BIOREMEDIATION OF CONTAMINATED SOIL

A Thesis presented in partial fulfilment of the requirements for the degree of Master of Technology in Environmental Engineering at Massey University.

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ABSTRACT

The release of contaminants into the environment is inevitable. Contaminants are released through manufacture and use of products and as a result of treatment and disposal of wastes. Upon release to the environment, contaminants move and respond to a number of interrelated natural and man made factors.

Penta-chloro-phenol (PCP) is one such contaminant that has been released into the environment and is known to have serious long term environmental effects. The objective of this study was to determine the effectiveness of biological processes to remediate soil contaminated with Penta-chloro-phenol (PCP). This thesis reviews mechanisms by which soil is contaminated, processes available to remediate soils, and in particular, process requirements for successful bioremediation.

The abilities of bacteria to degrade PCP from soil contaminated with PCP was evaluated. Solid phase and slurry phase experiments were examined for their effect on PCP concentration over a four month period at the Department of Technology, Massey University. The objectives of this study were (1) To determine if aeration and inoculation of soil in-situ could produce significant removal of PCP. (2) Determine the effect of concentration on bioremediation rates. (3) Compare in-situ treatment with bio-slurry treatments.

The experiments showed that it is possible to remove up to 95 % of PCP from contaminated soil by inoculation with bacteria. Inoculum size and aeration were shown to be critical factors in affecting the rate of degradation. The larger the initial inoculum the greater the rate of degradation. Without aeration the inoculum was unable to significantly degrade PCP. The bio-slurry confirmed that PCP could be removed readily from soil to an aqueous state. In an aqueous state PCP is degraded at a faster rate than when it is incorporated into the soil matrix.

The results of this work is to show that soil rehabilitation by way of biodegradation is a feasible and attractive process.

ABSTRACT

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1. INTRODUCTION

The total land area on Earth is approximately 14500 million hectares of which 13250 million hectares is ice free. Land use patterns are mainly determined by interactions between climate, geography, geology, human and economic processes. Land can be categorised into four categories: arable and permanent cropland, permanent pasture, forests and land for general use including land unused in urban areas, and waste and barren land. The latter encompasses a third of the earths surface, of which more than 3,000 million hectares is suspected to be exposed to chemicals[1].

All categories of land are open to pollution and consequently are hazardous to the environment, attention has been focussed on land categorised under general use because this represents a more direct risk to human health and thus receives the greater publicity in the case of incidents.

Contaminated land results from a wide range of human activities including industrial discharge processes and the disposal of waste. New Zealand has an advantage over old world countries such as the United Kingdom and United States of America in that there is not a legacy of nearly two centuries of industrial operation. Despite this New Zealand has sites which are as heavily contaminated as some overseas industrial sites. Remedial technique experience with respect to contaminated land is brief, dating back only to the 1970's. Its is important in the long term to develop new and effective means of soil decontamination.

Bioremediation of soils is a novel technique that is slowly gaining acceptance despite having significant cost advantages over other remediation technologies. The main reason for this lack of acceptance being a reluctance on the part of engineers to utilise a treatment option that was generated outside their field.

The aim of this thesis is to add to this body of knowledge

2. CHEMICALS IN SOIL

2.1 INTRODUCTION

The release of contaminants into the environment is inevitable. Contaminants are released through manufacture and use of products and as a result of treatment and disposal of wastes. Upon release to the environment, contaminants move and respond to a number of interrelated natural and man made factors. They may move quickly or slowly to living receptors in their original or altered form. The pathway may be direct or tortuous.

Understanding how contaminants are released and their transport and fate in the environment is necessary for successful hazardous waste management. In this context this chapter presents the fundamental mechanics of contaminant transport and describes the fate of contaminants in the environment.

2.2 PHYSICAL PROCESSES CONTROLLING THE TRANSPORT OF CONTAMINANTS IN THE AQUEOUS PHASE

Interphase transfer potential for waste constituents among oil (waste or NAPL), water, air and solid (organic and inorganic) phases of a soil system is affected by the relative affinity of the waste constituents for each phase, and may be quantified through calculation of partition coefficients. Distribution coefficients are calculated as the ratio of the concentration of a chemical in the soil (aquifer), oil, or air phase to the concentration of chemical in the water phase.

A waste chemical, depending on its tendency to be associated with each phase, will distribute itself among the phases, and can be quantified in terms of distribution coefficients.

The distribution coefficients are available for a wide variety of chemicals and can be expressed as ratios of the concentrations of a chemical between two phases in the sub-surface.

$$K_d = \frac{\text{Concentration in solid phase}}{\text{Concentration in aqueous phase}}$$

$$K_o = \frac{\text{Concentration in oil phase}}{\text{Concentration in aqueous phase}}$$

$$K_h = \frac{\text{Concentration in air phase}}{\text{Concentration in aqueous phase}}$$

K_h is also known as Henry's Law constant. When distribution coefficients are not available, they can be estimated using structure activity relationships (SARs) or can be determined by laboratory tests. Concerning toxic metallic contamination of soils, metal concentrations in the soil aqueous phase are governed by the following interrelated processes

1. inorganic/organic complexation.
2. acid base reactions
3. redox reactions
4. precipitation/dissolution reactions
5. interfacial reactions.

The ability to predict the concentration of a given toxic metal in the soil solution depends on the accuracy with which the multiphase equilibria can be calculated, evaluated or predicted.

Arsenic, selenium and chromium are metals that can exist as anions. Because of their anionic nature, their behaviour in soil will differ from other toxic metals. However, since metals distribute among the phases of the soil systems described previously, distribution coefficients may be used, along with metal speciation, to evaluate metal distribution in a contaminated soil system.

The partition coefficient of a chemical between soil and water is given by:

$$K_d = \frac{C_s}{C_w}$$

where K_d is the soil/water partition coefficient, C_s is the concentration of chemical in the soil phase, and C_w is the concentration of chemical in the aqueous phase. K_d values for a soil can be estimated from K_{oc} values if the organic fraction for the soil f_{oc}, is known, assuming that hydrophobic interactions dominate.

$$K_d = K_{oc} f_{oc}$$

Where K_{oc} is the organic carbon normalised soil/water partition coefficient.

The partition coefficient of a chemical between water and oil is given by.

$$K_o = \frac{C_o}{C_w}$$

Where K_o is the oil water partition coefficient, C_o is the concentration of chemical in the oil phase, and C_w is the concentration of chemical in the water phase.

The partition coefficient of a chemical between air and water can be written as.

$$K_h = \frac{C_a}{C_w}$$

Where K_h is the air water partition coefficient, C_a is the concentration of chemical in the air phase, and C_w is the concentration of chemical in the aqueous phase.

2.2.1 Transport of Waste Constituents

Retardation of the downward transport (leaching potential) and upward transport (volatilisation potential) is referred to as immobilisation of waste constituents. Immobilisation of organic chemicals has been related to soil organic content, soil moisture and presence of concentration of organic solvents.

A means of predicting the rate of contaminant transport through the sub-surface is to describe its mobility by predicting its retardation. The retardation factor describes the relative velocity of the constituent compared to the rate of movement of water through the sub-surface.

$$R = \frac{V_w}{V_c}$$

Where R is the retardation factor, V_w is the average water velocity and V_c is the average constituent velocity. A retardation factor greater than one indicates that a constituent is moving more slowly than water through a system. A factor developed from a transport model combined with a description of sorption processes as described by linear Freundlich isotherm, can be calculated from the following equation for retardation in unsaturated soil.

$$R = \left(\frac{\rho K_d}{\Theta} \right)$$

Where ρ = soil bulk density. K_d = soil water partition coefficient, which describes the partitioning between the solid phase and soil water, and Θ = volumetric moisture content. For a saturated system, Θ is replaced by the porosity of the system.

This information can then be used to evaluate treatment techniques for a contaminated soil system (for example through control of soil moisture, changes in bulk density, or addition of amendments to soil that will affect soil water partition coefficients) so that constituents can be captured or contained in the system, thus allowing time for degradation at the site or for

engineering implementation and performance of other remediation treatment techniques, such as soil washing. Linear retardation of chemicals in the vapour phase can be expressed as

$$R = 1 + \frac{P_b}{O_a K_h} + \frac{O_w}{O_a K_h}$$

Where P_b is bulk density, K_p and K_h represent partition coefficients between soil and water and between air and water respectively, and O_a and O_w represent air and water content respectively. The second term in the equation represents partitioning into soil organic matter, and the third term represents partitioning in soil water.

2.3 TRANSPORT OF NON-AQUEOUS PHASE LIQUIDS IN THE SUB SURFACE

Liquids that do not readily dissolve in water and can exist as a separate fluid phase are known as non-aqueous phase liquids (NAPLs). Generally, NAPLs are subdivided into two classes:

1. Those that are lighter than water (LNAPLs);
2. Those with a density greater than water (DNAPLs).

Most LNAPLs are hydrocarbon fuels such as gasoline, heating oil, kerosene, jet fuel, and aviation gas. Most DNAPLs are chlorinated hydrocarbons such as 1,1,1-trichloroethane, carbon tetrachloride, chlorophenols, chlorobenzenes tetrachloroethylene and PCBs.

Concern about NAPLs exists because of their persistence in the subsurface and their ability to contaminate large volumes of water. For example 7 L (10 kg) of trichloroethylene (TCE) can contaminate10^8 L of ground water at 100 ppb (Feenstra and Cherry[2], 1987). NAPLs are ubiquitous throughout the industrialised world and have been identified at 4 out of 5 hazardous waste sites in New Zealand. Greater understanding of the transport and dissolution of NAPLs is necessary to implement cost effective techniques for the cleanup of these contaminants.

2.3.1 Transport and Dissolution of NAPLs

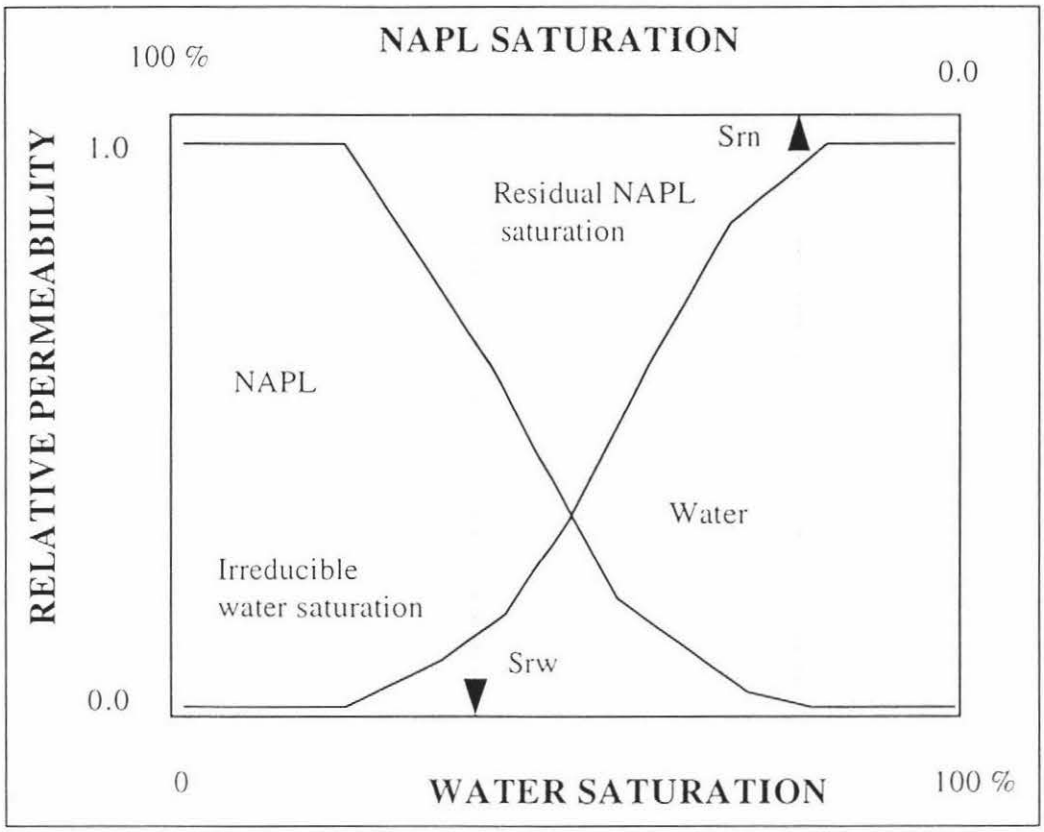
As NAPLs move through geologic media, they displace water and air. Because water is the wetting phase relative to both air and NAPLs, it tends to line the edges of the pores and cover the sand grains. The NAPL is the non-wetting phase and tends to move through the central portions of the pores. Neither the water nor the NAPL phase occupies the entire pore. Because of this, the permeability of the medium with respect to these fluids is different than when the pore space is entirely occupied by a given phase. This reduction in permeability depends on the medium and often is described in terms of relative permeability, k_{ri}, for phase i, which is defined as:

$$k_{ri} = \frac{k_i(S)}{k_{si}}$$

Where S_i is the fraction of pore space occupied by phase i, $k_i(S_i)$ is the permeability of the medium to phase i at saturation S_i, and k_{si} is the permeability of the medium at complete saturation with phase i. Thus, the relative permeability varies from 1.0 at 100 percent saturation to 0.0 at 0 percent saturation.

A plot of relative permeability versus water saturation for a hypothetical medium (Figure 1) reveals some important features about multiphase flow. At 100 percent water saturation, the relative permeability of the water and the NAPL are 1.0 and 0.0, respectively. As the fraction of the pore space occupied by the NAPL (S_n) increases, a corresponding decrease occurs in the fraction of water within the pore space (S_w). As S_w decreases, the relative permeability with respect to the water phase decreases to zero.

Figure 1 Relative permeability versus water saturation



Zero relative permeability is not obtained at zero S_w, but at the irreducible water saturation (S_{rw}). At this water saturation, the water phase is effectively immobile and there is no significant flow of water. The relative permeability of the NAPL behaves in a similar manner. At 100 percent NAPL saturation, the relative permeability for the NAPL is equal to 1.0, but as the NAPL saturation decreases, so does the relative permeability. At the residual NAPL saturation (S_{rn}), the relative permeability for the NAPL is the non-wetting phase and tends to move through the central portions of the pores. Neither the water nor the NAPL phase occupies the entire pore. Because of this, the permeability of the medium with NAPL is effectively zero and the NAPL is considered to be immobile.

These immobile fractions of NAPL cause great concern because they cannot be easily removed from the pores except by simple dissolution by flowing ground water. An example of this could be a cubic meter of soil with a 35 percent porosity and containing TCE at a 20 percent residual saturation. This situation implies that there is 0.07 me or 103 kg of TCE within the soil.

If the solubility of the TCE is 1,100 mg/L and if ground water flows through the soil at a rate of 1.7 cm/day, it would take 15.4 years for the TCE to be removed by dissolution. If the contaminated aquifer is twice as long (2 m), 30.8 years are necessary. If a NAPL with a lower solubility than TCE is spilled, the rate of mass removal is lower, requiring even more time for dissolution. Thus, NAPLs that enter the sub-surface can remain there for decades and can contaminate large volumes of ground water.

Understanding how an NAPL moves within a porous aquifer can be useful. The movement of petroleum products in the sub-surface is described in detail by Schwille[3] (1967), and the movement of DNAPLs is examined closely by Schwille[4] (1988), Feenstra and Cherry (1987), Kueper and McWorter[5] (1988), and Anderson[6] (1988).

2.3.2 Light Non-Aqueous Phase Liquids

As a spilled LNAPL enters the unsaturated zone, it flows through the central portion of the unsaturated pores. If the amount of product released is small, the product flows until residual saturation is reached (Figure 2) Therefore, a three-phase system consisting of water, product, and air is created within the vadose zone. Infiltrating water dissolves within the LNAPL (eg, benzene, toluene, and xylene) and carries them to the water table. These dissolved constituents then form a contaminant plume emanating from the area of the residual product.

Many of the components commonly found in LNAPLs are volatile and, as a consequence can partition into the soil air and be transported by molecular diffusion to other portions of the aquifer. As these vapours diffuse into adjacent soil areas, they partition back into the water phase and spread contamination over a wider area. If the surface is not covered with an impermeable material, these vapours diffuse across the surface boundary and into the atmosphere.

However, if a relatively impermeable boundary covers the area, no mass transfer occurs with the atmosphere and the concentrations of contaminants in the soil atmosphere may build up to equilibrium concentrations.

Figure 2 LNAPL distribution after spillage

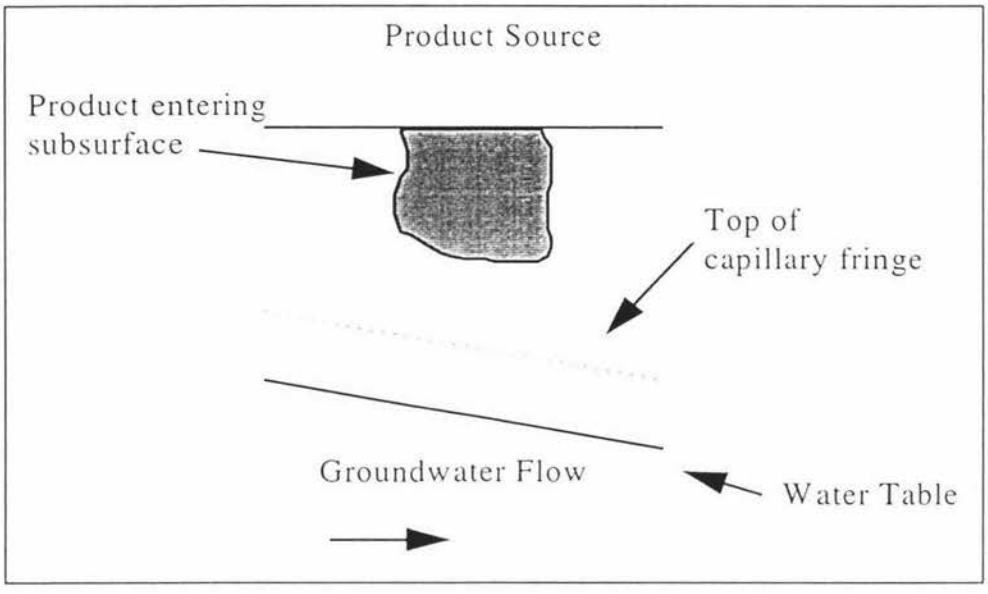


Figure 3 Depression of capillary fringe and water table

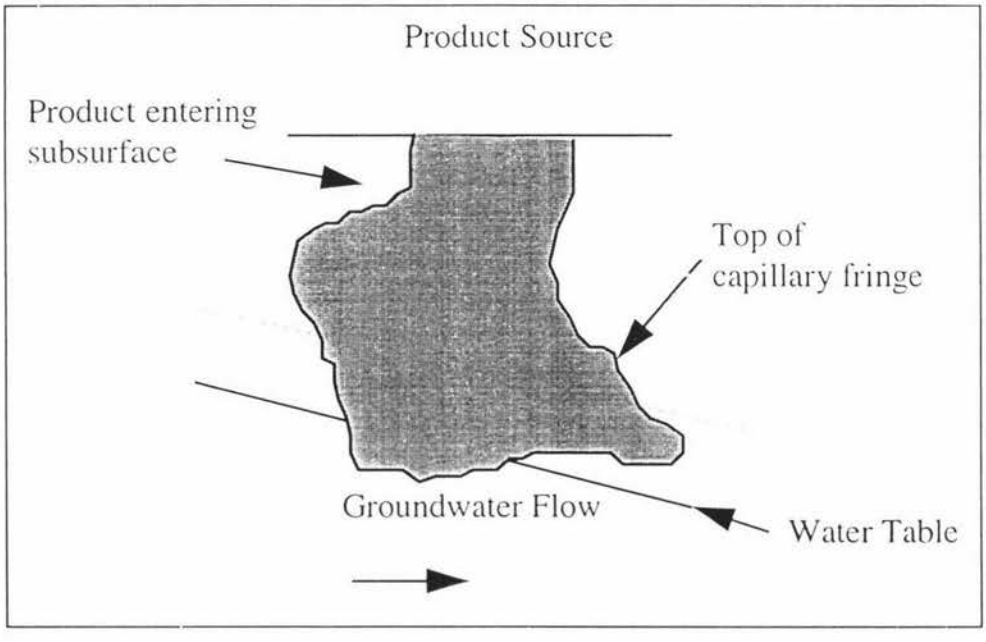
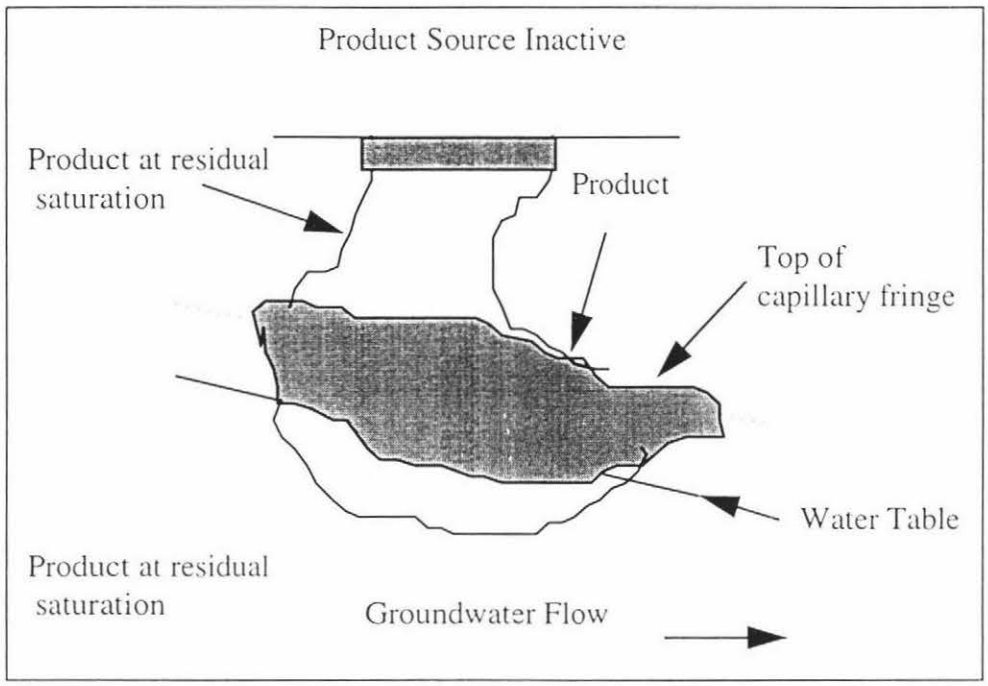


Figure 4 Rebounding of water table



If large volumes of product are spilled (Figure 3) the product flows through the pore space to the top of the capillary fringe. The dissolved components of the infiltrating product precede the product and may change the wetting properties of the water, causing a reduction in the residual water content and collapse of the capillary fringe.

