ENHANCED BIOREMEDIATION OF PETROLEUM CONTAMINATED SOILS USING AN ENGINEERED BIOREACTOR DESIGN CFB PETAWAWA PETAWAWA, ONTARIO

by

DAN McNICOLL, B.Sc.

A thesis submitted to the Faculty of
Graduate Studies and Research in partial fulfilment
of the requirements for the degree of
Master of Science, Department of Earth Sciences

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Thesis Supervisor

Chairman, Department of Earth Sciences

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ABSTRACT

An engineered, bioreactor system was designed and constructed to bioremediate approximately 1,800 m^3 of petroleum contaminated soil at CFB Petawawa, Petawawa, Ontario. The bioremediation facility was constructed in late fall of 1992 and operated for a six month period between May 1993 and November 1993.

The bioremediation facility consists of four above ground bioreactors each incorporating a network of aeration piping and a two-tiered water/nutrient delivery system. The aeration piping is connected to a central vacuum pump which draws air through the bioreactor providing a source of oxygen essential for the proliferation of the hydrocarbon-degrading bacteria. The nutrient delivery system provides a means of uniformly introducing nutrients to the hydrocarbon-degrading bacteria and allows the user to control and maintain soil pH and moisture content within the bioreactor in the optimum range for the bacteria. An elaborate leachate collection system enables the leachate to be amended as required and recirculated back into the bioreactor from which it originated. The bioreactors are covered with an opaque vapour barrier to minimize volatilization and enhance solar heating.

An elaborate monitoring program was implemented over the six month operating period. The monitoring program involved the collection of soil, water and air samples on a weekly and bi-weekly basis and various field measurements. The field and analytical results obtained were used to optimize conditions within the bioreactors during the course of the study to enhance natural biological degradation of the predominantly diesel contaminants within the soil. In addition to the organic and inorganic analyses performed, a detailed microbial monitoring program was implemented to track biological activity and to provide evidence supporting biological degradation.

Based on the field and analytical results obtained, the total petroleum hydrocarbon concentrations in the bioreactor soils were found to have been reduced by 97% at the end of the six month operating period. Mass balance calculations indicate that 99% of the degradation was attributable to biological degradation, with the remaining 1% due to volatilization.

Strong correlations between soil temperatures and the initial Toluene mineralization rates indicate that temperature has had an effect on the rate of petroleum biodegradation. Conversely, little or no evidence was observed in this project to suggest that the continuous addition of nutrients to the soil had a significant effect on the rate of biodegradation.

The estimated treatment cost for this project was calculated to be in the range of 70 - $90 per tonne. This facility however, has the added benefit of being reusable and hence the potential exists to lower the net treatment cost to the 20 - $40 per tonne range. This project has shown that diesel contaminated soil can be efficiently and effectively treated to meet the most stringent federal and/or provincial criteria in a cost effective manner over a typical Canadian summer.

The very favourable results obtained from this project should encourage the widespread use of this technology on military bases across Canada and/or any other sites where time and space constraints are not a prime concern. Future research emphasis should be placed on the bioremediation of different contaminants and/or soil conditions.

ACKNOWLEDGEMENTS

Financial support for this study was provided by the Department of National Defence and by Environment Canada under the National Contaminated Sites Remediation Program and the Office of Environmental Stewardship. Additional support was provided by the Biotechnology Research Institute, the University of Ottawa and Agriculture Canada.

The enthusiastic supervision, guidance and prompt reviews of my work by Michel J.L. Robin was much appreciated. Valuable insight was also provided by good discussions with John A. McKee, Anar Baweja, Charles Greer, Walter Zebchuk, and Dan Reynolds.

Many thanks go to Blaine Coons, Wade Kornik, and Ramona Bietlot who all assisted in the laborious task of data compilation and report preparation.

Finally, it is with enormous gratitude that I acknowledge the contribution of my wife, Vicki, who provided me with endless support and encouragement, and displayed extreme patience throughout this endeavour.

ORIGINAL CONTRIBUTION

The following will describe the extent of my involvement in this project. I was fortunate enough to act as Project Manager for the subsurface investigation which was performed during the summer of 1991. My involvement as Project Manager involved organizing and planning a borehole drilling investigation, selecting representative soil and groundwater samples for petrochemical analysis, data compilation, and the preparation of a report which presented available options for various soil remediation technologies including above ground bioremediation.

Upon submission of the report to the Department of National Defense, I had limited involvement in the decision making process and once the formal decision was made to proceed with the above ground bioremediation technique, I arranged the laboratory feasibility study which was performed by the Bio-Technology Research Institute of the National Research Council of Canada. Upon receipt of the laboratory feasibility results, I proceeded to design the bioreactor system which is discussed in this report. The design was conducted during the winter of 1992 after which our company was retained to provide limited supervision of the construction phases and to perform the monitoring program discussed in this report.

I supervised the construction phases during the Fall of 1992 and organized and oversaw all of the monitoring program which was undertaken between May 1993 and November 1993. Although a large portion of the actual field sampling program was undertaken by a technician with our firm, I was closely involved with the sampling program and undertook all data compilation and interpretation. All analytical work was performed by certified environmental laboratories and I relied on the resources of our company for all drafting and typing associated with the preparation of this report. All data compilation, interpretation, statistical analyses and writing was performed by the author.

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1. INTRODUCTION

On June 20, 1991 a gasoline spill occurred at a bulk petroleum storage facility located on Canadian Forces Base (CFB) Petawawa. The firm of Oliver, Mangione, McCalla & Associates Ltd. was immediately retained by the Department of National Defence (DND) to perform an investigation to determine the extent of subsurface contamination. Based on the results of that investigation it was determined that a significant amount of native soil had become contaminated with diesel and gasoline from a combination of recent and past spill events (McNicoll and McKee, 1991).

Several in-situ and ex-situ soil remediation options were presented to DND. After much consideration DND, in collaboration with Environment Canada, decided to proceed with a remedial strategy which involved treating the soil using bioremediation principles in the form of an above ground bioreactor. The bioreactor which was subsequently designed, was intended to provide the user

with maximum control over the conditions necessary to enhance natural biodegradation of the petroleum contaminants within the soil.

DND, recognizing the potential of using this technology at other facilities across the nation, agreed to fund the design, construction, and a limited monitoring program. Environment Canada decided to fund a more extensive monitoring program in order to obtain reliable data which would determine the effectiveness of this technology for soil remediation in a typical Canadian climate.

The following paper will present the bioreactor design, the monitoring program which was undertaken, and the results obtained.

1.1 Study Objectives

The primary objectives of the project were to:

1. Design a bioreactor system which would enable the user to control and optimize conditions within the bioreactor to enhance the natural biological degradation of the petroleum contaminants within the soil;
2. Utilize the analytical and field results obtained during the study to optimize conditions affecting biodegradation;
3. Assess whether petroleum degradation is predominantly a result of biodegradation or volatilization;

4. Assess whether temperature or nutrient adjustments have the greatest effect on the rate of petroleum degradation; and
5. Determine whether the use of bioreactors is a safe, cost effective, method of remediating petroleum contaminated soils in a typical Canadian environment.

1.2 Study Procedure

Prior to the design of a bioreactor system, a laboratory feasibility study was performed on representative soil samples to assess the biodegradation potential of the contaminated soil. Based on the favourable results obtained, the design of a full scale bioremediation facility incorporating four bioreactors was initiated. The facility was constructed during the fall of 1992 and operated over a six month period between May 1993 and November 1993. Weekly and bi-weekly site visits were performed to collect field measurements and to obtain representative soil, water and air samples. All samples were submitted to environmental laboratories for organic, inorganic and bacteriological analyses.

The field measurements and analytical results obtained were used to adjust or amend conditions within the bioreactors during the course of the study in order to optimize the natural biodegradation process.

2. BACKGROUND INFORMATION

2.1 Site Location

Canadian Forces Base (CFB) Petawawa is a major Land Forces Base used for military formation training and mounting of government missions throughout Canada and the world. The base was established in 1906 and occupies approximately 30,440 hectares of land. The base is located immediately north of the Village of Petawawa, which is situated on the banks of the Ottawa River approximately 200 km northwest of Ottawa, Ontario (Figure 1).

2.2 Site Geology

The Base is situated on what is locally known as the Petawawa Plain. The Plain consists of a thick accumulation of fine-to-medium grained sand of deltaic origin. The sand was deposited during the Pleistocene Period when sand laden glacial and post glacial streams flowed into the deep Ottawa Valley. The sand deposits exceed 25 metres in thickness in this vicinity and directly overlie Precambrian bedrock formations (Gadd, 1962).

The groundwater table in the overburden material was encountered at a depth of approximately 23 metres below grade. The local groundwater flows northeasterly, towards the Ottawa River with an average hydraulic

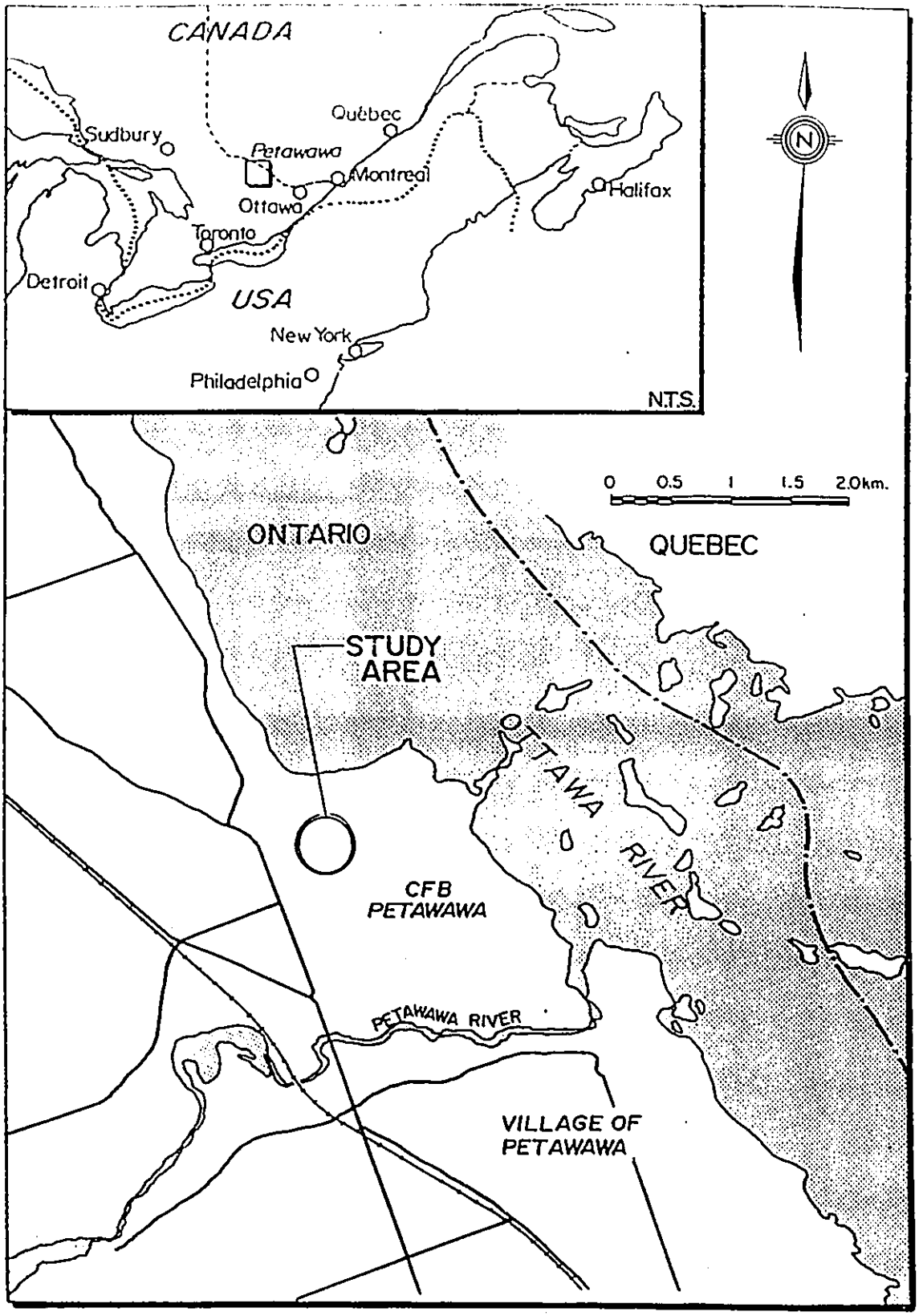


FIGURE 1. LOCATION MAP OF CFB PETAWAWA, PETAWAWA, ONTARIO

gradient of 0.02 m/m (Figure 1) (McNicoll and McKee, 1991). Assuming a typical hydraulic conductivity for medium grained sand of 1 x 10^{-4} m/sec and a porosity of 40% (Freeze and Cherry, 1979), the average linear groundwater velocity was estimated at 0.4 m/day.

2.3 Site Climate

The climate at CFB Petawawa is temperate with cold winters and warm, humid summers. Average winter and summer temperatures are in the range of -10 to -20°C and 20 to 30°C, respectively. The average annual rainfall, based on a 30 year mean (1951 to 1980), is 612 mm, while the average annual snowfall is 208 cm (AES, 1993).

2.4 Site History

A large bulk petroleum storage facility is centrally located at CFB Petawawa and is referred to as the P.O.L. Compound. The P.O.L. Compound has the capacity to store in excess of 2 million litres of petroleum products including gasoline, diesel and aviation fuel.

On June 20, 1991, two above ground storage tanks were accidentally overfilled resulting in the discharge of approximately 7,000 litres of gasoline into the subsurface. The firm of Oliver, Mangione, McCalla & Associates

Ltd. was retained by DND to perform a subsurface investigation to determine the extent of subsurface contamination and to provide recommendations for site remediation. Based on the results of the investigation it was determined that approximately 500 to 1,000 m^3 of soil was contaminated with gasoline and diesel from a combination of recent and past spill events (McNicoll and McKee, 1991). Total Petroleum Hydrocarbon concentrations as high as 12,020 mg/kg were measured in the contaminated soil. The zone of soil contamination was found to extend to a depth of approximately six metres below grade.

The surface of the overburden groundwater regime was encountered at a depth of approximately 23 metres. Laboratory analyses of representative groundwater samples collected directly beneath the P.O.L. compound found no detectable Total Petroleum Hydrocarbons (TPH), Benzene, Toluene, Ethyl-Benzene and/or Xylene (BTEX).

Several remedial options were presented to DND for the remediation of the contaminated soil. These included, but were not limited to, insitu soil vapour extraction, low temperature thermal desorption, soil washing, and bioremediation. After much consideration and discussions with Environment Canada, DND decided to explore the potential of treating the contaminated

soil using bioremediation technology in the form of an above ground bioreactor. Some of the factors leading to this decision were:

1. DND's intention to decrease the petroleum storage capacity of the POL Compound by decommissioning the tanks in the vicinity of the contaminated zone;
2. The lack of time and space constraints; and
3. The potential of having a permanent facility where contaminated soil from other parts of the base could be treated in the future.

Prior to the design of the bioreactor system, a laboratory feasibility study was performed to assess the biodegradation potential of the petroleum contaminated soil. The feasibility study was performed during the winter of 1991/92.

3. FACTORS AFFECTING BIODEGRADATION

Hydrocarbons are highly reduced substrates and require an electron acceptor such as oxygen, nitrate, and/or sulphate in order to undergo biodegradation (Zobel, 1947). In the presence of oxygen, the hydrocarbon substrate is converted to carbon dioxide (CO_2), water (H_2O) and cellular biomass. In general, the reaction can be depicted as;

$$\text{Hydrocarbon} + O_2 \rightarrow CO_2 + H_2O + \text{Biomass}$$

Oxygen is the most abundant and energetically favourable electron acceptor and can often become a limiting factor in all types of petroleum degradation (Moulna and Grubbs, 1990; Bossert and Bartha, 1984; King et al, 1992; Major, 1990; Nyer, 1992; Sims et al, 1989; TRI, 1982; Staps, 1989; Irvine et al, 1993; Freeman et al, 1987). Hence, a continuous supply of oxygen is generally required to optimize the rate of biodegradation.

In addition to oxygen, macro nutrients such as nitrogen and phosphorus are generally required to correct the nutritional imbalance created when a large quantity of hydrocarbons are added to a soil (Fogel et al, 1986). Laboratory experiments by Dibble and Bartha (1979) showed C:N ratios of 60:1 and C:P ratios of 800:1 to be optimum for most hydrocarbon-utilizing bacteria. Calabrese and

Kostecki (1989) recommend maintaining nitrogen levels in excess of 5 ppm at all times and phosphorous levels of 1 ppm or more in order to avoid impeding microbial activity.

Soil water serves as the transport medium through which many nutrients and organic constituents diffuse to the microbial cell (Sims et al, 1989; Moulna and Grubbs, 1990). As a result, soil moisture content can be an important parameter in the biodegradation process (Hinchey and Arthur, 1991; Barnhart and Myers, 1989; Sims et al, 1989). Optimum soil moisture levels have been reported in the range of 10 to 20% by weight or 40 to 75% of the moisture holding capacity of the soil (King et al, 1992; Riser-Roberts, 1992).

Temperature and pH also play an important role. Studies have shown that the majority of hydrocarbon degrading bacteria are most active in the range of 20°C to 30°C (TRI, 1982; Bradford and Krishmanoorthy, 1991). Similarly, the rate of hydrocarbon biodegradation is greater under neutral to slightly alkaline conditions (Grubbs, 1986; King et al, 1992; Riser-Roberts, 1992).

By maintaining all of the above noted parameters within the optimum range of the hydrocarbon degrading bacteria, one can expect to enhance or accelerate the natural biodegradation process. One system which allows the user maximum

control of the above noted parameters is an above ground, self contained, bioreactor design.

4. LABORATORY FEASIBILITY STUDY

Prior to designing the bioreactor system, a laboratory feasibility study was performed to determine whether indigenous hydrocarbon degrading bacteria were present within the contaminated soil and whether these bacteria could be stimulated through the addition of commercially available inorganic nutrients. The laboratory study was performed by the National Research Council's Biotechnology Research Institute.

Representative contaminated soil samples were collected from the site and homogenized prior to being subdivided into numerous microcosms. Varying amounts of nutrients and water were added to each microcosm. The microcosms were incubated at room temperature and moisture levels were maintained with the addition of sterile water at weekly intervals over a six week period. Soil microcosms were monitored for Total Viable Bacteria - using the Spread Plate Technique, and for specific micro-organisms with the genetic potential to degrade hydrocarbons - using gene probes and molecular biology techniques such as DNA Hybridization (Greer et al, 1992). In addition, Total Petroleum Hydrocarbon concentrations within the soil microcosms were monitored at specific intervals. A summary of laboratory findings follows:

1. The soil was found to contain indigenous bacteria with the genetic potential to degrade hydrocarbons;
2. The total viable bacteriological population was found to increase with the addition of commercially available inorganic nutrients (mainly nitrogen, phosphorous and potassium);
3. The population of bacteria with the genetic potential to degrade hydrocarbons was found to increase in response to nutrient amendments; and
4. A decrease in the Total Petroleum Hydrocarbon concentration was observed in the soil microcosms containing the highest nutrient amendments.

Based on these favourable results, the potential of enhancing natural biodegradation processes was considered high. As a result, the design of a bioremediation facility incorporating four above ground bioreactors was initiated.

5. BIOREACTOR DESIGN AND CONSTRUCTION

5.1 Bioreactor Details

The bioremediation facility consists of four rectangular bioreactors, each being approximately 27 metres long and 14 metres wide. A longitudinal cross-section through one of the bioreactors is shown in Figure 2.

Each bioreactor cell, was constructed to a height of two metres and contains approximately 450 m^3 of contaminated soil. The base of each cell is sloped towards one end and lined with an impermeable membrane (Figure 2). Rounded, peastone material was placed on top of the liner to form a highly permeable subdrain. The peastone material was subsequently covered with a geosynthetic fabric (Figure 2).

The petroleum contaminated soil was placed on top of the fabric in layers or lifts of approximately 0.3 metres in thickness. After each lift, a nutrient solution was applied to the soil surface. The lift was lightly compacted to promote uniform density. After two successive lifts were in place, the lower tier of the Nutrient Delivery System, which consists of perforated tubing with pressure compensative valves behind each perforation, was installed (Figure 2). The Nutrient Delivery System was designed to promote uniform moisture/nutrient application over the entire lift area.

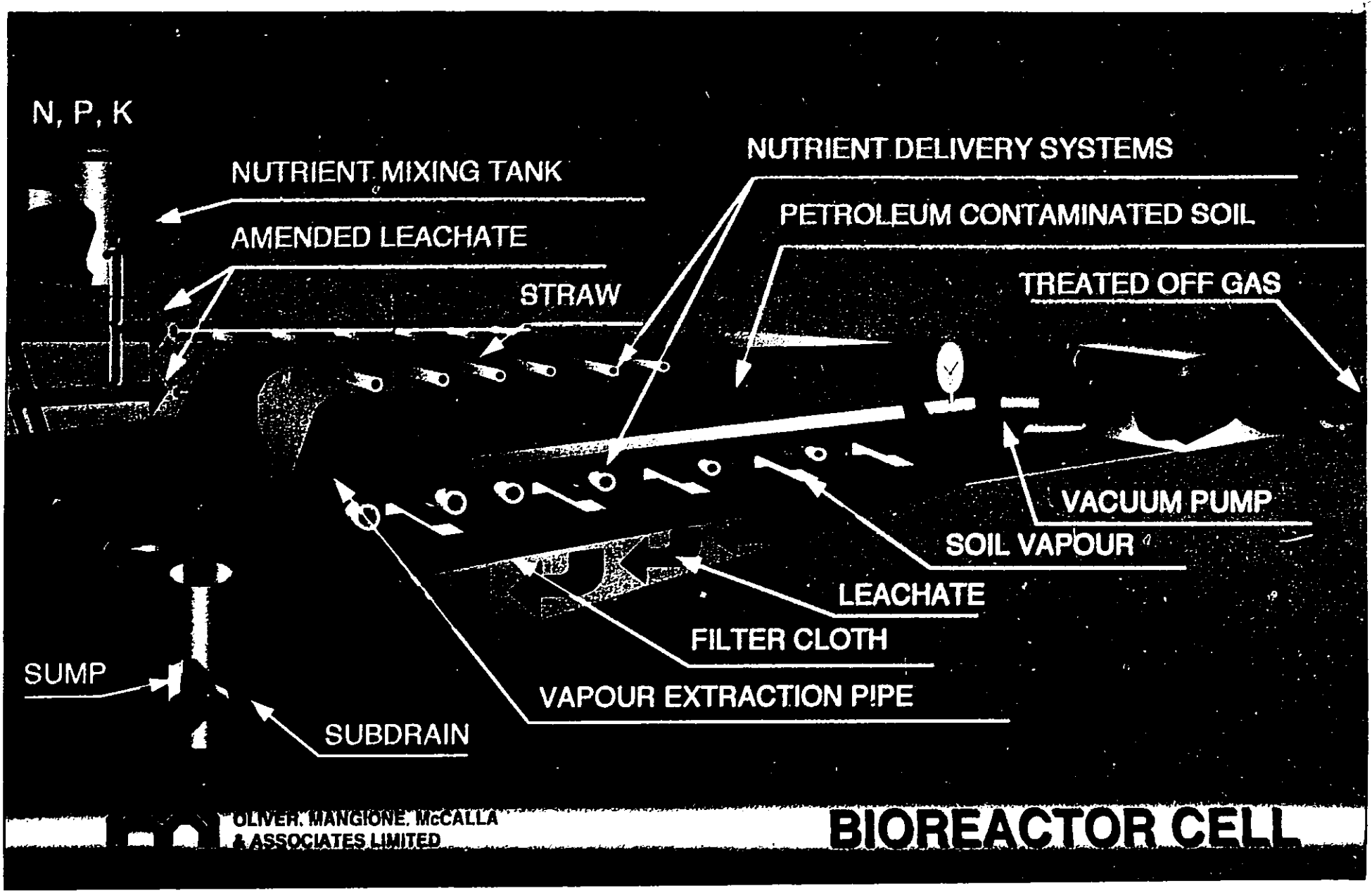


FIG. 2 **LONGITUDINAL CROSS SECTION THROUGH ONE OF THE FOUR ABOVE GROUND BIOREACTORS**

After the third lift, or at approximately mid height, two active 100-mm diameter soil vapour extraction (SVE) pipes and one 100-mm diameter passive air intake pipe were installed (Figure 2). The two SVE pipes were connected to a central vacuum pump while the passive intake pipe was left unconnected and temporarily capped.

Three more lifts of petroleum contaminated soil were placed in each cell, attaining a total height of approximately two metres. At the top of each cell an upper Nutrient Delivery System similar to the lower one was added. Each cell was then covered with approximately 100-mm of straw and draped with a black polyethylene vapour barrier (Figure 2).

5.2 Design Objectives

The various components of the bioreactors were designed specifically to attain certain objectives. Some of the more significant components and their operating objective are discussed hereafter.

The Nutrient Delivery System permits the controlled application of inorganic nutrients and provides a means of maintaining optimum soil moisture content and pH for the hydrocarbon-degrading bacteria within the bioreactor soils.

The Soil Vapour Extraction system provides a continuous source of oxygen to the bacteria by continuously replacing soil vapour with fresh oxygenated air. The contaminated soil vapours are directed to a central treatment facility which consists of a moisture separator, a sand filter, and a series of Granular Activated Carbon units. The volatile organic contaminants, present within the vapours, are adsorbed onto the carbon and the treated vapours are discharged to the atmosphere.

The subdrain/leachate collection system at the base of each cell permits excess moisture to pass through the cell and collect in the frontal sump area. The water is then amended with nutrients and lime, and recirculated through the cell from which it originated.

The layer of straw over the cells serves as insulation and allows air flow over the entire surface area of the pile, while the black vapour barrier minimizes volatile emissions and attracts solar radiation to promote soil heating.

5.3 Bioreactor Construction

The design of the bioremediation facility was undertaken during the winter (January - February) of 1992. The original intent was to have the facility constructed during the months of April or May 1992 and to operate it from

May to October of that same year. Due to construction delays, however, excavation and removal of the contaminated soil at the P.O.L. compound did not commence until July/August, 1992.

During excavation activities, distinguishing contaminated sand from uncontaminated sand was difficult due to the presence of gasoline vapours which migrated laterally from the zone of soil contamination. As a result a significant amount of uncontaminated material was unavoidably removed with the contaminated sand effectively diluting the petroleum contamination. The excavated material was transported approximately 2 to 3 kilometres to an abandoned airfield where the bioremediation facility was to be constructed.

The soil was stockpiled and covered with a plastic membrane to minimize volatilization and surface water infiltration. Emplacement of the soil within the prepared bioreactor cells did not occur until October with final completion at the end of November 1992 (Photo 1).

Towards the completion of the facility, DND requested that some diesel-contaminated soil, which resulted from a recent winter spill on the base, be

Photo 1 **- Aerial view of bioremediation facility, CFB Petawawa**

brought to the bioremediation facility for treatment. The diesel-contaminated soil was placed as an additional lift on the top of each cell. Unfortunately, the relatively high concentration of petroleum contamination within this soil created contaminant heterogeneity within the bioreactors.

Due to the delay in the completion of the facility and taking into account the inefficiency of operating such a system during the cold winter months, it was decided to leave the system dormant over the winter months and to operate it from May 1993 to November 1993. An initial soil sampling round was performed on November 27, 1992 to determine initial conditions.

6. OPERATION OF THE BIOREMEDIATION FACILITY

6.1 Remediation Objectives

At the initiation of the project, it was decided to evaluate the level of remediation attained at the end of the project in terms of the Alberta MUST Criteria (MUST, 1991). The Alberta MUST Guidelines were selected due to the absence of provincial and/or federal criteria for TPH concentrations in soils at that time.

The MUST guidelines establish the maximum Total Petroleum Hydrocarbon (TPH) concentration acceptable within soil on the basis of the potential risk of exposure and the potential for impact on human health and environment. Based on this risk assessment, three levels of soil contamination have been established. The established levels for soil TPH concentrations, ranging from least to most stringent, are Level I - 40 ppm, Level II - 400 ppm, and Level III - 1,000 ppm (MUST, 1991).

The ultimate use of the treated soil following completion of this project would depend upon the level of remediation attained. For example, if the soil attained the Level I criteria (40 ppm), it would be considered decontaminated and would have unrestricted reuse potential. If, however,

the soil attained Level III criteria (1,000 ppm) it would have restricted reuse potential such as subgrade material for a paved parking lot and/or road.

The ultimate goal of this project, however, was to remediate the contaminated soil by encouraging biological degradation of the petroleum contaminants to the point where the soil would no longer be considered contaminated. This level is signified by the most stringent TPH criteria of 40 ppm (i.e. Level I MUST Criteria).

6.2 Bioreactor Operation

On November 27, 1992, construction of the bioremediation facility was completed. For the purpose of this study, time '0' was considered to be November 27, 1992 which represented our first soil sampling event and the first application of nutrients to the soil.

The second set of soil samples was collected on May 6, 1993 (day 160). The cells were then sampled weekly for the first four months and bi-weekly for the next two months until November 18, 1993 - the date at which the experiment was terminated. The details of the sampling and monitoring program are discussed in Chapter 7. The operation of the nutrient delivery and soil vapour extraction (aeration) systems will be discussed hereafter.

a) Nutrient Delivery System

On May 6 (day 160), all four cells were doused with lake water through the upper nutrient delivery system. The objective of this initial dousing was to attempt to homogenize the contaminant distribution within the soil piles. It is estimated that approximately 1 to 2 pore volumes of water was flushed through the cells at this time. Following the initial dousing, the leachate from each individual cell was amended with nutrients and recirculated back into the cell from which it originated.

As previously mentioned, nutrients were initially added to the soil in November of 1992. A commercial fertilizer (Plant Prod: 35-5-10) with nitrogen, phosphorus and potassium in the form of N (total), P_2O_5, and K_2O was added to the soil during cell construction. Urea and lime were also added to obtain the desired nitrogen concentrations and pH levels.

On May 25, 1993 (day 179), nutrients were added to the sumps of cells A, C and D in order to attain the recommended soil C:N and C:P ratios of approximately 60:1 and 800:1, respectively. The liquid nutrients were periodically circulated throughout the cells. On July

14 (day 230), the electronic timer, which controlled the operation of the sump pumps, was activated so that the pumps would operate for 15 minutes every hour and douse the cells with nutrient enriched waters. Although the cells were predominantly doused through the upper nutrient delivery system, the lower nutrient supply system was periodically activated when the lower portions of the cells were found to be relatively dry. The timer was adjusted on August 16 (day 262), to 30 min/hour in an attempt to increase the soil moisture content and nutrient concentrations.

On August 23 (day 269), aqueous ammonium hydroxide (NH_4OH) was added to the sumps of Cells A, C and D in an effort to raise soil nitrogen concentrations.

On October 20, (day 320) the sump pumps were shut off and drained to avoid freezing.

b) Soil Vapour Extraction System

On May 19, 1993 (day 174), the soil vapour extraction system (SVE) was activated. Regulating valves and vacuum gauges at the front and back of each cell were adjusted to maintain a uniform vacuum

and air extraction rates among all four bioreactors. The SVE system remained in operation 24 hours a day, 7 days a week except for the periods of June 17-23 and August 6-12 when the system was inoperative. The SVE was shut off on November 18, 1993 (day 355) marking the end of the experiment.

A summary of operating events that occurred during the operation of the bioremediation facility is presented chronologically in Table 1.

5.3 Control/Treatment Cells

One objective of the project was to assess whether temperature or nutrient amendments had the greatest effect on the rate of petroleum biodegradation. The effect of oxygen on the rate of petroleum degradation was not explored as it has been well documented in the literature that oxygen is often the rate limiting factor in biological degradation (Dupont et al, 1991; Floodgate, 1973; Zobel, 1973; Moulna and Grubbs, 1990). In order to assess the relative importance of temperature and/or nutrients, two of the four cells were randomly selected to serve as control/treatment cells.

Table 1

Summary of Major Operating Events

Date	Day	Event
Nov. 27/92	0	• Completion of bioreactor cell construction. First set of soil samples collected.
May 6/93	160	• Second set of soil samples collected. Initial dousing of all four cells with lake water.
May 19/93	174	• Vacuum pump started.
May 25/93	179	• Sump pumps started. Nutrients added to Cells A, C and D.
June 17-23/93	202-209	• Vacuum pump shut off.
June 30-July 5/93	216-221	• Sumps pumps shut off.
July 14/93	230	• Timer for sump pumps activated and set for 15 min/hr.
Aug. 6-12/93	252-258	• Vacuum pump shut off.
Aug. 16/93	262	• Timer for sumps adjusted to 30 min/hr.
Aug. 23/93	269	• Added Ammonium Hydroxide to sumps A, C and D.
Sept. 7-21/93	284-298	• Sumps shut down due to power failure.
Sept. 30-Oct. 4/93	306-311	• Sump pumps working continuously.
Oct. 20/93	327	• Sump pumps turned off due to freezing.
Nov. 18/93	355	• Vapour extraction system shut down.

Cell B was randomly selected as the control cell for nutrients (Photo 1). Aside from the initial application of nutrients during cell construction, no nutrient amendments were supplied to this cell throughout the six month monitoring program. Lake water was however applied to the cell at the same rate as the remaining three cells to maintain soil moisture levels.

Cell D was randomly selected as the low-temperature cell. In order to save costs, it was decided to proceed with a passive method of creating a temperature difference between cell D and the remaining three cells. For this purpose, a white 5 mm filter cloth material was placed over this cell in an attempt to reflect some solar radiation and hence reduce soil heating (Photo 1). Since the temperature was not controlled directly, the experimental design actually tested the hypothesis that a white covering would have an effect (through temperature) on biodegradation.

All factors being equal, any differences in the rate of petroleum degradation between these two cells and the remaining two cells could then be attributed to the effect of cell cover colour (temperature) and/or nutrient supplementation.

A summary of the treatments applied to the four cells is given in Figure 3.

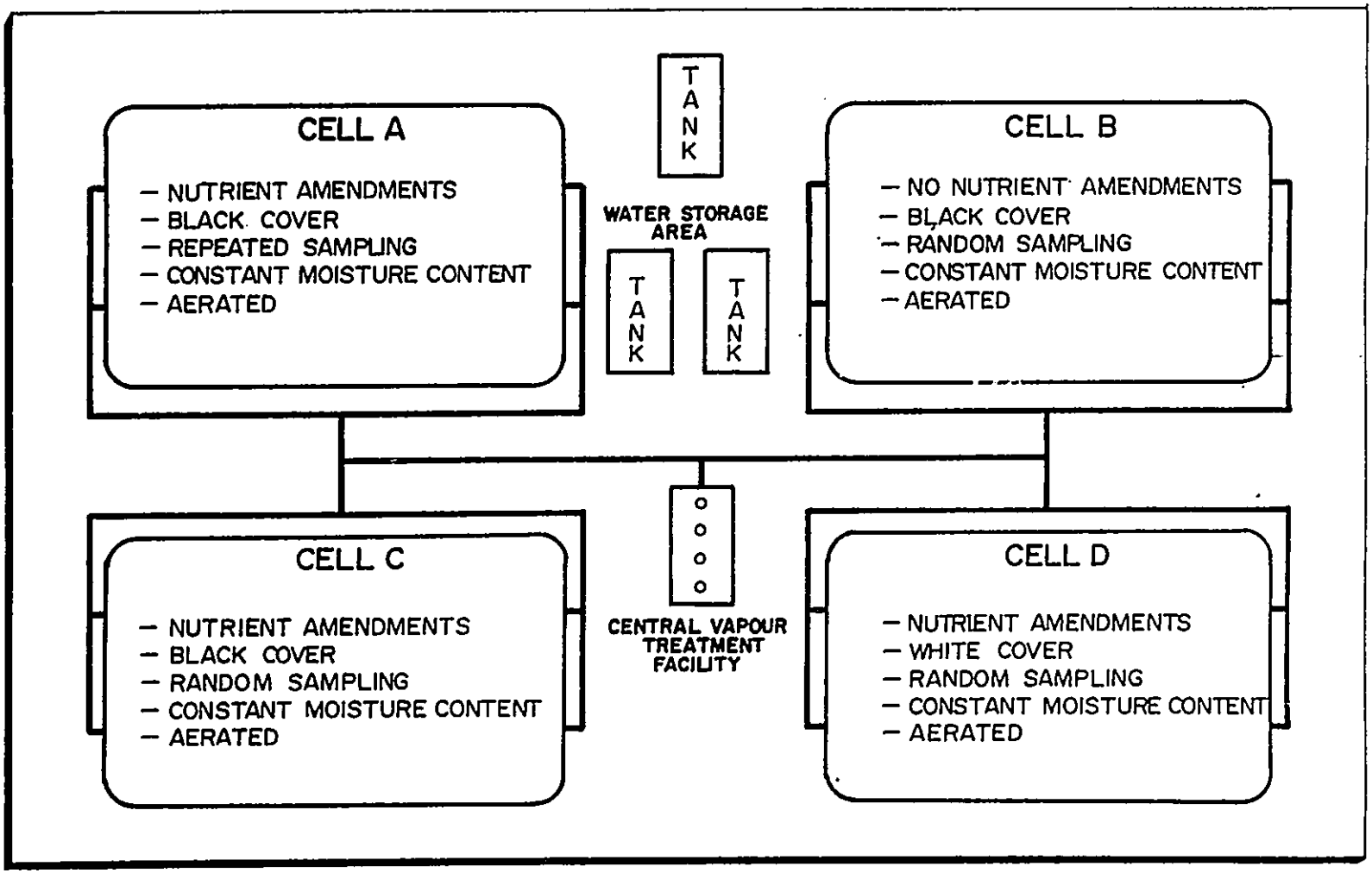


FIG3. SUMMARY OF TREATMENTS & SAMPLING METHODS APPLIED TO ALL FOUR CELLS

7. MONITORING PROGRAM

Due to the dynamic nature of biodegradation processes, an extensive monitoring program was implemented. The objective of the monitoring program was to obtain reliable field and analytical data to confirm, quantify and optimize biodegradation processes within the soil bioreactors. The program involved soil, water and air sampling, for subsequent laboratory analyses, and field measurements of insitu soil vapour, temperature and moisture conditions. All field measurements were recorded on specially prepared field data sheets - examples of which are included as Appendix "A".

A summary of the sampling and monitoring program is presented in Table 2 and will be discussed in more detail hereafter.

7.1 Laboratory Samples

7.7.1 Soil Sampling

Two composite soil samples were collected from each bioreactor on a weekly basis for the first four months of the operating period and bi-weekly thereafter. Each composite soil sample consisted of three subsamples at randomly selected locations and depths (Table 2, Appendix "A"). The rational of the sampling approach used will be discussed in more detail in Section 8.1 of this report.

Table 2

Summary of Sampling and Monitoring Program

A. Laboratory Samples

Sample Matrix	Sample Location	Sample Frequency	Analyses Performed
Soil	• 6 random locations in each bioreactor (2 composite samples)	• weekly (May-Aug.) • bi-weekly (Sept.-Nov.)	• TPH, N, P, K, pH, moisture content, total bacteria (May-Nov.) • BTEX (May) • Detailed microbiology (monthly May to Nov.)
Water	• bioreactor sumps	• weekly (June-Aug.) • bi-weekly (Sept.-Nov.)	• TPH, N, P, K, pH (May-Nov.)
Air	• at each bioreactor • at central vapour treatment facility	• bi-weekly	• TPH (May-Nov.) • BTEX (May-June)

B. Field Measurements*

Sample Matrix	Type of Measurement	Measurement Location	Measurement Frequency
Soil	• soil temperature	• four random locations per bioreactor	• twice weekly (May-Sept.)
	• Soil Moisture (TDR)	• bioreactor cells A & C only	• bi-weekly (June-Nov.)
Water	• flow rate - volume added to each bioreactor	• sump of each bioreactor • upper and lower nutrient delivery system	• twice weekly (May-Sept.)
Air	• flow rate - volume extracted from each bioreactor	• front and back of each bioreactor	• twice weekly (May-Sept.)
	• soil vapour - O_2/CO_2 concentrations	• two repeated sampling locations per bioreactor	• bi-weekly (June-Nov.)

* - See Appendix "B" for examples of Field Data Sheets

To meet the stringent sampling requirements of the project, an innovative soil sampling device was designed and constructed to permit retrieval of contaminated soil at a specified depth without generating excess contaminated soil. The sampling probe consists of a two metre length of 35 mm I.D. galvanized steel pipe casing with a removable centre core. The probe was driven to the desired depth using a hammering device and the centre core was then removed leaving the hollow casing in place. A second galvanized pipe, of slightly smaller diameter, was then inserted into the casing and pushed into the soil approximately 15 cm. The pipe was then retrieved yielding an intact soil core. A plunger type device was then used to extract the soil core into a stainless steel bowl after which the soil was immediately covered with aluminum foil to prevent volatilization.

The above soil sampling procedure was completed a total of six times within each bioreactor cell. The soil from three sub-samples was mixed in a stainless steel bowl to promote homogeneity and transferred as a composite sample to two or three food grade glass jars and stored in a cooler for subsequent laboratory submission.

Between cells, the sampling device was pressure washed and/or thoroughly wiped in order to avoid cross contamination. All samples were stored in coolers at approximately 4°C and submitted to an analytical laboratory within 24 hours of sample collection. Chain of Custody forms were used at all times.

All soil samples were analyzed for Total Petroleum Hydrocarbons (TPH), Benzene, Toluene, Ethyl-Benzene, Xylene (BTEX), Total Bacteria, Nitrate, Phosphorus, Potassium, pH, and Moisture Content. In addition, detailed microbiological analyses using gene probes and molecular biology techniques were performed on a monthly basis (Table 2).

7.1.2 Water Sampling

Water samples were collected from the frontal sump of all four cells on a weekly and bi-weekly basis (Table 2). All samples were analyzed for TPH, Nitrate, Phosphorus, Potassium, and pH.

A concentrated nutrient solution was periodically added to cells A, C and D during the first several months of operation. The nutrients consisted of a commercial fertilizer (Plant Prod: 35:5:10) which was supplemented with urea and lime to obtain targeted C:N and C:P

ratios of 60:1 and 800:1, respectively. These ratios are reported in the literature as being optimum (Dibble and Bartha, 1979).

7.1.3 Air Sampling

Air samples of the soil vapour extracted from each of the four cells were collected on a bi-weekly basis to determine the amount of petroleum being volatilized from the soil within each bioreactor cell.

The samples were collected from each of the four cells, and between the granular activated carbon units at the central Vapour Treatment Facility (Figure 3). They were obtained by inserting small charcoal tubes intc sampling ports and withdrawing vapours through the sampling tube using a low flow air sampling pump. Petroleum vapours within the air stream were adsorbed to the charcoal media which was subsequently submitted for laboratory analysis. Air samples were analyzed for TPH and BTEX (Table 2).

Measuring between the carbon units ensured that the carbon filter at the downstream end of the vapour treatment chain was contaminant-free thereby minimizing the potential for contaminant breakthrough. A new carbon unit was added to the series of carbon

filters when TPH and/or BTEX levels were detected before the last carbon unit of the Vapour Treatment System.

7.2 Field Measurements

7.2.1 Soil Temperature Measurements

Insitu soil temperature measurements were obtained using a specialized thermometer with a two meter long probe. The location and depth of temperature measurements were randomly selected using a random number generator. At each location, the temperature probe was inserted to the desired depth and the soil temperature was recorded after one minute.

In total, four temperature measurements were obtained from each of the four cells two or three times weekly (Table 2). Temperature measurements were recorded on field sheets - an example of which is included in Appendix "A".

7.2.2 Soil Moisture Content

Insitu volumetric soil moisture measurements were obtained in the field on a bi-weekly basis between June and November, 1993 using Time Domain Reflectometry (TDR). These

measurements were used to determine whether the need for adjustments to the rate of water being introduced into the bioreactors were required (Table 2).

A total of eight TDR probes were constructed and installed in two of the four cells (Cells A & C, Photo 1). Each TDR probe consists of two 5 mm diameter stainless steel rods approximately 50 cm in length. One end of the rod is soldered to a coaxial cable and set in a hardened plastic resin in such a manner that the two rods are perfectly parallel and approximately 40 mm apart.

The TDR probes were inserted approximately 2 m into the upper and lower portions of the cell with the coaxial cable coming out of the bioreactor. Field measurements were obtained by connecting a portable TDR cable tester to the coaxial cable. The cable tester transmits a step voltage pulse, or signal, along the coaxial cable and measures the signals propagation velocity through the soil. Signal velocity is dependant upon the dielectric constant of the soil which in turn is dependant upon the soil's moisture content (Topp and Davis, 1985; Topp et al, 1984; Yanuka et al, 1988). Knowing

the time it takes to travel the length of the probe and the length of the probes themselves, one can compute the soil moisture content over the length of the probe.

7.2.3 Volume of Added Water

The volume of water added to each bioreactor was monitored by periodically recording readings from flow totalizing water meters. Water meters were installed immediately after the sump pump, and on both the upper and lower nutrient supply systems of each bioreactor (Figure 2). All water meter readings were recorded on Field Data Sheets (Appendix "A").

Water meter readings were collected on average twice weekly from May to September (Table 2). Based on the readings obtained, adjustments to regulating valves were made to ensure a uniform amount of water was being added to all four bioreactors.

7.2.4 In Situ Soil Vapour Measurements

Insitu soil vapour oxygen (O_2) and carbon dioxide (CO_2) concentrations were measured at two locations within each bioreactor.

These measurements were obtained from fixed insitu vapour monitoring wells. The wells consisted of 6 mm diameter copper tubing which were installed to a depth of approximately 2 metres in the upper and lower portions of each bioreactor.

Soil vapour measurements were obtained bi-weekly using an explosive gas detector for O_2 measurements and a Dräger air sampling pump for CO_2 measurements. The stagnant air within each monitoring well was purged with a pump prior to sampling. Purging continued until O_2 measurements stabilized (i.e. after 1-2 wellbore volumes removed). O_2 and CO_2 measurements obtained were recorded on Field Data Sheets (Appendix "A").

8. QUALITY ASSURANCE AND QUALITY CONTROL (QA/QC) PROCEDURES

8.1 Field QA/QC

The importance of a thorough Quality Assurance and Quality Control (QA/QC) program in a project of this magnitude is well documented (Env. Can., 1992; Phillips, 1992; E.R.G., 1993; NWWA, 1986). In order to ensure that the field and analytical data obtained during this project were of good quality in terms of being both reliable and representative of site conditions, sound QA/QC procedures were implemented in the field and laboratory. In general, field QA/QC protocols closely followed the new federal guidelines for Sampling, Analysis and Data Management (Env. Can., 1992).

In order to negate aspects of cell heterogeneity, orientation, sample depth, time of day etc., a classical statistical random sampling technique was implemented. The sampling approach uses the theory of random chance probability to choose representative sample locations and depths (Env. Can., 1992). Random sampling locations were selected using a computerized random number generating program. Each cell was subdivided into eighteen imaginary plan-view sectors and four sampling depths (0.5, 1.0, 1.5, 2.0 metres) (Figure 4).

