The Use of Electrokinetics to Enhance the Degradation of Organic Contaminants in Soils

A thesis submitted for the degree of Doctor of Philosophy by

Michael John Harbottle
St Hugh's College
University of Oxford

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Abstract: The Use of Electrokinetics to Enhance the Degradation of Organic Contaminants in Soils

Michael Harbottle, St Hugh's College

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The application of an electric field to contaminated soil specimens and the resulting electrokinetic phenomena have been combined with the degradative actions of bacteria to create a novel method for the remediation of contaminated land. Currently, the vast majority of remediation projects involve disposing of contaminated soil in landfill. With the introduction of, and increases in, landfill tax in the UK, this option is becoming less desirable, and so robust, more sustainable techniques are required, such as bioremediation. A major problem with the implementation of bioremediation is the bioavailability of contaminating chemicals. Reduced accessibility of bacteria to the chemical(s) they are attempting to degrade can lead to significant increases in required remediation times, as well as the possibility of significant residual contamination, the levels of which cannot be easily further reduced.

This thesis addresses the problem of bioavailability of contaminants in soils, and has investigated the use of electrokinetic phenomena as tools to bring about an increase in this factor, leading to improved biodegradation. Soil contaminated with pentachlorophenol (sodium salt) was subjected to an electric field in a number of experiments, with significant transport of the initially ionic chemical noted. The transport and fate of this chemical were tracked throughout each experiment, along with properties of the soil pore fluid. Significant changes in soil chemistry were noted (particularly pH or moisture content, depending on the experiment). The effect of pH change was found to be particularly important in this respect, with acidic conditions hindering both movement and bioavailability. A method of applying an electric field to contaminated soil containing degrading bacteria was developed which minimised the changes to these parameters within the soil. A significant increase in the effectiveness of the remediation was noted with this technique, with substantially faster degradation found to occur.

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1. Introduction

The sudden increase in the industrial use of land from the 17th century onwards has left a legacy of derelict and polluted sites across much of the developed world. In some areas of the developing world, rapid commercial and technological growth is increasing the extent of the problem. Often, industrial processes have led, accidentally or otherwise, to chemical spills or leaks. These chemicals would then escape into the ground, and from there could contaminate groundwater supplies or remain in the soil until disturbed at a later date. In recent years, due to a variety of reasons such as demand for building land and stricter environmental regulations, more attention has been given to the remediation of contaminated land, and so research into suitable technologies to bring this about has increased. This project has investigated one particular technique: the combination of bioremediation with electrokinetics.

Contaminated land is defined in Section 78A(2), Part IIA of the Environmental Protection Act 1990 as:

"Any land which appears to the local authority in whose area it is situated to be in such a condition, by reason of substances in, on or under the land that:

- significant harm is being caused or there is a significant possibility of such harm being caused; or
- pollution of controlled waters is being, or is likely to be caused"

The extent of the problem in the U.K. is difficult to measure, as there is no official register of contaminated land. However, under Part IIA of the Environmental Protection

Act (1990), local governmental authorities are required to identify such sites in their area and ensure that they are remediated. As of May 2003, few had been identified and categorised, although the Environment Agency estimated that there may be as many as 100,000 sites in England and Wales (covering almost 1% of the land area of Great Britain). Maini *et al* (2000b) quoted an estimated 200,000 contaminated sites. There will also be much variation in the type and extent of pollution at these locations. Worldwide, a similar picture is presented. In the United States, the National Priorities List (NPL) is a published list of hazardous waste sites currently undergoing remediation; there were, as of August 2001, 1235 sites on this list (source: US Environmental Protection Agency). However, there are many more sites that are not currently involved in that program – the Comprehensive Environmental Response, Compensation, and Liability Information System (CERCLIS) contained some 44,000 hazardous waste sites as of July 2001 (source: US Environmental Protection Agency).

The U.K. is currently undergoing something of a housing crisis – there is a large demand for housing, but land is relatively scarce upon which to build, especially non-green belt land. To attempt to minimise urban sprawl, the U.K. Government has indicated that by the year 2010, at least 60% of new housing should be constructed on so-called brownfield sites - areas that have previously been developed (Parliamentary Office of Science and Technology, 1998). A good source of brownfield sites is the wealth of former industrial land, often located in the centre of cities and large towns. However, there is a greater risk of this land being contaminated due to its previous uses, and so before construction can begin, the land may have to be remediated. By not remediating, the risk of exposing construction workers or the general public to a formerly ‘dormant’ chemical reservoir is greatly increased. Studies have been performed to investigate the effect of such chemical sources on human health. An investigation into the links between different environmental

lead sources and child blood lead levels in urban areas of the USA showed that water, soil and house dust are important sources (Lanphear *et al*, 1998). A prime example of the need to remediate prior to building is that of the Millennium Dome, built on the Greenwich Peninsula in London. The site formerly housed a gasworks and was contaminated with a variety of different chemicals; the entire site was remediated prior to construction of the Dome itself, plus further commercial development and three thousand houses.

There is huge variation in soil conditions at potential remediation locations. Consequently, a range of different remediation technologies has been developed in order to overcome the different problems associated with different sites. Currently, the most popular is to remove the soil concerned to landfill (commonly known as 'dig and dump'). Although this is cheap, it does have environmental problems associated with it – the soil is not treated as such, but is simply moved elsewhere, and landfill sites take up a lot of space and are unpopular. An investigation by Staples *et al* (2003) described a link between gaseous hexachlorobutadiene emissions from an industrial landfill site and an increased incidence of renal disorders in those living in close proximity to the site. An extensive study on birth disorders in areas close to all known landfill sites in the UK was performed by Elliot *et al* (2001), and showed a slight increase in some types of birth abnormalities. In order to encourage more sustainable waste treatment, a landfill tax has been applied in the U.K. since 1999, whereby any waste going into landfill is taxed and the resulting proceeds used to further development on more sustainable waste technologies. Many land remediation projects now employ a multi-faceted approach, but will often involve a certain amount of dig and dump, at least for the most contaminated material, simply because it remains comparatively inexpensive. The range of other technologies is too broad to list here, but there are two main categories – *in situ* treatment

and *ex situ* treatment. The main distinctions are that *ex situ* processes are relatively easy to control but require excavation (i.e. increased cost), whereas with *in situ* techniques it can be difficult to ensure a uniform removal of contaminants. The decision as to which technique or suite of techniques to use is usually made on a cost basis; certain technologies will be more expensive in certain soil types than in others. The price of dealing with contamination can, of course, vary tremendously from location to location, due to the wide variety of factors that impinge on cost. Amounts of 10-80 £/m^3 for bioremediation, 100-400£/m^3 for incineration and 30-75£/m^3 for landfill are quoted by Jackman *et al* (2001).

The technologies investigated in this project are electrokinetics and bioremediation, both of which can be implemented *in situ* or *ex situ*. Briefly, electrokinetics (in this context) involves the application of an electric field to soil. The movement of ions within the soil pore fluid completes the circuit. This current through the soil mass leads to a number of phenomena that form the basis of the usefulness of electrokinetics. These are largely due to the movement of charged particles within the soil mass, and include the movement of pore water ions due towards the electrode of opposite charge (termed *electromigration*), and, only in a soil containing clay particles, the flow of water from one electrode to another (*electroosmosis*). The soil chemistry can be subject to significant changes due to the application of this field (for example, significant pH changes occur in the region of the electrodes due to the electrolysis of water).

Bioremediation harnesses the action of natural organisms in degrading unwanted chemicals. Almost all chemicals, natural and manmade, are susceptible to degradation by bacteria or fungi, or even higher organisms such as plants (although often this apparent

degradation will be due to microbial communities associated with the plant), given the correct conditions. In soil, temperature, pH, the presence of natural predators of the degrading organism and many other factors can affect the efficacy of the bioremediation process. The *bioavailability* of a chemical in soil describes how accessible it is to a degrading organism, and many factors play a role in determining this. Many organic chemicals exhibit water insolubility to some degree, and so will preferentially associate with the soil itself rather than remain in the aqueous pore solution. This is particularly prominent in fine grained and clayey soils, where the soil particle surface area (and therefore the number of possible bonding sites) is large. In addition, the presence of significant amounts of organic matter within the soil matrix can lead to sequestration of the pollutant. The degrading organism may therefore not be able to access the pollutant in order to degrade it, leading to recalcitrance of a certain proportion of the chemical in the soil (dependent of course on its water solubility). Bioavailability can be further reduced by variability of contaminant concentration within an area (for example, a relatively low average concentration may contain highly contaminated hotspots). In sufficiently fine-grained soils, the pore spaces may not be large enough to give bacteria access and as such the pollutant within these spaces will persist.

Currently, bioremediation and, to a lesser extent, electrokinetics are employed in the contaminated land industry, although they are not widely used. They both have the advantage of being applicable *in situ*, meaning that excavation is not necessarily required, but taken individually, it may be difficult to achieve a satisfactory level of removal. They are also both time consuming, and can take months or even years to complete. Electrokinetics, however, is an extremely manageable technique, with the ability to treat difficult-to-reach areas – it has been proposed that it may even be used to clean up soils underneath existing buildings without disturbing the foundations, due to its directional

nature. Electroosmosis has been used for several decades to strengthen problematic soils – in the right conditions, electroosmotic water flow leads to consolidation and an increase in soil strength results. The electromigratory effects of applying an electric field have been successfully employed to remediate metal-contaminated soils in many laboratory studies, and several field scale investigations have shown promise. However, remediation of organic contaminants is generally less successful. Organic chemicals can strongly associate with soil organic matter, so becoming less susceptible to drag by any electroosmotic water flow, and also, if polar, unaffected by electromigration. These contaminants can be removed, but it can take months or even years to achieve a suitable reduction in concentration. Bioremediation is more commonly used to remove organic chemicals, but can be difficult to apply *in situ*, as uniform distributions of the degrading organism are difficult to achieve over the entire contaminated zone. The bioavailability of a contaminant often hinders progress also, with recalcitrant molecules remaining inaccessible to degrading organisms. A truly robust and reliable *in situ* remediation technology is desirable for the remediation industry, especially for organic contaminants in low permeability soils such as clays, which are traditionally difficult to remediate with current methods.

Whilst the application of both electrokinetics and bioremediation have been practised for a number of years, the combination of the two is a novel remediation method. This new method will retain its advantages of being easily manageable and versatile, as well as remaining applicable *in situ*. The problems of bioavailability can be overcome by the use of electrokinetics. The ability of the electric field to move the contaminant molecules even a small distance will significantly increase access, by helping to move it out from inaccessible pores, by redistributing high concentrations and also by desorbing the contaminant from the soil. The alliance of these two techniques gives greater scope for *in*

situ remediation in fine-grained soils. The problems of dealing with the often-strong association between pollutant and clay soil are usually overcome by removing either all the soil or at least the finer portions of it. This new technology would allow a more rapid and efficient remediation of fine-grained soils without the need to dig and dump.

The aims of the work described here were to assess the feasibility of directly increasing contaminant bioavailability using an electric field, thereby offering scope to improve the process of bioremediation by reducing remediation time and increasing the rate of degradation. The first objective was the design of simple, robust apparatus that would allow ease of use in complex experiments. Simple, safe protocols to prepare contaminated, inoculated soil specimens were also required, in order to ensure the repeatability of experiments. The next goal was the determination of the basic properties of the soil and contaminant, particularly with respect to the behaviour of the contaminant in the soil. This could then be extended with the introduction of the electric field, to establish the contaminant transport behaviour within the system – movement of PCP (on a micro- or macro-scale) was required to improve bioavailability. Once the contaminant behaviour was characterised, and a method of inducing movement had been found, the introduction of degrading bacteria and their effect on the pollutant could be studied with and without the electric field. Further studies on the effect of ageing (increased recalcitrance with time) of the contaminant in soil, the behaviour of other contaminants and the effect of the electric field and contaminant on bacteria were additional targets.

2. Literature Review and Theory

2.1. The Theory of Electrokinetics

Reuss (1809) discovered that fluid flow across a clay soil could be induced by the application of a D.C. electrical field. This was later termed electroosmotic flow, and is defined as the flow of pore fluid relative to the solid matrix. This is one of the major phenomena associated with the term 'electrokinetics'.

The phenomenon of electroosmotic flow in clay soils may be explained with reference to the electrical charge on the surface of clay particles. This is well explained in Gray and Mitchell (1967). Generally, clay particles are of the form of flat plates, with an excess of negative charges on the large, flat areas and positive charges on the ends. This is due to the construction of these particles from interleaved sheets of silicon oxides and magnesium or aluminium oxides, as shown in the example in Figure 2-1.

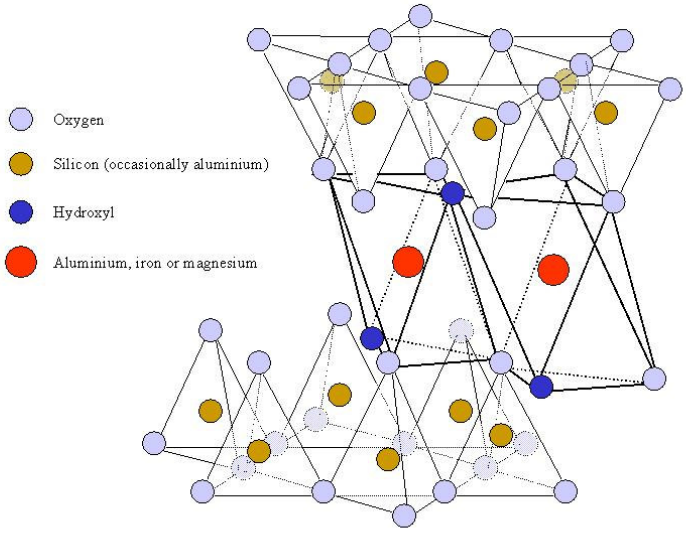

Figure 2-1: Atomic structure of clay minerals

Therefore, pores in a clayey soil are largely bounded by faces exhibiting a net negative charge. This affects the distribution of ionic or polar chemical species in the pore water, leading to what is known as the 'double layer' theory in clays. A diagram of the effect of these charges around an idealised (spherical) particle is shown in Figure 2-2 and a qualitative graph of the variation of electrical potential with distance from particle surface is presented in Figure 2-3, along with an idealised distribution of charge:

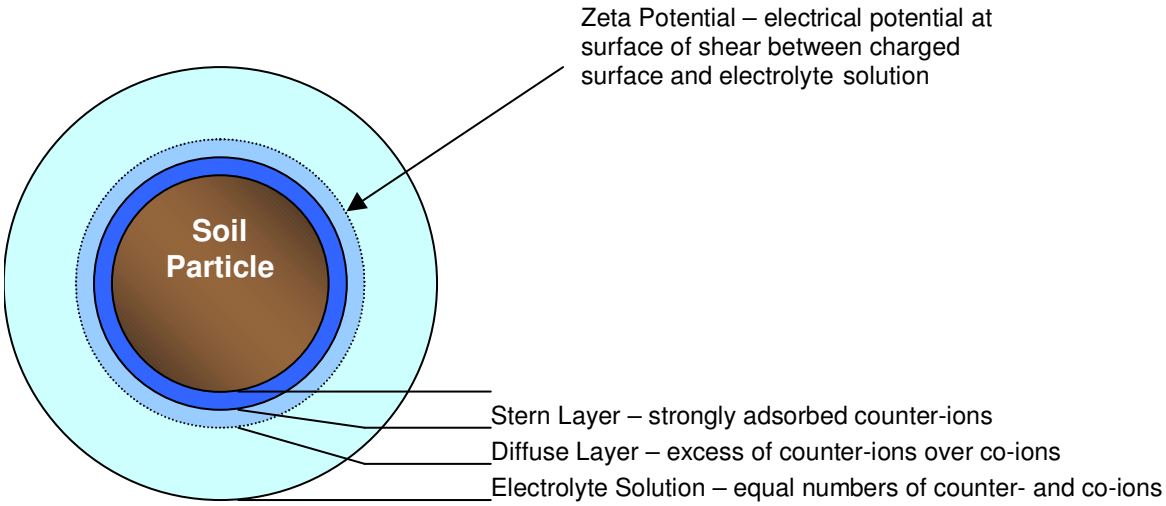

Figure 2-2: Idealised representation of water around a clay particle

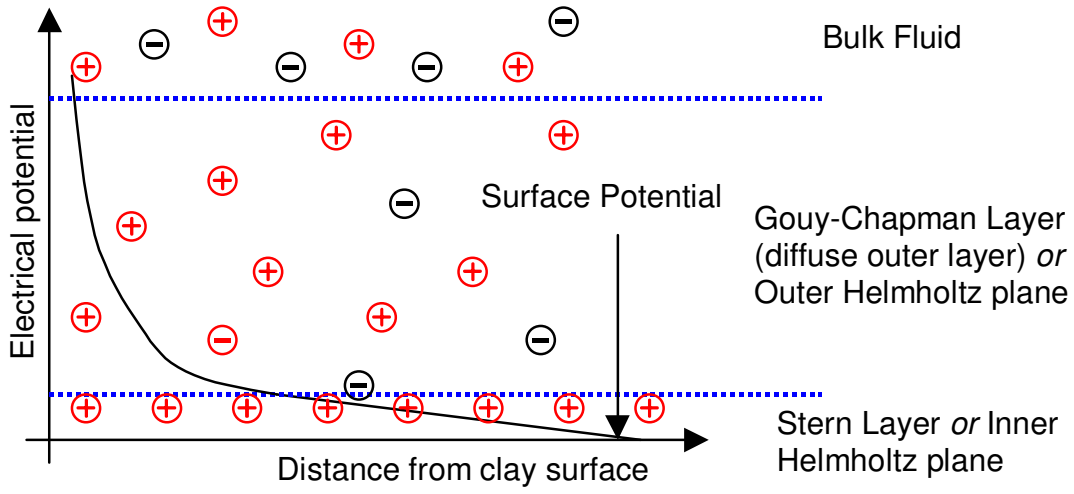

Figure 2-3: Typical variation of electrical potential with distance from clay surface

To balance the charge on the pore walls, there will be an excess of positive ions in a region close to the face, together with 'bound' water molecules. The model used to describe the variation in electrical potential around a particle is the Gouy-Chapman-Stern model (Atkins, 1990), as shown on Figure 2-3. This was created by taking the Gouy-Chapman model, which provided a realistic model of the diffuse outer layer but led to unrealistically large surface concentrations of ions, and modifying it by combining it with Helmholtz' theory of the distribution of ionic species at a solid-solution boundary. The electrical potential around an object with surface charge such as this may be quantified using the zeta potential. This is the potential at the boundary between immobile water molecules sorbed to the soil and mobile water molecules in the free pore fluid. The species of cations present will vary depending on variables such as the soil constituents and history. When an electric field is applied, cations in the fluid flow towards the cathode and anions in the fluid flow towards the anode, but the excess of cations generates an overall friction on water molecules, creating a net flow of water, which is known as the *electroosmotic flow*. This flow is usually towards the cathode, due to the negative zeta potential and therefore excess of cations in the surrounding double layer. However, under certain conditions (notably in highly acidic regions), an excess of small, positive ions can become tightly bound to the soil due to the electrostatic attraction. This has the net effect of reducing the effect of negative charges on the soil surface, and may even make the net charge positive. In this case, as the zeta potential will now be positive, an excess of negative ions will be required in the double layer for charge balance, and so application of an electric field will lead to flow towards the anode. This effect is described by Eykholt and Daniel (1994).

Electroosmosis is a phenomenon that occurs only in soils containing these particles with charged surfaces. It is a function of the excess of positively charged ions over negatively charged ones, or *vice versa*. It therefore does not occur in sands, silts or gravels unless an appreciable amount of clay particles is present.

One of the first analytical models of electroosmotic behaviour was proposed by Helmholtz (1879) and modified by, amongst others, Smoluchowski (1921), which gave the velocity of fluid flow in such a porous medium:

$$v_e = \frac{\varepsilon.\zeta.\Delta E}{4\pi\eta\Delta L}$$

Equation 1 – the Helmholtz-Smoluchowski Equation (after Gray and Mitchell, 1967)

ε – dielectric constant of pore fluid
ΔE – applied voltage
ΔL – electrode spacing
ζ – zeta potential of material
η – viscosity of pore fluid

This theory assumes that the pore fluid is fresh water or a dilute electrolyte, and that the radii of the pores relative to the thickness of the double layer surrounding the material particles are large. It is based on capillary theory, and so does not take into account the tortuous water flow pathways through the soil mass. The porosity of the system will also have an effect on the electroosmotic flow. Gray and Mitchell (1967) describe more recent work by Schmid (1950 and 1951) that accounted for fine-grained soils where the assumptions made by the Helmholtz – Smoluchowski model do not hold. It takes into account pore fluid parameters and pore dimensions (in this case, the electric double layer would be significant with respect to pore size). The equation below was derived:

$$v_e = \frac{r^2 A_0 F \Delta E}{8\eta\Delta L}$$

Equation 2 (after Gray and Mitchell, 1967)

r – pore radius
A_0 – volume charge density in pore
F – Faraday Constant (96500 C/mol)

Further major phenomena that occur when an electric field is applied to any soil sample are electromigration and a related aspect, electrophoresis. The pore space in a soil matrix is an extremely heterogeneous environment, and will contain a wide range of chemical

constituents, including a large number of charged particles. These will range from ions in solution and located in the diffuse layer around solid particles to larger objects such as bacteria (which can have a variable charge that depends on factors such as pH) and colloidal particles (such as very fine clay material or organic matter) which exhibit a net charge. Electromigration is the term that describes the transport of ions in the pore fluid due to electrostatic attraction. When the electric field is applied across the soil, positive ions will be attracted to the cathode, and negative ions to the anode. This is a major constituent of the current carried by soil. Electrophoresis describes the transport of the larger colloidal or other charged material through the soil mass. The effects of the three major phenomena associated with electrokinetics are illustrated in Figure **2-4**:

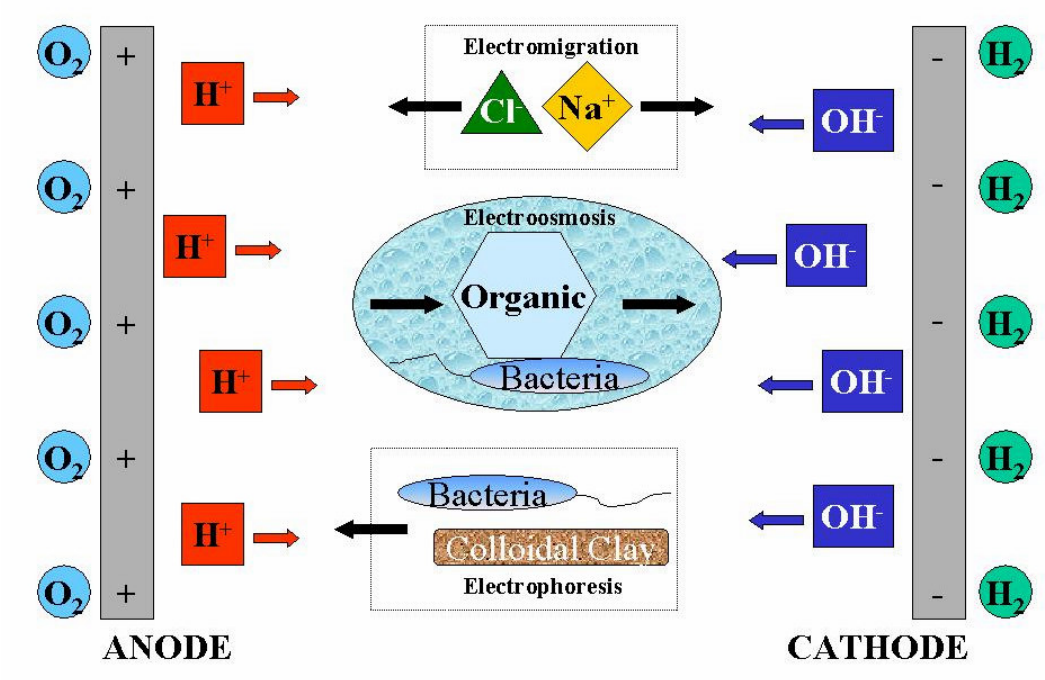

Figure 2-4: Electrokinetic phenomena

Objects such as pollutant molecules or bacteria in the soil are therefore subject to a range of conditions and forces. For example, bacteria may be affected by electrophoresis as

well as the electroosmotic water flow, although they will naturally become attached to soil particles or organic matter and so may resist these forces.

One further major effect of applying an electric field across a soil sample is the electrolysis of water molecules at the electrodes. As shown in the diagram above (Figure 2-4), hydrogen gas and hydroxyl ions are produced at the cathode, and oxygen gas and hydrogen ions produced at the anode. These reactions are explained by the following equations:

$$2H_2O + 2e^- \rightarrow H_2(g) + 2OH^-$$

Equation 3 - cathodic reaction

$$2H_2O - 4e^- \rightarrow O_2(g) + 4H^+$$

Equation 4 - anodic reaction

These reactions lead to an acidic environment around the anode and a basic environment around the cathode. In time, the hydrogen and hydroxyl ions will migrate into the soil due to the electric field and diffusion, creating a pH gradient through the sample. The effects of this phenomenon are discussed further in section 2.2.

2.2. The Effect of Electrokinetic Phenomena on Soils and Geotechnical Problems

The application of electrokinetics to soils in field applications was first performed by Casagrande in the 1930s to 1950s (Casagrande *et al*, 1947 and 1952). The initial applications were for stabilisation of soft soils, but these have since expanded somewhat, moving into geo-environmental engineering and contaminated land remediation (see Section 2.4). The most important of the electrokinetic phenomena in terms of geotechnical engineering is electroosmosis, due to the water displacement effect. Gray and Mitchell (1967) related this to water content and pore water chemistry (specifically the type of electrolyte used as the clay pore fluid and the relative amounts of positive and

negative ions). They made a range of predictions for electroosmotic flow in soil based on the ratio of positive to negative ions within the soil mass, and performed experiments on a variety of clays, attempting to show that it is this that determines the magnitude of flow. Their results showed that the effect of pore water chemistry is very important in understanding the events involved, and that a high ratio of positive to negative ions did indeed increase the flow. The effect of water content on flow was also investigated, with higher water contents shown to give higher electroosmotic permeability.

Ballou (1955) made predictions of electroosmotic flow and the effect of various physicochemical properties of the soil and pore fluid. He prepared a variety of homo-ionic clays and subjected them to electric fields, measuring the initial electroosmotic flows (in time, the creation of hydrogen ions through electrolysis of water at the anode may have led to hydrogen ions exchanging onto the clay surfaces, thereby altering the zeta potential and hence electroosmotic flow). This allowed an investigation of the effect of different cations on electroosmosis; different cations led to different zeta potentials as expected (with hydrogen ions showing a strong shift from the natural value). However, the water flow noted with these different cations was not accounted for solely by water that would be expected to associate with the ion by hydration – other effects such as friction were expected to play a part. Electrochemical changes in soil have a marked long-term effect on the properties of the soil, with for example the introduction of different cations allowing changes in the soil structure, and possible improvements in soil behaviour. Substantial changes of pH, Atterberg Limits, water content and shear strength of an illitic clay were shown by Esrig and Gemeinhardt (1967) in research into electrokinetic strengthening of soils. The introduction of calcium ions was used to illustrate the effect of cation exchange on electrochemical hardening. Variation of all these factors was noted across the sample, showing that the whole treated soil mass cannot be expected to attain the same levels of improvement, as position relative to the electrodes is an important factor. Indeed, shear strength and Atterberg Limits increased

from cathode to anode. This has implications for electrokinetic treatment of real sites, where suitable electrode positioning in three dimensions (rather than one dimension as in many laboratory tests) would be required. For example, in terms of electrokinetic remediation, Maini *et al* (2000a) investigated positioning of electrodes in two dimensions, and showed that removal of contaminants was substantially greater in a direct line between electrodes than it was away from this line. Alshawabkeh *et al* (1999) analysed a variety of different electrode configurations in terms of remediation efficiency, cost and other parameters.

The fundamental role of ion exchange in electrochemical soil treatment was investigated by Gray and Schlocker (1969), who looked at the introduction of aluminium ions into a range of clays. Often, the use of iron or aluminium electrodes will cause the introduction of the particular metal ions into the soil, and they examined the improvement in strength brought about by ion exchange rather than dewatering. The effect was found to be soil sensitive, with certain clays undergoing substantial changes of structure due to build up of hydroxy-aluminium inter-particle layers, whereas others were unaffected with aluminium remaining mainly as a constituent of the pore water.

More recent work by Lo *et al* (1991a) has concentrated on strengthening of soft clays in the laboratory with substantial increases in shear strength over short periods of time. They followed this up with fieldwork (Lo *et al*, 1991b), using a variety of electrode arrangements, and again showed an improvement in soil strength, although this was somewhat less than that achieved in the laboratory, an example of the problems of scaling up to prototype sizes. In a similar vein, Micic *et al* (2001) and Abdel-Meguid *et al* (1999) strengthened marine sediments using electrokinetics, in studies to develop a method for the improvement of offshore foundation capacity. In addition, the latter tested piled foundation capacities following electrokinetic treatment and found significant increases of up to 30% from a single treatment.

The effect of pH is extremely important in the soil, and can cause large changes in soil properties. Also, any pH change is likely to allow different species of soil flora and fauna to flourish (the distribution of micro-organisms particularly will tend to quickly adapt due to the relatively short reproductive period – see section 2.5). However, the effect of pH change may be counteracted by any natural buffering capacity of the soil for hydrogen and / or hydroxyl ions, due to factors such as chemical reaction with or sorption to soil matter. The effect of pH on the zeta potential of soils (the electrical potential at the outer edge of the bound water layer on clay particles) was discussed by Eykholt and Daniel (1994), with reference to the effect that low pHs can have (described in section 2.1). Such effects are also described in depth by Yeung *et al* (1997), who particularly focus on zeta potential variation due to both pH and metal sorption, but also describe the resistance of soil to changes of pH (acid / base buffering capacity).

There is substantially more literature concerning the laboratory application of electrokinetics (largely electroosmosis) to soil improvement than on a field scale. However, specific field projects that have been presented include:

- The improvement of foundation (pile) capacity through electroosmosis (Milligan, 1995), where the increased shear strength due to dewatering and ion exchange effects led to an increase in pile friction capacities;
- Reduction of high pore water pressures below the West Branch Dam, Ohio, USA by electrokinetic dewatering (Fetzer, 1967) stopped rapid movement of the near-completed earthworks and allowed completion of the project;
- Foundation improvement for a proposed sewage treatment plant (Bjerrum *et al*, 1967). Electroosmotic treatment of a slope allowed the excavation of the toe for construction purposes, without endangering a main railway line;
- The improvement of ground for a railway and a dam in British Columbia (Casagrande *et al*, 1981).

2.3. Interaction between Soils and Contaminating Chemicals

In general, soils are extremely heterogeneous, with many different 'micro-environments' possible in a small region. In clay soils especially, the physical and chemical structure can be complex, with soil particles, pore liquid, pore gas, and organic matter providing locations for sequestration and sorption of chemical species. This sets up a number of interacting chemical equilibria between chemical species sorbed to soil matter and in solution. An important factor in determining the state and location of any particular contaminant is that of solubility at the particular soil chemical conditions involved, and its affinity for organic matter or soil particles. Different chemicals can form a wide range of different complexes and bonds with soil matter. Figure 2-5 (taken from Wild (1988)) illustrates this point, by showing a number of different ways in which ions can become associated with an idealised clay mineral surface.

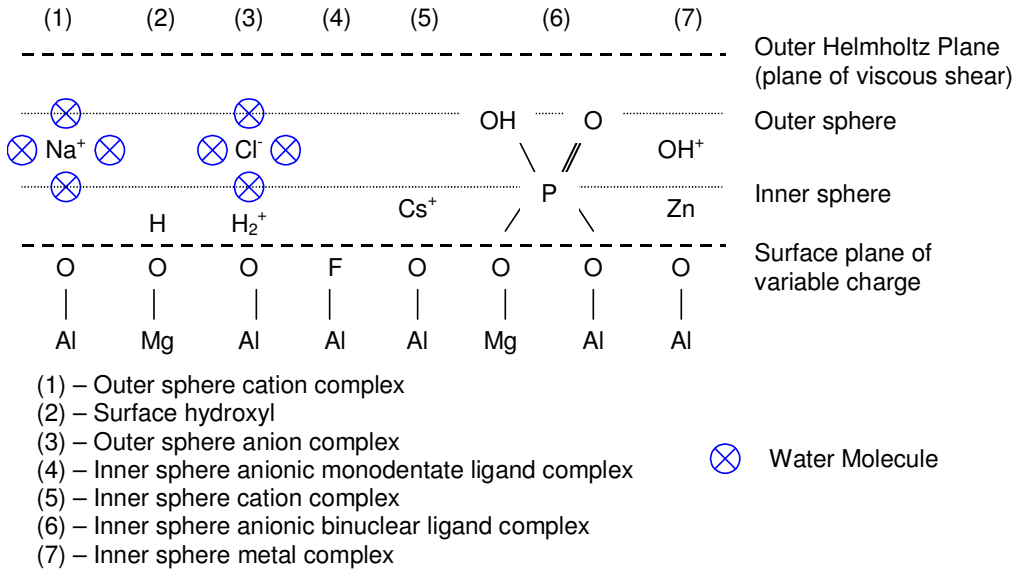

Figure 2-5: Surface complexation on an idealised clay mineral (Wild, 1988)

Organic contaminants in soils will sorb to both soil particles themselves and organic soil matter. The complex structure of the organic material (which includes a large proportion

of complex polymeric molecules, formed from the partial breakdown of plant or animal tissues) leads to a variety of different environments within even one molecule. Organic molecules with a degree of insolubility in water may preferentially sorb to hydrophobic sections of organic matter. As time goes on, molecules such as this may migrate further into organic matter (where greater hydrophobicity may exist) making them more resistant to degradation and so more persistent. Such an effect is known as ageing. Reid *et al* (2000) discussed the factors involved in bioavailability and ageing and the link between the two. They quote results from, for example, Kelsey *et al* (1997) showing how biological availability (to both earthworms and bacteria) and chemical extractability decrease with time. The effect of interaction between organic matter and contaminants was illustrated by Mayes *et al* (2002), who exposed rhesus monkeys to soil containing polychlorinated biphenyls (PCBs) and measured dermal absorption. Of the order of 4% of PCBs were absorbed through skin in this study (with a soil organic content of 8.7%), which was compared to 14% recovered in a previous investigation (soil organic content 0.9%) – the authors draw the link between organic matter content and dermal absorption from these results.

The interaction between soil system and contaminants is of course dependent on soil conditions other than organic matter content. For example, the solubility of metals can vary with pH - lead solubility increases as pH drops (Alshawabkeh and Açar, 1996), for instance.

2.3.1. Pentachlorophenol

Pentachlorophenol is an ionisable organic compound – it is commonly found either in its protonated form or as a sodium salt (sodium pentachlorophenate), as shown in Figure 2-6.

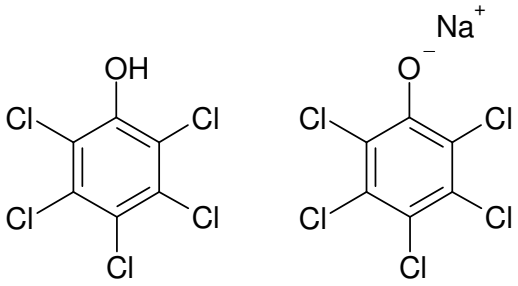

Figure 2-6: Molecular structure of Pentachlorophenol and Sodium Pentachlorophenate

Pentachlorophenol in its undissociated form has a low solubility in water at room temperature, of the order of 1x 10^{-5} M. For dissociated PCP, this value is much higher (of the order of 1x 10^{-3} M). The dissociation of PCP in water is highly pH dependent, as the following equation shows:

$$C_6Cl_5OH \Leftrightarrow C_6Cl_5O^- + H^+$$

At higher pH, the equation shifts to the right, indicating that more undissociated PCP can be dissolved. At a low pH, the reverse is true. The log of the dissociation constant (K_a) is approximately 4.75 (Lee *et al*, 1990). Therefore, the pH conditions in a soil will be vital to understanding how PCP will behave within the soil mass. Stapleton *et al* (1994) investigated the effect of soil pH on PCP sorption and showed that a high proportion of PCP sorbed to the soil at low pH, when the protonated form was prevalent.

2.4. Geoenvironmental Uses of Electrokinetics

Whereas the use of electroosmosis for stabilisation of soft clays is at least well established, if not extensively used, the use of the range of electrokinetic phenomena to remediate contaminated land is a relatively recent development. As with the geotechnical work, there are substantially more laboratory studies presented than work in the field. The attraction of this particular method of remediation is based on it being an *in situ*

technique - it would be possible to decontaminate fine-grained soils without recourse to dig and dump or other methods.

Heavy metals are common pollutants in soils that have previously supported a wide range of industrial processes. Highly toxic species such as cadmium and arsenic pose a health hazard (Pearce, 2003), and so will have to be remediated. The process of electromigration is one choice for this task. However, the soil environment is a complex one, especially once the effects of electrokinetics are imposed (changes in the soil chemistry, such as the development of a pH gradient as discussed earlier, are particularly important). The simplicity of the basic technique then becomes vastly more complicated. There is a significant collection of research on the remediation of heavy metals, as the effects and processes will change from soil to soil. Açar and Alshawabkeh (1993) demonstrated and explained many of the effects that occur due to pH changes in soil subjected to an electric field. They describe lead and calcium removal from kaolinite and associated problems with precipitation of the contaminant at high pH near the cathode. Pamukcu and Wittle (1992) tested an extensive range of different contaminants and soils, and reported good removal efficiencies up to around 90% (in sandy soils). This depended on soil, contaminant type and initial concentration, and, of course, pH development. The probable existence of negatively charged metal complexes (e.g. hydroxides) was considered as a possible cause of inefficiency, seen in many metal removal projects. Probstein and Hicks (1993) also looked at metal contaminant removal and the dominance of pH effects. They noted the problems of metal precipitation and the advantage of metal solubilisation by extremes of pH, and noted the increasing effect of diffusion as concentration gradients increase with increasing movement of the pollutant. Chemistry of the electroosmotic system in a heavy metal contaminated soil was further assessed by Eykholt and Daniel (1994), examining the major influences of pH and changes to pore water chemistry on the efficiency of pollutant removal. They used copper contaminated soil to show that pH has a dominant effect on the overall processes, with high pH leading

to high electroosmotic flow, but also leading to precipitation of metal contaminants. This is shown in many of the above publications, with a high concentration of contaminant recovered from high pH regions of the soil. This shows an intriguing problem with the development of pH in many cases. A decrease in pH near the anode helps to mobilise certain metal contaminants, whereas a high pH near the cathode precipitates them and reduces drastically the effect of electromigration (although for certain metal complexes, for example chromate, a high pH leads to higher mobility). A compromise may therefore be needed to assist in complete removal of metal contaminants, rather than redistribution.

Metal contamination has been shown to be effectively remediated by proper application of electrokinetic techniques. Problems of adsorption of metal ions to soil particles can be countered by effective pH control, or simply through the effect of the electric field. The technique may also be applicable to contamination by organic molecules in soils, for example, oil spills, pesticide accumulations etc, although success again depends on soil properties and good project design.

Bruell *et al* (1992) demonstrated removal of a range of water-soluble organic contaminants through electroosmosis, although their testing regime was limited. It was noted that removal of more water-soluble contaminants such as benzene and xylene was quicker than that of species such as hexane with a lower solubility. They also modelled the transport of contaminants through the soil incorporating adsorption effects. A similar approach was followed by Probstein and Hicks (1993). In both cases, experiments were performed for relatively short times but extrapolation of the data using models they developed indicate good removal efficiencies over time. Again, as with metals, the effect of pH can be significant, as it can affect the zeta potential of the soil and have detrimental effects on the electroosmotic flow. Saichek and Reddy (2003) used pH control at the anode in order to maintain electroosmotic flow (by preventing the reduction in zeta potential due to an influx of hydrogen ions), in an unsuccessful effort to move PAHs – a

notable increase in electroosmotic flow (by a factor of four) was achieved however. Because many organic contaminants may be largely undissociated, and therefore uncharged, in the soil, electroosmosis tends to be the dominant mode of contaminant transport, and so reduction of flow due to extremes of pH can be deleterious to the remediation process. Often, some form of pH control is introduced, such as by buffering at anode and / or cathode with suitable chemicals. A further hindrance to the efficacy of remediation of organic contaminants by this method may be the adsorption of organic contaminants to soil matter. This will be especially true for low solubility contaminants that will sorb to the soil preferentially rather than dissolve in the aqueous pore fluid. However, there are examples of movement of such species. Polycyclic aromatic hydrocarbons (PAHs) are a prime example of such chemicals, and Maini *et al* (2000a) describe movement of these pollutants under the effects of electrokinetics in a historically contaminated soil (contaminated with a range of metals and other organic chemicals). Açar *et al* (1992) demonstrated using phenol that there appears to be no retardation of the contaminant while it desorbs from the soil. This indicates that the electric field helps to make this an almost instantaneous process, although phenol is a relatively soluble organic compound. High levels of organic removal (this time using pentachlorophenol as well as phenol) were demonstrated by Kim *et al* (2000), again indicating that sorption and low solubility of pollutants does not appear to hinder substantially the electrokinetic remediation technique in the soil used.

An example of this technology used in the field for organic contaminant removal is the so-called Lasagna Process. The principles of this are presented by Ho *et al* (1999a and 1999b) in two papers in 1999 where small and large-scale field tests were performed to remediate a trichloroethylene (TCE) contaminated site in the USA. It is termed the Lasagna Process due to its layered arrangement - rows of parallel anodes and cathodes with several rows of carbon treatment zones between them, meaning that the TCE did not have to be moved far before it could be trapped by the carbon filters. Removal

efficiencies on the small-scale test were again high, generally over 90% within the test zone, which is certainly substantial for a field process. In the large-scale testing, *in situ* degradation was employed, using zero-valent iron to dechlorinate the TCE. High reductions in TCE levels were achieved in certain locations, although others only exhibited a 40% removal. This work was a successful example of the scale up from laboratory to field, and is one of the few examples of published field results using electrokinetic techniques. Electrokinetic remediation as a commercial venture has shown some promise in the United States and in the Netherlands. Lageman *et al* (1989) describe the successful remediation of heavy metals from two test sites, and for a commercial project where more than 50% of total arsenic was removed from a contaminated timber processing site.

2.5. Bacteria and Soil

The structure of soil is such that it often leads to a very heterogeneous arrangement of particles, pores, organic matter and living organisms. There may be areas where the soil is entirely saturated with pore fluid, along with areas containing only soil matter and gas-filled voids, or a combination of gas and liquid. Microbe numbers in this environment are extremely variable. In a typical surface soil (with good access to both oxygen and water), there may be of the order of 10^8-10^9 bacteria per gram of dry soil, along with several hundred metres of fungal hyphae (Prescott *et al*, 1999). Bacteria are consumed by organisms such as protozoa, and so may more commonly be found in smaller pores within the soil (2-6μm (Prescott *et al*, 1999)) which will afford them some protection. High concentrations tend to be found in immediate proximity to plant roots, due to symbiotic relationships between plant and microbes.

The application of an electric field to a soil is likely to change the soil chemistry, notably moisture content, conductivity and pH. Moisture content changes in soil are known to have an effect on indigenous bacteria – Cho *et al* (2000) showed considerable variation of chlorophenol degradation with differences in moisture content, and showed clearly that anaerobic degradation is considerably slower than aerobic degradation (the former would be more common with increasing levels of moisture). Large changes in pH are often reported in electrokinetic processing (Açar and Alshawabkeh, 1993, and Eykholt and Daniel, 1994). Each individual species of bacteria has its own optimal pH for growth, although most prefer a range around neutral (fungi tend to prefer a slightly more acidic value, e.g. 4-6) (Prescott *et al*, 1999). Changes in pH will allow different microbe species to flourish. At extremes of pH in soil (whether natural or induced by outside means), few microbes will be able to take advantage, and so only a few species will dominate, leading to possible changes in soil biomass. Differences in pH in natural soils were shown to have an effect on the bacterial community in a range of natural soils (Bååth, 1996), although it was difficult to separate this from the effect of organic matter concentration.

2.6. Bioremediation in Soils

Bioremediation is a well-established method of cleaning up contaminated land, but the remediation of soils using micro-organisms is a complex process. Gaining understanding or effective use of the technique involves in depth analysis of soil physical, chemical and biological properties and knowledge of how different disciplines will interact, in order to achieve the best outcome. Many of the theories involved, problems encountered and practical techniques used are described by Alexander (1999), who has looked at biodegradation and bioremediation of organic and inorganic pollutants in air, soil and groundwater, and describes various technologies currently used in industry. The two major approaches to bioremediation involve either addition of specific micro-organisms,

whose behaviour is already known and catalogued, or the stimulation of the behaviour of the natural bacterial community, through addition of nutrients, oxygen etc. Both may be used either *in situ* or *ex situ*. Bacteria grown in the laboratory, however, will have disadvantages when inoculated into soil as the existing soil microbial communities will be well adapted to their environment and can out-compete the new species, or possibly even consider them as a food source. Examples of *in situ* techniques include phytoremediation (higher plants commonly have diverse microbial populations associated with their root system, which can help to deal with pollutants. The plants themselves may also achieve uptake of certain chemicals), and land farming (pollutants are added to soils to allow indigenous microorganisms to degrade them). *Ex situ* methods can be more expensive due to the necessity of removing the contaminated land and storing it during the remediation process, but usually a greater degree of control can be exercised on soil conditions and monitoring of the soil can be performed more easily. Bioreactors and biofiltration techniques are common examples of this. An important concept explored in Alexander (1999) is that of bioavailability, which underpins a large proportion of this current project. Bioavailability is a term that describes the accessibility of a contaminant to a particular degrading organism, taking into account factors such as chemical sorption, sequestration and concentration. It can vary from organism to organism for a particular chemical, and will vary with time as well as location. The soil environment is a very complex one, with solid, liquid and gaseous phases, with soil particles and extremely complicated molecules of organic matter, with many different forms of plant and animal life, of both microscopic and macroscopic scale. The introduction of a pollutant to such a system is just one more variable. The bioavailability, and therefore fate, of the pollutant is determined by the presence or absence (and to what degree) of a combination of these factors. Contaminant chemicals in soil can become resistant to microbiological degradation in a variety of ways. They may become sorbed to soil particles or organic matter, sequestered within microscopic pores in the soil structure (clay particle sizes, and therefore the size of pores between them, are of the order of microns), or be distributed

unevenly on a macroscopic scale, leading to areas of high (and therefore toxic) concentration, and areas of low concentration. These factors are by no means the only ones affecting the bioavailability of chemicals, but they do indicate the possibility for chemical recalcitrance in soil.

