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A LABORATORY STUDY ON THE DEVELOPMENT OF A BIOLOGICAL POLLUTION CONTROL SYSTEM FOR CONTAMINATED SOILS

By

Benjamin U. Ugwuegbu

A thesis submitted to the Faculty of Graduate Studies and Research in Partial Fulfilment of the Requirements for the Degree of

Doctor of Philosophy

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ABSTRACT

Ph.D. Benjamin U. Ugwuegbu **Agricultural and Biosystems Engineering**

This study describes a laboratory scale development of an in-situ bioremediation method, which uses a water table management system to supply nutrients to subsoil microorganisms, for biostimulation and subsequent biodegradation of pollutants such as fertilizer-nitrate and hydrocarbons (e.g., diesel oils), in the unsaturated zone of the soil. The study, which was divided into two parts: first nitrate bioremediation and secondly diesel biodegradation, was carried out on packed soil columns.

For the nitrate study, different levels of glucose were introduced into packed soil columns, 1,000 mm long x 200 mm diameter, via subirrigation in order to supplement the organic carbon levels in the soil. Two sandy soils were used, with 1.6% and 3.4% organic matter content, respectively; and the water table in the soil columns was maintained at a depth of 350 mm below the surface. Fertilizer-nitrate was applied to the soil surface at a rate of 180 kg/ha nitrate-N. Simulated rainfall was used to leach nitrates to lower depths. The efficacy of using the subirrigation system, as a method for nutrient delivery in the bioremediation of leached nitrate, was monitored with time and with reference to the nitrate residue, redox potential of the soil solution, and solubilized Fe and Mn.

Leached nitrate was denitrified to less than 10 mg/L nitrate-N, which is the limit permitted in drinking water. The ideal organic carbon range was considered to be the glucose level (20 mg/L glucose-C) that reduced most nitrate and gave redox potential and soluble Fe and Mn levels, similar to the control soil solution, when subjected to 96 days of subirrigation. Successful delivery of nutrient for the bioremediation of nitrate, within the farm

boundaries, will be considered a "break through" toward nitrate residue control if this novel approach to nitrate control is demonstrated in the field. The delivery method will offer a technical solution to on-farm nitrate pollution. It is inexpensive, easy to adopt, and does not require major changes in the current farm practices.

In the second part of the study, a diesel contaminated sandy soil was packed in columns, 2,000 mm long x 200 mm diameter. The subirrigation method was used to supply two different combinations of treatments to the microorganisms in the soil for the biodegradation of the diesel contaminant, namely: air, water and nutrients (N, P etc.), and air and water. The success of using subirrigation, to deliver nutrients to the soil in the columns, was monitored by measuring the trend in the reduction of soil diesel-TPH (diesel-total petroleum hydrocarbon) residue with time. Results obtained from the treated columns were compared with each other, and with the control columns undergoing passive biodegradation.

The study showed that subirrigation can be used as a method of nutrient delivery in the bioremediaton of diesel contaminated soil. The TPH in the contaminated soil decreased, from an initial 670 mg diesel TPH/kg soil to an acceptable level of 40 mg diesel TPH/kg soil, in 82 days in the columns subjected to a combination of nutrient, air and water treatments. If this method of delivering biostimulants to the subsoil microbial population is demonstrated in the field, it will be invaluable to in-situ bioremediation of contaminated soils.

RESUME

Ph.D. Benjamin U. Ugwuegbu **Génie Rural et Biosystémes**

Cette étude décrit le développement d'une méthode de biorestauration *in situ*, celle-ci utilisait le système de gestion de la nappe phréatique pour fournir, dans la région non saturé du sol, des éléments nutritifs aux micro-organismes et ainsi stimuler la dégradation biologique des polluants tels le nitrate (provenant d'engrais) et les hydrocarbures (huiles, diésel). L'étude, réalisée sur des colonnes de sol, comportait deux parties: la médiation du nitrate et la dégradation biologique du diésel.

Pour l'étude du nitrate, différentes quantités de glucose furent introduites par irrigation souterraine dans des colonnes de sol (1000 mm de long x 200 mm de large) afin d'augmenter le niveau de carbone organique. Deux sols sablonneux contenant respectivement 1,6% et 3,4% de matière organique, furent utilisés. La nappe phréatique fut maintenue à une profondeur de 350 mm en dessous de la surface du sol. Un application d'engrais de 180 kg NO_3^--N/ha en surface du sol fut suivie d'une pluie simulée afin de lessiver le nitrate et de l'amener jusqu'aux régions plus profondes. L'efficacité du système d'irrigation souterraine pour fournir les éléments nutritifs permettant la biorestauration du nitrate fut suivie dans le temps par la mesure des résidus de nitrate, du potentiel d'oxydoréduction

d'une solution de sol et du Fe et du Mn solubles.

Le nitrate lessivé fut dénitrifié jusqu'à moins de 10 mg NO_3^--N/L, ce qui est la limite tolérable pour l'eau potable. Le niveau idéal de carbone organique correspondait à une concentration de 20 mg glucose-C/L puisque cette concentration permettaint de réduire le plus de nitrate et d'atteindre des potentiels d'oxydoréduction ainsi que des niveaux de Fe et de Mn solubles semblable à ceux du témoin, et ce, après 96 jours d'irrigation souterraine. Si l'efficacité de l'irrigation sauterraine comme méthode d'approvisionnement en éléments nutritifs permettant la biorestoration du sol contaminé du nitrate est démontrée également sur le terrain, elle sera considéré comme une méthode de pointe pour le contrôle des résidus de nitrate. Cette méthode offrira une nouvelle solution pour le contrôle de la pollution par le nitrate sur la ferme. Elle est peu coûteuse, facile à appliquer et ne nécessite aucun changement majeur dans les pratiques courantes de la ferme.

Dans la seconde partie de l'étude, un sol sablonneux contaminé par du diésel fut entassé dans des colonnes de PVC (2000 mm de long x 200 mm de large). La méthode d'irrigation souterraine permettait d'exposer les microorganismes du sol à deux types de traitements différents pour la biodégradation du diésel : un premier type constitué d'une combinaison air, eau et éléments nutritifs et un second, d'une combinaison air et eau seulement. L'efficacité de l'irrigation souterraine pour fournir des éléments

nutritifs dans les colonnes fut suivi en mesurant l'évolution de la réduction des résidus diésel-HPT (niveau total d'hydrocarbures de pétrole) en fonction du temps. Les deux types de traitements furent comparés entre eux ainsi qu'avec un traitement témoin constitué de colonnes soumises à un régime de dégradation biologique passif.

L'étude démontra dans la biorestauration de sols contaminés avec du diésel que l'irrigation souterraine peut être utilisée comme méthode d'approvisionnement d'éléments nutritifs. Le HPT dans le sol contaminé diminua d'un niveau initial de 670 mg diésel HPT/kg de sol à un niveau acceptable de 40 mg diésel HPT/kg dans le cas des colonnes soumises à la combinaison air, eau et éléments nutritifs. Si cette méthode d'approvisionnement en biostimulants de la population microbienne du sous-sol s'avère également efficace sur le terrain elle sera d'une grande valeur dans la biorestauration *in situ* de sols contaminés.

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DEDICATION

I dedicate this work to my Hero and Saviour, JESUS THE CHRIST, for all that He means to me.

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CONTRIBUTION TO KNOWLEDGE

In-situ Bioremediation of Nitrate Residue

1. A new and innovative method of delivering nutrient to the subsoil to enhance nitrate-N dissipation in agricultural soils has been developed. The method uses a water table management system to deliver readily available organic carbon to the subsoil for bioremediation of nitrate-N residues.

2. Water table management, as currently practiced for nitrate reduction, is insufficient over a long period, without readily available organic carbon augmentation. This study has shown that there is a rapid decline in the potential of water table management, as currently practiced, to sustain denitrification in soils whose organic matter has been repeatedly leached and subjected to microbial decomposition.

3. The study has shown that the redox potential, Mn and Fe levels of the soil solution can be used in designing or predicting organic carbon supply management strategy for on-farm pollution control of nitrate pollution.

In-Situ Bioremediation of Diesel Contaminated Soils

1. A new and innovative method that delivers and manages nutrients and oxygen during in-situ bioremediation has been developed on a laboratory scale. The delivery system developed may be operated simultaneously or alternately, under both aerobic and anaerobic conditions. Sequential aerobic/anaerobic conditions are suitable for dehalogenation and biodegradation of halogenated hydrocarbons.

2. A treatment method that can isolate and treat any soil depth zone has been developed for in-situ bioremediation. This implies that contaminated soils, below leaky underground storage facilities, can be bioremediated without excavating the tank. After treatment, the remediation facility could be left intact as a biopreventive system, in the event of a leakage reoccurrence. Also, the water buffer system can protect the water table from the contaminants in the subsoil and the nutrients used. The method will permit the use of nitrate as a potential alternative electron acceptor for in-situ bioremediation. Difficulty in maintaining low oxygen conditions in-situ, and the strict control of nitrate-N not to exceed 10 ppm in ground water, resulted in under utilization of nitrate as alternate electron acceptors.

CHAPTER 1: GENERAL INTRODUCTION

Soils polluted either from nonpoint sources (agricultural farms) or point sources (industrial activities) lead to the contamination of ground water by the process of leaching. This ultimately contaminates the surface waters which are recharged by ground water; hence, diminishing land and water resources.

The ecosystem has the ability to destroy or transform pollutants to maintain a state of equilibrium. In some cases, the presence of contaminants (e.g. xenobiotics) lead to inhibition of the biogeochemical cycle, thus prolonging the capacity of the ecosystem to restore itself. The inability of the system to bioremediate the contaminated soils has also been attributed to unavailability of essential nutrients to the resident microorganisms responsible for mineralization and transformation.

This study focuses on the development of a nutrient delivery system for in-situ bioremediation of contaminated soil. The first part of the introduction will address pollution originating from an agricultural source (nonpoint source), specifically from the use of fertilizer-nitrate. The nature of the problem and the objectives of the nitrate study will be stated in sections one and two of part 1, respectively.

The second part of the introduction will discuss pollution arising from

diesel contamination of soils (point source). The nature of the problem and the objectives of developing a delivery system to aid bioremediation of such a contaminated soil will also be stated in sections one and two of part 2, respectively. The third part of the introduction will define the scope of the entire study.

1.1 Nitrate Pollution

Sharpley and Meyer (1994) state that agricultural nonpoint sources now account for a larger portion of pollution than point source pollution. The public is now increasingly aware of the role of agriculture and its associated chemicals in nonpoint source pollution (Oberle and Burkart, 1994; Sharpley and Meyer, 1994). Pimental (1993) estimated that 30 to 50% of the earth's land is affected by nonpoint source contamination from pesticides, organic manure and fertilizer-nitrate. Nitrate is estimated to be the most common agrochemical contaminant of the world's aquifer (Spalding and Exner, 1993). In humid regions, drain effluent, carrying fertilizer-nitrate residues from agricultural farms, contribute significantly to the pollution (Patni et al., 1992, Gilliam et al., 1985 and Skaggs, 1989).

The increased awareness of the detrimental effects of nitrate pollution has led to the development of water quality management programs for the

control of nitrate pollution (Malik et al., 1994).

1.1.1 Problem definition (In-situ bioremediation of nitrate residue)

Agricultural chemicals have significantly increased the production and protection of food, feed and fibre in Canada (McRae, 1989). For example, it is estimated that the corn yields quadrupled between 1950 and 1985, largely due to: the effective use of fertilizers, better control of weeds, diseases, insects, improvement in crop varieties and expanded irrigation schemes (Bock and Hergert, 1991). Bock and Hergert (1991) also indicated that these increases were mainly due to fertilizer-N inputs.

Although nitrate is essential for efficient plant growth, excessive ingestion of water containing more than the stipulated 10 mg/L nitrate-N limit is harmful to humans. Effects of high nitrate consumption include: hypertension (Malberg et al., 1978) and increased infant mortality (Super et al., 1981). Central nervous system birth defects have been reported, as also methaemoglobinaemia in infants less than six months old (Dorsch et al., 1984). Fletcher (1991) indicated that domestic animals, such as ruminants may also be poisoned with nitrate contaminated well water. Public awareness of the effect of nitrate consumption has made the use of fertilizer-nitrate a controversial issue since nitrate is being leached from the farms into the ground water. This concern has been supported by researchers who have

reported detection of a significant amount of nitrate in our water resources.

Gilliam et al. (1985) noted that major losses of nitrate to surface water occurred on subsurface drained farmland, cultivated for several years in North Carolina. Skaggs (1989) reported that a soil with poor subsurface drainage loses about 17 kg/ha/yr of nitrate-N in North Carolina. However, Skaggs (1989) also indicated an increase in nitrate loss from about 22 to 34 kg/ha/yr, with improved soil drainage. In another study, leachate from cropped land, intercepted by tile drains before discharging to surface waters, had about 20-100 kg nitrate-N/ha per year (Logan et al., 1980). Thus, nitrate is reaching the water resources, and the nitrate leaching problem is evidently becoming a global issue.

The ground water of several agricultural areas in southern Ontario, Canada, already exceeds the United Nations' 10 mg/L N limit for drinking water (Gilliam, 1991). Similar reports from other parts of the world, especially the European Community (EC), indicate that the groundwater supplies of some member nations are exceeding the EC limit of 11.3 mg/L N (Fried, 1991). It is estimated that if the current input of N from agricultural land in EC continues, the nitrate level in ground water will double the EC limit of 11.3 mg/L N (Spalding and Exner, 1993). Spalding and Exner (1993) summarized the nitrate threat to water resources by stating that no catchments have pristine nitrate-N levels.

Evans et al. (1990) cited a nitrate-N leachate load of between 4 and 32 kg/ha/year nitrate-N to surface water, under a water table management of low to high intensity subsurface drainage. However, the nitrate-N load decreased by about 45% (10 kg/ha/year) under controlled drainage practice (Evans et al., 1989). Kalita and Kanwar (1993) investigated nitrate-N levels in ground water under different water table depths. They found nitrate-N concentrations ranging from less than 10 to 20 mg/L. If infants in a population of about 100,000 consume 10-20 mg/L nitrate-N continually, it is projected that about 17 deaths will occur. This mortality estimate, based on a world wide 8% rate for infants with methaemoglobinaemia, is significant even in a North American context (Giraldez and Fox, 1994). As cited by Fletcher (1991), about 8% of 2,000 reported cases, of infants suffering from methaemoglobinaemia, have died since 1945 in North America and Europe.

Currently, water table management (WTM) is the best management practice (BMP) for nitrate pollution control (Evans et al., 1989, Kalita and Kanwar, 1993; Madramootoo et al., 1993). But, Kalita and Kanwar (1993) investigated nitrate-N levels in ground water under different water table depths, and found nitrate-N level ranging from 10 to 20 mg/L, which is considered unacceptable in potable water.

Water table management systems would reduce environmental

pollution from nitrate by increasing the denitrification process. However, the speed of the process may not be sufficient to eliminate the risk associated with nitrate-N environmental pollution. Denitrification of nitrate, under water table management, depends upon the availability of organic carbon from the surface of the soil. This assists in the depletion of oxygen in the soil and creates suitable conditions for denitrification. Depletion of carbon in the saturated zone will inevitably decrease the rate of denitrification, and also increases the associated risk of high nitrate-nitrogen in our water resources. Denitrification capacity, under water table management, depends upon organic carbon leaching from the soil surface. Such a capacity will obviously decrease with an inadequate supply of organic carbon.

The major source of nitrate, in farm drains, originates from residual nitrate remaining in the unsaturated zone at the end of the farming season. After crop harvest, WTM is not in operation to control or eliminate the nitrate residue in the unsaturated zone, below the root zone. Rather, the subirrigation network reverts to a drainage mode that quickly carries any nitrate leachate from the unsaturated zone to a receiving surface water body. Also, the period between harvest and the onset of winter is very short and does not allow complete microbial transformation of all the residual nitrate in the unsaturated zone. Therefore, prompt management is necessary to remove nitrate in leachate during the growing season (Wright et al., 1992),

as well as after the harvest.

The extent and speed of denitrification in the soil relates to the microbial biomass, and the biomass varies with: oxygen, organic carbon, pH, and temperature (Drury et al., 1991). A rich carbon environment establishes conditions for quick depletion of oxygen, allowing the continuation of denitrification in the soil (Burford and Bremner, 1975). Under subirrigation management, if a saturated condition exists at the depths requiring cleanup, a readily available carbon supplement will improve the denitrification capacity of the deficient soil.

This study intends to introduce a readily available carbon as a nutrient in subirrigation water. The nutrient will enhance the activity of the naturally-occurring denitrifying bacteria in the subsoil, and thus accelerate nitrate removal in the saturated subsoil during the growing season. The distribution of the nutrient in the soil profile will be accomplished using the subirrigation system.

Raising the water table, just below the root zone, immediately after harvest, will alter the current routine practice of WTM and distribute readily available carbon to the unsaturated zone. Simultaneously, the raised water table saturates the unsaturated zone, causing an anoxic condition favourable to the denitrification process. Consequently, this prevailing denitrifying condition allows microbial dissipation of any residual nitrate in the soil

below the root zone; therefore reducing the nitrate load in the fall leaching and in the spring snow-melt.

Adoption of this method will alleviate the pollution problem of fertilizer-nitrate, especially on sandy soils which are prone to higher nitrate leaching; also crop yield may not be compromised.

1.1.2 Objectives for the nitrate study

Nitrate pollution from fertilizers is technically challenging due to the variability in hydrogeologic conditions, agronomic practices and the diffused nature of the pollution process (Fletcher, 1991). Fletcher (1991) indicated that the agricultural, environmental and public health authorities hold conflicting opinions on how to address the nitrate problem, since it involves both agricultural production and economic viability. Some farmers in humid areas have adopted subirrigation for its agricultural benefits. If the same system is adapted to include organic carbon supplementation in the subsoil, for enhanced denitrification of nitrate leached below the root zone, then this contribution will help to sustain agriculture in humid regions.

One major objective of this study is, therefore, to develop an inexpensive and easily adaptable in-situ nitrate bioremediation technique. This technology will supplement soil organic carbon via subirrigation, in order to enhance denitrification. More specifically, the objectives are:

1. To determine the impact of organic carbon on the soil nitrate-N residue, using subirrigation as the method of water and organic carbon supply to the subsoil,
2. To evaluate changes in soil solution redox potential and soluble Fe and Mn content, because of added organic carbon,
3. To make recommendations on water table management practices in order to sustain agricultural activities in humid regions.

The aforementioned objectives were accomplished using sandy soils, packed in columns, and subirrigated with different levels of glucose solution while monitoring the loss of nitrate residue in the soil solution.

1.2 Hydrocarbon Pollution

It has been reported that since the Industrial Revolution (1945), the waste generated from industrial activities has increased by fifteen times (Hawkes, 1989). Generation of wastes continues and increases, with many sites being contaminated. Canada and U.S.A. have about 43,000 contaminated (chemical) sites, with 2,200 of them classified as high risk (Smith, 1991). In U.S.A., there are over 750,000 underground storage facilities with over two million tanks (Caplan, 1993). Caplan (1993) also reported that about 50% of these tanks are leaking, and the estimated

cleanup cost is between $10,000 and $20,000 per tank. As the inventory of contaminated sites in industrialized countries (Table 1.1) expands into a health hazard, nations are redirecting resources toward remediation efforts (Hrudey and Pollard, 1993).

Table 1.1: Contaminated sites in some industrialized countries (Hrudey and Pollard, 1993)

Country	Contaminated sites
Canada	10,000
United States	33,000
United Kingdom	100,000
Netherlands	110,000
Germany	100,000
Norway	2,441
Finland	20,000
Denmark	20,000

In the past, inadequate management of point source pollutants has sometimes exposed society to hazardous substances which have been disposed without regard to public health or environmental effects (Hrudey and Pollard, 1993; Tadesse et al., 1994). For instance, excavation and removal to a burial location is the most common method of remediating contaminated soils. Cases are reported of leaking and contaminating leachate from these burial sites.

Recently, Hrudey and Pollard (1993) reported that remedial action has shifted to treatments that reduce the volume, toxicity and mobility of the

contaminants permanently; thus avoiding the cost and risks associated with excavation and relocation. It is further stated that in-situ remedial actions are generally preferable, and it is doubted that any single method can handle the cleanup of contaminated sites, as each site dictates its own treatment requirement.

A treatment process, consisting of different methods, such as biological and physical, is advocated for heterogeneous constituents and non-uniformly distributed contaminants so that the remediation goal can be accomplished within a given time. This part of the study deals with sites contaminated with petroleum hydrocarbons. It will focus on developing a method for the remediation of diesel contaminated soils.

Currently, available technology for remediating contaminated soils includes burying in landfills or thermal, physical and biological treatments. However, biological treatment employing ex-situ (on-site/off-site treatments which may involve the use of either bioreactors, biopiles or land farming), or alternatively in-situ bioremediation is the method of choice. The in-situ method is preferred over ex-situ, although there is a lack of technology to ascertain optimum conditions for in-situ remediation in the field. A delivery system is needed that could supply nutrients, oxygen and moisture throughout the soil profile in order to optimize the physical, chemical and biological conditions that eventually accelerate the bioremediation process.

1.2.1 Problem definition (In-situ bioremediation of hydrocarbons)

The major sources of environmental pollution include: wastes, industrial landfill sites, petroleum operations, coal gas production/utilization, mining industries, military activities and accidental spills of industrial chemicals. The U.S.A. is currently spending approximately $10 billion annually on the cleanup of contaminated sites (Bredehoeft, 1994). Some of the contaminants consist of compounds such as polycyclic aromatic hydrocarbons (PAH), which may be carcinogens and may also resist bacterial mineralization or may be physically, chemically or biologically transformed to more hazardous compounds, and thus cause environmental pollution.

The health risks, posed by these contaminants, resulted in the development of remediation methods (physical, chemical and biological) for contaminated sites. Currently, contaminated soil may be cleaned in four possible ways: thermal treatment which needs off-site transport and involves risk and is also quite expensive; burying in landfills which is a temporary solution and requires long-term monitoring of the site; physical treatment methods which include washing the contaminated soil and are also very expensive options; and bioremediation which employs the use of indigenous or implanted microorganisms to remediate contaminated sites. The bioremediation option can be effected either on-site/off-site or, better still in-situ.

Recent trends in hydrocarbon contaminated site remediation focus on on-site and in-situ treatment techniques linked to a process train (e.g. sequential combination of biological and physical methods of soil treatment) that can handle heterogeneous mixtures and sites (Sims, 1990). Sims (1990) stated that the process train (a combination of different methods applied to a specific cleanup operation) might include physio-chemical and/or biological methods.

The biological method components of the process train may require biostimulation, bioaugumentation and maintenance of the resident microbial populations of the contaminated soil. More specifically, an in-situ bioremediation operation requires that all additives, including nutrients and oxygen, should be delivered to the subsoil in a controlled closed-loop system (Hopper, 1989). Lee et al. (1988) stated that the main constraint of in-situ bioremediation is the inadequate nutrient delivery system to the subsurface microorganisms.

Oxygen delivery to subsoil is either by diffusion from the atmosphere or via the oxygen dissolved or bonded in the percolating nutrient. Oxygen is necessary in the microbial metabolism as an electron acceptor, especially at the initial stages of oxidation of hydrocarbons. Thereafter, the degradation process can progress slowly using bound oxygen in compounds which include nitrate and sulphate as an alternate electron acceptor. Oxygen is

preferable to other electron acceptors since its utilization is favoured thermodynamically. The amount of oxygen, supplied by unaided diffusion, readily depletes because of increased metabolic activity in the subsoil. Therefore, less oxygen implies a decrease in microbial oxidation of the contaminants, consequently reducing the rate of biodegradation.

The slow degradation of xenobiotics, imposed by oxygen limitations, may be overcome by injecting hydrogen peroxide via pipes into the subsoil. However, injection wells have been abandoned before completing some in-situ bioremediation projects, because of biofouling that bioplug the wells (Shouche et al., 1994).

Thus, in-situ bioremediation requires the sustenance of microorganisms with adequate nutrients, to permit break down of the contaminants, if other environmental factors relevant for microbial growth are present. Although biological degradation of contaminants is effective and more natural than other soil remediation methods, some problems must be addressed by researchers (Alexander, 1991). Such problems include ensuring contaminants are bioavailable to the degrading consortium organisms (Hrudey and Pollard, 1993; Alexander, 1991). Compounds that would have been easily degraded in subsoils are not readily bioavailable because they are strongly adsorbed to the soil. Alexander (1991) indicates that the polluting compounds could be sorbed, dissolved in non aqueous-phase liquids, or in

a physically inaccessible state difficult for microbial enzymatic attack. He suggests that the behaviour of some pollutants in the soil indicates a need to design processes to overcome poor bioavailabilty. It is also asserted that a good design process should overcome scale-up problems, from successful laboratory bench experiments to field in-situ bioremediation operations (Alexander, 1991).

Another identified problem area is the poor availability, or non uniform distribution of nutrients and oxygen, to microorganisms in the subsoil. Achieving optimal nutrient conditions in the subsoil is very difficult. For example, in most ex-situ bioremediation practices, the contaminated soil is uniformly premixed with nutrients before placing the conditioned contaminated soil on biopiles for treatment. After setting up the biopile or spreading the soil on the farm, maintenance of the soil moisture content is effected by sprinkling water on the surface. Frequent tilling allows for oxygen diffusion into the biopile or land farm. The design of ex-situ bioremediation operations provides for enough water/organic interfaces, which makes the contaminants accessible to the microorganisms.

Current in-situ treatment provides nutrients and oxygen, injected via wells which flow by gradient or applied suction, through the contamination plume or through the contaminants present in the bulk organic phase compartments (non aqueous phase liquids). However, the hydrophobic nature

of the non aqueous phase liquid pockets diminishes the water/organic interface, where most microbial activities occur. The low water/organic interface imposes a limitation, on the mass transfer of low soluble contaminants to the aqueous phase, for enzymic transformation. Therefore, it takes a long time for "proper clean up" of contaminated soil.

To accelerate biodegradation by microorganisms, nutrients and oxygen should also be made available just as in a laboratory bench bioremediation setting. The nutrient supply design must encourage the simultaneous or alternate aerobic, anaerobic and cometabolic biotransformation of the pollutants. The design should also increase the water/organic interface, and encourage formation of biosurfactants (if none are added). This will improve transfer of contaminants from the organic phase to the aqueous phase. The processes, when systematically manipulated, will culminate in a large contaminant mass transfer to the aqueous phase.

Together with these challenges for in-situ bioremediation, there is a demand for a delivery system to uniformly deliver nutrients, including, oxygen, in the soil profile. Thus, microbial metabolism may be accelerated and successful bioremediation effected. Subirrigation systems, used exclusively in agriculture, could be employed to ensure uniform distribution of nutrients and oxygen. Subirrigation is practiced in humid regions of North America and some parts of Western Europe, in order to irrigate below the

surface with a subsurface drainage system. In this study, the use of the subirrigation system is proposed, as an invaluable tool for organic waste in-situ bioremediation protocol, for hydrocarbon contaminated sandy soils. This will achieve uniform delivery of surfactant, nutrients, and oxygen to any desirable depth, while also protecting any leachate from reaching the ground water. The large water body, together with water table management, will provide sufficient contact between non-aqueous phase liquids and microorganisms.

The in-situ treatment cell/site design will be a closed system, allowing for the recycling of effluent; it will be possible to adequately adjust the pH and the temperature of the subsoil to mimic laboratory conditions. Frequent lowering and raising of the water table, and continuous passing of air (when fully saturated) through the treatment cells, will slowly etch or emulsify low soluble pockets of non-aqueous organic compounds. The changes in the level of water table (by raising and lowering it) will also help redistribute both nutrients and microorganisms within the soil profile (Shouche et al., 1994). It is anticipated that the proposed laboratory delivery design can easily be "scaled up" from a laboratory to a field operation.

It is also proposed that the design will have application as a preventive biological seal, for under ground storage hydrocarbon tanks and other such facilities which are prone to accidental leakage. It has the

advantage of installation and operation, beneath already leaking underground storage facilities, without tank excavation. Channelling for subirrigation network under the tanks could be done with water or horizontal drilling. The method could be used as a preventive system for new sites, where it could be installed prior to the construction or burying of the storage tank.

The hypothesis in the second part of this research is that delivery and availability of nutrients, and bioavailability of contaminants in the subsoil increases microbial metabolism. Therefore, proper manipulation of these three factors will accelerate the biodegradation of soil organic contaminants. Thus, the delivery system in a confined field situation will be designed to simulate laboratory conditions, by uniformly distributing nutrients and maintain optimal growth conditions in the subsoil, which will enhance the microbial metabolism and consequently improve in-situ bioremediation.

1.2.2 Objectives (In-situ bioremediation of hydrocarbons)

The second major objective of this study is to develop a biological in-situ pollution control technique, for hydrocarbon-contaminated soils, using subirrigation as the delivery system to enhance in-situ bioremediation of a diesel contaminated soil; and to understand the method and management of such a system, which delivers nutrients and oxygen for in-situ bioremediation of a diesel contaminated soil. More specifically, the objectives are:

1. To evaluate water table management as a system to improve the efficacy of an in-situ bioremediation process, by conducting a laboratory study on long soil columns and measuring the diesel total petroleum hydrocarbon (TPH) loss with time in the soil.
2. To evaluate the sustenance of the system for complete soil remediation; to verify its effectiveness as a method of delivering nutrients and oxygen for in-situ bioremediation.
3. To evaluate some of the possible management alternatives of the system in terms of stepwise and full column treatments.

The above objectives were accomplished using a diesel contaminated sandy soil, packed in columns, and subirrigated with water containing essential nutrients required to biostimulate microbial degradation of the diesel hydrocarbon. Performance of the delivery system was assessed by monitoring the decrease, with time, of total petroleum hydrocarbon (TPH) in the contaminated soil. Diesel oil contamination of soil is an environmental concern, since it is reported in Song et al. (1990) as having the highest content of PAH and total aromatics compared to other fuels used on land.

This entire study focusses on developing an in-situ method of bioremediating nitrate and diesel contaminated soils. Chapter 2 includes a literature review on methods of remediating nitrate and hydrocarbon contaminated soils. Emphasis is placed on in-situ methods and their

limitations. Chapter 3 describes the experimental methodology, and chapter 4 presents the results and discussion. The summary and conclusions are presented in chapter 5.

1.3 Scope of the Project

A nutrient delivery system, for in-situ bioremediation of nitrate fertilizer residue and diesel contaminated soils, was developed in the laboratory using packed soil columns. The results apply to sandy soils with a history of elements that are not inhibitory or toxic to microorganisms. Samples and data collections were restricted to laboratory conditions. Efficacy of the delivery system was determined by monitoring the decrease in contaminant concentrations, in solution and soil for the nitrate and diesel studies, respectively. Nutrient distribution in the soil profile was assessed by comparing reduction of contaminant with time and depth, and also by measuring soil profile N and P (for the diesel study). Nutrient levels were not optimized for diesel biodegradation using the delivery system, and ecotoxicological tests were not employed to assess ecologically significant toxicity of bioremediated soils. Also metabolites of contaminants were not monitored. The results obtained applied to column conditions where the water table was supported by an impermeable base or layer.

CHAPTER 2: LITERATURE REVIEW

This chapter reviews the literature on bioremediation of fertilizer-nitrate and hydrocarbon contaminated soils. It is divided into three major sections, covering: methods of controlling nitrate pollution in farms, remediation of hydrocarbon contaminated soils and finally the concluding remarks on the literature review.

2.1 In-Situ Bioremediation of Nitrate Residue

This review emphasises the role of nitrate in boosting agricultural production, together with the health and environmental dangers resulting from excessive, yet unavoidable, use of nitrate in agriculture for improved crop yield. The sections below refer to: the importance and sources of nitrate, its fate in the soil, and some available methods of controlling nitrate residue in the soil.

2.1.1 Importance of nitrate and its sources

Increase in population and industrial activities have caused the diversion of most agricultural lands for habitation and other societal needs. As a result there is less land for farming but more mouths to be fed. Hence,

limited arable lands are being subjected to intensive cultivation, to ensure an increased crop yield and to reduce production cost also.

To achieve higher crop yields, external chemical nutrients in form of fertilizers, e.g., nitrate-fertilizers, to boost agricultural production have been introduced. Unfortunately, farmers often oversupply nitrate fertilizers as an insurance for a better yield (Addiscott et al., 1991). The wanton use of the chemical (fertilizer-nitrate), therefore, has caused accumulation of excess nitrate residue in the subsoil. The accumulation of nitrate in the subsoil is even more severe in areas with intensive cropping and livestock systems (especially confined animal operations such as dairies). Intensive agricultural operators augment nitrate-fertilizer application with an unlimited amount of organic manure from agricultural wastes. Unlimited application of organic manure further increases the organic nitrogen budget of the soil (Sharpley et al., 1993). Microbial processes in the soil subsequently transform the excess organic nitrogen into nitrate, thus increasing the soil nitrate residue.

2.1.2 Fate of fertilizer-nitrate residue

Nitrate applied to the soil may be subjected to either one or a combinations of the following (Paul and Clark, 1989; Payne, 1981):

(i) General biological uptake which decreases the level of nitrate in the soil. The amount of nitrate taken up by the biota depends on, among

other factors, the carbon nitrogen (C:N) ratio requirement of the species in the soil.

(ii) Incorporation of the nitrate (assimilatory pathway) into the soil biomass and finally soil organic matter, until the microorganisms release the immobilized nitrate to the soil by the process of mineralization. Immobilization by assimilation of nitrate during denitrification, under anaerobic conditions, usually is not significant at high concentrations of nitrate.

(iii) The microbial denitrification process (dissimilatory pathway) reduces the amount of nitrate remaining in the soil to nitrous oxide and dinitrogen gases according to the following process:

$$2NO^-_3 \rightarrow 2NO^-_2 \rightarrow [2NO] \rightarrow N_2O \rightarrow N_2$$

The ratio of nitrous oxide to dinitrogen produced depends on the soil chemical properties, including: redox potential, moisture content, nitrate and oxygen concentrations, and time of day (Tate, 1995). Letey et al. (1981) found in their incubation study that, at a redox potential higher than 300 mV, no denitrification occurred. Nitrous oxide production was shown to be time dependent; and at 200 mV, in the absence of oxygen, nitrous oxide reached its maximum in approximately three days, and decreased to zero in five to seven days

after initiation of incubation. It was pointed out that the rate of nitrous oxide emission from land surface depends on: the rate of nitrate reduction to nitrous oxide, the rate of nitrous oxide diffusion to the atmosphere, and the rate of reduction of nitrous oxide to dinitrogen. The study showed that, after appropriate incubation of the soil, nitrate was completely converted to dinitrogen and nitrous oxide was not formed. An earlier study, however, suggested that nitrous oxide is not reduced to dinitrogen until most of the nitrate in the system is consumed (Cooper and Smith, 1963). Also, nitrate at high levels encourages the production of nitrous oxide (Paul and Clark, 1989).

Studies suggest that the soil may serve as a sink for nitrous oxide rather than as a source, under prolonged anoxic conditions (Blackmer and Bremner, 1976, Letey et al., 1981). Letey et al. (1981) showed that, under fluctuating redox potentials (wetting and drying cycles), the rate of emission of nitrous oxide was higher than under a continuously low (wet-to-saturated conditions) redox potential.

As cited by Tate (1995), not all nitrous oxides result from the activity of denitrifiers. Nitrifiers and some other soil organisms can also produce nitrous oxide as a metabolic product or by-product.