In order to assess the effect of fully randomized versus repeated sampling approaches, a repeated sampling approach was used in one of the

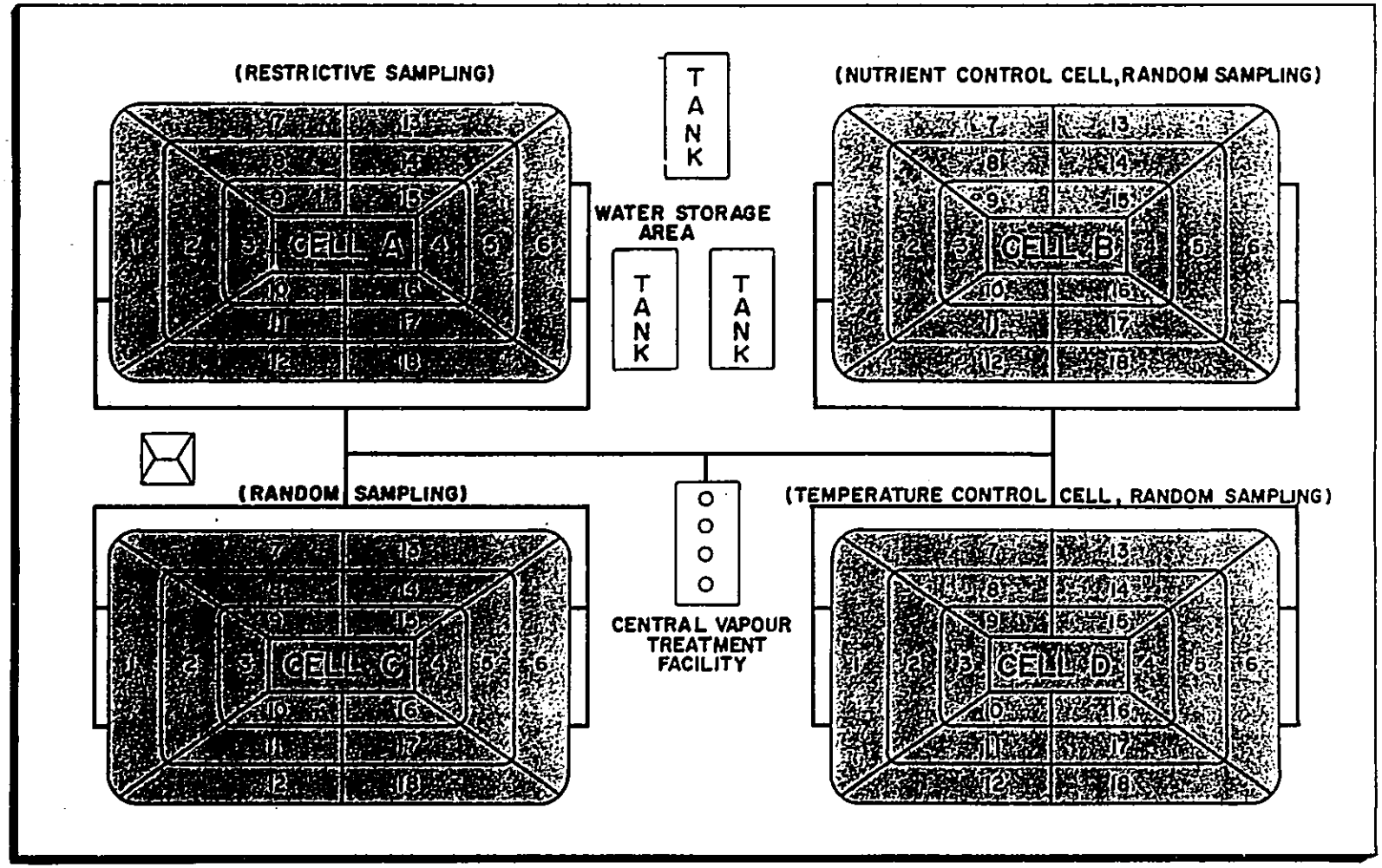


FIGURE 4. PLAN VIEW OF BIOREMEDIATION FACILITY SHOWING IMAGINARY SAMPLING SECTORS

bioreactors. Cell A was randomly selected for this approach as were its repeated sampling locations. Once selected, however, Cell A soil samples were collected at the same locations and depths throughout the six month monitoring program - hence the term "repeated-sampling".

Composite soil samples, each consisting of three subsamples, were collected throughout the monitoring program in order to obtain a representative sample of the extent of petroleum contamination within each bioreactor.

All sampling equipment was thoroughly cleaned using pressure washing and/or wiping procedures to minimize cross-contamination between cells and sampling events. All field instrumentation (i.e. O_2/CO_2 meter, air sampling pump etc.) were calibrated prior to use to ensure accuracy and reliability of readings.

All soil, water and air samples were collected in laboratory supplied bottles and/or food grade containers. Careful measures were taken during sampling procedures to minimize volatilization, photodegradation and/or biodegradation. As previously mentioned, all samples were appropriately labelled and documented in the field and immediately stored in a cooler and kept at approximately 4°C. Chain of Custody forms were used to track all

samples from point of collection to point of analysis. Samples were submitted to the analytical laboratory within 24 hours of sample collection.

Field Blanks and duplicate samples were collected throughout the monitoring program to determine the potential of sampling environment contamination and to verify laboratory procedures used. Duplicate samples were sent to two different laboratories for inter-laboratory verification of results obtained (Env. Can., 1992). Details of the laboratory QA/QC procedures are discussed in the following section.

8.2 Laboratory QA/QC

Two laboratories, certified by the Canadian Association of Environmental Analytical Laboratories (CAEAL), were used for all organic and inorganic analysis. Duplicate and blank samples were analyzed by both labs as part of the inter-laboratory verification measures. Microbiological analyses were performed by the Biotechnology Research Institute of the National Research Council of Canada. Details of the QA/QC procedures and analytical methods used are presented as Appendix "B".

Laboratory QA/QC measures for inorganic and index parameters involved running standards with each set of samples, standards and lab blanks for

all inorganic analysis; calibrating the pH electrode/meter with three commercially available buffer solutions; and zeroing the gravimetric scale before each moisture content determination (Appendix "B").

Organic analyses (TPH, BTEX) were performed using Gas Chromatography/Mass Spectrometer (GC/MS) and Gas Chromatography/Flame Ionization Detector (GC/FID) methods. The method for TPH extraction was validated with several types of soils. In addition, seven soil samples from this project were analyzed in triplicate, as submitted, and also analyzed after a known volume of diesel fuel was added. QA/QC of the GC system was charted using the detector response of a known volume of varsol. Varsol was used as there is currently no reference soils for TPH analysis.

9. FIELD AND ANALYTICAL RESULTS

All of the field and analytical results obtained throughout the six month monitoring program have been compiled into tables and graphs and are presented in Appendix "C" of this report. Upon reviewing the data obtained, numerous trends have been observed. The important findings are summarized hereafter.

9.1 Soil Temperatures

Four soil temperature measurements were obtained from each of the four cells frequently throughout the monitoring program. A graph of the mean soil temperatures for each cell and the mean daily air temperature at CFB Petawawa is presented in Figure 5 and Appendix "C" (Table C12).

Based on the soil temperature measurements, the white covering which was placed over Cell D to inhibit solar heating was not effective as soil temperatures in Cell D were not significantly different from those of Cell B or C. Cell A, on the other hand, consistently had slightly lower soil temperatures throughout the monitoring program. This is considered to be the result of obtaining soil temperatures at the same locations (i.e. repetitive measurement locations). As a result, these measurement locations were subject to factors such as location within the cell, orientation of the cell, time

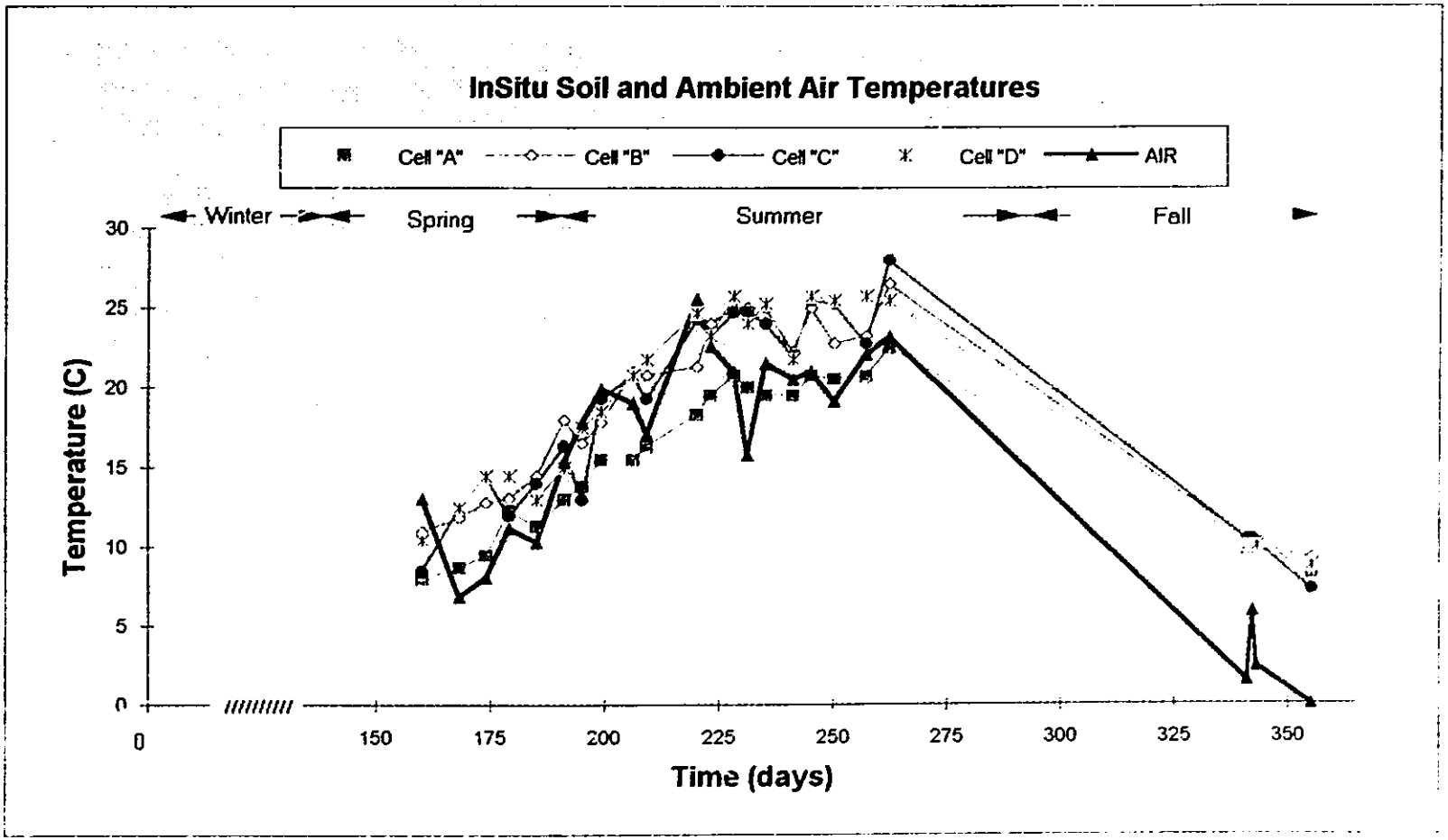


Figure 5 Comparison of Insitu Soil Temperatures for Cells A, B, C and D and Ambient Air Temperatures

of day etc., all of which may have contributed to the lower cell temperature measurements obtained.

A strong positive correlation was found to exist between the mean daily air temperature and the mean daily soil temperatures. Linear correlation coefficients were calculated to be 0.86, 0.89, 0.87 and 0.90 for Cells A, B, C and D, respectively. This strong correlation and the absence of any lag time, suggests that the operation of the soil vapour extraction system, which promoted ambient air to circulate through the soil piles, had a controlling effect on the soil temperatures. Approximately 3 to 5 air filled pore volumes were circulated through the cells daily.

In light of these results, Cells A, C and D can be considered as having the same treatment (with Cell A undergoing repeated or restricted random sampling).

9.2 Soil TPH Concentrations

The soil TPH concentrations obtained from each sampling event are shown in Figure 6 for all four cells. Each point on the graph is the mean of all measurements for a given cell and sampling session. It is apparent from the graph that a substantial degradation occurred in all four cells.

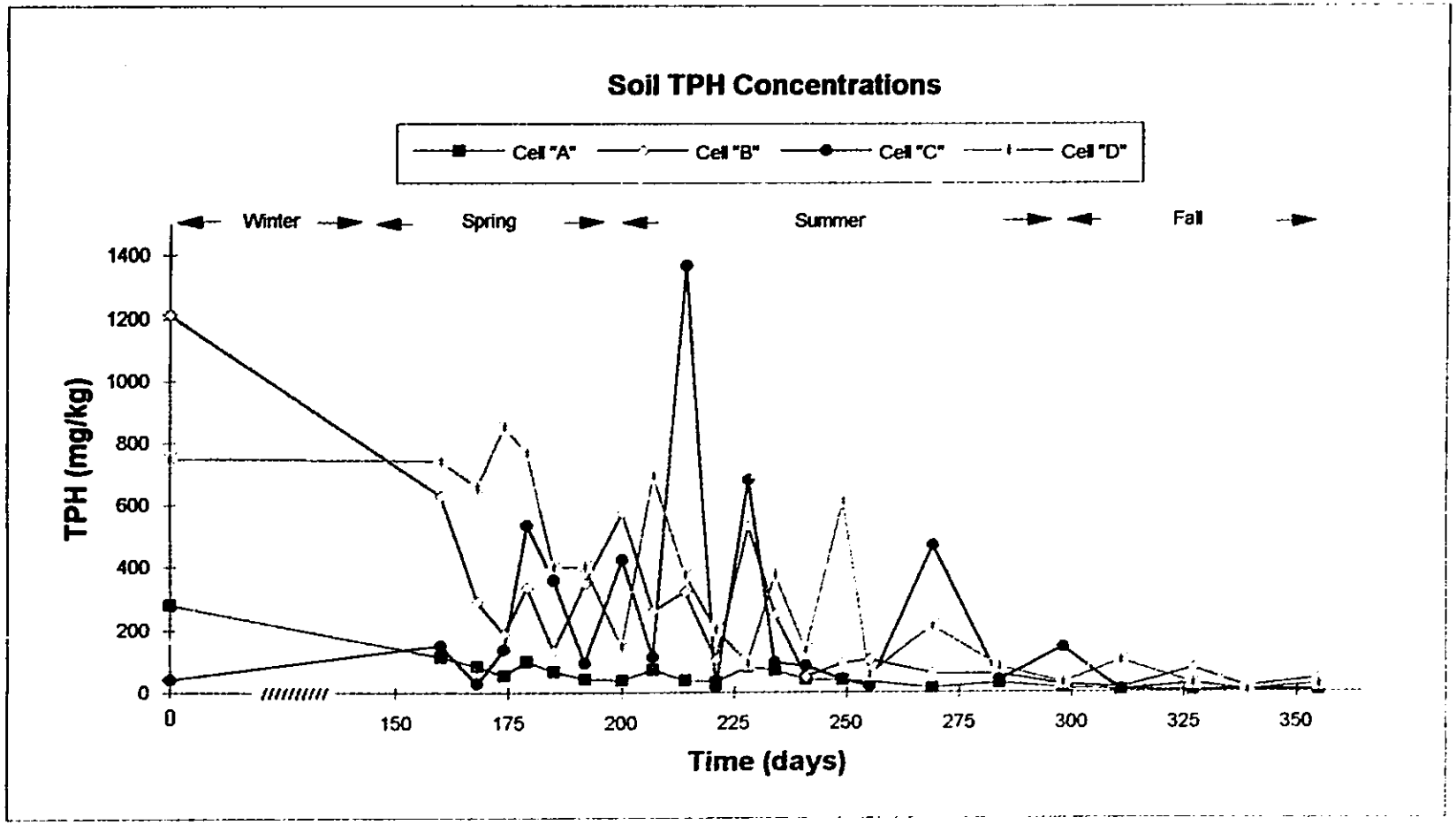


Figure 6 Mean Soil TPH (Total Petroleum Hydrocarbon) Concentrations in Cells A, B, C and D

The mean TPH concentrations in Cell C were somewhat erratic, reflecting the heterogeneous distribution of the petroleum contaminant. The heterogeneous contaminant distribution in this cell is believed to be the result of the addition of the relatively high concentration of diesel contaminated soil on top of the cell during the later phases of cell construction (winter diesel spill).

As can be observed from Figure 6, the variability (and concentration level) in TPH measurements decreased drastically over time with variance measurements of approximately 20,000 ppm^2 at early times (May - August, 1993) to 260 ppm^2 at later times (August - November, 1993). The homogenization of the contaminant distribution is believed to be a combination of leaching effects from the moisture supply system and biological activity.

The amount of petroleum degradation observed in each of the four cells was calculated using the initial and final mean soil TPH concentrations for each cell. Based on these results, the percent reduction in total petroleum hydrocarbons was approximately 97%, 97%, 94% and 98% for Cells A, B, C and D, respectively. This corresponds to an average petroleum degradation of approximately 97% over all four cells.

The soil in all four bioreactors was found to have final TPH concentrations well below the most stringent federal and/or provincial criteria of 40 ppm (Level I criteria) as demonstrated by the mean soil TPH concentrations obtained during the final month of monitoring (November, 1993).

9.3 Microbiological Results

A microbiological program was undertaken to monitor biological activity within the cells. This program was considered essential in providing evidence to demonstrate that biological degradation was in fact occurring within the cells. The program involved monthly enumeration of total viable and probe positive bacteria; and monitoring the mineralization activity of the bacteria using radio-labelled petroleum products. Due to the sophisticated instrumentation and techniques required to perform these analyses, the Biotechnology Research Institute was retained to perform the microbiological analyses. The analytical procedures used and results obtained are included in Appendices "B" and "C", respectively. A summary of the major findings are discussed hereafter.

9.3.1 Total Viable Bacteria

To determine the total number of Viable Bacteria, soil bacteria were incubated on a nutrient rich base (YTS medium) and on a selective

medium containing gasoline and diesel (DYG medium). The media were incubated at room temperature and colonies were counted after two weeks (Appendix "B").

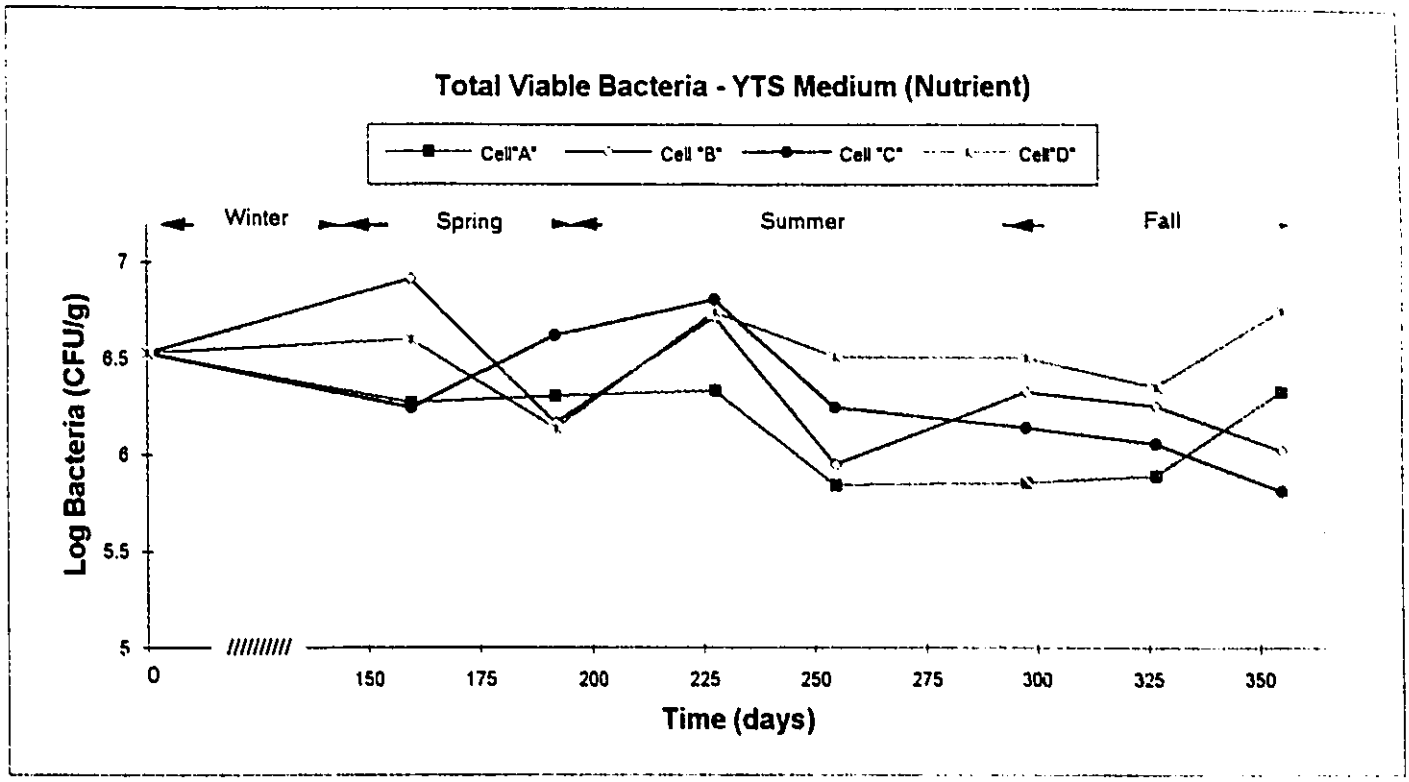
Based on the results presented in Figure 7 (see also Appendix "C" - Table C2), it is interesting to note that for any given cell and sampling session, the population counts on the DYG medium (containing diesel and gasoline) were as high or higher than those on the YTS medium (nutrient enriched) (Table 3).

Table 3

Difference in Log Counts Between Total Viable Bacteria on DYG and YTS Media

Date	Day	Cell A (log CFU/g)	Cell B (log CFU/g)	Cell C (log CFU/g)	Cell D (log CFU/g)
June 6/93	192	+0.47	+0.74	+0.71	+0.83
July 12/93	228	+0.11	+0.04	-0.02	+0.14
August 9/93	255	+0.37	+0.42	+0.37	+0.22
Sept 21/93	298	+0.09	-0.08	+0.05	-0.05
Oct 20/93	327	-0.01	+0.15	+0.12	+0.03
Nov 18/93	355	+0.09	+0.27	+0.16	-0.02
	Avg	**+0.19**	**+0.26**	**+0.23**	**+0.19**

CFU - Colony Forming Units
\+ - Actual bacteriological counts for both media are presented in Table C2 of Appendix "C".



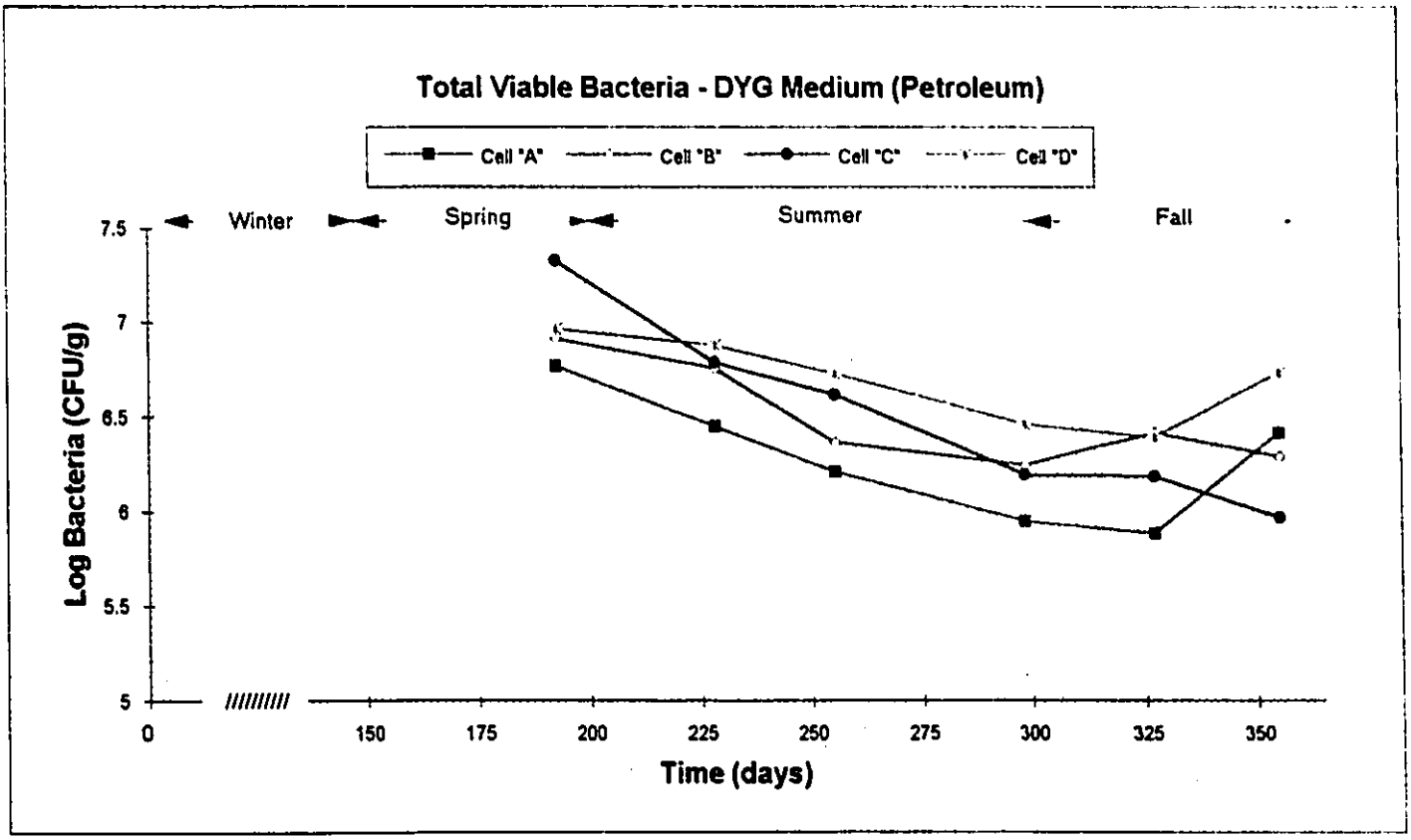


Figure 7 Total Viable Bacteria on YTS and DYG Media for Cells A, B, C and D

It should be noted that a difference of +0.2 on the log scale corresponds to a factor of 1.6 difference in the population counts. These results are somewhat surprising and would indicate that the bacteria were either quite resistant to any potential toxic effects from the gasoline and diesel products; and/or the bacteria were able to readily metabolize the petroleum products (Greer et al, 1994). Either of these potential traits is desirable in a bioremediation project.

A noticeable and steady decrease in the population of Total Viable Bacteria on the DYG medium (Petroleum rich) was observed from late spring onwards. Conversely, very little, if any, trend was observed in the bacterial population on the nutrient rich YTS medium (Figure 7).

The plots of Total Viable Bacteria counts versus soil temperatures in Appendix "D" show that although temperature had no significant effect on bacteria counts on the YTS medium (i.e. the slope of the log bacteria count versus temperature could not be declared significantly different than zero), a slight but significant effect was observed on the DYG medium (Tables D1 and D2).

The apparent increase in bacteriological population in Cells A and D in mid November, 1993 is unusual (Figure 7). Observations made during enumerations indicate that the distribution and diversity of the bacteria decreased at this point with select species becoming more prevalent (Greer et al, 1994). The information available is insufficient to indicate whether this increase in a few selective species was at the expense of other species competing for the nearly depleted hydrocarbons as their primary energy source.

No significant differences in Total Viable Bacteria population were observed between the nutrient treatment cell (Cell B) and the remaining three cells (i.e. the values for Cell B were almost always within the range of the other three cells).

9.3.2 Probe Positive Bacteria

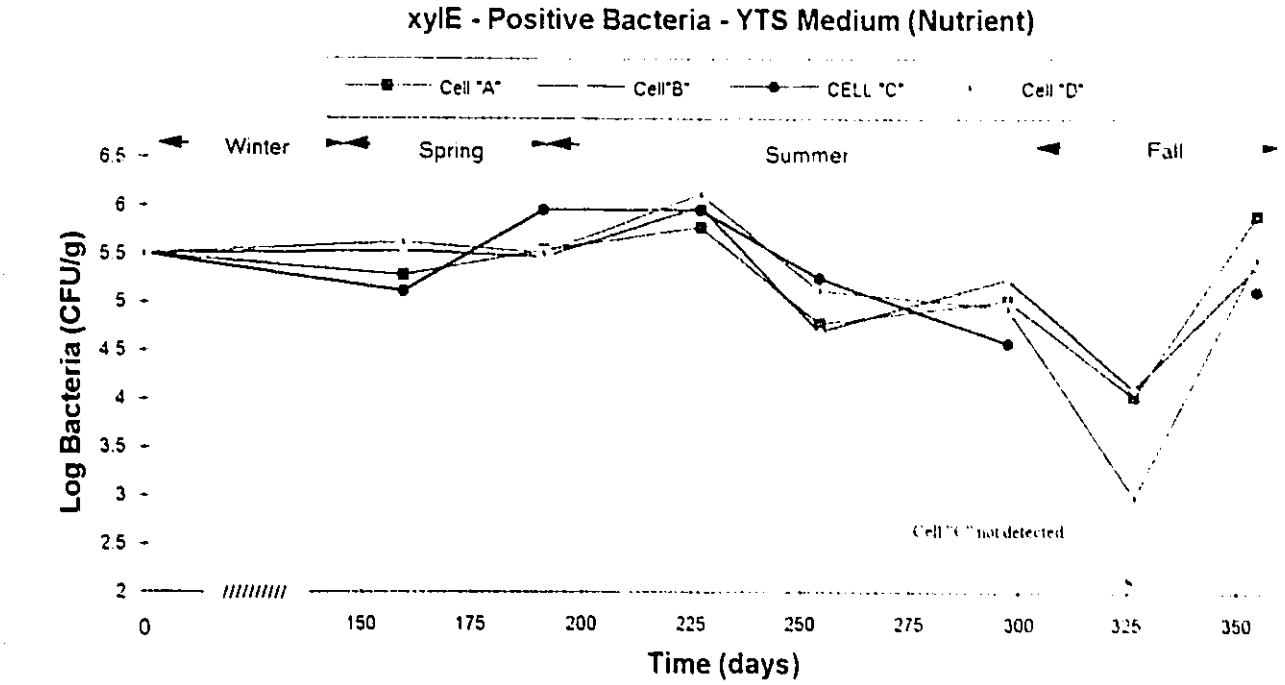
Bacteria with the genetic potential to degrade hydrocarbon compounds were identified as probe positive for the alkB and xylE gene probes. The alkB probe detects the bacteria which have the genetic potential to degrade simple alkanes present in diesel fuel (i.e. Hexane, Octane, Decane); while the xylE probe detects those with the potential to degrade aromatic hydrocarbons present in gasoline

(i.e. Toluene, Xylene). A summary of the bacterial counts is presented in Figures 8 and 9 (see also Appendix "C" - Tables C3 and C4).

Based on the probe positive counts, the sum of the alkB and xylE probe-positive bacteria were found to represent approximately 10% of the Total Viable Bacteria population. This proportion of probe-positive bacteria is considered to be relatively high (Samson et al, 1992).

Scatter plots showing the relationship between xylE and alkB bacteria counts versus soil temperature are presented in Appendix D. The level of significance of the calculated correlation coefficients and the slope of the linear regression are also included in Appendix "D".

Although the plots in Figures 8 and 9 reveal what appears to be a trend in which probe positive bacteria counts attain maximum levels during the warmer months of June and July (days 192 and 228) and decrease in the cooler fall months; the plots of probe positive bacteria versus soil temperature reveal a slightly different trend



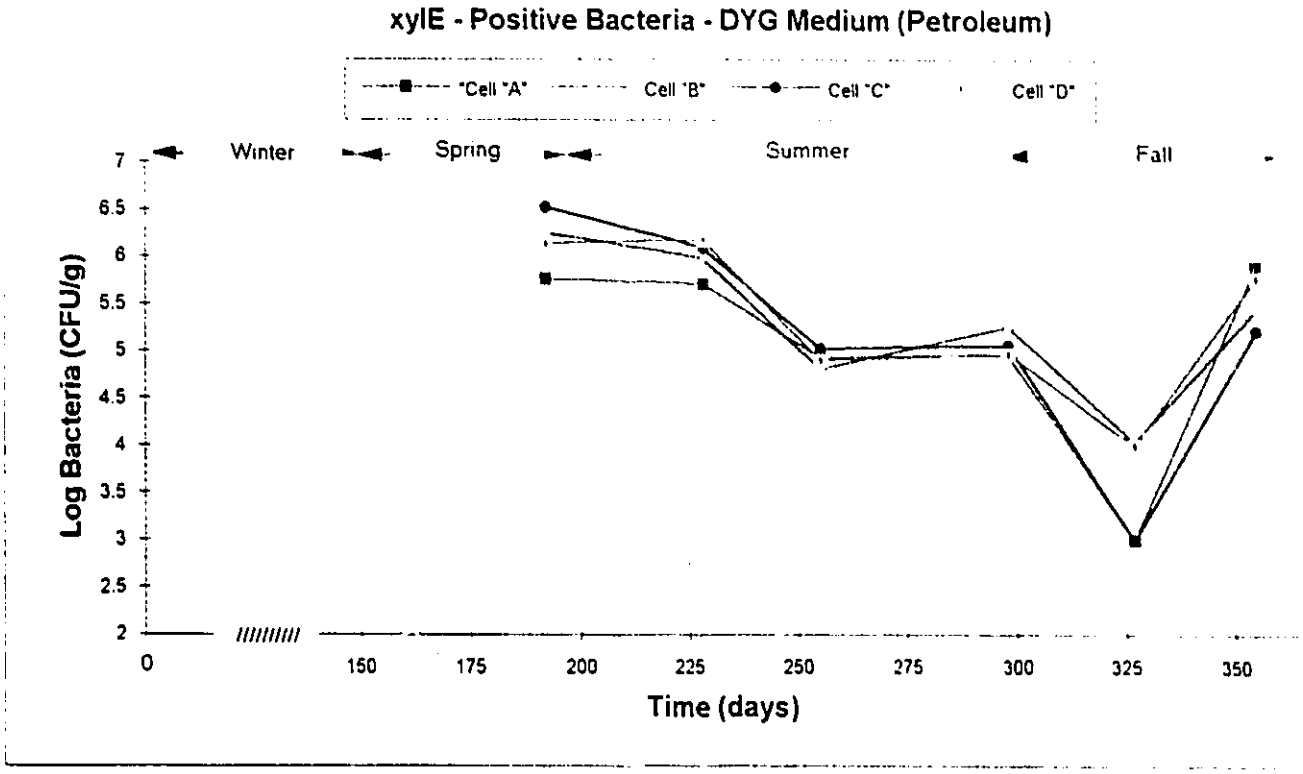


Figure 8 Population of xylE - Probe Positive Bacteria on YTS and DYG Media for Cells A, B, C and D

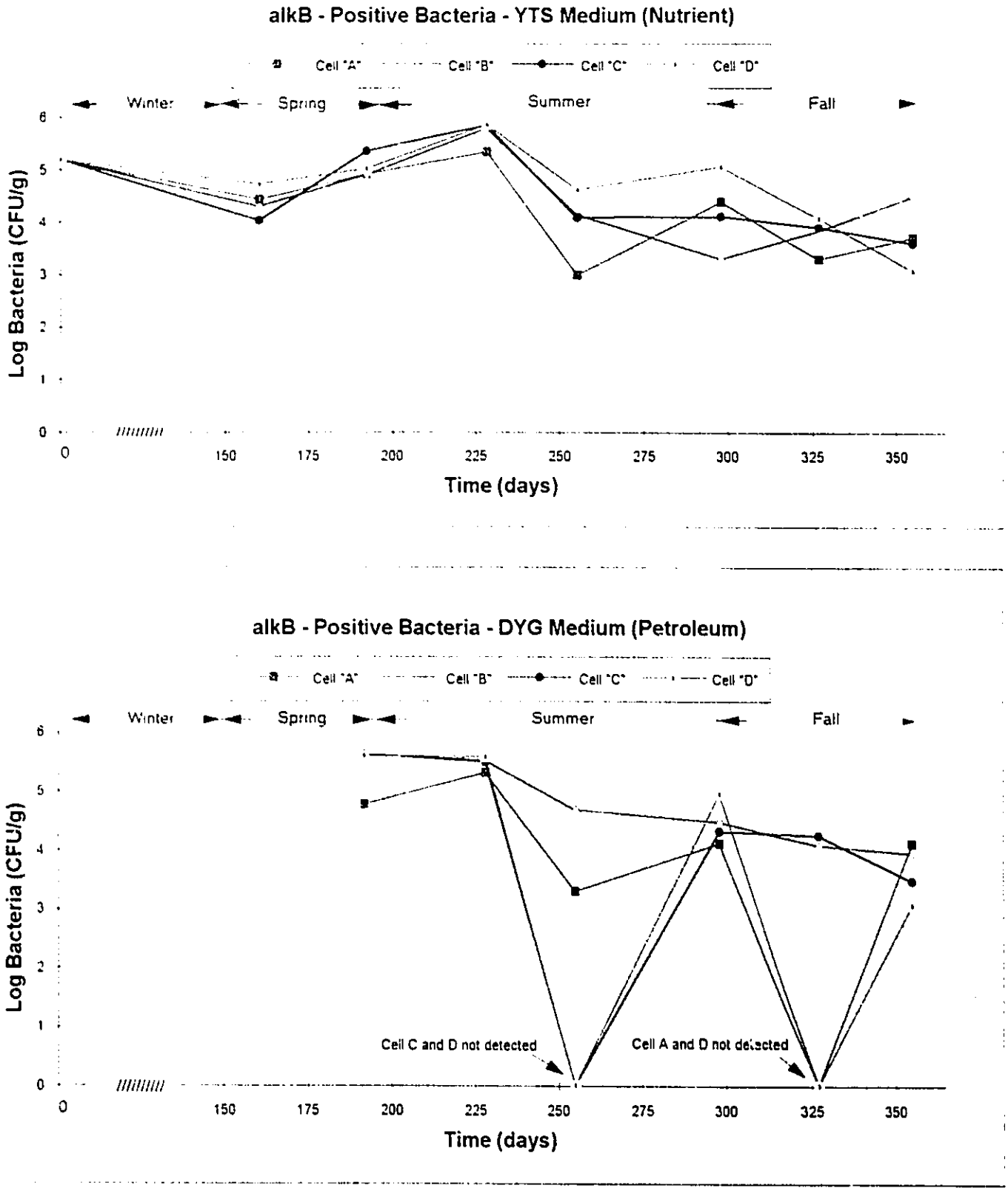


Figure 9 Population of alkB - Probe Positive Bacteria on YTS and DYG Media for Cells A, B, C and D

(Appendix "D", Tables D3-D6). In general, the population of both xylE and alkB probe positive bacteria were found to increase significantly with temperature for temperatures below approximately 10°C and were not affected significantly by temperatures in the range of 10° - 26°C.

The effect of temperature on biological activity is not unusual. From a bio-availability point of view as temperatures decrease, hydrocarbons - particularly the heavier aliphatic compounds, become more viscous and as a result are not as easily mobilized and hence mineralized by the bacteria (Greer et al, 1994; Sims et al, 1989).

Based on the probe positive trends observed in each of the four cells, no significant difference could be detected between the nutrient treatment cell (Cell B) and the remaining three cells (i.e. Cell B values were always within the range of the other cells).

9.3.3 Mineralization Activity

Mineralization activity was monitored monthly by adding a known volume of ^{14}C-labelled Toluene and Hexadecane to soil samples from each cell. The rate of Toluene and Hexadecane mineralization was monitored in the lab over three week periods by recovering ^{14}C-

labelled carbon dioxide (Appendix "B"). The objectives were to determine whether the hydrocarbon degrading bacteria, that were identified in the soil were active in mineralizing (i.e. degrading) the petroleum contaminants into carbon dioxide, and to determine the biodegradability of the contaminants themselves.

The mineralization rates of Toluene and Hexadecane are shown in Graphs C27 to C34 in Appendix "C". In general, approximately 60% of the added radio-labelled Toluene or Hexadecane was recovered as CO_2 after 21 days. This value is normal as approximately 40% of the labelled carbon typically ends up as cellular biomass while the remaining 60% serves as an energy source and is converted to CO_2 (Greer et al, 1994; King et al, 1992; TRI, 1982). Although these results indicate that the ^{14}C-labelled petroleum components added to the soil samples were successfully mineralized by the bacteria over the 21 day monitoring period, the initial rates (first three days) of mineralization were found to vary.

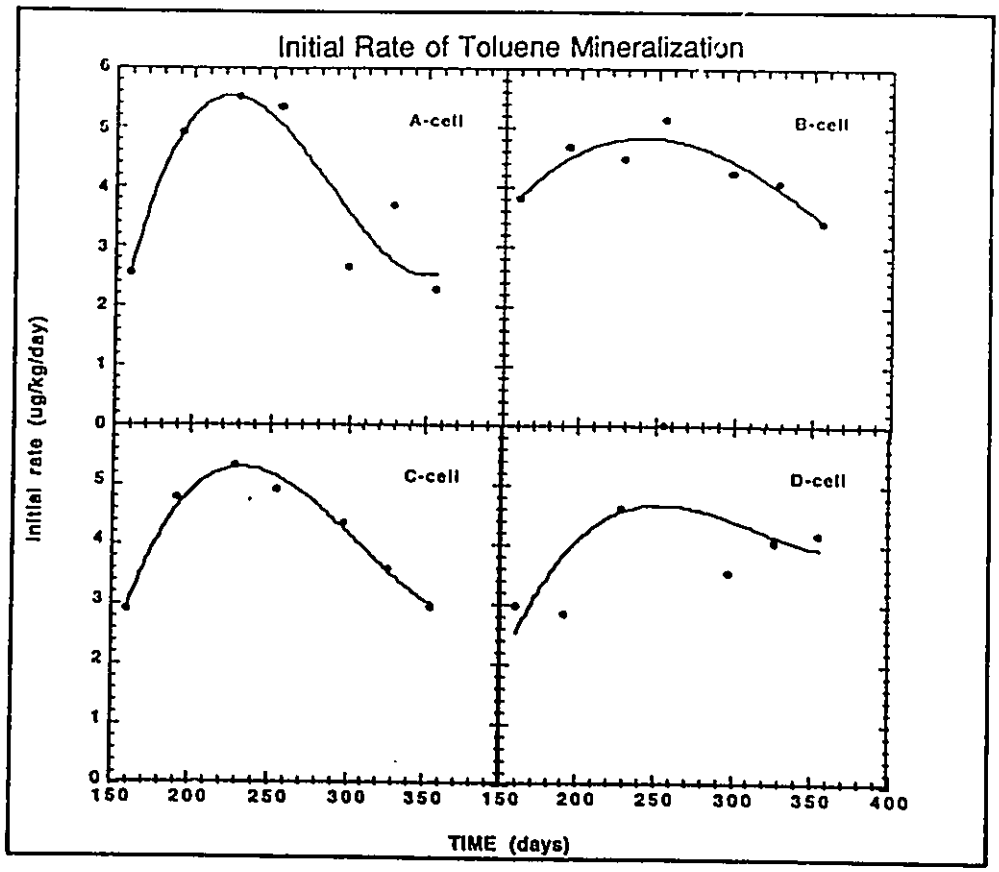
Initial mineralization rates are considered to be indicative of the instantaneous state of biological activity within the soil. The initial mineralization rates of Toluene and Hexadecane (after 2 or 3 days)

are presented in Tables C5 and C6 of Appendix "C" and are shown graphically in Figure 10. For visual purposes a 3rd degree polynomial curve was fitted to the data for each cell.

Based on the results obtained, the initial mineralization rates of Toluene peaked during the summer months of June - August (days 192 - 255) which coincided with the maximum soil temperatures (Table C12). Significant positive correlations were found to exist between the initial rate of Toluene mineralization and soil temperatures (Appendix "D", Tables D7 and D8).

No significant correlation, however, was found between the initial Toluene mineralization rates and soil TPH concentrations (Appendix "D", Tables D11 and D12).

The initial mineralization rates of Hexadecane were a little more erratic than those of Toluene (Figure 10). Nevertheless, the polynominal curve fitted to the data shows a trend which increases to a maximum and then decreases. The peak in mineralization occurred during August and September (days 255 to 298).



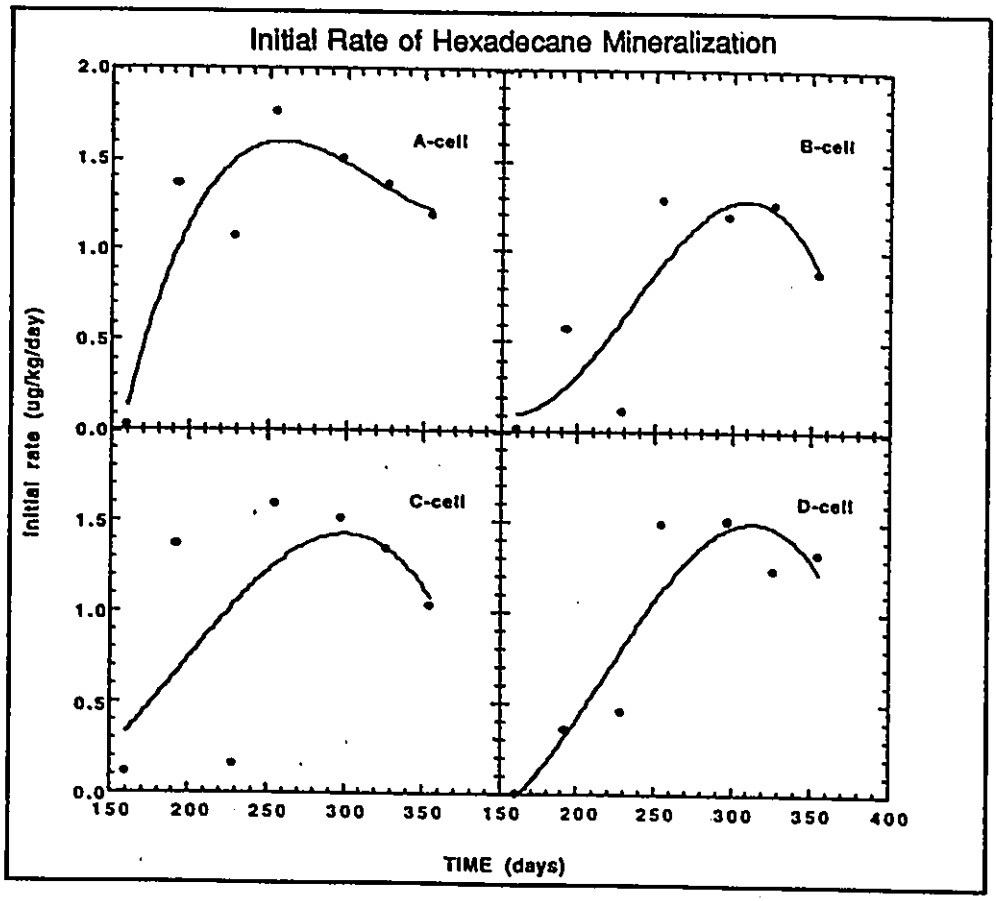


Figure 10 Initial Rates of Toluene and Hexadecane Mineralization in Cells A, B, C and D

Although the rate of Hexadecane mineralization was found to have no significant correlation with soil temperatures, it was found to have a significant negative correlation with soil TPH concentrations (Appendix "D", Tables D10 and D14). The strong negative correlation shows that large initial Hexadecane mineralization rates correspond to low soil TPH concentrations (Figure 6, Table C1 and Tables D13 and D14).

