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DISAPPEARANCE OF PAHs IN A CONTAMINATED SOIL FROM MASCOUCHE, QUEBEC

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INTRODUCTION

Of the 365 sites classified by the Québec Ministry of the Environment in 1984 and 1985 as contaminated, 62 are considered hazardous. The site chosen for this study is one of these 62 sites, and is located in the Mascouche municipality north of the city of Montreal. Site records indicate that it was used as a solid waste dump during the 1950s and 1960s. The northern side of the site was used as a dumping ground for semiliquid wastes from the Montreal refineries.

In 1969, permission was received to operate an incinerator for liquid wastes from the petroleum refineries. However, it was discovered that after a year of operation the incinerator did not perform well and spilled liquid wastes into the surrounding soil. In 1974, the incinerator was shut down and all operations ceased. In addition to the incinerator, two open-air storage basins and numerous solid waste deposits occupy the present site. The two open-air concrete basins on the site originally contained a total of 660 m^3 of liquid petroleum wastes. These basins were fissured at several places and consequently leaked liquid petroleum wastes into the groundwater. In periods of heavy rain, the basins overflowed onto the surrounding soil terrain. In 1986, the liquid waste inside the basins was tranferred into large metal containers and left on site, and the basins and the incinerator were then covered with sand.

The site, which occupies an area of approximately 30 hectares, is mainly flat, with a few marsh-like areas. The first

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Mechanistic evaluation of mitigation of petroleum hydrocarbon contamination by soil medium

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Present *in situ* chemical treatment technologies for mitigation of petroleum hydrocarbon contamination are in the developmental stage or being tested. To devise efficient strategies for restricting the movement of petroleum hydrocarbon (PHC) molecules in the contaminated soil, it is proposed to utilize the sorption–interaction relationships between the petroleum contaminants and the soil substrate. The basic questions addressed in this paper are as follows (*i*) What are the prominent chemical constituents of the various petroleum fractions that interact with the soil substrate? (*ii*) What are the functional groups of a soil that interact with the contaminants? (*iii*) What are the bonding mechanisms possible between the soil functional groups and the PHC contaminants? (*iv*) What are the consequent changes brought about the soil physical properties on interaction with PHC's? (*v*) What are the factors influencing the interactions between PHC molecules and clay particles of the soil substrate? (*vi*) What is the possibility of improving the soil's attenuation ability for PHC's? The development of answers to the basic questions reveal that petroleum hydrocarbons comprise a mixture of nonpolar alkanes and aromatic and polycyclic hydrocarbons, that have limited solubility in water. The bonding mechanism between the nonpolar PHC's and the clay surface is by way of van der Waals attraction. The adsorption of the nonpolar hydrocarbons by the clay surface occurs only when their (i.e., the hydrocarbon molecules) solubility in water is exceeded and the hydrocarbons exist in the micellar form. Dilute solutions of hydrocarbons in water, i.e., concentrations of hydrocarbons at or below the solubility limit, have no effect on the hydraulic conductivity of clay soils. Permeation with pure hydrocarbons invariably influences the clay hydraulic conductivity. To improve the attenuation ability of soils towards PHC's, it is proposed to coat the soil surface with "ultra" heavy organic polymers. Adsorption of organic polymers by the clay surface may change the surface properties of the soil from highly hydrophilic (having affinity for water molecules) to organophilic (having affinity for organic molecules). The organic polymers attached to the clay surface are expected to attenuate the PHC molecules by van der Waals attraction, by hydrogen bonding, and also by adsorption into interlayer space in the case of soils containing swelling clays.

Key words: petroleum hydrocarbons (PHC's), bonding mechanisms, functional groups, PHC–soil interaction, permeation, hydraulic conductivity, attenuation, van der Waals, organic polymers, organophilic.

Les technologies actuelles de traitement chimique pour diminuer la contamination aux hydrocarbures du pétrole sont au state du développement ou présentement testées. Afin de concevoir des stratégies efficaces pour restreindre le mouvement des molécules des hydrocarbures de pétrole (HCP) dans le sol contaminé, l'on propose l'utilisation des relations sorption-interaction entre les contaminants de pétrole et le sous-sol. Les questions fondamentales qui sont traitées dans le présent article sont (*i*) Quels sont les principaux constituants chimiques des diverses fractions du pétrole qui réagissent avec le sous-sol? (*ii*) Quels sont les groupes fonctionnels d'un sol qui réagissent avec les contaminants? (*iii*) Quels sont les mécanismes possibles de liaison entre les groupes fonctionnels du sol et les contaminants HCP? (*iv*) Quelles sont les conséquences sur l'interaction avec les HCP des changements apportés aux propriétés physiques des sols? (*v*) Quels sont les facteurs qui influencent les interactions entre les molécules de HCP et les particules du sous-sol? (*vi*) Quelle est la possibilité d'améliorer la capacité qu'a un sol d'atténuer les effets des HCP? L'élaboration de réponses aux questions fondamentales révèle que les hydrocarbures de pétrole comprennent un mélange d'hydrocarbures non polaires, alkanes, aromatiques et polycycliques qui ont une solubilité limitée dans l'eau. Le mécanisme de liens entre les HCP non polaires et la surface de l'argile se fait par l'intermédiaire de l'attraction de van der Waals. L'adsorption des hydrocarbures à la surface de l'argile se produit seulement lorsque la solubilité dans l'eau des molécules d'hydrocarbure est dépassée et que les hydrocarbures existent sous la forme mi-cellulaire. Des solutions diluées d'hydrocarbures dans l'eau, i.e. des concentrations d'hydrocarbures à ou sous la limite de solubilité, n'ont aucun effet sur la conductivité des sols argileux. La percolation d'hydrocarbures purs influence invariablement la conductivité hydraulique de l'argile. Pour améliorer la capacité du sol d'atténuer les effets des HCP, l'on propose de recouvrir la surface du sol avec des polymères organiques ultralourds. L'adsorption des polymères organiques à la surface de l'argile peut changer les propriétés de surface du sol d'un état fortement hydrophile (ayant une affinité pour l'eau) à un état organophile (ayant une affinité pour les molécules organiques). L'on s'attend à ce que les polymères organiques attachés à la surface de l'argile atténuent l'effet des molécules de HCP par l'attraction de van der Waals, par les liens d'hydrogène, et aussi par l'adsorption dans les espaces inter-couches dans le cas de sols contenant des argiles gonflantes.

Mots clés : hydrocarbure de pétrole (HCP), mécanismes de liens, groupes fonctionnels, interaction HCP-sol, percolation, conductivité hydraulique, atténuation, van der Waals, polymères organiques, organophile.

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TABLE 3. Physicochemical properties of monocyclic aromatics

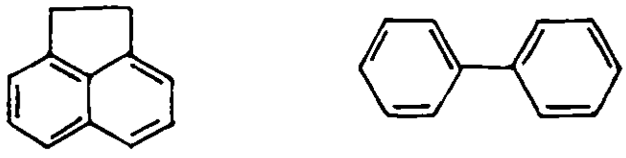
Compound	Formula	Molecular weight	Boiling point (°C)	Solubility in water at room temperature (ppm)	Density (g/mL)	Dielectric constant	Dipole moment Debye
Benzene	C_6H_6	78.11	80.1	1780	0.88	2.28	0
Toluene	$C_6H_5CH_3$	92.1	110.8	515	0.87	2.44	0.36
Ortho-xylene	$C_6H_4(CH_3)_2$	106.17	144.4	175	0.88	2.57	0.62
Meta-xylene	$C_6H_4(CH_3)_2$	106.17	139.09	175	0.86	2.37	0.30
Para-xylene	$C_6H_4(CH_3)_2$	106.17	138.4	198	0.86	2.27	0

moments less than one (1) and dielectric constants less than three (3). Hoffman and Brindley (1960) have shown that adsorption of nonionic organic compounds by soil clays is governed by the CH activity of the molecule; the CH activity arises from electrostatic activation of the methylene groups by neighboring electron-withdrawing structures, such as C = 0 and C = N. Molecules possessing many C = 0 or C = N groups adjacent to methylene groups would be more polar and hence more strongly adsorbed than those compounds in which such groups are few or absent (Theng 1974).

Examination of the chemical structures of the various petroleum hydrocarbons (Figs. 1–4) indicates that these molecules have no electron-withdrawing units such as C = 0 and C = N. Based on Hoffman and Brindley (1960), it is apparent that the petroleum hydrocarbons molecules would be weakly adsorbed (mainly by van der Waals adsorption) by the soil functional groups and do not involve any strong ionic interaction with the soil. Indeed, Meyers and Quinn (1973) in their studies on association of hydrocarbons and mineral particles in saline solutions obtained heats of adsorption for eicosane ($C_{20}H_{42}$, a normal straight-chained alkane molecule) and anthracene ($C_{14}H_{10}$, polycyclic aromatic hydrocarbon) onto bentonite clay of −2.1 and −9.1 cal/mol, respectively (1 cal = 4.1868 J). These heats of adsorption indicate weak adsorption by van der Waals attraction.

Changes in soil physical properties on interaction with PHC molecules

On release to the soil media, besides weakly interacting with the clay surface by van der Waals attraction, the nonpolar PHC molecules in the soil voids could influence the soil physical properties. The soil physical parameter of importance in soil–contaminant interaction is the soil hydraulic conductivity (for soils with $k > 10^{-8}$ m/s), as it controls the fluid movement through the soil. There have been a number of studies of the effects of nonpolar alkanes (heptane, cyclohexane) and aromatic hydrocarbons (benzene, xylene) on the hydraulic conductivity of clays (Green *et al.* 1983; Acar *et al.* 1985; Anderson *et al.* 1985*a*, 1985*b*; Bowders 1985; Fernandez and Quigley 1985; Uppal and Stephenson 1989). Mitchell and Madsen (1987) have reviewed the various investigations and arrived at the following conclusions about the effect of hydrocarbons on the hydraulic conductivity of clay soils. Dilute solutions, i.e., concentrations at or below the solubility limit of the hydrocarbone in water, have no effect on the hydraulic conductivity of clay soils. Permeation with pure hydrocarbons invariably influences the clay hydraulic conductivity. The degree of effect depends on the method of hydraulic conductivity testing used. Permeation with pure hydrocarbons leads to a decrease in hydraulic conductivity of clay soils when flexible-wall permeameters are used. Permeation with pure hydrocarbons can lead to hydraulic conductivity increases of up to three orders of magnitude when rigid-wall permeameters are used. The marked increase in hydraulic conductivity in rigid-wall tests on passage of pure hydrocarbons is attributed to shrinkage of soils, leading thereby to the formation of cracks and macropores. Flow of concentrated hydrocarbons through clay soils is predominantly through the cracks and macropores. Most of the water in the micropore remains in the soil.


Acenaphthene
Biphenyl


Anthracene
Napthalene

FIG. 4. Chemical structure of selected polycyclic hydrocarbons.

Factors influencing interactions between PHC molecules and clay-mineral particles

Meyers and Quinn (1973) and Meyers and Oas (1978) have studied the associations of different hydrocarbons with clay particles in a saline medium. The concentrations of hydrocarbons used in these studies were well above the solubilities of the organic molecules in the aqueous solution (Table 5). The results of Peake and Hodgson (1956) had earlier indicated that water medium has the capacity to accommodate higher levels of hydrocarbon than that would be predicted by simple solubility considerations by way of micelle formation; the presence of these colloidal micellar particles in water results from aggregation of the amphiphiles (i.e., an ionic or molecular hydrocarbon species) to form small "ionic" or large "neutral" micelles. These micelles enhance water solubility of the hydrocarbons by providing hydrocarbon-like regions within the water in which the hydrocarbons preferentially dissolve (Baker 1962).

FOREWORD

The following selection of papers on PHCs AND BIOSTIMULATION STUDIES has recently been published:

1. Yong, R.N. and Mourato, D.
   "Stimulation of Microbial Biodegradation in a Jet Fuel Contaminated Soil"
   Proceedings, Second International Conference on New Frontiers for Hazardous Waste Management, Pittsburgh, Penn. September 1987.

2. Yong, R.N., Tousignant, L.P., Leduc, R. and Chan, E.C.S.
   "Disappearance of PAHs in a Contaminated Soil from Mascouche, Quebec"
   Proceedings, International Symposium on In Situ and On Site Bioreclamation,
   San Diego. March 1991.

3. Yong, R.N. and Rao, S.M.
   "Mechanistic evaluation of Mitigation of Petroleum Hydrocarbon Contamination by Soil Medium"
   Canadian Geotechnical Journal, 28:84-91 (1990)

Second International Conference on
NEW FRONTIERS FOR HAZARDOUS WASTE MANAGEMENT
Pittsburgh, Penn. September 1987

STIMULATION OF MICROBIAL BIODEGRADATION IN A JET FUEL CONTAMINATED SOIL

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ABSTRACT

Previous studies have indicated the marked effect of aeration and nutrient additions on the ability of indigenous soil microbial populations to biodegrade intruding organic contaminants. This phenomenon was utilized in this study for enhancing the biodegradation of jet fuel contaminated soils through stimulation of the soil's indigenous microoranisms. This study was carried out as a preliminary investigation to establish the necessity to develop in-sity aeration technology.

Jet fuel biodegradation was enhanced by supplying air, nitrogen, phosphorus and trace minerals to the contaminated field soils. Jet fuel biodegradation was monitored by CO_2 evolution, as by following hydrocarbon transformation through gas chwomatography and infra-red spectroscopy studies.

Comparative studies were conducted between aerated and non-aerated soils. Whilst aeration was observed to significantly enhance CO_2 evolution and hydrocarbon degradation, non-aerated soils have also displayed some jet fuel biodegradation. The rates of CO_2 evolution and hydrocarbon degradation were, however, shown to be significanlty slower in these soils, and the addition of external sources of nitrogen and phosphorus, more important for degradation to occur. Jet fuel degradation was only initiated after a lag time in the non-exposed soils, whereas the contaminated soils showed immediate response. This phenomenon appeared to be related to the presence of jet fuel adapted microbial species in the soils which have been contaminated for a period varying between 5 to 10 years.

INTRODUCTION

Contamination of soils and groundwater with hydrocarbon products is a concern of environmentalists, since petroleum products are toxic compounds known to be harmful to aquatic life at concentrations equivalent to 90 ppm (1). The problem of leaking buried gasoline tanks as sources of groundwater contamination is indeed a real and pressing issue.

Contaminated soils could either be excavated, displaced and treated, or treated in-situ. There are advantages and disadvantages in all cases, but for large volumes of contaminated soils, in-situ de-toxification/decontamination is an alternative to be considered. In these cases, techniques of land-farming used for oily sludges become applicable.

Gasoline products are composed of a variety of aromatic hydrocarbons. Common gas hydrocarbons comprise toluene, xylene, benzene, ethyl benzene, intermediary hydrocarbons, etc. The aromaticity of these hydrocarbons renders biodegradation a more difficult task, but partial degradation of such compounds under aerobic conditions in soils has been referenced (2, 3). Hydrocarbons are known to be biodegraded by a variety of bacterial species. Low molecular weight aromatic hydrocarbons, in particular, have been observed to undergo significant biodeusadation under aerobic conditions (3, 4). In contrast, anaerobic biodegradation of aromatic hydrocarbons has been largely discounted over the years. More recent studies have, however, shown hope for anaerobic biodegradation of polyaromatic hydrocarbons, although an equivalent concentration of these compounds were shown to be biodegraded more than 20 times as rapidly under aerobic conditions, and the lag time for initiation of anaerobic biodegradation was of more than 270 days (3,4).

PURPOSE

Initially, the reported herein studies were carried out as a preliminary investigation to establish the usefulness of soil aeration for in-situ soil self-decontamination of an actual jet fuel contaminated site. Results have, however, shown that although aerobic treatment was faster and more efficient, anaerobic biodegradation appeared to occur, and this could be enhanced through enrichment of the soils with non-carbon nutrients. The objectives of this study included gaining a better understanding of natural soil-related phenomena available for in-situ biodegradation (and possibly reactor biotechnology) of hydrocarbon-contaminated soils, and evaluating metabolic stimulation procedures for further field application.

