BIOREMEDIATION TECHNOLOGY FOR THE TREATMENT OF CONTAMINATED SOIL IN SOUTH AFRICA

by

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EXECUTIVE SUMMARY

MOTIVATION AND BACKGROUND

Contaminated soils have a negative impact on the environment, through impairing ground- and surface water quality. They are thus a risk to human health. A practice often utilized to remove hazardous waste is to landfill the contaminated soil. An alternative option, however, is bioremediation which offers a relatively inexpensive yet efficient solution to remove toxic organic chemicals from the contaminated soil. It is a biological technique which harnesses and enhances the natural ability of microorganisms to degrade hazardous organic chemicals into innocuous forms. Despite many opportunities for its use, bioremediation has not been utilized to its full potential in South Africa.

PROJECT AIMS

The aims of the project were:

To evaluate bioremediation technologies on a laboratory scale as an appropriate and viable technology. These evaluations were to include soil systems simulation, slurry digestion and volatile organics biotower reactors. In each case biological treatment was to be applied employing selected microorganisms for specific pollutants. The model pollutants included mineral hydrocarbon oils, aromatic organics and aromatic halogenated organics.

To optimize treatment conditions and scale-up for pilot scale evaluations of bioremediation at contaminated sites. The economics of the technology were to be evaluated and process design criteria provided to successfully remediate contaminated soils.

A survey was to be undertaken to determine the nature and extent of contaminated sites in South Africa. The objective was to determine whether contaminated sites are an area for concern, what the most prevalent contaminants are, and to assess whether bioremediation is seen as an appropriate and viable technology. Needs within the bioremediation technology arena in South Africa were to be identified.

ACHIEVEMENT OF OBJECTIVES

A simulation of a soil system was undertaken using soil columns. Laboratory scale slurry reactors were evaluated as a bioremediation technology to remediate phenol contaminated soils. A further bioremediation technology, landfarming, was investigated at pilot scale for the clean up of petroleum contaminated soil. Factors affecting the rate of biodegradation of phenol and petroleum products were established.

A survey was done, using a multi-faceted approach, to determine the nature and extent of contaminated sites in South Africa. Due to the sensitivity of the information required, many respondents were unwilling or unable to provide a comprehensive response. As such, the survey gives a confined perspective, thought to be indicative of the broader situation.

THE NATURE AND EXTENT OF CONTAMINATED SITES IN SOUTH AFRICA

The most common contaminated sites identified from the survey were of an industrial nature. Contaminants identified included organic (from petrochemical, solvent and wood treating

chemical manufacturers etc.), and inorganic (from plating, fertilizer, explosives manufacturers etc.) chemicals. Further contaminated sites included railway sidings, harbours and waste disposal sites of various origins.

Fifty percent of the questionnaires were returned. However, due to the sensitive information required, inadequate detail being known about the sites and unreliable and infrequent monitoring, questions were not always answered comprehensively. Most expressed confidence in the bioremediation technology, seeing it as viable and cost-effective. Landfarming and soil vapour extraction are best known and most frequently used bioremediation techniques.

Seventy eight actual contaminated sites were reported, which can be taken with certainty as an underestimate of the number of sites requiring remediation. Of these, 24 indicated that groundwater pollution had occurred and 23 of the sites were in the vicinity of surface water.

The survey identified 28 sites in South Africa where bioremediation has been used. Where details were given, all reported the treatment as being cost-effective.

Little response was obtained on companies possessing the expertise to implement bioremediation projects.

LABORATORY STUDIES

At laboratory scale a number of aspects were investigated. These included the factors affecting the rate of bioremediation of phenol using batch reactors and the viability of slurry reactors to remediate phenol contaminated soil. A simulation of a soil system using soil columns was undertaken. A satisfactory analytical method of phenol extraction and determination was developed for soil matrices.

High recoveries of phenol from soil adsorption studies indicated that minimal adsorption of phenol to soil surfaces occurs under the conditions used. Thus, any decrease in phenol concentration obtained in the laboratory studies could be attributed to degradation rather than adsorption. Some losses of pure phenol may have occurred due to volatilization.

The rate of phenol degradation was enhanced in batch reactors with the addition of nutrients. Where no nutrients were added, biosupplementation increased phenol breakdown.

Addition of nutrients and biosupplementation in the slurry reactors resulted in no significant advantages in the rate of phenol degradation. This may have been due to adequate nutrient concentrations and bacterial populations already existing in the soil.

The results of the soil column experiment showed that some breakdown of phenol in contaminated seepage water occurred as it percolated through the soil. Anaerobic conditions decrease degradation rates. It was demonstrated that increased oxygen levels in the seepage water improved the rate of degradation.

PILOT SCALE DEMONSTRATION OF LANDFARMING

Petroleum contaminated field samples were used to demonstrate landfarming and investigate parameters affecting the rate of degradation. Parameters that were investigated in isolation and in conjunction with each other included addition of moisture, nutrients, oxygen (through

turning the soil and by addition of hydrogen peroxide) and biosupplements. The pH of the soil was adjusted and maintained at 6-7. The effect of a commercial biosupplement was compared with that of a biosupplement cultivated from indigenous microorganisms in the soil. A comparative study showed that application of moisture, nutrients, air and a biosupplement resulted in the fastest rate of degradation of total petroleum hydrocarbons (TPHC). A decrease of 94% from initial levels of 320 g/kg soil to 18 g/kg TPHC soil over a period of 10 weeks was achieved. A control enabled differentiation between a decrease in TPHC due to volatilization, chemical or photo-oxidation and biodegradation. Volatilization contributed largely to the initial reduction of TPHC. However, after the volatile fractions had been lost, the microorganisms then degraded the heavier fractions of the oil. There was no significant difference in the ultimate performance between the two biosupplements, although the indigenous biosupplement initially showed quicker degradation. This may have been due to an acclimatization period of the commercial microorganisms to the specific soil conditions.

Over a ten week period, application of moisture and oxygen resulted in increased rates of biodegradation when compared to a natural control, indicating these to be limiting factors in bioremediation. Both parameters affect the ability of microorganisms to grow and thrive. Moisture levels may also affect the mass transport and bioavailability of the contaminant to the microorganisms.

Measurement of subsequent growth of wheat seedlings in the soil after bioremediation was not indicative of successful remediation. Possible reasons include alteration of the soil structure, and an unsuitable choice of indicator seedlings.

FULL SCALE BIOREMEDIATION

Important factors impacting on the strategy and design of a full scale bioremediation project are highlighted, and demonstrated with a case study. Sufficient data to design the project is essential. Data requirements include the location and history of the site, its physical characteristics, the nature and extent of contamination and risks associated with the contamination. It is important at the outset to establish closure goals, to help with the assessment and choice of a suitable clean up technology. A comprehensive design and costing should be undertaken for any full scale project. After installation, bioremediation must be maintained and controlled using a well designed sampling and monitoring programme. Analyses should be of a chemical and biological nature and should include determinations of contaminant levels, nutrient concentrations, pH, and microbiological plate counts. Bioremediation is considered complete when target levels have been achieved. Rehabilitation of the bioremediation site (should treatment have occurred *in-situ*), or of the excavated site, should follow.

A case study of an on-going full scale bioremediation project, following the strategy outlined above is presented. Bioremediation of the excavated contaminated soil containing high concentrations of weathered petroleum oils was performed on site using landfarming. Total petroleum levels were reduced from 7400-23000mg TPHC/kg soil to 820-2335 mg TPHC/kg soil over 168 days. Depressed moisture levels due to the low water retention capacity of the soil necessitated frequent application of water, which was essential to enhance rate of degradation. Low moisture retention, a larger fraction of more recalcitrant and weathered petroleums, and less intensive treatment compared to the pilot scale, resulted in a slower TPHC degradation rate when compared to pilot scale investigations.

GENERAL CONCLUSIONS

Bioremediation is a viable technology for the treatment of contaminated soils when used correctly. Slurry reactors, although effective, are not seen as an appropriate technique for widespread use in South Africa due to high initial capital requirements. Landfarming, on the other hand, is feasible, requiring no technologically advanced infrastructure.

Parameters influencing the rate of biodegradation include moisture, oxygen, pH, nutrients and microorganism strains and population levels.

It is unrealistic to expect pilot scale degradation rates under full scale conditions, due to less intensive treatment, different conditions and weathering of contaminants.

TECHNOLOGY IMPLEMENTATION

Bioremediation is a viable technology applicable to many organically contaminated sites in South Africa. Landfarming, either *in-situ* or off-site, is a relatively simple technology to implement, and cost-effective when used under the correct conditions and in the appropriate manner. Care must be taken, however, to implement treatment on a scientific, site specific basis, rather than by rule-of-thumb.

Where landfarming is not suitable, due to toxic volatile emissions, or to high concentrations of contaminants which require more intensive and controlled treatment, other technologies such as enclosed bioventing, and/or slurry reactors should be investigated.

BIOREMEDIATION NEEDS IDENTIFIED

Further research is needed to increase rates of degradation and to determine which bioremediation technique and treatment is applicable for specific contaminants and under certain conditions. Here a collaborative approach between remediation technologists would be advantageous.

Different sources of nutrients and their application should be investigated to enhance nutrient availability to the microorganisms.

Often complex mixtures of contaminants need to be analyzed, making extraction and analyses problematic. Further development work is therefore needed on methods of soil analyses which need to be reliable, reproducible and accurate.

Suitable target levels should be set by regulatory bodies to encourage clean up of contaminated sites.

Sites contaminated with inorganics are a concern. However, very little data on feasible and cost effective remediation technologies for inorganic contaminants is available. Consequently this aspect merits further attention.

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LIST OF SYMBOLS AND ABBREVIATIONS

CFU	= Colony Forming Units
DWAF	= Department of Water Affairs and Forestry
EPA	= Environmental Protection Agency
g	= gram
HC	= Hydrocarbon
HPLC	= High Performance Liquid Chromatography
kg	= kilogram
ℓ	= litre
m	= metre
MAP	= Mono Ammonium Phosphate
mg	= milligrams
mℓ	= millilitre
NA	= Nutrient Agar
nm	= nanometre
OECD	= Organization for Economic Cooperation and Development
PCP	= Pentachlorophenol
ppm	= parts per million
TPHC	= Total Petroleum Hydrocarbon
UST	= Underground Storage Tank
UV	= Ultra violet

1. INTRODUCTION

1.1 INTRODUCTION TO BIOREMEDIATION

Soil has served mankind in many areas, from being a crucial element in agriculture to being the receptacle of our waste products. Man's often careless utilization of this precious resource results in the continuous introduction of unnatural man-made substances into the soil. Contamination of water and soils by organic chemicals is widespread and is increasingly receiving attention, due to its potential impact on public health and the environment. The presence of these contaminants is frequently due to inadequate disposal methods as well as leaks and spills. Halogenated hydrocarbons and organic aromatic compounds are mentioned most regularly as priority pollutants since many of these are toxic to a broad spectrum of organisms and man.

To solve many of the environmental problems facing us today, innovative technologies are required. Bioremediation is a biological treatment involving the controlled use of microorganisms to break down hazardous organic chemicals into innocuous forms, degrading them aerobically to carbon dioxide and water, or anaerobically to carbon dioxide and methane. Nature has been using bioremediation to recycle organic compounds since time began. However, the innovation to harness this energy to degrade hazardous and recalcitrant hydrocarbons, in a confined and controlled environment, came relatively recently (Schneider & Billingsley, 1990). Bioremediation offers a comparably inexpensive, yet highly efficient, method of removing toxic chemicals from contaminated soils. It can be used exclusively or in tandem with other physical and chemical treatment strategies.

1.2 MOTIVATION AND BACKGROUND

South Africa faces a number of environmental challenges, some of which can be directly addressed through harnessing the process of bioremediation. A comparatively low, and highly variable rainfall, averaging about 502 mm per annum, as compared to a world average of 802 mm per annum (Department of Water Affairs, 1986) makes South Africa a relatively arid country. It is thus important not only to develop water resources but also crucial to protect the quality of water. Although use of groundwater is currently limited, it is expected to increase in future and hence this valuable resource must also be protected. Contaminated soil in the vicinity of either surface or groundwater may have adverse impacts on the water quality.

In South Africa, a growing industrial sector contributes to an increasing number of contaminated sites, requiring treatment. Currently, the most frequently utilized practice is to landfill contaminated soil. Not only is this a short-sighted option, since the availability of space in a hazardous waste landfill site is diminishing rapidly and suitable new sites are not easily found, but it is really a displacement of the problem. In contrast, bioremediation technology can provide a more environmentally friendly and cost effective solution to the problem. Opportunities for the bioremediation of contaminated soil are therefore increasing.

Bioremediation of contaminated soil is not unknown in South Africa, although it is not nearly as prevalent as in Europe and America where it is used extensively as a means

of reducing the negative impact of pollution by undesirable solids, liquids and gases on the environment. Bioremediation techniques are proving to be economic methods for the effective treatment of effluents and rehabilitation of polluted sites (Jespersen, *et al.*, 1993).

Full scale bioremediation has had most application within the petrochemical industry. The South African Oil Industry agreed at the beginning of 1994 (Camp, 1994, in print) that

"No liquid hydrocarbons or soil polluted with liquid hydrocarbons shall be disposed of to a landfill site, waste dump or Class 1 or A site

Liquid hydrocarbons shall be recovered and be reused whether before or after reprocessing as conditions require

Polluted soil shall be bioremediated, whether *in-situ* or at an approved location, or treated in another acceptable manner so as to render it acceptable to the environment".

Although awareness of bioremediation as a treatment technology is growing, full scale application thereof is not utilized to its full potential (Pearce & Oellermann, 1994, in print). The first documented use of bioremediation in South Africa was approximately 1980 when a refinery established an area to treat oily wastes through "landfarming". Since that time, various refineries have followed suit, as did the storage and handling sections of the oil industry.

To date a number contaminated sites arising from industrial activities, service stations, vehicle accident spills, bulk storage facilities and railway sidings have been treated in South Africa using bioremediation.

A further risk of oil contamination occurs on the beaches as South Africa lies on one of the world's major shipping routes. South Africa has a coastline of about 3000km, which exacerbates the risk. It was estimated in 1993 that approximately 120 million tons of Middle East oil exports passed the Cape of Good Hope. Although this is a considerable decrease compared to the 635 Million tons that were shipped in 1977, due to alternative shipping routes opening up, it still represents a major risk to ecologically sensitive coastal areas (A.Moldan, Sea Fisheries Research Institute, 1994, personal communication). This was highlighted in June 1994 when the Apollo Sea lost 2500 tons of heavy fuel oil, just off the Western Cape coastline. Apart from the many scenic beaches that were contaminated with oil, the marine life and penguins were also adversely affected.

1.3 PROJECT OBJECTIVES

The original objectives of this project were :

(i) To evaluate bioremediation technologies on a laboratory scale as an appropriate and viable technology. These evaluations were to include soil systems simulation, slurry digestion and volatile organics biotower reactors. In each case biological treatment was to be applied employing selected microorganisms for specific pollutants. The model pollutants aimed at included mineral hydrocarbon oils, aromatic organics and aromatic halogenated organics.

(ii) To optimize treatment conditions and scale-up for pilot scale evaluations of bioremediation at contaminated sites. The economics of the technology were to be evaluated and process design criteria provided to successfully remediate soils contaminated with hazardous and recalcitrant pollutants.

1.4 ACHIEVEMENT OF OBJECTIVES

The overall objectives of the project were achieved, with the exception of the volatile organics biotower which was decided by the steering committee to be beyond the scope of the project.

A survey was done to determine the nature and extent of contaminated sites in South Africa and to direct research towards relevant contaminants. Due to the sensitive nature of the information required, a comprehensive survey was not possible. The information obtained therefore can only be used as an indicator of the situation in South Africa.

At laboratory scale, slurry reactors were evaluated as a bioremediation technology for degradation of phenol in soil. Parameters affecting enhanced bioremediation were investigated using batch reactors. A soil system was simulated using soil columns, however, problems were experienced in establishing a sterile control and in repeatability.

Using the landfarming technique, a pilot scale evaluation of the parameters affecting the degradation rate of TPHC was undertaken. Bioaugmentation (addition of enhanced concentrations of microorganisms which may or may not be pollutant specific) and biostimulation (optimization of soil conditions to stimulate activity of existing microorganisms) were evaluated. Other factors investigated included addition of air, moisture and nutrients. Highest rates of degradation were obtained when nutrients, moisture, aeration were regulated and a biosupplement added. A 94% (m/m) TPHC reduction was obtained from initial levels of 320g/kg soil to 18g/kg over a period of ten weeks.

Following the success achieved at pilot scale, full scale bioremediation was implemented. The results to date of the ongoing full scale bioremediation project are discussed. The process design of the project is provided, and pertinent economical factors, are discussed. In many situations, bioremediation can be implemented as an economically viable process.

2. LITERATURE SURVEY

2.1 TREATMENT TECHNOLOGIES FOR CONTAMINATED SOIL

During the last few years, use of bioremediation as a treatment technology for contaminated soils has steadily increased. Concomitantly, much has been published in the form of books, journal articles and conference proceedings, as remediation technologists continue to research and improve on methods to clean contaminated sites. This literature review is by no means meant to be exhaustive. Instead it aims to bring an overview of remediation technologies before focusing on bioremediation as a means to remove hydrocarbons from soil.

Treatment technologies can be categorized into a number of groups, each of which may have subgroups and may be used in isolation or in conjunction with each other. These include

- Chemical
- Physical
- Stabilization
- Thermal
- Biological

Alternatives to treatment of contaminated soil include direct landfilling or co-disposal with municipal waste. These are not preferred options as the life of the landfill site is reduced. Furthermore, there is a potential threat to groundwater because as temperatures increase in a landfill, the viscosity of the contaminant may decrease and percolation rates increase. Care must therefore be taken to control the volumes of contaminated soil being disposed of in a landfill (Dehrmann, 1991b).

2.1.1 Chemical

Chemical processes can be used either to remove or reduce concentrations of chemical contaminants in soil.

Purely chemical technologies used in isolation include solvent extraction and supercritical fluid extraction. Solvent extraction uses a solvent that is mixed with the soil to extract the contaminant. The solvent can then be treated to remove or concentrate the contaminant. The solvent may be recycled.

Supercritical fluid extraction is a technique where the properties of a pure substance, such as water or carbon dioxide, above its critical point are used advantageously to solubilize organic contaminants from environmental matrices and sludges (Rubin & Mon, 1994; Akgerman, 1993).

Chemical processes are known to be used for petroleum wastes and sludges, although they are not widely accepted, possibly due to cost considerations. Limited data for chemical treatment technologies, especially at full scale, is available (Rubin & Mon, 1994).

2.1.2 Physical

Physical treatment does not remove or destroy contaminants, its function is rather to separate, concentrate or to immobilize contaminants. It can be used in conjunction with biological treatment to increase the bioavailability of the contaminants to the microbes.

Typical physical technologies include soil washing, froth flotation, coal tar agglomeration and soil vapour extraction.

Soil washing can be done *in-situ* or *ex-situ* using a chemical surfactant or extraction agent to mobilize the contaminant which was chemically or physically attached to the soil particles (Raghavan *et al.*, 1991). The technology is found to be most successful in sand or gravel soils (Rubin & Mon, 1994), where adsorption properties of the soil are lower than those found in clay soils.

Coal tar agglomeration uses a solid rather than a liquid adsorbent to remove contaminants from waste. Oil contaminants are strongly adsorbed onto the surface of fine coal adsorbents, which are then separated from the soil in an aqueous slurry (Rubin & Mon, 1994).

Soil vapour extraction is a technology whereby an airflow is provided in the vadose zone to vaporize and transport volatile organic pollutants upward from the subsurface to more amenable media for degradation or disposal. This stimulates *in-situ* volatilization, and contaminant vapours are drawn to the extraction point where they are treated either by activated carbon or biofilters (Hoeppel *et al.*, 1991).

2.1.3 Stabilization

Being a non-destructive technique, stabilization is in effect a combination of chemical fixation and physical consolidation, and can be used not only as an aid to storage and transportation, but also as a means of treatment in its own right. Stabilization is used to produce a fixed blend of the soil matrix and contaminant that will not leach into the environment. An inert product can be formed by binding the contaminant with an inorganic substance such as quicklime (CaO). Cement and fuel ash are further possibilities (Dehrmann, 1991b).

This method can also be used for inorganic contaminants (Olfenbuttel, 1991). An uncertainty associated with this method is whether the contaminant would leach from the matrix with time, or should the local physico-chemical conditions be changed in any way.

2.1.4 Thermal

Heat can be applied at various temperatures to a soil matrix to either incinerate or to volatilize the organic contaminants. The contaminants are thus either destroyed or they may be collected, condensed and recovered. Thermal technologies can be classified as, amongst others, thermal desorption, incineration and pyrolysis (Rubin & Mon, 1994), and may take place in asphalt batching and cement kilns.

These techniques are used for treating fuel contamination *in-situ* and include heated

gas or steam injection and radio frequency heating of in-place soils. Heated air or steam injection technologies can be energy and equipment intensive, resulting in large costs. These methods are not used routinely to remediate hydrocarbon spillages (Hoeppel *et al.*, 1991).

Radio frequency heating is a means by which energy is directed into the soil, where it is efficiently converted into thermal energy. Price *et al.* (1994) showed at pilot scale the viability of this technique to enhance the recovery rate of fuel oil in a silty soil at a depth of 20 feet.

