capabilities among alfalfa genotypes has also been detected, indicating the possibility for breeding manipulation in order to maximize HC-degrading potential (Wiltse et al. 1998).

The growth characteristics of forages, microbial metabolic response patterns to added substrate and the evidence from previous research suggest a potential for enhanced land remediation of petroleum pollutants through application of carbon amendments. The objective of this investigation was to monitor the effects of forage and fallow treatments on crude oil concentrations in an agricultural soil. The hypothesis tested was that forage crop growth or straw incorporation into fallow would enhance the degradation of crude oil constituents relative to unamended landfarmed soil.

5.3 Materials and Methods

The study spanned two field seasons at the site of a pipeline rupture and subsequent release of crude oil onto agricultural land in October, 1994. Soil profile and landscape data for the spill and control experimental sites is presented in Table 5.1. The plot location in the spill area was selected for two main reasons. The soil exhibited relatively high HC levels in the spring of 1996 (0.92% mean total oil and grease in the 0-30 cm depth and 0.60% in the 30-60 cm depth) and generally successful forage crop growth. Four treatments were established: meadow bromegrass (*Bromus biebersteinii* Rohman and Schult), algonquin alfalfa (*Medicago sativa* L.), fallow with wheat straw incorporation (SF) and fallow left unamended to simulate landfarming (UF). The spill area had received approximately 3500 kg N ha^{-1} of fertilizer nitrogen, as part of remediation activities, one year before the experimental trials began. Treatments were arranged roughly north-south side by side in the field in four replicates, each plot 1.5 m by 5 m in dimension.

Table 5.1 Properties of typical soil profile for study plots.

Research Site	Horizons	Depth (cm)	Texture	Surface Expression	Comments on Profile
Spill	Apk	0-18	CL	Level	Moderately fine lacustrine parent material throughout Evidence of disturbance Most gypsum found at 70-90 cm
	AC	18-32	CL		
	Ckgjs	32-90	SiC		
	Ckgs	90-120	SiCL		

The forages were seeded in June, 1996 at a rate of 7 kg ha^{-1}. Straw was incorporated in August, 1996 at a rate of 4100 kg ha^{-1}. This rate of straw application is similar to that applied to farm fields at harvest and so should allow for adequate decay in soil. The SF plot was tilled at the same time as the unamended fallow plot in the second year. Tillage passes in the fallows numbered approximately ten in each year which matched the landfarming tillage regime.

Composite samples from three equidistant holes in each replicate were taken in August, 1996 (t=0) and in October, 1997 (t=1). Samples were submitted to Norwest Labs for two analyses: a gravimetric determination of total oil and grease by CH_2Cl_2 extraction (McGill and Rowell 1977) and total extractable HCs by the Alberta Environment method G108.0 (total extractable volatile/nonvolatile HCs using GC/FID) (Norwest Labs Environmental Analytical Service Description 1995).

To confirm the results from the two initial HC analyses, samples were submitted to Norwest Labs for a carbon group assessment. This latter procedure lists the amount of each petroleum component from C_{10} to compounds with greater than sixty carbons in chain length. A subsample from one replicate belonging to the grass and unamended fallow treatments was taken for each depth.

5.4 Results and Discussion

Growth in the forage plots was generally successful but not uniform for both crops. The grass emergence and growth was better than alfalfa throughout the replicates, and one alfalfa replicate in particular showed very poor emergence. Mean mid-season

aboveground biomass in the spill experimental site was 3240 and 2023 kg ha^{-1} for grass and alfalfa, respectively. Mean biomass in the control experimental site was 2477 and 3760 kg ha^{-1} for grass and alfalfa, respectively. These differences in growth indicate that the phytoremedial effect of grass in the spill site was not limited by the productivity of the stand. By contrast, agronomic potential was a limiting factor for the effect of alfalfa growth on HC levels in the spill site.

Initial total oil and grease concentrations in soil ranged from 0.80-0.92% total oil in the 0-30 cm depth and from 0.60-0.74% in the 30-60 cm depth (Figure 5.1). These starting total oil levels were not significantly different (Tables 5.3 and 5.4). Figure 5.2 shows the amounts of total oil and grease in the plots at t=1. Initial TEH levels in the top 30 cm ranged from 3300 to 7100 ppm and in the lower 30 cm from 2800 to 6800 ppm (Figure 5.3). These starting TEH concentrations were not significantly different (Table 5.3). Figure 5.4 shows the TEH levels in soil at t=1.

Table 5.2 One-way ANOVA results for total oil and grease levels in the 0-30 cm depth at t=0.

Source	Sum-of-Squares	df	Mean-Square	F-ratio	P
Treatment	0.034	3	0.011	0.207	0.890
Error	0.651	12	0.054		

Table 5.3 One-way ANOVA results for total oil and grease levels in the 30-60 cm depth at t=0.

Source	Sum-of-Squares	df	Mean-Square	F-ratio	P
Treatment	0.043	3	0.014	0.495	0.693
Error	0.346	12	0.029		

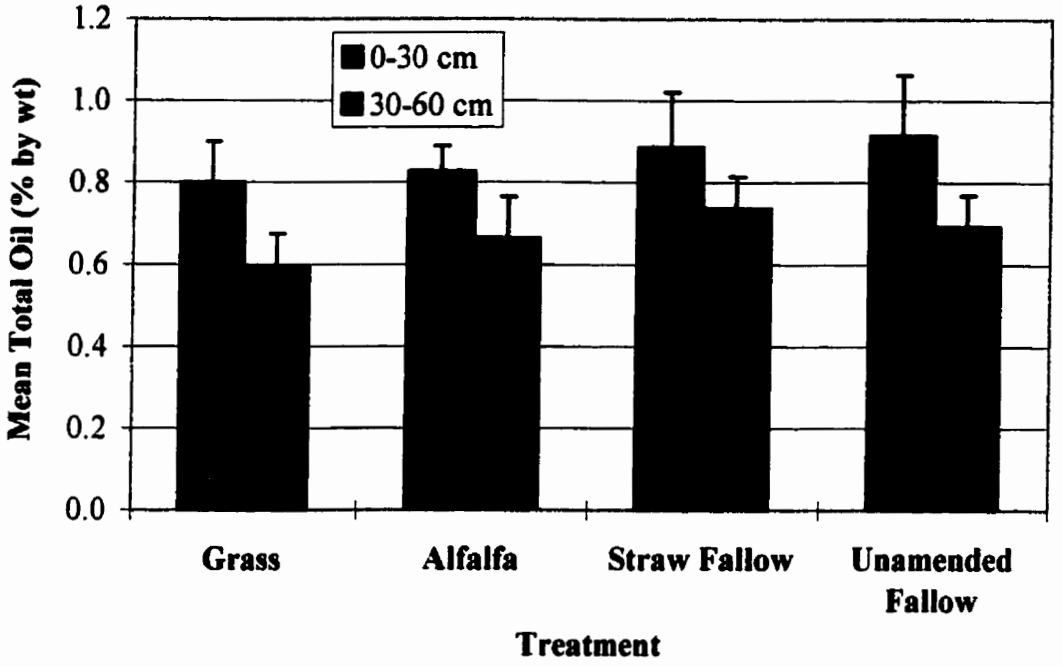

Figure 5.1 Total oil and grease levels in SP1 phytoremediation plots in 1996.

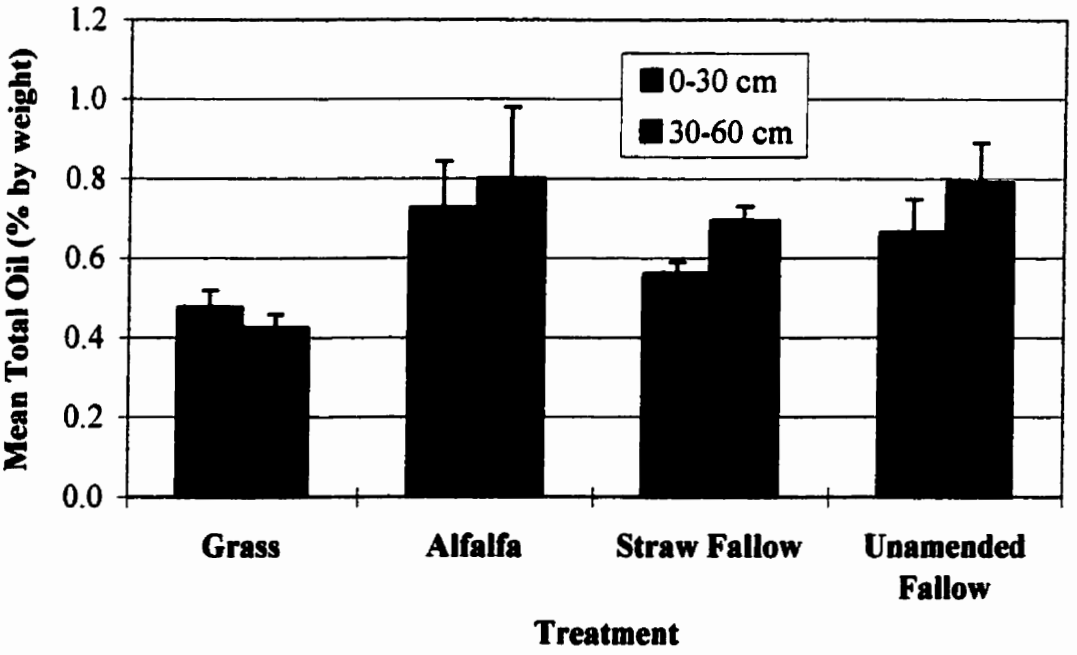

Figure 5.2 Total oil and grease levels in SP1 phytoremediation plots in 1997.

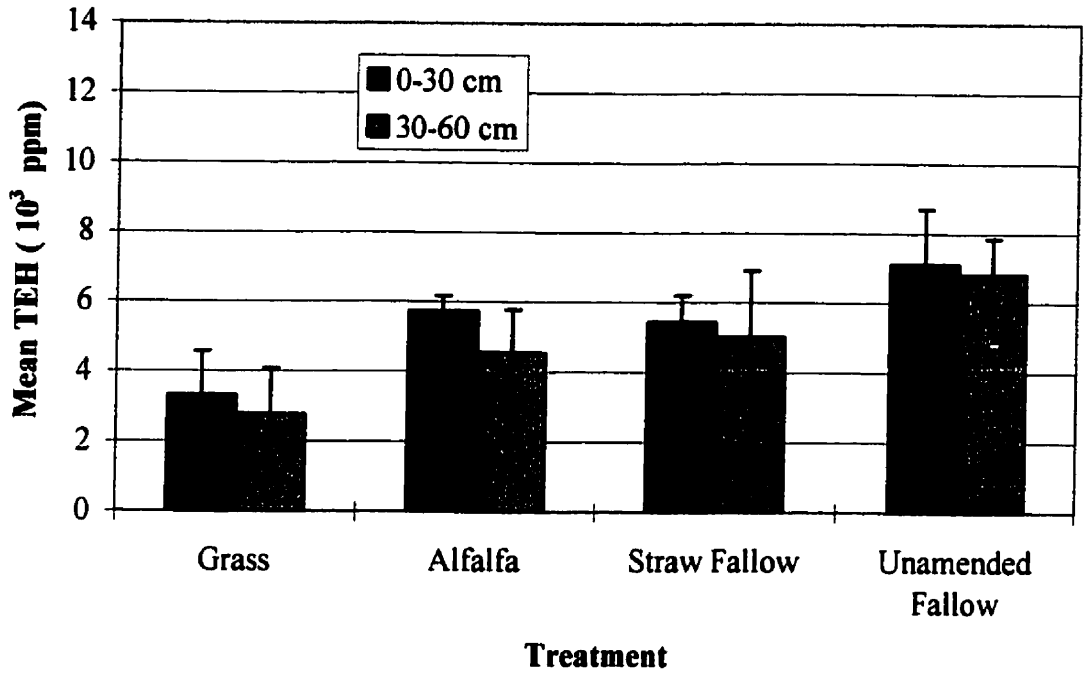

Figure 5.3 Total extractable hydrocarbons (TEH) in SP1 phytoremediation plots in 1996.