The LNAPL product is lighter than water and tends to float on top of the capillary fringe. As the head created by the infiltrating product increases, the water table is depressed and the product begins to accumulate in the depression. If the source of the spilled product is then turned off, the LNAPL within the vadose zone continues to flow under the influence of gravity until reaching residual saturation. As this drained product continues to recharge the product pool, it spreads laterally on the top of the capillary fringe (Figure 4). The draining of the upper portions of the vadose zone also reduces the total head at the interface between the product and the ground water, causing the water table to rebound slightly.

The rebounding water can only displace a portion of the product because the latter remains at residual saturation. Ground water passing through this area of residual saturation dissolves the components within the residual product, creating a contaminant plume. Water infiltrating from the surface also can dissolve the residual product and vapours within the vadose zone, thereby contributing to the overall contaminant load to the aquifer.

If the water table drops because of seasonal variations or pumping, the pool of product also drops. If the water table rises again, part of the product is pushed upward, but a portion remains at residual saturation below the new water table. Thus, variations in the water table can spread the product over a greater thickness of aquifer, causing increased volumes of soil to be contaminated.

2.3.3 Dense Non-Aqueous Phase Liquids

DNAPLs can have great mobility in the subsurface as a result of their relatively low solubility, high density, and low viscosity. The sparingly soluble DNAPLs do not readily mix with water and therefore remain as separate phases. The relatively high density of these liquids provides a driving force that can carry product deep into aquifers. The combination of this high density and low viscosity is particularly important with regard to the transport of DNAPLs in the subsurface. When a high density, viscosity fluid (DNAPL) displaces a lower density, higher viscosity fluid (water), the flow is unstable" and viscous fingering occurs (Saffman and Taylor[7], 1958; Homsy[8] 1987, Kueper and Frind[9], 1988).

During a spill (Figure 5), DNAPL flows through the unsaturated zone under the influence of gravity toward the water table. If only a small amount of DNAPL is spilled, it flows until reaching residual saturation in the vadose zone. If there is water within the unsaturated zone, the DNAPL exhibits viscous fingering during infiltration No viscous fingering is observed if the vadose zone is dry.

The DNAPL can partition into the vapour phase and these dense vapours may sink to the capillary fringe. Infiltrating water can dissolve the residual DNAPL or the vapours and transport these contaminants to the water table, creating a dissolved chemical plume within the aquifer. If a greater amount of DNAPL is spilled (Figure 6), the DNAPL flows until it reaches the capillary fringe and, once there, begins to penetrate into the aquifer. However, to do this, the DNAPL must displace the water by overcoming the capillary forces between the water and the medium.

$$Z_c = 2\gamma\cos(\theta)\left(\frac{\left(\frac{1}{r_t} - \frac{1}{r_p}\right)}{\delta\rho g}\right)$$

The critical height of DNAPL required to overcome these capillary forces (Z_c) can be calculated from:

$$z_c = 2\gamma \cos(\theta)\left(\frac{1}{r_t} - \frac{1}{r_p}\right) / \Delta\rho g$$

where γ Is the interfacial tension between the water and the DNAPL, cos(θ) is the contact angle between the fluid boundary and the solid surface, rt is the radius of the pore throat, rp is the radius of the pore, $\delta\rho$ is the difference in the density between the water and the DNAPL, and g is the acceleration of gravity (Villaume[10], et al., 1983).

As an example, calculated critical heights required for perchloroethylene to penetrate saturated porous media of different grain size range from a few centimetres for coarse grains to tens of meters for clays (Table 1). Thus, unfractured, saturated clays and silts are effective barriers to the migration of DNAPL's, provided critical heights are not exceeded.

Table 1 Critical Height for Perchloroethylene to Penetrate Water Saturated Media

Material	Diameter	Critical Height cm
Coarse Sand	1.0	13
Fine Sand	0.1	130
Silt	0.01	1,300
Clay	0.001	13,000

Calculated for $\delta\rho = 0.62$ g/cm^3

Y= 47.5 dynes/cm, cosθ =1

After penetrating the aquifer, the DNAPL continues to move through the saturated zone until it reaches residual saturation. The DNAPL then is dissolved by ground water passing through the contaminated area, resulting in a contaminant plume that can extend over a great thickness of the aquifer. If finer-grained strata are contained within the aquifer, the infiltrating DNAPL accumulates on top of the material, creating a pool. At the interface between the ground water and the DNAPL pool, the solvent dissolves into the water and spreads vertically by molecular diffusion.

As water flows by the DNAPL pool, the concentration of the contaminants with the ground water increases until saturation or the down gradient edge of the pool is reached.

The relative density of pools and fingers of within the aquifer is important when controlling the measured concentrations of dissolved contaminants derived from DNAPLs. The existence of fingers and pools of the DNAPL, rather than relatively continuous distributions, in the subsurface accounts for the observation that the concentration of many of the DNAPL compounds in ground water are far below their saturation limit (Anderson, 1988). If even larger amounts of DNAPL are spilled (Figure 7), the DNAPL can, in principle, penetrate to the bottom of the aquifer, forming pools in depressions.

If the impermeable boundary is sloping, the DNAPL flows down the dip of the boundary. This direction can be up gradient from the original spill area if the impermeable boundary slopes in that direction. The DNAPL also can flow along bedrock troughs, may be oriented differently from the general direction of groundwater flow. This flow along low permeability boundaries can spread contamination in directions that would not be predicted on the basis of hydraulics.

Figure 5 Distribution of DNAPL after small volume spilled

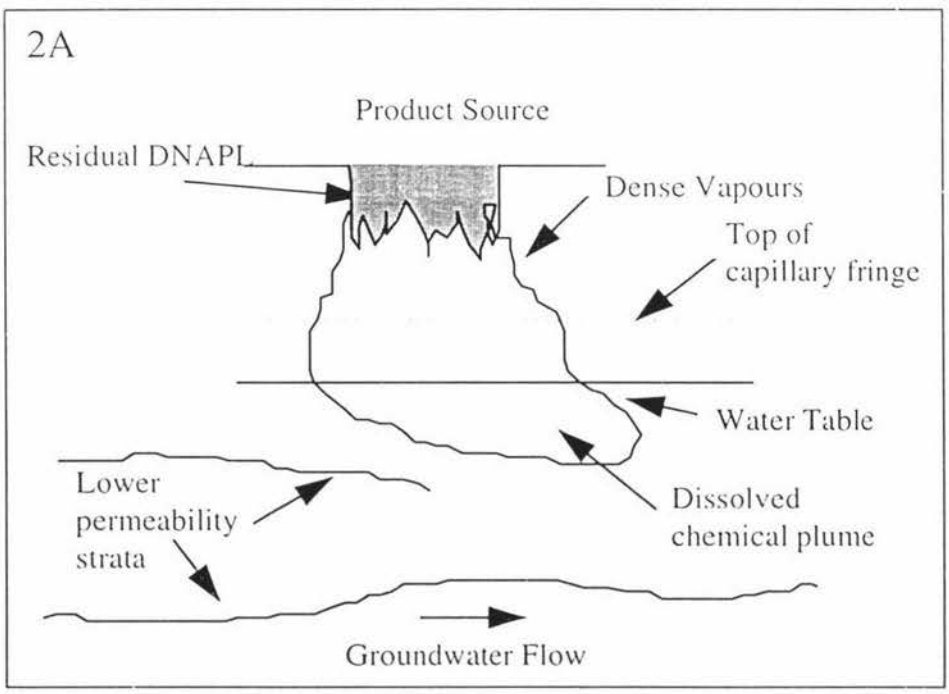


Figure 6 Distribution of DNAPL after moderate volume spilled

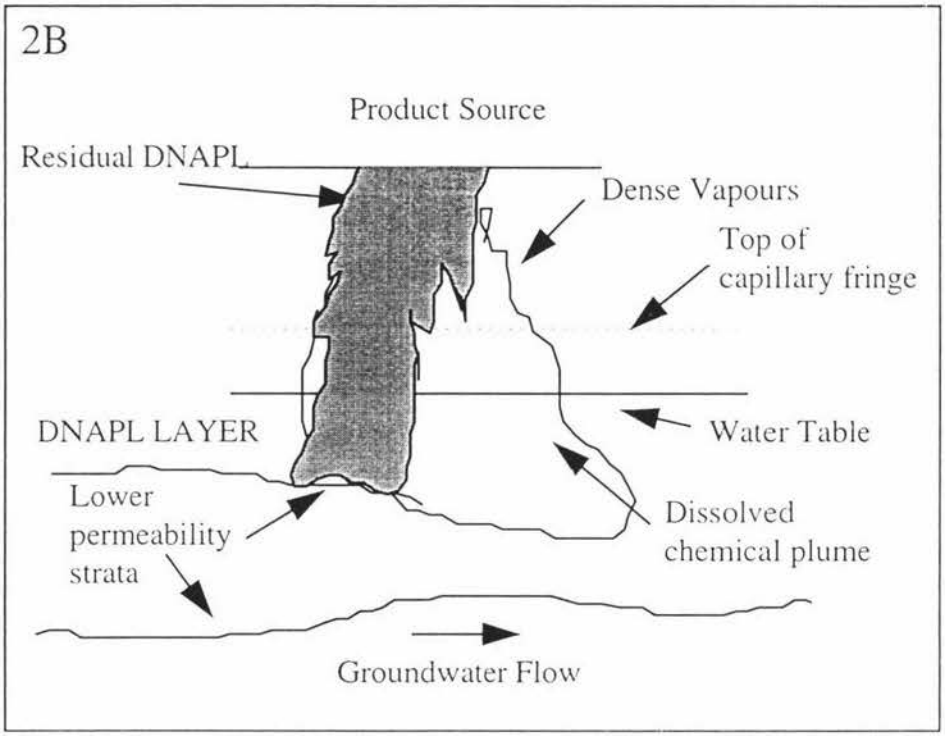
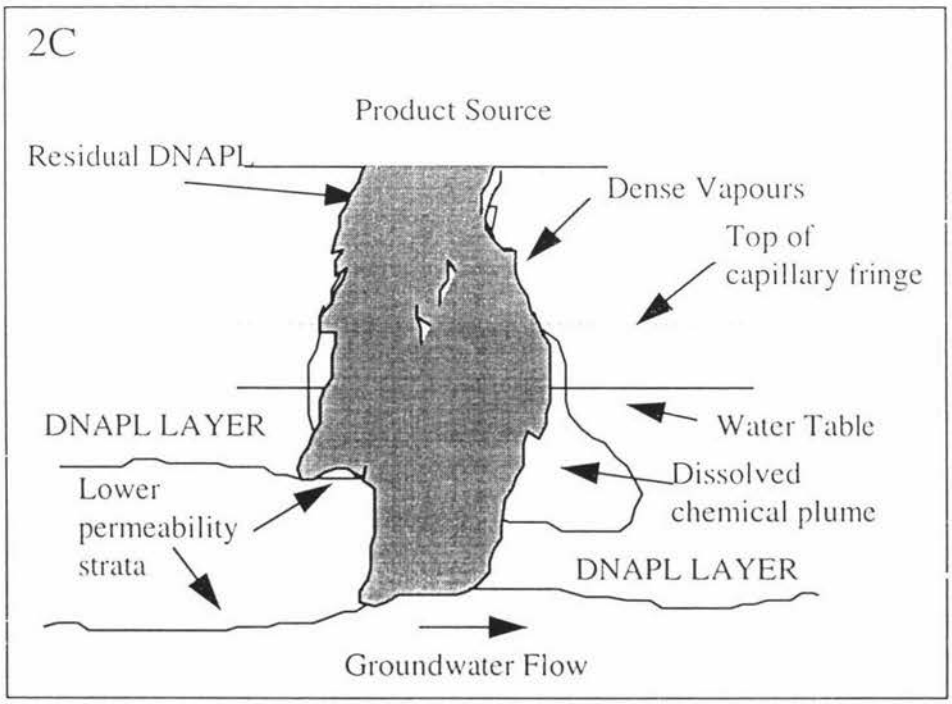


Figure 7 Distribution of DNAPL after large volume spilled



The transport of DNAPLs in physical models of fractures illustrates the importance of fracture aperture and roughness. Schwille (1988) found that if fracture apertures are greater than 0.2 to 0.5 mm the DNAPL moves directly to the capillary fringe where it spreads out. Eventually the DNAPL penetrates the capillary fringe and is transported to the bottom of the aquifer. There is very little residual DNAPL for fractures larger than 0.2 mm. If the fracture aperture is less than 0.2 mm and is smooth, the DNAPL spreads out near the surface and a few fingers migrate down to the capillary fringe.

At the capillary fringe, the DNAPL spreads out further and a few relatively wide fingers penetrate below the capillary fringe. If the fracture is rough, there is a great amount of fingering and the DNAPL penetrates below the capillary fringe in small, scattered fingers. Thus, for fractures with apertures less than 0.2 mm, there can be a large volume of DNAPL that remains at residual saturation in the fractures both above and below the capillary fringe. While similar behaviour is expected to occur in fractured rock, the statistical distribution of fracture aperture and fracture roughness may preclude the use of such a simple categorisation based on the mean apertures

3. REMEDIATION TECHNIQUES

3.1 INTRODUCTION

In nations with developed market economies contaminated and polluted land and its remediation have become major environmental concerns. This concern has led to research and development of commercially viable remedial techniques for soil decontamination. A wide range of remedial techniques have been considered and many of them are summarised in this section. These techniques aim to protect the environment from exposure to hazards by dealing with the problem at source. Remediation of soil requires a multi-disciplinary approach and advances have occurred due to input from microbiology, soil science, chemistry, environmental engineering, and civil and chemical engineering.

Remediation techniques can basically be divided into three modes Sims[11] 1990

- *In Situ*
- *Prepared Bed and*
- *In Tank reactors*

Within these techniques the separation process involved can be physical, chemical or biological. These methods and their appropriate processes aim to achieve separation, volume reduction, immobilisation and detoxification where possible. An in situ treatment system consists of treating the contaminated soil in place, where the contamination is located.

This approach is the most favourable to site owners as it causes the least disruption to site activities and avoids the movement of large volumes of fill both onto and off site as is the case with prepared bed and in tank processes.

The prepared bed method deals with contaminated soil in one of two ways:

1. By physically moving the soil for on site treatment to avoid transport of contaminants

2. By provisional removal of soil from the site in order to prepare the site for use then return of the soil after the treatment is complete. The bed contains the soil to be treated within an impermeable barrier such as plastic or clay to prevent outward migration of contaminants. It can also be achieved by adding clean soil to provide a favourable medium for treatment. As with the in situ method, chemical, physical or biological methods or any combination of these can be used to improve treatment.

The in tank treatment utilises industrial processes in which the soil is removed off site to be treated in an enclosed reactor. The soil may be in an unsaturated or a saturated form, and treatment includes composting, slurry phase and solid phase methods.

It should be noted that these techniques are limited to the remediation of soil that lies near or at the surface. However, this pollution is followed by pollution of the deep lying strata of the soil and ground water, and that this is even more difficult and complex to deal with. Ground water pollution will remain in the aquifer for longer due to slow movement and the impact of the desorption of the contaminants.

3.2 PHYSICAL TECHNIQUES

3.2.1 Excavation

Excavation is the simplest and oldest prepared bed method. It's aim is to transfer the contaminants elsewhere or to prepare the contaminated soil for on site and off site treatments. Process drawbacks are that it is difficult to locate the boundary of contamination, the identification of a suitable location for disposal, the requirement for large quantities of clean fill material and the increase in ground water pollution which results from disturbance of the soil column. Other problems include traffic movement, noise, atmospheric and surface water pollution.

3.2.2 Entombment

This is prepared bed method which is used to collect most of the contaminated soils together at one or more places on the site and to construct tombs to prevent dispersion. It can be applied to soil contaminated with a range of contaminants. The drawbacks are contamination of the collection area, movement of contaminants and the fact that an effective structure has to be constructed. Entombment is a popular method particularly in the United Kingdom.

3.2.3 Cover

This applies to in situ and prepared bed methods and has been widely applied in the UK, although it is considered an inappropriate method by the environmental cognoscenti. The cover materials vary from asphalt and concrete to clean soil. The drawbacks of this method is that the covered soils remain contaminated and there can be lateral contamination from the material.

3.2.4 Soil washing

This is an in tank method for separation and volume reduction of contaminants. It is used for the removal of organic and inorganic contaminants , but is most suited for soils polluted with non volatile hydrophobic and hydrophobic organic compounds and heavy metals. The washing process is less effective with soil that contains high amounts of clay and organic materials and semi volatile substances. Apart from the overall ineffectiveness of the soil washing process, treatment of the washing fluid presents another problem with its high toxicity, low treatability and its effect on soil permeability.

3.2.5 Soil flushing

This is an in situ method that aims to separate and reduce the volume of the contaminants. Flushing solutions that may be used include water, acidic and basic solutions, surfactants and solvents. The main difference between soil washing and flushing methods is that the former involves in situ application and the latter is basically an on site and/or off site process. Thus the problems posed in soil washing also occur here.

3.2.6 Soil vacuum extraction

This applies to in situ and prepared bed methods and uses air stripping to extract contaminants from unsaturated soil by injecting air into the unsaturated zone. The close contact causes a mass migration of contaminants from the soil matrix into the air. The difficulties of this method is inherent site heterogeneity and saturated soil materials.

3.2.7 Super critical extraction with Adsorption

This applies to in tank methods. Organics are continuously extracted using a supercritical fluid. However this process is not viable for soils contaminated with very high levels of organics because the volume reduction achieved by the transfer of the organic from soil to the activated carbon is not significant.

3.2.8 Electro-reclamation

This is an in situ method of electro kinetics which occurs when the soil is electrically charged with direct current by one or several electrode arrays. The method also requires the insertion of a water reticulation system to facilitate collection at the cathode. The method is based upon the movement of soil moisture containing metals from the anode to the cathode, and the effectiveness of the technique depends on the chemical composition of the parent soil. The drawbacks of this method are the limitations of the electric current that can be applied to the soil and the length of time required for the process.

3.2.9 Particle size separation

This is an in tank method for particle size separation by gravity. It involves the removal of the finest particles from the site and is based on the assumption that the contaminants will be associated with the finest soil particles. The drawback of this method is its complexity and the difficulty of treating fine particles.

3.3 CHEMICAL TECHNIQUES

3.3.1 Neutralisation

This is a detoxification and immobilisation process designed to reduce the reactivity and corrosiveness of acid- alkali containing systems. It can be carried out by in situ, prepared bed and in tank methods. The main shortcoming of this process is the difficulty of achieving compatibility of contaminants and treatment chemicals to prevent the formation of more toxic or hazardous compounds.

3.3.2 Oxidation

This process can be used as in situ, prepared bed and in tank methods for the detoxification of oxidisable contaminants. A major hazard to this process are the possibility of explosive reactions, the production of more toxic or hazardous products and the non selective nature of this process.

3.3.3 Photolysis

This is a prepared bed method which uses a photochemical reaction and generally involves using sunlight in natural systems. It is used in the detoxification of dioxins and nitrated contaminants. The inability of light to penetrate deep into the soil is the major drawback of this technique.

3.3.4 Precipitation

This technique can be carried out in three ways, in situ, prepared bed and in tank methods. The main function of the method is the separation, volume reduction and immobilisation of metals and certain anions, by the formation of insoluble precipitates. The long term effect of the precipitation technique has not yet been tested, and its use could have unfavourable effects on soil permeability.

3.3.5 Reduction

This is a detoxification process for the heavy metals chromium, silver and mercury, using in situ, prepared bed and in tank methods. The drawbacks of this process are the risk of explosive reactions and the production of more toxic and hazardous substances and its possible effect on other substances.

3.3.6 Carbon adsorption

This a separation and immobilisation technique, most suitable for organic contaminants and those contaminants with high molecular weight, high boiling point, low solubility and polarity. It can be used in situ and in prepared bed methods but the long term stability of the adsorbed complexes is unknown. A major disadvantage of this process is processing of the deactivated carbon.

3.3.7 Ion exchange

This is a separation and immobilisation technique, suitable for metal contaminants. It uses in situ and prepared bed methods. The drawbacks are that its use is to a few organic contaminants in suitable soils and that it requires the control of pH.

3.4 THERMAL TECHNIQUES

Thermal techniques are in tank processes that use high temperatures as the primary method of volume reduction, destruction and or detoxification of contaminants.

3.4.1 Fluidized Bed

This is a thermal process of volume reduction and detoxification for halogenated and non halogenated organic and organic cyanides. the drawbacks of this process are the high maintenance requirements, and the necessity to use homogenous soil which has small particle sizes and a low content of sodium and other metal.

3.4.2 Rotary kiln

This is a volume reduction and detoxification process for combustible organic and inorganic contaminants. The drawbacks are that it only produces high particulate emissions and the fact that only small particles can be treated, which necessitates the additional use of size reduction equipment.

3.4.3 Pyrolysis

This is a volume reduction and detoxification process for contaminated soil which is unsuitable for conventional incineration and soils containing volatile metals or recoverable residues. Experience to date has shown that this process can only handle small amounts of treatable material at any one time. No significant industrial usage has been described.

3.5 SOLIDIFICATION AND STABILISATION

3.5.1 Cement solidification

This is a storage and immobilisation technique carried out in tank and in situ for metal cations, latex and solid plastic wastes. It is carried out by mixing cement with the soil to form a hardened matrix. The drawbacks are its incompatibility with large amounts of dissolved sulphate salt or metallic anions such as arsenate or borates and difficulty in achieving cement hardening in a short time when organic matter, lignite sand and clay are present. Moreover the long term stability of the method is unknown, in particular it may eventually increase the soil permeability and therefore increase run-off.