2.1.5 Biological

Biological treatment technologies harness the metabolic utilization of organic compounds by microorganisms. Under the appropriate conditions, these biological processes are capable of breaking down hazardous contaminants to carbon dioxide, water and cell mass under aerobic conditions, or to methane, water and cell mass under anaerobic conditions.

2.2 BIOREMEDIATION TECHNOLOGIES

2.2.1 Aerobic bioremediation technologies

The 1986 Superfund Amendments Act of the United States made fundamental changes in the approach to hazardous waste site remediation by clearly indicating that permanent solutions are preferred. Treatment technologies fall within all five groups (section 2.1 above). However, bioremediation was rated second in status as a treatment in the Superfund sites in America, as a result of its technical feasibility and effectiveness, and its low cost as compared to other treatment alternatives (Kovalick, 1992).

Bioremediation collectively stands for a number of treatment technologies which can be broadly classified as *in-situ* or *ex-situ*; the latter entails excavation and treatment on-site or off-site. Two broad methodologies can be employed, biostimulation and bioaugmentation. Biostimulation targets the microorganisms already present in the particular environment, and aims to enhance their activity, by the addition of suitable additional nutrients which otherwise limited their activity. Degradation of contaminants can also be enhanced by increasing microbial numbers, i.e. bioaugmentation, so that their activity can be more effectively asserted in the particular system (Mason *et al.*, 1992; Bradford & Krishnamoorthy, 1991). In bioaugmentation, specially adapted or engineered microorganisms are introduced to the contaminated soil. The basis of this technique is to target the contaminant with a microorganism known for its ability to degrade that particular compound (Mason *et al.*, 1992). According to Rubin *et al.* (1992) most U.S.A. remediation experts avoid the use of engineered microorganisms, noting strict federal rules on their use and the fact that natural microbes can perform comparably well on most contaminants.

The methodology used depends on a number of factors. Some considerations that need to be assessed are risk to health, further contamination of soil and groundwater, as well as factors such as the availability of space for the treatment. Generally, these can only be decided upon after a detailed site assessment.

2.2.1.1 *In-situ* bioremediation technologies

The contaminated soil is treated in place and essentially remains undisturbed during treatment. The most common form of *in-situ* treatment is the biodegradation of contaminants within the saturated zone of the soil (Wilson & Jones, 1993). *In-situ* technologies which have been used to remediate contaminated sites include soil-washing, low-temperature thermal treatment, soil bioventing and enhanced bioremediation or landfarming.

Soil washing

This involves the injection of a synthetic surfactant or solvent into the contaminated zone to promote greater release of hydrophobic contaminants to the aqueous phase (Hoeppel *et al.*, 1991). Arthur *et al.* (1989), according to Hoeppel *et al.* (1991), claimed that this technology had been implemented with limited success. Arthur *et al.* (1989) hoped to increase bioavailability by adding surfactants. However, 53 synthetic surfactants were screened and tested for their ability to enhance natural biodegradation rates in jet-fuel contaminated soils, and neither this effect nor inhibition could be noted.

Bioventing

The aim of bioventing is to promote *in-situ* aerobic biodegradation rather than to vaporize hydrocarbon fuels.

Bioventing has specific application to low volatility fuels which are not removed efficiently by soil vapour extraction. Most limiting to the degradation of these compounds is the lack of sufficient oxygen to the microbes and the poor accessibility of the microbes to the hydrophobic contaminants (Kittel *et al.*, 1994). Increased oxygen levels in the soil, from bioventing, should increase degradation rates of all biodegradable organic compounds. An airflow is established through the soil, through pulling a vacuum in the vadose zone. This provides oxygen for the microbial degradation process (Kampbell *et al.*, 1992). Vapour extraction wells or dewatering points are placed in the contaminated zone to aid in this.
Bioventing can also be used for enhancing degradation of a wide range of hydrocarbons from the subsurface, especially from the unsaturated vadose zone above the groundwater table.

Enhanced bioremediation

Enhanced bioremediation (Bradford & Krishnamoorthy, 1991), also known as landfarming, involves the controlled management and manipulation of microbial processes in the subsurface. The contaminated land is farmed, by regularly loosening the ground using a plough or rotovator. This allows mixing and oxygen penetration into the soil. Nutrients (10 parts nitrogen and 1 part phosphorus to 100 parts hydrocarbon/oil) must be applied and worked into the soil, the pH should lie between 6-7. The latter can be adjusted with agricultural lime. Enhanced bioremediation systems utilize aerobic processes, which can be added as air, oxygen gas or hydrogen peroxide. In this manner, the metabolic capabilities of soil microorganisms which degrade or detoxify contaminants residing within the soil or groundwater are stimulated.

In-situ bioremediation has the virtue of simplicity and consequent modest capital and operational costs. It is often applicable when other techniques cannot be employed, for example, when excavation is impossible. A further advantage is that the treatment can move with the plume of contamination in the groundwater.

Dehrmann (1991b) evaluated alternative options for the disposal of oily waste after an oil spill, and found landfarming techniques suitable for the treatment of large quantities of oily sand or sludge. It was suggested that a combination of techniques may be needed, and care must be taken to limit environmental contamination.

In-situ bioremediation cannot be used where land availability is a problem, nor where there is threat of further contamination of water, air and soil (Savage *et al.*, 1985). The effectiveness of *in-situ* bioremediation can be highly dependant on the permeability of the soil. Treatment conditions are difficult to control, due to difficulties in nutrient delivery and maintenance of adequate consortiums and levels of microorganisms. Sufficient mixing may also be a problem. Due to these factors, bioremediation would take longer *in-situ* than would be expected *ex-situ* (Saps, 1989).

However, *in-situ* bioremediation remains the most cost-effective method for contaminants that are easily degraded, but which have low solubilities in water (Ryan *et al.*, 1991).

2.2.1.2 *Ex-situ* bioremediation technologies

Where the disadvantages of *in-situ* bioremediation outweigh the advantages, *ex-situ* treatment will need to be considered. The greatest advantage of *ex-situ* treatment is that a closer control over parameters can be exercised. Most of the *in-situ* treatments can also be employed *ex-situ*, such as bioventing and landfarming. In these cases biocells or bioreactors are used to contain the contaminated soil and treatment manifolds.

If landfarming is the selected treatment technology, the excavated soil should be placed in a lined biocell. If bioventing is used an aeration system should be included.

Slurry phase bioremediation is a further alternative. The contaminated soil is treated as an aqueous slurry in a large bioreactor system usually situated close to the contaminated site (Ryan *et al.*, 1991). The technology enables intimate mixing and contact of microorganisms with the contaminants and allows for the creation of optimum environmental conditions for microbial biodegradation (Stegman *et al.*, 1994). Britto *et al.* (1992) developed an effective continuous flow bioreactor treatment for petroleum contaminated soils.

Microorganisms employed for decontamination can include the indigenous microorganisms or the bioreactors may be inoculated with specially selected organisms capable of rapidly and extensively degrading target pollutants. Addition of inorganic and organic nutrients, oxygen and acid/alkali for pH control is possible, so that an optimum environment for bacterial growth is created. The use of this process can also provide control over volatile emissions, when they are of concern. Once biodegradation is completed, the soil slurry is dewatered and the soil is returned.

A disadvantage of the system is that the contaminated soil has to be excavated and handled.

Economic factors often favour solid-phase systems, however, costs associated with slurry bioremediation are still about 50% less than alternative treatments such as incineration.

2.2.2 Anaerobic/anoxic bioremediation technologies

Aerobic bioremediation uses oxygen as a terminal electron acceptor for the oxidation of organic compounds. It is possible, however, that alternate terminal electron acceptors, such as the ferric, nitrate and sulphate ions, may be used under anaerobic or anoxic conditions. Most energy is released when oxygen is used, and hence, this is the preferred treatment as there is a direct link between energy released and microbial activity and growth. However, the amount of free energy released under Fe(III)-reducing conditions is nearly equal to that under aerobic conditions and thus the ferric ion may be an important electron acceptor under anaerobic conditions (Pollard *et al.*, 1994). Significant anaerobic degradation is thought to occur in the clay soil aggregates with anaerobic centres.

Anaerobic treatment is found to have distinct advantages for the treatment of highly chlorinated compounds resistant to aerobic biodegradation. This occurs through a reductive de-halogenation mechanism which is able to remove chlorine atoms residing on recalcitrant compounds. In this manner, anaerobic bioremediation can be used beneficially in conjunction with aerobic bioremediation, to further bioremediate the de-halogenated compounds.

2.3 FACTORS INFLUENCING BIOREMEDIATION

Pollard *et al.* (1994) has identified four major categories of constraints which may limit bioremediation;

a) the contamination may occur in more than one medium (soil, soil vapour, groundwater and free phase)
b) the contamination may be a mixture of organic and inorganic compounds with a range of environmental and toxicological properties
c) heterogenous subsurface properties may be difficult to characterize
d) sub-optimal conditions may exist for on-site and *in-situ* treatment

Very little can be done about the first three categories. However, as they influence bioremediation, as much information about these factors should be known by the remediation technologist. It is important to know whether any toxic compounds, that may inhibit biodegradation, are present and whether the contaminant is at all biodegradable. Greater control can be exercised over the last category. Sub-optimal conditions can be improved by addition of soil amendments.

Environmental conditions have an impact on bioremediation on two levels - firstly, they influence microbial numbers and activity; and secondly, they have an influence on the physical/chemical properties of the contaminant. These effects can be interactive, thus predictions are difficult to make. The many factors contributing to the environmental conditions have been discussed and reviewed by a number of authors (Schneider &

Billingsley, 1990; Providenti *et al.*, 1993 and Pollard *et al.*, 1994 amongst others), some of the which are summarized and discussed below.

2.3.1 Contact/Bioavailability

Intimate contact is needed between the microorganisms and the contaminant to increase biodegradation rates. Degradation occurs most readily in the aqueous phase, which is limited by oil-phase partitioning, adsorption and diffusion processes (Pollard *et al.*, 1994). This in turn influences the bioavailability i.e. the fraction of substrate available for microbial attack.

2.3.2 Oxygen

Aerobic degradation is known to be faster than anaerobic for many organic compounds, hence it is often the preferred method of treatment.

In landfarming, aeration of the soil can be improved by tilling, composting with bulking agents to increase porosity or by venting. In slurry or other aqueous systems, oxygen levels can be increased by sparging with air or addition of hydrogen peroxide (Providenti *et al.*, 1993). The dissolved oxygen concentration can either be supplied continuously using a diffuser (external source), or introduction of oxygen (air) can be achieved through the method of mixing, e.g. tumbling.

Peroxygen compounds can also be added directly to the soil as hydrogen peroxide or in the form of calcium or magnesium peroxide which are more stable. However, application of peroxide may oxidize the contaminant chemically. A further disadvantage is that high concentrations of hydrogen peroxide may sterilize the soil. Stability of hydrogen peroxide is a limiting factor. However, this may be increased by adding chemicals such as polyphosphates, stannate or phosphate (which decrease the catalytic action of iron), sodium pyrophosphate (which precipitates or sequesters iron), citrate (which reduces decomposition from enzymatic catalysts) amongst others (Elizardo, 1993).

2.3.3 Moisture

Moisture is essential for microbial degradation, as inadequate hydration decreases microbial metabolism, as well as microbial movement through the soil. Lack of moisture also decreases contaminant as well as nutrient transport through the soil (Providenti, 1993).

2.3.4 Contaminant composition and concentration

Chemical wastes are inherently mixtures of a large number of individual compounds. Recalcitrant compounds, such as complex aromatic structures, and some xenobiotic compounds are resistant to microbial degradation. Highly chlorinated compounds are typically resistant to aerobic degradation and may be treated under anaerobic conditions.

The degree of weathering or ageing also affects the ease of degradation. Weathering includes such processes as evaporation, photolytic loss, hydrolysis and

biotransformation which selectively reduces the concentration of readily biodegradable substrates and leaves behind refractory compounds resistant to further microbial attack (Pollard *et al.*, 1994) making further bioremediation difficult.

Certain wastes may also be toxic above certain concentrations. Crawford & Mohn (1985), according to Pollard *et al.* (1994), showed that mineralization of pentachlorophenol (PCP) at concentrations of 500ppm was suppressed, whereas lower concentrations were readily reduced. Additionally, increasing concentrations of PCP resulted in decreased rates of degradation. Pollard *et al.* (1994) suggests that a treatability concentration range may exist, above which metabolic activity is inhibited, and below which the microorganisms may switch to alternative substrates. This is corroborated by Providenti *et al.* (1993) who refers to a threshold concentration necessary for biodegradation, which is dependant on both the contaminant and the microorganisms present.

High salinity and appreciable concentrations of metals, often associated with petroleum and wood-treating wastes may cause inhibition of metabolic activity.

2.3.5 Strain and population level of microorganisms

Soil microorganisms have a large diversity in their metabolic activities and are thus able to degrade a wide range of contaminants. The usual response of a microbial consortium to a waste is a large increase in the waste-utilizing component of the community, for example, of hydrocarbon degraders when exposed to petroleum-contaminated soil. An alternative is to use bioaugmentation, and thus to supplement a microbial community adapted to a particular waste.

Corseuil & Weber (1994) suggest that delays in field scale bioremediation projects may be due to insufficient numbers of "contaminant-adapted" indigenous microbes. It is suggested that these microbes may need to achieve a critical population in order to yield demonstrable contaminant degradation.

Certain microorganisms may require a cometabolite in order to biodegrade certain contaminants. The cometabolite is seen to provide energy and the ability to degrade non-growth substrates. The cometabolite may also encourage production of enzymes needed to recognize contaminants and catalyze their degradation (Providenti *et al.*, 1993). An example of cometabolism is the requirement of glucose to degrade high molecular weight polyaromatic hydrocarbons.

2.3.6 Nutrients

To degrade any substrate, microorganisms need essential nutrients, such as nitrogen and phosphorus. In general, 10 parts nitrogen and 1 part phosphorus is needed to every 100 parts carbon (Dehrmann, 1991b). Research done by Wren *et al.* (1994) indicated that the source of the nitrogen affects the degradative ability of oil-degrading cultures. Results showed that KNO_3 permitted greater degradation than NH_4NO_3, possibly due to the absence of the pH decline often associated with ammonia. Thus successful bioremediation may depend as much on appropriate source of nutrients as on the concentration of the nutrient itself.

Trace nutrients such as K, Mg, Ca, Na and S may also be required.

2.3.7 pH

Many industrial wastes are highly alkaline or acidic, whereas the optimum pH for metabolic activity lies between pH 6 - 8. Fungi tend to be more tolerant of acidic conditions than bacteria (Schneider & Billingsley, 1990). The pH of the soil should be monitored regularly as it may change due to bioremediation products in the soil. The water solubility and contaminant sorption to soil particles may also vary with pH and hence should be taken into consideration (Providenti *et al.*, 1993).

2.3.8 Temperature

Optimum metabolic activity is commonly found between 15° C and 35° C. Depending on the strain of bacteria, many are unable to thrive at temperatures above 40° C. Similarly, few microorganisms are able to be active below 0° C. As a result, any bioremediation programme must take into account the operating temperatures. Further affects of temperature include equilibrium and kinetic constants, as well as the viscosity and solubility of hydrocarbons.

Temperature of enhanced bioremediation projects can be influenced by application of mulches or by irrigation to increase the soil heat capacity. In this way, fluctuations of temperature can be minimized (Pollard *et al.*, 1994).

2.3.9 Soil structure and texture

Moisture, porosity, temperature and workability of soil are often influenced by soil texture. The success of bioremediation is therefore also affected since infiltration of water, retention thereof and ease of aeration play a vital role. Much is still unknown in this area, and conflicting evidence exists as to what soil type or structure is amenable to bioremediation (Schneider & Billingsley, 1990). Research has shown that increased surface area in certain cases facilitated increased degradation rates (Pollard *et al.*, 1994).

In soils with high clay contents, aggregation can cause entrapment of microorganisms and adsorption of contaminants to soil surfaces thus lowering the bioavailability. This may lead to a decreased rate of biodegradation. Clay soils however, have an advantage in that their water retaining capacity is greater than that of sandy soils. In general terms, however, clay soils are not suited to enhanced landfarming techniques and more intensive treatments, such as occur in slurry reactors, are more suitable due to the increased agitation.

Conversely, sands have excellent water permeation characteristics, however, their water retaining capacity is low. Problems to maintain adequate moisture in the soil may therefore arise. Sorption of the hydrocarbons to sandy soils is usually not as extreme as that found in clay soils (Schneider & Billingsley, 1990).

In conclusion, many factors influence bioremediation, some of them simultaneously with interactive effects. It is thus difficult to predict the rate of contaminant degradation or even to determine how optimal conditions can be achieved. Every site will be different and needs to be assessed as such.

3. THE NATURE AND EXTENT OF CONTAMINATED SITES IN SOUTH AFRICA

3.1 INTRODUCTION

South Africa's economic growth continues to increase with a concomitant industrial growth. Due to this and a number of other factors pertinent to South Africa, such as the long transport hauls by tankers, inadequate concern for the environment, and inadequate environmental protection measures, it can be presumed that many contaminated sites exist. However, the nature and extent of contaminated sites in South Africa is largely unknown. Table 1 shows the origin of contaminated sites commonly found, and possible causes thereof.

Table 1. Origin of contaminated sites and possible causes

Nature of Contaminated Site	Cause
Industrial - Petrochemical e.g. refinery service station	Spills Leaks from UST's* Pipeline bursts Tanker mishap Poor housekeeping
Industrial - Organic chemicals e.g. solvent manufacturing wood treating pesticide/herbicide	Spills Tanker mishap Poor housekeeping
Industrial - Inorganic chemicals e.g. plating companies fertilizer producers	Spills Poor housekeeping Tanker mishap
Railway sidings	Spills Poor housekeeping
Harbours	Spills Accidents

* UST = Underground storage tank

It is thought that the majority of sites with soil and water contamination are due to waste disposal sites and industrial sites. A survey done by Lombard & Associates on the status of waste management operations in South Africa (Lombard *et al.*, 1991) indicated that landfilling is the predominant technology for waste disposal. Few sites, however, are permitted. The survey indicated that only 17,9% of the 542 recorded sites had permits or were awaiting the outcome of permit applications. This could indicate the potential for poor management practices which increases the potential for soil and water contamination. Many sites are not recorded at all, thus it may be realistically assumed that the situation is much worse than documented.

In order to determine the applicability of bioremediation, the potential for this treatment technology in South Africa, and also to direct research in this area, it was necessary to obtain an overview of the following:

- Whether contaminated sites are a cause for concern in South Africa
- The nature, origin and location of contaminated sites
- The most prevalent contaminants and associated concentrations
- Extent and success of treatment, specifically of bioremediation

Due to the sensitive nature of the information requested, it was realized that there would be difficulties in obtaining a representative overview. A multi-faceted approach was thus used.

3.2 METHODOLOGY

The investigation was done through interviews, questionnaires and liaison with the Department of Water Affairs and Forestry (DWAF).

3.2.1 Interviews and Questionnaires

The Regional Offices of DWAF were contacted to obtain information on the nature and extent of contaminated sites.

In an attempt to obtain information from a broader spectrum of interested and affected parties, a questionnaire (Appendix 1) was drawn up and sent to managers in

- Waste Management Companies
- Petrochemical Industries
- Consulting Engineering Firms

Where needed, informal interviews were conducted with individuals involved in waste management and/or bioremediation of contaminated sites.

3.2.2 Liaison

The DWAF was simultaneously carrying out a survey to determine the number and nature of landfill sites, and to what extent pollution had already been caused or had the potential to occur. A number of interviews were held with the personnel at DWAF and the information from these questionnaires was combined with the above survey.

3.3 RESPONSE

Response to the questionnaires sent out by the Division of Water Technology is summarized in Table 2.

Table 2. Response to questionnaire

Number of questionnaires sent out	70
Number of questionnaires returned	35
Number of questionnaires returned containing information on contaminated sites	18
Number of respondents claiming information relevant to contaminated sites to be confidential	17

3.4 RESULTS

Due to the sensitivity of the information required in the questionnaire, and the need to protect confidentiality, some questions by the respondents were answered inadequately. Further reasons for supplying insufficient information included

- inadequate detail known about sites
- unreliable and infrequent analyses

All respondents were aware of the need to treat polluted sites, while 91% knew of bioremediation as a means to clean contaminated sites. An overwhelming majority would consider bioremediation as a clean-up option, under suitable conditions. *In-situ* landfarming, soil vapour extraction and/or excavation were the most common methods of dealing with contaminated soil, according to the response obtained.

The perception of the cost of bioremediation is summarized in Table 3.

Table 3. Summary of perception of cost feasibility of bioremediation

Perception of cost	No. of responses
Inexpensive	3
Reasonable	22
Reasonable to expensive	1
Unknown	3

Thirteen respondents were unwilling to give comment on whether landfilling is a more economic option, as this depends largely on transport costs. Six respondents were of the opinion that it was a more economic option, while fifteen saw bioremediation as more cost effective.

The efficiency of bioremediation on reaching required target levels was seen overwhelmingly as "acceptable" to "very acceptable", with only one respondent having the opinion that it was unacceptable.

3.4.1 *Type of site and extent of contamination*

A total of 78 contaminated sites were identified through the survey. Results are shown in Figure 1.