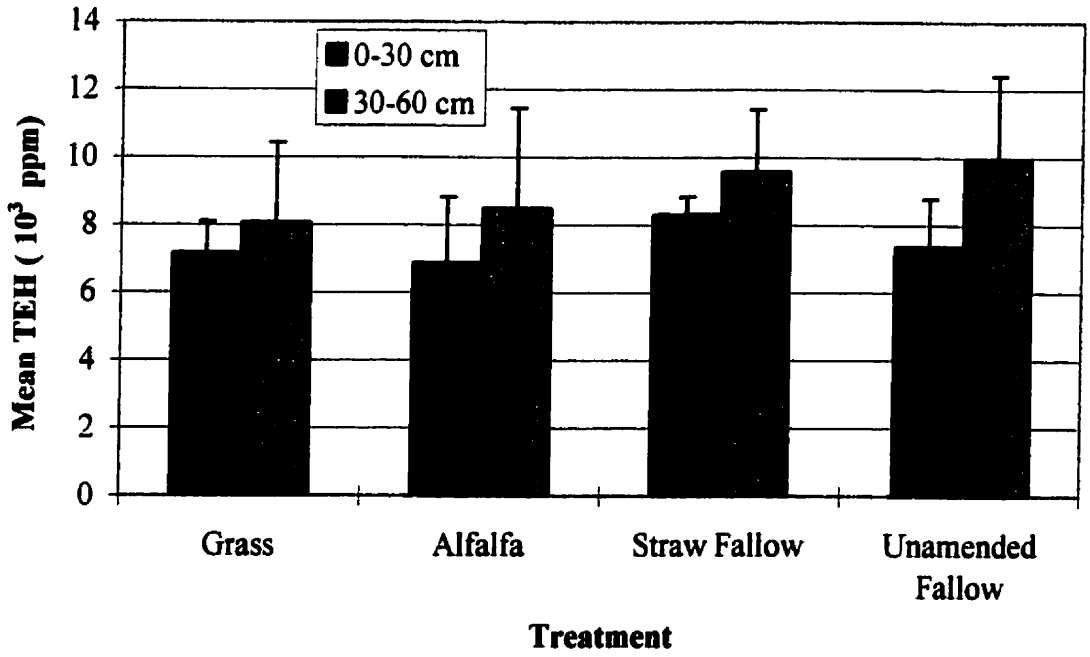

Figure 5.4 Total extractable hydrocarbons (TEH) in SP1 phytoremediation plots in 1997.

Table 5.4 One-way ANOVA results for TEH levels in the 0-30 cm depth at t=0.

Source	Sum-of-Squares	df	Mean-Square	F-ratio	P
Treatment	2.95000E+07	3	9833331.000	2.018	0.165
Error	5.84622E+07	12	4871851.833		

Table 5.5 One-way ANOVA results for TEH levels in the 30-60 cm depth at t=0.

Source	Sum-of-Squares	df	Mean-Square	F-ratio	P
Treatment	3.31388E+07	3	1.10463E+07	1.373	0.298
Error	9.65430E+07	12	8045249.646		

Mean total soil oil contents in the 0-30 cm depth were lower in all four treatments at t=1 than at t=0 (Figure 5.5). There were no statistically significant differences between treatment means for the 0-30 cm soil depth (Table 5.6). However, there was a basic trend of greater declines in total oil levels in the grass plot than in all other plots. For the 30-60 cm depth, the probability value for differences between treatment means was 0.115 (Table 5.7). Considering the high variability of crude oil distribution in soil and the weak statistical power of the experiment, this result is not unacceptably low. Under this premise, the pairwise comparison probabilities for the grass and alfalfa pairing as well as the grass and UF pairing were 0.039 in both cases for the 30-60 cm depth (Table 5.8). This supports the general trend of larger declines in total oil under grass than under other treatments.

Table 5.6 One-way ANOVA results for change in total oil and grease levels in the 0-30 cm depth.

Source	Sum-of-Squares	df	Mean-Square	F-ratio	P
Treatment	0.146	3	0.049	1.386	0.295
Error	0.421	12	0.035		

Table 5.7 One-way ANOVA results for change in total oil and grease levels in the 30-60 cm depth.

Source	Sum-of-Squares	df	Mean-Square	F-ratio	P
Treatment	0.519	3	0.173	2.434	0.115
Error	0.853	12	0.071		

Table 5.8 Matrix of pairwise comparison probabilities for change in total oil and grease concentrations in the 30-60 cm depth based on Fisher's Least Significant Difference Test.

	Grass	Alfalfa	SF	UF
Grass	1			
Alfalfa	0.039	1		
SF	0.231	0.309	1	
UF	0.039	0.995	0.312	1

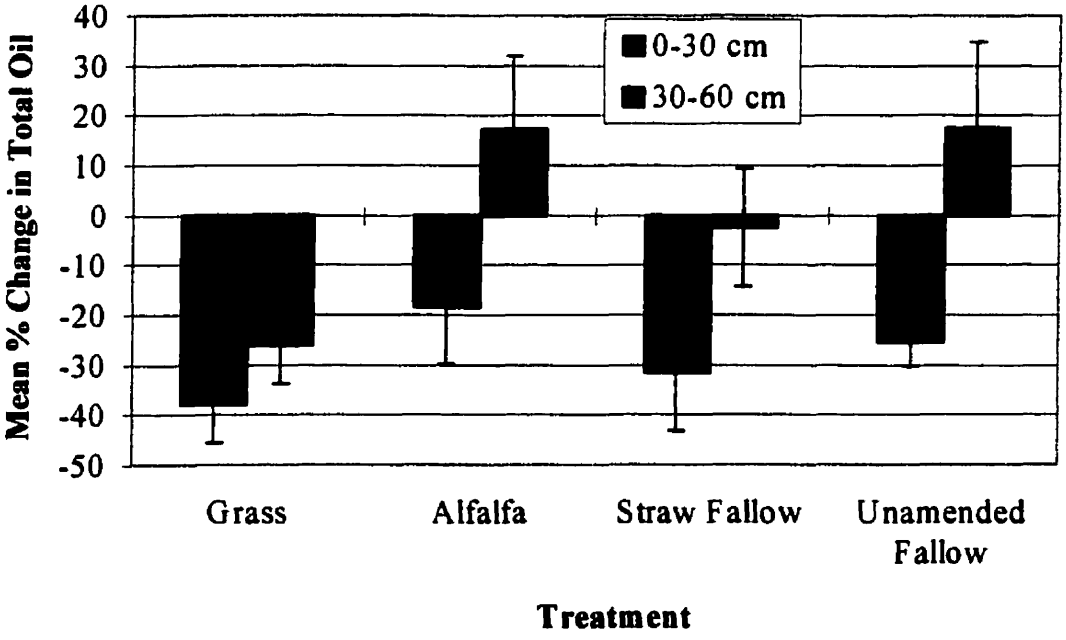

Figure 5.5 Change in total oil and grease levels in phytoremediation plots between 1996 and 1997.

In both soil depths, mean TEH levels were higher in all treatments at t=1 than at t=0 (Figure 5.6). Treatment means were not statistically significantly different from each other (Tables 5.9 and 5.10). However, the general trend for both depths was greater increases in TEH in the grass plot than in any other plot. This trend was more evident in the 30-60 cm depth than in the 0-30 cm depth.

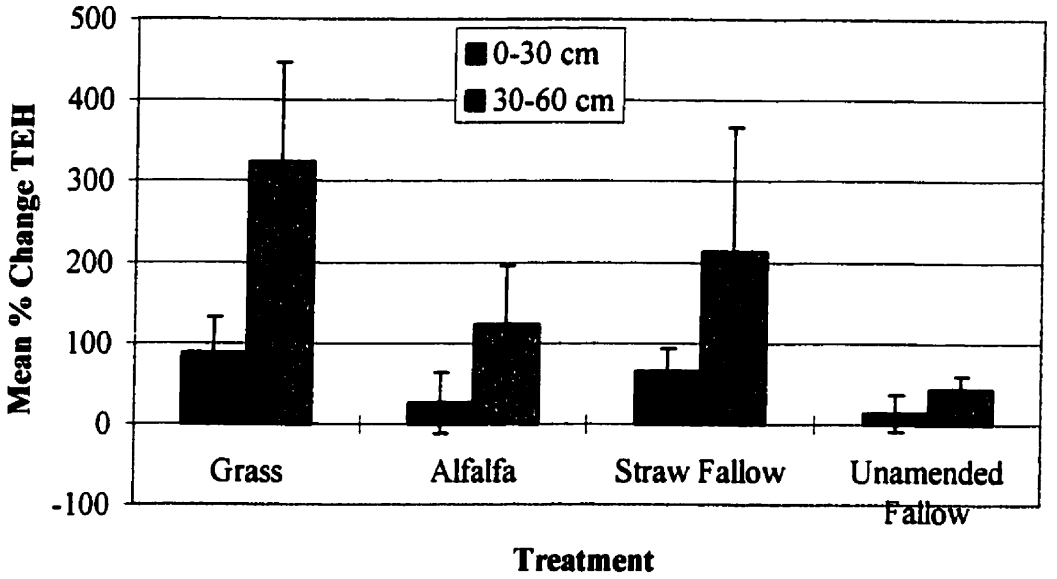

Figure 5.6 Change in total extractable hydrocarbons in phytoremediation plots between 1996 and 1997.

Table 5.9 One-way ANOVA results for change in TEH levels in the 0-30 cm depth.

Source	Sum-of-Squares	df	Mean-Square	F-ratio	P
Treatment	1.232	3	0.411	0.920	0.463
Error	4.909	11	0.446		

Table 5.10 One-way ANOVA results for change in TEH levels in the 30-60 cm depth.

Source	Sum-of-Squares	df	Mean-Square	F-ratio	P
Treatment	17.431	3	5.810	1.308	0.317
Error	53.303	12	4.442		

Results from the non-replicated carbon group assessment were in part contrary to the other HC analyses (Figure 5.7). Changes in the amounts of each of the three carbon groups followed the same trends in total oil and grease change. The increased levels in all carbon groups in the 30-60 cm depth of the unamended fallow followed the same trends as the changes in both total oil and grease and TEH. However, concentration increases in the TEH range (C_{10}-C_{30}) for both grass depths and the upper unamended fallow depth only occurred for compounds with 10 to as high as 16 carbon-length chains. Carbon chains of length above this range actually decreased in concentration. The net result was substantial concentration decreases in the TEH range. Thus, the expected shift in abundance to the TEH range of compounds from groups above C_{30} was not observed.

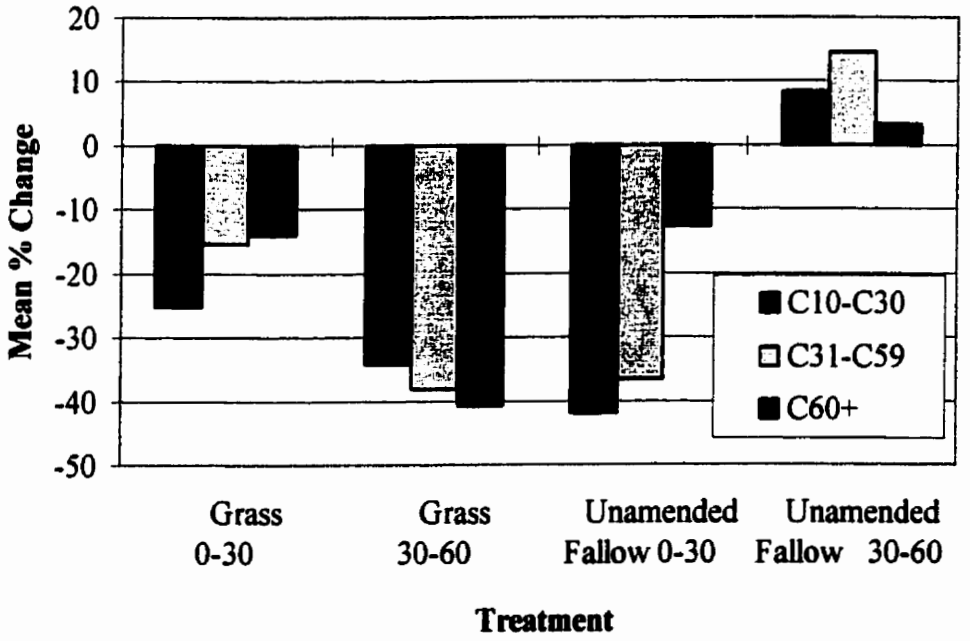

Figure 5.7 Change in concentration of three carbon groups in soil in select phytoremediation plots.

As the starting HC levels in the study were relatively similar in the four treatments, it is assumed that any toxic effects on soil microorganisms are similar. Changes in total oil and grease, at least in the 0-30 cm depth, occurred even at the highest concentration. This evidence suggests that oil degraders were not prevented from acting on the crude oil.

In instances where changes in total oil and grease in the soils were observed, crude oil constituents, as an entire group, were altered by soil microorganisms. The gravimetric determination of HC content in soil captures the entire suite of crude oil compounds present in the samples. Therefore, the results reveal a change in the full spectrum of compounds, from lightweight, short-chain volatiles to heavy, long-chain substances. However, the relative changes may be different for lighter and heavier constituents, especially considering the time that has passed since the spill during which volatiles could have escaped.