3.5.2 Pozzolanic solidification

This is a storage and immobilisation technique which is carried out in tank and in situ for metals, waste oils and solvents. It is based on the reaction of lime with fine grained siliceous material. The drawbacks of this technique are that a complete and uniform mixture of soils and reagents must be used and that it reduces soil permeability

3.5.3 Thermoplastic stabilisation

This is a volume reduction, storage, immobilisation technique carried out in tank and in situ for complex soils that are difficult to treat. Basically it involves sealing the soils in the matrix such as asphalt bitumen, paraffin, or polyethylene. This technique has very limited use in soil with high water content or which contains strongly oxidising contaminants, anhydrous inorganic salts, tetraborates, iron and aluminium salts, or organics with low molecular weight and high vapour pressure.

3.5.4 Vitrification

This storage and immobilisation technique is carried out in a tank and in situ for inorganics and some organics. The major drawback of this process is its high energy requirement especially in soils with high water contents.

3.6 BIOLOGICAL TECHNIQUES

Biological techniques use micro-organisms to break down organic molecules to simpler compounds and or detoxify contaminants provided the soil is not so polluted that it is biocidal. It is mainly effective with organic contaminants, but it has been utilised in certain circumstances to oxidise or reduce inorganic contaminants through biochemical reactions.

3.6.1 Aerobic bioremediation

This is a technique for detoxification of biodegradable contaminants by aerobic microorganisms using in tank, prepared bed and in situ methods. The drawbacks are the difficulty in the maintenance of conditions conducive to biodegradation and the high scientific requirement for staff.

3.6.2 Anaerobic bioremediation

Technique for detoxification of certain halogenated organic contaminants by the use of anaerobic microorganisms. Drawbacks are the production of toxic intermediates the length of time required and the need for aerobic degradation step to complete the degradation process.

3.6.3 Land farming

Land farming is a bioremediation process that is performed in the upper soil zone or in biotreatment cells. The process consists of the controlled degradation of contaminated materials in an above ground system or with in situ treatment near the soil surface. Land farming is widely used and has been successfully applied to many wastes. Equipment requirements are typical to agricultural operations. These land farming activities cultivate and enhance microbial activity thereby increasing degradation of the target contaminants

3.6.4 Phytoremediation

This is an in situ technique. Phytoremediation is the use of vegetation to remove contaminants by uptaking them and storing them in plant tissue where it can be metabolised as with organics or stored in tissue and collected in mulch and then processed as with some heavy metals. Phytoremediation is most effective at sites with shallow contaminated soils, where nutrient and organic contaminants can be treated in the rhizosphere and by root uptake. Although deep contaminated sites and those with deep pools of non aqueous phase liquids are not good applications, deep groundwater may be treated by pumping and drip irrigation on plantations of trees. However the technology is only recent and there has been few full scale applications at this stage.

3.7 REMEDY SLECTION

The parties having a stake in the selection of a remedy come from diverse interests representing the local community, industry, environmentalists, the scientific and engineering professions, and government. Their concerns differ as do their values. They will inevitably disagree about what is the best remedy, and even as to what level of protection is needed. However, even if decision making waer confined to the technical community, engineers and scientists with experience in the same field would probably offer differing opinions regarding the needed level of protection and the best remedy. Four technical reasons explain the difficulty in selecting a remedy.

1. Site conditions can be complicated.

2. Remediation is not a straight forward but takes place over a long period of time.

3. A great number of alternatives exist, each having its own technical tradeoffs.

4. Experience does not yet exist to show clearly how best to proceed.

Because of these complicating factors, the selection of a remedy involves a great deal of judgment. The process of how this is done is important to ensure that relevant information and opinions are collected and considered. Selecting a remedy is equivalent to problem solving, and the literature is replete with models usually embodying a classic process as follows.

Define Problem→Establish Objectives→Develop Alternatives→
Select Alternatives→Implement and monitor.

4. BIOREMEDIATION OF PCP CONTAMINATED SOIL

4.1 INTRODUCTION

Pentachlorophenol (PCP) has been in the New Zealand marketplace for approximately forty years. PCP is a widely used biocide used mainly by the wood preserving industries. During the course of operation on timber treatment sites widespread PCP contamination has occurred.

The typical PCP wood preserving process includes dissolving 1 to 5 percent PCP in a heavy fuel oil and then forcing the mixture into wood using high temperature pressure treatment. Waste is produced when water and extractable components from the wood mix with the PCP and its petroleum carrier during pressure treatment, creating an unusable mixture. PCP solution bleeding from treated wood products after processing, plus spillage generate additional waste streams.

PCP is a highly toxic and persistent organochlorine. The United States Environmental Protection Agency (USEPA) has determined that PCP poses carcinogenic, fetotoxic and tetragenic risks. The presence of PCP in the environment therefore is of extreme concern.

PCP has been demonstrated to degrade through aerobic biodegradation, photodegradation and anaerobic biodegradation. Photodegradation is negligible in comparison to microbial action. Anaerobic treatment yields slower rates of degradation than aerobic metabolism of PCP. In addition, anaerobic treatment of PCP can result in non mineralised products, such as lesser chlorinated compounds, making aerobic treatment the preferred approach for PCP waste stabilisation at most sites.

In most sub-surface systems the availability of oxygen limits the rate of biodegradation. Soil venting the process of moving air from blowers or compressors through soil via air ducts or injection wells, can overcome oxygen availability problems and hence increase the rate of biodegradation.

The practice of inoculation is based on the assumption that contamination has persisted in the sub-surface because the micro-organisms present are unable to degrade the contaminant or that the biomass present is insufficient. The practice further assumes that nutrients or oxygen are not rate limiting or that they can be provided in significant amounts to ensure degradation.

OBJECTIVES

The objectives of the thesis was to

1. Determine if venting in addition to inoculation of microorganisms is effective for the remediation of PCP contaminated soil

2. Investigate the influence of biomass on the rate and extent of biodegradation of PCP in soil.

3. Evaluate efficiency gains in using bioslurry treatment of PCP contaminated soil.

4.2 MICROBIOLOGICAL PRINCIPLES INFLUENCING BIOREMEDIATION

4.2.1 Environmental fate of contaminants and treatment options

It may seem unusual to consider the environmental fate of a contaminant together with various options for the abatement of that contaminant. However, these topics are covered by the same two phenomena-the transport characteristics of a pollutant and the reaction of the contaminant with the environment. Table 2 generally predicts the fate of a contaminant in a specific environment in terms of movement, retention, and reaction processes as a function of the properties of both the environment and the contaminant.

Table 2 Mechanisms influencing the fate of contaminants in environments

Process	Environmental conditions	Contaminant
Movement	Water flow rate Formation permeability Water motion Gravity Surface tension	Amount of material Physical state Solubility Viscosity
Retention	Soil/Sediment Organic matter content Sorptive capacity	Type solubility Ionic character
Reaction	pH Redox status Microbial communities	Chemical transformation Biodegradability

The fate of the contaminant is largely a function of the chemistry of the pollutant in its environment. Similarly, the reaction mechanisms that a pollutant may undergo for example hydrolysis, precipitation, oxidation/reduction etc, are a function of that chemical and the existing environmental conditions. These various processes are interrelated. For instance, a chemical may be inherently susceptible to microbial attack, but it is deposited in an environment where the production of low molecular weight acids from the microbial metabolism of other forms of organic matter results in the decrease in overall pH. The immediate pH conditions may prevent the continued metabolism of that chemical.

4.3 ABIOTIC TRANSFORMATION REACTIONS

Hydrolysis, substitution, elimination, and oxidation-reduction are the abiotic reactions that will be discussed in this chapter. These reactions produce a variety of end products whose presence may play a role in decisions made to select compounds for the remedial investigation phase. The results of an abiotic reaction may enhance the biological degradability of a compound and provide possible treatment of the parent compound.

4.3.1 Hydrolysis

Hydrolysis reactions are those reactions where an organic chemical reacts with either water or a hydroxide ion to produce an alcohol. The following equations represent these reactions:

$$R\text{-}X + H_2O \rightarrow R\text{-}OH + H^+ + X^-$$

$$R\text{-}X + OH^- \rightarrow R\text{-}OH + X^-$$

In these reactions, either H_2O or OH^- act as a nucleophile and attack the electrophile , R-X, to displace the leaving group, X. This type of reaction is referred to as a nucleophile displacement reaction mechanism. The rate of hydrolysis reactions is typically first order with respect to the concentration of the compound. The rate of a first order reaction increases as the concentration of the organic compound increases. The first order rate constant k can be calculated as:

$$k = \frac{(2.303)}{t} \log\left[\left(\frac{C_0}{C_0 - C_t}\right)\right]$$

where t is time, C_0 is initial concentration, and C_t is concentration at t. The time required for half of the concentration of the compound to degrade is known as the half life, ($t_{1/2}$), and is calculated as:

$$t_{1/2} = 0.693k$$

Examples of hydrolysis half lives are given in Table 3 . A more extensive listing of half lives can be found in Dragun[12] (1988).

Table 3 Selected hydrolysis half lives for a variety of organic compounds	
Organic Compound	**Hydrolysis Half life**
Atrazine	2.5 hours
Chloroethane	38 days
Chloromethane	339 days
Dichloromethane	704 years
Ethyl Acetate	136 days
Diazinon	9.5 days
Iodoethane	49 days
Trichloromethane	3500 years
Trimethylphosphate	1.2 years

The rates of hydrolysis vary from compound to compound and can be on the order of hours to years. The rates of hydrolysis also indicate the susceptibility of the compounds to hydrolysis. Some examples of organic chemicals that are subject to hydrolysis are alkyl halides, carbamates, chlorinated amides, esters and epoxides. Examples of chemicals that are more resistant to hydrolysis are aldehydes, alkanes, alkenes, , and compounds with carboxy or nitro substituents. Once an organic compound enters the subsurface, environmental factors can decrease or increase the hydrolysis half life that may be expected from the results of laboratory evaluation

4.3.2 Substitution

Hydrolysis reactions are classified as a type of substitution reaction but they are presented first because of the predominance of water, which causes the reactions to occur. Other chemicals in the subsurface can cause substitution reactions to occur. An example of a substitution reaction involves hydrogen sulphide acting as the nucleophile agent to attack organic compounds, which result in the production of sulfur containing compounds.

4.3.3 Elimination

Elimination reactions cause the loss of two adjacent groups from within the molecule resulting in the formation of double bond. The reaction occurs as:

$$R\text{-}CHX_1\text{-}CH_2X_2 \rightarrow R\text{-}CH{=}CH_2 + X_1 + X_2$$

One example of an elimination reaction is the formation of 1,1-dichloroethane from 1,1,1-trichloroethane. An additional formation product of an abiotic reaction was the detection of acetic acid formed as a result of substitution. The ratio of acetic acid to 1,1-dichloroethane was about 3:1 Cline[13] et al 1988. Elimination can also result in the formation of bromoethene from 1,2 dibromoethene and bromopropene from 1,2 dibromopropane Dragun 1988.

4.3.4 Oxidation-Reduction

Oxidation is the net removal of electrons from an organic compound, while reduction is the net gain of electrons by an organic compound. These reactions are coupled by the transfer of electrons from one compound to another. The oxidation reduction couples in soil systems are complex and multiple. In many instances, if a biological response to an organic compound occurs, the biological system will tend to become predominant.

Abiotic reactions may occur in the subsurface by a variety of mechanisms and at varying rates. The use of abiotic reactions as a remediation technology has hitherto not received a lot of attention , but may provide an alternative treatment in some instances. Abiotic reactions may occur in conjunction with biological reactions and make some compounds susceptible to biodegradation. Abiotic reactions may not always provide extensive treatment of the organic compound but the treatment that does occur may produce a compound that is of less environmental concern.

4.3.5 Electron Acceptors

The catabolic reaction involve a transfer of electron from the waste to an electron acceptor, and the biological process by which this occurs is termed respiration. In aerobic respiration, bacteria utlise oxygen as the terminal acceptor of electrons removed from oxidised organic compounds. Typically, in the case of biotransformation, oxygen is added to the organic molecule and hydrogen is removed (oxidation of an alcohol to an acid). Upon complete mineralisation, oxygen is reduced to water and and organic carbon is oxidised to carbon dioxide. The mass of oxygen required by aerobic systems can be calculated based on stoichiometric equation or laboratory determinations.

Anaerobic processes may be defined as treatment that occurs in the absence of oxygen. This definition greatly simplifies a complicated system of multiple reactions resulting in the conversion of hydrolyzed organics to higher organic acids. These are subsequently degraded to acetic acid, hydrogen and carbon dioxide which are metabolised by methanogenic bacteia, therby producing methane. Unlike aerobic processes oxygen is not the terminal electron acceptor. Instead, the terminal electron acceptor in anaerobic respiration can involve any of several inorganic oxygen bearing compounds in the following order of preference.

Nitrates	Reduced to nitrogen: Also referred as anoxic rather than anaerobic.
Sulphates	Reduced to hydrogen sulphide.
Carbon dioxide	Reduced to methane.

The rate of anaerobic degradation is usually lower than aerobic degradation. However anaerobic degradation offers key advantages when degrading high strength wastes. By not requiring free oxygen the expense of aeration is eliminated. By converting more of the substrate to methane the process produces less biomass per unit of organic waste removed.

4.4 BARRIERS TO BIODEGRADATION

4.4.1 Environmental barriers to biodegradation

In order to grow, micro-organisms need a suitable physical and chemical environment. Extremes of temperature, pH, salinity, osmotic or hydrostatic pressures radiation, free water limitations, contaminant concentration, and/or the presence of a heavy metal or other toxicant materials can adversely influence and even limit the rate of microbial growth and/or substrate utilisation. Often two or more environmental factors interact to limit microbial decomposition processes, and, in fact, environmental barriers can act to render normally labile substances persistent.

This explanation was used by Woods Hole researchers (Jannasch[14], et al 1971) who found food substances from an accidentally sunken and subsequently recovered submarine to be almost preserved after 10 months exposure to deep sea conditions. However, when the food sample was incubated in sterile sea water at in situ temperatures (3 C), the materials putrefied after a period of only a few weeks. The authors suggested that the hydrostatic pressures of the sea (150 ATM) effectively raised the minimal temperature necessary for microbial proliferation. Once this increase exceeded incubation temperatures, microbial activity slowed 10 to 100 fold.

Recognition of the nature of the limiting environmental factor(s) and a consideration of its practical application to subsurface environments will help dictate which type of bioreclamation procedures to use. For example, the temperature of the subsurface 20-30 metres below the surface probably could not be significantly altered to stimulate in situ microbial growth and metabolism. However, the same is not true for a surface biological treatment process.

4.4.2 Physiological Barriers to Biodegradation

In addition to the immediate environment, various microbial physiological factors can influence the bio-transformation of pollutant chemicals. Like all forms of life, the requisite micro-organisms present in the soil are primarily composed of carbon, hydrogen, oxygen, nitrogen, phosphorous, sulfur, and a variety of trace elements. These substances are required in varying degrees for soil micro-organisms to proliferate. Soil micro-organisms can utilise such substances to the point where one or more of the requirements are exhausted and effectively limit further microbial growth or metabolic activity. For soil remediation efforts to succeed, these materials must already be present in the soil or be supplied in the proper form.

Ideally, the organic pollutants in the soil represent an appropriate supply of carbon and energy necessary for heterotrophic microbial growth. However that supply can be too high or too low. Too high a substrate concentration can limit microbial metabolism due to toxicity of the substrate to the requisite microflora. In contrast, contaminants also can be present at concentrations that are too low to allow microbial response and/or may not be suitable growth substrates. Growth and energy sources need not come from the same carbon substrate. The growth and metabolism of degrading microbes can sometimes be stimulated by providing them with a non harmful primary carbon substrate so that the rate and extent of pollutant decomposition can be proportionally increased (McCarty[15], 1985).

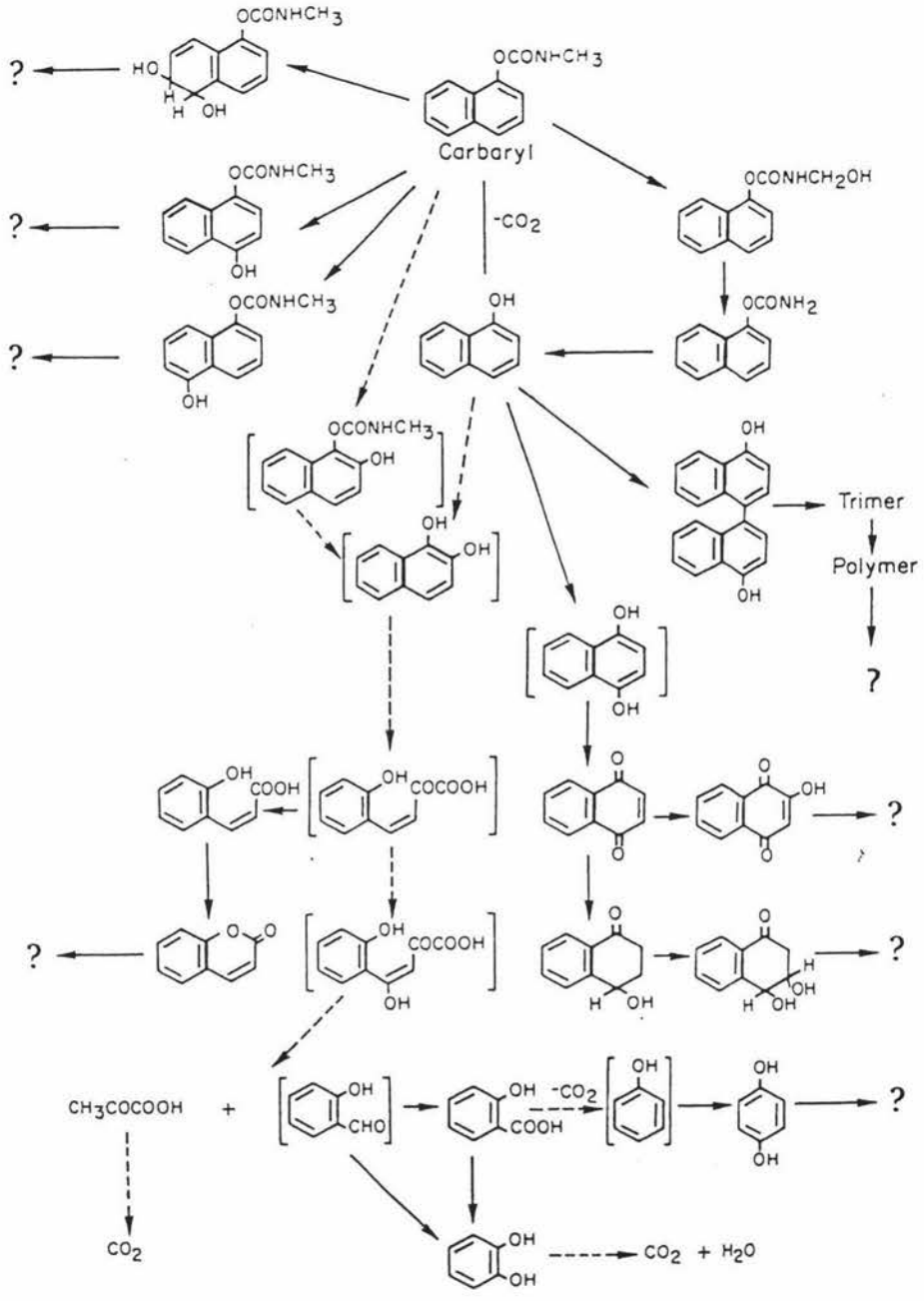
A chemical will also be metabolised poorly if it is unable to enter microbial cells. This may occur with either natural or anthropogenic polymers. While the monomeric units may be inherently amenable to microbial destruction, the larger molecular weight polymers persist because they often fail to gain access to intracellular catabolic enzymes. A substrate will also persist if it fails to depress the enzymes necessary for its destruction. It may be possible to induce the appropriate enzymes with an alternate chemical compound. Occasionally, initial biochemical reactions result in metabolites that tend to inhibit the degradation of even the parent molecule and can adversely affect the biodegradation of some pollutants.

Lastly, the absence of other necessary micro-organisms can limit the destruction of a contaminant. Often, several microbial groups are needed for the destruction of a pollutant. In anaerobic environments, this type of relationship is a prerequisite for the completion of the carbon cycle. The anaerobic mineralisation of organic matter is critically dependant on obligate microbial consortia; if any of the individual members of a consortium are absent, the biodegradation of the parent material effectively ceases.

4.4.3 Chemical Barriers to Biodegradation

One of the most important factors that influence the degradation of a contaminant in soil is the contaminants structure, which can dictate the pollutants physical state (for example soluble, adsorbed, conjugated etc) and thereby alter its tendency to biodegrade. However it is important to be specific when referring to biodegradation. When a compound undergoes primary attack, initial metabolic events often result in reaction products with their own environmental impact and persistence characteristics. An example of this is the fate of the pesticide carbaryl. Carbaryl is widely used as a substitute for DDT and is touted as being readily degradable. However this chemical is known to have a myriad of environmental fates as shown below in Figure 8.

Figure 8 Pathway of carbaryl degradation



Examination of Figure 8 reveals that some metabolic routes do lead to the ultimate conversion of carbaryl to carbon dioxide, while others result in the formation of complex aromatic metabolites, the fate of which is still unknown.

Therefore, when evaluating the scientific literature, the distinction must be made between "biodegradation" and "mineralisation". The mere loss of a chemical from an environment may or may not be a desirable consequence of bio-transformation processes. If biodegradation results in the production of undesirable metabolites, it may be best to choose a non-biological strategy for a soil remediation project.

The example above illustrates the need to understand the overall fate of a contaminating chemical in the soil and how informed decision making must be based on solid metabolic information.

Soil pollutants may contain various chemical linkages that tend to favour or hinder microbial attack. However, broad generalisations on the biodegradability of various linkages tend to be of limited value since substitute effects drastically alter the susceptibility of even simple organic molecules to bio-transformation. The number, type and position of the substituents must be considered when evaluating the metabolic fate of particular contaminants in soil.

The effect of branching is illustrated in Table 4 which is adapted from a USEPA seminar publication.