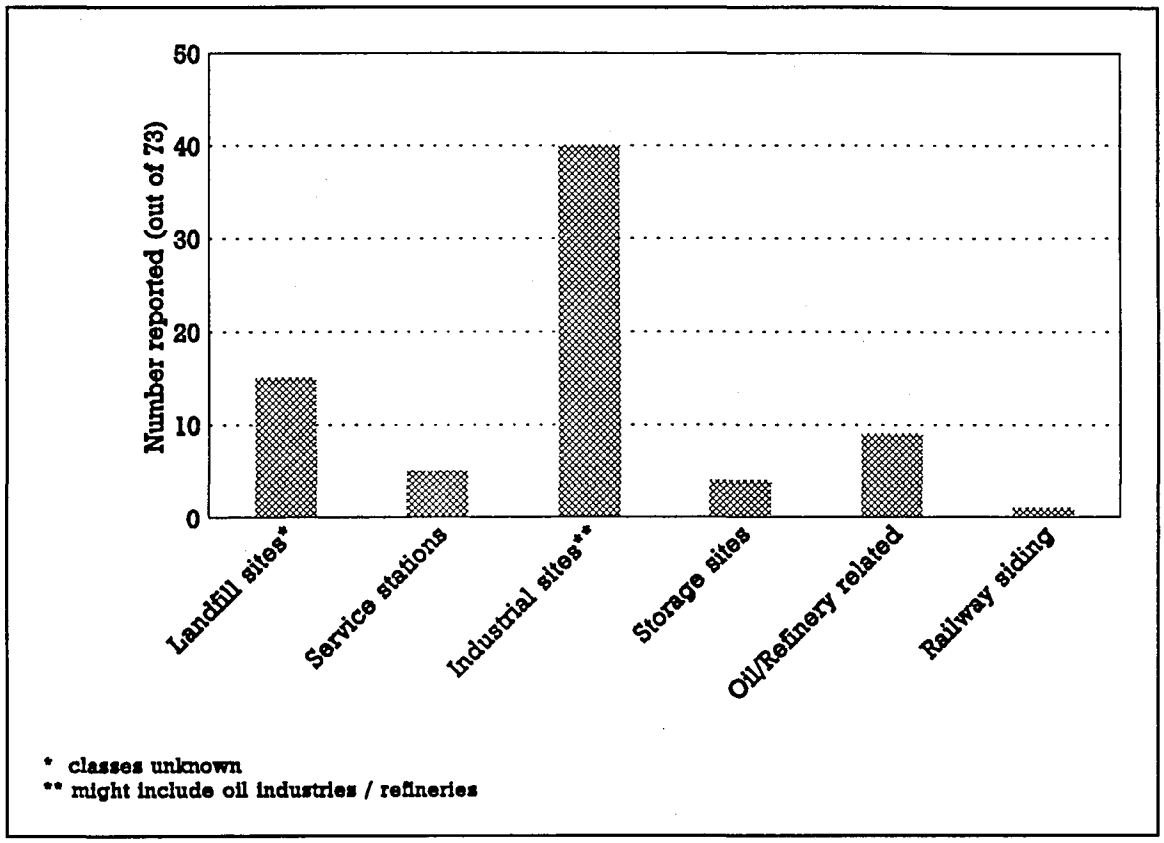


Figure 1. Types and number of sites identified

The majority of sites were industry related, followed by others such as domestic waste sites, railway sidings and harbour areas. Twenty three of the sites recorded in the survey were situated near surface water, indicating a potential threat of water pollution. All of these sites had an age of 20 years or more, indicating long term pollution has most probably occurred, and precautions against polluting the environment are probably absent. Newer waste disposal sites are usually designed with precautionary measures such as leachate collection systems, monitoring boreholes etc.. The survey showed that pollution of the groundwater had occurred for 24 of the 78 sites. A further 43 sites indicated uncertainty with regard to groundwater pollution, while for 11 sites, no groundwater contamination was claimed.

3.4.2 Nature of pollutants

The most frequently occurring pollutants are shown in Figure 2.

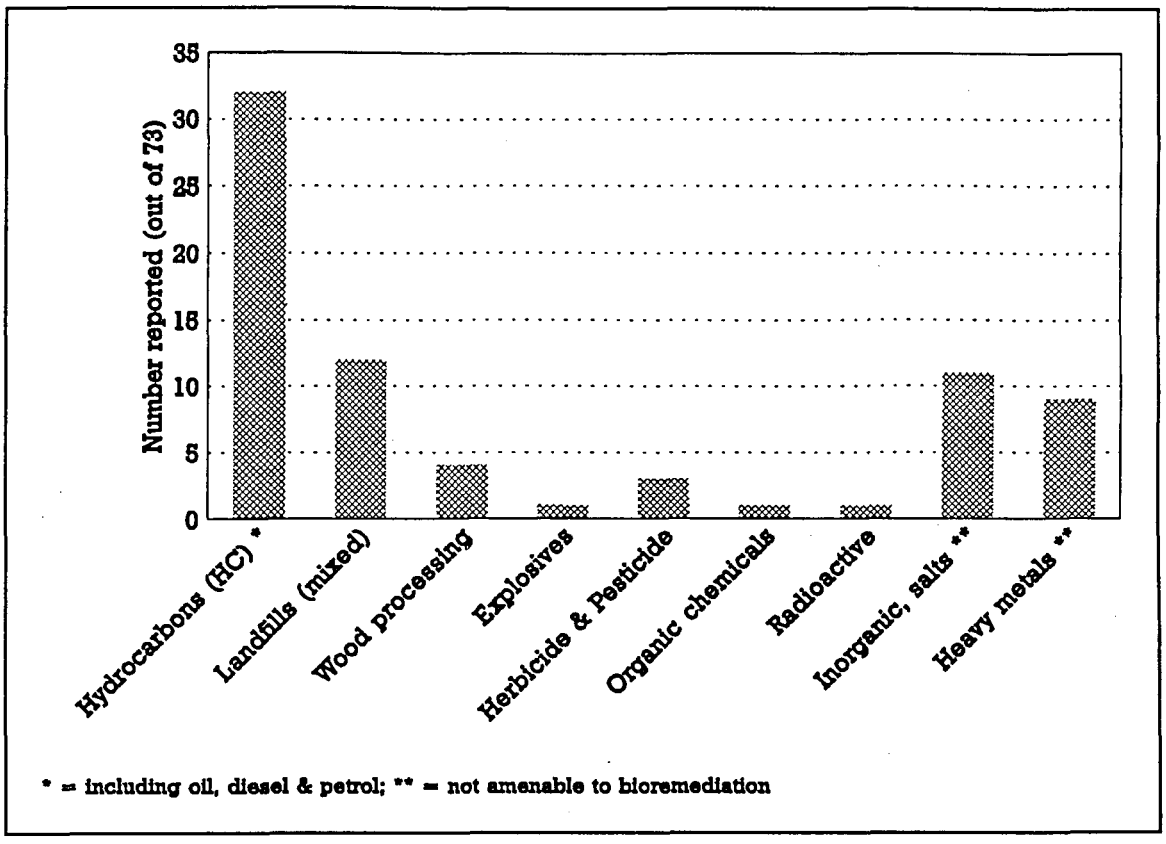


Figure 2. The nature and occurrence of specific polluting agents

Contamination from petroleum products was most prevalent. Of further concern is the number of sites contaminated with wood processing chemicals, such as creosote, and contamination with pesticides and herbicides. Isolated cases of sites contaminated with organic solvents were recorded.

Contamination of soil with high concentrations of salts and heavy metals were a frequently observed concern, especially where contamination of groundwater is possible. These contaminants cannot be treated using bioremediation. Other methods of remediation for such contaminants need to be assessed, such as soil washing and immobilization.

3.4.3 Bioremediation of contaminated sites

It is estimated that, in total, contaminated soil from approximately 30 service stations, 40 vehicle accident spills and 11 bulk storage facilities have been treated in South Africa using bioremediation (C.R.Camp, 1994, personal communication).

A large environmental clean-up operation undertaken by the South African Oil Industry involved a tank farm which had been established in 1914, when there was little concern about environmental pollution. The tanks stored a variety of chemicals. Clean up actions started in 1987 and involved the excavation of old tanks and pipes and the bioremediation of contaminated soil. The soil was remediated to a level of 1000ppm or less (C.R.Camp, 1994, personal communication).

Bioremediation of oil contaminated beach sand occurred following the Petingo oil spill on the North-eastern seaboard in 1990, when approximately 1650 tonnes of heavy fuel oil contaminated 5500m^3 of sand (Dehrmann, 1991a).

Response to the questionnaire identified 28 sites in South Africa where bioremediation has been used. Table 4 gives data recorded for 12 of these sites. No details were given for the remaining 16 sites. Contaminants specified for these sites were reported mainly as "hydrocarbons" (6 cases) and "oil" (8 cases), but no concentrations were specified. The majority indicated that the bioremediation treatment was cost effective.

Table 4. Contaminated sites cleaned by means of bioremediation

Type of site	Major contaminant	Concentration levels		Volume of soil	Cost	Cost effective?	Levels set by
		Initial	After				
Industrial	Oil	59*	12	50 000 m^3	R200 000	Yes	DWAF
Oil storage site	TPHC	40 000 ppm	<1000 ppm	7 000 m^3	R1 x 10^6	Yes	Oil Industry, DWAF
Service station	Gasoline	10 000 ppm	<1000 ppm	1 000 m^3	R100 000	Yes	Oil Industry, DWAF
Oil storage site	Diesel	5 000 ppm	<1000 ppm	3 000 m^3	R 50 000	Yes	Oil Industry, DWAF
Spill	Diesel	6 000 ppm	<1000 ppm	150 m^3	R 2 000	Yes	Oil Industry, DWAF
Vacated storage site	Hydrocarbons	>10 000 ppm	<1000 ppm	100 000 m^3	R2,5x10^6	Yes	DWAF
Oil company	Diesel, fuel, oil	25 000 ppm	<500 ppm	200 m^3		Yes	Local auth
Construction site	Motor oil	25 000 ppm	<500 ppm	500 m^3		Yes	Local auth
Building site	Oil	3 500 ppm	16 ppm	100 m^3		Yes	Local auth
Oil loading site	Crude oil	600 000 ppm	500 ppm	50 000 m^3		Yes	Local auth
Fuel storage site	Petrol/ Diesel	14 000 ppm	200 ppm	2 000m^3		Yes	Client
Service station	Petrol/ Diesel	7000 ppm	500 ppm	1 000m^3		Yes	Client

* no units were provided on the returned questionnaire

A framework defining clean-up procedures or the specification of concentration targets for hazardous compounds in soil, has not been legally formulated in South Africa. Guidelines, set by the Oil Industry Environment Committee (Camp, 1994, in preparation), recommend a target concentration of 1000 ppm TPHC above background values for soil, and 100 ppm TPHC in water. In all of the above

bioremediation projects, target limits for contaminants were set mainly by the Department of Water Affairs and Forestry and the Oil Industry, others were set by local authorities or by the industry or client themselves.

The future of bioremediation as a technology appears positive, with respondents expecting an increase in use ranging from 1-100% over the next year, and 2-500% over the next five years.

3.5 CONCLUSIONS

The information on the nature and extent of contaminated sites is sparse, due to a reluctance to pool information and also due to lack of knowledge. Information obtained from the survey indicated the following:

- Many contaminated sites are known and are a cause for concern, especially where water pollution is a possibility.

- Ninety one percent of the respondents knew of bioremediation, and accept it as a treatment technology. Use of bioremediation is expected to increase, indicating a confidence in the technology.

- Landfarming, followed by soil vapour extraction, are the best known and most frequently used bioremediation technologies in South Africa.

- The majority of the respondents would use bioremediation, under suitable conditions, seeing it as a cost-effective technique.

- Sites contaminated with petrochemicals appear to be the most prevalent, although inorganic contaminants (salts and heavy metals) also need attention.

- Not many companies capable of implementing bioremediation are known to the respondents. This indicates that there may be a lack of expertise in this area. A number of companies, especially the larger petrochemical industries, perform bioremediation in-house.

4. LABORATORY STUDIES : BIODEGRADATION OF PHENOL

4.1 INTRODUCTION

A number of factors were investigated at laboratory scale. However, before experimentation could start, two aspects needed consideration. A satisfactory method of soil analyses for the determination of phenol needed to be developed. Secondly, soil adsorption studies were performed so that a decrease in contaminant concentration due to sorption to soil surfaces was not erroneously contributed to biodegradation.

Once these two aspects had been covered, factors affecting the rate of biodegradation could be researched. This was followed by investigating the viability of using slurry reactors to remediate phenol contaminated soil. Soil columns were used to simulate phenol contaminated seepage water percolating through the soil. The aim was to monitor the breakdown of phenol occurring through the soil columns and to investigate the effects of nutrients and biosupplementation.

4.2 PHENOL ANALYSES

Preliminary experiments to determine phenol degradation in soil slurries indicated that the extraction of phenol from soil samples and the analyses thereof were not accurate enough, nor were they reproducible.

Phenol in soil is known to be an extremely difficult contaminant to quantify, unlike its chlorinated or methylated counterparts (Bengston, 1985; Chriswell *et al.*, 1975 and Realini, 1981). This is mainly due to the inherent properties of phenol such as its high polarity and low vapour pressure. Generally, compounds having strong hydrogen binding groups such as OH^- or NO_2^- remain more strongly associated with water than phenols substituted with chlorine or methyl groups. These characteristics make it difficult to extract the phenol into an organic solvent. Many published methods for analyses of phenol report low recoveries such as 35% (Anonymous, 1992) using HPLC and solvent extraction efficiencies of 60-66% (Bengston, 1985).

Two methods for determination of phenol concentrations were investigated. Firstly, a solvent extraction method was tested using various organic solvents to optimize recovery and minimize losses. The second method involved soil venting at elevated temperatures. Further details on both the solvent extraction and soil venting are in Appendix 2 and 3, respectively.

Initially the soil venting gave the highest recoveries of phenol, however, reproducibility was a concern. The method based on aqueous extraction of phenol using pH control, with no organic extraction step, was finally decided upon for use in the remainder of phenol soil extractions, which gave satisfactory results.

4.3 SOIL ADSORPTION STUDIES

It was a concern that a decrease in phenol concentration during the experiments may be erroneously attributed to biodegradation when the decrease may in fact be due to soil adsorption. The phenol adsorption characteristics of the soil were therefore investigated.

4.3.1 Aim

The overall aim of these experiments was to determine the extent of phenol adsorption on the soil used for bioremediation laboratory experiments under two different conditions, namely for a slurry reactor and for a soil column reactor. A further aim was to determine the maximum capacity of the soil to adsorb phenol and finally a test was done to obtain an indication of how volatile phenol was at ambient temperatures under a stream of air simulating wind under natural conditions.

4.3.2 Experimental procedure

4.3.2.1 Simulated slurry tests

Ten grammes of sieved garden soil were spiked with 50mg phenol. The closed container with the soil was shaken for 10 minutes, after which it was centrifuged and the supernatant was filtered. The aqueous supernatant was made to volume using distilled water and analyzed for phenol. A control was done with soil and water only, no phenol, to determine background interferences. A further control containing only a phenol solution with no soil was included to quantify adsorption to container walls, if any.

4.3.2.2 Simulated soil column tests

Ten grammes of soil were put into a fritted porcelain crucible. Ten mℓ of a 5g/ℓ phenol solution, representing 50mg of phenol was filtered under gravity through the soil. The soil was then rinsed with 100mℓ of distilled water and the filtrate made up to 250mℓ in a volumetric flask before analysing for phenol. The soil was extracted for phenol twice using the method detailed in Appendix 3.

4.3.2.3 Soil adsorption capacity

A further test was done to determine the capacity of the soil to adsorb phenol without a subsequent rinse with water. The experiment was done as before (see section 4.3.2.2). However, 40mℓ of a 20g/ℓ phenol solution was allowed to filter through the soil before being made up to volume in a volumetric flask. This was followed with a second aliquot of phenol solution which was collected separately.

4.3.2.3 Volatility of phenol

Soil, artificially contaminated with phenol, was left to air dry. A sample of the contaminated soil was put into a round-bottomed flask and a stream of air was passed over the soil, simulating wind under natural conditions. The air leaving the flask was bubbled through a solution of sodium hydroxide which was analyzed for phenol after several days.

4.3.3 Results and discussion

4.3.3.1 Simulated slurry tests

Results obtained can be seen in Table 5. The controls showed that there were no background interferences, and very little adsorbance of phenol occurred on the container walls. High recoveries of phenol showed that no significant amount of phenol adsorbed irreversibly to the soil surfaces during the experimental run.

Table 5. Phenol recovery after soil slurry adsorption tests

Reactor	Phenol spiked (mg)	Phenol recovered (mg)	Recovery (%)
1	50	42.8	85.5
2	50	43.1	86.3

4.3.3.2 Simulated soil column tests

Results (Table 6) show high recoveries, indicating that very little, if any, adsorption of phenol to the soil surface occurs.

Table 6. Phenol recovery after soil column adsorption studies

Reactor	Sample matrix	Phenol spiked (mg)	Phenol recovered (mg)	Recovery (%)
1	Aqueous soil (1st extract) soil (2nd extract) Total:	50	49.6 1.1 0.5 ----- 51.2	 102.4
2	Aqueous soil (1st extract) soil (2nd extract) Total:	50	47.0 1.4 0.7 ----- 49.1	 98.2

4.3.3.3 Soil adsorption capacity

Soil capacity for phenol adsorption was as in Table 7. Results showed, that after two aliquots had been filtered through, recoveries of 95 to 100% of the phenol is recovered, indicating minimal adsorption.

Table 7. Soil adsorption capacity for phenol

Sample	Phenol dosed (mg)	Phenol recovered (mg)	Phenol recovered (%)
1 - 1st aliquot	800	526	66
2nd aliquot	800	758	95
2 - 1st aliquot	800	800	100
2nd aliquot	800	839	105

4.3.3.4 Volatility of phenol

Although the initial concentration of the phenol in the soil was not known, analyses of the NaOH solution after a number of days showed that 10.6 and 12.2 mg of phenol had been trapped in duplicate experiments.

4.3.4 Conclusions

High recoveries of phenol in the above preliminary experiments indicate that minimal adsorption to the soil occurs during the time that the phenol solution was in contact with, or percolates through, the soil. Furthermore, results indicated that most of the adsorbed phenol is released from the soil surface during soil extractions. Although surfactants can be used to further increase the release of phenol from the soil, the results obtained indicate that this was not necessary.

The preliminary experiment to test the volatility of phenol in soil shows that losses of phenol from soil do occur.

4.4 PARAMETERS AFFECTING ENHANCED BIOREMEDIATION

4.4.1 Aim

To determine conditions under which the phenol degradation rate increases.

4.4.2 Experimental procedure

Batch reactors were used. Eight 250mℓ Erlenmeyer flasks were set up as in Table 8, each holding a 100mℓ of either sterilized water or mineral medium containing 50mg of phenol. The batch reactors were stirred for the duration of the experiment. Uncontaminated soil obtained from the grounds at CSIR, Pretoria was used. Bacteria isolated from phenol contaminated soil were used where supplementation is indicated.