Based on general trends, grass appears to have been the most effective treatment in enhancing degradation of total oil and grease in both soil depths, although more clearly in 30-60 cm of soil. All grass treatment values for per cent change were negative and fell within a relatively tight range. A large segment of the soil microbial community is associated with growing plant roots (Curl and Truelove 1986). Rhizodeposition of readily metabolized substrates by plant roots promotes the activity of these microbes resulting in increased breakdown of HCs in the soil (Reilley et al. 1996). The addition to soil of root exudates and other substrates, which serve as primary sources of carbon, may promote the indirect breakdown of HCs, a secondary carbon source. This latter process is known as *cometabolism* (Hornick et al. 1983).

The overall trends in the data demonstrated that basic cultivation had a reduced effect on HC degradation compared to meadow bromegrass growth, especially in the 30-60 cm depth. This is consistent with studies on the abilities of other grass species to enhance the breakdown of individual HCs in soil. Fescue, sudangrass and switchgrass all promoted the disappearance of anthracene and pyrene from both previously contaminated and uncontaminated soils over a 24 week period (Reilley et al. 1996). Aprill and Sims (1990) reported significantly greater declines in the concentrations of four PAHs in soil planted with a variety of prairie grass species compared to unvegetated soil. Another persistent compound, pentachlorophenol, has been shown to dissipate to a greater extent in soil planted with hycrest crested wheatgrass than in unplanted soil (Ferro et al. 1994). Seeding contaminated field plots to Prairie Buffalograss (*Buchloe dactyloides* cv. Prairie) led to greater reductions in concentration of low molecular weight PAHs in surface soil than in unseeded plots (Qui et al. 1997). No effect was observed in deeper soil due to inhibited root growth caused by excessive moisture levels. The absence of an effect of grass growth on high molecular weight PAHs revealed a potential limitation of the phytoremedial approach. However, in a another phase of the same study by Qui et al. (1997), success in enhancing the disappearance of both light and heavy-weight HCs was achieved in soil planted with Kleingrass (*Panicum coloratum* cv. Verde) compared to unvegetated soil. The fact that other grass species could only promote the dissipation of light-weight compounds indicated that the ability of grass to enhance contaminant breakdown varies among species.

The growth of grass roots created better soil conditions for microbial degrading activity than those found in the UF. Establishing forage crops such as grass or alfalfa has

been shown to increase soil microbial biomass relative to uncropped soil by the third year of growth (Drury et al. 1991). Peaks in microbial biomass carbon (MBC) were associated with times during the growing season when the forages were actively growing. MBC has been shown to be relatively stable throughout a growing season under perennial crops (Chantigny et al. 1996). This stability may be attributed to the root systems of perennials which grow continuously and release rhizodeposits from spring to autumn following the establishment year. Chantigny et al. (1996) also found a significant correlation between MBC and plant-derived water-soluble organic C. This result indicated that the size of the microbial community associated with a given rhizosphere was a function of the rhizodeposition patterns of the particular type of vegetation. The amendment of soil with plant roots significantly increased soil dehydrogenase enzyme activity relative to that of unamended soil (Christensen et al. 1992). Dehydrogenase activity estimates the oxidative activity of soil microorganisms (Friedel et al. 1994), and oxidation steps are prevalent in HC transformation (Rowell 1977). Dehydrogenase is an intracellular enzyme and therefore associated with only living cells (Skujins and Burns 1976). Thus, it reflects total microbial metabolic activity in soil. In this study, grass growth exhibited a larger soil microbial biomass and a higher dehydrogenase activity in the 0-15 cm depth than that of the UF as measured in August, 1997 (Chapter 4). This probably contributed to differences in the dissipation of crude oil between the grass and UF plots.

Rhizodeposition provided additional substrates to the soil microbial community in the grass plot which were absent in the UF (Curl and Truelove 1986; Reilley et al. 1996). This process extended to the lower soil depth because of the expanding grass rhizosphere. Deep root growth enables microbe-HC contact at greater soil depths (Qiu et al. 1994).

Soil microbial growth and activity were then enhanced in both soil depths under grass while tillage improved soil conditions in the 0-30 cm depth only. This was the approximate depth reached by the tillage equipment. The increased aeration and microbe-HC contact caused by cultivation as part of a landfarming approach to remediation is limited to the depth of tillage (Bollag et al. 1994).

Total oil levels from 30-60 cm actually increased in the UF. These increases were probably due to the vertical migration of more soluble HCs, which may have been partially altered, into a zone of low degradative activity. Similar reasoning was adopted by Oudot et al. (1989) to explain the detection of generally intact lightweight HCs below the 30 cm depth of soil onto which fuel oil was spilled. The limited degradation of HCs in the deeper soil was primarily attributed to reduced aeration. Certain alkanes and fatty acids can become mobile by reacting with humic and fulvic acids, resulting in increased water solubility (Ogner and Schnitzer 1970; Khan and Schnitzer 1972). More hydrophobic compounds can be adsorbed to or joined within the structures of humic materials which can serve as transport media in the soil profile. Both biotic and abiotic processes can produce stable emulsions which can also enable leaching of otherwise insoluble materials. Some remediation measures, extensive tillage for example, can enhance the formation of such emulsions (Rowell 1977). Such contaminant movement combined with poor degradation may also account for the increases in total oil and grease in the lower depth of the UF.

The basic trends in HC dissipation in soil indicate that grass had a greater influence than alfalfa on oil levels in soil. The apparent difference in influence between

the two forages was most noticeable in the 30-60 cm depth in which total oil and grease actually increased under alfalfa.

Alfalfa growth has been shown to significantly enhance HC dissipation in soil containing 2% (w/w) crude oil relative to unvegetated soil (Wiltse et al. 1998). However, this result was only obtained with two of twenty genotypes tested. Agronomic variables such as aboveground biomass yield, plant height, root mass and time to maturity all indicated that alfalfa growth was significantly depressed in contaminated soil. Presumably due to oil degradation, alfalfa growth rebounded as the experiment progressed. This occurred for all aspects of growth except for root mass. If alfalfa root growth in this study was impeded by the presence of crude oil in soil, the rhizosphere effect may have been reduced. This may have led to less HC degradation in the alfalfa plot. However, Wiltse et al. (1998) found weak correlations between HC dissipation and either root mass or total forage yield. This indicates that other aspects of the plant and particularly root growth may have a greater bearing on phytoremediation, such as root structure. The authors suggested that more soil would be influenced by the larger root surface area of a fibrous system than by the smaller surface area of a massive tap root system. The soil was a very fine sandy loam, a coarser textured soil than that employed in this study. No indication was made as to the depth of soil in the pots used in the experiment. It is possible that plant genetic and soil factors in this study were not suitable for augmenting crude oil degradation in soil under alfalfa. In addition, that the experiment by Wiltse et al. (1998) was a pot study and not a field investigation may account in part for the differing results.

Reilley et al. (1996) also observed an enhancement of HC dissipation under alfalfa. After 24 weeks, anthracene concentrations in alfalfa pots were significantly less than those in unplanted pots; the soil in both sets of pots was previously contaminated with petroleum HCs and spiked with a mixture of anthracene and pyrene. In the same period of time, pyrene degradation was significantly greater under alfalfa than in the absence of vegetation in uncontaminated soil spiked with the PAH mixture. The difference between pyrene levels in alfalfa planted and unplanted pots with contaminated soil + PAHs was not statistically significant but still considerable. The enhanced degradation of individual HCs found in crude oil should indicate the potential for enhanced degradation of crude oil in general under alfalfa. Soil and genetic factors may affect results.

Research has found that root exudation and other types of rhizodeposition vary among plant species and types (Curl and Truelove 1986; Bachmann and Kinzel 1992). Plant rooting patterns and root morphology may contribute to this variation as well as determine the volume of soil influenced by root growth (Walton et al. 1994; Shann and Boyle 1994). The higher surface:volume ratio of the fibrous grass roots results in a larger surface area than that of the alfalfa tap root system. This increases the area over which the size and activity of the soil microbial community can be enhanced by rhizodeposition. Crop type has been shown to affect soil microbial biomass. Drury et al. (1991) measured a larger microbial biomass under reed canarygrass than several other forage and annual crops throughout the summer, but greater than alfalfa only in July. Larger microbial biomass C amounts in soil have been observed under bromegrass than alfalfa (Perfect et al. 1990). Grass crops have been shown to generate more root growth than legumes

several months following seeding (Stone and Buttery 1989). Differences in the quantity and quality of rhizodeposits from roots exist among plants which influences the nature and size of each rhizosphere microbial population (Lynch and Bragg 1985). Grass and alfalfa microbial communities were statistically of the same size in this study, but biomass was measured in 0-15 cm of soil (Chapter 4). This similarity may not have been the case below the examined depth. Among the stimulated microorganisms in the grass plot were HC degraders which were able to more greatly reduce the amount of total oil and grease in the soil than those under alfalfa. The effect of root surface area was probably most pronounced below 30 cm where the overall trend in HC degradation was much greater under grass than under alfalfa. The tap-root system of alfalfa may have also reached the deeper soil but may not have provided the extensive surface area needed to create a significant rhizosphere effect. This potential drawback was cited by Wiltse et al. (1998) as a possible limitation for the use of alfalfa in phytoremedial applications compared to a type of plant equipped with a fibrous root system. The reduced effect under alfalfa is apparent in the lower depth where a mean increase in total oil and grease was recorded. Again, movement of somewhat more soluble HCs from higher in the profile into the 30-60 cm depth could explain this result (Ogner and Schnitzer 1970; Khan and Schnitzer 1972).

The growth of grass roots may have also modified other aspects of the soil environment to a greater degree than did the alfalfa roots. Prairie grasses increase soil aeration through the proliferation of their fibrous roots at considerable depths (Qiu et al. 1994). The grass plot may have developed better soil structure through the growth and subsequent death of a large number of fine roots which created channels for the passage

of air and water. In a grass rhizosphere, from one quarter to one half of roots in the upper portion of the soil profile are dead or dying (Newman 1985). An improvement in structure could enhance the biodegradation of oil in soil. A larger volume of soil would have been thus affected under grass than under alfalfa because of differences in root morphology.

Incorporation of straw also enhanced crude oil breakdown relative to unamended fallow, but to a lesser extent than grass. Variation in contaminant disappearance was greater in the SF. In the affected depth, the straw served as additional substrate for microorganisms which may have increased cometabolism of crude oil compounds. The enhancement in the SF plot was restricted to the top depth as incorporation did not extend beyond 30 cm. Rowell (1977b) advised the removal of straw or similar absorbents that have been incorporated into oil contaminated soil because of the potential for further reduction of available nutrients. This appeared not to have occurred in the SF plot. The depth limitation also occurred in the UF for the same reason. No tillage effect on HC levels would be expected below the 30 cm depth.

The elevated TEH levels across all treatments indicated the breakdown of long carbon chain compounds ($>C_{30}$) into shorter chains which fall within the detection range of the TEH procedure (C_{10}-C_{30}). This type of shift in HC abundance out of an undetectable range towards a detectable one was also observed by Harmsen (1994). He recorded an initial decline in C_{10}-C_{40} *n*-alkanes after a summer of biodegradation. The following winter and spring, the levels of this fraction increased before again declining the subsequent summer. He made the assumption that heavier weight compounds were partly degraded over winter and spring, generating lighter weight compounds which fell

within the detection range of the analytical procedure used. He attributed this phenomenon to the heterogeneity of crude oil and the difficulty in analytically defining it. In this study, the increases in TEH complimented the declines in total oil and grease. However, the TEH increases are larger in the 30-60 cm depth which is contrary to the larger total oil decreases in the 0-30 cm depth. This is possibly attributable to the vertical migration of more soluble degradation products to the lower depth, adding to those already present in the 30-60 cm depth. The trends of greater change in TEH concentrations in the grass and SF treatments substantiates the reasoning behind elevated TEH levels. These two treatments had the most degradation of total oil which led to the greatest increases in concentration of C_{10}-C_{30} compounds. It also confirms that, based on general trends, grass was most effective in enhancing biodegradation of crude oil, particularly in the lower depth reached by roots. For the 30-60 cm depth, the prevalent trends consisted of larger declines in total oil and larger increases in TEH under grass than any other treatment.

The carbon group assessment failed to show the expected shift in abundance to shorter chain compounds. However, this single piece of contradictory evidence is insufficient to undermine the assertions made regarding HC degradation in this study. That the data were not replicated seriously reduces the integrity of the results. This individual subsample may not have been representative of the HC composition of the other samples examined in the HC analysis of this study.

