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Optimization of soil physical and chemical conditions for the bioremediation of creosote-contaminated soil

H.I. Atagana[1,2,*], R.J. Haynes[2] & F.M. Wallis[2]
[1]*School of Earth Sciences, Mangosuthu Technikon, P.O. Box 12363, Jacobs, Durban 4026, South Africa;* [2]*School of Applied Environmental Sciences, University of Natal, Private Bag X01, Scottsville, Pietermaritzburg 3209, South Africa (*author for correspondence: e-mail: atagana@yahoo.com)*

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Abstract

Mispah type soil (FAO : Lithosol) contaminated with >250 000 mg kg^{-1} creosote was collected from the yard of a creosote treatment plant. The soil's carbon, nitrogen and phosphorus contents were determined. Due to creosote contamination, the carbon content of the soil was found to be 130,000 mg C kg^{-1}. This concentration was found to greatly affect the nitrogen content (0.08%). The phosphorus content was less affected (4.5%). It was estimated that a nutrient amendment to bring the soil to a C : N 10 : 1 would be adequate to stimulate microbial growth and creosote degradation. The soil was amended with a range of C : N ratios below and above the estimated ratio. In one of the treatments, the phosphorus content was amended. Sterile and natural controls were also set up. The soil was incubated at 30 ± 2 °C on a rotary shaker at 150 rpm in the dark for six weeks. Water content was maintained at 70% field capacity. The lowest nitrogen supplementation (C : N = 25 : 1) was more effective in enhancing microbial growth (3.12E + 05) and creosote removal (68.7%) from the soil. Additional phosphorus was not very effective in enhancing the growth of microorganisms and removal of creosote. The highest nitrogen supplementation (C : N = 5 : 1) did not enhance microbial growth and creosote removal. Phenolics and lower molecular mass polycyclic aromatic hydrocarbons (PAHs) were observed to be more susceptible to microbial degradation than higher molecular mass compounds. Nutrient concentration, moisture content and pH were thus observed to play very significant roles in the utilization of creosote in soil. These results are being used for the development of a bioremediation technology for the remediation of creosote contaminated soils in a treatment plant in South Africa.

Introduction

The supply of nutrient elements in the soil, apart from carbon, usually exceeds the needs of the resident microbial communities. Indeed, the growth limiting nutrient in natural environments such as soil, sediments and water is commonly carbon (Alexander 1999). In the event of the introduction of an organic pollutant that has the potential to be utilized by the resident microorganisms in the environment, this situation may change considerably. This will occur were the concentration of the polluting substance is high enough to make one or more of the previously non-limiting nutrients become limiting. Most often, the nutrients that become limiting are nitrogen and phosphorus (Alexander 1999). The introduction of creosote into the soil environment thus creates an imbalance in the nutrient ratio in the soil because of the high level of carbon supplied. The addition of N and P to soil contaminated with hydrocarbons is known to stimulate the biodegradation of such compounds and increase the abundance of microbial species (Bossert & Bartha 1984; Baker & Herson 1994; Alexander 1999).

The amount of N and P needed for the biodegradation of hydrocarbons is usually assumed to reflect the amount that must be incorporated into the biomass that is formed as the microorganisms use the carbon source for growth. The C : N : P ratio in bacterial biomass is quoted by Zitrides (1983) to be 100 : 15 : 3 and by Alexander (1977) to be 120 : 10 : 1. From such estim-

ates, it is possible to estimate the amount of N and P that will be necessary to sustain bacterial growth if the amount of carbon from the pollutant that ends up as bacterial biomass can be estimated (Thibault & Elliot 1980). To ensure that these nutrients do not limit microbial activity, sufficient nitrogen and phosphorus should be applied (Alexander 1981).

Mobilization of nutrients and incorporation of these nutrients into microbial biomass requires adequate amounts of water. The density and texture of the soil determines the water-holding capacity, which in turn affects the available oxygen, redox potential and microbial activity (Parr et al. 1983). The microbial species composition of a soil is often dependent upon water availability (Alexander 1977; Baker & Herson 1994; Tate 1995). Although, the capacity of soils to hold water under field conditions varies from one to another, microbial activity have been reported to proceed optimally in the presence of between 50% and 70% of field capacity (Alexander 1999).

Soil pH has been widely reported to affect microbial growth and the degradation of hydrocarbons in soils (Dibble & Bartha 1979; Baker & Herson 1994; Tate 1995). A pH of between 5 and 7 has been found to be optimal for bacterial growth (Alexander 1977; Tate 1995). However, organisms are known that grow in habitats with very low and high pH. Thus the soil pH partially determines the organisms that are present and thus the extent of bioremediation of a given compound that can be carried out.

The focus of this study was to optimize soil conditions such as aeration, moisture, pH levels and nutrients for the degradation of creosote in soil with a view to developing a treatment technology for creosote contaminated land in South Africa. It was particularly aimed at determining a suitable nutrient amendment by varying the levels of N in the soil that will be optimal for the growth of microorganisms and degradation of creosote in the contaminated soil.

Based on the C : N ratio estimation of Zitrides (C : N 100 : 15) and Alexander (C : N 120 : 10) it was estimated that a C : N ratio of between C : N 7 : 1 and 12 : 1 would be adequate for effective growth of soil microorganisms and subsequent degradation of the contaminant creosote. Thus it was hypothesised that at C : N 10 : 1 microbial growth and creosote degradation would be optimal.