(iv) An alternative fate of nitrate residue in the soil is that it leaches beyond the root zone into the subsoil. The leachate eventually reaches

the subsurface drains, and subsequently the surface water bodies, where it contributes to algal bloom and consequent eutrophication of the water bodies (Cooper, 1993). The nitrate leachate may also contaminate ground water.

Leaching of the applied nitrate depends upon timing of rainfall events after application (Bengtson et al., 1988), and also on irrigation scheduling. In addition, soil properties, such as infiltration rates and soil drainage, affect downward percolation of nitrate. The ability of the percolating nitrate load to cause a hazard depends on how fast the load reaches shallow ground water or the drain tiles that transport the nitrate load to surface water bodies. The depth of the water table also dictates the pace of groundwater contamination by the leaching nitrate.

2.1.3 Methods of controlling nitrate residue

Nitrate is harmful to man when ingested above the 10 mg/L NO_3-N (nitrate-N) limit. The most affected are communities with water supplies (wells) from a shallow aquifer contaminated by nitrate. Fletcher (1991) states that when the contamination results from diffused land mass usage for agriculture, it is virtually impossible to treat the ground water either technically or economically, or both. It is further stated that when well-water

users have high nitrate in their water, the alternatives are bottled drinking water or the location of a new source of water supply. Since agricultural nitrate contributes to the eutrophication of water bodies and the contamination of ground water, it is inevitable that practices will be adopted which relieve the hazard posed by nitrate to the environment. Methods for controlling nitrate residue in the soil include: fertilizer management and public policy, crop management and water table management as detailed below:

2.1.3.1 Fertilizer management and public policy

Lee (1992) outlines some farm-management practices and public policy options that can prevent excessive accumulation of nitrate in the subsoil; also measures are presented that can prevent nitrate from reaching drain tiles or ground water. The farm management guiding principle is reduction, to its lowest level, of the amount of nitrate in the rooting zone, especially during periods when leaching is likely to occur (Keeney and Follett, 1991).

Minimizing the amount of nitrate in the root zone can be achieved by: multiple fertilizer applications, employing cover crops or deep rooted crops, use of slow-release fertilizer, and proper irrigation scheduling that will not allow applied nitrate to leach beyond the reach of the plants, etc. (Keeney

and Follett, 1991). Though nitrate budgeting, for effective nitrate pollution management, might be adequate, some farmers apply more than the recommended optimum nitrate rates. (Their action presumably "assures" them that any low yield cannot be attributed to inadequate supply of nitrate nutrient to the crops). The other method of nitrate control is the public policy options. These include strict compliance regulations, pollution taxes and subsidies to encourage less pollution (Lee, 1992). However, such options are difficult to enforce.

2.1.3.2 Crop management

The crop management (crop rotation, cover cropping and multiple cropping) assists in limiting nitrate loss in drain water, since the cropping practice may reduce the amount of nitrate escaping from the root zone. Cropping practices for nitrate reduction in the drain outflow include: intercropping, crop rotation and use of catch crops (Russelle and Hargrove, 1989; Schepers and Fox, 1989). For example, corn has a limited root system that cannot easily intercept percolating nitrate in the soil. In contrast, the alfalfa plant has a more extensive and deeper root system that can intercept the nitrates (Owens, 1990); thus, the alfalfa root system retains the nitrate within the root zone, permitting more efficient nitrate uptake. The simultaneous or alternate cropping of the two crops might therefore reduce

the nitrate loss. Unfortunately, success in controlling nitrate leaching by cropping practices is limited because leachate still contains high levels of nitrate, beyond that of root system absorption. Leachate under corn (*Zea mays L.*) may have 5-120 mg/L NO_3-N (Gast et al., 1978; Logan et al., 1980). Corn rotation with oat (*Avena sativa L.*) or soybean (*Glycine max L.*) produced 10-70 mg/L NO_3-N in leachate (Baker et al., 1975). In a multiple cropping system, the leachate contained 3-51 mg/L NO_3-N (Zwerman et al., 1972); thus indicating the inefficiency of cropping practices for nitrate loss control in the soil.

Appropriate fertilizer rates and timing, which match crop uptake, usually reduce nitrogen transport and leaching (Evans et al., 1989), especially on coarse textured soils. However, the application timing and low rates necessary for economic crop production still result in a significant amount of nitrate loss to the surface and/or ground waters (Skaggs et al., 1994). Besides the above agricultural management practices, used to reduce nitrate pollution, water table management (WTM) has had a great impact on both surface and ground water quality.

2.1.3.3 Water table management

WTM reduces the amount of agrochemical pollution reaching the

ground water, since WTM involves controlled drainage (CD) and controlled drainage-subirrigation system (CD/SI). Both systems use submerged drain pipes to modify moisture status of agricultural plots. In CD, rain water is not allowed to leave the soil profile, thus raising the water table elevation. In contrast to CD, SI involves pumping of water into the field through the subsurface drains.

More specifically, subirrigation involves submerging drain outlets with water so that the water table rises in the field to a height that is adequate for moisture supply to crops. The water body/column within the farm plot can be maintained by a trenchless installation of a vertical plastic barrier, to a depth of two meters below the soil surface (Madramootoo et al., 1994). The system is designed such that, in heavy rainfall or storm events, there are drain outlets that allow excess water to drain off the field. While the field is draining, the WTM simultaneously maintains the water table at a desired height. Sometimes, lowering of the water table assists quick drainage of the plot that is under subirrigation (Skaggs, 1979).

Controlled drainage-subirrigation or controlled drainage uses flashboard risers to raise the water level (Gilliam et al., 1979). The raised water level then initiates the anaerobic condition, suitable for denitrification. Controlled drainage-subirrigation systems have been particularly effective in pollution control of regions with high water table conditions (Wright et al.,

1992; Gilliam and Skaggs, 1986; Gilliam et al., 1979; Skaggs and Gilliam, 1981). It is reported that about 50% of nitrate-N reduction in drainage waters may be obtained using flashboard risers, during the fallow winter months in North Carolina (Gilliam et al., 1979).

The extent to which subirrigation affects the reduction of the nitrate load in the soil is dependent on the depth of the water table. A shallow water table results in low nitrate concentrations in the drain effluent (Kalita and Kanwar, 1989 and 1993). However, shallow water table management is not always a suitable method for decreasing the nitrate residue in the soil, since not all crop types thrive well under shallow water table management.

Evans et al. (1989) attributed about a 45% loss of nitrate-N, under controlled drainage water table management, to denitrification. This report is consistent with other findings, stating that denitrification is largely responsible for the removal of most NO_3-N contained in leachate from agricultural land (Daniels et al., 1975; Gambrell et al., 1975; Jacobs and Gilliam, 1985). This microbial denitrification process occurs because of existing anaerobic conditions, established by WTM, in the presence of an available organic carbon, and favourable pH etc.

Microbial oxidation of organic carbon provides energy for growth in the soil. The energy results from electron transfer via a series of intermediates to a terminal acceptor (Paul and Clark, 1989). The organic

carbon provides electrons for the electron transport chain, while oxygen is the most important electron acceptor because it is easy to reduce. Also oxygen yields the highest energy, is inexhaustible in the atmosphere, and readily diffuses into the soil (Patrick and Jugsujinda, 1992). When oxygen is not available, under anaerobic conditions, other redox components, NO_3^-, Mn^{4+} compounds, Fe^{3+} compounds, SO^{2-}_4, and CO_2 serve sequentially as electron acceptors (Turner and Patrick, 1968).

Anaerobic conditions arise when the microbial metabolism depletes the dissolved oxygen present in a water-saturated soil. Water-saturation thus restricts further oxygen diffusion into the saturated zone. Consequently, oxygen limitation causes some microorganisms (denitrifiers) to use nitrate as an alternate terminal electron acceptor, in the electron transport chain responsible for energy production. This energy process results in the denitrification of nitrate.

The rate of denitrification varies in soils because of the existence of microsites which might contain varying amounts of organic matter and pockets of oxygen (Parkin, 1987). Once other factors are in place, organic carbon content in the soil is the key factor controlling denitrification rate.

It has been shown that the denitrification capacity of soils is highly correlated ($r=0.99^{***}$) to the available carbon in the soil (Burford and Bremner, 1975), and to the type of organic carbon (Bremner and Shaw,

1958a). Denitrification is more rapid with glucose and cellulose than with sawdust or lignin. The denitrification rate increases in the subsoil when soluble carbon increases (Weier et al., 1991), and it has been found to be more in the rhizosphere than other zones of the soil (Woldendorp, 1962). This is attributed to the abundance of organic matter at the surface, and within the root zone; however, organic matter decreases with depth (Kalita and Kanwar, 1993).

At low depths, microbial denitrification accounts for most losses of nitrate under a subirrigation method, and depends on the organic matter that leaches to the lower soil depths. Leaching and accumulation of organic matter at lower depths probably occurs before the introduction of subirrigation to the field. It is suspected that the organic carbon reserve at the lower depth might readily deplete with time (as denitrification progresses under subirrigation practice). Thus, the future supply of soluble carbon will depend on the decay processes on the soil surface. Nevertheless, the organic carbon supply from the surface to the lower depth may not quickly provide the organic carbon requirement for residual nitrate denitrification within a short time interval. Anaerobic conditions could exist at lower depths, but the rate of nitrate denitrification would be too slow or non existent. This condition arises from the absence or depletion of the readily available organic carbon (Kalita and Kanwar, 1993).

An Australian study (Myers and McGarity, 1971) stated that soil horizon A is high in organic matter and that soluble organic compounds leach from horizon A to horizon B, where the leached organic compounds accumulate. The B horizon, which is the subsoil below the A horizon, has little organic matter content, few plant roots, and small population of microorganisms compared to the A horizon, which is a reservoir of microbial food. The A horizon is rich in organic carbon coming from plant exudate and decaying plant debris. The A horizon is, thus, the source of readily available carbon which is transported by water down to the B horizon. The water table may be located in the B horizon or below it in the C horizon. The C horizon contains the parent material of the soil and has a very low organic matter content.

The extent of denitrification of nitrate, in either the B or C horizon, is determined by the amount of available organic carbon present in either of the horizons. Also, the ease of organic carbon replenishment, through leaching from the soil surface, will further decide the sustainability of the denitrification process at the B or C horizon. Table 2.1 shows a typical distribution of organic carbon, which decreases with depth, in the profile of three soil types from Iowa (Pearson and Simonson, 1939).

Organic carbon in the soil is exhaustible and can be reduced remarkably by microbial activities and cultivation, which enhances organic

matter consumption (Alexander, 1977).

Table 2.1: Organic carbon in several Iowa soil profiles

Soil Type	Depth (cm)	Organic C (%)
Carrington silt loam	0-15	2.40
	15-23	2.28
	31-38	1.43
	46-53	0.81
Grundy silt loam	10-15	2.84
	15-25	2.36
	35-46	1.64
Garwin silty clay loam	0-13	5.11
	13-35	1.74
	35-56	0.68

(Source: Pearson and Simonson, 1939).

He reported that the organic matter content of 28 soils in Georgia decreased from 3.29 to 1.43%, after 25 years of cultivation. It is, therefore, imperative to supply additional organic carbon to the soil if quick management is required to reduce nitrate residue in the subsoil. This is especially important below the root zone, during the fall, immediately after harvesting, or before the winter fallow season (Wright et al., 1992).

Although anaerobic conditions established by WTM is important for denitrification, its rate is also dependent on temperature described by Arrhenius equation (Troy et al., 1994):

$$K_T = K_{23°C}\theta^{(T-23°C)}$$

where θ is a temperature coefficient. The activity of denitrificants increases with increasing temperature within the range of enzyme activity.

The nitrate remaining in the unsaturated zone, after harvest, is responsible for the nitrate load in drainage waters from agricultural fields, during the fall season and the spring snow melt. In a particularly dry year, or when there is a poor nitrate uptake by plants, most applied nitrate remains close to the surface. This excess nitrate residue becomes a potential source of nitrate pollution in ground water and drainage water, during subsequent heavy rainfall events (Kalita and Kanwar, 1993), or during spring snow melt.

2.2 In-Situ Bioremediation of Diesel Contaminated Soils

In-situ bioremediation involves the stimulation of biological activity in the subsoil, causing microorganisms to biodegrade contaminants (GASReP, 1990). The GASReP (1990) reported a general principle indicating that all types of bacteria are available at all times everywhere; thus, if the right combination of circumstances is applied to the indigenous microbial population, their biological activity might be stimulated to degrade contaminants.

Methods for stimulating biological activity include: improvement and/or modification of geochemical or physical conditions, or addition of nutrients or microorganisms (GASReP, 1990). This study employs nutrient addition, in order to achieve stimulation of biological activity of the indigenous microbial populations, for the purpose of cleaning up a diesel contaminated soil. The literature review includes some non-biological methods of hydrocarbon remediation which are also useful to consider. The first section of the review deals with the feasibility of a contaminated site for bioremediation, while the second and third sections describe principles of biodegradation and methods of bioremediating contaminated sites, respectively.

2.2.1 Feasibility of in-situ bioremediation

In-situ bioremediation requires an initial confirmation of the possibility of modifying a hostile environment, in a practical and cost effective manner, before implementation (Kaufman, 1994). Some of the required information includes: identification, distribution and concentration of contaminants, characterization of the contaminated site, and assessment of environmental conditions such as pH, redox, moisture content and nutrient status, especially nitrogen and phosphorous needed by the microbial populations. The most suitable mode to biodegrade the contaminants (e.g. aerobic, anaerobic,

cometabolism and analog or gratuitous process) is also investigated before commencing the in-situ bioremediation project (GASReP, 1990).

Some of the site characteristics assessed include soil texture, permeability, and proximity of ground water to the contaminated soil (Kaufman, 1994; Norris, 1994). Permeability of the site affects the distribution of nutrients and electron acceptors, while the soil type influences the extent of sorption of both contaminants and nutrients by the soil. Sand and gravel soil textures favour nutrient and electron acceptor transport more than clays. Nutrient mobility is also restricted by the presence of high mineral content, especially of calcium, magnesium and iron, which may precipitate the nutrients. Another important site characteristics is the species of iron present. Ferrous ions consume oxygen in the soil, thus decreasing the redox potential of the environment.

The proximity of the contaminated site to ground water is given special consideration. In-situ soil bioremediation does not pose the problem of transferring the contaminants from soil to ground water, when both ground water and soil are being treated simultaneously. Lateral and vertical migrations of nutrients and contaminants can be tolerated during the treatment. But, when only the soil is contaminated, the risk of contaminating the ground water imposes a restriction on the use of in-situ technology (Kaufman, 1994).

Identification of contaminants involves a knowledge of their physical and chemical properties, which may affect their biodegradation. The characteristics assessed include: solubility, viscosity, sorption, volatility, solubility, biodegradability, toxicity, hydrolysis, photolysis, and chemical transformation.

When all the environmental site conditions and characteristics of the contaminants are favourable, the nutrient status determines the viability of the microbial populations of the site. Nitrogen and phosphorus are the major nutrients added to the soil to biostimulate the activities and possible growth of indigenous populations. Nitrogen is required mainly for protein synthesis and it is a constituent of nucleic acids. Sometimes, oxides of nitrogen are used as alternate electron acceptors in energy production.

Phosphorus (P) as phosphate is required for the generation of ATP (adenosine triphosphate) which is the energy storage and transferring compound of most biological systems. It is also a constituent of nucleic acids, phospholipids, etc. When phosphate is present in a high concentration, it may be inhibitory to microbial growth (Atlas and Bartha, 1987). Solubility and availability of phosphate are pH dependent, and its mobility in soils is limited. Its availability decreases below pH 5.5 and above pH 7.0 (Dineen et al., 1989). They also reported that the availability of phosphate was decreased by its precipitation as calcium and magnesium phosphates in a

southern California soil with a pH range of 7.5 to 8.5.

Substantial amounts of mineral nutrients (N and P) are required to enhance biodegradation of wastes containing high organic carbon. The required ratio of oily waste-carbon to fertilizer-N is cited by Rasiah et al. (1992) to vary from 17 to 161. A C:N and a C:P ratio of 10:1 and 100:1, respectively, were cited by Frankenberger (1991) as requirements for 100% conversion of petroleum hydrocarbons into microbial mass. Dibble and Bartha (1979) found a C:N ratio of 60:1, and a C:P ratio of 800:1. Dineen et al. (1989) reported that 1 kg of reduced nitrogen is required per 160 kg of hydrocarbon degraded. The composition of nutrient solution applied in field and laboratory bioremediation of contaminated soils varies among researchers. Aggarwal et al. (1991) cited about twelve different formulations which had different C:N ratios.

While nutrients (N, P) are necessary for the stimulation of microbial activity to degrade the organic contaminants, oxygen is usually the most limiting factor in the degradation process due to its poor diffusion from the atmosphere into the contaminated soils (Dineen et al., 1989). A most efficient delivery system is expected to overcome this limitation. In a well aerated soil, the oxygen level is about 20% or 200,000 ppm, while under saturated conditions, it is about 8 ppm. The level of dissolved oxygen can be increased up to 200 - 800 ppm using hydrogen peroxide dissolved in water

(Ward et al., 1989). Hydrogen peroxide is expensive and decomposes rapidly resulting in the loss of oxygen (Brown and Crosbie, 1994). To effectively deliver air in the vapour state, the permeability of the soil to air (K_{air}) (which is a function of soil texture, moisture, and bulk density) is important (Dineen et al., 1989). An in-situ permeability assessment can be conducted by monitoring the breakthrough curve of an injected sulphur hexafluoride tracer gas (Dineen et al., 1989). Oxygen is required at a rate of approximately 3 kg of oxygen per kg of hydrocarbon degraded (Dineen et al., 1989).

Lastly, biological treatability is affirmed at the beginning of any in-situ project and hydrocarbon degraders should be present in all of the area being treated (Dineen et al., 1989). In soils that have been contaminated over a long period of time, the soil microbial ecology adjusts such that the indigenous microorganisms increase their petroleum degraders by 100 to 1,000 times from 0.1 to 1.0% of 10^7 to 10^9 microorganisms per gram of surface soil (Bossert and Bartha, 1984).

2.2.2 Principles of biodegradation

Understanding the fundamental principles of biodegradation helps to evaluate current practices in bioremediation of contaminated sites (GASReP, 1990). Biodegradation is the ability to biologically decompose/mineralize organic materials such that the residues are non toxic and will not

accumulate in food chains (Jones et al., 1990). A general process equation for biological degradation of a substrate is given as:

$$Substrate + Electron\ acceptor \rightarrow Biomass + CO_2 + H_2O + Residues$$

For an environmental polluting substrate, the residues and their characteristics determine the extent of degradation and detoxification of the contaminant. In bioremediation, as the term implies, the polluted environment should be "cured" of the contamination.

The substrate in this study is diesel oil which consists of 53.7% saturates, 45.0% aromatic, and 1.3% polar compounds, and has a carbon range between C_9 to C_{23} (Song et al., 1990). Diesel oil has the highest aromatic contents (including polyaromatic hydrocarbon (PAH)) compared to other fuels used in the terrestrial environment (Song et al., 1990). Because of the carcinogenic property of PAH and their relative persistence in the soil, diesel spill is treated as an important terrestrial pollution. The biodegradation residues from diesel substrate are not toxic as per Ames nor Microtoxicity tests (Wang et al., 1990), therefore, a diesel contaminated soil can be remediated.

The other form of degradation by microorganisms, called biotransformation or bioconversion, results in toxic or non toxic modification to another compound. Both biodegradation and biotransformation may occur simultaneously or sequentially. The environmental conditions required for

successful biodegradation or biotransformation include both aerobic and anaerobic conditions.

Biodegradation by microorganisms is enzymic and can take place aerobically or anaerobically. Aerobic biodegradation is characterized by the oxidation of reduced compounds, such as hydrocarbons, to produce energy by electron transfer along the electron transport chain to the final electron acceptor, molecular oxygen (GASReP, 1990). Similarly, anaerobic metabolism produces energy by oxidation reduction reactions, but uses alternate electron acceptors such as CO_2, SO_4^{2-}, and NO_3^- as the terminal electron acceptor (GASReP, 1990).

While all kinds of hydrocarbons (aliphatic branched and unbranched hydrocarbons, cyclic hydrocarbons and aromatic hydrocarbons) are easily biodegraded aerobically, halogenated hydrocarbons tend to persist under aerobic environments (GASReP, 1990). However, they are dehalogenated under anaerobic conditions. Thus, complete biodegradation of such halogenated hydrocarbons often requires sequential anaerobic/aerobic conditions.

Aerobic and anaerobic metabolism utilize hydrocarbons as a primary or secondary substrate for supply of energy and carbon. In some cases, specific enzymes (induced only in response to the presence of their analogue compounds) are required for degradation of a particular hydrocarbon

(xenobiotic). This is referred to as analogue or gratuitous degradation (GASReP, 1990). Degradation of xenobiotics has also been accomplished by cometabolism. This involves simultaneous/gratuitous biotransformation of the xenobiotic compound without deriving energy, carbon or any other nutrient from the transformation (GASReP, 1990; and Vogel et al., 1987). All the microbial metabolic processes described can occur simultaneously or alternately in any bioremediation project.

2.2.3 Monitoring bioremediation

The main requirements for bioremediation are conditions that allow for the biodegradation of contaminants; these include: a supply of oxygen, nitrogen and phosphorous as nutrients, the adjustment of temperature, pH and moisture content to stimulate activities or growth of microorganisms needed for biodegradation (Blackburn and Hafker, 1993). Sometimes, the appropriate microorganisms are added to further accelerate the biodegradation process (bioaugmentation). Surfactant may also be added to improve the bioavailability of contaminants. The biodegradation process is then monitored to assess its effectiveness. Monitoring strategies include soil sample analysis before, during and after treatment. Components analyzed include:

1. TPH level.
2. Nutrients and dissolved oxygen supplied to the soil.

3. Microbial population. Their enumeration (hydrocarbon-degrading bacteria) gives information on the presence or absence of the bacteria, but not on their activity or non-activity. GASreP (1990) advocates caution in the interpretation of biomass increase during bioremediation, since the increase could be due to sample variation. Furthermore, the report indicated that microbial activity does not necessarily imply growth since levels of contaminants or metabolites can activate or inhibit bacteria, and when the bacteria is cultured in the laboratory, the inhibition is often removed, allowing the bacteria to grow.

4. CO_2 evolution. Compared to biotransformation that might also reduce both the toxicity and concentration of contaminants, this shows the extent of mineralization of the contaminant.

Methods of confirming biodegradation include: the mass balance of contaminants, nutrients and end products; the presence of expected end-products and/or intermediates; the utilization of electron donor/acceptors; an increase in biomass; and the activity of hydrocarbon degraders. Control experiments are also used to confirm biodegradation.

2.2.4 Remediation methods for contaminated soils

Treatment methods for contaminated sites may be classified into three

broad headings: namely containment or immobilization, mobilization and destruction. Any of these methods may be performed in-situ or ex-situ (Hrudey and Pollard, 1993). Ex-situ treatment involves excavating the contaminated soil and transferring it to another specially prepared site, for treatment or permanent storage. The receiving site for an excavated soil may be a prepared bed (land fill, biopile or in-tank reactor for soil washing or destruction (incineration or biodegradation)). In-situ bioremediation involves the installation of pipes, for nutrient delivery in the subsoil.

2.2.4.1 Containment or immobilization

Containment or immobilization (Hrudey and Pollard, 1993) of contaminated soil involves the isolation of the contaminated site by capping or covering the site with a solidification/stabilization material, such as cement or sometimes only with fresh soil.

Some drawbacks of the land fill method of disposal include: identification of a suitable burial site, scarcity of fill materials, traffic movement, noise, and atmospheric and surface water pollution (Anon. 1990). Land burial also requires long term monitoring of leachate in the ground water. Hrudey and Pollard (1993) stated that containment/immobilization did not eliminate the hazard posed by the contaminant. Thus, it is asserted that isolating, concentrating, or relocating a contaminated soil provides only a

short term remedy since the environmental risks posed by the contaminant remain over a long term.

2.2.4.2 Mobilization and destruction

Mobilization technology of soil contaminants includes: the "pump and treat" method for ground water, soil vapour extraction, soil heating and soil washing (Hrudey and Pollard, 1993). Soil vapour extraction and/or soil heating is suitable for volatile and low water soluble contamination. It is difficult to apply vapour extraction and soil heating to a soil contaminated by hydrocarbons with high boiling points. Soil washing or flushing has been applied to water soluble contaminants, but for less water soluble hydrocarbons, surfactant added to the wash solutions help dissolve the contaminants. Recovery of wash solution and contaminant is by the "pump and treat" method. Sometimes, groundwater contamination occurs because of poor recovery or lateral and vertical seepage during an in-situ soil washing operation.

Destruction, involving incineration (thermal destruction) or bioremediation, leads to complete removal, or an exceptionally high reduction in environmental risks posed by contaminants in the soil (Hrudey and Pollard, 1993). Thermal destruction is an in-tank method requiring excavation and high temperature treatments for the incineration of the

combustible hydrocarbon. Its major disadvantages include the cost of heating voluminous inorganic soil containing very little organic matter (Hrudey and Pollard, 1993). The emission of particulate matter during burning or incineration is of concern as well. The particulate matter emitted contaminates the atmosphere (Sims, 1990). The bioremediation option (as means of destruction) is most recommended, since it involves microbial degradation of the contaminant.

Bioremediation exploits microorganisms to detoxify or degrade the contaminant. This may be effected under aerobic and anaerobic conditions. Major requirements for effective bioremediation are: a biodegradable organic substrate, an appropriate and active microbial community (consortium), bioavailabilty of the polluting substrate to the microorganisms, and the creation of optimal conditions for microbial metabolism (Hrudey and Pollard, 1993). Sometimes, bioremediation requires further biostimulation with nutrients or some specific analogue substrate; it may also require bioaugumentation of the microbial community if the site does not have an appropriate indigenous biodegrader population. The biodegradation of the contaminant is effected by complete mineralization or biotransformation into non toxic, less toxic, or more toxic daughter compounds. Sometimes, the biotransformation by-products polymerize and/or react with humic substances to become recalcitrant and therefore persist in the environment for a long

time.

Land-farming is the simplest aerobic biodegradation (destruction) technique where the contaminated soil is spread on an agricultural field for biodegradation. Its drawback is the difficulty in optimizing the performances of the microorganisms; there is also the possible contamination of subsoil by leachate. This method also requires excavation, transportation, etc. (Sims, 1990). Other popular aerobic and anaerobic methods are mostly carried out on prepared beds or in tank reactors (Norris 1994; King et al., 1992).

In prepared beds, cell surfaces are lined with impermeable barrier boundaries before placing the contaminated soil in the cells. The soil is also conditioned by adjusting the pH and the nutrient status to optimal levels suitable for biodegrading the contaminants. Subsequent addition of supplementary nutrients to the soil may be carried out by sprinkling. Oxygen supply to the biopile is often by diffusion, aided by frequent tilling. Sometimes the prepared bed is instrumented with a network of pipes that receive drain effluent; these pipes are also often used for aerating the biopiles as well. Biopiling may be a treatment or a biopreventive measure; when used for prevention, there is usually no impermeable lining (Hater and Goldsmith, 1989).

Hater and Goldsmith (1989) have located prepared beds below storage tanks, which contain viable or dormant organisms, capable of degrading the

organic compounds of interest. Vertical pipes supply nutrients or nutrient vapours, including air and steam, to the prepared bed located below the ground. Distribution of nutrients and air in the contaminated zone is accomplished by a vacuum, applied on or close to the soil surface. The ground water is not protected from receiving seepage during nutrient addition, or if there is a rainfall event occurring within the treatment period.

This method of nutrient and air supply, via pipes, to prepared bed or biopile is also applicable to bioventing. In bioventing, vertical pipes carry nutrient vapour and air into the contaminated subsoil region below the contaminated zone, while vacuum suction applied to extraction wells, at different space intervals, forces the nutrient and air to diffuse across the contaminated region, before being drawn upwards. As the nutrient vapours and air (or steam) are infusing through the soil, low boiling hydrocarbons are stripped and recovered via extraction wells. All nutrients and additives cannot be supplied in the vapour phase. This poses a limitation to in-situ bioventing since non vapour nutrients are also required to biostimulate and to create optimal conditions for the biodegrading indigenous microorganisms. Another limitation, envisaged in bioventing, is the poor bioavailability of the organic contaminant to the microorganisms.

Bioavailability of contaminants to biodegrading organisms can be increased by allowing sufficient contact time between the contaminant and

the microorganisms. In the presence of optimal moisture content, some microorganisms produce biosurfactants that bring the organic compound into solution. Optimum moisture content, as in a bench slurry microcosm study, should make both transport and metabolism easy. Microbial uptake of substrate is more efficient in solution. However, in bioventing, poor contact time and insufficient dissolution of contaminants results in poor bioavailability of contaminants to the microbial degraders.

Hazen and Fliermans (1994) patented an in-situ bioremediation apparatus that can inject nutrient fluids periodically and oxygenated fluid continuously, via horizontal or vertical pipes, installed below a contaminant plume. The fluids are then drawn upwards or horizontally under suction, across the plume, so that the nutrient fluid stimulates growth of the indigenous microorganisms within the plume. The nutrient fluid is essentially methane or propane, utilized by methanotrophs. Other nutrients can also be incorporated into the nutrient fluid. The nutrient fluid specifically causes an increase in the indigenous methane degrading population. Afterwards, the nutrient fluid is stopped, allowing the increased population to starve and consequently forcing the organisms to cometabolize the contaminants in the plume. Constituents of the oxygenated fluid may be: air or oxygen-nitrogen mixtures, water vapour, or steam.

Methane is metabolized by specialized bacteria that cannot grow on

other simple compounds. Furthermore, the methane utilizing organisms are inactive to larger hydrocarbon molecules. However, certain mycobacteria are known to degrade both methane and other compounds (Alexander, 1977). Hazen and Fliermans (1994) suggested an in-situ biodegradation method which may be effective for the degradation of TCE (Trichloroethylene) contamination. It is doubtful whether the cometabolism process, by methane utilizing organisms, can alone effectively decontaminate a soil, polluted by a complex mixture such as diesel and crude oil at high contamination levels.

The degradation potential of the soil biota is not fully harnessed by using only methanotrophs, since the soil contains a diverse range of microorganisms having a high mineralization and biotransformation potential (Hrudey and Pollard, 1993). Thus, it would be unwise to overlook the degradation potential in other organisms, by only biostimulating the activity of methane cometabolic degraders.

Hrudey and Pollard (1993) further assert that microorganisms, adapted to degrade only a specific trace contaminant, may face competition from other organisms growing on the bulk of organic matter. This means that, although conditions are made favourable for the increase of methane degraders, other diverse organisms present in the same site may out-compete the methane degraders for other essential nutrients; thus prolonging the remediation time.

It is further indicated that complex mixtures of organic pollutants have been successfully degraded by using microbial consortia adapted to the pollutant. Use of all such adapted organisms ultimately reduces the lag time between enzyme production and actual degradation of the pollutants (substrate) (Hrudey and Pollard, 1993). Reduction in lag time reduces the in-situ bioremediation treatment time.

The apparatus, designed by Hazen and Fliermans (1994), did not make provision for the protection of ground water from leachate which are likely to result from a combination of rainfall and treatment of the soil above the ground water. Hazen and Fliermans (1994) patented method presumes that the contaminant plume is immobilized without the possibility of further lateral or vertical migration.

In another in-situ bioremediation method, the nutrients were introduced via pipes and they percolated through the soil profile, based on the geological gradient of the terrain (Molnaa and Grubbs, 1989). The percolating nutrients, and sometimes metabolic byproducts from contaminants, were recovered downstream via extraction wells. Since this method relies on the geological gradient, nutrient distribution may not be uniform because physical obstructions can divert the flow of the percolating nutrient away from densely contaminated regions. Some nutrients, including phosphates, are needed to optimize in-situ bioremediation and are not easily

mobile under normal gradient flow. In addition to poor distribution of nutrients in the soil for in-situ bioremediation, oxygen supply also limits microbial degradation of hydrocarbons in the soil.

2.2.5 Remediation costs

Factors that contribute to the cost of bioremediation include: site conditions, soil characteristics, mass and type of contaminant, depth to water table, remedial goals, the design of the system, operating and monitoring schedule, etc. (Norris, 1994). These factors also include types of nutrient and electron acceptors (oxygen or hydrogen peroxide or an alternative electron acceptor). The more oxygen supplied per unit of time, the greater is the contaminant load that will be treated (Brown and Crosbie, 1994).

In-situ bioremediation of a light petroleum product leakage at an underground site costed from one to 1.5 million dollars for a 0.2 to 0.4 ha field. The time frame for completion ranges from one to five years (Norris, 1994). An infiltration and recovery well on a 0.4 ha site contaminated by gasoline to a depth of about 3 m cost 250,000 US$. Another gasoline contaminated 0.1 ha site, also using an infiltration gallery and injection wells, costed about 600,000 US$ and needed a time frame of 1.5 years to decontaminate.

A breakdown of various technologies, including bioremediation, is

given in Tables 2.2 and 2.3 (Molnaa and Grubbs, 1989; Lemme, 1996; Brown and Crosbie, 1994).

2.3 Concluding Remarks

Our water resources are being contaminated by agricultural and industrial chemicals. Nitrate is one of the main agricultural contaminant. Without compromising crop yield, there is currently no cost-effective method of controlling this pollution. WTM has been recommended as the best management practice for the control of nitrate pollution. Water table management systems can reduce environmental pollution from nitrate due to increased denitrification. However, the pace of reduction is not sufficient to eliminate the risk associated with nitrate pollution. Denitrification of nitrate under water table management depends on the availability of organic carbon. When the available organic carbon is consumed, there is an inevitable decrease in denitrification, concomitant with an increased risk of high nitrate in our water resources.

Industrial chemicals, such as diesel from leaking storage facilities and other petroleum operations, are posing a significant threat to our water and soil ecosystems. Because of the health risks associated with diesel contamination, remediation methods have been developed to decontaminate

such pollution. In-situ bioremediation is a method that is currently used to treat diesel contaminated soils. The method requires the sustenance of microorganisms, aerobically or anaerobically, for detoxification of the contaminated soil. So far, this method has met limited success because of inadequate nutrient delivery systems, especially in deeper soil layers.

The major objective of this study is to develop a method of introducing nutrients to the subsoil for the biostimulation of the indigenous microbial population to bioremediate nitrate and diesel contaminants. The study will be on packed soil columns, engineered to simulate field conditions. The system's performance will be assessed by determining the reduction of the contaminants with time and depth.

Table 2.2 Operating and cost-effectiveness comparison for oxygenation of a highly contaminated matrix

System	Addition rate	Oxygen kg/day	% Site treated	System utilization efficiency	Treatment time (days)	Contaminant treated $/kg
In-well aeration	15 wells @ 0.06 m^3/min	2.7	57	70	1716	40
Water inject	270 Lpm	3.6	75	80	987	35
Vent/Sparge	4.5 m^3/min	1800	98	5	132	16
Peroxide	270 Lpm	86	95	15	330	24
Nitrate	270 Lpm[a]	96	85	13	335	36

[a] 454 L/min to ensure capture of added water.
Adapted from Brown and Crosbie (1994).

Table 2.2 contd. Operating and cost-effectiveness comparison for oxygenation of a highly contaminated matrix

System	Capital $	Operation ($/month)	Maintenance ($/month)	Total $
In-well aeration	35,000	800	1,200	150,000
Water inject	77,000	1,200	1,000	149,000
Vent/Sparge	94,000	1,500	500	103,000
Peroxide	30,000	10,000	1,000	151,000
Nitrate	120,000	6,500	700	200,000

Adapted from Brown and Crosbie (1994).