This paper summarizes studies conducted to evaluate the ability of soils to undergo self-decontamination under aerated and non-aerated conditions. More precisely, emphasis was put on the influence of soil microbial activity on the decontamination process. Enhanced hydrocarbon degradation and microbial activity are studied through aeration and supply of non-carbonaceous co-nutrients to soils exposed to the contaminant for a period of 5 to 10 years and to soils which have not been previously exposed hydrocarbons.

APPROACH

Two main experiments were conducted, namely a jet fuel contaminated soil biodegradation assay, and an adaptability assay.

Soils: The contaminated soils sampled consisted of a mixture of gravel and silt (fill material), contaminated with jet fuel that has overflowed from reservoirs over the last 10 years. The soil's hydrocarbon content was shown to be equivalent to 5% w/w and water content of 10%. A laboratory processed soil, consisting primarily of illitic minerals with some chlorite and kaolinite, was utilized in the adaptability studies.

Biodegradability assay: 320g of jet fuel contaminated soils (5% w/w jet fuel) were continuously aerated with CO_2-free compressed air routed to a manifold system which distributed air to the culture vessels (refer to Fig. 1). Gas flow did no cause soil mixing in the vessels due to the density of the soil. The soils were not inoculated prior to the tests and any microbial activity was considered to be inherent to the soil. The CO_2 produced in the vessels could originate from two sources; chemical or biological processes. Although chemical oxidation of the hydrocarbons has to be considered, biodegradation appeared to be the most important generator of carbon dioxide.

Non-aerated reference tests were carried out in parallel by replacing the flowing air with argon. These soils were initially purged with argon to evacuate trapped oxygen. Non-aerated conditions were ensured by maintaining a positive argon flow throughout the duration of the test. Air evacuation was monitored with a Yellow Springs oxygen meter and an oxygen-free environment was insured throughout the test by incorporating a Gas Pack disposable anaerobic indicator in each vessel. These provided visual evidence that anoxic conditions were maintained.

All soils were enriched at various stages with minimal salts solution (20mL in 320g), consisting of the following:

20g/L NH_4Cl
4g/L HN_4NO_3
8g/L Na_2SO_4
4g/L KH_2PO_4
0.4g/L $MgSO_4$
12g/L K_2HPO_4

These salts could offer sources of exogenous electron acceptors, susceptible to induce oxygen-free hydrocarbon oxidation and produce carbon dioxide. This had to be taken in account in the interpretation of the CO_2 evolution curves.

To follow hydrocarbon transformation, two types of tests were conducted; gas chromatography of pore fluid extracts and infra-red spectroscopy of air-dried soils. Stripping of hydrocarbons had to be considered as a small contribution to hydrocarbon disappearance and was considered in the analysis of the results.

CO_2 evolution was monitored by 2 distinct tests:

- Barium hydroxide titration ($Ba(OH)_2$ - CO_2 trapping).

Carbon dioxide produced within the reaction vessels was directed towards a series of 125 mL flasks containing 100 mL of 0.024N $Ba(OH)_2.8H_2O$ (refer to Fig. 1). The carbon dioxide contained in the flowing gas was trapped by $Ba(OH)_2$ in the form of $BaCO_3$. The cumulated CO_2 trapped as $BaCO_3$ was determined daily in the 2 flasks by titration of each absorber with 0.05N HCl to a phenolphthalein end-point. The amount of carbon dioxide produced in a given time period was calculated as follows (5);

$$CO_2 = 0.025 \text{ mole/mL} \times \text{vol HCl} \times 44 \text{ mg}$$

- CO_2 analysis by infra-red detection.

At irregular time intervals, the gas tube leading to the $Ba(OH)_2$ flasks was diverted to an infra-red detector, part of a Beckman - Total Organic Carbon Analyzer IR detector, model 915. The CO_2 contained in the flowing gas which was measured directly represented the CO_2 produced.

Jet fuel analysis before and after biodegradation was performed using the following methods:

- Gas chromatography by flame ionization detection (FID)

2 microliter samples of acetone extracted pore fluid samples were injected in a 10% OV-351 Chromosrob W-HP 80/100, 20ft column. Carrier gas (Helium) was set at a flow equivalent ot 25 mL/min. Hydrocarbon analysis was carried out by means of a temperature program set as follows:

initial: 100°C for 4 mins.
program: 4°C/min
final: 150°C for 8 mins.

- Infra-red spectroscopy (Beckman Acculab 9)

IR analysis was carried out in a wavelength range between 4000 to 300 cm^{-1}. 5 mg air dried soil samples were mixed with 495 mg dried KBr, finely ground and pressed into a pellet. IR spectra were analyzed as a function of new peaks related to the soil or to the contaminating hydro- carbons.

Adaptability assay: These assays were carried out to investigate the potential of non-exposed soils to undergo hydrocarbon biodegradation in aerated and non-aerated conditions. Due to their nature, the adaptability tests allowed to study the initial steps of jet fuel biodegradation, since non-degraded jet fuel was admixed to non-exposed soils. In contrast, the biodegradability tests were conducted on contaminated soils in which in-situ biodegradation has been initiated. The adaptability assays compared CO_2 evolution and hydrocarbon biodegradation of aerated and non-aerated soils inoculated with top soil indigenous microbes, (never exposed to hydrocarbons).

The adaptability tests set-up (gas distribution and analysis) was similar to the biodegradation assays described above, but were conducted on laboratory processed sterile soils which were enriched with 50 mL jet fuel and 50 mL minimal salts solution, and specifically inoculated with 20g hydrocarbon-free top soil.

During the test period, CO_2 evolution was monitored by both $Ba(OH)_2$ and IR analysis. After 13 days of incubation, residual jet fuel in the soils was analyzed by IR and GC tests. Comparisons were established between aerated and argonated assays, and also with reference samples. Reference samples included jet fuel-free inoculated DS soils (aerated vs argonated) and sterile jet-fuel enriched soils (to investigate evaporation effects).

RESULTS:

Carbon dioxide Evolution

Biodegradability assays:

Both CO_2 evolution tests suggested that CO_2 was produced in all soils, but the rate at which CO_2 was synthesized varied in accordance with the treatment given. Aerated soils were characterized by elevated CO_2 evolution curves, whereas non-aerated soils had carbon dioxide levels of approximately half (refer to Figures 2 and 3). This was verified in both cumulative tests (by $BaCO_3$ titration) and by direct CO_2 analysis (IR spectroscopy).

It has only been recently that studies have indicated the existence of a slow anaerobic catabolism of hydrocarbons. In all cases, however, anaerobic hydrocarbon catabolism only started after a long period of time (270 days) (3, 4). This long adaptation time was not reflected in our experiments, but could occurred naturally over the years at the site. The CO_2 evolution curves do not represent the activity of fresh microbes in contact with the pollutant, but of microbes that had more than 5 years to adapt to the jet fuel.

Whilst CO_2 evolution is an acceptable tool to investigate metabolism, care needs to be exercised in interpretation of results - especially in relation to biodegradation of pollutants. Depending on the pathway taken to catabolize the contaminant (in this case, jet fuel), microbes will produce different quantities of carbon dioxide. Also, depending whether mineralization of cellular synthesis occurs, the ratio of CO_2 produced will be different. Finally, as mentionned above, carbon dioxide can also be produced as a result of chemical oxidation. For these reasons, CO_2 evolution curves were interpreted in view of the results generated by GC and IR tests.

Carbon dioxide evolution was used to evaluate the influence of minimal salts on both aerated and non-aerated soils. Both soils exhibited large increases in CO_2 production after addition of these co-nutrients (refer to Figure 5). Addition of salts could result in an increase in chemical oxidation and CO_2 evolution, even in the absence of oxygen, since the salts supply sources of electron acceptors. Nevertheless, results derived from the adaptability studies confirmed that this phenomenon was limited in the tests, and the measured CO_2 could be related to biological activity. Furthermore, the influence of salt addition to the soils reflected the lack of non-carbon nutrients in hydrocarbon rich soils, often limiting biodegradation to occur. These results agreed with studies conducted on oil biodegradation in soils (6, 7).

Carbon dioxide was also used for rapid screening of the nature of the metabolizing microbes. To assess whether the microbes in the non-aerated soils were strict anaerobes, the non-aerated soils were aerated for a period equivalent to 10 days after 30 days of non-aerated treatment. In all cases, the CO_2 produced was significantly enhanced after the first day (refer to Fig. 4).

Interpretation of these results could be made on the basis of a chemical oxidation phenomenon. Nevertheless, these results seem to indicate that the metabolizing microbes were not sensitive to air, unless the activity detected was attributed to fast growing aerobes. This suggests that the microbes were most probably facultative anaerobes undergoing anaerobic respiration. Biodegradation of hydrocarbons by facultative microbes through anaerobic respiration, utilizing nitrogen and/or sulfate as a final electron acceptor has also been demonstrated by other reseachers (8, 9).

Carbon dioxide Evolution
Adaptability assays:

Carbon dioxide evolution of the soil samples undergoing adaptability tests were similar to the ones presented for biodegradation studies (refer to Figs. 4 and 5). In summary, the non-aerated samples exhibited some carbon dioxide production, but this was significantly lower than the aerated soil samples. These results could not be interpreted without the support of GC and IR results, since the observed carbon dioxide synthesized could originate from the biodegradation of non-hydrocarbon organics present in laboratory soil or added as inoculum.

Gas chromatography
Biodegradability assays

Gas chromatography of the non-treated jet fuel revealed a large number of peaks related to the hydrocarbons present in the fuel (Fig. 6, curve a). No attempt was made to identify all hydrocarbons, but the dominating peaks (f, j and p) were identified as being benzene, toluene and xylene peaks respectively, 3 major compounds of jet fuel. Furthermore, analysis of jet fuel chromatograms showed that it is composed of products of a large molecular weight range.

Over the years, in-situ natural, anaerobic biodegradation appeared to have taken place, transforming the jet fuel into a product of decomposition. However, the periodic addition of jet fuel from continuous spills would indicate that the sampled soils would contain jet fuel at different states of biodegradation. This was indeed exhibited by a chromatogram of the soil's pore fluid (Fig. 6, curve a). Comparative analysis of the jet fuel and of this pore fluid extract suggests that in-situ natural biodegradation had indeed taken place, eliminating most hydrocarbon peaks. The presence of toluene, xylene and a hydrocarbon with similar molecular weight as xylene (peak o') (absent in the original jet fuel), is, however, noticeable. The results indicate that natural biodegradation is more effective in degrading the low molecular weight hydrocarbons, in accord with the results reported by Atlas (4).

As expected, biodegradation resulted in the appearance of an important number of peaks in the non-aromatic area of the chromatogram (first peaks to appear). These low molecular weight compounds would consist of by-products of biodegradation since the original jet fuel only exhibited insignificant peaks in this chromatrogram region. Similar results were reported for chromatography studies of biodegraded hydrocarbons (10, 11).

Non-aerated soils, exhibited pore fluid chromatograms very similar to the field extract. However, the intensity of high molecular weight compounds' peaks were further reduced, and low molecular weight compounds exhibited increased intensity. Furthermore, the o' peak exhibited in the in-situ soil's pore fluid extract was again clearly shown in these chromatograms. Overall, these results supported the CO_2 evolution curves, by demonstrating that even without aeration, biodegradation was succesfully continued in the soils with the introduction of low concentrations of nitrogen and phosphorus in the system.

Aeration of the nutrient-enriched contaminated soils for a period of 30 days, appeared to significantly reduce the presence of higher molecular weight compounds while building up a reserve of low molecular weight by-products of biodegradation (Fig. 6, curve c, peaks a, b, c and f). As had been noted previously, catechol, acetyl-COa, succinate, acetaldehyde and pyruvate are common by-products of biodegradation of aromatic hydrocarbons, and their synthesis depends on the metabolic pathway taken (4). These by-products are all of lower molecular weight than most hydrocarbons present in the jet fuel, and their synthesis would appear on the chromatograms as peaks with short retention times (eg. peaks a to e). Further studies are presently being conducted to identify and compare by-products from aerated and non-aerated soils.

Also of interest was the disappearance of peak o' (by-product of biodegradation), in the aerated soils. Development of equivalent peaks in the low molecular range seems to indicate that aeration is required to biodegrade the component related to peak o'. It would appear that catabolism of the jet fuel could also be due to non-aerated respiration from facultative microorganisms present in the soils. The oxygen present in air may be a required element to fully biodegrade the element related to peak o'.

Gas Chromatography
Adaptability assays

The results shown in Figure 7 indicate that in general larger reductions of peak heights were obatined in the aerated samples, confirming the CO_2 evolution results. The following points are noted:

- whilst similar trends were observed in the chromatograms, the peak intensities of non-aerated soils were higher than the aerated soils.
- in the incubation period of 13 days, the results indicate that only low molecular weight hydrocarbons were biodegraded in both aerated and non-

aerated soils, with a net decrease in the first peaks of the chromatogram.
- The j peak (toluene) in aerated soils was shown to be displaced to the left (shorter retention time). A study of the significance of this event has yet to be performed.
- the non-aerated soils' chromatograms indicated the presence of the o' peak discussed above and thought to be related to a by-product of biodegradation encountered in anaerobic soils. This shows to build up after only 13 days of treatment under anaerobic conditions.

In summary, therefore, gas chromatography studies indicate that the CO_2 evolution observed could be related to the biodegradation of non-aromatic low molecular weight compounds and probably to some short chain hydrocarbons, in non-aerated soils containing no adapted microbes. Furthermore, biodegradation appears to be accompanied by a build-up of an intermediary product common to the non-aerated soils (refer to peak o').

Aerated non-adapted microbes present in topsoils appear to exhibit some low and intermediate MW hydrocarbon biodegradation without build-up of the o' peak-related intermediary by-product.

IR spectroscopy
Biodegradability assay

The aerated and non-aerated spectra shown in Fig. 8 indicate the presence of absorption bands typical of both laboratory soil and jet fuel peaks (compare D and JF bands). However, the non-aerated spectrum shows a predominance of jet fuel bands (JF1, JF3 and JF4). These overshadow the presence of laboratory soil's characteristic D4 band (900 - 1100 cm^{-1} region).

Only trace bands of jet fuel were noticeable in the aerated soils. One might conclude from these results that the contribution of air enhances jet fuel degradation. We note that these results support the CO_2 evolution data generated and discussed above. From IR spectroscopy one can also conclude that the actual source of CO_2 produced in this assay is the result of catabolism of jet fuel hydrocarbons.

IR spectroscopy
Adaptability assays:

Analysis of soils derived from the adaptability assays by IR spectroscopy (refer to Figure 9) leads to the same conclusions as above. In general, the non-aerated soils exhibit IR absorption bands typical of both laboratory soil and the contaminating jet fuel (eg. peaks JF2, JF3, JF4 and JF5), whilst the bands from the aerated soils only reflect the soils' spectrum. Again, the jet fuel bands overshadowed the laboratory soil typical peaks in the 900 - 1100 cm^{-1} region in the non-aerated soils' IR spectrum. Since the adaptability tests were only run for 13 days, the state of degradation was less than in the biodegradability samples which were run for thirty days.

Conclusions and Applications

It would appear that it is possible for non-aerated field soils exposed for long periods of time to an aromatic hydrocarbon source, such as the jet fuel contaminated soil studied, to develop adapted microbial species that can utilize the contaminant as a source of carbon. The biodegradation rate would, however, be slower in comparison to aerobic catabolism. All hydrocarbons present would probably not be mineralized (especially the high MW molecules), and some by-products of biodegradation could accumulate in the soils. However, with external addition of a source of readily available nitrogen and phosphorus, to the non-aerated soils, enhancement of CO_2 evolution and hydrocarbon degradation (supported by both IR and GC results) occurs.

The results obtained indicate that the biodegradation monitored under non-aerated conditions appeared to be mainly attributed to facultative anaerobes. In the absence of oxygen, an intermediary by-product of biodegradation is shown to accumulate in the soil (o' peak), and that for further degradation of the by-product, oxygen is probably required. Studies on accumulation and toxicity of the synthesised by-products, are presently being conducted.