Table 8. Parameters used for the beaker experiments

Reactor	100mℓ solution	Soil added (20g)	Bacteria supplemented
1	distilled water	yes	yes
2	distilled water	yes	no
3	distilled water	no	yes
4 (control)	distilled water	no	no
5	mineral media	yes	yes
6	mineral media	yes	no
7	mineral media	no	yes
8 (control)	mineral media	no	no

4.4.3 Results and discussion

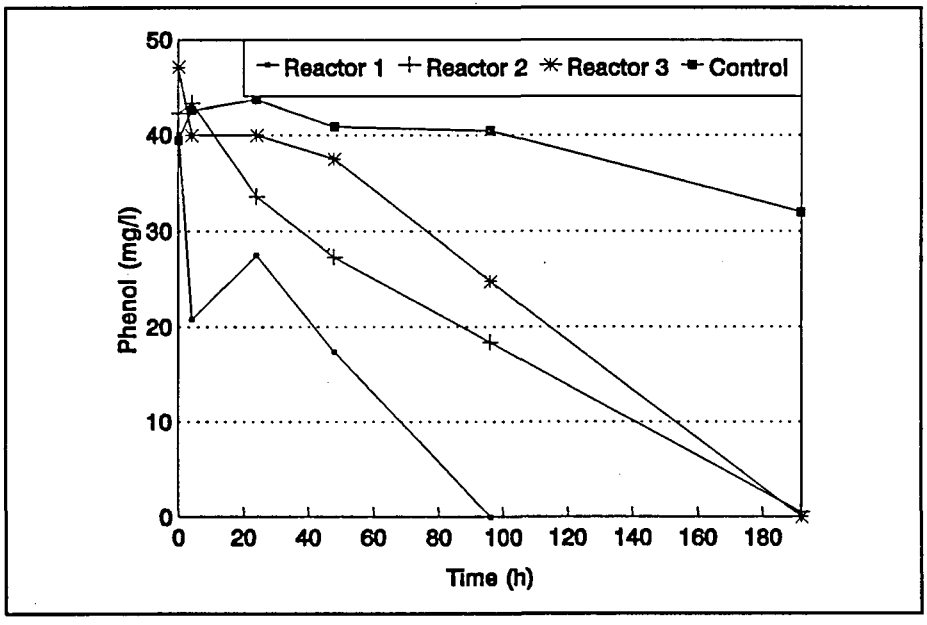


Figure 3a. Degradation of phenol in distilled water

Results of the phenol determinations can be seen in Figure 3a and b. Initial differences in phenol concentrations at time 0 were attributed to analytical variations. Where water was used as the background medium, soil solutions supplemented with bacteria degraded phenol faster than those containing only natural bacteria; thus reactor 1 with

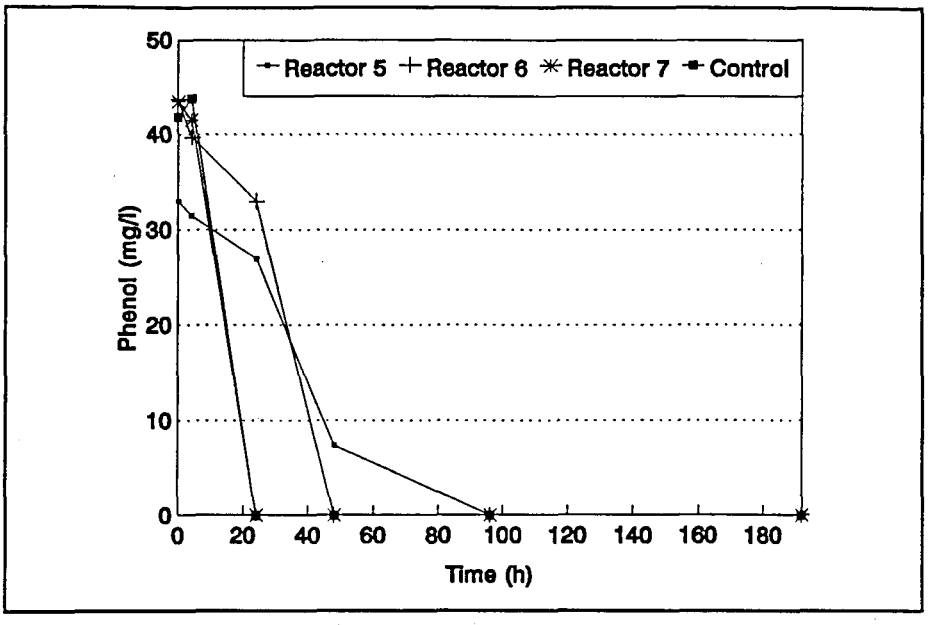


Figure 3b. Degradation of phenol in mineral media

supplemented bacteria and natural soil-bacteria degraded phenol at a greater rate than that observed in reactors 2, 3 and 4. In all instances, solutions containing mineral media degraded phenol to non-detectable levels faster than solutions containing only distilled water with no added nutrients.

The presence of soil seemed to increase degradation rates in those reactors containing distilled water. This could be due to the soil matrix providing additional microorganisms and nutrients, or the soil may act as an immobilization medium for the bacteria. This trend however, was not evident with reactors containing mineral media.

The control reactor without nutrients showed a slight decrease in phenol (39,5mg to 32,0mg) over 8 days indicating partial breakdown possibly due to photolytic oxidation, volatilization or biodegradation through bacterial contamination. Degradation of phenol in mineral media, occurred quickly in all reactors, including in the control. The latter is possibly due to bacterial contamination in the mineral media or bacterial contamination from the air. As became evident in the other experiments, keeping bacterial contamination out of any mineral media solution proved difficult.

4.4.4 Conclusions

Conclusions drawn are that additional nutrients increased the rate of phenol degradation under all conditions. Where no nutrients were added, biosupplementation and the presence of soil particles, increased phenol breakdown. Results in the distilled water run indicated that the presence of soil particles alone also increased rate of phenol degradation.

4.5 DEGRADATION OF THE PHENOL USING SLURRY REACTORS

4.5.1 Aim

To monitor phenol breakdown in contaminated soils using laboratory slurry reactors.

4.5.2 Experimental procedure

Two slurry runs were done. The first run used a mixed population of 16 bacteria isolated from activated sludge conditioned on phenol-containing liquid wastes (inoculum 1). The second run incorporated bacteria isolated from phenol contaminated soil (inoculum 2).

4.5.2.1 First run:

Four 1ℓ Schott bottles were used as reactors. Each contained 200g of artificially contaminated soil (14,3mg phenol/g soil) in a 40% w/v slurry. The reactors were agitated continuously on a rotary shaker. The mineral medium contained 5 mℓ 10% w/v ammonium sulphate solution, 5mℓ of 1M potassium dihydrogen orthophosphate, and 2mℓ of trace metals solution.

Reactor A: 40% w/v slurry only. To keep the volume constant with reactors B and C, distilled water was added each time a sample was taken.

Reactor B: 40% w/v slurry supplemented daily with mineral medium.

Reactor C: 40% w/v slurry supplemented daily with inoculum 1.

Reactor D: 40% w/v slurry supplemented daily with inoculum 1 and mineral medium.

Samples were taken at time 0, then after 4, 21, 27, 52, 100, 124 and 148 hours, and analyzed for phenol.

4.5.2.2 Second run

Experimental set-up was similar to run 1, except that a higher concentration of phenol was used (120mg phenol/g soil). Two types of biosupplement were used; inoculum 1 and inoculum 2.

Three 1ℓ Schott bottles were used as reactors, phenol contaminated soil was made up to a 40% w/v slurry using a phenol solution (to increase the concentration of phenol). The reactors were agitated continuously on a rotary shaker. The mineral medium used was as in run 1.

Reactor A: 40% w/v slurry only. To keep the volume constant with reactors B and C, distilled water was added each time a sample was taken.

Reactor B: 40% w/v slurry supplemented daily with inoculum 1 and mineral medium.

Reactor C: 40% w/v slurry supplemented daily with inoculum 2 and mineral medium.

Samples were taken at time 0, then after 3, 6, 22, 29, 48, 72 and 96 hours.

Phenol was extracted from the slurry samples and analyzed using HPLC. The soil fractions of the samples were dried and weighed so that the concentration of phenol could be reported as a mass per 25mℓ slurry and per gram of soil.

4.5.3 Results and discussion

Results for slurry run 1, shown in Figure 4a and 4b, show the trend of a fluctuating phenol concentration before it decreases to zero. Reactors B, C and D did not show a significant improvement in the rate of phenol degradation despite additional nutrients and biosupplements. Reactor A showed a slower rate of phenol degradation initially, however at 72 hours was comparable to the rest. All reactors had reached a concentration of 0 ppm after 124 hours.

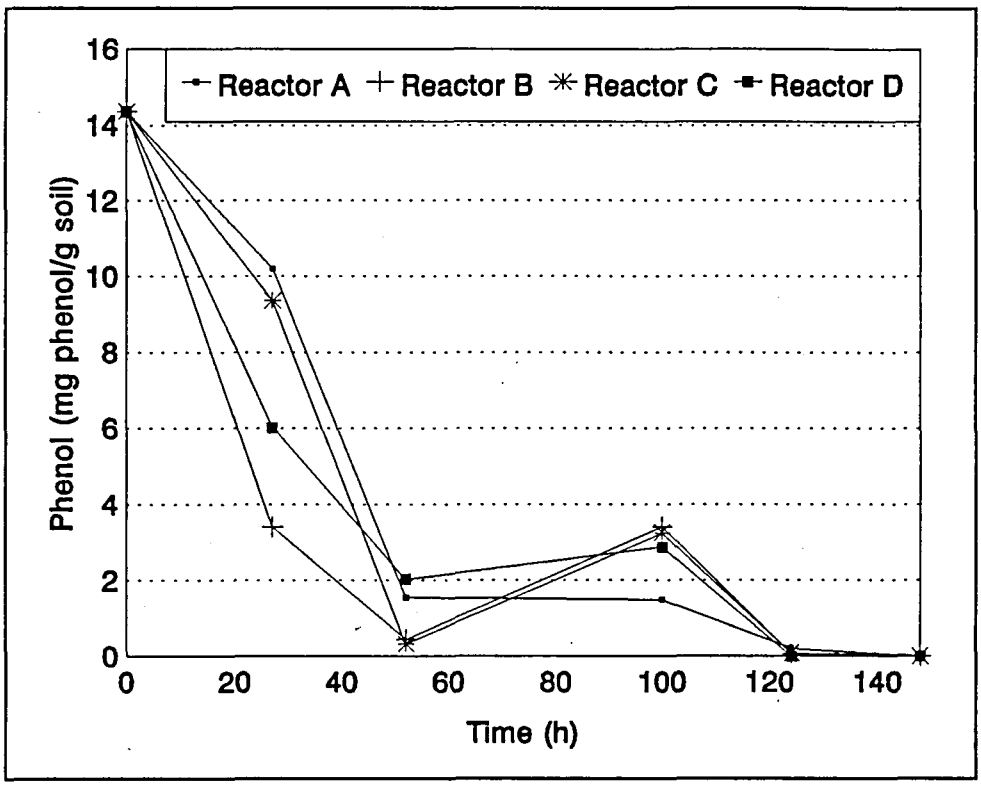


Figure 4a. Results of first slurry run reported per mass of soil

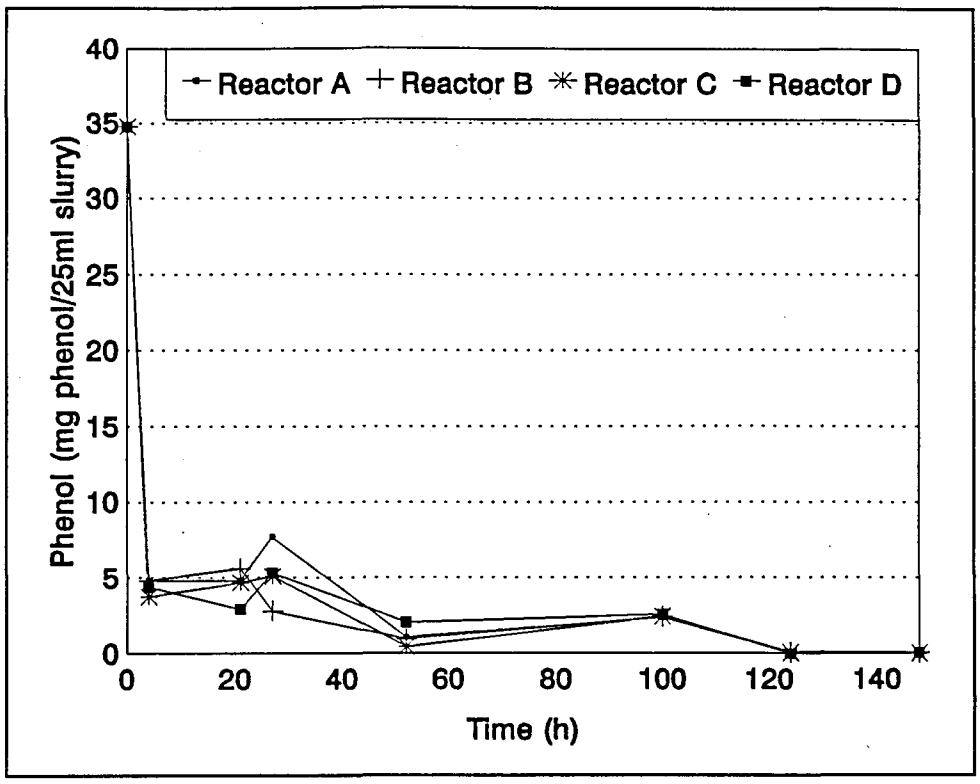


Figure 4b. Results for first slurry run reported per 25mℓ of slurry

Degradation of phenol in slurry run 2 (Figure 5a and b) occurred faster despite higher initial phenol concentrations. Possibly this was due to the contaminated soil being supplemented with a phenol solution, causing phenol as a carbon source to be more accessible to the bacteria than in run 1 where contaminated soil only was used. Again an initial fluctuation was observed in the phenol concentration. Two inocula were used as it was thought that bacteria in inoculum 1 might not be adapted to soil slurry conditions. In all three reactors, rates of degradation were very similar, showing no phenol remaining in any of the reactors after 72 hours.

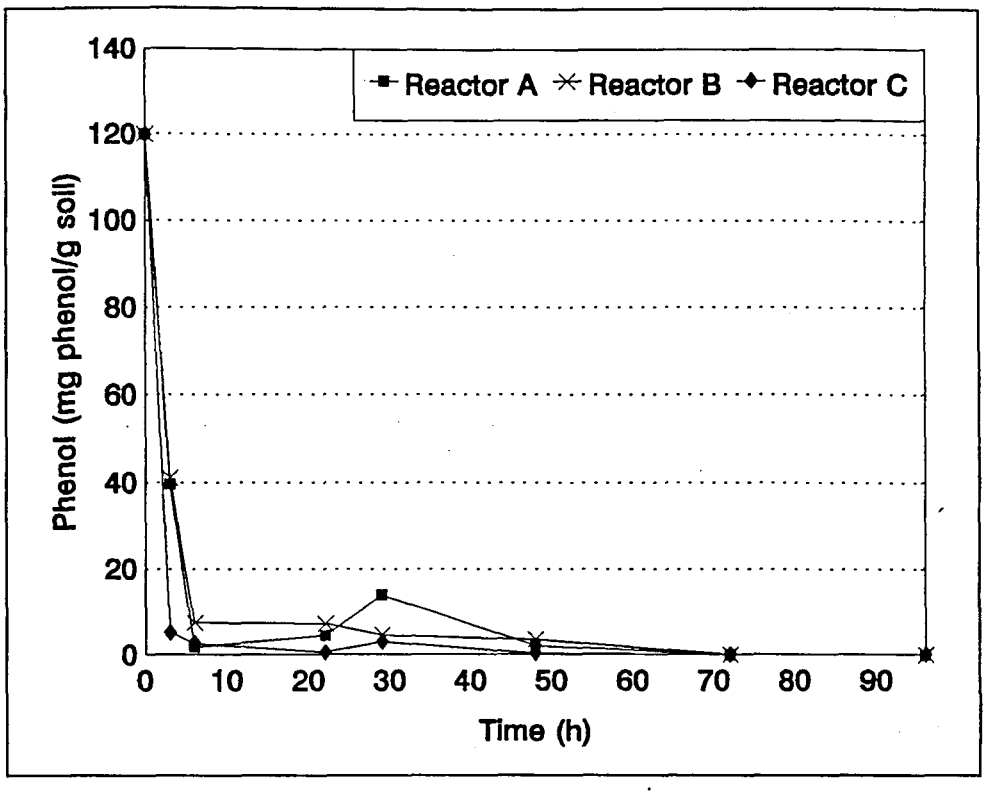


Figure 5a. Results of second slurry run reported per mass of soil

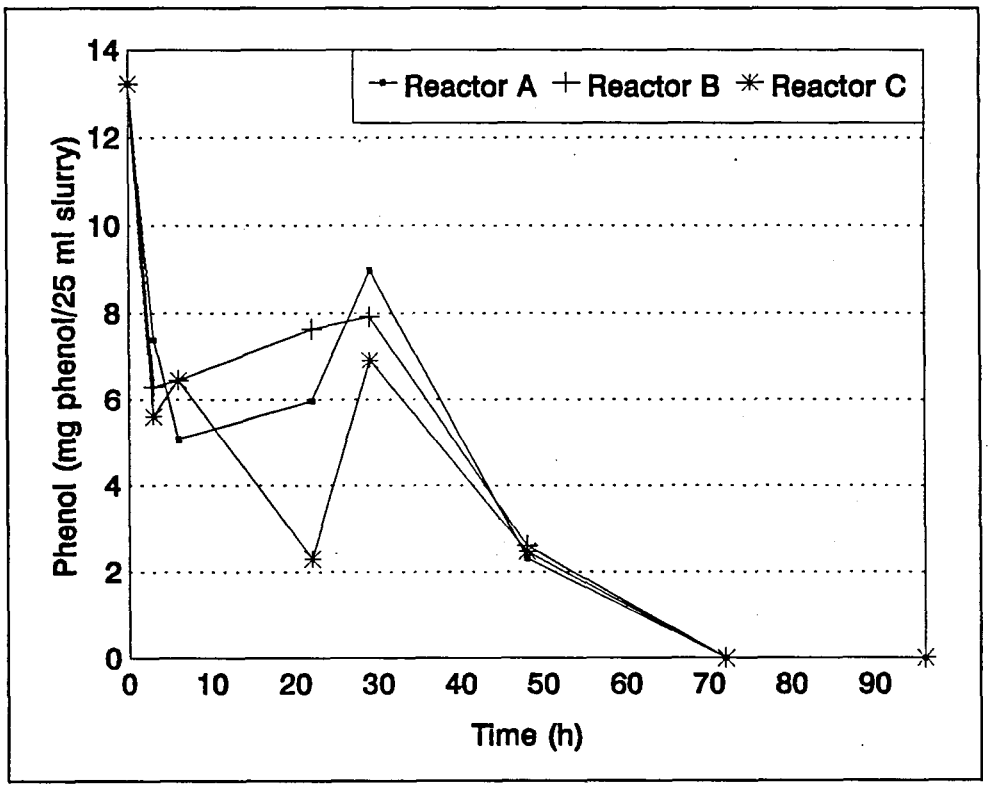


Figure 5b. Results of second slurry run reported per 25mℓ of slurry

There were difficulties in obtaining a representative 25mℓ sample of the slurry as the soil tended to settle very quickly once the reactor was taken off the rotary shaker. For this reason the analyses were reported as mg phenol/g soil and as mg phenol/25 mℓ slurry sample. However, it must be kept in mind that the mass of soil in each slurry sample varied.

The results show that no advantage was obtained by adding biosupplements and/or mineral media as there were an adequate number of indigenous bacteria already present. The soil, having been taken from grounds within CSIR, had most likely already been treated with fertilizers, thus nutrients such as nitrogen and phosphorus may have already been present in adequate quantities.

4.6 DEGRADATION OF THE PHENOL IN SOIL COLUMNS

4.6.1 Aim

The aim was to simulate phenol contaminated seepage water percolating through the soil using soil columns in order to monitor the breakdown of phenol and to investigate the effects of adsorption, nutrients and a biosupplement.

4.6.2 Experimental procedure

Four Sephadex columns were used, each of differing height. Columns were initially fed with solutions containing 50 mg/ℓ phenol, the concentration was subsequently increased to 500mg/ℓ. Column parameters can be found in Table 9. All columns had a diameter of 1.47cm.

Table 9. Parameters of soil columns

Column	Bed Height (cm)	Volume (cm^3)	Retention time (h)	Flow rate (mℓ/min)
A	38.5	65.34	32.0	0.034
AA	27.5	47.95	12.5	0.064
B	88.5	150.20	31.3	0.08
C	65.5	114.21	28.4	0.067

Column A, a control, contained sodium azide sterilized garden soil and was included to determine the extent of phenol adsorption to the soil. The effect of phenol degradation under natural conditions was investigated using column B. Column B was fed with phenol in sterilized water. The effect of a biosupplement was investigated in column C and the effect of a biosupplement and nutrient addition, in the form of mineral medium, was investigated using column D. Due to column blockage, the run using column D was discontinued after 8 days, and column AA, initially containing sterilized soil, was introduced. Although a 24 hour residence time was aimed at, the slow flow rates required were not easy to realize and hence various retention times were used, although flow rates were kept in the same range.

During the initial period of the experiment, several problems had to be addressed in preliminary experimentation. These problems as well as some of the solutions are discussed in Table 10. The feed of columns C and D was changed to phenol in water,

with no additional nutrients.

Using a 500mg/ℓ phenol feed for all columns, air was bubbled through the feed from day 7 in order to increase dissolved oxygen levels in the soil. Inoculum 1 was added at the start of the run, after day 27 bacteria isolated from column AA were added daily to all columns to determine whether phenol breakdown improved. Due to recurrent bacterial contamination in the feed, the feed was changed daily and a sample of feed was always taken the following morning simultaneously with a sample of the effluent to incorporate any decrease of phenol concentration that might have occurred overnight. However, as the retention time of the columns should also be taken into account the initial concentration of the phenol in the feed will most likely be higher than that shown in figures 6 to 8.

Table 10. Different operational problems in soil column technology

	Column type	'Column blockage	Contamination in the sterile control
Problem	Various problems were encountered using a downflow configuration such as uneven eluent rates and compaction, resulting in different soil column heights. Samples taken at different heights of the columns gave evidence of channelling. The uneven and also changing eluent rates of the columns, as well as total blockage, prompted use of smaller ion exchange columns, also using downflow. These, however, were also not successful. Stratification of the soil was also tried, thinking that it would help to equalize flow rates between the columns.	Columns receiving biosupplements tended to block within 48 hours, possibly due to excessive bacterial growth.	Bacterial contamination quickly set in giving spurious results for phenol adsorption to the soil. A major source of contamination was found to be the feed. Despite precautions being taken, e.g. sterilizing the feed bottle, sterilizing the deionized water to make up the feed, bacterial contamination occurred within hours. An attempt was made to sterilize the soil in the column by feeding a solution of NaN_3 (0.1%). The eluent was monitored until no microorganisms were evident. However, bacteria started growing rapidly again as soon as phenol was fed to the column. Although the feed was changed on a daily basis, contamination by external bacteria persisted, especially where nutrients were present.
Solution	Sephadex columns gave best performance using upflow, and the rest of the soil column run was done with these.	The columns were repacked and restarted. The inoculum was centrifuged and resuspended which was less viscous than the original solution.	Change all columns to feed phenol in sterilized water with no nutrient addition.

4.6.3 Results and discussion

Overall the effluent concentration showed a decrease in phenol, with column AA showing the greatest decrease. Typical results of phenol degradation of columns AA, B as well as C at the two different feed concentrations can be seen in Figures 6 to 8.