5.5 Summary and Conclusions

Changes in total oil and grease and total extractable HCs were observed in the forage and fallow treatments. Results were not statistically significant. However, considering the high variability in crude oil contamination of soil and the relatively poor statistical power of the investigation, the results are not unreasonable. The general trends in the data indicate that the presence of a grass stand enhanced the dissipation of crude oil compounds to a depth of 60 cm relative to a control fallow, particularly in 30-60 cm of soil. The probable cause of this enhancement was the stimulation of microbial activity by root growth. This stimulation could have been caused by the release of organic compounds from grass roots into the rhizosphere or by increased soil water movement and aeration through improved soil structure. An alfalfa crop did not promote biodegradation in the upper 30 cm of soil relative to basic tillage and was ineffective below 30 cm. The difference in rooting systems of these two crops may have been responsible for the differing results.

The general trend in oil level changes in the SF demonstrated that amendment of straw to contaminated soil also promoted HC disappearance in 0-30 cm of soil relative to cultivation alone. This trend was not as marked as in the grass plot. Crude oil degradation was generally greater in the SF than in the UF. The additional substrate may have enabled greater cometabolism of petroleum compounds. Such an amendment could be incorporated into a landfarming approach to remediation. Contaminant levels also declined in the UF between sampling times, but only in the upper 30 cm of soil which

was the tillage depth. No influence was observed on contaminants below the vertical extent of tillage in either of the fallow plots.

In cases of land contamination with crude oil and other heavy petroleum products, total oil and grease and total extractable HC results from sampling may compliment each other. As more complex compounds are broken down, the increased concentrations of simpler substances lead to elevated values for TEH in soil.

Establishing meadow bromegrass on crude oil contaminated land appears to enhance the degradation process relative to basic tillage. Phytoremediation may be a viable option in designing a reclamation strategy for a contaminated site, either as an initial or follow-up measure. Direct carbon amendments, such as straw, may also shorten the time required to reach remediation goals, but any effect would likely be limited to the depth of incorporation.

6. GENERAL DISCUSSION

6.1 The Effects of a Crude Oil Spill on Agricultural Land Productivity

6.1.1 Annual Crop Production

6.1.1.1 Wheat and Canola Growth and Productivity. Over the course of two field seasons, canola demonstrated a greater sensitivity in its growth to the presence of crude oil in soil than did wheat. This sensitivity was expressed at various stages of the growing season, including spring emergence, mid-season and harvest. Crop productivity with respect to seed production was assessed using harvest yields and indices. The results revealed that canola was less productive on the spill affected land than was wheat. Differences in crop growth and productivity between wheat and canola were most evident in the highly contaminated plots in which canola performed at a lower level than did wheat. The effect of crude oil in soil was most apparent at emergence, indicating susceptibility of the seed. Differences in seed architecture, size and shape were likely responsible for the differing responses of grain kernels and oilseeds to oil pollution. Such differences likely determined the degree to which each type of seed was able to germinate and each type of seedling to develop successfully in the presence of crude oil. However, contamination also hampered crop growth at later growth stages, indicating that altered soil conditions inhibited the maturation process.

The presence of crude oil had an increasing effect on emerged plants as the growing season progressed, based on the mid-season and harvest results. This was

evident both in wheat and canola plots which had successful emergence. As those crops which survived the early stages of growth developed through the season, they met with further adverse conditions possibly attributable to oil contamination. Plant growth may have been inhibited as roots encountered difficulty in extracting either nutrients, water or oxygen in soil below the seed bed containing more hydrocarbons. Nitrogen should not have been limiting as a result of heavy fertilization in the course of landfarming. However, differences in crop productivity within the spill area may have indeed resulted from greater competition for nitrogen between crops and soil microbes in some spill sites more than in others. Lower crude oil levels in some plots may have led to lower microbial activity and therefore a lower demand on soil nitrogen, allowing for better crop growth. Over time, as the intensity of microbial activity related to hydrocarbon degradation declines with diminishing residues, competition for nutrients will be alleviated (Rowell and Toogood 1977). Deficiencies of oxygen and water may have also contributed to depressed crop growth in the spill area relative to the control land.

6.1.1.2 Soil Salinity as a Limiting Factor for Annual Crop Productivity. The potential for soil salinity to be a limiting factor for crop growth existed in only two of the experimental sites. Electrical conductivities (ECs) for the soil in these two sites fell between 6 and 8 mS cm^{-1}. Values for all other sites, both spill and control, indicated salinity levels within the range of tolerance for the crops tested. Heavy fertilization and not the application of crude oil has been found to be responsible for elevated EC values (Toogood et al. 1977). This may have been the case in this study because of the large amount of fertilizer applied as part of the landfarming activities on the site. Multiple

regression analysis confirmed that soil salinity had a minor role in determining grain and oilseed yields over the course of the study. According to this analysis, crude oil concentrations in soil were significantly more important than soil salinity levels in determining yields in 1996. However, neither factor had a major influence on crop yields in 1997 according to multiple regression results. This substantiated the weaker relationships found between grain and oilseed yields and total oil and grease amounts in soil in 1997.

6.1.1.3 Critical Crude Oil Concentrations for Annual Crop Productivity. A consistently strong relationship between wheat grain yield and crude oil concentrations in soil could not be established. Although grain yields were related to soil oil levels in the first year, yields were quite variable in the second year over a range of hydrocarbon contents of soil. For the first year, the threshold for unaffected wheat production in the spill area was 0.2% total oil and grease by weight. This critical limit is substantially lower than previously recommended application rates of hydrocarbons for purposes of disposal or land improvement (Racz and Cansfield 1977; Biederbeck 1997).

A relationship between canola oilseed yield and total oil concentrations in soil appeared to persist through the two study years. The pattern of yield response to soil contaminant levels was less evident in the second year but still resembled that observed in the first year. The less apparent results of the second season were likely due to the influence of soil salinity on crop productivity in two of the spill canola plots. Oilseed yields dropped precipitously when soil hydrocarbon contents were above 0.1-0.2%. This concentration range is below the critical threshold found by other researchers (Toogood

et al. 1977; Overcash in Dueul 1990).

6.1.2 Perennial Forage Crop Production

Mid-season aboveground biomass was sampled to assess the impact of crude oil contamination of soil on the growth of meadow bromegrass and alfalfa. Grass growth in the spill area, in both relatively heavily and lightly contaminated soil, was generally competitive with that of the control land. This tolerance for hydrocarbons in soil is consistent with the findings of Overcash in Dueul (1990) who observed successful growth by perennial grasses in soil containing >3% oil. Rowell (1977) also characterized bromegrass as having a low sensitivity to hydrocarbons in soil. Alfalfa exhibited considerable sensitivity to relatively high hydrocarbon concentrations in the spill area. Alfalfa growth was reduced in plots having higher oil contents while growth was greater in soil with small amounts of oil than on the control land. It is possible that the growth of both forage crops was stimulated by slight residues of crude oil in soil. Forage crops, including bromegrass and alfalfa, have shown good tolerance to initial low oil levels in soil but only several years after the application of oil to soil (Toogood 1977). In a direct comparison, bromegrass performed better overall than alfalfa in the spill area and, in particular, in the more heavily contaminated soils.

6.1.3 The Remediation Status of the Study Area

The results of this study demonstrate that one year of landfarming prior to the study was insufficient to remediate certain portions of the spill area to a point where canola growth and productivity could match that of the adjacent uncontaminated land.

By contrast, wheat production had returned to near-normal levels by the end of the study.

It appears that the spill area has been remediated adequately to allow normal growth of meadow bromegrass but not alfalfa. Thus, the return of oil affected land to the production of livestock feed crops may be limited to certain types of crops depending on the degree to which a site has been remediated.

6.1.4 The Efficacy of Excavating Contaminated Soil

Highly contaminated soil from one end of the spill area was replaced with soil taken from another part of the spill area containing low oil levels. This excavation enabled crop growth and productivity at the former "hot spot" which was comparable or frequently better than that of the adjacent unaffected land. The mixing of polluted soil with less contaminated or uncontaminated soil has been used in other instances as a step in landfarming to aid in remediation (Smith et al. 1997). McGill (1977) stated that hydrocarbons in soil can improve soil quality and stimulate plant growth, but only at very low levels. Certain crude oil constituents are known promoters of plant growth (Gudin and Syratt 1975).

However, the cost of successful crop growth in the excavation site was the poor production at the site of deposition in the spill area. The negative impact of this measure on productivity at the deposition site was substantial. Both wheat and canola growth were depressed at this latter location. Mixing the extracted highly contaminated soil with soil containing minimal oil residues did not adequately dilute the hydrocarbons present to allow normal crop growth. Thus, the excavation and deposition of high-oil soil were an incomplete solution to the contamination problem at the original "hot spot". This

measure could be applied in instances in which a particularly important or sensitive portion of a spill area requires rapid cleanup and other less critical parts of the affected area are available for deposition of excavated, heavily oiled soil. Ultimately, the contamination must be dealt with regardless of its location.

6.1.5 Use of Crops as a Bioassay

The use of crop production as a bioassay of the impact of land contamination by a crude oil spill was appropriate for this agricultural site. Canola, in particular, exhibits a sensitivity to the presence of crude oil in soil which could be utilized to assess the severity of a spill in terms of crop production potential. The relative performance of a sensitive crop such as canola in one portion of a spill area versus another could enable the efficient allocation of remediation resources to where they are most needed. However, the relatively low tolerance of crops like canola to soil salinity reduces their suitability for bioassays on sites with salinity problems. Identifying a crop which exhibits susceptibility to oil effects but salinity tolerance would enable the use of crop bioassays to determine the extent of crude oil contamination of agricultural land.

6.2 Presence of BTEX and PAHs in Crop Tissue

Analysis of crop tissues for BTEX compounds and a number of PAHs was done to determine if such constituents of crude oil were present in the test crops. Composite annual crop samples were taken at harvest from the relatively highly contaminated experimental sites in both years of the study. The forage crops were sampled at mid-

season but only in the second year. BTEX levels in grain, oilseed, straw and forage aboveground biomass were consistently near or below the detection limit. The highest concentrations were still <1 mg kg^{-1}. All analyzed PAHs were below the detection limit of 0.1 mg kg^{-1} throughout the study. These findings agreed with those of previous research with agricultural crops for both natural background levels (Shabad and Cohan 1972) and experimental conditions (Wagner and Siddiqi 1970; Shabad and Cohan 1972; Wild et al. 1992; Wild and Jones 1992; Hulster et al. 1994; Chaineau et al. 1997). Harvesting these crops for use from land polluted with crude petroleum at concentrations found in this study may be a safe practice pending annual crop tissue analysis. This is based solely on the analytes examined in this study and does not account for breakdown products.

6.3 The Effects of a Crude Oil Spill on Soil Biological Quality

6.3.1 The Effect of the Spill on Microbiological Indices

The presence of crude oil in soil had a detrimental effect on the size of the microbial biomass based on total microbial biomass carbon. Previous research has found that microbial numbers and activity tend to rise in response to the addition of hydrocarbons to soil (Baldwin 1922; Plice 1948; Dobson and Wilson 1964; Jobson et al. 1972; Gossen and Parkinson 1974; Biederbeck 1990; Joergensen et al. 1995). These increases in microbiological activity variables were measured for both recently oil-treated and long-term contaminated soils. In this study, it is possible that the microbial biomass followed the typical growth pattern previously observed for oiled soils which ends in a

return to pre-exposure levels (Baldwin 1922; Matthews 1924; Plice 1948; Odu 1972; Gossen and Parkinson 1974; Biederbeck 1990; Chaineau 1996). However, due to the nature of this study, no evidence could be gathered to determine if this occurred. Regardless, the microbial biomass in the spill soil at the time of this investigation was at a size below that in a control soil. This new equilibrium size was all that could be sustained under the new physical and chemical conditions created by the presence of oil in soil or the continuing toxicity induced by certain oil constituents.

Based on general trends in the data, dehydrogenase activity (DHA) was depressed in the spill soil relative to the control soil. This result agrees with previous findings supporting the use of DHA as an indicator tool for assessing the impact of a disturbance on soil microbiology (Rossel and Tarradellas 1991). The results for the DHA index also indicated that microbial activity was altered by the presence of crude oil in soil. Based on the ratio of DHA/biomass, oil contamination caused the soil microbial community to be more active per unit biomass than was the community in the uncontaminated soil. This result compliments the observed decline in microbial metabolic diversity (MMD) in the spill soil compared to the control soil measured using the multiple-substrate microplate technique. Altered substrate utilization in microplates by soil microorganisms has been previously reported for both recently and long-term oil contaminated soils (Wunsche et al. 1995). This pattern change was attributed to a shift in soil microbial community composition towards the dominance of hydrocarbon degraders. In this study, the presence of crude oil caused a narrowing of the functional spectrum of the soil microbial community to a highly active fraction which was focused on hydrocarbon degradation. The high variability in DHA index and MMD in the spill soil further

reflected the instability of the contaminated soil system in comparison with the control. The introduction of crude oil was a source of major disturbance in the previous nature and functioning of the soil.