In soils not previously exposed to hydrocarbons, oleophilic microbes are not naturally present, and although some CO$_2$ evolution was observed, this can be related to the biodegradation of low molecular weight compounds of non-aromatic nature and hydrocarbon stripping. Fresh hydrocarbon (jet fuel) spills in non-preconditioned anaerobic soils may not undergo self-decontamination even if large amounts of nitrogen and phosphorus nutrients are added, because of the absence of non-adapted microbial species. As indicated in this study, one could expect that given sufficient time, adapted microbial species can develop, therefore permitting slow biodegradation/decontamination. The time required for such adaptation to occur remains, however, unknown. Inoculation of the site with adapted anaerobes, and a supply of additional nutrients, could also be a remediation solution technique to be considered if accelerated biodegradation is desired.

Aerated soils, have shown faster biodegradation rates by adapted and non-adapted soil microbes, with more complete hydrocarbon degradation. The results confirm the advantages of aerating contaminated soils, and suggest that development of aeration processes for soils would be a useful technology to implement for a faster and more thorough decontamination of soils. Incorporation of a mechanism to supply nitrogen and phosphorus solutions to the soil should allow for better treatment efficiency. Whilst aerobic biodegradation of hydrocarbons in-vitro has been well studied, field applications of aerated in-situ decontamination requires the development of proper technology.

Anaerobic in-situ treatment of hydrocarbons appears to be a relatively new subject. Laboratory investigations of metabolic enhancers including detailed studies to evaluate the influence of nitrogen, phosphorus and water content on biodegradation, steps of biodegradation, by-products of metabolism, and toxicity of these by-products need to be conducted.

ACKNOWLEDGEMENTS

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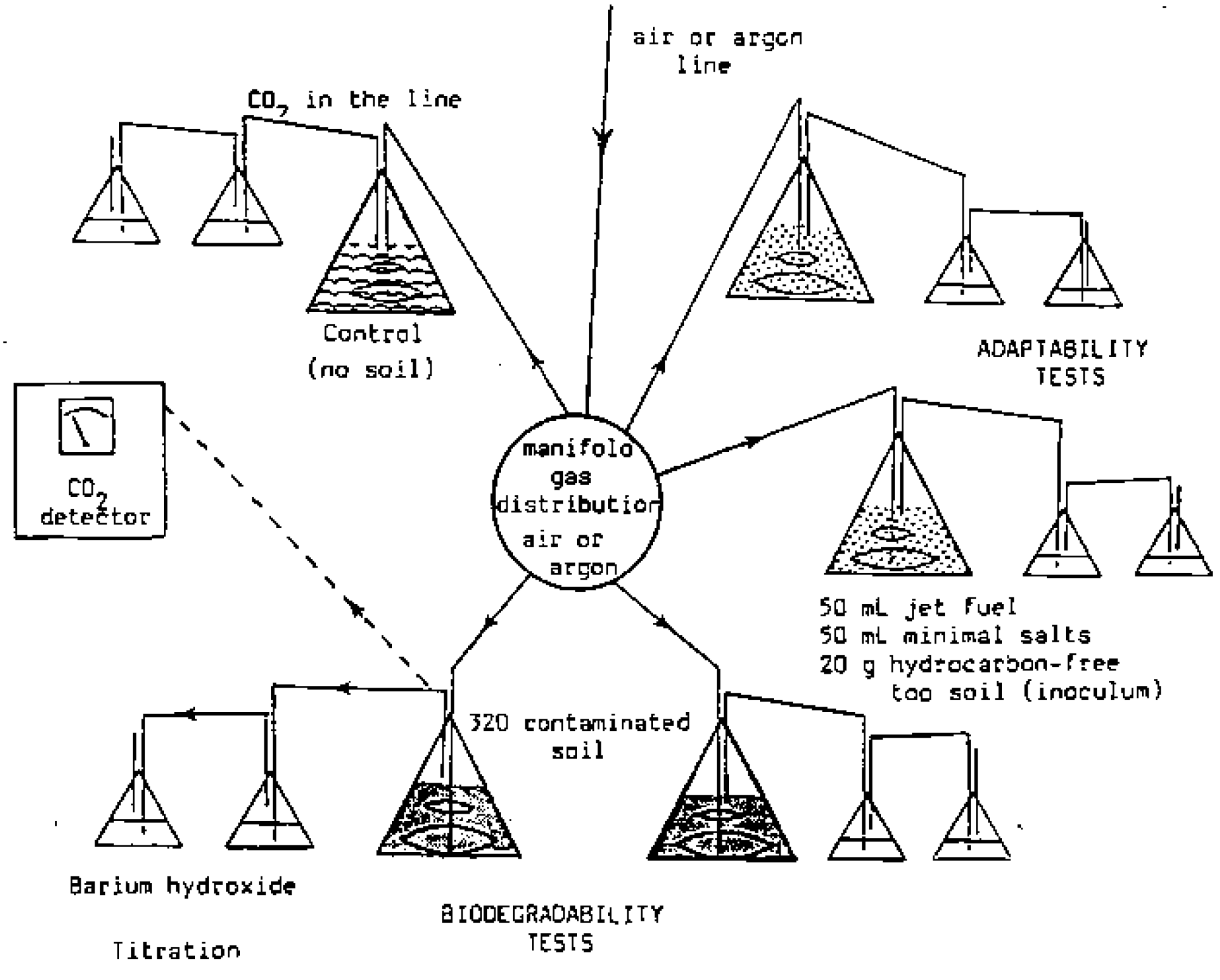


Figure 1, Experimental set-up for the biodegradability and the adaptability assays.

Figure 2 BIODEGRADABILITY ASSAY
CUMULATIVE CO_2 CURVES

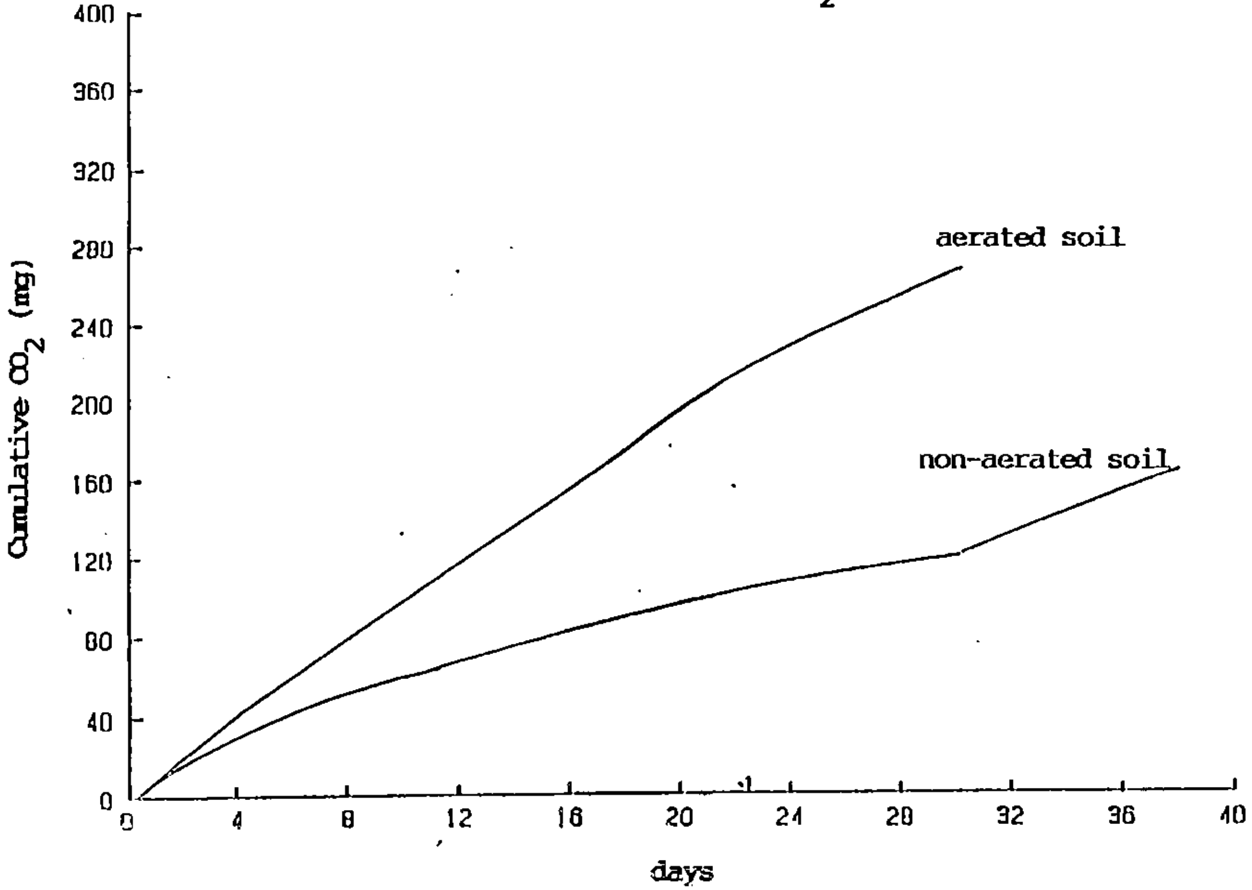


Figure 3 ADAPTABILITY ASSAY
Cumulative CO_2 Curves

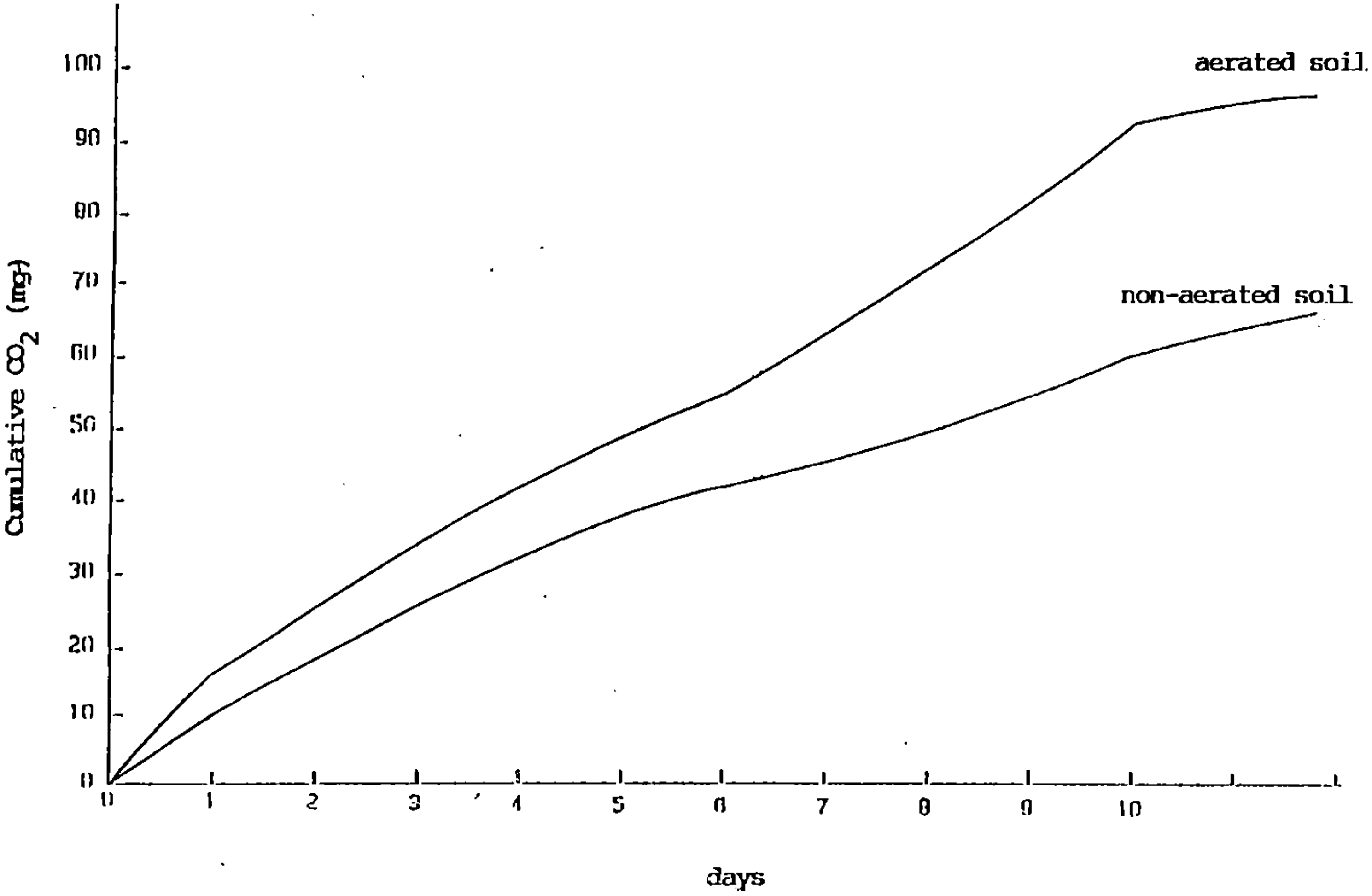
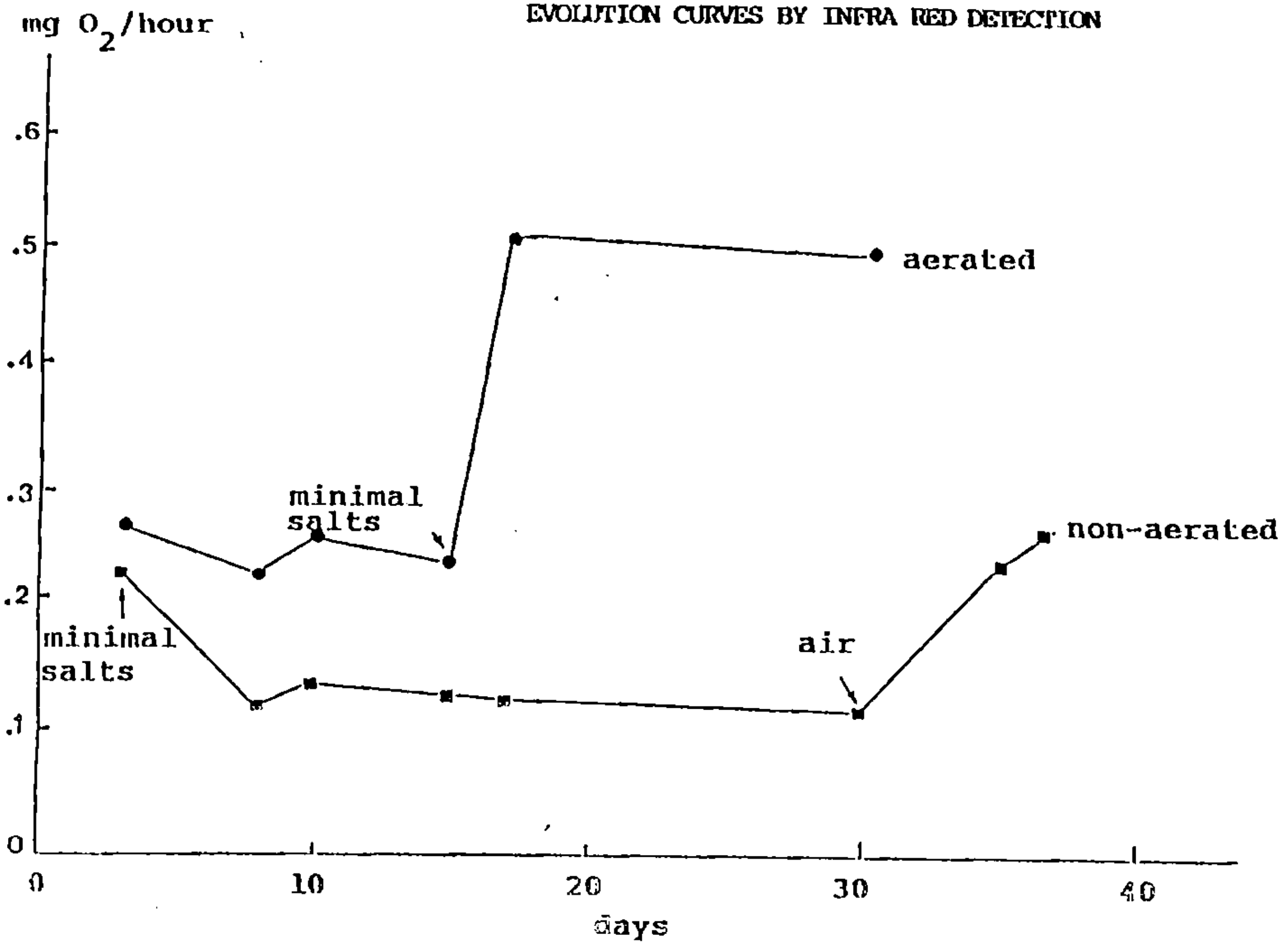