Table 4 Microbial Growth response to Phenyldecanes

Organism	Strain	1-phenyldecane	1-phenyl-4-methyldecane	1-phenyl-4-4-dimethyldecane
Micrococcus cerificans	H.O.1-N	2	0	0
	H.O.3	2	0	0
	H.O.4	2	0	0
	S-18.2	2	0	0
	S-14.1	2	0	0
Pseudomonas aeruginosa	119 JWF	2	0	0
	191 JWF	2	0	0
	Sol 20 JS	2	0	0
Mycobactrium phlei	No. 451	2	2	0
M. fortuitum	No. 389	2	2	0
M. rhodochrous	No. 382	2	2	0
M. smegmatis	No. 422	2	2	1
Nocardia opaca		2	2	1
N. rubra		2	2	1
N. erythropolis		2	2	1
N. polychromogenes		2	2	1
N. corallina		2	2	1

Where 2 = profuse growth, 1 = slight growth, 0 = no growth

Approximately 15 bacterial strains from several genera were evaluated for their ability to use a series of structurally related substrates. Cultures exhibiting profuse growth, slight growth, or no growth on a particular substance were assigned a qualitative index of 2,1, or 0, respectively. All cultures grew well with 1-phenyldecane, however a single methyl substitution on the side chain drastically influenced the susceptibility of the resulting structure to metabolism by the test organisms. All the myco-bacteria and nocardia were able to grow on 1-phenyl-4-methyldecane. However, the pseudomonads and micrococci were no longer able to do so.

When the substrate contained an internal quaternary carbon atom, as is the case for 1-phenyl-4-4-dimethyldecane, the molecule proved available to only a few organisms and permitted minimal growth at best.

This simple example illustrates that the chemical structure of the contaminant also influences its susceptibility to biodegradation. Despite the difficulty in generalisation, the scientific literature shows that the closer a contaminant structurally resembles a naturally occurring compound the better the possibility that the former will be able to enter into a microbial cell, depress the synthesis of catabolic enzymes, and be converted by those enzymes to central metabolic intermediates.

In addition, biodegradation is less likely, but not entirely precluded, for those molecules having unusual structural features only frequently encountered in nature. Ease of biodegradation can be viewed as a continuum ranging from very labile compounds to those that are recalcitrant.

When viewed in this context, it should not be surprising that xenobiotic compounds persist in nature because micro-organisms have not evolved the necessary metabolic machinery to attack those compounds. However as a group, micro-organisms are nutritionally versatile, have the potential to grow rapidly, and possess only a single copy of DNA. Consequently, any genetic mutation or recombination is immediately expressed. If the alteration is of adaptive significance, new species of micro-organisms can arise and proliferate. The polluted environment supplies selection pressure for the evolution of organisms with novel metabolic potential. Ultimately, the organisms may not only survive in the polluted environment, but also be capable of growing at the expense of the contaminating substance.

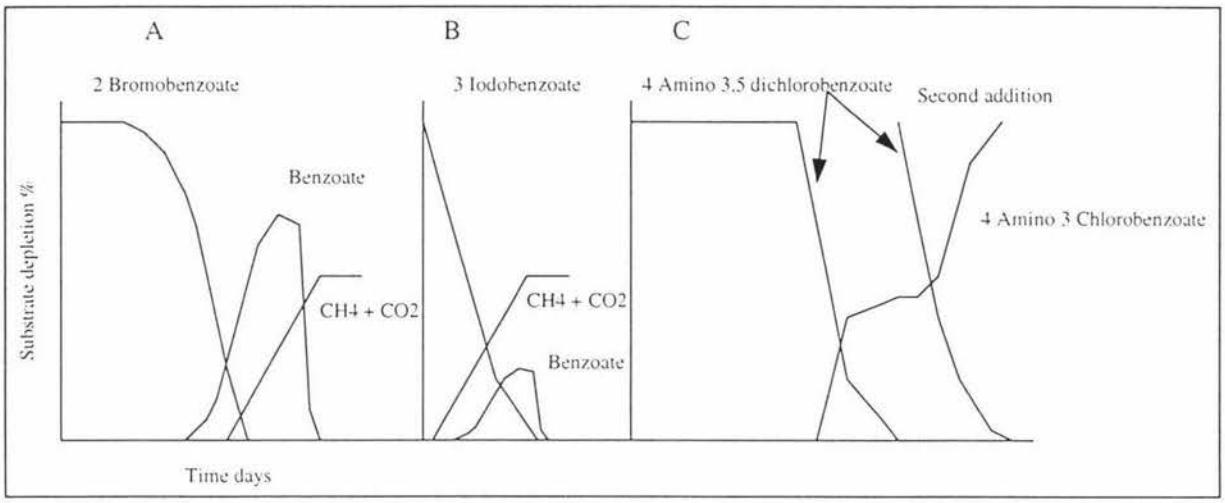
4.4.4 Lag, Adaptation, or Acclimation Periods

In biodegradation a period of time often is observed where very little substrate is turned over and correspondingly little product is formed. This phase of metabolism is potentially more environmentally significant than other phases because of its possible effects on the level of the exposure of humans and ecosystems to specific pollutants. The lag, adaptation, or acclimation phase is one the most frustrating portions of

bioremediation programs, since, despite all efforts, virtually nothing seems to be happening to the problem contaminant.

For example Figure 9A, shows the reductive dehalogenation of 2-bromobenzoate in anoxic sediment microcosms. The substrate lags for several weeks after which it is rapidly metabolised. The production of an intermediate catabolite, benzoic acid, occurs before its rapid degradation.

Figure 9 Lag Adaptation periods.



Ultimately the substrate and intermediate are converted to the gaseous end products, methane and carbon dioxide. At this point, the microorganisms are considered acclimated to the degradation of the parent substrate and subsequent substrate additions will be degraded without a lag.

This phenomenon is graphically illustrated for the reductive dehalogenation of a related substrate 3-iodobenzoate (Figure 9B). In this case, the second addition of 3-iodobenzoate is presented as time zero. Note the immediate consumption of the substrate, the much lesser accumulation of benzoic acid, and the more rapid conversion of the parent molecule to gaseous end products.

In the examples given in Figure 9A and 9B, acclimation might reasonably be expected since the microorganisms were able to mineralise the parent substrates. Some of the carbon and energy gained from the metabolism of those substrates was presumably used for the proliferation of the catalysing microbial communities.

If the concentration of the requisite microorganisms was initially very low, the lag period could conceivably be a function of the time required for these organisms to grow to sufficient density to effect some significant amount of substrate depletion relative to the large background added as the amendment. However, acclimation also is observed for substrates that are not mineralised.

As seen in Figure 9C, the substrate 4 amino-3,5-dichlorobenzoate can be reductively de-halogenated to form the mono-halogenated product following a long lag period. Even though the parent substrate is not mineralised and does not supply carbon for microbial growth, additions of the parent substrate following the removal of the initial amendment are metabolised without an additional lag period.

Examining and understanding the factors which influence the lag period can be difficult, but may ultimately lead to bio-restoration scenarios with controlled, reduced, or even eliminated adaptation times. The requirement for the growth of the requisite microorganism is most often touted as the operational reason for lag periods. While undoubtedly true, the example above illustrates that there may be other reasons for the delay in biodegradation.

The structure of the chemical itself is known to influence the rate of biodegradation. Figure 10 compares the adaptation period for a variety of halobenzoates in methanogenic sediment microcosms. Note that all the substrates were added at the same starting concentration and that they all possessed halogen substitutions at one or both meta (3 or 5) positions. When degraded, all of the substrates were metabolised via reductive dehalogenation reactions. The various substrates were degraded in a specific order; that is 3 bromo-bezoate (3 Br-Bz) degraded before 3-iodobenzoate (3-I-Bz) which in turn degraded faster than 3,5-dichlorobenzoate (3,5-diCl-Bz).

Therefore, chemical structure definitely influences the length of the lag period, and, perhaps more significantly, the length of the lag periods are relatively reproducible. Table 5 illustrates that a repeat experiment not only gives the same relative order of degradation, but also approximately the same length of the lag period. The lag period does not appear to correlate with whether the substrate is mineralised. This result implies that a specific physiological or chemical basis exists for the characteristic lag periods

Figure 10 Effect of chemical structure on adaptation periods.

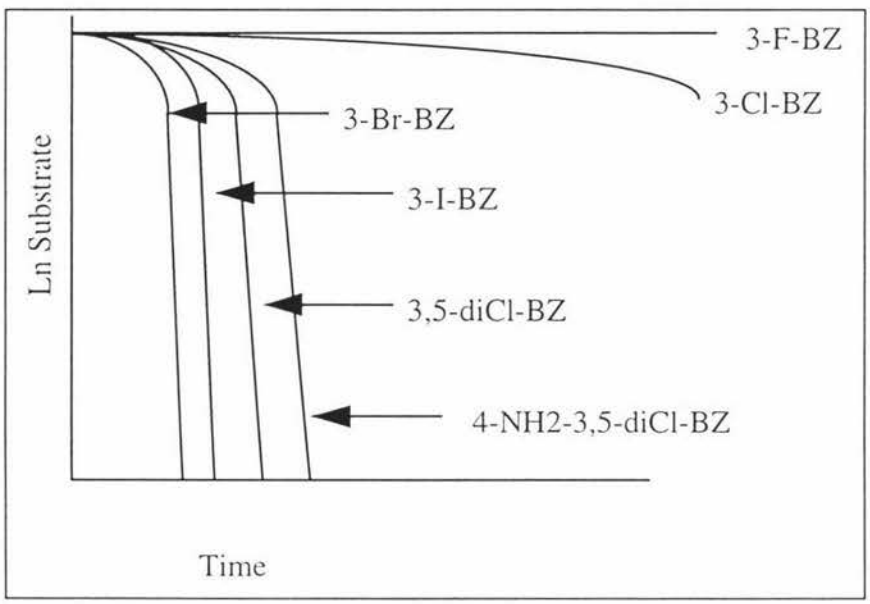


Table 5 Adaptation period (days)						
Expt	3-Br	3-I	3,5 diCl	4 NH2-3,5 di Cl	3-Cl	3-F
A	27	34	35	60-65	150-170	>170
B	21-24	28	29-35	37	125-148	>365

Figure 11 Effect of substrate concentration on length of adaptation period

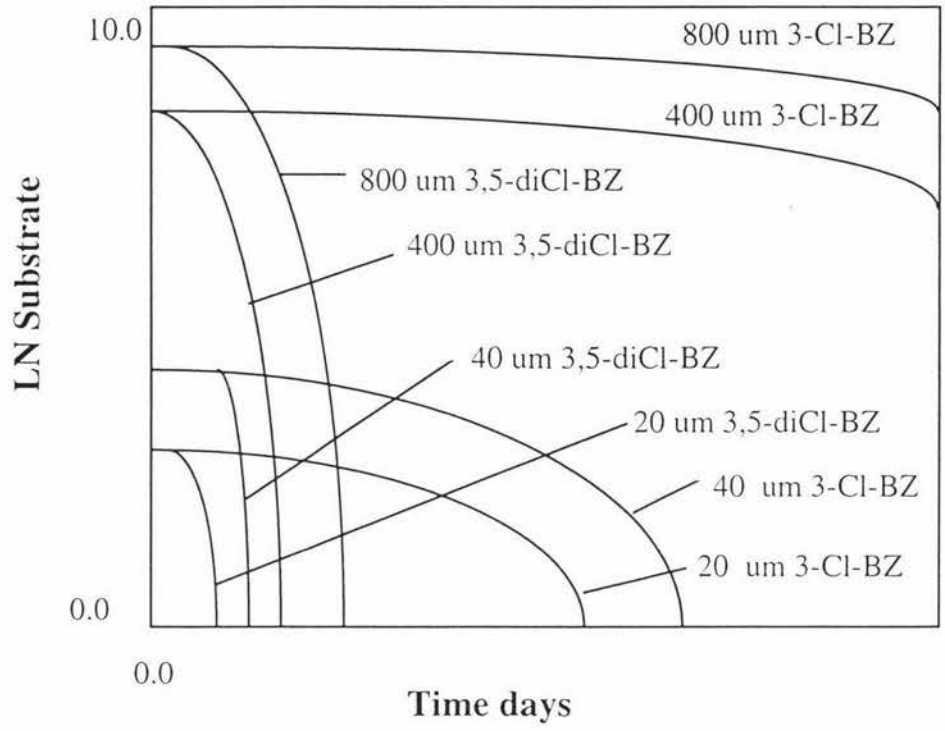
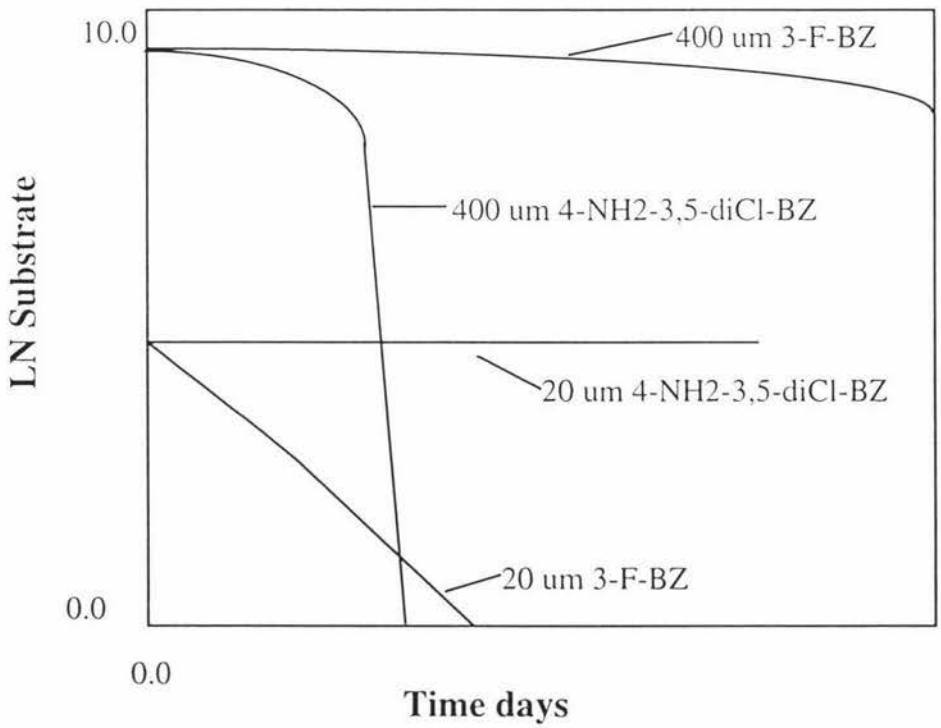


Figure 12 Differences in the adaptation period as a function of substrate concentration.



Other experiments show that concentration can markedly influence the length of the lag period. Figures 11 and 12 compare the reductive dehalogenation of several halobenzoates at various concentrations and illustrate a number of characteristic patterns. In the case of the anaerobic biodegradation of both 3,5-dichloro- and 3-chlorobenzoate at substrate concentrations ranging from 20-800 μM (Figure 11), the dichloro-substrate exhibits a characteristic lag time prior to rapid biodegradation regardless of the substrate concentration range.

However, the length of the lag period associated with the lower concentrations of monochlorobenzoate is much shorter than those observed with higher substrate concentrations. This perhaps is not surprising since the benzoates are known bacteriostatic agents and higher concentrations tend to inhibit microbial activity.

A similar situation also can be observed in the case of low vs high concentrations of 3-fluorobenzoate (Figure 12). However, the opposite result is observed when the substrate is changed to 4-amino-3,5 dichlorobenzoate. With this more complex substrate, concentrations $\geq$ 40 μM were degraded with a characteristic lag period. However, concentrations of $\geq$ 20 μM exhibited lag periods in excess of one year. If the concentrations of this substrate were only doubled, biodegredation would proceed in typical fashion and reach levels far below 20 μM. From these examples, one can see that when substrate concentrations are either too high or too low, biodegradation activity can be adversely affected and increased lag periods could result.

4.5 IN SITU BIOREMEDIATION

In situ bioremediation is the method of treating subsurface contaminants where they are found without excavating the overlying soil. That is, essentially all of the treatment actually takes place below the surface. As was discussed in Section 2.2, contaminants that have migrated into the subsurface from sources such as product spills and leaking disposal facilities exist in three pahses:

1.Free product
2.Adsorbed or otherwise bound into the soil matrix (sorbed phase)
3.Dissolved into the groundwater (solute phase)

4.5.1 Hydraulic Control

Isolation of the contaminated zone is the first design stage for remediation of a contaminated zone. Hydraulic control is required to either halt or remove groundwater flow, raise or drop the water table, and control the movement of the contaminated plume. The most frequently applied method of isolation is hydrological intervention. Hydrodynamic isolation systems are generally less costly than physical containment systems. They also increase system flexibility since pumping rates and flow patterns can be adapted as required over the remediation period. Hydrological control systems are designed such that groundwater is centrally withdrawn and reinjected at selected points.

It is impossible to achieve 100% isolation. However if the hydraulic configuration is too open the bioremediation may be unsuccessful or transport pollutants into previously uncontaminated zones. In many situations, hydraulic control can be improved by combination with physical methods. The hydraulic system is the means for operational control. Fluid flow provides delivery of appropriate nutrients, substrates, and electron acceptors within the sub-surface. The design goal is to provide a system of injection wells, recovery wells, and possibly barriers that allows for mass transfer into and out of the contaminated area.

The design of hydrological controls requires an understanding of the hydrogeology of the site. This includes details on the groundwater flow, interaction of multiple aquifers, and other aquifer abnormalities. Injection rates influence biodegredation rates, and an optimum flow rate is expected to exist for each site. Flow rates impact on solute, gas transfer rates, and contact times. As infiltration or recirculation rate changes, so does the flow velocity and dispersion. With increased infiltration the hydraulic detention time in soil decreases, and zones of influence usually increase.

Subsurface delivery systems usually rely on the hydraulic system used to isolate the treatment area. The goal of the delivery system is to deliver the bioremediation control agents to the zone of contamination in the most efficient manner. This requires the minimisation of losses, clogging, and promoting a uniform distribution within the zone.

Injection recovery systems rely upon the extent and location of the contaminants as well as the hydrological characteristics of the site. Injection systems can be divided into two groups gravity and force methods. In the case of gravity methods the required chemical can be applied directly to the contaminated soil or groundwater by flooding, spray irrigation, trenching. Forced delivery methods inject the necessary additives through a pressurised pipe.

The application of gravity feed systems is limited, as few installations are suitable for this technique.

4.5.2 Bioventing

In situ bioremediation was at its inception applied to contaminants in the saturated zone, since hydraulic control is necessary for the optimisation of the process. Bioremediation of the unsaturated zone has used water injection to raise the water table for hydraulic control. However, it is now recognised that significant bioremediation can occur in the unsaturated zone by forcing air into the soil through soil venting or air injection. Optimising bioremediation of the unsaturated zone with air is called bioventing.

One of the first projects to control soil venting for enhanced biodegredation was at the Hill Air Force Base in Utah (USA). During field clean up activities using soil vapour extraction for remediation of fuel, removal was also occurring from biodegredation. Bioremediation in the unsaturated zone has been reported for gasoline contaminated soil piles Conner (1988)[16], and PCP contaminated piles Valo and Salkinoja-Salonen [17] (1986).

Air venting provides an effective means to remove volatile organic compounds and induce oxygen. Air venting provides for multiple treatment modes. First, oxygen is supplied for biological remediation. Second, air venting removes significant levels of volatile organic compounds.

Bioventing is applicable to the unsaturated zones that have good gas permeability's. Gaseous permeability is related to hydraulic conductivity. However, soil moisture significantly affects gaseous permeability. As soil moisture increases, gaseous permeability drops. At 55% water saturation , gaseous permeability is decreased by approximately 80%.

Bioventing is applicable to contaminants that are degraded through aerobic metabolism and have vapour pressures less than 1 ATM. Highly volatile organic compounds will volatilise rather than degrade. For example, tetrachloroethylene is not amenable to bioventing since it is not degraded under aerobic conditions. Vinyl chloride which is easily degraded under aerobic conditions has been characterised as being moderately suited because of its high volatility. Significant portions may be volatilised rather than biodegraded. The BTEX compounds are good candidates for bioventing. They degrade readily under aerobic conditions and have a low enough vapour pressure that biodegredation is the main in removal mechanism.

When feasible, bioventing can provide a installation low in capital and maintenance costs. Oxygen can be delivered to the sub-surface with less energy than using water as a carrier. It also provides clean up of hydrocarbons that are not amenable to soil vapour extraction. Its limitations are that contaminants must be degradable by aerobic metabolism and the remediation of volatile compounds is slower than with soil vapour extraction.

4.5.3 Introduced Micro-organisms

Microbial movement through the sub-surface was first addressed as early as the mid 1920's for microbial enhanced oil recovery. At that time Beckmann[18] (1926) suggested that microorganisms that have the ability to produce emulsifiers or surfactants could be transported into an oil bearing formation to recover the oil that remains after a well has stopped flowing. At about the same time, research on the transport of micro-organisms through the sub-surface environment was being conducted to determine the effectiveness of on site wastewater disposal systems (for example pit latrines, septic tanks) in removing pathogens. More recently, the concept of transporting microorganisms with specialised metabolic capabilities has been proposed Thomas and Ward[19] 1989.

The addition of microorganisms to the sub-surface in remedial operations would be beneficial when contaminants resist biodegredation by the indigenous microflora, where evidence of toxicity exists, or when the sub-surface has been sterilised by the contamination event. Seed microorganisms have been added to the sub-surface to aid in contaminant biodegredation, however the role of introduced microorganisms has never been differentiated from that of indigenous microflora Thomas and Ward (1989).

For an added microorganism to be effective in contaminant destruction, it must be transported to the zone of contamination, attach to the sub-surface matrix , survive, grow, and maintain their degradative capabilities Thomas and Ward (1989). When injected into a non-sterile formation, the added organisms must compete with the indigenous microflora for limiting nutrients and escape predation. Transport through the sub-surface is a complex interaction between the sub-surface and the microorganism. Physical phenomena related to the composition of the sub-surface formation that affect transport include filtration and adsorption Gerba and Britten[20] (1984). Passage through the sub-surface will depend on grain size and related values of hydraulic conductivity and channels made by cracks and fissures. However, transport by channelling will probably result in uneven seeding of the formation.

The movement of bacteria and viruses in the subsurface is a significant problem. More than 50,000 individuals in the United States suffered from waterborne disease between 1971 and 1979 and about 45 percent of them involved ground water sources. In addition, increasing interest in the use of micro-organisms for in-situ remediation of aquifers contaminated with chemicals necessitates a greater understanding of the transport and fate of micro-organisms within the subsurface.

There are many processes that limit the movement of micro-organisms through geologic material. Some bacteria are large enough to be strained from the water. In comparison some viruses can pass through the pores, but their surfaces are charged and, like charged ions, may undergo adsorption under the proper chemical conditions. Like molecules micro-organisms are transported by diffusion. Some micro-organisms are motile and move in response to changes in chemical concentrations. Like other living organisms, microbes grow and die, and the rates of these processes must be included in the description of the transport of microbes in the subsurface.