The fact that this TPH trend was observed in the initial rates of Hexadecane mineralization and not in those of Toluene is not surprising considering that the predominant form of petroleum hydrocarbons within the soil were found to be alkanes (diesel fuel) similar to that of Hexadecane. Laboratory results have shown that no detectable aromatics (<0.1 ppm BTEX), were detected in any of the soil samples collected during the first month of operation (Tables C17 - C20).

9.4 Soil Vapour TPH Concentrations

Soil vapour TPH concentrations were obtained by withdrawing contaminated vapours from the SVE pipe of each cell through a dedicated charcoal tube using a low-flow air sampling pump. Charcoal tubes were then submitted

to an analytical laboratory for TPH analysis. The results are presented in Figure 11 and in Appendix "C" (Table C9).

The results show a significant decrease in soil vapour TPH concentrations during the first 100 days of operation of the soil vapour extraction system. During this time the average vapour concentration of approximately 200 ppb decreased to undetectable levels in all four cells (i.e. <0.3 ppb). It is interesting to note that none of the volatile components commonly associated with gasoline (i.e. Benzene, Toluene, Ethyl-Benzene and Xylenes) were detected in any of the soil samples after completion of the bioreactor facility in the fall of 1992 (Appendix "C", Tables C17 - C20). These volatiles, although present before excavation activities, are believed to have been lost during the excavation, transportation and construction of the bioremediation facility. The measured concentrations of volatiles adsorbed to the charcoal sampling tubes are probably the result of the accumulation of low concentrations (previously undetected) of volatile compounds, and/or semi-volatile compounds such as napthanlene and phenanthrene, which are not readily volatilized under normal conditions (Calabrese et al, 1989).

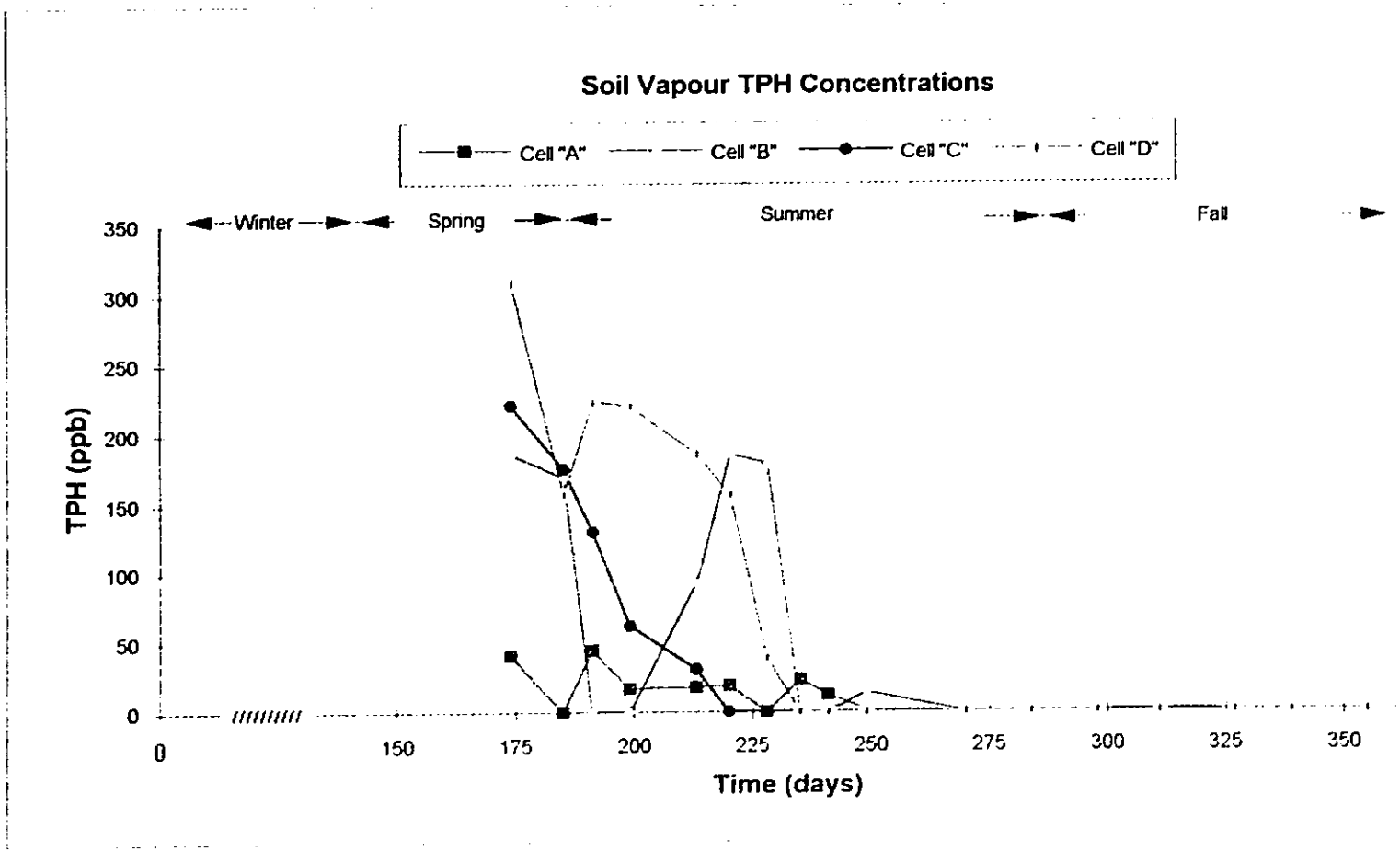


Figure 11 TPH Concentrations in Soil Vapour Extracted from Cells A, B, C and D

9.5 Soil Vapour O_2/CO_2 Concentrations

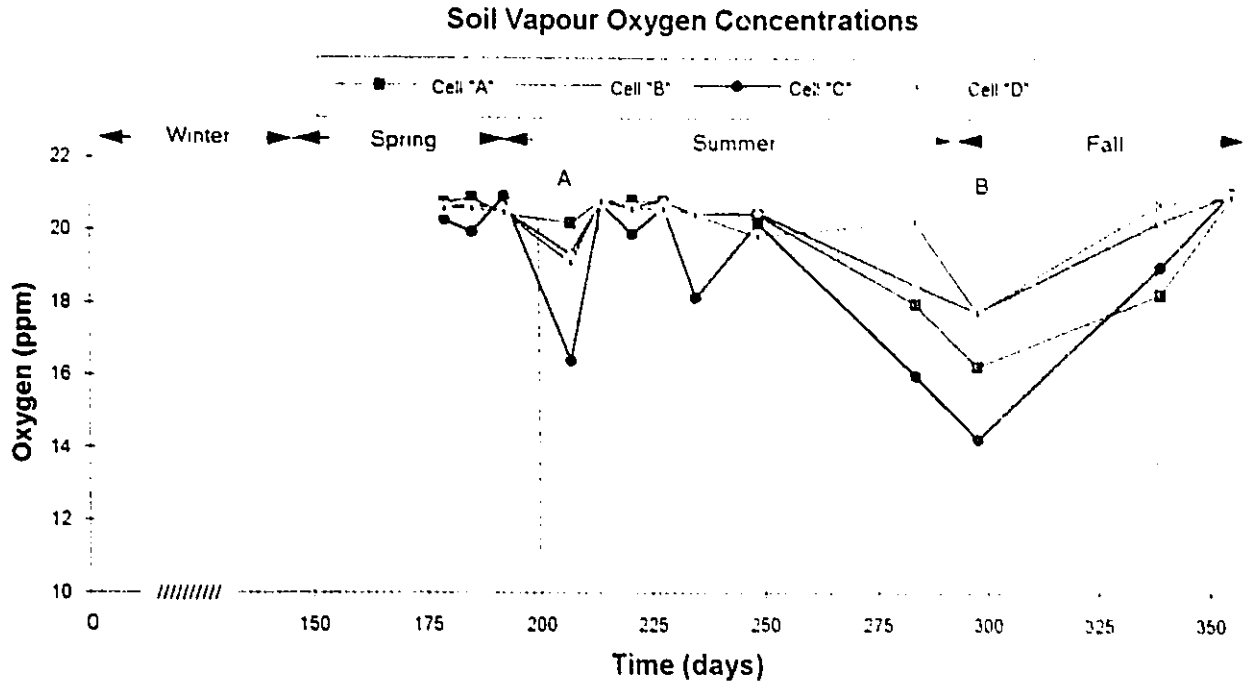
Insitu soil vapour O_2 and CO_2 concentrations were measured in each of the four cells on a bi-weekly basis during the six month monitoring program. The results are presented in Appendix "C" (Tables C10, C11) and in Figure 12.

As previously indicated, the mineralization of petroleum hydrocarbons can be expressed in the following form:

$$\text{Hydrocarbon} + O_2 \Rightarrow H_2O + CO_2 + \text{biomass}$$

In this reaction the mineralization of hydrocarbons results in oxygen (O_2) being consumed and CO_2 being produced.

Based on the measured O_2 concentrations, aerobic conditions were maintained within each of the four cells throughout the six month monitoring program. Of interest to note, however, is the O_2/CO_2 trend between days 200 and 210 (event A - Figure 12) when the soil vapour extraction system was temporarily shut down. During this shutdown period, the soil oxygen concentrations in all four cells decreased and the carbon dioxide concentrations increased. Once the soil vapour extraction system was



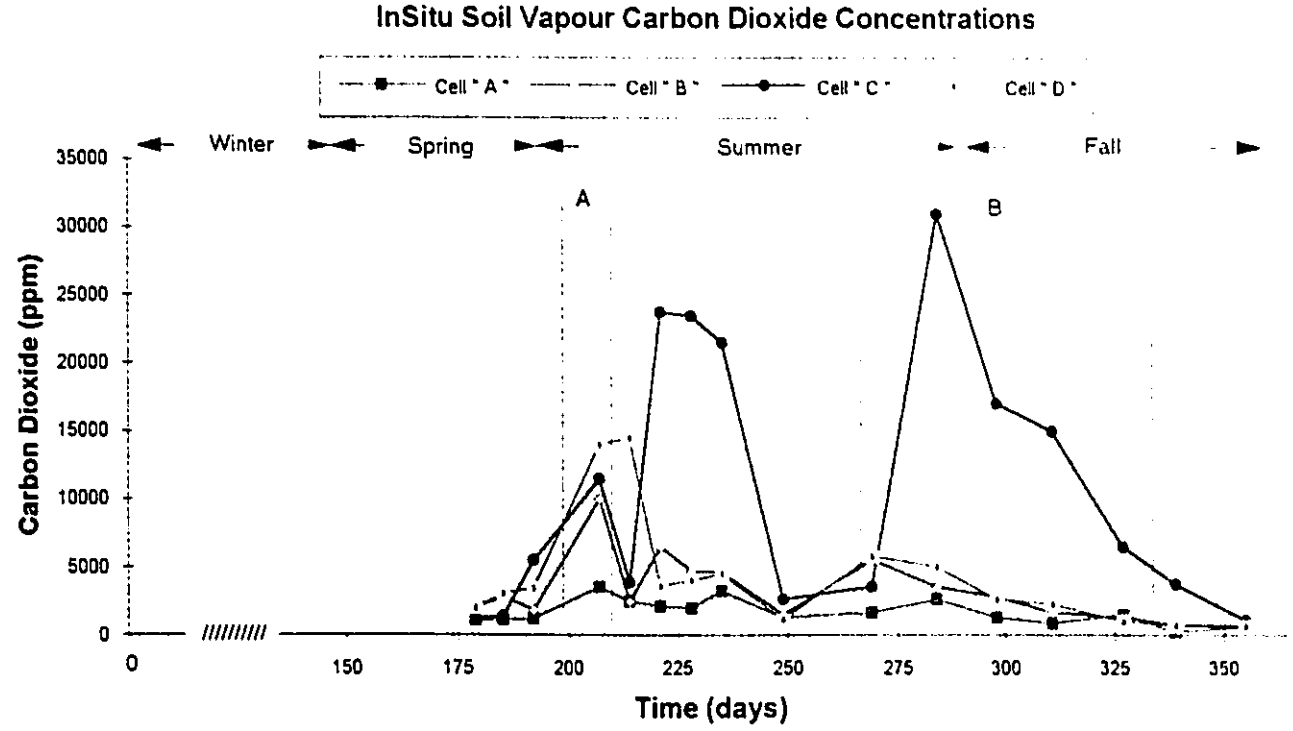


Figure 12 Comparison of Insitu Soil Vapour Oxygen and Carbon Dioxide Concentrations in Cells A, B, C and D

reactivated (day 210), the oxygen concentrations increased to their pre-shutdown levels and a corresponding decrease in CO_2 concentrations was observed for the most part. This O_2-CO_2 trend is what one would expect to see in a situation where aerobic biodegradation of the hydrocarbon contaminants was occurring.

The reason for the significant increases in CO_2 concentration in Cell C between days 220 - 240 and 270 - 320 is unknown. It was originally considered to reflect elevated biological activity in this one cell; however, the microbial results obtained do not support this conjecture.

The significant decrease in O_2 concentrations and lack of corresponding increase in CO_2 concentrations between days 270 and 339 (Event B - Figure 12) can be attributed in part to nitrification, or more specifically the oxidation of ammonia to nitrate. As previously indicated, Ammonium Hydroxide (NH_4OH) was added to Cells A, C and D on day 269 in an attempt to increase soil nitrate concentrations. It is well documented that the nitrification of ammonia can result in an increased oxygen demand and may lead to the depletion of oxygen (Brady, 1974; McRae, 1988; Vilenskii, 1960; EPA, 1993; Nyer, 1992). The oxidation of ammonia can be depicted as follows:

$$NH_4^+ + 2O_2 \rightarrow 2H^+ + NO_3^- + H_2O$$

Soil nitrate concentrations in Cells A, C and D peaked during this period with concentrations in the range of 10 to 20 mg/kg - well above the minimum recommended concentration of 5 mg/kg (Table C13). Although no NH_4OH was added to Cell B, it is interesting to note that soil nitrate concentrations in Cell B also peaked during this period at the relatively low concentration of 1.5 mg/L. No effect on pH could be detected (Table C16, Appendix "C") because lime was added to the soil at the same time as the NH_4OH.

As expected from the above reaction, soil nitrate showed a very strong negative correlation with soil vapour O_2 concentrations. Correlation coefficients of -0.98, -0.87, -0.99 and -0.95 were calculated for Cells A, B, C and D, respectively. The observed correlations were found to be statistically significant with only a 5% probability that the observed correlations arise merely as a result of chance.

9.6 Soil N, P, K Concentrations and pH

On August 23, 1993 (day 269) an effort was made to raise the soil nitrate levels in order to attain the minimum recommended concentration of 5 ppm.

Aqueous ammonium hydroxide ($NH_4.OH$) was added to the sumps of Cells A, C and D. Urea and lime were also added to the sumps to assist in increasing the nitrate concentration and the buffering capacity of the soil. Concentrations of soil nitrate, phosphorus, potassium and pH levels are presented in Appendix "C" (Tables C13 - C16).

A significant increase in the soil nitrate concentrations in Cells A, C and D was observed after the addition of NH_4OH compared to that of Cell B which did not receive any amendments. No significant trend in either phosphorus potassium or soil pH was observed in any of the three cells prior to or after this event.

Soil phosphorous concentrations remained above the minimum recommended level of 1 ppm in all four cells throughout the study period.

On October 20, 1993 (day 320) the sump pumps which recirculated nutrient enriched waters into Cells A, C and D were shut down due to freezing conditions. Termination of the supply of nutrients resulted in a significant decrease in soil nitrate concentrations and appears to have had a slight effect on the soil phosphorus and potassium concentrations (Appendix "C", Tables C13 - C15).

Throughout the monitoring program, soil pH in all four cells varied from 5 to 7.5 and showed no discernable trend irrespective of what was done during the monitoring program (Appendix "C", Table C16).

9.7 Soil Moisture Content

Soil moisture content was determined gravimetrically in the laboratory. The analytical results obtained are presented in Figure 13 and in Table C22 of Appendix "C".

The gravimetric soil moisture content within all four cells was found to have a positive correlation with the daily volume of water added to the cells (Table C21). Increases in soil moisture content occurred when the sump pumps were initially turned on (Event A - day 179), when the timer was programed to activate the pumps for 15 minutes every hour (Event B - day 230), and when the timer was adjusted to 30 minutes every hour on day 262 (Event C - Figure 13). Soil moisture content decreased when the sump pumps were temporarily shut down due to a power failure (Event D - days 284 to 298) and after the final shut down of the water/nutrient supply system on October 20, 1993 (Event E - day 327).

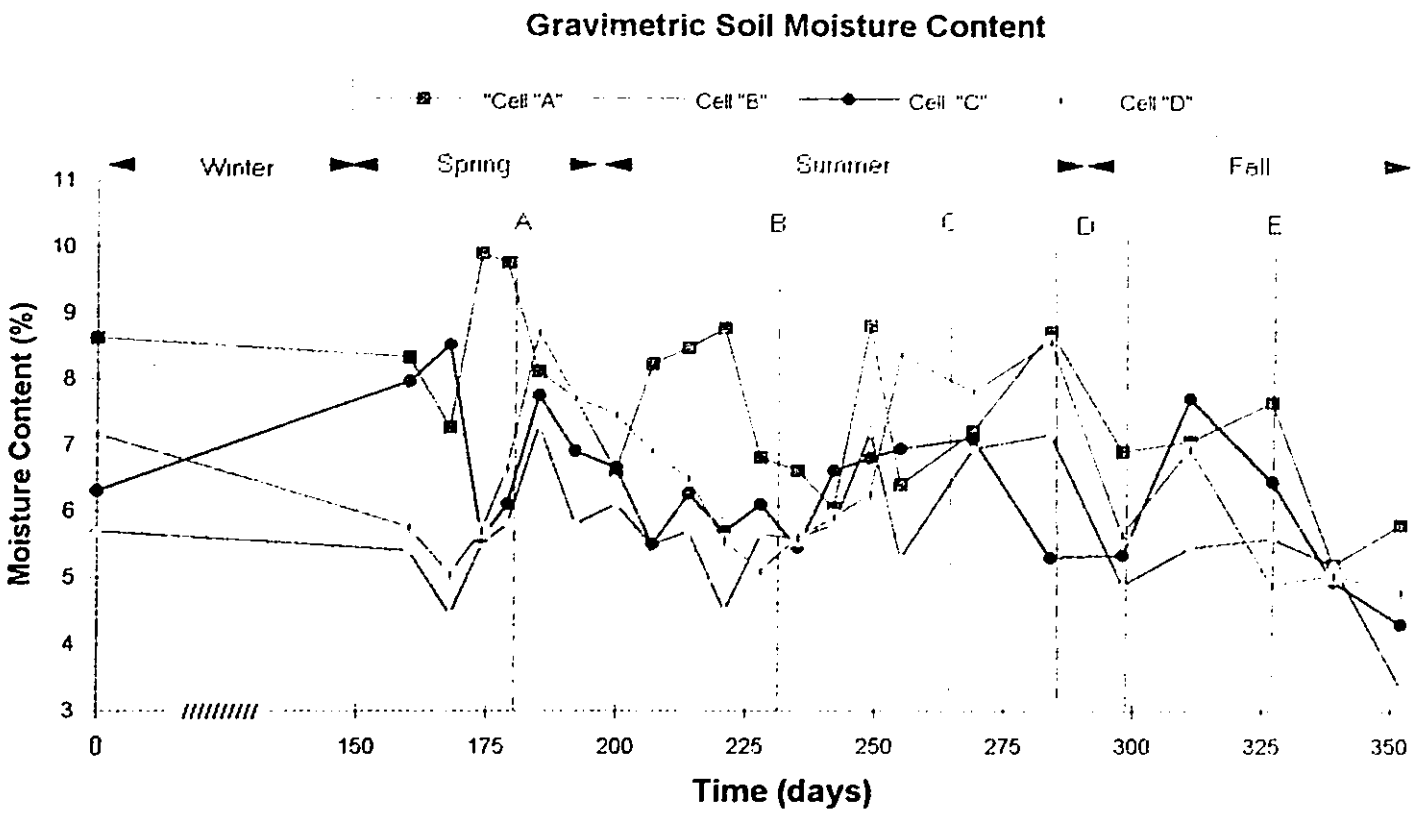


Figure 13 Relationship of Soil Moisture Content with Events A, B, C, D and E

This positive correlation supports the effectiveness of the nutrient delivery tubing, which incorporated pressure compensating valves behind each perforation, to promote uniform application of liquid over the entire cell area.

9.8 Repeated Versus Random Sampling

Two sampling approaches were used; a **repeated** sampling approach in which samples and measurements were always obtained from the same locations, and a **random** sampling approach in which samples and measurements were obtained from new, randomly selected locations each time. Cell A was chosen at random for the repeated sampling approach, whereas the other cells were sampled at random. The initial sampling locations within Cell A were selected at random.

The difference between the two approaches is that repeated sampling produces numbers that are correlated with one another in time, whereas random sampling produces independent measurements. The practical implication is that trends obtained from repeated sampling appear smoother but may have widely different shapes from one location to another (i.e. the variability is introduced in the trend rather than in the points), whereas random sampling will produce large variability in the points but not in the trends.

The only discernable differences which can be attributed to the method of sampling were observed in soil temperature, TPH, potassium, and pH measurements (Figures 5 and 6; Tables C15 and C16 - Appendix C, respectively).

Soil temperatures in Cell A were consistently lower than the remaining three cells by 3 - 5°C (Figure 5). The lower temperature measurements obtained in Cell A are considered to reflect the fact that temperature measurements were obtained from the same location throughout the monitoring program. The measurements obtained at these locations would be sensitive to cell orientation, time of day etc.

Soil TPH concentrations in Cell A showed a smoother curve (i.e. a more uniform rate of degradation) than the other cells (Figure 6). This trend is considered to be a result of the repeated sampling location which would cause a smaller soil TPH variability and hence the appearance of a smoother curve.

Similarly, soil potassium and pH levels were found to be consistently lower in Cell A than the others. This trend is may also be the result of the repetitive sampling strategy.

Although it is apparent that repetitive sampling can result in less variability in the analytical results obtained, (i.e. smoother curves), the price to pay for the better precision is a decreased accuracy (i.e. non-representativeness). In addition, repeated measurements produce a correlation between adjacent points and as a result, statistical comparisons are more difficult.

10. DISCUSSION

10.1 Level of Remediation Attained

One of the primary objectives of the project was to use the field and lab results to optimize conditions within the bioreactors to enhance biodegradation of the petroleum contaminants. This was undertaken by maintaining aerobic conditions and optimum moisture levels within each of the soil bioreactors. In addition, nutrient amendments were added to three of the four bioreactors. The importance of nutrient amendments will be discussed in Section 10.3.

The soil in all four bioreactors was found to have TPH concentrations below the most stringent federal and/or provincial (Alberta or Ontario) criteria of 40 ppm as demonstrated by the mean soil TPH concentrations obtained during the month of November 1993.

The TPH degradation is best illustrated by comparing the laboratory Gas Chromatograms of soil samples collected from the four cells at the beginning (May 6, 1993) and at the end (November 18, 1993) of the operating period (Figure 14). The peaks between 10 and 30 minutes in the May 6 soil samples identify the petroleum contamination as diesel fuel. The

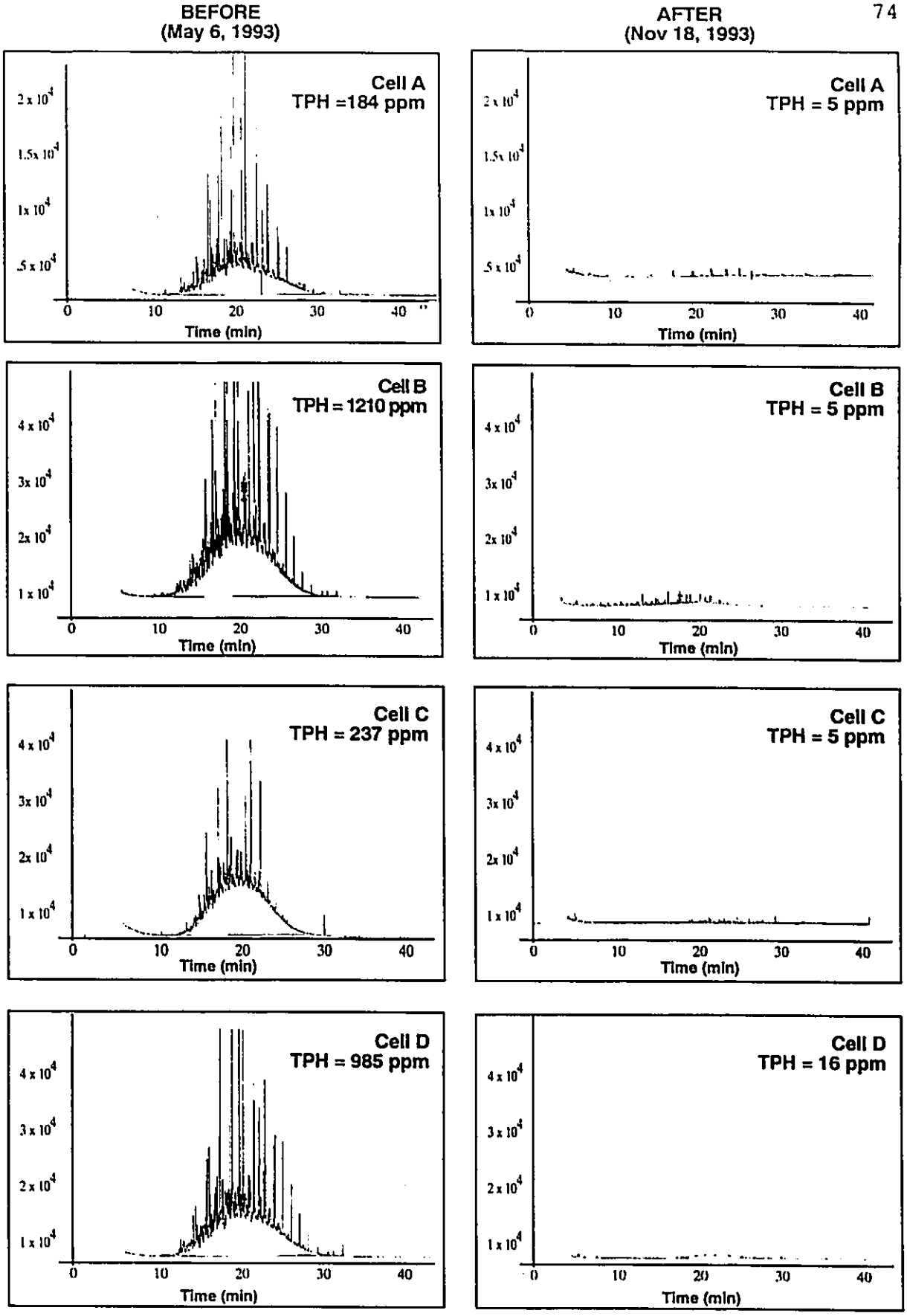


Figure 14. COMPARISON OF GAS CHROMATOGRAMS OF PETROLEUM CONTAMINATED SOIL SAMPLES

concentration of TPH within the soil is calculated by integrating the chromatogram (area under the curve).

By comparison, the chromatograms obtained from the soil samples collected on November 18, 1993 show the absence of peaks and hence visually depicts the amount of petroleum degradation that occurred over the six month operating program.

The average TPH degradation was approximately 97% or 5.6 kilogram per day over the six month operating period. Although degradation rates of this magnitude have been reported in the literature (Dupont et al, 1991), this rate is unique in that it essentially reflects the rate of biological degradation with very little volatilization contribution. This will be discussed in more detail in Section 10.2.

Based on the level of remediation attained, the soil in all four bioreactors can now be considered decontaminated and, as a result, the soil has unrestricted reuse potential.

10.2 Biodegradation Versus Volatilization

Biological degradation of petroleum contaminants is difficult and very expensive to prove. Therefore efforts to obtain good scientific data to support biological degradation claims are not generally undertaken in soil remediation projects (Madsen, 1991). Recognizing this, one of the prime objectives of this project was to attempt to quantify the amount of degradation that could be attributed to biodegradation and how much was due to volatilization. This was particularly important in this project as the primary objective was to promote biological degradation of the contaminants as opposed to volatilizing them and hence transferring the contaminants from one medium to another (i.e. desorption from soil and adsorption to carbon).

10.2.1 TPH Mass Balance Calculations

The amount of biodegradation that occurred during the operation of the project was determined by performing TPH mass balance calculations. In doing these calculations, the initial mass of TPH was taken as the mean of the November 27, 1992 and May 6, 1993 soil TPH concentrations. Similarly, the final mass of TPH remaining in the soil at the end of the operating period was taken as the mean of the November 1 and November 18, 1993 TPH concentrations.

The total initial mass of TPH in the contaminated soil was approximately 1096 kg while the remaining mass at the end of the six month operating period was approximately 30 kg; a reduction of approximately 1066 kg (97.4%).

Due to the self-contained nature of the bioreactor system, the reduction in TPH mass within the soil could result from one of the following three processes: a) leaching of hydrocarbons from possible biosolubilization reactions, b) volatilization of the hydrocarbon components with relatively high vapour pressures, and c) biomineralization of the hydrocarbons into carbon dioxide and water (biodegradation). A summary of the calculated reduction in TPH mass from each of these three processes is presented in Table 4 and will be discussed hereafter.

a) Mass Loss Due to Leaching

The mass of TPH that was leached from the soil was determined through analyses of water samples collected at the base of each of the four cells at the end of the operating period.

Table 4

Summary of TPH Mass Balance Calculations

Method 1 - Mass detected in soil vapour extracted from cells.

Bioreactor Cell	Initial Mass[1] of TPH	Remaining TPH Mass[2]	Leached TPH Mass[3]	Total Mass of TPH Degraded		Loss Due to Volatilization (Method 1)		Loss Due to Biodegradation	
	kg	kg	kg	kg	%	kg	%	kg	%
A	110	3	0.002	107	97.3	0.3	0.3	106.7	99.7
B	515	16	0.002	499	96.7	1.2	0.2	497.8	99.8
C	53	3	0.002	50	94.3	1.0	2.0	49.0	98.1
D	418	8	0.002	410	98.1	2.2	0.5	409.5	99.5
Total	1096	30	0.008	1066	97.4	4.7	0.4	1061	99.5

Method 2 - Mass adsorbed to granular activated carbon.

Bioreactor Cell	Initial Mass[1] of TPH	Remaining TPH Mass[2]	Leached TPH Mass[3]	Total Mass of TPH Degraded		Loss Due to Volatilization (Method 2)		Loss Due to Biodegradation	
	kg	kg	kg	kg	%	kg	%	kg	%
A, B, C, D	1096	30	0.008	1066	97.4	0.9	0.1	1065	99.9

Note: 1 Initial mass of TPH calculated from the mean TPH concentrations of Nov. 27/92 and May 6/93 sampling events.
2 Remaining mass of TPH calculated from the mean TPH concentrations of Nov. 1/93 and Nov. 18/93 sampling events.
3 Leached mass of TPH calculated from sump water at end of monitoring program.

Although detectable TPH concentrations were measured in the water during the operation of the bioreactors, no detectable concentrations were measured in any of the four cells at the end of the project. The dissolved TPH concentrations in tne leachate may be a result of microbial induced surfactants which solubilized adsorbed hydrocarbons at the onset of biostimulation. This phenomenon is often observed in bioremediation projects (King et al, 1992). The TPH concentrations detected in the water during the operating period are believed to have been degraded within the soil as the water was recirculated through the bioreactor.

For calculation purposes, TPH concentrations below the detection limit were assumed to be one half of the detection limit. The corresponding mass of TPH that may have been leached from the soil was 0.002 kg for each cell or 0.008 kg in total (Table 4).

b) Mass Loss Due to Volatilization

The amount of volatilization that occurred over the six month monitoring period was calculated using two methods.

The first method (Method 1) involved multiplying the petroleum hydrocarbon concentration measured within the soil vapour (bi-weekly) by the average volume of vapours extracted from that particular cell over the same period. The mass of the TPH that was volatilized from each cell was then calculated by summing the bi-weekly volatilization losses over the 6 month operating period. The mass loss due to volatilization using Method 1 was approximately 4.7 kg or 0.5% of the total TPH mass degraded (Table 4).

The second method (Method 2) used to estimate the amount of volatilization was to simply collect representative carbon samples from the central vapour treatment facility and have them analyzed. The TPH concentration was then multiplied by the total mass of carbon to obtain the total mass of TPH that was adsorbed to the carbon. The calculated TPH mass adsorbed to the carbon is considered to represent the mass which was volatilized from the soil. Using method 2, the calculated mass of TPH that may have volatilized from the soil was approximately 0.9 kg or 0.1% of the total degraded TPH mass.

The relatively small discrepancy in the calculated volatilized mass using these two methods is considered to reflect the assumptions that were made. In Method 1 it was assumed that the bi-weekly TPH concentrations measured in the soil vapour and the volume of air extracted from the individual cells were constant throughout the two week period. The assumption made in Method 2 was that the TPH concentrations measured in the carbon samples obtained from the central treatment facility were representative of the entire mass of carbon.

Allowing for some measure of error in these calculations due to the above noted assumptions, we still end up with extremely low levels of volatilization. This is not very surprising when one considers the fact that no detectable BTEX concentrations (aromatic hydrocarbons) were measured in any of the soil samples upon completion of the bioreactor in November of 1992 or upon start-up in May of 1993.

c) Mass Loss Due to Biodegradation

The reduction in TPH mass that can be attributed to biological degradation (biodegradation) processes was calculated by subtracting the sum of TPH mass loss due to leaching and volatilization from the total reduction in TPH mass (previously calculated to be 1066 kg - Section 10.2.1). The reduction in TPH mass that is attributable to biodegradation processes was approximately 99% or 5.6 kg/day over the operating period of the bioreactors (Table 4).

10.2.2 Evidence for Biodegradation

The evidence supporting biological mineralization (biodegradation) include the following:

a) Lab Results

i) The noticeable increase in soil bacteria with the genetic potential to degrade hydrocarbons over the operating period (DNA hybridization tests);

ii) laboratory mineralization tests using ^{14}C-labelled hydrocarbons showing that the hydrocarbon degrading bacteria were active and successful at mineralizing the petroleum products;

iii) the strong negative correlation (r = -0.33 to -0.95) between the initial rates of Hexadecane mineralization and soil TPH concentrations; and

vi) the overall decrease in soil TPH concentrations.

b) Field Results

i) The soil vapour O_2/CO_2 relationship observed in the field, which indicated that oxygen was being consumed and carbon dioxide was being produced. This is the expected relationship if hydrocarbons were, in fact, being biomineralized into carbon dioxide and water.

c) Mass Balance Calculations

i) Mass balance calculations showed a significant reduction in soil TPH and an insignificant reduction in TPH mass from volatilization and/or leaching processes.

Factors contributing to the significant amount of biological degradation that occurred during this project include:

a) the absence of aromatic hydrocarbons within the soil which would have had a tendency to volatilize,

b) the sandy nature of the contaminated geologic medium (relatively high hydraulic conductivity and air permeability), and

c) the design of the bioreactor system which permitted optimum moisture and oxygen contents to be easily maintained within the bioreactors.

10.3 Temperature Versus Nutrients

Another objective of the project was to assess the effects of temperature and nutrients on the rate of petroleum biodegradation. The importance of oxygen was not examined since it has been extensively documented in the literature that oxygen is the most important parameter affecting biodegradation. There appears to be less certainty with regards to the importance of temperature and nutrients.

The attempt to generate a temperature difference between the temperature control cell (Cell D) and the remaining three cells was not successful due to the relatively high air exchange rate (3-5 air-filled pore volumes/day). Nevertheless, a significant correlation between soil temperatures and initial Toluene mineralization rates was observed (Appendix "D"). This correlation

in itself is not proof of causation since it could be argued that bacterial activity can generate heat. However, the soil temperature was clearly controlled by the ambient air temperatures (Table C12). Therefore, the correlation between the initial Toluene mineralization rates and temperature indicates that temperature had an effect on the rate of petroleum biodegradation (mineralization). Similarly, the measured probe positive bacteria counts were found to have a signficant positive correlation with soil temperature for temperatures up to approximately 10°C.

Although the addition of nutrients to three of the four cells was successful in significantly increasing and maintaining the soil nitrate concentration in excess of the minimum recommended 5 ppm concentration throughout the last three months of the study period, no significant differences in the rate of TPH oegradation, mineralization activity, and/or population of probe-positive hydrocarbon degrading bacteria were noted. The nutrient treatment cell (Cell B) was supplied with the same amount of water as the remaining three cells. The water which was circulated into the cells was lake water (i.e. non-chlorinated) with a natural nitrate concentration of approximately 2 - 3 mg/L and trace phosphorus and potassium concentrations. As a result, low but none the less detectable nitrate concentrations (<1.0 mg/kg)

were measured in the soil and sump waters of Cell B throughout most of the monitoring program.

It is likely that the initial addition of nutrients to the soil in Cell B coupled with the minor amounts of nutrients inadvertently supplied through the addition of lake water, provided sufficient nutrients for biodegradation of the petroleum compounds over the six month operating period.

It is apparent from these results that commonly recommended nutrient concentrations - particularly C:N ratios of 60:1 and/or minimum nitrogen levels of 5 mg/kg were not critical in enhancing biological degradation of the petroleum hydrocarbons at this site. Maintaining proper aeration and moisture contents seemed to be more important than amending the nutrient supply (after the initial nutrient application). Temperature, as mentioned above, appears to have been a more important factor in the biodegradation process.

These findings are consistent with recent work in similar bioremediation projects (Miller and Hinchee, 1990; Miller et al, 1990).

10.4 Cost-Effectiveness

As in any remediation project, the cost-effectiveness of the method needs to be scrutinized. The costs to design, construct and monitor this particular bioremediation facility were estimated to be in the range of $70 to $90 per tonne of treated soil. These costs however, can be reduced to $20 to 40 per tonne range with continued re-use of the facility and a less stringent monitoring/sampling program.

The extensive monitoring/sampling program that was performed throughout the project was purposely undertaken to assess the rate at which soil and bacteriological conditions changed within the bioreactors during operation. Based on the results, it is recommended that future monitoring programs for similar bioremediation projects should include, as a minimum: weekly visits during the first month of operation, bi-weekly visits for the next one or two months, and monthly thereafter. This sampling frequency would permit adequate monitoring of the biological degradation process occurring within the cells and would provide sufficient information with respect to soil conditions and the need for adjustments. With the implementation of such a monitoring/sampling program, it is anticipated that future bioreactor treatment costs for similar soils and contaminants could be reduced to the $50 to $70 per tonne range for initial treatment.

One important consideration in this treatment cost exercise is the fact that this facility has the potential to be reused upon successful remediation of the first batch of contaminated soil. It is DND's intention to reuse their facility at CFB Petawawa two or three more times to treat additional petroleum contaminated soil generated on their property. By reusing this facility, DND can potentially reduce the ultimate soil treatment costs to the $20 to $40 per tonne range.

Other common methods of ex-situ soil remediation which would have been available for this site include low temperature thermal desorption - which would be comparable in cost, and landfilling of the petroleum contaminated soil in a municipal landfill site. Currently, municipal landfill tipping fees for contaminated soil range from $20 to $150 per tonne. It should be noted however, that landfilling petroleum contaminated soils is not generally considered a soil remediation method as it serves only to transfer the contaminated soil from one location to another.

In summary, bioremediation undertaken in the form of an above ground bioreactor can be safe, cost effective, and based on the high level of remediation attained, can be successfully undertaken during the summer months in a typical Canadian climate.

11. SUMMARY AND CONCLUSIONS

An engineered, above ground, bioreactor system was designed and constructed to bioremediate approximately 1,400 m^3 of petroleum contaminated soil at CFB Petawawa, Petawawa, Ontario. The bioremediation facility was constructed in the fall of 1992 and was operated for a six month period beginning in May 1993 to November 1993. The facility was closely monitored over the six month period. Field and laboratory results were used to optimize conditions within the bioreactors to enhance natural biological degradation of the petroleum contaminants within the soil. The following is a summary of the main findings and conclusions:

1. The white covering placed over Cell D to inhibit soil heating had no effect, as soil temperatures in Cell D were not significantly different than those of Cells B or C.

2. A significant decrease in the mean soil TPH concentrations was observed in all four bioreactors. The total reduction in soil TPH mass was approximately 1066 kg or 97% of the initial TPH mass.

   The mean TPH concentration during the final month of operation was well below the ultimate goal of 40 ppm in all four cells. As a result, the soils can

now be considered decontaminated and will have unrestricted reuse potential.

3. Total Viable Bacteria population counts were found to be greater than an order of magnitude higher on the petroleum rich (DYG) medium compared with those enumerated on the nutrient rich (YTS) medium. These results indicate that the bacteria were either quite resistant to any potential toxic effects from the gasoline and diesel products; and/or the bacteria were able to readily metabolize the petroleum products. Either of these potential traits are desirable in a bioremediation project.

4. A relatively large population of hydrocarbon-degrading bacteria, representing approximately 10% of the Total Viable Bacteriological population, was detected in the native contaminated soil. Both xylE and alkB bacteria counts were found to be strong functions of temperature up to approximately 10°C, but not between 10°C and 26°C.

No significant differences in the population of hydrocarbon degrading bacteria were observed between the nutrient treatment cell (Cell B) and the remaining three cells.

5. The ability of the hydrocarbon-degrading bacteria to mineralize the petroleum contaminants was monitored using ^{14}C-labelled Toluene and Hexadecane. The initial mineralization rates of Toluene had a significant positive correlation with soil temperatures. The initial mineralization rates of Hexadecane were found to have a strong negative correlation with soil TPH concentrations, showing high initial Hexadecane mineralization rates corresponding to low soil TPH concentrations.

6. Although the soil was contaminated initially with gasoline and diesel, no detectable aromatic hydrocarbons, commonly associated with gasoline (BTEX), were measured in the soil upon completion of the bioremediation facility. These results suggest that the gasoline component of the contamination was volatilized and/or biodegraded during the excavation and construction of the facility. Efforts such as covering the soil with vapour barriers were undertaken to reduce the potential for volatilization during construction.

7. Soil vapour TPH concentrations in all four cells decreased from approximately 200 ppb to less than 0.3 ppb within the first 100 days of operation of the soil vapour extraction system. Due to the absence of detectable aromatic hydrocarbons in the soil, the soil vapour TPH

concentrations probably represent the accumulation of minor aromatic hydrocarbons and/or semi-volatiles compounds in the diesel product.

8. A significant decrease in soil vapour O_2 concentrations and a corresponding increase in CO_2 concentrations were observed during temporary shutdown of the soil vapour extraction system. This trend in O_2/CO_2 concentrations is evidence that biological degradation of the petroleum contaminants into CO_2 and H_2O was occurring within the bioreactors.

   A strong positive correlation was observed between soil temperatures and ambient air temperatures. Correlation coefficients of 0.86, 0.89, 0.87 and 0.90 were calculated for Cells A, B, C and D, respectively. The strong correlation and absence of any lag time suggests that the rate of soil vapour extraction (3-5 pore volumes/day) had a significant effect on controlling soil temperatures.

9. The addition of Ammonium Hydroxide and Urea to Cells A, C and D was successful in significantly increasing the nitrate concentrations of the soil above the minimum recommended level of 5 ppm.

There was very little effect on the soil Phosphorus, Potassium and pH levels throughout the monitoring program.

10. The nutrient delivery system, which was designed to promote uniform application of liquid/nutrients over the entire cell area, was found to be effective.

11. Repeated sampling approach as opposed to a random sampling approach can result in less variability in the analytical results obtained; however, it may not provide the user with representative parameter measurements.

12. Based on TPH mass balance calculations, in excess of 97% of the original TPH mass was degraded over the operating period. Of this amount, approximately 99% can be attributed to biological degradation while the remaining 1% was due to volatilization.

13. Evidence for biological degradation include the documented increase in hydrocarbon-degrading bacteria within the soil; laboratory mineralization tests which indicated that the hydrocarbon-degrading bacteria were active; the significant negative correlation between the population of bacteria with

genetic potential to degrade diesel compounds and soil TPH concentrations; the significant decrease in soil TPH concentrations; the observed soil vapour O_2/CO_2 relationship; and the results of TPH mass balance calculations.

14. Although no significant temperature differences between the four bioreactors were observed, the analytical and microbiological results indicate that strong correlations exists between soil temperatures and the initial Toluene mineralization rates; and the initial Hexadecane mineralization rates and soil TPH degradation, suggests that temperature had an important effect on the rate of petroleum biodegradation.

Conversely, in spite of a significant nutrient imbalance (mainly nitrate) created between the treatment cells, no effect on microbial activity or TPH degradation was apparent. It is likely that the small quantities of nutrients originally supplied during cell construction and the trace quantities present within the lake water circulated through our nutrient treatment cell (Cell B) were sufficient to prevent nutrient concentrations from becoming a limiting factor in the rate of petroleum biodegradation. The absence of a significant effect between cells indicates that the recommended C:N ratios of 60:1 and

maintaining soil nitrate in excess of 5 ppm seem to be overly conservative for this type of soil and contaminant.

Based on these results, temperature seemed to be the more important of the two parameters affecting petroleum biodegradation at this site.

15. The treatment cost was estimated at $70 - $90 per tonne with the potential of reducing these costs to $20 - $40 per tonne if the system is re-used and/or if monitoring is reduced.

16. Enhanced bioremediation in the form of above ground bioreactors was found to be a safe, cost effective and environmentally friendly method of remediating petroleum contaminated soil. The system successfully enhanced the biodegradation of the diesel contaminants within the soil within the six month monitoring program which spanned a typical Canadian summer. The remaining petroleum concentrations within the soil are below the most stringent federal and/or provincial remediation criteria of 40 ppm.

12. FUTURE RESEARCH EMPHASIS

Based on the favourable results, it is hoped that the information obtained from this project will be used to stimulate additional interest in bioremediation and to increase the efficiency of biodegradation processes. Future bioreactors used to treat petroleum contaminated soils should focus on enhancing soil temperature in a cost-effective manner. In addition, this project has demonstrated the importance of minimizing handling of the contaminated soil to reduce volatilization especially when dealing with gasoline contamination. If possible, above ground bioremediation facilities should be constructed during late fall or winter conditions when biological activity and the potential of volatilization are at a minimum.

This project has demonstrated that sandy soils contaminated with diesel can be readily bioremediated in a relatively short time frame using an above ground bioreactor design. Future research should focus on obtaining good scientific data under different soil conditions (i.e. silty clay soils) and/or different contaminants. In addition, the effect of soil moisture content on the rate of hydrocarbon degradation should be examined. The information obtained from studying above ground bioreactors will greatly enhance the successful utilization of bioremediation principles to insitu applications where excavation of the contaminated material is not possible or desired.