Figure 4 BIODEGRADABILITY ASSAYS CO_2
EVOLUTION CURVES BY INFRA RED DETECTION



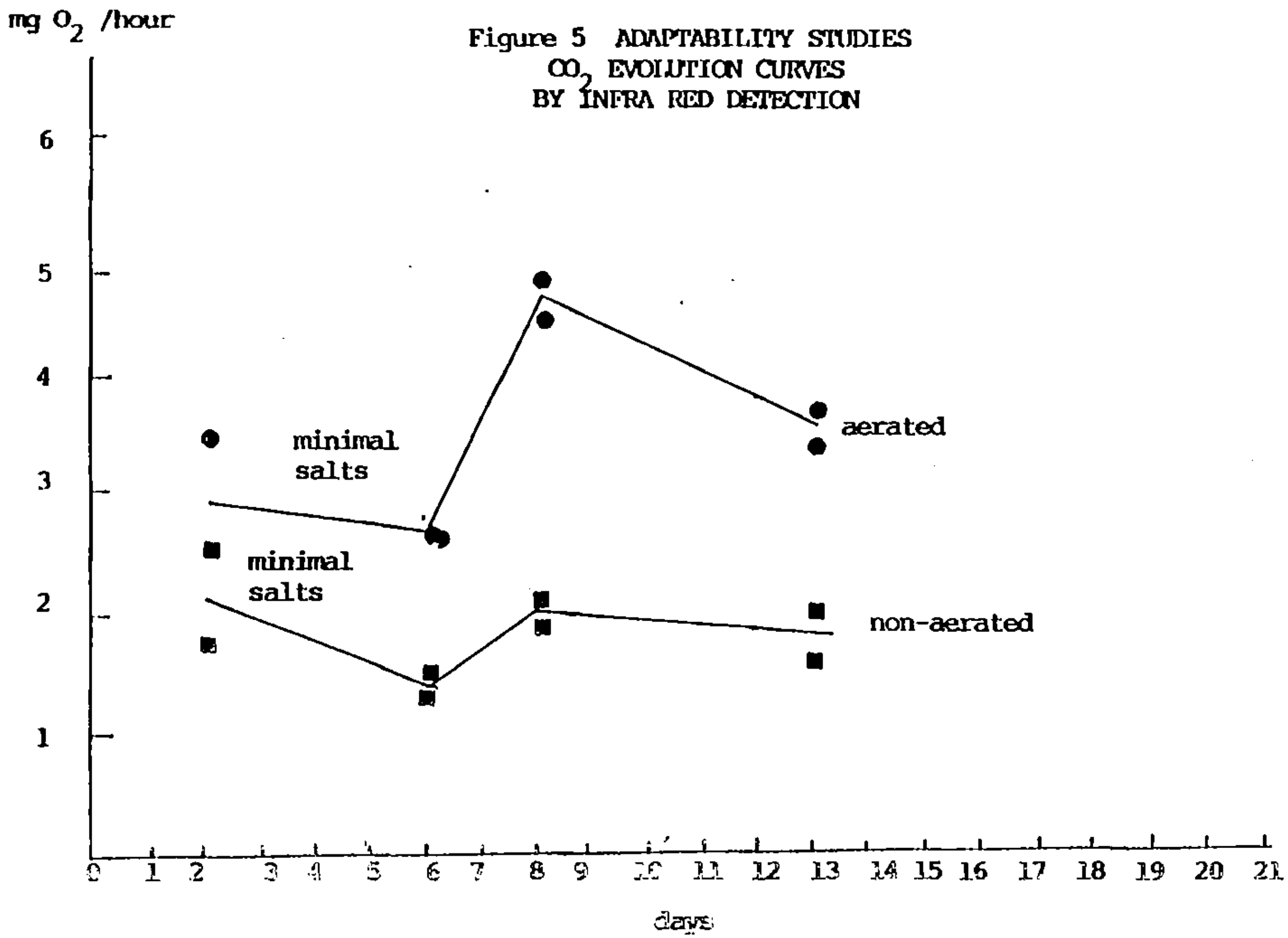


Figure 5 ADAPTABILITY STUDIES
CO_2 EVOLUTION CURVES
BY INFRA RED DETECTION

Figure 6 ADAPTABILITY ASSAY CHROMATOGRAMS

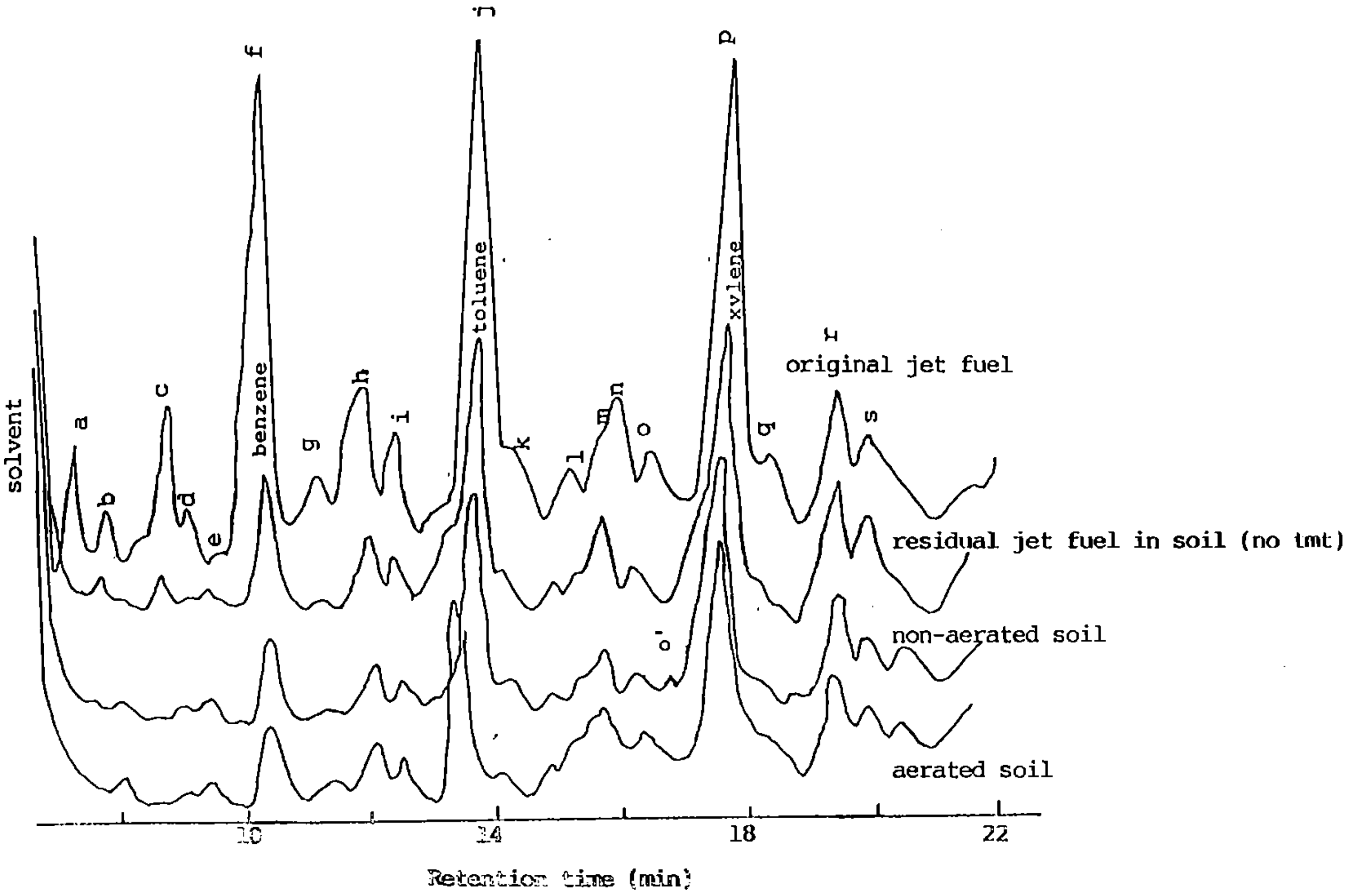


Figure 7 BIODEGRADABILITY ASSAY CHROMATOGRAMS

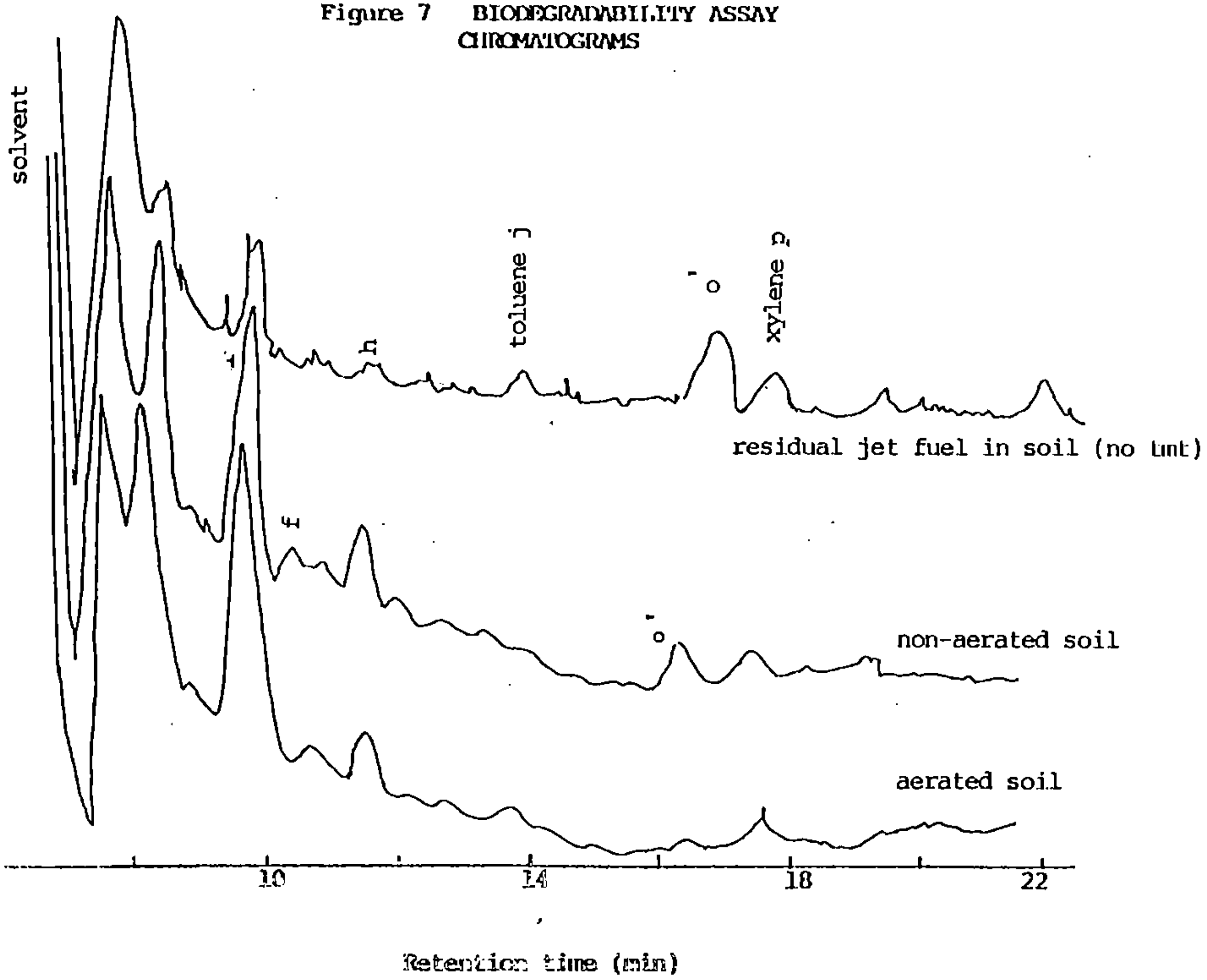


Figure 8 BIODEGRADABILITY ASSAY IR SPECTRA

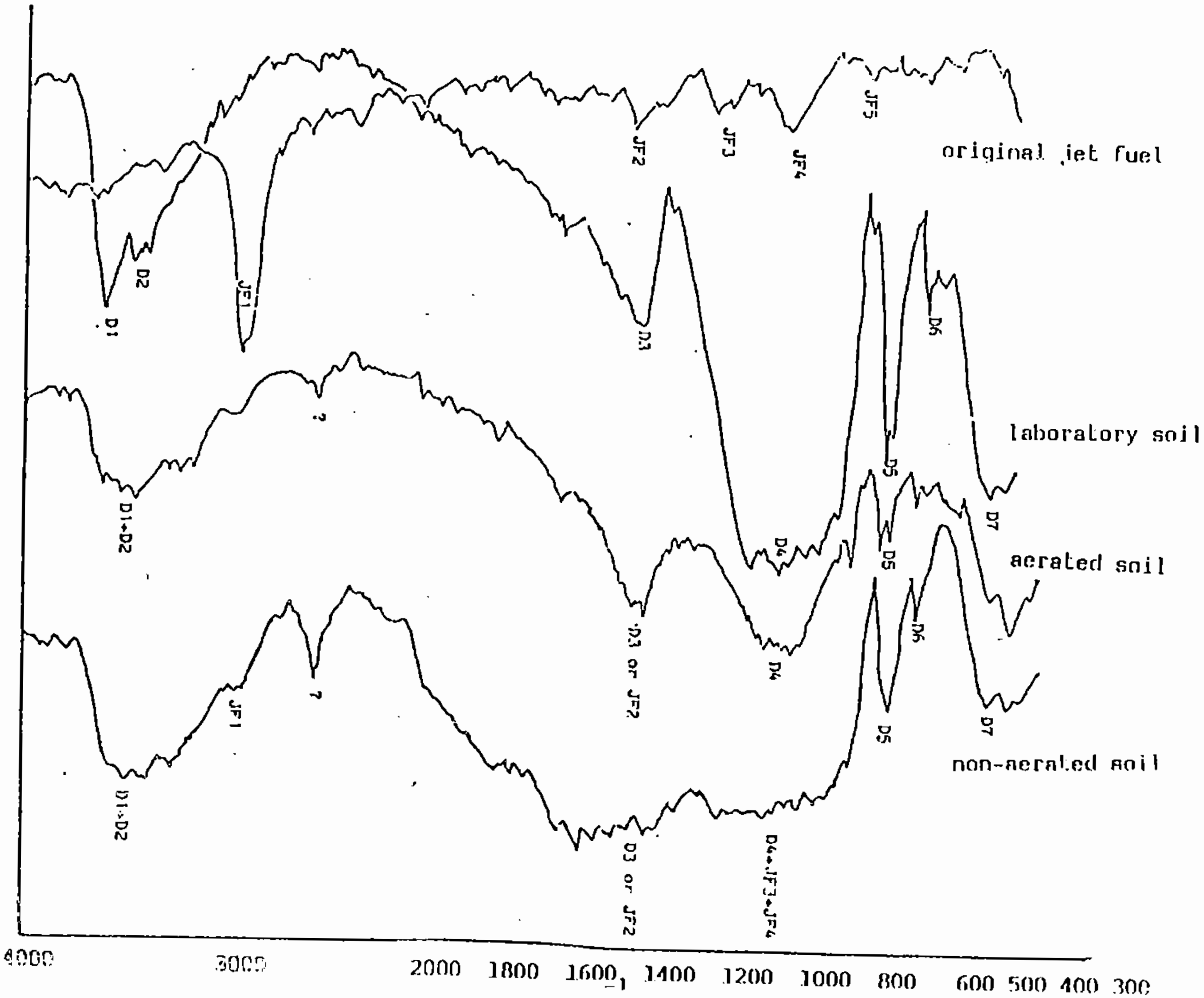
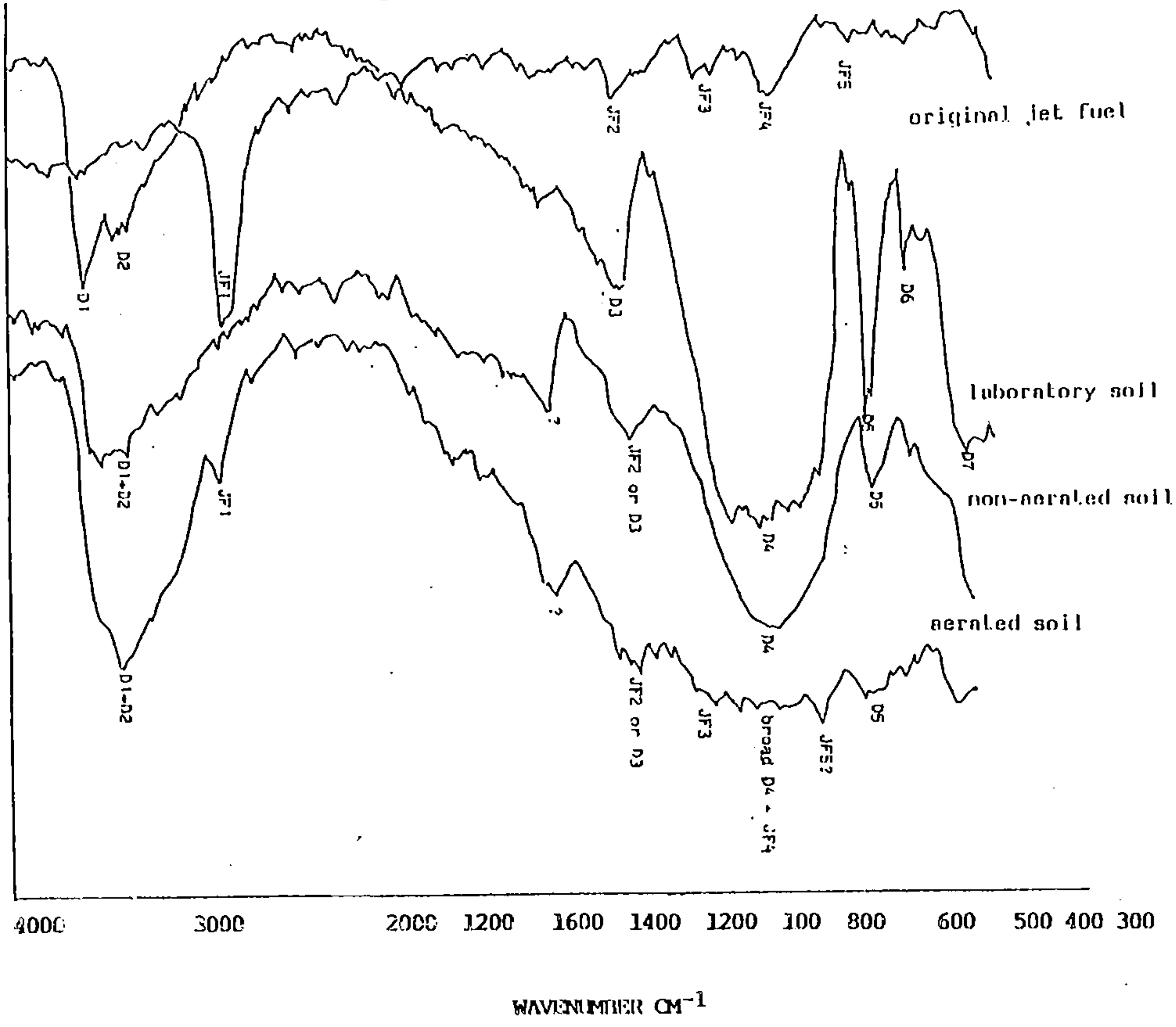