Table 2.3: Some technologies and costs

Treatment process	Cost ($) per $kg*10^3$
Landfill disposal fees	140 to 120
	+taxes
	+transportation
Mobile incineration	150 to 400
Stabilization/fixation	100 to 200
Bioremediation	15 to 70

CHAPTER 3: MATERIALS AND METHODS

Pollutants in the subsoil, which are susceptible to biodegradation, might persist for a long time if optimal conditions do not exist to accelerate a passive rate of degradation. Passive degradation can be hastened by establishing optimal conditions, through the delivery of essential nutrients that will biostimulate the activities of indigenous microbial populations. Chapter 3 presents the procedures developed for the delivery of biostimulants to subsoil microorganisms. The chapter is divided into two sections, with section one describing the materials and methods for in-situ bioremediation of nitrate residue, while the second section describes the materials and methods for the in-situ study of diesel contaminated soils.

3.1 In-Situ Bioremediation of Nitrate Residue

This section describes the experimental design to test the effect of external organic carbon on nitrate applied to two sandy soils, packed in columns. The section is subdivided into parts that cover: soils used in the experiment, the design, fabrication and packing of the columns with soil, experimental stages (setups), and the sampling and the analytical methods used for assessing the effect of treatment on nitrate residue.

3.1.1 Soils used

Two sandy soils, St. Amable and St. Benoit (hereafter referred to as soil-1 and soil-2, respectively), excavated from two different sites at the Macdonald Campus farm, were used in this study. The topsoil (5 to 10 cm) was scraped off before excavation. The main differences between the soils were the organic matter content, determined on three samples of each soil, by loss-on-ignition method (Ball, 1964, Davies, 1974). The organic matter content of soil-1 was 3.5% while soil-2 had 1.6% organic matter. The saturated hydraulic conductivity of the soil was determined by the constant-head method (Klute, 1965) on each of the soil column (Table 3.1).

3.1.2 Column fabrication (nitrate study)

Eighteen steel columns, 1000 mm long x 198 mm inside diameter, were endcapped with steel plates and sampling ports were drilled on the side of the columns at 400, 600 and 850 mm depths from the top. On each column, a nutrient delivery/subirrigation port was drilled at 980 mm from the top, and a delivery pipe was installed. Slits of 2 mm width were made at 25 mm intervals on the delivery pipe to transfer water and nutrients into the soil column. Before packing the fabricated columns, a geotextile filter of 5 μm pore size was placed over the slit delivery pipe. A schematic diagram of the packed soil is shown in Figure 3.1.

Table 3.1: Soil Characteristics

Soil Type		Sand (%)	Silt (%)	Bulk density (gm/cm^3)	Organic matter (%)	*Hydraulic conductivity (mm/h)
St Amable	Sandy soil	91.2	4.2	1.4	3.5	77.6±33.7
St Benoit	Sandy soil	97.4	1.0	1.4	1.6	142.0±16.6

*Measurement on the soil columns

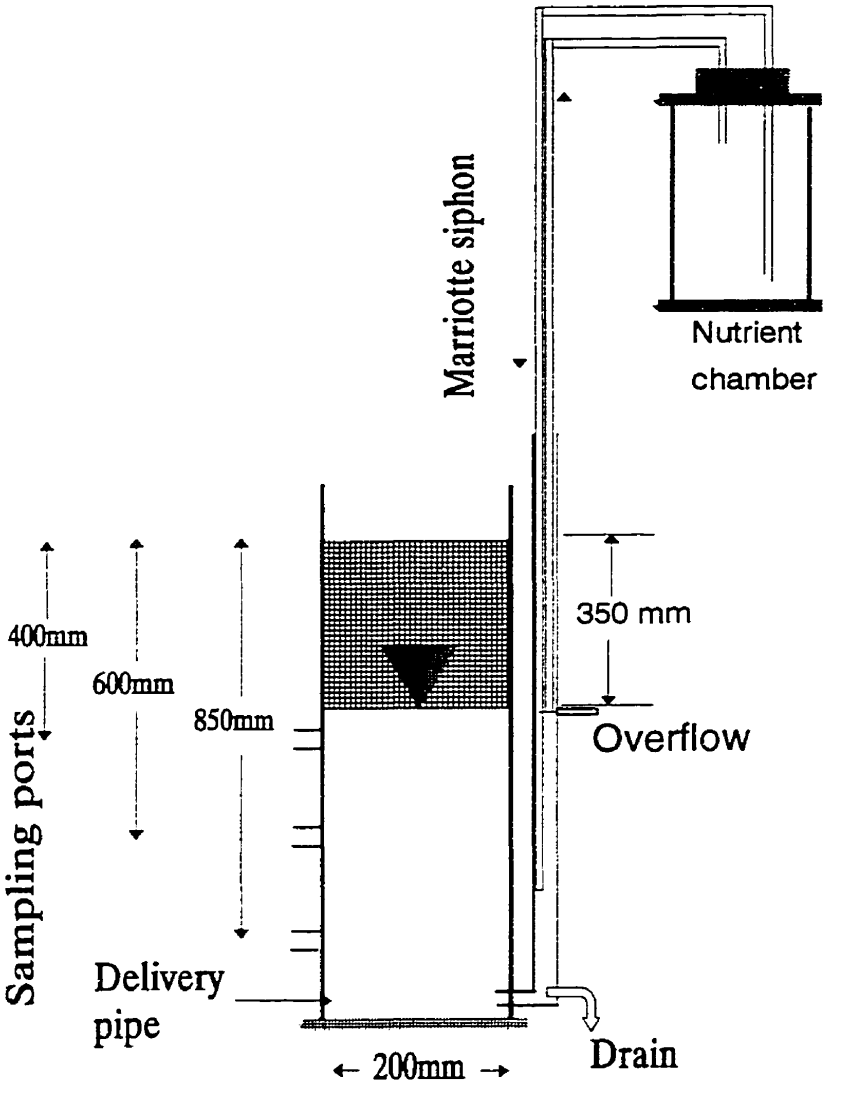


Figure 3.1: Schematic of a packed soil column for nitrate treatment.

Six columns were packed with soil-1, and the other twelve columns, with soil-2. All soil columns were packed to a bulk density of 1.4 gm/cm^3, similar to the bulk density found in the field. After packing the columns, perforated Teflon liquid sampling tubes were installed at sampling ports located on the side of each column.

A 1,000 mm-long riser, employed to supply nutrient and water to the soil column and to maintain water table at desired depths, was connected with an elbow to the nutrient delivery pipe. The other end of the riser was attached, via PVC tubing, to a 4-litre Marriotte bottle (nutrient or water) reservoir which was placed 300 mm above the columns. The columns were placed in a well-ventilated room (at a room temperature of 24°C). Heating lamps were installed 450 mm above each soil column to cause evaporation of water at the surface of the columns, thus inducing upward water flow in the columns.

3.1.3 Subirrigation of soil columns

Two treatments were used: one involving subirrigation with glucose solution, and the other with water only. Each treatment was randomly assigned to the columns. The influent flowed from a separate Marriotte siphon system, via the riser and the slit delivery pipes at the base of the columns, into the soil profile. The Marriotte siphon and the riser maintained

water level in the soil columns to any desired depth. The water table was kept at a depth of 350 mm from the surface at all stages; and water or nutrient continuously passed into the columns from the nutrient or water reservoir unless during simulation of some rainfall events used for leaching applied nitrate to lower depths. The reservoirs were replenished as the contents depleted.

3.1.4 Nitrate application and leaching

All the columns received fertilizer as calcium nitrate. About 4.7 g of calcium nitrate was dissolved in 50.0 ml of water and poured over a mesh of fibre glass, placed on the surface of each of the soil columns for uniform distribution and non-disturbance of the soil surface. The beaker was rinsed with 500 ml of water and poured over the soil surface.

Several rainfall events were simulated at different times to assist in the transfer of nitrate from the upper soil profile to the lower depths. Water application was arrived at considering that the amount of each rainfall in any given year cannot be predicted accurately. Rainfall events included (Smith et al., 1992): amounts (127 mm) equivalent to a once-in-25-year month of May in Montreal, the 103 mm rainfall depth represented a once in a 100 year storm such as that occurring in Montreal on July 1987, and a once-in-20-year storm was represented by 36 mm depth of rainfall. The disappearance trend

of leached nitrate was monitored within the saturated zone. During rainfall simulation, water delivery to the soil columns was interrupted and excess water was allowed to drain off the columns.

3.1.5 Experimental stages

Table 3.2 gives a summary of the four stages, indicating: the experimental stage and duration, organic matter content of soil, and the amount of nitrate applied to the columns. The table also shows the soil columns used in each stage (columns are identified by numbers), and the concentration and average volume of glucose-C introduced in the columns. Finally, the time (in days) and depth of rainfall simulated on the columns are shown. Each preceding stage determined modifications of glucose level required for the next stage. Soil columns, identified by numbers, were carried forward to the next experimental stage without any modification.The first two stages relate to soil-1 while the remainder relate to soil-2. In stages 1 to 4, fertilizer was applied to the surface of the columns at a rate of 180 kg/ha N. Simulated rainfall was used to leach the applied nitrate into the soil profile. Soil solutions were obtained before, between, and after the leaching events.

Table 3.2: Stages in the nitrate study

Stage and Duration	Soil and org. mat.	N Applied (kg/ha) per column	Columns, Glucose level and Volume of influent				Rainfall	
			treatment		control		time (days)	(mm)
			col. #	glu: mg/L and infl.vol. (L)	col. #	H_2O infl.vol (L)		
1 55 days	St. Amable (Soil-1) 3.4% org. mat.	180	1,2,3	970 19±1	4,5,6	18±1	1 2 5 8 12 15 18 30 43	6 11 31 6 6 25 11 127 63
2 13 days	St. Amable (Soil-1) 3.4% org. mat.	180	1,2,3	120 5±1	4,5,6	5±1	1	165
3 124 days	St. Benoit (Soil-2) 1.6% org. mat.	180	7,8 9,10 11,12 13,14, 15	20 28±3 70 30±4 150 28±2 300 21±1	16,17 18	23±3	8 12 15 34 48 51	36 36 103 37 36 70
4 38 days	St. Benoit (Soil-2) 1.6% org. mat.	180	7,8 9,10 11,12 13,14, 15	20 15±1 70 13±2 150 13±2 300 10±1	16,17 18	12±1	1 5 8 18 29 33	11 11 32 11 11 32

N: NO_3-N; col.: column; glu.: glucose; infl. vol: influent volume; org. mat.:organic matter

3.1.5.1 Stage 1: Effect of high glucose-C on nitrate residue

As stated above, nitrate was applied, in solution, to the columns at a rate of 180 kg/ha nitrate-N. Subirrigation commenced six days after fertilizer application. Three soil columns (St Amable soil), chosen at random, were subjected to subirrigation with 970 mg/L glucose-C (glucose treatment), while another three columns (control) of same soil were subirrigated with water. The nitrate was leached with different depths of rainfall, ranging from 6 to 127 mm, at different times (Table 3.2). The subirrigation process was interrupted during the leaching events. Soil solutions were obtained and analyzed for nitrate, redox and pH from the sampling at 400, 600, and 850 mm below the water table, before and after the leaching events.

Forty five days after the commencement of treatment, the water table was raised and maintained at 150 mm depth from the surface for ten days. On day 10, the saturated hydraulic conductivity was measured and the columns were drained completely for stage 2.

3.1.5.2 Stage 2: Effect of low glucose-C on nitrate residue

Columns used earlier, for previously described glucose treatments were carried over to stage 2. Nitrate was applied again, at the rate of 180 kg/ha N on the columns. A 165 mm amount of rainfall was simulated on each column on the same day (day 1). Treatment columns were subjected to

subirrigation after five days, with 120 mg/L glucose-C solution; and water applied to the three previous control columns. Soil solutions were sampled at different times and analyzed for nitrate residue and redox changes.

3.1.5.3 Stage 3: Effect of glucose on redox potential, Fe and Mn

In this stage, the long-term effect of a range of glucose levels (0, 20, 70, 150 and 300 mg/L) were investigated on another soil (St Benoit). The following information were noted: the nitrate residue reduction and redox potential changes, and soluble Fe and Mn content in the soil solution. Twelve columns were packed with soil-2 (St. Benoit soil) which is different from the soil (St. Amable soil) used in the previous two stages. A new soil was selected to test reproducibility of the observations in the previous two stages, as well as to determine desirable carbon range required for remediation of nitrate residue.

There were four levels of glucose treatment and a control treatment. Three columns each were randomly assigned to the control (0 mg/L glucose-C), and the 300 mg/L glucose-C treatments, respectively. The other treatments at 20, 70 and 150 mg/L glucose-C levels were carried out in duplicate, respectively. The columns were subirrigated with their respective solutions (water for the control) for five days before nitrate-N was applied at a rate of 180 kg/ha to the soil columns; meanwhile subirrigation

continued. Rainfall, with depths ranging from 36 to 103 mm (Table 3.2), was simulated on the columns between days 8 and 51.

Soil solutions were sampled and analyzed for nitrate residue at different times, from day 1 to 51. Within this period, the redox potential in some randomly selected columns was measured. However, the redox potential for treatments, receiving 0 and 150 mg/L glucose-C treatments, were consistently measured on days 1, 2, 3, 6, 8, 9, 12, 15, 18 and 26.

On day 96, soil solutions were sampled and analyzed for Fe, Mn, redox changes and pH in all the columns. The soil columns remained under subirrigation until day 124 before draining.

3.1.5.4 Stage 4: Desirable glucose range for nitrate reduction

Stage 4 utilized all the columns from stage 3. Subirrigation continued as in stage 3, and 180 kg/ha nitrate-N was again applied to the columns, same 20, 70 and 150 mg/L glucose-C levels, as in stage 3, were used. Rainfall depths ranging from 11 mm to 32 mm were simulated on different days (Table 3.2). Soil solutions were sampled at different times, as before, for nitrate analysis. On day 35, the columns were drained completely, and soil samples collected at depths of 100 mm (top) and 850 mm (bottom) in all the columns. A total microbial count estimate was carried out by plating on TYC (Tryptophan yeast extract calcium) agar medium (Beringer, 1974).

3.1.6 Sampling and analytical methods

Soil solutions were collected from below the water table, nine hours before and after every leaching, at depths of 400, 600 and 850 mm. The samples flowed freely into receiving vials, via the sampling tubes, located on the side of the columns. The first 5 to 10 mL of the effluent was allowed to go to waste, while the next 10 to 15 mL was collected for analysis. During leaching, 20 to 30 mL of drain effluent was collected for analysis.

Nitrate concentrations in the samples were determined by ion chromatography (Waters 510 HPLC pump and Star-Ion-A 300 Anion Peek column: 100 mm X 4.60 mm), equipped with a conductivity detector (Waters model 431). Fe and Mn analysis was carried out by using atomic absorption spectrophotometry. Each sample was filtered through a 0.45μm pore sized membrane filter, prior to analysis. Quantification was done by an external standard calibration curve. The effluent redox potential was measured immediately after collection using a platinum Eh electrode (Hanna Scientific); pH and temperature were determined directly with a combined pH-temperature probe (Hanna Scientific).

3.1.7 Total microbial count

Soil samples were collected from the top and bottom regions of the columns (after stage 4) in sterile tubes. One gram of the soil was added to

4 ml of sodium phosphate buffer and vortexed for 75 s. Serial dilutions up to 10^{-5} were prepared, and 100 µl of dilutions 10^{-3} and 10^{-5} were surface plated on TYC agar plates. All the inoculated plates were incubated at 29 °C and the counts were obtained (Ahmad, 1994).

3.2 In-Situ Bioremediation of Hydrocarbon-Diesel

This section is divided into subsections covering: the fabrication of columns, the packing of soil columns with contaminated soil, the experimental setup, the extraction and analysis of total petroleum hydrocarbon (TPH) residues. The soil, supplied by an ESTAC member company, was contaminated by diesel for more than 20 years, and was excavated from a site in Quebec. The excavation site did not have a history of heavy metal contamination, and the soil had 99% sand and 1% silt content.

The contaminated soil received three treatments: (a) Treatment A consisted of a combination of nutrients, air and water, (b) Treatment B was a combination of air and water, and (c) Treatment C served as a control. The experimental protocol further divided treatments A and B into two groups each; a full column treatment group, A_f and B_f, and a stepwise column treatment group, A_s and B_s.

3.2.1 Column fabrication (diesel study)

Figure 3.2 shows the schematic of a packed full soil column as the influent is forced up by air pressure. Twelve PVC pipes (schedule 80), 2,000 mm long x 200 mm inside diameter each, were end capped with PVC plates, and pairs of sampling ports, 100 mm apart, were drilled at depths of 500, 1000 and 1,500 mm from the top of each column. An overflow port was drilled close to the soil surface. A drain tap was also installed at a 300 mm height from the bottom. Another port (50 mm in diameter) to contain a nutrient delivery pipe (300 mm long) was bored close to the bottom of the column, at the 1,950-mm depth.

Starting at 125 mm from one end of the nutrient delivery pipe (PVC), holes were drilled at 50 mm intervals around the circumference and the length of the pipe. The pipe was inserted into a geotextile filter sock to cover the holes and the other end of the pipe, before installation at the nutrient delivery port (at 1,950-mm depth). The pipe was pushed, starting with the covered end, through the delivery port to touch the opposite side of the column, and the joint between the pipe and the column was sealed with a PVC sealant. The holes on the delivery pipe served to uniformly distribute influent at the bottom of the column. After installing the delivery pipe, the contaminated soil was homogenized with an electric driven rotary cement mixer, and packed into the columns to a bulk density of 1.4 g/cm^3.

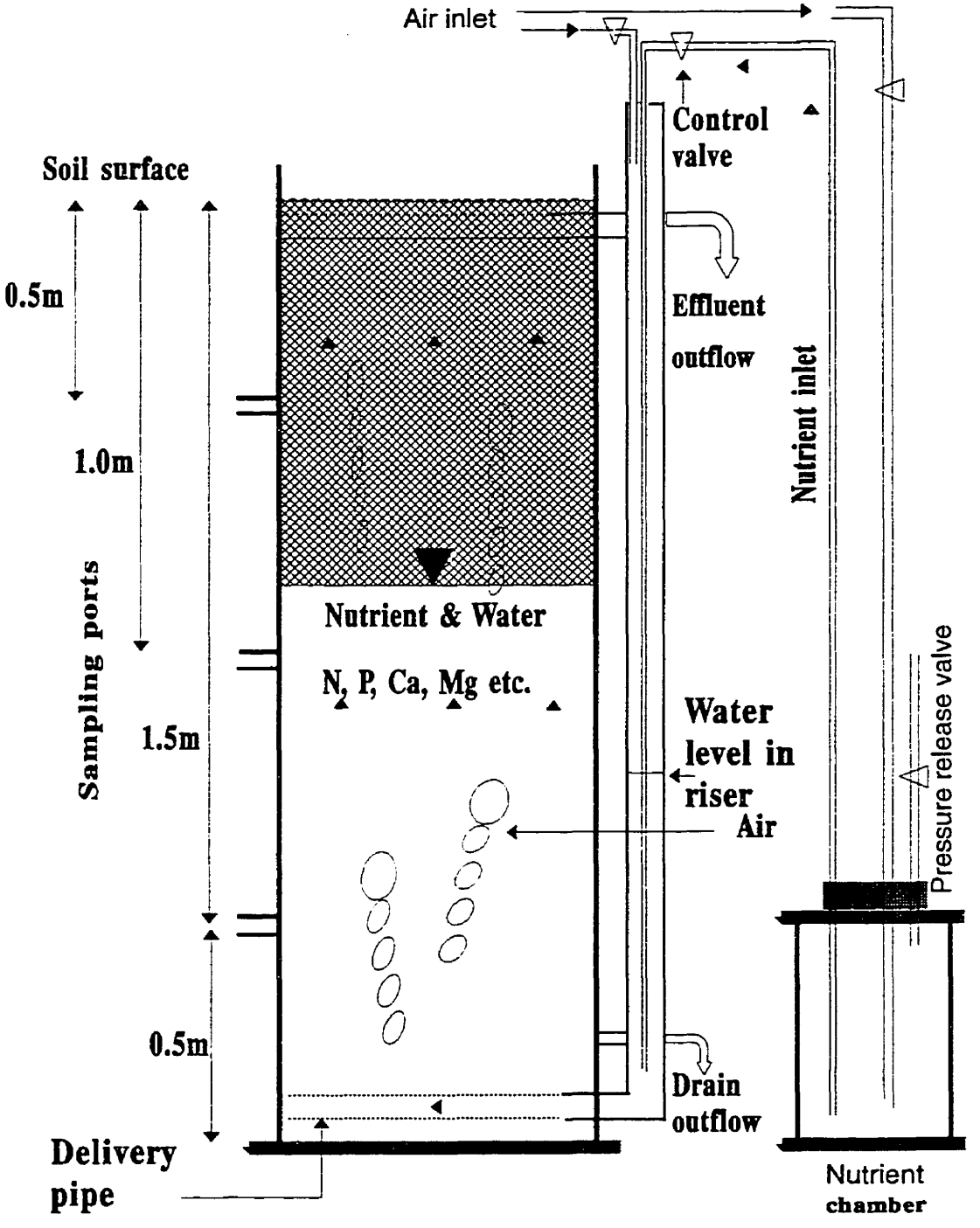


Figure 3.2: Schematic of a soil column for full column treatments.

On completing the packing of the soil, a vertical riser, 2,000 mm in length, was attached to the nutrient delivery pipe at 1,950-mm depth, while the other end of the riser was closed with a two-hole plug, carrying two transfer tubes. Each tube was 2 m long (PVC) with a flow control valve. One of the transfer tubes connected the riser to an air supply source, and the other to a nutrient chamber.

Each nutrient chamber was constructed from a 500 mm long by 150 mm wide clear PVC pipe. It was capped at the lower end, while the upper end was closed with a screw cap carrying an adapter for the nutrient transfer tube. Air was supplied from a network of copper tubing connected to a 2.5 hp air pump compressor. Each riser was hooked up to the network through the air transfer line.

Six of the packed columns were randomly selected for a stepwise column treatment (Treatments A_s and B_s) (Figure 3.3). On each of these columns another delivery pipe, similar in design and specification to the delivery port at 1,950-mm depth, was installed at a depth of 1,200 mm from the top of the column. A riser (1,200 mm long), similar to that attached to the delivery pipe located at 1,950-mm depth, was also attached to the delivery pipe. The delivery pipe at 1,200-mm depth divided the soil column into lower and upper soil zones. The control soil sample was packed in three 1,000 mm long columns; each column had sampling ports drilled at 150, 400

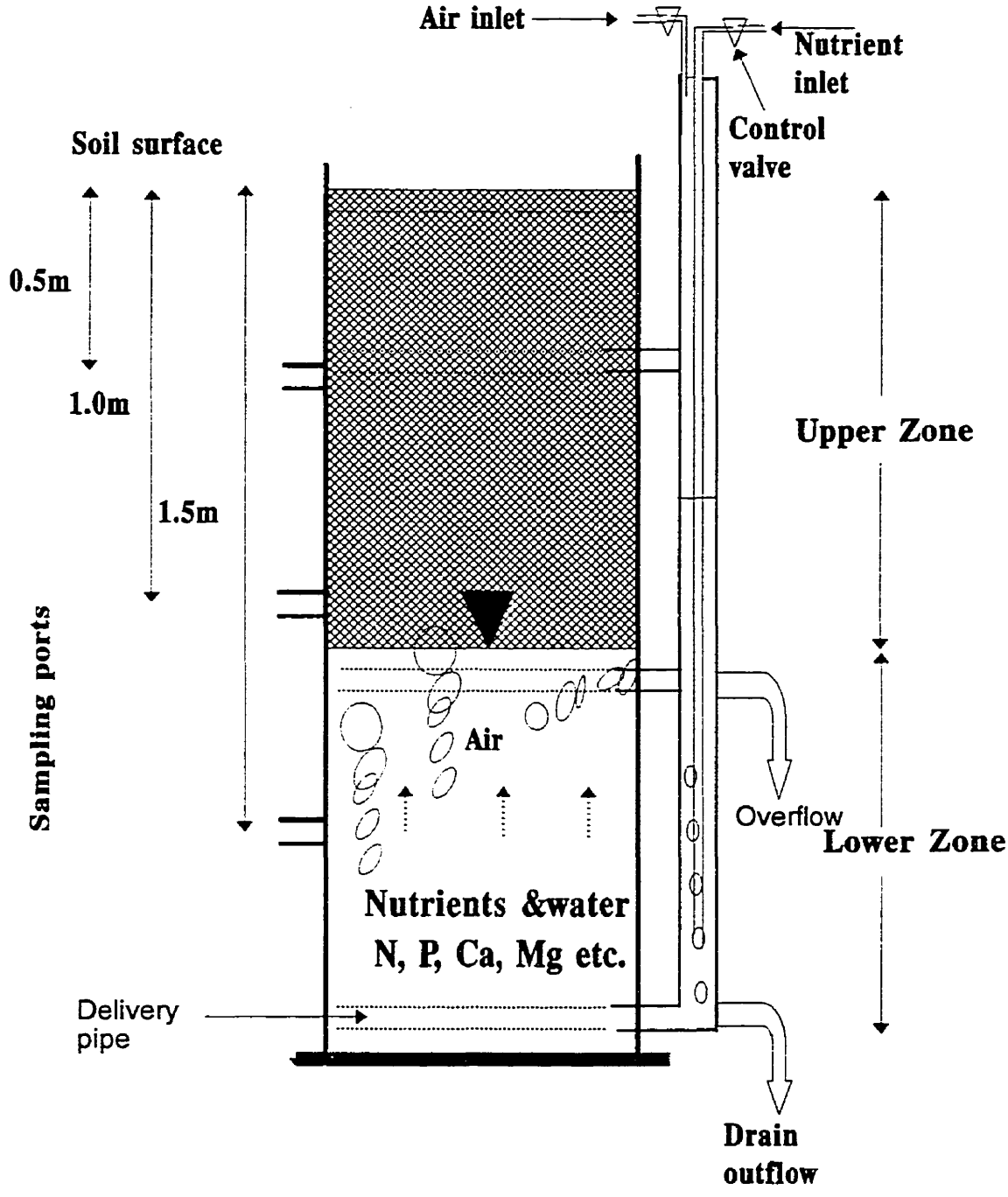


Figure 3.3: Schematic of a soil column for stepwise column treatments.

and 850-mm depths.

A wide bore syringe was used to collect about 5 g of soil samples from the columns, via the sampling ports; these were stored in amber bottles at 4°C for analysis. All the sampling ports were then stoppered with suba recess plastic plugs, supplied by Aldrich Chemical Company, U.S.A.

3.2.2 Experimental setup

The soil columns, with one delivery pipe each, were grouped into full column treatments (A_f and B_f). Other columns, divided into lower and upper soil zones by a second nutrient delivery pipe, were grouped into stepwise column treatments (A_s and B_s). The latter group was treated in segments, starting with the lower soil zone. Each treatment was carried out in triplicate. As stated earlier, treatments A and B generally refer to treatment combinations of nutrient, air and water, or air and water, respectively. Subscript f refers to full column treatment, in which the whole soil profile was treated in one shot while subscript s refers to a column divided into two lower and upper soil zones and treated stepwise.

3.2.2.1 Full column treatments

A nutrient solution containing N, P, S and trace metals (0.382 g NH_4Cl, 0.084 g $Ca(NO_3)_2$, 4.000 g K_2HPO_4, 4.000 g Na_2HPO_4, 0.200 g

$MgCl_2$, 0.001 g $CaCl_2$, 1.420 g Na_2SO_4, and 0.001 g $FeCl_3$ per litre of water (Ahmad, 1994)) was poured into the nutrient chamber and aerated for one hour. Dissolved oxygen and pH of the solution were measured using electrode probes. The nutrient chambers were then capped and pressurized by the air pump. As the pressure in the chambers increased, the aerated nutrient solution was forced through the nutrient transfer tube into the risers, down to the nutrient delivery pipes at the base of the soil columns, and finally into the soil. As more liquid passed into the soil column, the water table was raised, thus subirrigating the soil with liquid nutrients. More aerated nutrient solution flowed continuously into the soil until there was an overflow through the overflow pipe. The nutrient solutions in the chambers were replenished to allow for a continuous flow until there was an overflow of influent through the overflow pipe. Nutrient supply to the columns was stopped after four litres of overflow influent had been collected in a holding tank. The valve on the nutrient transfer line was closed and the air transfer line was opened.

Air was pumped into the columns for about two days; then, the air supply was stopped and the water table was lowered to about 300 mm depth, by opening the drain tap. The discharge from the drain tap was collected into the holding tank for aeration and nutrient adjustment The same initial amounts of nutrient constituents were compounded with the discharge liquid

and aerated for one hour; then transferred to the nutrient chamber for nutrient recycling. This process was repeated on days 2, 4 and 6, allowing a two-day resident time for the nutrient solution in each column.

After eight days, the water table was lowered, and samples of the effluent from each column were collected in 20 ml amber vials, and stored at 4°C for nutrient and TPH analysis. The air supply was continuous until day 16, avoiding water super saturation of the system. During this period, the nutrient effluent was adjusted with 0.057 g/L NH_4NO_3 and 2.500 g/L $(NH_4)_2HPO_4$, and aerated in the holding tank.

After the dry aeration cycle, soil samples were collected from the sampling ports at 500, 1,000 and 1,500 mm depths in all the columns, in order to determine the total petroleum hydrocarbon (TPH). The nutrient status of the effluent was augmented again with 0.057 g/L NH_4NO_3 and 2.500 g/L $(NH_4)_2HPO_4$, and pumped into the soil columns on day 16. The solution was allowed a holding period or residence time with continued aeration, and discharged again into the holding tank on day 22. Soil samples were collected on day 26, and subirrigation with nutrient adjusted effluent commenced simultaneously with aeration on day 30. The columns were drained, and soil and effluent samples collected on day 37; aeration continued until day 43, and the system remained dormant until day 58, when aeration commenced. On day 82, the last subirrigation with nutrient adjusted

effluent commenced, and lasted until day 90.

Treatment B_f was similarly carried out, with only air and water supplied to the columns. All the steps in treatment A_f were repeated for treatment B_f.

3.2.2.2 Stepwise column treatments

The stepwise design tested the speed of loss of TPH if nutrients, air and water or air and water were introduced into the soil columns from different depths (treatment of small soil column segments in "stepwise"). This treatment also tested the possibility of raising the water table to a certain depth, in order to act as a buffer or support for nutrient delivery to an upper soil profile, at some distance from the water table.

Stepwise column treatments were similar to those of the full columns, except that the lower zones in stepwise columns were treated first (Figure 3.3). The influent overflowed via the second delivery pipe at the bottom of the upper soil zone. Samples were only collected at 1,500 mm depth in the lower soil zone. But from day 58 to 82, depths of 500 and 1,000 mm in the upper soil zone were also sampled and analyzed for diesel-TPH. Subirrigation of the lower soil zone stopped after day 82. The resident liquid was drained and the lower zone of the column was filled with water. The riser supplying the lower zone was sealed, and the treatment shifted to the

upper zone (Figure 3.4). Influent flowed from the nutrient chamber via the second riser, the second delivery pipe, and into the soil column. Excess nutrient overflowed from the overflow pipe at 500 mm depth. Subsequent sampling took place at depths of 500 and 1,000 mm. This treatment lasted for another eight days after treatment of the lower depth ceased. Treatment B_s was similarly carried out with only air and water supplied to the columns. All the steps in treatment A_s were repeated for treatment B_s.

3.2.3 Extraction and analysis of TPH

A 3 g sample of soil was mixed with 0.3 g of florisil, and a 0.6 mL aliquot of a 20 μg/ml androstane was added as an internal standard to the mixture. The mixture was extracted with 5.4 ml of 1,1,2-trichloro-1,2,2-trifluoroethane for 4 h by flask shaking. The extract was decanted onto a 3 g sodium sulphate column, contained in the barrel of a 5 ml syringe with its needle endcapped by a 0.2 μm nylon filter. The dried and filtered extract was collected directly into autosampler vials for analysis by gas chromatography/flame ionization detector. A calibration curve was prepared with a diesel standard supplied by Imperial oil, Canada. The standards were spiked on garden soil and extracted and dried; the soil samples were similarly treated before injection.

Gas chromatography of extracts was carried out on a 30 m long

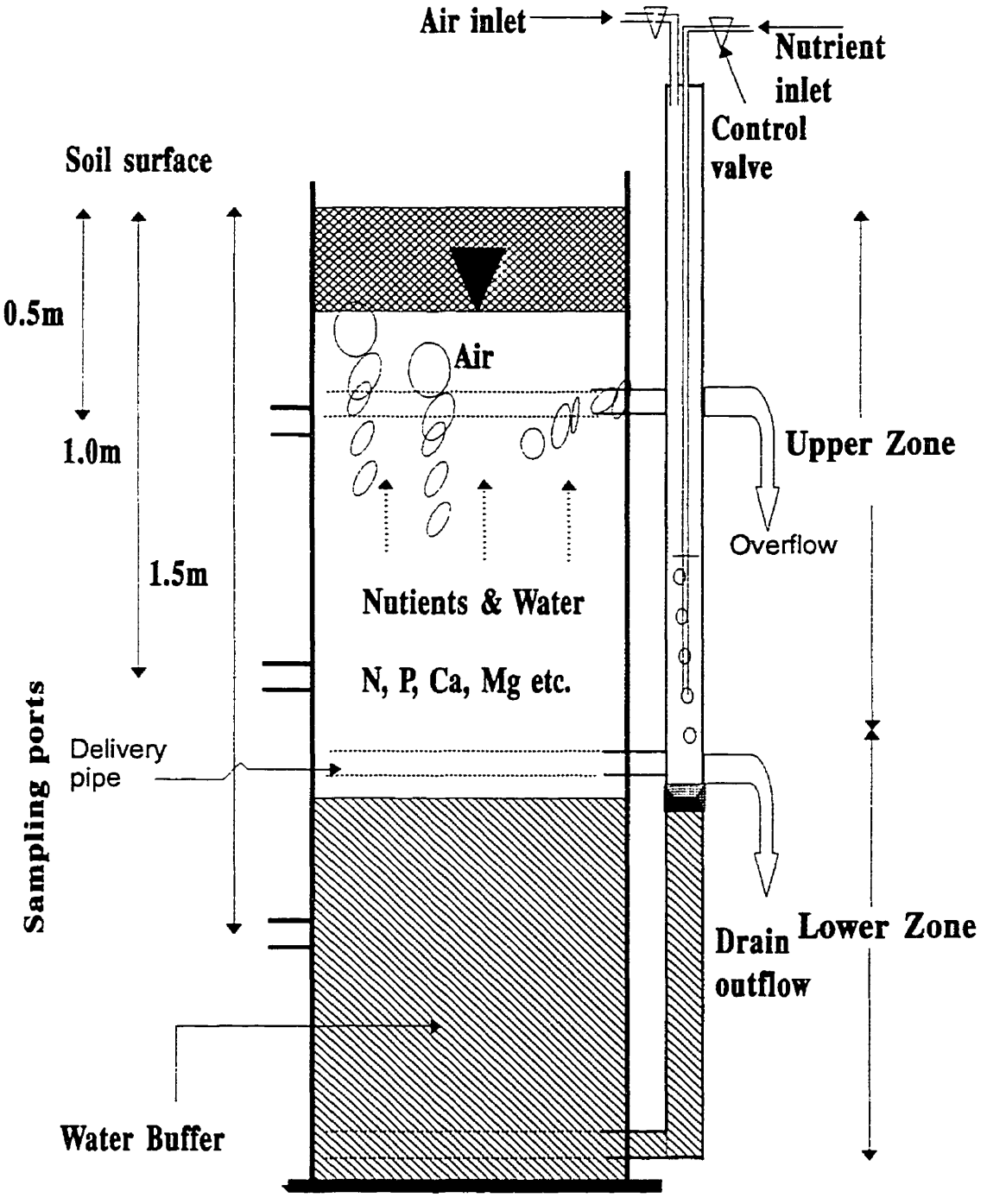


Figure 3.4. Schematic of a stepwise column treatment showing the lower zone acting as a water buffer or support for nutrient delivery to the upper soil zone.