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APPENDICES

APPENDIX "A" -	EXAMPLES OF FIELD DATA SHEETS
APPENDIX "B" -	ANALYTICAL METHODS AND QUALITY CONTROL PROCEDURES
APPENDIX "C" -	FIELD AND ANALYTICAL RESULTS
APPENDIX "D" -	SCATTER PLOTS AND LEVEL OF SIGNIFICANCE OF CORRELATIONS AND SLOPES

APPENDIX "A"

EXAMPLES OF FIELD DATA SHEETS

- Soil Sampling Data Sheet
- Temperature Measurements
- Vapour Extraction (vacuum, velocity)
- Vapour Monitoring (O_2/CO_2)
- Water Meter Readings

OLIVER, MANGIONE, McCALLA & ASSOCIATES LIMITED

CONSULTING ENGINEERS, HYDROGEOLOGISTS & PLANNERS

SOIL SAMPLING DATA SHEET
BIOREMEDIATION FACILITY - CFB PETAWAWA

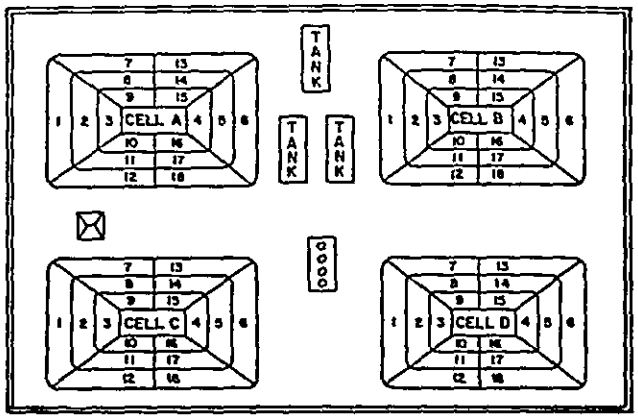
Job: 92-8731

DATE: June 7, 1993

WEATHER: Temp. (°C) 20 °C

Conditions: Sunny with some cloudy periods

COMMENTS: windy at times



CellA		Sector	Depth(m)	Observations
Composite A1	1	18	2.0	(LB) DB BLK SO MO STO (MT) SAT DRY
	2	3	1.5	LB DB (BLK) SO MO STO (MT) SAT DRY
	3	2	0.5	(LB) DB BLK (SO) MO STO (MT) SAT DRY
Composite A2	4	6	2.0	(LB) DB BLK SO MO STO MT (SAT) DRY
	5	7	1.5	(LB) DB BLK (SO) MO STO (MT) SAT DRY
	6	8	1.5	LB DB (BLK) SO MO STO (MT) SAT DRY

Cell B		Sector	Depth(m)	Observations
Composite B1	1	14	1.0	(LB) DB BLK SO MO STO MT SAT (DRY)
	2	8	1.0	(LB) DB BLK SO MO STO (MT) SAT DRY
	3	13	1.0	(LB) DB BLK (SO) MO STO (MT) SAT DRY
Composite B2	4	3	2.0	LB (DB) BLK SO MO STO (MT) SAT (DRY)
	5	10	1.5	LB (DB) BLK (SO) MO STO (MT) SAT DRY
	6	15	2.0	(LB) DB (BLK) SO MO STO (MT) SAT (DRY)

Cell C		Sector	Depth(m)	Observations
Composite C1	1	6	0.5	(LB) DB BLK SO MO STO MT SAT (DRY)
	2	18	1.5	(LB) DB BLK SO MO STO (MT) SAT DRY
	3	3	1.0	LB DB (BLK) SO MO STO MT (SAT) DRY
Composite C2	4	11	0.5	(LB) DB BLK (SO) MO STO MT SAT (DRY)
	5	3	1.0	LB DB (BLK) (SO) MO STO (MT) SAT DRY
	6	5	1.0	(LB) DB BLK SO (MO) STO MT SAT (DRY)

Cell D		Sector	Depth(m)	Observations
Composite D1	1	2	2.0	LB (DB) BLK (SO) MO STO (MT) SAT DRY
	2	15	0.5	(LB) DB BLK (SO) MO STO (MT) SAT DRY
	3	1	1.5	(LB) DB BLK SO MO STO MT SAT (DRY)
Composite D2	4	13	2.0	(LB) DB BLK (SO) MO STO (MT) SAT DRY
	5	9	1.5	LB DB (BLK) SO MO STO (MT) SAT DRY
	6	8	1.5	LB (DB) BLK (SO) MO STO (MT) SAT DRY

Comment Legend

COLOUR:	LB	light brown	ODOUR:	SO	slight odour	MOISTURE CONTENT:	MT	moist
	DB	dark brown		MO	moderate odour		SAT	saturated
	BLK	black		STO	strong odour		DRY	dry

TEMPERATURE MEASUREMENTS
BIOREMEDIATION FACILITY - CFB PETAWAWA

Job: 92-8731

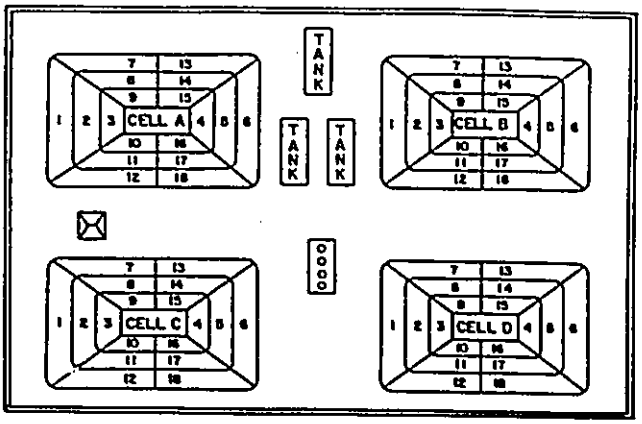
DATE: June 7, 1993

WEATHER CONDITIONS:

Temperature: 20 - 26 (°C)

Sunny, (Partly Sunny) Cloudy, Showers, Rain

COMMENTS: Windy



Cell A	Sector	Depth(m)	Temperature (°C)
	18	2.0	11
	3	1.0	15
	7	1.5	13
	6	2.0	13

Cell B	Sector	Depth(m)	Temperature (°C)
	14	1.0	18
	8	1.0	18
	13	1.0	18
	3	2.0	17

Cell C	Sector	Depth(m)	Temperature (°C)
	6	0.5	14
	18	1.5	13
	3	1.0	19
	11	0.5	19

Cell D	Sector	Depth(m)	Temperature (°C)
	2	2	12
	15	0.5	18
	1	1.5	12
	13	2.0	17

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VAPOUR EXTRACTION SYSTEM
BIOREMEDIATION FACILITY - CFB PETAWAWA

Job: 92-8731

DATE	CELL A									CELL B									Vacuum Pump	
	1 FRONT		2 FRONT		3 BACK		4 BACK		Total Cell Vel.	1 FRONT		2 FRONT		3 BACK		4 BACK		Total Cell Vel.		
	VAC	VEL	VAC	VEL	VAC	VEL	VAC	VEL		VAC	VEL	VAC	VEL	VAC	VEL	VAC	VEL		VAC	VEL
June 7									141									120		
June 8	2	59	8	44	2	85	6	132	156	7	37	8	58	6	58	4	63	440	98	860
June 9	2	47	8	43	4	84	6	31	135	8	46	9	58	8	31	6	40	133	96	1180

CELL C									CELL D									Vapour Treatment PID Readings (ppmv)		
1 FRONT		2 FRONT		3 BACK		4 BACK		Total Cell Vel.	1 FRONT		2 FRONT		3 BACK		4 BACK		Total Cell Vac.			
VAC	VEL	VAC	VEL	VAC	VEL	VAC	VEL		VAC	VEL	VAC	VEL	VAC	VEL	VAC	VEL		Before	Middle	After
								139									140	15	1	1
1	51	8	135	8	29	6	73	165	6	31	4	73	2	56	7	20	143			
1	45	8	49	10	37	7	62	136	6	30	7	58	2	28	8	22	142			

VAC - Vacuum (inches of water)
VEL - Velocity (ft/min)

NOTE:
- Front of cell where sumps are located
- Front 1 - closest extraction pipe to sump
- Back 1 - corresponds to other end of Front 1 vapour extraction pipe

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VAPOUR MONITORING WELLS
BIOREMEDIATION FACILITY - CFB PETAWAWA

Job: 92-8731

DATE: June 7, 1993

Atmospheric Pressure (AP) 1015

Corrected CO_2 = CO_2 X (101 3/AP)

Cell A	Pump Strokes	O_2 (%)	CO_2 (ppm)	Corrected CO_2(ppm)
A7	1	20.6	—	
	2		500	
	3		1000	
	4		1000	
	5	20.5	1500	1497

Cell A	Pump Strokes	O_2 (%)	CO_2 (ppm)	Corrected CO_2(ppm)
A16	1	20.7	—	
	2		500	
	3		—	
	4		—	
	5	20.4	1000	998

Cell B	Pump Strokes	O_2 (%)	CO_2 (ppm)	Corrected CO_2(ppm)
B7	1	20.4	250	
	2		250	
	3		1000	
	4		1250	
	5	20.5	2000	1996

Cell B	Pump Strokes	O_2 (%)	CO_2 (ppm)	Corrected CO_2(ppm)
B16	1	20.6	250	
	2		500	
	3		1000	
	4		1250	
	5	20.6	1750	1747

Cell C	Pump Strokes	O_2 (%)	CO_2 (ppm)	Corrected CO_2(ppm)
C7	1	21.0	1800	
	2	21.0	9000	
	3			
	4			
	5			

Cell C	Pump Strokes	O_2 (%)	CO_2 (ppm)	Corrected CO_2 (ppm)
C16	1	20.1	250	
	2		1000	
	3		1250	
	4		1750	
	5	20.9	2000	1996

Cell D	Pump Strokes	O_2 (%)	CO_2 (ppm)	Corrected CO_2(ppm)
D7	1	20.9	250	
	2		1000	
	3		1500	
	4		2500	
	5	20.7	3000	2994

Cell D	Pump Strokes	O_2 (%)	CO_2 (ppm)	Corrected CO_2(ppm)
D16	1	20.5	500	
	2		1000	
	3		2000	
	4		3000	
	5	20.3	4000	3992

OLIVER, MANGIONE, McCALLA & ASSOCIATES LIMITED
CONSULTING ENGINEERS, HYDROGEOLOGISTS & PLANNERS

NUTRIENT APPLICATION SYSTEM
BIOREMEDIATION FACILITY - CFB PETAWAWA

Job: 92-8731

Water Meter Readings (m^3)

DATE	TIME	CELL A			CELL B			CELL C			CELL D		
		sump	upper	lower	sump	upper	lower	sump	upper	lower	sump	upper	lower
June 8	10:15	43.083	12.4	0.275	26.779	22.516	0.273	73.807	87.523	0.275	83.466	88.768	0.273
June 9	12:40	46.265	12.4	0.275	27.354	23.192	0.273	82.493	96.544	0.275	93.943	89.013	0.273
June 10	8:25	48.566	12.4	0.275	27.931	23.669	0.273	88.758	99.723	0.275	101.944	89.013	0.273
June 11	12:20	51.741	12.4	0.275	28.504	24.249	0.273	88.758	99.723	0.275	102.076	89.013	0.273
June 14	9:00	59.720	12.4	0.275	30.231	25.979	0.273	88.758	99723	0.275	102.077	89.013	0.273

APPENDIX "B"

ANALYTICAL METHODS AND

QUALITY CONTROL PROCEDURES

- Inorganic/Organic
- Microbiology

B1

Inorganic/Organic
performed by

Accutest Laboratories
146 Colonnade Road South
Unit 8
Nepean, Ontario
K2E 7Y1

and

Analytical Services Unit
Queen's University
Kingston, Ontario
K7L 3N6

Analytical Methods and Quality Control for Inorganic/Organic Parameters

The following analytical methods and quality control procedures were used by Accutest Laboratories and the Analytical Services Unit at Queen's University.

pH

Equal quantities of soil (air dried) and water were mixed when measuring for pH. The gel-filled pH electrode/meter was standardized with the normal 3 commercially available buffer solutions (pH 4.01, 7.41 and 10.4).

% Moisture

This was determined gravimetrically by weighing before and after drying at 105°C.

Total Petroleum Hydrocarbon (TPH)

This was determined by extracting the wet soil with hexane after addition of anhydrous sodium sulphate and a chromatogram of the extract was obtained by running it through a Gas Chromatogram/Flame Ionization Detector (GC/FID) system equipped with capillary column (DB5). The GC was a Hewlett Packard 5890 series 11 with a Supelco SPB-1 column. The procedure has been validated with silica sand, potting soil and moistened bentonite and found to give recoveries in the range 75 to 115%.

Seven samples from this study were analysed in triplicate, as submitted, and another five were analysed with addition of 4,458 ug of diesel fuel. The results presented in Table B1, show that recoveries were in the range 84 - 115%.

The amount of diesel in the samples was calculated on a dry weight basis using the amount of moisture content to convert the actual weight used to a dry weight equivalent. Quantification of diesel fuel in the sample was measured against varsol standards; these

Table B1

Analytical Results of Triplicate Soil Samples

Sample	TPH (ug/g) - Sample	TPH (ug/g) - Sample + Added Diesel	% Recovery of Added Diesel	Date of Sample
B1	502	793	102	14 May
B1	394	773	93	14 May
B1	391	758	84	14 May
B1	214	579	92	22 June
B1	239	589	96	22 June
B1	156	698	112	22 June
C2	202	643	107	15 June
C2	233	659	102	15 June
C2	244	706	109	15 June
D2	694	1118	100	22 June
D2	676	1095	88	22 June
D2	812	1121	96	22 June
D2	276	688	99	15 June
D2	238	673	101	15 June
D2	239	737	115	15 June
D1	27	-	-	22 November
D1	25	-	-	22 November
D1	23	-	-	22 November
B2	17	-	-	22 November
B2	33	-	-	22 November
B2	20	-	-	22 November

standards give an identical detector response to diesel fuel or home heating fuel and the detector response is linear over a wide range of concentrations. QA/QC of the GC system was charted using the detector response for a QC sample with 710 ug of varsol per gram of soil. Table B2 gives the responses and calculated concentrations in this QC sample based on the average area (4733) obtained over many years of operation. No reference soils for TPH are available.

Extractable Phosphorus

This was determined using the method of Olsen and Dean (1965) by extracting dried soil with a solution of 0.5 M sodium bicarbonate in the presence of carbon black. After agitation for 30 minutes, a 5-mL aliquot of each sample was filtered for the determination of phosphorus. This was done colorimetrically by the molybdenum blue method. QA/QC for the final determination was achieved by the use of a long term 0.15 ppm standard which was run with each set of samples, standards and blanks. Values obtained for this standard were: 0.14, 0.14, 0.14, 0.15, 0.14, 0.15, 0.15, 0.15, 0.14, 0.16, 0.14, 0.16, 0.14, 0.15, 0.14 with mean = 0.15, variance 5×10^{-5} and a coefficient of variability of 4.9%. No reference soils for extractable P were available.

Extractable Potassium

Potassium was extracted from air dried soil with 1 N ammonium acetate and determined by atomic absorption spectrophotometry. A 1 mg/L long term standard was run with each set of samples to ensure good QA/QC along with the usual blanks and standards. Values obtained for this long term standard were: 0.95, 1.00, 1.04, 0.98, 0.97, 1.03, 1.01, 0.99, 1.10, 0.95, 1.00, 1.00, 1.00, 0.93, 0.98 with mean = 1.00, variance 1.6×10^{-3} and a coefficient of variability of 4%.

Table B2

Response and Calculated Concentrations of
Quality Control Sample

Date	Varsol Area	Varsol Concentration (ug/g)
7 May 93	5113	767
14 May 93	4952	743
20 May 93	4530	680
26 May 93	4711	707
1 June 93	4650	698
7 June 93	4535	680
15 June 93	4546	682
22 June 93	4650	698
29 June 93	5128	769
6 July 93	5080	762
20 July 93	4728	709
27 July 93	5073	760
4 August 93	5013	752
10 August 93	5281	792
24 August 93	5226	784
9 Sept 93	5000	750
22 Sept 93	4810	722
5 Oct 93	5268	790
22 Oct 93	5170	776
2 Nov 93	4815	722
22 Nov 93	4880	732

Total Nitrogen

Total nitrogen was determined by the Kjeldahl method using a Buchi 425 digestor block and Buchi 321 distillation unit. A 12 mg/L solution was used to periodically check the performance of the instrumentation; blanks are also run, one per three samples, and the blank values (titration volumes) subtracted as part of the calculation. Values obtained for this long term standard were; 11.76, 12.04, 12.32, 11.76 with mean = 11.97, variance = 0.05 and a coefficient of variability of 1.9%.

Extractable Nitrate

This was determined by extracting air dried soil with an equal amount of water and determining the extracted nitrate by ion chromatography. Values obtained for the laboratory 0.50 mg/L long term standard were; 0.45, 0.49, 0.50, 0.50, 0.50, 0.53, 0.45, 0.61, 0.49, 0.45, 0.49, 0.56, 0.50, 0.47, 0.47, 0.50, 0.49, 0.50 with mean = 0.497, variance = 1.5×10^{-7} and a coefficient of variability of 8.4%.

Benzene, Toluene, Ethyl Benzene and Xylenes (BTEX) (Lab 1)

This was determined by purge and trap GC/FID using a Tekmar LSC 2000 attached to a HP 5890 gas chromatograph. Quantification of BTEX in the samples was achieved by using freshly prepared BTEX standards. QA/QC of the GC system was charted using the detector response to a 10ng/mL standards.

B2

Microbiological Enumeration Methods and Quality Control Procedures Performed by:

Biotechnology Research Institute
National Research Council of Canada
6100 Royalmount Avenue
Montreal, Quebec
H4P 2R2

The microbiological analyses were performed by the Biotechnology Research Institute of the NRC in Montreal, Quebec. The following is an excerpt from their report (Greer et al, 1994) which describes the methodologies used.

Soil Samples

Soil samples were received in glass jars the day following on-site sampling. They were shipped on ice and processed immediately upon arrival or overnight at 4°C.

Sample identification

At each sampling, two soil samples were collected from each cell, and numbered A1, A2, B1, B2, etc...

Enumeration of microorganisms

Prior to any soil manipulations, a sample was aseptically removed from each glass jar for enumeration of total bacteria. A mass of ≈5 g of soil was weighed and aseptically mixed with a volume of 0.85% (w/v) saline solution equivalent to three times the sample weight, in a tube containing 2.5 g of previously sterilized glass beads (diameter 3 mm). The sample was vigorously mixed (by vortexing) for 2 min. Serial dilutions were then prepared in saline, and 0.1 mL aliquots were spread on agar gels (BBL) containing 250 mg/L each of yeast extract (BBL), soluble starch (Sigma) and tryptone (Difco) in a mineral salts base (YTS medium). Samples were also spread on a more selective medium (DYG medium) containing 50 mg/L yeast extract, 250 mg/L gasoline and 250 mg/L diesel fuel. The gels were incubated inverted at room temperature (20-23°C) on the bench (YTS) or in dessicators (DYG) for 1 week and the colonies were counted. The plates were re-incubated for another week and additional colonies were counted. The total number of colonies was recorded.

DNA hybridization analysis

Isolated bacterial colonies on solid media, obtained from spread plating the dilution series from the soils were transferred directly onto nylon membranes by a colony lift technique. The membranes were then treated to lyse the cells and fix the DNA (Membranes were floated on 1.5 ml 0.5 M NaOH for 5 min, blotted dry, then floated on 1.5 mL 1 M TRIS.HCl, pH 7.5 for 1 min, blotted dry, then floated on 1.5 mL 0.5 M TRIS.HCl, 1.5 M NaCl, pH 7.5 for 5 min, and blotted dry. The membranes were air dried 30 min, and baked at 80°C for 30 min. Bacterial debris was subsequently removed by soaking the membranes in 6x SSC salts, 0.05% Triton X-100, and scraping off debris with a razor blade).

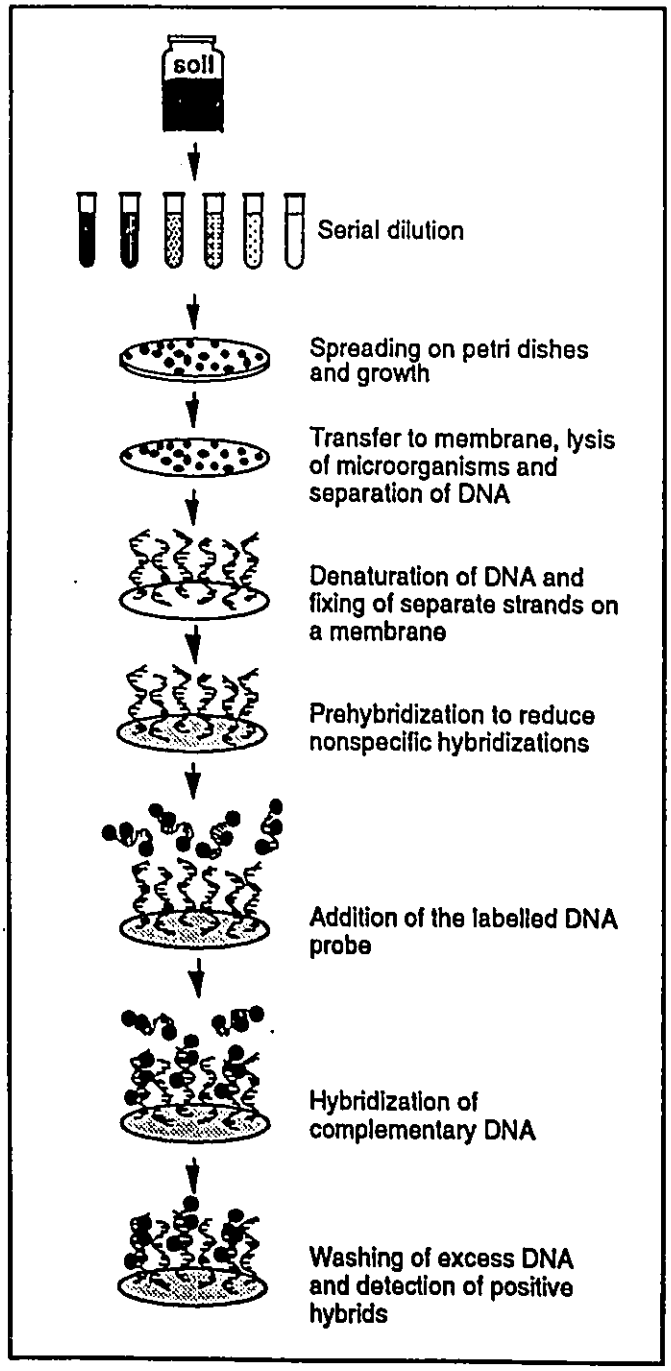


Figure 2: DNA-DNA (Southern) hybridization technique

The *xylE* gene probe was an 823 base pair fragment from the internal coding region of the *xylE* gene, encoding the enzyme catechol-2,3-

dioxygenase, on the Tol plasmid (from *Pseudomonas putida*, ATCC 33015). This enzyme is a key enzyme in the bacterial biodegradation of compounds such as toluene, xylenes, and naphthalenes . This probe was prepared by the polymerase chain reaction (PCR) using two 30-mer synthetic oligonucleotide primers derived from the published sequence data of the *xyl*E gene. The fragment was separated by agarose gel electrophoresis, and isolated from the gel using the "Geneclean" kit (BIO 101 Inc.). The fragment was labelled with ^{32}P-ATP using the "Multiprime DNA labelling system," (Amersham).

The *alk*B gene probe is an 870 base pair fragment for the internal coding region of the alkane hydroxylase gene of the Oct plasmid (from *P. oleovorans*, ATCC 29347). The enzyme, alkane hydroxylase, is the first enzyme in the bacterial biodegradation pathway for simple straight chain alkanes with from six to twelve carbons. This probe was also prepared by PCR and purified as outlined above.

Membranes prepared using the techniques above were pre-hybridized and hybridized using the standard protocol for "Zeta-probe blotting membranes," (Bio-Rad Laboratories). Hybridized membranes were sealed in blotting bags, and exposed to Kodak X-AR2 films at -80 °C. The technique of DNA-DNA hybridization (Southern) is presented schematically in Figure 2. Following exposure, the films were developed, and black spots corresponding to the positions of colonies on the original media plates were counted. The numbers of probe-positive bacteria were expressed per gram of soil (dry weight) or as a percentage of the total viable bacterial population.

Mineralization of control contaminants

In order to determine the biodegradability of the pollutants in a contaminated soil, control compounds representative of the type of pollution present were placed in a microcosm to determine whether microorganisms bring about mineralization.

A mass of 20 ± 0.1 g of soil was placed in a 100 ml microcosm (serum bottle). ^{14}C-Hexadecane and ^{14}C-toluene were dissolved separately in hexane and 25 µl of the solution was injected into individual microcosms to obtain final concentrations in the soil of 8.65 mg/kg and 21.36 µg/kg, respectively. Each microcosm was equipped with a CO_2 trap consisting of 1 ml of 0.5 M KOH placed in a tube inside the bottle (Fig. 3).

The microcosms were filled with air and sealed to prevent the loss of CO_2. Air was added at each sampling during the course of the experiment. The microcosms were incubated without agitation at the room temperature.

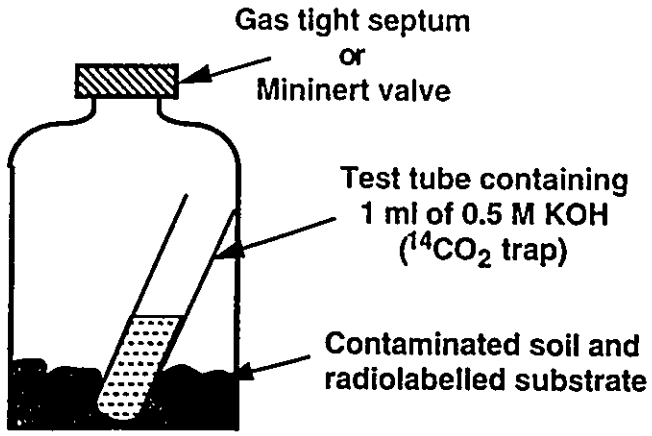


Figure 3: Microcosm used for the hexadecane and toluene mineralization tests

Samples of the KOH were taken by piercing the rubber septum or ball of the mininert valve with a syringe and aspirating the KOH solution, with air being added through a 0.22µm membrane (Millex GS) to compensate for the vacuum created. The tube containing the trap was rinsed with 1 ml of 0.5M KOH, and refilled with 1 ml of 0.5M KOH. The two fractions of KOH were combined and placed in a scintillation vial with 18 ml of scintillation fluid (ACS, Amersham). The radioactivity was measured in a scintillation counter (Packard model Tri-Carb 4530). The amount of $^{14}CO_2$ trapped was measured and recorded as the percentage of ^{14}C-substrate added to the microcosm originally.

B3

Standard Microbiological Methods and Procedures

Performed by:

Accutest Laboratories Ltd.
146 Colonnade Road South
Unit 8
Nepean, Ontario
K2E 7Y1

APPENDIX "B"

Standard Microbiological Methods

and Procedures

Method for Bacteria in Soil

Take 1 gram of soil sample (as submitted) and put into dilution bottle containing 99 mL of sterile buffer.

Shake for 2 - 3 minutes, let settle.

Remove 0.1 mL and filter through 0.45 micron membrane.

Incubate at 35°C on Standard Plate Count Media for a period of 48 hours.

Total Bacteria in Diesel/Water Mixture

Prepare 2 x 300 mL of Stand Plate Count Media in Media bottled.

Pour into 10 cm plates and let solidify.

Pipet directly 0.1 mls of sample and use hockey stick spreader to spread the sample.

Incubate at 35°C for a period of 48 hours.

Kingston MOEE Laboratory Procedure for the Bacteriological Analysis of Sediment Samples by Membrane Filtration

1. Pour off any supernatant from the sediment sample.
2. Remove 9 mL from a 99 mL dilution blank of sterile phosphate - $MgCl_2$, buffered dilution water (Standard Methods 16th ed., page 855) and add 10 ± 0.1 g of sediment to give a 1 x 10^{-1} dilution.
3. Shake the sediment-buffer mixture vigorously 5 - 10 times. Allow the mixture time (5 - 10 min) to settle until a clear supernatant is obtained.
4. Make further solutions of the clear supernatant into buffered dilution water. For example, 1 mL of the 1 x 10^{-1} into 99 mL is a 1 x 10^{-3} dilution.
5. Filter the supernatant using standard membrane filter techniques for total coliform, fecal coliform, E.Coli, or fecal streptococci analysis.
6. Calculate the count per gram of sediment (wet weight) as follows:
   a) If 1 ml of the 10^{-1} dilution was used and a count of 20 FC were obtained the count should be reported as,
      20/1 mL x 0.1 g per mL
      = 200 FC per gram
   b) If 10 mL of the 1 x 10^{-3} dilution was plated and a count of 20 FC were obtained report the count as,
      20/10 mL x .001 g per mL
      = 2,000 FC per gram

Summary of calculations to determine the counts per gram for the initial sediment dilution of 1:10:

1. For 10 ml - report what you count
2. For 1 ml - multiply by 10
3. To report 0 FC counts - for 10 mL report < FC/gram
   - for 1 mL report < FC/gram

APPENDIX "C"

FIELD AND ANALYTICAL RESULTS

Page

TABLE C1
SOIL TOTAL PETROLEUM HYDROCARBON (TPH) CONCENTRATIONS

DATE	DAYS	CELL A AVG. TPH (ppm)	CELL B AVG. TPH (ppm)	CELL C AVG. TPH (ppm)	CELL D AVG. TPH (ppm)
NOV 27/92	0	281	1211	41	751
MAY 6/93	160	113	628	149	743
MAY 14/93	168	83	291	28	658
MAY 20/93	174	54	189	137	853
MAY 25/93	179	99	335	535	768
MAY 31/93	185	69	134	360	401
JUNE 6/93	192	44	348	93	404
JUNE 14/93	200	40	574	425	146
JUNE 21/93	207	73	262	112	694
JUNE 28/93	214	40	322	1368	377
JULY 5/93	221	34	104	15	203
JULY 12/93	228	80	534	682	94
JULY 19/93	234	70	250	95	378
JULY 26/93	241	42	49	83	136
AUG 3/93	249	38	93	42	615
AUG 9/93	255	33	103	18	58
AUG 23/93	269	13	59	470	213
SEPT 7/93	284	29	56	38	82
SEPT 21/93	298	14	23	140	32
OCT 4/93	311	5	14	5	100
OCT 20/93	327	5	74	25	28
NOV 1/93	339	5	15	5	5
NOV 18/93	355	5	41	5	23

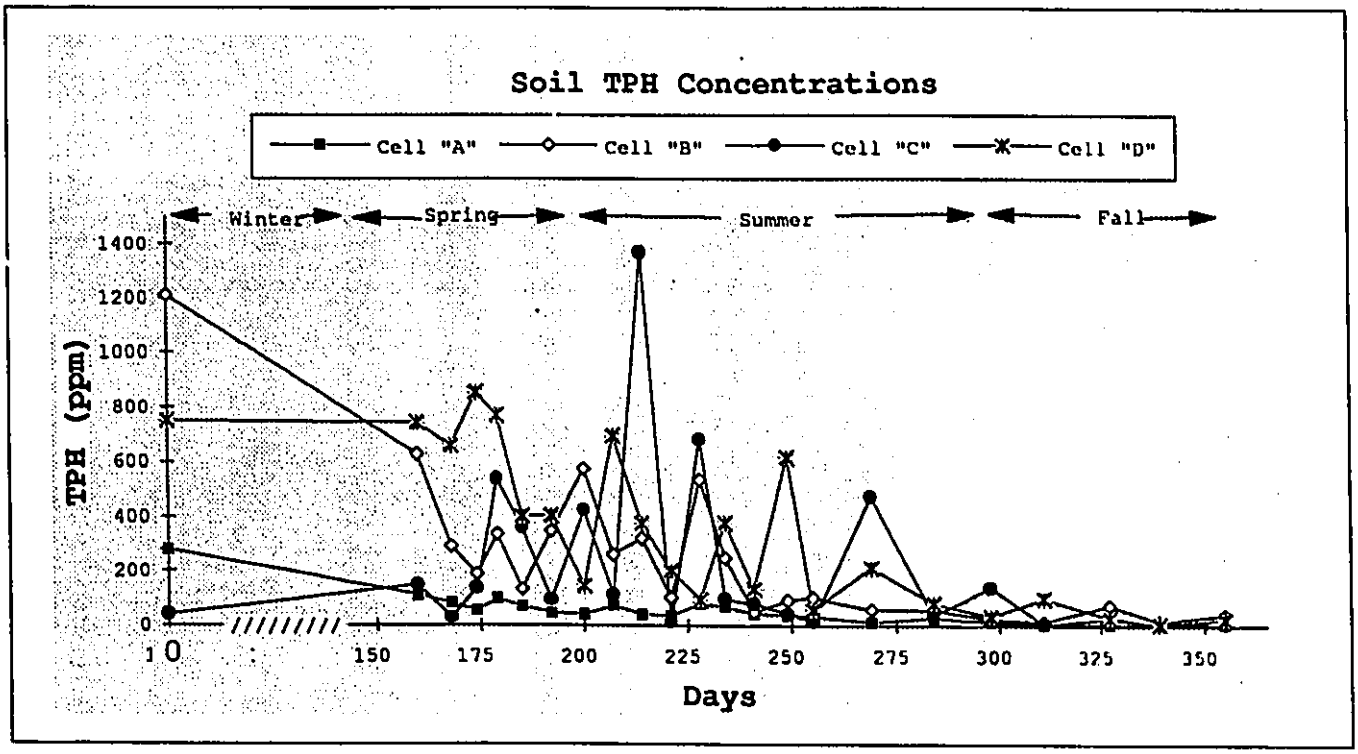
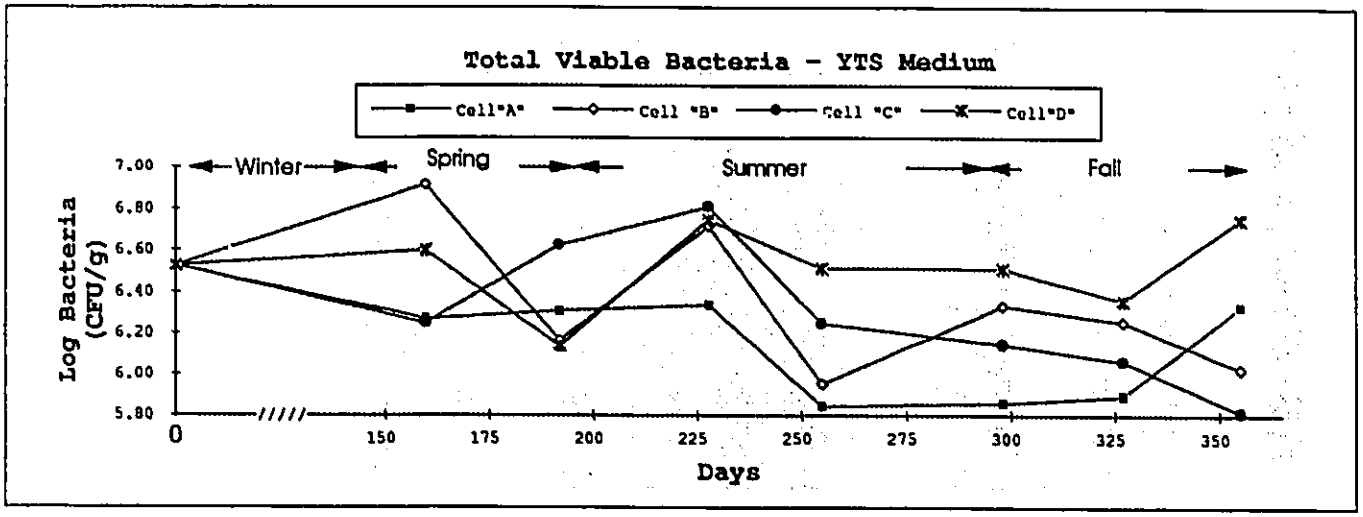


TABLE C2

TOTAL VIABLE BACTERIA - YTS AND DYG MEDIA

YTS Medium is nutrient rich, DYG medium is petroleum rich

DATE	YTS MEDIUM (CFU/g)					DYG MEDIUM (CFU/g)			
	DAYS	CELL A log bact	CELL B log bact	CELL C log bact	CELL D log bact	CELL A log bact	CELL B log bact	CELL C log bact	CELL D log bact
Nov. 27/92	0	6.53	6.53	6.53	6.53				
May 6/93	160	6.27	6.91	6.25	6.60				
June 6/93	192	6.30	6.17	6.62	6.14	6.77	6.91	7.33	6.97
July 12/93	228	6.34	6.72	6.81	6.74	6.45	6.76	6.79	6.88
Aug. 9/93	255	5.84	5.95	6.25	6.51	6.21	6.37	6.62	6.73
Sept. 21/93	298	5.86	6.33	6.14	6.51	5.95	6.25	6.19	6.46
Oct. 20/93	327	5.89	6.26	6.06	6.36	5.88	6.41	6.18	6.39
Nov. 18/93	355	6.33	6.02	5.81	6.75	6.42	6.29	5.97	6.73



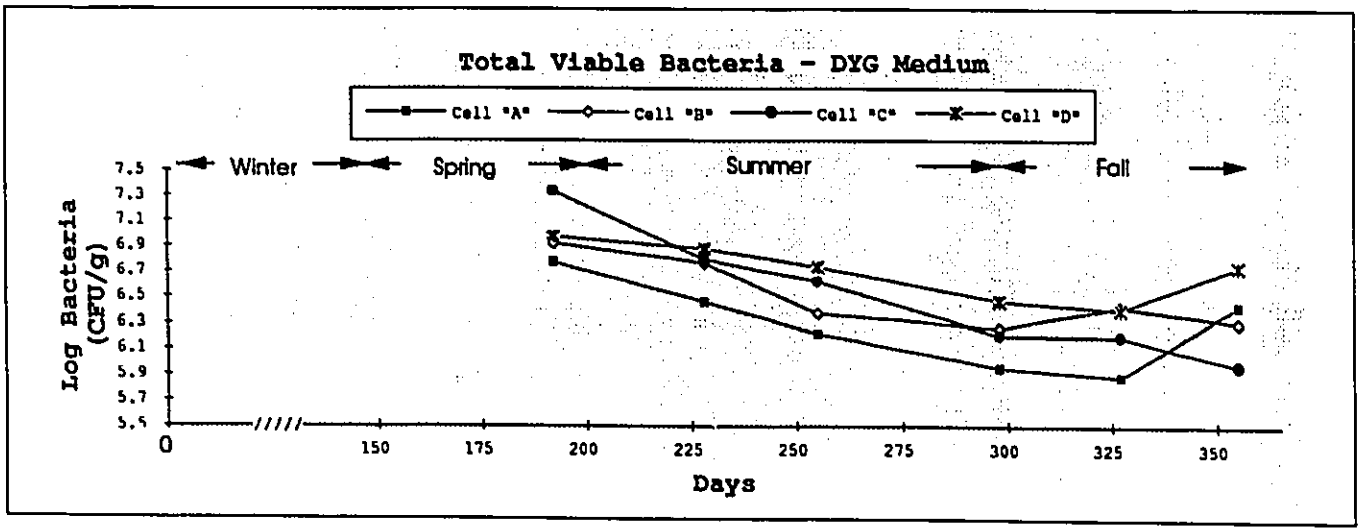
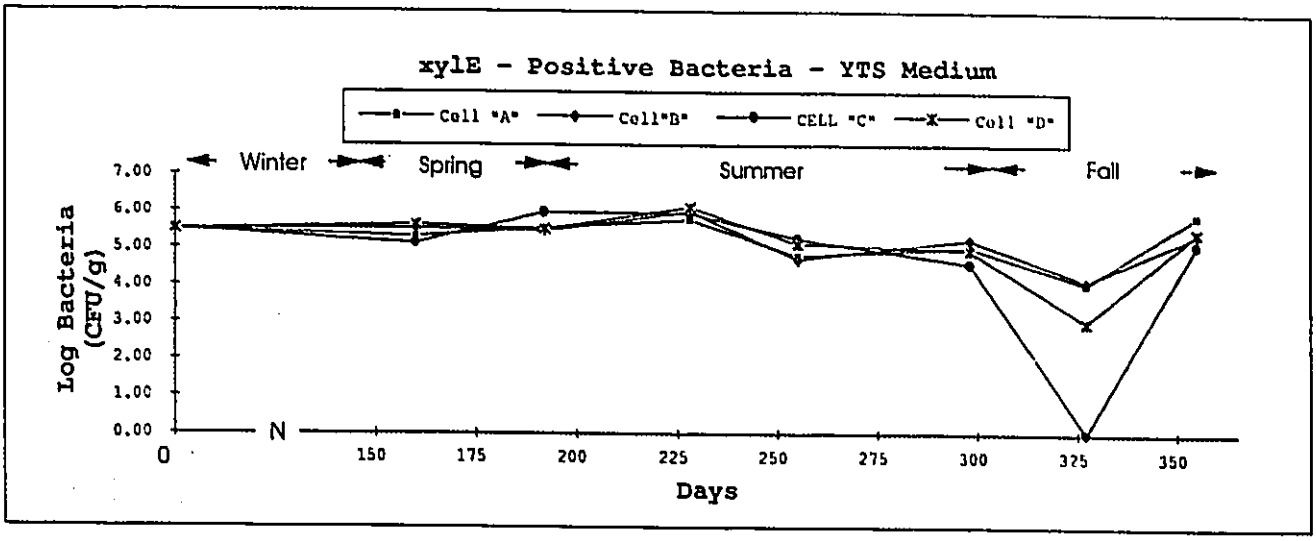


TABLE C3
xylE - POSITIVE BACTERIA: YTS AND DYG MEDIUMS

DATE	DAYS	YTS MEDIUM (CFU/g) CELL A log bact	CELL B log bact	CELL C log bact	CELL D log bact	DYG MEDIUM (CFU/g) CELL A log bact	CELL B log bact	CELL C log bact	CELL D log bact
Nov. 27/92	0	5.50	5.50	5.50	5.50				
May 6/93	160	5.29	5.54	5.12	5.63				
June 6/93	192	5.54	5.47	5.97	5.51	5.76	6.24	6.52	6.13
July 12/93	228	5.78	6.00	5.96	6.13	5.70	5.98	6.10	6.18
Aug. 9/93	255	4.79	4.71	5.26	5.13	4.93	4.81	5.03	4.90
Sept. 21/93	298	5.03	5.25	4.59	4.95	4.96	5.26	5.06	4.97
Oct. 20/93	327	4.04	4.11	0.01	3.00	3.00	4.04	3.00	4.00
Nov. 18/93	355	5.92	5.39	5.13	5.46	5.90	5.43	5.21	5.78



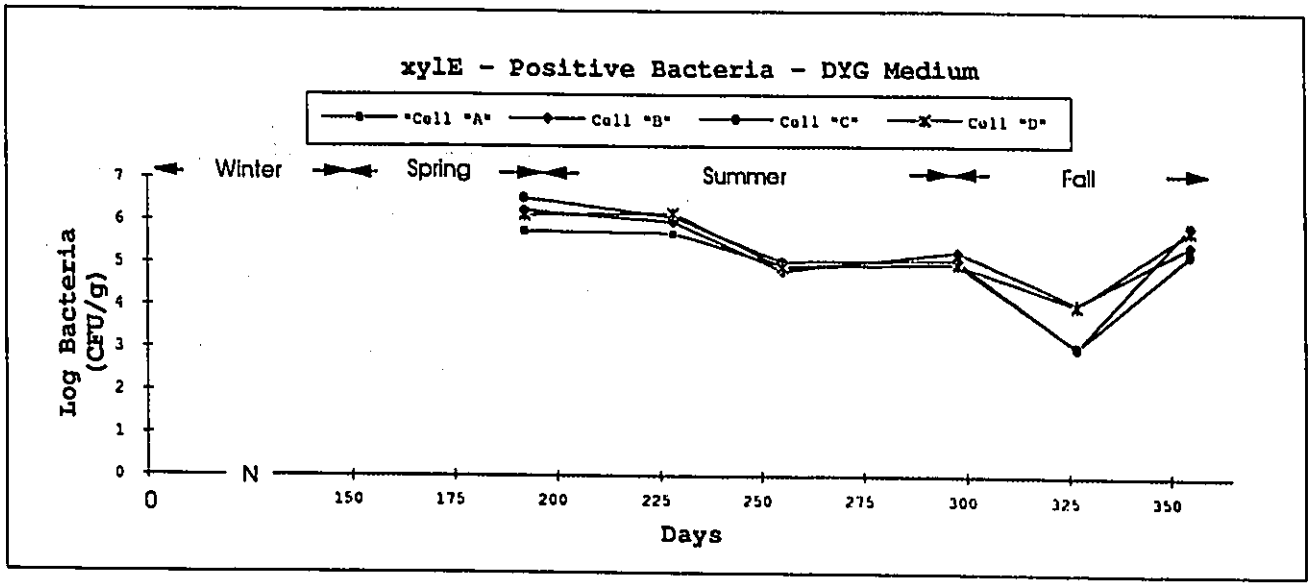
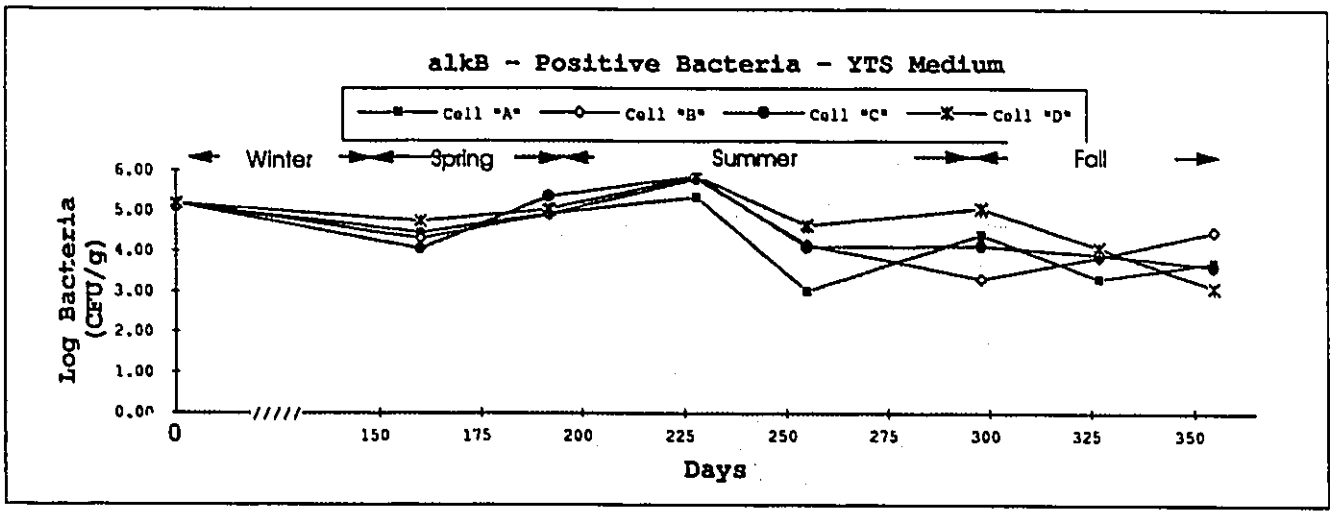