Figure 9 ADAPTABILITY ASSAY IR SPECTRA



2 m of soil depth in this area consists of a sandy soil (Figure 1) underlain by 12 to 15 m of clay. The perched water table is located in the sandy soil layer and flows toward the southeast and southwest into the Mascouche River and the Milles-îles River. These rivers eventually join the des Prairies River and ultimately the St. Lawrence River. The "permanent" water table, situated in the rock underneath the clay, shows no trace of contamination yet.

Since contamination appears to be confined to the sandy soil layer, with traces in the top portions of the underlying clay, it appeared that *in situ* bioremediation using forced aeration to a nutrient-enhanced sandy soil system could yield significant benefit. However, before any field experiment could be conducted, it was necessary to undertake laboratory studies with the field soil to determine if forced aeration could produce effective results. In addition, the laboratory program was considered essential inasmuch as base values had to be established with respect to initial and boundary conditions. Accordingly, the disappearance of PAHs was monitored in the laboratory under forced aeration conditions, and isolation and identification of PAH-degrading microorganisms were attempted. The results will be used to evaluate field implementation requirements, especially in regard to accountability for the parameters controlling the various aspects of the forced aeration technique.

MATERIALS AND METHODS

The sandy soil samples obtained from an area adjacent to the foundations of the old incinerator, at a depth of one meter, were stored at 4 C. Analysis of contamination consisted of determining:

- Concentration of heavy metals (Zn, Cr, Pb, Cu, Ni, Cd) and potassium from atomic adsorption measurements on digested soil using the digestion technique described in HACH (1987)
- Concentration of nitrogen using the Total Kjieldahl Nitrogen test (TKN), and concentration of phosphorus using the ascorbic acid method (HACH 1987)
- Total organic carbon (TOC) using the wet oxidation test (U. S. D. A. 1954)
- pH of the samples according to the method of Dunn and Mitchell (1984)
- Grain size distribution of the sandy soil according to ASTM D421 and D422 (Bowles 1986)

The disappearance of PAHs and PCBs (polychlorinated biphenyls) was monitored at room temperature over two periods, 10 and 20 days, after initiation of forced aeration conditions. The contaminated soil was first placed in capped plexiglass cylinders (cells) provided with holes at both top and bottom caps. For the aeration procedure, compressed air at 0.0352 kg/cm^2 (0.5 psi) was introduced at the top (cap) and allowed to freely exit through the holes in the bottom cap. The (compressed) air from the compressor was forced through a gauze filter and humidified with sterile deionized water. Preliminary experiments designed to examine pressure effects indicated that pressures greater than 0.0352 kg/cm^2 caused excessive moisture loss in the test samples. Hence to avoid rapid desiccation of the test samples, it was decided to maintain an air pressure limit value of 0.0352 kg/cm^2. At this pressure, and for the type of soil tested, the average flow was approximately 0.79 L/min. Air flow was monitored every week for every cell using a rotometer.

Nine cells were used in the course of the laboratory study to assess the various parameters affecting the disappearance of PAHs. These cells were kept in the dark throughout the study period to avoid photodegradation of the samples. Cells A_1 and A_2 were filled with approximately 170 g of the sandy soil obtained from the site, with no added nutrients (subscripts 1 and 2 represent measurements after 10 and 20 days, respectively). The soil in each cell was subjected to forced air during the prescribed test period, and the concentration of PAHs was determined thereafter.

Cells AN_1 and AN_2 were identical to cells A_1 and A_2, but were supplemented with specificed amounts of nutrient salts (0.114 g NH_4NO_3 and 0.174 g KH_2PO_4 per cell), determined according to Parkinson (1974). Cells C_1 and C_2 were control cells containing the test soil samples, but without benefit of forced aeration and without nutrients. Cells AC_1 and AC_2 were control cells containing autoclaved test soil samples subjected to forced aeration. Autoclaving was conducted at 120 C and at standard pressure for 20 min. This procedure was adopted since it has been shown to have no effect on the concentration of the PAHs in the soil (Herbes & Schwall 1978).

Determination of PAH and PCB concentrations were performed by Analchem Laboratories, St. Hubert (Québec) according to the standards of the Québec Ministry of the Environment (1985). The only PAHs and PCBs measured were the ones listed on the PAH priority pollutants listed by the Québec Ministry of the Environment (1988).

Soil extract medium was used to isolate the microorganisms (Parkinson *et al.* 1971). After the specified test periods of 10 nd 20 days, 1 g of the test soil was taken from each cell and dissolved in 100 mL sterile deionized water. Dilutions ranging from 10^{-2} to 10^{-5} g/mL were made, and 0.1 mL of each dilution spread on soil extract agar (1.5%) (Parkinson *et al.* 1971). The plates were incubated at room temperature until distinct colonies formed. Isolated colonies were transferred to fresh soil extract agar plates and stored at 4 C.

The biodegrading activity of the isolated microorganisms in the presence of any contaminant was determined via optical density (O.D.) measurement (using a spectrophotometer at 420 nm), and population growth comparisons made with control samples. The isolated microorganisms were grown in soil extract broth, and 0.1 mL was transferred to 10 mL of minimal salt solution (Bailey & Coffey 1986). Duplicate tubes of each microorganism were then singly supplemented with a final concentration of 50 ppm of either phenanthrene, anthracene (American Chemical Co.), or fluorene (Eastman Kodak Co.). These hydrocarbons were first dissolved in acetone (Bumpus 1989). The controls contained only the Minimal Salt Solution and the chemical. Assessment of growth of the microorganisms was by O.D. measurement. After a 28-day incubation period, the extent of growth was determined also by a plate count of microorganisms in the presence and absence of the above-described single contaminants.

RESULTS AND DISCUSSION

The grain size distribution for the sandy soil samples obtained from the Mascouche site and used for the study is given in Figure 1. The particle size analysis indicates that the soil is a silty sand. Figure 2 shows the concentration of heavy metals present in the test soil compared with the background concentration for a typical Québec soil and together with the level considered "contaminated" by the Québec Ministry of the Environment (1988). The results show that Pb and Cd far exceed the contamination levels established by the Québec government. The measured concentration of nutrients in the soil were: phosphorous 693 mg/kg, potassium 765 mg/kg, and nitrogen 220 mg/kg. These concentrations were increased to 903 mg/kg, 1,031 mg/kg, and 430 mg/kg, respectively, after the addition of nutrient salts in the AN cells.

Figure 3 shows the variation of the soil pH over the duration of the PAH disappearance experiments. All samples showed a linear decrease in pH with the exception of the control sample C. Sample AN demonstrated the greatest drop in pH. The sterile control sample C showed a slight increase in soil pH with time. The slightly acidic pH levels are not expected to affect the potential for biodegradability since the values are not below the limit conditions discussed by Leahy and Colwell (1990). Since most heterotrophic bacteria and fungi prefer near-neutral pH values, with fungi being more tolerant to acidic conditions (Leahy & Colwell 1990), fungi would be expected to be the main biodegrading agent.

The initial water content (27%) of the soil dropped significantly (to 3.1%) after one week of forced aeration, but only slightly thereafter. Since water content is essential for growth and metabolism of microorganisms (Leahy & Coldwell 1990), it was considered essential to maintain favourable water content conditions throughout the tests. Although pressure from forced aeration on the soil may have a slight inhibiting effect on the activity of the microorganisms (Leahy & Colwell 1990), this effect is considered minimal and insignificant for the pressure levels used. The total organic carbon (TOC) at the start of the experiment was approximately 2.76 percent. The small drop observed in the TOC for sample AN (2.66 percent) could mean that natural selection of microorganisms, as well as full development of the microbial population, may not have had time to occur (Kosson *et al.* 1987).

Disappearance of PAHs

The results shown in Figure 4 indicate biodegradation of PAHs in cell AN (the AN curve is lower than the AC curve) and the importance of added nutrients. A significant portion of the disappearance of PAHs occurred over the first 10 days. As shown in Figure 5, the AN curve is much lower than all the others, indicating that phenanthrene and/or anthracene was easier to biodegrade than the other contaminants. In Figure 6, the sample without added nutrients showed a much greater susceptibility to biodegradation of fluorene than the other samples.

These results indicate that the major factors in the disappearance of PAHs were nonbiological in nature. Approximately 80 percent of the PAH disappearance was due to physical or chemical parameters; only 20 percent was microbially transformed. The disappearance of anthracene and phenanthrene, however, demonstrates the effectiveness of bioremediation. The results in Figure 5 show that 52.4 percent of phenanthrene and anthracene was biodegraded. The results in Figure 6 indicate that microbial activity was responsible for the disappearance of fluorene. Since phenanthrene and anthracene show similar peaks in gas chromatograph measurements, they were added together in the results. However, phenanthrene is easier to degrade since it is approximately 20 times more soluble in water than anthracene (Leahy & Colwell 1990). Also, because of the bay area (Chakrabarty 1982) inherent in the biochemical structure of phenanthrene, it is exposed to enzymatic attack on three surfaces at the same time whereas anthracene exposes only two of its surfaces. Fluorene demonstrated positive biodegradation as well. This is to be expected since it is more soluble in water than phenanthrene (CRC Handbook 1984), and mineralization rates of PAHs are related to aqueous solubilities rather than to total substrate concentration (Leahy & Colwell 1990).

Volatilization seems to be the major factor affecting the disappearance of PAHs in this experiment. Under natural conditions (i.e., no forced aeration), volatilization would have been negligeable (Park *et al.* 1990). Forced aeration, combined with the decrease in water content and the low percentage of TOC (which increases the amount of contaminants exposed to air), stripped and volatilized most of the PAHs in the soil. PCBs such as Arochlor 1242, 1248, 1254, and 1260 were unaltered during the experiment and remained at 0.8, 0.2, 1.5, and 0.9 mg/kg, respectively, in the sandy soil.

Biological Tests

Of the 21 strains of microorganisms isolated from samples AN, A, and C, only 13 were actually tested for their degrading capacities. This is mainly because some of the strains were molds that could have been contaminants, and some strains could not be maintained. Only the results of strains that demonstrated positive degradation are included in this paper (Figures 7, 9, 11, 13, 15, 17, and 19). In Figures 7, 9, 13, 15, and 19, the plate count was higher for the minimal salt solution contaminated with phenanthrene (black bar graph) than for the control containing no contaminant (striped bar graph). This demonstrates that the microorganisms utilize phenanthrene as a source of carbon, and therefore, phenanthrene increases it's population. In Figures 11, 13, and 18, the fluorene bar graph (black) is higher than the control bar graph (striped), showing that fluorene is utilized by those strains. In Figures 13 the bar graph for anthracene is higher than the control bar graph, indicating that this strain can use anthracene as sole source of carbon. However, from Figures 9, 11, 15 and 19, the bar graphs indicate that anthracene has an inhibiting effect on growth of these strains.

Figures 8, 12, 14. 16, 18, and 20 show the variation in optical density over aperiod of 43 days. In the case of phenanthrene, the optical density decreased slightly before increasing. This was observed in most cases where phenanthrene was biodegraded. However, strains that showed no positive degradation, but some tolerance to phenanthrene, had the same shape of curve. In the cases where fluorene was biodegraded, the curve was mainly flat, so prediction of biodegrading capacity was not possible. In the case of anthracene, O.D. techniques proved impractical. O.D. curves must be compared to the control curve to avoid confusing a natural increase in population with growth of the microorganisms in the presence of a contaminant. Dean-Ross (ASMB 1990) reported that growth of a phenanthrene-degrading, gram-negative bacteria could be measured by observing an increase in the optical density and in colony forming units in a liquid inoculum. However, Amador, Alexander, and Zika (ASMB 1988) observed a decrease in optical density when monitoring the biodegradation of organic molecules in the presence of sunlight. Therefore, the initial drop in the O.D. curve for phenanthrene could indicate the emulsifying effect the bacterial or fungal enzymes may have on the chemicals (Leahy & Colwell 1990), as well as a certain degree of photodegradation and possibly adaptation (Leahy & Colwell 1990) during which many of the intolerant microorganisms die. The subsequent increase in optical density of the samples that demonstrates population increase could result from the growth of the microorganisms that increases the turbidity of the broth culture.

From the results of the biological tests performed on the individual strains (Figure 7, 9, 13, 15 and 19), phenanthrene seems to be the chemical that supported the most growth, thus is most susceptible to microbial attack. Anthracene showed the least susceptibility to microbial attack and had some inhibiting effects on certain strains (Figures 9, 11, and 15). This could explain the asymptotic return of curve AN in Figure 5, since most of the phenanthrene disappeared, while the more resistant anthracene remained in the soil. In Figure 15, no results are shown for fluorene because the plates were found to be contaminated during the count. Contamination also negated results for anthracene after 28 days.