DB-5 megabore fused silica column, installed in a GC Varian 3400 model, equipped with an FID detector and an integrator. The flow rate of the carrier gas (helium) was 6 ml/min. The FID air and hydrogen flow rates were 300 and 30 mL/min, respectively, and that of the makeup gas (nitrogen) was 30 mL/min. The injector and detector temperatures were 280 and 310°C, respectively. Oven temperature programming was as follows: initial oven temperature was 40°C with a holding time of 2 min., increased at a rate of 10°C per min to 270°C and held for 5 min. This was further increased to 300°C, at a rate of 12°C per min and held for 20 min. Data acquisition was made on a Varian electronic integrator, using its valley to valley baseline function. Hydrocarbon quantification was effected by an external calibration curve drawn from the total peak heights of a diesel standard.

Total Kjeldahl nitrogen was determined by Nessler method (Hach, 1992). Phosphorus content was analyzed by the Phosver 3 method (Hach, 1992). Available phosphorus and potassium in the contaminated soil were extracted with Mehlich III (Tran and Simard, 1993).

3.3 Statistical Analysis

The assessment of delivering nutrients, using subirrigation for in-situ bioremediation, was made by monitoring the decrease in nitrate and TPH

levels at different depths in the soil columns. The experimental design was mixed factorial with spatio-temporal repeated measures. There were three levels of the spatial repetition factor, depth, and different levels (in each stage of nitrate and diesel studies) for the temporal repetition factor, time duration, after commencing each segment of the study.

In stages 1 and 2 of the nitrate study, one group of 3 soil columns received the treatment and another three served as a control. In stages 3 and 4, the treatment group received 4 levels of treatment to determine the desirable range of carbon supplement which would not have adverse impact on the soil solution, while facilitating enhanced reduction of nitrate.

For the diesel study, one group of 3 soil columns received air, water and nutrients, while another three, serving as control, received only air and water. An additional set of 3 columns was introduced to serve as passive remediation control. The biological activity in the treatment, with and without nutrients, was further assessed by comparing the dissolved oxygen in the effluent immediately after aeration and after 12 h elapsed without aeration.

Preplanned statistical analysis was formulated before data collection commenced, and it involved individual pair wise comparisons between the treatment and the control (Zolman, 1993) using GLM (general linear model) contrast procedure, and the option repeated (SAS Institute, 1985). The

univariate approach to the analysis of variance (ANOVA) of repeated measures was adjusted due to the heteroscedasity (inequality of variances) and autocorrelation (lack of independence) of data, by applying a correction factor to the number of degrees of freedom of the F-statistics, involving either of the repetition factors, namely time and depth. More details on the methodology can be found in Dutilleul (1997). The true value of the correction factor, known as epsilon (ϵ), is between $0 \prec \epsilon \succeq 1$, and its effect is to deflate or reduce the number of degrees of freedom, according to the size and magnitude of heteroscedasticity and autocorrelation. The significance criterion was at the 5% level.

CHAPTER 4: RESULTS AND DISCUSSIONS

The results and discussion chapter has been divided into two main sections. The first deals with the results for enhanced bioremediation of nitrate residues in soils, while the second discusses the nutrient delivery system for in-situ bioremediation of diesel contaminated soils. Measured raw data (nitrate, redox, Mn, Fe and diesel levels) are given in the appendices.

4.1 In-Situ Bioremediation of Nitrate Residues

Organic matter in soil was augmented with organic carbon in the form of glucose to accelerate the removal of nitrate residue in the soil. Other impacts of this augmentation treatment, such as redox potential, and Fe and Mn contents in the soil solution, were evaluated, and a desirable glucose-C range, required for nitrate reduction under the present study condition, was determined.

4.1.1 Effect of high carbon supplement on nitrate residue

This section discusses trends in nitrate disappearance with time and depth, and also the changes observed in the redox potential of the soil solution after subirrigating the soil with a 970 mg/L glucose-C.

4.1.1.1 Nitrate reduction with time and depth

Figure 4.1 presents the trend of nitrate-N disappearance in soil solution with time. The error bars in the figure and all the other subsequent graphs show the estimated standard deviation showing that 68% of the data obtained (assuming a normal distribution) lie within ± one standard deviation from the mean (Zolman, 1993). Figure 4.1 also shows the time of rainfall simulation and the amount of rainfall employed for each leaching, and the respective nitrate load that reached the 400-mm depth. The amount of nitrate present at the 400-mm depth was 71±37 mg/L nitrate-N and 66±25 mg/L nitrate-N in both treatment and control columns, respectively (Figure 4.1). Both levels dropped with time, and by day 14, they decreased to 4±2 mg/L nitrate-N and 11±4 mg/L nitrate-N, respectively. This represents an average loss of 94% and 83%, respectively. The next three rainfall events did not leach any significant amount of nitrate into the water table (Figure 4.1).

Another rainfall (127 mm), on day 30, transported 169±102 and 93±72 mg/L nitrate-N to the 400-mm depth of the treatment and the control columns, respectively. In six days, the nitrate-N in the treatment column decreased significantly (at the 5% level) to 2±1 mg/L nitrate-N level, but the one in the control columns increased to 157±21 mg/L nitrate-N. The level of nitrate in the control column was still 141±55 mg/L nitrate-N on day 42, meanwhile the treatment column was less than 1 mg/L nitrate-N. Another

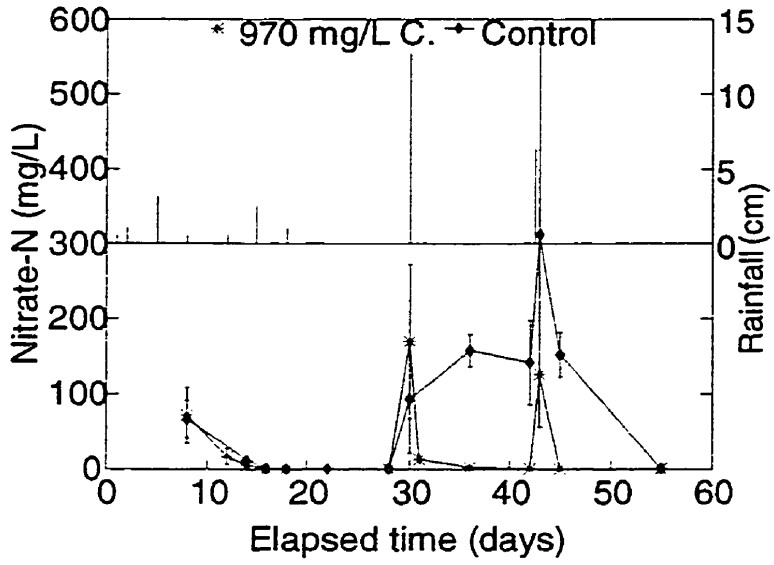


Figure 4.1: Decrease of nitrate-N residue in soil solution at the 400-mm depth with time, after periodic leaching (stage 1).

rainfall (63 mm) increased the nitrate level in the control columns to 312±256 mg/L nitrate-N on day 43; by day 45, the nitrate level then decreased to about 152 mg/L nitrate-N, and finally, to less than 1 mg/L by day 55. The rainfall event on day 43 leached about 125 mg/L nitrate-N in the treatment columns, which then dissipated to less than 1 mg/L nitrate-N in about two days. In contrast, the nitrate-N level in the control column decreased slowly to 1 mg/L in about 10 days. Such a slow decrease could be due to depletion of readily available carbon in the saturated zone, which took some time to hydrolyse enough electron donors (organic carbon) from the organic matter present in the soil.

The nitrate loads that entered the 600-mm depth, during the leaching events described above, are shown in Figure 4.2. Less nitrate leached to the 600-mm depth compared to the upper depth of 400 mm (Figure 4.1). Nitrate amounts leached on day 8 (4th rainfall) from the upper soil zone to the 600-mm depth were 27±16 and 66±25 mg/L nitrate-N for the treatment and the control columns, respectively. Both declined to less than 1 mg/L nitrate-N in four days. The 8th rainfall leached 151±16 and 174±41 mg/L nitrate-N, to the 600-mm depth of the treatment and control columns, respectively. In six days (day 36), the nitrate levels reduced significantly to less than 1 and 139±65 mg/L in the treatment and the control columns, respectively. The latter decreased to 14±11 mg/L after another six days (day 42). The last leaching, on day 43, increased the nitrate load to 26±22 mg/L nitrate-N and 101±78 mg/L nitrate-N in both treatment and control columns, respectively. In about two days, the treatment and control column nitrate concentrations decreased to about 1 mg/L N and 46±34 mg/L nitrate-N, respectively. In contrast to the control columns, there was sufficient readily available carbon that served as electron donors for the denitrification of nitrate leached into the treatment columns. The rapid decrease of nitrate concentration in the treatment columns is attributed to the readily available carbon required for denitrification.

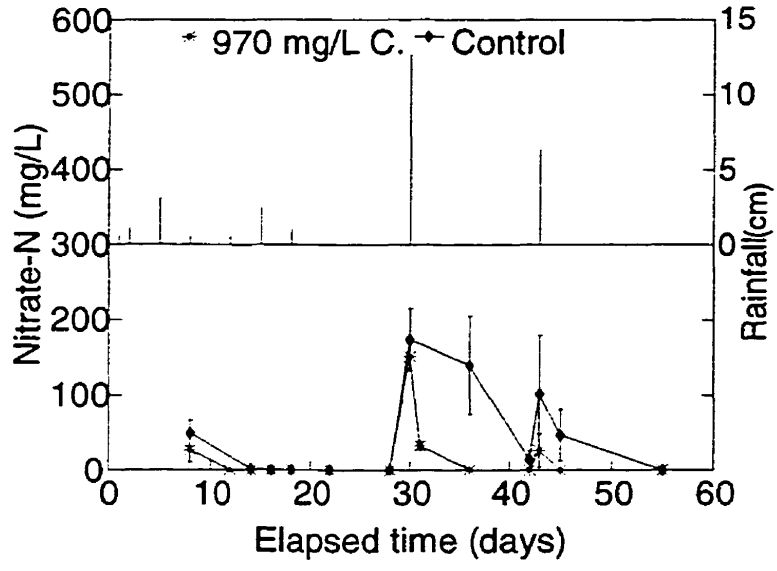


Figure 4.2: Decrease of nitrate-N residue in soil solution at the 600-mm depth with time, after periodic leaching (stage 1).

Less than 10 mg/L of nitrate-N was leached to the 850-mm depth in both treatment and control columns (Figure 4.3). Thus, the nitrate load that reached this depth disappeared quickly.

Rainfall simulations in stage 1 leached different nitrate levels into the the saturated zone. Although the depths of rainfall were similar in all columns, equal amounts of nitrate were not leached to corresponding depths. For instance, the amount of nitrate that leached on day 30 to the 400-mm depth, in both the treatment and control columns varied by 72 mg/L nitrate-N to 102 mg/L nitrate-N (Figure 4.1). Uniform packing of soil to the same bulk density was expected to permit equal transport of solutes in the columns,

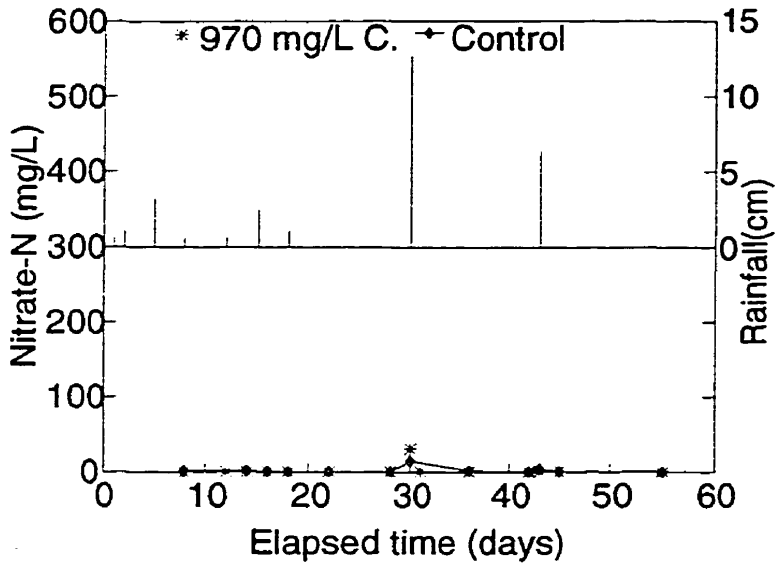


Figure 4.3: Decrease of nitrate-N residue in soil solution at the 850-mm depth with time, after periodic leaching (stage 1).

but this was not the case. One reason is that the soil was not homogenous when the columns were packed, since it is very hard to homogenise a large amount of soil. Another explanation may be the presence of active organisms, burrowing non-systematically through the soil, which in turn created macropores (Hole, 1981) which might have been non-uniformly distributed, and caused unequal leaching of nitrate to different depths in different columns.

The results in stage 1 suggest that nitrate reduction occurred in both the treatment and control soil columns (Figures 4.1 and 4.2). This reduction could be attributed to the denitrification process, since an anoxic condition

existed in the saturated zone of both sets of soil columns. Although there was nitrate reduction in both the treatment and control columns, analysis of variance for nitrate-N concentration in the soil solution showed that carbon treatment main effect was significant at 5% level on day 36; the treatment receiving 970 mg/L glucose-C dissipated the nitrate loads faster at all depths, especially from day 36 onward (Figures 4.1 and 4.2). The absence of a distinct difference in nitrate reduction between the treatment and the control soil columns, in the first 30 days of the study, is explained by the small amount of nitrate leached to all the depths (Figures 4.1, 4.2 and 4.3). The least amount leached to the 850-mm depth which was followed by the 600-mm depth.

Another reason for the low nitrate concentrations, at lower depths in the first 30 days, might be explained by the presence of sufficient initial organic matter (3.4%) in the treatment and control soil columns at the beginning of the study. The aforementioned would facilitate rapid denitrification of nitrate as it leached into the water table. Therefore, the extra organic carbon added in the form of glucose probably did not make much difference in the nitrate reduction process in the first 30 days.

As more nitrate was leached into the water table on day 30 onwards, significant differences (at the 5% level) in the trend of nitrate reduction, between treatment and control columns, were observed at the 400 and 600-

mm depths (Figures 4.1 and 4.2). These results relate to the effect of external/supplemental organic carbon. In the columns receiving 970 mg/L glucose-C solution, the nitrate loads at all depths declined rapidly. Those in the control columns seemed to increase rather than decrease with time, from days 30 to 43 (Figure 4.1). Bremner and Shaw (1958b) described this phenomenon when they showed that denitrification was faster in the presence of a readily available carbon, rather than with organic matter which had been previously subjected to decomposition and leaching. Induction of nitrate loss was faster in the treatment columns receiving 970 mg/L glucose-C, in comparison to the control columns (Figures 4.1 and 4.2).

Weier et al. (1993) proved that the level of organic carbon content in the soil controls the rate of denitrification. Therefore, the increased amount of nitrate, leached in the 8th and 9th rainfall events, placed a high demand on the organic carbon content in the columns; the treatment columns, supplemented with high carbon, indicated a greater loss of nitrate (within a given time lapse) than the control columns.

The water table in stage 1 was raised to a depth of 150 mm from the surface after 45 days of experimentation. It was maintained at that level for 10 days in order to create saturated anaerobic conditions, conducive to denitrification. On draining the soil columns after 10 days, the nitrate concentration in the effluent was less than 10 mg/L N in all the columns.

This result was anticipated since, with the water table raised, the unsaturated zone became anaerobic and thus conducive to denitrifying activity of microorganisms. The microorganisms used the carbon present in the saturated zone to reduce the nitrate residue. Supplementing the zone with a readily available carbon ensured a faster reduction of the residual nitrate, thus decreasing the time necessary to maintain a high water table. Furthermore, augmenting the indigenous organic carbon with readily available glucose prevented depletion of the native organic carbon.

4.1.1.2 Changes in redox potential due to high carbon supplement.

Figures 4.4 shows the redox changes in the soil solution at 400-mm depth, for the treatment and control soil columns. The redox levels fluctuated between sampling days in the treatment columns (Figure 4.4). Initially, on the first three sampling days the level was above 100 mV. By day 16, the level was close to zero and fluctuated between zero and about 100 mV on subsequent sampling days. At the 400-mm depth (Figure 4.4), the soil solution redox potential remained above 300 mV in the control columns throughout the experimental period.

The redox changes in the treatment and control columns at a depth of 600 mm, are given in Figures 4.5. The redox potential in the treatment columns varied between -200 and 200 mV from days 8 to 16. On day 31, the

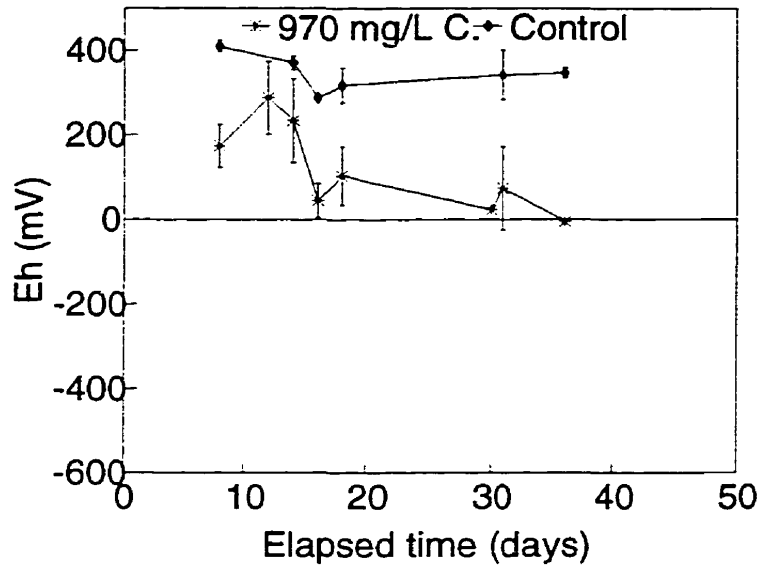


Figure 4.4: Changes in redox potential at the 400-mm depth (stage 1).

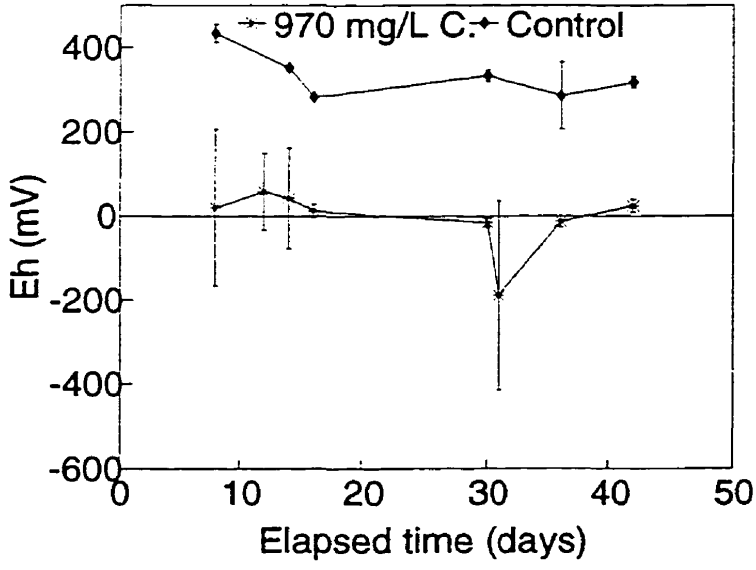


Figure 4.5: Changes in redox potential at the 600-mm depth (stage 1).

variation shifted to a lower level between -400 and 0 mV, and by the next sampling date (day 36), it returned to about 0 mV. The redox change at this depth (600 mm) in the control (Figure 4.5) was similar to the 400-mm depth (Figure 4.4).

Figures 4.6 presents the changes in redox potential at the 850-mm depth, in both treatment and control columns. The redox potential of the treatment columns at the beginning of sampling was about -520 mV, with very little variability (Figure 4.6). This remained constant on subsequent two sampling, and changed to about 70 mV on day 16. The redox potential further increased to about 170 mV on day 30 sampling. Day 30 sample indicated an average variability between -210 and 29 mV. The control presented very different results, since the redox potential was stable at 300 mV (Figure 4.6). The trend in redox change followed the pattern observed in the control columns at depths of 400 and 600 mm (Figures 4.4 and 4.5 respectively).

Generally, the redox potential in all the control columns, at all depths, did not show much variation; this contrasted with the observation in the treatment columns where the redox potential varied at all depths.

Saturated conditions can limit oxygen diffusion into a waterlogged soil, and microbial respiration may further reduce the oxygen content. Addition of extra organic carbon to the waterlogged soil enhanced the

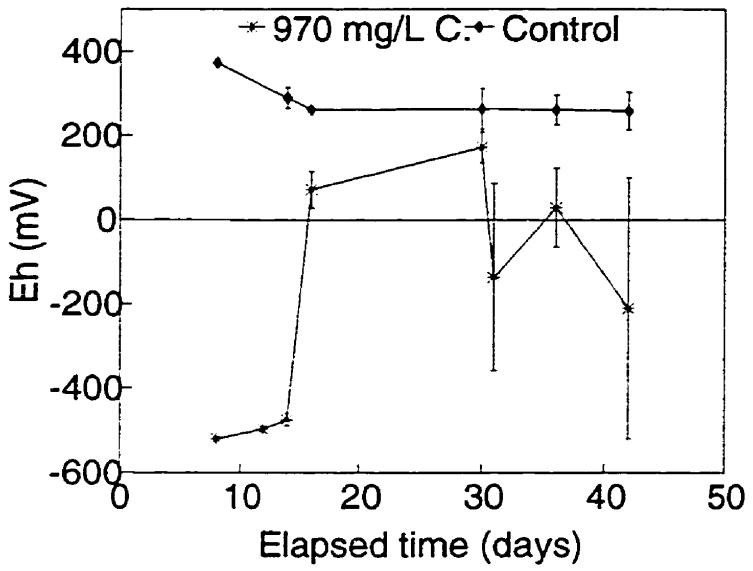


Figure 4.6: Changes in redox potential at the 850-mm depth (stage 1).

depletion of the oxygen, and thus provided a more reduced state. The redox potential of the soil solution in the treatment columns dropped most when compared to the control columns. This was attributed to the effect of additional 970 mg/L glucose-C supplied to the treatment columns. The reduction was more pronounced at the bottom of the column, increasing to higher redox potential as depth decreased (Figures 4.4, 4.5 and 4.6).

Factors that maintained a high redox potential at the 400 mm depth in the treatment columns, could have been: the influence of air diffusion from the surface, and also the dissolved oxygen in the simulated rain water. Also, the 400-mm depth received less carbon, since the carbon content of the

subirrigation water was decreased due to consumption by microorganisms, as the glucose solution passed through the soil profile at lower depths to the 400-mm depth.

Soil profile around the 850-mm depth zone was most reduced and sensitive to the influx of carbon because that zone was in contact with the highest level of in-flowing organic carbon (Figure 4.6). However, on day 30 sampling, the 850-mm depth had the highest redox potential, which was contrary to expectation. Increase in redox potential at this depth is mostly explained by the percolating rainfall water, which displaced resident soil solution and redox components via draining.

As observed in the control columns, the redox potential remained close to 300 and 400 mV on all the sampling days; thus, it is probable that the energy source from the organic matter was small and not readily available. However, in columns treated with 970 mg/L glucose-C, microbial activity was high, and this resulted in a significant (at the 5% level) drop of the redox potential.

The redox potential of ground water gives an indication of the type of reductant present, e.g., organic matter and redox couples (NO^-_3 / NO^-_2, MnO_2/Mn^{2+}, Fe^{2+}/Fe^{3+} etc.) (Anon., 1994). In this study the drain effluent was observed during rainfall simulation; the treatment columns exuded a smell of decomposing organic matter and a greenish hue, likely due to Fe^{2+}. The

drain effluent when exposed to air in the laboratory produced a brownish precipitate (likely to be Fe^{3+}). Dissolution of Fe suggests a reduced state (low redox potential); in this study, the interpretation of redox potential level served to indicate that an oxygen free status existed in the soil (Tate, 1995). The redox potential of the soil solution may also be an indicator of the soil component which is acting as electron acceptor during a prolonged anaerobic condition. Redox potential measurement in this study monitored the general effect of adding extra organic carbon to a saturated soil. The redox potential also helped to determine when to reduce the level of organic carbon added to the treatment columns so that soil property is not disturbed (e.g. metal mobilization) and denitrification is still efficient.

4.1.1.3 Impacts of subirrigation with a high glucose level

The slow nitrate reduction trend in the control columns (Figures 4.1 and 4.2), supports the hypothesis that the soil organic matter, under continuously saturated conditions, will not readily satisfy the soluble organic carbon requirement for quick nitrate removal. Moreover, the rapid drop in nitrate concentration in the treatment columns indicates a need to supplement the soil organic carbon with a readily available source of organic carbon. However, the low redox potential (-500 mV, Figure 4.6) shows that the amount of glucose added to the soil might lead to the adverse effect of

increased Fe^{2+}in the soil solution.

Redox potential and pH of a solution can give an indication of the species of Fe or Mn in a soil solution. During the study, the pH at the sampling ports, for both the control and treatment columns, stayed at an average value of 6.1 and 5.8, respectively. Superimposing the Eh and pH data from this study on a stability field (plot of Eh vs. pH) of iron and manganese (Collins and Boul, 1970), the form of Fe and Mn was shown to fall within the Fe^{2+} and Mn^{2+} species for the treatment columns, while the values for the control were found in Fe^{3+} and Mn^{2+} fields. This affirmed the observation that the organic carbon load in the subirrigation water might have caused more iron to come into solution, and on exposure to air in the laboratory, the reduced iron was reoxidized to produce brownish precipitates, which from the stability diagram, is indicated to be $Fe(OH)_3$.

If these mobile forms of Fe and Mn are transported upwards by a rising water table and subsequently exposed to a more oxidizing conditions as the soil desaturates, coatings of $Fe(OH)_3$ will be formed on the mineral surfaces in the desaturated zone. Such alternation between reducing and oxidizing conditions might lead to the formation of mottles and concretions in the soil solum in a subirrigated farm. Courchesne et al. (1996) hypothesized that such coatings could act as a barrier, limiting contact between the soil solution and fresh mineral surfaces.

The results, after subirrigation of the soil columns with glucose, clearly indicate that a water table management practice may be used to introduce supplementary nutrients to any part of the saturated zone. However, the amount of organic carbon content added must be controlled in order to avoid the adverse effect of low negative redox potential. Tate (1995) indicated that a flooded soil might not be oxygen free, but that the depletion of oxygen might create an anaerobic condition that induces facultative metabolism (e.g., fermentation), anoxic metabolism (e.g., denitrification), or a strictly anaerobic process (e.g., methanogenesis). Production and accumulation of fermentation products (organic acids), in the presence of high organic carbon, may result in a drop in pH and subsequent mineral dissolution. Precipitation of iron was observed in the effluent from the treatment columns. This suggests some adverse effect in a soil subjected to the prolonged addition of 970 mg/L glucose-C.

Reduction occurs in a soil environment saturated with water, especially below the water table, where oxygen supply is low and biological oxygen demand is high (Boul et al., 1989). Cate (1964) noted that the effect of such an environment is the reduction of iron compounds to the highly mobile ferrous; the ferrous form is then lost from the system if there is a net downward or upward and outward movement of the ground water. If the water is removed from the soil, precipitation or deposition of the dissolved

compounds will not occur. But wherever the iron or manganese is reoxidised and precipitated, soft masses or hard concretions or nodules are formed.

This movement of iron and manganese due to redox processes in a soil might result in the mobilization of heavy metals e.g. arsenic and phosphate, and the formation of redoximorphic features (Anon., 1992). The mobile ions, which include reduced forms of manganese may be transported upwards by water as the water table is raised. Desaturation results in oxidation of iron and manganese compounds, thus leaving iron and manganese coatings on the mineral surfaces. However, if the reduced iron lingers in the system, in the presence of relatively high contents of organic matter, it might react to form sulfides and related compounds (Bloomfield, 1952; Jeffery, 1960). Subirrigation water containing a high amount of dissolved carbon might, therefore, lead to mobilization, transportation and precipitation of iron and manganese compounds leading to redoximorphic features. Because of the adverse effect of excessive organic carbon in the soil, the organic carbon concentration was, therefore, decreased from 970 to 120 mg/L glucose-C and the effect of the reduction was tested in stage 2.

4.1.2 Effect of low carbon supplement on nitrate residues

In the previous study, a high glucose level denitrified more nitrate residues in the treatment columns than in the control columns. However, the

redox potential of the soil solution was very low and Fe dissolution occurred. Therefore, the concentration of glucose was reduced to 120 mg/L glucose-C to ascertain if the observed adverse effect, associated with low redox potential and high organic carbon in the preceding experiment, could be controlled by a lower glucose concentration. This section is divided into two subsections. Subsection one describes the trend of nitrate disappearance with time and depth; subsection two describes the changes observed in the redox potential of the soil solution.

4.1.2.1 Nitrate reduction due to low carbon supplement

Figure 4.7 depicts the changes in nitrate levels leached into the 400-mm depth, in both the treatment and control columns after the rainfall simulations. About 154±120 and 82±79 mg/L nitrate-N leached into the 400-mm depth of the treatment and control columns, respectively. The nitrate in the treatment columns on day 13 significantly declined (at the 5% level) below 4±2 mg/L, i.e. more than 95% nitrate-N was dissipated. However, the level in the control columns only decreased to 79±49 from 82±79 mg/L nitrate-N.

The nitrate load and its disappearance at the 600-mm depth is shown in Figure 4.8. About 184±90 and 115±103 mg/L nitrate-N leached into the 600-mm depth of the treatment and control columns, respectively. On day 8,

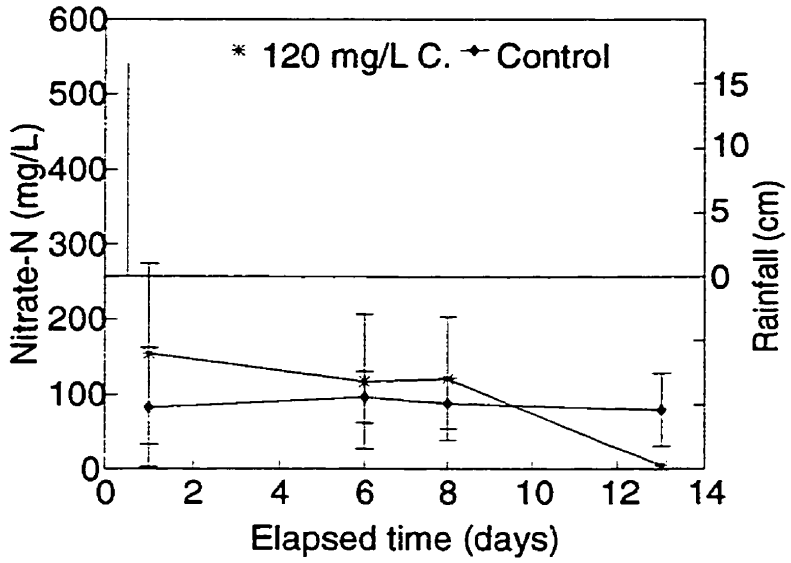


Figure 4.7: Decrease of nitrate-N residue in soil solution at the 400-mm depth with time, after leaching (stage 2).

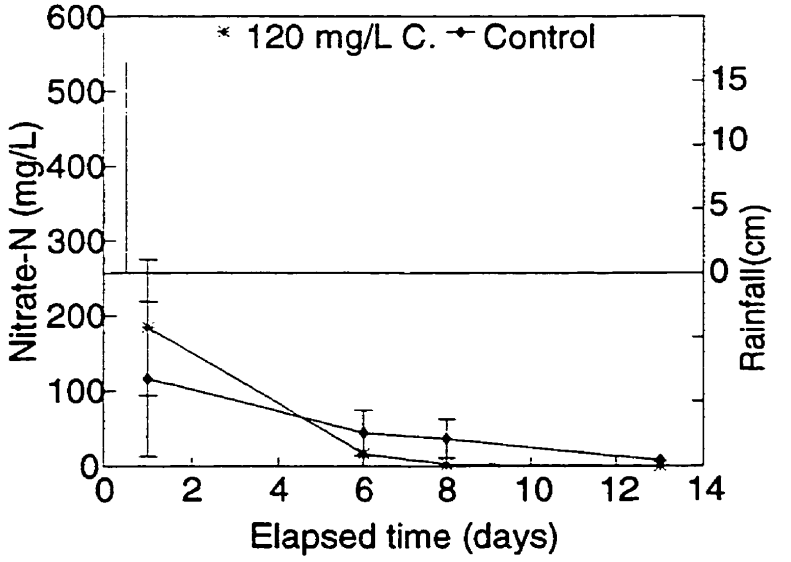


Figure 4.8: Decrease of nitrate-N residue in soil solution at the 600-mm depth with time, after leaching (stage 2).

the levels in the treatment and control columns also decreased significantly (at the 5% level) to 2±1 and 36±25 mg/L nitrate-N, respectively. Nitrate in the treatment columns dissipated to less than 1 mg/L nitrate-N by day 13, while the control nitrate level by day 13 was 7±4 mg/L nitrate-N.

Figure 4.9 indicates the nitrate load transported to the 850-mm depth in both the treatment and control columns. Both received about 44±37 and 58.5±37.98 mg/L nitrate-N, respectively. The nitrate level in the treatment columns diminished by 98% in four days, leaving about 1 mg/L nitrate-N. The level in the control columns reduced to 16±7 mg/L N in the same period.

Nitrate denitrification of the control in stage 2 was lower than that of the previous stage. This confirms previous research (Bremner and Shaw, 1958b), which reported that the rate of denitrification, with previously decomposed soil organic matter, was slower than with the original soil organic material. There is a decrease in the denitrification rate if the organic matter in the soil had been leached. This was the case here, where the soil used in this stage was the same soil (St. Amable) used in stage 1. For more than 50 days, the soil used in stage 1 was subjected to a reducing condition with intermittent leaching by simulated rainfall (as effected in stage 1). From 30 days onwards in stage 1, the nitrate reduction in the control significantly dropped because of inadequate supply of readily available organic carbon.