The negative impact of crude oil contamination on soil biological quality may have implications for the agricultural quality of the affected land. Disturbing the state of the soil microbial community may interfere with the many roles of soil microorganisms in the development and functioning of soil in the environment. Soil microbes make vital contributions to nutrient cycling, soil organic matter decomposition, soil development, maintenance of tilth and structure, and pollutant stabilization and degradation (Weil et al. 1993; Turco et al. 1994). If these contributions are diminished, the ability of a soil to function in an agroecosystem may also be diminished. Reduced crop growth and productivity, as observed in this research, may be in part due to the altered and/or depressed functioning of the soil microbial community.

6.3.2 The Effect of Remediation Treatments on Soil Biological Quality

The presence of forage crops on crude oil affected land improved the soil biological quality of crude oil contaminated soil relative to unvegetated soil. The improvement was made chiefly in terms of microbial biomass carbon and dehydrogenase activity which were restored to levels similar to those in unvegetated, uncontaminated soil and greater than those in unvegetated, contaminated soil. Establishing forage stands on crude oil contaminated soil was found to be the best means of restoring soil biological quality to control levels. Plant growth elevated the resiliency of the soil microbial community in response to disturbance.

A likely mechanism for this phenomenon is rhizodeposition, the continuous release of readily metabolizable organic compounds by growing roots into the root zone (Rovira and Davey 1974; Rovira et al. 1979; Curl and Truelove 1986). They serve as additional substrate for soil microorganisms in the rhizosphere, stimulating their growth and activity.

Straw incorporation failed to stimulate a recovery of the biological quality of crude oil contaminated soil except in terms of microbial metabolic diversity. Amending the soil with this type of organic material did not provide adequate or appropriate substrate for enhanced microbial growth and activity relative to unamended soil.

6.4 Phytoremediation and Direct Carbon Amendment as Means of Enhancing the Reclamation of Crude Oil Contaminated Soil

The growth of meadow bromegrass appeared to have been the most effective treatment in enhancing degradation of total oil and grease relative to an unplanted soil, a result consistent with research on individual hydrocarbons (Aprill and Sims 1990; Ferro et al. 1994; Reilley et al. 1996). The trend of greater dissipation under bromegrass was likely attributable to enhanced microbial community size and activity in the rhizosphere stimulated by the release of organic substrates from roots (Reilley et al. 1996). Greater microbial biomass carbon and dehydrogenase activity were measured under grass than in either of the fallow plots. The degradative capacity of a soil should be related to its biological quality because of the dominant role of soil microorganisms in the breakdown process. Improved physical conditions, such as increased aeration, may have also been present under grass and contributed to elevated crude oil degradation under that treatment

(Qiu et al. 1994).

The basic trends in hydrocarbon dissipation in soil indicate that bromegrass had a greater influence than alfalfa on oil levels in soil, particularly at a greater depth. Differences in rooting pattern and therefore the extent of a rhizosphere effect were probably responsible for the differences in crude oil degradation under the two treatments.

Incorporation of wheat straw had a reduced effect on crude oil levels in soil relative to bromegrass based on general trends. Any enhancement through straw addition was limited to the depth of incorporation. Distribution in soil of the substrate found in the straw was determined by the reach of the tillage equipment used to mix in the straw.

The elevated TEH levels across all treatments at the end of the trial indicated the breakdown of long carbon chain compounds (>C30) into shorter chains which fell within the detection range of the TEH procedure (C10-C30). The increases in TEH generally matched up with decreases in total oil and grease, indicating that the two analyses may in fact compliment each other in their results.

Establishing bromegrass on crude oil contaminated land appears to be the best remedial alternative to landfarming among the treatments examined. Elevated microbial growth and activity in the rhizosphere of grass plants may lead to enhanced hydrocarbon degradation. In addition, the presence of a grass crop offers soil stabilization on sites which may have their soil stability compromised as a result of intensive landfarming or other remedial treatments. Phytoremediation should prove to be a less expensive and less intrusive method of managing crude oil contaminated land.

7. SUMMARY AND CONCLUSIONS

7.1 The Effect of the Spill on Agricultural Productivity

The pipeline spill at the study site continued to affect agricultural productivity of the land two and three years following initial contaminated by crude oil. In 1996, the effect of the oil on wheat growth varied somewhat during the growing season. By harvest, the oil had no detectable effect on yields of grain and straw. Nevertheless, a relationship between wheat grain yield and the crude oil content of soil within the spill area was found. By contrast, canola growth was hampered throughout the growing season in soil with the highest hydrocarbon levels. Emergence counts, mid-season above-ground biomass and harvest yields of oilseed and straw were all lower in the most heavily contaminated plots than in the control plots. Allocation of carbon to seed was also inhibited in the high oil canola plots. Oilseed yield exhibited a strong relationship with total oil and grease in 0-30 cm of soil, with yield dropping off precipitously when soil oil content was above 0.1-0.2%.

In 1997, the effect of the oil on wheat production was not evident. At mid-season, wheat growth was lower in several of the spill plots relative to the controls, but salinity and disease factors make it difficult to determine the cause of reduced growth in some of the spill plots. Grain and straw harvest yields were not appreciably affected by the presence of oil in soil. Wheat grain yield was independent of hydrocarbon concentrations

in soil based on regression analysis. Dry soil conditions confounded the results for canola to a certain degree. Despite this circumstance, similar trends were observed as in the previous year for canola with the exception of two experimental sites which exhibited higher salinity than all other sites. At these two sites, canola productivity was seriously depressed at both mid-season and harvest. In the most heavily contaminated plots, in which salinity was not a factor, the canola crop was virtually non-existent. The relationship between canola oilseed yield and crude oil concentrations was not clear possibly because of the confounding factor of salinity in two of the plots. Nevertheless, the decline of yield with rising hydrocarbon levels followed a pattern similar to that of the previous year.

Over the course of the study, canola exhibited a greater sensitivity to the presence of oil within the root zone than did wheat. A critical crude oil concentration above which crop yield was reduced could only be repeatedly estimated for canola, and only with a correction for salinity effects in the second year. This threshold was in the range of 1000 to 2000 mg kg^{-1}. Wheat yield was related to soil hydrocarbon levels only in the first year when the threshold concentration for yield reduction was approximately 2000 mg kg^{-1}. Establishing a critical crude oil concentration at which crop growth and yield begins to be negatively affected may enable the calculation of what amount of contaminated soil can be safely spread without depressing the plant productivity of a site.

The results of this research suggest that canola may be a suitable bioassay crop on crude oil contaminated sites. The growth and yield of canola may potentially serve as measures of remediation. Used as a screening tool, canola productivity could be used to identify portions of a spill area which require further remedial treatments. This would

help to enable the most efficient allocation of remediation resources to portions of the spill area that merit the most attention.

The presence of BTEX and PAHs which are found in crude oil in wheat, canola, bromegrass and alfalfa plant tissue was minimal to non-existent. Accumulation of such compounds in the tissue of the crops tested does not appear to be a prominent environmental fate for these constituents of crude oil.

The St. Leon spill site appears to be approaching a level of productivity for wheat which would be expected in that area on land with no crude oil contamination. This assertion is based on the most recent results of this study. Evidence supporting this conclusion consists of: 1) the competitive performance of research plots within the spill area compared to control plots on adjacent land and 2) the lack of relationship between grain yield and total oil and grease in 0-30 cm of soil. However, site productivity with respect to canola remains well below that which is typical for the affected field. In both years of the study, canola exhibited a sensitivity to crude oil in soil. Thus, determining the endpoint of remediation for agricultural land depends on the types of crops to be grown and their respective sensitivities to the presence of crude oil or other hydrocarbons in soil.

7.2 Effect of the Spill on Soil Biological Quality

The spill had a significant effect on soil biological quality based on microbial biomass carbon, dehydrogenase enzyme activity and microbial metabolic diversity. The soil microbial community was inhibited by the presence of crude oil in fallowed soil

approximately three years after exposure to oil. The introduction of crude oil has caused potentially long-term changes in the physical and chemical soil environment or has exerted prolonged toxic effects on the soil microbes themselves.

Of the five treatments tested, meadow bromegrass had the greatest effect on soil biological quality. Algonquin alfalfa exhibited a slightly reduced effect compared to grass based on general trends, an observation likely attributable to differences in rooting pattern and deposition of organic substrates into the root zone. No statistically significant differences were found, however, between the two forage crops among the examined parameters. The incorporation of wheat straw had a reduced effect on soil biological quality in the spill soil. Any initial effects on the microbial indices examined which may have occurred following straw amendment had diminished by the time of sampling. The single application may have also been insufficient to stimulate the soil microbial community. The establishment of perennial forage crops, meadow bromegrass in particular, or repeated applications of straw to soil are recommended as potential means of restoring the soil biological quality of a crude oil contaminated soil.

7.3 Phytoremediation Potential of the Spill Affected Soil

The general trends in the data indicated that meadow bromegrass growth was the most effective treatment in reducing crude oil levels to a depth of 60 cm relative to a control unamended fallow (UF), particularly in 30-60 cm of soil. The probable cause of this enhancement was the stimulation of microbial activity by root growth through the release of organic substrates or improved soil structure. An alfalfa crop did not promote

biodegradation in the upper 30 cm of soil relative to basic tillage and was ineffective below 30 cm. The difference in rooting systems of these two crops may have been responsible for the differing results. The ability of alfalfa to fix nitrogen which would have been advantageous in a nitrogen limited system was perhaps nullified by the high soil N levels in the spill area.

Oil concentrations also tended to be lower in the wheat straw fallow (SF) demonstrated that amendment of straw to contaminated soil also promoted hydrocarbon disappearance in 0-30 cm of soil relative to cultivation alone. This trend was not as marked as in the grass plot. Crude oil degradation was generally greater in the SF than in the UF. The additional substrate may have enabled greater cometabolism of petroleum compounds. Contaminant levels also declined in the UF between sampling times, but only in the upper 30 cm of soil which was the tillage depth. No influence was observed on contaminants below the vertical extent of tillage in either of the fallow plots.

In cases of land contamination with crude oil and other heavy petroleum products, total oil and grease and total extractable hydrocarbon results from sampling may compliment each other. As more complex compounds are broken down, the increased concentrations of simpler substances lead to elevated values for TEH in soil.

Establishing meadow bromegrass on or straw fallowing of crude oil contaminated land appears to enhance the degradation process relative to basic tillage. However, any effect of a direct carbon amendments like straw is likely to be limited to the depth of incorporation, while the bromegrass effect is limited in depth by the extent of root growth. Phytoremediation may be a viable option in designing a reclamation strategy for a contaminated site, either as an initial or follow-up measure.

8. CONTRIBUTION TO KNOWLEDGE

This research demonstrated that the introduction of a large quantity of crude oil from a pipeline rupture onto agricultural land can have a deleterious effect on site agronomic productivity. This study confirmed that canola is more sensitive to the presence of crude oil in soil than is wheat. The oil-affected area has been sufficiently remediated to allow for normal wheat production. However, certain locations within the spill area have yet to be reclaimed to the point where canola can be grown as successfully as on adjacent uncontaminated land. This greater sensitivity exhibited by canola may enable its use as a screening tool for the degree of contamination of an agricultural site. A critical oil concentration for unaffected canola production was approximated, although further experimentation is recommended to more clearly define such a value. Rigorously establishing this type of threshold would provide an endpoint goal for remediation of similarly oil-exposed sites.

Findings for BTEX and PAH analysis of crop tissue confirmed that uptake by the test crops of hydrocarbons is not a prevalent environmental fate for crude oil constituents. Testing crop residues generated on crude oil polluted lands will ensure that such materials are safe to use for livestock feed or human food production.

The presence of crude oil in soil also affects the biological quality of soil. This impact can be negative as was shown in this study for the size, activity and functional diversity of the soil microbial community. Microbiological indices such as microbial

biomass carbon, dehydrogenase activity, a dehydrogenase activity index for microbial biomass and microbial metabolic diversity can be used to measure the impact of crude oil contamination of soil. Establishing forage stands can restore microbial biomass carbon, dehydrogenase activity and the dehydrogenase activity index to control levels.