4.5.4 Matrix Properties

Bioremediation of the sub-surface is limited to soils with hydraulic conductivities less than 10^{-4} cm/sec, because of the difficulties involved in pumping through material with lower hydraulic conductivities. Laboratory studies have tended to concentrate on sieved soils, however this artificial homogeneity has led to underestimates of microbial transport. The use of intact cores will provide a better estimate of microbial transport.

4.5.5 Moisture

Biodegradation requires moisture for two reasons:

1. For cellular growth, because cellualr tissue is 75-80% moisture.
2. As a medium for movement of the microorganisms to the subsurface, or vice versa, for non motile species.

Biodegradation in soil systems can occur at moisture levels well below saturation. Most bacteria fail to grow if the water content of the medium falls below 92% relative humidity.

However, it is generally accepted that the minimum moisture content necessary for treatment of wastes such as contaminated soil is 40% of saturation.

4.5.6 Temperature

Temperature has a major influence on growth rate. Cellular activity, particulary enzyme systems, responds to heat so that the rate of cell growth increases sharply with increasing temperature until the optimum is reached. Increases in temperature just a few degrees above the optimum can slow growth dramatically by inactivating enzyme systems and reducing reproductive capability. Continued exposure to high temperature results in denaturation of enzymes and death. Unlike high temperatures, low temperatures are rarely lethal. Instead the cells become dormant. Activity decreases when dropping below the optimum because of reduced enzyme activity and a loss of fluidity of the cell membranes

4.6 SLURRY TREATMENT

Slurry-phase reactors have been successfully applied to the decontamination of solids and sludges. Hazardous substances treated by slurry phase systems include polynuclear aromatic hydrocarbons, pesticides, petroleum hydrocarbons, and heterocyclic and chlorinated aromatics associated with wood preservation facilities. The ability to handle a broad range of contaminants at higher initial soil concentrations is an advantage of this treatment process.

4.6.1 Advantages over solids phase

Field bioremediation projects have demonstrated several advantages for slurry reactor systems over solid phase systems. Slurry reactor systems increase degradation rates compared with rates observed with solid systems. Desorption of the contaminant from soil particles must take place before biodegradation can occur. The exception is the case where waste exists as droplets to which microorganisms have attached. Desorption is particularly important with the soil-water suspensions characteristic of slurry-phase treatment.

The rate at which the pollutant desorbs from the soil particles is influenced by the physical and chemical properties of both the soil and the contaminant. Essentially, the same factors control desorption as control sorption. A portion of each pollutant is bound in a stable position along micropores within soil aggregates and particles. The relatively large size of microorganisms excludes them from the micropores, preventing their direct access to the sorbed contaminant.

However, some microorganisms do attach to the outer surfaces of the soil particles. To be accessible the contaminant must desorb to the micropore water and then move by diffusion to the outer surface of the soil particle where the solute is either degraded by attached microorganisms or passes to the bulk aqueous phase. Local equilibria may develop within the soil particle and act to slow further desorption. The high affinity of many compounds for soil (e.g., PAHs have a high affinity for clay minerals) will also inhibit desorption.

Investigations in the Netherlands and United States[21] claim that desorption-diffusion from the sorbed phase to the solute phase is the rate limiting factor for slurry phase treatment. This conclusion derives from observations in laboratory tests that:

1. The rate of degradation is a function of the concentration of the contaminant in solution rather than the total sorbed mass, and

2. Microbial populations grow at a linear rather than logarithmic rate.

Another investigator observes that this is true for lower molecular weight compounds even for those with fairly low solubility, however, for higher molecular weight PAH compounds, the degradation rate is the limiting factor[22]. With regard to the higher, unsubstituted PAHs, their solubility may be too low to support significant biological activity, again identifying desorption as the rate limiting factor.

It was noted in the Dutch research projects that when experimenting with different mechanical mixing techniques and the same pollutant, the mixing technique which reduced the soil particle size to 30 microns resulted in degradation rates several times greater than

the technique which yielded a particle size of 60 microns. Thus, an important consideration in pretreatment and mixing is the reduction of particle size. Similarly, washing the soil with a surfactant or other suitable chemical could facilitate transfer of contaminants into the aqueous phase.

For example the half life of phenanthrene has been reported at 8 days in slurry based systems compared with 32 days in solid phase systems LaGrega[23] et al (1994). Similar enhanced rates have been demonstrated for other compounds that have a high soil adsorption affinity.

The enhanced rates for degradation are the direct result of improved contact between the microorganisms and the hazardous compounds. The water phase provides greater homogeneity and reduces the concentration of the hazardous compounds. Typical applications include pesticides, PAHs, clay and silty soils and halogenated hydrocarbons.

Slurry phase systems do however suffer from several disadvantages. These by and large are related to additional process requirements and materials handling, causing higher costs for facilities. Slurry treatment is more costly than land farming and composting. It is, however usually less costly than incineration, solvent extraction and thermal desorption. Significant quantities of wastewater can result from solids separation and de-watering after slurry treatment. This wastewater may require treatment before discharge. However these disadvantages are often offset by greater removal efficiencies over solid phases systems.

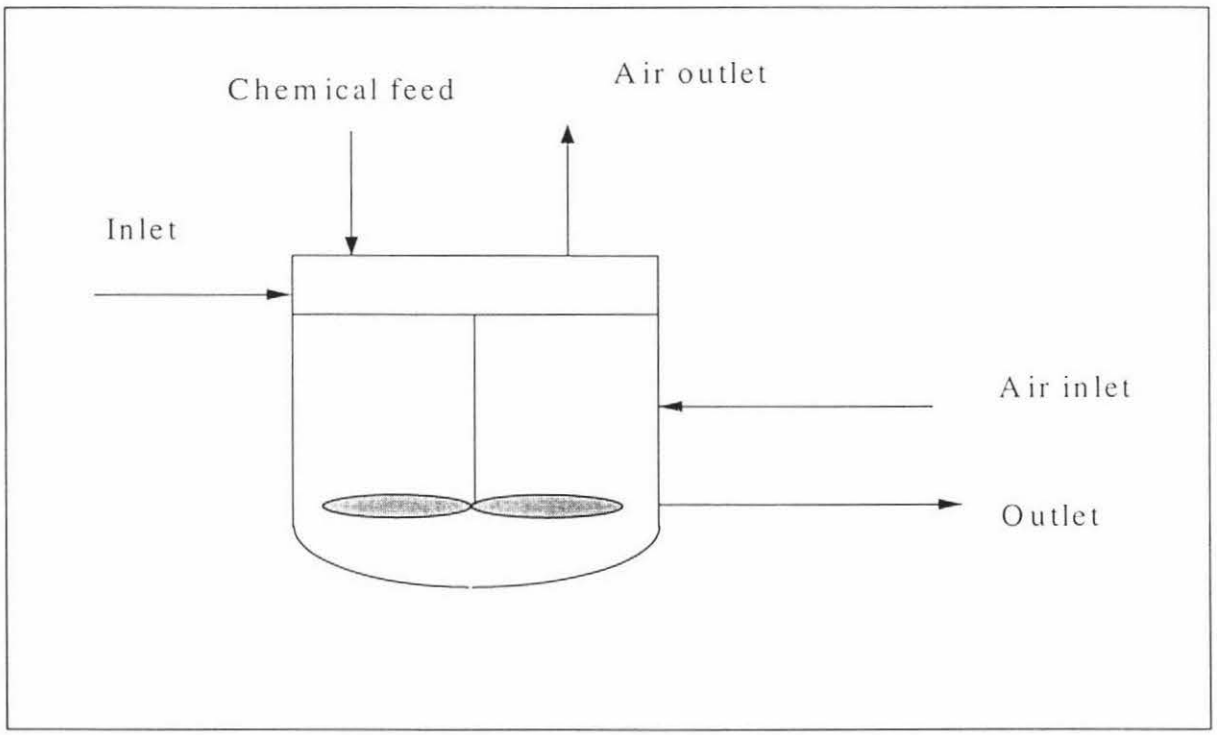
The use of nutrients and microbial augmentation normally improves degradation rates for slurry treatment. Augmentation with specific microorganisms and co-metabolism has been evaluated in pilot studies. Slurry treatment of soils contaminated with chlorinated compounds can be enhanced by augmentation with organisms having degrading capabilities for halogenated hydrocarbons.

4.6.2 Process Equipment

Slurry phase treatment is handled predominantly as a batch process. The reactors include lagoons, open vessels, and closed systems. The first step involves abstraction of the soil from the contaminated site, this is the followed by screening to remove gross solids and the creation of an aqueous slurry. The slurry phase is often 60 to 90% water by weight, depending on the nature of the reactor.

Many reactor configurations exist the differences arise simply in the methods of aeration and mixing of the suspension. The main disadvantages of slurry reactors is the high energy requirement to maintain the suspension and the potential for significant volatilisation. An example of a typical bio-slurry reactor is shown in Figure 13.

Figure 13 Slurry phase bioreactor



Many facilities have used existing open lagoons as holding ponds for waste sludges. They vary in size from small lagoons to lagoons of several acres. These existing lagoons are often the source of hazardous chemicals and can often be employed after modification (prevention of leakage) to serve as a slurry bioremediation facility. The benefits of this approach is that it

minimises the handling of toxic substances thus reducing the risk encountered when transporting large volumes of hazardous wastes.

The disadvantage to this approach is that the engineering is made more complex as lagoons are often the result of uncontrolled discharges over in some cases several decades. This often results in a lack of homogeneity due to production/process changes over the years. These discharges often remain as pockets of different chemicals leading to differential rates of remediation.

4.7 CONCLUDING REMARKS

Critical evaluation of biorestoration

Bioremediation efforts seek to increase the activity of microorganisms. The confidence in bioremediation technologies would be greater if the increase in microbial numbers were quantitated relative to:

1. Plume areas prior to treatment
2. Areas within the plume that did not receive any treatment
3. Control areas outside the plume.

The latter will give some indication of background levels of microorganisms and allow comparisons to be made before, during and after a remediation programme.

Often, the production of microbial catabolites can be measured in areas that receive treatment but not in contaminated areas left untreated. For instance, the production of a variety of lesser halogenated PCB congeners in river sediments contaminated with Aroclors was previously noted (USEPA 1987[24]). Many of the congeners produced did not comprise a significant portion of the original contaminant Aroclors. Their production was suggested as field evidence that the original PCB materials were metabolised by anaerobic micro-organisms and reductively dehalogenated (USEPA 1987).

Laboratory evidence in microcosm experiments designed to test this hypothesis confirmed that suspicion (Quensen[25] et al 1988) Similarly, the production of metabolites like hydrogen sulphide or methane often are testament to the metabolic activities of microorganisms. If the degradation pathways of particular contaminants are known prior to a remedial effort, It is sometimes possible to specifically assay for the production of likely catabolites.

Many times, biorestoration programs rely on the contaminant itself to supply the electron donors necessary for microbial growth and proliferation. One of the objectives of the remediation is to supply the necessary electron acceptor. The consumption of added terminal electron acceptors could possibly be measured. For instance, if oxygen is added to help stimulate hydrocarbon metabolism in aquifers, its depletion in the treatment area should be relatively easy to measure.

Finally, rapid microbial biodegradation typically seems to be preceded by a variable length of time where little or no activity is measured. This length of time is referred to as the adaptation or acclimation period. So far, adaptation seems to be a unique biological response. Observation of this type of phenomenon in response to a biorestoration effort can be taken as presumptive evidence that microbial action is operative.

It is important to emphasise that all of the techniques and measurements suggested above must be made relative to appropriate controls. For some techniques, that may mean the assay of untreated areas or areas outside the plume. Many clients may not be willing to invest the necessary time and financial resources to gather this level of information. Even with controls, the evidence garnered in the above fashion tends to be largely circumstantial. However, the stronger the evidence, the greater the degree of confidence in the technology.

5. MATERIALS AND METHODS

5.1 SOIL

The uncontaminated soil used in all the trials was abstracted from the top 30 cm near Himitangi Beach Road, New Zealand. The soil is characterised[26] as Hokio-Waitarere Association sandy soil. The important soil characteristics are shown in Table 6 and Table 7. The most distinguishing feature of the soil type is that it is very low in organic matter and has high hydraulic conductivity and intrinsic permeability. Before use in the experiments the soil was thoroughly mixed by hand and then passed through a 5 mm mechanical sieve to remove large organic and inorganic solids.

Table 6 Soil Characteristics

pH	5.8
Organic Carbon	0.3 mg/kg
Phosphorous	14 mg/kg
Total N	0.03 mg/kg
C/N ratio	10
Cell count	1 x 10^6 cells/g

Table 7 Cation Exchange Properties of soil

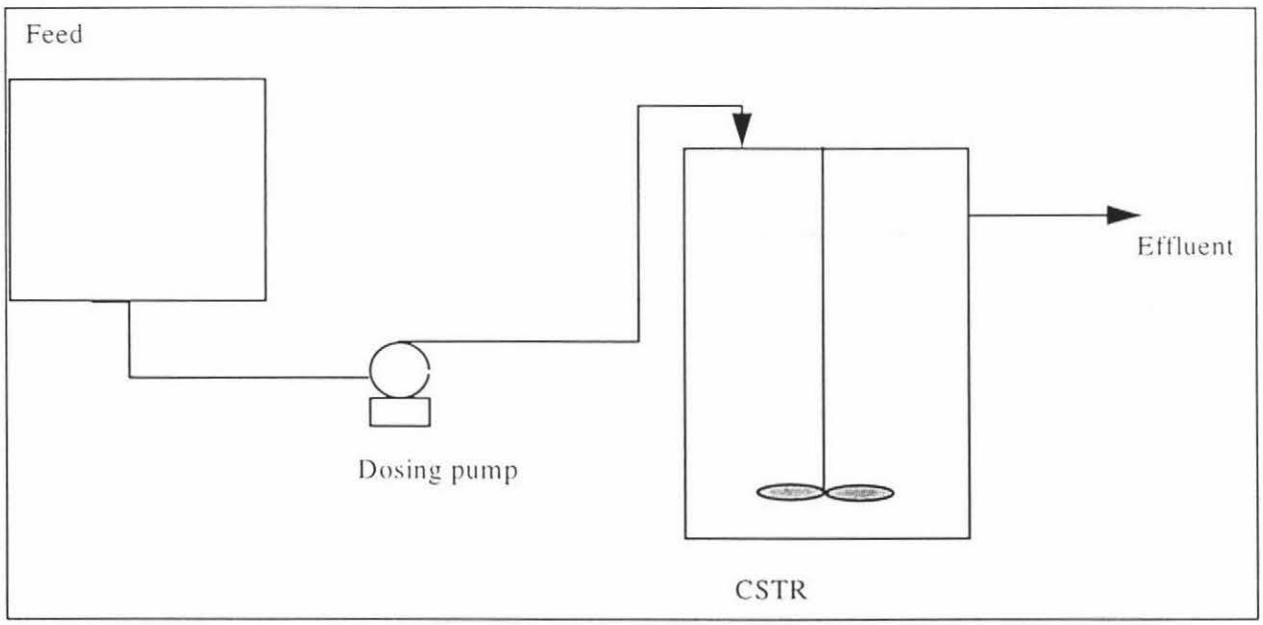
Cation exchange capacity	Base saturation	Calcium	Potassium	Magnesium	Sodium
2.4	64	1.0	0.35	0.3	0.1

5.2 BACTERIA

The bacterial consortium used throughout the study was cultured in a chemostat in the Department of Process and Environmental Technology, Massey University, New Zealand. The operational conditions are shown in Table 8. Figure 14 shows a basic schematic of the chemostat. The bacterial consortium was *Pseudomonas sp.* that had been previously isolated from soil sites contaminated with PCP.

Table 8 Chemostat operational conditions	
Volume of Reactor	1.5 litres
Temperature	30 C
PCP concentration in Feed	100 ppm
Specific Growth Rate	0.1 h^{-1}

Figure 14 Schematic of chemostat



5.3 MEDIA

The media employed to cultivate the micro organisms prior to inoculation was a mineral salts minimal medium the composition of which is shown in Table 9. Methanol was also added to increase the chemostat biomass Manderson[27] 1995.

Table 9 Composition of Feed	
Component	**Concentration g/l**
Na_2HPO_4	2.4
KH_2PO_4	2.0
NH_4NO_3	0.1
$MgSO_4.7H_2O$	0.01
$Ca(NO_3)_2$	0.01
PCP*	50, 75 and 100 ppm
Yeast extract	50, 75 and 100 ppm
C_2H_3OH*	75, 100 ppm

5.4 CHEMICALS

The PCP used throughout these trials was added as an aqueous solution of the sodium salt from a 1:1 mol mixture of NaOH and 99% purity PCP Sigma Chemical Company, St Louis, United States of America.

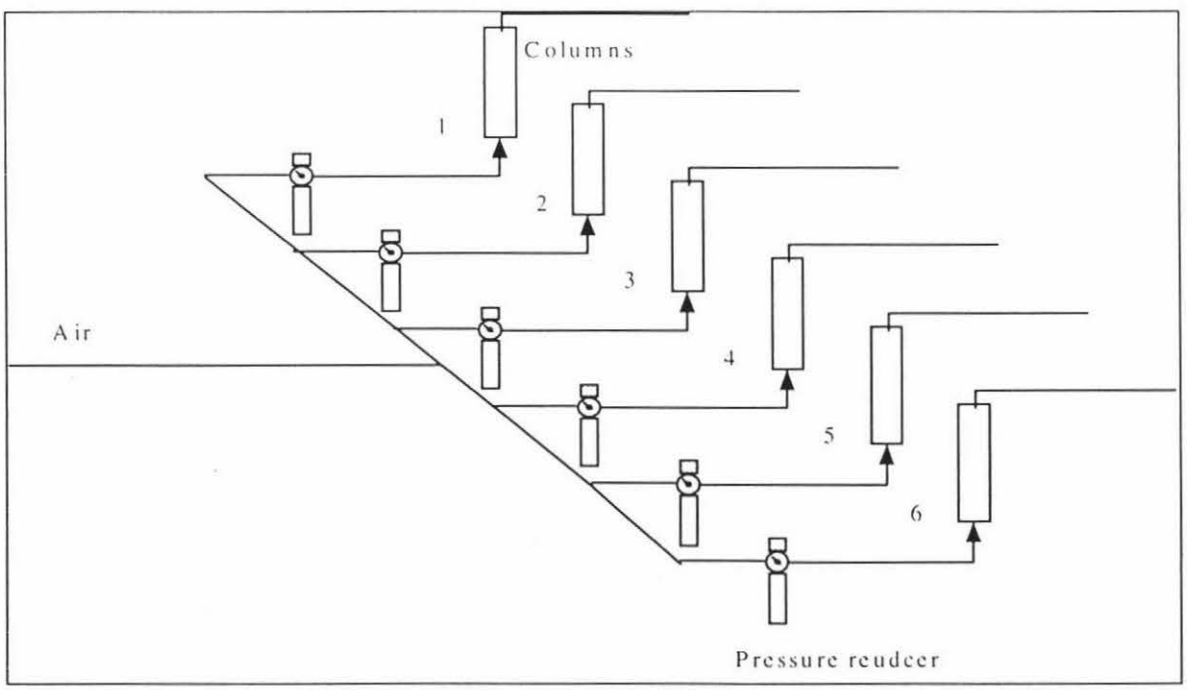
5.5 AERATION EQUIPMENT

The column materials were purchased from MICO-WAKEFIELD Palmerston North, the air lines used was standard 5mm OD polyethylene. The sintered plastic used in all columns was 10 um POREX INDUSTRIES Ltd. Perspex columns were fabricated in the Department of Process and Environmental Technology workshop. The system layout throughout these experiments are shown in Figure 15. For the purpose of aeration compressed was used, the supply pressure was 80 p.s.i. Pressure reducing valves (0-120 p.s.i.) MASTER PNEUMATIC DETROIT INC were placed on line to prevent excessive pressure build up in the columns. Compressed air flow controllers were manufactured by SHAKO CO JAPAN. The air flow volume l/min was measured using a standard calibrated flow meter.

The following features were incorporated into the column design:

1. Cleaning. Column was constructed as three separated pieces this enabled unhindered application and removal of soil and bacteria.
2. Distribution. System constructed with sintered plastic to ensure uniform air flow.

Figure 15 Experimental layout



5.6 GLASSWARE

Standard laboratory glassware obtained from commercial sources were used throughout this work. All glassware was routinely washed in hot water containing commercial cleaning agents, rinsed three to four times with distilled water and air dried at about 50°C.

5.7 SLURRY REACTOR

A slurry reactor similar to that shown in Figure 13 was used throughout the experiment. The reactor is able to be kept at a constant uniform temperature throughout the reactor by means of a temperature controller. Aeration was provided via compressed air. Mixing was provided by dual impellors that were able to suspend the entire slurry in solution.

6. ANALYTICAL PROCEDURES

6.1 SOIL PH

Approximately 10 g of soil was placed in a 250 ml screw capped Duran glass bottle (Schott, Germany) containing 50 ml of deionised water. After shaking the bottle for 5 minutes, the pH of the sample was determined using a pH meter (ORION RESEARCH model 701A digital analyser).

6.2 SOIL MOISTURE

Samples of soil (4.0 - 5.0 g weighed to precision of ±0.0001 g) were weighed on an aluminium plate and oven dried (CONTHERM OVEN, SALMON SMITH BIOLAB) at approximately 105 C to constant weight after 48 hours.

6.3 TOTAL NITROGEN

6.3.1 Sample preparation

A portion of 10 g of sample was dried in an oven at 65 C for at least 48 hours to avoid volatilisation of nitrogen occurring.

6.3.2 Digestion Preparation

250 g K_2SO_4 (BDH Chemicals Ltd, Poole, UNITED KINGDOM) and 2.5 g selenium powder (Ajax Chemicals, AUSTRAILIA) were added to 2.5 l concentrated H_2SO4 (BDH Chemicals Ltd, Poole, UNITED KINGDOM) in a 5 l pyrex beaker. The mixture was heated to 300 C for about 3 hours until it became clear.

6.3.3 Digestion

The digestion procedure of Bolan and Hadley[28] (1987) was used for the total nitrogen determination. This procedure was based on the work of Mc Kenzie and Wallace[29] (1954).