TABLE C4

alkB - POSITIVE BACTERIA: YTS AND DYG MEDIA

YTS medium is nutrient rich, DYG medium is petroleum rich

DATE	YTS MEDIUM (CFU/g)					DYG MEDIUM (CFU/g)			
	DAYS	CELL A log bact	CELL B log bact	CELL C log bact	CELL D log bact	CELL A log bact	CELL B log bact	CELL C log bact	CELL D log bact
Nov. 27/92	0	5.19	5.19	5.19	5.19				
May 6/93	160	4.45	4.30	4.04	4.73				
June 6/93	192	4.92	4.90	5.36	5.03	4.78	5.62	5.62	5.60
July 12/93	228	5.35	5.81	5.86	5.87	5.32	5.52	5.49	5.58
Aug. 9/93	255	3.00	4.15	4.09	4.62	3.30	4.69	0.01	0.01
Sept. 21/93	298	4.40	3.30	4.11	5.07	4.11	4.48	4.32	4.95
Oct. 20/93	327	3.30	3.85	3.90	4.08	0.01	4.08	4.26	0.01
Nov. 18/93	355	3.70	4.48	3.60	3.07	4.11	3.95	3.48	3.07



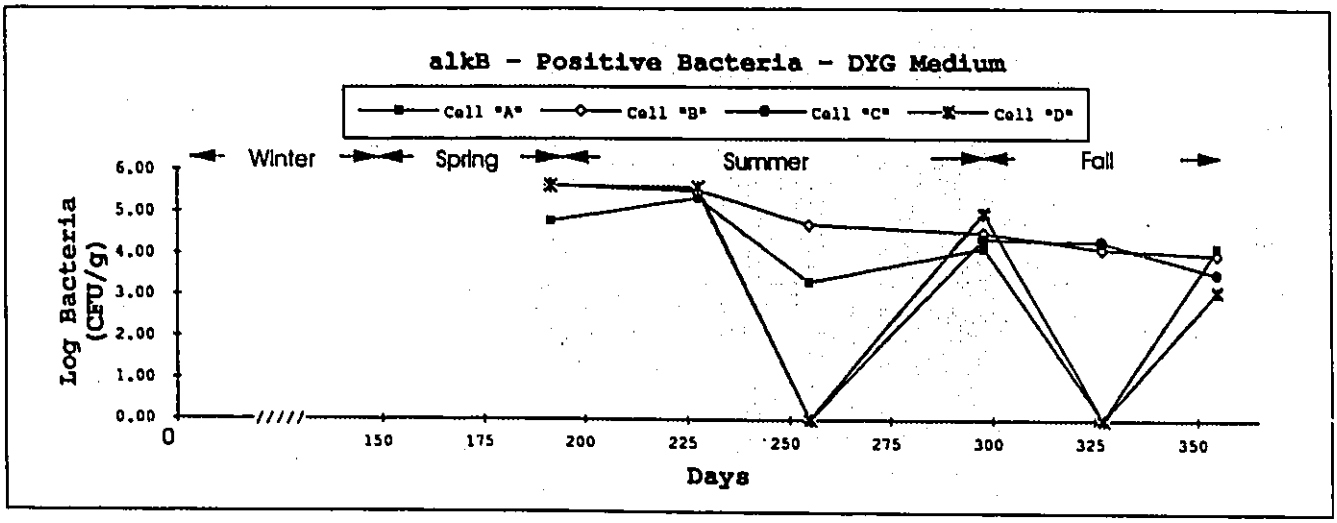


TABLE C5
INITIAL TOLUENE MINERALIZATION RATES

DATE	DAYS	CELL A	CELL B	CELLC	CELL D
MAY 6/93	160	2.5632	3.816	2.8943	2.9834
JUNE 6/93	192	4.9181	4.6832	4.7793	2.8249
JULY 6/93	228	5.5376	4.4803	5.3186	4.6084
AUG 9/93	255	5.372	5.1691	4.9235	5.9915
SEPT 21/93	298	2.654	4.256	4.3468	3.5511
OCT 20/93	327	3.6793	4.1065	3.5564	4.0531
NOV 18/93	355	2.2748	3.4229	2.9530	4.1652

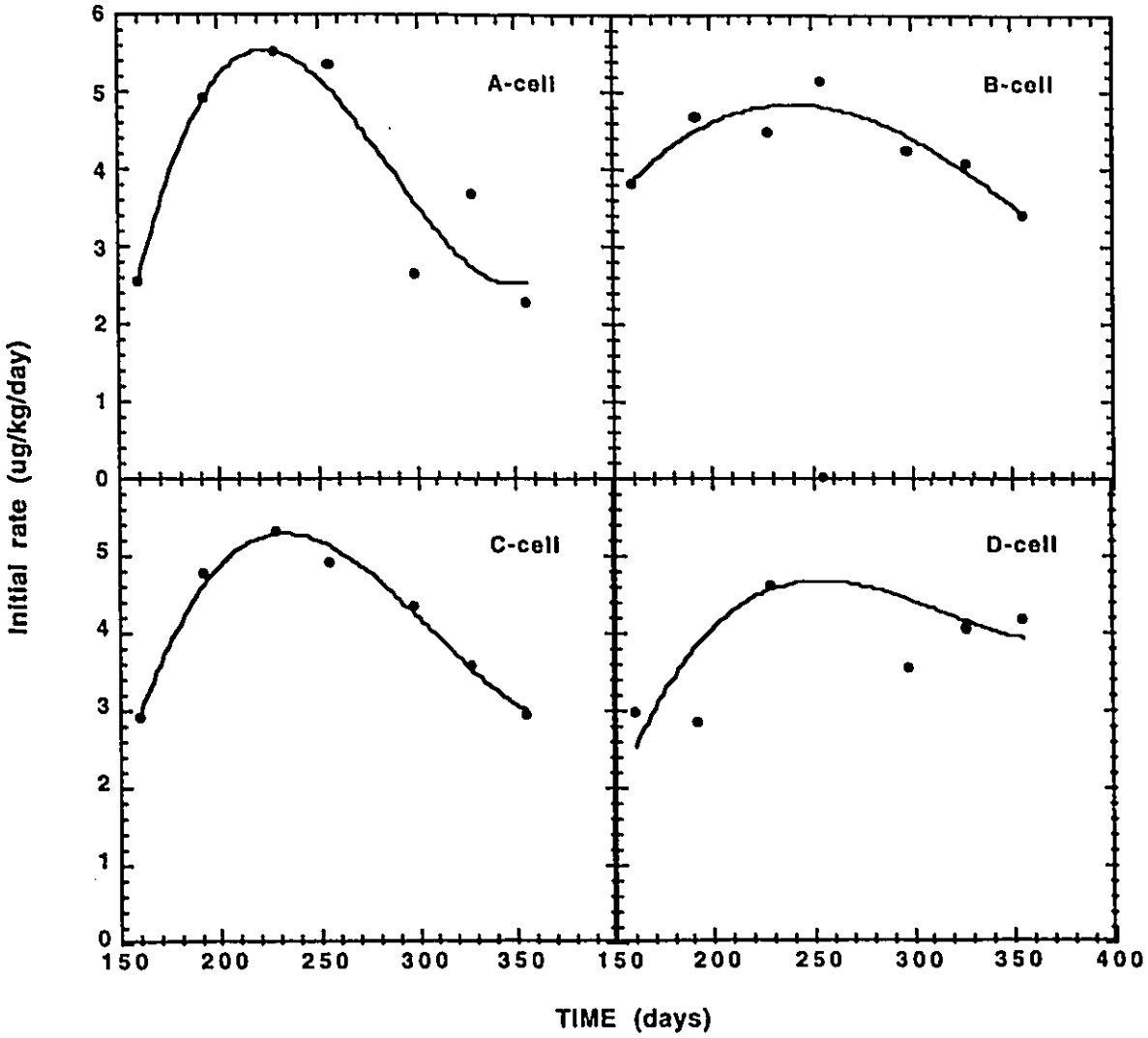


TABLE C6
INITIAL HEXADECANE MINERALIZATION RATES

DATE	DAYS	CELL A	CELL B	CELLC	CELL D
MAY 6/93	160	0.0303	0.020167	0.12254	0.0153
JUNE 6/93	192	1.3645	0.5796	1.3732	0.3655
JULY 6/93	228	1.0726	0.1168	0.1644	0.4649
AUG 9/93	255	1.7668	1.2867	1.60003	1.4943
SEPT 21/93	298	1.5073	1.1959	1.5181	1.5116
OCT 20/93	327	1.3645	1.2672	1.3537	1.2413
NOV 18/93	355	1.2002	0.8888	1.0445	1.3299

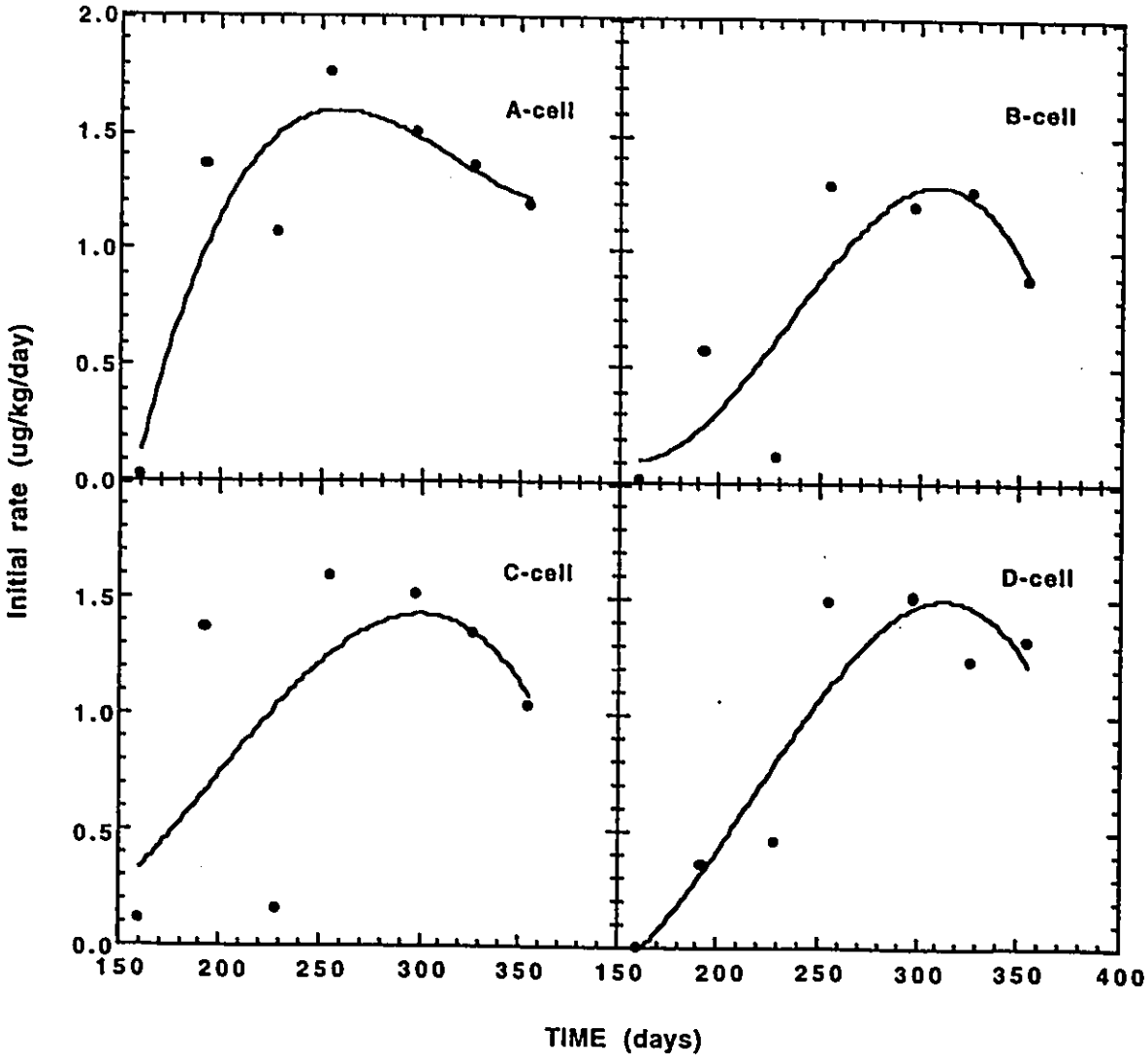


TABLE C7
Total Soil Bacteria
(Standard Procedure)

DATE	DAYS	Cell "A"		Cell "B"		Cell "C"		Cell "D"	
		Avg. Bacteria Counts/gm	Log	Avg. Bacteria Counts/gm	Log	Avg. Bacteria Counts/gm	Log	Avg. Bacteria Counts/gm	Log
NOV 27/93	0	7500	3.88	50500	4.70	54000	4.73	31000	4.49
MAY 6/93	160	73000	4.86	130000	5.11	56000	4.75	87500	4.94
MAY 14/93	168	67500	4.83	131000	5.12	86000	4.93	67000	4.83
MAY 20/93	174	94000	4.97	152000	5.18	75500	4.88	178500	5.25
MAY 25/93	179	61500	4.79	71500	4.85	56500	4.75	45500	4.66
MAY 31/93	185	60500	4.78	72500	4.86	180500	5.26	48000	4.68
JUNE 6/93	192	38500	4.59	81000	4.91	44000	4.64	56000	4.75
JUNE 14/93	200	73000	4.86	57500	4.76	60500	4.78	69500	4.84
JUNE 21/93	207	28000	4.45	92000	4.96	92000	4.96	92000	4.96
JUNE 28/93	214	54000	4.73	84000	4.92	84000	4.92	84000	4.92
JULY 5/93	221	71000	4.85	108000	5.03	108000	5.03	108000	5.03
JULY 12/93	228	54500	4.74	70500	4.85	70500	4.85	70500	4.85
JULY 19/93	234	41000	4.61	74000	4.87	74000	4.87	74000	4.87
JULY 26/93	241	55000	4.74	57500	4.76	57500	4.76	57500	4.76
AUG 3/93	249	107000	5.03	34750	4.54	48500	4.69	87000	4.94
AUG 9/93	255	70000	4.85	74500	4.87	79000	4.90	59500	4.77
AUG 23/93	269	43000	4.63	57500	4.76	62000	4.79	43000	4.63
SEPT 7/93	284	50000	4.70	59500	4.77	91500	4.96	107000	5.03
OCT 20/93	327	69000	4.84	69000	4.85	68500	4.84	123500	5.09
NOV 1/93	339	38000	4.58	33500	4.53	42000	4.62	28500	4.45
NOV 18/93	355	69500	4.85	65000	4.83	57500	4.76	76500	4.88

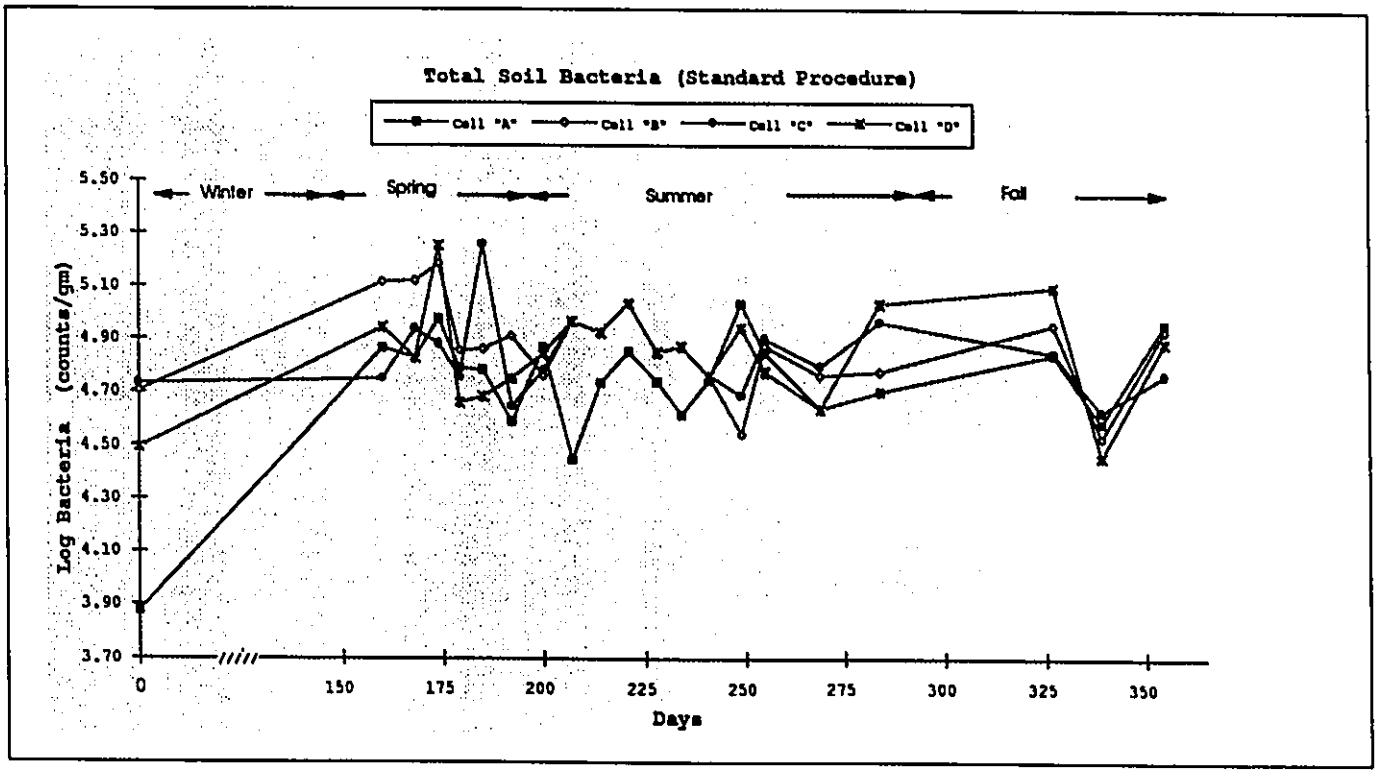


TABLE C8
DAILY VOLUME OF AIR WITHDRAWN FROM CELLS

DATE	DAYS	CELL A (M^3)	CELL B (M^3)	CELL C (M^3)	CELL D (M^3)	DATE	DAYS	CELL A (M^3)	CELL B (M^3)	CELL C (M^3)	CELL D (M^3)
NOV 27/92	0					JULY 15/93	231	424	390	473	542
MAY 25/93	179	521	559	580	573	JULY 16/93	232	421	355	393	583
JUNE 7/93	193	486	414	480	483	JULY 19/93	235	338	366	355	462
JUNE 8/93	194	538	1518	569	493	JULY 20/93	236	355	386	328	455
JUNE 9/93	195	464	459	469	490	JULY 21/93	237	283	359	569	380
JUNE 10/93	196	435	390	386	380	JULY 22/93	238	400	278	462	707
JUNE 11/93	197	414	493	404	362	JULY 23/93	239	338	317	466	359
JUNE 14/93	200	435	462	362	311	JULY 26/93	242	286	173	421	466
JUNE 15/93	201	297	725	380	335	JULY 27/93	243	235	242	331	573
JUNE 16/93	202	221	742	255	431	JULY 28/93	244	331	345	328	449
JUNE 17/93	203	0	114	0	145	JULY 29/93	245	273	155	387	466
JUNE 18/93	204	0	0	0	0	JULY 30/93	246	304	328	321	725
JUNE 23/93	209	0	0	0	0	AUG 3/93	249	404	290	342	449
JUNE 25/93	211	673	863	673	845	AUG 4/93	250	449	393	500	483
JUNE 28/93	214	442	486	493	587	AUG 5/93	251	362	138	449	404
JUNE 29/93	215	459	690	528	759	AUG 12/93	258	497	273	N/A	331
JUNE 30/93	216	455	476	514	538	AUG 13/93	259	N/A	269	N/A	338
JULY 5/93	221	459	794	445	531	AUG 16/93	262	N/A	N/A	N/A	404
JULY 6/93	222	449	362	442	576	AUG 17/93	263	N/A	162	N/A	262
JULY 7/93	223	449	404	459	566	AUG 18/93	264	N/A	204	N/A	248
JULY 8/93	224	424	362	549	490	AUG 19/93	265	N/A	242	N/A	255
JULY 9/93	225	373	393	676	555	NOV 4/93	343	983	742	1173	966
JULY 12/93	228	442	411	580	449	NOV 5/93	344	1035	966	1811	1001
JULY 13/93	229	438	369	438	514	NOV 10/93	355	1311	1035	949	1380
JULY 14/93	230	458	473	493	573						

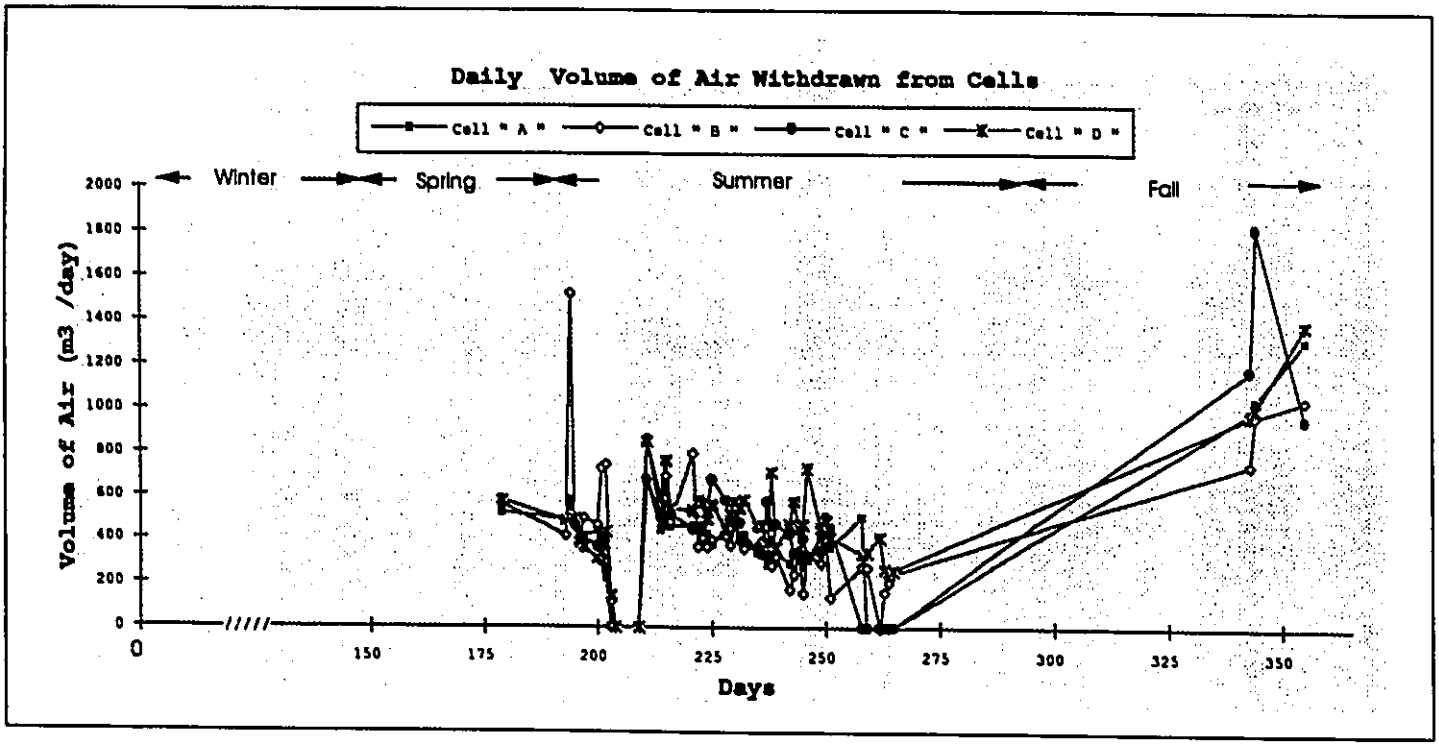


TABLE C9
SOIL VAPOUR TPH CONCENTRATIONS

DATE	DAYS	CELL A (ppb)	CELL B (ppb)	CELL C (ppb)	CELL D (ppb)
MAY 20/93	174	40.310	186.047	220.775	308.527
MAY 31/93	185	0.155	169.302	175.504	157.209
JUNE 6/93	191	44.031	0.155	130.233	223.256
JUNE 14/93	199	16.434	0.155	62.016	220.155
JUNE 28/93	213	17.674	93.023	31.008	186.047
JULY 5/93	220	18.915	186.047	0.310	157.209
JULY 13/93	228	0.310	179.225	0.310	39.070
JULY 20/93	235	23.256	0.310	0.310	0.310
JULY 26/93	241	12.403	0.310	0.310	0.310
AUG 3/93	249	1.643	13.953	0.310	0.310
AUG 24/93	270	0.310	0.310	0.310	0.310
SEPT 7/93	284	0.310	0.310	0.310	0.310
SEPT 21/93	298	0.310	0.310	0.310	0.310
OCT 5/93	311	0.713	0.310	1.271	0.310
OCT 20/93	327	0.310	0.310	0.310	0.310
NOV 1/93	339	0.310	0.310	0.310	0.310
NOV 18/93	355	0.310	0.310	0.310	0.310

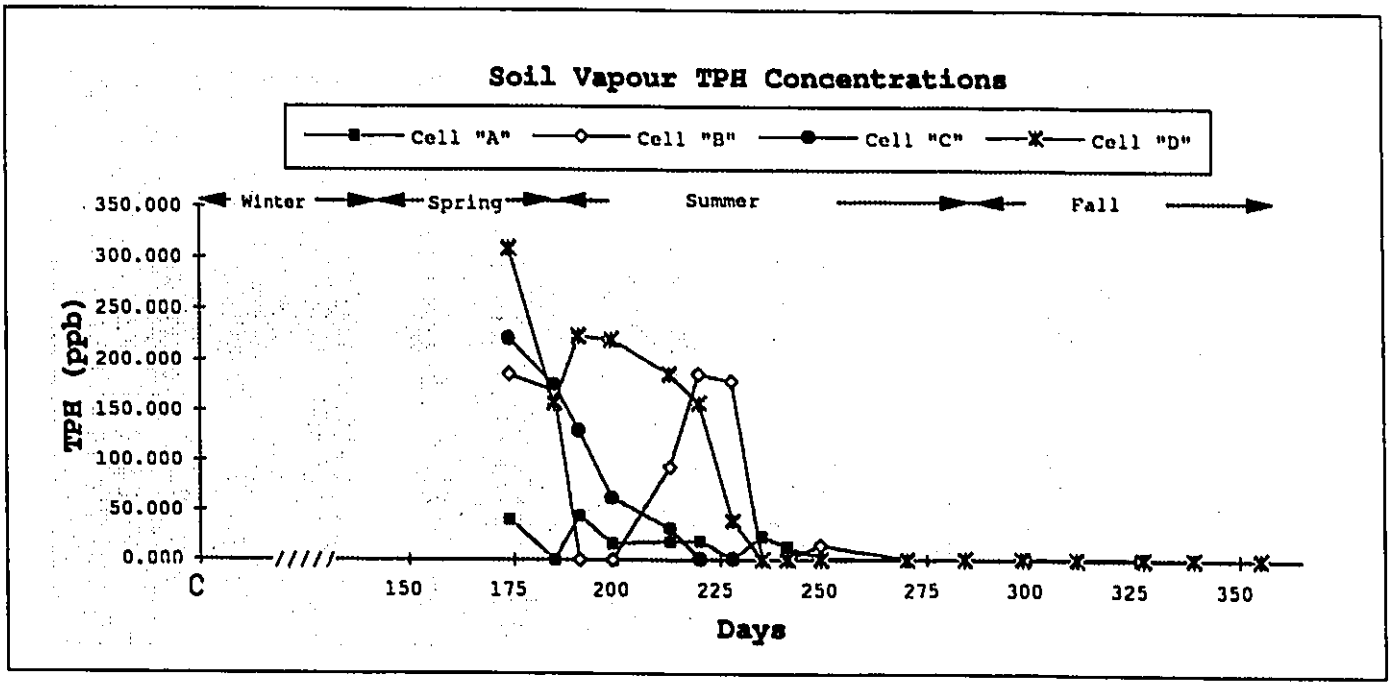


TABLE C10
INSITU SOIL VAPOUR OXYGEN (O2) CONCENTRATION

DATE	DAYS	CELL A AVG. O2 (%)	CELL B AVG. O2 (%)	CELL C AVG. O2 (%)	CELL D AVG. O2 (%)
MAY 25/93	179	20.75	20.60	20.30	20.60
MAY 31/93	185	20.90	20.70	19.95	20.60
JUNE 8/93	192	20.45	20.55	20.95	20.50
JUNE 21/93	207	20.20	19.35	16.40	19.10
JUNE 28/93	214	20.80	20.80	20.75	20.80
JULY 5/93	221	20.85	20.65	19.90	20.60
JULY 12/93	228	20.80	20.80	20.65	20.60
JULY 19/93	235	20.45	20.45	18.15	20.45
AUG 3/93	249	20.45	20.50	20.20	19.85
SEPT 7/93	284	18.00	18.50	16.00	20.25
SEPT 21/93	298	16.25	17.75	14.25	17.75
NOV 1/93	339	18.25	20.25	19.00	20.75
NOV 18/93	355	20.90	21.00	21.05	20.95

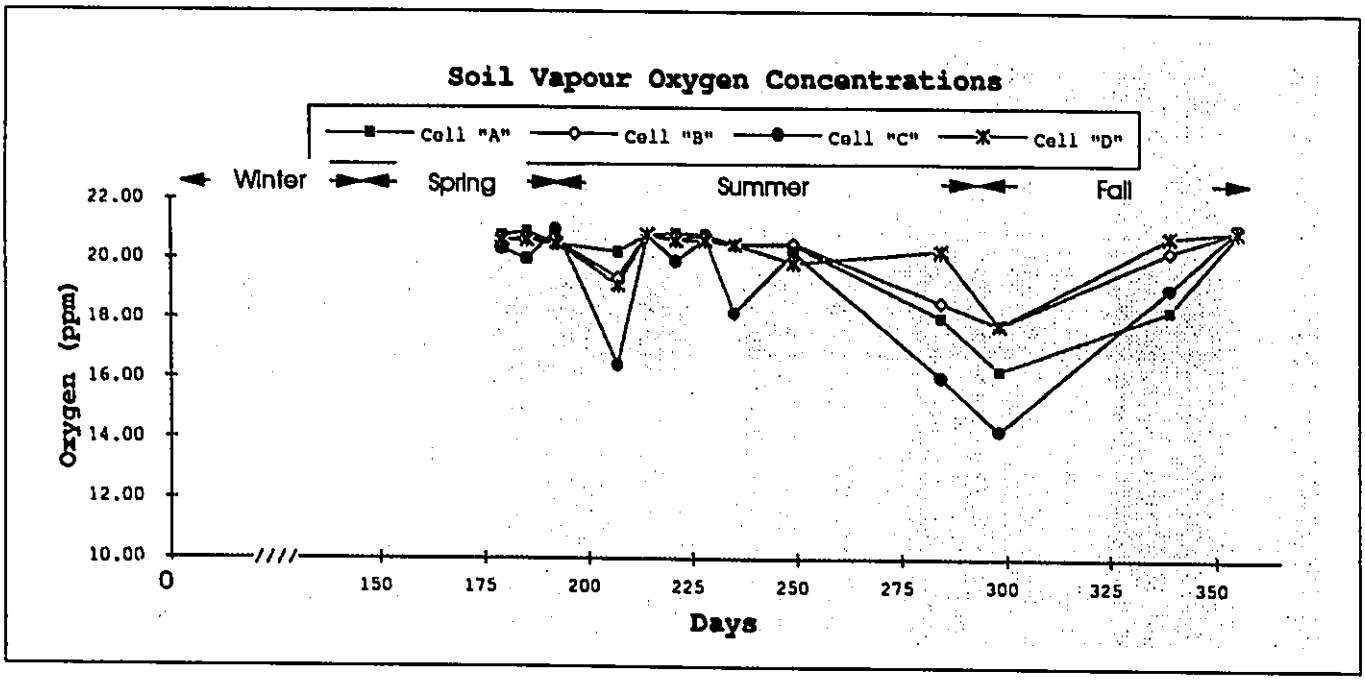


TABLE C11

INSITU SOIL VAPOUR CARBON DIOXIDE (CO2) CONCENTRATIONS

DATE	DAYS	CELL A AVG. CO2 (PPM)	CELL B AVG. CO2 (PPM)	CELL C AVG. CO2 (PPM)	CELL D AVG. CO2 (PPM)
MAY 25/93	179	1058	2016	1235	2016
MAY 31/93	185	1143	2794	1372	3048
JUNE 8/93	192	1248	1872	5498	3493
JUNE 21/93	207	3500	10000	11500	14000
JUNE 28/93	214	2500	2500	3875	14500
JULY 5/93	221	2125	6500	23750	3625
JULY 12/93	228	2000	4750	23500	4000
JULY 19/93	235	3250	4625	21500	4500
AUG 3/93	249	1250	1500	2625	1125
AUG 23/93	269	1625	5500	3500	5750
SEPT 7/93	284	2625	3500	31000	5000
SEPT 21/93	298	1250	2750	17000	2625
OCT 4/93	311	875	1625	15000	2250
OCT 20/93	327	1500	1250	6500	1000
NOV 1/93	339	250	750	3750	750
NOV 18/93	355	550	800	1100	650

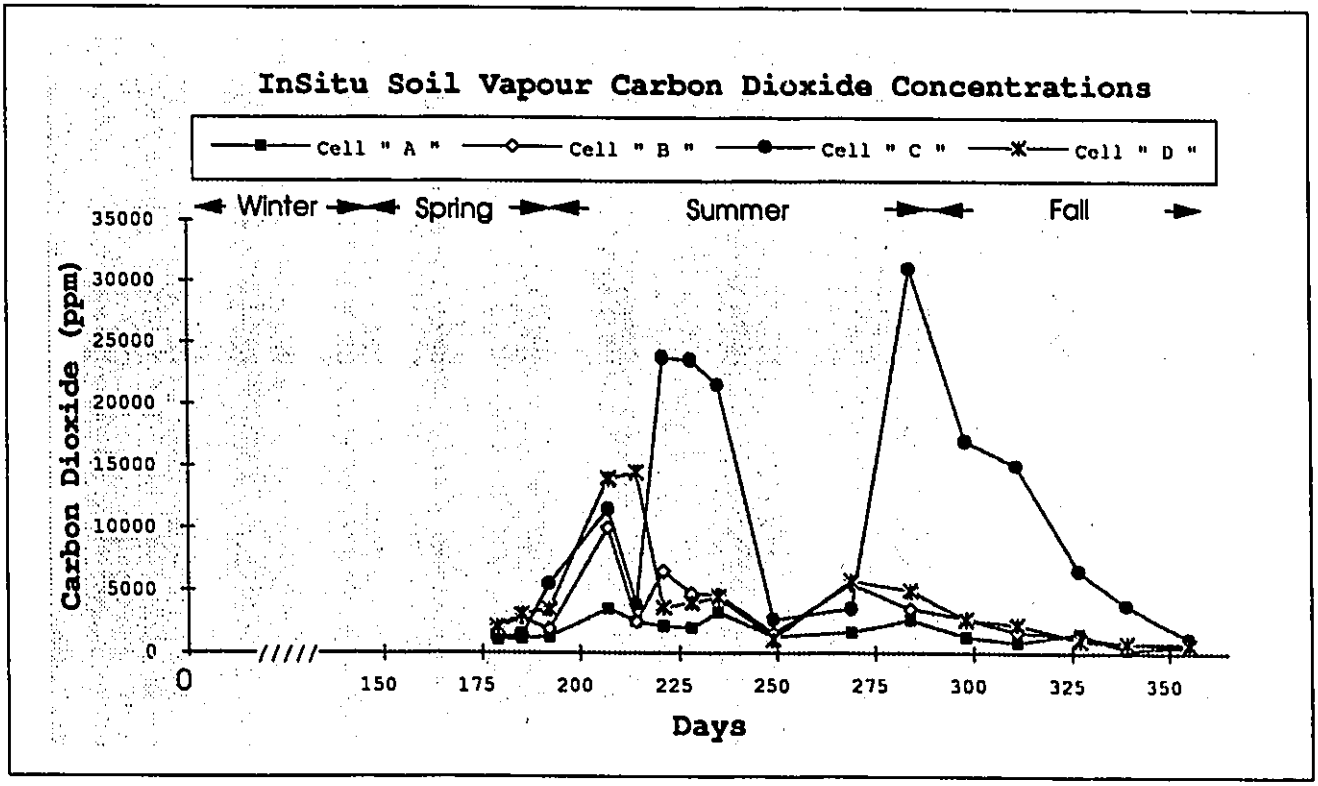


TABLE C12
INSITU SOIL AND AMBIENT AIR TEMPERATURES

DATE	DAYS	CELL A	CELL B	CELL C	CELL D	AIR
MAY 6/93	160	8.0	11.0	8.5	10.5	13.1
MAY 14/93	168	8.7	11.9	12.5	12.5	6.9
MAY 20/93	174	9.5	12.8	14.5	14.5	8.1
MAY 25/93	179	12.3	13.1	12.0	14.5	11.2
MAY 31/93	185	11.3	14.5	14.0	13.0	10.3
JUNE 6/93	191	13.0	18.0	16.3	15.0	15.4
JUNE 10/93	195	13.8	16.5	13.0	17.5	17.8
JUNE 14/93	199	15.5	17.8	19.3	18.5	19.9
JUNE 21/93	206	15.5	21.0	20.8	20.8	19.0
JUNE 24/93	209	16.3	20.8	19.3	21.8	17.1
JULY 5/93	220	18.3	21.3	24.3	24.7	25.6
JULY 8/93	223	19.5	24.0	23.3	23.3	22.6
JULY 13/93	228	20.8	25.0	24.8	25.8	21.0
JULY 16/93	231	20.0	25.0	24.8	24.0	15.8
JULY 20/93	235	19.5	25.0	24.0	25.3	21.5
JULY 26/93	241	19.5	22.3	22.0	21.8	20.5
JULY 30/93	245	20.8	25.0	25.5	25.8	21.0
AUG 4/93	250	20.5	22.8	25.3	25.5	19.1
AUG 11/93	257	20.7	23.3	22.8	25.8	22.1
AUG 16/93	262	22.5	26.5	28.0	25.5	23.2
NOV 3/93	341	9.8	10.4	10.4	10.0	1.5
NOV 4/93	342	10.0	9.7	10.4	9.8	5.9
NOV 5/93	343	10.1	10.0	10.1	10.0	2.4
NOV 18/93	355	8.3	9.3	7.3	8.8	0.0

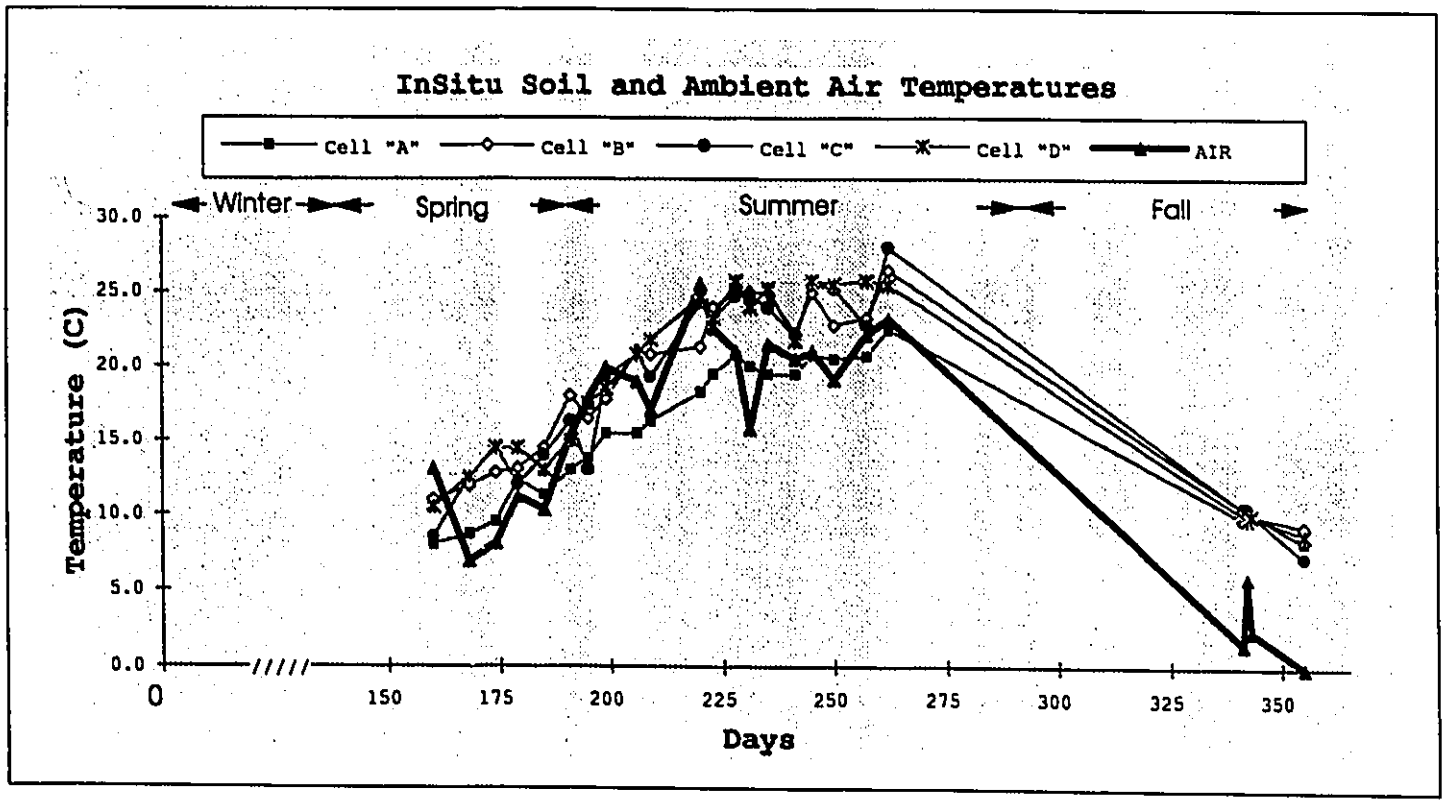


TABLE C13
AVERAGE SOIL NITRATE (NO3) CONCENTRATIONS

DATE	DAYS	CELL A AVERAGE NO3 (ppm)	CELL B AVERAGE NO3 (ppm)	CELL C AVERAGE NO3 (ppm)	CELL D AVERAGE NO3 (ppm)
JULY 5/93	221	0.3	0.0	0.5	0.3
JULY 12/93	228	0.3	0.1	0.1	0.2
JULY 19/93	235	0.4	0.1	0.1	0.4
JULY 26/93	242	0.6	0.1	0.5	0.3
AUG 3/93	249	0.9	0.1	0.9	0.3
AUG 9/93	255	0.9	0.3	4.1	3.1
AUG 23/93	269	1.6	0.2	0.8	1.3
SEPT 7/93	284	8.3	0.3	2.0	12.0
SEPT 21/93	298	18.0	1.5	11.2	9.3
OCT 4/93	311	17.8	0.9	24.5	16.5
OCT 20/93	327	9.0	0.3	5.8	4.7
NOV 1/93	339	8.2	1.0	8.3	11.8
NOV 18/93	355	7.2	0.6	3.3	2.7

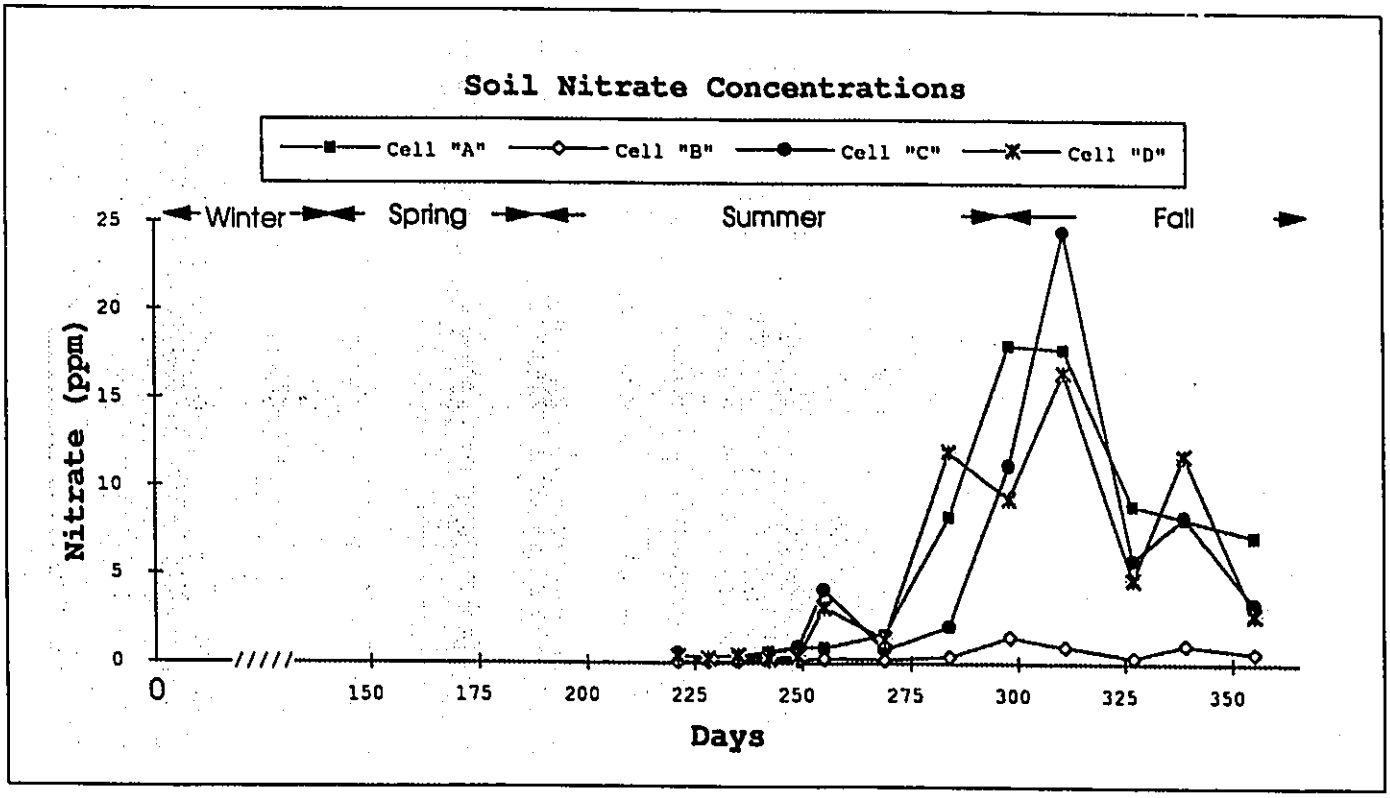


TABLE C14
SOIL PHOSPHOROUS (P) CONCENTRATIONS

DATE	DAYS	CELL A AVG P (ppm)	CELL B AVG P (ppm)	CELL C AVG P (ppm)	CELL D AVG P (ppm)
NOV 27/92	0	7.2	5.9	7.8	11.6
MAY 6/93	160	5.4	1.7	3.7	7.0
MAY 14/93	168	2.9	2.6	2.3	3.2
MAY 20/93	174	3.3	2.7	4.5	1.8
MAY 25/93	179	3.8	4.7	1.9	3.9
MAY 31/93	185	3.9	1.6	1.8	2.5
JUNE 6/93	192	3.6	5.1	3.5	2.4
JUNE 14/93	200	6.6	1.9	3.7	4.2
JUNE 21/93	207	3.8	2.8	1.9	1.3
JUNE 28/93	214	7.7	5.3	3.4	4.0
JULY 5/93	221	4.4	3.1	4.0	3.5
JULY 12/93	228	5.1	2.5	3.1	4.0
JULY 19/93	235	4.3	3.7	3.9	2.7
JULY 26/93	242	5.5	4.3	5.7	6.2
AUG 3/93	249	4.8	3.8	4.0	2.9
AUG 9/93	255	4.9	10.9	6.2	7.4
AUG 23/93	269	3.8	1.3	3.1	2.6
SEPT 7/93	284	5.6	4.9	2.2	6.4
SEPT 21/93	298	2.7	2.8	2.0	2.4
OCT 4/93	311	6.6	7.5	6.0	6.4
OCT 20/93	327	5.0	3.2	4.1	3.0
NOV 1/93	339	4.2	3.1	2.8	3.0
NOV 18/93	355	6.2	3.4	5.7	4.5