The bioremediation process involves micro-organisms using organic chemicals as a source of carbon, nitrogen, energy or other component in order that they may further grow and reproduce. Commonly, bacteria will have genes in their chromosome which encode for a range of degradative enzymes. Each enzyme may perform one part of the degradation process, transforming the target chemical into another form, in a chain that may end in mineralisation of the chemical (i.e. complete decomposition to carbon dioxide and water). The following diagram, Figure 2-7, illustrates the degradation pathway of pentachlorophenol (PCP – the model pollutant used in this study) under aerobic conditions.

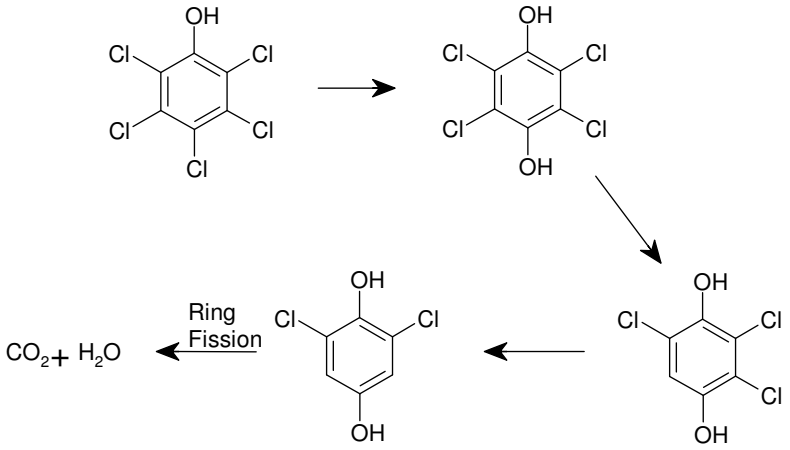

Figure 2-7: Aerobic degradation pathway of pentachlorophenol

Atlas and Bartha (1998) make the point that, in some cases, degradation may not lead entirely to mineralisation, or sometimes the intermediate degradation products such as those illustrated above may persist for a period of time, due to greater hydrophobicity, for example. If a particular metabolite is also a toxic chemical, then the situation may not be improved, or may even deteriorate. McGrath and Singleton (2000) performed a

toxicological assessment of soil during the breakdown of PCP by white-rot fungi (*Phanerachaete chrysosporium*) to test for the presence of toxic breakdown products. The creation of various other chlorophenols in the degradation process was thought to lead to maintained toxicity levels within the soil.

Atlas and Bartha (1998) also make note of the need for testing the efficiency of bioremediation, by monitoring both bacterial or fungal growth and decline, and the levels of chemicals remaining in the soils. Many techniques exist for the determination of microbiological activity, ranging from detecting the activation of certain genes to counting the numbers of bacteria in a known mass of soil. Jansson *et al* (2000) created biomarkers by engineering different bacteria with a *luc* gene (which encoded for firefly luciferase, leading to the ability to track changes through counting luminescent bacterial colonies) or a green fluorescent protein (*gfp*) gene (which performs a similar role, although in this case no energy source or addition of substrate is required, other than the provision of blue light). Details of methods available for the determination of levels of chemicals in soils (specifically pentachlorophenol in these cases) are given by McGrath and Singleton (1997) and by Wall and Stratton (1991).

The model pollutant used in the current work was pentachlorophenol (PCP). There is substantial information available on the fate of this particular compound in soils and other media, as it is a relatively common contaminant, used for such purposes as wood and canvas preservation, and also as a biocide. It is toxic to many living organisms, including humans, and behaves in a recalcitrant manner, and so is an ideal target for research into its removal. A study on a range of chlorophenols in soil indicated both microbiological and non-microbiological degradation (Baker and Mayfield, 1980). The position of chlorine atoms relative to the hydroxyl group on the benzene ring in chlorophenols was identified as a possible cause for the apparent toxicity of some compared to others; this was also a possible cause of differing rates of non-biological decomposition of the

chemicals, the occurrence of which was attributed to autooxidation or to catalysis at the surfaces of soil particles. Most, if not all, organic contaminants in soil will be susceptible to microbiological degradation to some degree, and Leung *et al* (1997b) isolated one particular PCP-targeting organism from soil on the site of a former wood treatment facility in Canada (as reported in Shaw *et al* (1997)). It is this bacterium (*Sphingobium* sp. UG30, formerly *Sphingomonas* sp. UG30, formerly *Pseudomonas* sp.UG30) that is used in the current project. Shaw *et al* (1997) then performed experiments in solution to examine the effect of various parameters on PCP degradation in a relatively ideal situation. As would be expected, overall speed of degradation was proportional to the initial amount of bacteria, with an increased lag phase (acclimation time) for lower densities. These lag periods also increased with increasing initial PCP concentration and increasing levels of chloride ions in the solution due to PCP mineralization led to inhibition of the degradation. As with many bacteria, the optimal temperature for degradation for this *Sphingobium* is in the region of 30º Celsius. However, in real bioremediation situations, temperatures may well be encountered that are much lower. Shaw *et al* (1997) also investigated the effect of temperature, and, as would be expected, although PCP was degraded at 10º Celsius, it took substantially longer than at the optimum temperature. The effect of temperature on biodegradation of PCP was also investigated by Cort and Bielefeldt (2000), in conjunction with the action of a range of surfactants. Surfactants are chemicals that have a hydrophilic end and a hydrophobic end – they form structures in aqueous environments called micelles, which arrange the hydrophilic ends outwards. They have been used to trap hydrophobic organic contaminants (for example, PCP) in the hydrophobic centres of these micelles, which enables greater mobility of the contaminant and can help desorb it from soil, thereby increasing its bioavailability. A pictorial representation of a micelle in aqueous solution is given in Figure 2-8 below, with yellow strands representing hydrophobic sections and blue spheres representing hydrophilic ends. This arrangement therefore presents a hydrophilic outer surface to the aqueous solution.

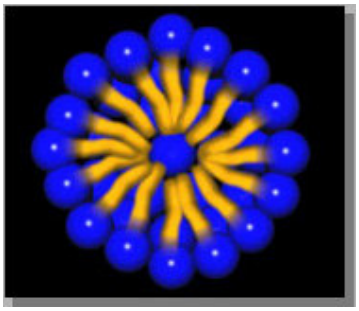

Figure 2-8: Schematic of a micelle

Toxic effects of surfactants were seen at high concentrations; better biodegradation results were obtained at concentrations around the critical micellar concentration (CMC – the concentration of surfactant or detergent at which micelle creation begins). In addition, ionic surfactants inhibited degradation to a greater extent than non-ionic examples. With the particular bacteria involved (*Sphingomonas chlorophenolicum* sp. Strain RA2), temperature increase again led to an apparent increase in growth of bacteria.

A practical example of the use of bioremediation is the clean up after the Exxon Valdez oil spill in Prince William Sound, Alaska, in 1989, as reported in Atlas and Bartha (1998). Eleven million gallons of crude oil was leaked, contaminating over one thousand miles of shoreline. It was determined that addition of fertiliser to the sites would improve bioremediation, due to a lack of nutrients. This decision was borne out by the reduction of predicted clean up time for the worst affected sites from 10-20 years to 2-3 years.

2.7. Using Electrokinetics to Enhance Bioremediation

It may be possible to solve or at least ameliorate many of the problems involved with bioremediation by applying electrokinetics, especially for sites containing organic contaminants. The simplest approach is to attempt to introduce suitable nutrients or other stimulatory chemicals to the soil using electrokinetic movement, which then encourage a natural microbiological community to go about cleaning up the site. Budhu *et al* (1997) and Thevenayagam and Rishindran (1998) have shown that nitrates can be injected into a

low permeability soil using the electrokinetic technique in a relatively short space of time, by injecting them at the cathode and utilising the electromigration effect (although a hydraulic pressure gradient may be necessary to counteract the force of the electroosmotic flow on the chemicals). The latter also make the important point that many bacteria operate in an optimum pH range, often around neutral. Therefore, the development of a pH gradient through the application of an electric field could prove detrimental to their efforts, and so it would be necessary to incorporate some form of pH control. A similar investigation by Açar *et al* (1997) showed the possibility of using the same techniques to introduce sulphate (a negative ion) and ammonium (a positive ion) from either end of the sample using electromigration, achieving a flow of these nutrients through kaolinite soil. The use of ammonium hydroxide and sulphuric acid as sources of these chosen nutrients meant that pH at both electrodes could be controlled, with the hydroxyl ions neutralising hydrogen ions at the anode and *vice versa*. Such work was taken further by Rabbi *et al* (2000) by injecting a cometabolite to encourage biodegradation of an organic contaminant. A cometabolite is the target of bacteria in the soil, whilst the contaminant itself is degraded too, as part of the process.

Electrokinetics can also be used to effect movement, not only of the contaminant, but of the bacteria as well (Deflaun and Condee, 1997). This movement is pH-dependent, due to the ionisable nature of certain polymers in the cell membranes, which have different charges at different pH. Different polymers in different bacterial strains lead to a strain-specific variation in flow rate under an electric field. Deflaun and Condee's experiments compared a wild-type strain of *Burkholderia cepacia* G4 with a variant that was not as susceptible to adhesion to soil particles - bacteria will often attach strongly to solid particles, which can cause problems for bioremediation. Results showed that under a hydraulic gradient only, the original strain showed little movement in relation to the variant, but under an electrokinetically imposed flow, flow rates were similar. It was also

demonstrated that these micro-organisms can degrade trichloroethylene (TCE) whilst under the influence of the electric field, and that the field helps to desorb TCE itself, an additional bonus. A typical combination of electrokinetics and bioremediation is described by Jackman *et al* (2001), who used the applied electric field to move an organic contaminant into areas where micro-organisms were at work. They demonstrated that the bacteria in question were active and metabolising under the D.C. electric field, although little movement was noted by electrophoresis. This may be due to sorption or the effect of several forces acting upon them in different directions.

2.8. Conclusions

Electrokinetics as a tool for soil improvement has been investigated in numerous studies, with both dewatering and ion exchange being presented as methods for increases in soil strength. Practical applications of this method have been employed successfully in a number of cases.

The behaviour of contaminants in soil has also been extensively studied, both with and without the presence of electrokinetic effects, and in terms of microbial degradation. The properties of pentachlorophenol, the contaminant used in this work, have been particularly well characterised in terms of sorption to soil and organic matter, and it has been the pollutant used in numerous bioremediation studies, with some success. In particular, the work by Shaw *et al* (1997) and Leung *et al* (1997a and 1997b) have provided a great deal of information regarding the interaction between the particular micro-organism used in this work (*Sphingobium* sp. UG30) and the PCP. It has been shown that this bacterium will degrade PCP in soil, and therefore is a valid combination for this work.

There have been many studies on the transport of contaminants, both metallic and organic, in soil due to electrokinetic phenomena. The important effects of changes to soil chemistry (especially pore water pH, moisture content and zeta potential) have also been demonstrated. Movement of these chemicals has been presented due to both electromigration and electroosmotic water flow, with the latter seen to move uncharged organic molecules (e.g. Ho *et al*, 1999a and 1999b) in some soils. Significant removal of contaminants has been seen, especially with metals, using electrokinetics alone. Similarly, movement of the target contaminant (PCP) had been shown by Kim *et al* (2000). These studies provide evidence that the movement of PCP (and other contaminants) is certainly possible in soil, and so the use of electrokinetics to induce movement in this case was reasonable. However, many investigations have been performed using an idealised, kaolinite soil, which can behave in a very different way to a natural material with organic matter. To fully understand the behaviour of contaminants, bacteria and electrokinetics in real applications, the use of a real soil would be desirable.

There is no apparent evidence in the literature of electrokinetics being used to increase the bioavailability of a contaminant, and thereby increasing the efficiency of bioremediation. The proposed technique, therefore, is novel, although the combination of electrokinetics and bioremediation has been made in the past. Electrokinetic transport has been used to move pollutants to bacterial 'treatment zones' (e.g. Jackman *et al*, 2001) or to introduce nutrients into a soil mass to encourage biodegradation (e.g. Budhu *et al* 1997), but amendment of bioavailability has yet to be investigated.

3. Experimental Apparatus, Materials and Methods

3.1. Development of Experimental Apparatus

The apparatus designed for and used in the experiments detailed in this thesis has of course evolved over the course of the project. The aims of the project meant that there were several criteria that had to be incorporated in a successful design, and these are listed here.

- Unsaturated soil specimens were required, to allow aerobic bacteria a supply of oxygen in order that they might be able to degrade the target contaminant. However, in order to maintain an electric current, there would have to be continuous water pathways between electrodes as well.
- Any design should incorporate the facility to prepare and store numerous soil specimens for long periods of time. The storage containers should be able to be incorporated into the test set-up, in order to minimise soil disturbance.
- Preparation and contamination of soil specimens should follow a procedure whereby the contaminating chemical is added to a ready-prepared sample. This will ensure that the chemical itself will not interact with the soil structure in an unnatural manner, and that the experimental sample conditions would mimic a real chemical spill. Otherwise, the behaviour of the chemical under an electric field may be affected by unnatural interaction with the soil structure.

The apparatus can be described in terms of two major designs, which are shown below.

3.1.1. Design 1

Figure 3-1, Figure 3-2, Figure 3-3 and Figure 3-4 show the two major elements to the first design. The first of these was termed the cartridge; it was a very simple object, with two acrylic walls (250x120x6mm) separated by four plastic bars (100x10x10mm) at each corner (Figure 3-1 and Figure 3-2). Hydrophobic Vyon membranes (Porvair Plastics, UK) of thickness 2mm were secured across each end as shown, until experiment PCP_5, when Daramic (see next section) was introduced. Soil was compacted into a cartridge and stored; many specimens could be prepared at once, due to the simplicity and low cost of this design.

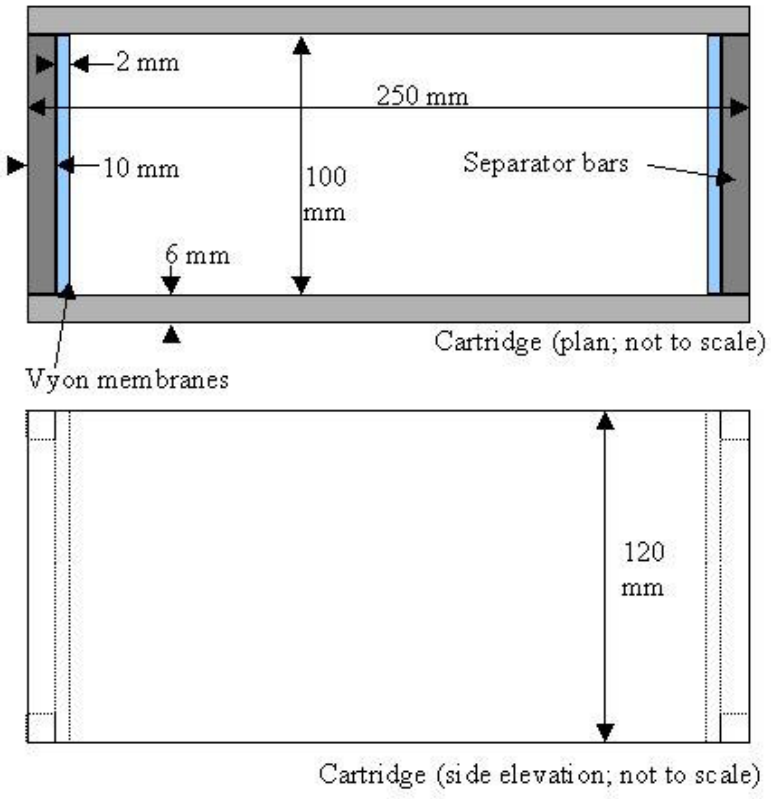

Figure 3-1: Large cartridge design diagrams (not to scale)

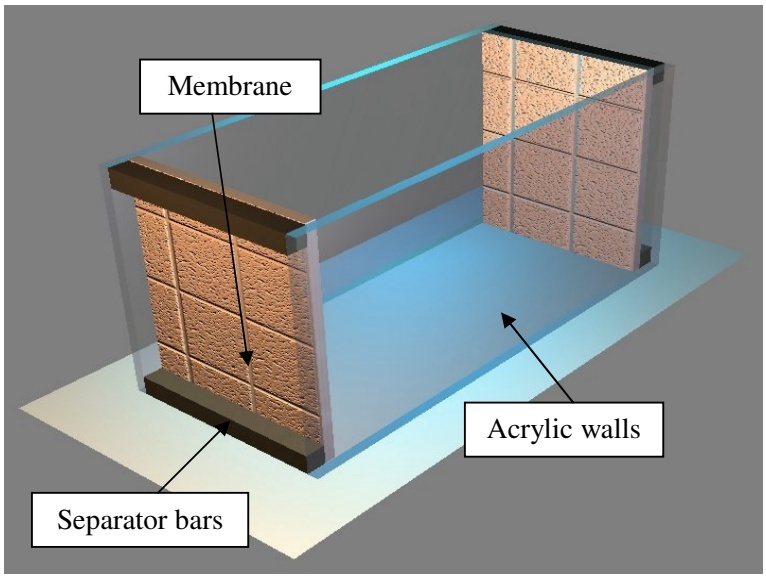

Figure 3-2: Large cartridge

The second portion of the apparatus was known as the tank (Figure 3-3 and Figure 3-4). It was constructed entirely of acrylic, and had several ports drilled for voltage probes and electrode fluid control. The tank was large enough to contain a cartridge with sufficient room at either end to house an electrode. A ring of silicone grease was used to seal the base of the cartridge, and strips of rubber along the side of the cartridge isolated the electrode chamber fluids from one another. The electrodes themselves were constructed from compressed graphite, with a regular array of holes drilled to allow flow of electrolyte around them. Their dimensions were approximately 100x100x10mm, similar to that of the soil specimens, in order to ensure that all areas of the soil experienced a uniform electric field. Power was supplied by a bench-top power supply (maximum 60V). Voltage was measured at five points along the soil sample, using stainless steel probes placed in ports along the bottom of the tank, as well as at the electrodes themselves. Electroosmotic flow (if generated) was measured using an overflow port at the cathode end of the tank, draining into a glass beaker.

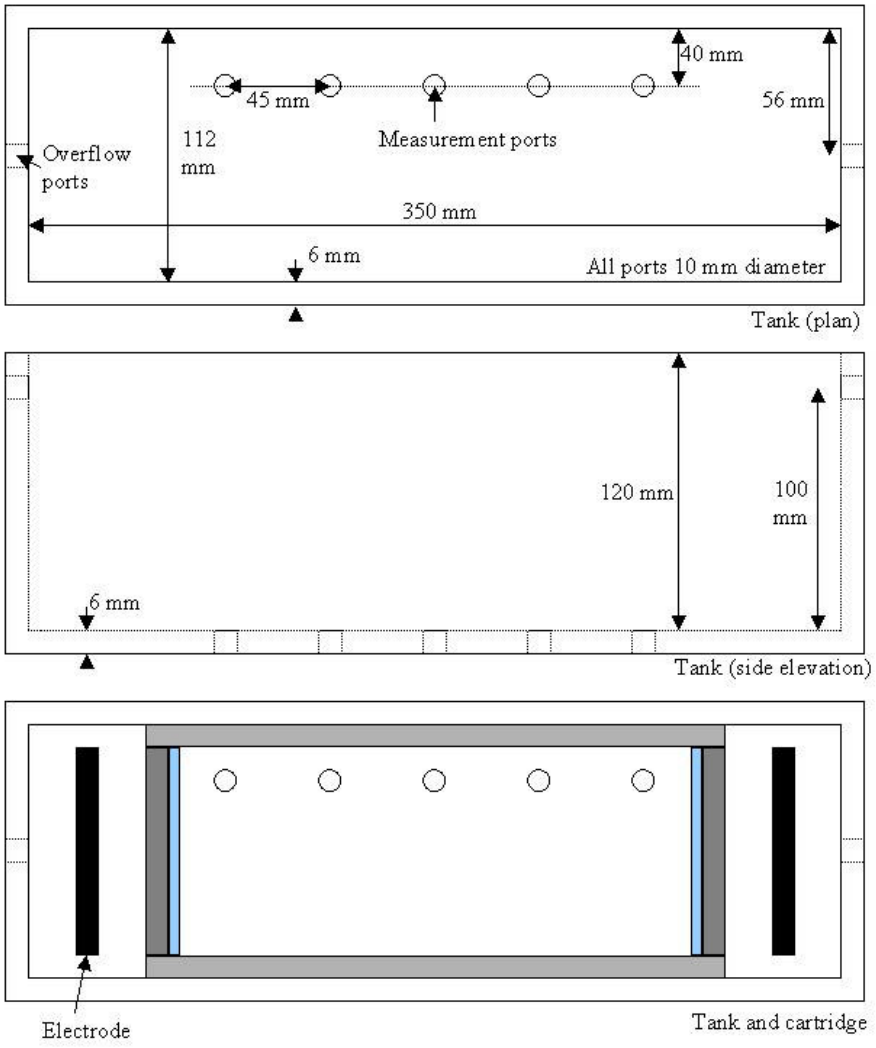

Figure 3-3: Tank design diagrams and combined apparatus schematic plan (not to scale)

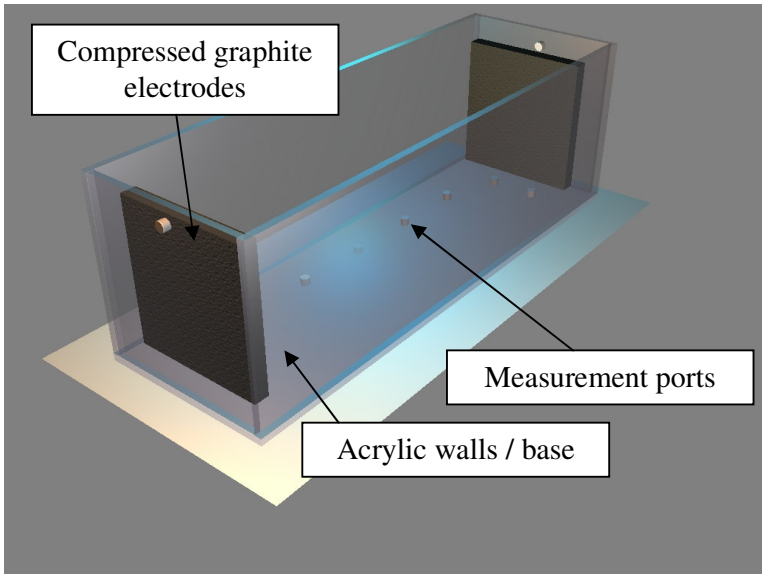

Figure 3-4: Tank design

The apparatus was redesigned after several experiments, as it became clear that it was extremely difficult to prevent the soil from taking up water from the electrode chambers.

3.1.2. Design 2

The second design retained many of the elements of the previous construction. The cartridge system was entirely retained, but new electrode chambers were constructed. Figure 3-5 is a photograph of the assembled apparatus. Design diagrams are presented in Figure 3-6, Figure 3-7, and Figure 3-8, whilst an idealised diagram in Figure 3-9 shows how the components fit together. The electrode chambers were designed to be entirely self-contained and separate from one another. To perform a test, a soil specimen was placed on a baseplate and the chambers plugged into either end of the cartridge. The protruding face on the chambers was permeated by approximately 30 small holes (approximately 4mm diameter) and covered by a semi-permeable membrane, and was long enough to ensure the membrane made good contact with the soil. The holes in this

face allowed electrolyte fluid to make contact with the membrane over a substantial portion of its area, enabling a uniform water flow through the membrane. The membrane was a hydrophobic semi-permeable material called Daramic (Daramic Inc., North Carolina, USA). It was sufficiently hydrophobic that it did not permit the passage of water under working hydraulic gradients (i.e. when the chamber was filled with water) but allowed water to flow due to external forces (for example, that of electroosmosis). This was fixed in position using Araldite glue. Each chamber had several ports to allow fluid and pH control and the baseplate had provision for voltage probes, as in the previous design.

Each experiment using this apparatus was performed with six soil specimens, all in cartridges and all prepared identically. Three were situated in the electrokinetic apparatus and subjected to an electric field, whilst the remaining three were used as control experiments. Testing in triplicate in this way provided replication of results.

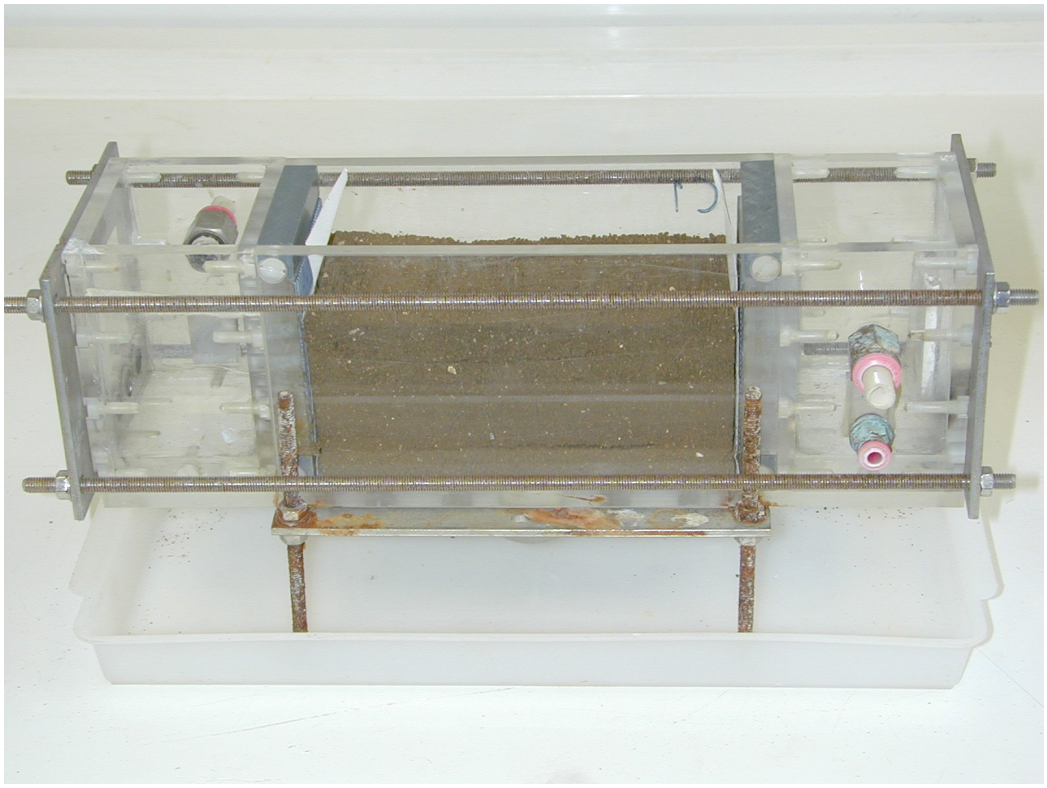

Figure 3-5: Photograph of small apparatus

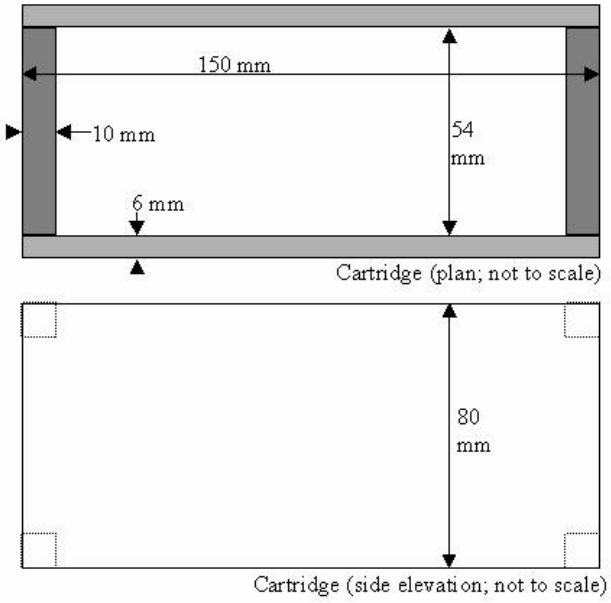

Figure 3-6: Cartridge design (small apparatus; not to scale)

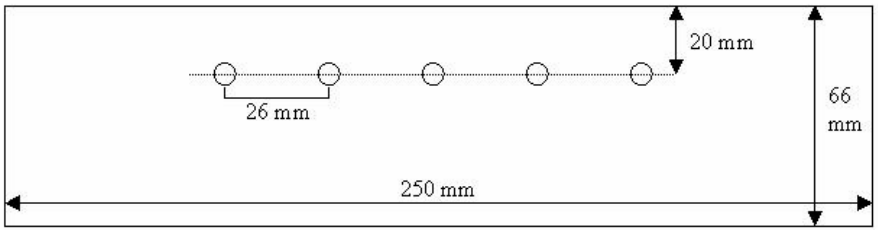

Figure 3-7: Base design (small apparatus; not to scale)

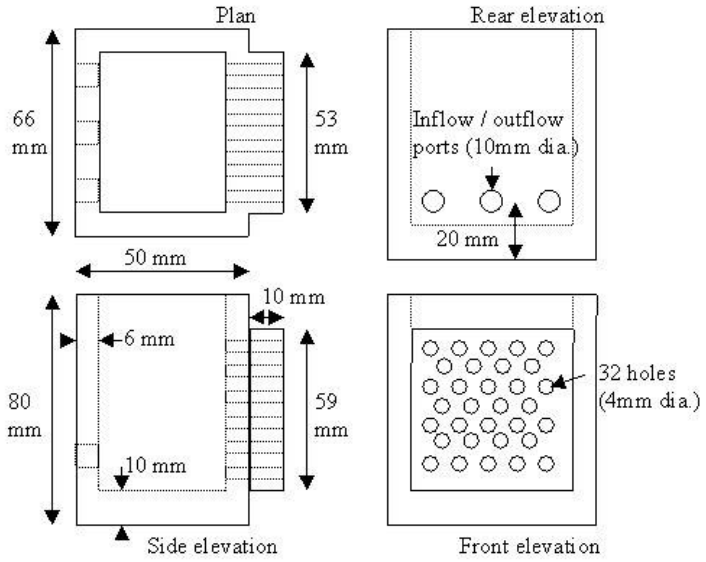

Figure 3-8: Electrode chamber design (not to scale)

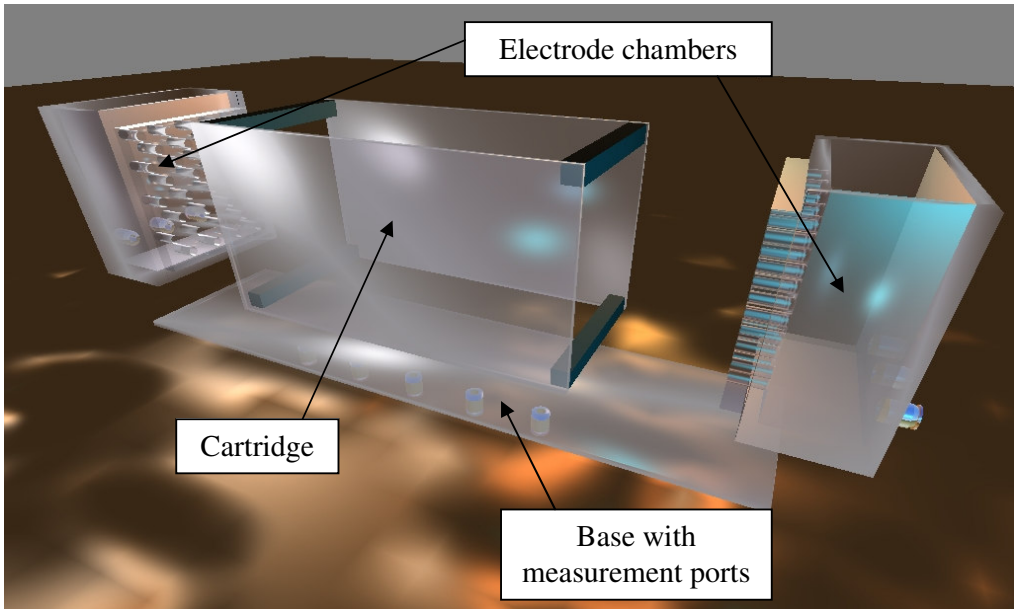

Figure 3-9: Second apparatus design

3.1.3. Control of pH and Electrolyte Fluids

The electric field induced significant pH changes at both cathode and anode. In order to prevent, or control, the resulting changes in soil chemistry, the pH of either electrolyte could be controlled. Early attempts at this used manual addition of either sulphuric acid (at the cathode) or sodium hydroxide solution (at the anode). For the majority of experiments where pH control was implemented, however, commercial electronic pH controllers were used. Their design was such that when the pH was outside a specified range, the power supply was routed to an external device which could allow introduction of suitable chemicals (sulphuric acid or sodium hydroxide solution). The devices used were either solenoid valves or peristaltic pumps. This process took place in an external control chamber, as pH electrodes do not function well in the presence of an electric field. Fluids were pumped into this chamber using peristaltic pumps, with the pH-adjusted fluid allowed to flow back to the electrode chambers under gravity. In experiments where the pH at both anode and cathode was required to be unchanged, all electrode fluids, both anolyte and catholyte, were pumped to the same chamber, with flow under gravity used to keep electrode chambers full. As the number of hydrogen ions produced equalled the number of hydroxyl ions, the combination of these fluids allowed recombination of these ions to reform water molecules (this method is similar to one used in Lee and Yang (2000)). This procedure also allows ions and other material removed from the soil under the electric field to be reintroduced.

Electroosmotic flow in a clay soil involves the flow of water from the anode to the cathode, except for under highly acidic conditions. This may lead to a drop in fluid level at the anode, which, in addition to fluid loss by evaporation and electrolysis, can cause significant water loss. In order to maintain water levels in anode chambers, a device known as a Mariotte bottle (Figure 3-10) was used.

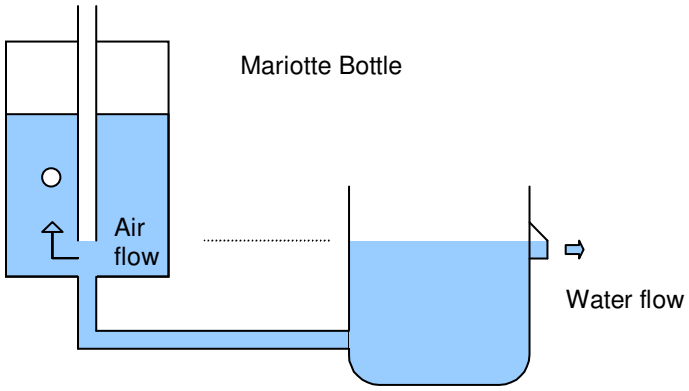

Figure 3-10: Schematic of Mariotte Bottle

The Mariotte bottle is an almost sealed chamber with a tube in the centre. When it is filled with a liquid and connected to a container through a port in the base, the level of water maintained in the container is that at the base of the tube, as this is the level at which atmospheric pressure is applied. This will hold true for as long as there is liquid in the reservoir above the level of the base of the central tube.

3.1.4. Measurement of Physical Parameters

Physical parameters that have been logged in experiments in this project include voltage, current, temperature, pH, and electroosmotic flow. In several later experiments, voltage and current were monitored every 15 minutes using an automated data acquisition system. It was decided in experiments involving radiolabelled PCP not to take readings of electrode chamber pH and temperature, as this would have required the sealed containers to be opened frequently, leading to errors in monitoring of carbon dioxide emissions.

Voltage was measured *via* stainless steel probes inserted into the soil specimen from beneath, at five locations along the specimen, either using a portable voltmeter or through the data acquisition system. Current measurements were made using digital meters connected in series with the experiments themselves (although with the automated system, the voltage across a small resistor was used to calculate this). A hand-held

temperature probe was used in early experiments to monitor temperature at the same locations as voltage measurement. The pH of electrolyte fluids could readily be measured with a standard combination pH electrode, although the electric field had to be removed to prevent damage or incorrect readings. Within the soil cartridges, pH was only measured in certain experiments using residual fluid from a contaminant extraction technique, as described in section 3.8.2. Electroosmotic flow was measured in early experiments, using water level drop in the anode chamber as a measure of this. This could be determined by the drop in water in the attached Mariotte bottle, when present.

3.2. Soil Classification Testing Methods

The soil used in the experimental portion of this research was a natural material taken from a site at the Oxford University Field Station at Wytham, near Oxford, from a depth of twenty to fifty centimetres below ground. It was classified using a range of tests, which are described below. Tests performed according to the methods presented in Head (1980a) are taken from BS1377: 1990. The results themselves are presented in section 4.1.

3.2.1. Natural Moisture Content

The natural moisture content of soil was calculated using the standard oven method (section 2.5.2, Head, 1980a), relating the mass of water present to the mass of the dry soil.

3.2.2. Specific Gravity

Specific gravity (G_s) of soil grains was determined using the density bottle method (section 3.6.2, Head, 1980a).

3.2.3. Atterberg Limits

The plastic limit (moisture content at which a soil begins to behave in a plastic manner) was determined by rolling threads of soil on a glass plate (section 2.6.5, Head, 1980a). Liquid limit (the moisture content at which the soil exhibits liquid properties) was determined using the fall cone penetrometer method (section 2.6.4, Head, 1980a).

3.2.4. Particle Size Distribution

The particle size distribution of soil has been calculated using two methods. The proportion of the soil particles falling within the larger grain sizes (above 63μm) was determined by wet sieving through a range of sieve sizes (2mm, 600, 425, 300, 212, 125 and 63μm), using techniques described in Head, section 4.6.6 (1980a). The distribution of the finer clay and silt size particles was determined using a Cilas 920 automatic laser particle size analyser (School of Geography and the Environment, University of Oxford).

3.2.5. Maximum Dry Density / Optimum Moisture Content

The maximum dry density and corresponding optimum moisture content of this soil were determined using the Proctor Compaction Test (section 6.5.2, Head, 1980a). Soil was compacted into a mould of known volume using a manually applied ram, at a range of moisture contents. The variation in dry density achieved was plotted against moisture content. The maximum point on the trend line was taken to be the maximum dry density; the moisture content at which this may be achieved was the optimum.

3.2.6. pH Buffering Capacity

The acid buffering capacity of soil was investigated using the following method. Fifty grams of soil were placed in a glass beaker, and 50ml deaired deionized water added, before thorough mixing. After allowing the soil to settle, the pH of the overlying fluid was measured using a standard combination pH electrode. Aliquots of acidic solution

(sulphuric acid, approximately 0.1M) were added and thoroughly mixed with the soil slurry before being allowed to equilibrate. Once the pH had stabilised (remained constant for one day), the pH of the overlying fluid was measured as above. This whole procedure was performed numerous times, until the pH no longer changed with the addition of further acid.

A very similar procedure was carried out using sodium hydroxide (approximately 0.1M) to determine soil behaviour with respect to hydroxyl ions.

3.2.7. Organic Content

The organic content of the soil was calculated as the percentage loss in soil mass when the soil was combusted in a muffle furnace at 550º Celsius.

3.2.8. Cation Exchange Capacity

Cation exchange capacity (CEC) was determined using ammonium ions as an exchangeable cation source (Black, 1965). The method is outlined below.

- 25g soil (wet weight) and 125ml ammonium acetate (1M) were shaken together overnight in a 250ml flask, on an orbital mixer. The resulting slurry was filtered through a Büchner funnel and filter paper under light suction, ensuring the filtrate was clear.
- Four 25ml aliquots of ammonium acetate were added, allowing each to filter through before the next was added (the soil was not allowed to dry or crack). The leachate was discarded.
- Eight separate additions of 96% ethanol (sufficient to cover the soil) were then applied to remove excess ammonium acetate. The leachate was discarded and the receiving flask cleaned.
- Eight separate 25ml aliquots of potassium chloride (1M) were used (in the same

manner as with the ammonium acetate) to extract the ammonium ions adsorbed to the soil. The leachate was diluted to 250ml with further potassium chloride and the ammonium content of this solution was determined using the spectroscopic method outlined below.

Spectroscopic Ammonia Assay

Three replicates and one control (using water instead of ammonium acetate) were tested in the above manner. Ammonium concentration in the final leachate was determined using the following assay:

1) Dilutions of each sample (100 and 400 times) were prepared
2) 100µl of diluted sample added to 400µl double distilled water
3) 500µl of reagent A (see below) added
4) Vortex
5) 500µl of reagent B (see below) added
6) Vortex
7) Incubate at 37º C for 15 minutes
8) Read optical density at wavelength of 570nm in spectrophotometer

Reagent A: 55.6g Phenol (99%)
0.3g Sodium Nitroprusside
Make up to 1 litre with double distilled water

Reagent B: 20g Sodium Hydroxide
35.4g Sodium Hypochlorite
Make up to 1 litre with double distilled water

Concentration of ammonium was determined from a range of standards of known concentration. The cation exchange capacity (CEC) was calculated using the following formula:

$$CEC = \frac{[NH_4^+] - [NH_4^+]_{control}}{18}$$

3.2.9. Variation of Zeta Potential with pH

The zeta potential of a clay soil can vary with pH; at low pH, hydrogen ions associate with the negatively charged binding sites on the clay particle surface, changing the zeta potential from negative to positive. The variation in zeta potential with pH was determined using a very dilute solution of fine soil particles (passing a 63μm sieve) – one gram soil was diluted with approximately 1.25 litres of deaired deionized water. The pH was amended using either concentrated sulphuric acid or sodium hydroxide solution. Once equilibrium was reached, the samples were tested in a Malvern Instruments Zetamaster automated zeta potential analyser.

3.2.10. X-ray Diffraction and X-ray Fluorescence

X-ray diffraction / x-ray fluorescence was performed by X-Ray Minerals Limited of Betsw-y-coed, North Wales, UK, on samples of prepared Wytham soil (see section 3.3 for preparation method). The soil passing 2μm was used for the analyses. X-ray diffraction techniques were used to identify the clay structure within the soil, whilst major and minor trace elements were determined using x-ray fluorescence.

3.2.11. Pore Fluid Electrical Conductivity

The electrical conductivity of soil pore fluid was measured by preparing three soil cartridges as detailed in section 3.3, then contaminating them (see section 3.4) followed by centrifugation of soil samples from these cartridges at 4000rpm. Supernatant fluid was

taken off, and the conductivity of this fluid was measured using a Hanna 8633 conductivity meter.

3.2.12. Tortuosity

Flow of liquid or gas through a particulate material such as a soil will not be in a straight line (especially in a clay soil, such as is used here, due to the fine size of particles). Flow is more likely to follow a tortuous path, and therefore involve further travel than if the soil was not present. This effect is exacerbated by the unsaturated nature of the soil in this case, as separate air and water flow paths will exist. Tortuosity is a factor that describes the effective path length of an element flowing through the soil relative to the actual length of the specimen, and so is important in the transport of contaminants through soils.

$$\tau = \frac{L}{L_e}$$

τ is the symbol given to the tortuosity factor. L is the specimen length, and L_e is the effective path length. The method used to determine an approximate value for this factor for, in this case, the soil pore water phase, uses electrical signals generated in experiments. The pore fluid largely carries the electric current in soil, with the soil particles behaving as insulators. Therefore, if the pore fluid followed a tortuous path, the voltage required to pass a known current would be larger than that expected for the path length involved, if it were assumed that the electrical flow paths are analogous to the hydraulic flow paths. Using the electrical conductivity determined as above, the voltage required to pass a known current was calculated, on the assumption that the path length was straight and of known length. Using the same conductivity, the path length that required the level of voltage noted in experiments (the effective path length) for the same current was calculated. The tortuosity of the soil specimen was then calculated using the above formula.

Tortuosity will vary during the experiment, with changes in moisture content and saturation ratio. However, it is not possible to keep track of this variation *via* electrical measurements, as the moisture content and pore fluid electrical conductivity will vary considerably with time.

3.2.13. Hydraulic Permeability (saturated)

Hydraulic permeability of a saturated sample of processed Wytham soil was determined using the falling head permeameter method, as described in section 10.7, Head, 1980b. One kilogram of soil, prepared in the way described in section 3.3, was compacted at 50kPa into a 100mm-diameter perspex tube. This was sealed by saturated Vyon membranes and steel end caps (which allowed for water throughflow).

3.3. Soil Specimen Preparation

A consistent method of soil specimen preparation has been employed throughout this work. It was decided from the outset to prepare soil specimens prior to contamination, in order to ensure a realistic soil structure was present before any pollutant was added.

Soil was air dried for at least three days before it was broken up in a mortar and pestle, and passed through a 2mm-aperture sieve. This dry soil was then mixed thoroughly with deaired deionised water in the ratio fourteen millilitres of water to one hundred grams of soil. This gave a moisture content similar to the optimum moisture content (see page 3-12), at approximately 19%. The mixture was allowed to homogenise overnight. With earlier experiments (using larger cartridges (page 3-2)), 2.5kg of soil were used. This was compacted in five additions of 500 grams under a static load of 50 kPa, leading to specimen dimensions of 230x100x100 mm. A similar procedure was used in the smaller

apparatus (page 3-5), although only 500 grams of soil was used in total (specimen dimensions 130x54x59 mm).