Materials and methods

Soil samples

Seven sample cores constituting approximately 5 kg of Mispah type (Lithosol : FAO) soil contaminated with $>250{,}000$mg kg^{-1} creosote were taken up to depths of about 30 cm with a spade from the experimental site. The samples were mixed in an electric concrete mixer before use. A 1 kg sub-sample (field moist) was sterilized by gamma irradiation at a dosage of 2.5 Mrad.

Elemental analysis

Analyses of carbon, nitrogen and hydrogen contents of contaminated soil, were carried out in a Perkin Elmer 2400 CHN Elemental Analyser in the University of Natal chemistry department. Phosphorus content of the soil was determined by standard methods at the KwaZulu-Natal Department of Agriculture, Cedara.

Inorganic nutrient treatments

Based on the organic carbon and nitrogen content of the soil samples, five different ratios of C : N and one of C : N : P were established in the different soils in the jars. The C : N and C : N : P nutrient amendments were added as NH_4NO_3 and K_2HPO_4 respectively. The treatment combinations of C : N were : 25 : 1; 20 : 1; 15 : 1; 10 : 1; 5 : 1, and the C : N : P was 10 : 1 : 2. This range of C : N ratios was chosen to accommodate differences in the delivery of nutrients between the soil system and the microbial cell as it is known that 100% assimilation may not be achievable (Baker & Herson 1994; Lees 1996) and also that high nutrient supplementation may be inhibitory to microbial growth. A sterile control was set up for each of the treatments above using the gamma(γ)-irradiated soil. One biological control, which received only water, was set up. All treatments, including the controls, were duplicated.

Jar microcosms

Shallow glass jars of about 100 ml in volume and a height of about 5 cm were used to allow air to reach every part of the sample. Each 100 ml glass jar was soaked in Extran^{TM} for two hours rinsed with a 1M solution of HNO_3 and then rinsed twice with deionised water before drying overnight at 110 °C. The jars were then autoclaved at 121 °C for 15 minutes. Forty

grams (fresh mass) of the contaminated soil. Duplicate samples with all the treatments described above were added separately to 96 glass jars. For each jar the nutrients were dissolved in 3.5 ml of deionised water, corresponding to approximately 80% of the field capacity of the same volume of soil packed at a bulk density of 1370 kg m^{-3}. This was to ensure retention of the nutrients in the microcosms without water limitation or water logging. The content of each jar was then mixed thoroughly, weighed, sealed with parafilm to prevent water loss but allow oxygen diffusion before incubating at 30 °C ± 2 °C in the dark. The jars were re-weighed on a weekly basis and replenished with deionised water when necessary. This method was adapted from Lees (1996). The duplicate jars were sampled destructively every seven days. The soils were sub-sampled for microbial counts, infrared analysis to determine change in total creosote concentration, gas chromatography for changes in concentration of selected creosote components and pH measurement. The sub-samples for microbial counts were used immediately and the remainder was heat-sealed in a cellophane bag and stored in the freezer at −17 °C until required for analyses.

The moisture content of the soil was determined as described by Forster (1995). The pH of the soil was determined by air-drying soil samples from the jars for 72 hours in the dark at room temperature (25 °C). The soil was then ground and passed through a 2 mm sieve. Ten grams of each soil sample were put in 50 ml beakers into which 25 ml of sterile distilled water were added. The slurry was stirred for one minute and allowed to stand for 15 minutes. The pH of the supernatant was measured with a standard pH metre (Crison Micro pH 2000). Adjustment of soil pH was made by the addition of lime at the start of the experiment as the soil pH was found to be originally acid (<5.0). Soil extract agar and mineral salts agar overlaid with filter sterilized creosote were used for enumeration of creosote degraders.

Determination of changes in concentrations of creosote and selected creosote components by infrared spectrophotometry (IRS) and gas chromatography (GC)

Total creosote concentration in the soil was analysed using the USEPA 418.1 (1983) method as follows: creosote contaminated soil (2 g) and 2 g anhydrous Na_2SO_4 were placed in a 30 ml amber glass vial. Carbon tetrachloride (10 ml) was added before sealing the vial with a teflon-lined screw cap. The sealed vial was vortexed for 15 seconds and placed in a sonicating bath (Whaledent Biosonic) for 15 minutes before remixing on the vortex mixer for about 15 seconds. It was then placed in the sonicating bath for another 15 minutes. The solvent was transferred to a clean, dry vial containing 1 g activated Florosil™ (Sigma) and 0.6 ml water [i.e. 6% water (w/w)]. The sealed vial was shaken for one minute and allowed to stand overnight at ambient temperature. This silica "clean-up" procedure was used to remove interfering humic materials (EPA 1985). The extract was finally filtered through a Whatman GF/C glass fibre filter. The filtrate was made up to 10 ml in a volumetric flask and the absorbance determined with a Nicolet Avater 320 Infra-red Spectrophotometer at wave numbers between 400 and 4000 cm^{-1}. Calibration of the reference creosote was done by diluting commercial grade creosote with carbon tetrachloride to a series of five working standards (10; 100; 250; 500; 1000 mg L^{-1}) and a calibration curve was derived by determining the absorbance of each standard. A calibration plot of the absorbance versus mg creosote (100 ml L^{-1}) solution was generated with Omnic™ software. The concentration of creosote in each extract was determined by comparing the response with the calibration plot. To calculate the results, a linear equation ($y = 1.192x + 1.363$) was generated by Omnic™ software, where x = creosote concentration of sample (kg^{-1} soil) and y = peak area. The actual hydrocarbon concentration in mg kg^{-1} soil was then calculated by multiplying x by a factor of 50 which compensates for the cell pathlength, sample size and dilution factor. This formula was then programmed into a spreadsheet which automatically calculated the creosote concentration in mg kg^{-1} from the infra-red absorbance values as they were entered.