Samples of dried screened soil (0.1 - 0.2 g weighed to precision of +/- 0.0001 g) were weighed on piece of rice paper and placed in a pyrex tube (100 ml), which was previously calibrated to 50 ml. Digestion mixture was then added to the sample which was then heated in an aluminium digestion block (350 C for four hours). After cooling the samples were diluted to 50 ml with distilled water and then thoroughly mixed on a vortex mixer and finally transferred into screw capped glass containers previously cleaned with chromic acid. During storage the bottles were kept undisturbed, so sedimentation of undissolved particles could occur. The supernatant was used for the determination in the auto analyser. A blank using rice paper alone as well as a herbage standard sample of known N content were run with each set of samples

6.3.4 Determination

In this study reference was made to total nitrogen concentration and phosphorous concentration in the sample. This was taken to mean total nitrogen concentration equals total kjeldahl nitrogen concentration excluding nitrate.

The content of the total nitrogen in the supernatant after digestion was measured by a colorimetric method, using a Technicon Auto analyser.

6.4 PCP EXTRACTION

The PCP was extracted from soil samples (total weight 1-2 grams) by at least two hours of mixing at 25 C with ten ml of acidified (pH < 2) distilled methanol. The soil-methanol slurry was then allowed to stand at 4 C for 2-3 hours . The supernatant was removed and centrifuged at 13000 rpm for five minutes to separate the liquid and solid phases it was then filtered using 0.2 μm filter paper. The extraction efficiency rates were in excess of 85 % for the duration of the trials. To ensure no concentration occurred due to evaporation of the solvent several samples were weighed before and after mixing at 25 C.

6.4.1 PCP Analysis

The extracted PCP was derivatized by High Performance Liquid Chromatography (HPLC). The chromatographic system consisted of a Waters 600E system controller equipped with a Waters U6K injector and Waters 486 Tunable absorbance detector. The detector was set to a wavelength of 308 nm. The integration was performed using the Baseline 810 programme Waters on a 386 computer. The injection volume was fixed at 50 μl and the Hamilton syringe used was washed three times with this volume before injection. A Waters RCSS guard pak column was used before the Radial-PAK eight mm column. The mobile phase was 90 % methanol, 9.9 % water and 0.1 % glacial acetic acid. The column flow rate used for analysis of PCP varied between 2.5 and 5.0 ml/min. Before analysis the column was calibrated using four standard PCP solutions 5, 25,125, and 250 mg/m^3, that had been diluted from a stock solution of 10,000 mg/m^3.

6.4.2 PCP Volatilisation

PCP is an organic chemical and is therefore susceptible to volatilisation. In order to verify that no PCP was volatilised during the trials the exhaust air from the experimental columns was passed through a 2 M NaOH solution. The NaOH will trap any volatilised PCP. A sub sample was removed from this solution (replenished weekly) and freeze dryed using a rotovap device until solid.

This solid was then dissolved in analytical grade methanol and assayed by the same method as for the PCP in soil (3.3.1). To test the efficacy of this method several control samples were dosed with known concentrations of PCP and analysed for PCP.

6.5 CHLORIDE EXTRACTION

To measure chloride, soil samples were extracted in the field moist state with 0.05 K_2SO_4 BDH Chemicals Ltd, Poole, UNITED KINGDOM (equivalent to 5 g oven dry soil solids to 15 ml extractant). The samples were shaken for 2 hours, centrifuged for 5 minutes at 4500 revolutions per minute, and then filtered through Whatman No. 41 filter paper.

6.5.1 Chloride Analysis

Soil chloride ion concentrations were determined by the direct potentiometric method using three calibration standards. Water was added to 10 mls of soil and the chloride ion concentration was measured with a Cl^- specific ion electrode and an Orion 720A meter.

6.6 MICROBIOLOGICAL ANALYSIS

6.6.1 Extraction

The method as described by Dudley[30] et al 1980 was followed. Soil samples weighing approximately one gram were abstracted from each of the soil columns after twenty four hours of incubation. For each sample a dilution blank containing 99 ml of Peptone water DIFCO LABORATORIES Detroit Michigan USA and 15 to 20 glass beads was made. In addition to this seven 90 ml dilution blanks with Peptone water was also prepared. The bottles were capped and autoclaved at 121 C for 20 minutes and then allowed to cool. The soil was transferred to the 95 ml bottle and shaken vigorously in a mechanical shaker for 10 minutes. Immediately thereafter, transfer 10 ml of the soil suspension taken from the centre of the suspension to a fresh 90 ml blank. This established a 10^{-3} dilution. The bottle was capped and vigorously shaken, and 10 ml of the suspension was removed as previously described. The sequence was continued until a dilution of 10^{-8} was reached.

6.6.2 Microbiological Count

Standard agar was prepared according to manufacturing instructions. About 15 ml of media was distributed onto each plate and allowed to cure for 2 days. Four dilutions 10^{-5}, 10^{-6}, 10^{-7} and 10^{-8} were selected for the initial characterisation. Three 0.1 ml aliquot's were transferred to separate plates from each dilution. The suspension was then spread on the agar surface using a sterile glass spreader (Hockey stick) for each plate. The spreader was alcohol flamed between each use. The plates were then inverted and incubated at 30 C for 48 hours. At the end of the incubation period the dilution that yielded 30 - 300 colonies per plate was selected and used to calculate the number of colony forming units per gram of soil.

6.7 ISOLATION MEDIA

Mineral salts medium as used for the chemostat feed was used to isolate bacteria from the soil samples. To this medium Agar was added at 1.8 % w/v. PCP and methanol were added to give a concentration of 50 ppm.

6.8 STATISTICAL ANALYSIS OF DATA

The results obtained were subjected to statistical analysis. The methods used were one-way analysis of variance and two way analysis of variance. The obtained F- values were compared with tabulated F- values on the 90 % level and they were considered to be significant at a given level if they were equal to or greater than the tabulated values.

In carrying out the statistical analysis it was assumed that the response of the treatment (for example time) to the contents of the variable (PCP concentration) was independent of the position from which the sample was collected

The statistical analysis was performed using the MICROSOFT EXCEL™ statistical computing system MICROSOFT LABORATORIES.

7. EXPERIMENTAL PROCEDURE

7.1 EXPERIMENT ONE

This experiment had three aims:

- Assess the effect of aeration on bioremediation rates.
- Examine the effect of increasing contaminant chemical concentration on bioremediation rates.
- Assess the effect of innoculation

To do this six columns were set up in the fashion presented shown in Figure 15.

Soil

To each column 2 kg of sandy soil was added. The soil was collected from the top 30 cm of an agricultural field at Himitangi Beach, Himitangi, New Zealand. The soil was used for the production of livestock for the previous 40 years and as far as the author could determine had no history of pesticide application.

Inoculation

PCP

In all treatments inoculation was performed at day 0 with PCP as an aqueous solution of the sodium salt from a 1:1 mol mixture of NaOH and 99% purity PCP. The standard stock solution used during the experiments was 10,000 ppm. The concentrated solution was diluted appropriately as required.

Bacteria

Bacteria was harvested from the effluent stream of a chemostat. The cell density in the chemostat was determined by direct counting of cells and by dilution plating of sub-samples. Prior to inoculation one half litre of the effluent stream was collected and allowed to stand for 30 minutes the supernatant was then carefully decanted and the bacterial suspension was then used as the stock solution. The stock solution was then diluted to appropriate levels prior to soil inoculation.

Preparation

The PCP and bacterial solutions were thoroughly mixed into the soil in the appropriate concentrations. To ensure the system components were homogenous throughout the soil matrix initial samples were abstracted and analysed for PCP concentration before the experiment proceeded. If PCP levels were significantly (greater than ±5 ppm) different between sub samples the soil matrix was remixed and reanalysed until the PCP concentration was uniform.

Column conditions

Throughout the experiment soil moisture contents were kept at approximately 20 % w/w using distilled water. Air flow rates for the columns that were supplied with air were kept at 2 l/min for the duration of the experiment. The environmental conditions in the room used for bioremediation trials were not temperature or humidity controlled. They were subjected to normal ambient fluctuations. The trials were conducted indoors in an attempt to minimise these fluctuations.

For determination of PCP concentrations in the soil samples were taken to a depth of 5-10 cm and were analysed in triplicate for PCP using the protocol outlined in the sample analysis section.

Sampling

Samples were taken with a core sampler to a depth of 5-10 cm. The soil from each sample was homogenised and a sub-sample weighing approximately 1 g was taken. Residual soil was then returned to the area from which it was taken. If not assayed immediately soil samples were stored at -20C for 1-2 days prior to analysis for PCP and Cl^-. At the end of the trial each column was sampled at various depths to determine if stratification of the column had occurred.

8. EXPERIMENT 2

This experiment had one aim:

- Assess the effect of increased biomass concentration on the rate of disappearance of PCP in soil

To do this six columns were set up in the fashion presented shown in Figure 15

Soil

To each column 2 kg of sandy soil was added. The soil was collected from the top 30 cm of an agricultural field at Himitangi Beach, Himitangi, New Zealand. The soil was used for the production of livestock for the previous 40 years and as far as the author could determine had no recent history of pesticide application.

Inoculation

PCP

In all treatments inoculation was performed at day 0 with PCP as an aqueous solution of the sodium salt from a 1:1 mol mixture of NaOH and 99% purity PCP. The standard stock solution used during the experiments was 10,000 ppm. The concentrated solution was diluted appropriately as required.

Bacteria

Bacteria was harvested from the effluent stream of a chemostat. The cell density in the chemostat was determined by direct counting of cells and by dilution plating of sub-samples. Prior to inoculation one half litre of the effluent stream was collected and allowed to stand for 30 minutes the supernatant was then carefully decanted and the bacterial suspension was then used as the stock solution. The stock solution was then diluted to appropriate levels prior to soil inoculation.

Preparation

The PCP and bacterial solutions were thoroughly mixed into the soil in the appropriate concentrations. To ensure the system components were homogenous throughout the soil matrix initial samples were abstracted and analysed for PCP concentration before the experiment proceeded. If PCP levels were significantly (greater than 5 ppm) different between sub samples the soil matrix was remixed and reanalysed until the PCP concentration was uniform.

Column conditions

Throughout the experiment soil moisture contents were kept at approximately 20 % w/w using distilled water. Air flow rates for the columns that were supplied with air were kept at 2 l/min for the duration of the experiment. The environmental conditions in the room used for bioremediation trials were not temperature or humidity controlled. They were subjected to normal ambient fluctuations. The trials were conducted indoors in an attempt to minimise these fluctuations.

For determination of PCP concentrations in the soil samples were taken to a depth of 5-10 cm and were analysed in triplicate for PCP using the protocol outlined in the sample analysis section.

Sampling

Samples were taken with a core sampler to a depth of 5-10 cm. The soil from each sample was homogenised and a sub-sample weighing approximately 1 g was taken. Residual soil was then returned to the area from which it was taken. If not assayed immediately soil samples were stored at -20C for 1-2 days prior to analysis for PCP and Cl^-. At the end of the trial each column was sampled at various depths to determine if stratification of the column had occurred.

9. EXPERIMENT 3

This experiment had one aim:

- Assess the effect of an in tank remediation strategy using a slurry reactor.
- Compare slurry reactor with in the in-situ simulations

Soil

To the slurry reactor 2 kg of sandy soil was added. The soil was collected from the top 30 cm of an agricultural field at Himitangi Beach, Himitangi, New Zealand. The soil was used for the production of livestock for the previous 40 years and as far as the author could determine had no history of pesticide application.

Inoculation

PCP

In all treatments inoculation was performed at day 0 with PCP as an aqueous solution of the sodium salt from a 1:1 mol mixture of NaOH and 99% purity PCP. The standard stock solution used during the experiments was 10,000 ppm. The concentrated solution was diluted appropriately as required.

Bacteria

Bacteria was harvested from the effluent stream of a chemostat. The cell density in the chemostat was determined by direct counting of cells and by dilution plating of sub-samples. Prior to inoculation one half litre of the effluent stream was collected and allowed to stand for 30 minutes the supernatant was then carefully decanted and the bacterial suspension was then used as the stock solution. The stock solution was then diluted to appropriate levels prior to soil inoculation.

Preparation

The PCP and bacterial solutions were thoroughly mixed into the soil in the appropriate concentrations. To ensure the system components were homogenous throughout the soil matrix initial samples were abstracted and analysed for PCP concentration before the experiment proceeded. If PCP levels were significantly (greater than 10 ppm) different between sub samples the soil matrix was remixed and re-analysed until the PCP concentration was uniform. The soil was then added to the chemostat in a ratio of 2 kg soil: 14 l H_2O MMS + 40 ppm MeOH.

Slurry reactor conditions

Throughout the experiment the slurry reactor was held at a constant temperature of approximately 25°C. The aeration rate was set up to give excess oxygen. The impellor speed was set to keep the slurry in suspension to ensure the reactor was homogenous.

Sampling

Three 50 ml samples were taken from the reactor using a pipette. The sample was collected from the reactor and centrifuged at 3000 rpm to separate the slurry into solid and liquid phases. After this separation had occurred the soil sample was assayed in the same manner as in Experiments 1 and 2. The water sample was filtered through 0.2 μm filter paper and assayed directly

10. RESULTS AND DISCUSSION

10.1 PRELIMINARY REMARKS

The main purpose of these trials was to examine the feasibility of mineralising PCP by adding microorganisms able to degrade PCP and manipulating environmental conditions to enhance bioremediation. Inhibitory chemicals associated with timber treatment (Diesel, Chlorinated Copper Arsenic (CCA) compounds) were not present. The disappearance of PCP concentration was the focus of the experimental studies.

10.2 EXPERIMENT 1:RESULTS

Effect of PCP concentration on PCP mineralisation rates.

Prior to inoculation from the operational chemostat an assay was performed on the bacterial consortia. The microorganisms isolated were gram negative, oxidase positive, catalase positive, aerobic motile rods that liquefy gelatin. On the basis of these traits the bacterium was identified as a strain of *Pseudomonas*. The bacterium grew in the salts solution containing 100-150 mg of PCP per litre and mineralised the chlorinated compound.

To determine if PCP was being volatilised during the aeration of the columns, a PCP trap containing NaOH solution was assayed throughout the the trials. Analysis of the NaOH solution failed to detect any PCP. It was concluded that PCP was not being volatised during aeration of the columns.

At the termination of experiments all columns were sampled for PCP at differing depths to determine if stratification had occurred, with the exception of column 5 (discussed further below) the results of the PCP analysis were uniform throughout the column.

The results of the first biodegradation experiments are presented in Figures 1.1-1.6. Raw data from the experiment are presented in Appendix I. The biodegradation rate was most rapid in all columns (other than controls) for the first 10 days after inoculation. The rate of PCP disappearance was most rapid in Column 2 which had an initial PCP concentration of 92 mg/kg (shown in Figure 1.3). The next highest biodegradation rate was Column 1 (shown in Figure 1.4) which had an intial PCP concentration of 132 mg/kg. The replicate columns 3 and 4 shown in Figure 1.1 and Figure 1.2 (initial PCP concentrations of 50 mg/kg) had significantly slower initial biodegradation rates in comparison to columns 1 and 2.

Column 5 (Figure 1.5) which received an inoculation but no aeration had a greatly reduced PCP removal rate and at the termination of trials (42 days) had removed little of the PCP initially present. To determine if stratification had occurred during the experiment soil samples were taken from the bottom of each column. Table 10 shows the vertical extent of degradation in the columns at the termination of the experiment.

Table 10 Vertical extent of PCP degradation.

	Column number					
Depth	1	2	3	4	5	6
0 mm	9.5	9.4	10.3	9.8	32	27.5
75 mm	ND	9.5	9.8	ND	42.5	42.5
150 mm	ND	ND	ND	1.8	38.07	41.1

As soil depth in the column increased the PCP concentration was observed to be approximately the same concentration as the initial PCP concentration ie at day zero. Column 6 shown in Figure 1.6 (was fully aerated but was not inoculated with PCP degrading bacteria) displayed slightly improved removal of PCP than that found in column 5. The removal of PCP was significantly less than the columns receiving inoculation and aeration.

The disappearance of PCP slowed markedly in columns 1,2,3 and 4 after 28 days. The overall removal of PCP at this time was approximately 80%. The final residual concentration in these columns displaying appreciable disappearance was between 10 and 20 mg/kg.