3.4. Specimen Contamination

The model contaminant used in this project was pentachlorophenol (PCP). This model pollutant was used as there are few bacteria known to have the capability to degrade it, and so the effect of the inoculation of specific degrading bacteria could be investigated.

The contaminant was used in an ionic form, specifically its sodium salt (sodium pentachlorophenate, $Na^+ C_6Cl_5O^-$, Sigma Aldrich Ltd., Poole, UK). Uniformly contaminated soil specimens were desirable in order to give reliable results. Experiments showed that the protonated form of the chemical was of sufficient hydrophobicity that it was impossible to distribute the chemical evenly in any of the methods attempted. The PCP would sorb to the soil at the point of introduction and not travel any further. In addition, it was desired that the contamination of the soil would be of the order of 100ppm. Experiments (results not presented) have shown that the insolubility in water of this chemical meant that it would have been very difficult to attain this level of contamination.

In work presented in the literature, it is common for a uniform contaminant distribution to be achieved by thoroughly mixing the contaminant and soil (Reid *et al*, 1998). In this work, this technique was not applied, for reasons already given (page 3-1). Investigations were performed into a variety of different contamination methods. The most successful was used for all experiments involving contaminated soil; the procedure was as follows:

- A solution of PCP (sodium salt) was applied to the top surface of the soil specimen, and allowed to percolate through. Any effluent was collected at the base.

- It was found that, even using the sodium salt of PCP, the contaminant would accumulate at the surface of the specimen due to its high affinity for soil organic matter (log K_{ow} = 5.24 (Schellenberg *et al*, 1984)). The effluent was reapplied to the top of the specimen and again allowed to flow through the soil. This procedure was repeated three more times. The reapplication of the PCP-containing effluent was found to redistribute the PCP within the soil mass in a more uniform manner.

There were small variations in applying this technique to the two different apparatus types. With larger soil cartridges, the soil specimen was rested on a thin bed of glass beads (0.4mm diameter) wrapped in filter paper, inside the 'tank'. This permeable layer allowed the contaminating solution to exit the soil and flow easily to the voltage measurement ports where it was collected. A dedicated aluminium contamination platform was constructed along with the second set of apparatus, and was used for all other experiments. Specimens were placed on a stainless steel mesh (with filter paper to prevent loss of soil) on the top of this platform, and the solution applied. Figure 3-11 indicates the procedure followed.

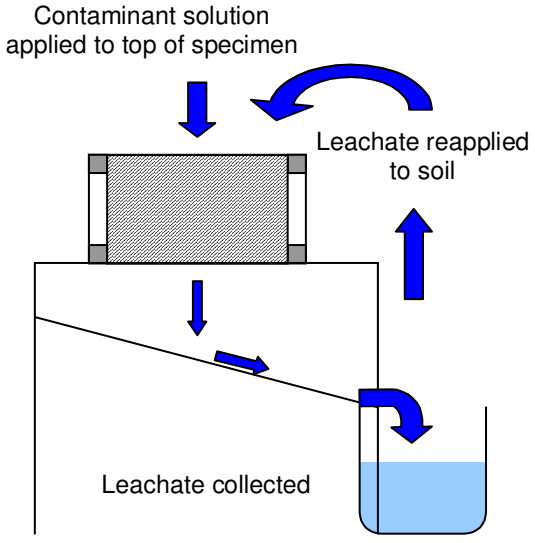

Figure 3-11: Contamination Procedure

Following the addition of the contamination solution, the soil specimen would have a saturation ratio (ratio of volume of water to volume of voids) of between 95 and 100%. It was desired that the saturation ratio would be of the order of 70% during experiments. This was envisaged to allow continuous pathways of water (to allow electrical conduction and transport of chemicals) along with continuous pathways of air (to give an oxygen supply for aerobic degradation of PCP). Therefore, this initially almost-saturated soil was allowed to stand for 24 hours, with evaporation of water bringing saturation to approximately 70%. The specimen was then sealed in a plastic bag for a further five days to allow the moisture to attain an even distribution within the soil. Concentration of PCP in the soil at the start of experiments was found to be in the range 90 to 160ppm.

Later experiments incorporated radiolabelled PCP (each molecule containing one carbon-14 atom). The desired amount of this material was added to the correct amount of non-labelled PCP prior to soil contamination.

3.5. Bacterial Inoculation

The pentachlorophenol-degrading organism used was *Sphingobium* sp. UG30 (formerly *Sphingomonas* sp. UG30, (Leung *et al*, 1997a and 1997b). This is a facultative anaerobe (does not require oxygen to survive, but grows better in its presence) and was obtained from the culture collection at the Centre for Ecology and Hydrology (Oxford). An antibiotic-resistant strain of the UG30 was isolated by Lear (2004) in order to simplify preparation, and tracking of the bacteria in soil (the antibiotic used was Rifampicin). This strain was used in all experiments, and was cultured in a minimal salts medium, the details of which are given in Table 3-1 (taken from Leung *et al* (1997b), originally from Seech *et al* (1991)).

Potassium dihydrogen phosphate (KH_2PO_4)	0.19g
Dipotassium Hydrogen Phosphate (K_2HPO_4)	0.65g
Magnesium Sulphate ($MgSO_4 \bullet 7H_20$)	0.10g
Sodium Nitrate ($Na\ NO_3$)	0.50g
Sodium Glutamate ($NaC_5NH_8O_4$)	4.00g
One litre of double distilled water was added and the mix autoclaved at 121ºC. The following were then added aseptically:	
Rifampicin (antibiotic)	2.0ml
Iron Sulphate ($FeSO_4$)	2.0ml

Table 3-1: Growth Media Preparation for *Sphingobium* sp. UG30

A small amount of bacteria, either taken from an existing liquid culture or from a sample stored at –80ºC, was added to this medium, and the culture grown up over a 24-hour period in a shaking incubator at 30ºC. The amount of cells present in the solution after this time was estimated using a spectrophotometric method, with absorbance measured at a wavelength of 660 nanometres. The number of viable cells was determined from the resulting optical density (O.D.) using a standard curve determined by Wall and Stratton (1995). This was for a PCP-degrading *flavobacterium* - the bacterium used was formerly labelled as a *flavobacterium*, and the curve was found to give a sufficiently accurate value for cell numbers. The formula for this curve is given below.

$$O.D._{660} = \left(1.08 \times 10^{-3}\right) + \left(1.90 \times 10^{-9}\right) \times cell\ numbers$$

The volume of culture that contained the required number of cells could then be calculated, and this amount was spun for 25 minutes at 4000rpm in a Beckman J2-HS centrifuge. The supernatant was discarded and the bacterial pellet was washed and resuspended in phosphate-buffered saline solution (PBS; NaH_2PO_4, 2mM; Na_2HPO_4, 8mM; NaCl, 130mM; pH 7.2) to give the desired bacterial concentration.

Initial bacterial experiments had concentrations of 10^6 colony-forming units (cfu) per gram of dry soil. The bacterial suspension was point-inoculated using a syringe, with 0.1ml introduced at forty-two points on two levels within the soil. This amount of extra fluid would not change the moisture content of the soil significantly. It was considered after these experiments that the initial bacterial concentration was insufficient, and so a concentration of 10^8 cfu per gram of dry soil was used in later experiments. The solution was then inoculated at 96 locations on three different levels (at depths of ~0.5, 3.0 and 5.5cm below the soil surface), again with 0.1ml of solution, in order to give a more uniform distribution of the UG30. The distribution of these inoculation points is shown in the cut-away diagram in Figure 3-12:

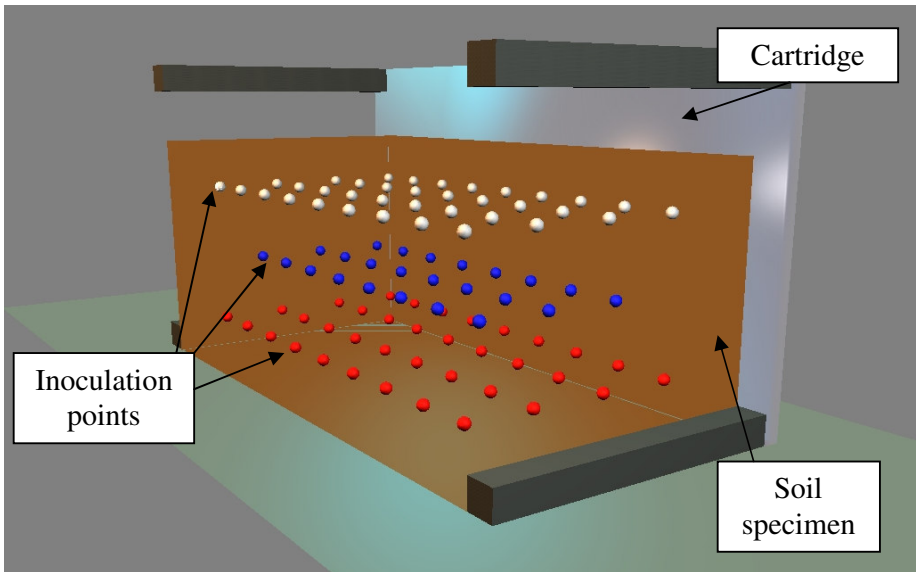

Figure 3-12: Cut away diagram showing points of inoculation

The initial concentration of bacteria in the soil was more accurately determined after inoculation, using a standard Miles-Misra technique (Miles *et al*, 1938). Serial dilutions of the initial suspension were created (generally of concentrations from 10^{-1} to 10^{-8} of the initial solution). Plates containing standard plate count agar (with 2ml rifampicin per litre) were divided into segments (one segment per dilution). Three 20µl drops of each of

dilution were placed on each segment, and the number of individual colonies from each of these drops was counted after sufficient growth time. The concentration of the original solution could then be calculated.

3.6. Preparation of Aged Specimens

A total of thirty-six contaminated specimens were prepared as detailed in sections 3.3 and 3.4 above at an early stage, in order that the ageing effect of the contaminant in the soil might be investigated. Half of these specimens were inoculated as described in section 3.5 at this time also. It was intended to test a set of six of each of these specimen types (inoculated and non-inoculated) at intervals until the end of the project. However, at the time of testing of the first inoculated specimens, it was found that there was no detectable PCP remaining. One test was performed on the non-inoculated specimens (experiment API_1).

At a later date, six soil specimens were contaminated with radiolabelled PCP and aged for eight months before testing (experiment API_21)

3.7. Experimental Procedure and Sampling

The duration of the experiments varied substantially. Information on individual experiments is given in section 4.2. Soil sampling was performed in contaminated soil specimens at a range of time points throughout each experiment. In early experiments, the specimen was destructively sampled at the end of the experiment. In other experiments, soil cores were taken using thin-walled stainless steel corers at several locations between anode and cathode as well as at several time points in order to

determine the behaviour of the contaminant both temporally and spatially. Figure 3-13 shows a plan of a typical arrangement of soil cores extracted during an experiment.

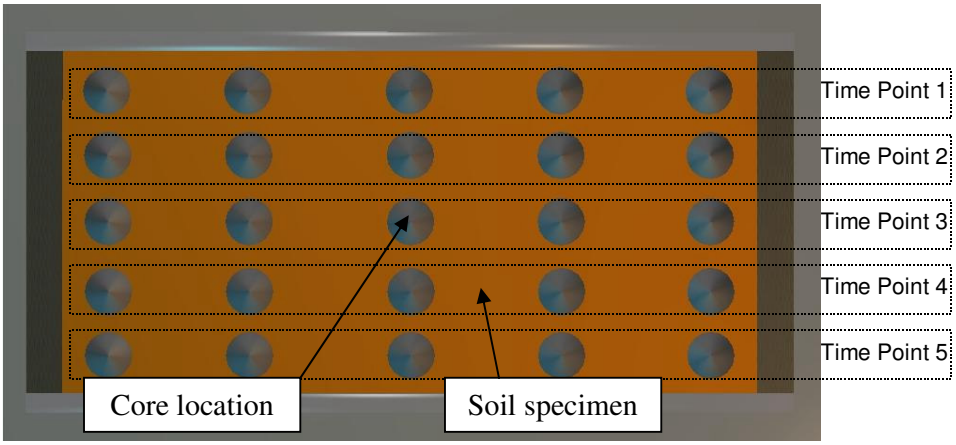

Figure 3-13: Typical soil core locations

Samples were taken in 'lanes' at each time point, in order to ensure that the reduction in effective soil area was minimised for the remainder of the experiment.

Soil cores were extruded and at least one gram taken for chemical and/or biological analysis. The remainder of the soil was used to determine the soil moisture content (method as on page 3-11). The majority of experiments tracked PCP by analysing soil concentrations at five points along the specimen; these points were labelled according to sampling location (numbered from 1 (anode end) to 5 (cathode end)). In one experiment, nine samples were taken, in order to provide sufficient soil for a range of bacterial analyses (see section 3.9). These were labelled from a (cathode end) to i (anode end) – the difference in numbering direction was due to this test being planned by G. Lear.

3.8. Chemical Analyses

3.8.1. Determination of non-radiolabelled PCP in soil

The transport and fate of (non-radiolabelled) pentachlorophenol in soils was tracked using a solvent extraction method. The method was based on a technique investigated by Wall and Stratton (1991), following work by Mikesell and Boyd (1986). A volume of 0.5ml of acetonitrile (CH_3CN) was added to 1 gram of wet soil, and vortexed for four minutes. The resulting slurry was then centrifuged at 10,000rpm, and the supernatant decanted and retained. The process was repeated a further two times. The collected supernatant fluid from all three extractions was combined and analysed by HPLC. Samples of 1ml were used for HPLC analysis, with a mobile phase of 70:30 acetonitrile / 5% acetic acid aqueous solution (Wall and Stratton, 1991) at a flow rate of 0.8ml/min. The detector wavelength used for these analyses was 254 nanometres. A Supelco Discovery 150 x 4mm reverse phase C18 column (5µm particle size) was used, together with a Dionex UVD170S HPLC system and Chromeleon v6.10 software (Dionex, UK). Standard samples of PCP in phosphate buffered saline were used to calibrate the results with each run of samples. This buffered solution was used to allow the production of high concentration solutions of PCP for standards. Resulting concentrations were used to determine a mass balance for each specimen.

3.8.2. Determination of Radiolabelled PCP in Soil

The fate of radiolabelled PCP was tracked in four ways. In each, liquid scintillation counting was performed (using a LKB Wallac Rackbeta 1217 Liquid Scintillation counting unit) instead of HPLC to determine the quantity of radiolabelled PCP present.

1. Firstly, carbon dioxide was trapped in sodium hydroxide traps (the acidic CO_2 reacts with NaOH to form sodium hydrogencarbonate – $NaHCO_3$). PCP mineralised by bacteria would have produced radiolabelled carbon dioxide as a degradation product. Three millilitres of sodium hydroxide were placed in a 20ml liquid scintillation vial and placed in the container with the soil specimen. Sixteen millilitres of Ultima Gold liquid scintillation cocktail (PerkinElmer Life and Analytical Sciences Inc., Boston,

USA) was added to the sodium hydroxide and the mixture shaken thoroughly until it became clear. Scintillation counting was then performed.

2. Half a gram of soil taken in sampling operations as described above (page 3-23) was placed in a Teflon Oak Ridge centrifuge tube (30ml). 10ml of deaired deionized water was added, and the resulting slurry was spun on a slowly rotating rotary mixer (approximately 6rpm) for 24 hours. This was then spun down in a Beckman L8-M ultracentrifuge at 5000rpm for 15 minutes and the supernatant extracted and retained. Six millilitres of this supernatant were shaken with twelve millilitres of Ultima Gold XR scintillation cocktail (PerkinElmer Life and Analytical Sciences Inc., Boston, USA) until it became clear, before performing scintillation counting. The use of water was intended to provide an indication of the amount of PCP that was bioavailable. In certain experiments, the pH of each water sample was measured after the material for scintillation testing was removed, in order to determine the changes in pH both with time and across the soil cartridges over the experiment.
3. 10ml of acetonitrile was then added to the soil, and the whole process repeated. Scintillation analysis was performed in an identical fashion to that with water samples. The harsher solvent extraction with acetonitrile was to recover as much of the remaining PCP as possible.
4. The amount of radiolabelled PCP remaining after these extractions was determined in a number of samples using an oxidation method. The soil remaining after extraction was placed in a paper thimble, to which was added 200µl of a combustion assisting fluid ('Combustaid', Packard Bioscience Ltd, Pangbourne, UK). The thimble was then placed in a sample oxidizer (Packard model 307, Packard Bioscience Ltd, as above) and combusted at a temperature exceeding 1500ºC. Any radiolabelled carbon dioxide produced from the oxidation of radiolabelled PCP was trapped in 10ml of Carbosorb E (Packard Bioscience Ltd, as above) which was then passed to a 20ml

scintillation vial. 10ml of scintillation fluid (Permafluor E+, Packard Bioscience Ltd, as above) was added also, and the vial passed through the scintillation counter as above.

Results from all extractions / detection methods were then calibrated to give actual amounts of PCP using the initial ratio of counts per minute per gram of PCP, and then used to calculate mass balances and PCP concentrations.

3.9. Bacterial Analyses

The combination of electrokinetic field, bacteria and pollutant has been investigated in several experiments, using the analyses described below. Several of these were performed in conjunction with or, where indicated, by, Lear (2004).

3.9.1. Plate Counts

Counts of viable organisms from soil were performed using plate counts. Attempts to track inoculated *Sphingobium* sp. UG30 were performed using a Miles-Misra technique similar to that described previously (section 3.5). Plate Count Agar (PCA) with 2ml rifampicin and 1ml cyclohexamide (anti-fungal agent) per litre was again used. Soil samples were diluted by weight with phosphate buffer solution (K_2HPO_4, 0.65g/litre; KH_2PO_4, 0.19g/litre) and serial dilutions plated out.

Total numbers of culturable bacteria and fungi were determined in some experiments. Soil was diluted with phosphate buffer solution to get serial dilutions of 10^{-1}, 10^{-2} and 10^{-3} times. Tests for bacteria used 50µl of the 10^{-2} and 10^{-3} dilutions spread on plates of PCA (containing 100mg/litre cyclohexamide). Tests for fungi were performed in a similar manner using 10^{-1} and 10^{-2} dilutions on Potato Dextrose Agar (PDA) containing

320mg/litre Aureomycin® (chlortetracycline hydrochloride - anti-bacterial agent). After 24 hours (bacteria) and 48 hours (fungi) incubation time at 28ºC, individual colonies were counted.

3.9.2. Soil Microbial Respiration (performed by Lear (2004))

The method used was a modified version of that developed by Öhlinger (Schinner *et al*, 1993). Two grams of soil were placed in individual glass screw-top vessels and the moisture content adjusted to 50% with sterile water. A smaller vessel containing 15ml of 0.05M sodium hydroxide was also placed in this container and petroleum jelly used to seal the lids. Control tests (glass vessels set up as above, but containing no soil) were also performed. After eighteen hours incubation at room temperature, the fluid in the sodium hydroxide trap was combined with 2ml barium chloride ($BaCl_2$). Five drops of phenolphthalein solution (0.1% in 60% ethanol) were added and the solution titrated with 0.1M hydrochloric acid until the solution changed from pink to cloudy white. The following formula was used to determine carbon dioxide respiration:

$$CO_2 \ respired \ per \ gram \ dry \ soil \ (mg) = \frac{V_{HCl} x 2.2 x 100}{Mass \ of \ dry \ soil}$$

(V_{HCl} is the volume of hydrochloric acid required; 1ml of 0.1M HCl = 2.2mg CO_2)

3.9.3. Soil Dehydrogenase Activity

The behaviour of soil microflora and microfauna are strongly influenced by environmental parameters, such as pH and temperature. The analysis of dehydrogenase activity in the soil can be an indicator of microbial activity, and therefore of soil conditions (Sinclair *et al*, 1997; Margesin *et al*, 2000; Quilchano and Marañón, 2002). In this case, it was used to determine the effects of pollutant, pH changes and electric field on the soil micro-organisms. It is possible for a significant amount of enzymatic activity in soil to be due to extracellular enzymes emitted by flora and fauna in the soil, which can

then be supported and protected by the soil matrix itself (Prescott *et al*, 1999; Li and Pariente, 2003). However, these enzymes will also be subjected to the same effects as micro-organisms in soil, and so dehydrogenase activity is a suitable indicator of conditions within the soil.

The method used to determine dehydrogenase activity was based on that described in Sinclair *et al* (1997), and used 2,3,5-triphenyltetrazolium chloride (TTC) as an artificial electron acceptor. This was reduced to insoluble, red 2,3,5-triphenyltetrazolium formazan (TTF) by dehydrogenase activity, the amount of which could then be determined using a spectrophotometer.

Soil samples (0.5g wet weight) were placed in 2ml microcentrifuge tubes, containing 5mg of calcium carbonate and 0.7ml 0.75% TTC solution (Sigma Aldrich Ltd, Poole, UK). After vortexing thoroughly, they were incubated at 30ºC for 24 hours in the dark. Following this period, 1.0ml of ethanol was added, the slurry vortexed and then spun at 10,000rpm in a microcentrifuge. The extraction process was repeated with two further additions of ethanol (1.5ml and 1.0ml), and the total supernatant combined. One millilitre of this solution was then analysed in a spectrophotometer at 485 nanometres. Results were calibrated against standards produced by dissolving 1,3,5-triphenyltetrazolium formazan (Sigma Aldrich Ltd, Poole, UK) in ethanol, and analysing as above.

3.10. Adsorption Isotherms

The interaction between pentachlorophenol and soil has been investigated using adsorption and desorption isotherms. The protocol followed is based on that described in test guidelines given by the United States Environmental Protection Agency (USEPA, 1996), and is described below.

Individual experiments were performed in six 1.5ml microcentrifuge tubes, arranged in three pairs, each with identical conditions. All were performed at a temperature of 22º Celsius. 0.20 grams of soil and 1.0ml of the desired PCP solution were combined in each tube, before vortexing and allowing to equilibrate for ten days. A control tube was also set up, with solution but no soil, in order to determine the amount of chemical sorbed to the container itself. The samples were then spun in a microcentrifuge at 10,000rpm for 15 minutes, and the supernatant extracted. The supernatants of each pair were combined in order to present sufficient fluid for analysis, leaving triplicate samples for each particular set of conditions. One millilitre of each sample was then taken for HPLC analysis, using HPLC conditions as described in section 3.8.1. The amount of PCP remaining in solution could then be calculated, and the amount sorbed to soil derived.

The experiment was performed for a range of PCP concentrations (up to 700μM), in order to determine the profile of sorbed PCP with respect to solution concentration. This was repeated with the addition of sulphuric acid (pH ~ 1.0, 0.05ml and 0.1ml in each 1ml of solution) and sodium hydroxide solution (pH ~13, 0.1ml in each 1ml) in order to investigate the effect of pH on sorption. The effect of the soil organic matter was also investigated; a large proportion of this material was removed using the hydrogen peroxide method (Head, 1980a), and isotherms performed as above.

Kinetics of sorption and desorption were investigated, in order to determine their time scales relative to the effects occurring in the electrokinetic experiments. Experiments were performed as above, the only difference being that all the sets of samples were identical, and were allowed to equilibrate for different amounts of time. With desorption

experiments, all sets were identical and allowed to equilibrate for the full ten days. Following this, 1ml of water was added to each sample (after supernatant removal), and equilibration allowed for a variety of different times, before analysing the PCP content of the solution.

3.11. Statistical Methods

Statistical analyses on experimental data were performed using SPSS Standard Version software (release 11.5.1 - SPSS Inc., Chicago, USA). Univariate and multivariate analyses of data from individual experiments were performed using analysis of variance (ANOVA), in order to compare several means of triplicate samples at once. Results are presented with error bars indicating 95% confidence intervals, unless indicated otherwise. Significance is indicated as suggested in Fowler *et al* (1998):

- An outcome which is predicted to occur fewer than 1 trial in 20 ($P<0.05$) is considered to be *unlikely* or *statistically significant*.
- An outcome which is predicted to occur fewer than 1 trial in 100 ($P<0.01$) is considered to be *very unlikely* or *statistically highly significant*.
- An outcome which is predicted to occur fewer than 1 trial in 1000 ($P<0.001$) is considered to be *extremely unlikely* or *statistically very highly significant*.

These terms are used to describe, in this case, how likely it is that one group of sample data (for instance, from soil samples with an electric field applied) is indistinguishable from another (soil samples without an electric field applied). The lower the probability of this being so, the higher the significance of the results.

4. Experimental Programme and Results

4.1. Soil Properties

The soil used in the experiments detailed here was obtained from the Oxford University Field Station at Wytham, near Oxford, UK. It was taken from a depth of between twenty and fifty centimetres below the ground surface. The soil was classified using a range of analyses as described in section 3.2.

4.1.1. Moisture Content

The natural moisture content of the soil was determined, using the method in section 3.2.1, to be 22%.

4.1.2. Specific Gravity

The specific gravity (G_s) of this soil has been calculated to be 2.63 (using the method in section 3.2.2).

4.1.3. Atterberg Limits

Using the method in section 3.2.3, the plastic limit was determined to be at a moisture content of 27%, and the liquid limit 58%. These values give a plasticity index of 31% and a liquidity index of approximately 0.42 after soil cartridge preparation and contamination. The plasticity chart (Figure 4-1) shows the position of this soil in terms of liquid limit and plasticity index, and allows the classification of the soil as a high plasticity clay.

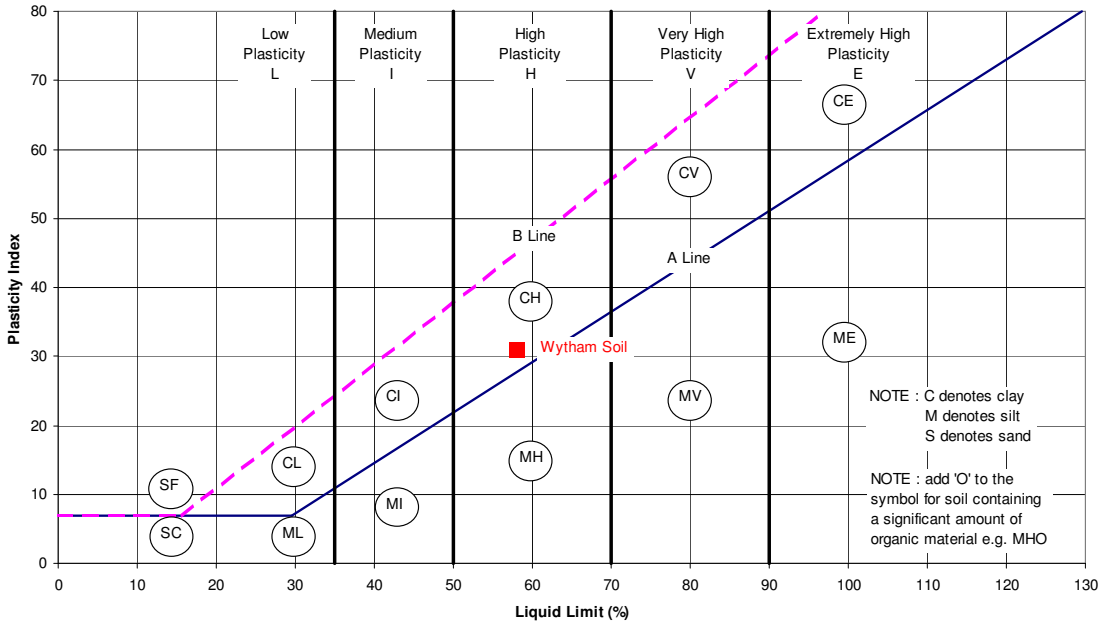

Figure 4-1: Plasticity Chart (Wytham Soil)

4.1.4. Particle Size Distribution

The particle size distribution of Wytham soil is shown (Figure 4-2). From this information, it has been classified as a well-graded silty clay. Details of the techniques used to obtain this information may be found in section 3.2.4

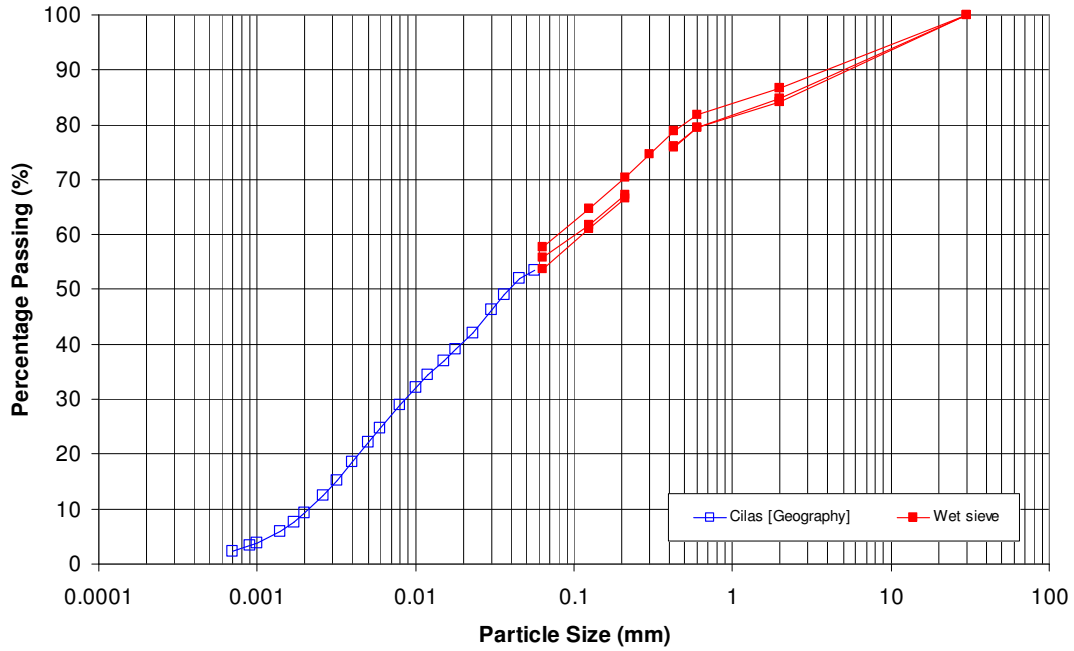

Figure 4-2: Particle Size Distribution (Wytham soil)

4.1.5. Maximum Dry Density / Optimum Moisture Content

The maximum dry density and corresponding optimum moisture content of the soil were 1.65g/cm^3 and 20% respectively. The information is taken from Figure 4-3, and the test method is given in section 3.2.5.

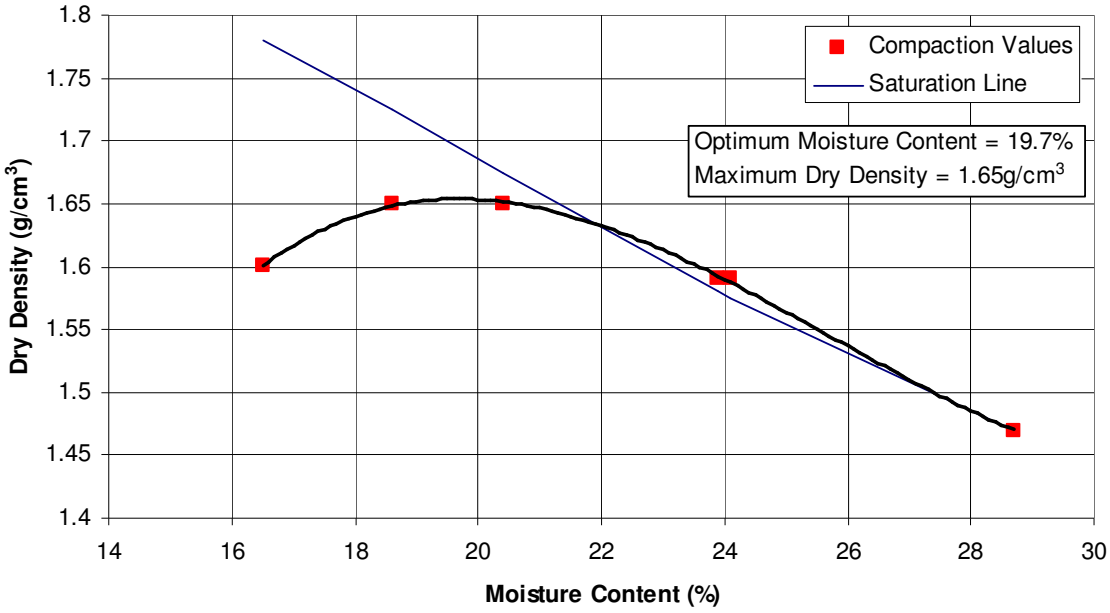

Figure 4-3: Optimum Moisture Content / Maximum Dry Density

4.1.6. pH Buffering

Figure 4-4 and Figure 4-5 show the equilibria between H^+ and OH- ions associated with the soil (either through sorption, chemical reaction or otherwise) and those in solution (method as in section 3.2.6). It was difficult to obtain a sensible result for OH- ions – the experiment was performed three times, and each time a dark brown solution was produced at higher pH. Measured pH values at these points often did not make sense – the best results set is presented.

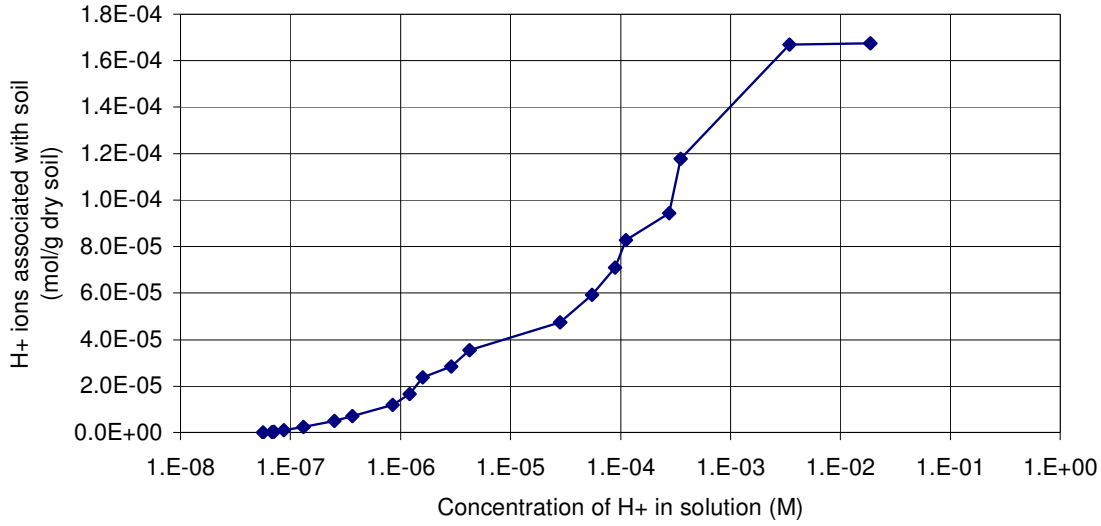

Figure 4-4: Buffering of H+ ions in Wytham soil

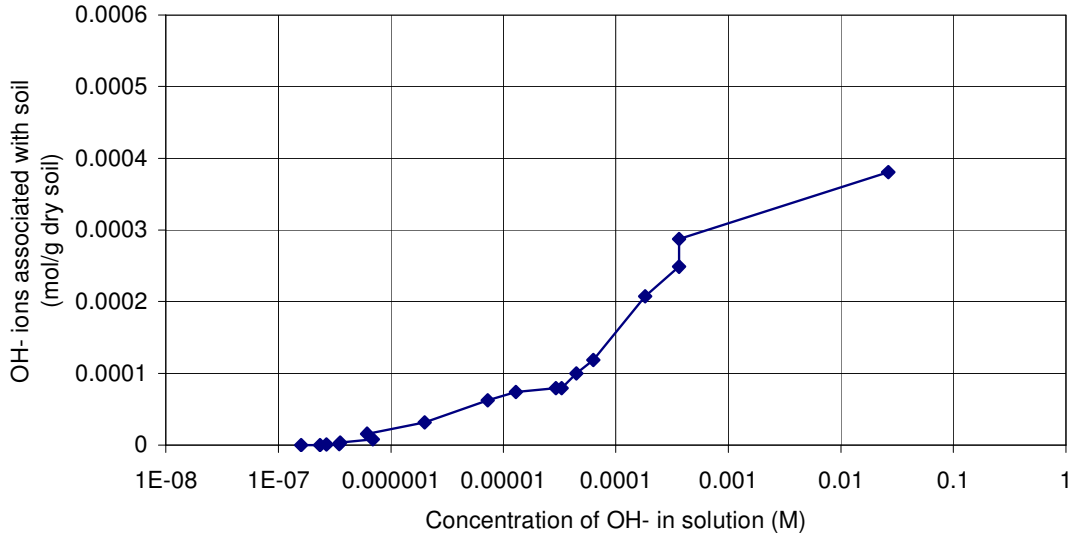

Figure 4-5: Buffering of OH- ions in Wytham soil

4.1.7. Organic Matter Content

The organic matter content of the soil was 8.1% (see section 3.2.7).

4.1.8. Cation Exchange Capacity

The cation exchange capacity of this soil is 22 meq / 100g dry soil (method – section 3.2.8).

4.1.9. Effect of pH on Zeta Potential

The profile of zeta potential versus pH for Wytham soil is shown on Figure 4-6 (method described in section 3.2.9).

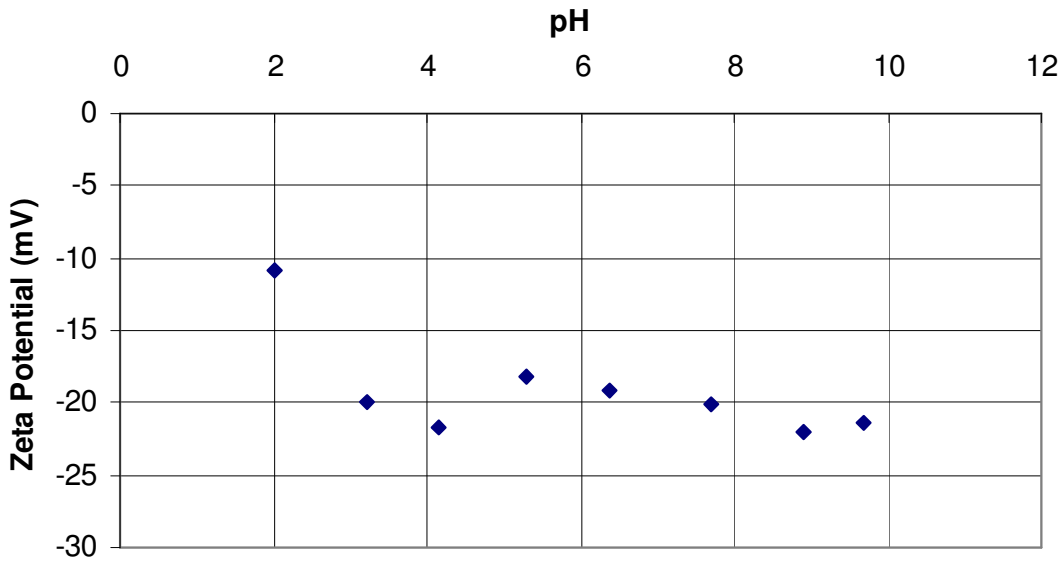

Figure 4-6: Correlation between clay particle zeta potential and pH (Wytham soil)

4.1.10. X-ray Diffraction and X-ray Fluorescence.

The data obtained from x-ray analysis of Wytham soil are presented in Table 4-1, Table 4-2 and Table 4-3 (see also section 3.2.10):

Wt % <2µm	Illite / Smectite			Illite		Kaolinite		Chlorite		Quartz	Calcite
	Wt%	Order	% Illite	Wt%	Cr	Wt%	Cr	Wt%	Cr	Wt%	Wt%
31.70	53.8	O	70-80	20.1	P	13.5	M	7.6	P	4.9	0.0

Table 4-1 - Clay structure (x-ray diffraction analysis)

Wt% - percentage of <2µm fraction by weight

Cr – Crystallinity (VW – very well crystallised, W – well crystallised, M – moderately crystallised, P – poorly crystallised, VP – very poorly crystallised)

Mixed-layer ordering – RI (randomly interstratified), O (ordered interstratification), LR (long-range ordering)

Na_2O %	MgO %	Al_2O_3 %	SiO_2 %	P_2O_5 %	SO_3 %	Cl %	K_2O %	CaO %	TiO_2 %	MnO %	Fe_2O_3 %	LOI
0.19	0.60	8.48	62.23	0.52	0.08	0.04	1.52	2.91	0.55	0.15	8.54	14.3

Table 4-2 - Major elements (x-ray fluorescence analysis)

(LOI – loss on ignition)

V ppm	Cr ppm	Ni ppm	Cu ppm	Zn ppm	Ga ppm	As ppm	Se ppm	Br ppm	Rb ppm	Sr ppm	Y ppm	Zr ppm
128	105	49	42	169	9	31	<1	11	105	80	34	265

Nb ppm	Mo ppm	Ag ppm	Cd ppm	Sn ppm	Ba ppm	La ppm	Ce ppm	Pb ppm	Bi ppm	Th ppm	U ppm	
13	2	<0.5	1	5	300	31	73	59	<1	8	<1	

Table 4-3 - Minor elements (x-ray fluorescence analysis)

4.1.11. Pore Fluid Electrical Conductivity

The electrical conductivity of the pore fluid in soil cartridges of prepared and contaminated Wytham soil was found to be 2.62 milliSiemens per centimetre (see section 3.2.11 for method).

4.1.12. Tortuosity

The initial tortuosity of prepared and contaminated soil cartridges was calculated as described in section 3.2.12.

$$\tau = 0.57$$

4.1.13. Hydraulic Permeability (saturated)

Hydraulic permeability of prepared Wytham soil was found to be approximately $4x10^{-5}$ metres per second, using the method described in section 3.2.13.

4.1.13.1. PCP Extraction

In the majority of experiments, the total recovery of PCP, either with the acetonitrile extraction technique or the combined water / acetonitrile techniques used with radiolabelled PCP, yielded on average approximately 80% of that thought to have been introduced. This compared PCP recovered from extractions on day 0 of an experiment with that calculated to be in the initial solution. It is likely that the remaining PCP was distributed between that strongly sorbed to the soil, and that which was 'lost' during contamination, for example due to association with the contamination apparatus (especially filter paper). Combustion of the soil (in experiments PI13u and PI13i only – see sections 4.7 and 4.8) following water and acetonitrile extractions recovered all the remaining PCP in soil, which was found to be only a small percentage of the missing contaminant. Therefore, it is thought that the majority of unaccounted-for PCP was lost during contamination, and soil extraction results give an accurate picture of soil conditions.

4.2. Main Experiment Summary Table

Table 4-4 gives brief details of all the main electrokinetic experiments whose results are presented later in this chapter. The numbering system began with experiments A_1-A_4, which were studies on electrokinetic behaviour in soil, as well as test runs for different apparatus types. The large apparatus set (see section 3.1.1) was introduced with experiment A_4. Following this, investigations of the effect of electric field on pentachlorophenol in soil were performed, in experiments PCP_1 to PCP_6. Experiments in the small apparatus (see section 3.1.2) began with experiment P_1, and continued up to PI_22. The labelling system for these experiments is as follows:

- 'P' denotes specimens contaminated with pentachlorophenol

- 'I' denotes specimens inoculated with *Sphingobium* sp. UG30
- 'A' denotes specimens aged

Experiment	Concentration of PCP (ppm)	*Sphingobium* sp. UG30 (cfu / gram dry soil)	pH Control (anode / cathode)	Electric Field	Duration (days)	Sampling Times (days) and number of cores	Other details
A_4	0	0	None	60V (cv)	12	None	1 large cartridge
PCP_1	142	0	C	60V (cv)	6	EOT (5 samples)	1 large cartridge
PCP_2	164	0	C	60V (cv)	4	EOT (5 samples)	1 large cartridge
PCP_3	126	0	C	60V (cv)	8	6, 8 (5 samples each)	1 large cartridge (voltage reversal after 6 days)
PCP_4	~140	0	C	60V (cv)	4	EOT (5 samples)	1 large cartridge
PCP_5	280*	0	C	30V (cv)	10	0,2,4,6,8,10 (7 cores each)	* Central section only. 1 large cartridge
P_1	160 ±3	0	None	30V (cv)	6	0,2,4,6 (5 cores)	6 small cartridges (3 without field)
PI_2	87 ±3	~10^6	C	30V (cv)	10	0,3,6,10 (5 cores)	6 small cartridges (3 without field)
PI_3	102 ±5	~10^6	C	10mA (cc)	25	0,7,16,25 (5 cores)	6 small cartridges (3 without field)
PI_4	~90	10^6 *	None	None	104	0,19,47, 104 (5 cores)	6 small cartridges (* 3 uninoculated)
PI_5	118 ±8	3.8x10^2 ±9x10^1	C	10mA (cc pulsed)	25	0,7,16,25 (5 cores)	6 small cartridges (3 without field)
API_1	100 ±5 (aged 7.5 months)	4.8x10^6 ±6.5x10^5	None	10mA (cc pulsed)	25	0,7,16,25 (5 cores)	4 small cartridges (2 without field)
PI_7	140*	10^6 in flasks 10^6 in cartridges 10^8 in flasks	None	None	22	0,1,2,3,4,8,12,15,19,22 (1 core)	6 containers each (flasks - unstructured soil; cartridges – structured soil) *3 containers with PCP, 3 without
I_9	0	0	C	10mA (cc)	27	0,7,16,27 (9 cores)	9 small cartridges (3 with electric field, 3 water-amended, 3 controls)
PI_13u	148 ±15 (^{14}C)	None	C	10mA (cc)	36	0,7,14,26,36 (5 cores)	6 small cartridges (3 without field)

Experiment	Concentration of PCP (ppm)	*Sphingobium* sp. UG30 (cfu / gram dry soil)	pH Control (anode / cathode)	Electric Field	Duration (days)	Sampling Times (days) and number of cores	Other details
PI_13i	106 ±19 (^{14}C)	$1.5x10^{9}$ $\pm 7x10^{7}$	C	10mA (cc)	36	0,7,14,26,36 (5 cores)	6 small cartridges (3 without field)
PI_14	115 ±32 (^{14}C)	$3.5x10^{7}$ $\pm 2.2x10^{7}$	C	10mA (cc pulsed)	36	0,7,14,26,36 (5 cores)	6 small cartridges (3 without field)
PI_16	106 ±12 (^{14}C)	$3.8x10^{8}$ $\pm 1.4x10^{8}$	C	10mA (cc)	36	0,7,14,26,36 (9 cores)	6 small cartridges (3 without field)
PI_17	104 ±12 (^{14}C)	$3.8x10^{8}$ $\pm 1.4x10^{8}$	C	1mA (cc)	36	0,7,14,26,36 (5 cores)	6 small cartridges (3 without field)
PI_18	109 ±24 (^{14}C)	$3.8x10^{8}$ $\pm 1.4x10^{8}$	A+C	10mA (cc) after 66 days	95	0,66,73,85,95 (5 cores)	6 small cartridges (3 without field)
PI_20	133 ±11 (^{14}C)	$1.0x10^{7}$ $\pm 2.4x10^{6}$	A+C	10mA (cc)	36	0,7,14,26,36 (5 cores)	6 small cartridges (3 without field)
API_21	~130 (^{14}C)aged 10months	$1.9x10^{8}$ $\pm 1.0x10^{8}$	C	10mA (cc)	42	0,7,14,26,42 (5 cores)	6 small cartridges (3 without field)
PI_22	113 ±22 (^{14}C)	$1.9x10^{8}$ $\pm 1.0x10^{8}$	None	10mA (cc reversed daily)	56	0,7,14,26,56 (5 cores)	6 small cartridges (3 without field). No electrolyte fluid – electrodes in contact with soil

Table 4-4: Summary of test conditions

The terms 'cc' and 'cv' represent constant current and constant voltage conditions respectively. Pulsed current experiments had a constant current field applied for 2 hours per day. Confidence intervals for concentration of PCP and of inoculated bacteria are presented as ±1 standard deviation.