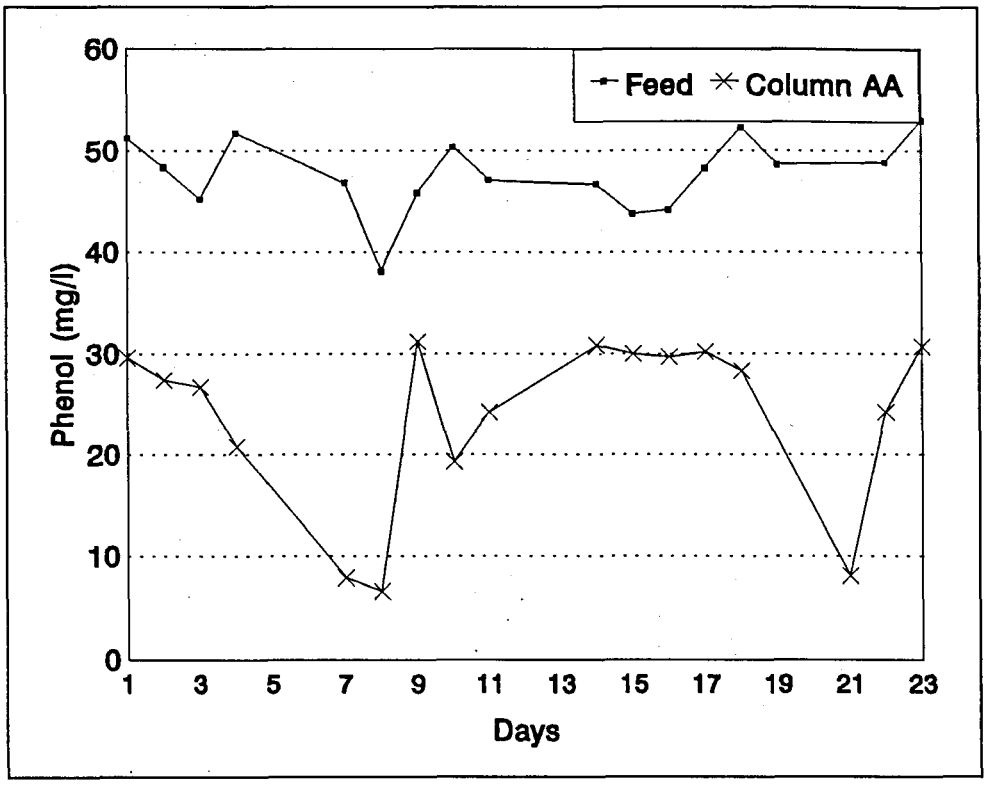


Figure 6. Degradation of phenol in soil column AA with feed containing 50mg/ℓ phenol

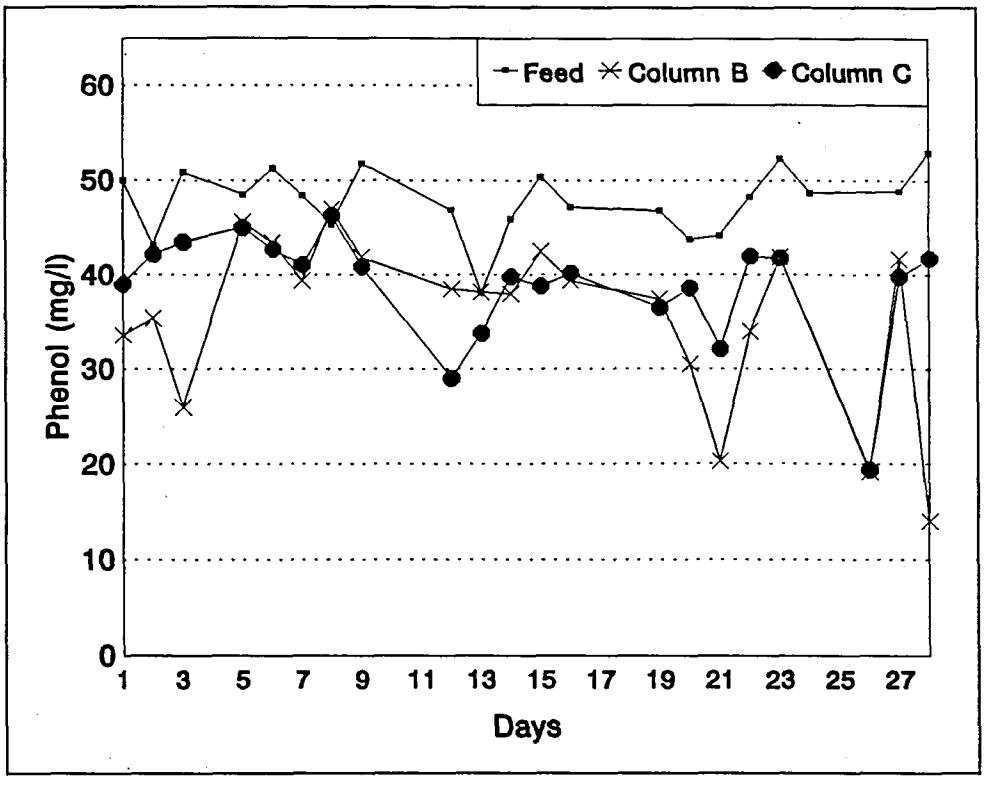


Figure 7. Degradation of phenol in soil columns B and C with feed containing 50mg/ℓ phenol

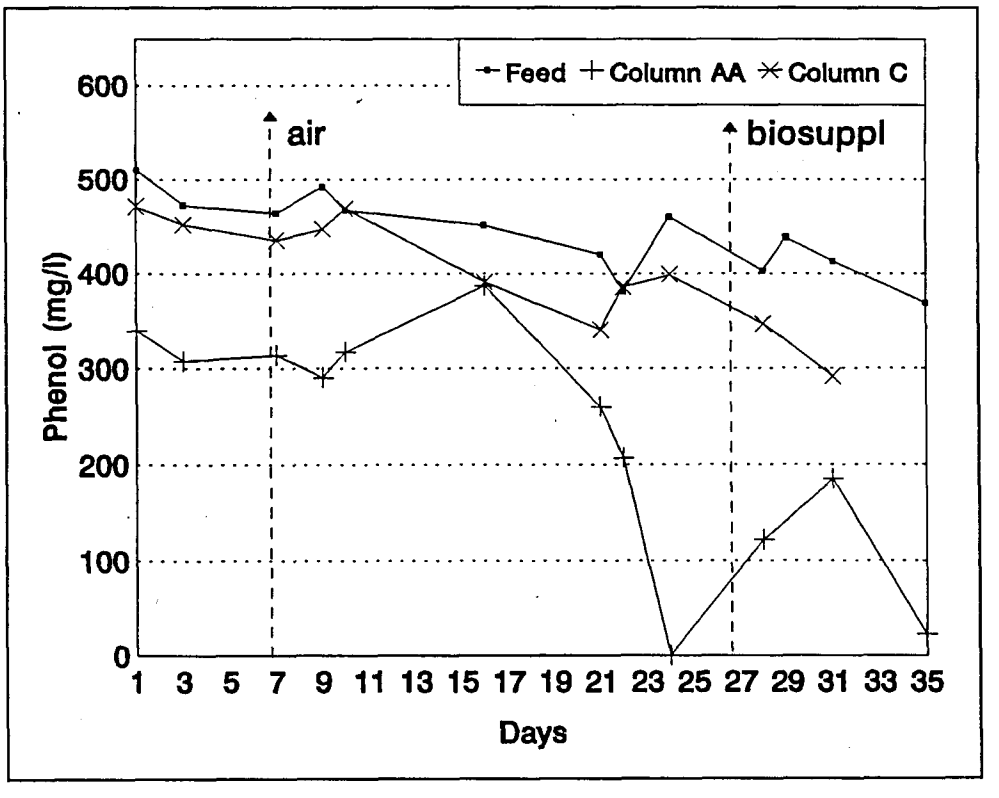


Figure 8. Degradation of phenol in soil columns AA and C with feed containing 500 mg/ℓ phenol

The effluent concentration mimicked the previous days' influent concentration. Despite having the shortest retention time, column AA performed satisfactorily, as can be seen in Figures 6 and 8. This was evident for both feed concentrations of 50 and 500mg/ℓ phenol. Columns A (results not shown), B and C performed more or less equally with an average decrease of about 10mg/ℓ phenol from an initial feed concentration of 50mg/ℓ, and about 25-50mg/ℓ for an initial concentration of 500mg/ℓ.

The microbiology of column AA differed from the others, in that large populations of *Pseudomonas* were noted. An unidentified fungus was also noticed in column AA, which was absent in the other columns.

In Figure 8, a decreased effluent concentration can be seen after the addition of air in column AA. However, it cannot be said with certainty that an increased dissolved oxygen level is the cause, as the major portion of the column would still remain anaerobic. Preliminary experiments indicated that the bacteria supplemented to the columns were facultative, however, this would need to be confirmed. The addition of a biosupplement to column B and C made up from bacteria isolated from column AA did not significantly improve phenol breakdown.

From the above it is evident that the phenol concentration decreased over the length of the soil column. As the soil adsorption studies had shown that minimal adsorption occurs, this decrease can be ascribed to biodegradation by the soil microorganisms. It could not be determined whether addition of biosupplement and/or nutrients affected phenol degradation rates. Increased dissolved oxygen levels in the feed improved the rate of phenol degradation.

4.7 CONCLUSIONS

From the laboratory experiments the following conclusions can be made:

- It is imperative that accurate and reproducible analytical methods are available. A satisfactory method of determination of phenol was developed.

- In the experiments conducted, minimal adsorption of phenol occurred on the soil surfaces, surfactants hence were not thought to be necessary under these conditions.

- Additional nutrients increased the rate of phenol degradation investigated at laboratory scale using beaker experiments. Where no nutrients were added, biosupplementation increased phenol breakdown.

- Addition of nutrients and biosupplementation in the slurry reactors showed no significant advantages. This could possibly be due to adequate nutrient concentrations and bacterial populations already existing in the soil.

- The results of the soil column experiment allow one to conclude that some breakdown of phenol in contaminated seepage water occurs as the water percolates through the soil. Increased oxygen levels in the seepage water improved the rate of degradation.

5. PILOT SCALE DEMONSTRATION OF BIOREMEDIATION

5.1 INTRODUCTION

From the survey to determine the nature and extent of contaminated sites in South Africa, it became apparent that petroleum contaminated soil was the most prevalent. The laboratory batch experiments had indicated that nutrients and biosupplements increased the rate of degradation of phenol. The pilot scale was used to do a similar study on a larger scale using petroleum contaminated soil. A comparative study of the rate of TPHC degradation under various conditions using landfarming as the bioremediation technique was undertaken. Real field samples that had been contaminated during an oil spill were used.

5.2 AIMS

To demonstrate bioremediation of petroleum contaminated soil on pilot scale using landfarming.

To regulate parameters and determine under which conditions the fastest degradation of petroleum compounds occurs.

To determine whether growth of plants on treated versus untreated contaminated soil can be related to the concentration of total petroleum hydrocarbons (TPHC) still in the soil.

5.3 EXPERIMENTAL PROCEDURE

5.3.1 Preparation of soil

Seven plastic reactors were filled with 25kg of base oil contaminated soil. Lime (175g) was added to all reactors in order to increase the soil pH. The soil of the control was treated with 57g/ℓ NaN_3 in order to establish a sterile control i.e. where no biological degradation can take place.

5.3.2 Experimental set up for treatment of soil

Reactors were placed in semi-hot house (no temperature control). This enabled the study to be run in a controlled manner (without rain) with weather conditions simulating the full-scale situation. The set up is detailed in Table 11.

Table 11. Set up for soil reactors simulating bioremediation by landfarming

Reactor	Addition of water	Addition of MAP	Soil turned daily	Addition of H_2O_2	Addition of commercial biosupple-ment	Addition of enriched microbial suspen-sion
Control (sterile)	no	no	no	no	no	no
1	no	no	no	no	no	no
2	yes	no	yes	no	no	no
3	yes	yes	yes	no	no	no
4	yes	yes	yes	yes	no	no
5	yes	yes	yes	no	yes	no
6	yes	yes	yes	no	no	yes

Relevant reactors received water in amounts varying from 250mℓ to 500mℓ every second day as needed (water need determined by visual inspection). Mono ammonium phosphate (MAP)(25g) was added to all relevant reactors weekly until phosphorus and nitrogen levels were sufficient. Hydrogen peroxide was added to reactor 4 at 500mg/ℓ of water added. The commercial biosupplement and enriched microbial suspension were dosed at 50mℓ per reactor (10^8 cfu/mℓ stock solution).

5.3.3 Analyses

5.3.3.1 Microorganisms

Samples (1g) were collected weekly from each soil reactor and analyzed for the total number of indigenous microorganisms present by dilution series plating on nutrient agar (NA) (Wollum, 1982). The soil was also analyzed for the total number of petroleum hydrocarbon degrading bacteria by plating on OECD minimal media containing 30mℓ of base oil as sole carbon source. OECD/base oil media was prepared by adding 4mℓ of $FeCl_3$ (0,25g/ℓ), 1mℓ of each $MgSO_4.7H_2O$ (22,5g/ℓ), $CaCl_2$ (27,5g/ℓ) and $(NH_4)_2SO_4$ (40g/ℓ) to 2mℓ of the following mixture: KH_2PO_4 (8,5g/ℓ), K_2HPO_4 (21,75g/ℓ), $NaHPO_4.7H_2O$ (33,4g/ℓ) and NH_4Cl (1,7g/ℓ). After adding 17g of agar and 1ℓ of distilled water the mixture was autoclaved and after cooling the base oil was added and the agar sonified, before pouring the plates.

5.3.3.2 Soil analyses

Samples (1kg) were taken from the untreated contaminated soil and sent for total petroleum hydrocarbon analysis. The test method used was the EPA 418.1 method (U.S.Environmental Protection Agency, 1979). Nitrogen and phosphorus were determined using standard methods (Soil Science Society of South Africa, 1990).

5.3.4 Seedling experiment

Wheat seed with a germination efficiency of 96% was used for the experiment. The *Triticum aestivum* cv. Betta (wheat seed) harvested in Reitz (1991) was sown in reactor 6 (received nutrients, water and aeration) and reactor 1 (natural control). Seedling trays with soil from reactors 1 and 6, were sown with the same seed. One seed per segment was planted. The pH of the soil had been adjusted to pH 6. Both control and experiment seedling trays and reactors received water. Seedlings were harvested and rated, according to shoot and root lengths, after 32 days. Soil pH was determined and the seedling roots and foliage length was measured. The germination efficiency was determined in the seedling trays.

5.4 RESULTS AND DISCUSSION

5.4.1 Bioremediation

Figures 9 to 11 illustrate the decrease in total petroleum hydrocarbon concentration with time. The performance of each reactor was compared to the sterile control. The decrease in TPHC in the sterile control, from 32% to 11.7% mass/mass, was used as a measure of the petroleum hydrocarbons lost through volatilization as no microbiological activity was possible. Decrease of TPHC in the reactors 1 to 6 beyond 11.7% could therefore be attributed to bioremediation. Volatilization thus contributed largely to the reduction of petroleum hydrocarbons in the beginning, however, after the volatile fractions had been lost, the bacteria then degraded the heavier, less volatile fractions of the oil.

Reactor 6 (indigenous microorganism enrichment) showed the largest decrease of 85% TPHC during the first four weeks, followed by reactor 4 (supplemented with H_2O_2) and reactor 5 (commercial biosupplement addition), with decreases of 79.6% and 78.4% respectively. In week 10, reactor 5 and 6 have similar residual TPHC concentrations of 1.8% TPHC. As non-indigenous microorganisms can have difficulty initially to acclimatize to new environments, the initial lag time in reactor 5 was to be expected. An overall reduction of petroleum hydrocarbons of 94% was achieved in reactors 5 and 6. Reactor 4 followed closely with residual levels of 2.24% TPHC.

The natural control, as simulated by reactor 1, showed a residual TPHC level of 9.6%. When compared with results obtained in reactors 2 - 6, it gave an indication of the extent to which regulation of the parameters to produce a favourable environment for the microorganisms, can increase the rate at which bioremediation proceeds.

The similarity in the residual TPHC levels of reactors 2 and 3 show that in this particular soil, the level of nutrients was not a limiting factor. Existing nutrient concentrations levels in the soil were within an acceptable range. The addition of MAP increased the phosphorus concentration from 6,8 to 43,9mg/kg and total nitrogen concentration from 0,14 to 0,16%.

A comparison of the residual TPHC levels in reactors 3 and 4, appears to verify that the additional oxygen supplied to reactor 4, through use of H_2O_2, enhanced bioremediation rates.

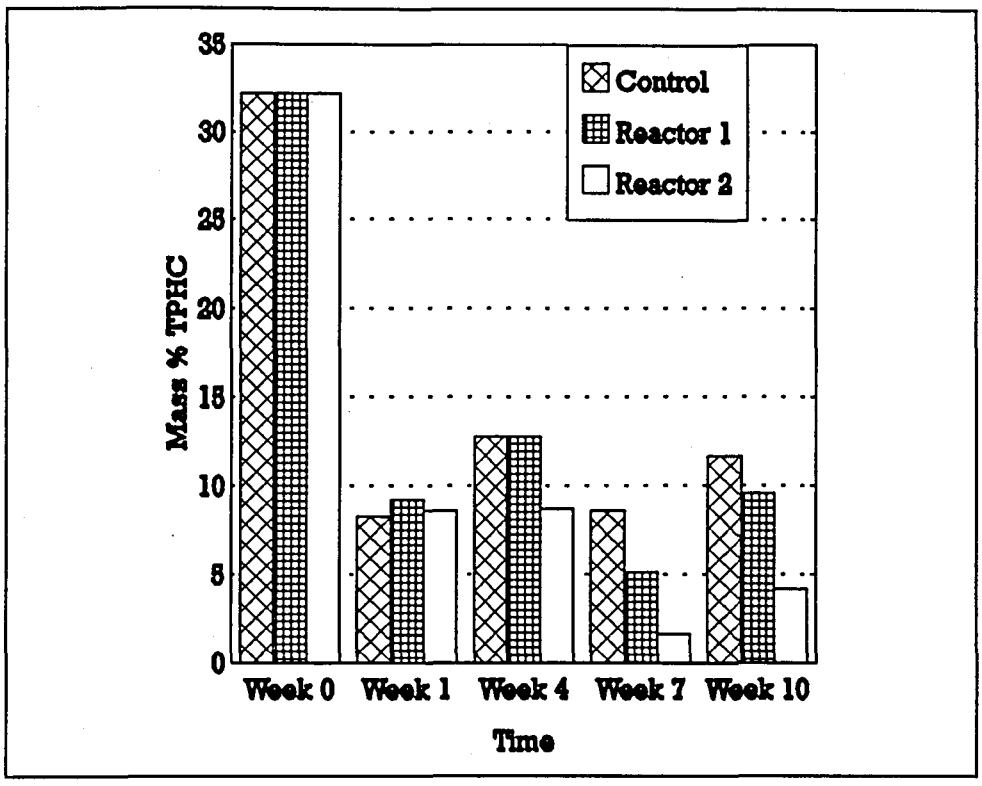


Figure 9. TPHC concentrations in reactor 1 (natural control) and reactor 2 (moisture and oxygen) vs sterile control

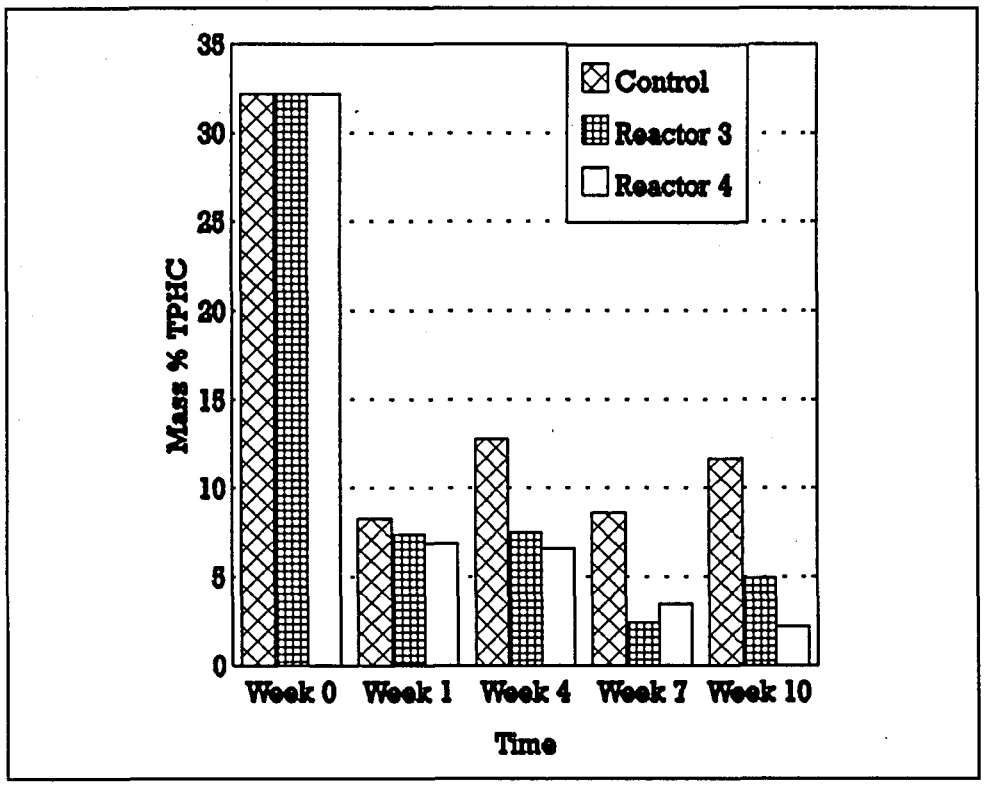


Figure 10. TPHC concentrations in reactor 3 (moisture, MAP & oxygen) and reactor 4 (moisture, MAP, oxygen & H_2O_2) vs sterile control