The growth of meadow bromegrass is capable of enhancing crude oil dissipation in soil relative to landfarming based on the general trends observed in this study. The creation of a soil environment in the root zone more condusive to microbial growth and activity was responsible for the trend of greater oil degradation under grass than in the unamended fallow. The likely mechanism for this apparent effect was the release of readily metabolizable organic substrates from roots which stimulated the hydrocarbon-degrading portion of the soil microbial community Incorporation of straw into soil enhanced hydrocarbon breakdown compared to basic landfarming to a lesser degree than did grass and only in the depth of incorporation. Alfalfa growth showed no potential for enhancement of the degradation process, possibly due to differences in rooting patterns compared to bromegrass. Through nitrogen fixation, an alfalfa stand may potentially influence crude oil concentrations in soil which is more limited in nitrogen than was the study soil. Phytoremediation approaches, particularly those using grass, may be a feasible alternative to managing crude oil contaminated sites.

REFERENCES

Alexander, M. 1961. Pages 139-162, 248-271, 425-441. *in* Introduction to soil microbiology. John Wiley and Sons, Inc., New York.

Allison, M.F. and Killham, K. 1988. Response of soil microbial biomass to straw incorporation. J. Soil Sci. **39:** 237-242.

Amakiri, J.O. and Onofeghara, F.A. 1984. Effects of crude oil pollution on the germination of Zea mays and Capsicum frutescens. Envrion. Pollut. **35:** 159-167.

Anderson, T.A., White, D.C. and Walton, B.T. 1995. Degradation of hazardous organic compounds by rhizosphere microbial communities. *in* V. Singh (Ed.). Biotransformations: Microbial Degradation of Health-Risk Compounds.

Angers, D.A. 1992. Changes in soil aggregation and organic carbon under corn and alfalfa. Soil Sci. Soc. Am. J. **56:** 1244-1249.

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

Bachmann and Kinzel 1992. Soil Biol. Biochem. **6:** 543-552.

Baldwin, I.L. 1922. Modification of the soil flora induced by applications of crude petroleum. Soil Sci. **14:** 465-478.

Baker, J.M. 1970. The effects of oils on plants. Environ. Pollut. **1:** 27-44.

Baker, J.M. 1971. Growth stimulation following oil pollution. Pages 72-77. *in* E.B. Cowell (Ed.). The Ecological Effects of Oil Pollution on Littoral Communities. Institute of Petroleum, London.

Bassio, D.A. and Scow, K.M. 1995. Impact of carbon and flooding on the metabolic diversity of microbial communities in soils. Appl. Environ. Microbiol. **61:** 4043-4050.

Biederbeck, V.O. 1990. Using oily waste sludge disposal to conserve and improve sandy cultivated soils. Pages 1025-1038. In Proceedings 1st International Symposium on Oil and Gas Exploration/Production. Sept. 10-13, 1990. New Orleans, LA.

Biederbeck, V.O., Larney, F.J. and Eilers, R.G. 1997. Improvement of marginal farmland with effective use of urban, industrial and agricultural wastes. Pages 1-26. *in* Proceedings of the 40th Annual Manitoba Society of Soil Science Meeting Jan. 21 and 22, 1997.

Bollag, J., Mertz, T. and Otjen, L. 1994. Role of microorganisms in soil bioremediation. Pages 2-10. *in* T.A. Anderson and J.R. Coats (Eds.). Bioremediation Through Rhizosphere Technology. American Chemical Society, Washington, D.C.

Bossert, I., Kachel, W.M. and Bartha, R. 1984. Fate of hydrocarbons during oily sludge disposal in soil. Applied and Environ. Microbiol. **47:** 763-767.

Bossert, I. and Bartha, R. 1985. Plant growth in soils with a history of oil sludge disposal. Soil Sci. **140:** 418-422.

Bremner, J.M. and Tabatabai, M.A. 1973. Effect of some inorganic substances on TTC assay of dehydrogenase activity in soils. Soil Biol. Biochem. **5:** 385-386.

Brown, K.W. and Deuel, L.E., Jr. 1983. An evaluation of sub-surface conditions at refinery land treatment sites. EPA-600/52-83-096.

Buddin, W. 1914. Partial sterilization of soil by volatile and nonvolatile antiseptics. J. Agric. Sci. **6:** 417-451.

Canadian Council of Ministers of the Environment (CCME). 1997. Recommended Canadian soil quality guidelines. 129 pp.

Carnes, A. 1934. Soil crusts – methods of study, their strength and a method of overcoming their injury to cotton stands. Agr. Eng. **15:** 167-171.

Carr, R.H. 1919. Vegetative growth in soils containing crude petroleum. Soil Sci. **8:** 67-68.

Carter, M.R. 1986. Microbial biomass as an index for tillage-induced changes in soil biological properties. Soil Tillage Research. **7:** 29-40.

Casida, L.E., Klein, D.A. and Santoro, T. 1964. Soil dehydrogenase activity. Soil Sci. **98:** 371-376.

Chaineau, C.H., Morel, J.L. and Oudot, J. 1996. Land treatment of oil-based drill cuttings in an agricultural soil. J. Environ. Qual. **25:** 858-867.

Chaineau, C.H., Morel, J.L. and Oudot, J. 1997. Phytotoxicity and plant uptake of fuel oil hydrocarbons. J. Environ. Qual. **26:** 1478-1483.

Chander, K. and Brookes, P.C. 1991. Effects of heavy metals from past application of sewage sludge on microbial biomass and organic matter accumulation in a sandy loam and a silty loam U.K. soil. Soil Biol. Biochem. **23:** 927-932.

Christensen, S., Griffiths, B.S., Ekelund, F. and Ronn, R. 1992. Huge increase in bacterivores on freshly killed barley roots. FEMS Microbiology Ecology. **86:** 303-310.

Cole, M.A., Liu, X. and Zhang, L. 1994. Plant and microbial establishment in pesticide-contaminated soils amended with compost. Pages 210-222. *in* T.A. Anderson and J.R. Coats (Eds.). Bioremediation Through Rhizosphere Technology. American Chemical Society, Washington, D.C.

Collins, H.P., Rasmussen, P.E. and Douglas, C.L., Jr. 1992. Crop rotation and residue management effects on soil carbon and microbial dynamics. Soil Sci. Soc. Am. J. **56:** 783-788.

Constantinides, G. and Arich, G. 1967. Nonhydrocarbon compounds in petroleum. Pages 109-176. *in* B. Nagy and V. Columbo (Eds.). Fundamental Aspects of Petroleum Geochemistry. Elsevier, New York.

Conway, G.R. 1985. Agricultural ecology and farming systems research. *in* J.V. Remenyi (Ed.). Agricultural Systems Research for Developing Countries. Australian Centre for International Agricultural Research, Canberra.

Cook, F.D. 1977. Microbiological aspects: ten months after oil applied. Page 95. *in* J.A. Toogood (Ed.). The Reclamation of agricultural soils after oil spills. Part 1: Research. Alberta Institute of Pedology Pub. No. M-77-11.

Cook, F.D. and Westlake, D.W.S. 1975. Biodegradability of Northern Crude Oils. INA Pub. No. QS-8048-000-EEE-A1. Dept. of Indian Affairs and Northern Development. Ottawa.

Cooper, J.M. and Warman, P.R. 1997. Effects of three fertility amendments on soil dehydrogenase activity, organic C and pH. Can. J. Soil Sci. **77:** 281-283.

Cuddington, K.S. and Lowther, N.F. 1977. The character of crude oil. Pages 208-221. *in* P.A. Stockil (Ed.). Our Industry Petroleum. British Petroleum Co., London.

Cunningham, S.D. and Lee, C.R. 1995. Phytoremediation: plant-based remediation of contaminated soils and sediments. Pages 145-156. *in* H.D. Skipper and R.F. Turco (Eds.). Bioremediation Science and Applications. Soil Science Society of America Special Publication No. 43. SSSA, Inc. Madison, Wisconsin.

Cunningham, S.D., Shann, J.R., Crowley, D.E. and Anderson, T.A. 1997. Phytoremediation of contaminated water and soil. Pages 2-17. *In* E.L Kruger, T.A. Anderson and J.R. Coats (Eds.). Phytoremediation of Soil and Water Contaminants. American Chemical Society, Washington, D.C.

Curl, E.A. and Truelove, B. 1986. Root exudates. Pages 55-91 *in* The rhizosphere. Springer-Verlag, New York.

Deuel, L.E., Jr. 1990. Evaluation of limiting constituents suggested for land disposal of exploration and prduction wastes. Pages 411-430. *in* Proceedings 1st International Symposium On Oil and Gas Exploration and Production Waste Management Practices. Sept. 10-13, 1990. New Orleans, LA.

Dick, R.P. 1994. Soil enzyme activities as indicators of soil quality. p. 107-124. *in* Defining soil quality for a sustainable environment. SSSA Special Publication no. 35. Soil Science Society of America, Madison, WI.

Dick, R.P., Breakwell, D.P. and Turco, R.F. 1996. Soil enzyme activities and biodiversity measurements as integrative microbiological indicators. *in* Defining soil quality for a sustainable environment. SSSA Special Publication no. 35. Soil Science Society of America, Madison, WI.

Dobson, A.L. and Wilson, H.A. 1964. Respiration studies in soil treated with some hydrocarbons. Proc. Soil Sci. Soc. Am. **31:** 536-539.

Drury, C.F., Stone, J.A. and Findlay, W.I. 1991. Microbial biomass and soil structure associated with corn, grasses and legumes. Soil Sci. Am. J. **55:** 805-811.

Duffy, J.J., Mohtadi, M.F. and Peake, E. 1977. Subsurface persistence of crude oil spilled on land and its transport in groundwater. Pages 475-478. *in* J.O. Ludwigson (Ed.). Proceedings 1977 Oil Spill Conference, New Orleans, LA. API Washington, D.C.

Elliot, L.F., Gilmour, G.M., Lynch, J.M. and Tittemore, D. 1984. Bacterial colonization of plant roots. Pages 1-16 *in* R.L. Todd and J.E. Giddens (Eds.). Microbial-plant interactions. Soil Science Society of America, Madison, Wis.

Ellis, R.E., Jr. and Adams, R.S., Jr. 1961. Contamination of soils by petroleum hydrocarbons. Avances in Agronomy. **13:** 197-216.

Enright, R.J. 1963. Oil-field pollution – and what's being done about it. Oil Gas J. **61:** 76-86.

Evans, G.B., Jr., Deuel, L.E., Jr. and Brown, R.W. 1980. Mobility of water soluble Organic constituents of API separator waste in soils. EPA Grant No. R805474010 Report.

Expert Committee on Soil Survey. 1982. The Canada Soil Information System (CanSIS) Manual for describing soils in the field. Research Branch, Agriculture Canada, Ottawa, Ontario.

Falih, A.M.K. and Wainwright, M. 1996. Microbial and enzyme activity in soils amended with a natural source of easily available carbon. Biol. Fertil. Soils. **21:** 177-183.

Fattah, Q.H. and Wort, D.J. 1970. Effect of light and temperature on stimulation of vegetative and reproductive growth of bean plants by naphthenates. Agron. J. **62:** 576-577.

Fauci, MF. and Dick, R.P. 1994. Microbial biomass as an indicator of soil quality: effects of long-term management and recent soil amendments. p. 229-234 *in* Defining soil quality for a sustainable environment. SSSA Special Publication no. 35. Soil Science Society of America, Madison, WI.

Ferro, A.M., Sims, R.C. and Bugbee, B. 1994. Hycrest crested wheatgrass accelerates the degradation of pentachlorophenol in soil. J. Environ. Qual. **23:** 272-279.

Follett, R.F. and Schimel, D.S. 1989. Effect of tillage preactices on microbial biomass dynamics. Soil Sci. Soc. Amer. J. **53:** 1091-1096.

Foster, J.W. 1962. Hydrocarbons as substrates for microorganisms. Antonie van Leeuwenhoek. **28:** 241-274.

Foster, R.C. and Bowen, G.D. 1982. Plant surfaces and bacterial growth: the rhizosphere and rhizoplane. Pages 159-185. *in* M.S. Mount and G.H. Lacy (Eds.). Phytopathogenic prokaryotes. Vol. 1. Academic Press, New York.

Frankenberger, W.T., Jr. and Dick, W.A. 1983. Relationships between enzyme activities and microbial growth and activity incices in soil. Soil Sci. Soc. Am. J. **47:** 945-951.

Fraser, D.G., Doran, J.W., Sahs, W.W. and Lesoing, G.W. 1988. Soil microbial populations and activities under conventional and organic management. J. Environ. Qual. **17:** 585-590.

Friedel, J.K., Molter, K. and Fischer, W.R. 1994. Comparison and improvement of methods for determining soil dehydrogenase activity by using triphenyltetrazolium chloride and iodonitrotetrazolium chloride. Biol. Fertil. Soils. **18:** 291-296.