Figure 16 Column 1 Experiment 1, PCP 130 mg/kg

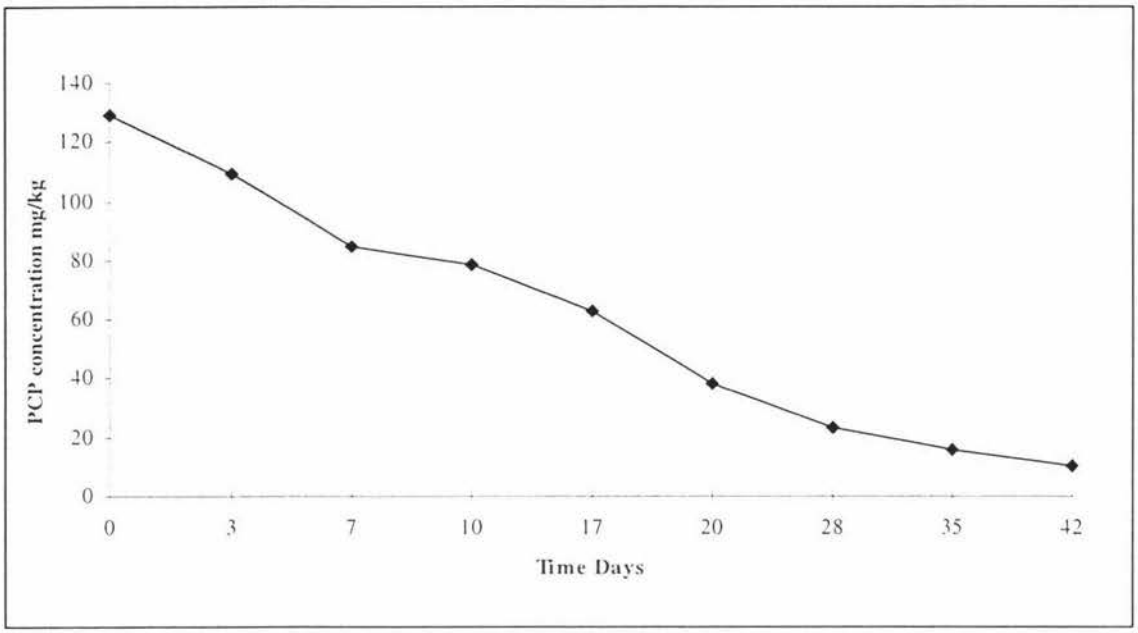


Figure 17 Column 2 Experiment 1, PCP 92 mg/kg

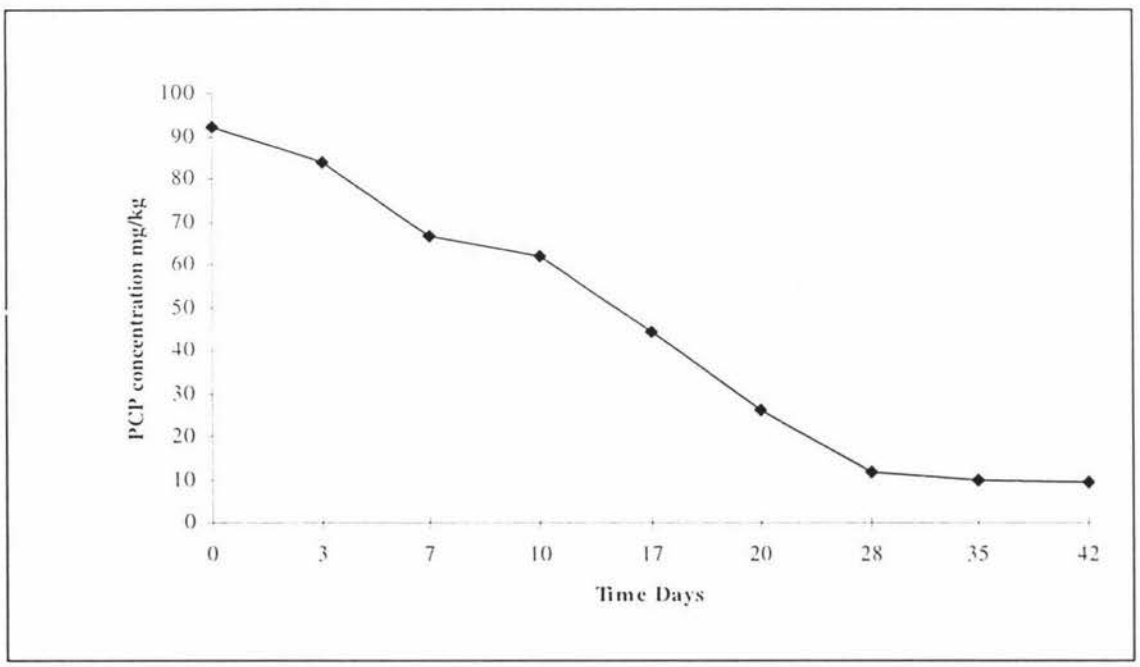


Figure 18 Column 3 Experiment 1, PCP 50 mg/kg

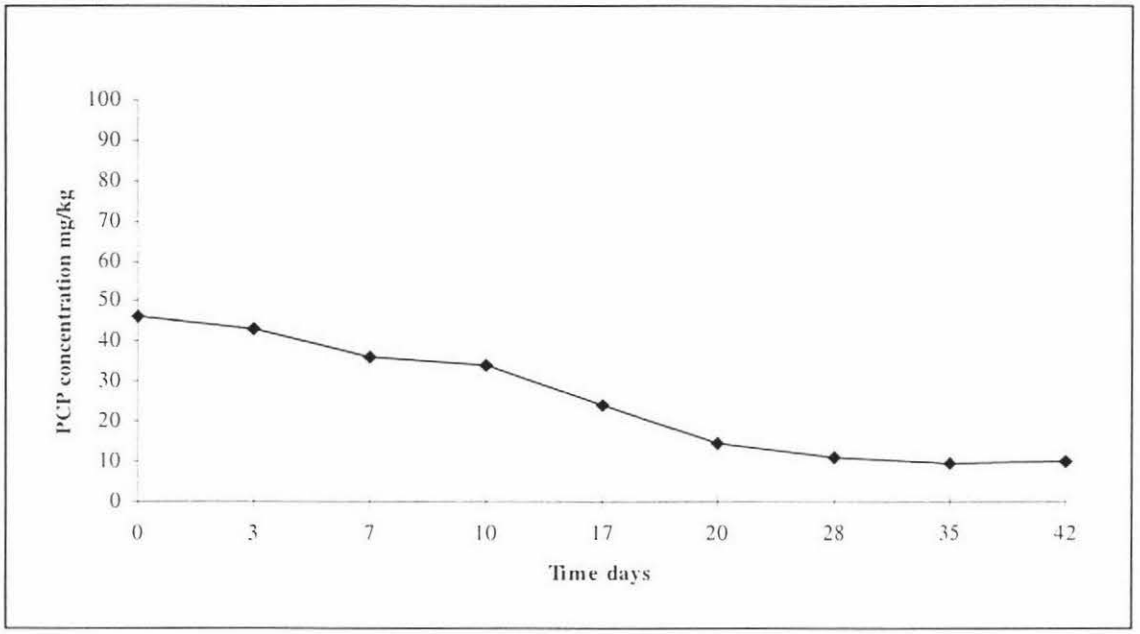


Figure 19 Column 4 Experiment 1, PCP 45 mg/kg

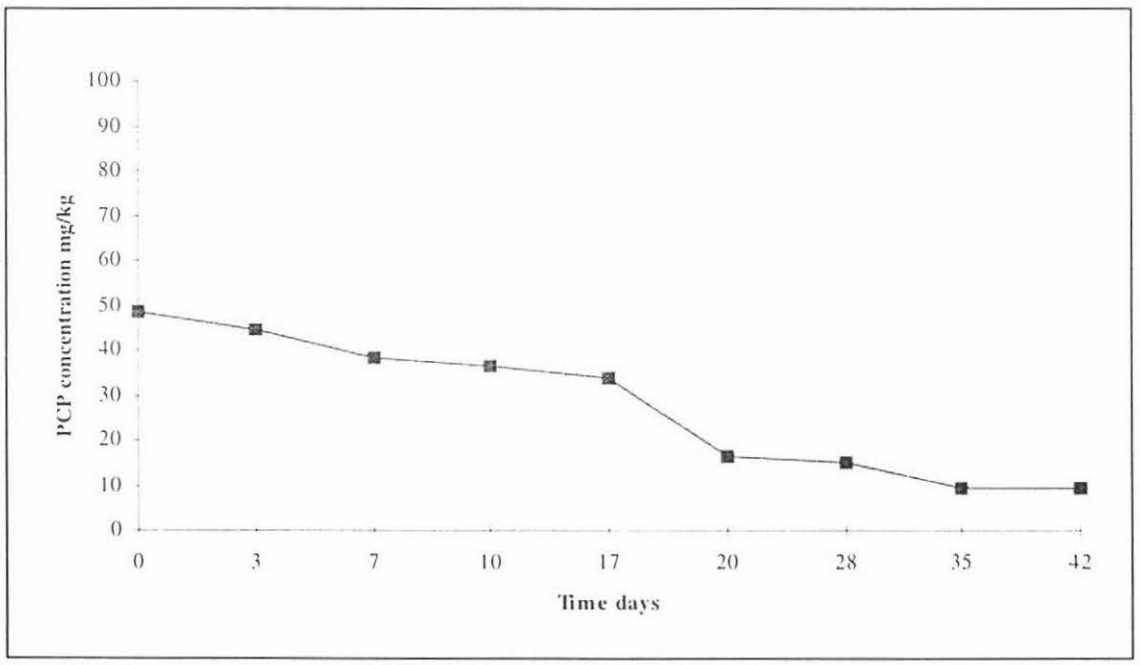


Figure 20 Column 5 Experiment 1, PCP 48 mg/kg no aeration

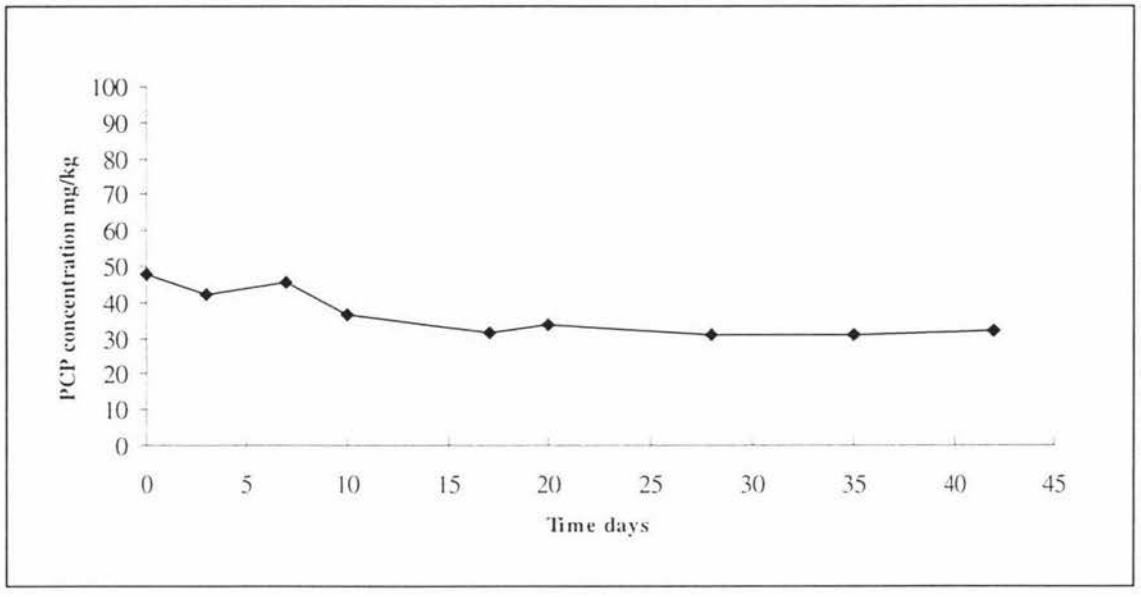
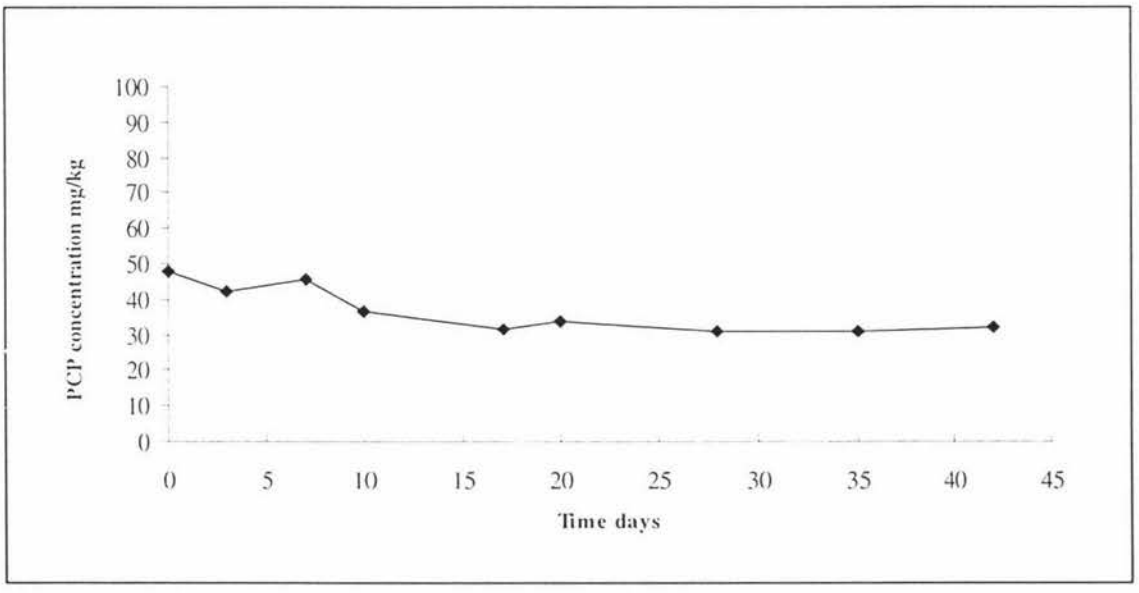


Figure 19 Column 5 Experiment 1, PCP 51 mg/kg no inoculum



10.3 DISCUSSION EXPERIMENT 1.

The bacteria was a mixed culture of predominantly *Pseudomonas sp.* *Pseudomonas* bacteria was selected as it has been previously reported in the literature to degrade a variety of herbicides. The bacteria was available in large quantities from a 10 l chemostat operating at Massey University. The bacterial consortium had been initially isolated from PCP contaminated soil and was known to have significant degradative capabilities. There was no volatilisation of PCP during the trials. This result was expected as PCP has low volatility and is a very stable compound when contained within the soil matrix.

Statistically significant changes in PCP concentration was detected after three days in all columns receiving inoculum and aeration. The reason for this short lag period was that the inoculum was actively degrading PCP in the chemostat prior to soil inoculation. The microorganisms therefore were not required to synthesise new enzymes or to use alternative metabolic pathways to degrade PCP.

The rate of degradation was greatest in column 2 (92 mg/kg) with slight increases in degradation rates for columns 1, 3 and 4. The effect of initial concentration on the rate of PCP disappearance is statistically significant and is related to availability of the target compound. Goldstein et al (1985)[31] reported that the success of adding non-indigenous microorganisms to the environment is dependant upon the concentration of the target compound and the preferential use of alternate substrates.

The low organic content present in the soil limits the choice of substrates. Thus whilst metabolically more favourable compounds were available in the soil matrix they were in amounts likely to be rate limiting, thereby making the utilisation of PCP the most energy efficient carbon source. This is evidenced to a certain extent by lower degradation rates as the introduced microorganisms were unable to utilise the target compound as effectively as at higher concentrations. Table 11 below presents the PCP half lives for each of the columns.

Table 11 PCP half lives		
Column number	Initial PCP concentration	Half life
1	130 mg/kg	11 d^{-1}
2	92 mg/kg	9 d^{-1}
3	50 mg/kg	13 d^{-1}
4	45 mg/kg	16 d^{-1}
5[1]	48 mg/kg	38 d^{-1}
6[2]	51 mg/kg	44 d^{-1}

1 No aeration.

2 No inoculation.

The biodegradation rates obtained are similar to much of the reported literature on biodegradation rates of PCP. Valo and Salkinoja-Salonen (1986) reported approximately 80% disappearance within 40 days. Similarly Seech et al[32] (1990) reported 60% PCP removal after 28 days.

After approximately 28 days the rate of degradation dropped off significantly and no further removal of PCP was observed after this time. The residual PCP concentration was between 10 and 20 mg/kg. A similar residual result was been obtained by Valo and Salkinoja-Salonen (1986) and Crawford and Mohn[33] (1985). This can also be explained by the availability of the compound being rate limiting. The residual PCP in the soil matrix was probably the remaining bound portion and was therefore unavailable for degradation. The enzymes synthesised for PCP degradation were suppressed and alternative substrates consumed.

The unaerated control (column 5) exhibited some removal of PCP. This may be due to the fact that while air was not actively supplied to the column natural convectional forces were still able to supply air to the column. Thus soil in the top layers of the column were still exposed to sufficient amounts of oxygen such that it was not a limiting factor. This may be the reason why degradation was not observed to have occurred in the column below the soil surface.

In the un-inoculated control (column 6), PCP disappearance lagged behind that seen in soils that received both inoculation and aeration. However some reduction in the initial concentration of PCP was observed.

PCP was not volatilised during the trials. The NaOH solution designed to trap volatilised PCP was found to was unable to detect the presence of PCP. The observed disappearance of PCP indicates that natural microbial communities present in the soil were responsible for the disappearance of PCP. The capability of indigenous micro-organisms in soil to adapt to degrade chlorophenols has been widely observed Kaufman[34] 1978, Watanabe[35] 1977, Crawford and Mohn 1985. This observation has led many researchers to question the efficacy of inoculation of introduced micro-organisms as opposed to manipulation of environmental conditions to favour compound degradation

10.4 EXPERIMENT 2

This experiment was set up and run in an identical manner as experiment 1. This second experiment had one aim:

- Assess the effect of increased biomass concentration on the rate of disappearance of PCP in soil.

10.5 RESULTS

The results of the second biodegradation experiment are presented in Figures 22-27. The raw results are presented in Appendix I. Biodegradation was most rapid in Columns 1 and 2 (Figure 16 and 17 respectively) both were inoculated with 10^7 cells per gram of soil. The biodegradation rate was most rapid for the first 10 days after inoculation.

The column with the next highest biodegradation rate was Column 3 (shown in Figure 1.9). This column received an inoculum of 3 x 10^6 cells per gram. Replicate columns 4 and 5 which were both inoculated with 10^5 cells per gram (Figure 1.10 and Figure 1.11) had significantly slower initial biodegradation rates in comparison to columns 1,2 and 3. Column 6 (Figure 1.12) received an inoculation of 10^4 cells per gram had a significantly reduced PCP removal rate in comparison to columns 4 and 5.

The residual levels of PCP are shown in Table 13 below. The residual levels for the columns receiving 10^7 cells are lower than those observed in the other columns. There appears to be a relationship between inoculation and final residual concentration. The higher the initial concentration of cells in the inoculum the lower the residual value.

Table 3 shows the residual levels of PCP degrading bacteria at the conclusion of the experiment. It can be seen that the higher the initial inoculation the higher the amount of PCP degrading bacteria at the termination of the experiment.

Table 12 PCP degrading bacteria concentrations		
Column number	**Day 0**	**Day 45**
1 (10^7)	2×10^7	1×10^7
2 (10^6)	5×10^6	3×10^6
3 (10^5)	3×10^5	2×10^5
4 (10^7)	1×10^7	1×10^7
5 (10^5)	4×10^5	ND
6 (10^4)	4×10^4	ND