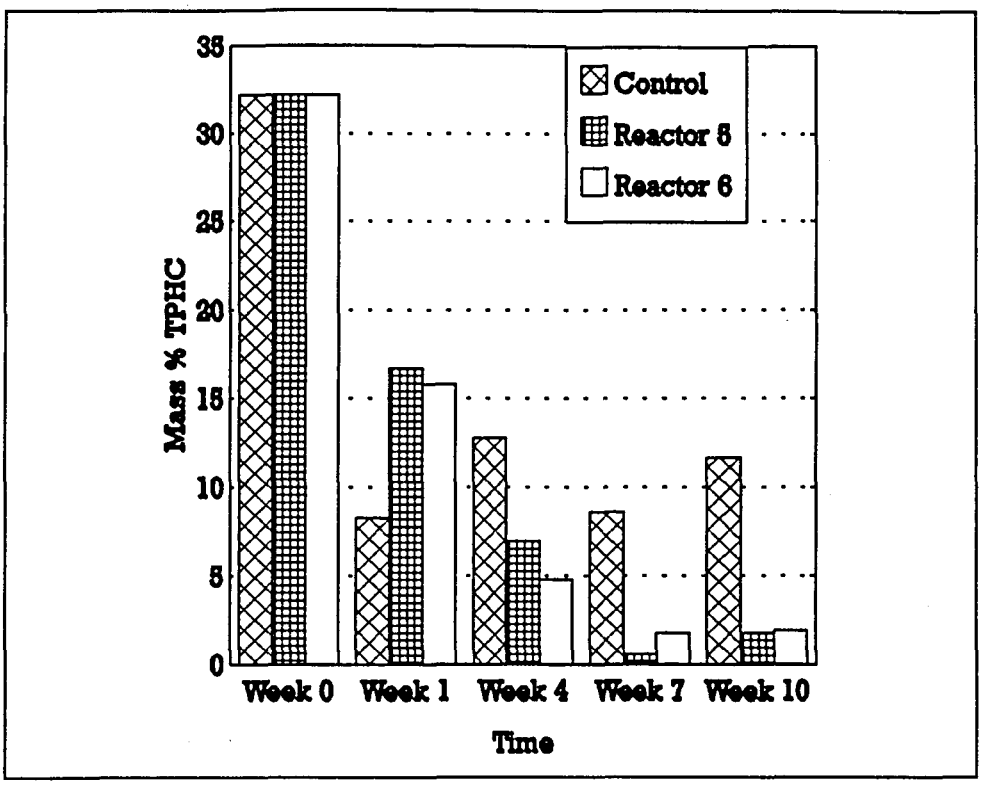


Figure 11. TPHC concentrations in reactor 5 (moisture, MAP, oxygen & commercial biosupplement) and reactor 6 (moisture, MAP, oxygen & indigenous microbial biosupplement) vs sterile control

Preliminary identification indicated the presence of 8 different indigenous microorganisms in the soil, including the *Pseudomonas* species. Fungal species isolated from the contaminated soil included *Aureobasidium pullulans*, an *Eurotuim* spp. and several species belonging to the Penicillium and Aspergillus families.

In healthy soil, bacterial and fungal counts should be in the order of 10^{8-9} and 10^{6-7} cfu/g soil respectively (Wollum II, 1982). The low counts in the contaminated soil indicated a stress situation. The fact that the soil did not contain fungi in the Pythium and Fusarium families further indicated the toxic nature of the soil to natural soil-borne organisms.

Figures 12 and 13 show the hydrocarbon-degrading microorganism

plate counts and total bacterial plate counts for each soil reactor determined weekly during the study. Data points in Figure 12 include only bacterial colonies, however Figure 13 shows both bacterial and fungal colonies.

The concentration of hydrocarbon-degrading microorganisms was similar to the total colony forming unit concentration in the initial soil. Thus only the microorganisms capable of degrading the oil are evident in the soil, indicating that the contaminated soil may be toxic to other soil microorganisms.

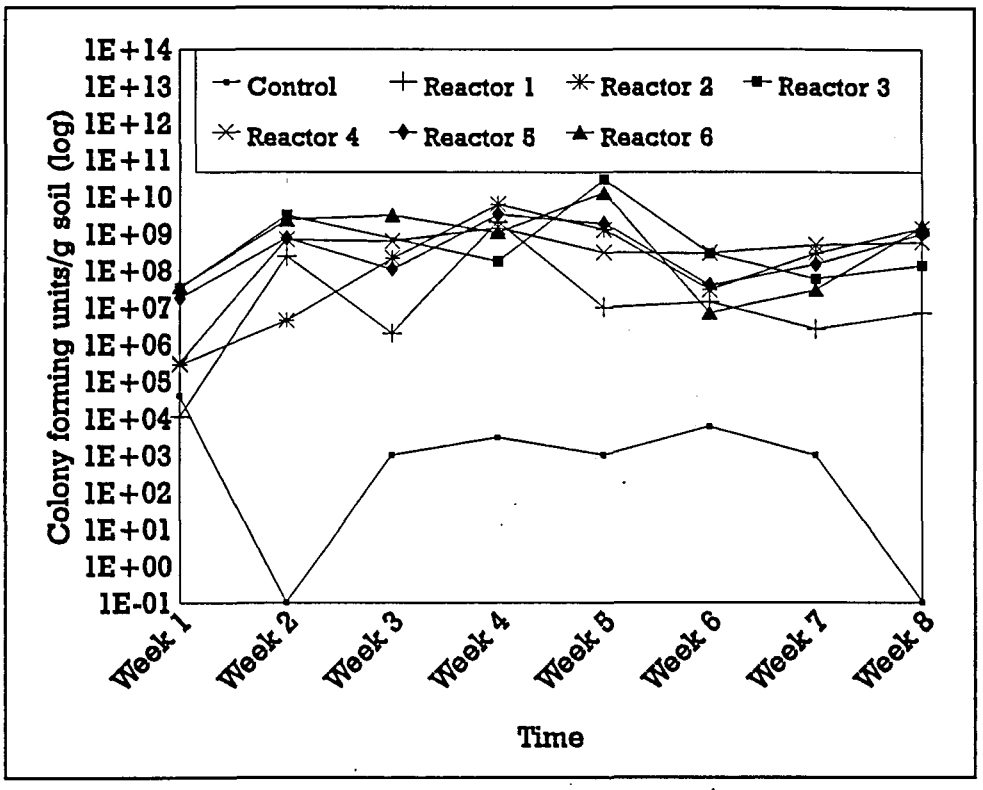


Figure 12. Hydrocarbon-degrading microorganism plate counts on OECD minimal media

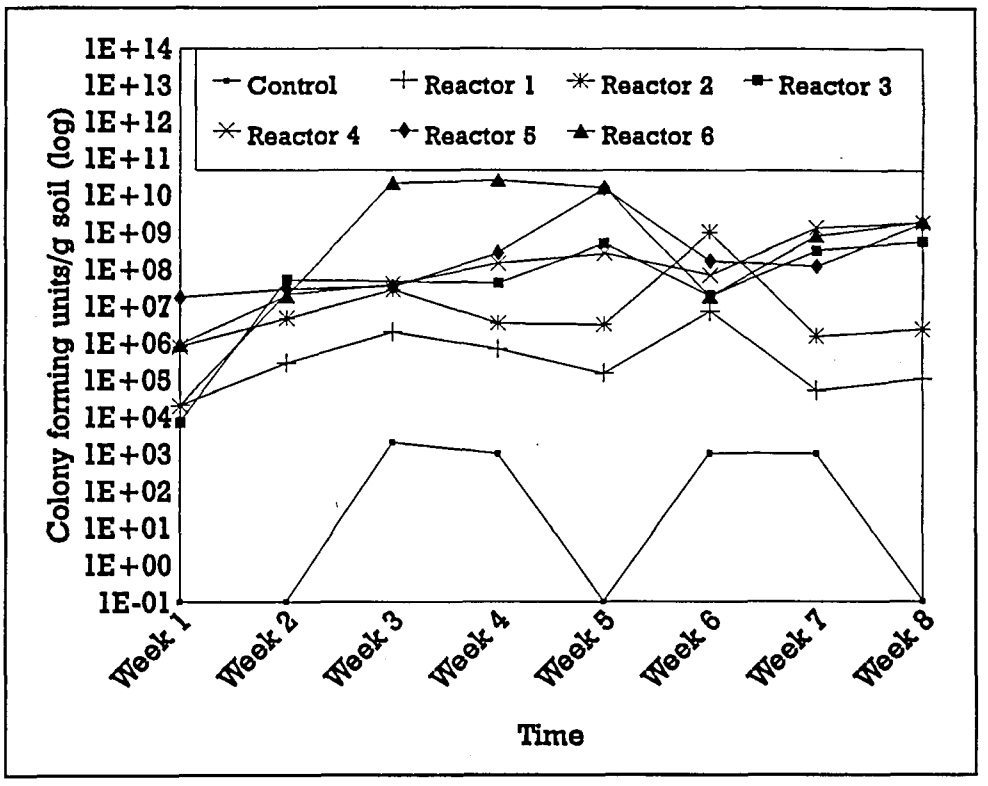


Figure 13. Total plate count data on nutrient agar

It is not known what percentage of the hydrocarbon breakdown can be attributed to fungal species. However, it is suspected that they do play a substantial role in conjunction with the bacteria. Total fungal counts increased, while bacterial counts decreased, in the reactors which tended towards an acidic pH. Fungi prefer acidic environments (Parkinson *et al.*, 1971) and have been known to create these. Alternatively, the decrease in pH in certain reactors may be due to the fungi or to the formation of phosphoric acid from the addition of the MAP. It is speculated that the latter may be a more feasible explanation, as the pH decrease was greater in the reactors receiving MAP.

5.4.2 Seedling experiment

The results of the growth in seedling trays and reactors is shown in Table 12 and 13 respectively. The pH of the soil in all cases was between 6 and 6.5 after termination of the experiment.

Germination efficiency of the wheat seed in the seedling trays containing bioremediated soil (from reactor 6) was found to be 95,8%, with 8% exhibiting stunted growth. Germination efficiency in the untreated contaminated soil (from reactor 1) was 87.5%. Thus there is little difference in the germination efficiency between the treated and untreated soil.

Table 12. Statistical analyses from ratings of seedlings in seedling trays

	Treated Soil Shoot length	Untreated soil Shoot length	Treated Soil Root length	Untreated Soil Root length
Number of Replicates	19	22	19	22
Mean length (cm)	10,82 cm	12,72 cm	2,3 cm	5,26 cm
Standard deviation	3,6 cm	3,42 cm	1,22 cm	3,6 cm

According to the Student's t test there is 95% certainty that the shoot lengths differ and a 99.5% certainty that the root lengths between the treated and untreated soils differ significantly.

Table 13. Statistical analyses from ratings of seedlings in reactors

	Treated Soil Shoot length	Untreated Soil Shoot length	Treated Soil Root length	Untreated Soil Root length
Number of Reps	83	73	83	73
Mean length (cm)	10,6 cm	15,25 cm	1,34 cm	5,71 cm
Standard deviation	3,06 cm	3,38 cm	0,75 cm	2,36 cm

In the case of the untreated soil (reactor 1) yellow tips were observed which was absent in the case of reactor 6. Although it is possible that this could be due to a nutrient deficiency, toxic effects cannot be ruled out.

In both experiments, seedling growth in the trays followed the same trend as growth in the reactors. Contrary to initial expectations, the untreated contaminated soil propagated healthier seedlings than the treated soil. However, certain explanations can be offered. Firstly, the soil that has been bioremediated has received intensive treatment with application of nutrients, water and aeration. This altered the soil structure making it difficult for small primary roots to establish. Secondly, although the nutrient conditions are favourable for the remediating bacteria, they are not necessarily optimum for plant growth. Thirdly, it may well be that wheat seeds are not necessarily an appropriate indicator of the state of the soil. In future experiments, other seeds should be chosen, possibly a leguminous crop, such as lupins, clover or lucerne. A very sensitive crop could also be tried. Lastly, the phenomenon of "hormesis" may be occurring, which is the stimulatory effect sometimes observed due to subinhibitory concentration of a toxic substance (Stebbing, 1982).

5.5 CONCLUSIONS

From this study, the following conclusions can be made

1. Bioremediation at pilot scale using landfarming is capable of TPHC reduction of 94%(m/m) from initial levels of 320g/kg soil to 18g/kg soil over a period of 10 weeks.

2. Reactors receiving biosupplements showed greater rates of bioremediation than the others, reaching final concentrations of 1.8% TPHC (m/m). There was no significant difference in TPHC decrease between addition of a commercial biosupplement and using an indigenous microbial supplement. However, the commercial biosupplement showed a lag phase initially, during which it is thought that the microorganisms acclimatized to the soil conditions.

3. Results indicated that addition of oxygen, either by turning the soil regularly or through addition of H_2O_2, and water enhanced degradation rates. The large improvement obtained in reactor 4 as compared to reactor 3 indicates that oxygen levels in the soil may be an important limiting factor, and can significantly affect bioremediation rates achievable.

4. Bioremediation can be enhanced through regulation of nutrients, oxygen and moisture to create a favourable environment for increased degradation rates, and through addition of biosupplements.

5. Growth measurements of wheat seedlings may not have been appropriated to indicate successful bioremediation. This may have been the result of the alteration of soil structure during bioremediation, a point which may need future attention. It may therefore be necessary to select more suitable seeds as indicator of "soil health".

6. FULL SCALE BIOREMEDIATION

6.1 STRATEGY AND DESIGN FOR FULL SCALE BIOREMEDIATION

6.1.1 Introduction

In the following section important factors for the strategy and design of a full scale bioremediation project will be highlighted. These aspects will then be illustrated by means of a case study of a full scale project.

Each contaminated site requiring bioremediation will be different. It is thus difficult to give a comprehensive strategy for every situation. Notable differences will include, amongst others, factors such as: whether surface and/or underground contamination is involved; whether contamination of surface water and/or groundwater has occurred or is likely to occur; and whether it has occurred on a developed or undeveloped piece of land. Listed below are factors that need to be considered, though it may not be feasible to cover them all in every single case.

6.1.2 Data requirements

Insufficient data can cause failure in the remediation of a site before the onset of the activity. This can result in great expense, both in terms of manpower and capital costs. The collection and analyses of data is perhaps the most important task in the development of a site assessment and a remediation plan. The data must include accurate and sufficient data about the geology and hydrology of the site. The type, nature and concentration of the contaminant, as well as the area of the contamination, is also needed (Russel, 1992). Collection of data can be subdivided into three classes; location of the site, physical characteristics of the site and the nature and extent of contamination. Closure goals must also be decided on. However, as always, the needs of the project must be balanced against the cost of obtaining the data.

6.1.2.1 Location and history of the site

The location of the site must be outlined i.e. the magisterial area in which it falls. Background history of the site should be obtained, such as the historical and current use of the land, whether it is developed or undeveloped, to whom the land belongs and the extent of this property. The history of the spill is important as to how it happened, whether the contamination occurred over a short or lengthy period of time. Information on nearby boreholes and underlying sewage/water pipes may also be necessary. One of the reasons for including borehole information is to assist in developing cleanup objectives at a site in terms of proximity of neighbours and the potential for human exposure to the chemicals at the site. Maps of the area should be obtained, these can include topographical and aerial maps.

6.1.2.2 Physical characteristics

The geology and hydrology of the site must be known in detail. Geological profiles, soil and rock borings and geological cross sections may be needed. Important are the surface features of the area, the topography, characteristics of the soil, as well as surface drainage. A site exploration should include the direction and movement of the groundwater, as well as levels of the groundwater table in the area and its seasonal fluctuations. Local and regional aquifer characteristics should be determined, if necessary, and preferential pathways of migration established.

6.1.2.3 Nature and extent of contamination

A complete description of the extent of the contamination is needed which includes the chemicals of concern and their characteristics e.g. their toxicity, mobility, etc.. The surface area and vertical distribution and limits of the contamination need to be determined through sample taking and analyses. The location and quantification of any free product, especially where there is contact with the groundwater table, is needed. The extent of the plume of dissolved contaminants is vital information. From the information gathered one should be able to estimate the total mass of contaminants in the site, and the distribution between various phases and matrices.

6.1.2.4 Risk assessment

Ecological risk assessment is becoming an important parameter in site remediation. Contaminated soils are a potential source of toxicity to the environment and to man, therefore ecological risk assessments should form part of the holistic approach to a contaminated site and its remediation. The aim of a risk assessment is to determine to what extent the contamination has changed the structure, function or interactions of the biological populations, communities or affected ecosystems (Durda, 1993). Bioassessment techniques forming part of the risk assessment can be used to assist determination of the spatial extent and severity of the contamination.

Debate surrounding risk assessment is rife and the tools to assess ecological impacts are not yet fully developed. The choice of bioassessment techniques depends largely on the type of contaminant, as well as its biological population and community. One technique is to use bioassays, which evaluate the toxicity of the contaminant to organisms. An alternative is to use exposure biomarkers which may be chemical residues in biological tissues or physiological or biochemical responses in individual organisms to the contaminant. Exposure biomarkers evaluate the degree of exposure, rather than the toxic effect of the contaminant and hence are limiting in the information that can be provided. A third bioassessment technique is a biological survey which is used to compare biota at the contaminated site with an uncontaminated reference site (Durda, 1993).

Bioassessment techniques not only have a place in the initial assessment of the site, but also in the monitoring of the bioremediation process, and may also be used as an indication of completion of bioremediation. The breakdown of contaminants may lead to the formation of intermediate metabolites that may be just as toxic, or even more so, than the parent compound. Alternatively, bioremediation practices may make the contaminant more bioavailable to organisms, hence the overall exposure is increased. Chemical analyses cannot predict the toxicity of these products. Thus bioassays have the potential to be used in conjunction with chemical analyses as a powerful tool to study relative toxicity, in that physical chemical methods cannot predict the toxicity due to complex chemical interactions (Slabbert, 1988).

6.1.2.5 Establish closure goals

Goals could be based on various factors. Firstly, legislation could stipulate what levels should be obtained. This is common in the United States of America where every state has their own clean up standards. Secondly, the closure goals could be risk based. Factors influencing this include the possible contamination of groundwater (boreholes), and future use of the land. In the establishment of these goals, the various environmental matrices must be dealt with individually and as a whole, i.e. water (both surface and groundwater), soil and air.

6.1.3 Bioremediation

6.1.3.1 Assessment of clean up methodologies

Biological treatment can either be aerobic or anaerobic. Generally, aerobic treatment is preferred as the biodegradation of contaminants is much faster than when anaerobic conditions prevail. Bioremediation can either take place *in situ* or the soil can be excavated and treated off site. The contaminated soil can be landfarmed, either *in situ* or off site, by means of adding water, nutrients and oxygen, as well as other supplements that would optimize degradation rates. Off site treatment could also take place in slurry reactors, where contaminated soils are treated as an aqueous slurry in a large bioreactor. Once the technology has been chosen, it may be necessary to determine optimum conditions through a laboratory study. Although time-consuming, it can be done during the period when the site is still being characterized, and may considerably shorten the bioremediation time span, and hence also positively influence the bioremediation costs.

The selection of the process would obviously vary depending on the characteristics of the site. It may not always be feasible to treat *in situ*, for example there may be overhead power lines, on the other hand in some cases it may not be possible to excavate, e.g. if the contamination is under a building. Selection of the technology to be used should always be chosen such that the goals are achieved at minimum cost.

6.1.3.2 Design and costing of full scale bioremediation

The design of the bioremediation programme includes determining the volume of soil to be treated, together with the technical practical details of how bioremediation should proceed. Amount and frequency of dosing (nutrients, lime, water, oxygen) should be decided on. It may be necessary to install a sprinkler system. Prevention of leachate, and contaminated surface run-off from the bioremediation site should be given attention, bund walls surrounding the site may be all that is necessary, depending again on the nature of the site and the contaminant in question.

Various costs need to be considered, from the initial site characterization through to rehabilitation of the bioremediated site. Specific costs include those originating from excavation, ploughing and turning the soil, soil supplements (such as biosupplements, fertilizer, lime, etc.), equipment (pumps, sprinkler systems etc), project management and chemical analyses. An example of costing, including excavation and bioremediation of 1000m^3 contaminated soil, can be seen in Appendix 4.

6.1.3.3 Implementation of bioremediation

This includes the installation and operation of the bioremediation programme as it was designed, as well as the maintenance thereof.