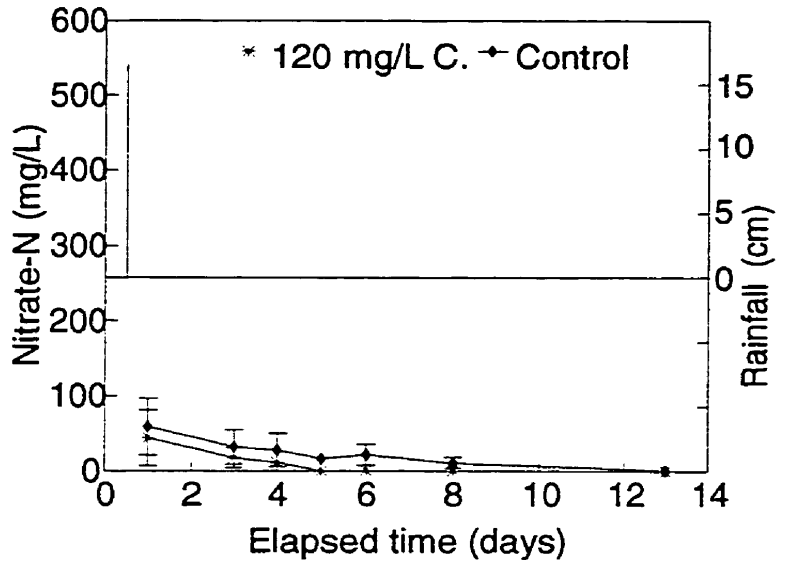


Figure 4.9: Decrease of nitrate-N residue in soil solution at the 850-mm depth with time, after leaching (stage 2).

Reduction of nitrate resumed after possibly new available carbon was hydrolysed from the organic matter (3.4%) originally present in the soil. Therefore, without a soluble organic carbon supplement, the denitrification rate in the control in stage 2 will continuously decrease, and with time lead to high levels of nitrate in shallow ground water or be carried by the drain tiles to surface water bodies were it will contribute to the process of eutrophication.

Therefore, the slow decrease in nitrate level in the control soil columns in stage 2 of the experiment suggests a rapid decline in the effectiveness of water table management to sustain denitrification in soils.

This highlights the fact that water table management, as currently practiced for nitrate reduction, may not provide a long-term solution for denitrification without carbon augmentation.

4.1.2.2 Changes in redox potential due to low carbon supplement

Figure 4.10 presents the redox changes over the thirteen days of stage 2 study, at depths of 400 mm in both the treatment and control columns. The redox trend in both treatment and control remained between 200 and 400 mV. The average redox potential, at the 600-mm depth for the treatment columns, remained at about 150 mV (Figure 4.11); while the control (Figure 4.11) exhibited an average redox of about 300 mV. Redox change in the treatment at a depth of 850 mm started at an initial value of about 0 mV, and increased with time to about 100 mV (Figure 4.12). In the control (Figure 4.12), the redox stayed at an average of 300 mV.

The redox trend, at all depths in stage 2, was similar to that observed in stage 1. As in stage 1, the redox potential increased as the depth decreased. Although the values were higher in stage 2, this is explained by a glucose-C concentration reduction from 970 to 120 mg/L. However, the redox potential in the treatment columns did not fluctuate as much as in stage 1 (Figures 4.4, 4.5 and 4.6); instead, there was a general upward trend of redox potential within the thirteen day period. Although, the redox

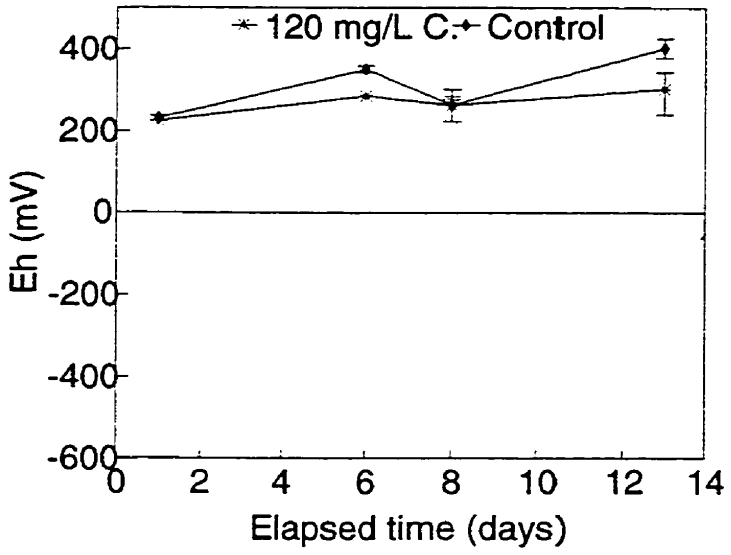


Figure 4.10: Changes in redox potential at the 400-mm depth (stage 2).

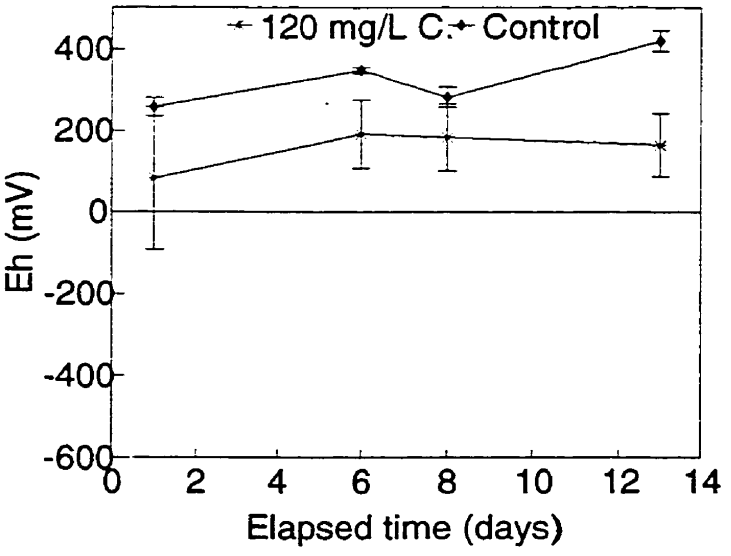


Figure 4.11: Changes in redox potential at the 600-mm depth (stage 2).

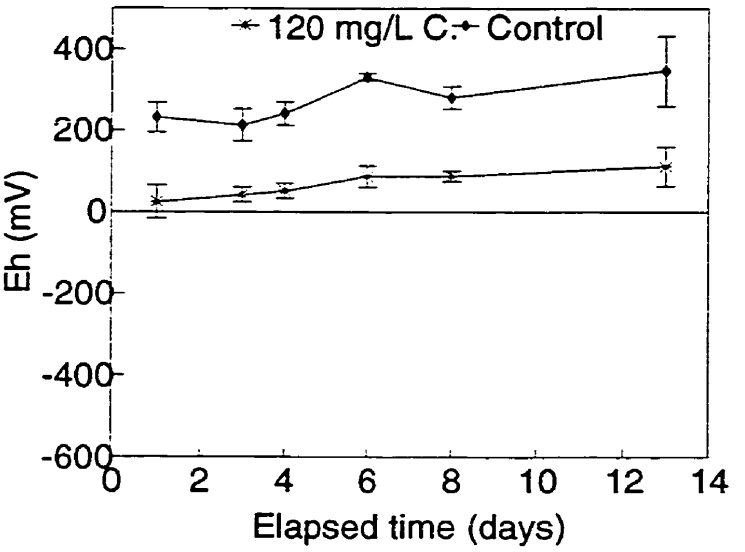


Figure 4.12: Changes in redox potential at the 850-mm depth (stage 2).

potential showed a general upward trend which remained above zero throughout the experimental period, the redox potential at corresponding depths in the treatment and control columns were significantly different (at the 5% level), except on days 1 and 3 at 400 mm depth, and on days 1, 2 and 3 at depth of 600 mm (Figures 4.10 to 4.12).

The high redox potential of the treatment compared to the redox potential values in stage 1, suggests that there could be a carbon concentration range that might influence nitrate dissipation without necessarily resulting in a big drop in redox potential. Higher redox potential in the treatment columns, after reducing the glucose level, further suggests

the importance of redox potential in the design and monitoring of a carbon additive management strategy. If the higher desirable range is not exceeded, this would drastically avoid a drop in the redox potential, while also maintaining conditions that would encourage faster denitrification.

To achieve this favourable redox potential range, a desirable glucose-C concentration range was determined in the next stage using another soil (St. Benoit soil) with a lower organic matter content.

4.1.3 Impacts of different carbon levels on St. Benoit soil solution

Saturating a soil decreases oxygen diffusion into the soil; as a result microorganisms, in the process of decomposing organic matter, switch to alternate electron acceptors (Patrick, Jr. and Jugsujinda, 1992). Alternate electron acceptors include NO_3^-, Mn^{4+} compounds, Fe^{3+} compounds, SO_4^{2-} and CO_2. However, O_2 is preferred since it is abundant in the atmosphere and it diffuses easily into soils and sediments that are not saturated, and it is also easily reduced (Patrick, Jr. and Jugsujinda, 1992).

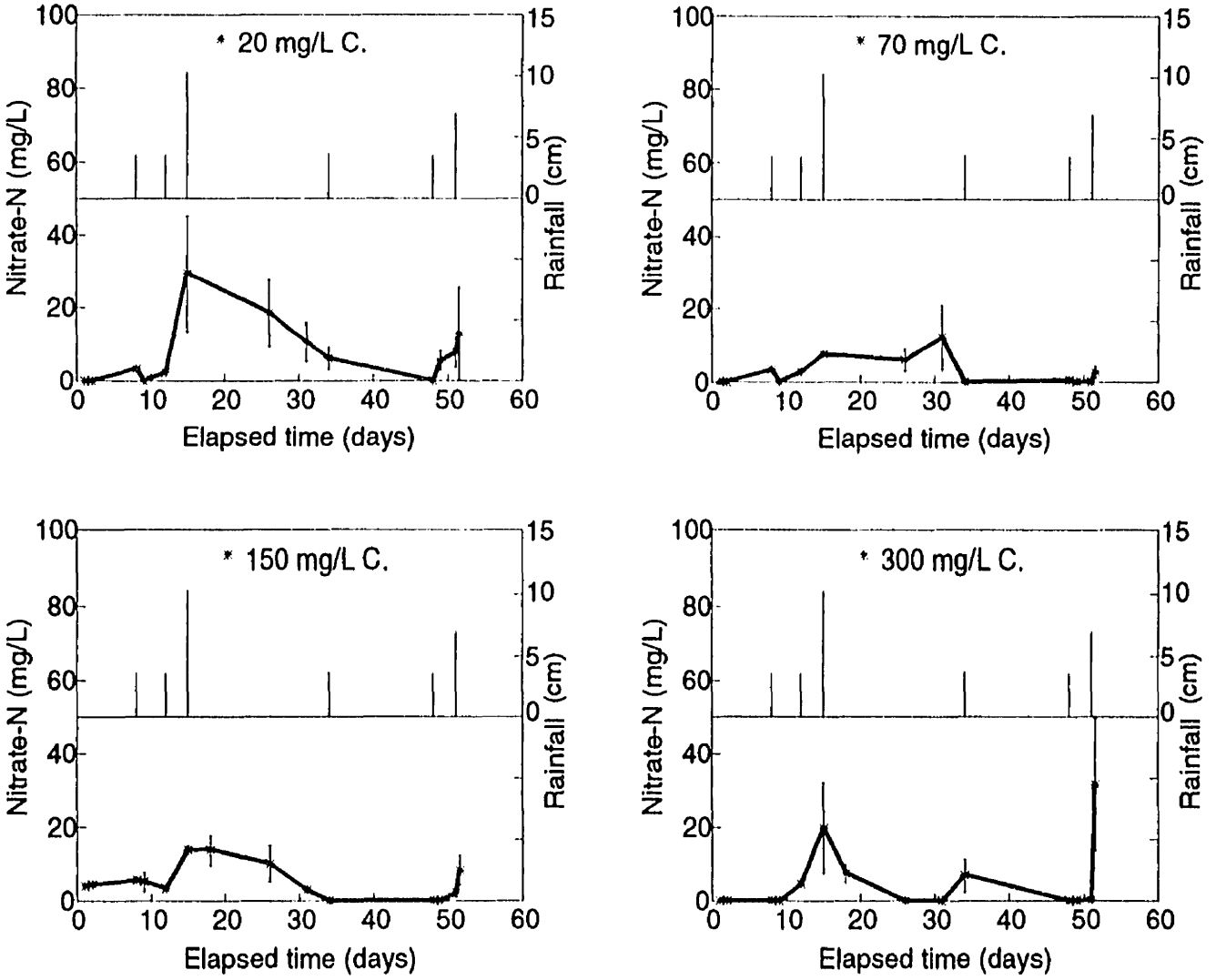
Among the alternate electron acceptors, nitrate is more likely to be utilized by microorganisms since the energy yield is greater. The order of preference of alternate electron acceptors and sequential reduction is as follows: NO_3^-, Mn^{4+} compounds, Fe^{3+} compounds, SO_4^{2-} and CO_2; however, Patrick, Jr. and Jugsujinda (1992) found that overlapping in the reduction

of electron acceptors (utilization of electron acceptors) occurs, because of the rapid onset of reducing condition after O_2 depletion. This section of the study tests the extent to which different levels of decomposing organic carbon can cause the redox potential to drop; more specifically, it determines the amount of soluble Mn^{2+} and Fe^{2+} formed, after subjecting the soils to ninety six days of subirrigation with water containing different levels of carbon.

The first part of this section briefly presents the trend of nitrate appearance after it has been leached, and also its disappearance with time. The second part discusses changes in redox potential monitored from some of the soil columns prior to day 96, when the redox potential as well as the soluble Fe and Mn in the soil solution were sampled and analyzed.

4.1.3.1 Nitrate disappearance with time and depth

Figure 4.13 presents the appearance and disappearance of nitrate after each rainfall event at the 400-mm depth, in glucose-C treatment levels of 20, 70, 150 and 300 mg/L, respectively. The different leaching times of nitrate and also its disappearance in the control columns are shown in Figure 4.13a. The first two rainfall events, 36 mm each time, on days 8 and 12 leached less than 5 mg/L nitrate-N to the 400-mm depth, in all columns. The third rainfall (103 mm) on day 15 leached 29±15 and 7±1 mg/L nitrate-N to the 400-mm depth in the 20 and 70 mg/L glucose-C treatments, respectively



Figures 4.13: Decrease of nitrate-N residue in soil solution at the 400-mm depth with time after periodic leaching (stage 3).

(Figure 4.13). Columns treated with 150 and 300 mg/L glucose-C, received about 13±1 and 19±12 mg/L nitrate-N loads, respectively, at similar depths of 400 mm (Figure 4.13). In the control columns (Figure 4.13a), 14±3 mg/L nitrate-N was leached by the third rainfall event (103 mm).

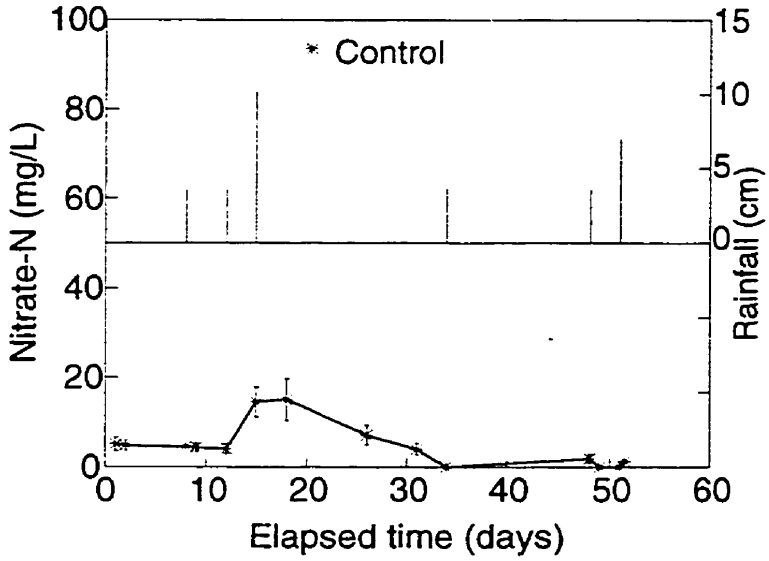


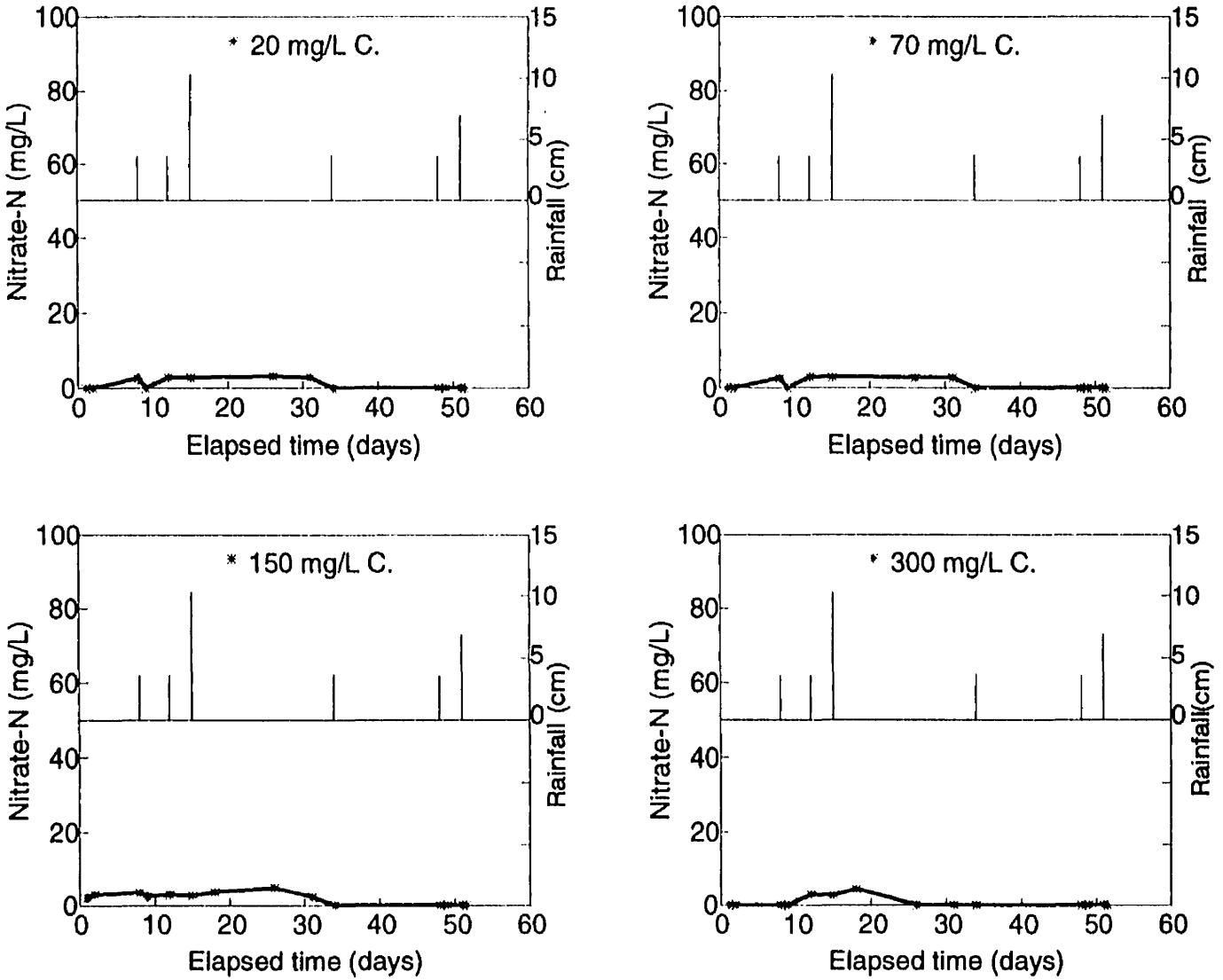
Figure 4.13(a): Decrease of nitrate-N residue in soil solution at the 400-mm depth with time after periodic leaching (stage 3).

The nitrate loads in all columns gradually decreased to less than 10 mg/L nitrate-N by day 34. Columns treated with 300 mg/L glucose-C decreased fastest, reaching below 1 mg/L nitrate-N by day 26 (Figure 4.13). Subsequent three rainfall events on days 34, 48 and 51 did not leach much nitrate into the water table.

The trend in nitrate appearance and disappearance in the 600-mm depth, in all the soil columns, is shown in Figure 4.14 for the 20 and 70 mg/L glucose-C treatments, respectively. Figure 4.14 also indicates leaching for the 150 and 300 mg/L glucose-C treatments, respectively, and Figure 4.14a shows the control results.

The trend in nitrate leaching and reduction, with time, at a depth of 850 mm for 20, 70, 150 and 300 mg/L glucose-C treatments, respectively, is shown in Figure 4.15, and Figure 4.15a presents the results for the control columns at the same depth. Each of the treatments and the control soil columns received less than 5 mg/L nitrate-N during each leaching and the nitrate load disappeared very quickly.

The amount of nitrate leached in stage 3 was low, suggesting that more rainfall was required to transport more nitrate to the saturated zone. At the 400-mm depth, the 20 mg/L glucose-C treatment received a maximum of about 30 mg/L nitrate-N while the others, including the control, received between 15 to 20 mg/L nitrate-N. Thus, it was difficult to assess the effect of the treatments vs. the control at such levels of nitrate. Since the concentration was low, the leached nitrate reduced easily to below 10 mg/L nitrate-N.



Figures 4.14: Decrease of nitrate-N residue in soil solution at the 600-mm depth with time after periodic leaching (stage 3).

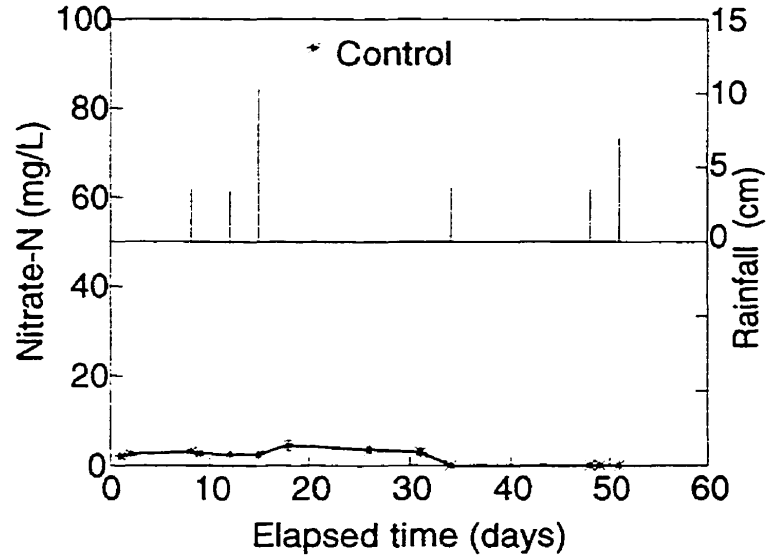


Figure 4.14(a): Decrease of nitrate-N residue in soil solution at the 600-mm depth with time after periodic leaching (stage 3).

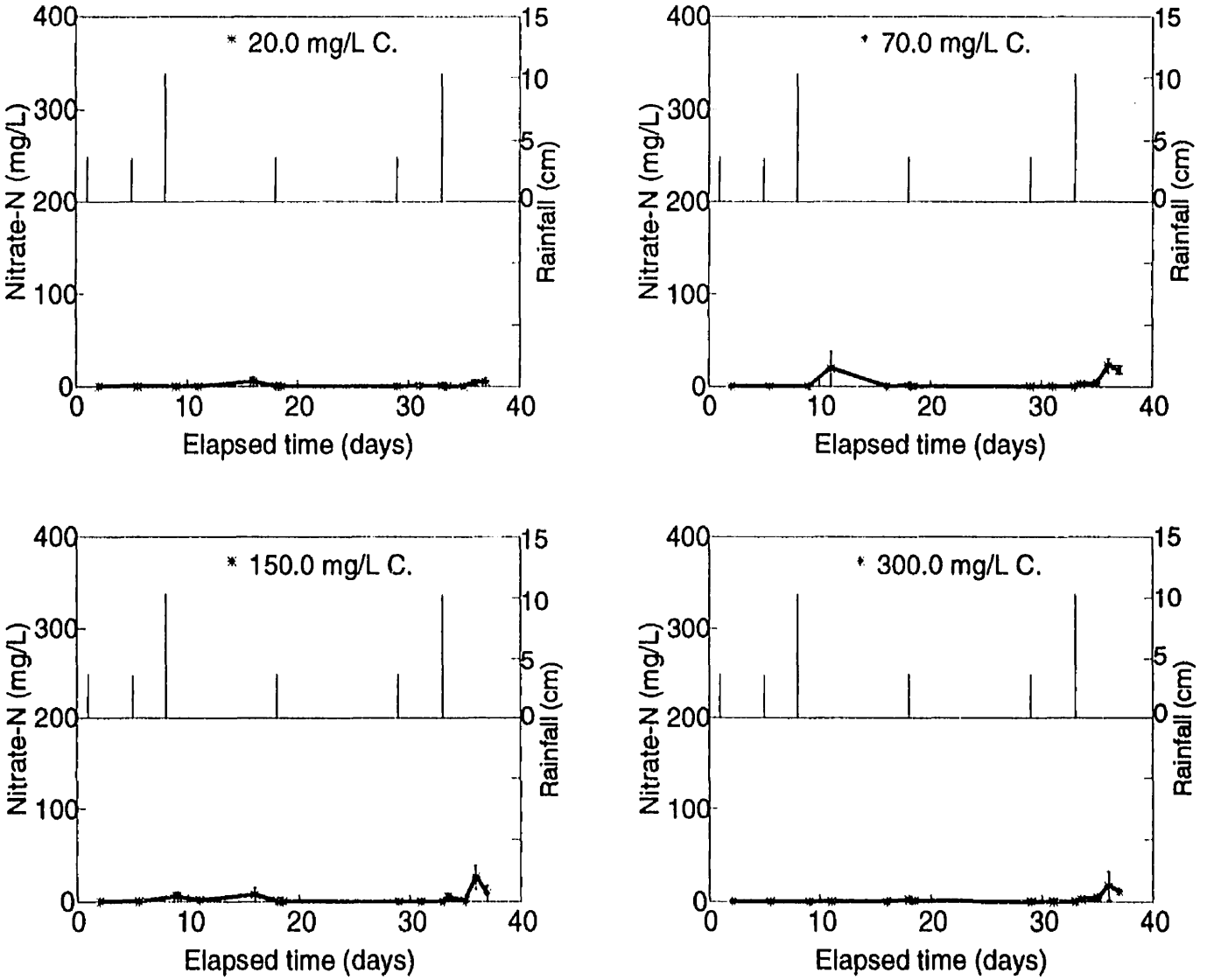


Figure 4.15: Decrease of nitrate-N residue in soil solution at the 850-mm depth with time after periodic leaching (stage 3).

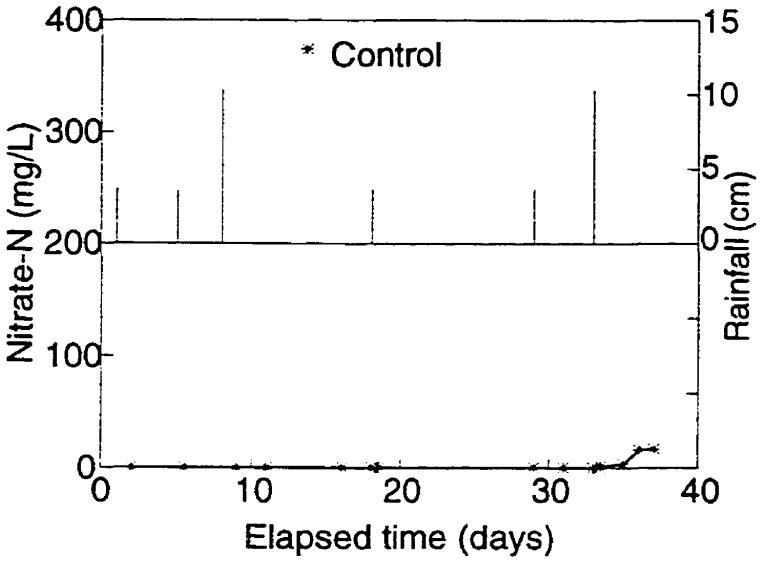


Figure 4.15(a): Decrease of nitrate-N residue in soil solution at the 850-mm depth with time after periodic leaching (stage 3).

4.1.3.2 Changes in redox potential, Fe and Mn in soil solution

The results of redox changes in the soil solution are presented in Figures 4.16 to 4.21. At the 400-mm depth, the redox potential in the treatment (150 mg/L glucose-C) and the control, decreased from a mean high of about 310 mV to 100 mV (Figure 4.16a). The redox potential values of the other treatments (20, 70 and 300 mg/L glucose-C) at the same depth, are superimposed on the curve from the 150 mg/L glucose-C level and the control results, as shown in Figure 4.16b. The measured redox potential values of the treatments fitted closely to the decreasing redox trend, from

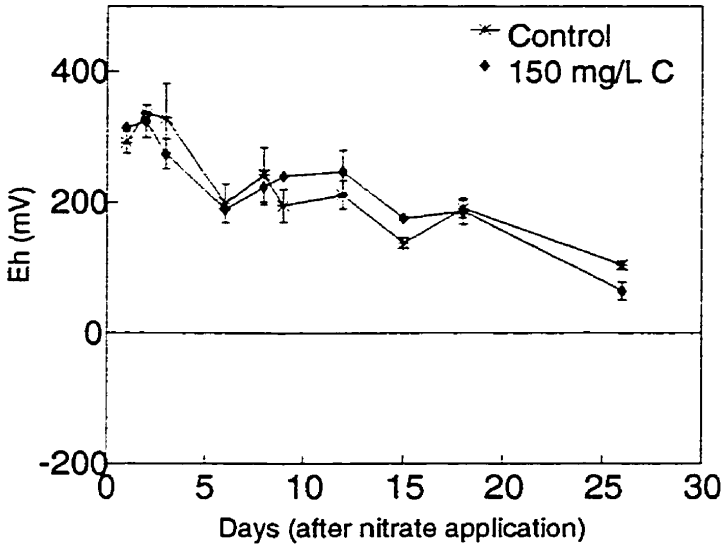


Figure 4.16a: Redox potential in soil solution at the 400-mm depth (stage 3).

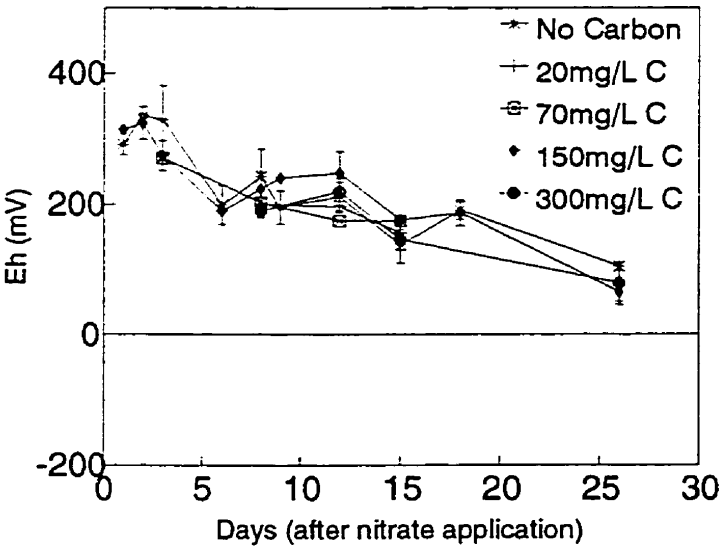


Figure 4.16b: Superimposed redox potential values of the other treatments on the 150 mg/L C treatment and control curves, at the 400-mm depth (stage 3).

about 300 to 100 mV observed at the 150 mg/L glucose-C level and the control, respectively.

Figure 4.17a shows the redox changes, at the 600-mm depth, in the 150 mg/L glucose-C treatment and control columns. The superimposed

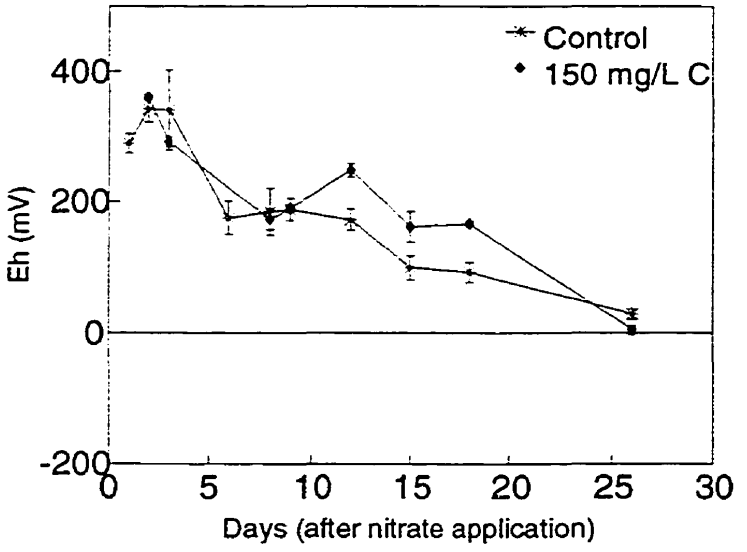


Figure 4.17a: Redox potential in soil solution at the 600-mm depth (stage 3).

curves of the other treatments (20, 70 and 300 mg/L glucose-C), on the 150 mg/L glucose-C treatment and the control, are shown in Figure 4.17b. The data points of the redox changes, from all the columns at the 600-mm depth, seem to indicate a general decreasing trend (as observed at the 400-mm depth) from about 300 to 0 mV.

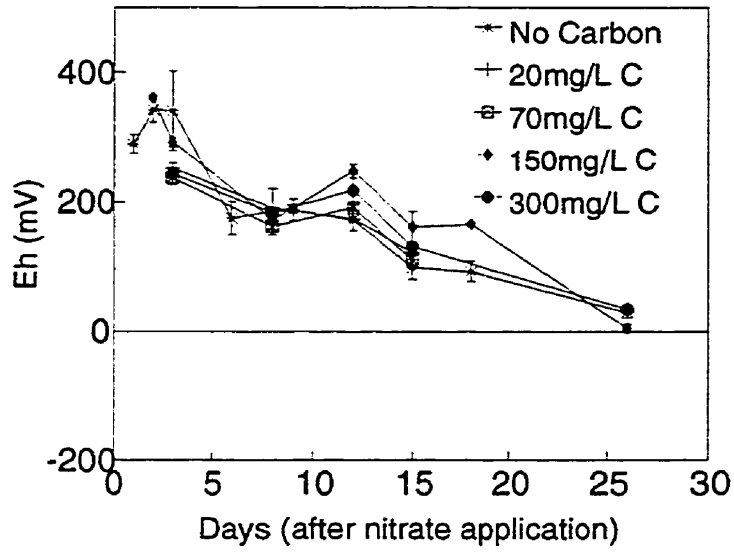


Figure 4.17b. Superimposed redox potential values of the other treatments on the 150 mg/L C treatment and control curves, at the 600-mm depth (stage 3).