The following table (Table 4-5) has been included to give further information about the design and purpose of each experiment, in addition to the specific data given in Table 4-4.

Experiment ID	Details
A_4	Effect of electrokinetic phenomena on soil
PCP_1	PCP movement under electric field
PCP_2	PCP movement under electric field

Experiment ID	Details
PCP_3	PCP movement under electric field
PCP_4	PCP movement under electric field
PCP_5	PCP movement under electric field
P_1	PCP movement under electric field (small apparatus)
PI_2	Combination of PCP, *Sphingobium* sp. UG30 and electric field (constant voltage)
PI_3	Combination of PCP, *Sphingobium* sp. UG30 and electric field (constant current)
PI_4	Long-term fate of PCP in *Sphingobium* sp. UG30 inoculated soil
PI_5	Effect of pulsed current on PCP and inoculated *Sphingobium* sp. UG30
API_1a	Effect of pulsed current on aged contaminated specimens and inoculated *Sphingobium* sp. UG30
PI_7	Survival of *Sphingobium* sp. UG30 in PCP contaminated soil
I_9	Analysis of effect of electric field on background microbial populations in soil
PI_13u	Effect of electrokinetics on PCP in soil (radiolabelled PCP)
PI_13i	Effect of electrokinetics on PCP in inoculated specimens (radiolabelled PCP)
PI_14	Effect of pulsed current on radiolabelled PCP and inoculated *Sphingobium* sp. UG30
PI_16	Analysis of effect of PCP (radiolabelled) and electrokinetics on *Sphingobium* sp. UG30 and background microbial populations
PI_17	Effect of reduced constant current on radiolabelled PCP and inoculated *Sphingobium* sp. UG30
PI_18	Effect of constant current on radiolabelled PCP and inoculated *Sphingobium* sp.UG30 – electric field applied after ~2 months
PI_20	Effect of electric field on radiolabelled PCP and *Sphingobium* sp. UG30 (pH control of both electrolyte fluids)
API_21	Effect of electric field on radiolabelled PCP aged in soil for eight months, and inoculated *Sphingobium* sp.UG30
PI_22	Effect of regular current reversal and prevention of water flow on radiolabelled PCP and *Sphingobium* sp.UG30

Table 4-5: Main Experiment Summary Table

The results are presented under the following headings:

- Effects of Electrokinetics on Soil
- Interaction between Contaminant and Soil

- Action of PCP degraders in PCP-contaminated soil
- Effect of Electrokinetics on Microbes in Soil
- Behaviour of PCP under an Electric Field
- Effect of electric field on PCP degradation
- Effect of Ageing of the Contaminant in Soil

4.3. Effects of Electrokinetics on Soil

Data are presented that illustrate typical effects of applying an electric field to soil on changes in soil properties (such as pH, moisture content and temperature) as well as voltage, current and electroosmotic flow.

4.3.1. Voltage and Current

The voltage gradient across soil samples was found to vary substantially with time and also between experiments. In early experiments (A_4, PCP_1 to PCP_5, P_1 and PI_2), a constant voltage was applied across the electrodes. It was found that, whilst the voltage between the electrodes was kept constant, the potential difference across the soil was variable and impossible to control in this manner. This effect was due to the movement of ions and changes in moisture in the soil system, as well as effects such as the degree of saturation of Vyon membranes used to separate the soil from the electrode fluids. Results from two experiments are presented with constant voltage conditions, indicating the difficulty of creating repeatable voltage profiles across soil specimens in different tests. Experiments PI_3 to PI_22 employed constant current conditions, as this gave a greater degree of control over the electric field conditions in the soil itself. One example plot of such a current profile is presented.

Figure 4-7 shows the voltage profile versus time for experiment PCP_3. This experiment ran initially for 140 hours, after which the voltage was reversed and applied for a further 50 hours. Initially there was seen to be a large voltage drop across the Vyon membrane at the anode. It is thought that this was because of the membrane being only partially saturated at the time. Over the course of the test, the potential difference over the soil increased, and the potential difference across the anode membrane dropped. This may have been due to changes in the saturation of the membrane with time, as well as chemical changes in the soil adjacent to it. Large changes in pH (see section 4.3.2) were found in electrolyte fluids, and these would be expected to affect the soil also; for example, high pH can lead to the formation of insoluble metal hydroxides, which could block pores and hinder fluid flow, thereby increasing resistance. There was a significant change in behaviour at the reversal of voltage at 140 hours, with the resistance across the anode membrane dropping substantially whilst the potential difference across the soil increased. Such a sudden change is difficult to explain, as the conditions in the soil remained constant across the reversal, and so the voltage conditions should have remained the same. It is thought that the reversed current might have quickly affected the pH at the electrodes, and perhaps reduced the resistance across the membrane now at the cathode, leading to more of the potential difference being applied to the soil.

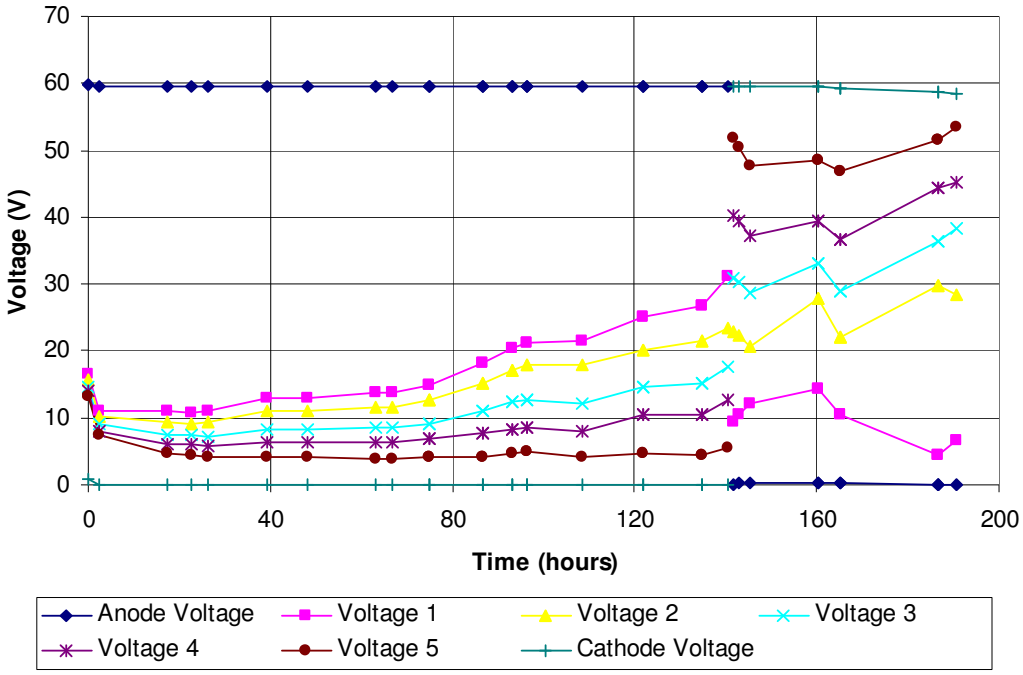

Figure 4-7: Voltage regime across specimen versus time (experiment PCP_3)

Experiment PCP_4 had identical initial conditions to PCP_3, although its duration was only 90 hours and there was no voltage reversal. The voltage profile across the soil (Figure 4-8) can be seen to be similar to that seen after the voltage reversal in experiment PCP_3, but when compared at similar times there is very little agreement. A gradual increase of potential difference across the soil was seen during the test (from approximately 20 volts to 40 volts). This can again be explained by changes in the state of saturation, and therefore electrical resistance, of the electrode membranes. One possible reason for the difference between the two experiments initially may be 'contamination' from reused Vyon membranes, which may have been charged with ions from a previous experiment. It is clearly difficult to attempt to decouple the effects of different parameters in the soil mass, as each impinges on the others. Therefore, it is likely that the causes for these differences are a combination of the different effects described here.

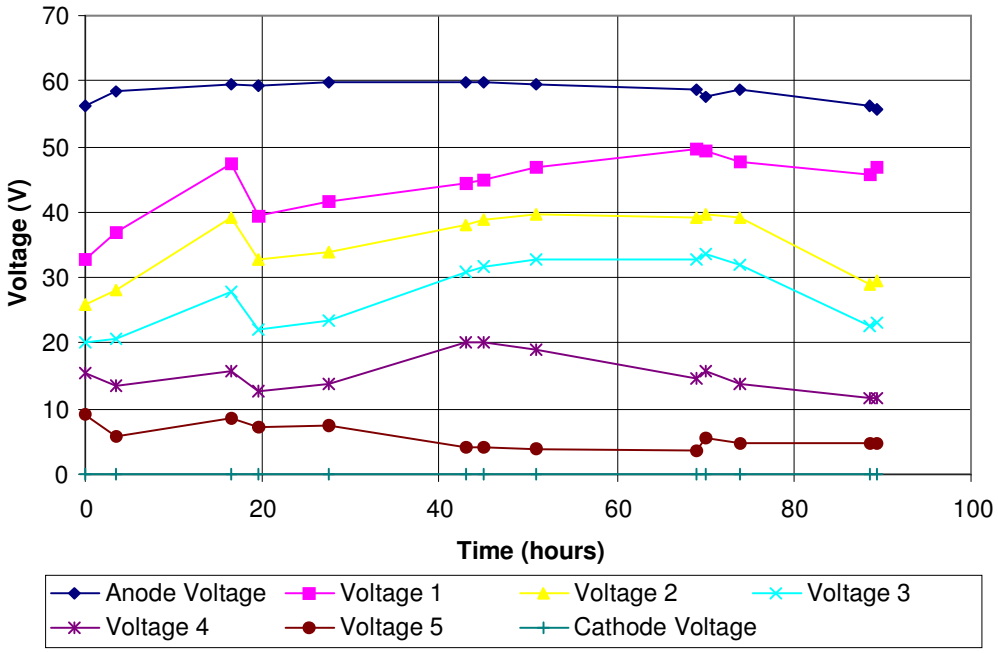

Figure 4-8: Voltage regime across specimen versus time (experiment PCP_4)

Other experiments performed under constant voltage conditions showed similar effects. Whilst the voltage across the electrodes could easily be controlled, the voltage across the soil depended on the relative resistance of the soil, the membranes and the electrolyte fluid, which acted as resistors in series. Any change in one affected the others, and so it was found to be impossible to control conditions in the soil itself. There was therefore found to be a great deal of variability in the voltage applied to the soil, from a minimum of a few volts to up to 45 volts. For this reason, experiments from PI_3 onwards used constant current conditions, as changes elsewhere in the apparatus generally did not have a detrimental effect on the current through the soil. Whilst it was still not possible to control potential difference within the soil, the current within the soil mass could easily be maintained at a constant level. In addition, it is the current that determines effects such as the extent of water electrolysis, and the force due to electromigration, and so by keeping these constant parallels could more easily be drawn between experiments.

Figure 4-9 is a plot of electric current versus time for the first constant current experiment, PI_3. Initial problems in operating power supplies led to high initial currents, but from 50 hours onwards, constant current conditions were implemented.

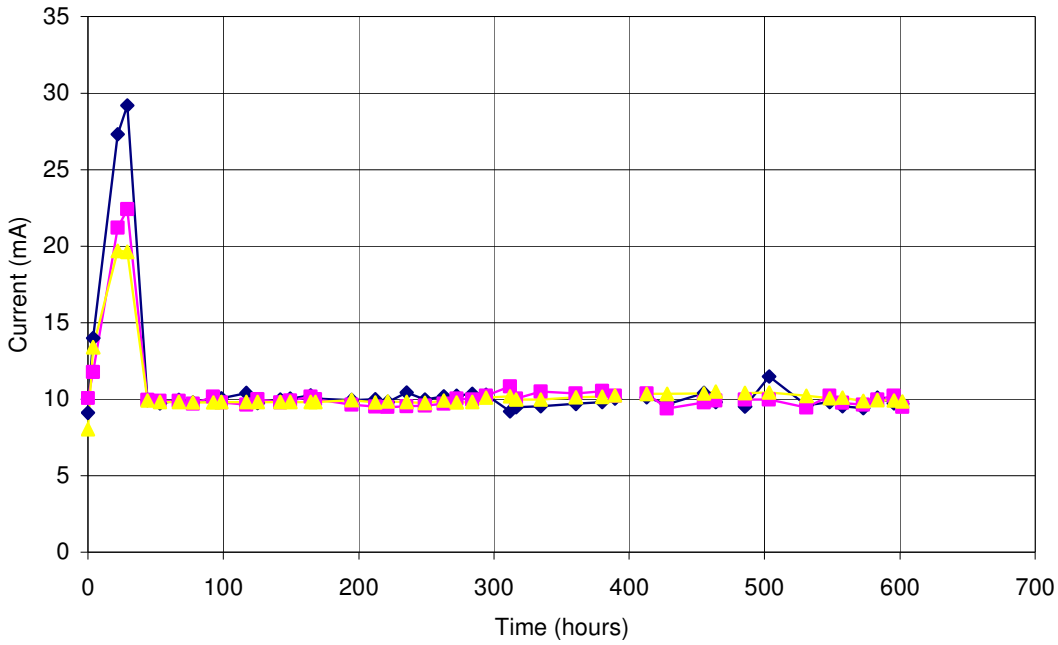

Figure 4-9: Electric current versus time (experiment PI_3)

All experiments following PI_3 had constant current conditions imposed although, infrequently, high resistance led to the current dropping. For instance, in experiment PI_22, the soil around the electrodes had to be wetted from time to time to allow a current to pass. It is thought that electroosmotic flow led to water being moved away from the electrodes and increasing the local resistance.

4.3.2. Changes in pH

Changes in the pH of the electrolyte fluids were found in all experiments, due to water electrolysis at the electrodes. It was expected that the hydrogen and hydroxyl ions produced would migrate into the soil, primarily through electromigration and diffusion, changing the pH of the soil pore fluid, and this was observed many times. Typical results

from a range of different experiment types are presented here. An example of uncontrolled pH changes is shown, followed by an investigation into the effect of manually controlling the pH through chemical addition. The effect of cathodic pH control on the pH within the soil pore fluid is then demonstrated using results from two experiments, followed by the effect of two types of pH control at both electrodes - either mixing of electrolyte fluids or through current reversal. These results show the success of pH control (both chemical control and through current reversal) in later experiments in preventing substantial changes in the soil. The considerable changes that occurred without such controls illustrated the important effect of electrolysis in the use of electrokinetics.

A typical pH plot versus time, from experiment P_1, is shown in Figure 4-10; the catholyte pH rapidly increased to approximately pH 12, whereas the anolyte pH rapidly decreased to approximately pH 2. These changes occurred in approximately ten hours, and this behaviour was seen in all experiments without pH control. Both constant current and constant voltage conditions were found to produce very similar pH conditions at the electrodes, in a relatively short space of time.

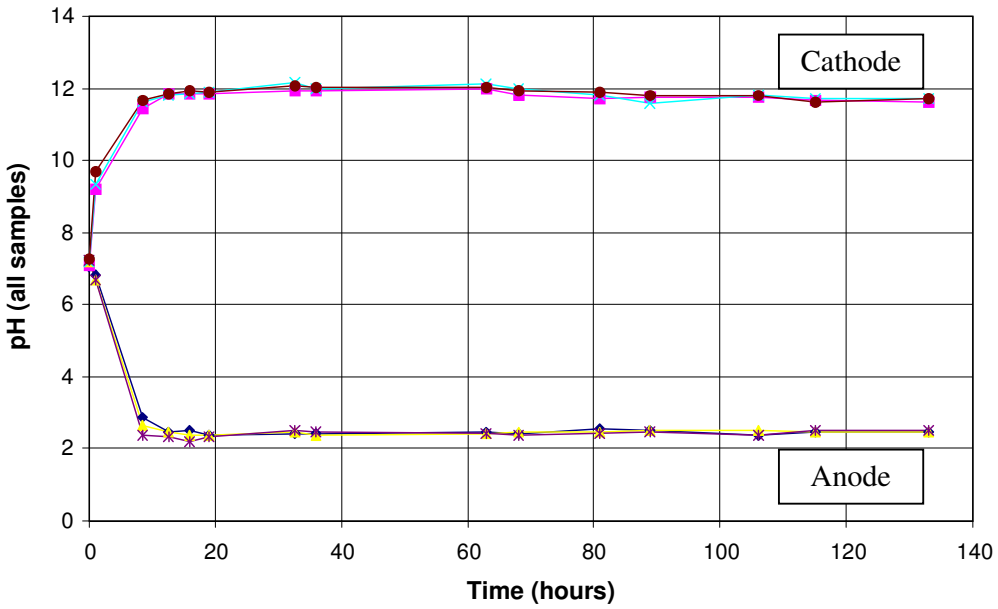

Figure 4-10: Electrolyte pH versus time (experiment P_1)

The pH of the catholyte fluid was controlled in most experiments, for reasons given later in this chapter. In earlier experiments, this was performed manually, through addition of acid, as in Figure 4-11 (experiment PCP_5). It can be seen that this did not produce a uniform pH, as only a few additions could be performed each day, and the pH rapidly returned to its former levels in between these times.

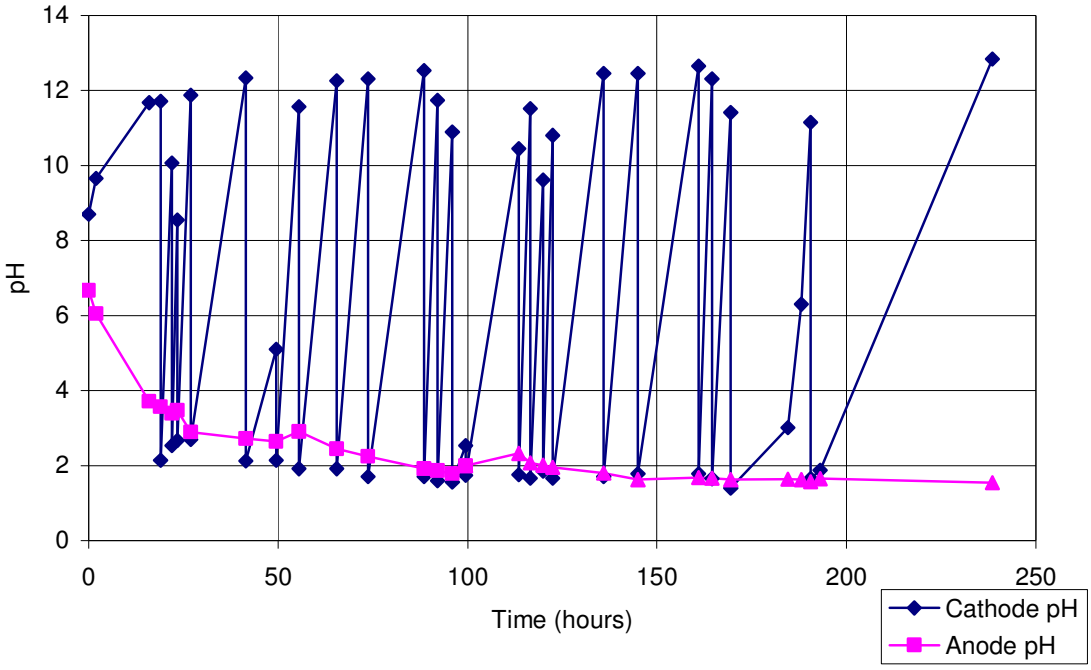

Figure 4-11: Electrolyte pH versus time (experiment PCP_5)

In experiments involving radiolabelled PCP, pH was not monitored at the electrodes, in order to minimise the opening of the sealed containers in which experiments were performed. It was expected, however, that changes similar to those seen in all previous experiments would occur at the electrodes, and so pH control was again employed, at the cathode and in some case at both electrodes. However, the pH of soil pore fluid was measured at every sampling location in several of these tests, giving a complete profile of pH changes versus position and time. This was done using the water extraction fluid used to determine PCP content (see section 3.8.2) and so does not represent the exact pH, as it measures the pH of a diluted sample of the pore fluid. It does, however, provide an indication as to changes. Back-calculations were not performed in order to determine the original pH, as the volumes of pore fluid involved were small and so large errors could have been introduced.

Figure 4-12 and Figure 4-13 illustrate how an acidic pH front moved through the soil in experiments PI_13u and API_21, with the average pH at the anodes (these experiments were performed in triplicate) decreasing by up to 5 pH units by the end of testing. Similar profiles were found in other constant current experiments. It can be seen that the pH control at the cathode in these cases led to little change in the pH at this point, and so no alkaline front moved into the soil. These graphs also show that the pH measured in control samples did not change with time or position during these tests, in contrast to the electrokinetic specimens. There was a greater pH effect in experiment API_21, which may have been because of the ageing of the samples and resulting chemical changes.

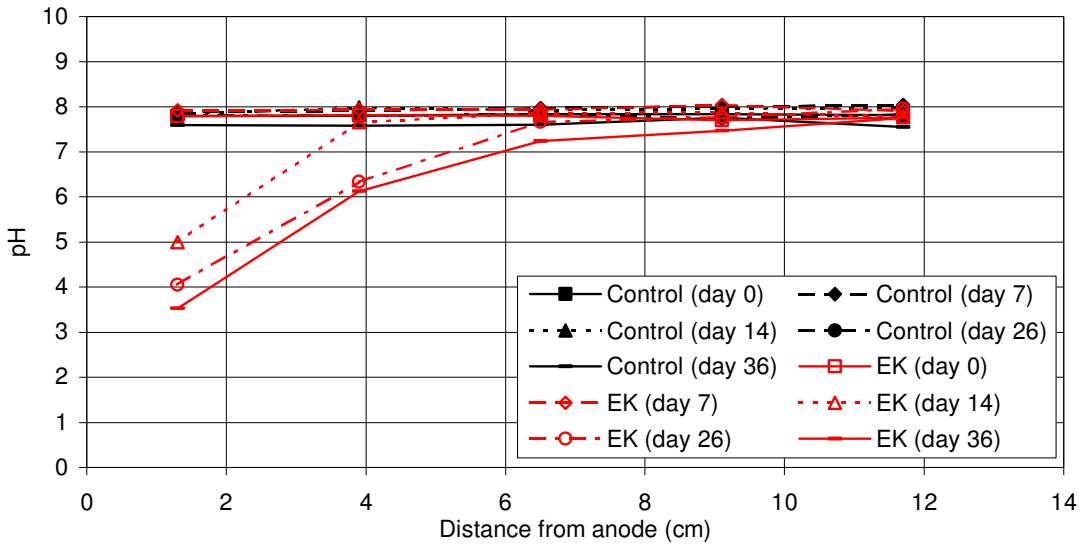

Figure 4-12: Variation of pH profile with time (experiment PI_13u)

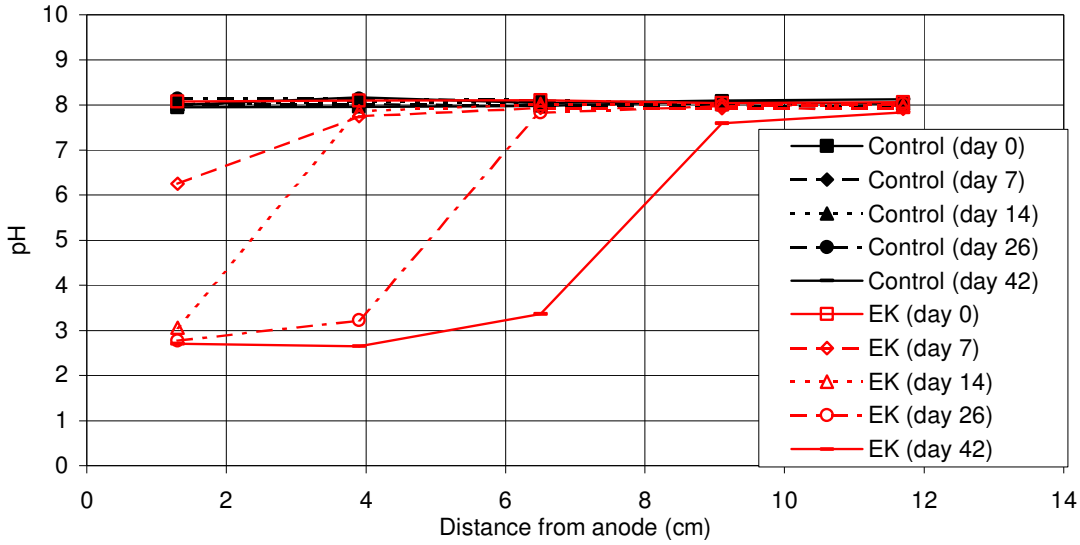

Figure 4-13: Variation of pH profile with time (experiment API_21)

Experiments PI_18 and PI_20 had pH control at both electrodes. The effect of this can be seen on Figure 4-14; very little change in pH (approximately one pH unit at most) was noted throughout the testing. This graph shows the results from experiment PI18 (the corresponding graph in PI_20 was very similar) - in this test an electric field was only applied after 66 days.

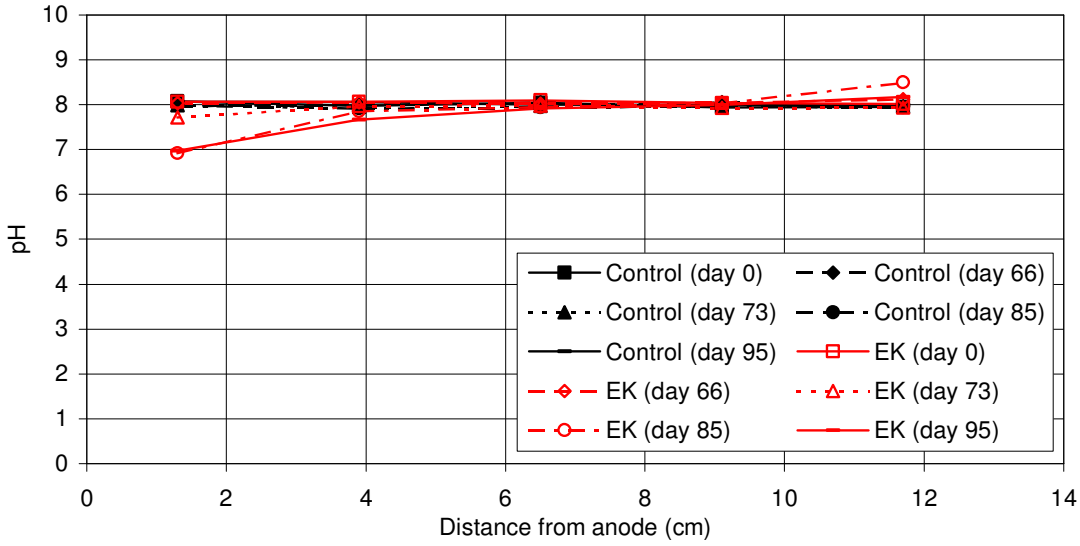

Figure 4-14: Variation in pH profile with time (experiment PI_18)

Experiment PI_22 (Figure 4-15) was designed so that a regular reversal of current could control pH in the soil (as there was no electrolyte fluid, pH could not be controlled by any other means). This can be seen to have worked, as there are only very small drops in pH at either end due to an electric current, after 56 days of testing.

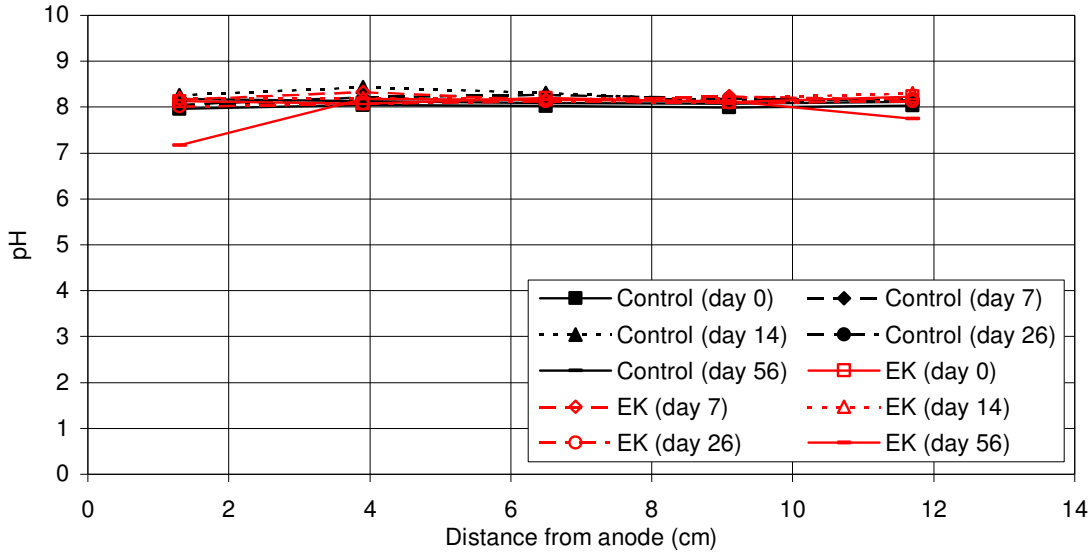

Figure 4-15: Variation in pH profile with time (experiment PI_22)

4.3.3. Temperature

Changes in temperature within the soil mass during electrokinetic processing were monitored in experiments with non-radiolabelled PCP. These were compared to the ambient temperature measured at the time of testing (also shown on the plots), or to control specimens in the case of experiment P_1. Temperature profiles from three experiments are presented here, showing the variability in temperature effects. It should be noted that the overall variability (including that of the ambient temperature / control) was due to the laboratory in which these tests were performed, which was not temperature controlled. It would be expected that the heating effect of the electric current would have

a large part to play in any increase in the temperature of electrokinetic specimens, and it was generally noted that the occurrence of temperatures higher than the ambient / control coincided with higher currents. In general, the highest temperatures were found to be associated with the anode end of soil specimens – this may be an effect of the current, or it could possibly have been due to exothermic chemical reactions induced by pH changes there. Temperature measurements were discontinued in later experiments as it was found that the constant current conditions at 10mA did not induce noticeable temperature variations.

Very little deviation from the ambient temperature was seen in experiment PCP_1 (Figure 4-16), whereas in PCP_4 (Figure 4-17) changes of up to five degrees were found, over a shorter period of time.

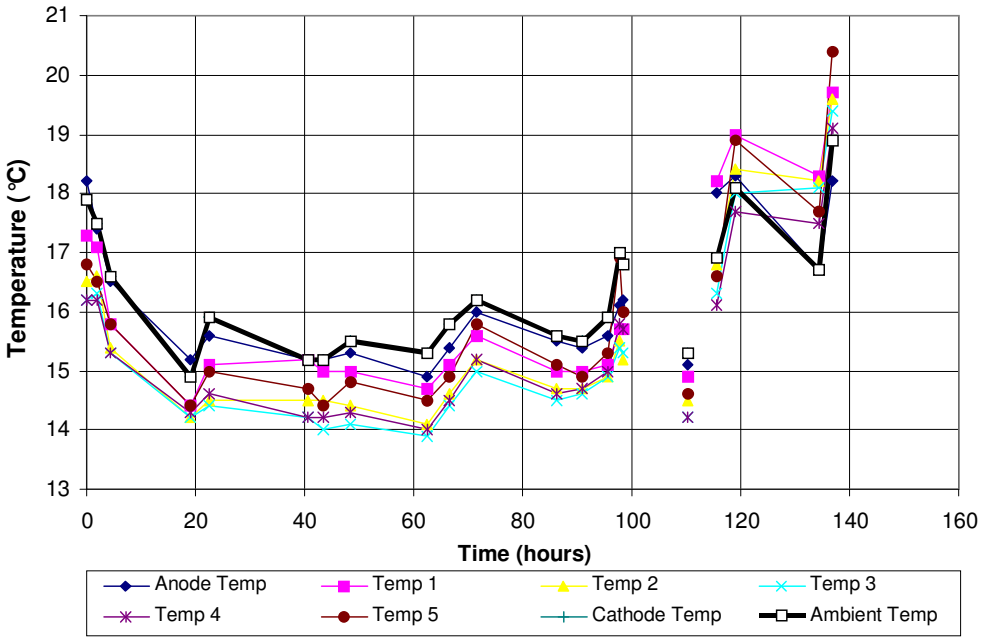

Figure 4-16: Temperature regime across specimen versus time (experiment PCP_1)

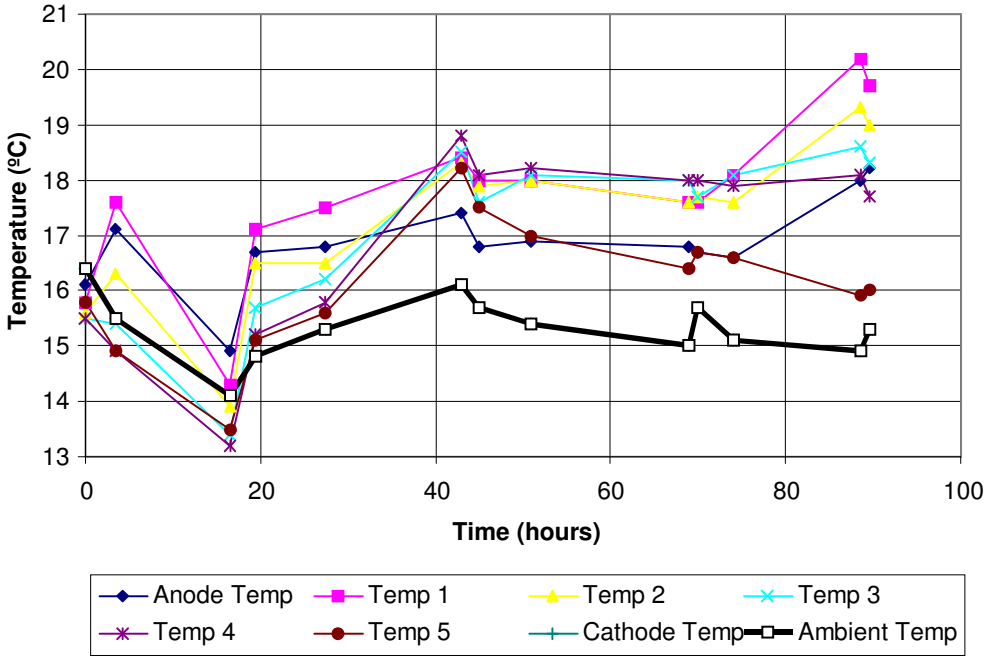

Figure 4-17: Temperature regime across specimen versus time (experiment PCP_4)

The temperature profile across samples in smaller cartridges in experiment P_1 can be seen in Figure 4-18. Little change or variation was noted either within individual samples or between control and electrokinetics samples, and this behaviour was found in other experiments in small cartridges.

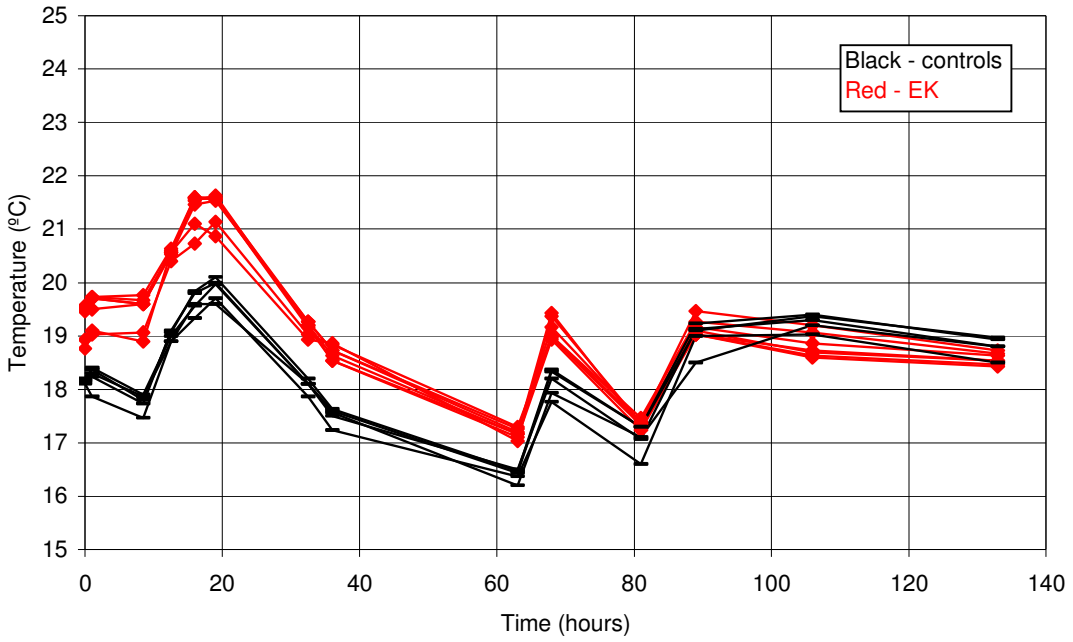

Figure 4-18: Temperature variation versus time (experiment P_1)

4.3.4. Electroosmotic Flow

Flow induced by the presence of an electric field was found to have a certain amount of repeatability between experiments, in that flow would initially be slow to start, then accelerate substantially, before reducing or stopping entirely. The results of three different experiments are shown. Reasons for the slow initial flow may include lack of saturation of Vyon membranes (which was used in all the experiments presented) hindering flow. The reduction or cessation in flow is dealt with later in this section.

In experiment A_4 (Figure 4-19) the majority of the electroosmotic flow detected took place between 50 and 100 hours, at a rate of approximately 4.5ml per hour. Similar behaviour was seen in experiment PCP_1 (Figure 4-20), with a fifty-hour period (from 20 to 70 hours) of flow followed by a cessation. It was hypothesised that the high pH at the cathode end might be blocking flow paths, through the formation of insoluble metal hydroxides. Indeed, a white powdery material was noted on the membrane in the cathode

chamber in experiment PCP_1. Therefore, the pH was adjusted to pH7 at the cathode, by the addition of acid after 100 hours. Electroosmotic flow restarted immediately and the rate increased still further after a second addition of acid at 112 hours. The reduction in electroosmotic flow rate after 120 hours could not be reversed with further acid addition. In subsequent tests, the cathodic pH was controlled in order to maximise electroosmotic flow and thereby to maximise the potential for movement of contaminants in the soil.

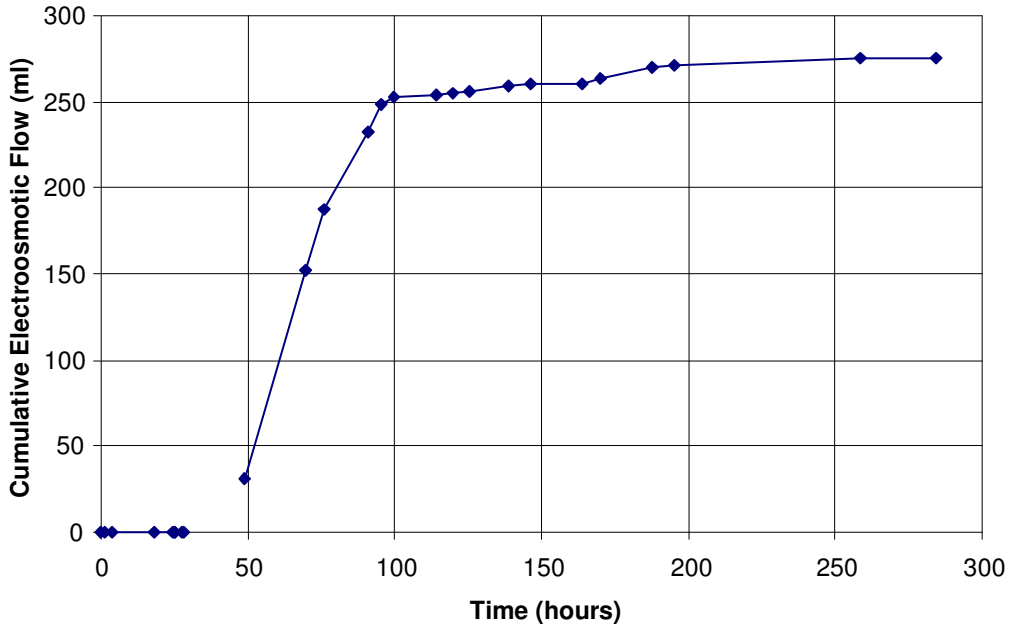

Figure 4-19: Electroosmotic flow versus time (experiment A_4)

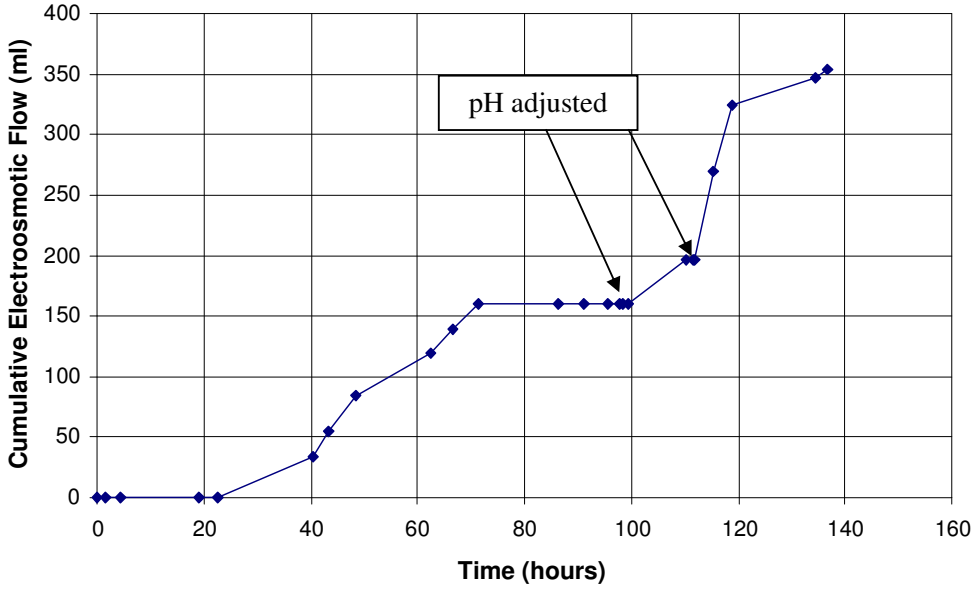

Figure 4-20: Electroosmotic flow versus time (experiment PCP_1)

The addition of acid did not guarantee unlimited electroosmotic flow, however. Figure 4-21 (experiment PCP_5) shows a cessation in flow after 170 hours, which corresponded to a large increase in resistance about the cathode end of the soil, even though pH control at the cathode was employed throughout the experiment (Figure 4-11). It is possible that the intermittent addition of acid was not the best way of controlling pH, and that a build-up of precipitate was allowed in this case. Control of pH in later tests was performed automatically, and therefore allowed a continuous adjustment of the pH.

A drying effect was noted in certain constant current experiments (section 4.3.5), when pH was not controlled at the anode. The results presented in this section are all from constant voltage tests, and such a drying effect may also have occurred here. This may be part of the reason for a cessation in electroosmotic flow, which could not be restarted by cathode pH control.