6.1.3.4 Sampling and monitoring programme

In order to assess the success of the bioremediation programme, it is vital that a monitoring programme be drawn up. A detailed monitoring schedule of system parameters should be devised in order to determine whether bioremediation is occurring and when closure goals have been reached. Fundamental biological prerequisites should be analyzed for on a routine basis. Analyses should include nutrient levels (nitrogen and phosphorus), pH and plate counts of microorganisms. Failure to do so may result in severe retardation of the remediation process, for example inhibition of the microorganisms due to a low pH.

6.1.3.5 Completion of bioremediation and rehabilitation of site

Bioremediation is considered complete once the closure goals have been reached. However, depending on the bioremediation technology chosen, the remediated soil still needs to be backfilled or spread. The contaminated site needs to be rehabilitated back to an acceptable state. This can be achieved by planting and reestablishing indigenous vegetation. Growth of this nature makes the area not only aesthetically pleasing but is also proof that bioremediation of the area has been successful.

6.2 CASE STUDY: FULL SCALE BIOREMEDIATION OF WEATHERED PETROLEUM OIL

6.2.1 Data requirements

6.2.1.1 Location and history of the site

A spill occurred from a storage tank containing oil. The mixture of oil spilled into the stormwater drain, which firstly leads into a concrete/rock culvert and then into a stream. It was gauged that a total of 120 000ℓ went into the stormwater system. A retainer wall at the stormwater drain outlet retained some of the oil, and a further fraction was recovered from an old building foundation further downstream which intercepted the oil. Pumps were used to recover any free product and an absorbent was used to dry out the soil. It is estimated that 10 000 ℓ of oil were not recovered and remained in the stream and surrounding soil.

The stream is currently on an undeveloped piece of land bordering an industrial area. At the head of the stream is a stormwater outfall, thus the stream has been exposed to many different industrial effluents and stormwater runoff over the years.

Topographical maps, as well as aerial and ortho photographs were obtained of the area, to determine the slope of the area and obtain other pertinent geographical data, such as topographical beacons, proximity of residential areas, and surface water bodies in the vicinity.

6.2.1.2 Physical characteristics

The stream had a total length of 500m. After about 200m the stream split into three branches which each flowed into the main river at different entry points. The remains of a stone wall belonging to an old kraal, held back a lot of the oil, on one of the stream branches.

The type of soil was identified from a Land Type map series (1:250 000) as being widespread red soil. The slope of the stream area was approximately 1:0.04.

The bedrock of the stream was very close to the surface in places, causing difficulties in excavating the contaminated soil in between the bedrock, and with the rehabilitation and restabilization of the stream once the excavation had been completed.

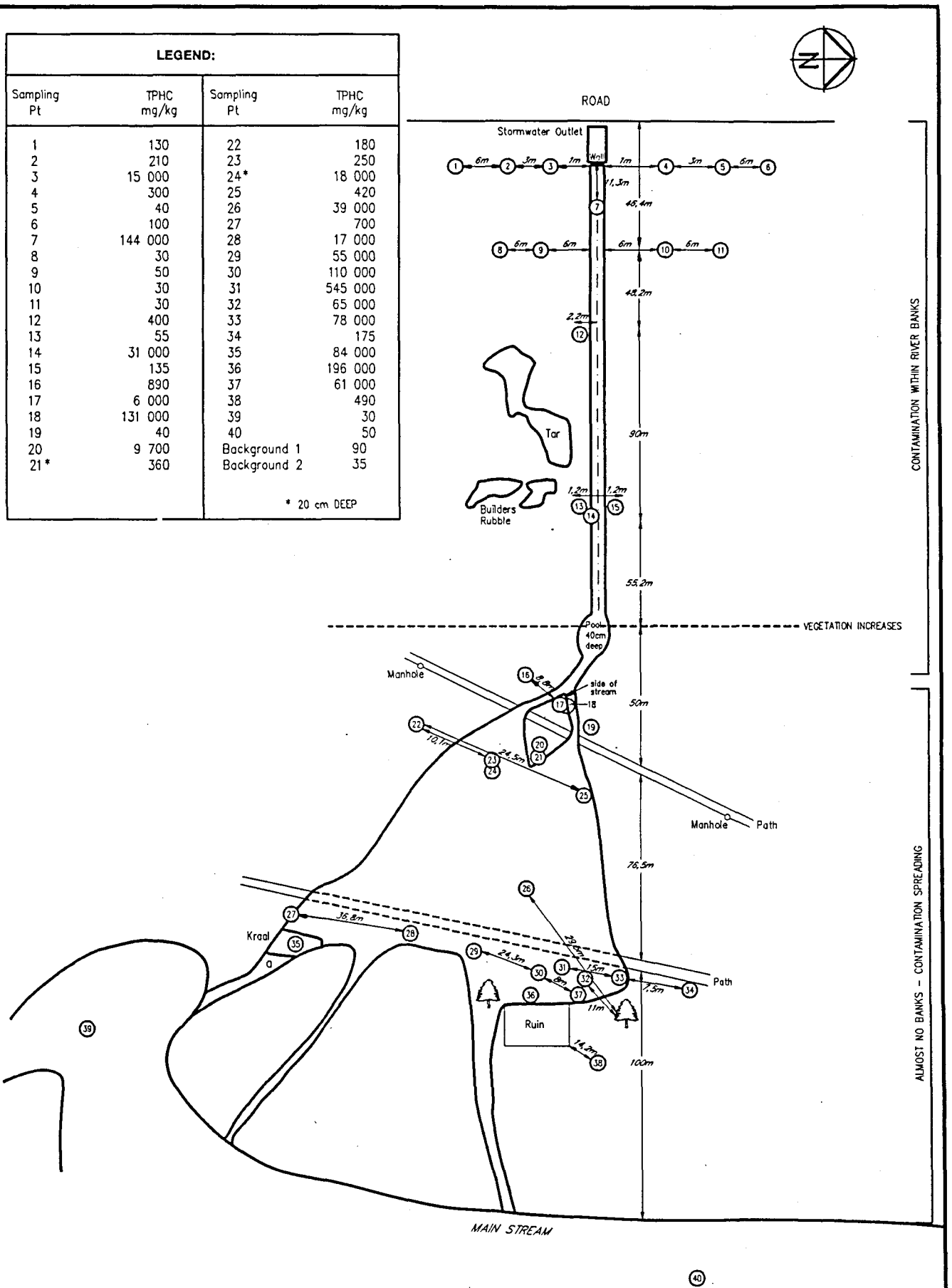
There were no boreholes in the direct vicinity of the stream. On average, the groundwater table was estimated from boreholes in the area to be deeper than 13-15m. As the pollutants were relatively heavy petrochemicals, these would be adsorbed to a large extent to the soil surfaces, hence reducing the risk to the groundwater. This was corroborated by taking samples at different depths which showed that the major proportion of the contamination was in the first 10-20cm from the surface. No complaints of contaminated borehole water were received.

6.2.1.3 Nature and extent of contamination

At the head of the stream, rock and concrete surfaces of the culvert were blackened with oil.

A visual inspection of the site of the spill showed substantial soil surface contamination from petroleum hydrocarbons for a distance of about 500 metres down the stream, where it then joined a larger river running east to west. The main contaminant in the stream was thought to be the oil which has a viscosity at 40° C of 103,69 cST, a specific gravity (20° C) of 0,879 kg/ℓ and a flash point at 214 °C. The sides of the stream were blackened, as well as the soil surface area just above the level of the water. The immediate vegetation was black with oil, some dead vegetation was evident, possibly due to the layer of oil covering the vegetation which would inhibit photosynthesis. A considerable area was affected downstream where the stream broadens into a larger clearing. It appeared that a recent fire also contributed to the death of the vegetation there. Free product floating on the water was visible. Few signs of aquatic life (fish or frogs) were noted in the stream. Very little oil appeared to have entered the main river, however the river showed signs of other chemical contamination and eutrophication. The banks of the main river were severely eroded.

A total of thirty eight samples were taken over the area. Samples were initially taken at cross sections to the stream to determine lateral width of the contamination. Where heavy contamination was visually seen or where analyses of initial samples indicated as such, further samples were taken. The results of the sampling can be seen in Figure 14. Contamination from previous spills became apparent through the sampling, which had not been evident before. The contaminant of concern here was a variety of very weathered heavy petroleum products, which had flooded the area on a previous occasion and collected in various areas. The area between the three smaller branches of the stream had been flooded and hence was contaminated. The results of the sampling were used as guidelines for excavations. Samples were again taken after excavation and areas still indicating high levels of contamination were excavated further. Figure 14 shows the stream area, with TPHC concentrations before excavation. The total stream area indicated was excavated with the exception of the area marked "a". Concentrations of TPHC after excavation are indicated in Figure 15.



Sampling Pt	TPHC mg/kg	Sampling Pt	TPHC mg/kg
1	130	22	180
2	210	23	250
3	15 000	24*	18 000
4	300	25	420
5	40	26	39 000
6	100	27	700
7	144 000	28	17 000
8	30	29	55 000
9	50	30	110 000
10	30	31	545 000
11	30	32	65 000
12	400	33	78 000
13	55	34	175
14	31 000	35	84 000
15	135	36	196 000
16	890	37	61 000
17	6 000	38	490
18	131 000	39	30
19	40	40	50
20	9 700	Background 1	90
21*	360	Background 2	35

B685-1a SCALE 1=1

FIGURE 14. STREAM AREA BEFORE EXCAVATION

LEGEND:	
Sampling Pt	TPHC mg/kg
1	3 359
2	2 340
3	1 290
4	1 745
5	3 520
6	1 630
7	2 930
8	65
9	25
10	8 745
11	2 510
12	80
13	40
14	230
15	240
16	1 120
17	24 125
18	75
19	1085

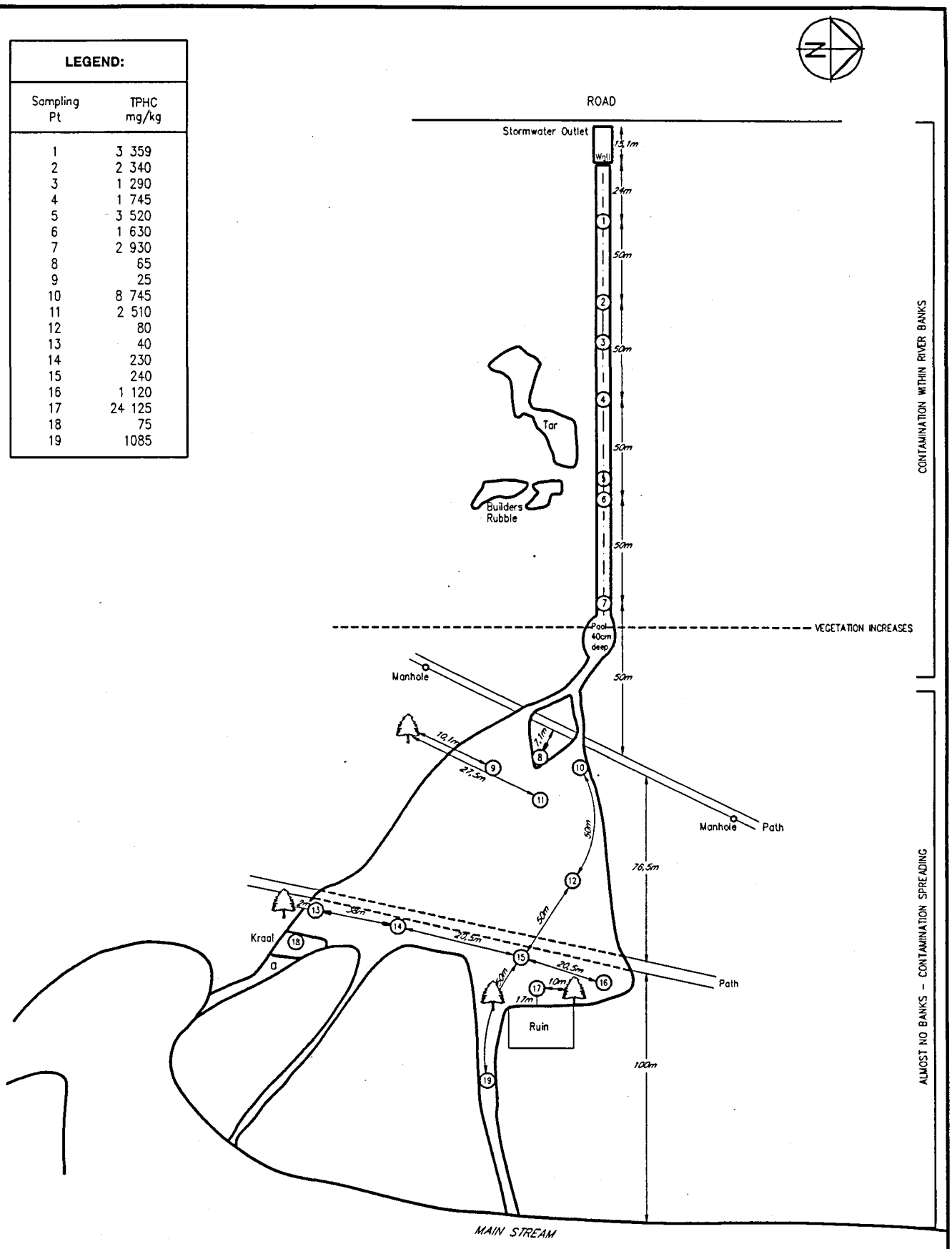


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FIGURE 15. STREAM AREA AFTER EXCAVATION

6.2.1.4 Risk assessment

No formal risk assessment was done. As the groundwater table was relatively deep and samples indicated that the contamination had at most penetrated 2m, it was decided that there was little risk in this respect.

Due to the informal settlement in the stream area and for users of the water downstream, cleanup of the stream was necessary. No life was evident in the stream, which can be taken as indicative of contaminant toxicity to water organisms.

Plate counts of the sediment were done to determine the stress experienced by the indigenous organisms. Levels to the order of $1,6x10^6$ and $3,7x10^5$ cfu/gram of soil were found for bacteria and fungi respectively. Healthy soil shows typical microorganism levels of 10^{8-9} and 10^{6-7} cfu/g of soil for bacteria and fungi respectively (Wollum II, 1982). This indicates a stress situation in the sediment.

6.2.1.5 Establish closure goals

Together with the relevant town council and the DWAF, it was agreed that soil would be bioremediated to a level of 2000mg/kg soil. The target would be set taking into account the industrial area, the future use of the land and what is realistically feasible.

6.2.2 Bioremediation

6.2.2.1 Assessment of clean up methodologies

As most of the contamination occurred in the spruit area, *in situ* bioremediation was not feasible, as the disturbed soil would wash away. Thus the soil needed to be excavated and moved to a "bioremediation site", established 50 m from the banks of the stream, having easy access to the road. As the land was available and there was no immediate time pressure, landfarming could be used.

The initial concrete and rock culvert needed to be cleaned as well. Here a combination of steam cleaning and rock chipping was used to remove the oil. Runoff from the steam cleaning was contained using adsorbent booms and fibres.

6.2.2.2 Design and costing of full scale bioremediation

Results from sampling indicated that approximately $1500m^3$ of contaminated soil needed to be excavated, on average, to a depth of 0.2-0.5 m. A bioremediation site (50x70m) was cleared of vegetation to a depth of 0.1 m and levelled. The soil from clearing the site was used to construct bund walls to contain any runoff. No lining was used as the product was relatively immobile and the groundwater table deep. The contaminated soil was levelled and is tended on a regular basis until target levels are reached. Maintenance of the site include dosages of lime for pH control, dosing of nutrients by way of fertilizer, addition of a biosupplement grown from the indigenous microorganisms in the soil, and keeping the moisture content at an optimum level.

Costing of the project was done according to the phases of the project. The first phase was to determine the nature and extent of the contamination and included cost of sampling and analyses.

The second phase of the project was to steam clean the initial culvert, and thus involved the hire of a steam cleaning machine and labour, as well as the cost of the adsorbent fibres used to contain the runoff caused from the cleaning.

A large proportion of the costs lies in the excavation, which includes hire of earthmoving equipment.

The costing of bioremediation is difficult should the period needed to reach target levels not be known. In such a case, it would be advisable to determine a monthly cost, based on the material and application costs associated with the soil supplements (nutrients, lime, biosupplement) and water. The latter may be dosed either by using sprinklers or by water tanker.

Lastly, but also of importance, is the rehabilitation of the stream area and bioremediation site, as well as the stabilization of the banks. In undeveloped areas it is usually acceptable to reestablish indigenous plants. Stabilization of banks may need planting of grass blocks, incorporation of stone blocks or stone packing to prevent erosion.

6.2.2.3 Implementation of bioremediation

The contaminated soil was excavated and placed on the remediation site to a depth of ca. 0,3-0,4m after levelling.

The full scale treatment was based on the results obtained during the pilot scale study. A 750ℓ biosupplement solution containing indigenous microorganisms ($>10^{12}$ CFU/mℓ) was applied fortnightly. MAP (0.4 kg/m^3) and lime (14 kg/m^3) were applied at the start of the project, the lime dosage was repeated after two months, and a further 350 kg of MAP was dosed after 3 months when sampling results indicated that levels of nitrogen and phosphorus had decreased substantially. Plate counts were done monthly to monitor biological activity in the soil. Rainfall was supplemented by spraying to increase soil moisture. The soil was ploughed on a weekly basis for mixing and aeration.

6.2.2.4 Sampling and monitoring programme

At certain intervals, 6 composite samples were taken, each made up of 3 individual samples collected along a predetermined grid system on the treatment site. The results of samples taken over a period of 4 months can be seen in Table 14.

6.2.2.5 Completion of bioremediation and rehabilitation of site

Bioremediation of the site will be considered complete when the target levels are reached. It is planned that the soil in the site will be spread over the undeveloped area to a depth of 0,1m and will be seeded with indigenous plants. However, vegetation, in particular khaki bos, poplar and black wattle trees are being naturally reestablished in areas adjacent to the stream that had been disturbed through excavation work.

Various options may need to be considered to stabilize the bed and banks of the stream. Due to the bedrock, lining the stream bottom and banks with grass blocks is not feasible, as a heavy flow would wash these away, because the roots are not able to get a good hold. A second option, that of stone casting in concrete or stone packing at regular intervals, was investigated and deemed to be a more effective option, albeit more expensive.

To contain any further future spills, the stream course was restructured after excavation to give it a more defined route and to prevent the area flooding again, should a similar incident occur. However, this makes the rehabilitation more difficult as it is better to spread the flow and hence lower the velocity of the water, thereby reducing erosion.

6.2.3 Results and discussion

The results of the TPHC analyses and microbiological plate counts from the remediation site are illustrated in Table 14.

Table 14. Total petroleum hydrocarbon analyses and plate counts of soil samples from the full scale site

Grid Line	TPHC mg/kg soil							
	Time 0	26 days	56 days	78 days	96 days	168 days	Average decrease from 26 days (%)	Average degradation rate (mg/kg.month)
A	7 425	9 270	7 555	2 280	1 560	1 050	88,7	1468
B	7 845	10 905	8 555	3 310	2 900	820	92,5	1800
C	20 720	19 575	18 460	5 250	8 330	1 770	91,0	3179
D	9 490	10 610	21 410	5 960	8 620	2 335	78,0	1478
E	5 260	10 755	7 985	2 560	5 180	1 135	89,4	1718
F	22 990	21 350	20 215	4 915	5 680	1 240	94,2	3591
Average						1 392	89,0	2206
Hydrocarbon-degrading microorganism (CFU/g soil)*	6×10^6	2×10^6	8×10^6	-	7×10^7	5×10^7		

* average of three plate counts on OECD agar.

Due to the unavailability of specialized mixing equipment the contaminated soil volume was not homogeneous from the start. Thus ongoing mixing occurred whenever the soil was ploughed, therefore the fluctuation observed in the TPHC concentrations is the combined effect of bioremediation and mixing. The initial TPHC concentrations was therefore taken as the concentration on day 26.

Decrease in TPHC was initially slow during the initial 56 days, with the limiting factor considered to be low levels of moisture in the soil due to the lack of rain, the low moisture retaining capacity of the stream sediment and high evaporation rates. The low moisture levels were probably responsible for lower soil microbial numbers, and consequently slower degradation rates. Mass transfer of the oil into the aqueous phase could also have been affected, thereby resulting in decreased bioavailability. Increased water dosages, as well as the start of the rainfall season, enhanced soil moisture, and hence degradation rates was seen between 56 and 78 days. However, a decrease in rate was again evident from 78 days. Mineralization of the more easily biodegradable compounds is expected in the initial stages, thus leaving the heavier fractions, which have a slower rate of breakdown, for the later stages of treatment. Treatment was stopped after 168 days when concentrations below the target were reached. The overall degradation rate for the 5,5 month period was 2206 mg/kg per month.

As the contaminated soil had undergone a lengthy period of weathering, it was assumed that loss through volatilization was not significant.

6.2.4 Conclusions

- In the full scale application, bioremediation reduced levels of TPHC by approximately 90% over a 5,5 month period.
- Using the landfarming technique, bioremediation reduced TPHC levels from 7400 - 23000 mg TPHC/kg soil to 820 - 2335 mg TPHC/kg soil over a period of 168 days, resulting in an average biodegradation rate of 2206 mg/kg.month.
- Low moisture retention of the contaminated soil contributed to the initial slow rates of biodegradation. Increased application of moisture improved this.
- The rate of degradation decreases as more recalcitrant contaminants remain in the soil.
- At full scale, the larger fraction of more recalcitrant and weathered petroleums, and the less intensive treatment compared to pilot scale, resulted in a slower rate of TPHC reduction.
- These results of the full scale bioremediation indicate the difficulty of achieving degradation rates comparable to pilot scale rates.