Gram stains were performed on the strains that showed increased growth (positive degradation) in the presence of the three chemicals studied (Figures 7, 9, 11, 13, 15, 17 and 19).

Strains 4, 8, and 21 were gram-negative rods, with strains 8 and 21 being pleormorphic. Strains 9 and 16 were yeasts. Strains 12 and 18 contained two microorganisms, which were difficult to separate at the start of the tests. Strain 12 consisted of a yeast and another fungus, and strain 18 consisted of a yeast and a gram negative-rod. All the yeast in these tests were of the same genera.

All the yeasts were identified as being *Candida parapsilosis* by the Royal Victoria Hospital Clinical Laboratories in Montreal. This type of yeast had been isolated from a soil in 1965 by Asahi Kaisha (Jong & Gantt 1987) and patented for its ability to degrade hydrocarbons. Equally, *Candida parapsilosis* has also been patented by Bioteknika International (Jong & Gantt 1987) for its ability to degrade petroleum. Parkinson (1974) stated that a yeast of the *Candida* genus could degrade petroleum in arctic soils. *Candida parapsilosis* can also produce citric acid (Jong & Gantt 1987), which could explain the decrease in soil pH in the sample that showed the most biodegradation, as well as in the other samples. The other strains of microorganisms are presently being identified.

CONCLUDING REMARKS

This study reinforces the view that base-value characterization of test technique -- for assessment of effectiveness of treatment procedures and parametric evaluation -- prior to scaled up field experiments is necessary. The importance of nonbiological parameters in a forced aeration system has been demonstrated. During decontamination procedures involving this type of technique, a transfer of contaminants from the soil to the atmosphere could occur if no precautions are taken. If byproducts and pathways of biodegradation are not fully documented, contaminants could be masked and may not be readily identified by gas chromatography. Nutrient salts appear to have made a difference in the biodegradation process in the sandy soil tested.

ACKNOWLEDGEMENT

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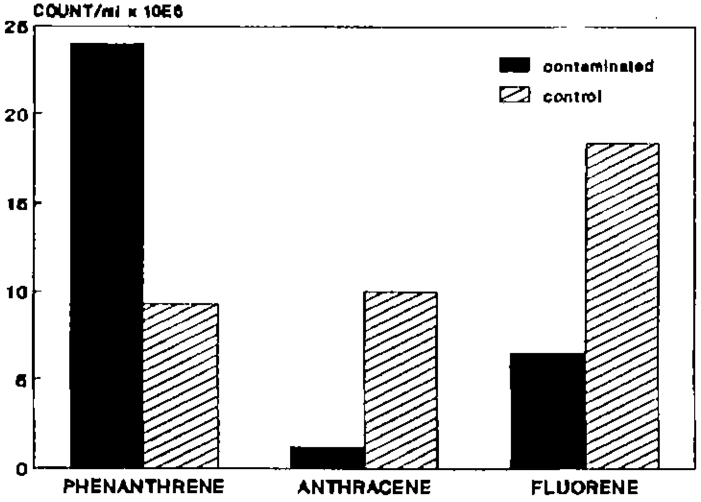

FIGURE 9 - PLATE COUNT (Strain #8)

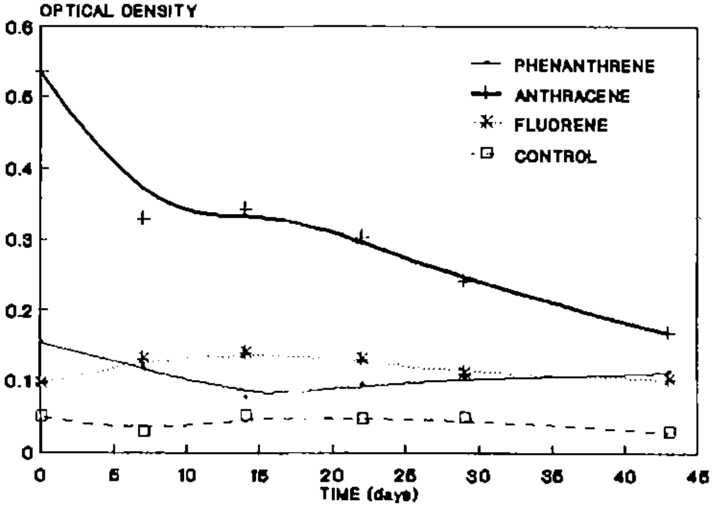

FIGURE 10 - OPTICAL DENSITY (Strain #8)

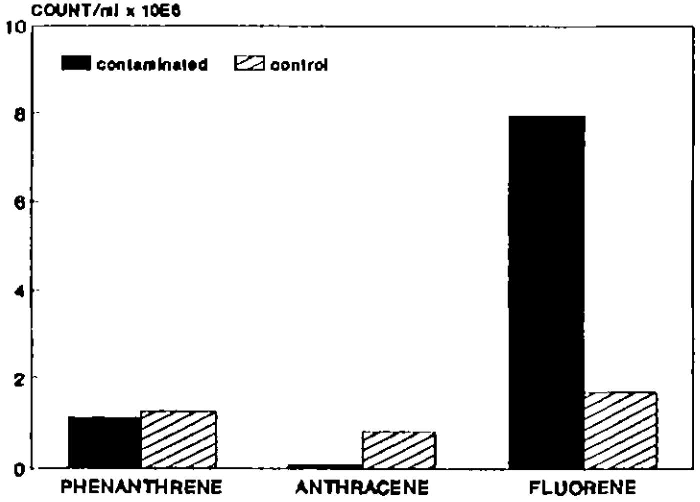

FIGURE 11 - PLATE COUNT (Strain #9)

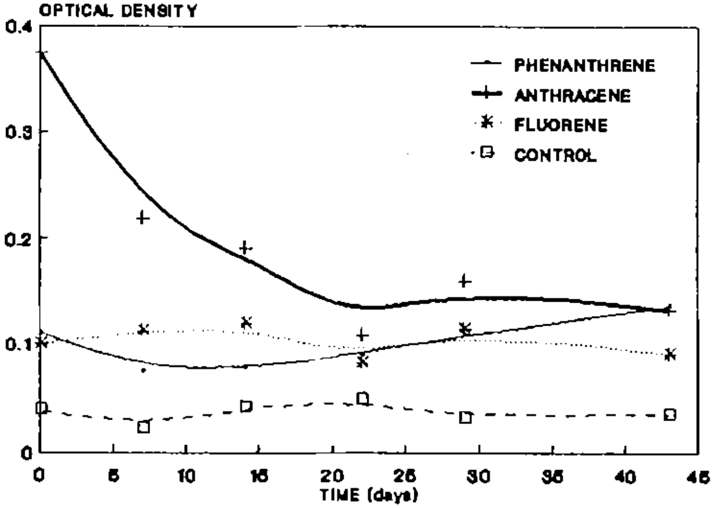

FIGURE 12 - OPTICAL DENSITY (Strain #9)

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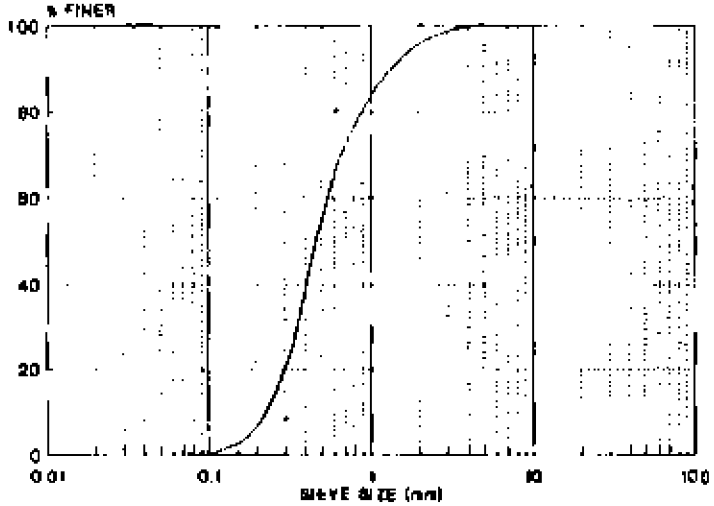

FIGURE 1 - GRAIN SIZE ANALYSIS OF SOIL

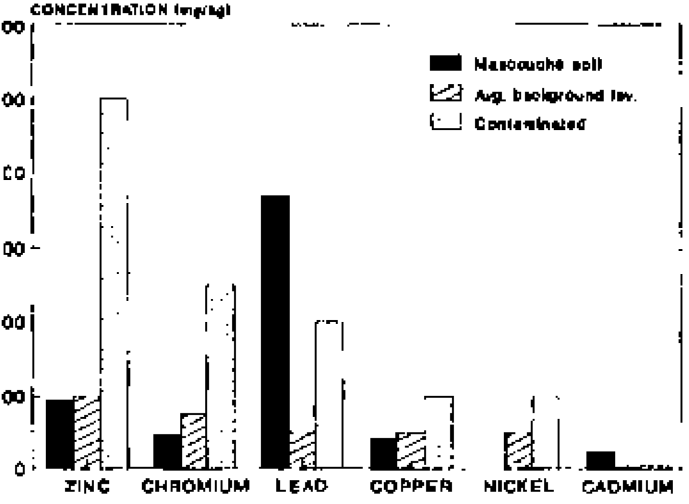

FIGURE 2 - HEAVY METALS CONCENTRATION

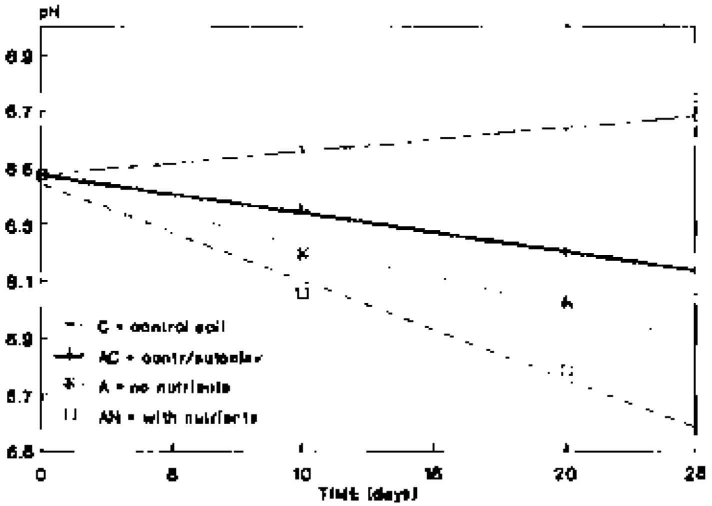

FIGURE 3 - pH vs TIME

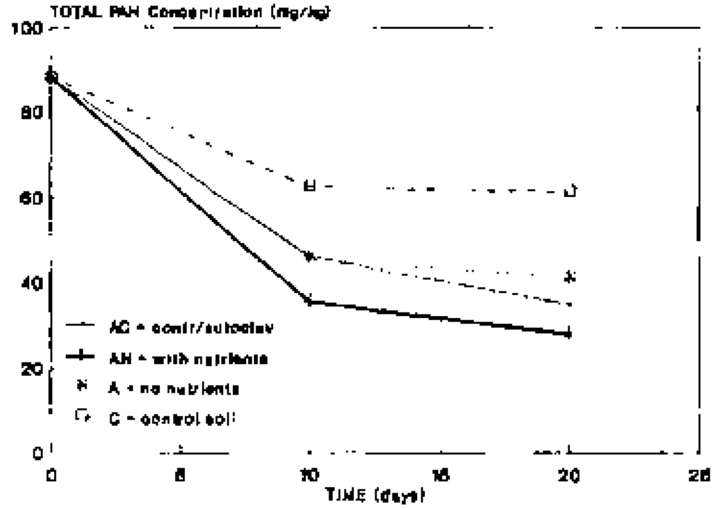

FIGURE 4 - DISAPPEARANCE OF PAHs

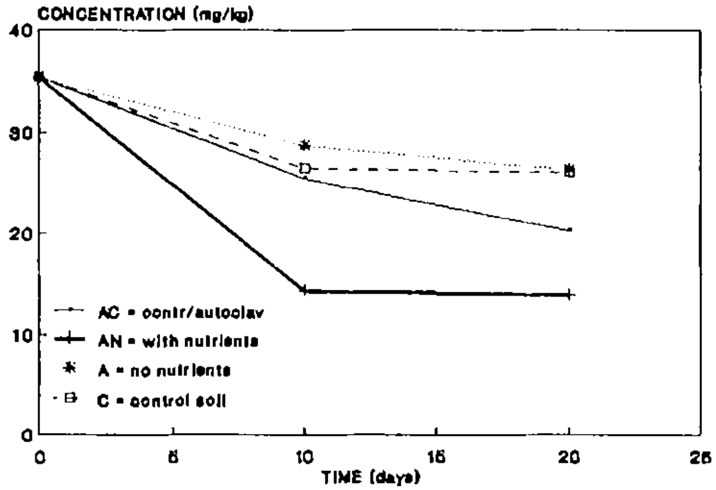

FIGURE 5 - DEGRADATION OF ANTHRACENE AND PHENANTHRENE

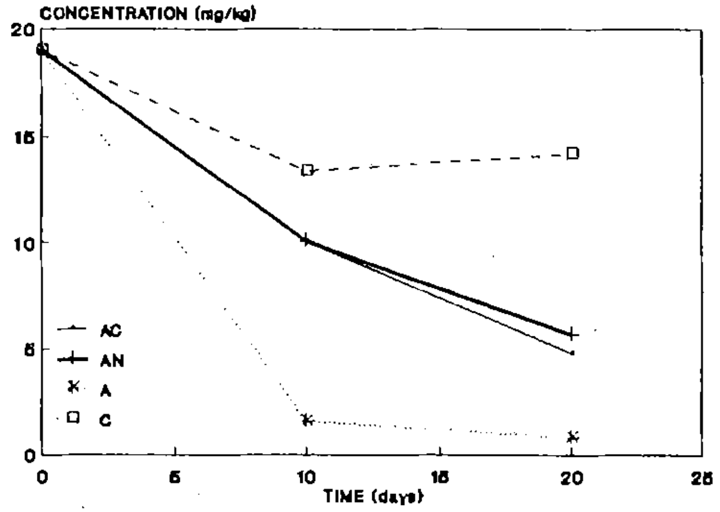

FIGURE 6 - DEGRADATION OF FLUORENE

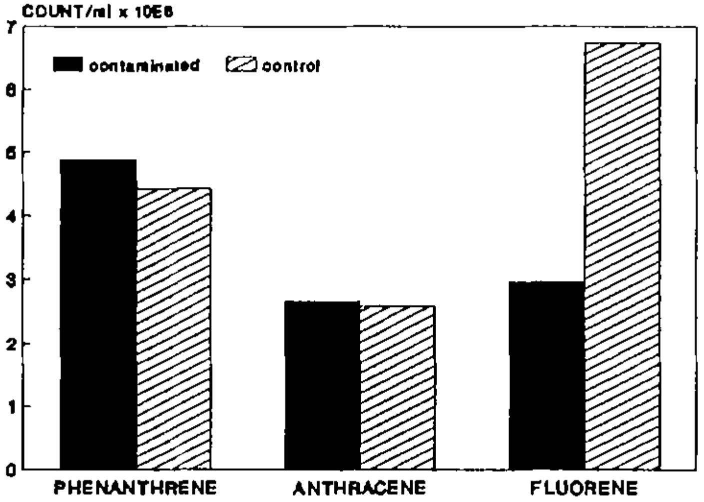

FIG. 7 - PLATE COUNT (Strain #4)