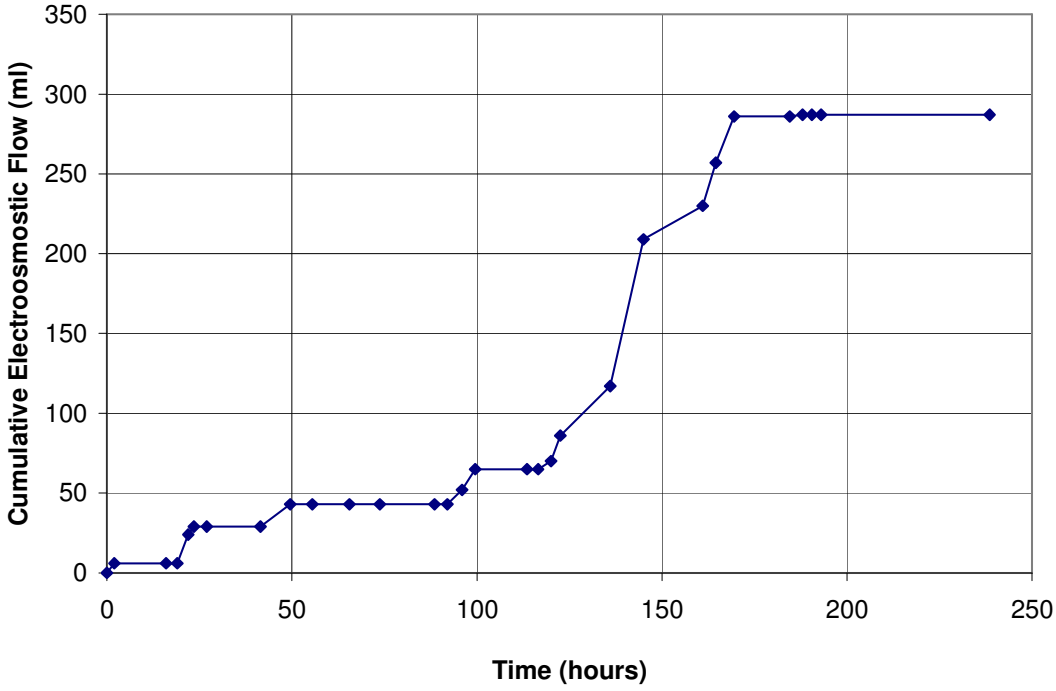

Figure 4-21: Electroosmotic flow versus time (experiment PCP_5)

4.3.5. Moisture Content

An unsaturated soil state was considered important in these experiments as aerobic bacteria were being used to degrade the contaminant. The soil was designed to be unsaturated at the start of each experiment, and so it was expected that changes in moisture content would be seen due to the action of electroosmotic water flow induced by the electric field. It is also possible that diffusion through membranes at the electrode chambers would have had an effect (although this would have been hindered by the extremely low hydraulic conductivity of the membrane material when Daramic was used). The suction present in the unsaturated soil would be expected to take up and hold the moisture presented by any of these methods, and so the moisture content would be expected to rise. Conflicting behaviour may have been generated, however, by such factors as the heating effect of an electric current or evaporation drying out the soil.

A variety of behaviour was observed, a summary of which is presented in this section - pH change appears to have had a greater impact than expected, as is shown here. Data are presented showing changes in moisture content between the start and end of testing for several groups of tests. Tests with a constantly applied current showed either no change or a decrease in moisture content whilst those with a low or pulsed current exhibited increases. Experiments incorporating pH control at both electrodes saw large moisture content rises, and the final test, which took steps to control moisture content by preventing any moisture access to the soil, showed no change.

The moisture content of soil during experiments was determined for each soil sample taken, in order to calculate contamination levels per dry weight of soil. Some small errors in its calculation may have been introduced due to the effect of compaction during sampling, as the soil corer was pushed in. However, these are not thought to be significant in terms of the conclusions drawn.

4.3.5.1. Constant Current Experiments

Typical results from experiments with a constant current of 10mA are shown in Figure 4-22, Figure 4-23, Figure 4-24 and Figure 4-25 (experiments PI_13i and PI_16). It can be seen that, in both cases, there was no initial significant difference between control and electrokinetic specimens, and that there was good uniformity across the soil. In experiment PI13i, there was no significant difference in moisture content after 36 days of electrokinetic processing, although on average electrokinetic specimens were slightly drier than controls (by no more than a few percent). No change was seen in control samples over the test. A similar result was obtained in PI_13u and API_21.

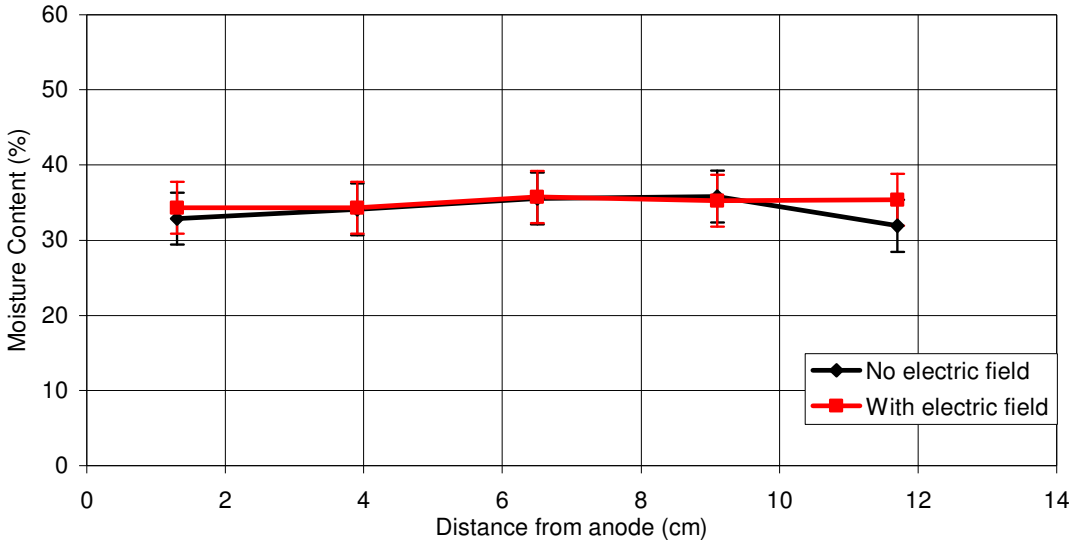

Figure 4-22: Moisture content profile - day 0 (experiment PI_13i)

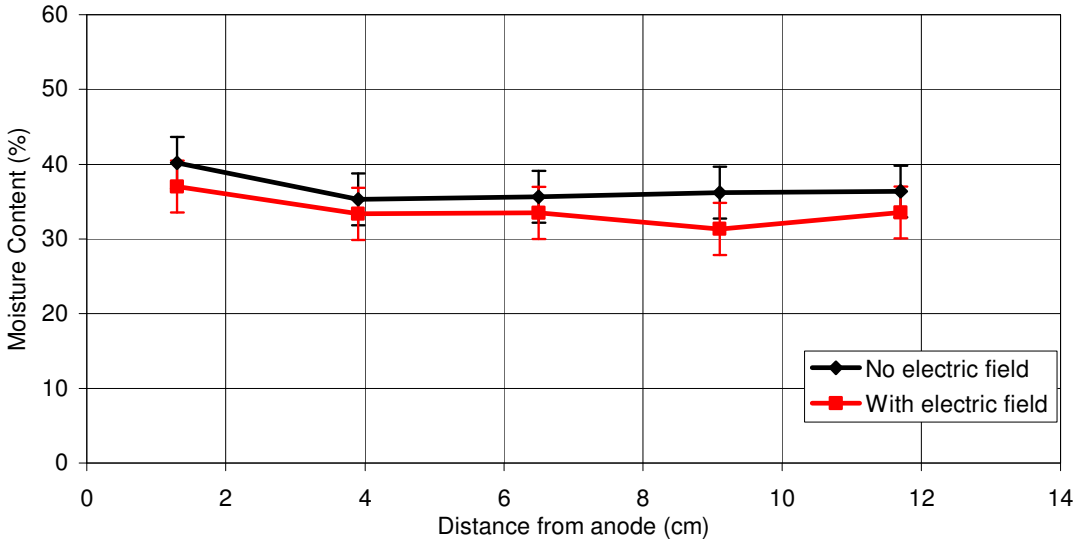

Figure 4-23: Moisture content profile - day 36 (experiment PI_13i)

Experiment PI_16 showed different behaviour in that over 36 days of testing, a statistically significant ($p<0.03$) difference in moisture content was noted between controls and electrokinetics specimens, with the former having on average a 10% higher

moisture content. The control specimen average moisture content increased by approximately 5% on average, whilst that in the electrokinetic specimens decreased by a few percent. As no fluids were supplied to the control specimens, then this increase may have occurred naturally, through biological activity (the soil contained organic matter that would have been the target of microbial degradation, producing carbon dioxide and water). However, it is not known why this effect was not seen in the other experiments discussed earlier.

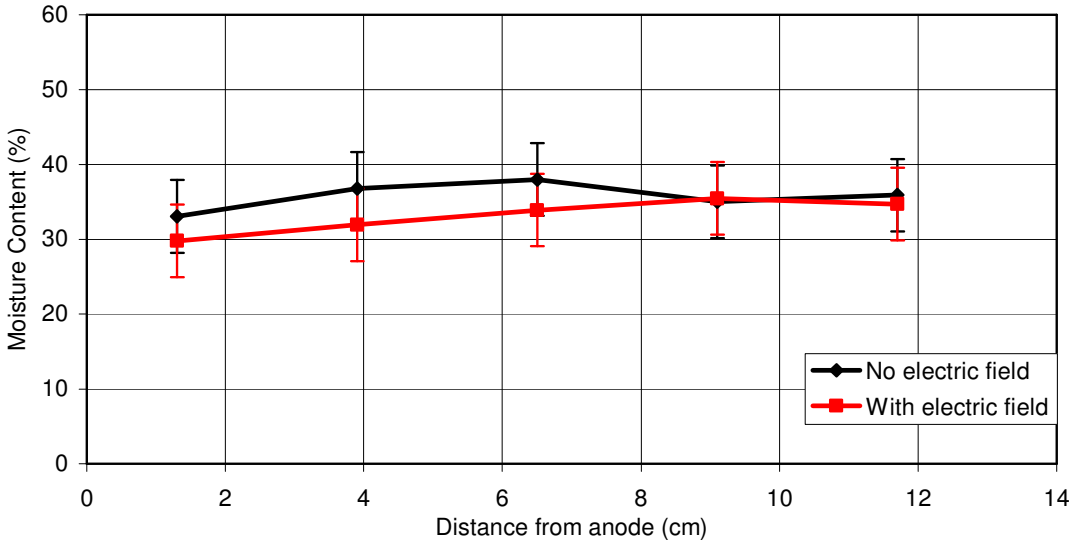

Figure 4-24: Moisture content distribution - day 0 (experiment PI_16)

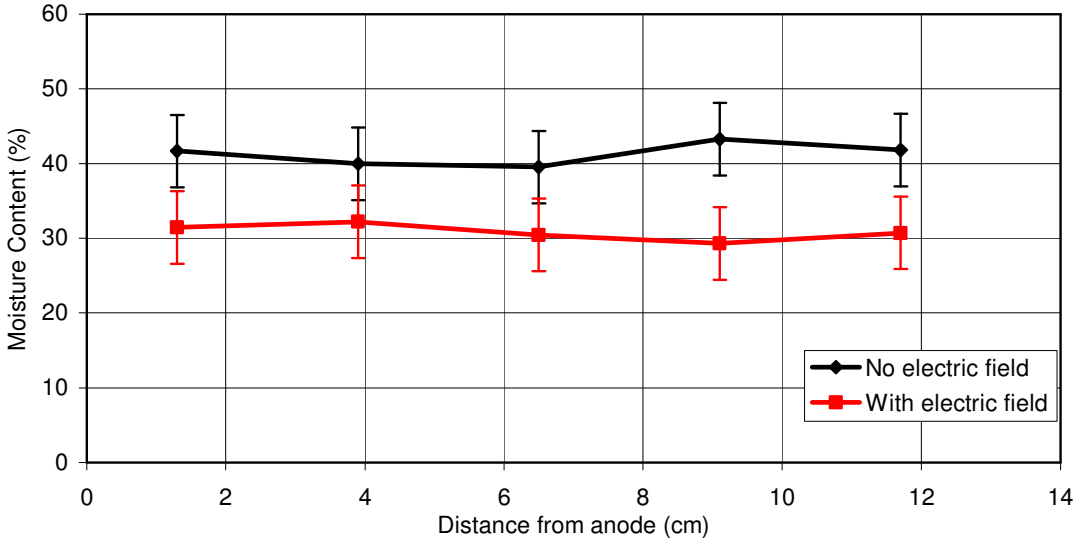

Figure 4-25: Moisture content distribution - day 36 (experiment PI_16)

A constantly applied current of 10mA may therefore be expected to dry out the sample (relative to controls), but it is difficult to predict to what extent.

4.3.5.2. Pulsed and Low Current Experiments

Despite drier conditions initially in electrokinetic specimens compared to controls, pulsed-current electrokinetic processing in experiment PI_14 (Figure 4-26 and Figure 4-27) increased the moisture content in the former specimens. It was significantly higher ($p<0.002$) at positions 2 to 5 after 36 days than corresponding positions in control experiments.

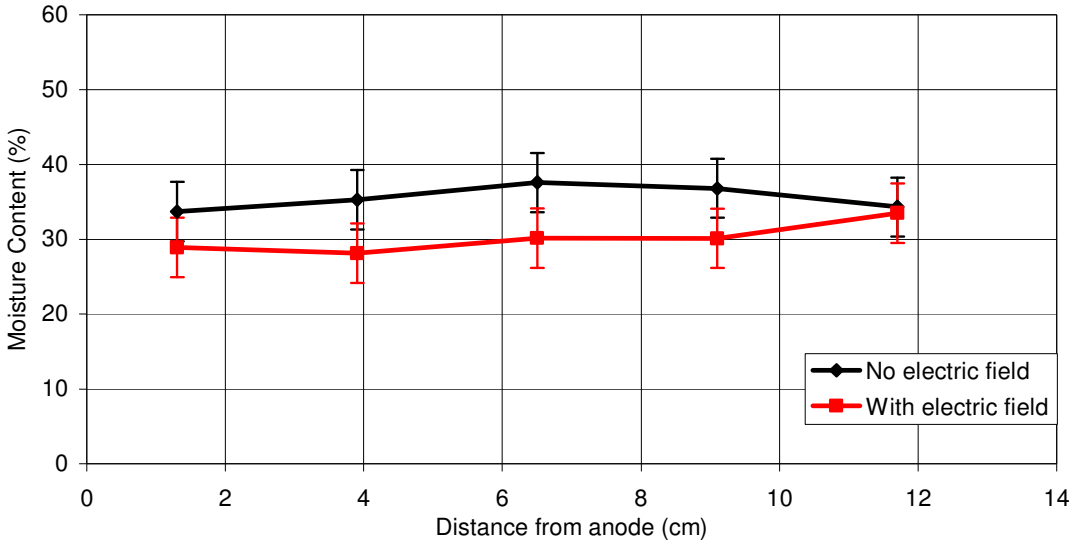

Figure 4-26: Moisture content profile - day 0 (experiment PI_14)

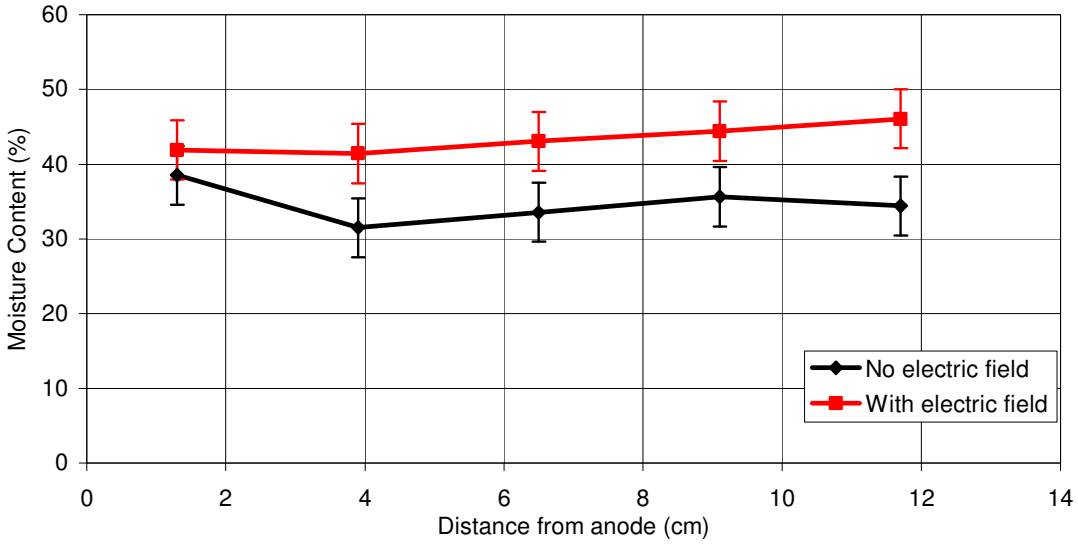

Figure 4-27: Moisture content profile - day 36 (experiment PI_14)

Testing in experiment PI_17 was under a constant current regime, although the current was reduced to 1mA, such that the power input might be compared with experiment PI_14. A similar total charge was applied (approximately 3000 Coulombs in this case,

compared with 2600 Coulombs in PI_14 – 10mA constant current tests had a total of approximately 30,000 Coulombs for the same duration). An initially uniform moisture content (Figure 4-28) across all specimens was noted, but after 36 days of testing (Figure 4-29), it had increased in both sets of specimens. In the electrokinetic specimens it was higher on average than in the controls, but not significantly so. This effect was unexpected, but was also seen in the constant current experiment PI16, where it was suggested that control moisture content rises may have been due to organic matter degradation. A low total power (compared to 10mA constant current experiments) can be seen to have increased the soil moisture content over the testing period, as compared to a possible drying effect with a higher current.

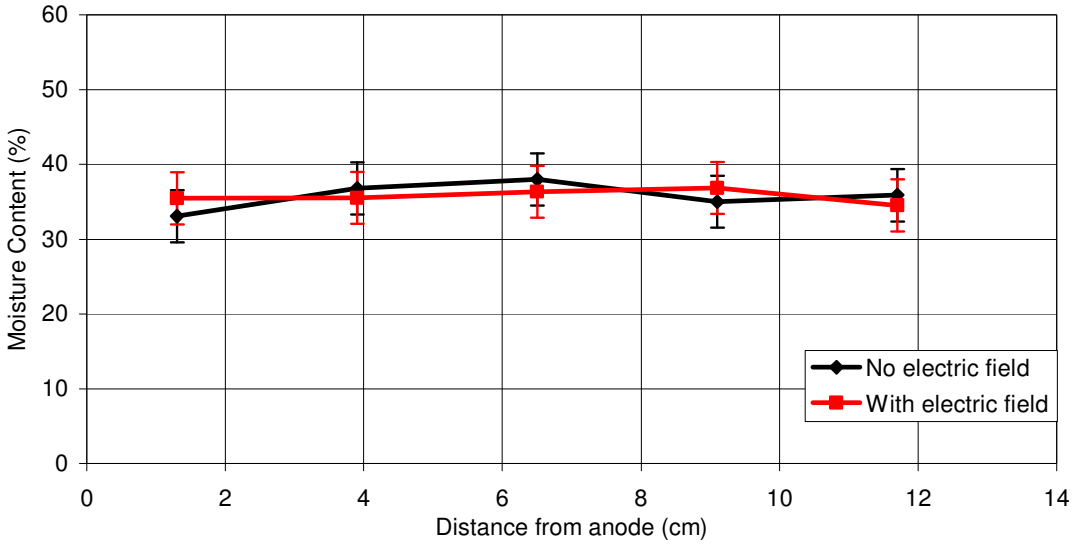

Figure 4-28: Moisture content profile - day 0 (experiment PI_17)

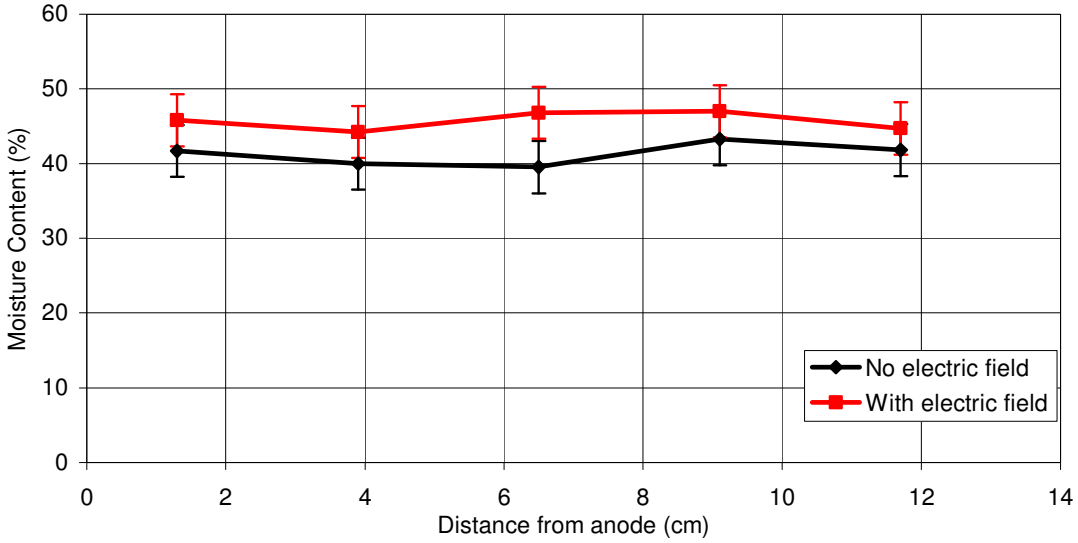

Figure 4-29: Moisture content profile - day 36 (experiment PI_17)

A low input of energy appeared to produce an increase in moisture content, contrasting with the decrease observed with approximately ten times the total applied charge (as seen in the previous section).

4.3.5.3. Effect of pH control at both electrodes

In the experiments described above, the pH was controlled at the cathode chamber, but not at the anode. In experiments PI_18 and PI_20, it was controlled at both electrodes, and a constant current of 10mA was applied in each. Over the initial 66-day period in experiment PI_18 (during which time no field was applied), there was a slight increase in moisture content on average over all samples (Figure 4-30 and Figure 4-31). With the application of an electric field for 29 days, however, a significant ($p<0.02$) increase of approximately 15% was seen across the electrokinetic specimens (Figure 4-32). A very similar result was obtained over the 36 days of experiment PI_20. These experiments can be compared to tests such as PI_13i and PI_16, which had the same applied current (over

similar times) but did not have pH control at the anode. The control of pH at both electrodes, therefore, can be seen to have been an important factor in large moisture content changes when an electric field was applied.

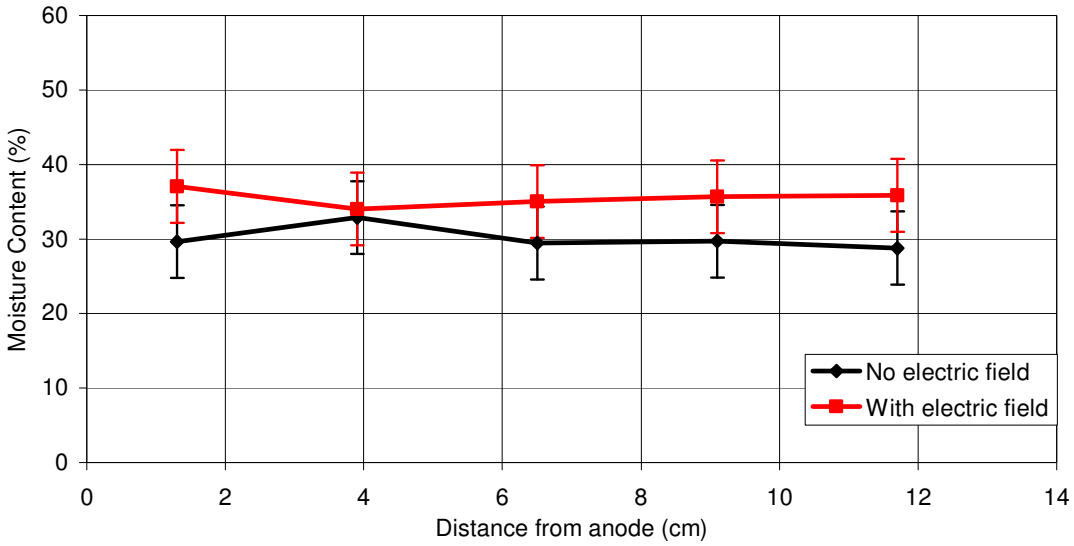

Figure 4-30: Moisture content profile - day 0 (experiment PI_18)

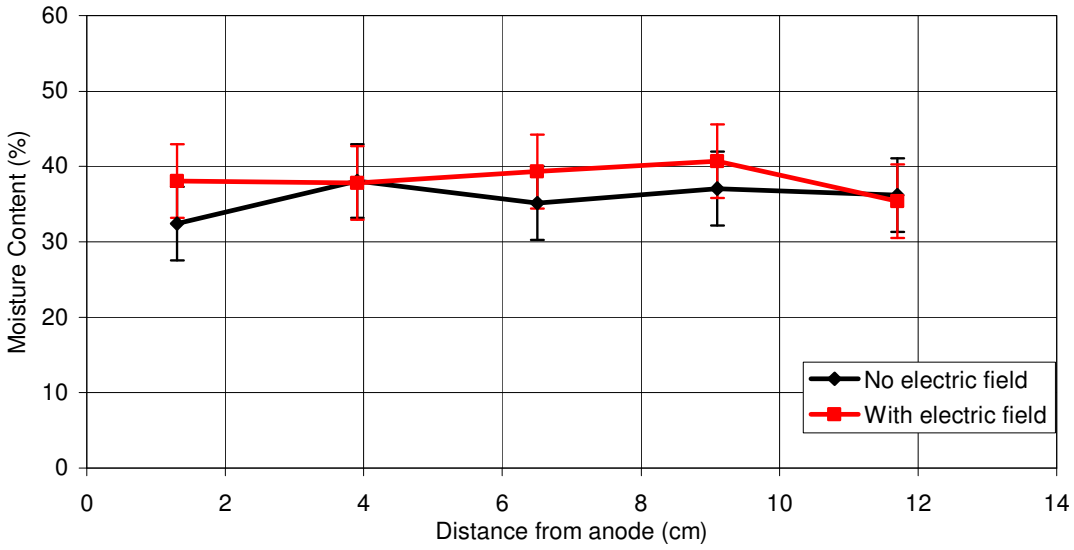

Figure 4-31: Moisture content distribution - day 66 (experiment PI_18)

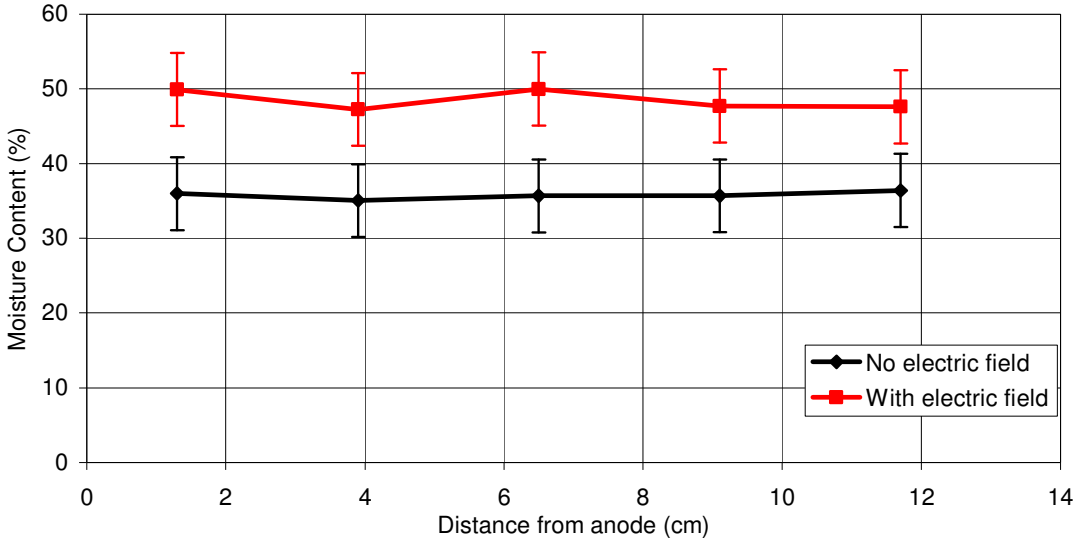

Figure 4-32: Moisture content profile - day 95 (experiment PI_18)

4.3.5.4. Control of Moisture Content and pH

The design of experiment PI_22 incorporated methods to prevent the saturation of the soil, as was seen in experiments PI_18 and PI_20 (see above). This was done by removing the need for electrolyte fluids – electrodes were placed in direct contact with the soil. Despite regular additions of water to the soil adjacent to the electrodes in electrokinetic specimens (necessary to maintain current flow), no change in the initially identical control and electrokinetic specimens' moisture content profiles was seen after 56 days of testing (Figure 4-33 and Figure 4-34).

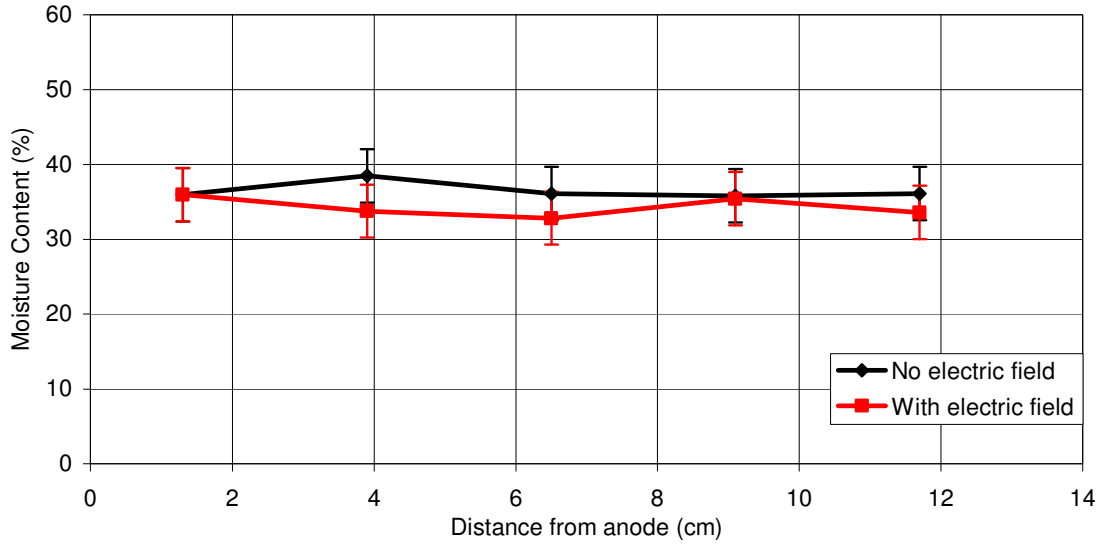

Figure 4-33: Moisture content profile - day 0 (experiment PI_22)

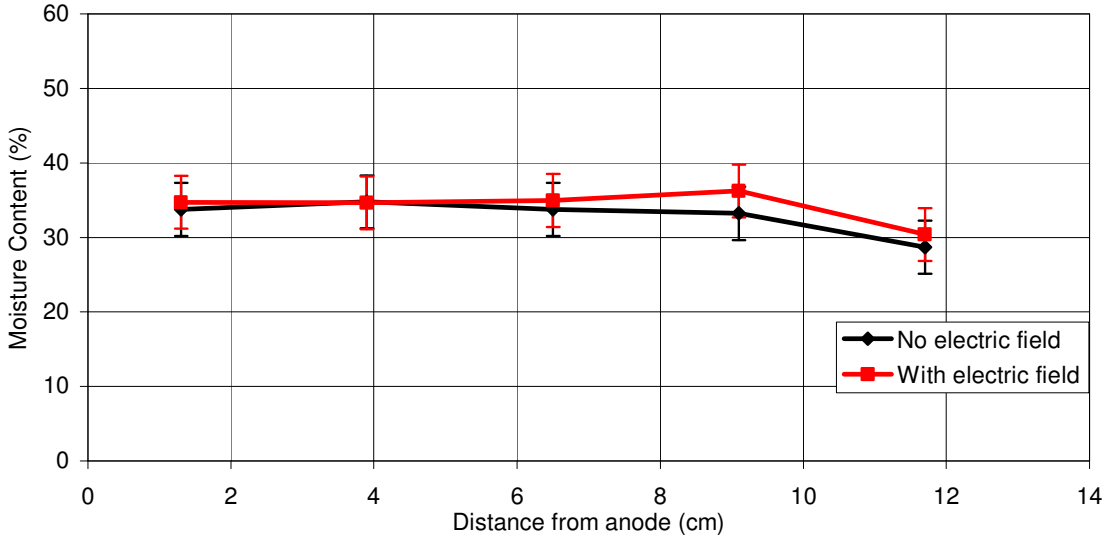

Figure 4-34: Moisture content profile - day 56 (experiment PI_22)

4.3.6. Organic Matter

In several experiments (notably those with a constant applied field), the anolyte fluid became a very dark brown colour over time, thought to be due to negatively charged

humic acids being moved by electromigration. In experiments PI_18 and PI_20, where anolyte and catholyte were mixed to control pH, both were dark brown by the end of testing.

4.4. Interaction between Contaminant and Soil

The sorption of PCP to Wytham soil was investigated under a variety of different conditions. Figure 4-35 and Figure 4-36 present the equilibria between PCP (sodium salt) in solution and sorbed to soil. It can be seen that substantially more PCP remained sorbed to soil material when organic matter was present, and as the pH dropped, so did the proportion of PCP in solution.

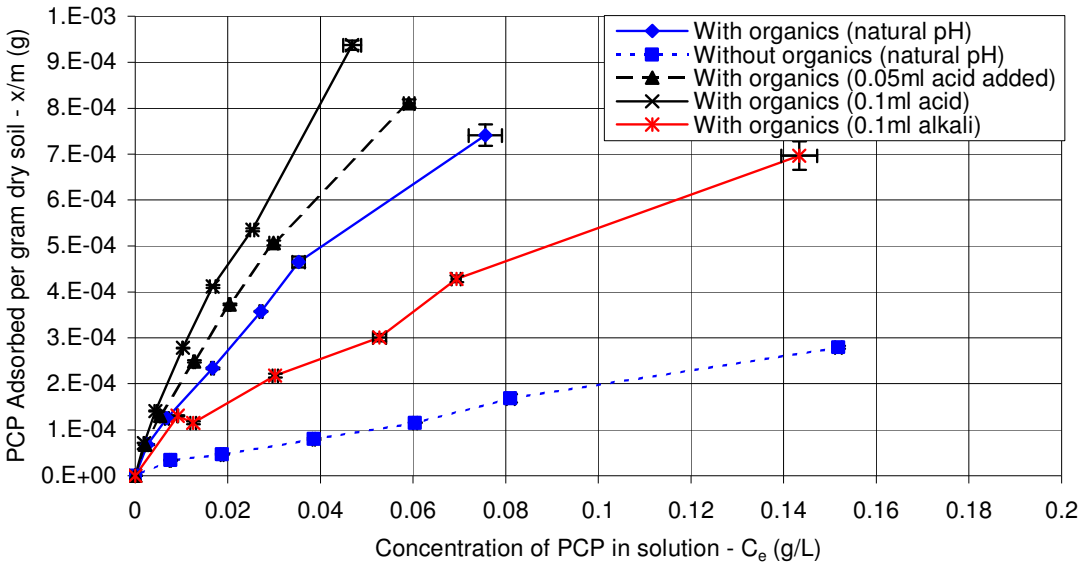

Figure 4-35: PCP Adsorption Isotherms

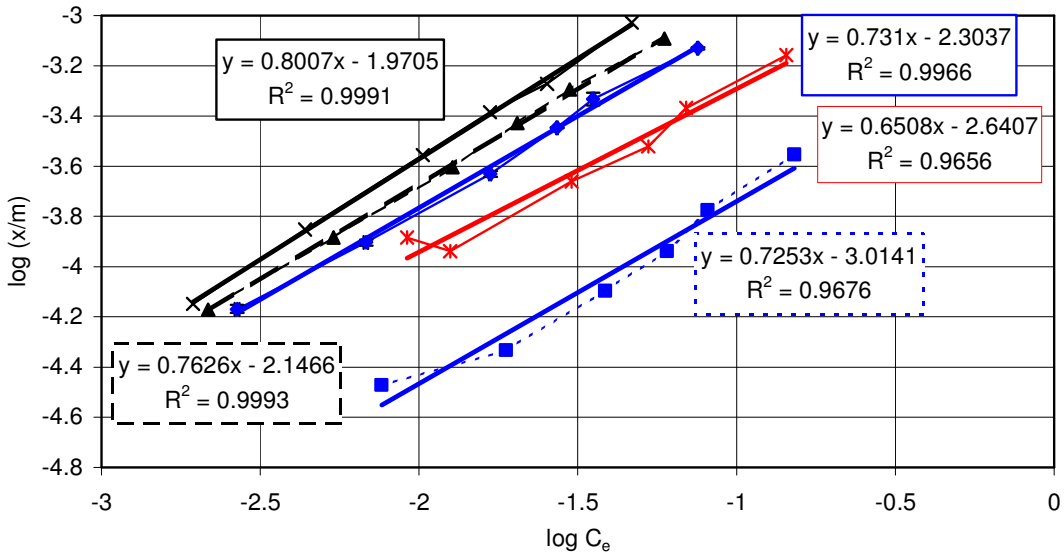

Figure 4-36: PCP adsorption isotherms (logarithmic plots)

The speed of PCP sorption onto Wytham soil (with organic matter, no pH change) is illustrated in the next plot (Figure 4-37), showing that the majority of PCP that would sorb to the soil did so within the first few hours. This effect was exacerbated when acid (100µl in each sample) was added. Figure 4-38 shows that in this case the time required to process the first sample was too long to detect the initial adsorption curve – all of the sorbing PCP had sorbed by the time of the first sample.

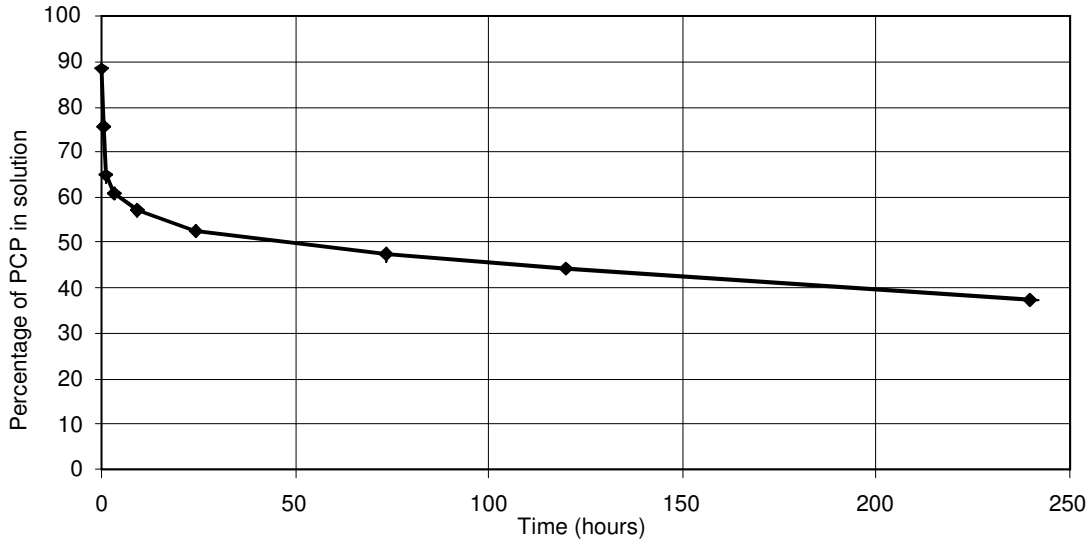

Figure 4-37: PCP adsorption versus time

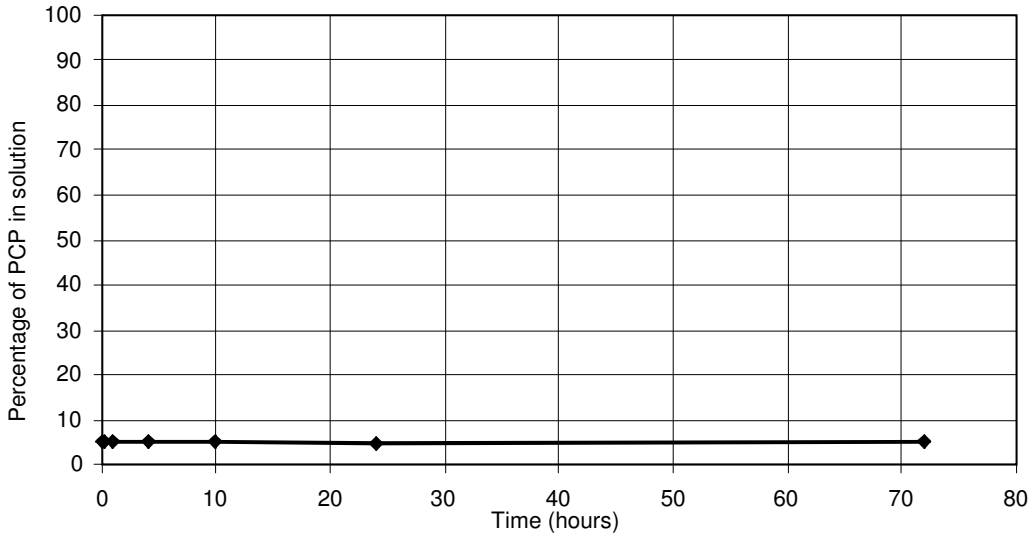

Figure 4-38: PCP adsorption versus time (with acid)

It is shown in Figure 4-39 that the desorption of PCP from soil (with organic matter and at natural pH) occurred at a comparable rate to the sorption – within the first few hours, most of the PCP that would desorb had done so.

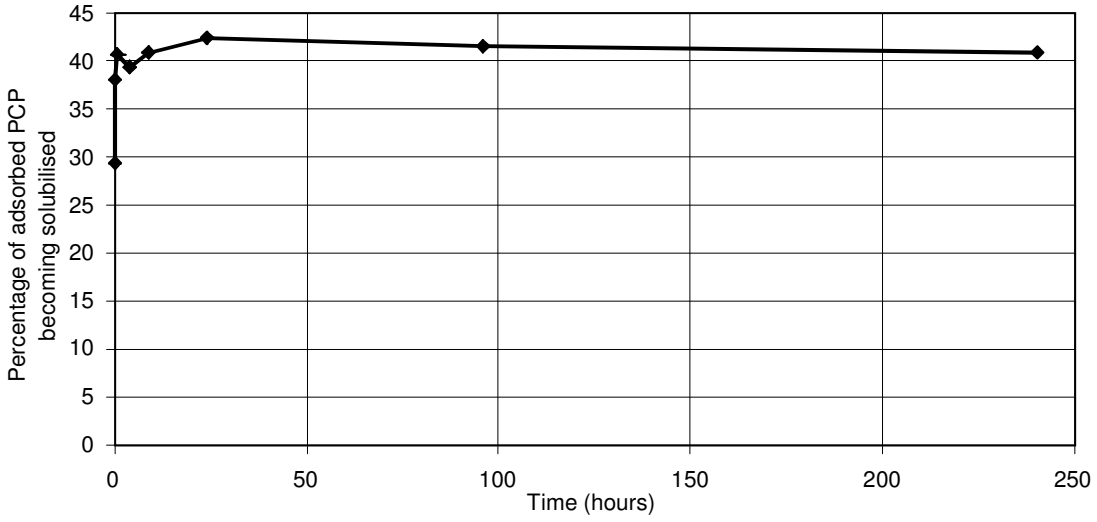

Figure 4-39: PCP desorption versus time

4.5. Action of PCP degraders in PCP-contaminated soil

The experiments presented in this section investigated the behaviour of inoculated PCP-degrading bacteria (*Sphingobium* sp. UG30) in soil, without the presence of an electric field but in contact with the PCP. The effect of the contaminant on survivability was investigated, as was the degradation of PCP, showing that lower concentrations of bacteria had difficulty in surviving and consequently did not degrade PCP efficiently. In addition, the effect of soil structure was tested, with experiments performed in both flasks (unstructured soil) and cartridges (structured soil, as seen in the majority of other experiments). The long-term behaviour of degradation was shown also, with unexpected degradation of PCP in uninoculated specimens. This occurred throughout soil specimens in a relatively uniform fashion, and so might have been due to naturally occurring micro-organisms that acquired the ability to degrade PCP rather than contamination with the degraders used in other specimens.

Experiment PI_7 was an investigation into bacterial survival in PCP-contaminated soil. It also investigated differences between the cartridge system used in this work (which was designed to produce a realistic soil structure) and flask-based experiments commonly used in microbiological experiments (in which soil, contaminant and bacteria were thoroughly mixed). Three different experiments were performed: in cartridges with 10^6 bacterial cfu / g dry soil (inoculated at 96 uniformly distributed points), in flasks with 10^6 bacterial cfu / g dry soil and in flasks with 10^8 bacterial cfu / g dry soil. Each of these was subdivided into tests with and without PCP. The moisture content was adjusted to be approximately the same (around 40%) in each test, whilst PCP concentration (where necessary) was approximately 140ppm. Flask experiments used thirty grams of wet soil, whereas cartridges had approximately six hundred grams in each.

Figure 4-40 shows the recovery of PCP from the different experiments, versus time. Each line represents one of the six types of experiment (i.e. with and without PCP, and the three different inoculum concentrations). The three control plots (without PCP) presented are zero for the duration of testing, as would be expected. The recovery efficiency was approximately 85% at the start of testing, presumably due to the inability of the extraction technique to retrieve all the contaminant. There was no apparent difference between flask and cartridge experiments with 10^6 cfu / g dry soil. Overall, no discernible PCP degradation was seen, although a sudden drop in recoverable PCP on 8 days in flask experiments with 10^6 cfu / g dry soil was noted. As this recovered at later time points, it is thought that this was an error in sampling or in uniformity in the soil. With 10^8 cfu, however, a rapid reduction in extractable PCP was noted, with only approximately 10% residual PCP remaining after 22 days of testing.