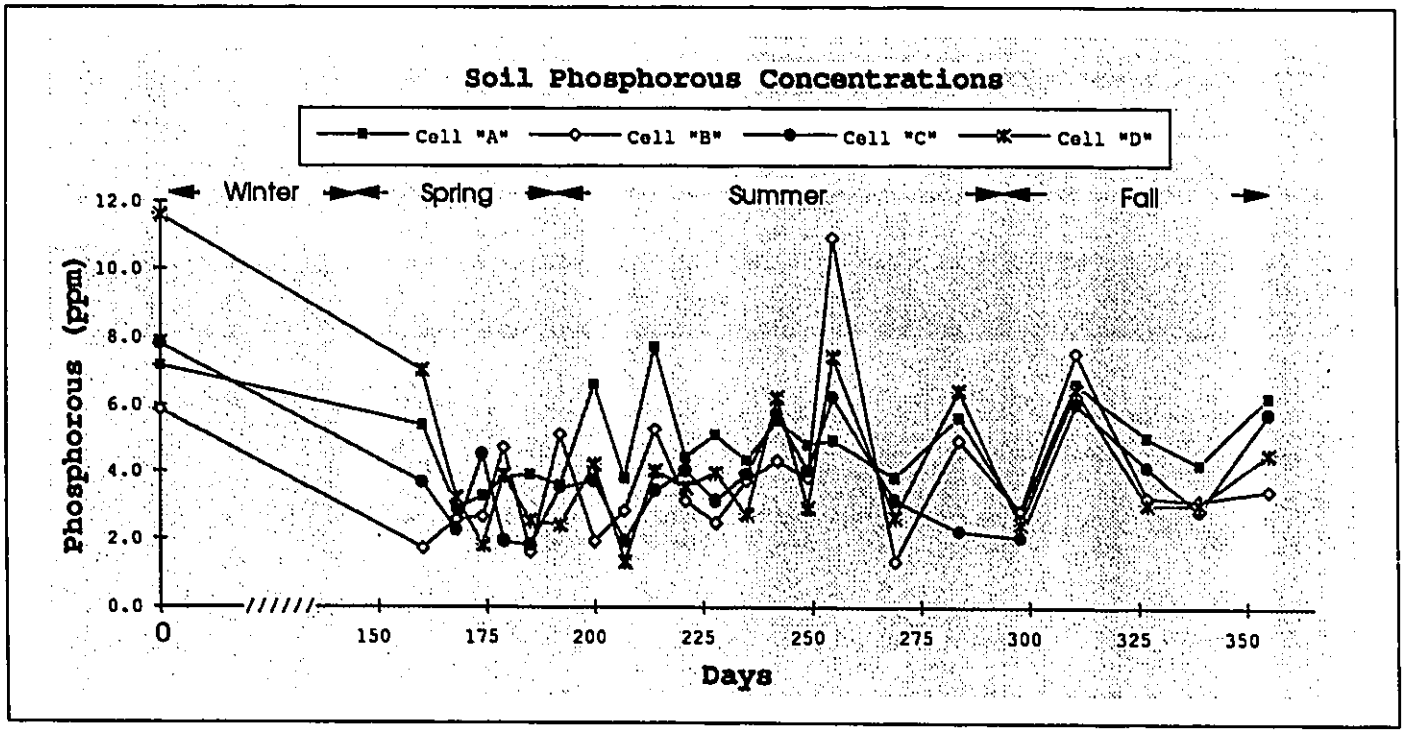


TABLE C15
SOIL POTASSIUM (K) CONCENTRATIONS

DATE	DAYS	CELL A AVG K (ppm)	CELL B AVG K (ppm)	CELL C AVG K (ppm)	CELL D AVG K (ppm)
NOV 27/92	0	16.0	22.0	19.0	22.5
MAY 6/93	160	18.0	23.5	25.5	19.5
MAY 14/93	168	17.0	18.0	20.5	25.5
MAY 20/93	174	17.0	22.0	21.0	27.0
MAY 25/93	179	14.0	18.0	18.0	19.5
MAY 31/93	185	15.0	16.5	23.5	23.0
JUNE 6/93	192	15.0	23.5	24.5	25.5
JUNE 14/93	200	17.0	22.5	20.0	36.5
JUNE 21/93	207	16.0	26.5	25.5	25.5
JUNE 28/93	214	18.0	17.0	21.0	31.0
JULY5/93	221	16.5	20.0	27.5	25.0
JULY12/93	228	15.0	23.5	30.0	23.0
JULY 19/93	235	15.0	14.5	15.0	24.5
JULY 26/93	242	21.0	24.0	26.0	35.0
AUG 3/93	249	17.0	23.0	23.0	22.5
AUG 9/93	255	18.0	21.5	31.5	32.0
AUG 23/93	269	20.0	24.5	24.0	30.0
SEPT 7/93	284	18.8	23.5	22.0	31.0
SEPT 21/93	298	19.8	31.5	22.5	34.5
OCT 4/93	311	23.4	26.0	24.0	27.5
OCT 20/93	327	16.0	18.0	19.5	21.5
NOV 1/93	339	18.0	23.5	22.0	24.0
NOV 18/93	355	18.5	16.0	23.0	28.0

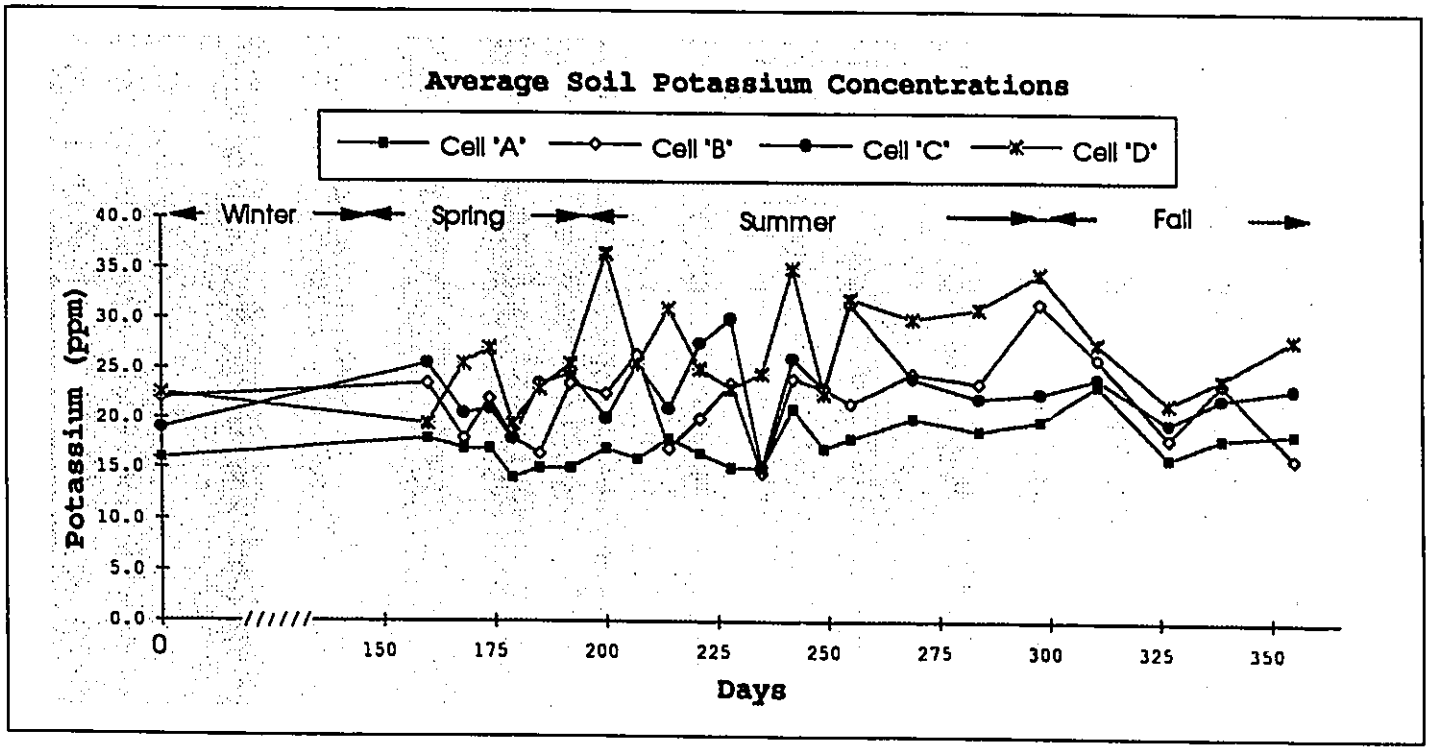


TABLE C16
SOIL pH LEVELS

DATE	DAYS	CELL A AVG. pH	CELL B AVG. pH	CELL C AVG. pH	CELL D AVG. pH
NOV 27/92	0	5.80	6.40	6.05	6.30
MAY 6/93	160	7.40	7.30	6.95	7.10
MAY 14/93	168	6.35	6.40	6.25	6.80
MAY 20/93	174	6.40	6.95	6.35	6.60
MAY 25/93	179	6.00	6.20	6.05	6.45
MAY 31/93	185	6.20	6.50	6.45	6.50
JUNE 6/93	192	6.10	6.20	6.75	6.70
JUNE 14/93	200	5.85	6.55	6.40	6.35
JUNE 21/93	207	5.90	5.00	6.35	6.40
JUNE 28/93	214	5.95	6.55	6.65	6.70
JULY 5/93	221	5.50	5.80	5.65	6.25
JULY 12/93	228	5.95	6.75	6.40	6.50
JULY 19/93	235	5.85	6.00	6.25	5.95
JULY 26/93	242	6.45	5.10	6.25	6.85
AUG 3/93	249	5.65	7.10	6.15	6.70
AUG 9/93	255	5.60	6.45	6.10	6.20
AUG 23/93	269	5.25	6.35	6.05	5.90
SEPT 7/93	284	5.70	6.25	6.20	6.20
SEPT 21/93	298	5.80	6.90	6.25	6.60
OCT 4/93	311	5.65	6.80	5.50	6.45
OCT 20/93	327	5.70	6.35	6.20	6.65
NOV 1/93	339	6.00	6.65	6.10	6.20
NOV 18/93	355	5.45	6.70	6.10	6.35

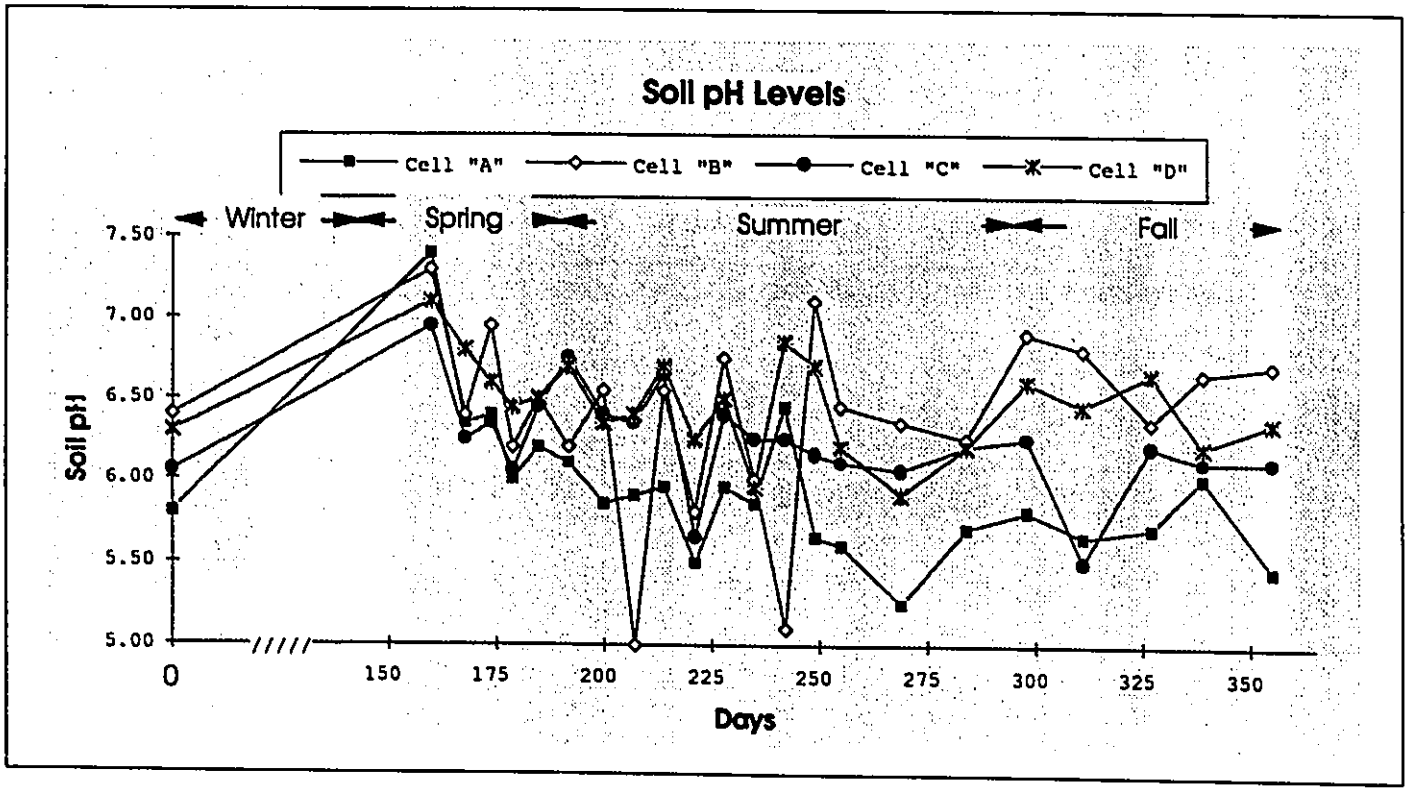


TABLE C17

CELL A
SUMMARY OF SOIL ANALYTICAL RESULTS
BIOREMEDIATION FACILITY - C.F.B. PETAWAWA

DATE	DAYS	SAMPLE	TOTAL BACTERIA (cts/gm)	TPH (ppm)	AVERAGE TPH (ppm)	BTEX (ppm)	MOISTURE CONTENT (%)	pH	T.K.N. (%N)	NITRATE (ppm)	EXTRACTABLE PHOSPHOROUS (ppm)	EXTRACTABLE POTASIUM (ppm)
NOV 27/92	0	A1	9000	479	281	N/A	6.4	5.8	0.028	N/A	7.4	17
		A2	6000	82		N/A	10.8	5.8	0.033	N/A	6.9	15
MAY 6/93	160	A1	108000	42	113	0.05	9.3	7.7	0.023	N/A	5.4	20
		A2	38000	184		0.05	7.2	7.1	0.018	N/A	5.1	16
MAY 14/93	168	A1	56000	48	83	0.05	8.8	6.6	0.016	N/A	2.6	18
		A2	79000	118		0.05	5.7	6.1	0.021	N/A	3.2	16
MAY 20/93	174	A1	45000	24	54	N/A	8.7	6.2	0.017	N/A	3.1	18
		A2	143000	83		N/A	11.1	6.6	0.009	N/A	2	19
MAY 25/93	179	A1	55000	66	99	0.05	9.4	6.0	0.012	N/A	4.2	15
		A2	68000	132		0.05	10.1	6.0	0.013	N/A	3.4	13
MAY 31/93	185	A1	47000	78	69	N/A	7.2	5.9	0.017	N/A	3.6	14
		A2	74000	60		N/A	8.9	6.5	0.012	N/A	2.2	16
JUNE 6/93	192	A1	46000	17	44	N/A	8.7	6	0.026	N/A	4	14
		A2	31000	70		N/A	6.7	6.2	0.012	N/A	3.2	16
JUNE 14/93	200	A1	86000	25	40	N/A	6.5	5.7	0.016	N/A	5.4	16
		A2	60000	54		N/A	6.7	6	0.013	N/A	7.8	18
JUNE 21/93	207	A1	30000	51	73	N/A	8.6	5.6	0.022	N/A	5	16
		A2	26000	95		N/A	7.8	6.2	0.011	N/A	2.6	16
JUNE 28/93	214	A1	56000	42	40	N/A	8.2	5.9	N/A	2.29	9.6	20
		A2	52000	38		N/A	8.7	6	N/A	2.11	5.8	16
JULY 5/93	221	A1	52000	18	34	N/A	7.1	5.4	0.015	0.29	5	17
		A2	90000	50		N/A	10.4	5.6	0.016	0.38	3.8	16
JULY 12/93	228	A1	52000	12	80	N/A	7.5	5.9	0.019	0.41	5.6	15
		A2	57000	147		N/A	6.2	6	0.018	0.24	4.6	15
JULY 19/93	234	A1	42000	50	70	N/A	7.1	5.6	N/A	0.62	4	16
		A2	40000	90		N/A	6.2	6.1	N/A	0.12	4.6	14
JULY 26/93	241	A1	37000	31	42	N/A	5.7	6.2	N/A	0.64	6	23
		A2	73000	52		N/A	6.4	6.5	N/A	0.54	5	19
AUG 3/93	249	A1	25000	15	38	N/A	11.2	5.6	N/A	1.09	5.4	19
		A2	189000	62		N/A	6.4	5.7	N/A	0.67	4.1	15
AUG 9/93	255	A1	68000	5	33	N/A	7.1	5.2	N/A	1.3	5	19
		A2	72000	61		N/A	5.7	6	N/A	0.4	4.8	16
AUG 23/93	269	A1	47000	5	13	N/A	7.5	5.2	N/A	1.5	4	20
		A2	39000	20		N/A	6.9	5.3	N/A	1.6	3.6	19
SEPT 7/93	284	A1	52000	28	29	N/A	9.7	5.9	N/A	10.1	5.8	20
		A2	48000	29		N/A	7.7	5.5	N/A	6.5	5.4	17.5
SEPT 21/93	298	A1	NA	15	14	N/A	8.1	5.4	N/A	24	3.6	19.5
		A2	NA	13		N/A	5.7	6.2	N/A	12	1.8	20
OCT 4/93	311	A1	NA	5	5	N/A	6.1	5.9	N/A	16.3	6.2	18.6
		A2	NA	5		N/A	8	5.4	N/A	18.6	7	28
OCT 20/93	327	A1	58000	5	5	N/A	7.9	5.8	N/A	11.5	5.6	16.5
		A2	80000	5		N/A	7.2	5.6	N/A	6.4	4.4	15.5
NOV 1/93	339	A1	25000	5	5	N/A	5.1	6	N/A	11.8	5	19.3
		A2	51000	5		N/A	5.3	6	N/A	4.6	3.4	16.8
NOV 18/93	355	A1	60000	5	5	N/A	6.1	5.4	N/A	9.9	7.6	19
		A2	119000	5		N/A	5.5	5.5	N/A	4.4	4.8	18

TABLE C18

CELL B
SUMMARY OF SOIL ANALYTICAL RESULTS
BIOREMEDIATION FACILITY - C.F.B. PETAWAWA

DATE	DAYS	SAMPLE	TOTAL BACTERIA (cts/gm)	TPH (ppm)	BTEX (ppm)	AVERAGE TPH (ppm)	MOISTURE CONTENT (%)	pH	T.K.N. (%N)	NITRATE (ppm)	EXTRACTABLE PHOSPHOROUS (ppm)	EXTRACTABLE POTASIUM (ppm)
NOV 27/92	0	B1	40000	46	N/A	1211	4.9	6.5	0.006	N/A	6.3	22
		B2	61000	2375	N/A		6.5	6.3	0.013	N/A	5.4	22
MAY 6/93	160	B1	130000	1210	0.05	628	5.3	7.1	0.009	N/A	1.4	21
		B2	130000	46	0.05		5.5	7.5	0.014	N/A	2.2	26
MAY 14/93	168	B1	158000	524	0.05	291	4.1	6.2	0.007	N/A	2.2	23
		B2	104000	58	0.05		4.8	6.6	0.008	N/A	2.9	13
MAY 20/93	174	B1	160000	278	N/A	189	6.1	7.2	0.011	N/A	2.7	26
		B2	144000	100	N/A		4.9	6.7	0.012	N/A	2.6	24
MAY 25/93	179	B1	72000	85	0.05	335	6.1	6.2	0.018	N/A	5.2	19
		B2	71000	585	0.05		5.5	6.2	0.012	N/A	4.2	17
MAY 31/93	185	B1	99000	50	N/A	134	6.9	7	0.009	N/A	1.8	15
		B2	46000	217	N/A		7.7	6	0.01	N/A	1.4	18
JUNE 6/93	192	B1	59000	613	N/A	348	4.7	6.2	0.02	N/A	3.6	24
		B2	103000	83	N/A		6.9	6.2	0.005	N/A	6.6	23
JUNE 14/93	200	B1	54000	58	N/A	574	4.5	6.6	0.008	N/A	2.4	22
		B2	61000	1090	N/A		7.7	6.5	0.009	N/A	1.4	23
JUNE 21/93	207	B1	28000	374	N/A	262	5.4	5.8	0.021	N/A	4.2	20
		B2	156000	150	N/A		5.6	4.2	0.009	N/A	1.4	33
JUNE 28/93	214	B1	139000	580	N/A	322	6.6	6.4	N/A	1.22	5	22
		B2	29000	64	N/A		4.8	6.7	N/A	1.15	5.5	12
JULY 5/93	221	B1	155000	143	N/A	103.5	4.6	5.9	0.013	0.05	3.2	22
		B2	61000	64	N/A		4.4	5.7	0.009	0.025	3.0	18
JULY 12/93	228	B1	86000	913	N/A	533.5	5.9	7.1	0.01	0.08	2.2	28
		B2	55000	154	N/A		5.4	6.4	0.01	0.06	2.7	19
JULY 19/93	234	B1	94000	300	N/A	250	6.8	5.9	N/A	0.09	4.8	23
		B2	54000	200	N/A		4.4	6.1	N/A	0.11	2.6	6
JULY 26/93	241	B1	92000	47	N/A	49	5.5	5.8	N/A	0.2	3.6	25
		B2	59000	51	N/A		6	4.4	N/A	0.06	5	23
AUG 3/93	249	B1	63000	120	N/A	93	8.3	6.8	N/A	0.15	5.3	22
		B2	6500	65	N/A		6.1	7.4	N/A	0.13	2.4	24
AUG 10/93	255	B1	51000	68	N/A	103	6.2	6.6	N/A	0.2	8.2	24
		B2	98000	138	N/A		4.4	6.3	N/A	0.3	3.6	19
AUG 23/93	269	B1	52000	34	N/A	59	7.1	6.3	N/A	0.24	1.4	28
		B2	63000	83	N/A		6.8	6.4	N/A	0.11	1.2	21
SEPT 7/93	284	B1	61000	16	N/A	56	8.5	6.4	N/A	0.47	7	25
		B2	58000	96	N/A		5.8	6.1	N/A	0.22	2.8	22
SEPT 21/93	298	B1	NA	20	N/A	23	1.2	6.9	N/A	1.3	2	24
		B2	NA	26	N/A		8.6	6.9	N/A	1.6	3.6	39
OCT 4/93	311	B1	NA	22	N/A	14	5.8	6.8	N/A	1.3	6.6	33
		B2	NA	5	N/A		5.1	6.8	N/A	0.5	8.4	19.3
OCT 20/93	327	B1	50000	37	N/A	74	6	6.2	N/A	0.3	4.2	17
		B2	128000	110	N/A		5.2	6.5	N/A	0.3	2.2	19
NOV 1/93	339	B1	23000	5	N/A	15	4.3	6.6	N/A	0.64	3.8	22
		B2	44000	24	N/A		6.1	6.7	N/A	1.41	2.4	25
NOV 18/93	355	B1	105000	5	N/A	41	1.1	6.7	N/A	0.76	2.4	20
		B2	65000	77	N/A		5.6	6.7	N/A	0.26	4.2	12

TABLE C19

CELL C
SUMMARY OF SOIL ANALYTICAL RESULTS
BIOREMEDIATION FACILITY - C.F.B. PETAWAWA

DATE	DAYS	SAMPLE	TOTAL BACTERIA (cfs/gm)	TPH (ppm)	AVERAGE TPH (ppm)	BTEX (ppm)	MOISTURE CONTENT (%)	pH	T.K.N. (%N)	NITRATE (ppm)	EXTRACTABLE PHOSPHOROUS (ppm)	EXTRACTABLE POTASIUM (ppm)
NOV 27/92	0	C1	20000	61	41	N/A	7.5	6	0.024	N/A	8.5	11
		C2	88000	20		N/A	5.1	6.1	0.013	N/A	7.1	27
MAY 6/93	160	C1	52000	237	149	0.05	10.2	6.7	0.051	N/A	2.9	23
		C2	60000	60		0.05	5.7	7.2	0.025	N/A	4.4	28
MAY 14/93	168	C1	97000	50	28	0.05	11.9	6.2	0.01	N/A	2.2	22
		C2	75000	5		0.05	5.1	6.3	0.006	N/A	2.3	19
MAY 20/93	174	C1	98000	73	137	N/A	4.8	6.3	0.037	N/A	4	21
		C2	53000	200		N/A	6.4	6.4	0.034	N/A	5	21
MAY 25/93	179	C1	45000	190	535	0.05	5.9	6.3	0.007	N/A	2	18
		C2	68000	880		0.05	6.3	5.8	0.013	N/A	1.8	18
MAY 31/93	185	C1	298000	84	360	N/A	7	6.5	0.013	N/A	1.8	20
		C2	63000	635		N/A	8.5	6.4	0.016	N/A	1.8	27
JUNE 6/93	192	C1	60000	36	93	N/A	6.1	7	0.012	N/A	2.6	26
		C2	28000	150		N/A	7.8	6.5	0.025	N/A	4.4	23
JUNE 14/93	200	C1	54000	635	425	N/A	7.1	6.2	0.015	N/A	2.6	19
		C2	67000	215		N/A	6.2	6.6	0.014	N/A	4.8	21
JUNE 21/93	207	C1	82000	5	112	N/A	5.4	6	0.004	N/A	1.2	23
		C2	32000	218		N/A	5.6	6.7	0.01	N/A	2.6	28
JUNE 28/93	214	C1	58000	1065	1367.5	N/A	7.3	6.7	1.26	N/A	4	22
		C2	135000	1670		N/A	5.2	6.6	2.33	N/A	2.8	20
JULY 5/93	221	C1	115000	5	15	N/A	4.3	5.5	0.012	0.48	5.4	20
		C2	59000	25		N/A	6.1	5.8	0.036	0.45	2.6	35
JULY 12/93	228	C1	43000	304	682	N/A	6.1	6.4	0.012	0.09	3.2	34
		C2	38000	1060		N/A	6.1	6.4	0.01	0.06	3	26
JULY 19/93	234	C1	53000	28	95	N/A	4.6	6.1	N/A	0.09	4.4	11
		C2	54000	162		N/A	6.3	6.4	N/A	0.11	2.4	19
JULY 26/93	241	C1	56000	118	83	N/A	6.5	7	N/A	0.12	4.4	25
		C2	16000	47		N/A	6.6	5.5	N/A	0.88	7	27
AUG 3/93	249	C1	32000	46	42	N/A	5.8	6	N/A	0.99	3.9	19
		C2	65000	38		N/A	7.8	6.3	N/A	0.73	4	27
AUG 9/93	255	C1	50000	16	17.5	N/A	6.9	6.3	N/A	3.8	5.2	30
		C2	108000	19		N/A	7	5.9	N/A	4.4	7.2	33
AUG 23/93	269	C1	87000	76	470	N/A	7	6	N/A	0.6	4.4	22
		C2	37000	863		N/A	7.2	6.1	N/A	0.9	2	26
SEPT 7/93	284	C1	101000	43	38	N/A	5.7	6.1	N/A	3.7	3	20
		C2	82000	32		N/A	4.9	6.3	N/A	0.34	1.4	24
SEPT 21/93	298	C1	NA	93	140	N/A	5	6.7	N/A	6.2	1	20
		C2	NA	187		N/A	5.7	5.8	N/A	16.2	3	25
OCT 4/93	311	C1	NA	5	5	N/A	7.1	5.7	N/A	26	4.4	26
		C2	NA	5		N/A	8.3	5.3	N/A	23	7.6	22
OCT 20/93	327	C1	92000	23	25	N/A	7.5	6	N/A	9.5	3.8	21
		C2	47000	26		N/A	5.4	6.4	N/A	2	4.4	18
NOV 1/93	339	C1	41000	5	5	N/A	4.2	6.2	N/A	7.4	2.4	17
		C2	43000	5		N/A	5.7	6	N/A	9.1	3.2	27
NOV 18/93	355	C1	43000	5	5	N/A	4.3	5.9	N/A	3.1	7.6	21
		C2	72000	5		N/A	4.3	6.3	N/A	3.4	3.8	25

TABLE C20

CELL D
SUMMARY OF SOIL ANALYTICAL RESULTS
BIOREMEDIATION FACILITY - C.F.B. PETAWAWA

DATE	DAYS	SAMPLE	TOTAL BACTERIA (cts/gm)	TPH (ppm)	AVERAGE TPH (ppm)	BTEX (ppm)	MOISTURE CONTENT (%)	pH	T.K.N. (%N)	NITRATE (ppm)	EXTRACTABLE PHOSPHOROUS (ppm)	EXTRACTABLE POTASIUM (ppm)
NOV 27/92	0	D1	38000	591	751	0.05	7.6	6.2	0.018	N/A	11.5	20
		D2	24000	911		0.05	6.7	6.4	0.022	N/A	7	25
MAY 6/93	160	D1	75000	500	743	0.05	5.9	7.2	0.007	N/A	3.6	18
		D2	100000	985		0.05	5.6	7	0.015	N/A	2.6	21
MAY 14/93	168	D1	61000	1210	658	0.05	5.4	6.9	0.021	N/A	2.6	25
		D2	73000	105		0.05	4.7	6.7	0.009	N/A	3.8	26
MAY 20/93	174	D1	161000	865	853	N/A	5.2	6.5	0.013	N/A	2	28
		D2	196000	840		N/A	6.2	6.7	0.01	N/A	1.6	26
MAY 25/93	179	D1	21000	180	768	0.05	6.4	6.3	0.014	N/A	4.8	15
		D2	70000	1355		0.05	6.9	6.6	0.017	N/A	3	24
MAY 31/93	185	D1	32000	97	401	N/A	7.4	6.6	0.016	N/A	3	18
		D2	64000	705		N/A	10	6.4	0.011	N/A	2	28
JUNE 6/93	192	D1	53000	277	404	N/A	8.2	6.9	0.007	N/A	2.2	21
		D2	59000	530		N/A	7.2	6.5	0.009	N/A	2.6	30
JUNE 14/93	200	D1	48000	52	146	N/A	6.3	6.6	0.018	N/A	5.2	26
		D2	91000	240		N/A	8.6	6.1	0.012	N/A	3.2	47
JUNE 21/93	207	D1	41000	865	694	N/A	6.6	6.7	0.055	N/A	1.4	28
		D2	92000	522		N/A	7.2	6.1	0.021	N/A	1.2	23
JUNE 28/93	214	D1	65000	645	376.5	N/A	7.7	6.7	N/A	1.5	4	40
		D2	49000	108		N/A	5.3	6.7	N/A	1.44	4	22
JULY 5/93	221	D1	64000	155	202.5	N/A	5.3	6.2	0.014	0.39	3.5	24
		D2	73000	250		N/A	5.8	6.3	0.015	0.27	3.5	26
JULY 12/93	228	D1	30000	94	93.5	N/A	4.8	6.5	0.012	0.41	3.7	28
		D2	27000	93		N/A	5.4	6.5	0.013	0.07	4.2	18
JULY 19/93	234	D1	41000	570	377.5	N/A	6.7	5.9	N/A	0.75	2	26
		D2	57000	185		N/A	4.5	6	N/A	0.07	3.4	23
JULY 26/93	241	D1	43000	73	135.5	N/A	6.5	7	N/A	0.41	5.6	26
		D2	42000	198		N/A	5.3	6.7	N/A	0.09	6.8	44
AUG 3/93	249	D1	130000	255	615	N/A	5.4	6.7	N/A	0.43	2.8	23
		D2	44000	975		N/A	7.1	6.7	N/A	0.23	2.9	22
AUG 9/93	255	D1	64000	13	58	N/A	8.8	6.1	N/A	3.6	10.4	29
		D2	55000	103		N/A	7.9	6.3	N/A	2.6	4.4	35
AUG 23/93	269	D1	61000	25	213	N/A	9.5	5.7	N/A	2.2	3.8	34
		D2	25000	400		N/A	6.2	6.1	N/A	0.4	1.4	26
SEPT 7/93	284	D1	133000	124	82	N/A	8.5	6.1	N/A	13.5	4.8	28
		D2	81000	40		N/A	8.6	6.3	N/A	10.4	8	34
SEPT 21/93	298	D1	NA	39	32	N/A	5.3	6.6	N/A	12.2	2.4	34
		D2	NA	25		N/A	6	6.6	N/A	6.4	2.4	35
OCT 4/93	311	D1	NA	37	100	N/A	6.6	6.3	N/A	16.6	3.8	28
		D2	NA	163		N/A	7.3	6.6	N/A	16.4	9	27
OCT 20/93	327	D1	135000	37	28	N/A	4.5	6.9	N/A	0.3	1.8	17
		D2	112000	18		N/A	5.3	6.4	N/A	9.4	4.2	26
NOV 1/93	339	D1	26000	5	5	N/A	3.7	6.4	N/A	5.9	1.6	21
		D2	31000	5		N/A	6.3	6	N/A	17.8	4.4	27
NOV 18/93	355	D1	113000	29	23	N/A	5.1	6.2	N/A	2.4	5.2	34
		D2	40000	16		N/A	4.5	6.5	N/A	3	3.6	22

Table C21
DAILY VOLUME OF AMENDED WATER ADDED TO CELLS

DATE	DAYS	CELL A FLOWRATE (M^3/DAY)	CELL B FLOWRATE (M^3/DAY)	CELL C FLOWRATE (M^3/DAY)	CELL D FLOWRATE (M^3/DAY)	DATE	DAYS	CELL A FLOWRATE (M^3/DAY)	CELL B FLOWRATE (M^3/DAY)	CELL C FLOWRATE (M^3/DAY)	CELL D FLOWRATE (M^3/DAY)
NOV 27/93	0	0.000	0.000	0.000	0.000	JULY 19/93	234	4.650	5.120	0.200	5.370
MAY 6/93	160	0.000	0.000	0.000	0.000	JULY 20/93	235	3.990	4.690	2.460	4.790
MAY 14/93	168	0.000	0.000	0.000	0.000	JULY 21/93	236	3.890	4.620	2.390	4.710
MAY 20/93	174	0.000	0.000	0.000	0.000	JULY 22/93	237	4.170	4.560	2.360	5.180
MAY 25/93	179	1.962	1.906	2.150	1.966	JULY 23/93	238	4.470	4.890	2.530	5.390
JUNE 8/93	193	0.185	0.928	5.484	5.638	JULY 26/93	241	4.027	4.737	1.880	5.133
JUNE 9/93	194	0.000	0.676	9.071	0.245	JULY 27/93	242	4.980	5.120	4.770	5.870
JUNE 10/93	195	0.000	0.477	3.129	0.000	JULY 28/93	243	4.640	4.680	2.760	5.280
JUNE 11/93	196	0.000	0.580	0.000	0.000	JULY 29/93	244	4.480	4.600	3.950	5.220
JUNE 14/93	199	0.000	0.577	0.000	0.000	JULY 30/93	245	4.500	4.730	3.930	5.290
JUNE 15/93	200	0.000	15.271	0.000	0.001	AUG 3/93	249	4.368	4.800	4.068	5.363
JUNE 16/93	201	0.000	8.771	0.000	0.000	AUG 4/93	250	4.250	4.340	3.820	4.830
JUNE 17/93	202	0.000	8.396	0.000	0.000	AUG 5/93	251	4.020	4.240	3.500	4.770
JUNE 22/93	207	1.349	7.648	2.542	0.000	AUG 9/93	255	4.490	5.123	9.500	5.665
JUNE 23/93	208	1.954	0.010	0.003	0.000	AUG 10/93	256	5.120	4.800	4.600	5.100
JUNE 24/93	209	9.143	10.694	0.001	0.001	AUG 11/93	257	4.780	4.600	3.900	5.100
JUNE 25/93	210	4.446	0.590	0.000	0.000	AUG 12/93	258	4.400	0.000	3.700	4.900
JUNE 28/93	213	12.844	0.352	0.000	0.000	AUG 13/93	259	5.200	9.900	2.000	5.500
JUNE 29/93	214	11.778	0.794	0.000	16.886	AUG 16/93	262	4.867	5.067	4.667	5.500
JUNE 30/93	215	13.990	1.760	0.000	1.400	AUG 17/93	263	4.900	4.800	3.800	5.300
JULY 5/93	220	0.582	0.148	0.000	0.980	AUG 18/93	264	4.700	5.700	5.800	10.600
JULY 6/93	221	1.000	0.000	0.000	0.100	AUG 19/93	265	4.800	5.400	3.900	10.600
JULY 7/93	222	1.100	0.000	0.000	0.000	AUG 20/93	266	5.000	6.100	4.600	10.400
JULY 8/93	223	2.900	0.000	0.000	0.000	SEPT 28/93	305	7.249	7.177	6.364	2.018
JULY 9/93	224	0.400	0.000	0.000	0.000	SEPT 29/93	306	0.000	0.000	0.000	0.000
JULY 12/93	227	0.600	0.000	0.000	0.000	SEPT 30/93	307	8.900	8.800	7.100	0.000
JULY 13/93	228	0.100	0.240	0.180	0.180	OCT 1/93	308	3.500	4.000	3.300	0.000
JULY 14/93	229	0.170	0.210	0.150	0.300	OCT 12/93	319	2.100	2.000	1.645	0.000
JULY 15/93	230	3.880	4.540	1.290	4.230	OCT 13/93	320	0.000	0.000	0.000	0.000
JULY 16/93	231	3.900	3.980	0.000	4.140						

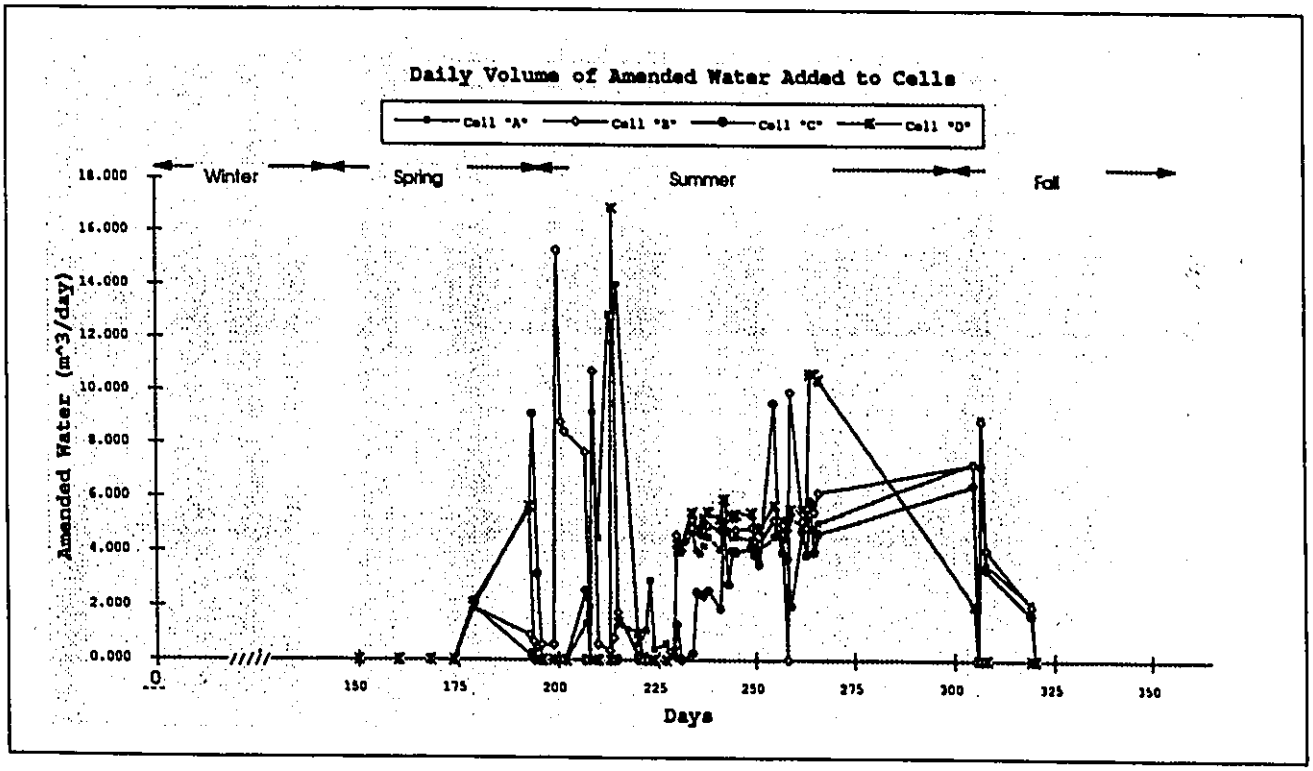


TABLE C22
GRAVIMETRIC SOIL MOISTURE CONTENT (M.C.)