Analysis of selected creosote components was carried out in a gas chromatography (Varian-3800) fitted with a flame ionization detector (GC/FID). Argon was the carrier gas. A 30 m capillary column with 0.25 mm internal diameter and 0.25 μm film thickness was used. Two temperature programmes were run in order to obtain a good separation and quantification of the more volatile compounds. The first temperature programme was: 60 °C, 4 min., ramped at 10 °C/min., up to 200 °C, and kept at this temperature for 40 min; injector temperature 220 °C. The second temperature programme which was used for analysis of the more volatile compounds was 20 °C, 1 min: 40 °C, 1 min, ramped at 10 °C/min up to 200 °C, and kept at this

temperature for 20 min; injector temperature 220 °C (Ericksson et al. 2000).

Results and discussion

Soil carbon content and biodegradation

The analyses of the soil samples collected from the contaminated sites showed that the soil was contaminated with >250 000 mg kg^{-1} creosote. The mean carbon content of the samples was 13.02% and hydrogen, 0.85% (w/w). This percentage of carbon represents approximately 130,000 mg C kg^{-1} soil. This amount of carbon greatly affects the carbon : nitrogen : phosphorus (C : N : P) ratio in the soil (130 : 0.08 : 4.5). This C : N : P ratio shows a great deficiency in nitrogen which may affect the requirement of microorganisms growing in the medium. As was earlier stated, the C : N : P ratio of the bacterial biomass is estimated at 100 : 15 : 3 (Zitrides 1983) and 120 : 10 : 1 (Alexander 1977). This suggests that additional nitrogen would be required in the medium to effectively support microbial growth. However, by both estimates, additional phosphorus would not be necessary since the phosphorus content of the soil was comparatively adequate.

The imbalance in C : N ratio resulting from the polluting carbon source possibly accounts for the reason why there still remained such a large amount of hydrocarbon (>250,000 mg kg^{-1}) in the soil in spite of a period of shut-down at the site. Nutrient imbalance in the soil between carbon, nitrogen and phosphorus, resulting from the excess carbon supplied by the pollutant hydrocarbon is known to limit the extent of hydrocarbon degradation in contaminated soils. This phenomenon has been widely reported in the literature (Bartha & Atlas 1977; Raiser-Roberts 1992; Alexander 1999). Reports of stimulation of hydrocarbon degradation by mineral fertilizer supplementation have been published (Jobson et al. 1974; Verstaete et al. 1975; Raymond et al. 1976), however no systematic effort has been made to determine the optimal concentrations of mineral fertilizer required. In theory, approximately 150 mg of nitrogen and 30 mg of phosphorus are consumed in the conversion of 1 mg of hydrocarbon to cell material (Rosenberg et al. 1983). This kind of complete assimilation of hydrocarbon into biomass is not achievable under natural conditions, given the fact that some of the compounds found in the hydrocarbon complex are recalcitrant or are only slowly metabolised over long periods and such components may constitute the major part of the carbon load (Lees 1996). This situation can be expected in hydrocarbon complexes with a large percentage of high molecular mass fractions which will only slowly degrade, and under conditions where such hydrocarbons are strongly sorbed to soil or sediment particles. Thus the conversion of substrate carbon into cellular biomass will only very rarely achieve 100% efficiency (Baker & Herson 1994; Alexander 1999).

Soil microbial activity and hydrocarbon utilization

The Duncan's Range Test at p 0.05 showed that counts of microorganisms by the end of the sixth week in the different treatments supported microbial activities in the following increasing order: sterile control < C : N 5 : 1 < natural control < C : N : P10 : 1 : 2 < (C : N15 : 1 & C : N 20 : 1) < (C : N 10 : 1 & C : N 25 : 1). However, the LSD test (p0.05) shows there was no significant difference between C : N ratios 10 : 1, 15 : 1, 20 : 1 and 25 : 1. Colony counts were found to be very low (75 cfu g^{-1}) in the sterile control (Figure 1). The few colonies observed may have resulted from contamination during the plating or incubation process since the soil sample was stringently sterilized.