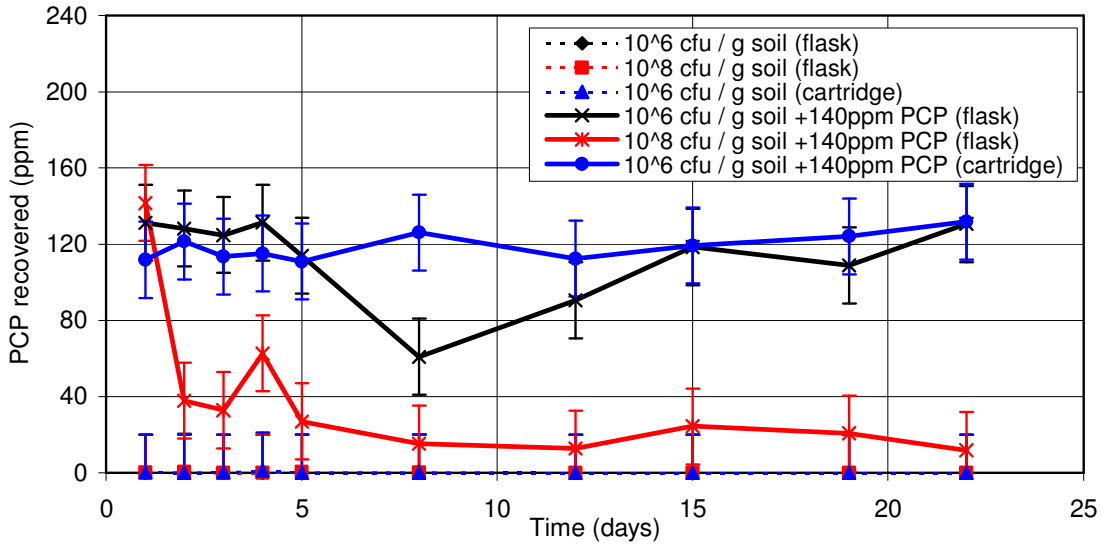

Figure 4-40: PCP recovered versus time (experiment PI_7)

Bacterial numbers are presented in Figure 4-41. With 10^6 cfu (flask), the number of bacteria countable from 5 days onwards was significantly lower ($p<0.05$) when PCP was present than when it was not, apart from on 22 days (when there are very low numbers of

UG30 present in both cases). A similar result was obtained with 10^8 cfu (flask), although the two sets of samples (with and without PCP) were very highly significantly different ($p<0.001$) from day 4 until the end of testing. Numbers of bacteria in cartridges with PCP were initially (for the first two days) much lower than those without access to the contaminant ($p<0.001$). This trend was repeated from day 5 onwards ($p<0.006$), although the last two time points showed similar bacterial numbers, as there were few left in either set of specimens.

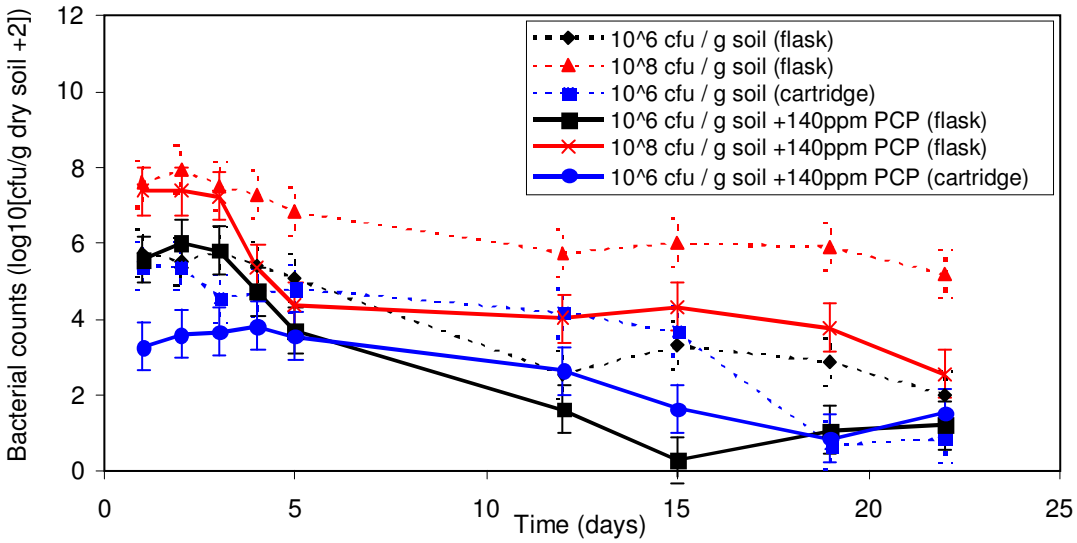

Figure 4-41: Number of *Sphingobium* sp.UG30 detected versus time (experiment PI_7)

The persistence of PCP in soil cartridges with and without inoculated degrading bacteria was investigated in experiment PI_4 by contaminating six cartridges in the usual way and inoculating three with UG30 (10^6 cfu/g dry soil). The remaining three were not inoculated. Figure 4-42 shows the mass balance of this experiment with time, over a period of 15 weeks. By the end of the experiment, there was very little PCP remaining in either set of specimens, but with degrading bacteria inoculated the PCP was degraded much faster. The removal of PCP without inoculated bacteria was unexpected, as it had

been thought that this chemical was particularly recalcitrant to degradation by most indigenous bacteria.

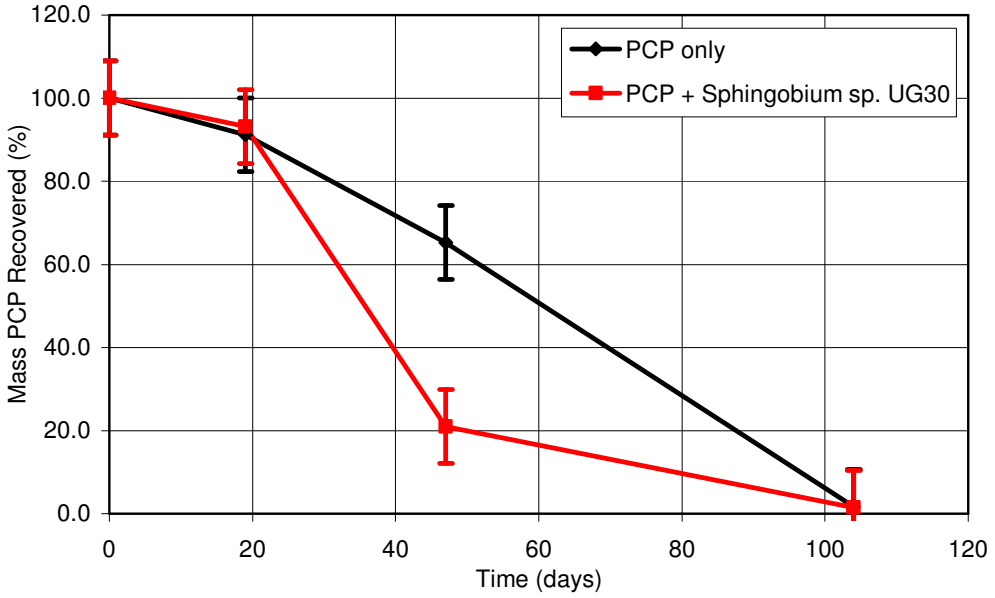

Figure 4-42: Mass balance (experiment PI_4)

4.6. Effect of Electrokinetics on Microbes in Soil

Experiments were performed to investigate the effect of an applied electric field on both inoculated *Sphingobium* sp. UG30 and natural micro-organisms in the soil. Experiment I_9 was performed in conjunction with Lear (2004). The effect of the electric field was to acidify the anode region of the soil specimens (pH control was employed at the cathode), as seen in other tests. Information obtained from I_9 included an increase in pore fluid conductivity, a lack of distinct changes in soil respiration (carbon dioxide output), and a significant reduction in bacterial numbers at the anode (although fungi numbers were not affected as much) with an applied electric field.

In other experiments, the analysis of enzyme (dehydrogenase) activity gave information as to the effect of electric field and PCP on bacteria in soil, both inoculated *Sphingobium*

sp. UG30 and naturally occurring species. Changes in pH due to the electric field led to decreases in activity at the anode in electrokinetic specimens, although other effects such as a corresponding increase in activity at the cathode could not easily be explained.

The behaviour of the microbial community in soil was investigated using a range of techniques in experiment I_9, which looked at the effect of both applied electric field and added water. Electroosmotic flow was expected to have led to an increase in moisture content in the electrokinetic specimens, and the added water tests were performed to isolate the effects of the electric field from those of the water influx. The amount of water added was equal to that noted to have entered the soil in electrokinetic specimens (calculated from water level drops in Mariotte bottles). Nine soil cores were taken from each cartridge at each sampling time, labelled a to i (from cathode to anode).

The final conductivity of the soil pore fluid is shown in Figure 4-43. Conductivity was generally elevated across the electrokinetic specimens relative to that found in water-adjusted and control specimens, although not significantly so. The pH was found to decrease with proximity to the anode, but with little change in pH at the cathode, as it was controlled there (results not presented).

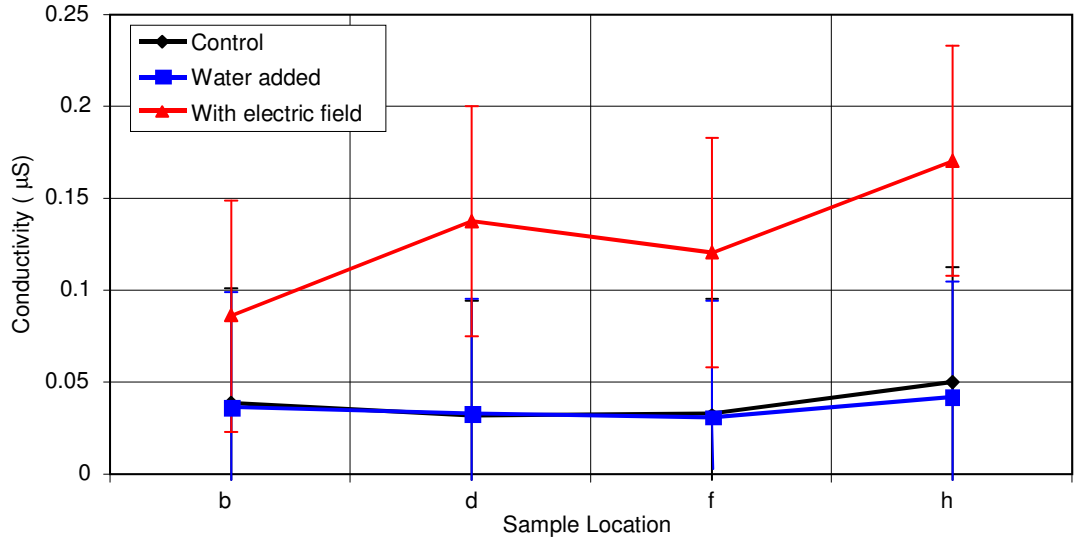

Figure 4-43: Conductivity across specimens at end of test (experiment I_9)

The degree of soil respiration (amount of carbon dioxide given off) was measured. In the data presented in Figure 4-44 and Figure 4-45, it can be seen that there are no trends evident, and that there were no significant differences where an electric field was applied or where specimens had water added.

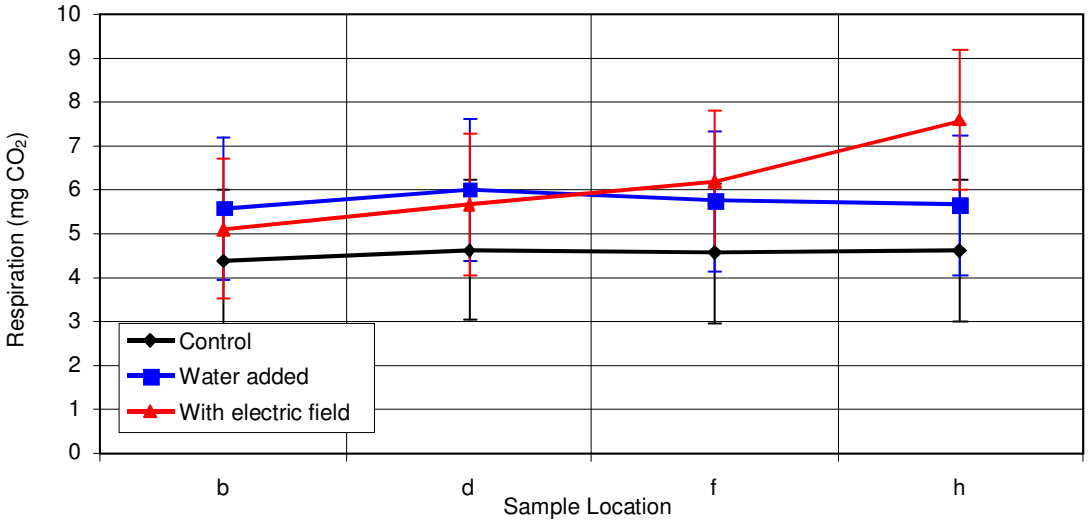

Figure 4-44: CO_2 respiration - day 7 (experiment I_9)

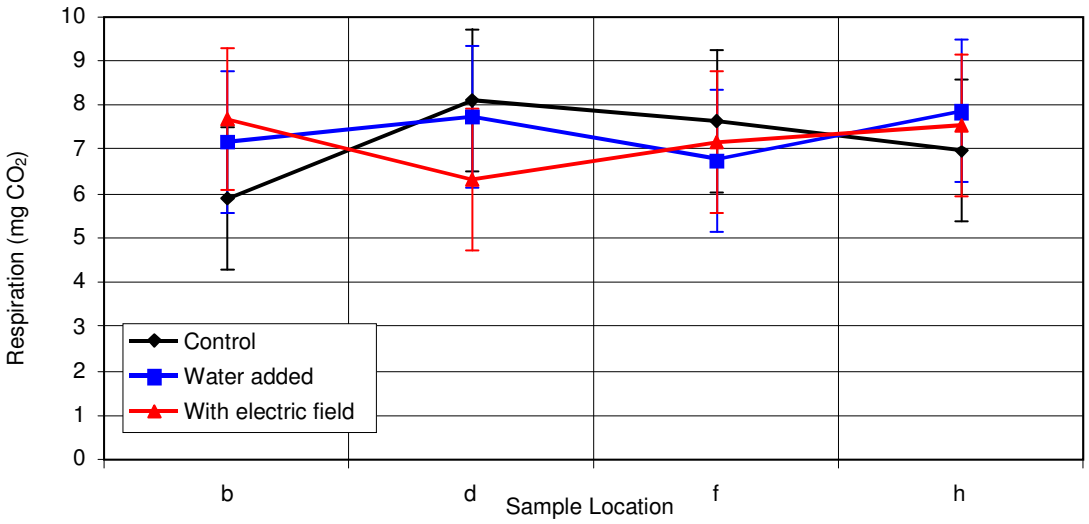

Figure 4-45: CO_2 respiration - day 27 (experiment I_9)

Counts of bacteria and fungi are presented on a logarithmic scale for time points 0 and 27 days (Figure 4-46 and Figure 4-47 are bacterial counts, Figure 4-48 and Figure 4-49 are fungal counts). There is very little difference between any of the curves on each graph. A statistically significant point is at position i on day 27 of the bacterial counts, where there was a drop in numbers in the electrokinetic specimens ($p \leq 0.001$ compared to control specimens).

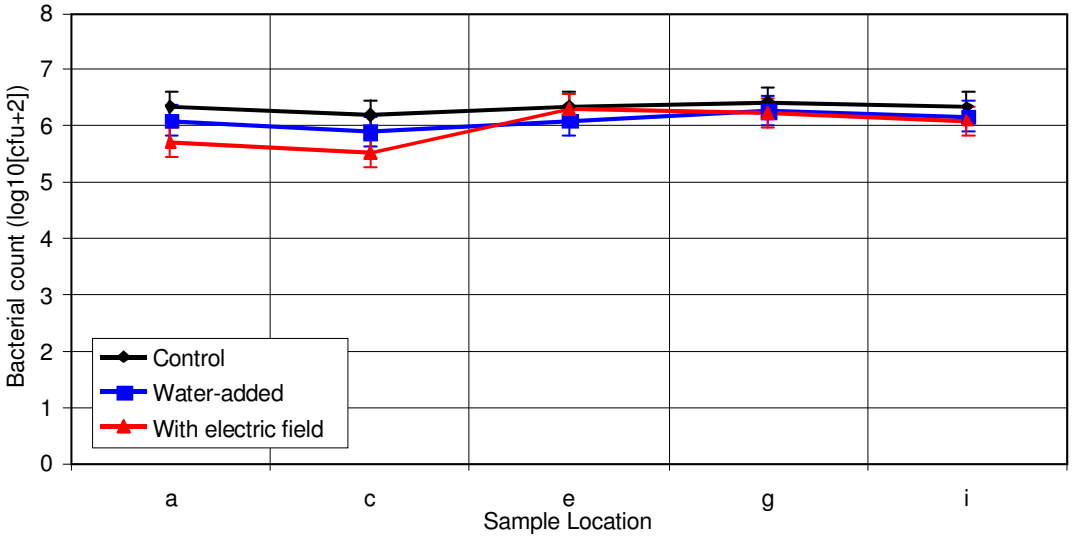

Figure 4-46: Numbers of bacteria – day 0 (experiment I_9)

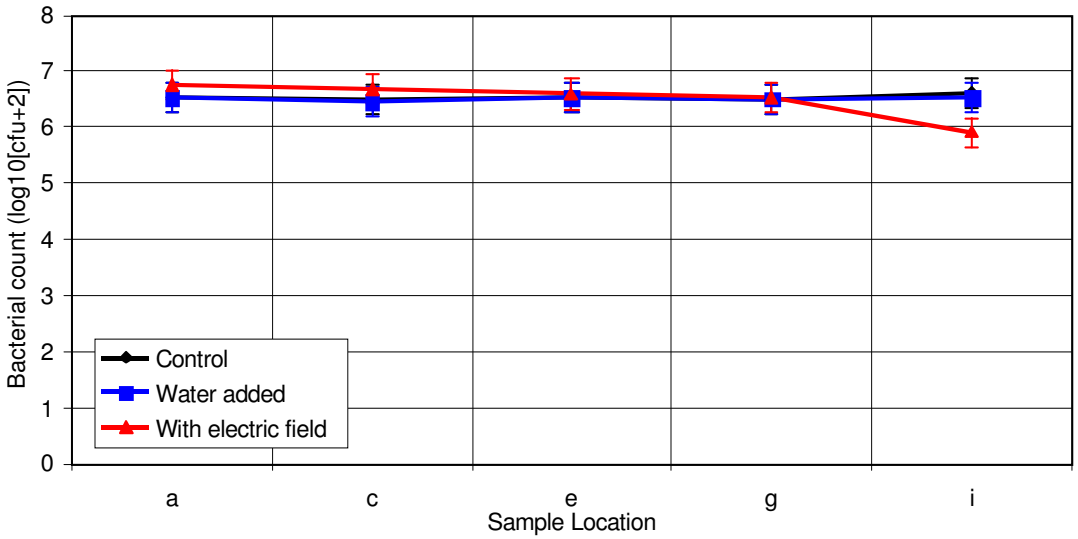

Figure 4-47: Numbers of bacteria - day 27 (experiment I_9)

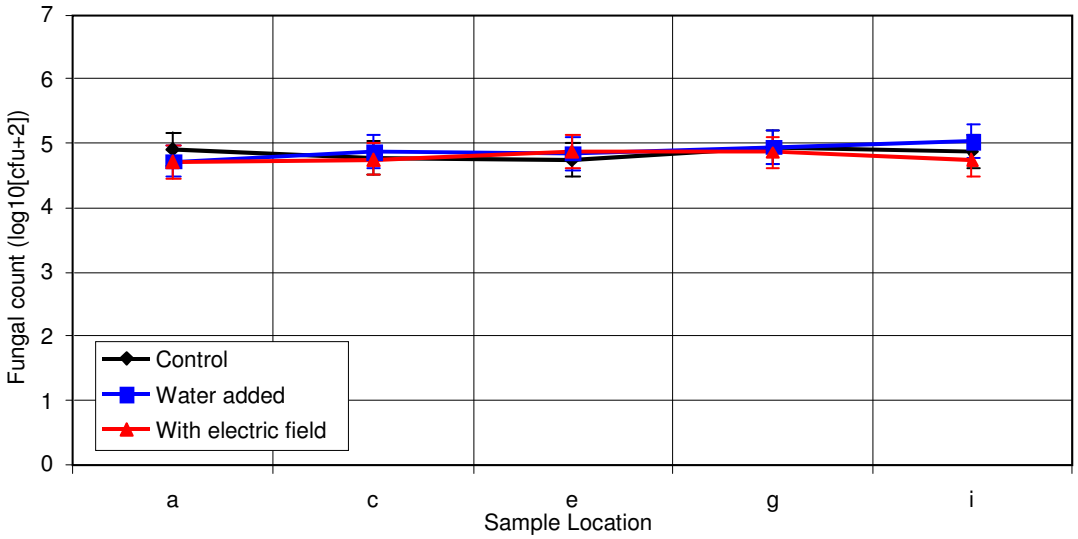

Figure 4-48: Numbers of fungi - day 0 (experiment I_9)

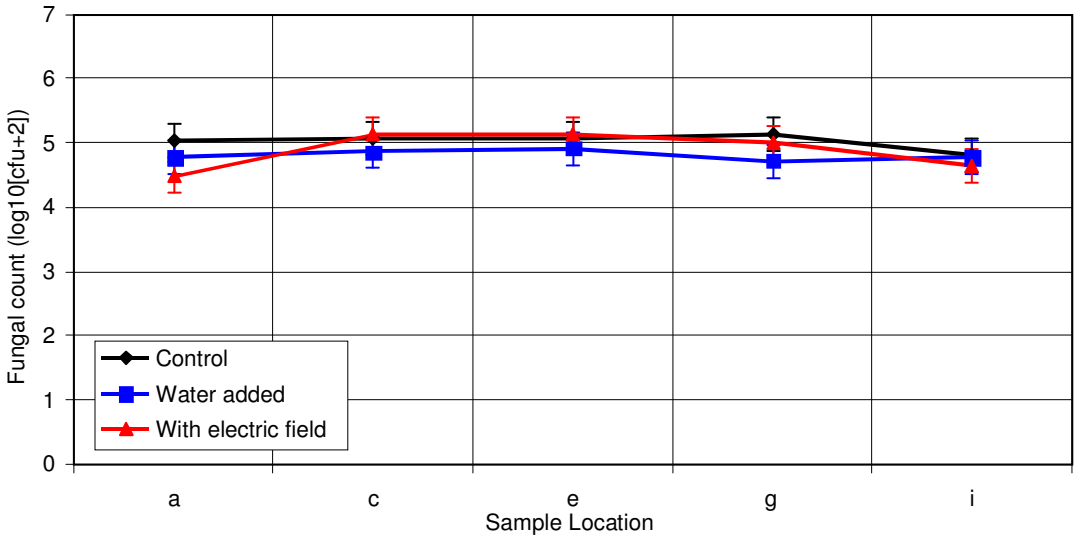

Figure 4-49: Numbers of fungi - day 27 (experiment I_9)

4.6.1. Results of Dehydrogenase Analysis

Dehydrogenase analysis was performed on soil samples from several experiments in order to investigate the effect of electrokinetics and the presence of PCP on enzyme activity in the soil. Comparisons have been made between experiments with constant current and pulsed current, as well as those with pH control at both electrodes rather than just the cathode. A graph is also presented from experiment PI_22, where pH control using a periodically reversed current was combined with moisture content control. Similar effects were seen with constant current with and without pH control at the anode as well as in PI_22 (decreased activity at the anode, increased at the cathode, compared to generally decreasing control activities). Substantially less effect (compared to controls) was noted with a pulsed current and in experiment PI18, where anodic pH was controlled and a two-month degradation period included prior to applying the electric field.

Results from dehydrogenase assays on experiment PI_13i are presented in Figure 4-50. Activity in control specimens did not differ greatly across the specimens. However, an electric field led to substantial changes. At 14 days, the activity recorded at the anode end of the soil dropped to almost zero, and was significantly lower than controls ($p<0.002$) for the remainder of the experiment. A similar drop happened in the centre of the electrokinetic specimens also, but only at day 36 ($p<0.001$). In contrast, activity at the cathode end of the soil was significantly higher than controls at days 26 and 36 ($p<0.001$).

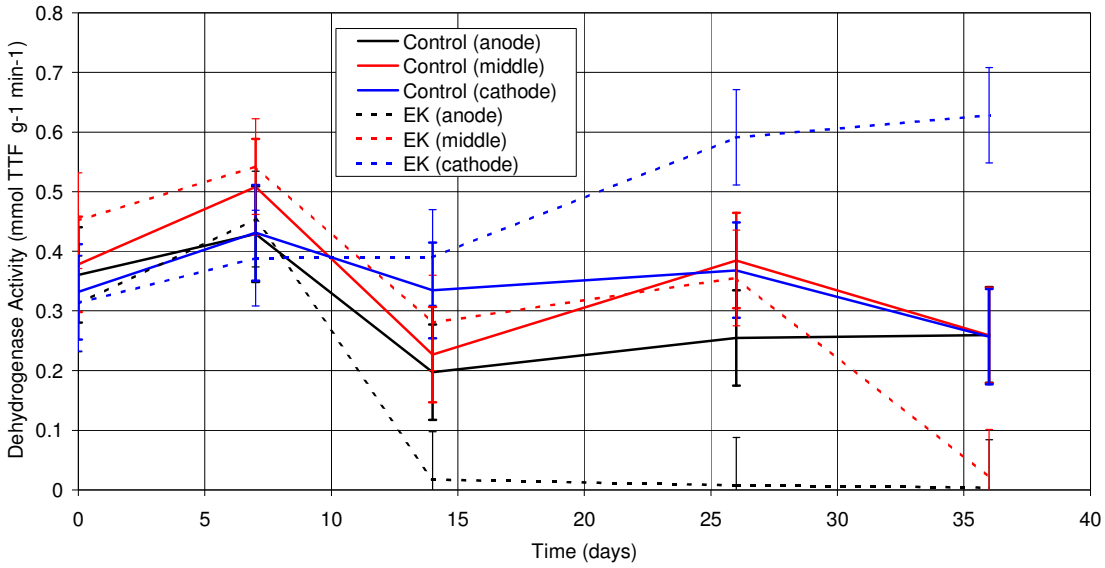

Figure 4-50: Dehydrogenase activity versus time (experiment PI_13i)

In experiment PI_14 (Figure 4-51 - applied pulsed current), dehydrogenase activities in control specimens gradually dropped with time, and there was little variation between the different positions. Those specimens with an electric field applied showed a little divergence after 14 days, although this was not significant. As in experiment PI_13i, activity at the anode dropped, but here cathode activity remained comparable with controls and the central activity increased slightly. In general, there was less divergence between control and electrokinetic experiments with the pulsed current employed here than was seen with a constant current, indicating less effect on soil enzymatic activity.

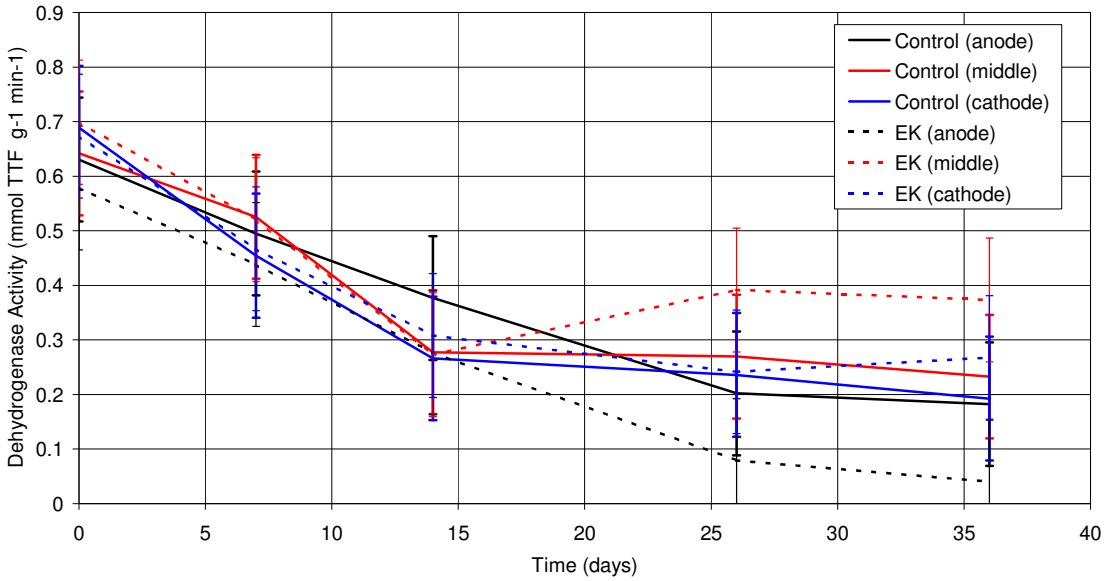

Figure 4-51: Dehydrogenase activity versus time (experiment PI_14)

No obvious patterns could be determined from dehydrogenase activity versus time (Figure 4-52) in experiment PI_18 (with pH control at both electrodes). There were no significant differences between control and electrokinetic specimens at any point. The 66-day period of waiting prior to application of the electric field meant that conditions in both control and electrokinetic samples were identical for a considerable length of time. As has been seen in previous graphs (control lines in Figure 4-50 and Figure 4-51), dehydrogenase activity tended to decrease with time naturally. By the time the electric field was applied, the activity had decreased to such a level that inhibition due to the electric field would not play an important role.

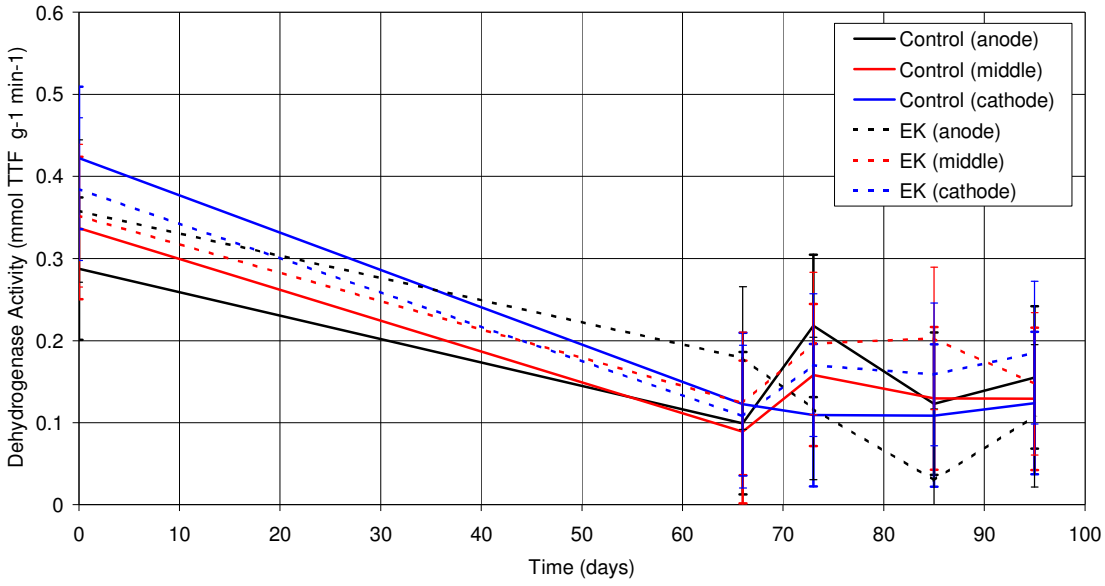

Figure 4-52: Dehydrogenase activity versus time (experiment PI_18)

Dehydrogenase activities presented in Figure 4-53 (experiment PI_20, with pH control at both electrodes) show that an electric field could induce large differences at the ends of soil specimens despite there being no large pH changes. Control specimens exhibited little or no variation in activity from one electrode to the other, but gradually decreased with time. In electrokinetic specimens, the activity in the central section was raised but not significantly so, whilst near the cathode it was significantly higher than controls ($p<0.001$) at days 26 and 36. A significant drop at the anode was recorded from day 7 onwards ($p<0.02$) although at day 36 this was not considered significant, possibly because both control and electrokinetic activity were very low.

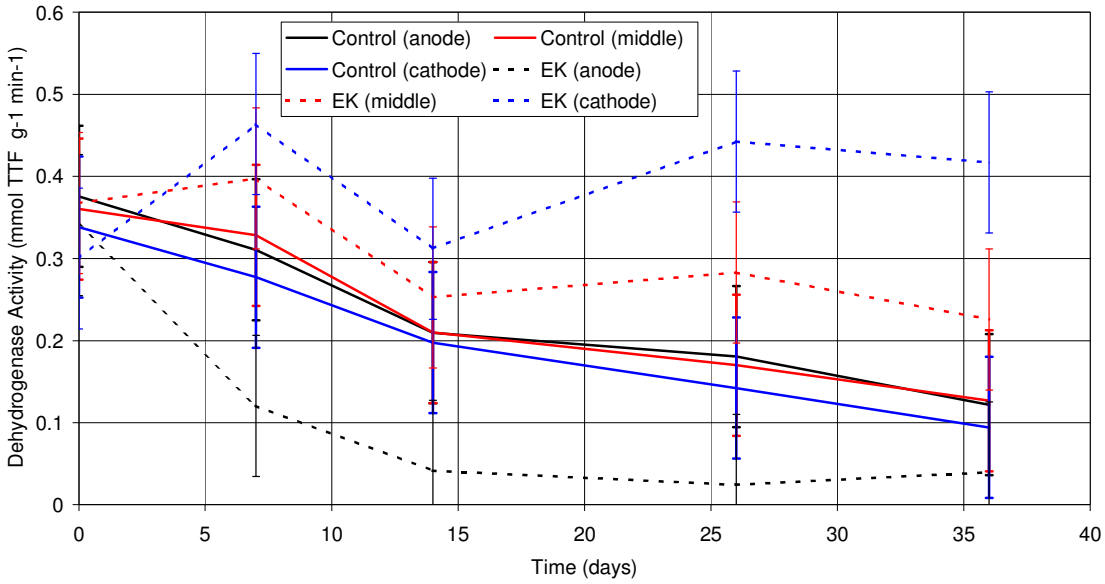

Figure 4-53: Dehydrogenase activity versus time (experiment PI_20)

Experiment PI_22 (Figure 4-54) had similar pH conditions to PI_18 and PI_20 (there was little change, due to the regularly reversed current). Dehydrogenase activities at day 56 in positions at the centre and near the cathode of the electrokinetic specimens were found to be significantly higher ($p=0.019$ and $p<0.001$ respectively) compared to control specimens. There was very little difference between the two sets of specimens at previous time points, however. Despite controlling the physical parameters of the experiment well, there was still a decrease (albeit insignificant) near the anode end of the soil with the electric field. It may be that the small drop in pH noted there at day 56 can account for this.

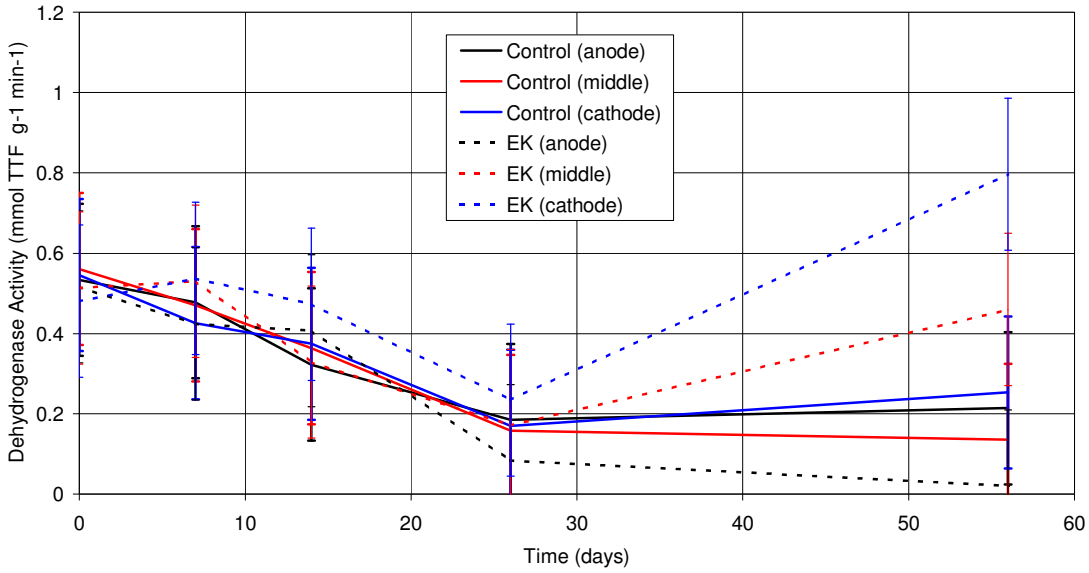

Figure 4-54: Dehydrogenase activity versus time (experiment PI_22)

4.7. Behaviour of PCP under an Electric Field

The work presented in this section describes the movement of PCP under an electric field, subject to a range of different conditions such as varying pH, moisture content and electric field. All results are shown as graphs of PCP recovered (in parts per million (ppm), equivalent to µg PCP per g dry soil) versus distance from the anode end (in centimetres). Many of these experiments were inoculated with PCP-degrading bacteria, and so degradation would have occurred concurrently with movement. In these tests, it was often difficult to distinguish between the two in terms of what caused a decrease in PCP at any one point, but it has been possible to draw general conclusions based on knowledge of what happened in uninoculated experiments.

This section is subdivided into different types of experiment, namely constant voltage experiments, constant current experiments, pulsed or low current experiments, the effect of pH control at both electrodes, and the effect of controlling both pH and moisture

content. The behaviour of the initially negatively charged PCP was found to be generally similar over many of these tests, with movement towards the anode *via* electromigration commonly seen, as was expected. However, the effect of pH change in the soil played an important role in the extraction of PCP. In tests using radiolabelled PCP, the sequential extractions using water and acetonitrile showed important changes to behaviour, with water-extractable PCP (indicative of the bioavailable fraction of the chemical) decreasing substantially in areas of low pH. The presence of electroosmotic flow did not have any apparent effects on the transport of the contaminant on a large scale in any experiments. However, it was maintained for as long as possible by applying cathodic pH control in all experiments, as it would be expected to have at least a small effect on a microscopic scale in helping to desorb and move contaminants.

Whilst movement of PCP was seen within the soil in many experiments, no PCP, either radiolabelled or not, was picked up in the electrolyte fluids, or in the Daramic membranes (using acetonitrile extraction). It was found that a piece of Daramic sorbed up to 80% of PCP when placed in a solution of the chemical of concentration 500μM, and a subsequent extraction of this material with acetonitrile yielded almost 90% of that which had been sorbed. Therefore, it is likely that the majority of PCP remained within the soil during testing, otherwise it would have been picked up in the Daramic. If any PCP was transported to the electrolyte fluids, it was not found. As it would have been expected to be in the anolyte fluid, which was usually dark with organic matter (see section 4.3.6), then the optical techniques used to determine PCP (HPLC and liquid scintillation counting) would have been of little use.

PCP transport results from earlier experiments in the large apparatus (PCP1 to PCP5) are not presented, as they largely repeat data obtained from experiments presented later in

this section. As these tests were only performed with one soil specimen, they were therefore less rigorous than those shown here, which involved six (or more) specimens per experiment.

4.7.1. Constant Voltage Experiments

Only two constant voltage experiments were performed in the smaller apparatus before the decision was made to switch to constant current conditions. One of these tests is presented here, showing movement of PCP towards the anode over a six day period, with a constant level of PCP recovery throughout the test.

Results are presented showing the transport of PCP at the start and end of testing during experiment P_1. Results from intermediate time points are not presented, as they do not provide any extra information. Initially it may be seen that control and electrokinetic specimens were very similar (Figure 4-55), with a very uniform PCP distribution.

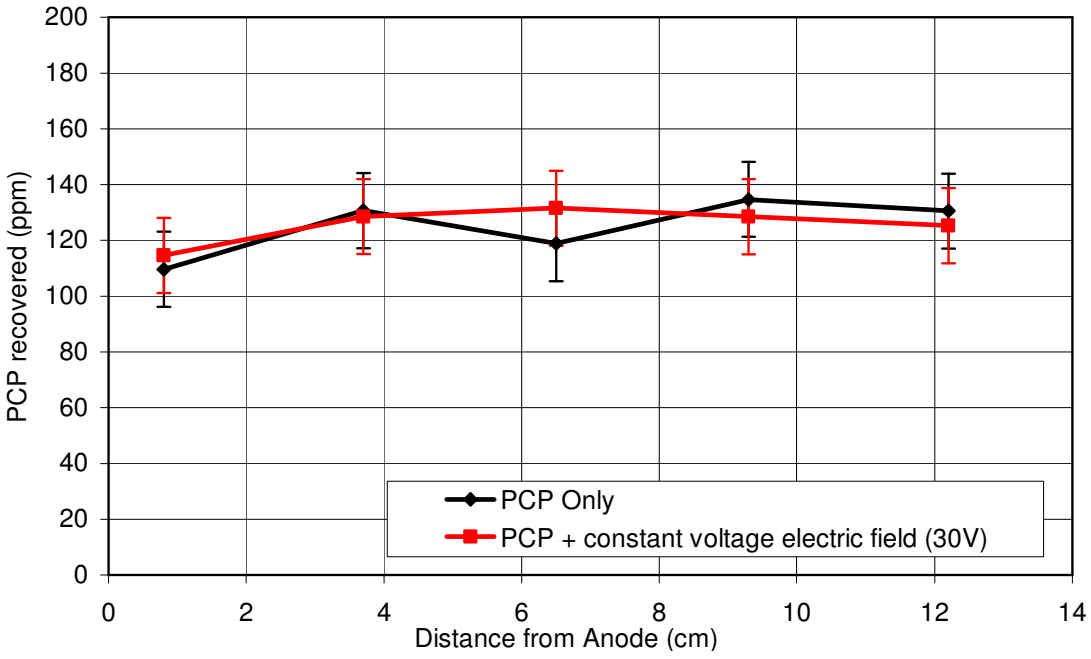

Figure 4-55: PCP distribution at day 0 (experiment P_1)

Following 6 days of electrokinetic processing, the resulting distribution (Figure 4-56) was very different. There was a significant decrease in PCP ($p<0.001$) at the cathode, with corresponding increases at the centre ($p=0.001$) and the anode end ($p<0.001$). This indicated movement of the PCP towards the anode, as would be expected with a negatively charged molecule in an electric field.

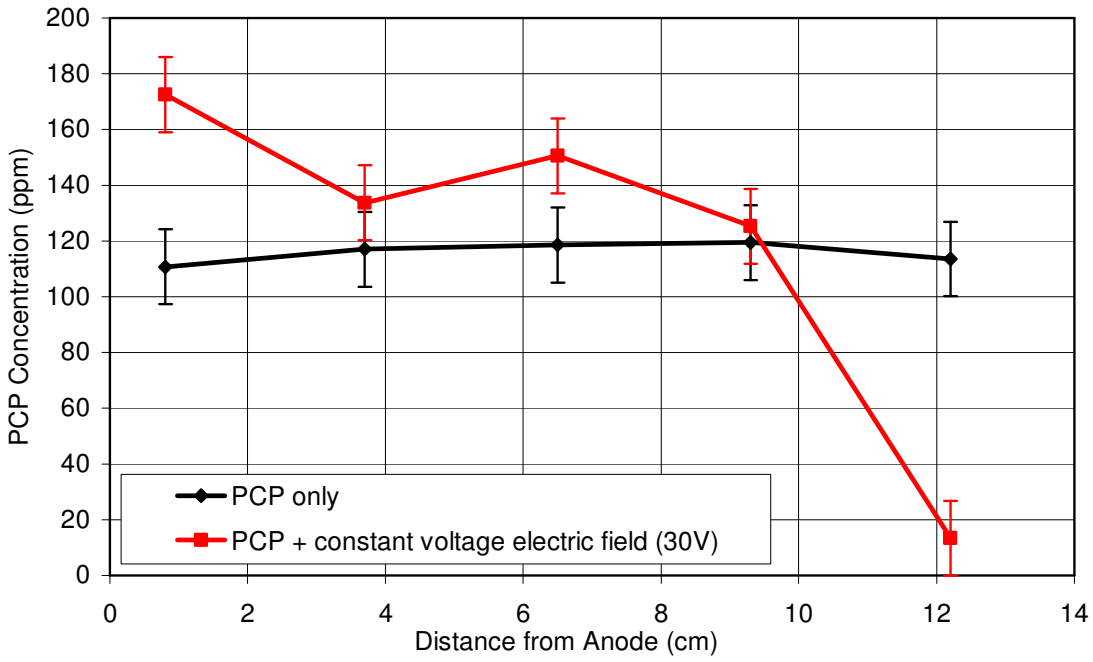

Figure 4-56: PCP distribution at day 6, end of test (experiment P_1)

An apparently similar mode of transport was seen in experiment PI_2 (not presented).

4.7.2. Constant Current Experiments

Experiments with a constant current (10mA) electric field exhibited PCP movement apparently consistent with experiment P_1 (see previous section). The results of four experiments are presented here, showing movement towards the anode as well as a large decrease in water-extractable radiolabelled PCP near this electrode (for the latter three experiments). Degradation of PCP was seen in all experiments except PI_13u, which was

not inoculated, but showed movement similar to that seen in experiment P_1. Despite the complication of the results by the combination of degradation and transport seen in several of these tests, the apparent motion is very similar to that seen in the constant voltage experiments shown earlier.

In experiment PI_3, the distribution of (non-radiolabelled) PCP across the specimens was initially uniform (Figure 4-57). After 25 days of applying the electric field, however, PCP concentration had decreased at the cathode (Figure 4-58), and there was a corresponding increase towards the anode end. Highly significant differences between specimens with and without an electric field were seen in positions 1, 2, 4 and 5 by the end of this experiment ($p \leq 0.003$). It can be seen that there was a substantial decrease in recoverable PCP over this test, most likely to be due to degradation of the PCP. However, it is difficult to decouple the effects of degradation and of movement. For example, at the anode end of the soil the degradation would have been hindered by the low pH. This may partially explain the peak seen towards the anode, although it is expected that PCP transport was the major cause of this. Control of cathodic pH meant that soil conditions were more comparable with and without an electric field at the cathode end of the samples, and the significant drop seen with a field at this point is therefore more likely to be due to movement, based on previous experimental results. It is possible that the electric field had improved the bioavailability and thereby enhanced degradation at this location.