7. GENERAL CONCLUSIONS

7.1 NATURE AND EXTENT OF CONTAMINATED SITES IN RSA

As the information required in the questionnaires was of a sensitive nature, many respondents were unwilling or unable to give details especially with regard to existing contaminated sites. Hence the survey provides a limited perspective of the situation in South Africa, but it is thought to be indicative of the broader state of affairs.

The following became evident from the survey

- Many contaminated sites are known, and are a cause for concern, especially where water pollution is a possibility.
- A large proportion of the respondents (91%) knew of bioremediation as a treatment technology for treatment of contaminated sites.
- Landfarming, followed by soil vapour extraction, are best known and the most frequently used bioremediation technologies in South Africa.
- The majority of respondents would use bioremediation to clean sites, under suitable conditions, seeing it as a cost-effective technique.
- The majority of contaminated sites reported were of an industrial nature. Of the industrial sites, contamination with petroleum products was most prevalent. Inorganic contaminants were frequently expressed as a concern.
- The bioremediation industry is expected to expand in the near future.
- Target clean-up concentrations for the contaminants were most frequently set by the Department of Water Affairs and Forestry and the local authority. Guidelines recommended by the Oil Industry Environment Committee are also used.
- Not many companies capable of implementing bioremediation projects were known to the respondents.
- Better communication between remediation technologists and affected parties on the advances made in bioremediation, especially results of full scale application is advocated in order to increase technology dissemination.

7.2 BIOREMEDIATION AS A TREATMENT TECHNOLOGY

Experiments on laboratory and pilot scale were used to give an indication of the viability and appropriateness of bioremediation technologies, as well as to determine the influence of various factors on the rate of biodegradation of contaminants. The following findings were obtained:

- Slurry reactors appear to be a viable bioremediation technology, at laboratory scale. However, it is envisioned that it would be a costly technology to use at full scale application and as such not suitable for use South Africa.
- Soil columns, used to simulate degradation of phenol contaminated seepage water in an *in-situ* soil system, brought a number of operational problems and hence was not assessed further.

- The pilot scale landfarming experiment gave good results with a decrease of 94% from initial levels of 320 g/kg to 18 g/kg TPHC over a period of 10 weeks. As no technologically advanced equipment is needed, it is a readily applicable technology. Should *ex-situ* landfarming be the appropriate bioremediation technique, a relatively large, inexpensive piece of land is required close to the contaminated site. This helps to reduce transport costs.

- A number of factors were found to affect the rate of biodegradation.

  Moisture

  Adequate moisture is essential, both as a means to solubilize the contaminant and hence increase contaminant bioavailability to the microorganisms, and as a parameter vital to microbial growth. Moisture levels close to field capacity of the soil should be aimed at. Care should be taken, however, not to saturate the soil, as leaching of the contaminant may occur. Covering the bioremediation site and/or soil conditioners can be used to improve moisture retaining capacity of the soil.

  Oxygen

  Sufficient and frequent application of oxygen to promote aerobic conditions, under which bioremediation occurs much faster, is just as important. An exception is treatment of highly chlorinated compounds, which degrade faster under anaerobic conditions. Even without addition of nutrients, the rate of biodegradation clearly showed a marked increase with application of water and air.

  Nutrients

  The application of nitrogen and phosphorus enabled a faster decrease in TPHC concentrations. A hydrocarbon to nutrient ratio of 100:10:1 as C:N:P was used at laboratory scale. At pilot scale, mono ammonium phosphate was used to supplement the nitrogen and phosphorus concentrations. Phosphate concentrations, that resulted from application of the fertilizer, were higher than needed, which may have led to a lowering of pH in some of the pilot scale reactors. Lower pH values can inhibit bacterial activity. Alternative sources of nutrients may be more suitable.

  Biosupplements

  Application of biosupplements showed an increase in biodegradative rates. However, it appeared that the biosupplement consisting of indigenous bacteria performed as well as the commercially available biosupplement over the total 10 week period of the pilot scale. The indigenous biosupplement initially showed better results, this may have been due to an acclimatization period of the commercial biosupplement to the specific soil conditions. Levels of between 10^7 and 10^8 of cfu/g soil should be aimed at using total plate counts. However, to obtain a better idea of specific hydrocarbon degraders, more specialized plate counts should be done.

  pH

  Application of lime can be used to increase the pH of the soil. The pH of the soil should be monitored regularly as it may change due to the degradation

products in the soil or due to soil amendments added. The pH may also affect solubility of the contaminant, as well as adsorption characteristics.

- Application of landfarming at full scale, showed slower rates of degradation than were seen at pilot scale. This may be ascribed to less intensive treatment being applied, for practical reasons, and also to bioremediating a more weathered mixture of petroleum products. At full scale it is also more difficult to explain fluctuations in TPHC concentrations as every time the soil is ploughed a certain amount of mixing occurs.

- Accurate analyses are vital to carry out comparative studies and determine rates of degradation. This project has highlighted analyses of soil constituents and contaminants that may be problematic, especially during solvent extraction. Analyses need to be reliable, reproducible and accurate.

- Further research is needed on different sources and methods of applying nutrients to the soil, to reach correct concentrations and to increase the bioavailability to the microorganisms.

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APPENDIX 1

SURVEY: QUESTIONNAIRE

THE NATURE AND EXTENT OF SOIL CONTAMINATION IN SA

Should you wish to expand on any of the questions asked, please make use of the attached sheet at the end of the questionnaire.

1. Is there a need for treatment of contaminated sites ?

Yes ☐

No ☐

2. Are you aware of bioremediation for treating contaminated sites ?

Yes ☐

No ☐

3a. What types of bioremediation are you aware of ?

Landfarming ☐

Slurry reactors ☐

Vapour extraction ☐

In situ ☐

Soil excavation ☐

None ☐

Other ☐

Specify .....

.....

3b. What types of bioremediation are you aware of that have been used in SA?

Landfarming ☐

Slurry reactors ☐

Vapour extraction ☐

In situ ☐

Soil excavation ☐

None ☐

Other ☐

Specify .....

.....

4. What contaminated sites are you aware of ?

Type of site (Industrial, Municipal etc)	Contaminants	Concentrations of contaminant	Volumes of contaminated soil	Groundwater contamination (Yes/No)	History of the site (age of site, closed, etc)	Situation of site (near rivers etc)

5. How many full scale bioremediation projects are you aware of ?

1 ☐

1-5 ☐

5-10 ☐

more than 10 ☐

Type of site where bioremediation has been done	Major contaminant	Levels (concentration)		Volume	Cost	Cost Effective? Yes/No Why?
		Initial	After treatment			

6. For each of the above projects, who set target contaminant levels?

	Project 1	Project 2	Project 3	Project 4
Local authorities	☐	☐	☐	☐
Department of Water Affairs	☐	☐	☐	☐
Department of Environment	☐	☐	☐	☐
Department of Health	☐	☐	☐	☐
Self	☐	☐	☐	☐

Other
Specify .....
.....

Levels set for major contaminants .....
.....

7. Would *YOU* consider bioremediation as a clean-up option ?

Yes ☐
No ☐
In some cases ☐

8. Under which circumstances would YOU consider/recommend bioremediation ?

.....
.....
.....

9. What do you see as alternatives ?

.....
.....
.....

10. What is your opinion on the cost effectiveness of bioremediation?

Extremely inexpensive ☐
Inexpensive ☐
Reasonable ☐
Expensive ☐
Exorbitant ☐

11. **Do you see landfilling as a more economical option?**

Yes ☐

No ☐

12. **What is your opinion on the efficiency of bioremediation in terms of reaching target levels?**

Very acceptable ☐

Acceptable ☐

Unacceptable ☐

13. **Do you have any particular problems/likes/dislikes with respect to bioremediation ?**

. . . . . .
. . . . . .
. . . . . .

14. **How many bioremediation projects do you foresee in the next year/5 years ?**

	Next year	Next 5 years
% increase with reference to (5) above	☐	☐
% decrease with reference to (5) above	☐	☐

15. **Who do you feel would have the capabilities of implementing a bioremediation project? Give company names where possible.**

. . . . . .
. . . . . .
. . . . . .

16. **How do you become aware of potential applications for bioremediation?**

Tenders ☐

Personal communication ☐

Other ☐

Specify . . . . . .
. . . . . .

17. How do you award a bioremediation contract?

Tenders ☐
Direct from service provider ☐
Personal referral ☐
Other ☐

Specify .....
.....

FURTHER COMMENTS

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APPENDIX 2

DEVELOPMENT OF SOLVENT EXTRACTION METHOD FOR PHENOL DETERMINATION

During preliminary experiments, it became evident that a loss of phenol occurred during the solvent extraction process. Experiments were therefore carried out, changing various parameters sequentially, to determine at what stage of the extraction procedure losses occurred, and concomitantly to improve the efficiency of extraction.

(a) Aims

To improve recovery efficiencies of phenol from soil using solvent extraction.

To compare methods of detection of phenol using UV with and without HPLC.

(b) Experimental Procedure

The initial method was as follows:

Twenty grams of phenol contaminated soil were weighed out into a glass beaker. To this 0.15g of $Na_2S_2O_4$ were added. Distilled water was added to make the volume up to 50mℓ. The pH of the soil and water mixture was increased to pH13 using 10M NaOH. This slurry was then transferred quantitatively to a centrifuge bottle. The bottle was closed tightly and shaken for 10 minutes, after which it was centrifuged @ 3000rpm for a further 10 minutes. The supernatant was filtered using vacuum. The soil was extracted a second time, following the above method, starting from the addition of NaOH. The soil after both extractions was dried overnight at 37°C before weighing. The filtered supernatents from both soil extractions were kept separate and the pH of each decreased to 2.5-3.0 using concentrated phosphoric acid. The solutions are then centrifuged @ 3000 rpm for 5 minutes. Each supernatents was extracted three times with 50 mℓ aliquots of dichloromethane. The dichloromethane aliquots were combined and 4mℓ glycerine was added. The total volume was evaporated to ca. 5mℓ volume using a rotovapor vessel. The residual dichloromethane was blown off using N_2. The residual in the flask was made up to volume in a volumetric flask using distilled water. The concentration of phenol was determined using high performance liquid chromatography (HPLC) with an isocratic acetonitrile:water mobile phase and a C18 reverse phase column.

Parameters that were changed included the pH of the aqueous phase, the organic solvent used (methylene chloride, ether, ethyl acetate, and a hexane:acetone mixture), the temperature of evaporation of the organic solvent (it was decreased as much as possible using a vacuum pump rather than a water vacuum), the method of evaporation of the solvent (Kuderna-Danish apparatus vs the rotary evaporator), and the purity of the phenol used for spiking the soil.

Detection and quantification of phenol with and without a preceding HPLC step was compared. A Waters HPLC, consisting of an automatic injector: WISP (Waters Intelligent Sample Processor), a resolve C-18 column, a detector and a recorder, was used for the phenol analyses. The mobile phase was pumped through the system by a high pressure pump (model 590). The mobile phase was 40% acetonitrile:60% deionized water. The mobile phase was filtered and degassed before use, and kept under sterile conditions. The samples were filtered through 0,45 nm filters, before injection.

(b) Results and discussion

Very little of the phenol was left in the aqueous phase after extraction with the organic solvents, hence extraction of the phenol, from the aqueous to the organic phase, was efficient. Through a process of elimination it was found that a large percentage of the phenol was lost while evaporating off the organic solvent. It appeared that the phenol volatilized with the organic solvent. This problem was largely solved through the addition of a retainer in the organic phase, such as glycerol (as indicated in the above method). The addition of the retainer increased efficiencies of recovery from an aqueous solution of phenol from generally below 50% to up to 80%.

Figure A2-1 compares the recovery of phenol from soil and aqueous samples using a retainer. Although acceptable recovery of phenol was obtained from aqueous samples, the recoveries from phenol-spiked soils remained unacceptable.

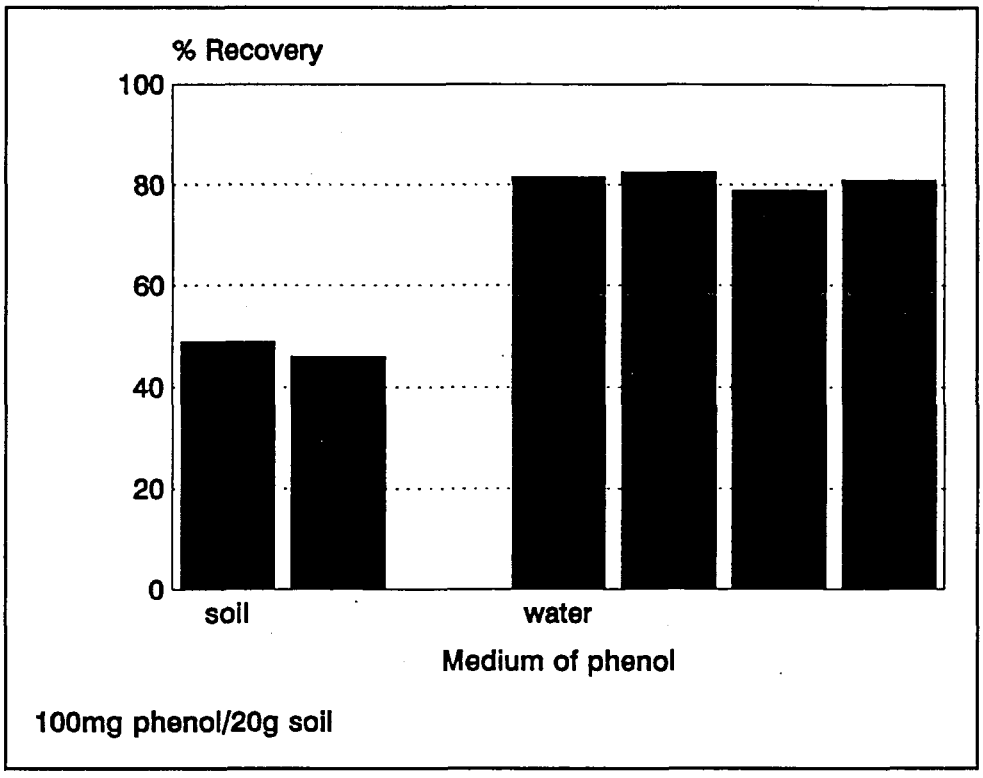

Figure A2-1. Recovery from aqueous and soil phenol-spiked samples using solvent extraction

This efficiency was improved by leaving out the organic extraction step totally and injecting the aqueous supernatant of the soil directly into the HPLC after pH adjustment and filtration. The method used for subsequent experiments and the remainder of the soil slurry runs was as follows:

Twenty grams of phenol contaminated soil were weighed out into a glass beaker. To this 0.15g of $Na_2S_2O_4$ were added. Distilled water was added to make the volume ca. 50mℓ. The pH of the soil and water mixture was increased to pH13 using 10M NaOH. This slurry was then transferred quantitatively to a centrifuge bottle. The bottle was closed tightly and shaken for 10 minutes, after which it was centrifuged @ 3000rpm for a further 10 minutes. The soil was extracted again, following the above method, starting from addition of NaOH. Both supernatants from the soil extractions were combined are made up to volume with distilled water. The pH was then increased to pH 7, to protect the HPLC column and injected into the HPLC, after filtration through a 0.45 μm filter. The soil was dried overnight at 37°C overnight before weighing, so

that the phenol could be reported as mass phenol/mass soil.

The concentration of phenol was determined using HPLC with an isocratic acetonitrile:water mobile phase and a CIB reverse phase column.

Table A2-1 shows that results obtained using UV spectroscopy only are comparable to that obtained when using HPLC combined with UV, varying at most by ca 1mg/ℓ. It was thus accepted that UV spectroscopy is an accurate method for determination of phenol concentrations.

Table A2-1. Comparison of phenol concentrations using UV spectroscopy and HPLC

Sample	UV Analyses mg/ℓ	HPLC Analyses mg/ℓ
1371	16,0	15,0
1372	9,0	8,9
1373	13,2	12,3
1374	9,5	8,9

y

APPENDIX 3

METHOD OF SOIL VENTING TO DETERMINE PHENOL IN CONTAMINATED SOIL

(a) Aim

Development of phenol determination using elevated temperature soil venting

(b) Experimental procedure

A soil slurry at elevated temperatures was continuously flushed for a period of time with nitrogen gas. The phenol was volatilized from the soil and was trapped in an alkaline solution, which was then analyzed using ultra-violet spectroscopy (UV).

The method was tested for recovery efficiencies using different concentrations of phenol (50mg - 5000mg phenol/kg soil). A further parameter investigated was the length of time of flushing needed for acceptable recovery of phenol.

All samples were analyzed in duplicate.

The absorption spectrum of phenol in an alkaline solution was analyzed to determine at which wavelength peak absorbance occurred. A standard curve was drawn up using standard alkaline solutions of phenol. Samples from the soil slurries were diluted so that the absorbance was within the limits of the straight line of the standard curves. At a higher absorbance instrumental error occurs leading to a skewed absorbance reading. The accuracy of UV spectroscopy for determination of phenol was checked by comparison with duplicate analyses done using the HPLC. Before the sample was analyzed using HPLC, the pH of the sample was decreased to pH 6-7 using phosphoric acid.

(c) Results and discussion

Figure A3-1 illustrates the results of the experiment done to determine the minimum amount of time needed for flushing to obtain an acceptable recovery. The experiment was done with 4.6mg phenol in a 20g sample of soil. It can be seen that the major portion of the phenol is recovered within 2 hours with very little further phenol being recovered after 4 hours. An acceptable length of time for flushing thus appears to be between 3-4 hours.

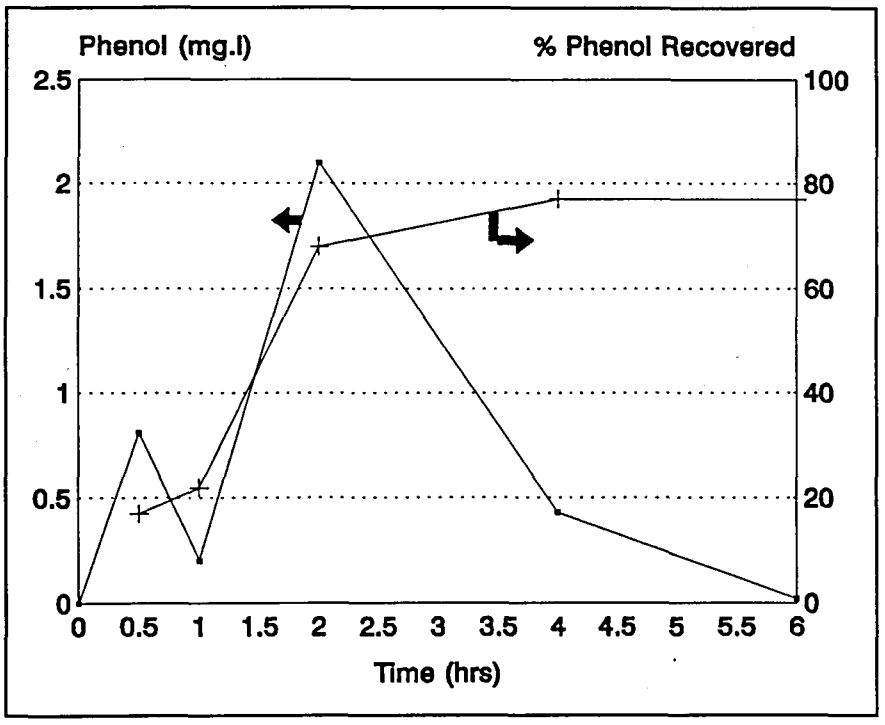

Figure A3-1. Recovery of phenol versus time of nitrogen flushing

The cumulative percentage recovery of phenol is also reflected in Fig. A3-1. Further work, however needs to be done to increase the reproducibility of the results. It is thought that fluctuations in the results occurred through nitrogen gas escaping from the system before having bubbled through the alkaline solution, as well as through slight differences in the flow rate of the gas in duplicate samples, which is difficult to monitor.

APPENDIX 4
COSTING OF BIOREMEDIATION

Costs for a volume of 1000 m^3 over a period of 5 months:

Excavation (@ R30/m^3)	
of soil (1 000m^3)	R30 000
of soil & vegetation to make bioremediation site (335m^3)	R10 000
to spread remediated soil back onto land (1 000m^3)	R10 000
	R50 000
Grass blocks (R5,00/m^2)	
to rehabilitate excavated area (1000m^2)	R 5 000
	R 5 000
Ploughing	
aerating the soil by ploughing (R 500/time)	R10 000
	R10 000
Soil Supplements	
Enriched biosupplement	R 6 000
Mono ammonium phosphate (5 tons @ R1 248/ton)	R 6 200
Agricultural lime (124 tons @ R46.70/ton)	R 5 800
	R18 000
Sprinkler equipment	
Pump	R 5 000
Sprinkler system	R 3 000
	R 8 000
Analyses	
Monthly analyses of total petroleum hydrocarbons by EPA 418.1	
(10 samples per time @ R180/sample)	R 9 000
	R 9 000
Project Management	
Project leader	
Biochemist	
Engineer	
Technical assistance	
Travelling expenses	
	R 40 800
Rehabilitation	
Planting and establishment of vegetation	R40 000
	R40 000

	R180 800
Contingency (10%)	R 18 000

	R198 800
TOTAL	**R198 800**