The results of these experiments showed that reductions in creosote concentrations are related to increases in microbial population. Counts of the microbial populations showed that the increase in the numbers of hydrocarbon degraders at C : N ratios of 25 : 1; 20 : 1; 15 : 1 and 10 : 1 reaching heights of 3.12E + 05, 2.58E + 05, 2.53E + 05 and 3.07E + 05 respectively (Figure 1) were positively corelated to corresponding decreases in creosote concentration of 68.7%, 61.1%, 56.3% and 63.8% respectively (Table 1). Although the results from C : N 10 : 1 showed large microbial growth and high creosote degradation as expected, C : N 25 : 1 supported larger microbial growth and better creosote degradation (p 0.05) (Figures 1 and 2). Thus, in the present study, microbial activity and the corresponding creosote removal rate were more at lower nutrient supplementation. This could be due to the exposure of the soil microorganisms to high concentrations of creosote (>250 000 mg kg^{-1}) and low nitrogen content (0.08 mg kg^{-1}) for a long period of time, as only slight increases in the nitrogen status were adequate to promote the growth of the organisms and subsequent assimilation of the creosote carbon. This accounts for the more than double the amount

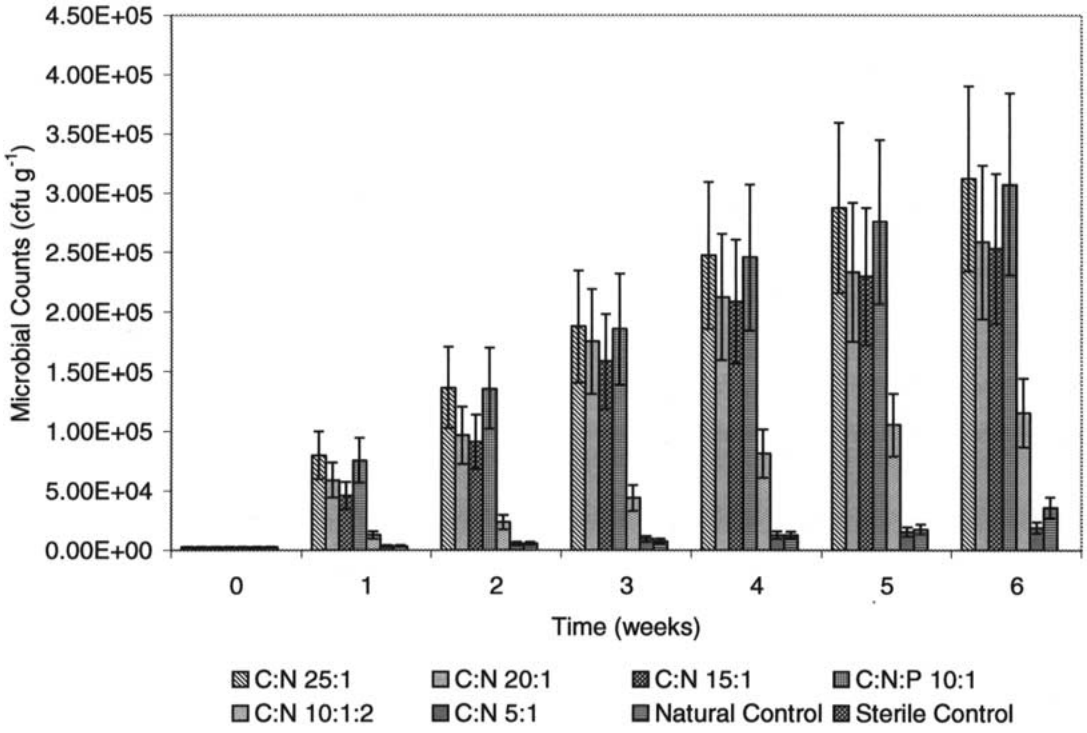

Figure 1. Counts of creosote degrading microorganisms after 24 hours incubation at 30 ± 2 °C. Values are means of two ± 1 Standard Error.

of carbon oxidised in the C : N 25 : 1 experiment. It is not expected that all the oxidised carbon would be incorporated into microbial biomass as some would be lost through biochemical processes. As earlier stated, it is not possible in nature to achieve a 100% assimilation of hydrocarbons into microbial biomass given the complexity of creosote. Other workers have previously reported increases in microbial populations and hydrocarbon degradation as concentration of supplementary nutrients decreased (Lees 1996). The highest nutrient supplementation used in this experiment (C : N 5 : 1), was found to be the least effective in supporting growth of microorganisms and consequently resulted in only 33% hydrocarbon removal (Table 1). This may be due possibly to the high concentration of the supplementary nutrient used being toxic to the microbial population.

Thus high levels of N and P supplementation did not enhance microbial degradation of creosote in soil. Approximately 18% creosote removal was recorded in the sterile control (Table 1). This must be attributed very largely to volatilization since only small numbers of microorganisms were present. In the natural control that received only water without any C : N supplementation, a total creosote removal of 46.6% was observed.

Table 1. Total creosote degradation in different C : N and C : N : P ratios during the period of incubation at 30 ± 2 °C. Values are means of two ± 1 Standard Deviation

Treatment	Creosote removal (%) ± 1 standard deviation
C : N 25 : 1	68.7
C : N 20 : 1	61.1
C : N 15 : 1	56.3
C : N 10 : 1	63.8
C : N 10:1 : 2	50.2
C : N 5 : 1	33
Sterile control	18.2
Natural control	46.6

This result suggests that C : N 5 : 1 was restrictive to creosote degradation possibly due to the inability of this treatment to sustain a continued growth of microorganisms through the experimental period. This also suggests that the high rates of removal of creosote at the lower C : N ratios was due to microbial metabolism rather than volatilization since the microbial counts in the two controls and C : N 5 : 1 were very low