Garland, J.L. and Mills, A.L. 1991. Classification and characterization of heterotrophic microbial communities on the basis of patterns of community-level sole-carbon-source utilization. Appl. Environ. Microbiol. **57:** 2351-2359.

Graf, W. and Nowak, W. 1966. Growth stimulation in lower and higher plants by carcinogenic polycyclic aromatic compounds. Arch. Hyg. Bakt. **150:** 513.

Gudin, C. 1973. Mise en evidence dans le petrole de regulateurs de la croissance des plants. International Meeting on on Organic Geochemistry, 6th ed. Rueil Malmaison, France.

Gudin, C. and Syratt, W.J. 1975. Biological aspects of land rehabilitation following hydrocarbon contamination. Environ. Pollut. **8:** 107-112.

Hanks, R.J. and Thorp, F.C. Seedling emergence of wheat as related to soil moisture content, bulk density, oxygen diffusion rate and crust strength. Soil Science Society Proceedings. **20:** 307-310.

Harmsen, J. 1991. Possibilities and limitations of landfarming for cleaning contaminated soils. Pages 255-272. *in* R.E. Hinchee and R.F. Olfenbuttel (Eds.). On-Site Bioreclamation: Processes for Xenobiotic and Hydrocarbon Treatment. Butterworth-Heinemann, Toronto.

Harmsen, J., Velthorst, H.J. and Bennehey, I.P.A.M. 1994. Cleaning of residual concentrations with an extensive form of landfarming. Pages 84-91. *in* R.E. Hinchee, D.B. Anderson, F.B. Metting and G.D. Sayles (Eds.). Applied Biotechnology for Site Remediation. Lewis Publishers, Boca Raton, FL.

Harmsen, J., Wieggers, H.J.J., van de Akker, J.J.H., van Dijk-Hooyer, O.M., van den Toorn, A. and Zweers, A.J. 1997. Intensive and extensive treatment of dredged sediments on landfarms. Pages 153-158. *in* Proceedings Fourth International In Situ and On-site Bioremediation Symposium. Vol. 2. Battelle Press, Columbus, Ohio.

Higa, T. 1991. Effective microorganisms: a biotechnology for mankind. *in* J.F. Parr, S.B. Hornick and C.E. Whitman (Eds.). Proceedings First International Conference on Kyusei Nature Farming. Khon Haen, Thailand, October 17-21, 1989. USAID, Washington, D.C.

Hitchon, B., Filby, R.H. and Shah, K.R. 1975. Geochemistry of trace elements in crude oils, Alberta, Canada. Pages 111-121. *in* T.F. Yen (Ed.). The Role of Trace Metals in Petroleum. Ann Arbor Science Publishing Inc., Ann Arbor, Michigan.

Holloway, J.D. and Stork, N.E. 1991. The dimension of biodiversity: the use of invertebrates as indicators of human impact. p 37-63. *in* D.L. Hawksworth (Ed.). The biodiversity of microorganism and invertebrates: its role in sustainable agriculture. CAB Iternational, Wallingford, Oxon, UK.

Hornick, S.B., Fisher, R.H. and Paolini, P.A. 1983. Petroleum Wastes. Pages 321-337. *in* Land Treatment of Hazardous Wastes.

Howard, A. 1947. The Soil and Health. Devin-Adair, New York, N.Y.

Howard, P.J.A. 1972. Problems in the estimation of biological activity in soil. Oikos. **23:** 235-240.

Hulster, A., Muller, J.F. and Marschner, H. 1994. Soil-plant transfer of polychlorinated dibenzo-p-dioxins and dibenzofurans to vegetables of the cucumber family(Cucurbitaceae). Environ. Sci. Technol. **28:** 1110-1115.

Hund, K. and Traunspurger, W. 1994. Ecotox-evaluation strategy for soil bioremediation exemplified for a PAH-contaminated site. Chemosphere. **29:** 371-390.

Husky Synthetic MSDS. 1993.

Ilangovan, K. and Vivekanandan, M. 1990. Evaluation of crop resistance to aqueous oil pollution. Applied Biochemistry and Biotechnology. **26:** 11-22.

IPL Crude Characteristics Booklet. 1994.

Janzen, H.H. 1993. Soluble salts. Pages 161-166. *in* M.R. Carter (Ed.). Soil Sampling and Methods of Analysis. Lewis Publishers.

Jobson, A., Cook, F.D. and Westlake, D.W.S. 1972. Microbial utilization of crude oil. Appl. Microbiol. **23:** 1082-1089.

Joergensen, R.G., Schmaedeke, F., Windhorst, K. and Meyer, B. 1995. Biomass and activity of microorganisms in a fuel oil contaminated soil. Soil Biol. Biochem. **27:** 1137-1143.

Karlen, D.L., Eash, N.S. and Unger, P.W. 1992. Soil and crop management effects on soil quality indicators. Amer. J. Altern. Agric. **7:** 48-55.

Klokk, T. 1984. Effects of oil pollution on the germination and vegetative growth of five species of valcular plant. Oil Petrochem. Pollut. **2:** 25-30.

Ladd, J.N. and Paul, E.A. 1973. Changes in enzymatic activity and distribution of acid-soluble, amino acid nitrogen in soil during nitrogen immobilization and mineralization. Soil Biol. Biochem. **5:** 825-840.

Leadbetter, E.R. and Foster, J.W. 1959. Oxidation products formed from gaseous alkanes by the bacterium Pseudomonas methanica. Arch. Biochem. Biophys. **82:** 491-492.

Lee, E. and Banks, M.K. 1993. Bioremediation of petroleum contaminated soil using vegetation: a microbial study. J. Environ. Sci. Health A. **28:** 2187-2198.

Lenhard, G. 1956. Die dehydrogenaseaktivitat des bodens als mass fur die mikroorganismentatigkeit im boden. Z. Pflanzenernahr. Dung. Bodenk. **73:** 1-11.

Lubchenco et al. 1991. The sustainable biosphere initiative: an ecological research agenda. Ecology. **72:** 371-412.

Lynch, J.M. and Bragg, E. 1985. Microorganisms and aggregate stability. Adv. Soil Sci. **2:** 133-171.

Matthews, A. 1924. Partial sterilization of soil by antiseptics. J. Agr. Sci. **14:** 1-57.

Maynard, D.G. and Kalra, Y.P. 1993. Nitrate and exchangeable ammonium nitrogen. Pages 25-38. *in* M.R. Carter (ed.) Soil Sampling and Methods of Analysis. Lewis Publishers.

McGill, W.B. 1977. Soil restoration following oil spills: a review. J. Can. Pet. Technol. **16:** 60-67.

McGill, W.B. 1978. Oil spills: a rapid way to remove oil and reclaim agricultural land. Crops and Soils Magazine. **31:** 6-9.

McGill, W.B. and Rowell, M.J. 1977. Extraction of oil from soils. Pages 69-78. *in* J.A. Toogood (Ed.). The Reclamation of agricultural soils after oil spills. Part 1: Research. A.I.P. Pub. No. M-77-11.

McGill, W.B., Rowell, M.J. and Westlake, D.W.S. 1981. Biochemistry, ecology, and microbiology of petroleum components in soil. Pages 229-296. *in* E.A. Paul and J.N. Ladd (Eds.). Soil Biochemistry. Volume 5. Marcel Dekker, Inc., New York.

Murphy, H.F. 1929. Some effects of crude petroleum on nitrate production, seed germination and growth. Soil Sci. 27(2): 117-120.

Nannipieri, P., Grego, S. and Ceccanti, B. 1990. Ecological significance of the biological activity in soil. Soil Biol. Biochem. **6:** 293-355.

Newman, E.I. 1985. The rhizosphere: carbon sources and microbial populations. Pages 107-121. *in* A.H. Fitter, D. Atkinson, D.J. Read and M.B. Usher (Eds.). Ecological Interactions in Soil: Plants, Microbes and Animals. Blackwell Scientific Publications, Oxford.

Norwest Labs. 1995. Environmental Analytical Service Description. p. 53.

Nuttall, W.F. 1982. The effect of seeding depth, soil moisture regime and crust strength on emergence of rape cultivars. Agron. J. **74:** 1018-1022.

Ocio, J.A. and Brookes, P.C. 1990. An evaluation of methods for measuring the microbial biomass in soils following recent additions of wheat straw and the characterization of the biomass that develops. Soil Biol. Biochem. **22:** 685-694.

Ocio, J.A., Brookes, P.C. and Jenkinson, D.S. 1991. Field incorporation of straw and its effects on soil microbial biomass and soil inorganic N. Soil Biol. Biochem. **23:** 171-176.

Ogner, G. and Schnitzer, M. 1970. Humic substances: fulvic acid-dialkyl phthalate complexes and their role in pollution. Science. **170:** 317-318.

Oil and Gas operation in Western Canada Map. 1998. Oil and Gas Department, Bank of Montreal, Calgary, AB.

Oudot, J., Ambles, A., Bourgeois, S., Gatellier, C. and Sebyera, N. 1989. Hydrocarbon infiltration and biodegradation in a landfarming experiment. Environ. Pollut. **59:** 17-40.

Overcash, M.R. and Pal, D. 1979. Design of land treatment systems for industrial wastes – theory and practice. Ann Arbor Science Publishers, Inc., Ann Arbor, MI. 684 pp.

Paul, E.A. 1984. Dynamics of organic matter in soils. Plant Soil. **76:** 275-285.

Perfect, E., Kay, B.D., van Loon, W.K.P., Sheard, R.W. and Pojasok, T. 1990. Factors influencing soil structural stability within a growing season. Soil Sci. Soc. Am. J. **54:** 173-179.

Pierzynski, G.M., Sims, J.T. and Vance, G.F. 1994. Soils and Environmental Quality. Lewis Publishers, Boca Raton. 313 pp.

Plice, M.J. 1948. Some effects of crude petroleum on soil fertility. Proc. Soil Sci. Soc. Am. **13:** 413-416.

Powlson, D.S., Brookes, P.C. and Christensen, B.T. 1987. Measurement of soil microbial biomass provides an early indication of changes in total soil organic matter due to straw incorporation. Soil Biol. Biochem. **19:** 159-164.

Qiu, X., Shah, S.I., Kendall, E.W., Sorensen, D.L., Sims, R.C. and Engelke, M.C. 1994. Grass-enhanced bioremediation for clay soils contaminated with polynuclear aromatic hydrocarbons. Pages 142-157. *in* T.A. Anderson and J.R. Coats (Eds.). Bioremediation Through Rhizosphere Technology. American Chemical Society, Washington, D.C.

Qiu, X., Leland, T.W., Shah, S.I., Sorensen, D.L. and Kendall, E.W. 1997. Field study: grass remediation for clay soil contaminated with polycyclic aromatic hydrocarbons. Pages 186-199. *In* E.L Kruger, T.A. Anderson and J.R. Coats (Eds.). Phytoremediation of Soil and Water Contaminants. American Chemical Society, Washington, D.C.

Racz, G.J. and Cansfield, P.E. 1977. Hydrocarbon degradation in soil. A report on experiments conducted by the Dept. of Soil Science, University of Manitoba to Shell Oil Ltd., Winnipeg, MB. 82 pp.

Rasmussen, P.E. and Collins, H.P. 1991. Long-term impacts of tillage, fertilizer and crop residue on soil organic matter in temperate semi-arid regions. Adv. Agron. **45:** 93-134.

Reilley, K.A., Banks, M.K. and Schwab, A.P. 1996. Dissipation of polycyclic aromatic hydrocarbons in the rhizosphere. J. Environ. Qual. **25:** 212-219.

Rice, C.W., Moorman, T.B. and Beare, M. 1996. Role of microbial biomass carbon and nitrogen in soil quality. p. 203-215. *in* Defining soil quality for a sustainable environment. SSSA Special Publication no. 35. Soil Science Society of America, Madison, WI.

Ritz, K., Griffiths, B.S. and Wheatley, R.E. 1992. Soil microbial biomass and activity under a potato crop fertilised with N with and without C. Biol. Fertil. Soils. **12:** 265-271.

Ross, D.J. 1973. Some enzyme and respiratory activities of tropical soils from Hebrides. Soil Biol. Biochem. **5:** 559-567.

Ross, D.J. and Cairns, A. 1982. Effect of earthworms and ryegrass on respiratory enzyme activities of soil. Soil Biol. Biochem. **14:** 583-587.

Rossel, D. and Tarradellas, J. 1991. Dehydrogenase activity of soil microflora: significance in ecotoxicological tests. Environmental Toxicology and Water Quality. **6:** 17-33.

Rothrock, C.S. and Hargrove, W.L. 1988. Influence of legume cover crops and conservation tillage on soil populations of selected fungal genera. Can. J. Microbiol. **34:** 201-206.