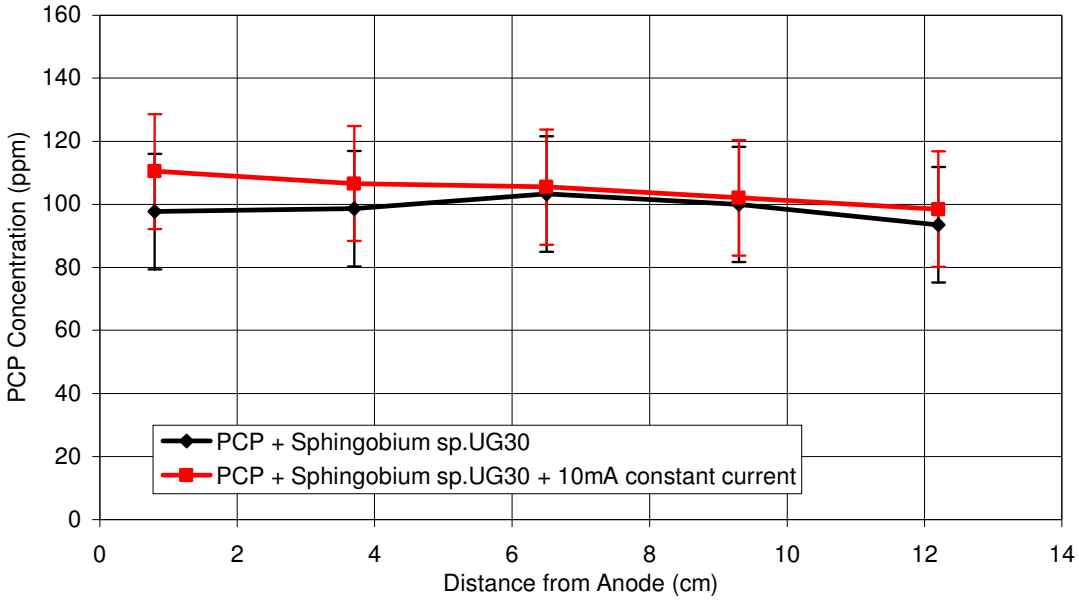

Figure 4-57: PCP distribution - day 0 (experiment PI_3)

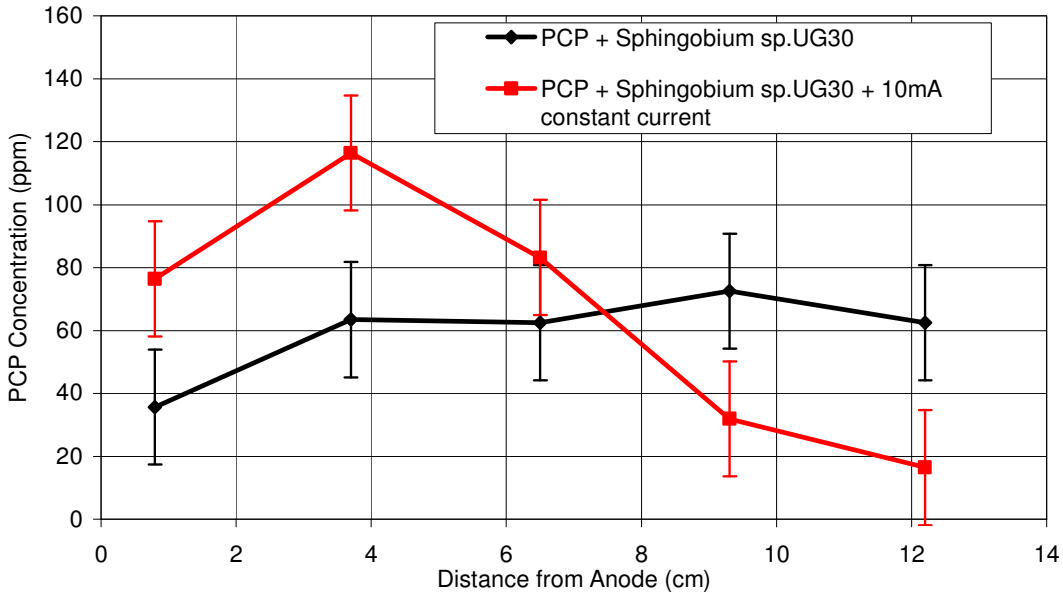

Figure 4-58: PCP distribution - day 25 (experiment PI_3)

From Figure 4-59 onwards, the graphs in this section are of the same format. Four lines are presented, two of which show the water-extractable PCP recovery (for control and electrokinetic specimens) whilst the remaining pair show total recovery (the sum of

water- and acetonitrile-extractable PCP, plus recovery from combustion in experiments PI_13u and PI_13i).

The spatial distribution of PCP across specimens in experiment PI_13u (an experiment without inoculated degrading bacteria) has been shown to be strongly affected by the constant current electric field. This experiment (Figure 4-59, Figure 4-60, Figure 4-61, Figure 4-62 and Figure 4-63) showed the progression of PCP towards the anode, and also how the proportion of water-extractable PCP decreased substantially in the same direction. By the end of testing, the water extractable fraction next to the anode accounted for only approximately 12% of the total PCP present, compared to over 70% initially. After 36 days, the water-extractable fraction was very highly significantly lower in positions 1 and 2 (due to acidification) and 5 (due to movement) with an applied electric field than without, whereas total PCP extract was only significantly lower at position 5 with the electric field (due to movement).

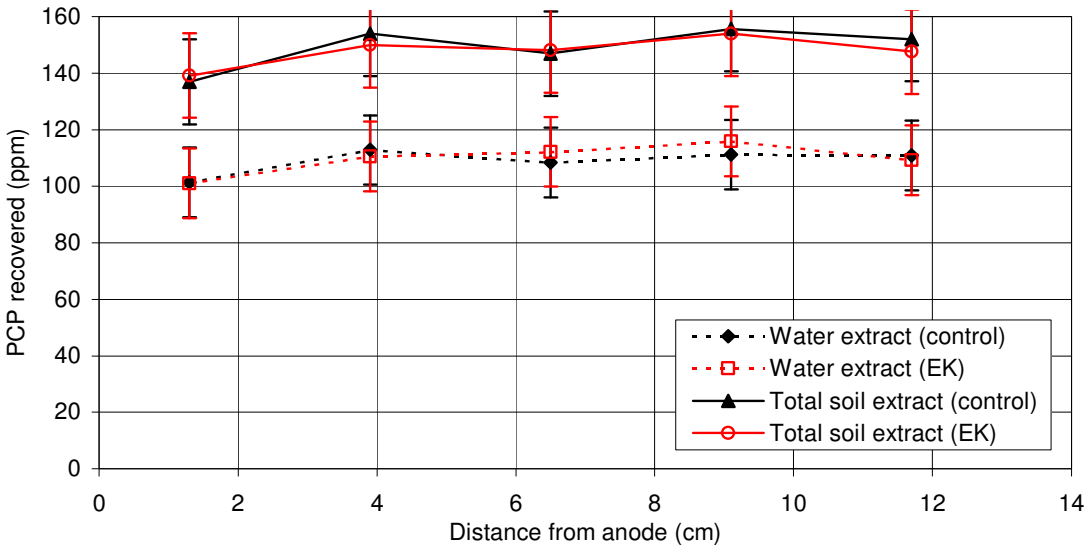

Figure 4-59: Spatial distribution of PCP - day 0 (experiment PI_13u)

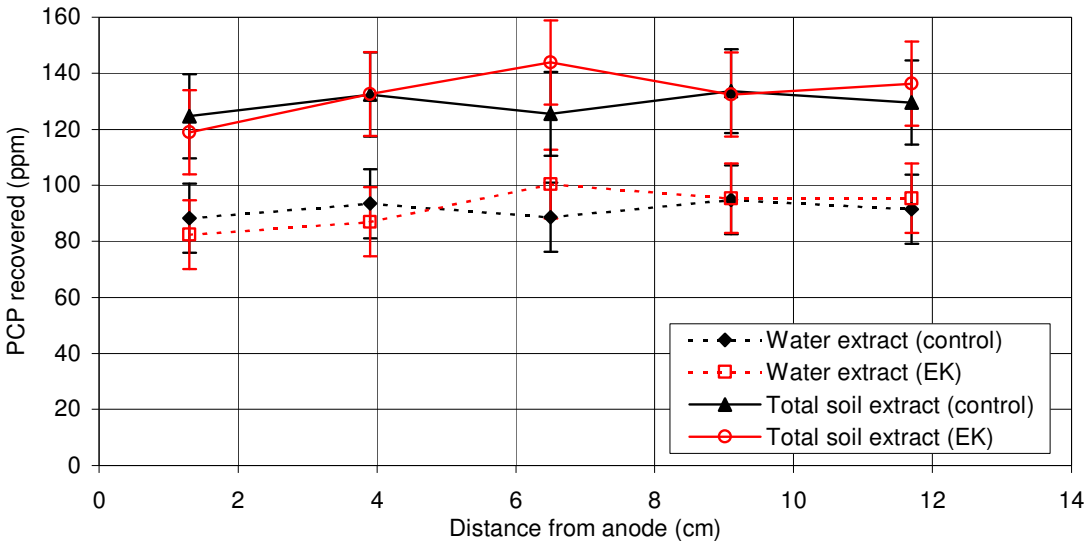

Figure 4-60: Spatial distribution of PCP - day 7 (experiment PI_13u)

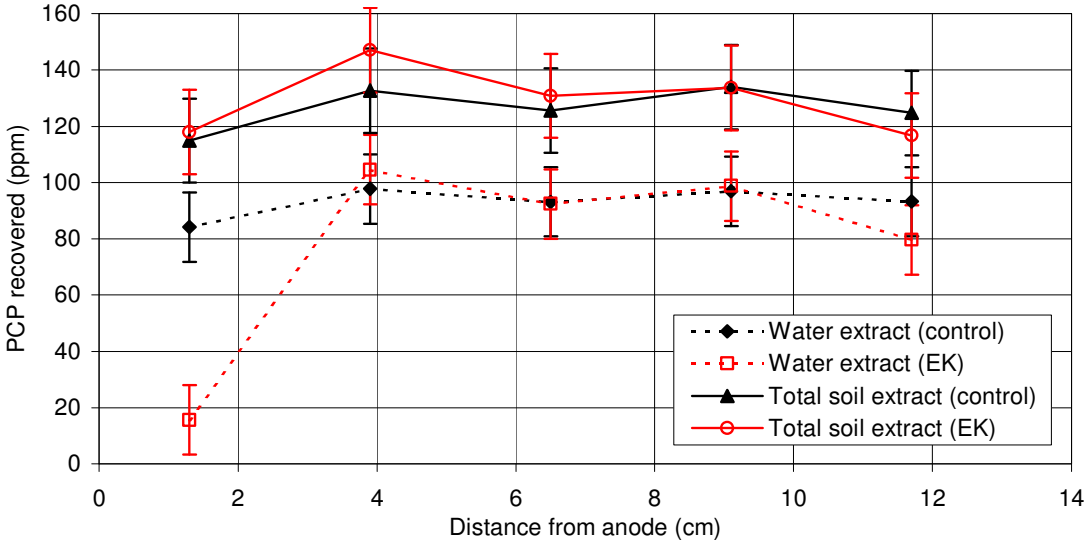

Figure 4-61: Spatial distribution of PCP - day 14 (experiment PI_13u)

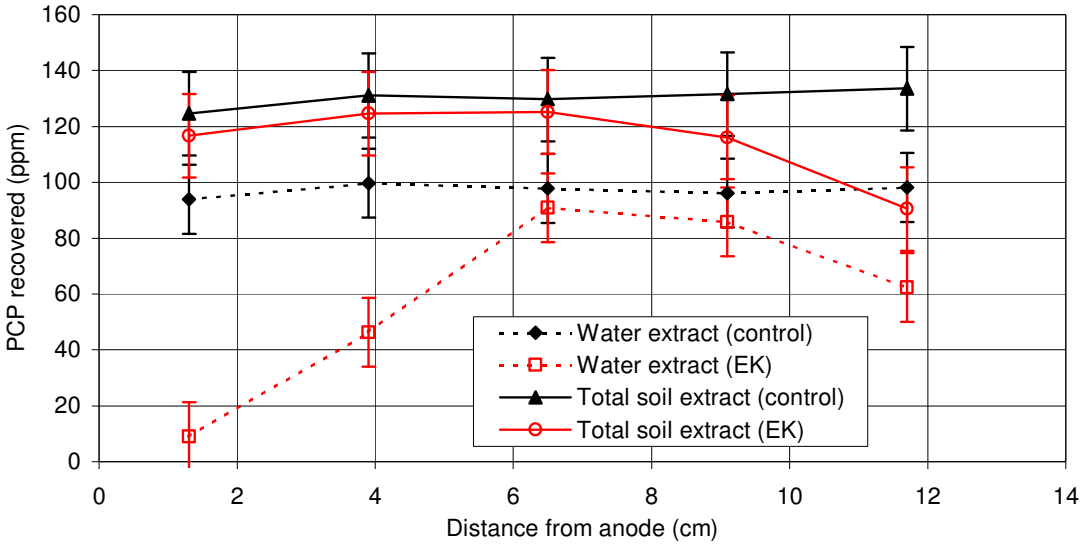

Figure 4-62: Spatial distribution of PCP - day 26 (experiment PI_13u)

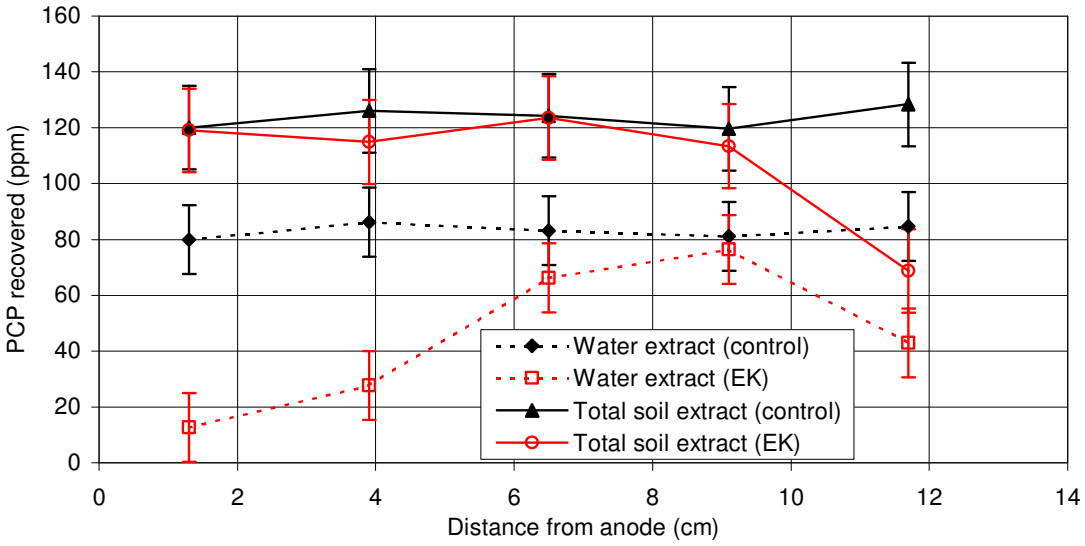

Figure 4-63: Spatial distribution of PCP - day 36 (experiment PI_13u)

In experiment PI_13i, the effect of inoculated *Sphingobium* sp. UG30 can be seen. Both water and total soil PCP extracts were initially higher on average in electrokinetic specimens (Figure 4-64). After 36 days (Figure 4-65), the PCP had once more shifted substantially towards the anode, with the water extractable fraction decreasing in the same direction as seen previously. Lower levels of PCP remained throughout the

specimens compared to experiment PI_13u, due to bacterial degradation of the contaminant. Total PCP levels at the anode (in the electrokinetic specimens) after 36 days are significantly higher ($p<0.001$) and significantly lower ($p=0.001$) at the cathode end, showing clearly the redistribution of PCP. The water-extractable fraction is significantly lower with the applied field ($p<0.05$) everywhere except the central sampling position. A combination of degradation and PCP transport would have occurred in this test, and the possible explanations for high recoveries at the anode, low at the cathode for experiment PI_3 apply here also.

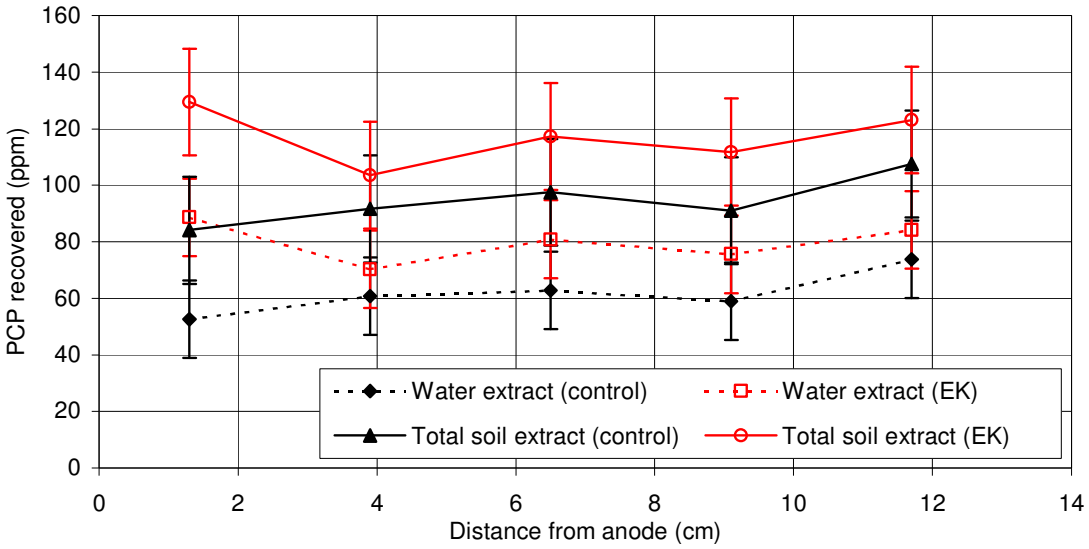

Figure 4-64: Spatial PCP distribution - day 0 (experiment PI_13i)

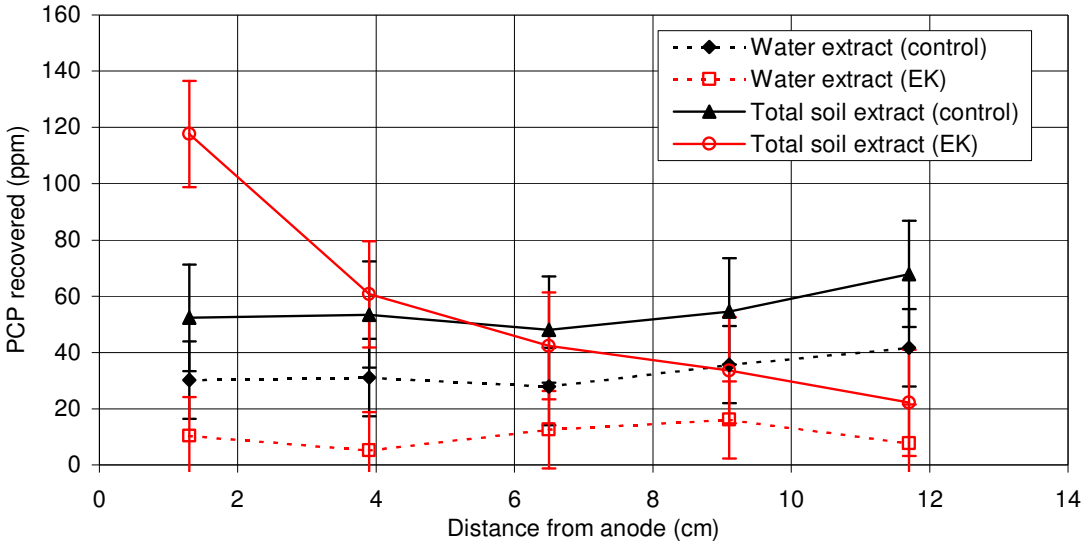

Figure 4-65: Spatial distribution of PCP - day 36 (experiment PI_13i)

The following charts (Figure 4-66 and Figure 4-67) describe the difference in PCP distribution across the specimens in experiment PI_16 (at day 0 and day 36). Results from this experiment are in good agreement with those from experiment PI_13i. There were obvious differences between specimens with and without the electric field both in terms of water and total extractions. At both ends, there were very highly significant ($p<0.001$) differences at day 36 (increase in PCP at the anode, decrease at the cathode), with significant differences ($p<0.017$) throughout the rest of the positions. Any differentiation between movement and degradation again cannot be made, but the possibilities discussed with experiment PI_3 would hold for this experiment also.

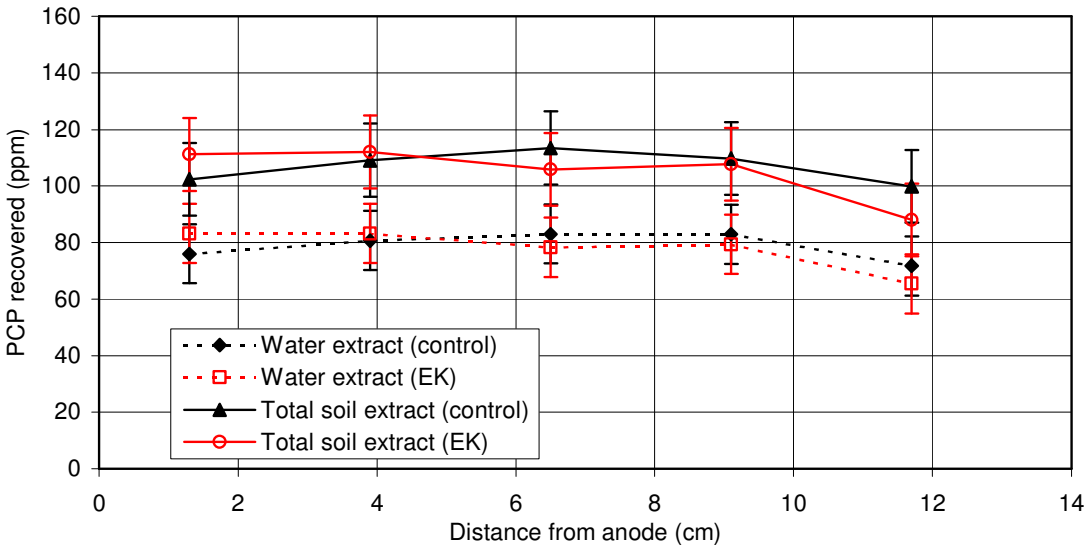

Figure 4-66: Spatial distribution of PCP - day 0 (experiment PI_16)

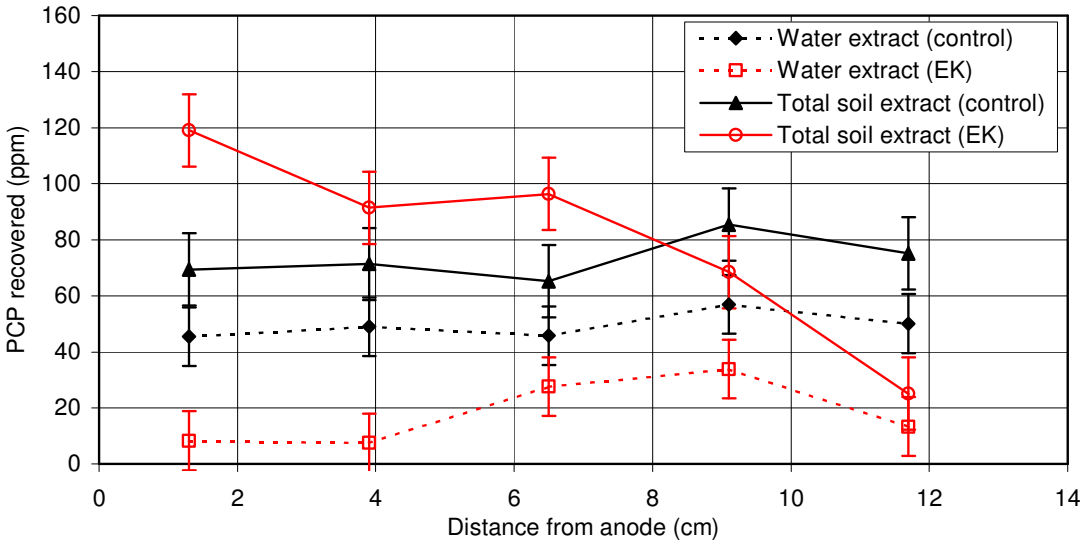

Figure 4-67: Spatial distribution of PCP - day 36 (experiment PI_16)

4.7.3. Pulsed and Low Current Experiments

The outcome of experiments with both pulsed and low constant current regimes were similar, in that there was no distinct movement or differences in degradation across the specimens in either of the experiments detailed here. These results are very distinct from the movement seen in constant voltage and constant current tests discussed earlier.

Experiment PI_14 had a pulsed current applied. There were no obvious trends or significant differences between control and electrokinetic specimens either at the start or the end of the test, in terms of PCP distribution across the specimens (Figure 4-68 and Figure 4-69). Results from experiment PI_5 back up those from this test (results not presented). However, there is a degree of initial variability in this test that was not expected based on the success of other experiments' contamination. At the central point, the average PCP concentration in both electrokinetic and control samples is lower than all the others, although not significantly so due to the relatively large variability in these results. It is difficult to explain why this occurred, although the similarity of electrokinetic and control specimens means that the experiment is still valid. After 36 days testing, the decrease is no longer present at this point, indicating that it might have been only present at those particular sampling locations, or that PCP movement has occurred during the testing period.

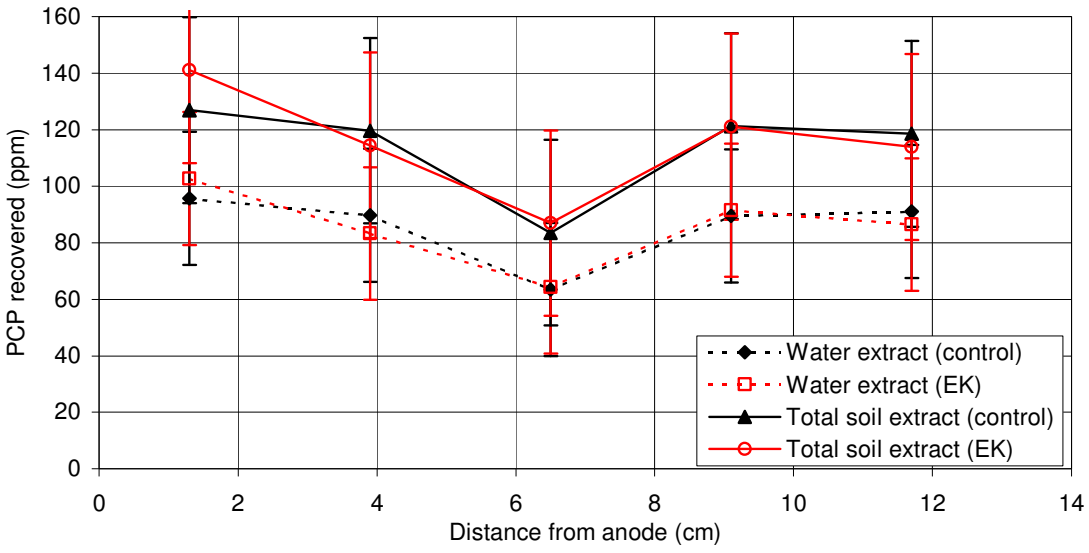

Figure 4-68: Spatial PCP distribution - day 0 (experiment PI_14)

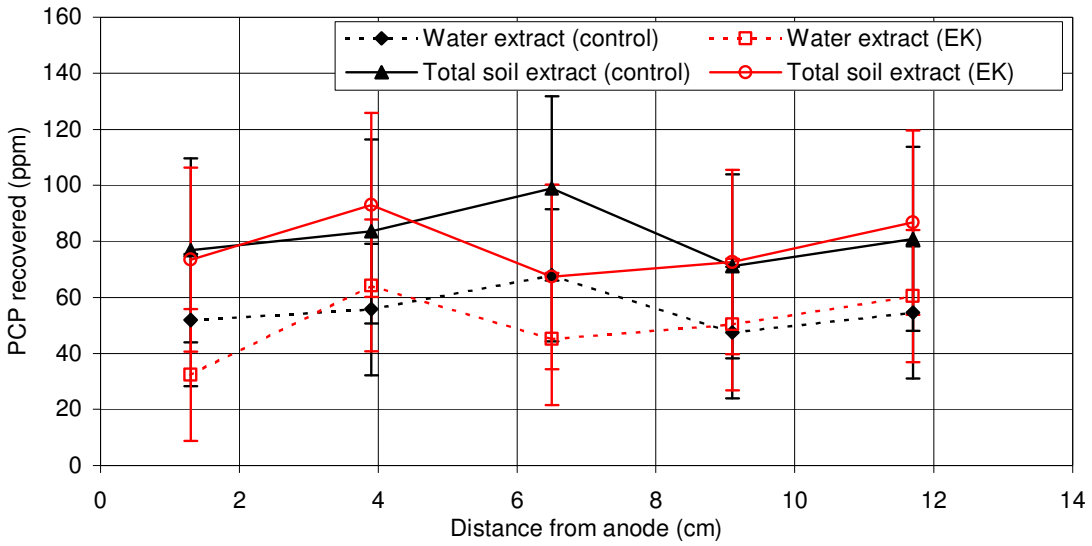

Figure 4-69: Spatial PCP distribution - day 36 (experiment PI_14)

As with pulsed current experiments, little change was seen in PCP distribution over the duration of testing in experiment PI_17 (Figure 4-70 and Figure 4-71), with a constant current of 1mA. No evidence of movement could be seen, and the proportion of PCP in different extractions remained the same.

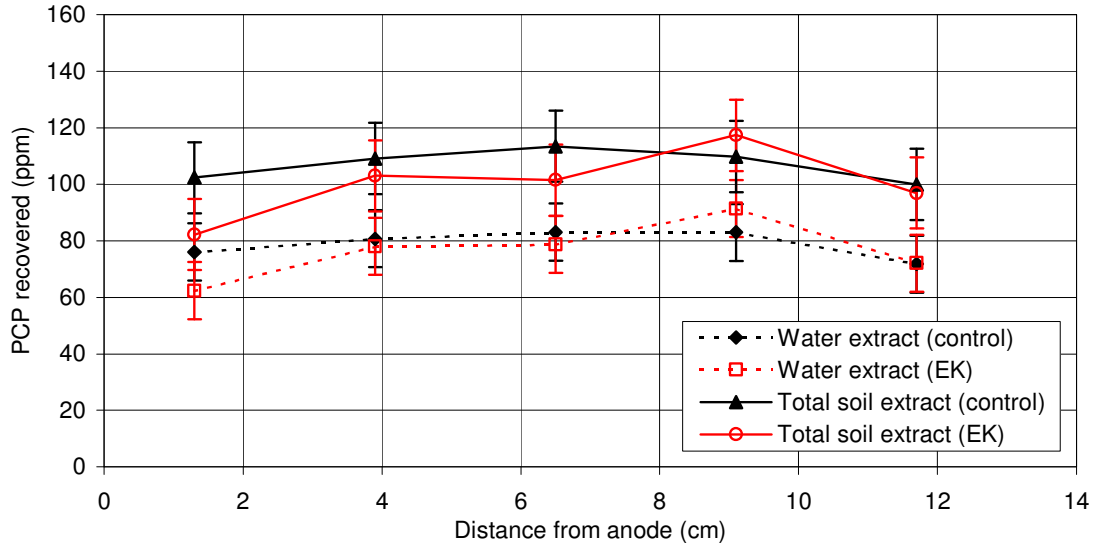

Figure 4-70: Spatial PCP distribution - day 0 (experiment PI_17)

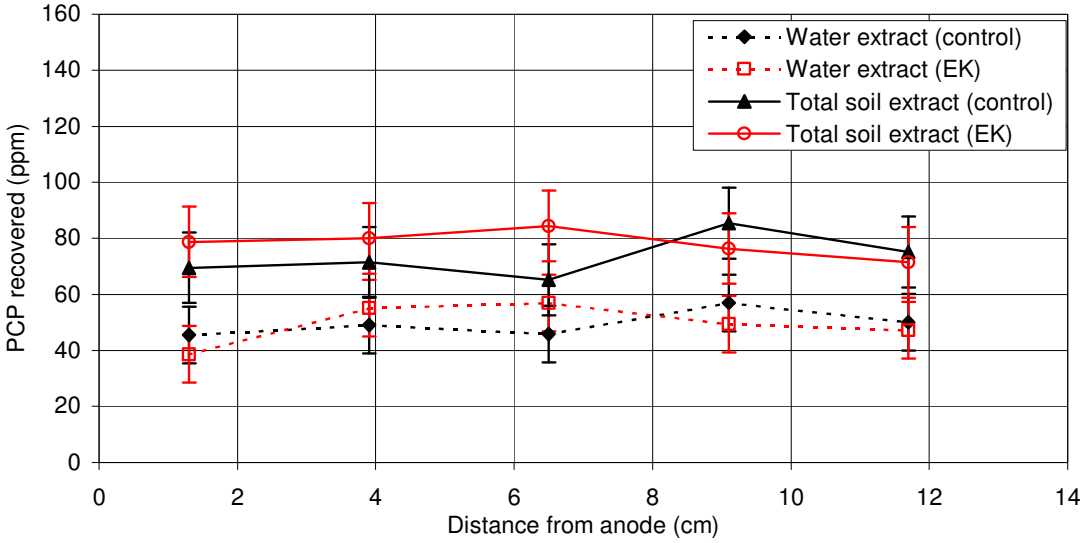

Figure 4-71: Spatial PCP distribution - day 36 (experiment PI_17)

4.7.4. Effect of pH control at both electrodes

The effect of pH control at both electrodes has been investigated in experiments PI_18 and PI_20, with data presented here showing profiles of PCP across soil specimens at different times during testing. These tests were performed in this way in order to combat the pH changes seen in previous experiments with constant current regimes (e.g. PI_16), in which acid build-up at the anode led to a severe reduction in water-extractable ('bioavailable') PCP. The behaviour of PCP in these tests was significantly different from that seen in these other experiments – no discernible change in the ratio of water- to acetonitrile-extractable PCP was observed due to the pH control. In the case of PI_18, where degradation was allowed for two months prior to the electric field being applied, no obvious movement was seen at any point in the experiment, whereas in PI_20 large decreases in PCP were initiated at the anode end of the soil. It will be shown later that this decrease was unlikely to be due to degradation, and therefore removal through transport was likely. Control of the pH was thought to be an important factor in the

effects seen, which led to the saturation of the samples, and possibly flushing out of the PCP. The lack of transport seen in PI_18 was thought to be because a large proportion of the water-extractable PCP had been degraded prior to the field being applied, and so, whilst more PCP may become available over time the amount that might easily be moved around was reduced.

The initial PCP distribution in experiment PI_18 (as shown in Figure 4-72) was less uniform than has been seen in other experiments. At later time points however there was greater uniformity across the specimens - although there was a 10mA current applied continuously during this test, there was little or no evidence of PCP movement in either direction.

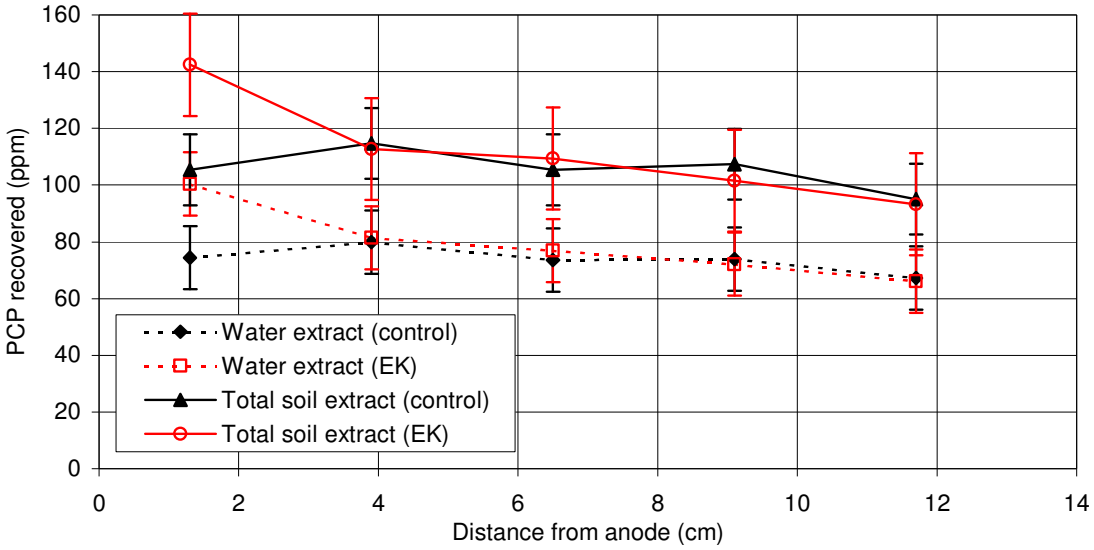

Figure 4-72: Spatial PCP distribution - day 0 (experiment PI_18)

The lack of observed PCP transport after 95 days (Figure 4-73) may be due to the substantial reduction in PCP concentration over the initial 66-day period prior to applying the electric field through bacterial degradation. The water-extractable ('bioavailable') fraction would have been preferentially removed due to its accessability, and whilst this

would have induced a certain amount of desorption of the more inaccessable PCP, the amount that could be moved might have been reduced. Therefore, movement would have been more difficult to detect.

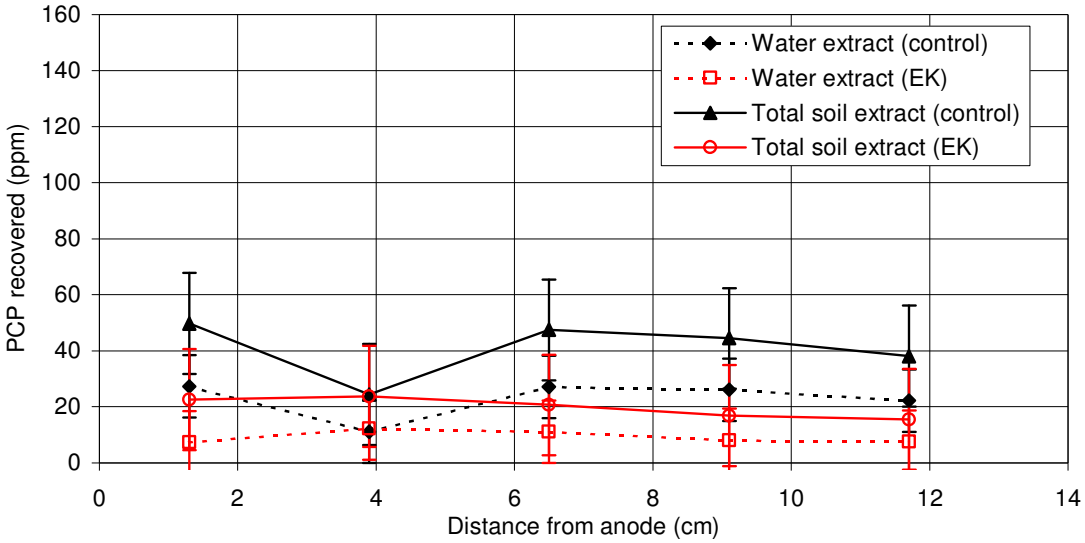

Figure 4-73: Spatial PCP distribution - day 95 (experiment PI_18)

The physical conditions (pH and moisture content) exhibited by experiment PI_18 and PI_20 were very similar (see sections 4.3.2 and 4.3.5). However, the fate of the PCP was quite different, due to the initial degradation period present in PI_18. In PI_20, there was no loss of PCP prior to application of the field, and so it is possible to see in more detail what happened to the PCP under the field. The PCP profiles across the samples were more or less uniform at the start and end of testing. It can also be seen (Figure 4-74, Figure 4-75, Figure 4-76 and Figure 4-77) that there was little increase in the proportion of acetonitrile-extractable PCP, with no apparent variations in time or space. Over the experiment, however, it can be seen that there was a very substantial decrease in PCP recovered, first at the anode end with the effect gradually moving all the way across the specimens. It may be that PCP was being moved from the soil, due to the pH control at the anode (more details are given later). Tests were performed to determine whether any

radiolabelled chemical remained in the electrolyte fluids or Daramic membranes at the end of testing. None could be found, although this may be because the electrolyte fluid was extremely dark due to organic matter extraction, and so would hinder luminescent detection methods. Only approximately 30% of the initial PCP could be found in electrokinetic samples by the end of testing – the proportion recovered as carbon dioxide does not account for this discrepancy.

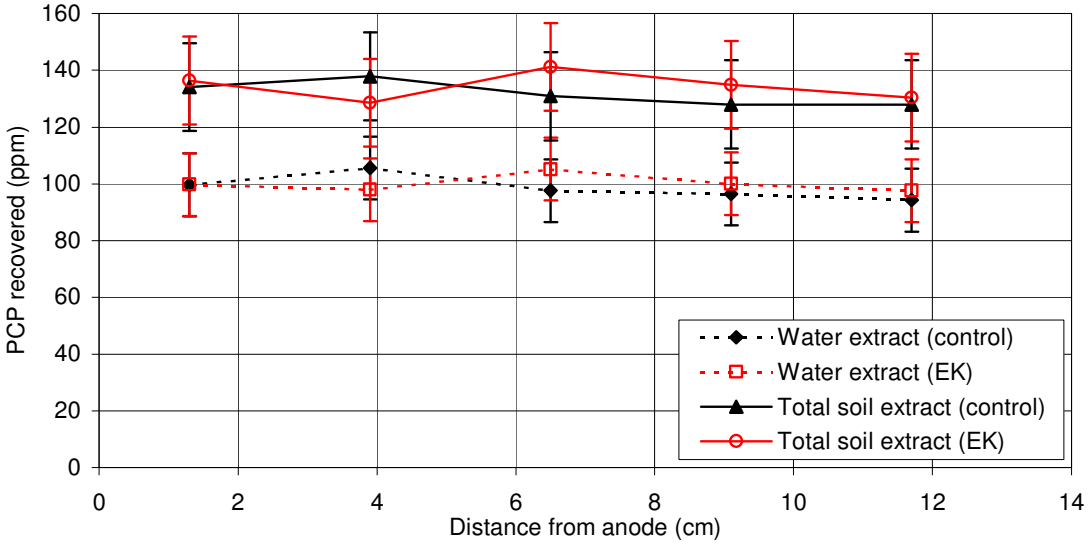

Figure 4-74: Spatial PCP distribution - day 0 (experiment PI_20)

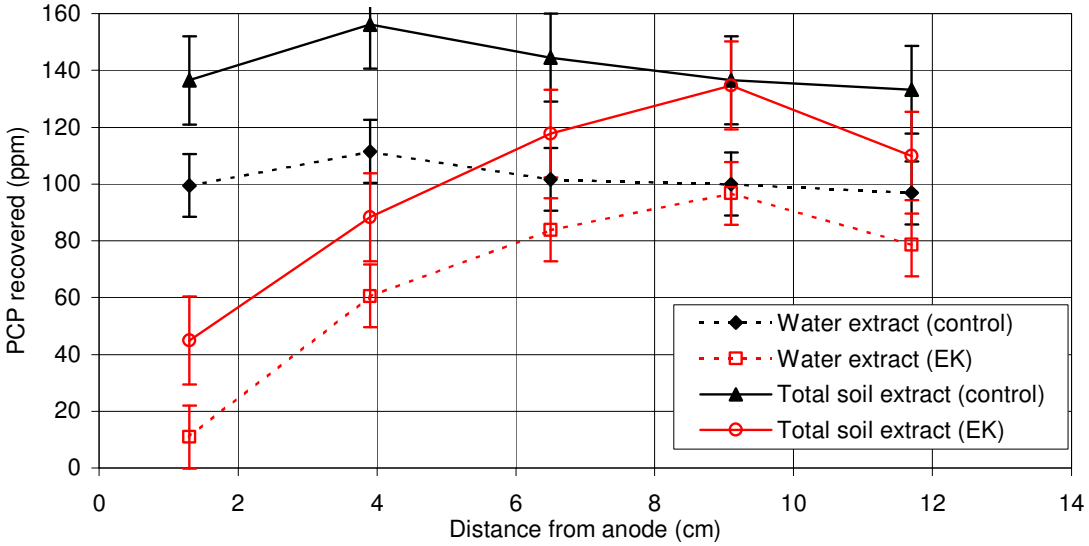

Figure 4-75: Spatial PCP distribution - day 14 (experiment PI_20)

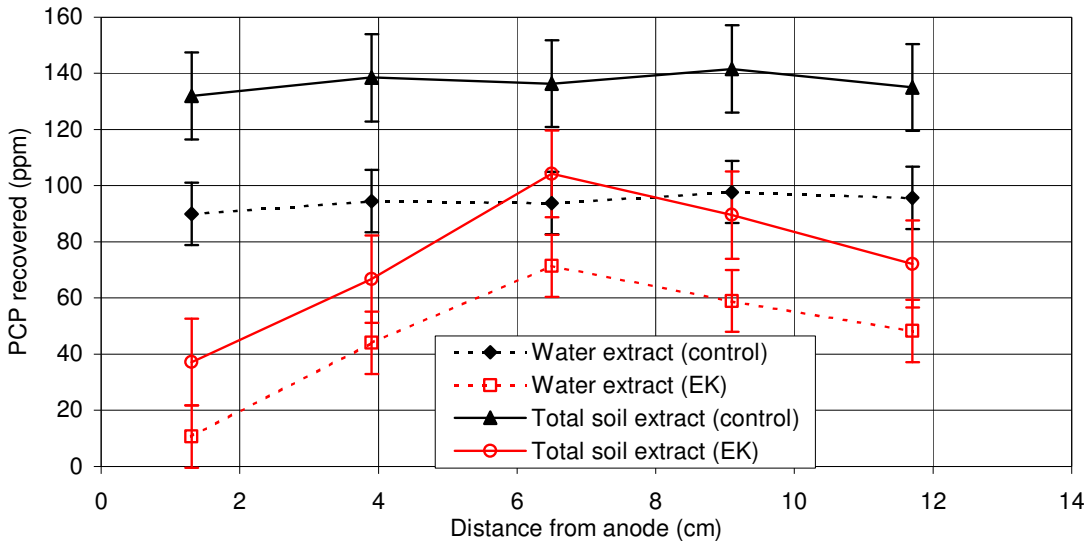

Figure 4-76: Spatial PCP distribution - day 26 (experiment PI_20)

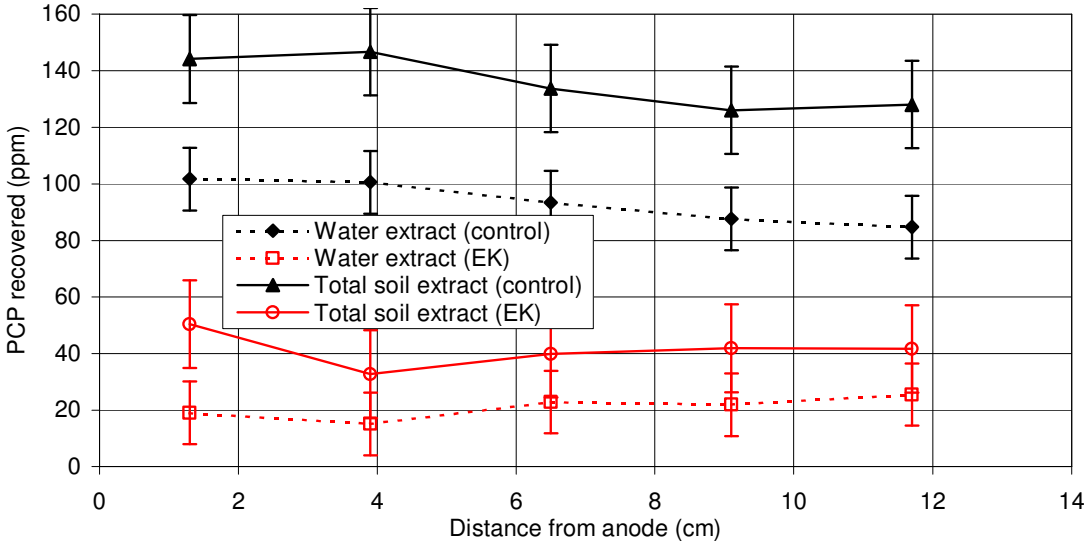

Figure 4-77: Spatial PCP distribution - day 36 (experiment PI_20)

4.7.5. Control of Moisture Content and pH

In this experiment, the term 'anode end' refers to the end of the soil at which the anode was located at the start of the experiment.