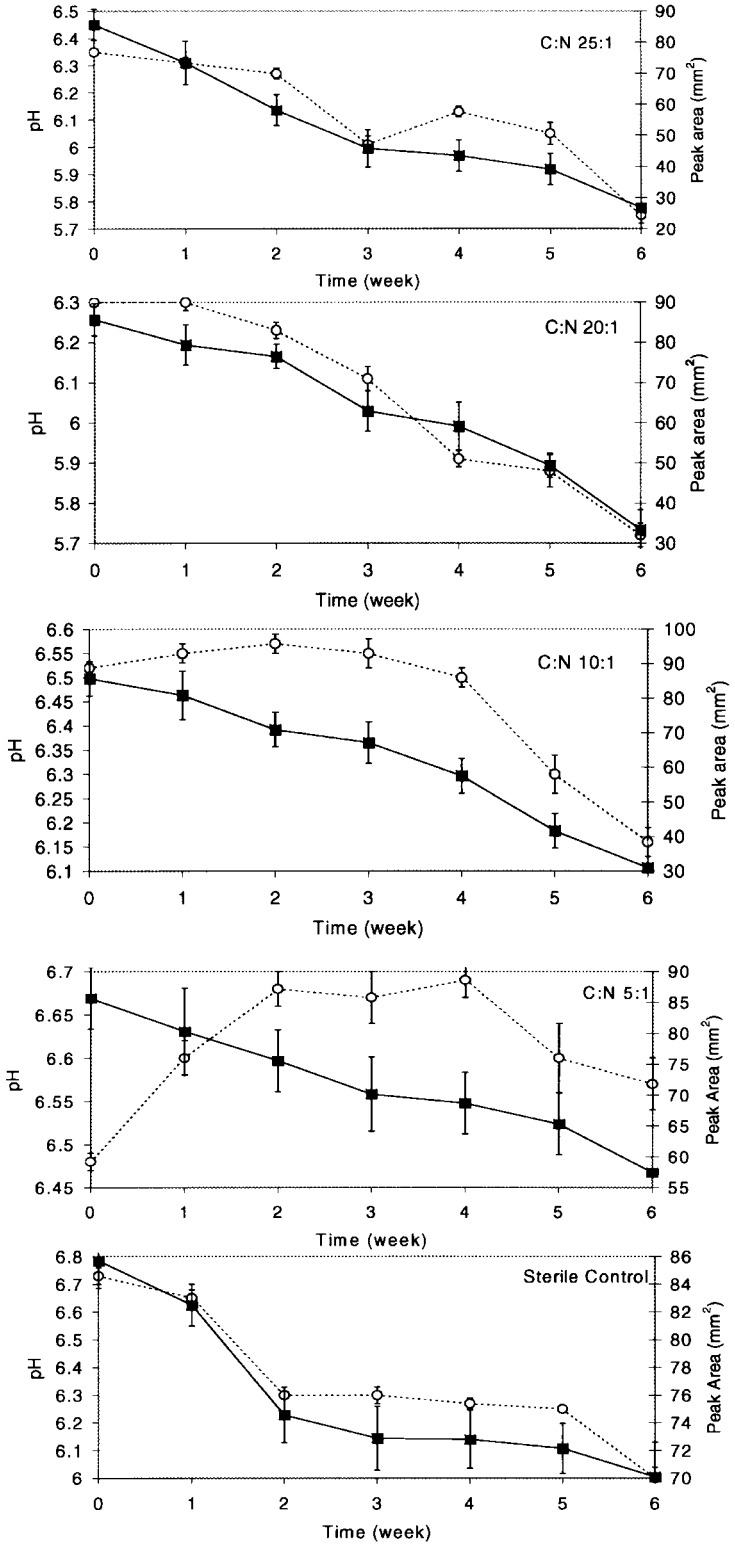

Figure 2. Changes in pH and corresponding changes in peak area of creosote in different C : N and C : N : P ratios. Values are means of two ± 1 Standard Error.

compared to the ones with lower N supplementation. However, it can be argued that the loss in creosote in the natural control could be partly due to volatilization. This argument further reduces the degradative potential of the C : N 5 : 1 treatment. The 46.6% creosote removal in the natural control is comparable with results from the treatment with C : N : P 10 : 1 : 2 (50.2% creosote removal) in which it could be seen that the addition of phosphorus did not enhance creosote removal (Table 1). Another explanation for the poor removal of creosote in the natural control was the low pH (4.79) compared to between 6.1 and 6.7 in other treatments. Such acid pH inhibit biodegradation of organic pollutants in the environment (Alexander 1999). Since the hydrocarbon content of the soil was very high (>250,000 mg kg^{-1} soil), and the nitrogen level was relatively lower than the required percentage, the N supplementation was necessary to boost the activity of degrading microorganisms to optimal levels.