The results from the 850-mm depth, for the 150 mg/L glucose-C treatment and control, are shown in Figure 4.18a; and the superimposition curves for the other treatments are shown in Figure 4.18b. The control curve had higher data points, ranging from an average high of about 270 to a low of 100 mV. Curves for the 150 mg/L glucose-C treatment started from a high of about 200 to a low of about -25 mV. (Figure 4.18a). All the fitted curves for the rest of the treatments (20, 70 and 300 mg/L glucose-C) fell between those of the 150 mg/L glucose-C treatment and the control curves. A wider variation was found within the mean values of the treatments, and between

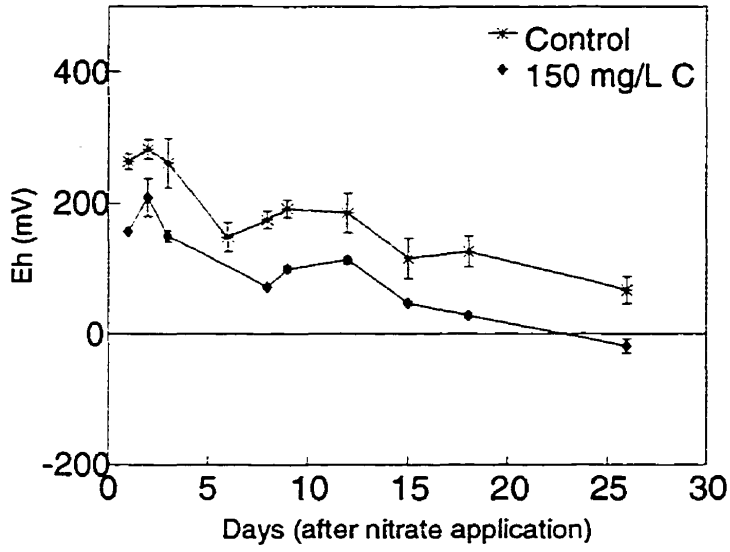


Figure 4.18a: Redox potential in soil solution at the 850-mm depth (stage 3).

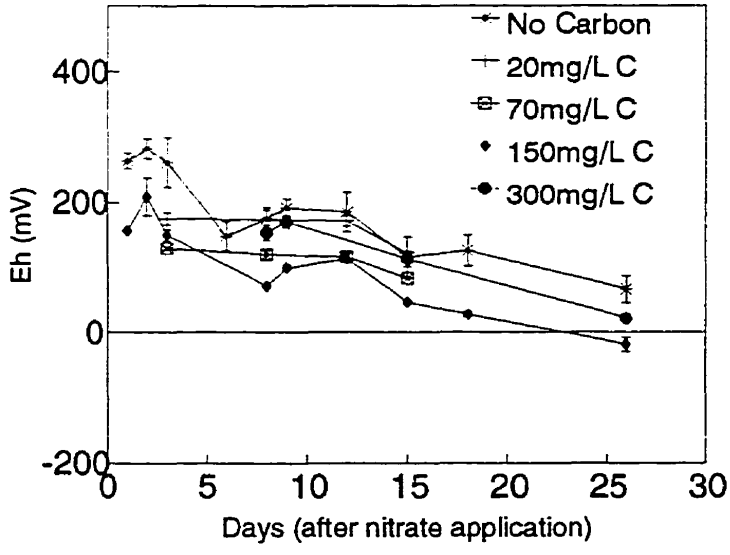


Figure 4.18b: Superimposed redox potential values of the other treatments on the 150 mg/L C treatment and control curves, at the 850-mm depth (stage 3).

the mean values of some treatment data points, and those of the control.

Figures 4.19 to 4.21 are the redox values measured on day 96 at the 400, 600 and 850-mm depths, respectively, for all the treatments including the control. At the 400-mm depth, the redox mean values for 70, 150 and 300 mg/L glucose-C treatments were significantly different (at the 5% level) from that of the control, which was above zero. The redox potential mean values for 70, 150 and 300 mg/L glucose-C treatment varied between -70 mV and -45 mV (Figure 4.19). For both the 20 mg/L glucose-C treatment and the control, which were also significantly different (at the 5% level), the redox mean values were about 12 mV and 60 mV, respectively (Figure 4.19).

Both the 20 mg/L glucose-C level treatment and the control had high redox potential values, when compared to the values for the other higher glucose level treatments. This suggests that the redox level attained in each of the treatments may be influenced by the organic carbon concentration.

A comparison of the redox values at 70, 150 and 300 mg/L glucose-C treatment levels, shows that the 150 mg/L glucose-C level decreased the redox potential more than the other glucose-C levels. Perhaps, at a glucose level of more than 150 mg/L glucose-C, substrate and product inhibition restrict microbial activities; thus preventing further lowering of the redox potential. Hence, it may be inferred that 150 mg/L glucose-C is close to the desirable for lowering redox potential when a soil is under continuous

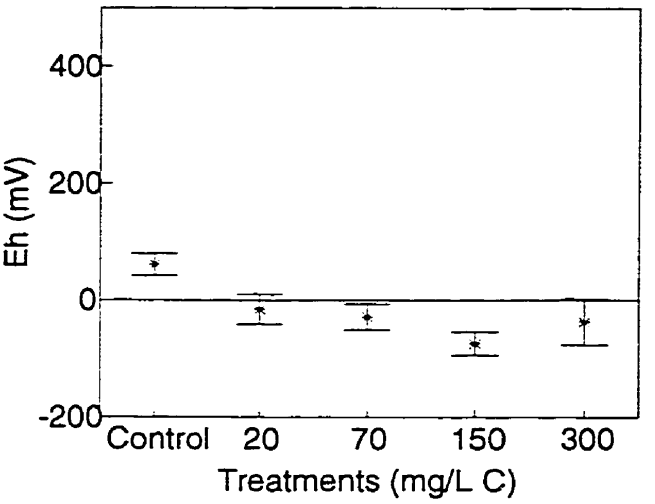


Figure 4.19. Redox potential after day 96 at the 400-mm depth (stage 3).

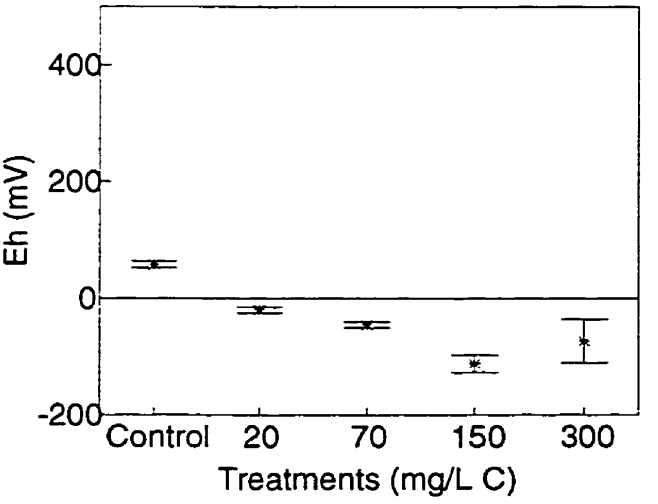


Figure 4.20: Redox potential after day 96 at the 600-mm depth (stage 3).

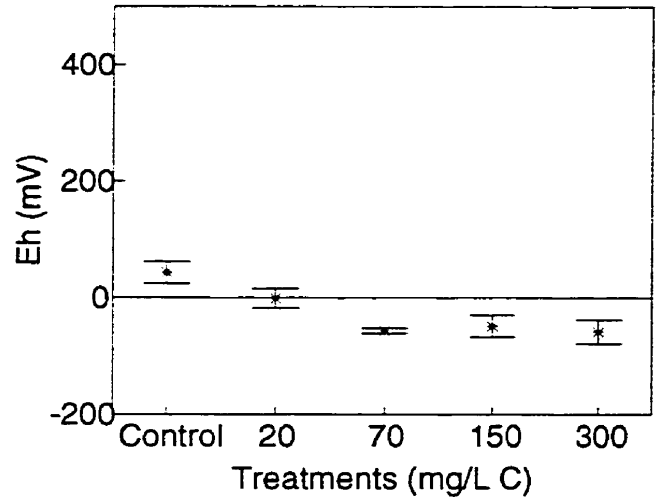


Figure 4.21: Redox potential after day 96 at the 850-mm depth (stage 3).

subirrigation with glucose solution.

Interpretation of the redox result is often qualitative (Anon., 1994). Therefore, the significance of the values for day 96 was further assessed by measuring the Fe and Mn content of soil solutions, from the three depths in all columns. Figures 4.22 and 4.23 indicate the levels of soil solution Mn and Fe, at depths of 400, 600 and 850 mm in all the columns. The control and the treatment columns that received 20 mg/L glucose-C had the least Mn and Fe content in their solutions, compared to the treatment columns with 70, 150, and 300 mg/L glucose-C concentrations. The concentration of Fe and Mn in both the control and the 20 mg/L glucose-C treatment soil solutions were not significantly different (at the 5% level) at the 400 and 600-mm

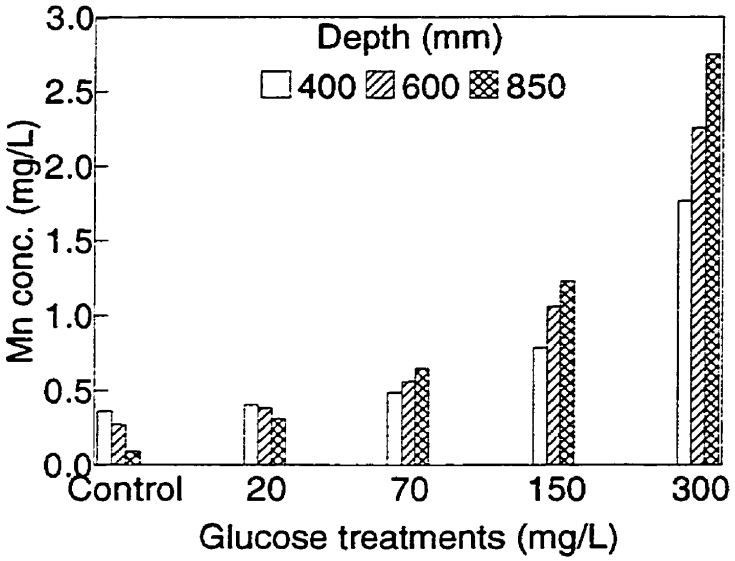


Figure 4.22: Mn concentration in soil solution on day 96 (stage 3).

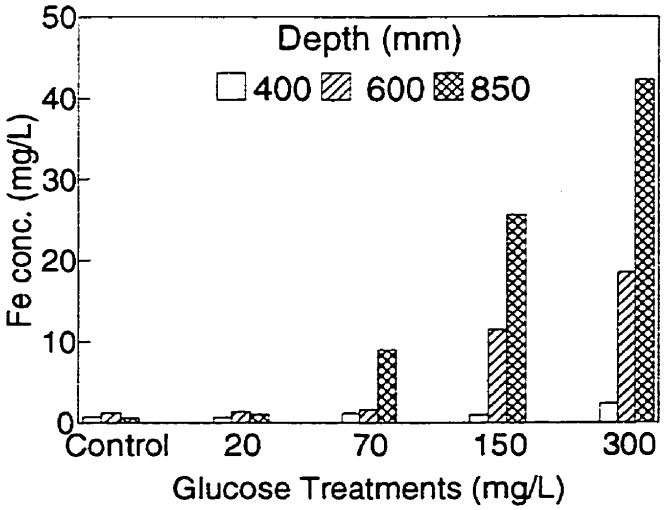


Figure 4.23: Fe concentration in soil solution on day 96 (stage 3).

depths. Fe concentration ranged from 0.5 to 1.4 mg/L, while the concentration of Mn ranged from 0.3 to 0.5 mg/L. Similarly, at the 850-mm depth, Fe concentration was not significant but Mn concentration was significant at the 5% level.

The 600 and 850-mm depths, in the columns that received the 70, 150 and 300 mg/L glucose-C levels, had a higher Mn and Fe dissolution than the 400-mm depth. The 850-mm depth had the highest Mn and Fe dissolution (Figures 4.22 and 4.23). The concentration of Mn and Fe increased in the soil solution, as the depth and concentration level of glucose-C increased. Fe dissolution seemed to be more sensitive to the addition of carbon to the soil (Figure 4.23). The decrease of Fe concentration at lower depths indicates that the glucose-C was gradually utilized before reaching the depth of 400 mm. This was indicated by lower concentration of Fe at depths of 400 and 600 mm, compared to the concentration at the 850-mm depth.

An organic carbon source, which does not lead to the dissolution of Fe at a lower depth, will be the most suitable source of reductant for denitrification. The sensitivity of soil, to external input of excess organic carbon, can be monitored by the redox potential and Fe dissolution in soil columns receiving carbon supplement. These two factors can be useful parameters in monitoring and implementing a management strategy, for supplementing soil with organic carbon for enhanced in-situ bioremediation

of nitrate.

According to Tate (1995), the redox potential remains close to a specific level, until the electron acceptor associated with that potential finishes. Subsequently, the redox potential drops allowing the use of the next available terminal electron acceptor. Patrick and Jugsujinda (1992) also observed that no overlap occurs in the oxidation or reduction of nitrate and manganese. However, little overlap was found in the redox of manganese and ferrous ions. Furthermore, it was noted that an overlap might occur between nitrate and Mn, if the soil was reduced enough to support Mn reduction before all the nitrate was reduced. Tate (1995) further pointed out that when nitrate or sulphate is utilized as the final electron acceptor, the organic carbon source of energy is completely oxidized to CO_2 and water. Therefore, byproducts of the carbon source, other than carbon dioxide, will not pose a threat to water resources if the nitrate pollutant, continuously leached by rainfall, is serving as the final electron acceptor.

Dissolution of manganese and iron compounds, shown in Figures 4.22 and 4.23, respectively, demonstrate that iron and manganese compounds, that play a role in contaminant mobility in the soil, will increase at a low redox potential. The extent of dissolution is determined by the level of glucose added to the soil; the higher the level of glucose added to the soil, the greater the adverse impact of the resultant Fe and Mn levels on the soil ecosystem.

Fe and Mn are included in the secondary drinking water standard, although they are essential minerals which become toxic in large doses (Csuros, 1994). The limit recommended for Mn in water by U.S. E.P.A. and Canadian Water Quality Guidelines is 0.05 mg/L, while the combined Mn and Fe limit is 0.30 mg/L, when based upon aesthetics and taste. The levels obtained in both the control and 20 mg/L glucose-C treatment were higher than the stipulated limit of 0.30 mg/L; this is primarily because of the high organic matter content of the soil. The high Fe concentration ranging from 1.3 mg/L to 44.6 mg/L obtained at 300 mg/L glucose-C, confirms the observation in stage 1 where the 970 mg/L glucose-C produced a solution of greenish hue and, after standing on the laboratory bench, formed a brownish precipitate.

The measured pH values of the solutions sampled did not vary much between treatments (Figure 4.24). The values ranged from 6 to 7. Although, the exact optimal pH for denitrification is variable, Parkin et al. (1985) observed two distinct optimal pH values close to the native soil pH of 3.9 and 6.3. The pH parameter was not very sensitive in discriminating between the different carbon levels. Generally, the denitrification rate decreases as the pH is lowered (Waring and Gilliam, 1983).

An attempt was made at this stage to estimate the glucose level that

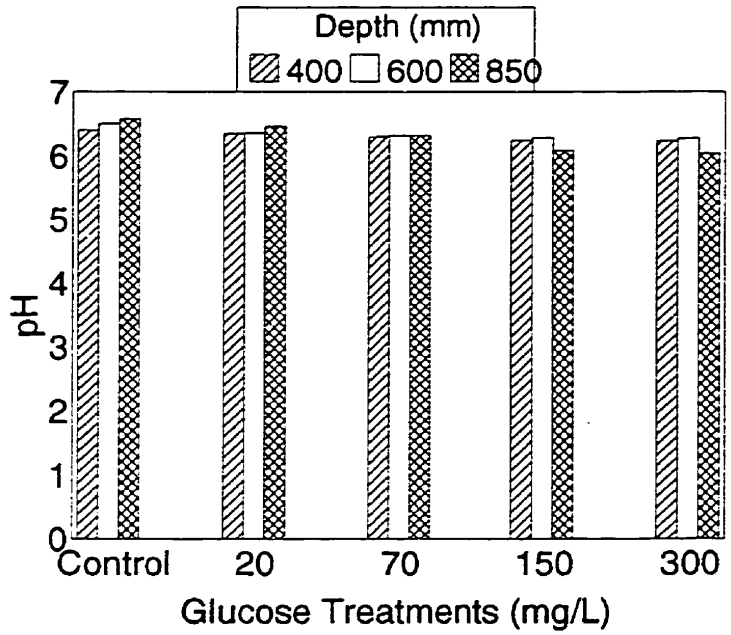


Figure 4.24: pH of soil solution at different depths on day 96 (stage 3).

had the least adverse impact on soil solution properties. More specifically, stage 3 of the experiments tested the long-term impact of continued carbon addition to a packed soil column undergoing subirrigation. It also assessed the desirable glucose-C concentration range which will not affect the soil solution Fe and Mn levels.

Prolonged addition of organic matter to the soil columns in stage one of this qtudy, caused a dramatic drop in the soil solution redox potential. In addition, effluent from the treatment columns in stage 1 formed a brownish iron precipitate when exposed to air; this brownish precipitate suggests Fe dissolution from the soil. It was, therefore, necessary to quantitatively compare the amount of Fe reduced in relation to the different glucose

treatments; and then to compare these values with the obtained redox values. It was decided to ascertain the Fe and Mn content when there was neither input of fresh water via rainfall simulation, nor loss of Fe and Mn during draining of the columns. Thus, on day 96, about 55 days after the last leaching event, Fe and Mn content of the soil solution was determined at the 400, 600 and 850-mm depths, in all the columns (Figures 4.22 and 4.23).

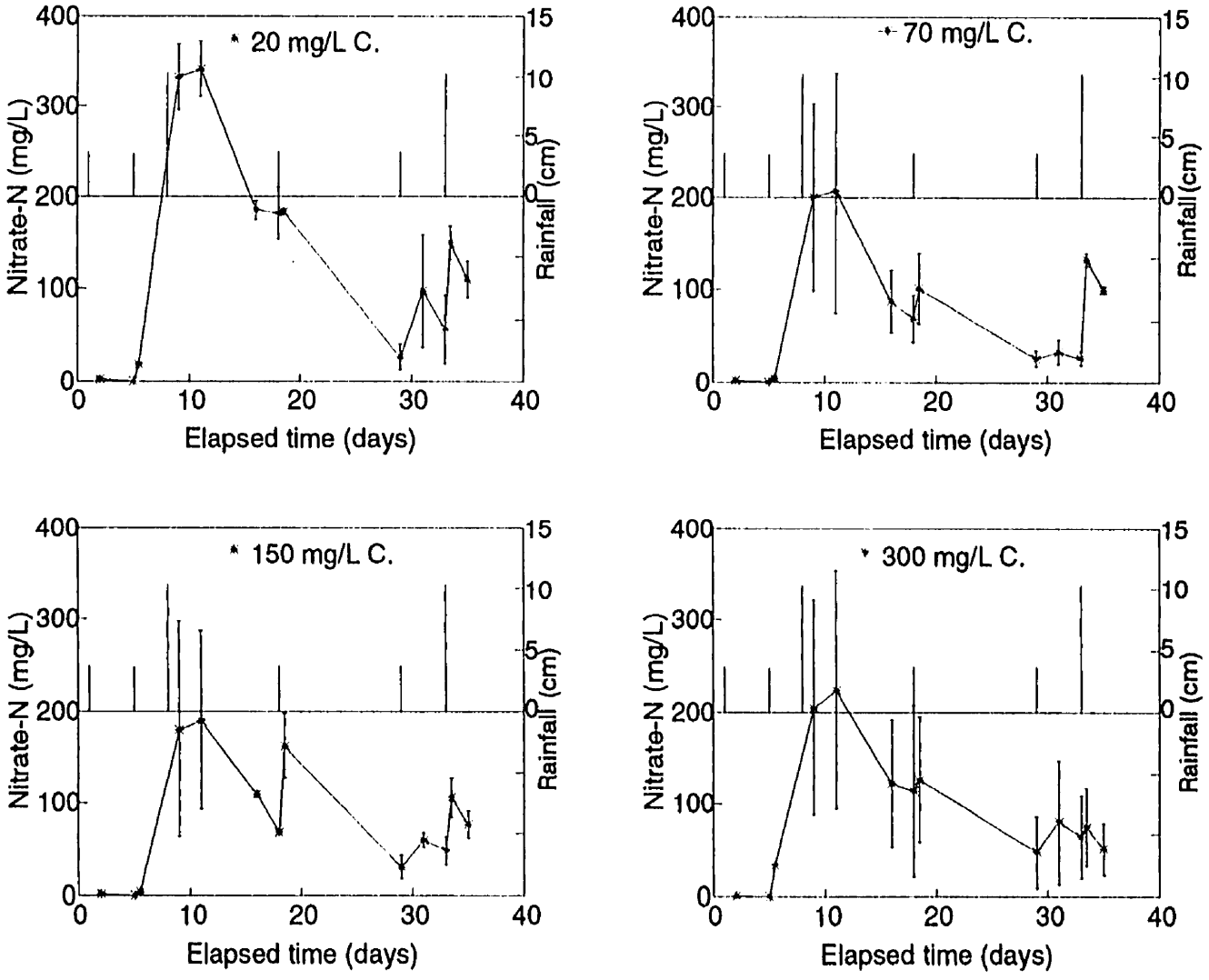
Input of carbon, especially at high levels, affected the constituents and properties of the soil solution chemistry. To further reduce the effect of addition of organic carbon to the soil, especially at input depths, an alternate feeding of organic carbon solution and water to the soil is suggested since the input zone of the soil had the most reduced state of all treatment levels, the organic matter content of the soil not withstanding. The latter would assist in minimizing an extreme drop of redox potential of the soil solution, while sustaining a reduced anaerobic condition without any adverse effect of solubilizing Fe and Mn compounds. Such an alternate feeding strategy would be conducive for denitrification throughout the planting season.

Leaching in stage 3 did not transport much nitrate to the lower depths, for an effective assessment of nitrate reduction; however, stage 3 helped to stabilize the packed soil column, after disturbing the soil with excavation and packing. Furthermore, stage 3 indicated that with prolonged subirrigation of the columns with different levels of organic carbon, the higher levels of

glucose caused dissolution and mobilization of soil minerals, such as Mn and Fe compounds and possibly other trace nutrients, which were not assessed in the present study. Stage 4 of the experiment therefore repeated stage 3 using the same levels of glucose-C. More specifically, stage 4 monitored the effect of organic levels on nitrate reduction. It determined the desirable range of glucose-C supplement which can maximize nitrate reduction, without compromising potable water quality beyond the effect elicited by the control treatment.

4.1.4 Desirable glucose range for nitrate reduction

Figure 4.25 shows the trend in nitrate appearance and disappearance at the 400-mm depth after rainfall simulation, on columns receiving 20, 70, 150 and 300 mg/L glucose-C, respectively; while figure 4.25a indicates the nitrate trend in the control columns. The initial two rainfall events on days 1 (11 mm) and 5 (11 mm) hardly leached any nitrate into the water table, because of the small depth of rainfall simulated. But the third rainfall event (32 mm) leached an average of 200 mg/L nitrate-N or more to the 400-mm depth in all the columns, including the control columns (Figures 4.25 and 4.25a). The subsequent rainfall events on days 18, 29, and 33 leached more nitrate into the lower depth. Throughout the experimental period, after the second rainfall event, the average was above 40 mg/L N at the 400-mm



Figures 4.25: Decrease of nitrate-N residue in soil solution at 400-mm depth with time after periodic leaching (stage 4).

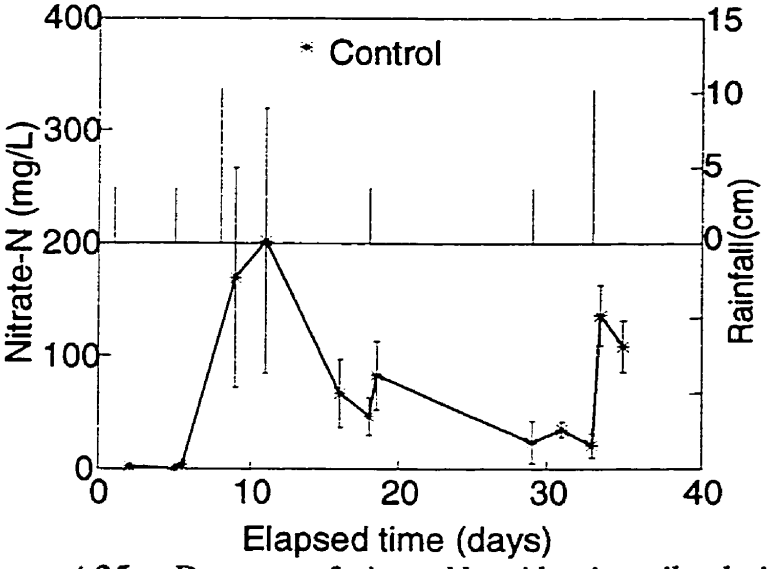


Figure 4.25a: Decrease of nitrate-N residue in soil solution at 400-mm depth with time after periodic leaching in the control (stage 4).

depth. The 400-mm depth operated at a maximum denitrification potential in each column, from days 9 to 31; denitrification was not limited by nitrate concentration in any of the columns. Therefore, a zero-order kinetics, which is independent of the amount of nitrate present, may be assumed since the lowest amount of nitrate was about 40 mg/L nitrate-N. The nitrate-N concentration has been shown to be sufficient to saturate the enzyme system, thus resulting in a reaction rate being determined by carbon availability rather than nitrate level (Paul and Clark, 1989).

The total nitrate losses at the 400-mm depth in all the columns

receiving carbon additive were estimated within 29 days of applying nitrate. At the 400-mm depth, the nitrate loss in the control was significantly different (at the 5% level) from the 20 mg/L glucose-C treatment. By the fifth rainfall simulation, on day 18, 82±30 and 183±3 mg/L nitrate-N load was leached to the 400-mm depth of the control and the 20 mg/L glucose-C treatment columns, respectively. The control nitrate load decreased from 82±30 to 23±18 mg/L nitrate-N by day 29, while that in the 20 mg/L glucose-C treatment diminished from 183±3 mg/L nitrate-N to a similar level (26±13 mg/L nitrate-N) within the same period (Figures 4.25 and 4.25a).

To determine the total nitrate losses for each treatment, nitrate concentration at the 400-mm sampling depth was assumed to represent a nitrate distribution spread which is 100 mm thick above and below the sampling port. This layer started from 50 mm above the water table, at 350 mm from the soil surface. Below the 400-mm layer is the 600-mm layer, that is also 200 mm thick. The lowest layer is the 850-mm depth zone, which has a thickness of 300 mm. The total nitrate losses were calculated for the depth of 400 mm using the average porosity of 0.4 (Volume × Porosity × Concentration (mg/L)). Figure 4.26 indicates the cumulative nitrate-N losses at 400-mm zone, at each level of the treatments including the control (control, 20, 70, 150 and 300 mg/L glucose-C, respectively). Figure 4.26 shows that the columns treated with 20 mg/L glucose-C significantly (at the

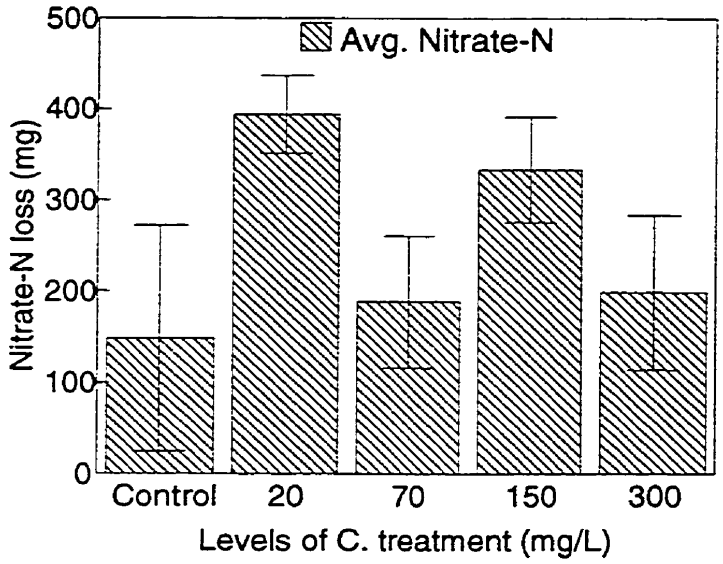


Figure 4.26. Cumulative nitrate-N losses between days 18 and 29 in soil columns at the 400-mm depth, after treatment with different glucose-C levels.

5% level) removed more nitrate from the leachate that reached the sampling zone, when compared to the control.

The poor performance of the 300 mg/L glucose-C level suggests that the presence of excess organic carbon decreased nitrate loss. This confirms the finding of Bremner and Shaw (1958a) that nitrate loss increased and then decreased with an increase in the amount of organic carbon. They explained the loss in reduction as a result of fixation of atmospheric nitrogen by other organisms which are utilizing the excess carbon. Alternatively, the reduction in nitrate loss could have resulted from excessive dissolution of mineral ions,

that may be toxic to microorganisms. It is known that at a high carbon level, microbial activity (fermentation) is increased, in turn leading to the formation of organic acids.

Table 4.1 gives the pH of drain effluents in all the treatments during nitrate leaching at stage 4. The table shows that the pH of the drain effluents for the control and 20 mg/L glucose-C treatment were very similar and remained above 6. In contrast, the rest of the treatments were lower by about one order. The organic acids formed by the microorganisms might have forced the pH to plunge to a lower value, thus initiating the dissolution of Fe and Mn in the system.

Tate (1995) confirms that Fe and Mn are more soluble at a low pH, and that acidic conditions are toxic to some microbial metabolism. Transport capacity of carbonaceous compounds, such as fatty acids and amino acids, are dependent upon the charge of the molecules. The charges on these compounds are pH dependent, and a reduction of soil pH below the pk_a value of the acids might convert negatively charged compounds to neutral compounds or alter the charge on zwitterion. Such alteration of the charge state of a compound changes the capacity of the cells or their catabolic enzymes to catalyze biotransformation and transportation of the carbonaceous materials (Tate, 1995).

Table 4.1 shows that the pH of drain effluent from the 150 and 300

Table 4.1. pH of drain effluent during nitrate leaching (stage 4).

	Treatment (mg/L glucose-C)				
	0	20	70	150	300
Time (days)			pH		
5	7.0	6.2	5.4	4.9	4.8
8	6.7	6.1	5.6	4.7	4.6
18	6.9	5.8	5.5	4.8	4.8
29	6.9	6.3	4.9	4.8	4.6
33	6.9	6.2	6.0	5.2	4.9

mg/L glucose-C treatment columns were about two orders lower than those from the 20 mg/L glucose-C and control columns. This might account for the high level of Mn and Fe (Figures 4.22 and 4.23, respectively) found at the 850-mm depth in the 150 and 300 mg/L glucose-C treatments. The additive effect of the lower pH and excessive dissolution of Mn and Fe resulted in the low performance of the 300 and 150 mg/L glucose-C treatments, in comparison with that of the 20 mg/L glucose-C treatment.

4.1.5 Assessment of bioplugging

Shouche et al. (1994) reported that biofouling occurs close to nutrient injection inlets in in-situ bioremediation, because of the high nutrient concentrations which result in the proliferation of organisms; consequently,

this leads to the bioplugging of the injection ports and the soil zone in the vicinity. When this occurs, it is accompanied by a decrease in the hydraulic conductivity of the soil. In this study, the saturated hydraulic conductivities in St. Amable and St. Benoit soils, initially 77.6±33.7 and 142.0±16.6 mm/h, respectively, did not change significantly (78.5±42.0 and 144.4±12.0 mm/h, respectively). A comparison of the biomass density, close to the surface and to the bottom of the soil columns, revealed that the microbial populations in the columns were close to the population distribution of 10^6/g often found in soil. In some columns, the density was less by one or two orders (Table 4.2). Further investigation of the low count, below normal estimate, was not undertaken since the aim of the count was to establish non-proliferation of biomass in the soil columns.

These results suggest that bioplugging did not occur at the bottom of the soil columns as a result of subirrigation of soil columns with glucose solution. The 850-mm depths in columns, receiving 150 and 300 mg/L glucose-C were an order higher than the rest. However, about the same microbial population was found at the 100 and 850-mm depths of the 300 mg/L glucose treatment.

Given the premise that bioplugging occurs closest to the point of injection, an increase in biomass was expected at the 850-mm depth; however, this did not occur. The 100-mm depth (top surface), which was

quite removed from this zone and should have shown a lower microbial population count, had the same biomass as the 850-mm depth.

Table 4.2 Microbial population (total count) distribution close to the surface and bottom of soil columns (stage 4).

Treatment mg/L glucose	Depth (mm)	Colony-forming units/g of soil
0	100	5.300E+05
	850	8.333E+05
20	100	1.550E+05
	850	5.800E+05
70	100	7.500E+04
	850	3.800E+05
150	100	5.350E+05
	850	2.500E+06
300	100	1.065E+06
	850	1.163E+06

Furthermore, Tate (1995) found that the accumulation of organic acids, under anaerobic conditions in an acidic environment, can limit microbial metabolism. Low pH values were observed in the drain effluent from the 150 and 300 mg/L glucose-C treatments (Table 4.1); such low values could limit microbial activities in these columns. Thus, the high concentration of organic carbon did not alter the microbial population density in the soil columns.

4.1.6 Evaluation of supplementing soil with organic carbon

The similarity of results, obtained from the columns receiving glucose carbon supplement and the control columns, may be due to the initial organic carbon content in the soil. This organic carbon level in the control will continue to decrease unless it is replenished; and denitrification will concurrently and gradually decrease with time, as shown in Figures 4.1, 4.2 and 4.26. There is a desirable carbon range which, when exceeded, could result in a decrease in denitrification capacity of the soil. Figure 4.26 clearly indicates the 20 mg/L glucose-C level as the desirable concentration in the St. Benoit soil used in the study.

Figure 4.26 shows that the treatment, employing 20 mg/L glucose-C solution, reduced more nitrate than the control by about 246 mg N; this represents a difference of about 81 kg/ha of nitrate-N. Evans et al. (1990) cited a nitrate-N leachate load of between 3.7 and 32.4 kg/ha/year to the surface water, under low to high intensity subsurface water table management practice. It was further stated that the nitrate load decreased by 45% (10 kg/ha/year nitrate-N) under controlled drainage. The average 81 kg/ha of nitrate-N loss, obtained from the 20 mg/L glucose-C treatment column at depths of 400 mm (Figure 4.26), is more than twice the loss (32.4 kg/ha nitrate-N) to the drains every year (Evans et al., 1990). Such a result shows that supplementing subirrigation water, with the desirable amount of

readily available carbon, will reduce nitrate loss to surface and groundwater bodies. Thus, this method can be used for sustainable agricultural practices in humid regions, whereby the use of nitrate fertilizer would not lead inevitably to nitrate pollution.

It has been shown that by raising the water table close to the surface with subirrigation water, supplemented with a readily available carbon, a quick reduction of the residual nitrate may be effected in the unsaturated zone. This has importance when controlling nitrate losses during the fall and the spring snow melt. In early spring, the major source of nitrate in field drains is the residual nitrate in the unsaturated zone. These nitrate residues could be removed by this system, immediately after harvesting. Thus, nitrate levels in the drain outflow in early spring would be less than the 10 mg/L nitrate-N limit.

The use of shallow water tables or CD-SI has been criticized since it may increase runoffs and consequently encourage the mobility of other applied agrochemicals, including herbicides and pesticides. It is, therefore, preferable to raise the water table for just a short period to permit clean up of nitrate residues in the unsaturated zone. Quick reversal to drainage mode will then allow infiltrations during rainfall events and thus reduce losses in runoff.

Measurements of the increase in biomass in the columns, in order to

ensure non plugging of the soil pore structure, did not show any significant proliferation of microorganisms after the introduction of organic matter. Vandevivere and Baveye (1992) showed that bioplugging of the soil profile could occur if oxygenated nutrients percolate through the soil profile. It was also shown that the extent of the plugging could be determined by the nutrient composition of the percolating liquid. In this study, however, the conditions for denitrification were anaerobic, and cell yields might have been limited by nitrogen once the denitrification had dissipated any leached nitrate. Nitrate reached the saturated zone in pulses, according to rainfall events rather than continuously, suggesting the possibility of intermittent nitrogen limitation. Therefore, the probability of soil pore-plugging was extremely low, since the biomass yield was low under anaerobic and nutrient limiting conditions (Semprini et al., 1991).