DATE	DAYS	CELL A AVG. M.C. (%)	CELL B AVG. M.C. (%)	CELL C AVG. M.C. (%)	CELL D AVG. M.C. (%)
NOV 27/92	0	8.6	5.7	6.3	7.2
MAY 6/93	160	8.3	5.4	8.0	5.8
MAY 14/93	168	7.3	4.5	8.5	5.1
MAY 20/93	174	9.9	5.5	5.6	5.7
MAY 25/93	179	9.8	5.8	6.1	6.7
MAY 31/93	185	8.1	7.3	7.8	8.7
JUNE 6/93	192	7.7	5.8	6.9	7.7
JUNE 14/93	200	6.6	6.1	6.7	7.5
JUNE 21/93	207	8.2	5.5	5.5	6.9
JUNE 28/93	214	8.5	5.7	6.3	6.5
JULY 5/93	221	8.8	4.5	5.7	5.6
JULY 12/93	228	6.8	5.7	6.1	5.1
JULY 19/93	235	6.6	5.6	5.5	5.6
JULY 26/93	242	6.1	5.8	6.6	5.9
AUG 3/93	249	8.8	7.2	6.8	6.3
AUG 9/93	255	6.4	5.3	7.0	8.4
AUG 23/93	269	7.2	7.0	7.1	7.8
SEPT 7/93	284	8.7	7.2	5.3	8.6
SEPT 21/93	298	6.9	4.9	5.4	5.7
OCT 4/93	311	7.1	5.5	7.7	7.0
OCT 20/93	327	7.7	5.6	6.5	4.9
NOV 1/93	339	5.2	5.2	5.0	5.1
NOV 18/93	352	5.8	3.4	4.3	4.8

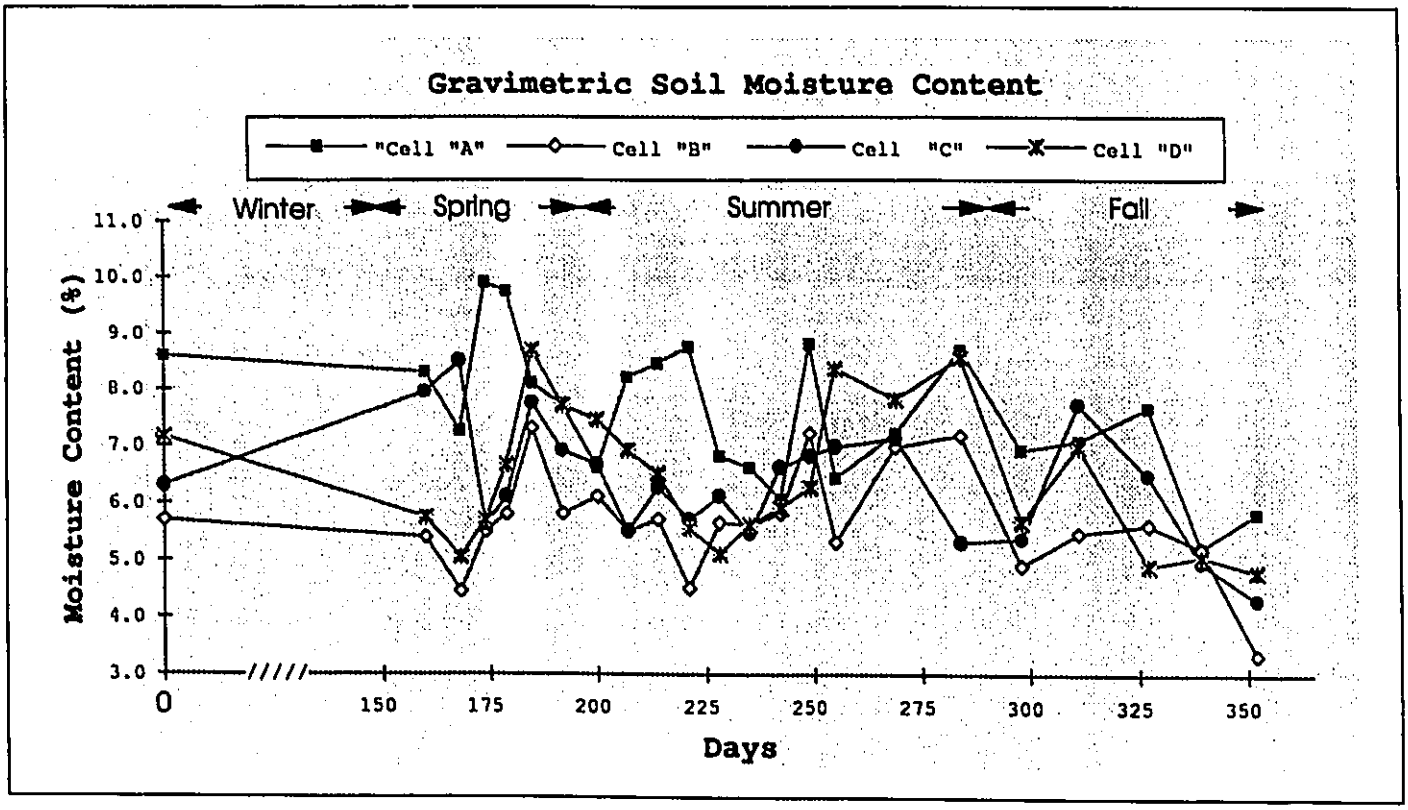


TABLE C23
NITRATE (NO3-N) CONCENTRATION OF AMENDED WATER ADDED TO CELLS

DATE	DAYS	SUMP A NO3-N (ppm)	SUMP B NO3-N (ppm)	SUMP C NO3-N (ppm)	SUMP D NO3-N (ppm)
JUNE 28/93	214	25	0.025	16.5	6
JULY 5/93	221	47	0.025	40	24
JULY 12/93	228	51	0.13	35	33
JULY 19/93	235	63	1.1	47	88
JULY 26/93	242	101	0.28	0.35	111
AUG 3/93	249	13.8	1.41	16	14.5
AUG 23/93	269	54	1.78	2.14	42
SEPT 7/93	284	197	1.7	365	310
SEPT 21/93	298	278	2.4	303	259
OCT 4/93	311	4.4	3.5	31	251

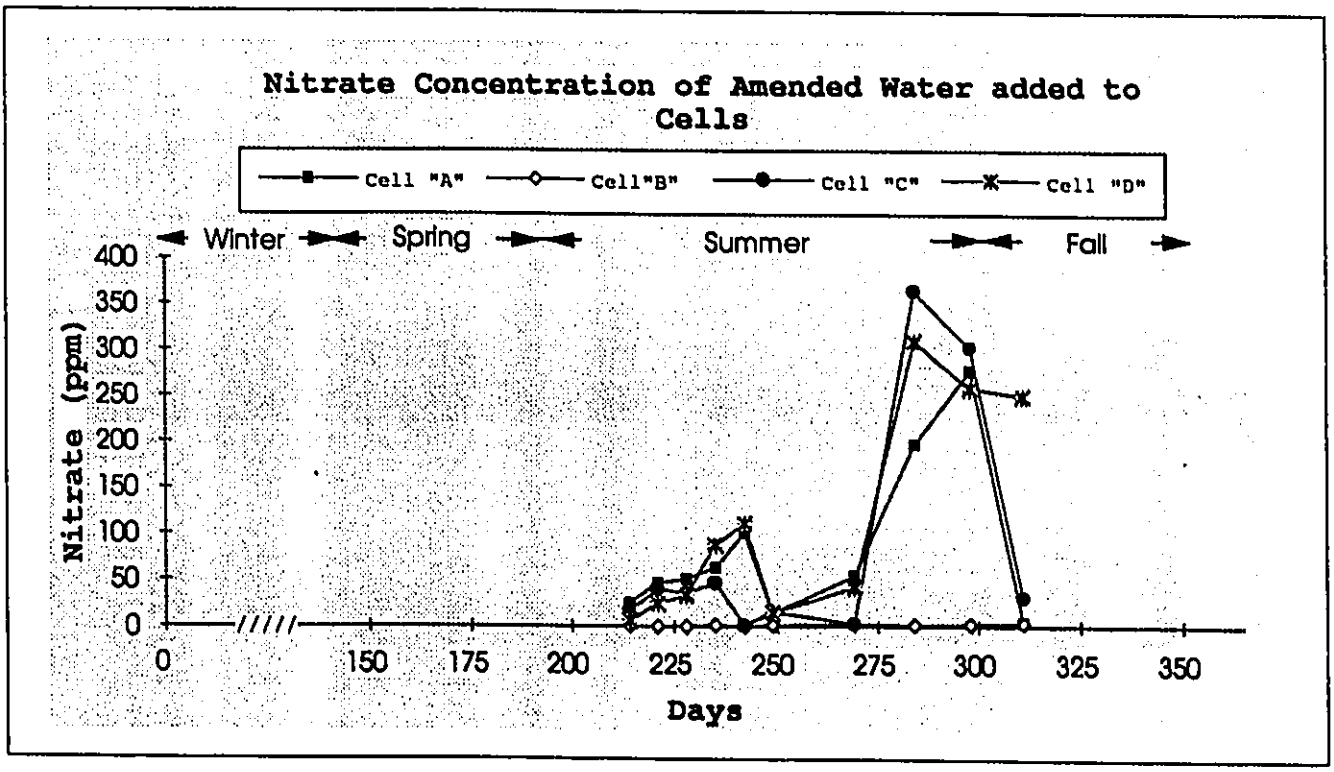


TABLE C24
PHOSPHORUS (P) CONCENTRATION OF AMENDED WATER ADDED TO CELLS

DATE	DAYS	SUMP A PHOS (ppm)	SUMP B PHOS (ppm)	SUMP C PHOS (ppm)	SUMP D PHOS (ppm)
MAY 31/93	185	0.04	0.03	0.09	0.06
JUNE 6/93	191	0.02	0.05	0.15	0.03
JUNE 21/93	207	0.03	0.04	0.04	0.02
JUNE 28/93	214	0.04	0.02	0.08	0.06
JULY 5/93	221	0.04	0.02	0.14	0.08
JULY 12/93	228	0.41	0.45	0.26	0.19
JULY 19/93	235	0.13	0.02	0.59	0.26
JULY 26/93	242	0.47	0.01	0.06	0.17
AUG 3/93	249	0.10	0.02	0.61	0.11
AUG 23/93	269	2.75	0.10	0.25	0.10
SEPT 7/93	284	0.55	0.08	0.75	0.20
SEPT 21/93	298	0.75	0.27	1.10	0.40
OCT 4/93	311	0.45	0.45	1.90	1.00

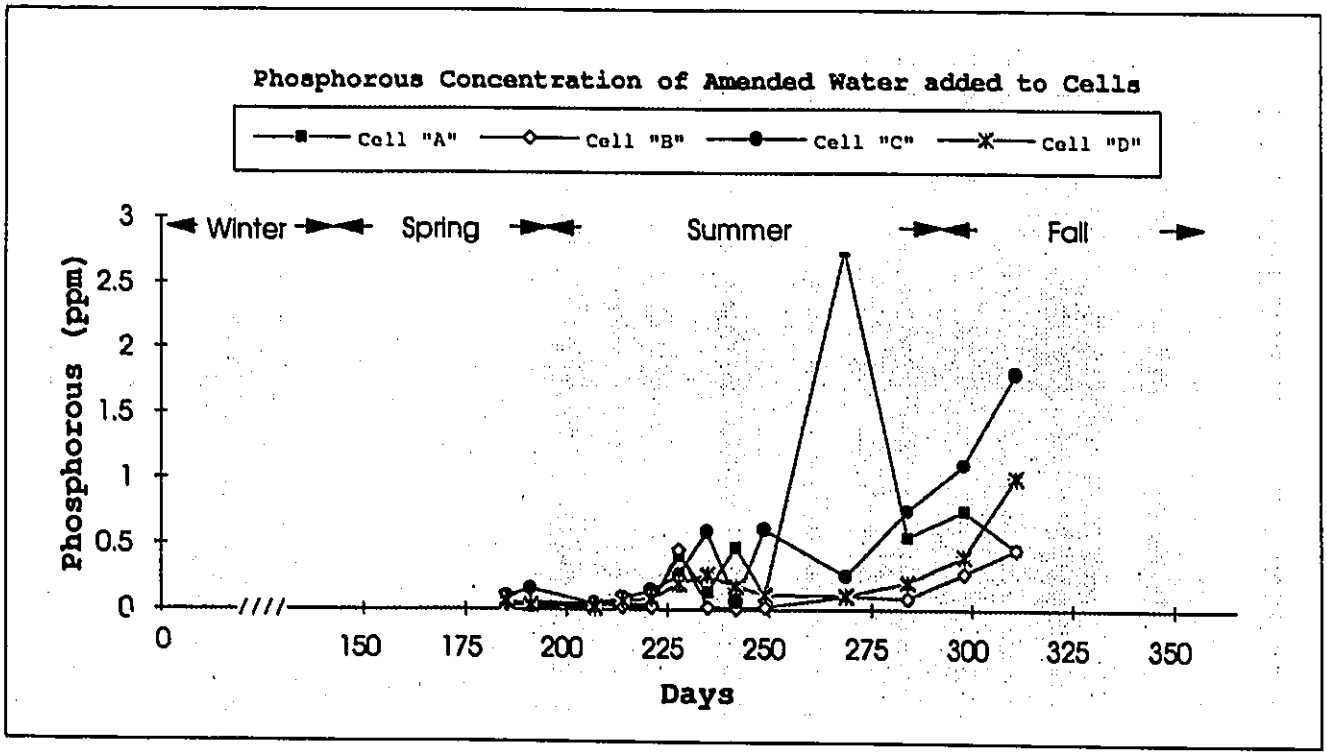


TABLE C25
POTASSIUM (K) CONCENTRATION OF AMENDED WATER ADDED TO CELLS

DATE	DAYS	SUMP A POTASSIUM (ppm)	SUMP B POTASSIUM (ppm)	SUMP C POTASSIUM (ppm)	SUMP D POTASSIUM (ppm)
MAY 31/93	185	3.0	2.3	4.9	4.2
JUNE 6/93	191	3.2	2.1	7.5	7.8
JUNE 21/93	207	2.7	6.5	3.1	2.7
JUNE 28/93	214	6.8	5.5	9.4	7.6
JULY 5/93	221	9.3	5.5	17.5	11.0
JULY 12/93	228	9.3	5.8	17.5	16.0
JULY 19/93	235	9.1	4.2	22.0	20.0
JULY 26/93	242	23.0	6.4	1.0	19.0
AUG 3/93	249	18.4	7.0	26.0	16.6
AUG 23/93	269	58.0	2.0	1.8	5.4
SEPT 7/93	284	23.0	6.2	31.0	24.0
SEPT 21/93	298	16.8	6.4	31.0	23.0
OCT 4/93	311	24.0	5.2	27.0	20.0

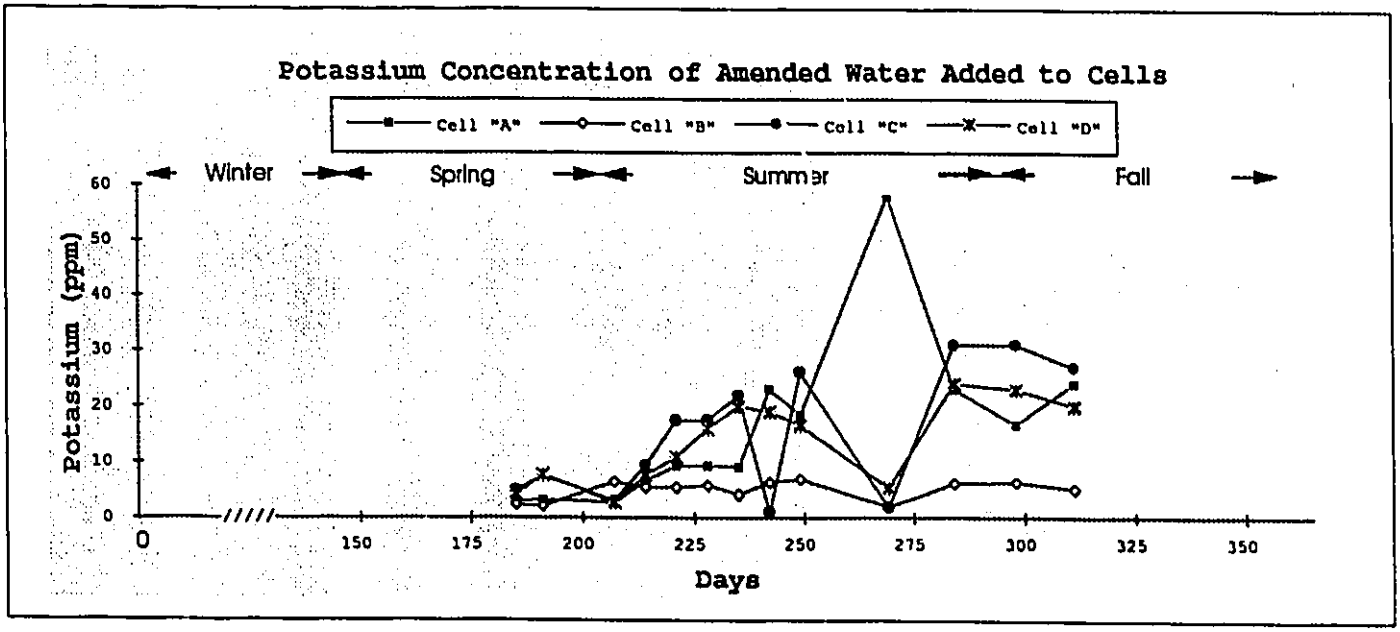
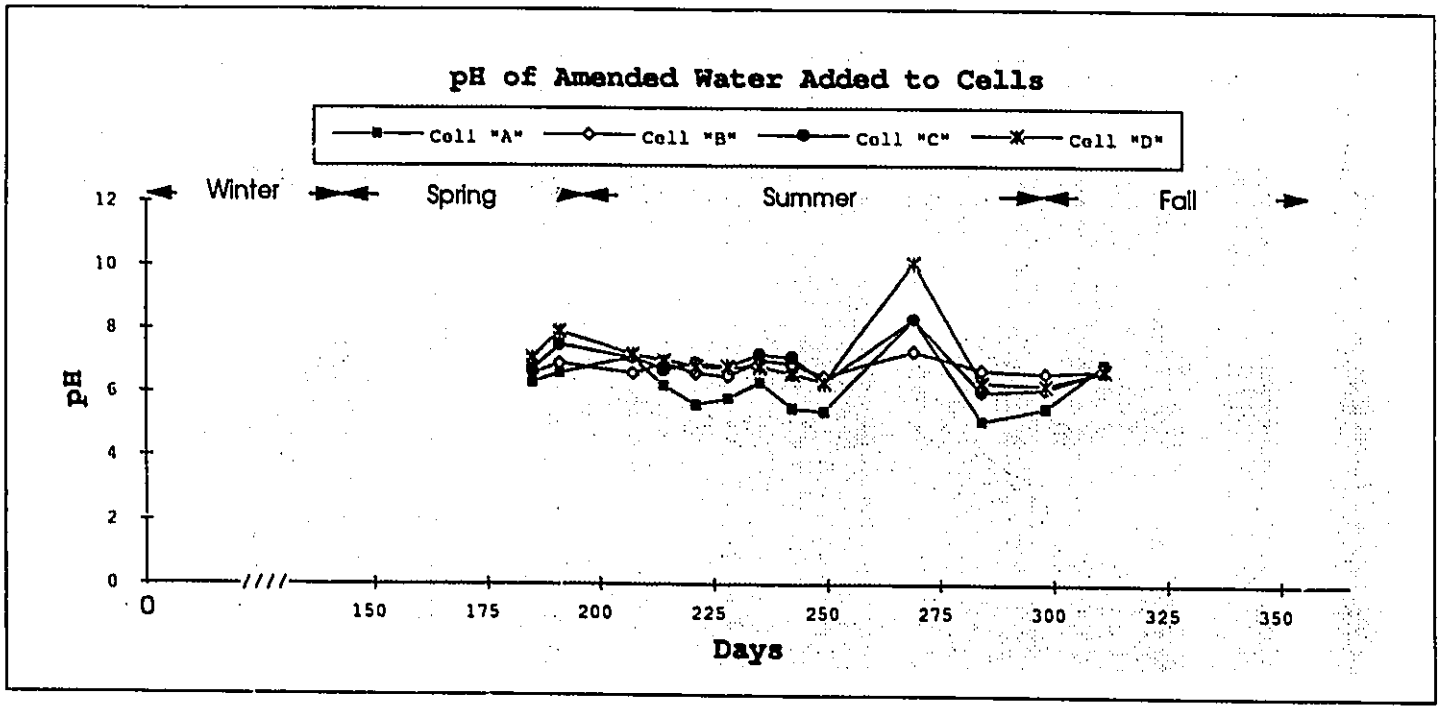


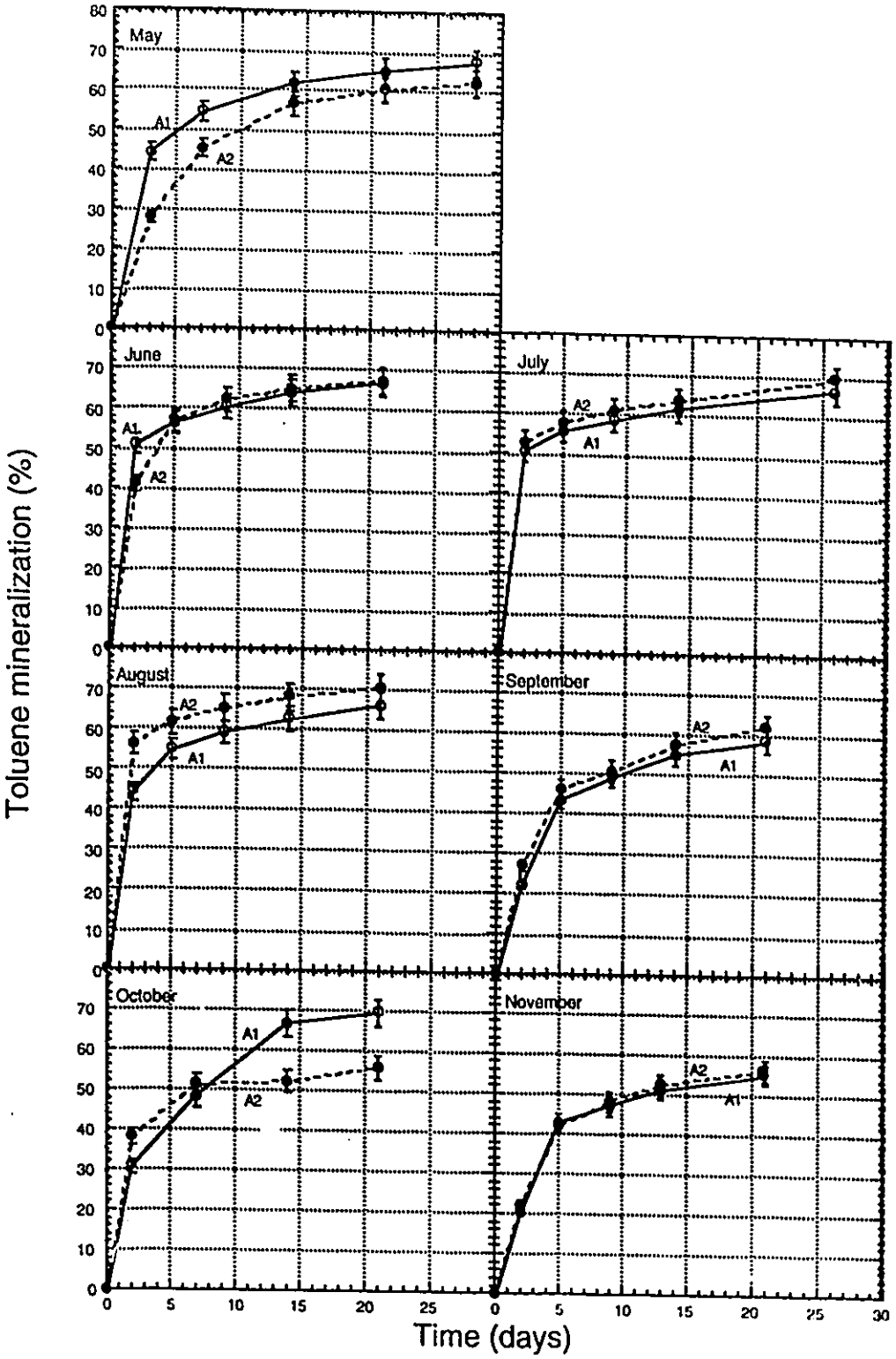
TABLE C26
pH OF AMENDED WATER ADDED TO CELLS

DATE	DAYS	SUMP A pH	SUMP B pH	SUMP C pH	SUMP D pH
MAY 31/93	185	6.3	6.6	6.7	7.1
JUNE 6/93	191	6.6	6.9	7.5	7.9
JUNE 21/93	207	7.1	6.6	7.1	7.2
JUNE 28/93	214	6.2	6.9	6.7	7.0
JULY 5/93	221	5.6	6.6	6.9	6.8
JULY 12/93	228	5.8	6.5	6.8	6.8
JULY 19/93	235	6.3	7.0	7.2	6.8
JULY 26/93	242	5.5	6.9	7.1	6.6
AUG 3/93	249	5.4	6.5	6.3	6.3
AUG 23/93	269	8.3	7.3	8.3	10.1
SEPT 7/93	284	5.1	6.7	6.0	6.3
SEPT 21/93	298	5.5	6.6	6.1	6.2
OCT 4/93	311	6.9	6.7	6.7	6.7



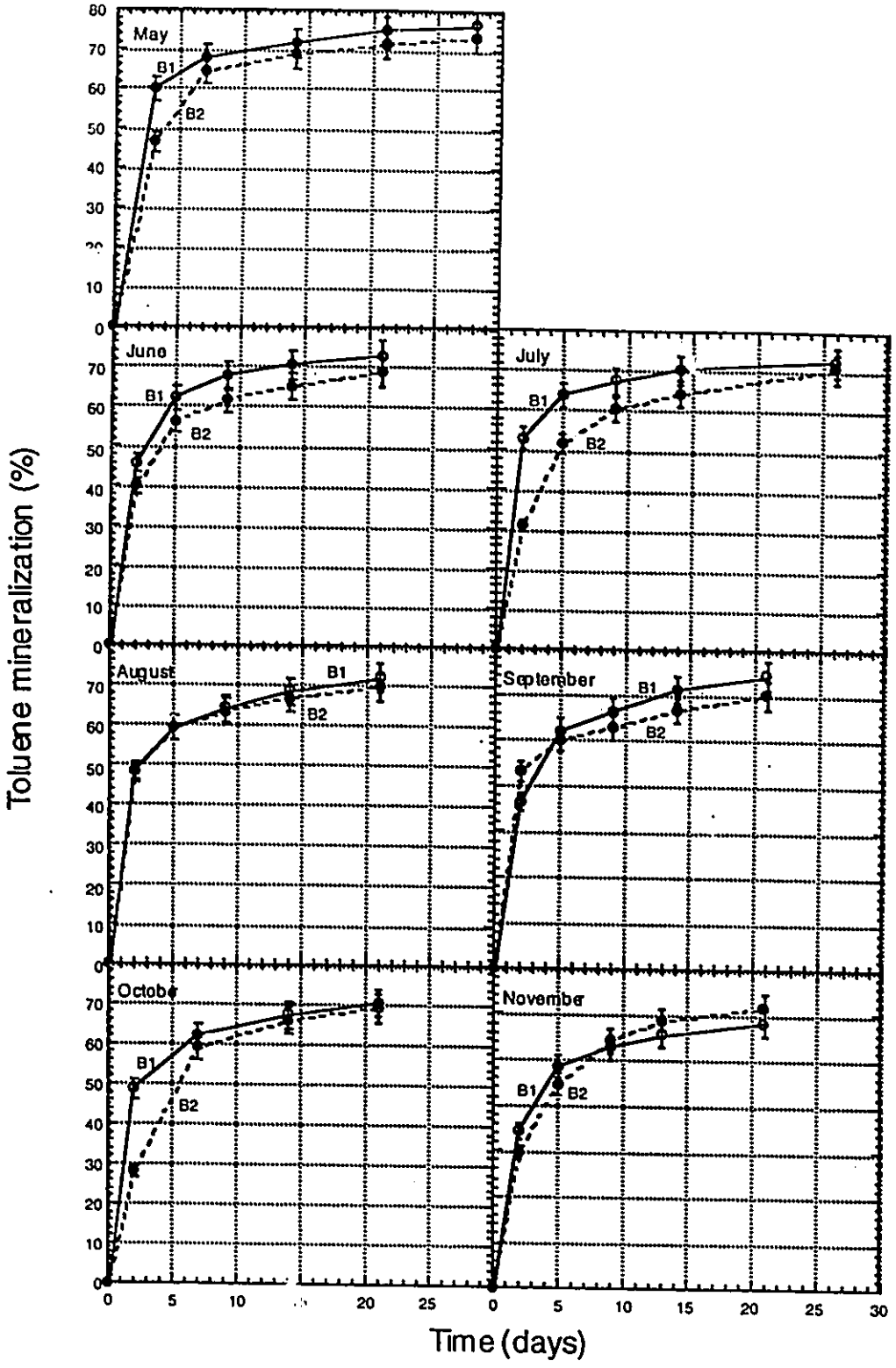
GRAPH C27

MINERALIZATION OF TOLUENE - CELL A



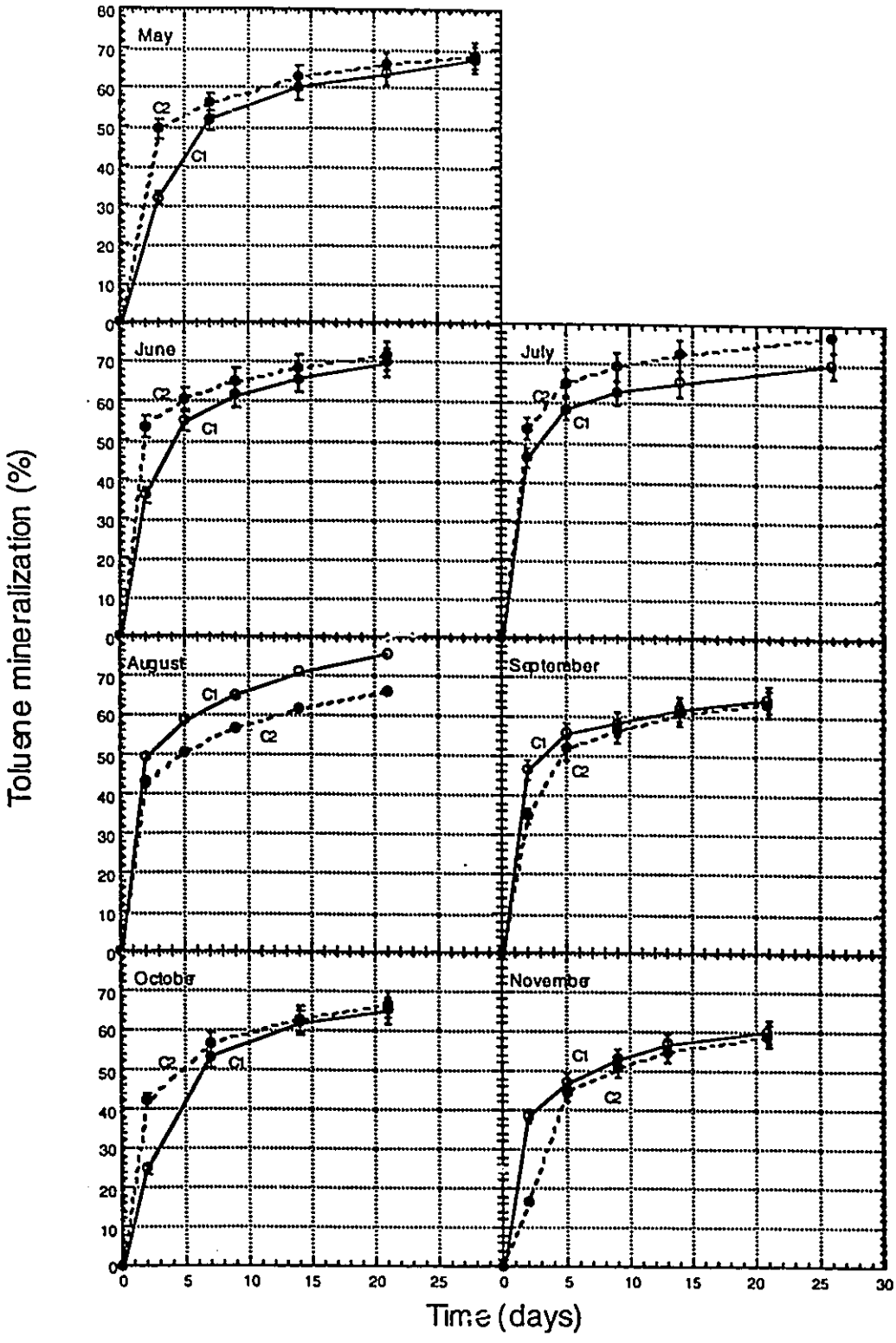
GRAPH C28

MINERALIZATION OF TOLUENE - CELL B



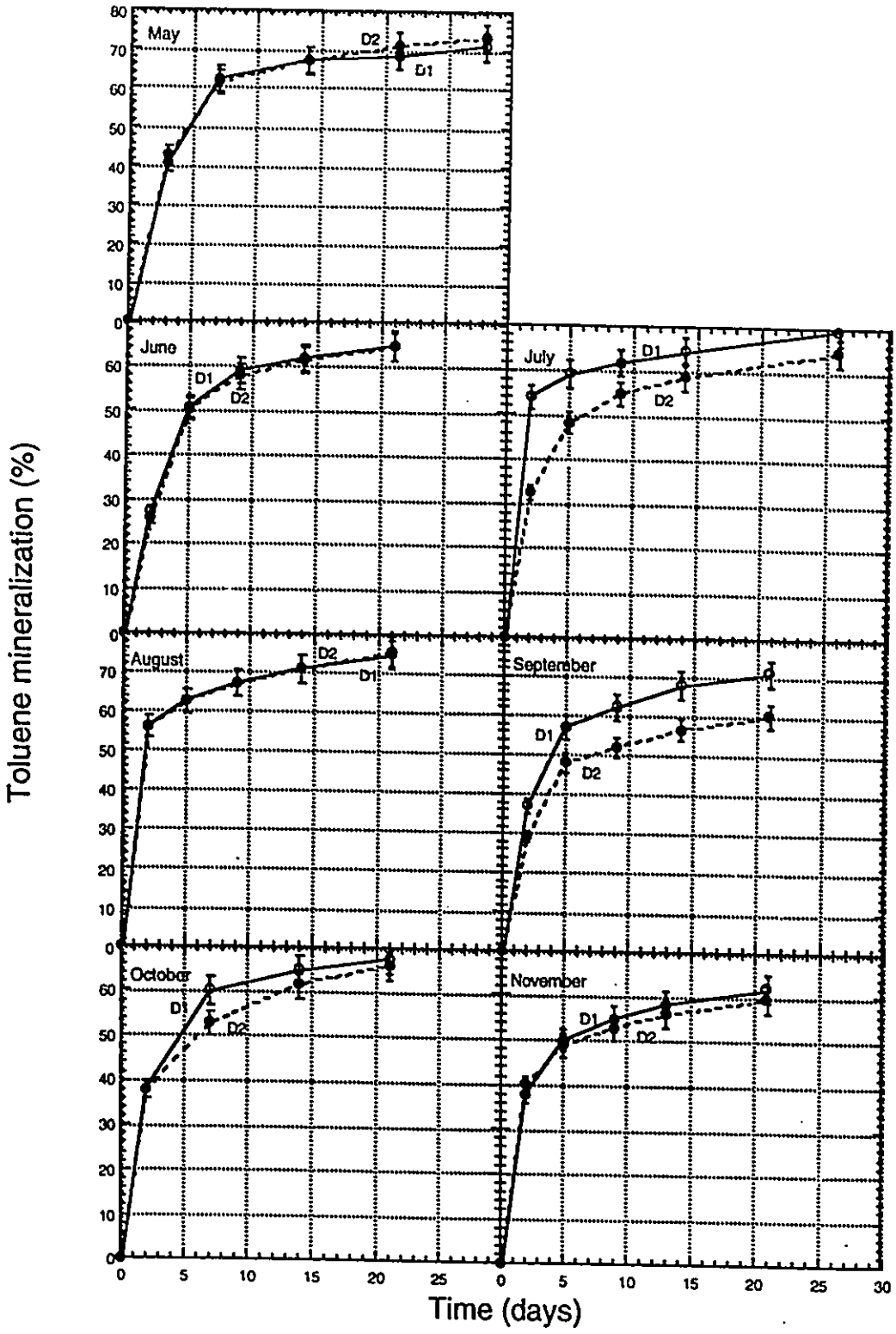
GRAPH C29

MINERALIZATION OF TOLUENE - CELL C



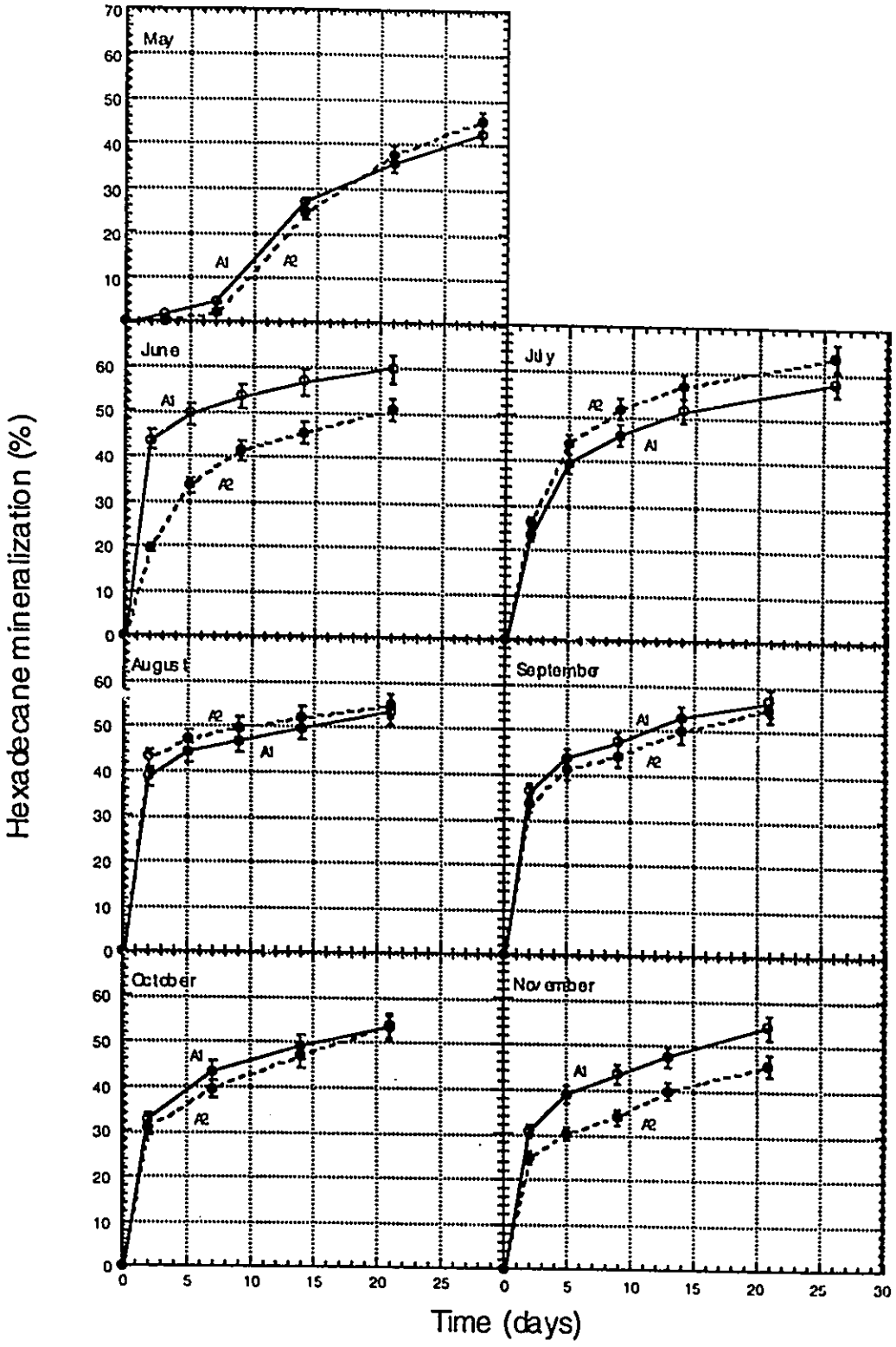
GRAPH C30

MINERALIZATION OF TOLUENE - CELL D



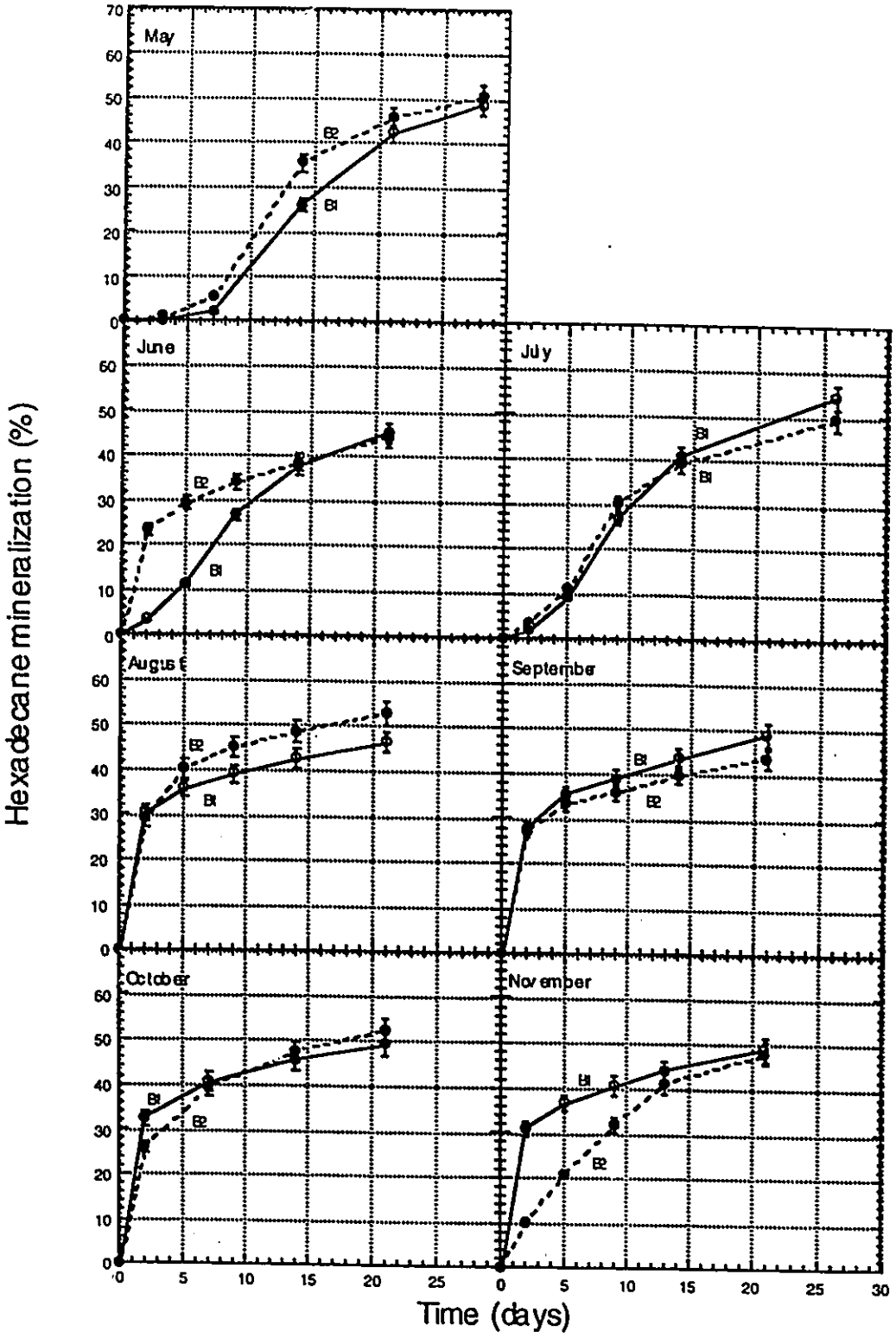
GRAPH C31

MINERALIZATION OF HEXADECANE - CELL A



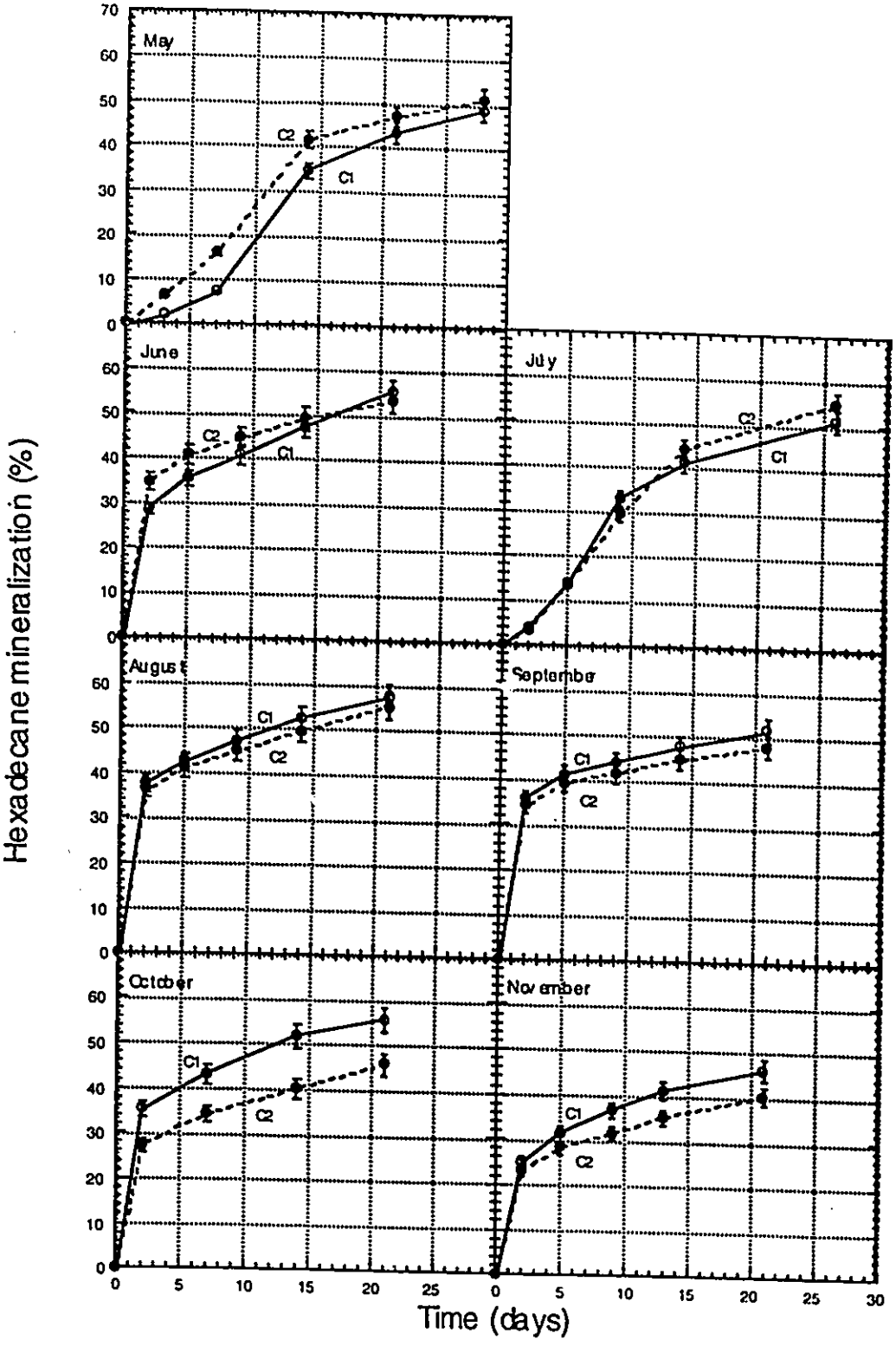
GRAPH C32

MINERALIZATION OF HEXADECANE - CELL B



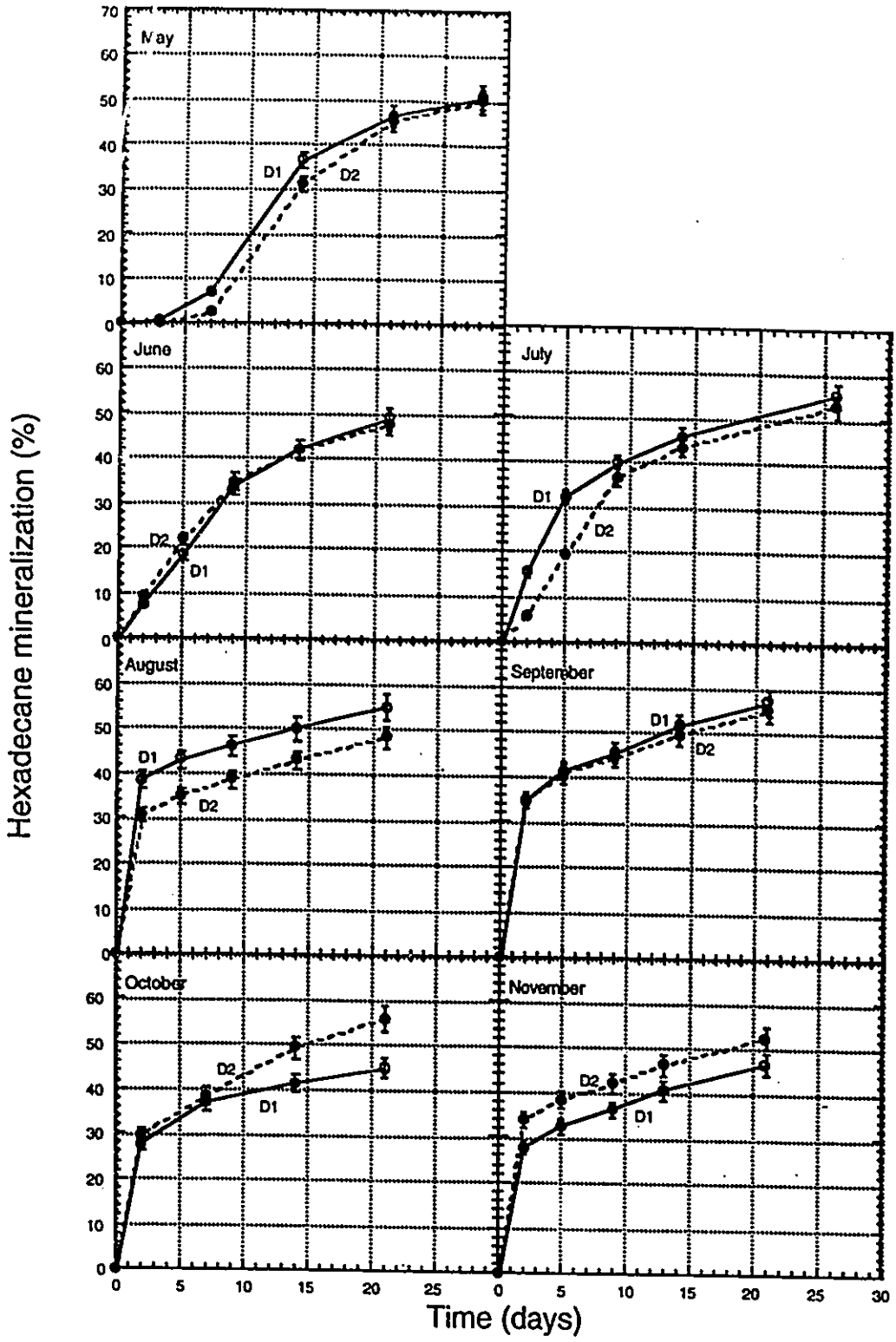
GRAPH C33

MINERALIZATION OF HEXADECANE - CELL C



GRAPH C34

MINERALIZATION OF HEXADECANE - CELL D



APPENDIX "D"

SCATTER PLOTS AND

LEVEL OF SIGNIFICANCE OF CORRELATION

COEFFICENTS AND SLOPES

APPENDIX "D"

DEFINITION OF STATISTICAL TERMS

- Correlation Coefficient (r) =

$$\frac{\frac{1}{(n-1)} \sum_{i=1}^{n} (X_i - \bar{X})(Y_i - \bar{Y})}{\sqrt{\frac{1}{(n-1)} \sum (X - \bar{X})^2} \sqrt{\frac{1}{(n-1)} \sum (Y - \bar{Y})^2}}$$

- Test of Correlation

  A t test for the significance of the correlation coefficient (r) is given by:

$$t = \frac{r\sqrt{n-2}}{\sqrt{1-r^2}}$$

where r = correlation coefficient
n = # of observations
t = test statistic

- Level of significance (L. of S.) of the **correlation** is the probability of rejecting the null hypothesis when it should be accepted (i.e. probability of being wrong). In this case, it is the probability of being wrong in saying that the correlation is significant.