Except in the C : N 5 : 1 and control jars, microbial population increased rapidly after the first week of incubation. Over subsequent weeks a slight but steady decreases in microbial population occurred. The natural control showed steady population increases from the first week up until the end of the incubation period. These increases in population were difficult to correlate with the decreases in creosote concentration in all the treatments. This can be attributed to utilization of the hydrocarbon substrate at certain times during the incubation period without such utilization manifesting itself in the size of the microbial biomass. If the bacterial cell density is high relative to the substrate concentration, little or no increase in cell numbers is possible (Alexander 1999). The relatively high creosote removal recorded during the period of incubation (six weeks) was high. However, the incomplete degradation of creosote in the soil was not unexpected considering the initial concentration of creosote (>250,000mg kg^{-1}) present in the soil. This result can be attributed to the soil type, the fact that the soil was well homogenized and other factors which may include aeration, moisture, pH, temperature and soil texture. Previous studies have shown high percentage reduction in concentrations of creosote and other hydrocarbons in soil. However, the length of the treatment period have been much longer and the initial concentration much lower in the reported cases. For example, Hansen et al. (2000) reported a decrease of about 96% from an initial creosote concentration of about14,790 mg kg^{-1} in six months. A removal of about 95% and 20% from initial coal tar oil concentrations of 25,000 and 100,000 mg kg^{-1} in twelve weeks was reported by Lajoie & Strom (1994). These reports are of experiments carried out in different soil types and environments.

Although pH in all the treatments showed slight decreases during the six weeks of incubation, microbial activity continued to increase during the period. The decrease in pH was found to be more evident in the C : N ratios 25 : 1, 20 : 1, 15 : 1. The highest nutrient supplementation, 5 : 1 showed an overall increase in pH from 6.48 to 6.57. The sterile control, natural control, 10 : 1 : 2 and 10 : 1 : 1 showed various levels of decreases. The decrease in pH was observed to be directly related to decrease in substrate concentration (Figure 2). This is believed to due to the production of microbial metabolites which resulted in decrease in pH as the microorganisms utilize the substrate. Microbial degradation of xenobiotic compounds have been reported to result in alteration of pH in soil and aquatic media (Baker & Herson 1994).

Phenol was observed to be completely removed by the end of the sixth week in all the treatments except the controls (Figure 3). The other phenolic compounds; o-cresol, m-cresol and p-cresol, were also completely degraded in all the treatments with the exception of m-cresol at C : N 5 : 1 which had 0.3 mg kg^{-1} left at the end of the sixth week. Concentration of o-cresol decreased beyond detectable limits by the end of the third week in all C : N treatments including the natural control. Phenolic compounds have been widely reported to be degraded by soil microorganisms at varying concentrations and at relatively short periods of time (Edgehill 1983, 1994; Vipulanandan et al. 1994; Dyreborg et al. 1995; Häggblom & Valo 1995). However, the total disappearance of most of the phenolic compounds at such high creosote concentration (>250,000 mg kg^{-1}) and short time (six weeks) was a very important development in this study.

The relative ease with which soil microorganisms were able to degrade phenolic compounds can be attributed to their simple structure when compared to the other aromatic compounds with more benzene rings. Those with simpler substitutions are also known to be more readily attacked by degrading microorganisms than those with complex substitutions (Gibson & Subramanian 1984; Sutherland et al. 1995). The position of substitution and the substituting group may have also plaid important roles in the susceptibility of the phenolic compounds to microbial degradation (Knackmuss 1992; Alexander 1999).