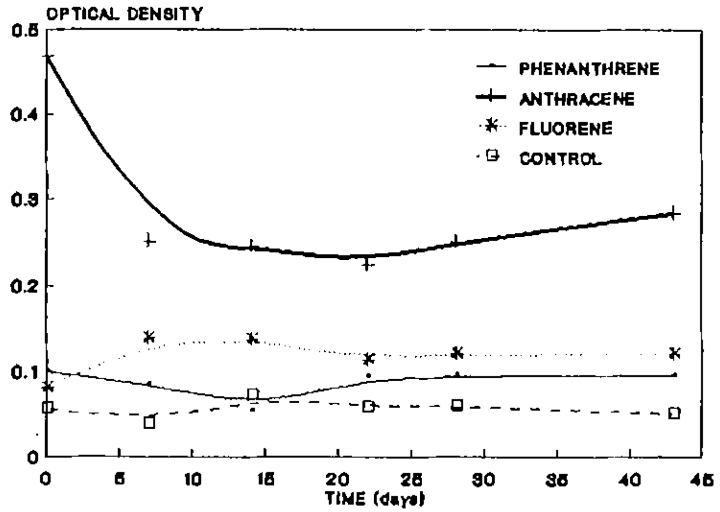

FIGURE 8 - OPTICAL DENSITY (Strain #4)

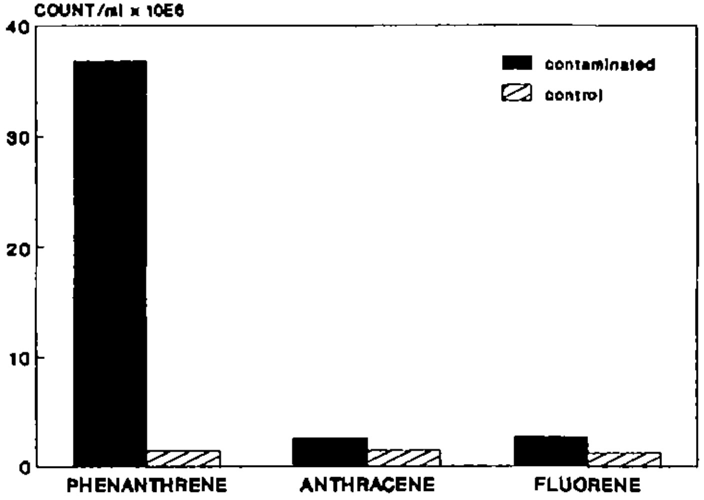

FIGURE 13 - PLATE COUNT (Strain #12)

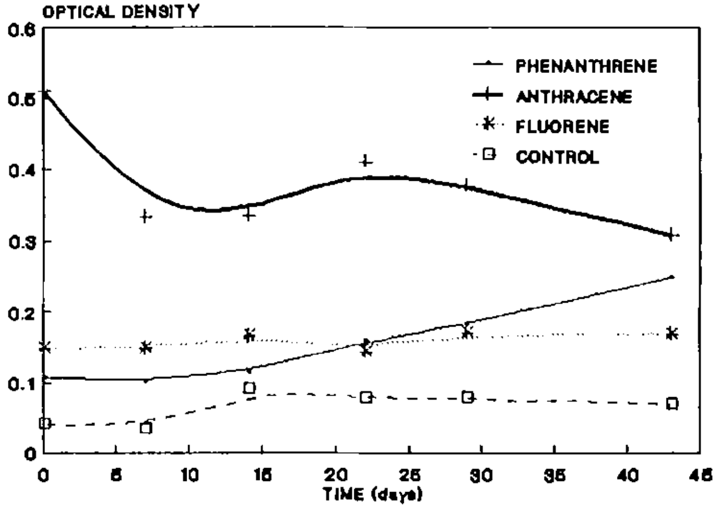

FIGURE 14 - OPTICAL DENSITY (strain #12)

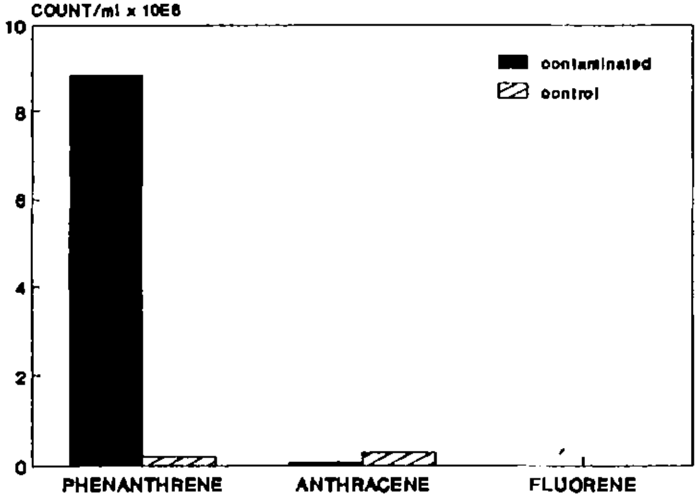

FIGURE 15 - PLATE COUNT (Strain #16)

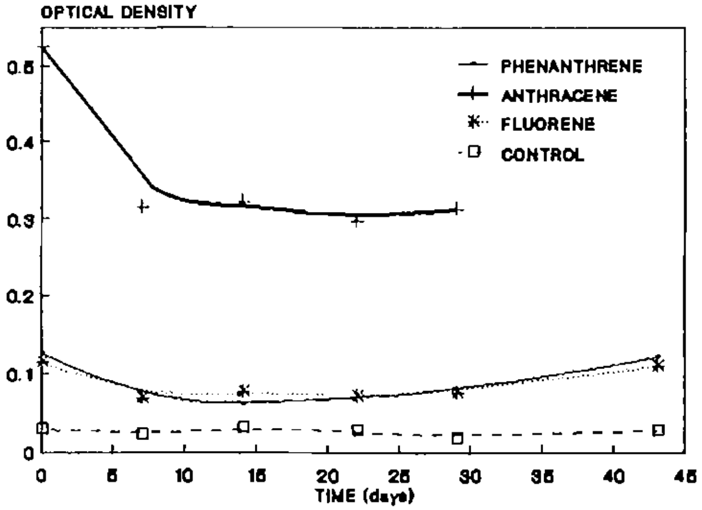

FIGURE 16 - OPTICAL DENSITY (Strain #16)

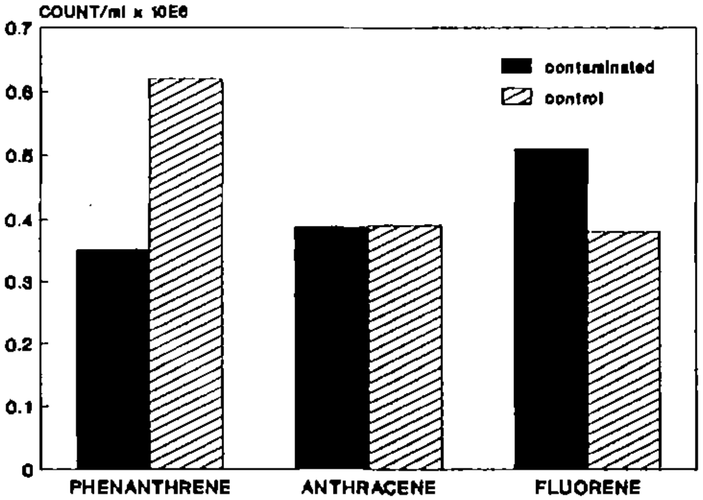

FIGURE 17 - PLATE COUNT (Strain #18)

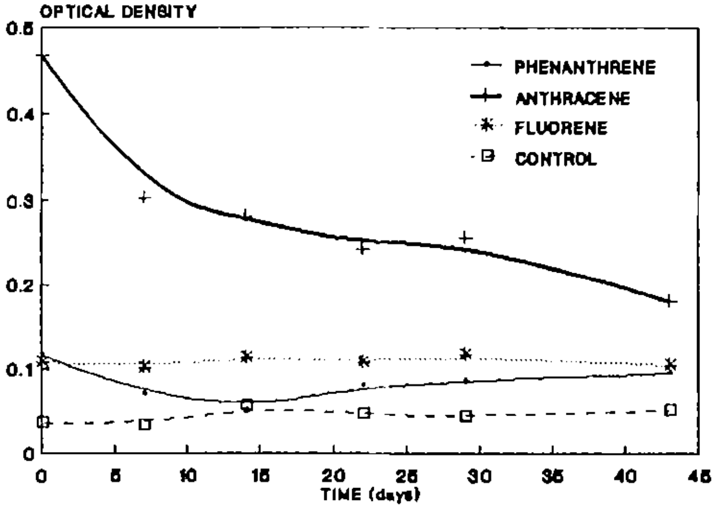

FIGURE 18 - OPTICAL DENSITY (Strain #18)

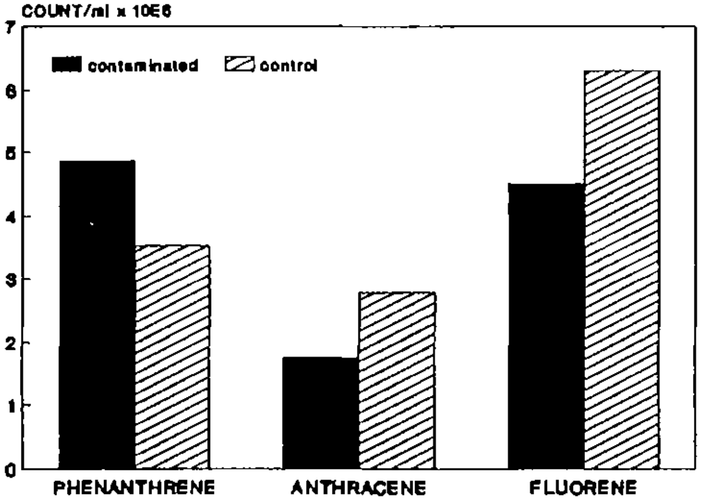

FIGURE 19 - PLATE COUNT (Strain #21)

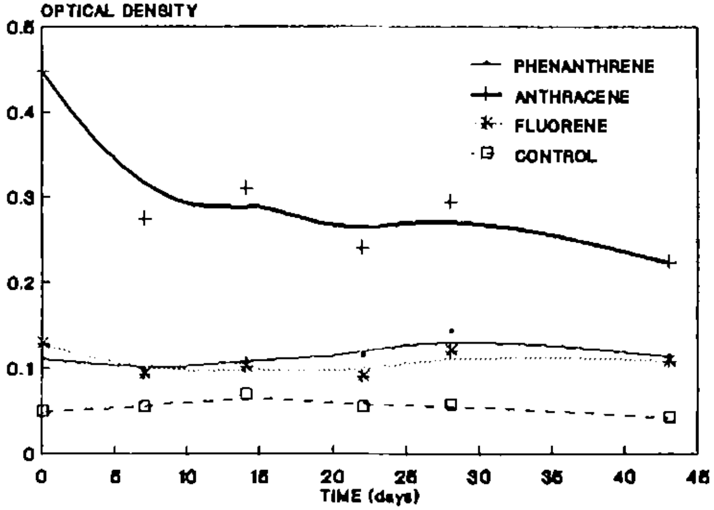

FIGURE 20 - OPTICAL DENSITY (Strain #21)

Introduction

Crude petroleum and its derivatives represent a complex mixture of hydrocarbons that are by and large inherently "toxic." Industry sources estimate that from 4 to 12% of the 5–8 million underground storage tanks in North America that store petroleum products and chemical substances are leaking and contaminating subsurface soils and groundwater systems (PEI) Associates Inc. 1988).

Present *in situ* (land) treatment technologies for mitigation of petroleum hydrocarbon (PHC) contamination from a contaminated site are either in the developmental stages or being tested. They involve physical, chemical, and biological methods or combinations thereof. *In situ* chemical treatments that attempt to immobilize the petroleum contaminants in the soil substrate involve precipitation, complexation, and polymerization of the contaminated subsurface soil. Sorption of the PHC's by the soil substrate is another method for restricting the movement of petroleum contaminants from a contaminated site. Sorption by soil can be improved upon by application of sewage, sludge, activated carbon, and agriculture products and byproducts (PEI Associates Inc. 1988).

Clay fractions of soils are electrically charged, have large surface areas, and have the ability to adsorb organic molecules. Theng (1974) and Raussell-Colom and Serratosa (1987) have reviewed the interaction of organic molecules with clays. Many polar organics (e.g., alcohols, amines, ketones) are adsorbed onto the external clay surface, interlayer (between unit layers) space, and probably also on the clay particle edges by electrostatic attraction and ion-exchange reactions. Adsorption of nonpolar organic molecules (e.g., alkanes, aromatic hydrocarbons) by clay occurs by the much weaker van der Waals attraction and is restricted to the external clay surface.

To devise efficient strategies for restricting petroleum migration from a contaminated site utilizing the attenuation ability of a soil substrate, it is necessary to establish the sorption–interaction relationships between the petroleum contaminants and the soil substrate. The basic questions that need to be addressed are as follows: (*i*) What are the prominent chemical constituents of the various petroleum fractions that interact with the soil substrate? (*ii*) What are the functional groups of a soil that interact with PHC contaminants? (*iii*) What are the bonding mechanisms possible between the soil functional groups and the PHC contaminants? (*iv*) What are the consequent changes in the soil physical properties on interaction with PHC molecules? (*v*) What are the factors influencing the interactions between PHC molecules and clay mineral particles of the soil substrate? (*vi*) If the petroleum contaminants are weakly adsorbed by the soil substrate, what is the feasibility of improving the soil's attenuation ability of PHC's?

The development of answers to the basic questions are undertaken herein with a view towards understanding the bonding mechanisms possible between PHC molecules and soil functional groups and the feasibility of improving the attenuation ability of the soil substrate for petroleum hydrocarbons on suitable chemical treatment.

Prominent petroleum chemical constituents

Based on their boiling point ranges, the various petroleum products may be grouped under (*i*) light gasolines (boiling point range 30–100°C), (*ii*) heavy gasoline (boiling point range 100–200°C), and (*iii*) diesel and kerosene (boiling point range 200–300°C) (Neumann *et al.* 1981). Each petroleum fraction comprises a mixture of hydrocarbons, i.e., compounds formed by combination of C and H atoms. The important hydrocarbons encountered in the light and heavy gasoline and diesel and kerosene petroleum fractions are described below.

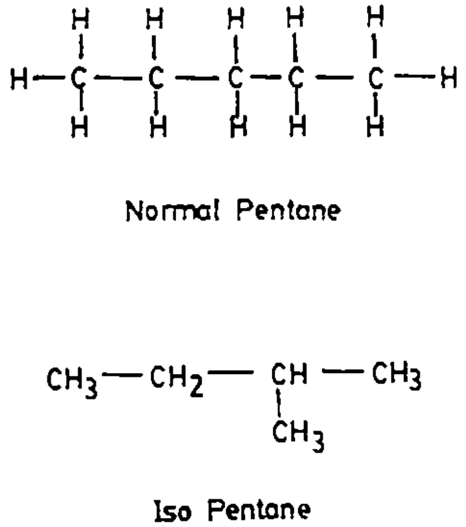

FIG. 1. Chemical structures of selected normal paraffin and isoparaffin.

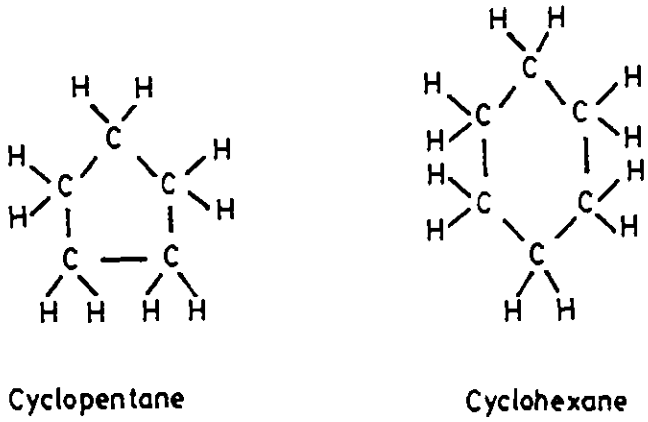

FIG. 2. Chemical structure of selected cycloalkanes.

Alkanes

These are saturated hydrocarbons and contain a single bond between two carbon atoms. All of the bonds of the tetravalent carbon, which are not bonded to other carbon atoms, are bonded to hydrogen atoms. The bonded carbon atoms can form chains (paraffins) or rings (cycloalkanes). Straight-chain structures are normal paraffins (*n*-paraffins). Branched-chain structures are isoparaffins. Examples of normal paraffins and isoparaffins are *n*-pentane and isopentane, respectively, whose structures are shown in Figs. 1 and 2.