Figure 22 Column 1 Experiment 2, 1 x 10^7 cells

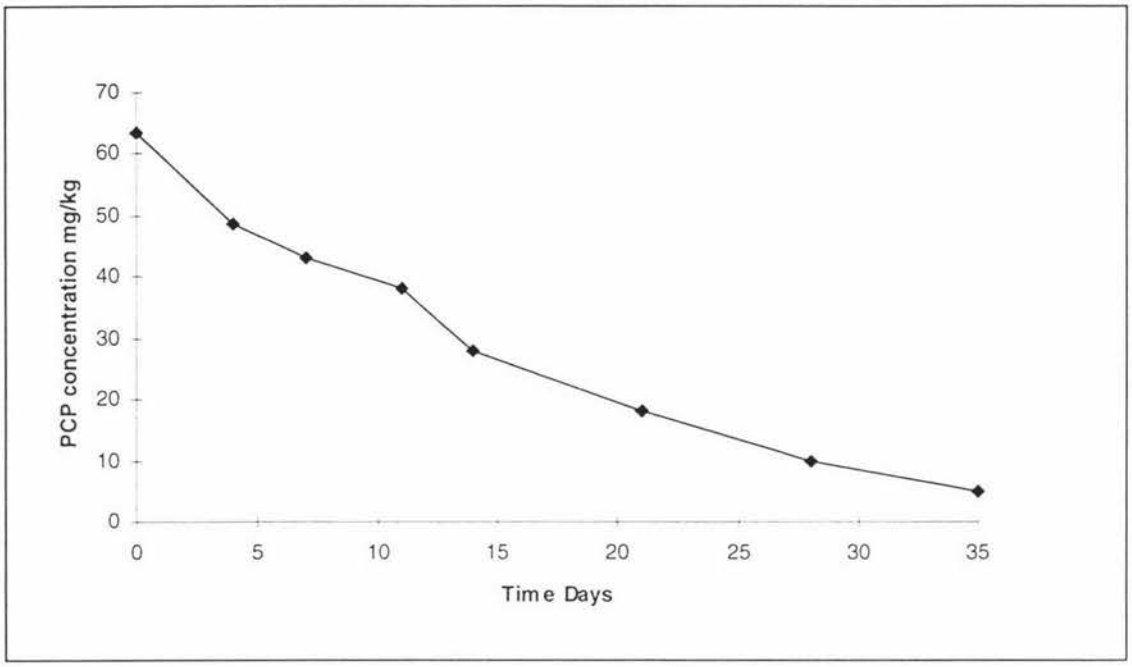


Figure 23 Column 2 Experiment 2, 1 x 10^6 cells

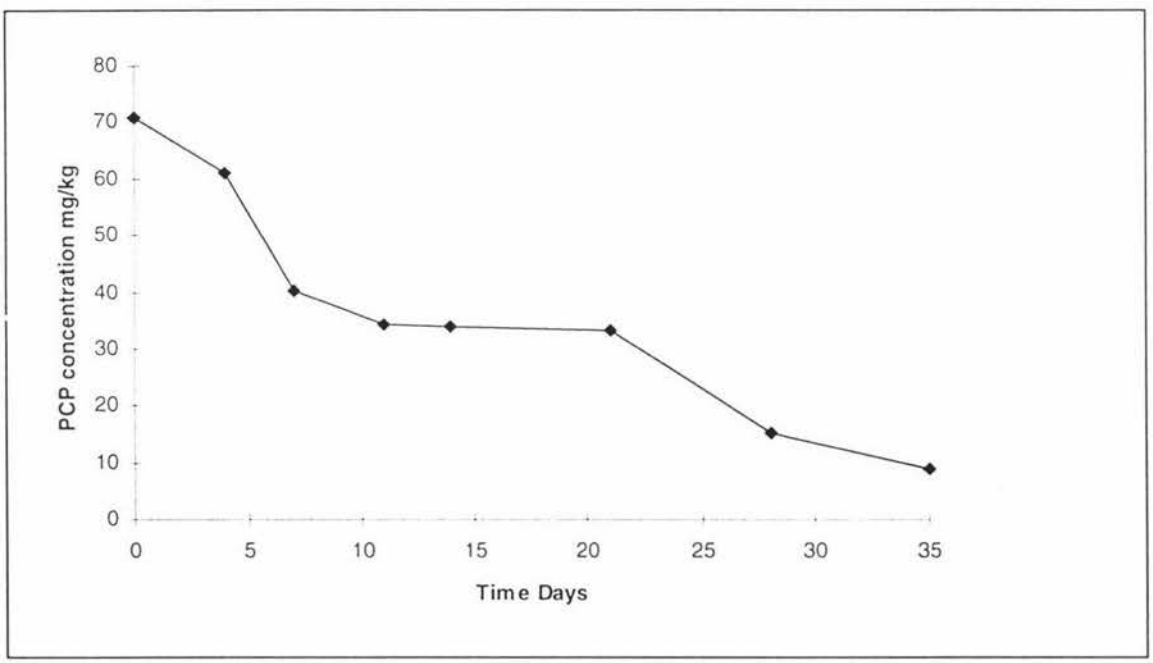


Figure 24 Column 3 Experiment 2, 1 x 10^5 cells

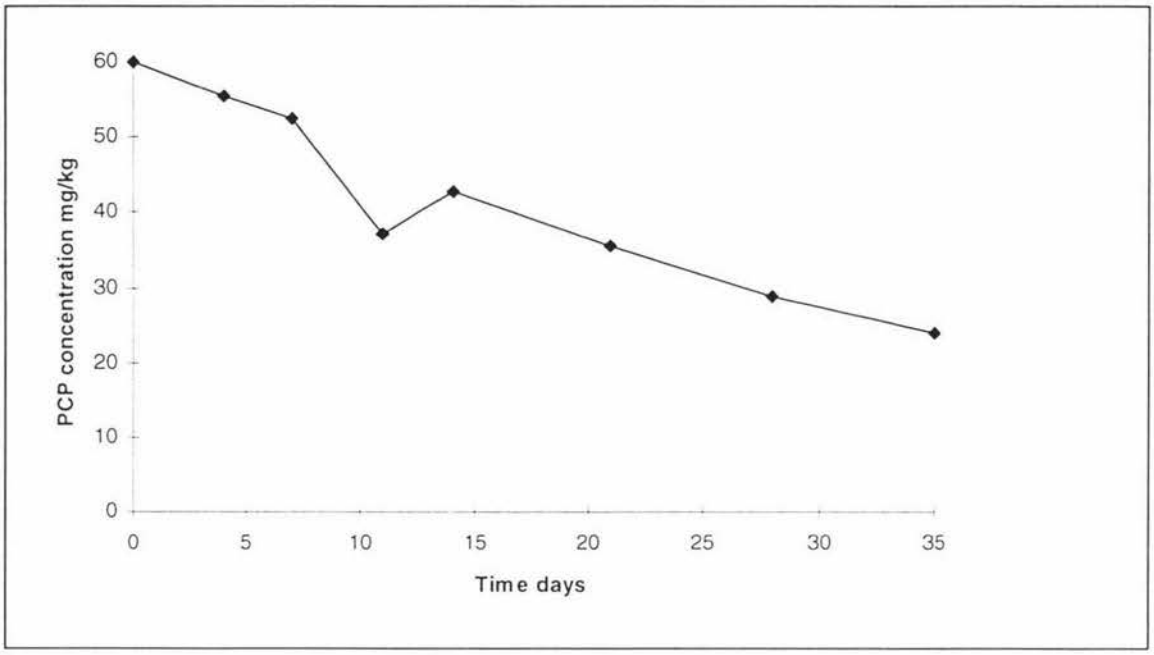


Figure 25 Column 4 Experiment 2, 1 x 10^7 cells

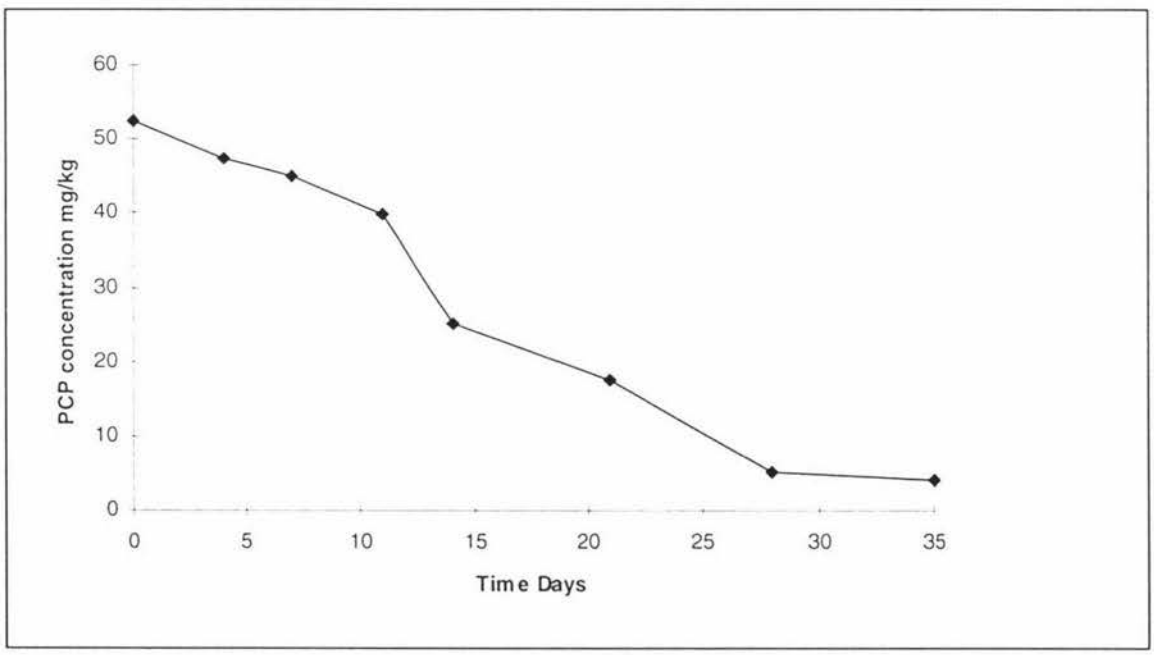


Figure 26 Column 5 Experiment 2, 1 x 10^5 cells

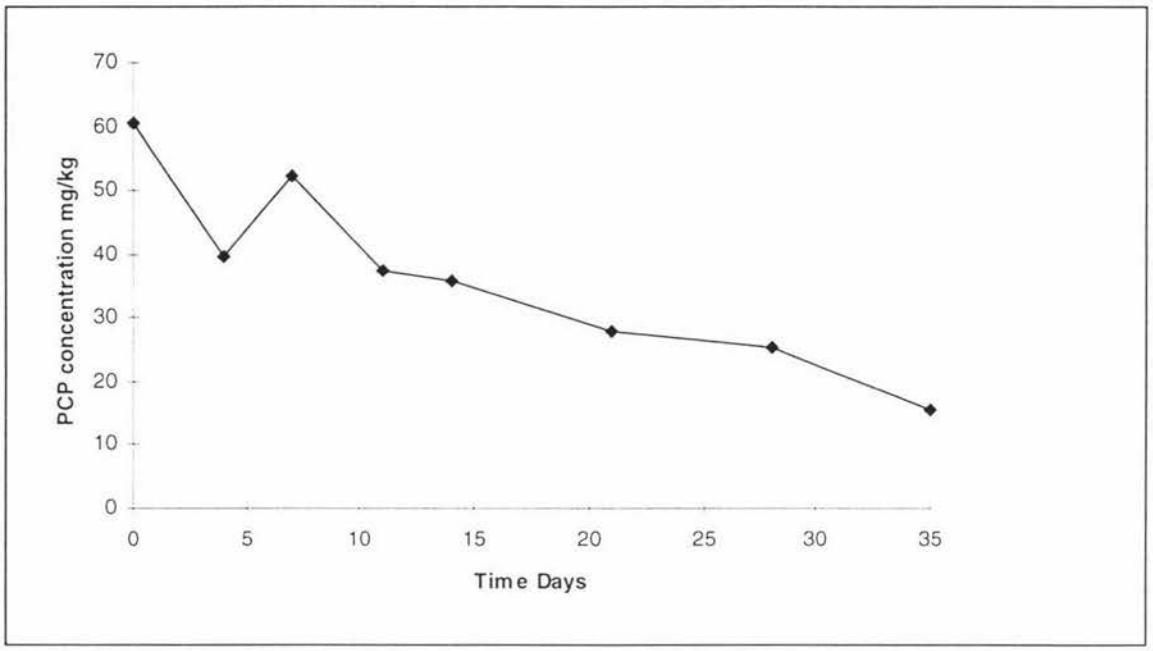
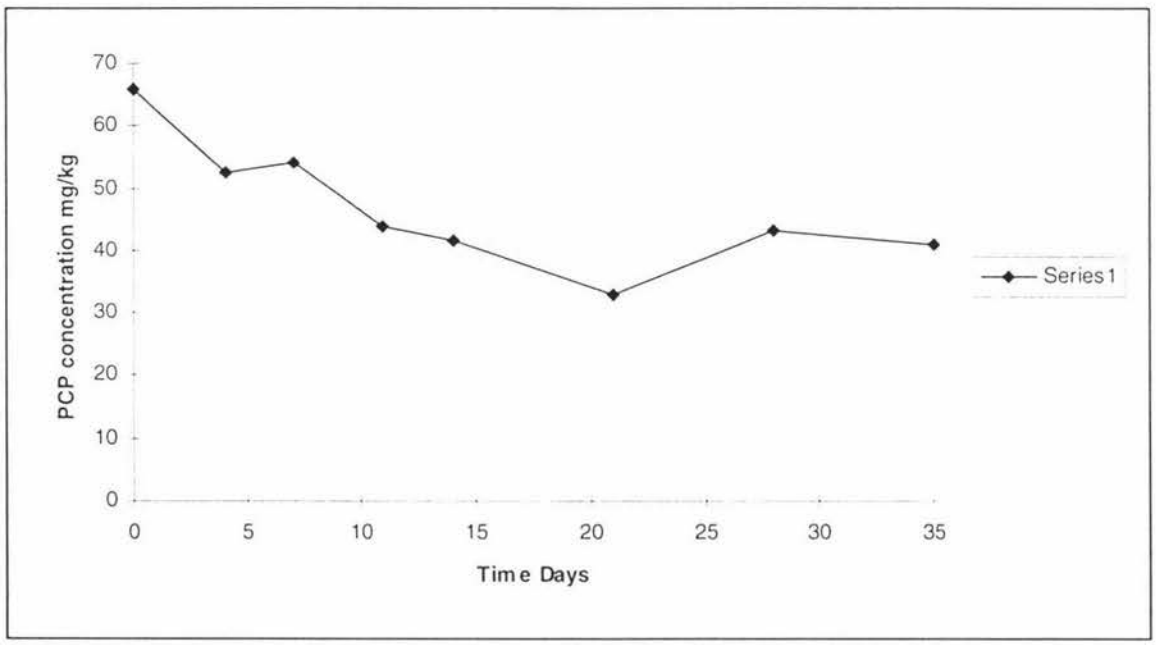


Figure 27 Column 6 Experiment 2, 1 x 10^4 cells



10.7 DISCUSSION EXPERIMENT 2

The data shows that a bacterium able to degrade PCP in pure culture is capable of destroying PCP when added to soil at relatively high cell densities but not at low ones. This experiment confirmed that the concentration of the initial inoculum affects the rate of disappearance and the final residual concentration. Ramadan et al[36] (1990) and Edgehill and Finn[37] (1983) also found that the rate of disappearance was related to the inoculum size.

The failure of the small inoculum to mineralise PCP in significant amounts could be a result of the failure of the bacterium to survive. Because more cells were added with the large inoculums, a marked population decline might not result in the total elimination of viable cells. The results demonstrate that survival and therefore degradation rate was affected by inoculum size. Thus when *Pseudomonas* was added to the soil at initial densities of 10^4 cells per gram, the population declined to a density below the detection limit. In contrast, when *Pseudomonas* was added at high cell density, the population was observed to remain relatively stable over the 45 day trial period.

Although antibacterial toxins are present in some soils, the decline is not thought to be the result of the presence of such toxins as the soil was removed from a remote rural location and was supporting vegetation. The presence of predators are known to affect the survival of inoculants. Zaidi et al[38] (1989) found that addition of eukaryotic inhibitor to lake water inoculated with a *Corynebacterium sp.* increased the extent of mineralisation of 26 ng/ml PNP, but did not increase mineralisation of higher concentrations of PNP, the authors suggested that the microorganisms were not able to replace those cropped by eukaryotic grazing at the lower concentrations of PNP.

Retention of biodegradative capabilities when other substrates predominate may be a reason for the PCP being left as a residual. When PCP concentrations become limiting, microorganisms adapt their metabolic pathways to utilise the most energy efficient substrate. Therefore at low PCP concentrations the utilisation of PCP becomes undesirable and is left as a residual.

Table 13 Residual PCP concentration		
Column number	Inoculum	Final concentration mg PCP/kg soil
1	10^7 cells/g	5 mg/kg
2	10^7 cells/g	3.5 mg/kg
3	10^6 cells/g	8 mg/kg
4	10^5 cells/g	24 mg/kg
5	10^5 cells/g	16 mg/kg
6	10^4 cells/g	41 mg/kg

11. EXPERIMENT 3

This experiment had two aims:

- Assess the effect of an in tank remediation strategy using a slurry reactor.
- Compare increases in process efficiency due to using a slurry reactor.

11.1 RESULTS

A desorption experiment was performed to determine the equilibrium concentration of PCP in the reactor. The results of this trial are shown in Figure 28. The concentration of PCP in the water phase moved rapidly to just under 40 mg/l within 4 hours. The slurry reactor illustrated in Figure was inoculated with the bacterial consortium and operated for 4 days.

The results of the third biodegradation batch experiments are presented in Figure 29. The raw results of both experimental runs are presented in Appendix I. The PCP concentration in the soil was below detection limits after approximately 12 hours. The concentration of PCP in the soil remained below detection limits for the remainder of the experiment. The concentration of PCP in the aqueous phase increased from 0.0 mg/l at time zero to 39 mg/l after 12 hours. The PCP concentration in the water phase was then reduced rapidly to below detection limits within 60 hours.

Figure 29 depicts the results of the second trial. The PCP concentration was below detection limits after 5 hours and remained at this level for the duration of the trial. The water had an associated increase in PCP concentration increasing from zero mg/l at time zero to 55 mg/l after ten hours. The PCP concentration in the water phase was then reduced to below detection limits within 60 hours.

Figure 28 PCP concentration in soil and aqueous phase

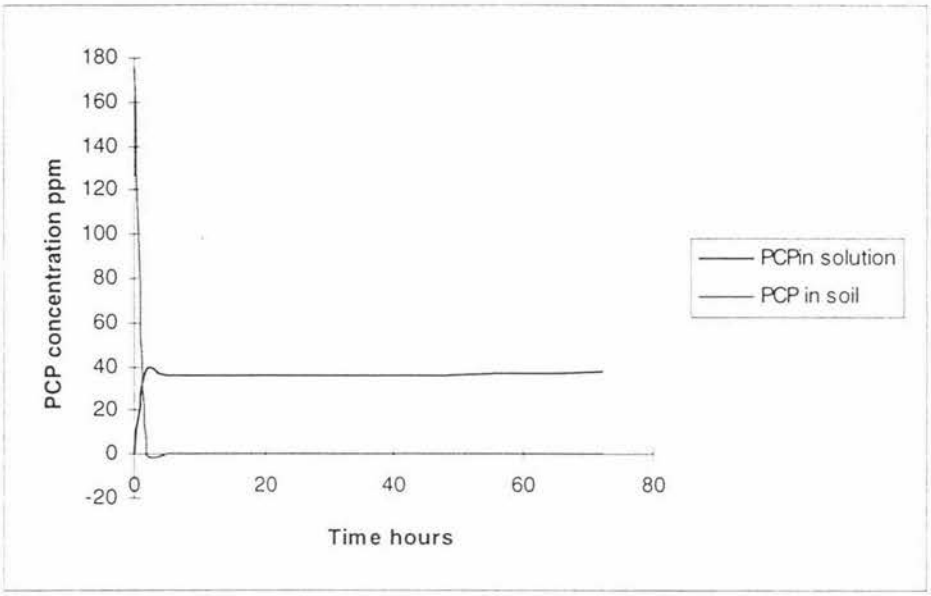
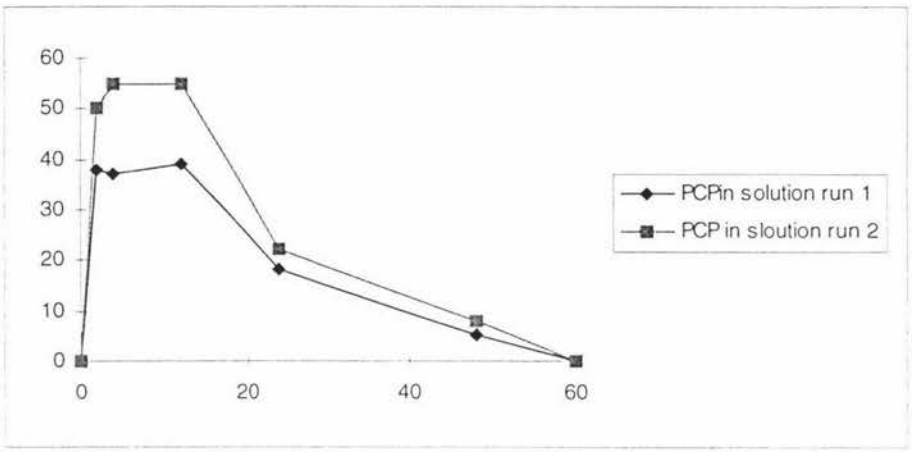


Figure 29 Disappearance of PCP in bioslurry reactor



11.2 DISCUSSION EXPERIMENT 3

Desorption of the contaminant from soil particles must take place before biodegradation can occur. Desorption is particularly important with the soil-water suspensions characteristic of slurry-phase treatment. The rate at which the pollutant desorbs from the soil particles is influenced by the physical and chemical properties of both the soil and the contaminant. Essentially, the same factors control desorption as control sorption.

PCP is a hydrophobic ionisable substance that in the pH range of natural systems (5-8) can exist either as a neutral molecule or as an anion (pentachlorophenate). The distribution of these two forms is a function of the pH of the system. Moreover, the parent molecule and its anion exhibit different sorption characteristics and have different mobility in the subsurface environment with the negatively charged ion being much more mobile than the neutral molecule (Christodoulatos[39] et al 1994). Although some of the mechanisms of PCP have been studied by several investigators Choi and Aomine[40], Warith[41] et al 1993, the effects of soil and solution properties are poorly understood and require further investigation.

By keeping the pH above neutral it was shown that PCP is readily removed from soil matrix into the aqueous and therefore bio-available phase. The half life of the PCP in the slurry phase is compared to the lowest observed half life for the degradation of PCP in the solid phase in Table 3 below.

Table 14 Comparison of PCP half life in solid and slurry phase

Experiment	**PCP half life**
Column experiment 1	216 hours
Bio-slurry run one	10 hours
Bio-slurry experiment run two	9 hours

As can be seen the reduction is half life time is approximately twenty fold. This is a large increase in the rate of degradation. The treatment performed more effectively than the solid

phase treatment at the two lower concentrations with the total PCP being removed to trace levels within 60 hours.

12. CONCLUSIONS

In this study it has been shown that conditions for activity of PCP degrading microbes could be created and that these microbes could lead to 90% removal of PCP within 2 months. The first 20 days being most effective, with a half life of 9-17 days.

The data presented here shows that it is possible to remove PCP from contaminated soil by inoculation with bacteria. This confirms the conclusion reached by Crawford and Mohn (1985). This study however showed that inoculation alone is not sufficient to significantly reduce PCP concentrations. It was shown that the introduced microbes require forced aeration to oxygenate the soil and that inoculum size is an important factor in bioremediation of soil.

The bioslurry experiment confirmed that slurry phase treatment has a large advantage with respect time over in situ systems. However efficiency improvements must be balanced against the extra effort required to prepare the soil to enable bioremediation.

13. FURTHER WORK

The study has shown that biological systems can be employed to remove PCP from soil. The next step in the development of reliable remedial systems is to determine the effect of other contaminants (creosote, arsenic, diesel oil) on the remediation of PCP contaminated soil.

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APPENDIX

Run one													
DATE	DAY	COLUMN 1			COLUMN B			COLUMN 6					
16	0	120	139	128	95	92	89	48	45	46			
19	3	105	113	110	80	85.6	86	40	43.5	46			
23	7	80.1	87.5	85.5	62.76	67.37	69.39	38.3	34.6	35.6			
26	10	81.9	75.3	78.6	59.4	61	65	34.5	36	32			
3	17	60.5	65	62.3	43.8	43.9	45.5	24.1	26.5	21.27			
6	20	36.8	39.2	37.8	25.36	28.65	24.12	12.76	16.04	15.26			
14	28	24.43	20.91	23.6	10.75	11.51	12.35	12.47	9.85	11.37			
21	35	15.5	15.3	16.5	9.8	10.35	9.75	10.3	8.8	9.5			
	DAY	COLUMN 5			COLUMN A			COLUMN 4					
	0	45	48	52	57	50	46	23	27	22			
	3	42	47	44	46	50	45	18	22.5	23			
	7	40.8	34	39.94	41	50	47	22.8	21.65	24			
	10	37	38	35	54.5	49	48	22.6	16.16	15.6			
	17	33.54	36	32.17	50.12	46.3	47.5	15.5	14.8	17.2			
	20	17.48	17.26	14.25	39.6	40.45	45.667	16.2	17.5	16.8			
	28	15.65	13.95	15.26	31.58	28.47	32.31	15.3	16.5	14.5			
	35	10.5	9.95	7.8	25.8	30.5	28.36	15.5	16.8	14.2			

Averaged results

COLUMN 1			COLUMN B			COLUMN 6			COLUMN 5		
DAY	AV	SD	DAY	AV	SD	DAY	AV	SD	DAY	AV	SD
0	129	9.539392	0	92	3	0	46.3333	1.527525	0	48.3333	3.511885
3	109.333	4.041452	3	83.8667	3.354599	3	43.1667	3.013857	3	44.3333	2.516611
7	84.3667	3.827967	7	66.5067	3.398269	7	36.1667	1.913984	7	38.2467	3.702774
10	78.6	3.3	10	61.8	2.884441	10	34.1667	2.020726	10	36.6667	1.527525
17	62.6	2.26495	17	44.4	0.953939	17	23.9567	2.617944	17	33.9033	1.940679
20	37.9333	1.205543	20	26.0433	2.341033	20	14.6867	1.713515	20	16.33	1.804688
28	22.98	1.840082	28	11.5367	0.800333	28	11.23	1.315599	28	14.9533	0.890524
35	15.7667	0.64291	35	9.96667	0.332916	35	9.53333	0.750555	35	9.41667	1.426826

	COLUMN A			COLUMN 4	
DAY	AV	SD	DAY	AV	SD
0	51	5.567764	0	24	2.645751
3	47	2.645751	3	21.1667	2.753785
7	46	4.582576	7	22.8167	1.175089
10	50.5	3.5	10	18.12	3.889884
17	47.9733	1.953493	17	15.8333	1.234234
20	41.9057	3.285018	20	16.8333	0.650641
28	30.7867	2.039224	28	15.4333	1.006645
35	28.22	2.353126	35	15.5	1.3

Day	Column 1		Column 1		Column 2		Column 2		Column 3					
0	63.29		63.29		70.9	72.8	70.9				60			
4	45.8		45.8		60.9	66.9	60.9		54.89	56.2	55.545			
7	36.76	49.45	43.105		40.1		40.1		51.5	53.67	52.585			
11	25.3		25.3		34.3		34.3		37.7	36.5	37.1			
14	37.5	23	30.25		33.7	37	33.7		41.9	43.6	42.75			
21	31.07	21.83	26.45		33.19	30.42	33.19		35.416		35.416			
28	7.3345		7.3345		29.51	33.22	29.51		37.12	20.38	28.75			
35					8.837		8.837		17.488	30.21	23.849			
Day	Column 4				Column 5				Column 6					
0	52.54		52.54		60.34		60.34		65.91		65.91			
4	47.38	44.8	46.09		39.5		39.5		52.5		52.5			
7	47.67		47.67		47.62	56.7	52.16		58.96	49.26	54.11			
11	39.75		39.75		37.22		37.22		44.43	43.42	43.925			
14	18	34.2	26.1		35.92		35.92		44.36	39	41.68			
21	17.47	20.348	18.909		26.35	29.144	27.747		32.01	33.82	32.915			
28	12		12		26.28	24.67	25.475		41.33	45.29	43.31			
35	10		10		11.3	20.04	15.67		40.8		40.8			
Cells														
107			106			105		107		105			104	
AV	SD		AV	SD		AV	SD	AV	SD	AV	SD		AV	SD
63.29	0.0		70.9	1.096966		60	0.0	52.54	0	60.34	0		65.91	0
45.8	0.0		60.9	3.464102		55.545	0.655	47.38	1.29	39.5	0		52.5	0
43.105	6.3		40.1	0.00		52.585	1.085	47.67	0	52.16	4.54		54.11	4.85
25.3	0.0		34.3	0.00		37.1	0.6	39.75	0	37.22	0		43.925	0.505
30.25	7.3		33.7	1.905256		42.75	0.85	18	8.1	35.92	0		41.68	2.68
26.45	4.6		33.19	1.59926		35.416	0	17.47	1.439	27.747	1.397		32.915	0.905
7.3345	0.0		29.51	2.141969		28.75	8.37	0	0	25.475	0.805		43.31	1.98
						23.849	6.361	0	0	15.67	4.37		40.8	0

COLUMN PROFILE		day 35					
depth mm	0	0	0	0	0	0	0
	50	9.8	9.5	0	0	42.56	42.5
	150	0	0	1.78	0	41.07	38.07
		MOISTURE %					
DATE	DAY	COL1	COLB	COL6	COL5	COLA	COL4
16	0	20	19.5	20	18.5	20	20
19	3	20	20	20	19	18	20
23	7	18	20	20	18	19	17.5
26	10	17.5	21	20	18	20	20
3	17	20	17.5	18.5	19.5	19	18
6	20	22	20	20	21	20	19
14	28	19	18	21	20	19	18
21	35	17	17	20	21	20	19
28	42	18	16	18	20	20	20
		19.056	18.778	19.722	19.444	19.444	19.056
Cl readings							
BLANK	COL1	COLB	COL6	COL5	COLA	COL4	
23.08	89	68.43	52.33	49.75	42.19	44.26	
26.3	96.7	69.57	49.57	50.17	40.11	43.39	
25	95	72	50.14	52.68	41.2	42.36	
27							
25.345	93.567	70	50.68	50.867	41.167	43.337	

	800 mls H2	200 g soil												
time	PCPin solu	PCP in soil												
0	0	175												
2	39	0												
5	36	0												
16	36	0												
40	36	0												
72	38	0												
	R1	R2												
time	PCPin solution run 1			PCP in sloution run 2										
0	0			0										
2	38			50										
4	37			55										
12	39			55										
24	18			22										
48	5			8										
60	0			0										
Soil was not detected														