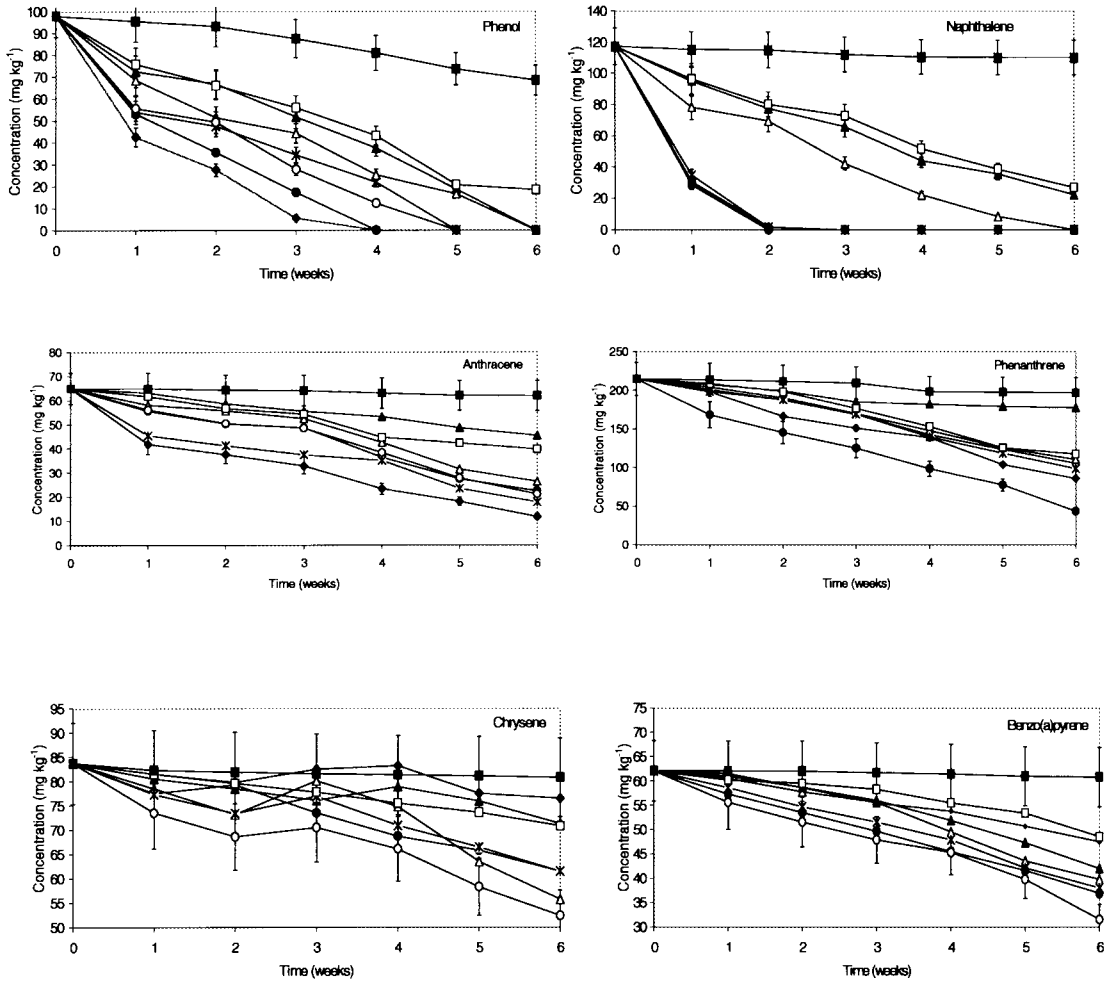

Figure 3. Changes in concentrations of selected creosote components in different C : N and C : N : P ratios. Values are means of two ± 1 Standard Error.

Among the PAHs studied, naphthalene was found to be the most susceptible to microbial degradation (Figure 3). Apart from the controls and C : N 5 : 1, naphthalene was completely removed by the end of the sixth week. The success with which naphthalene was degraded is attributed to its simple structure, consisting basically of two benzene rings, its low molecular mass and its high solubility in aqueous media (31 mg L^{-1} at 25 °C) (Alexander 1999; Eriksson et al. 2000). The degradation of naphthalene by biological processes has been well-studied and documented in the literature (Ghoshal et al. 1996; Ghoshal & Luthy 1998; Manohar & Karegoudar 1998; Annweiler et al. 2000). Results from this experiment demonstrates that high concentrations of naphthalene can be removed from contaminated soils over a short period of time by optimizing soil physical and chemical conditions. The removal of more than 70% of the compound from the contaminated soil in three different treatments by the end of the first week in comparison with the results from the controls as shown in Figure 3 demonstrates that soil nutrient manipulation can speed up the degradation of naphthalene in contaminated soils. Although, the intermediates of naphthalene degradation were not studied, it is assumed that intermediates produced were readily mineralized or biotransformed since there was no observed inhibition in microbial growth during the period of study. Intermediate products of the degradation of naphthalene are known to interfere with the further degradation of naphthalene if allowed to accumulate in the reaction medium (Goshal & Luthy 1998).

Figure 3 show that anthracene is much less susceptible to degradation than phenanthrene. Although, about 81% of anthracene was removed by the end of the sixth week of incubation in C : N 25 : 1, most of the other treatment still contained appreciable concentrations of the compound (Figure 3). This slow degradation is attributable to its low solubility (0.05 mg l^{-1}) in aqueous systems which renders it only slowly available for microbial attack. Phenanthrene on the other hand, was rapidly degraded (Figure 3) in most of the treatments in spite of its high initial concentration (215 mg kg^{-1}) in the contaminated soil. This is due to its higher solubility (1.1 mg L^{-1}) in aqueous media, making it more available for microbial attack than anthracene. Although, both compounds consist of the same number of rings and have the same molar mass, their structures are different. This difference in spatial arrangement of the ring structure possibly accounts for the difference in solubility. This difference in the rate of degradation of the two compounds has been reported previously in the literature (Mueller et al. 1991; Lajoie & Strom 1994).

Although the higher molecular mass compounds were observed to be more recalcitrant, nutrient amendment increased the rate of degradation of the compounds. The C : N ratio of 25 : 1 was found to be the most effective amendment ratio tested. The effectiveness of nutrient amendment decreased with an increase in molar mass, number of rings and the complexity of the structure i.e. phenols < naphthalene < phenanthrene < anthracene < pyrolle < fluorine < pyrene < chrysene < fluoranthene > benzo(a)pyrene. The highest supplementation C : N 5 : 1 was also found to be the least effective in enhancing degradation. Nutrient supplementation are known to be most effective when applied in close enough amount to the required concentration (Baker & Herson 1994).

Results from soils set at pH 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5 and moisture contents set at 45, 50, 55, 60, 65, 70, 75, 80% field capacity respectively, showed that microbial activity was highest between 60 and 70% of field capacity at p 0.05 (Figure 4). Previous studies have generally shown that soil microbial activity is optimum between 50 and 70% field capacity (Atlas & Bartha 1972; Lajoie & Strom 1994). The slight shift on the lower limit in this study could be an attribute of the soil type that was studied.