Saturated hydrocarbons having a ring structure are identified as cycloalkanes. Some members of this group are cyclopentane (C_5H_{10}) and cyclohexane (C_6H_{12}), which are formed from five or six CH_2 groups (Fig. 2). Some lower boiling point compounds in the paraffin and cycloalkane

TABLE 1. Physicochemical properties of normal paraffins

Compound	Formula	Molecular weight	Boiling point (°C)	Solubility in water at room temperature (ppm)	Density (g/mL)	Dielectric constant	Dipole moment Debye
Methane	CH_4	16.04	−162.0	24.4	0.555	1.70	0
Ethane	C_2H_6	30.07	−89.0	60.4	0.572	1.00	0
Propane	C_3H_8	44.11	−42.2	62.4	0.585	1.61	0.084
Butane	C_4H_{10}	58.12	−0.6	61.4	0.579	1.78	0.05
Pentane	C_5H_{12}	72.15	36.1	360	0.626	1.84	0

TABLE 2. Physicochemical properties of cycloalkanes

Compound	Formula	Molecular weight	Boiling point (°C)	Solubility in water at room temperature (ppm)	Density (g/mL)	Dielectric constant	Dipole moment Debye
Cyclopropane	C_3H_6	42.08	−33	37 000	0.72	Not available	0
Cyclopentane	C_5H_{10}	70.14	49.4	< 1 000	0.751	1.965	0
Cyclohexane	C_6H_{12}	84.16	80.0	55	0.779	2.02	0

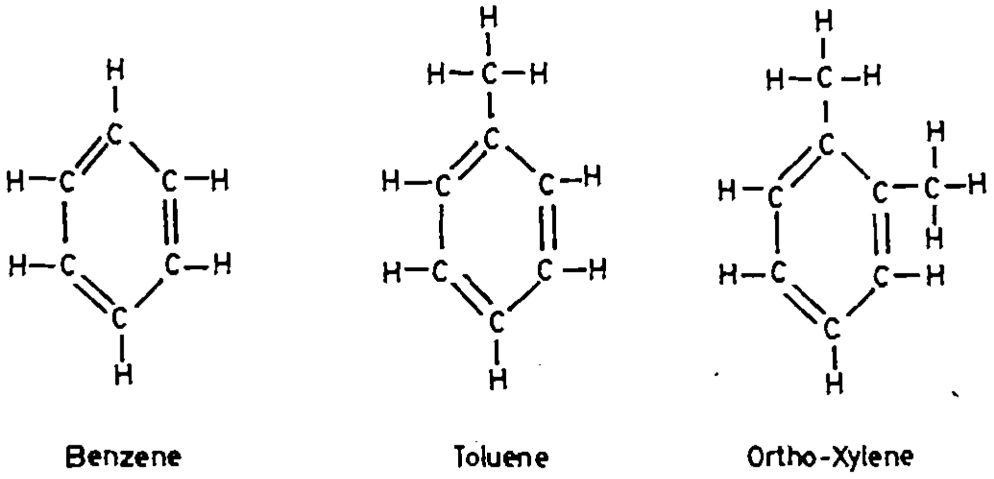

FIG. 3. Chemical structure of selected aromatic hydrocarbons.

groups which are encountered in the light gasoline fraction (group *i*) are listed in Tables 1 and 2, respectively.

Aromatic hydrocarbons

These are built on the 6-carbon benzene ring (Fig. 3). The constituent carbon atoms of the benzene ring do not contain the maximum possible number of hydrogen atoms and are therefore unsaturated. The bonds between the carbon atoms in the benzene ring are neither single nor double but of some hybrid type. Some properties of the aromatic hydrocarbons found in light and heavy gasoline fractions (groups *i* and *ii*) are identified in Table 3.

Polycyclic hydrocarbons

These are complex hydrocarbons and may contain aromatic as well as cycloalkane groups in the same molecule or may contain more than one aromatic ring (Fig. 4). Their polycyclic hydrocarbons are found in diesel and kerosene fractions (group *iii*). Some properties of these complex hydrocarbons are listed in Table 4.

PHC contaminant–soil interaction

Soil functional groups that can interact with PHC contaminants

The soil functional groups responsible for contaminant attenuation have been the subject of earlier detailed studies (Quigley 1987; Yong 1987). The reactive soil functional groups include both inorganic hydroxyl and organic surface functional groups. The inorganic hydroxyl surface functional groups can be found in crystalline clay minerals, amorphous silicate minerals, metal oxides, oxyhydroxides, and hydroxides. Organic surface functional groups include carbonyl, amino, sulphonyl, carboxyl, amine, and phenolic groups.

Bonding mechanisms possible between soil functional groups and PHC contaminants

A review of the properties of petroleum hydrocarbons (Tables 1–4) reveals that the various petroleum fractions are primarily constituted by nonpolar organics with dipole

TABLE 4. Physicochemical properties of polycyclic hydrocarbons

Compound	Molecular weight	Boiling point (°C)	Solubility in water at room temperature (ppm)	Density (g/mL)	Dielectric constant	Dipole moment Debye
Acenaphthene	154.21	279	3.47	1.02	na	na
Anthracene	178.24	342	0.075	1.283	na	0
Biphenyl	154.21	255.9	7.48	0.866	2.53	na
Naphthalene	128.19	218	31.3	1.025	2.54	0

On the basis of the above discussion, the hydrocarbon concentrations of Meyers and Quinn (1973) and Meyers and Oas (1978) refer to the accommodation concentration and not so much to the true dissolution level (concentration). The accommodation concentration of hydrocarbons in the water may be viewed to reflect the partitioning tendency of the organic substances between the aqueous and soil phases, respectively. Hydrocarbon molecules with lower accommodation concentrations in water would be partitioned to a greater extent onto the soil phase than in the aqueous phase, and vice versa. It is hence expected that the accommodation concentration of hydrocarbons in water has a bearing on the soil attenuation ability for hydrocarbons.

The results of Meyers and Quinn (1973) and Meyers and Oas (1978) for interaction of different hydrocarbon molecules at a concentration of 100 $\mu g \cdot L^{-1}$ with sodium smectite clay (50 mg) are shown in Table 5. These results show that there exists generally an inverse relationship between the accommodation concentration of the hydrocarbons and the percent adsorbed, i.e., the lower the accommodation concentration of the hydrocarbon in water, the greater the tendency of the organic compound to be associated with the (clay) mineral phase. Adsorption data of hydrocarbons indicate that only anthracene has substantial adsorption; presumably the higher accommodation concentrations of the aromatic hydrocarbons inhibits their association with the clay particles (Meyers and Das 1978). Data in Table 6 also show that the adsorption levels of the various hydrocarbons are low (≤ 1 g/kg of clay).

Contrary to the findings of Meyers and Quinn (1973), Button (1976) did not observe any removal of dissolved hexadencane molecules by smectite clay. The absence of adsorption is attributed to the use of a subsaturated solution where the preferred state of hydrocarbon molecule is in the true solution. Such a situation is possible because of the following reasons. A polar clay (mineral) surface is normally hydrated and has an ordered crystalline structure. When a nonpolar molecule dissolves in water at room temperature, it modifies the water structure in the direction of greater crystallinity, i.e., builds a microscopic "iceberg" around the solute molecule. This "freezing" of the water caused by the nonpolar molecule causes excessive heat and entropy to be lost (Frank and Evans 1945). Apparently the small heats of adsorption for a clay–hydrocarbon interaction (< -10 kcal/mol, Meyers and Quinn 1973) are inadequate to compensate the heat and entropy loss involved in desolvating the clay surface and the solute molecule, leading to a lack of hydrocarbon sorption from true solution.

Comparing the findings of Button (1976), Meyers and Quinn (1973), and Meyers and Oas (1978), it appears that adsorption of hydrocarbon by clay surfaces occurs only when the solubility of the hydrocarbon is exceeded and the hydrocarbon exists in the micellar form. The principal mode of interaction of the micelle with clay surfaces was inferred to be van der Waals attraction from the computed heats of adsorption values. It is known (van Olphen 1963) than van der Waals forces are small and rapidly decay with distance (inversely proportional to the 7th power of distance) for a pair of molecules. However, the van der Waals attraction force is additive, which leads not only to a large total force, but also to a less rapid decay with distance (inversely proportional to the 3rd power of distance between surfaces) for large particles. The aggregation of hydrocarbon molecules in micelle formation would thus lead to substantially enhanced van der Waals attraction of the clay surfaces and is presumably responsible for the removal of hydrocarbon molecules from the aqueous medium.

Improvement of soil attenuation ability for PHC's by chemical treatment of the soil

The bonding mechanism between the nonpolar PHC molecules and the clay surface is by way of van der Waals attraction. Data in Table 5 indicate that the adsorption levels of the hydrocarbons comprising the various petroleum fractions are low (≤ 1 g/kg of clay). Hence to improve the attenuation ability of a soil for PHC molecules, it is essential to strengthen the van der Waals attraction between the soil surface and PHC molecules. Adsorption of organic cations by van der Waals attraction on a clay surface becomes progressively significant as the molecular weight of the organic cation increases (Raussell-Colom and Serratosa 1987). Hence coating the soil surface with organic macromolecules should improve the attenuation ability of a soil for PHC's.

Organic polymers represent a class of macromolecules built up by repetition of small simple chemical units, termed repeat units. In some cases, the repetition is linear, much as a chain is built up from its links. In other cases, the chains are branched or interconnected to form a three-dimensional network. The length of an organic polymer chain is specified by the number of repeat units, also identified as degree of polymerization (DP). The molecular weight of a polymer is the product of molecular weight of the repeat unit and DP. Using polyvinyl chloride as an example, a polymer of DP 1000 has a molecular weight of 63 × 1000 = 63 000. In addition to being multisegmented and possessing very high molecular weights, organic polymers have several functional groups that allow them to interact with clay surfaces (Billmeyer 1984).

The use of organic polymers in improving soil aggregate stability is well known (Quirk and Panabokke 1962; Williams *et al.* 1967, 1968). However, attention seems to be

TABLE 5. Adsorption of different hydrocarbons from saline solutions at 25°C by sodium smectite (Meyers and Quinn 1973; Meyers and Oas 1978)

Compound	Formula	Solubility in distilled water at room temperature (ppm)	Adsorption (μg/mg of clay)	% adsorbed
Acenaphthene	$C_{12}H_{10}$	3.475	0	0
Pyrene	$C_{16}H_{10}$	1.480	0.39	19
Phenanthene	$C_{14}H_{10}$	1.70	0	0
Anthracene	$C_{14}H_{10}$	0.750	0.90	46
n-Octadecane	$C_{18}H_{38}$	0.0037	0.74	38
n-Eicosane	$C_{20}H_{42}$	0.0019	0.72	36
Hexadecane	$C_{16}H_{34}$	0.0009	1.12	56

focused on utilizing them to improve the attenuation ability of soils for organic contaminants.

The following discussion on the adsorption mechanisms of organic polymers by clay surface is based on a study of Greenland (1963) and a review of Theng (1982). Organic polymers may be neutral or positively or negatively charged. Adsorption of neutral polymers (e.g., polyvinyl alcohol) occurs via hydrogen bonds formed between the hydroxyl (OH) functional group of the polymer and oxygens of the clay surface. The adsorption of neutral polymers on clay surfaces is influenced by the valence of the exchangeable cation associated with the clay surface and the pore electrolyte concentration, i.e., an increase in exchangeable cation valence or increase in pore electrolyte concentration diminishes the amount of neutral polymer adsorbed by the clay surface.

Adsorption of positively charged polymers (e.g., cationic polysulfone) by clays occurs largely through electrostatic interaction between the cationic groups of the polymer and the negatively charged sites at the mineral surface.

Adsorption of negatively charged polymers by clays occurs under acid conditions (pH < 7) and (or) at high pore electrolyte concentrations, when the negative charge on the polymer is neutralized by H^+ ions from the acid medium and (or) is screened by the high pore electrolyte concentration. Adsorption is also promoted by the presence of multivalent cations on the clay surfaces which act as "bridges" between the anionic groups of the polymer and negatively charged sites on the clay.

Adsorption of organic polymers by the clay surfaces may change the surface properties of clay from hydrophilic (i.e., having affinity for water molecules) to organophilic (i.e., having affinity for organic molecules). In the natural or untreated state, the negative charge of clays is usually balanced by inorganic exchangeable ions, such as sodium and calcium, which are strongly hydrated in the presence of water. The hydration of these exchangeable metal ions and the presence of Si–O groups in clays impart a hydrophilic nature to the mineral surfaces. As a result, the adsorption of nonpolar hydrocarbons by clays is suppressed in the presence of water because relatively nonpolar organic molecules cannot effectively compete with highly polar water for adsorption sites on the clay surface. The adsorption of organic polymers by the clay surface would decrease the "free" alumiosilicate mineral surface area (i.e., the surface not covered by organic polymers) and the hydration of the clay surface. As a result, the surface properties of a clay may change from highly hydrophilic to increasingly organophilic, as the inorganic cations are progressively replaced by the organic macromolecules. In the absence of adsorbed water, the large surface area of a clay can provide a large adsorptive capacity for hydrocarbon contaminants (Lee *et al.* 1990). It is expected that the operative adsorption mechanism between the organic polymer attached to the clay surface and the petroleum hydrocarbon molecules would be of the van der Waals type. These forces would be of significant magnitude for interactions between the "ultra" heavy organic polymer attached to the clay surface and the PHC molecules. Adsorption of PHC molecules by the polymer attached to the clay surface should also be possible by way of hydrogen bond formation between the functional groups of the polymer (e.g., OH, COOH) and the hydrogens of the hydrocarbon molecules.

If the soil media to which the PHC molecules are released contain swelling 2:1 layer silicates (e.g., smectites), then besides the external surface, the interlayer space would also be available for molecular adsorption. Normally, the nonpolar alkanes and aromatic and polycylic hydrocarbons cannot enter the interlayer space of swelling 2:1 minerals, as they cannot overcome the attraction energy between clay unit layers (Green *et al.* 1983). However, the uncharged and positively charged organic polymers can enter the interlayer space of swelling clays (Theng 1982). The interlayer space created on treatment of the swelling clays with organic polymers can then accommodate the PHC molecules, resulting in an increase in the attenuation ability of the soil for petroleum contaminants.

The use of treated clays for removal of organic pollutants is not a new concept. McBride (1977), Wolfe *et al.* (1985, Boyd *et al.* (1988), Cadena (1989), and Lee *et al.* (1990) on treating smectites with organic cations found a significant increase in the adsorption capacity of the treated clays for aromatic hydrocarbons.

Concluding remarks

Petroleum hydrocarbons (PHC's) are composed of a mixture of nonpolar alkanes and aromatic and polycyclic hydrocarbons that have limited solubility in water. The water medium, however, has the capability of accommodating higher levels of hydrocarbons than that predicted by simple solubility considerations by way of micelle formation. The nonpolarity of the PHC molecules permits only a weak interaction with the clay surface (van der Waals type). The adsorption levels of the various hydrocarbons are consequently lower. The adsorption of the hydrocarbons by clay surface occurs only when their solubility (in water) is exceeded and the hydrocarbons are accommodated in the micellar form. To a good approximation, there exists an inverse relationship between the accommodation concentration of the hydrocarbon and the percent adsorbed by the clay surface. The important consequence of such a relationship is that the aromatic fraction of petroleum products that are the most "toxic" has the least affinity for the clay surface. Dilute solutions of hydrocarbons in water have no effect on the hydraulic conductivity of clay soils. Permeation with pure hydrocarbons invariably influences the clay hydraulic conductivity.

The adsorption behaviour of the petroleum hydrocarbons implies that once these contaminants are released into the environment, there would be little attenuation by the underlying soil substrate. The bonding mechanism between nonpolar PHC contaminants and the clay surface is by way of physical van der Waals attraction. Hence, to improve the attenuation ability of a soil for petroleum hydrocarbons, it is proposed to make the van der Waals forces (between the soil surface and hydrocarbon molecules) more effective by coating the soil surface with "ultra" heavy organic polymers. Adsorption or organic polymers by clay surfaces may change the surface properties of the clays from highly hydrophilic to increasingly organophilic. The organic polymers attached to the clay surface are expected to attenuate PHC molecules by hydrogen bonding (besides van der Waals attraction) and also by adsorption into the interlayer space in the case of soils containing swelling clay minerals.

Acknowledgments

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