4.1.7 Managing carbon supplement in subirrigation water

One of the primary goals of this study was to develop a pollution control system for nitrate residue that is inexpensive, and also easily adaptable to existing infrastructure in subirrigated fields. Management recommendations will be suggested for adopting the technique in the field and to minimize costs.

Management of carbon augmentation for in-situ bioremediation of

nitrate residue should be determined primarily by the nitrate pollution index of water resources in the area. The amount of carbon added, in a given farm situation, will depend on:

a) Readily available carbon content in the soil profile, especially within the water table, which gives an indication of the amount of organic carbon to be added to the soil. Organic carbon is correlated with denitrification potential. When a soil profile with a low organic carbon matter (e.g. Table 2.1) and previously subjected to decomposition and leaching can not support denitrification of nitrate in the leachate, organic carbon supplementation is recommended.

b) Maintaining a high water table elevation, which will need more water than a low water table. Augmenting organic carbon under shallow depths will require more carbon than under deep water table conditions because of increased volume.

c) The study showed that excessive organic carbon supplement to the soil might lead to adverse effects. This is indicated by low redox potential and Fe dissolution. In the summer, rainfall rarely cause drainage. Thus, the addition of carbon is not necessary.

d) Under coarse textured soil with a high hydraulic conductivity that facilitate efficient mass transfer of nitrate, there is more leaching of nitrate into the ground water; there is also more soluble organic

carbon leaching from tillable surface. Although carbon is simultaneously leached with nitrate, the amount reaching the lower depths may be small (Table 2.1). Evans et al. (1990) cited a nitrate-N leachate load of between 3.7 and 32.4 kg/ha/year to the surface water; Zhou (1996) showed that under WTM, nitrate-N levels ranging from 25 to 55 kg/ha were found at 750 mm depth after harvest. Thus, the carbon that leached was not sufficient, and some leached nitrate will remain within the water body if a low denitrification potential exists in the saturated zone. Furthermore, if the permeability of the soil barrier below the drains allows deep seepage, more nitrate will reach the ground water instead of being carried to the surface water by the drains. Under these conditions, namely: deep water table, low denitrification potential, and a permeable or a semi-permeable barrier below the drain together with a predicted wet cropping season, it is suggested that subirrigation water be supplemented with readily available carbon during the wet season.

e) Finally, climatic conditions determine if the cropping season will be wet (e.g. 800 mm/year rain) or dry (e.g. 480 mm/year rain). In a particularly wet year, more nitrate will leach into the water table. The facility to cope with such a load is dependent on denitrification potential within the water table. If the denitrification potential is low,

and the pollution index of ground water is not favourable, and nitrate pollution has been reported, the organic carbon in the saturated zone of the soil would need to be augmented throughout the wet season. However, in a dry year most of the nitrate would remain within the unsaturated zone. If the load poses such a danger, especially after harvest when the water table is lowered, it might be necessary to facilitate its removal by denitrification. This would regenerate a seemingly pristine condition. The decision, regarding future augmentation, would depend on how quickly newly applied nitrate reaches the water table in the next cropping season.

Zhou (1996) showed that under a water table depth of 700 to 800 mm, different levels of soil nitrate, which decreased with depth, will be distributed in the top 1000 mm soil profile during a consecutive fall, after harvest, and spring, before the planting season. In fall, nitrate-N residue ranging from 60 to 90 kg/ha was accumulated at 150 mm depth. At the depth of 500 mm, the nitrate residue was less, ranging from 35 to 60 kg/ha, except in the 800 mm water table depth where the nitrate residue exceeded 90 kg/ha. The soil nitrate residue at 750 mm depth ranged from 25 to 55 kg/ha nitrate-N, while the residue at the 1000 mm depth was between 15 and 30 kg/ha nitrate-N. By the next spring following winter, the

nitrate residue at the 150 mm depth decreased from an average of 75 kg/ha nitrate-N to an average of 35 kg/ha. At 500 mm depth, the nitrate residue after winter did not show much change for the 700 mm water table depth, accumulating an average of 48 kg/ha, while the 800 mm water table depth decreased from over 90 to 50 kg/ha nitrate-N. At 1000 mm depth, an average of 20 kg/ha nitrate-N residue remained in spring following winter.

These changes in nitrate-N residue most likely resulted from non-growing season precipitation which leached some of the nitrate residue out of the soil profile (Liang et al., 1991), and might pose a nitrate pollution threat to the environment. Losses of nitrate occur in fall and spring (Liang and MacKenzie, 1994; Zhou, 1996). Zhou (1996) study suggests that under water table management, nitrate pollution threat is possible after harvest. Therefore, a quick management decontamination of the soil profile below the root zone, before the onset of the sub-zero temperatures, likely to reduce microbial activities, should be undertaken.

In her study, Zhou (1996) showed that although the water table was maintained at 70 and 80 cm, the soil nitrate-N residue, after harvest at 75 cm depth, ranged from 25 to 55 kg/ha nitrate-N, while the residue at the 100 cm depth was between 15 and 30 kg/ha nitrate-

N. It could be deduced, therefore, that the water table during the growing season was not able to reduce all the nitrate that leached into the water table through denitrification.

Considering the above management possibilities, a region with a coarse textured sandy soil, which also has an adversely high nitrate pollution index of water resources, is recommended to adopt this technique. Repetition of treatment may vary depending on the amount of applied nitrate-N, climatic factors, depth of ground water, nitrate pollution index, etc. The most cost effective strategy would be a one time treatment of nitrate residue in the soil profile below the root zone immediately, as observed in Zhou (1996) study, after harvest especially in a particularly dry growing season; in a wet season, subirrigation water can be alternated with carbon solution.

Assuming the nitrate distribution in St. Benoit soil is similar to that employed by Zhou (1996), using sugar as source of organic carbon, it will cost about $113/ha to treat (after harvest) a 75 mm soil profile starting at 25 mm from the surface, and the subirrigation network located at 1000 mm from the surface. It is expensive and, therefore, more research is needed to identify a cheaper source of carbon rather than using a commodity product such as sugar.

4.2 In-Situ Bioremediation of Diesel

The results for the nutrient delivery system used for in-situ bioremediation of diesel contaminated soils are presented in this section. Different subsections present: a) assessments of the delivery system based on diesel-TPH reduction when supplying nutrient, air and water or air and water to the microbial population in the soil columns, using subirrigation water table management practice; b) diesel biodegradation rate; and c) comments on nutrient delivery to subsoil microorganisms. The last subsection discusses the management of oxygen and water by the delivery system.

4.2.1 Diesel-TPH reduction in the full column treatments

In the full column treatment, A_f (nutrient, air and water), the diesel-TPH reduction varied with time (Figures 4.27 to 4.29). By day 26 of treatment, the diesel-TPH in the columns receiving nutrients, air and water decreased from about 670 to 106±5, 159±52, and 175±30 mg/kg soil at depths of 1,500, 1,000 and 500 mm, respectively. The 1500-mm depth had the highest average decrease in diesel-TPH level, followed by the 1000-mm depth. The lowest decrease in diesel-TPH occurred in the uppermost depth of 500 mm. Although the three depths showed differences in their average diesel-TPH levels, the differences were not significant at the 5% level.

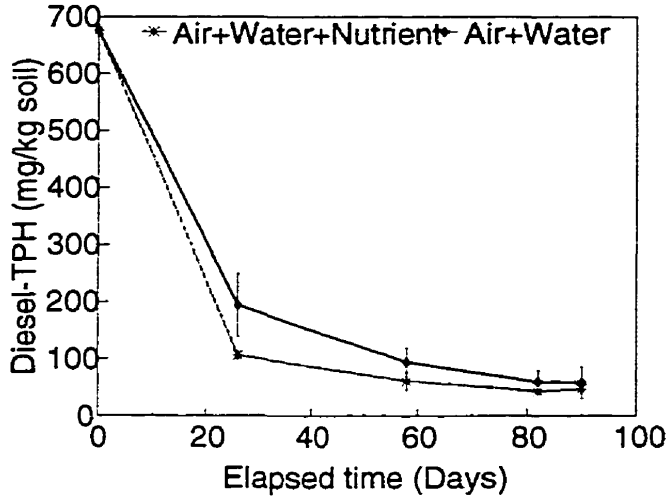


Figure 4.27: Diesel-TPH residue at the 1,500-mm depth, in full column treatments.

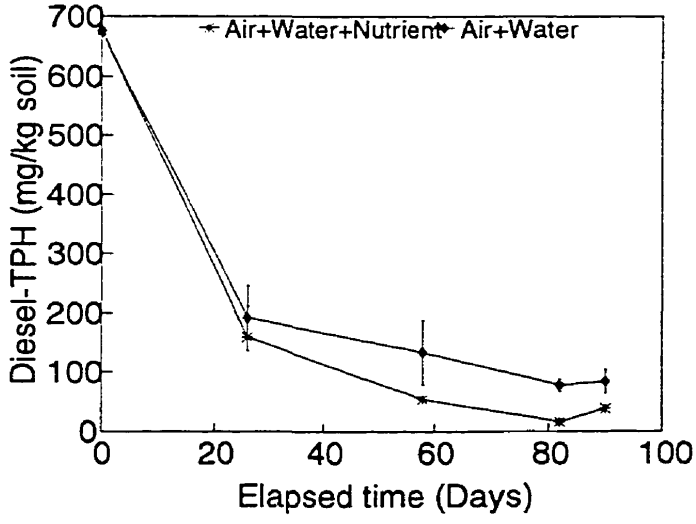


Figure 4.28: Diesel-TPH residue at the 1,000-mm depth, in full column treatments.

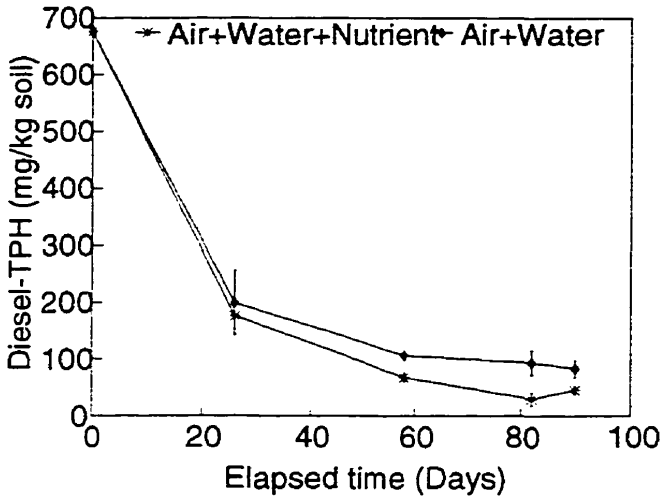


Figure 4.29: Diesel-TPH residue at the 500-mm depth, in full column treatments.

In the air and water full column treatment B_f, (Figures 4.27, 4.28 and 4.29), there was no significant difference, at the 5% level, observed in the diesel-TPH level at all depths on day 26. At the lowest depth of 1,500 mm, the diesel-TPH level was 193±55 mg/kg soil, and at 1,000 and 500-mm depths, the levels were 191±55 and 198±56 mg/kg soil, respectively.

Comparing B_f with A_f treatments at 1,500-mm depth by day 26, both were slightly significantly different ($0.05 \leq p < 0.10$). A combination of nutrient, air and water treatment (A_f) performed better than air and water only (B_f), due to the nutrient supplement in A_f. The decrease of Diesel-TPH in B_f treatment from the initial 670 mg/kg soil at all depths, could be

attributed to the native nutrients in the soil, and also to the external air supply to the contaminated soil.

By day 58, diesel-TPH level under treatment A_f decreased to 61±15, 54±4 and 67±6 mg/kg soil at depths of 1,500, 1000 and 500 mm, respectively. By day 90, it had decreased at the 1,500, 1,000 and 500-mm depths to 47±7, 38±5 and 45±6 mg/kg soil, respectively. Similarly, on day 58, the diesel-TPH in columns subirrigated with air and water only (Treatment B_f), also decreased to 94±25, 134±54 and 105±8 mg/kg soil at 1,500, 1,000 and 500-mm depths, respectively. The respective depths finally reached diesel-TPH level of 59±28, 85±20 and 83±15 mg/kg soil on day 82.

The lowest zone, the 1500-mm depth, was expected to indicate the best performance in diesel reduction since subirrigation of the soil columns with nutrients and air additives began at this depth, before reaching the upper soil zones at depths of 1,000 and 500 mm. Because some nutrient constituents, especially the phosphates and ammonium ions, have limited mobility in the soil, it was imperative that the lower portions should receive more; thus, the degradation of more TPH was expected. It may be deduced that the initial nutrient content of the soil masked the effect of supplementary nutrients to the soil microorganisms.

The combination of nutrient, air and water treatment showed significant difference (at the 5% level) from the air and water treatment only

at depths of 500 and 1,000 mm on days 58, 82 and 90. However, the diesel-TPH residue declines in both treatments were high at all depths, in the full column treatments, during the initial 26 days of treatment. But this decrease slowed as lower concentrations were approached. This rate of decrease may be attributed to the formation of recalcitrant products, not easily degraded by the organisms. Alternatively, the decrease might be due to a low substrate (diesel contaminant) concentration that could not saturate the enzyme system; consequently, this led to a slowed biodegradation rate.

The adjusted probabilities of significance for the observed F-value showed that time was highly significant ($P < 0.001$). However, the depth-time-treatment interaction tended to a lower significance ($P < 0.1$). The very high significance of time can be attributed to a high efficiency of the delivery system in supplying essential elements which enhanced the biodegradation of diesel in the soil. But the indigenous nutrient decreased the significance that would have been observed in the delivery system with respect to depth. That effect was further examined by using polynomial contrasts for depth. A slight significant ($P < 0.1$) quadratic depth contrast of the treatment was observed, suggesting that the quadratic component of the nutrient delivery along the soil profile made some difference in the biodegradation of diesel despite the masking effect of the indigenous constituents.

4.2.2 Diesel-TPH reduction in the stepwise column treatments

Treatment commenced with the lower soil zone (1,500-mm depth zone), and lasted for 82 days before the upper soil zone was treated . By day 26, the diesel-TPH residues dropped from about 670 to 112±9 and 102±3 mg/kg soil, in treatments A_s (nutrient, air and water) and B_s (air and water), respectively (Figure 4.30). In the subsequent days, 58 and 82, the A_s regime decreased further. After 82 days, the diesel-TPH residue in both treatments were 17±7 and 21±5 mg/kg soil, for A_s and B_s, respectively.

After day 82, the treatment shifted to the upper soil zone at the 1,000 and 500-mm depths. Prior to this period, the upper soil zones had not received any treatment. On day 58, the upper soil zone showed a reduction from 670 mg/kg soil to about 100 mg/kg (Figures 4.31 and 4.32). The already decreasing diesel-TPH residues in A_s and B_s were further reduced to 35±2 and 50±15 mg/kg soil at the 1,000-mm depth, respectively, (Figure 4.31). By day 90 at the 500-mm depth, A_s and B_s reduced to about 41±7 mg/kg soil each (Figure 4.32). The reduction of the upper soil zone, even when treatment of that zone had not started, suggests that if there is sufficient nutrient in the soil above where the water table is maintained in a sandy soil, it is not necessary to subirrigate the whole soil column at all times. This reduces the cost of treatment.

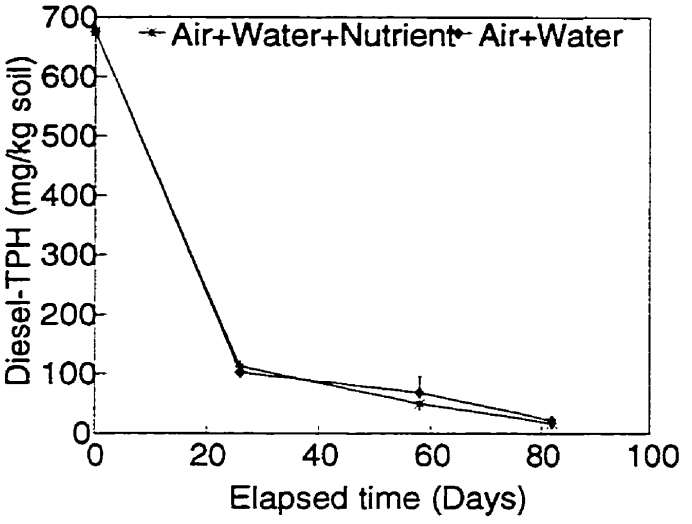


Figure 4.30: Diesel-TPH residue at the depth 1,500-mm, in stepwise treatments.

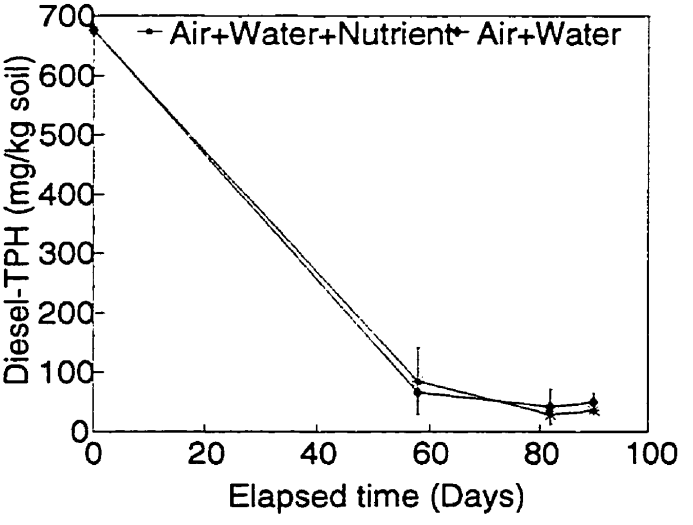


Figure 4.31: Diesel-TPH residue at the depth 1,000-mm, in stepwise column treatments.

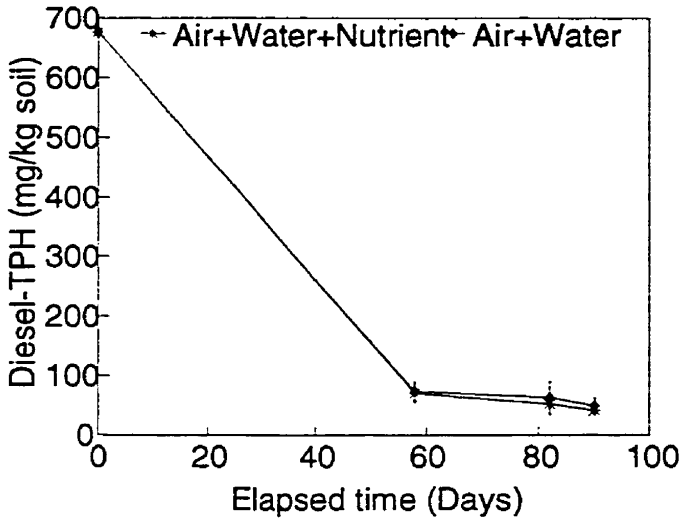


Figure 4.32: Diesel-TPH residue at the depth 500-mm, in stepwise column treatments.

The observed differences of the treatment combinations (Treatment A: air, water and other nutrients, and Treatment B: air and water) suggest that nutrients including air and water are essential for in-situ bioremediation (Figures 4.27 to 4.29). There was a greater reduction of diesel-TPH at all depths in the full column treatment, which received the air, water and nutrient combination, than in the full column treatment that received only air and water.

The reduction of the diesel-TPH (Figures 4.30, 4.31 and 4.32) in the stepwise treatment differed in extent from the full column treatment (Figures 4.27, 4.28 and 4.29). The remediation of the lower zone lasted for 82 days,

achieving a diesel-TPH reduction of about 17 and 21 mg/kg soil for the A_s and B_s treatments, respectively. At the same depth in the full column treatments, A_f and B_f, had average values of 43 and 60 mg/kg soil, respectively. The treatment of the lower zone affected the upper soil zone; the latter decreased before a direct treatment of the upper zone began on day 82. In the stepwise column treatment, since the overflows for the lower zone were located below the 1,000-mm depth, most of the influent and air flowed out of the stepwise column treatments without directly flowing through the upper soil zone. However, since the upper zone is a porous medium, some air and nutrient still reached the upper zone. The indirect nutrient and air supplies, to the upper soil zone from the lower zone of the soil, created conditions suitable for the bioremediation of the contaminated soil at the upper zone. Consequently, there was a general reduction in diesel-TPH before the treatment of the upper zone started on day 82.

The effect of the addition of nutrients, air and water for in-situ bioremediation using water table management, was assessed by making a comparison between the TPH residues in all the treatments, and also those from the non-intervention remediation and background soil. Figure 4.33 shows the diesel-TPH residues on days 58, 82 and 90, for the soil without any form of intervention (passive biodegradation). By day 90, the diesel-TPH residue was about 131 mg/kg soil. This performance is attributed to the

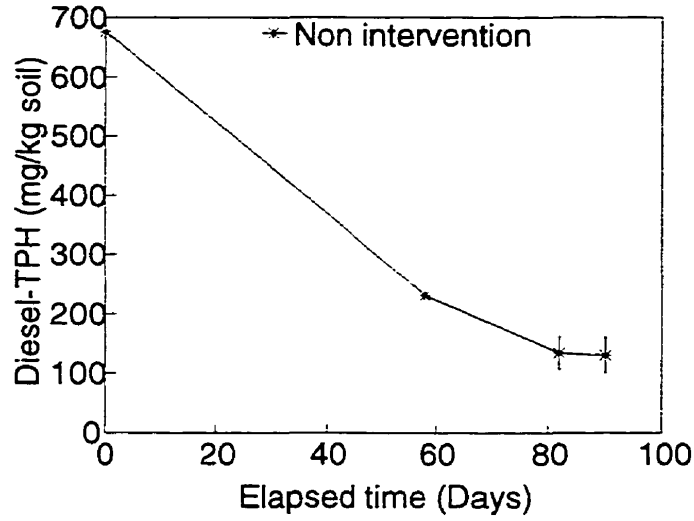


Figure 4.33: Diesel-TPH residue in the passive (non-intervention) treatment.

native nutrient and soil moisture content of 20% in the soil, and also air diffusion since the spaces were not filled by water. The total nitrogen, available phosphorus and potassium content of the soil were 74 mg/kg N, 8 mg/kg P and 18 mg/kg K, respectively.

The soil used to estimate the background TPH level (50 mg/kg soil) was St. Amable (Table 3.1). The Canadian remediation assessment criteria stipulate the attainment of approximation background levels and/or levels close to the analytical quantification limit of the instrument (Hrudey and Pollard 1993). The federal "clean up criterion" is 40 mg/kg soil (McNicoll et al., 1994). The water table nutrient delivery system, under investigation supplied nutrients to the microorganisms that reduced the contaminated soil

diesel-TPH to 40 mg/kg soil.

4.2.3 Determination of diesel biodegradation rate

Carbon dioxide, evolved from the soil as a metabolic end product, can be trapped and quantified at various intervals, and these values can be used to estimate the substrate biodegradation kinetics. However, the estimate will be more than the actual since carbon dioxide will also evolve from the metabolism of organic matter present in the soil and also the carbon dioxide introduced via aeration. The biodegradation kinetics in this study were estimated by monitoring the average loss of diesel-TPH at each depth in the soil columns by GC quantitative analysis. The biodegradation rate was obtained using the first order kinetic equation (Sims et al., 1989) given by:

$$\frac{dC}{dt} = -K_1 C$$

where C = substrate concentration (mg/kg)

t = time (days)

K_1 = First order biodegradation rate constant (day^{-1})

This kinetic model indicates that the removal rate, dC/dt, decreases as the substrate concentration (C) decreases. The integral form of the equation gives a linear relationship shown below:

$$lnC = lnC_o - K_1 t$$

where C_o = Initial concentration (mg/kg).

The slope of the equation (K_1) is the first order rate constant, determined by plotting ln C as a function of time (t), and the linearity assessed by linear regression analysis of the values based on time. K_1 derived from the slope of equation was used to determine the half-life of the diesel contaminant. The half-life is defined as the time required for the amount of diesel substrate to decrease by one half. It was determined using the equation:

$$t_{1/2} = \frac{ln2}{K_1}$$

Table 4.3 gives the first order rate constants, correlation coefficient and half-life for each treatment combination consisting of air, water and nutrients, and air and water, and the control (passive biodegradation). The first-order kinetics at each depth was derived from the average values of residual diesel-TPH at each depth, and that of the whole column was obtained from the average values for the entire column.

The half-life for the full column treatment, consisting of air, water and nutrients ranged from about 15 to 22 days at the depths sampled; with an average of about 19 days for all columns. The treatment consisting of only air and water gave an average half life of about 27 days for all columns, while the non intervention (passive) control column had a half life of about 35 days.

Table 4.3: First-order rate constant for biodegradation of diesel-TPH.

Treatment and depth	First-order kinetics ($-K_1$)	Correlation coefficient (R)	Half life (days)
Full column Treatment (air, water and nutrient)			
500	0.037	0.981	18.6
1,000	0.044	0.989	15.7
1,500	0.032	0.855	21.6
Total column	0.037	0.962	18.7
Full column Treatment (air and water)			
500	0.024	0.877	28.7
1,000	0.025	0.909	27.9
1,500	0.029	0.948	23.8
Total column	0.026	0.917	26.8
Stepwise column Treatment (air, water and nutrient)			
1,500	0.043	0.959	16.2
Stepwise column Treatment (air and water)			
1,500	0.039	0.917	18.0
Passive biodegradation			
	0.02	0.998	34.6

In the stepwise column treatment, the air, water and nutrients gave a half-life of about 16 days at the 1,500-mm depth, while the half life in the treatment consisting of air and water was 18 days. The result from the air and water treatment, which is lower than that of passive control by about 7 days, indicate that air was necessary for fast bioremediation of diesel contaminated soil. The better performance of the air, water and nutrient treatment shows that the supply of adequate nutrient and air to all depths enhanced biological activity, and thus led to a faster reduction in the level of the contaminant. Overall best performance of the stepwise treatment shows that if air and nutrients are supplied simultaneously to the soil column via different multilevel delivery ports, the rate constant of biodegradation of diesel contaminant would increase. Consequently, the time frame required for an in-situ bioremediation project would decrease.

Half-life of the diesel contaminant reduction was determined using a first-order rate constant; it can decrease or increase, depending on low or high environmental temperature. The prevailing average temperature during the study was about 23°C. The rate constant can be adjusted to a typical average summer temperature of 28°C using the Arrhenius equation (Troy et al., 1994):

$$K_T = K_{23°C}\theta^{(T-23°C)}$$

where θ is a temperature coefficient (= 1.088, experimentally determined for

hydrocarbon biodegradation by Troy et al. (1994)). Using the temperature adjusted first order rate constant, an accurate prediction of the time required for field in-situ bioremediation can be determined from laboratory soil column studies (Troy et al., 1994).

4.2.4 Nutrient delivery to the microbial population

The observation of TPH reduction at all depths, especially in the full column soil treatment, is an indication of nutrient transport and air diffusion in the soil columns receiving nutrients, air and water (Figures 4.30, 4.31 and 4.32). The nitrogen concentration (TKN) on day 82 at the top, at the 500, 1,000 and 1,500-mm depths of the soil columns that received full column treatment were 74±1, 100±21, 144±53 and 91±21 mg/kg N, respectively; while the initial N (TKN) concentration in the contaminated soil was 74±13 mg/kg N. These values are not significantly different (at the 5% level). Considering the lower half life (19 days) obtained for the full column (air, water and nutrient) treatment compared to the 27 days half life for the air and water treatment, it is possible that the difference between the two treatments resulted from nutrient addition. Because of nutrient addition, contaminants at all the depths in the air, water and nutrient full column treatment had lower half-lives than the equivalent depths in the air and water full column treatment (Table 4.3). This, therefore, implies that the nutrients

reached all sampling depths. Thus, water table management can effectively distribute nitrogen nutrient supplement to any depth in the soil profile during in-situ bioremediation.

Contrary to the uniform distribution of the readily soluble nitrogen nutrient, phosphate-P distribution was stratified from the bottom of the column to the top. The concentrations at the soil surface, at depths of 500, 1,000 and 1,500 mm were 0.27±0.02, 0.35±0.03, 0.36±0.01 and 0.71±0.09 mg/kg soil, respectively. The initial phosphate-P concentration in the soil was 0.16±0.01 mg/kg soil. Phosphate-P at the 1,500-mm depth was significantly different (at 5% level) from the amount of P at the higher depths, and in the control sample. However, the level of P at the 1,000 and 500-mm depths were not significantly different from each other, but significantly different from the control sample.

Since nitrogen and phosphate are essential macro nutrients for microbial metabolism, both nutrient supplements seem to have contributed collectively to the effective diesel substrate reduction in the soil treated with a combination of air, water and nutrients. The individual effects of the nutrients were not studied. The 1,500 mm depth, which is closest to the nutrient injection port, had the highest phosphate-P residue; this decreased at the 1,000 and 500-mm depths. This is primarily because phosphate can be adsorbed to the soil matrix, thus decreasing its mobility in the soil profile.

Multilevel injection of phosphate will help in the location of less mobile nutrient supplements to the soil profile.

4.2.5. Management of oxygen and water delivery to microorganisms

In this study, air was supplied continually during raising and lowering of the water table. In-situ measurement of dissolved oxygen at different depths was not undertaken; but the dissolved oxygen content of drain effluent, contained in the nutrient chamber, was monitored to indirectly determine the occurrence of biological activities in the columns with and without aeration. When the air supply to the nutrient chamber was cut off for 12 h after aeration, the dissolved oxygen in the effluent from both nutrient chambers dropped (Figure 4.34). The decrease in dissolved oxygen was greater in the drain effluents from the columns treated with nutrient, air and water than that from the air and water treatment. This suggests a high metabolic activity in the effluent which caused a decrease in dissolved oxygen concentration.

The concentration of dissolved oxygen in the columns was extrapolated from the data of dissolved oxygen estimation and indirect biological activity measured ex-situ. It is, however, believed that the dissolved oxygen in the soil zone close to the point of nutrient and air introduction would be higher than that at the 1,000 and 500-mm depths.

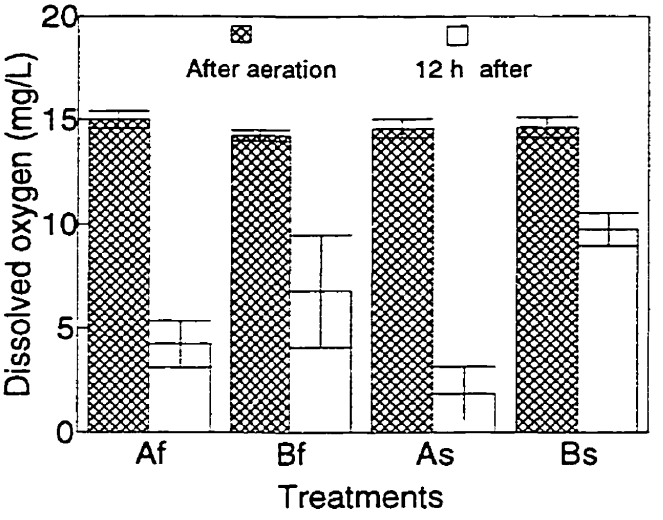


Figure 4.34: Dissolved oxygen concentration in drain effluents 12 h. after aeration. A_f and A_s are full and stepwise cmlumn treatment (air/water/nutrient), respectively. B_f and B_s are full and stepwise column treatment (air/water), respectively

Lower dissolved oxygen at the 1,000 and 500-mm depths would indicate depletion caused by a higher metabolic or biodegradation rate at the 1,500-mm depth, which reduced dissolved oxygen in the air passing through the soil columns. Oxygen was, therefore, not limiting the bioremediation process in the soil profile; in fact, it maintained aerobic conditions in the soil columns.

These observations show that a subirrigation network can serve as an effective method of oxygen supply during in-situ bioremediation of sandy soils. The air flow through the column to the surface was estimated, using a soap bubble meter during both dry and wet-cycles. The flow meter was

mounted on the plastic sheet, capping the soil columns. The air flow rate through the top of the full columns, during the wet-cycle was about 60 ml/sec.

The water table management system effectively delivered nutrients and air to various locations in the soil profile. The performance of the treated soil, compared to the passive control, shows that the water table was able to provide favourable conditions which made the diesel substrate bioavailable and biodegradable by the microorganisms. Hydrocarbon uptake can be facilitated by hydrophobilization of the cell envelope with biosurfactants produced in-situ, or by the emulsification of hydrocarbon with extracellular surfactants (Oberbremer and Müller-Hurtig, 1989). Since surfactant was not added to the soil columns, biosurfactants could have been produced in-situ (Oberbremer and Müller-Hurtig, 1989) and, thus enhanced the bioavailability of the contaminants to the microorganisms. Using the water table management method, the substrate-contaminant and the microorganisms were brought into close association so that, the limitation imposed by the mass transfer of low soluble contaminants to the aqueous phase for enzymic transformation, was overcome. It is, therefore, possible that the increased water/organic/microbial interface, maintained by water table management, encouraged the formation of in-situ biosurfactants which are known to improve the bioavailability of hydrophobic contaminants.

Bioremediation occurred as the TPH levels decreased by more than 85% over four weeks. The delivery system may be operated simultaneously or alternately, under aerobic and anaerobic conditions (an anaerobic cycle was not attempted in this study). Hydrocarbons, including aromatic compounds, may be degraded under both aerobic and anaerobic conditions. The saturated condition, without aeration, can provide an alternate mechanism of fermentative oxidation of aromatic compounds. Furthermore, sequential anaerobic/aerobic conditions are suitable for the dehalogenation and biodegradation of halogenated hydrocarbons which are otherwise known to persist in an aerobic environment.

Lowering and raising of the water table potentially offers the removal of biodegradation products and intermediates, that could be toxic and inhibitory to microbial metabolism. Most organic and inorganic compounds can be biotransformed or biodegraded, but excessively high concentrations may be toxic or inhibitory to the microorganisms. Kaufman (1994) indicated that solvents exceeding 7,500 ppm, non-solvents exceeding 25,000 ppm, and heavy metals in excess of 2,500 ppm in soil or water will reduce the potency of bioremediation, unless diluted prior to the biological treatment.

The capability of using the system to lower and raise the water table, if exploited, would facilitate decreasing concentrations and consequently reduce the toxicity and excessive substrate inhibition. Additionally, cleanup

levels established for specific sites may be lower than the maximum reductions that can be achieved by microorganisms. Levels of contaminants lower than 0.10 ppm in soil and 100 ppb in water may be difficult to achieve quickly and economically, if the biological process alone is relied upon. Therefore, additional "polishing" (final cleaning) steps may be required (Kaufman, 1994). Lowering and raising of the water table can be exploited for final polishing when the microbial activity becomes limited due to low carbon concentration in the soil.

In summary, it may be stated that subirrigation (water table management) practices may be used to distribute treatment additives (a combination of air, water and nutrients, or just air and water) to a diesel contaminated soil profile, packed in 2,000 mm long by 200 mm diameter columns. The study was designed for full and stepwise (column divided into lower and upper zones) column treatments. The decrease in diesel-TPH was monitored and compared for the two treatments, and also that of a control soil (natural intervention). Analysis of the results obtained from the two treatments showed that residual diesel-TPH levels decreased in all the soil columns. However, the TPH levels decreased faster in soil columns treated in a stepwise manner. Finally, the addition of a nutrient supplement significantly (at the 5% level) accelerated bioremediation in the full column treatment.