The microorganisms involved in the utilization of creosote hydrocarbons in the soil demonstrated a wide range of pH tolerance with the counts of microorganism continuing to increase up to the sixth week between the pH 5.5 and 8.0 although; maximum activity was recorded between pH 6.5 and 7.0. At more moderate pH values, biodegradation tends to be fastest. If a compound in a particular environment can be metabolized by a diverse group of organisms, the range of pH at which degradation occurs frequently is broader than if only one species can bring about the transformation (Alexander 1999). The optimal pH for microbial activity was however found to change with change in moisture content of the soil. For example, at the range of 60 to 70% moisture content, the pH range of 6.5 to 7.0 was found to be optimal. At lower water contents (45–55%), optimal activity was recorded at a pH range of 5.5 to 6.5. Microbial activity in this range was, however, found to be much less than was observed at 60 to 70% moisture content and pH 6.5 to 7.0. At higher moisture contents (70 to 80%) the pH range for optimal activity shifted to 6.0 to 7.5. At this moisture range microorganisms exhibited a wider pH tolerance but microbial counts were also found to be much lower than was recorded in the 60 to 70% moisture range. These responses are thought to be due to changes in the composition of microbial species prevalent in the soil with changes in pH and moisture content.

Conclusion

The results from the analyses of the carbon content of the soil confirmed that the soil was heavily contaminated with creosote oil >250,000mg kg^{-1} soil which resulted in a deficiency of nitrogen in the soil. Phosphorus was found to be less affected. Supplementation of nitrogen was observed to be necessary for effective growth of the microorganisms. The results further show that nutrient supplementation at lower concentrations of N (as was represented in the C : N ratio of 25 : 1) was most effective in enhancing biodegradation of the creosote and its components as against the expected C : N 10 : 1. The effectiveness of supplementation on microbial growth decreased with increases in the ratio of N to C. The highest N supplementation (C : N 5 : 1)did not enhance microbial activity. Decreases in creosote concentration during the study were found to correlate with increases in microbial counts with the highest decrease of 68.7% recorded at C : N of 25 : 1 and the least (32.7%) in C : N of 5 : 1. The decrease in creosote concentration was also found to directly correlate with a decrease in soil pH. It was also established that creosote degradation can

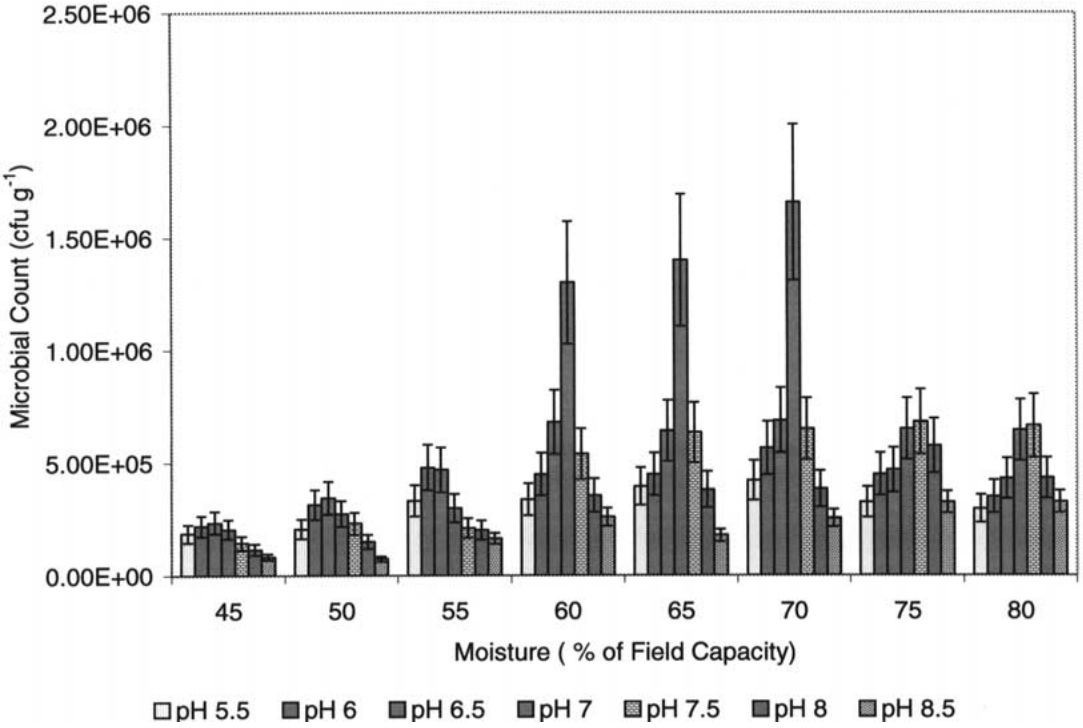

Figure 4. Effect of soil moisture and pH on microbial activity in creosote contaminated soil. Values are means of two ± 1 Standard Error.

proceed in the soil without nutrient supplementation but very slowly. However, moisture and oxygen must be adequate. The rate of degradation of creosote in the present study was high but the time used for the study was too short to allow for the total removal of such high concentration of creosote from the soil. However, the reduction in concentration of creosote achieved over such a short period (6 weeks) of incubation was high and of significant consideration in the remedial project being considered. Changes in soil water content were found to affect the pH optimum for creosote biodegradation. Optimal pH and moisture range for microbial degradation of creosote in soil were found to be 6.5–7.0 and 60 and 70% respectively. The phenolic compounds and the lower molecular mass PAHs studied were more susceptible to microbial degradation than the high molecular mass PAHs. It is concluded that nutrient supplementation can effectively enhance the degradation of creosote in contaminated soil and that management of aeration, moisture content and pH are also important considerations. These results, and results from other laboratory trials, will be considered during the land-farming project.

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