Rouatt, J.W., Katznelson, H. and Payne, T.M.B. 1960. Statistical evaluation of the rhizosphere effect. Soil Sci. Soc. Am. Proc. **24:** 271-273.

Rovira, A.D. and Davey, C.B. 1974. Biology of the rhizosphere. Pages 153-204. *in* E.W. Carson (Ed.). The plant root and its environment. University of Virginia Press, Charlottesville.

Rovira, A.D., Foster, R.C. and Martin, J.K. 1979. Note on terminology: origin, nature and nomenclature of the organic materials in the rhizosphere. Pages 1-4 *in* J.L. Harley and R.S. Russell eds. The soil-root interface. Academic Press, New York.

Rowell, M.J. 1977a. The effect of crude oil spills on soils – a review of literature. Pages 1-27. *in* J.A. Toogood (Ed.). The Reclamation of agricultural soils after oil spills. Part 1: Research. Alberta Institute of Pedology Pub. No. M-77-11.

Rowell, M.J. 1977b. Oil contaminated soil and some aspects of reclamation. Pages 150-166. In Proceedings of 1977 Annual Alberta Soil Science Workshop on Soil Conservation, Reclamation and Research. Feb. 1-2, 1977, Red Deer, AB. Alberta Agriculture.

Rowell, M.J. and Crepin, J.M. 1977. Brine spillage in the oil industry: the natural recovery of an area affected by a salt water spill near Swan Hills, Alberta. *in* Proceedings 2nd Annual Canada Land Reclamation Association Meeting, August, 1977, Edmonton, AB.

Rowell, M.J. and Toogood, J.A. 1977. Effect of oil spills on germination. Pages 91-93. *in* J.A. Toogood (Ed.). The Reclamation of agricultural soils after oil spills. Part 1: Research. Alberta Institute of Pedology Pub. No. M-77-11.

Scow, K.M., Somasco, O., Gunapala, N., Lau, S., Vennette, R., Ferris, H., Miller, R. and Shennan, C. 1994. Transition from conventional to low-input agriculture changes soil fertiltiy and biology. Calif. Agric. **48:** 20-26.

Serra-Wittling, C., Houot, S. and Barriuso, E. 1995. Soil enzymatic response to addition of municipal solid-waste compost. Biol. Fertil. Soils. **20:** 226-236.

Severson, J.G. 1974. Effect of naphthenic acid on ^{32}P incorporation in the organs of the bush bean, Phaseolus vulgaris L. Phyton **32:** 81-87.

Shabad, L.M. and Cohan, Y.L. 1972. The contents of benzo(a)pyrene in some crops. Arch. Beschwultsforsch. **40:** 237.

Shaykewich, C.F. and Williams. 1971. Resistance to water absorption in germinating rapeseed (Brassica Napus L.). J. Exp. Bot. **22:** 19-24.

Sims, J.T., Cunningham, S.D. and Sumner, M.E. 1997. Assessing soil quality for environmental purposes: roles and challenges for soil scientists. J. Environ. Qual. **26:** 20-25.

Sims, R.C. and Overcash, M.R. 1983. Fate of polynuclear aromatic compounds (PNAs) in soi-plant systems. Residue Reviews. **88:** 1-68.

Skujins, J. and Burns, R.G. 1976. Extracellular enzymes in soil. Crit. Rev. Microbiol. **4:** 383-421.

Smith, B. 1998. Personal communication.

Smith, J.R., Tabe, M.E. and Loehr, R.C. 1997. Active and long-term passive PCB/PAH bioremediation via land farming. Pages 159-165. *in* Proceedings Fourth International In Situ and On-site Bioremediation Symposium. Vol. 2. Battelle Press, Columbus, Ohio.

Soil Capability for Agriculture Map - 1:1 000 000 Map Series Manitoba. 1974. Environment Canada Lands Directorate, Agriculture Canada and Manitoba Soil Survey.

Soil Capability for Agriculture Map - 1:1 000 000 Map Series Saskatchewan. 1976. Environment Canada Lands Directorate, Agriculture Canada and Manitoba Soil Survey.

Solbrig, O.T. 1991. From genes to ecosystems: a research agenda for biodiversity. Report of a IUBS-SCOPE-UNESCO workshop. The International Union of Biological Sciences, Paris, France.

Sparling, G.P. 1981. Heat output of the soil biomass. Soil Biol. Biochem. **13:** 373-376.

Speers, G.C. and Whitehead, E.V. 1969. Crude petroleum. Pages 638-675. *in* G.Eglinton and M.T.J. Murphy (Eds.). Organic Geochemistry: Methods and Results. Springer, New York.

Stone, J.A. and Buttery, B.R. 1989. Nine forages and the aggregation of a clay loam soil. Can. J. Soil Sci. **69:** 165-169.

Stebbings, R.E. 1970. Recovery of salt marsh in Brittany sixteen months after heavy Pollution by oil. Environ. Pollut. **1:** 163-167.

Stevenson, I.L. 1959. Dehydrogenase activity in soils. Can. J. Microbiol. **5:** 229-235.

Stevenson, I.L. 1962. The effect of decomposition of various crop plants on the metabolic activity of the soil microflora. Can. J. Microbiol. **8:** 501-509.

Swader, F.N. 1975. Persistence and effects of light fuel oil in soil. Pages 589-593. *in* Proc. Conf. Prev. Control Oil Pollut.

Tabatabai, M.A. 1982. Soil enzymes. p. 903-945 *in* A.L. Page (Ed.). Methods of soil analysis. Part 2. 2nd ed. Agron. Monog. 9. ASA and SSSA, Madison, WI.

Toogood, J.A. 1974. Effects of light oil spills on crop growth. Pages 102-105. *in* Proceedings of the Workshop on Reclamation of Disturbed Lands in Alberta. Alberta Environment and Environment Canada.

Toogood, J.A. 1977. Effects of light oil spills. Pages 87-89. *in* J.A. Toogood (Ed.). The Reclamation of Agricultural Soils after Oil Spills. Part 1: Research. Alberta Institute of Pedology Pub. No. M-77-11.

Toogood, J.A. and McGill, W.B. 1977. The Reclamation of Agricultural Soils after Oil Spills. Part 2: Extension. Alberta Institute of Pedology Pub. No. M-77-11.

Toogood, J.A., Rowell, M.J. and Nyborg, M. 1977. Reclamation experiments in the field. Pages 34-64. *in* J.A. Toogood (Ed.). The Reclamation of agricultural soils after oil spills. Part 1: Research. Alberta Institute of Pedology Pub. No. M-77-11.

Trapp, S., Matthies, M., Schneunert, I. And Topp, E.M. 1990. Modeling the bioconcentration of organic chemical in plants. Environ. Sci. Technol. **24:** 1246-1252.

Turco, R.F., Kennedy, A.C. and Jawson, M.D. 1994. Microbial indicators of soil quality. pp. 73-90. *in* Defining soil quality for a sustainable environment. SSSA Special Publication no. 35. Soil Science Society of America, Madison, WI.

Udo, E.J. and Fayemi, A.A.A. 1975. The effect of oil pollution on germination growth and nutrient uptake of corn. J. Environ. Qual. **4:** 537-540.

v. Boguslawski, E., Zadrazil, F. and Debruck, J. 1976. Der einflub langjahriger stroh- und grundungung sowie stickstoffdungung auf faktoren der bodenfruchtbarkeit. I. Mitteilung dehydrogenaseaktivitat des bodens. J. Agron. Crop Sci. **143:** 249-258.

Visser, S. and Parkinson, D. 1992. Soil biological criteria as indicators of soil quality: soil microorganisms. Amer. J. Altern. Agric. **7:** 33-37.

Voroney, R.P., Winter, J.P. and Beyaert, R.P. 1993. Soil microbial biomass C and N. p. 277-283 *In* M.R. Carter (Ed.). Soil sampling and methods of analysis. Lewis Publishers.

Wagner, Van K.H. and Siddiqi, I. 1970. The metabolism of 3,4-benzpyrene and 3,4-benzfluoranthene in summer wheat. Z. Pflanzen Bodenkundle. **127:** 211.

Walker, J.D. and Calwell, R.R. 1975. Factors affecting enumeration and isolation of Actinomycetes from Chesapeak Bay and Southeastern Atlantic Ocean sediments. Marine Biol. **30:** 193-201.

Ward, J. and Shaykewich, C.F. 1972. Water absorption by wheat seeds as influenced by hydraulic properties of soil. Can. J. Soil Sci. **52:** 99-105.

Watkins, J.W., Sorensen, D.L. and Sims, R.C. 1994. Volatilization and mineralization of naphthalene in soil-grass microcosms. Pages 123-131. *in* T.A. Anderson and J.R. Coats (Eds.). Bioremediation Through Rhizosphere Technology. American Chemical Society, Washington, D.C.

Weil, R.R., Lowell, K.A. and Shade, H.M. 1993. Effects of intensity of agronomic practices on a soil ecosystem. American Journal of Alternative Agriculture. **8:** 5-14.

Whitehead, W.L. and Breger, I.A. 1963. Geochemistry of petroleum. Pages 248-332. *in* I.A. Breger (Ed.). Organic Chemistry. Monograph 16, Earth Sci. Ser. Macmillan, New York.

Wild, S.R., Berrow, M.L., McGrath, S.P. and Jones, K.C. 1992. Polynuclear aromatic hydrocarbons in crops from long-term field experiments amended with sewage sludge. Environ. Pollut. **76:** 25-32.

Wild, S.R. and Jones, K.C. 1992. Polynuclear aromatic hydrocarbon uptake by carrots grown in sludge-amended soil. J. Environ. Qual. **76:** 217-225

Wiltse, C.C., Rooney, W.L., Chen, Z., Schwab, A.P. and Banks, M.K. 1998. Greenhouse evaluation of agronomic and crude oil-phytoremediation potential among alfalfa genotypes. J. Environ. Qual. **27:** 169-173.

Wood, C.W., Westfall, D.G., Peterson, G.A. and Burke, I.C. 1990. Impacts of cropping intensity on C and nitrogen mineralization under no-till dryland agroecosystmes. Agron. J. **82:** 1115-1120.

Zak, J.C., Willig, M.R., Moorhead, D.L. and Wildman, H.G. 1994. Functional diversity of microbial communities: a quantitative approach. Soil Biol. Biochem. **26:** 1101-1108.

I. Soil profile description of the Joyale Series for the study area.

Experimental Site	Horizons	Depth (cm)	Description
SP 1 / SP 1-B	Apk	0-18	CL lacustrine material
	AC	18-32	Evidence of gypsum & disturbance; lacustrine material
	Ckgjs	32-90	Silty clay (SiC); most gypsum 70-90 cm
	Ckgs	90-120	Silty clay loam (SiCL)
SP 2	Apk	0-60 or 70	CL – SiCL; disturbed horizon; pockets of CO_3; Weakly carbonated at surface
	Ckgj	70-80	SiCL – SiC
	Ckg	80-120	SiCL – SiC; Weak evidence of gypsum 100-110 cm increasing sand content 110-120 cm return to moderately fine lacustrine materials
SP 3	Apkj	0-37	Moderately fine lacustrine; carbonated to surface but very weak
	ACk	37-45	Moderately fine lacustrine; evident gypsum;
	Ckgjs	45-70	Moderately fine lacustrine; abundant gypsum
	Ckg	70-95	Moderately fine lacustrine; weak evidence of gypsum
	Ckg	95-120II	Till
SP 4	Apk	0~20	Moderately fine lacustrine
	AC	20-85	Moderately fine lacustrine; disturbed Mixed material: calcareous with some gypsum; 70 cm – bright orange mottles (may be imported)
	Disturbed	85-110	Till
	Disturbed	110-120	Gravel
SP 5	Apk	0-24	Lacustrine
	Ckg	24-85	Lacustrine; 25-30 cm compacted zone (excavation) 45-60 cm evidence of gypsum
	CkgII	85-120	Till; Note: clear break b/w A & C likely due to excavation Rego Humic Gleysol but probably b/c of deposition (usually not farmed); should be a Gleyed Rego Black (like all other sites)

CON 1	Ap	0-26	Lacustrine
	AC	26-40	Lacustrine
	Ckgj	40-95	Lacustrine; 40-50 cm gypsum
	CkgjII	90-120	Till
CON 2	Apk	0-20/25	Lacustrine; carbonates at surface, decreasing intensity with depth
	AC	22-30	Lacustrine
	Ckgj	30-45	Lacustrine
	CkgjII	45-120	Till
CON 3	Apk	0-18	Lacustrine
	Ckgj	18-53	Lacustrine; ~35 cm evidence of gypsum
	Ckg	53-60	Lacustrine
	CkgII	60-120	Till; ~100 cm increasingly loamy