It is worth noting that the water table in this study was supported by a completely impermeable barrier; this might not be the case in a field in-situ bioremediation operation. In the latter case, however, it is possible to maintain a shallower water table (buffer zone) at a small distance below the contaminated horizon undergoing treatment.

CHAPTER 5: SUMMARY and CONCLUSIONS

The primary aim of this study was to develop biological in-situ pollution control systems for nitrate residue (non-point source pollution) in the soil, and also for a diesel (point source) contaminated soil. A common feature in the treatment approach of both types of pollution is the use of water table management practice, to introduce nutrients required by the remediating indigenous microorganisms in the soil.

The objectives of the nitrate study focused upon an on-farm pollution control system that used water table management as a delivery system to supply nutrients, specifically organic carbon, to microorganisms in the subsoil in order to enhance the denitrification of nitrate in the soil profile. The effect of organic carbon was tested on nitrate residue applied to two different sandy soils, packed in columns and subjected to subirrigation with different concentrations of glucose solution. Different amounts of glucose (ranging from 20 to 970 mg/L glucose-C) were added to the subirrigation water, while glucose was not added to the control. Fertilizer-nitrate was applied on the soil columns at the rate of 180 kg/ha nitrate-N, and leached by simulated rainfall events from the surface of the columns to lower depths in the soil profile. The study was carried out in four stages: the first two stages investigated the effects of a low (120 mg/L glucose-C) and a high

(970 mg/L glucose-C) glucose level on nitrate residue; and the changes in redox potential of the soil solution as a result of the external organic carbon addition to the soil. The redox potential served as a general indicator of soil health during the treatment. The third stage employed another sandy soil to investigate the effect of five glucose levels (0, 20, 70, 150 and 300 mg/L glucose-C) on nitrate residues in the soil columns. The long term impact on redox potential, pH, soluble iron and manganese soil solution content was determined after 96 days. The last stage determined the desirable glucose range required for nitrate residue reduction in the soil columns without disturbing the soil properties.

The results obtained, from the effect of a high glucose level on nitrate residue (stage 1), showed that nitrate reduction occurred in both the treatment and control soil columns, due to the initial 3.5% organic matter present. However, at all depths in stage 1, the columns receiving 970 mg/L C dissipated the nitrate loads faster than the control columns. This reduction of nitrate residues was due to anaerobic conditions in the saturated zone which supported the denitrification process.

As the control and treatment columns were subjected to more leaching (stage 2) and microbial decomposition of soil organic matter, the denitrification potential in the control columns decreased further. On all sampling days, at all depths, the redox values observed in the control

columns (stage 1) were higher than those in the columns treated with 970 mg/L glucose-C. When the glucose level was reduced to 120 mg/L glucose-C, the redox potential trend in both treatment and control remained similar. More Fe and Mn compounds came into solution at the higher glucose levels, but that from 20 mg/L glucose-C treatment and the control were similar. Biomass was also estimated in the columns to assess the extent of bioplugging of the soil pore; this did not reveal any significant proliferation of microorganisms, resulting from the introduction of organic matter.

The treatment using 20 mg/L glucose-C solution significantly reduced more nitrate than the control and some of the other glucose levels. The control performance was exceeded by more than 100 mg N, representing a difference of more than 33 kg/ha of nitrate-N. These differences are attributed to the extra organic carbon added, in the form of glucose, to the subirrigation water. The results from this study indicate that organic carbon, administered via the subirrigation water at a desirable range, caused a significant reduction (at the 5% level) in nitrate levels leached to the water table.

The changes in Fe and Mn concentrations in the soil solution, and their suggested influence on soil properties and microbial activity relating to nitrate reduction, must be carefully considered when contemplating the use of this technique to implement an in-situ bioremediation of nitrate in

agricultural farms. Although the changes in Fe and Mn were not monitored when the desirable carbon range for in-situ reduction of nitrate was determined, such a glucose effect was expected and extrapolated from the stage 3 study to the stage 4 carbon desirable range assessment.

To further assess the statistical significance of the treatment levels and the control, Fe and Mn occurrence in soil solution was employed to discriminate between the treatment levels, especially as it related to a possible adverse impact to the soil and the receiving surface water environment. Surface water bodies receive most of the drainage waters from agricultural farms.

Closely associated with the Fe, Mn and carbon treatment levels was the redox potential of the soil solution. Fe and Mn concentrations in 20 mg/L glucose-C treatment was not significantly different from that measured in the control; however, the redox potential was found to be significantly different between the two, but the range determined for the 20 mg/L glucose-C level was closer to that found in the control. Although the measurement of redox potential was considered to be a less expensive, indirect method of estimating the total redox couples contributing to redox potential of the soil solution, Fe and Mn were assumed to have more effect on the redox potential of the soil solution. Therefore, the significant difference between the control and the 20 mg/L C level was relegated, in favour of non-

significance in the Fe and Mn concentrations for both the control and the 20 mg/L C level. The desirable carbon range was defined as the range that has a similar Fe and Mn dissolution effect on the soil as the control, while maintaining a redox potential range very close to that of the control.

The success of applying water table management to distribute carbon to the subsoil, to biostimulate the subsoil microbial population, prompted the second goal in this study: that is to understand the method and management of the system for in-situ bioremediation of diesel contaminated soil. This section employed the subirrigation system as a nutrient delivery system for in-situ bioremediation of a hydrocarbon contaminated soil.

The objectives for diesel decontamination were directed towards developing subirrigation as an aeration and nutrient delivery network, for enhancing in-situ bioremediation of a diesel contaminated soil. The study was also carried out on packed soil columns. The soil (99% sand with 1% silt content), contaminated by diesel for more than 20 years, was excavated from a site in Quebec, and supplied by an ESTAC member.

Three treatments: (a) Treatment A: nutrient, air and water, (b) Treatment B: air and water, and (c) Treatment C: non intervention (control), on packed soil columns were employed in the assessment. The experimental profile further divided treatments A (nutrient, air and water) and B (air and water) into two groups, each with a full column treatment group, A_f and B_f,

and a stepwise column treatment group, A_s and B_s. The stepwise treatment was designed to test the rate of loss of TPH when either nutrient, air plus water or air plus water were introduced to the soil columns in short soil segments, by multiple level nutrient introduction ports, located along the length of the columns (from the bottom to the top - stepwise treatment). The stepwise treatment also tested the feasibility of raising the water table to an optional desirable height, to act as a buffer or support for nutrient delivery to the upper soil profile or when the soil below the drain is susceptible to deep seepage. Half of the study on the diesel contaminated soil employed full soil columns, while the other half studied soil columns in which the soil profile was divided into two lower and upper zones, to facilitate stepwise treatment, beginning with the lower soil zone.

Soil samples were taken from the columns and extracted and analyzed for diesel-TPH. The performance of subirrigation water table management was assessed for delivering nutrients for in-situ bioremediation, by monitoring the decrease in TPH (total petroleum hydrocarbon) levels at different depths in the soil columns. Columns receiving nutrient, air and water treatment were compared with those receiving only air and water, as well as the control or passive (non-intervention) bioremediation treatment. Columns receiving only air and water were also compared with untreated contaminated soil, undergoing passive biodegradation. The air and water

treatment served as an additional control, introduced to compare the effect of water and air addition, with and without nutrients.

The biological activity in the treatment with and without nutrients was assessed by comparing the dissolved oxygen in the effluent, which was kept without aeration. Biological activity was implied by the decrease in diesel-TPH in all the treated and untreated soil columns. This was confirmed by a decrease in the dissolved oxygen level of the effluent from the two treatments receiving water and air with or without nutrients.

The following conclusions are drawn from the study:

Nitrate Reduction Study

1. Soil nitrate was reduced to negligible levels in column studies as a result of added organic carbon, which enhanced denitrification in the soil profile. Thus, the effectiveness of subirrigation can be improved, in order to control nitrate pollution in agricultural fields, by supplementing soil organic carbon. If the in-situ bioremediation method developed in this study is applied, most of the nitrate load would be removed during the growing season and after the harvest.

2. There is a rapid decline in the potential of water table management, as currently practiced, to sustain denitrification in soils whose organic matter has been repeatedly leached and subjected to microbial

decomposition. This highlights the fact that water table management, as currently practiced for nitrate reduction, is insufficient over a long period, without readily available organic carbon augmentation.

3. Since the water table can be maintained at any depth, nitrate residue in the unsaturated zone below the root zone can be "cleaned up" after harvest, by raising the water table to just below the root zone for a short period.

4. The soil solution properties can be altered if excessive, readily available carbon is supplied to the soil by subirrigation. The treatment vs. control column studies have shown that the soil solution redox potential, soluble Fe and Mn content will not change much, if the amount of supplementary carbon applied does not exceed the desirable range that encourages dissolution of excessive Fe and Mn from the soil profile and thus, give rise to redoximorphic features.

5. The desirable readily available carbon level is defined as that leading to a maximum loss of nitrate residue (in this case 20 mg/L glucose-C treatment) which produces redox potential values and soluble Fe and Mn levels similar or close to that of the control soil solution, after

subjecting the soil to 96 days of subirrigation.

6. An alternate feeding of organic carbon solution and water to the soil was suggested since the input zone of the soil had the most reduced state of all treatment levels, the soil type not withstanding. This would strike a balance and would sustain a reduced anaerobic condition without any adverse effect; this would be conducive to denitrification throughout the planting season.

7. Measurement of the redox potential of the soil solution can be used in designing or predicting an organic carbon supply management strategy since the redox potential of the soil system is sensitive to carbon utilization and input into the soil.

8. Reduction of hydraulic conductivity or bioplugging of the soil pore structure due to organic carbon supplementation did not occur, since measurements on St Benoit soil did not show any significant proliferation of microorganisms after the introduction of organic matter.

9. This research sets a precedent since it attempts to minimize nonpoint

source pollution caused by nitrate, by in-situ bioremediation of the nitrate residue. This could be effected within agricultural plots by introducing nutrients via subirrigation water.

In-Situ Bioremediation of Diesel Contaminated Soil

1. In-situ bioremediation of diesel contaminated soil can be enhanced by using water table management for delivering nutrients, water and air to biostimulate the subsoil microbial population.

2. The water table management effectively delivered nutrients to all depths, since TPH residue reduction was greater in the soil columns receiving nutrients, air and water at all depths, than in those subjected to only air and water.

3. Multiple introduction of nutrients, air and water at different depths in a contaminated soil profile will lead to a faster bioremediation of the polluted subsoil, since the stepwise treatment led to faster in-situ bioremediation which was achieved within 82 days, instead of the normal 12 to 24 months.

4. The stepwise treatment further shows that any soil depth zone can be

isolated from the upper and lower soil zone for remediation. This implies that contaminated soils, below leaky underground storage facilities, can be bioremediated without excavating the tank. After treatment, the remediation facility could be left intact as a biopreventive system, in the event of a leakage reoccurrence.

5. Stepwise treatment study further indicated that the upper soil profile may not necessarily be saturated with nutrient solution since the treatment of the lower zone affected the upper soil zone; the latter decreased before a direct treatment of the upper zone began.

6. The observed differences of the treatment combinations (Treatment A: air, water and other nutrients, and Treatment B: air and water) suggest that nutrients including air and water are essential for in-situ bioremediation. There was a greater reduction of diesel-TPH at all depths in the full column treatment, which received the air, water and nutrient combination, than in the full column treatment that received only air and water.

7. Contrary to the uniform distribution of the readily soluble nitrogen nutrient, phosphate-P distribution was stratified from the bottom of the

column to the top. This is primarily because phosphate can be adsorbed to the soil matrix, thus decreasing its mobility in the soil profile. Multilevel injection of phosphate will help in the location of less mobile nutrient supplements to the soil profile.

5.1 Recommendations for Related Future Research

As a result of the study conducted and the limitations in the scope of this research, the following items are recommended for further research:

Nitrate Study

1. Field trial of an in-situ bioremediation of soil nitrate residues should be conducted on different types of soil textures, containing stratified organic matter content in soil profile. This is necessary to investigate the effect of organic matter supplement on the soil solution chemistry. During the field trial, the general impact of increased BOD (biological oxygen demand) on receiving surface water bodies should also be examined. Rate constants should be obtained to describe the various process interactions (nitrate movement, denitrification and carbon utilization rates etc.) occurring in the soil.

2. Upward transport of nitrate, leached below the root zone during subirrigation, should be assessed to determine if the in-situ process of accelerated reduction of nitrate is wasting essential nutrients that would otherwise be returned to the plants for utilization. The ultimate sink of the nitrate should be investigated, when subjected to accelerated reduction in the presence of readily available carbon. Furthermore, detailed experiments should be planned and conducted to investigate upward translocation of dissolubilized minerals (and associated pollutants such as phosphate, arsenic etc.) due to added carbon, and also the effect of organic carbon augmentation on root development and general crop yield.

3. It is important to identify the water table depth at which the added organic carbon will have the least adverse impact on the crops.

4. Pesticides, that have leached below the root zone, could be alternate sources of reductant for denitrifying microorganisms. Experiments should be conducted to establish that the pesticides are simultaneously degraded, if not enhanced; or that the addition of readily available carbon organic matter causes preferential utilization of the carbon, consequently leading to a higher pesticide residue in drainage water.

5. Succession and stability of the microbial community (at field scale) in the subsurface, in response to increased and continuously changing levels of organic carbon, redox potential, nitrate, and the general soil solution chemistry should be investigated to establish the impact of the treatment on the soil ecosystem.

6. Finally, the management options, suggested in the chapter on results and discussion, should be investigated during a field trial.

In-Situ Bioremediation of Diesel Contaminated Soils

1. A field trial of in-situ bioremediation, using water table management, should be conducted for different types of soil, particularly clay soils. Nutrient movement and uniform air diffusion in these soils should be carefully monitored. Data should be collected on the use of the system for sequential aerobic/anaerobic in-situ bioremediation of halogenated aromatic and aliphatic hydrocarbons.

2. Optimum moisture content during the dry cycle should be investigated. This, together with the optimum nutrient requirement, will determine the interval for dry cycle.

3. Effectiveness of water table management for in-situ bioaugmentation of recently contaminated soil should be investigated. Redistribution of indigenous microbial population as a result of lowering and raising the water table should also be documented.

4. Finally, the ecotoxicological and toxicological impacts of such a delivery system for bioremediation process should be studied.

APPENDICES

APPENDIX A

Effect of high carbon supplement on nitrate residue (Stage 1).

Depth	Nitrate-N (mg/L)								
	400mm			600mm			850mm		
Replica	I	II	III	I	II	III	I	II	III
Time (d)									
Treatment									
8	94.1	19.0	101.6	3.8	37.0	40.6	0.0	0.0	0.0
12	20.9	1.6	26.6	0.8	0.3	0.6	0.2	0.2	0.2
14	7.5	3.4	2.2	0.3	0.2	0.2	0.2	0.3	0.3
15	.	.	.	.	.	.	.	.	.
16	0.6	0.3	0.3	0.3	0.2	0.2	0.3	0.2	0.2
18	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
22	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
28	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
30	34.9	189.5	284.5	142.2	174.7	137.4	74.2	17.7	0.5
31	36.9	0.6	0.6	27.5	31.7	40.1	1.7	0.4	0.3
36	0.3	1.3	4.5	0.5	0.4	1.0	0.4	0.4	0.4
42	0.1	0.3	0.3	0.3	0.3	0.3	0.3	0.4	0.3
43	.	.	125.9	54.7	0.4	24.1	0.4	0.4	0.8
45	0.1	0.4	0.4	0.8	0.4	0.4	0.5	0.4	0.5
55	0.6	0.3	0.5	0.3	0.2	0.4	0.2	0.2	0.3
Control									
8	51.9	101.6	45.2	25.3	61.0	63.2	3.2	0.0	1.2
12	.	.	.	.	.	.	.	.	.
14	5.0	13.5	15.6	1.3	4.0	0.9	0.6	3.0	0.9
15	.	.	.	.	.	.	.	.	.
16	0.6	0.0	0.6	0.6	0.0	0.6	0.6	0.0	0.6
18	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
22	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
28	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
30	14.7	76.1	188.7	140.4	232.7	149.1	39.4	3.6	0.4
31	.	.	.	.	.	.	.	.	.
36	174.6	127.4	170.7	82.8	230.9	105.3	4.9	0.3	0.1
42	120.0	218.0	86.3	6.8	30.7	4.9	0.1	0.1	0.1
43	125.9	674.8	135.6	24.1	209.0	71.0	0.8	12.6	0.1
45	123.4	192.8	139.9	1.4	84.3	54.9	0.5	0.3	0.1
55	0.8	0.9	0.8	0.1	0.3	0.3	0.1	0.2	0.1

APPENDIX A (contd.)

Effect of low carbon supplement on nitrate residue (Stage 2).

	Nitrate-N (mg/L)								
Depth	400mm			600mm			850mm		
Replica	I	II	III	I	II	III	I	II	III
Time (d)									
Treatment									
1	15.2	37.7	194.4	262.3	38.4	46.9	36.6	112.0	27.0
3	.	.	.	.	.	.	18.7	63.6	12.2
4	.	.	.	.	.	.	15.2	58.6	9.1
5	.	.	.	.	.	.	6.3	40.7	2.1
6	54.8	95.1	138.8	86.8	22.3	23.1	2.5	26.7	35.6
8	40.8	118.4	.	71.6	26.3	11.0	0.1	14.8	16.4
13	99.5	11.6	126.8	2.3	19.7	0.2	0.1	0.2	1.7
Control									
1	306.3	144.0	11.6	266.2	230.2	58.3	24.8	95.9	11.5
3	.	.	.	.	.	.	15.2	34.6	3.0
4	.	.	.	.	.	.	4.0	28.2	1.6
5	.	.	.	.	.	.	0.2	0.3	0.2
6	239.7	86.8	25.4	3.5	45.6	0.2	0.2	0.2	0.3
8	176.6	181.6	4.4	0.2	6.8	0.2	0.3	0.2	0.3
13	1.9	10.9	0.1	0.2	0.2	0.2	0.2	0.1	0.1

. Data not available

APPENDIX B

Effect of high carbon supplement on redox potential (Stage 1).

Depth	Redox Potential (mV) 400mm			600mm			850mm		
Replica	I	II	III	I	II	III	I	II	III
Time (d)									
Treatment									
8	246	143	133	269	-180	-30	-516	-521	-524
12	339	167	359	166	66	-57	-501	-503	-488
14	258	102	342	80	167	-119	-456	-491	-477
16	55	-8	88	-10	23	24	102	10	105
30	11	126	174	-28	-3	-15	122	210	187
31	23	.	.	-28	-30	-507	82	-49	-444
36	.	173	-25	-25	5	-18	85	-103	106
42	.	-10	0	3	31	39	227	-391	-467
Control									
8	405	397	428	408	460	435	376	362	380
12	.	.	.	.	.	.	.	.	.
14	375	350	388	349	347	361	315	256	296
16	289	289	287	282	285	284	258	262	264
30	270	312	370	316	340	347	243	331	218
31	.	.	.	.	.	.	.	.	.
36	260	369	399	209	254	397	284	212	290
42	331	360	350	305	318	336	309	200	267

. Data not available

APPENDIX B (contd.)

Effect of low carbon supplement on redox potential

Depth	Redox Potential (mV)								
	400mm			600mm			850mm		
Replica	I	II	III	I	II	III	I	II	III
Time (d)									
Treatment									
1	177	333	164	-37	330	-45	-27	71	29
3	.	.	.	.	.	.	36	66	22
4	.	.	.	.	.	.	39	77	39
6	275	292	289	199	291	85	56	120	83
8	253	243	292	195	279	77	80	105	77
13	308	350	250	145	268	80	60	177	100
Control									
1	240	230	226	290	240	246	277	231	187
3	.	.	.	.	.	.	269	188	180
4	.	.	.	.	.	.	271	250	203
6	349	360	342	353	354	337	341	320	330
8	240	230	318	270	261	318	273	250	317
13	417	421	369	420	451	390	424	225	390

. Data not available

APPENDIX C

Changes in redox potential in soil solution (Stage 3).

Depth	Redox Potential (mV) 400mm			600mm			850mm		
Replica	I	II	III	I	II	III	I	II	III
Time (d)									
Treatment									
20 mg/L C.									
3	185	166		253	251		190	209	
8	193	156		190	.		205	188	
12	180	164		178	171		151	161	
15	.	123		.	122		-42	10	
96	-18	16		-24	-15				
70 mg/L C.									
3	133	127		230	244		273	267	
8	113	128		158	167		226	180	
12	123	111		196	187		174	175	
15	84	.		111	.		174	.	
96	-61	-52		-50	-40		-51	-6	
150 mg/L C.									
3	142	159		299	285		297	251	
8	70	74		189	158		249	197	
12	108	119		259	238		214	280	
15	44	50		185	138		173	178	
96	-68	-30		-126	-97		-55	-94	
300 mg/L C.									
8	168	156	139	266	239	225	190	190	190
9	183	158	171	180	180	190	225	240	192
12	.	.	.	227	246	182	95	182	160
15	122	121	97	162	147	85	11	-36	-86
96	-31	-63	-80	-23	-81	-114			
Control									
3	292	284	208	428	293	302	403	292	289
8	180	190	158	227	190	140	301	218	208
12	228	161	168	181	187	150	214	237	185
15	156	114	79	111	114	73	146	141	127
96	60	54	17	64	60	51	86	57	41

. Data not available

APPENDIX D

Mn and Fe concentration in soil solution (Stage 3).

Treatment (mg/L C.)	Depth (mm)	Replicates	Mn (mg/L)	Fe (mg/L)
0	400	1	0.3	0.7
		2	0.4	0.5
		3	0.4	1.1
20		1	0.3	0.5
		2	0.5	0.8
70		1	0.5	1.2
		2	0.5	1.1
150		1	0.8	0.8
		2	0.8	1.2
300		1	1.2	1.3
		2	1.9	1.3
		3	2.2	4.3
0	600	1	0.3	1.3
		2	0.3	1.0
		3	0.3	1.4
20		1	0.3	0.9
		2	0.4	1.8
70		1	0.5	1.9
		2	0.6	1.3
150		1	1.4	7.9
		2	0.8	14.9
300		1	2.5	19.3
		2	2.1	16.9
		3	2.2	19.2
0	850	1	0.1	0.5
		2	0.1	0.5
		3	0.1	0.8
20		1	0.3	0.8
		2	0.3	1.3
70		1	0.7	9.1
		2	0.6	8.8
150		1	1.3	20.4
		2	1.2	30.9
300		1	2.8	44.6
		2	2.7	43.0
		3	2.8	39.2

APPENDIX E

Determination of desirable glucose range for nitrate reduction (Stage 4).

Depth	Nitrate-N (mg/L) 400mm			600mm			850mm		
Replica	I	II	III	I	II	III	I	II	III
Time (d)									
Treatment									
20 mg/L C.									
0	.	.		.	.		.	.	
1	.	.		.	.				
2	3.9	0.9		0.0	0.1		0.0	0.0	
5	0.0	0.0		0.0	0.0		.	.	
5.5	17.5	19.7		1.1	0.0		0.0	0.0	
8	.	.		.	.		.	.	
9	369.2	296.3		75.6	22.5		0.0	0.0	
10	.	.		.	.		.	.	
11	372.7	311.1		86.1	35.8		0.0	0.0	
16	195.8	174.6		20.8	0.0		10.5	0.0	
18	153.1	209.9		0.0	0.0		0.0	0.0	
18.5	186.3	179.8		11.2	4.5		0.0	0.0	
29	12.4	40.1		0.1	0.0		0.0	0.0	
31	37.2	158.4		0.0	0.0		1.4	0.0	
33	19.6	93.7		0.0	0.0		0.9	0.0	
33.5	167.9	130.0		57.2	172.9		0.0	0.0	
35	128.6	90.1		44.6	121.7		0.0	0.0	
36	.	.		.	.		0.0	7.3	
37	.	.		.	.		0.1	9.7	
70 mg/L C.									
0	.	.		.	.				
1	.	.		.	.				
2	1.6	2.7		0.2	0.0		0.0	0.0	
5	0.0	0.5		0.0	0.0		.	.	
5.5	5.0	3.5		0.0	0.3		0.0	0.0	
8	.	.		.	.		.	.	
9	303.1	98.7		9.2	1.5		0.0	0.0	
10	.	.		.	.		.	.	
11	338.4	74.9		0.0	0.0		38.2	0.0	
16	120.5	53.8		61.8	0.0		0.0	0.0	
18	94.5	43.5		0.0	0.0		0.0	0.9	
18.5	138.5	64.0		49.8	12.3		0.0	0.0	

29	34.6	17.7		0.6	0.0		0.0	0.0	
31	46.3	19.9		31.0	0.1		0.0	0.0	
33	34.0	18.4		14.9	0.0		0.0	0.0	
33.5	138.7	124.5		51.3	34.9		3.0	1.7	
35	103.2	96.9		45.5	31.3		5.9	1.7	
36	.	.		.	.		30.1	14.9	
37	.	.		.	.		22.8	13.8	
150 mg/L C.									
0	.	.		.	.				
1	.	.		.	.				
2	2.4	1.0		27.7	0.1		0.0	0.0	
5	0.3	0.0		30.1	0.0		.	.	
5.5	4.3	2.9		12.5	0.0		0.0	0.0	
8	.	.		.	.		.	.	
9	63.2	297.3		1.3	5.8		10.2	0.0	
10	.	.		.	.		.	.	
11	92.8	287.2		0.0	18.2		2.2	0.0	
16	105.7	112.8		0.0	0.0		0.0	14.6	
18	70.0	65.9		0.0	0.0		0.0	0.0	
18.5	198.0	127.2		6.5	8.8		0.0	0.0	
29	42.3	17.5		0.0	0.0		0.0	0.0	
31	67.3	50.9		0.0	0.0		0.0	0.0	
33	62.6	32.3		4.4	0.0		0.0	0.0	
33.5	84.1	127.4		122.0	62.4		9.0	0.0	
35	61.6	91.2		93.0	48.4		1.2	0.0	
36	.	.		.	.		38.5	12.8	
37	.	.		.	.		16.9	0.0	
300 mg/L C.									
0	.	.	.	.	.	.	.	.	.
1	.	.	.	.	.	.	.	.	.
2	2.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1
5	0.0	0.0	0.0	0.0	0.0	0.0	.	.	.
5.5	94.6	3.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0
8	.	.	.	.	.	.	.	.	.
9	346.5	207.4	61.0	98.3	11.5	9.8	0.0	0.0	0.0
10	.	.	.	.	.	.	.	.	.
11	355.4	269.2	47.6	36.1	0.0	37.7	0.0	0.0	0.0
16	211.1	115.8	40.2	0.0	0.0	95.8	0.0	0.0	0.0
18	240.9	82.8	18.1	0.0	0.0	35.7	2.0	1.9	1.7
18.5	204.5	137.7	37.0	0.0	11.3	73.3	0.0	0.0	1.9
29	99.2	37.4	5.4	0.0	0.0	0.0	0.0	0.0	0.0
31	167.1	73.2	1.8	0.0	0.0	0.0	0.0	0.0	0.0

33	113.6	73.3	4.9	0.0	0.0	0.0	0.0	0.0	0.0
33.5	112.0	15.1	97.0	132.9	130.2	14.9	0.0	7.6	0.0
35	73.8	10.7	67.6	89.2	91.8	12.1	0.0	12.0	0.0
36	.	.	.	.	.	.	0.0	36.4	13.3
37	.	.	.	.	.	.	0.0	25.6	6.5
Control									
0	.	.	.	.	.	.	.	.	.
1	.	.	.	.	.	.	.	.	.
2	2.0	2.0	2.3	0.0	0.0	0.0	0.0	0.0	0.1
5	0.0	0.0	0.0	0.0	0.0	0.0			
5.5	2.5	3.7	3.6	1.9	0.7	0.0	0.0	0.0	0.0
8	.	.	.	.	.	.	.	.	.
9	137.8	69.1	300.9	8.6	13.4	0.2	0.0	0.0	0.0
10	.	.	.	.	.	.	.	.	.
11	148.0	92.1	365.4	44.0	47.7	0.5	0.0	0.0	0.0
16	51.5	39.9	108.9	8.5	22.4	0.0	0.0	0.0	0.0
18	69.1	39.1	30.5	0.0	0.0	0.0	0.0	0.0	0.0
18.5	81.1	46.0	120.6	17.5	22.6	0.0	0.0	0.0	0.0
29	23.6	46.7	0.6	0.0	0.0	0.0	0.0	0.0	0.0
31	39.5	40.1	26.0	0.0	1.9	0.0	0.0	0.0	0.0
33	26.0	31.1	6.2	0.6	1.6	0.2	0.0	0.0	0.0
33.5	133.9	104.7	170.1	63.4	79.5	49.1	1.1	2.1	0.0
35	108.5	79.8	136.0	55.6	64.1	45.5	1.1	9.2	0.0
36	.	.	.	.	.	.	2.3	46.6	0.0
37							6.4	44.2	0.0

. Data not available

APPENDIX F

Diesel-TPH reduction in columns (Diesel study).

	Diesel-TPH residue (mg/kg soil)								
Depth	1500mm			1000mm			500mm		
Replica	I	II	III	I	II	III	I	II	III
Time (d)									
Treatment									
Full Column									
Air/Water/Nutrient									
0	686.0	667.0	771.0	686.0	667.0	771.0	686.0	667.0	771.0
26	110.5	98.6	110.0	86.6	207.0	185.1	135.2	208.1	182.5
58	51.4	83.7	48.9	52.3	60.2	51.1	61.4	76.6	65.0
82	44.9	39.4	45.4	12.0	17.1	21.0	16.7	44.2	30.1
90	50.3	54.6	37.8	32.4	46.6	37.5	36.3	50.5	49.8
Air/Water									
0	686.0	667.0	771.0	686.0	667.0	771.0	686.0	667.0	771.0
26	212.9	117.8	250.0	219.5	114.4	241.3	273.1	135.7	185.8
58	99.4	61.7	123.6	209.3	80.0	113.0	113.3	94.2	110.0
82	82.8	34.0	63.6	85.9	62.6	87.1	113.4	63.6	104.3
90	52.6	28.6	96.5	70.2	72.0	114.5	99.6	62.4	87.8
Stepwise Column									
Air/Water/Nutrient									
0	686.0	667.0	771.0						
26	119.3	98.1	118.5						
58	49.3	46.6	53.8	45.4	45.7	165.9	59.9	60.1	91.7
82	6.4	22.6	22.2	10.5	28.3	49.1	61.3	49.3	45.9
90				33.0	35.6	39.0	34.9	37.7	52.0
Air/Water									
0	686.0	667.0	771.0						
26	99.6	.	105.8	85.9	63.0	50.7	90.9	53.5	78.1
58	49.2	108.1	48.8	84.1	23.4	19.9	83.8	23.5	81.9
82	29.7	17.3	18.2	63.0	59.7	29.1	44.7	35.8	67.1
90									
Control									
0	686.0	667.0	771.0						
58	228.9	234.4	.						
82	141.5	99.2	164.9						
90	168.4	93.5	132.2						
Garden 90	30.4	33.2	35.0						

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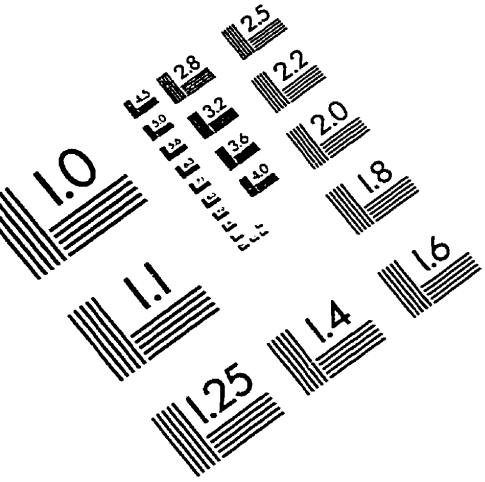
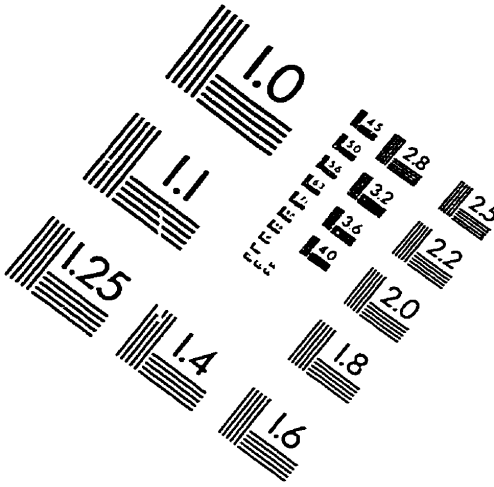
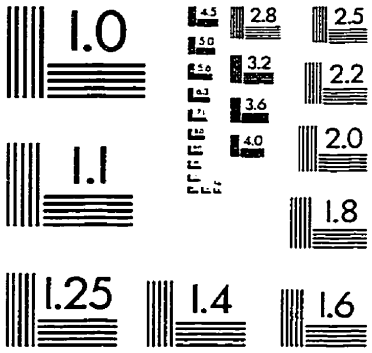
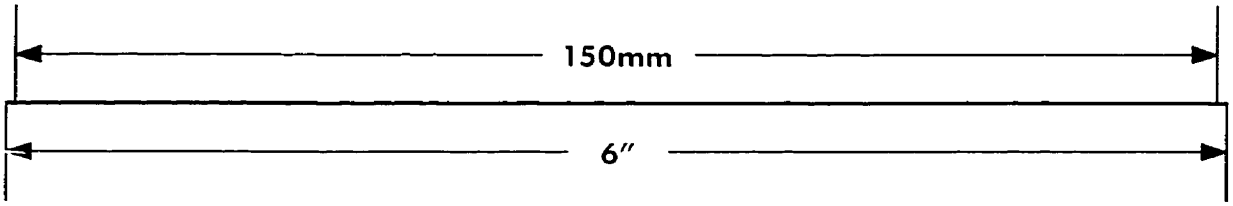
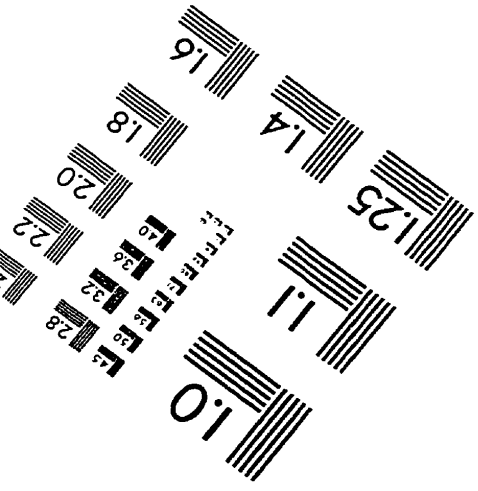
IMAGE EVALUATION TEST TARGET (QA-3)

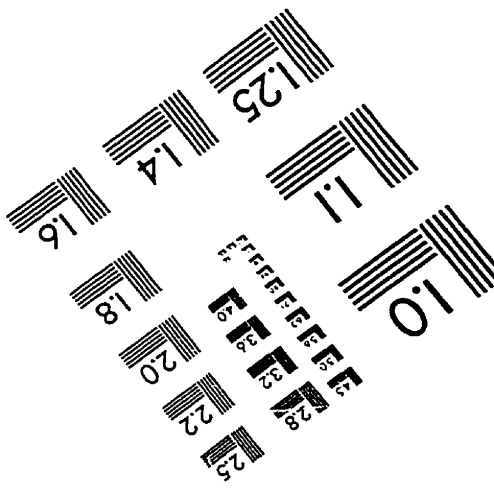












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