- Level of significance (L. of S.) of the **slope** is the probability of falsely rejecting the null hypothesis which states that the slope is zero (i.e. no linear correlation between the two variables), when it should be accepted. In this case, it is the probability of being wrong in saying that the slope is significantly different than zero.

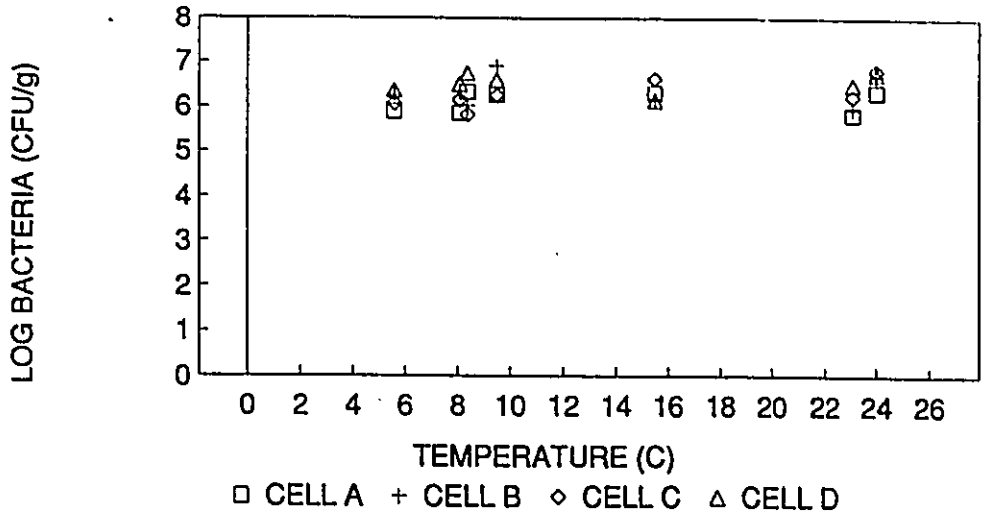
Table D1

Total Viable Bacteria versus Temperature

A. Significance of Correlations

	YTS Medium			DYG Medium		
Cell	r	t	L. of S.	r	t	L. of S.
A	0.1	0.2	85%	0.4	0.9	50%
B	0.0	-	-	0.6	1.5	20%
C	0.8	2.98	5%	0.7	2.0	20%
D	0.2	0.46	70%	0.6	1.5	20%

SCATTER PLOT 1
TOT BACTERIA VS SOIL TEMP. - YTS MEDIUM



SCATTER PLOT 2
TOT BACTERIA VS SOIL TEMP. - DYG MEDIUM

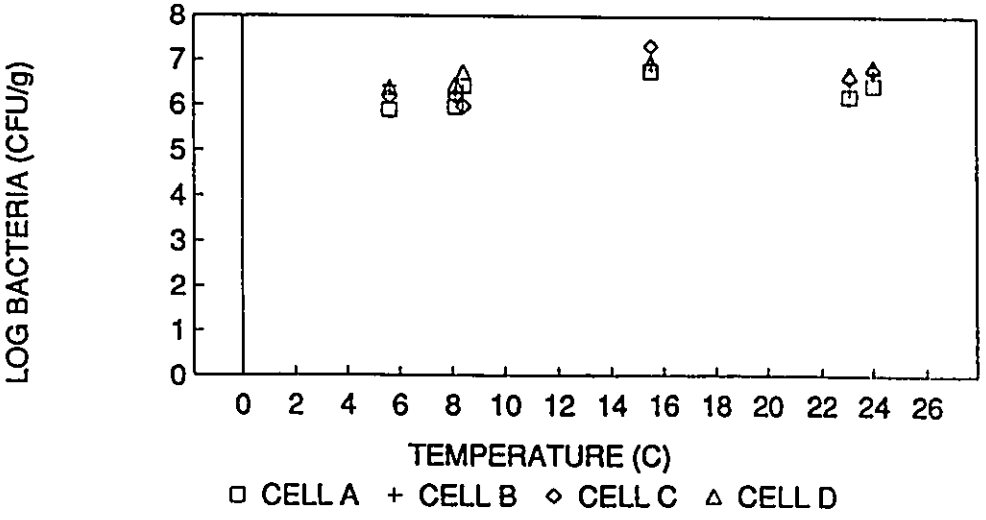


Table D2

Total Viable Bacteria versus Temperature

A. Significance of Slope

YTS Medium		DYG Medium	
Slope	L. of S.	Slope	L. of S.
1%	24%	2%	1%

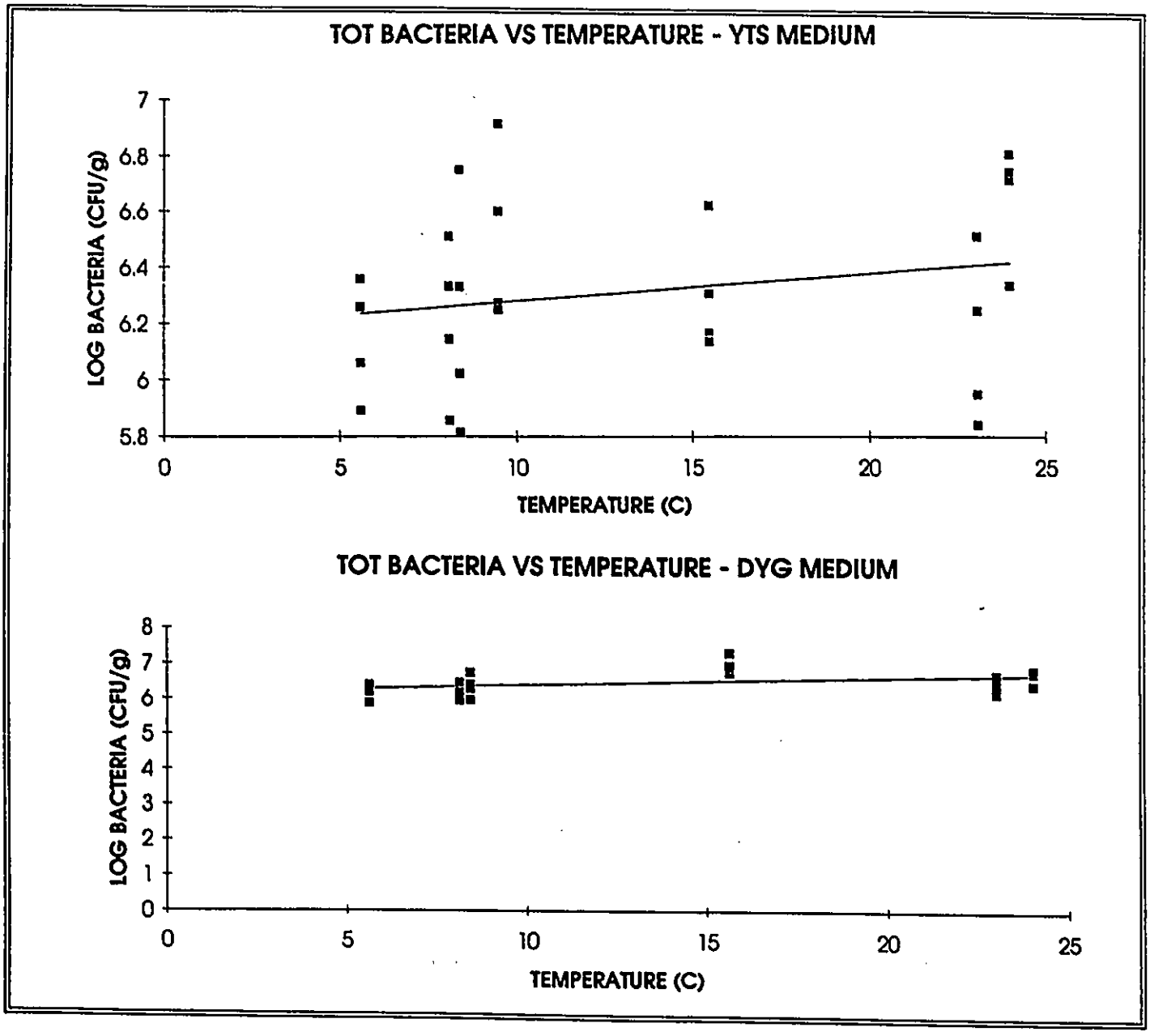
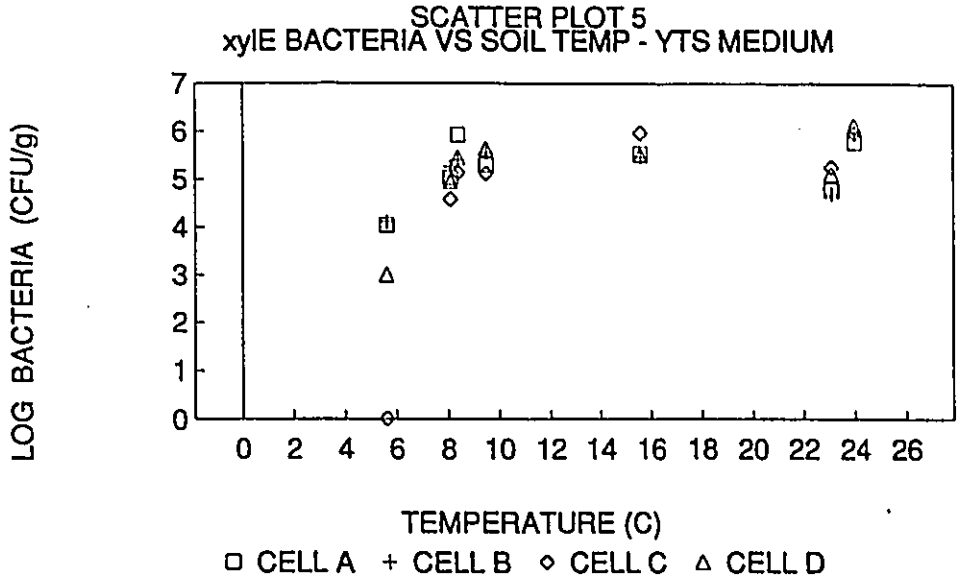


Table D3

xylE Probe Positive Bacteria versus Temperature

A. Signficance of Correlations

	YTS Medium			DYG Medium		
Cell	r	t	L. of S.	r	t	L. of S.
A	0.28	0.65	70%	0.44	0.98	40%
B	0.43	1.06	40%	0.51	1.19	30%
C	0.57	1.55	20%	0.60	1.50	20%
D	0.54	1.43	20%	0.43	0.95	40%



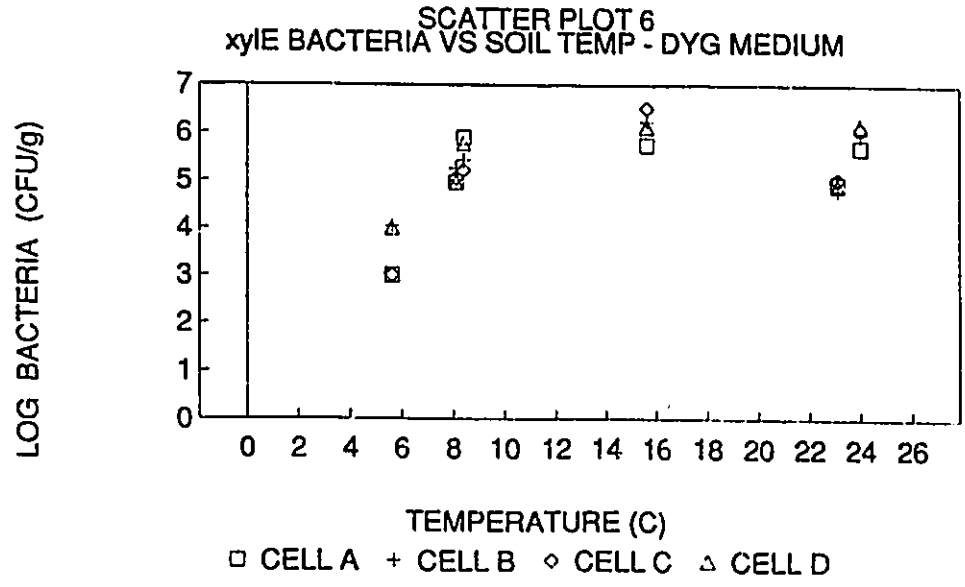


Table D4

xylE Probe Positive Bacteria versus Temperature

A. Significance of Slope

	YTS Medium		DYG Medium	
Segment	Slope	L. of S.	Slope	L. of S.
A	-0.2%	94%	-5%	26%
B	84%	0.2%	64%	0.006%

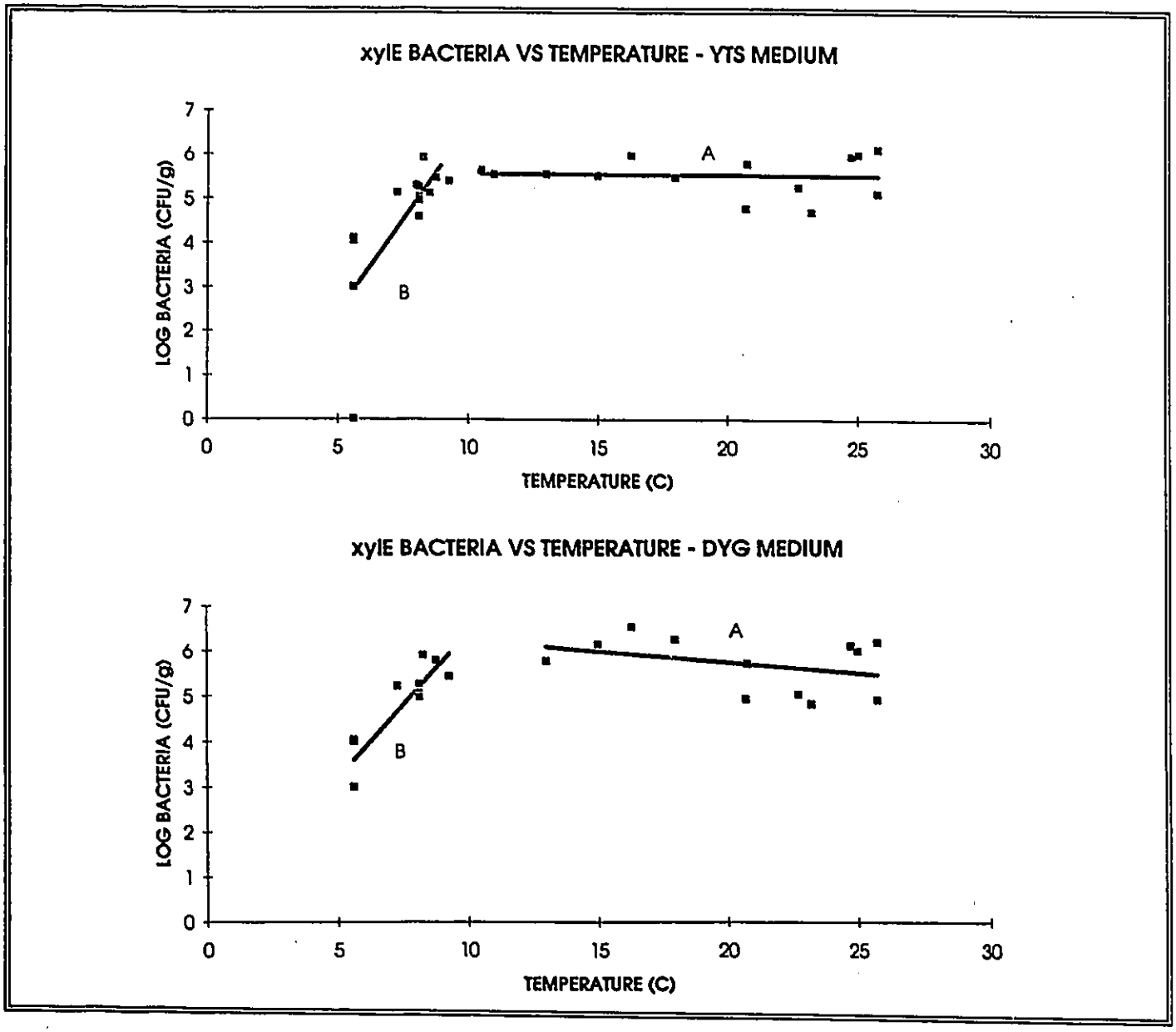
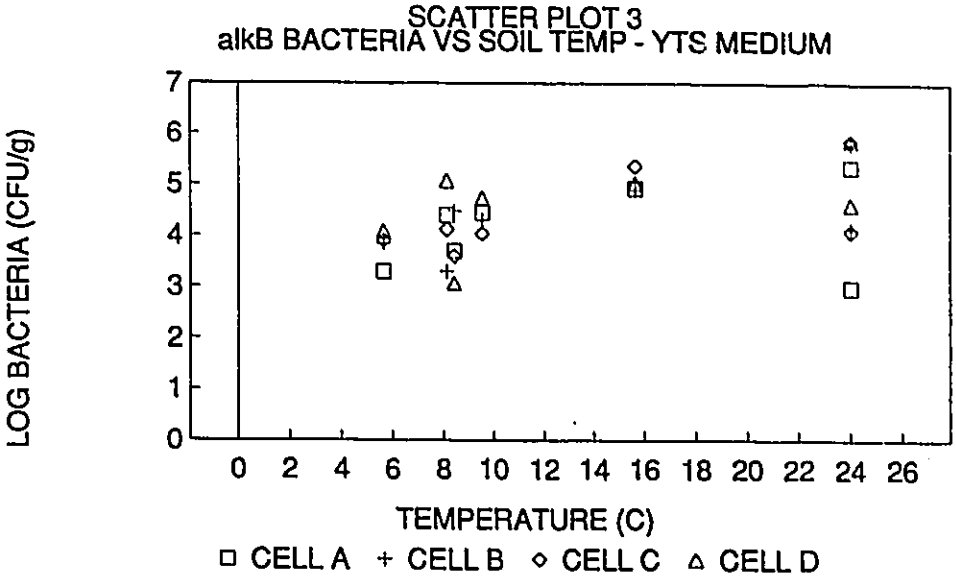


Table D5

alkB Probe Positive Bacteria versus Temperature

A. Significance of Correlations

	YTS Medium			DYG Medium		
Cell	r	t	L. of S.	r	t	L. of S.
A	0.19	0.43	70%	0.44	0.98	40%
B	0.71	2.25	10%	0.51	1.19	30%
C	0.72	2.32	10%	0.60	1.50	20%
D	0.56	1.51	20%	0.43	0.95	40%



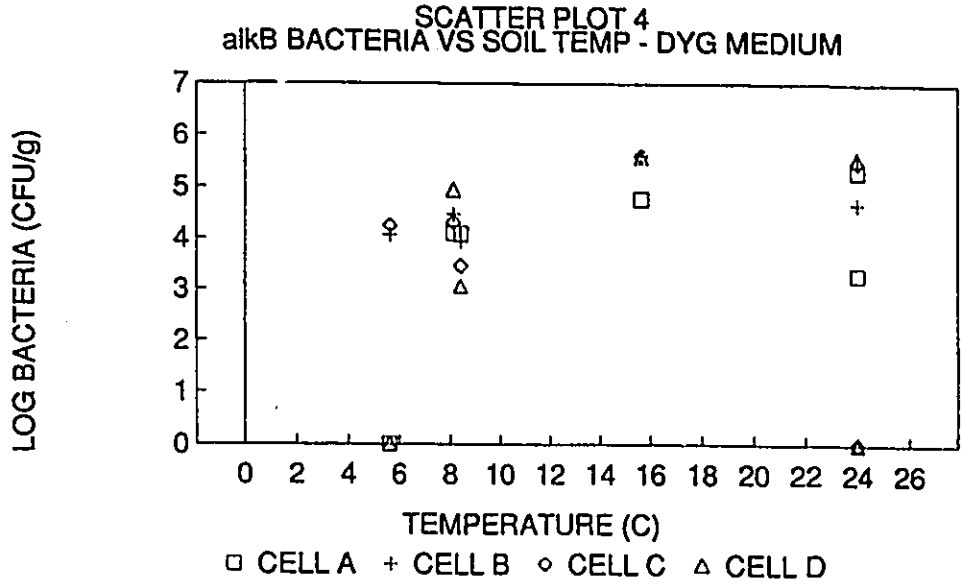


Table D6

alkB Probe Positive Bacteria versus Temperature

A. Significance of Slope

	YTS Medium		DYG Medium	
Segment	Slope	L. of S.	Slope	L. of S.
A	3%	49%	-16%	30%
B	9%	46%	65%	7%

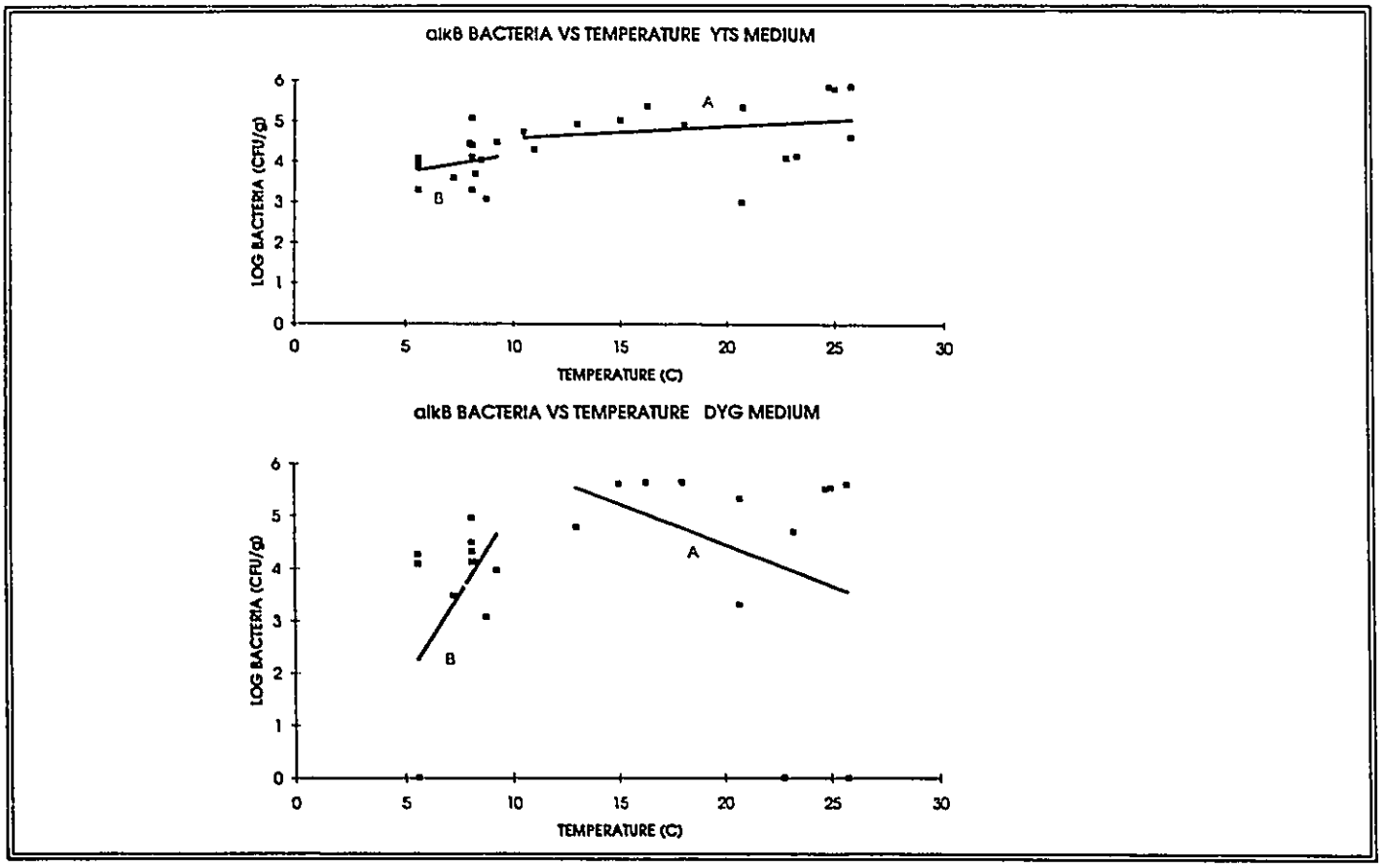


Table D7

Initial Rate of Toluene Mineralization versus Temperature

A. Significance of Correlations

Cell	r	t	L. of S.
A	0.86	3.77	2%
B	0.72	2.32	10%
C	0.85	3.61	2%
D	0.63	1.81	15%

Where r = correlation coefficient

t = test statistic

$$= \frac{r\sqrt{n-2}}{\sqrt{1-r^2}}$$

n = 7

L. of S. = Level of Significance

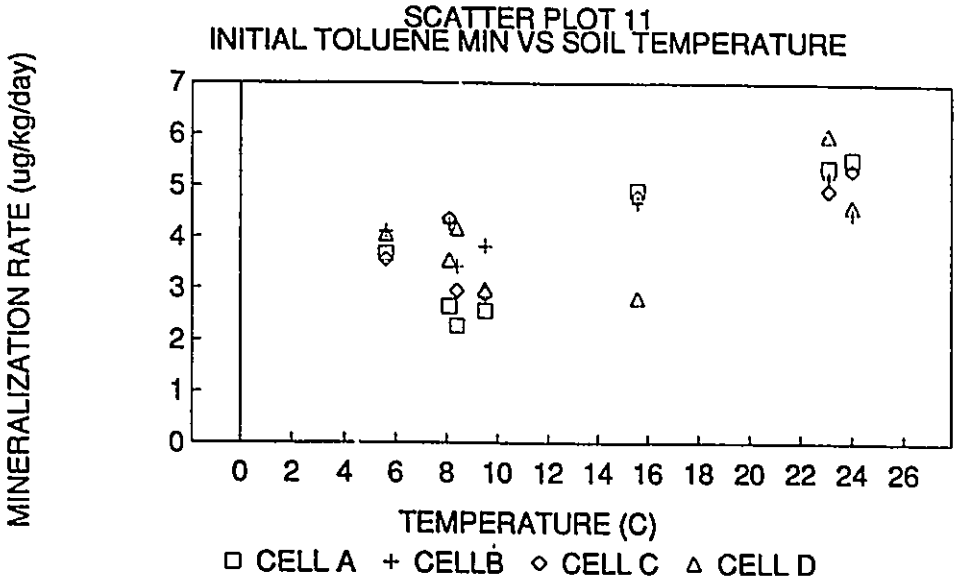


Table D8

Initial Rate of Toluene Mineralization versus Temperature

A. Significance of Slope

Slope	Level of Signficance
10%	0.001%

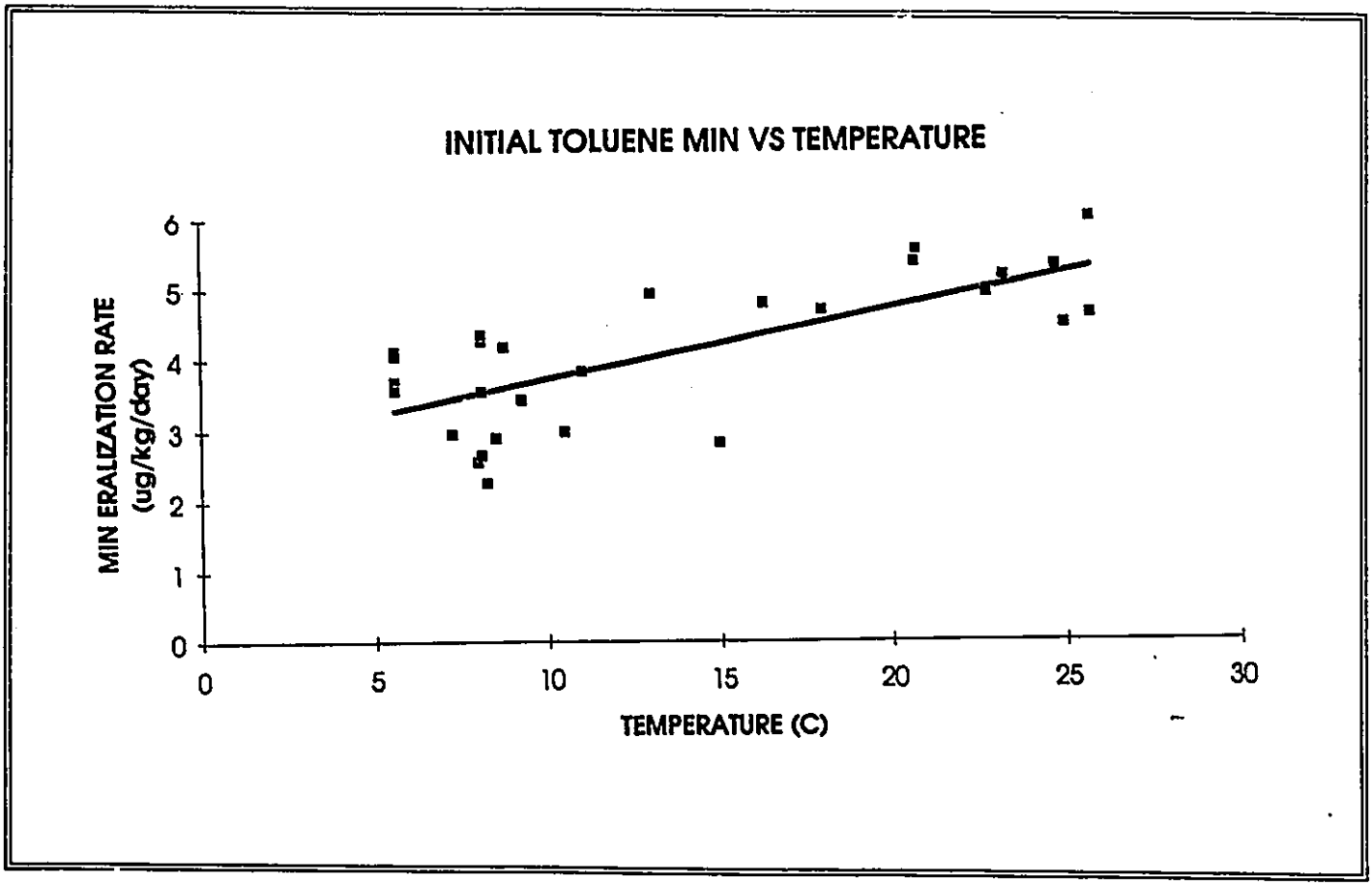


Table D9

Initial Rate of Hexadecane Mineralization versus Temperature

A. Significance of Correlations

Cell	r	t	L. of S.
A	0.51	0.73	50%
B	-0.33	-0.78	50%
C	-0.14	-0.32	80%
D	-0.14	-0.32	80%

Where r = correlation coefficient

t = test statistic

$$= \frac{r\sqrt{n-2}}{\sqrt{1-r^2}}$$

n = 7

L. of S. = Level of Significance

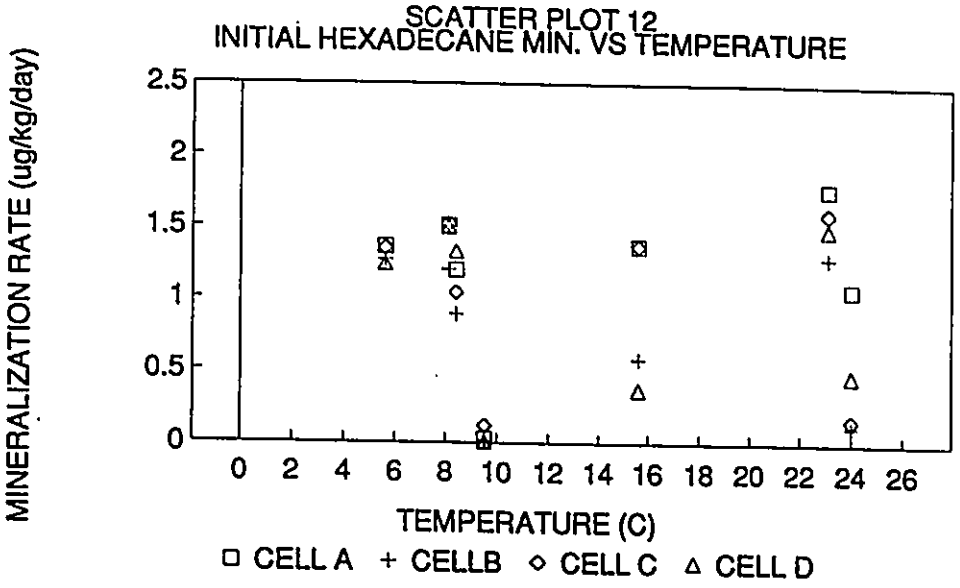


Table D10

Initial Rate of Hexadecane Mineralization versus Temperature

A. Significance of Slope

Slope	Level of Significance
-1%	53%

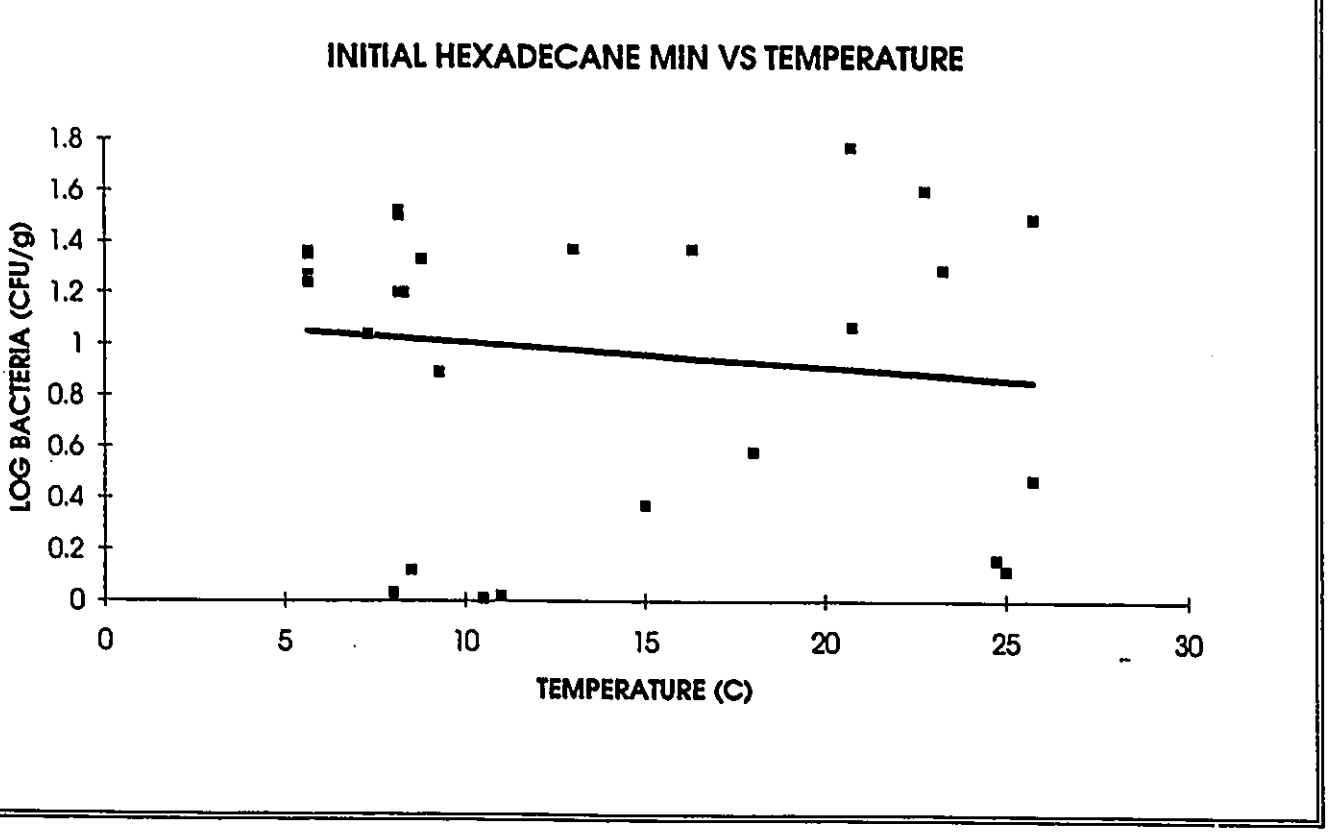


Table D11

Initial Rate of Toluene Mineralization versus Soil TPH

A. Significance of Correlations

Cell	r	t	L. of S.
A	0.12	0.27	80%
B	0.00	0.00	-
C	0.50	1.29	40%
D	-0.64	-1.86	20%

Where r = correlation coefficient

t = test statistic

$$= \frac{r\sqrt{n-2}}{\sqrt{1-r^2}}$$

n = 7

L. of S. = Level of Significance

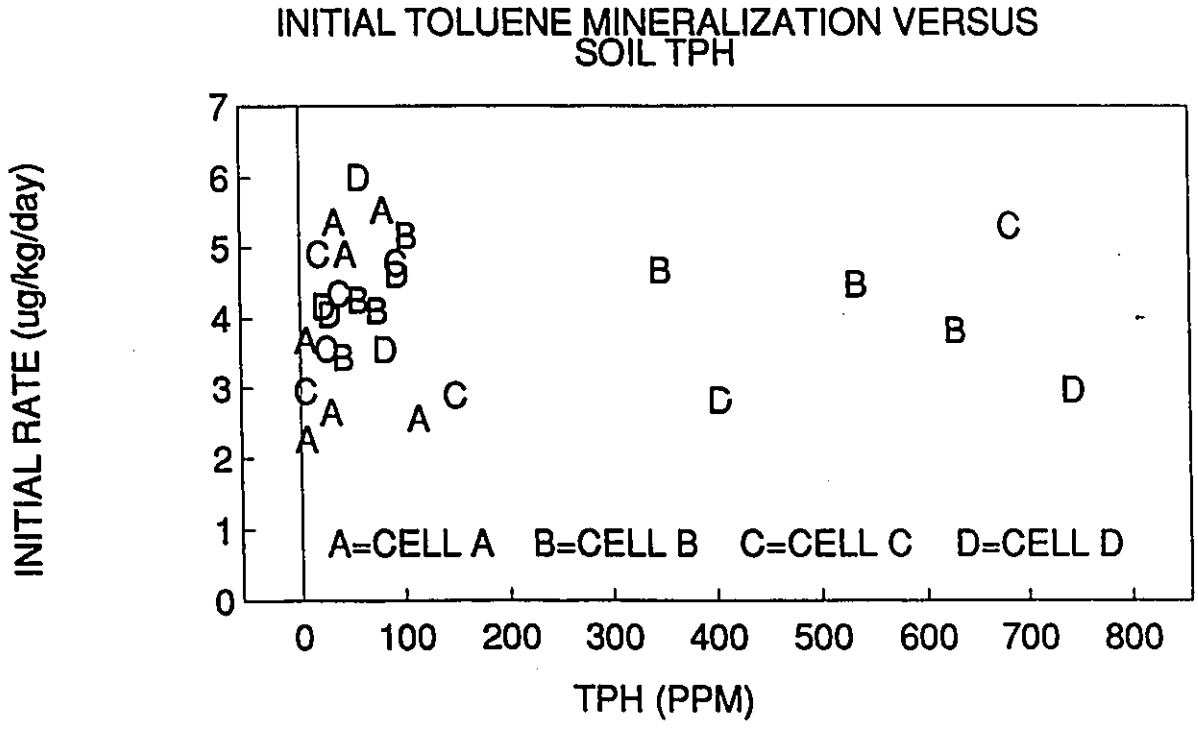


Table D12

Initial Rate of Toluene Mineralization versus Soil TPH

A. Significance of Slope

Slope	Level of Significance
-0.006	95%

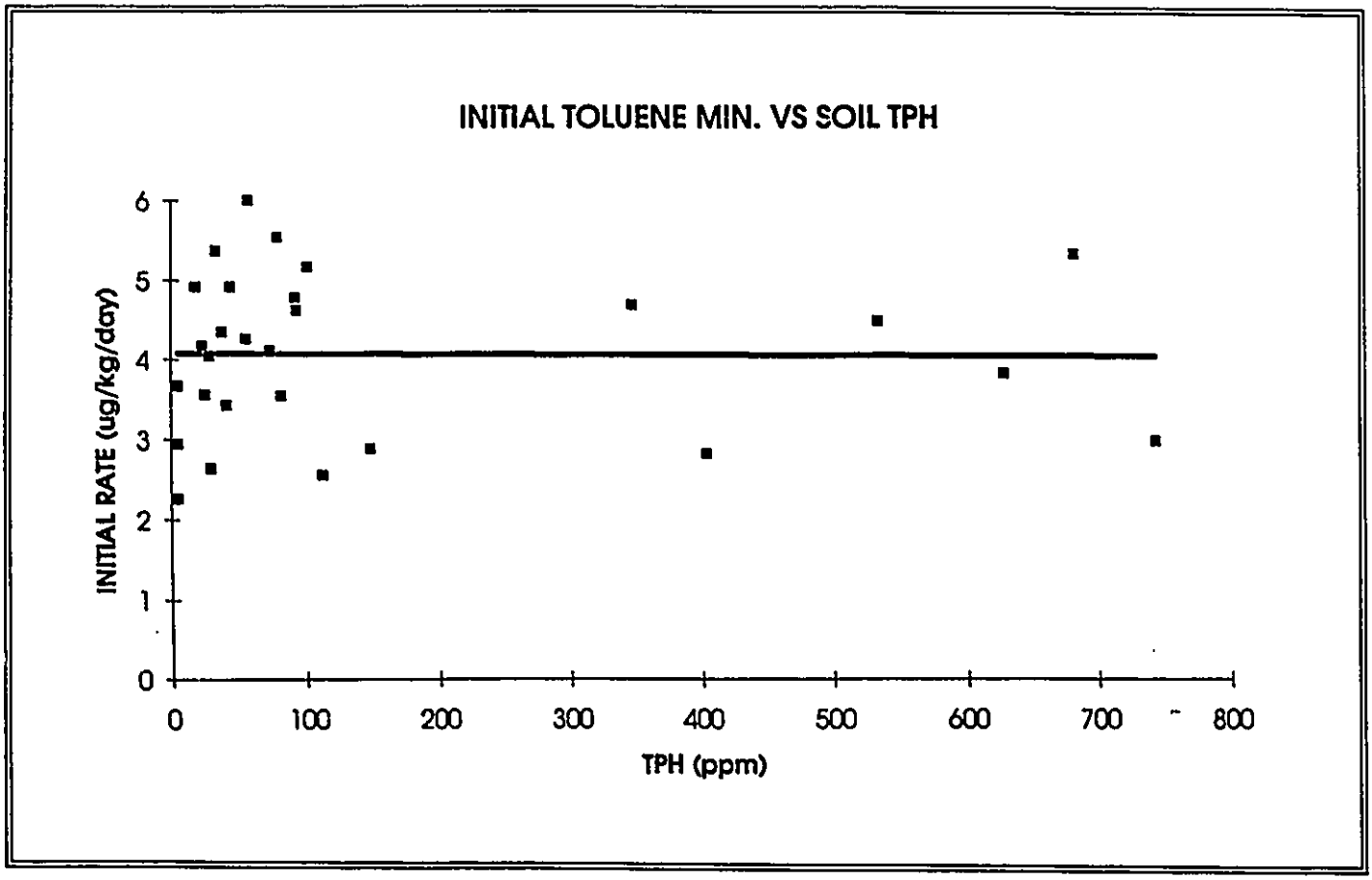


Table D13

Initial Rate of Hexadecane Mineralization versus Soil TPH

A. Significance of Correlations

Cell	r	t	L. of S.
A	-0.95	-6.80	1%
B	-0.33	-0.78	50%
C	-0.71	-2.25	10%
D	-0.83	-3.33	5%

Where r = correlation coefficient

t = test statistic

$$= \frac{r\sqrt{n-2}}{\sqrt{1-r^2}}$$

n = 7

L. of S. = Level of Significance

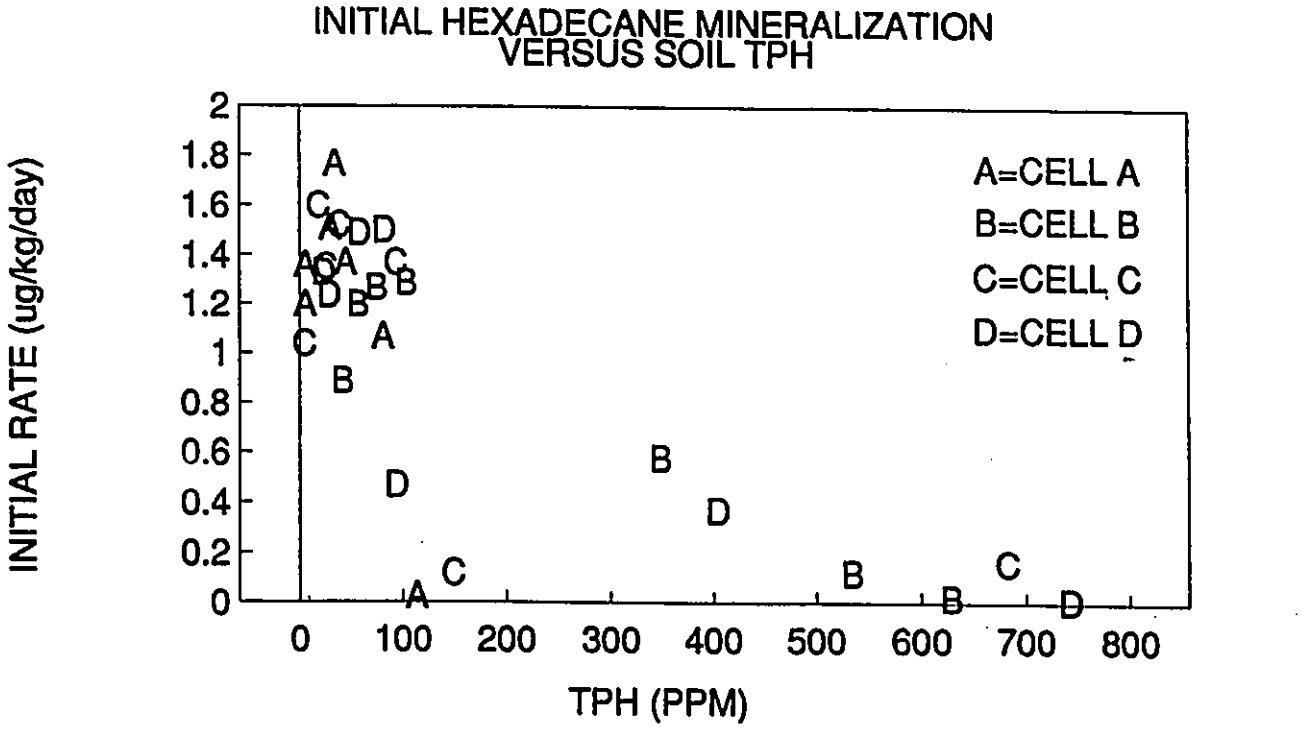


Table D14

Initial Rate of Hexadecane Mineralization versus Soil TPH

A. Significance of Slope

Slope	Level of Significance
-0.2%	0.0001%