The design of experiment PI_22 incorporated methods to control both pH and moisture content, through the application of a reversing electric field and the lack of electrolyte fluids. These methods have already been shown to be very successful. In the graphs presented here, it can be seen that there was no change in the proportion of water-extractable PCP over the experiment in either electrokinetic specimens or controls. Also, there was little or no movement of the PCP observed anywhere, largely due to the reversing field in the electrokinetic specimens. However, a great deal of degradation was observed with very little PCP remaining on average in electrokinetic specimens at the end of testing.

Over the first 26 days of experiment PI_22 it appears that most of the PCP remained where it was (Figure 4-78, Figure 4-79, Figure 4-80, Figure 4-81 and Figure 4-82), although there was a slight decrease at both ends of the electrokinetic samples. After 56 days, however, there was almost no PCP remaining in the central portions of the electrokinetic specimens, and a small amount at either end. At all five time points it is apparent that the PCP was not being moved at all on a large scale, due to the regular field reversal. Also, there was no change in the proportion of water-extractable (bioavailable) PCP recovered at any point, indicating that the pH control method (current reversal), considered in section 4.3.2, was successful.

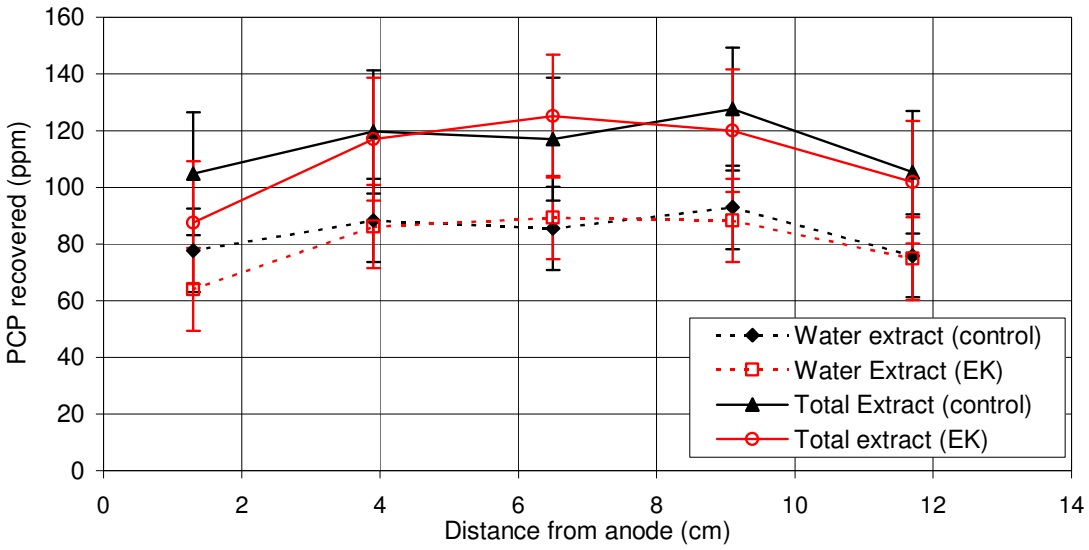

Figure 4-78: Spatial PCP distribution - day 0 (experiment PI_22)

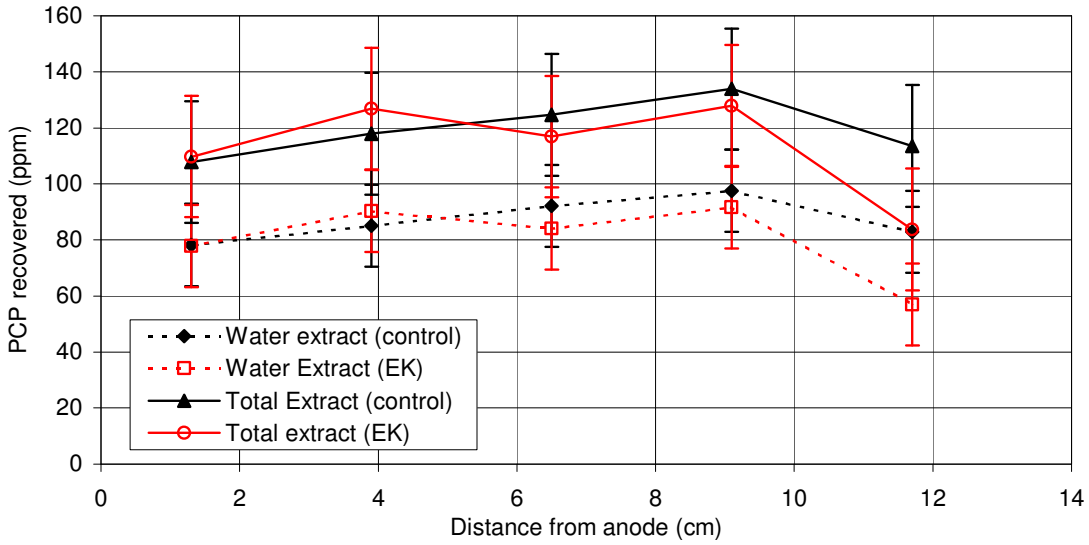

Figure 4-79: Spatial PCP distribution - day 7 (experiment PI_22)

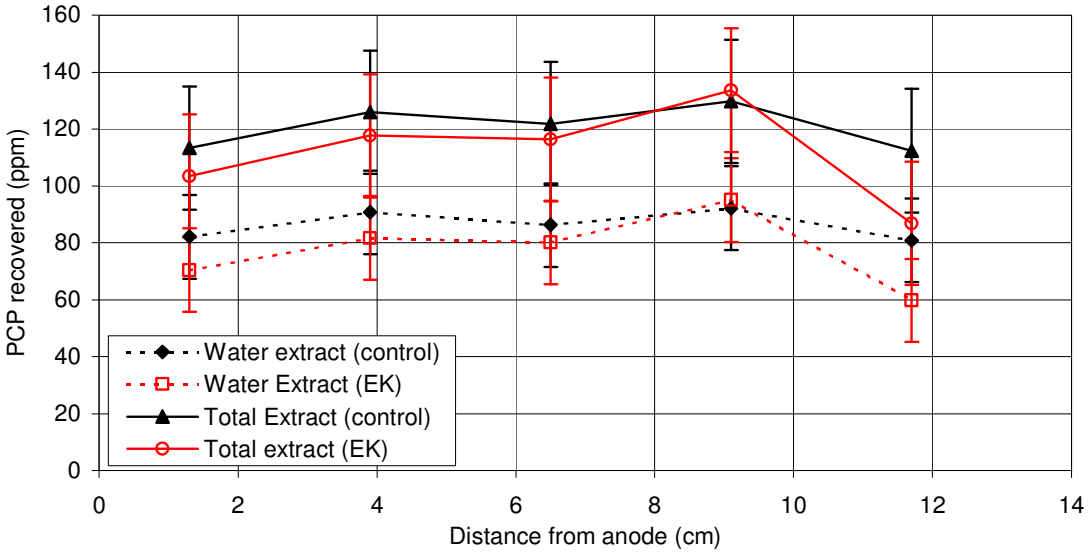

Figure 4-80: Spatial PCP distribution - day 14 (experiment PI_22)

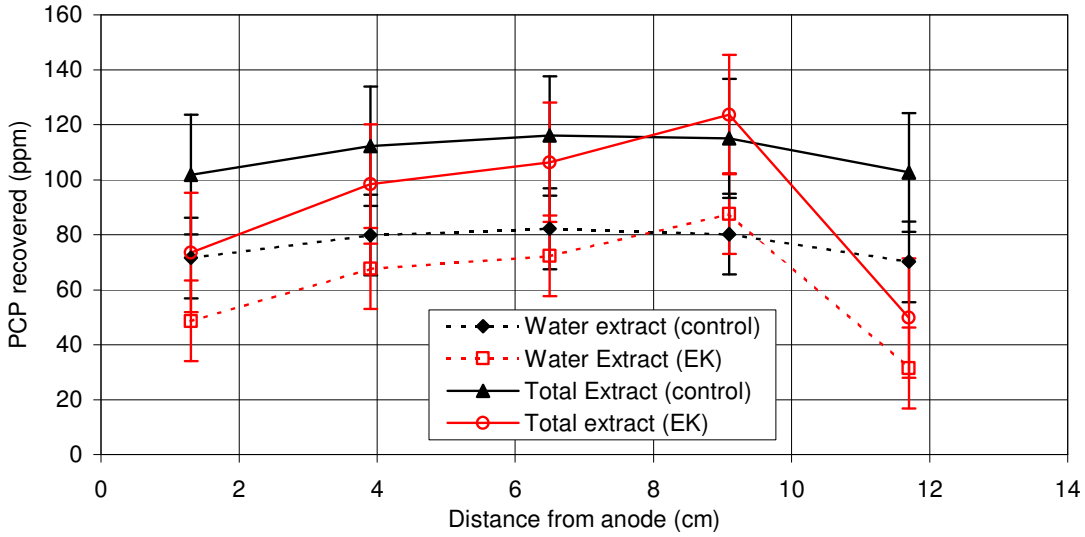

Figure 4-81: Spatial PCP distribution - day 26 (experiment PI_22)

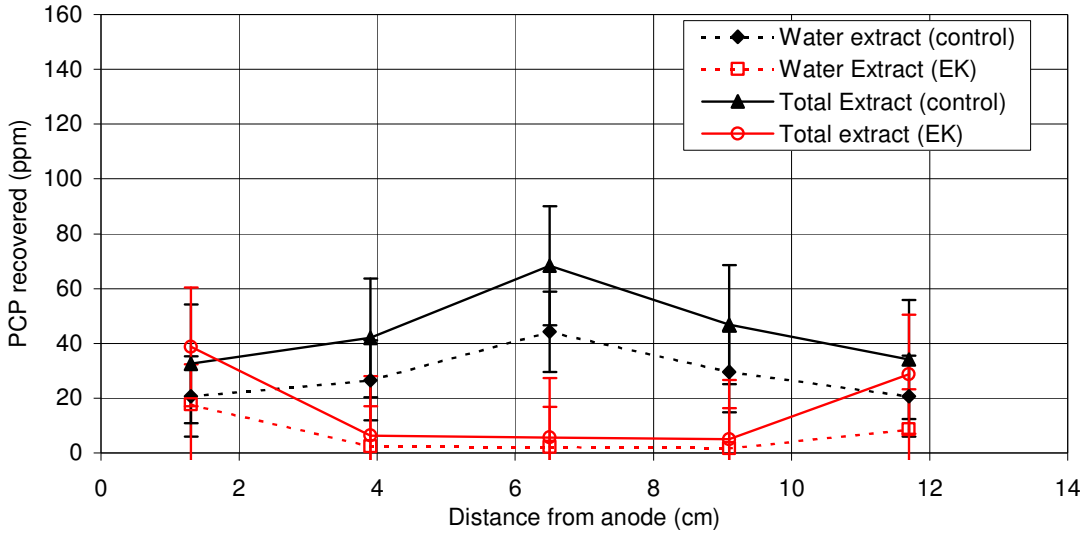

Figure 4-82: Spatial PCP distribution - day 56 (experiment PI_22)

4.8. Effect of electric field on PCP degradation

This section presents data recovered from experiments where degradation of PCP in soil was monitored. The majority of the experiments concerned were subjected to inoculation of degrading bacteria (*Sphingobium* sp. UG30), although a small number of tests were uninoculated. These data are often presented in the form of plots of mass balance versus time, and have been calculated as PCP recovery relative to that found at the start of testing (day 0). The combustion of soil in experiments PI_13u and PI_13i showed that the extraction of radiolabelled PCP with water and acetonitrile only left a small amount remaining in the soil, giving further confidence in the results from other radiolabelled PCP experiments.

As in section 4.7, the experiments have been divided up into several groups, many of which indicate that an electric field would have a detrimental effect on remediation efficiency. However, one experiment, which exerted a greater control over the effect of

the field on soil conditions, can be seen to have reversed this trend. Constant voltage and constant current experiments illustrate the fate of PCP and its interaction with the acidic pH generated at the anode in these cases. Experiments with non-radiolabelled PCP showed an overall decrease in recovered contaminant across the specimen by the middle of testing, although this did not continue. With radiolabelled PCP, the increase in the proportion of acetonitrile-extractable contaminant can be seen as the anode pH dropped, but differences between controls and electrokinetic specimens in total recoveries were small to non-existent. The emission of radiolabelled CO_2 in these tests showed that electrokinetics has a detrimental effect on the degradation of PCP, often with less than half the emission seen in control specimens.

Pulsed and low current experiments (with radiolabelled PCP) showed few differences in degradation with and without an electric field, reinforcing the lack of change seen in terms of movement in section 4.7. The experiments with anodic pH control as well as cathodic illustrate well the possible hindering effects of an electric field, with substantially lower $^{14}CO_2$ production with the field than without. It has not been determined whether these effects were due to increases in moisture content or detrimental effects of the field, or perhaps that the PCP may have been flushed out of the soil. The final experiment presented (experiment PI_22, incorporating both pH and moisture content control) is the only one where an increase in $^{14}CO_2$ production due to the field was found, which corresponded with a significantly lower PCP concentration in the soil at the end of testing. This test was designed to counteract any negative effects found in earlier tests from changes to soil parameters such as moisture content and pH.

4.8.1. Constant Voltage Experiments

The mass balance versus time for experiment P_1 is shown in Figure 4-83. There was no significant difference between the specimens with and without the electric field. There was no significant drop in PCP recovery throughout the duration of this experiment. There were no inoculated degrading bacteria in this test, and so it can be seen that, over a period of 6 days, electrokinetics does not lead to any noticeable decrease in PCP recovery through chemical or other means.

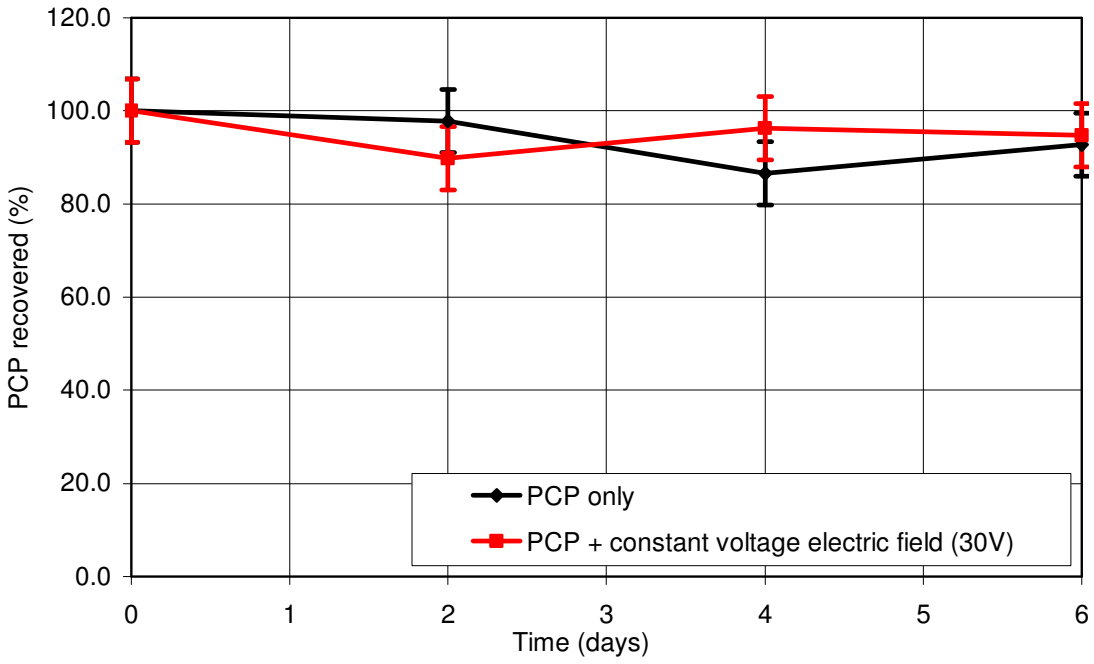

Figure 4-83: Mass balance (experiment P_1)

In contrast, the mass balance measured versus time (Figure 4-84) for experiment PI_2 (which included degrading bacteria) showed large changes at day 6 and day 10. At both these times the PCP recovered from specimens with an electric field was significantly lower ($p<0.001$ at day 6, $p=0.019$ at day 10) than that recovered from controls. The trends evident in both PCP distribution (see section 4.7) and mass balance plots are

similar. Day 6 showed a large decrease in extractable PCP with an electric field (approximately 30%), whilst four days later there was no further decrease in these specimens. Decrease in PCP in control specimens was less marked, but the apparent reduction began at the same time.

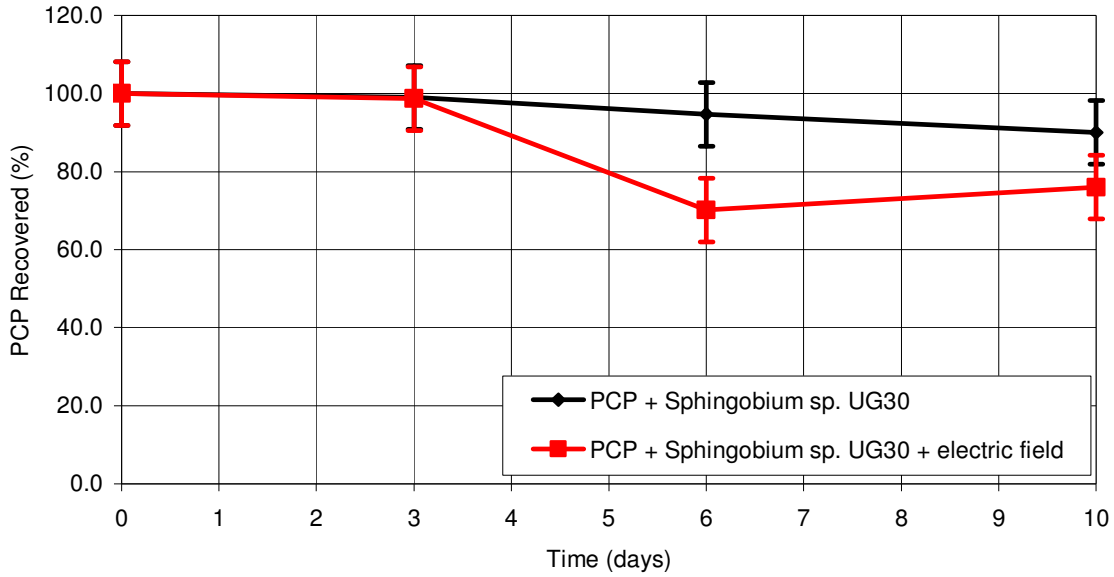

Figure 4-84: Mass balance (experiment PI_2)

4.8.2. Constant Current Experiments

Tests employing a constant current regime are presented in this section. All tests presented incorporated carbon-14 labelled PCP. Important effects here are the increase in the proportion of acetonitrile-extractable PCP (with $P^{14}CP$) and the lower amount of degradation noted with an electric field.

PI_13u is an experiment performed without the inoculation of PCP-degrading bacteria, which allowed identification of any other degradation sources in the system used. Any further radiolabelled PCP remaining in the soil following water and acetonitrile extracts

was measured by combusting the soil (combustion was only performed on soil from time points 0, 14 and 36 days – data at 7 and 26 days were obtained using linear interpolation).

A plot of recovered radiolabelled carbon dioxide is displayed (Figure 4-85), and shows a great deal more PCP mineralisation over the course of the experiment without an electric field rather than with one. However, it should be noted that there was very little PCP mineralised in total (less than 3% in the control specimens). The application of the electric field gave a significant difference between the two sets of specimens from 18 days ($p<0.02$) after the start of the test, becoming very highly significant ($p<0.001$) after 21 days.

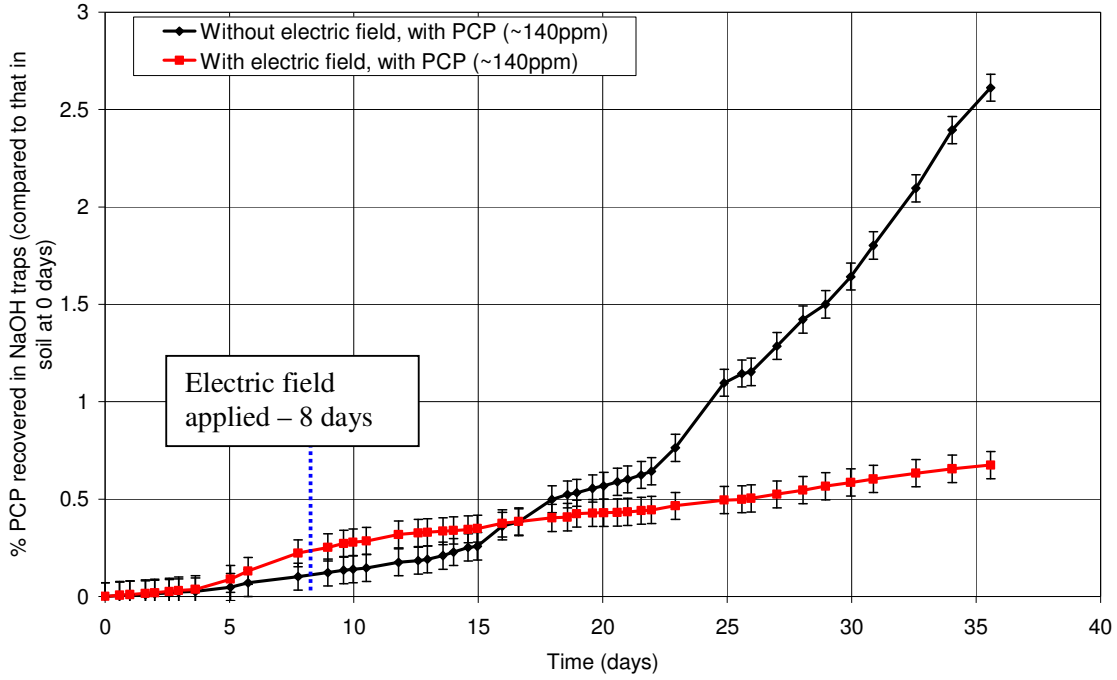

Figure 4-85: Mineralised PCP trapped as CO_2 versus time (experiment PI_13u)

Plots of recovered PCP (total (including carbon dioxide) and from soil) are given in Figure 4-86 for both control and electrokinetic samples. There was no significant difference between recoveries from soil at any point, but at 26 and 36 days there were

significant differences in total recovery between electrokinetic and control specimens ($p<0.028$). The graph shows that total recovery and soil recovery are almost identical in both cases, demonstrating the extremely low amount of mineralised PCP recovered as $^{14}CO_2$.

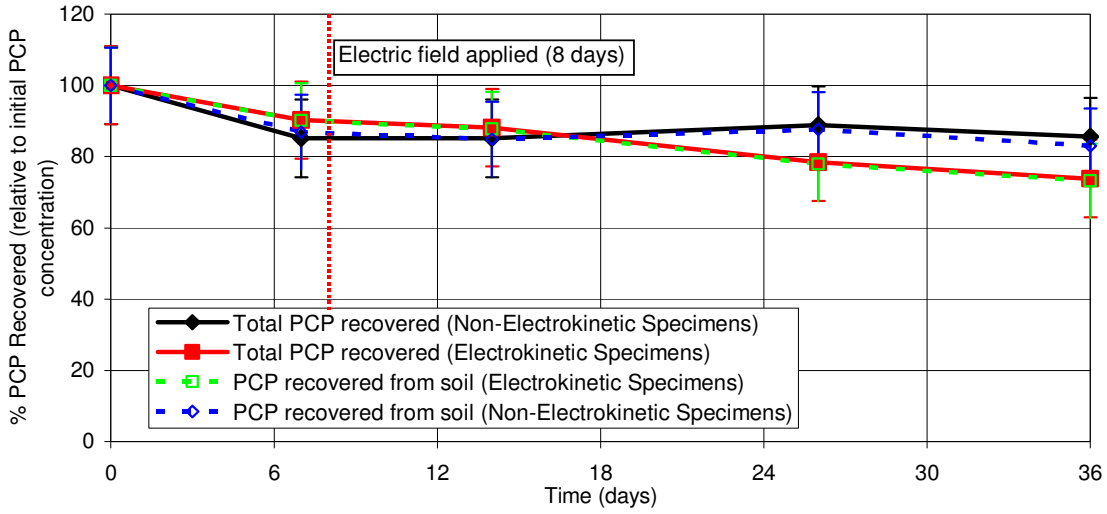

Figure 4-86: Total and soil PCP recoveries versus time (experiment PI_13u)

The graphs of PCP recovered from control and electrokinetic specimens (Figure 4-87 and Figure 4-88 respectively) overlap with Figure 4-86 in terms of the data presented. The latter is included, as it would be difficult to compare the data on the former two plots without it. The format of these two graphs is identical – each show as a shaded area the amount of PCP recovered versus time. The data from water and acetonitrile extracts, combustion of the soil and CO_2 recovery are presented as stacked data, such that the uppermost line represents the sum of all four sources, and is the same as one of the lines on Figure 4-86. The other line on Figure 4-86 is the total soil recovery, which is identical to this previous line minus the CO_2 recovery.

The very small amount of PCP mineralised without the presence of *Sphingobium* sp. UG30 noted on Figure 4-85 can be seen more clearly in Figure 4-87 and Figure 4-88. They also show the proportion of PCP remaining in the soil following water and acetonitrile extractions (determined by combustion) to be small throughout testing (a few percent), indicating that the extraction procedures work well. The electric field induced an increase in the proportion of acetonitrile-extractable PCP over water-extractable PCP, from 75% water extractable at day 0 to approximately 45% at the end of the test. The decrease in recoverable PCP over the experiment is not thought to be due to degradation, as very little radiolabelled carbon dioxide was recovered, whereas it is not due to increasing association with the soil, or it would have been picked up in combustion. The effect is seen both with and without electric field, and so it is not (entirely) due to movement of the contaminant from the soil by electrokinetic phenomena.

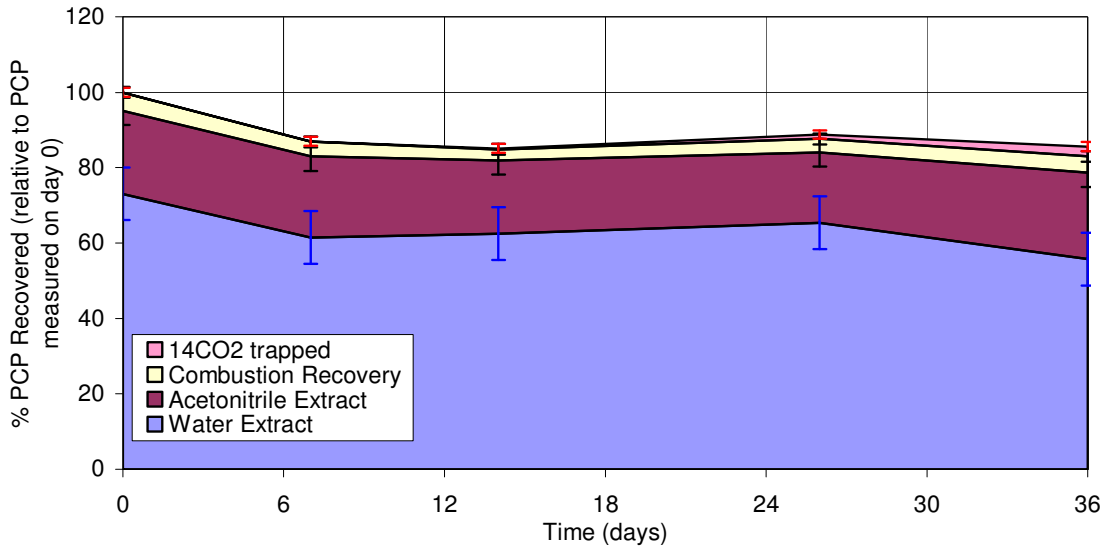

Figure 4-87: PCP recovery vs. time - control specimens (experiment PI_13u)

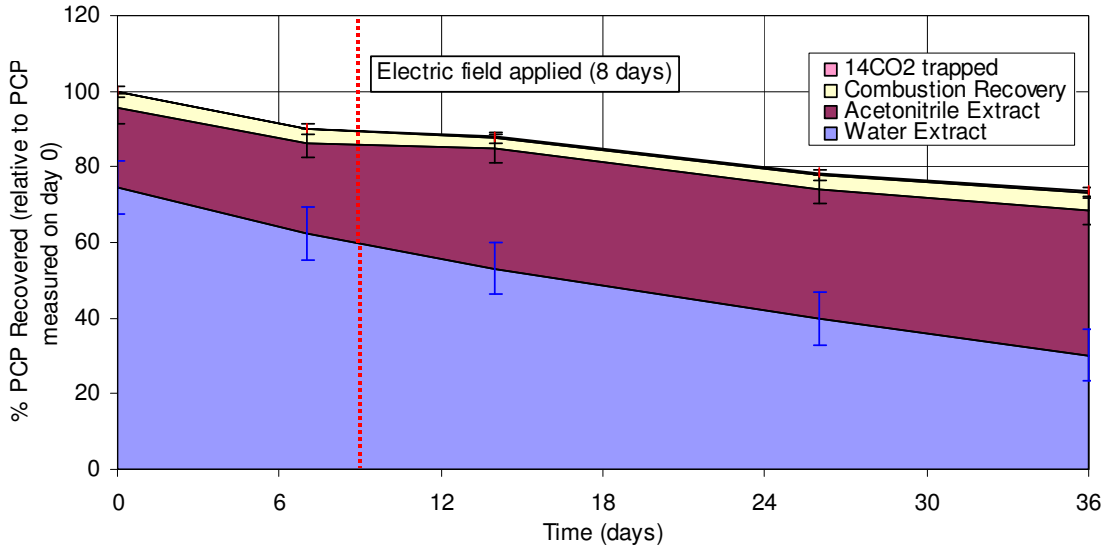

Figure 4-88: PCP recovery vs. time - electrokinetic specimens (experiment PI_13u)

Experiment PI_13i was identical to experiment PI_13u, apart from including the inoculation of *Sphingobium* sp.UG30. Again, combustion of the soil was employed to recover any PCP remaining in the soil following treatment with water and acetonitrile.

Figure 4-89 shows the amount of PCP that was mineralised and trapped as radiolabelled carbon dioxide. Similarities between this graph and the corresponding plot for experiment PI_13u are evident – after approximately 20 days the control specimens were producing labelled CO_2 at a much faster rate than electrokinetic specimens (the difference was significant after 19 days ($p<0.031$) and very highly significant ($p<0.001$) after 23 days). After 36 days, 20% of the PCP in the control specimens had been mineralised and trapped, compared to 10% in electrokinetic specimens.

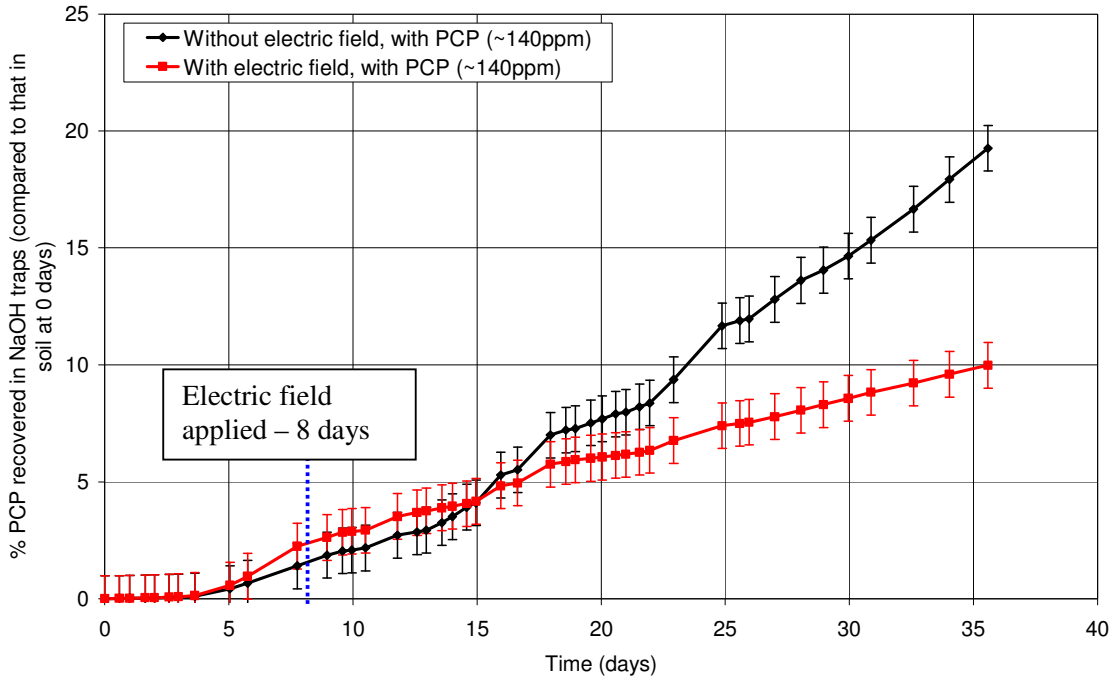

Figure 4-89: Mineralised PCP trapped as CO2 versus time (experiment PI_13i)

Not all PCP was recovered by the latter stages of this experiment, as is shown in the following three graphs (Figure 4-90, Figure 4-91 and Figure 4-92, which use the same format as those used for PI_13u). There were mass balances of approximately 80% and 60% for control and electrokinetic specimens respectively at the end of testing. PCP recovered from soil is not significantly different in control and electrokinetic specimens, but it is with total PCP recovery ($p<0.03$) at days 26 and 36. As in experiments PI_13u, the combustion of soil indicated that water and acetonitrile extractions were efficient, and so it may be that some PCP was being removed from the soil via electrokinetic phenomena. No PCP was recovered from the anolyte fluid, but as suggested previously it may be that either the Daramic membrane sorbed the PCP or the organic matter that darkened this solution prevented the optical detection methods used here from operating correctly. The electric field increased the acetonitrile-extractable portion of the PCP, as seen previously (changing from 30% to approximately 75% of the total).

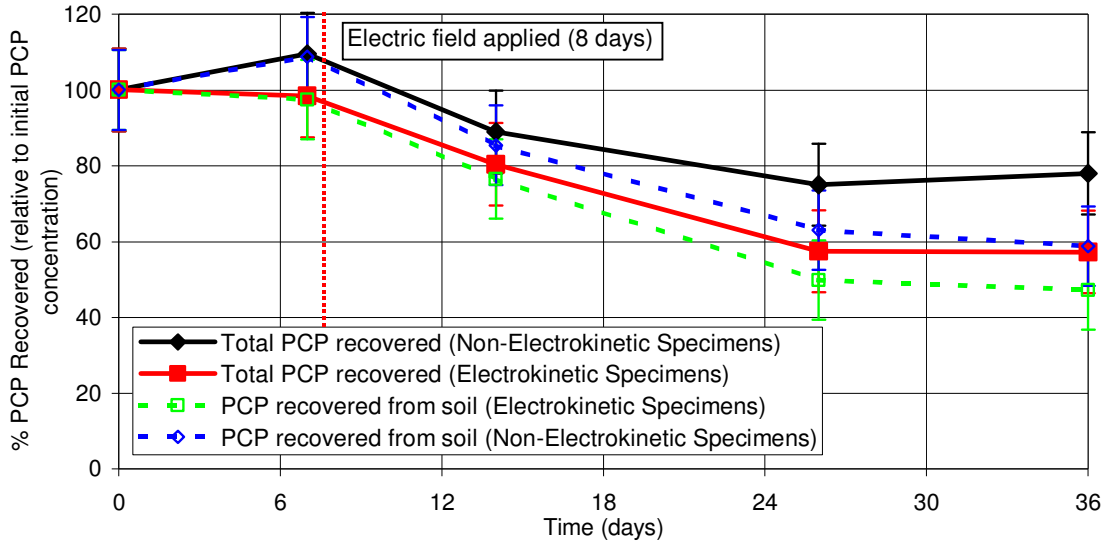

Figure 4-90: Total and soil PCP recoveries vs. time (experiment PI_13i)

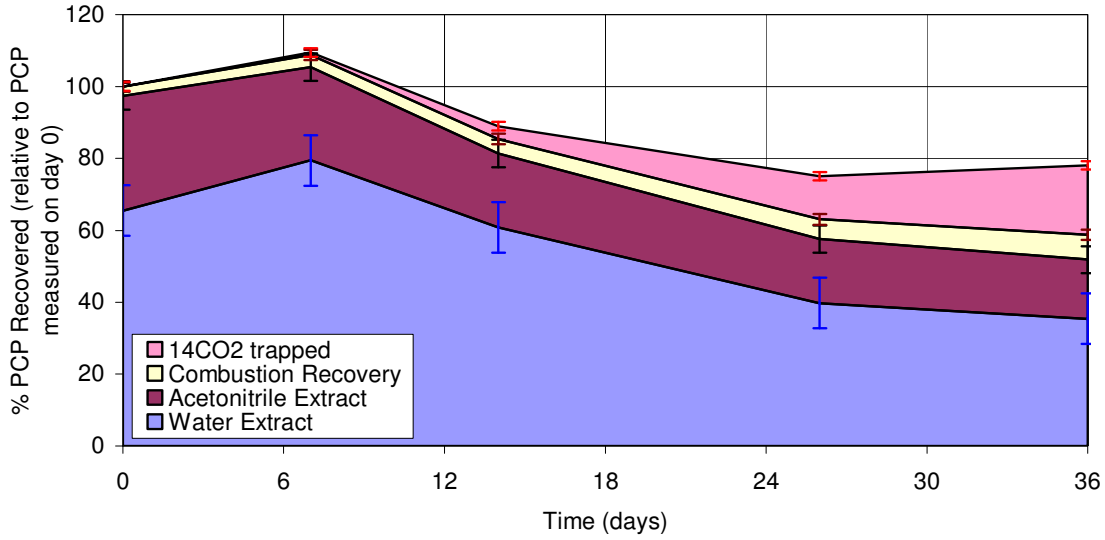

Figure 4-91: PCP recovery vs. time – control specimens (experiment PI_13i)

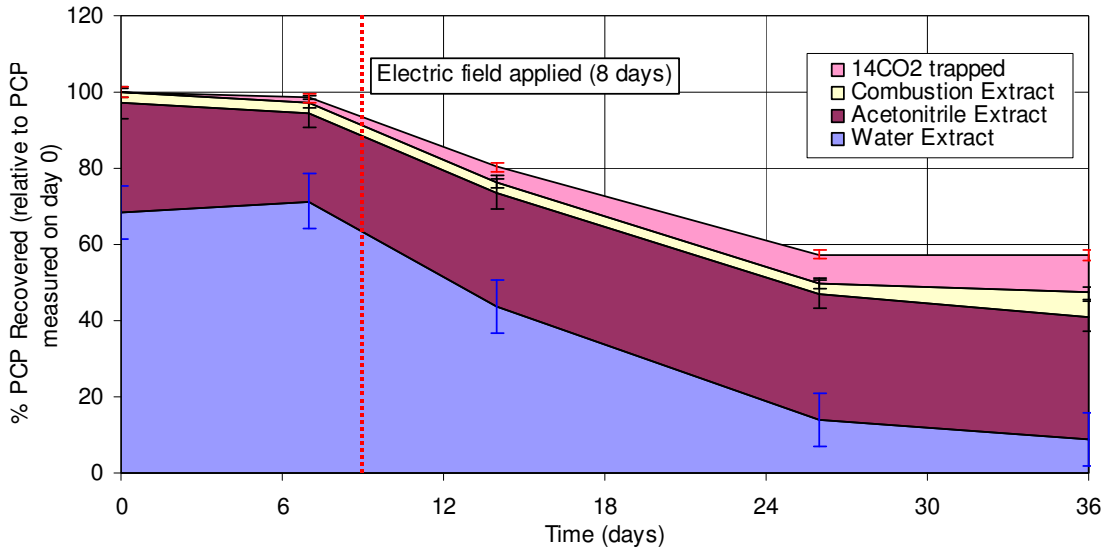

Figure 4-92: PCP recovery vs. time - electrokinetic specimens (experiment PI_13i)

The cumulative graph from experiment PI_16 of radiolabelled carbon dioxide trapped versus time is not presented as it shows similar trends to those presented for experiment PI_13i (Figure 4-89).

Recovery of PCP from the experiment is presented in the following three graphs. In Figure 4-93, the comparison between both total PCP recovery (including carbon dioxide emissions) and PCP recovery from soil only, from controls and electrokinetic cells, is shown. Neither of these can be seen to be significantly different due to the electric field, apart from at day 7. It is not known why a difference was seen at this point.

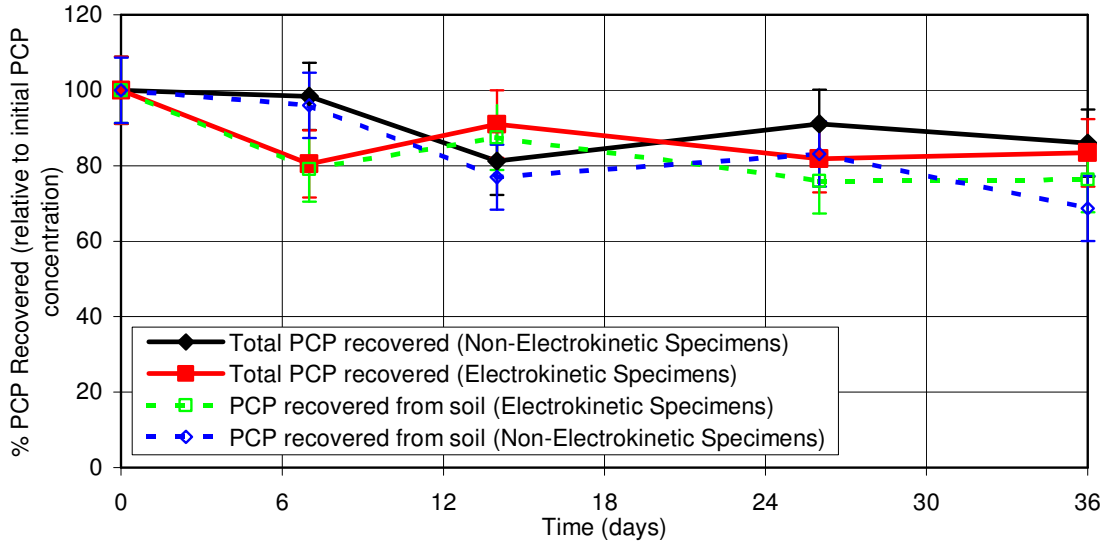

Figure 4-93: Total and soil PCP recoveries vs. time (experiment PI_16)

Figure 4-94 shows the PCP recovery from control specimens in more detail, and this is repeated in Figure 4-95 for electrokinetic specimens. The lower CO_2 output with an electric field can be seen here, as can a very highly significant increase ($p<0.001$) in the amount of acetonitrile-extractable PCP along with a corresponding decrease in that extractable by water. These data back up what has already been seen in experiment PI_13i, and, in terms of physical and chemical changes, PI_13u. There was no discernible difference between the unaccounted-for PCP in control and electrokinetic specimens – the slightly smaller amount of contaminant recovered from non-electrokinetic soil can be offset by the greater amount of PCP recovered as carbon dioxide.

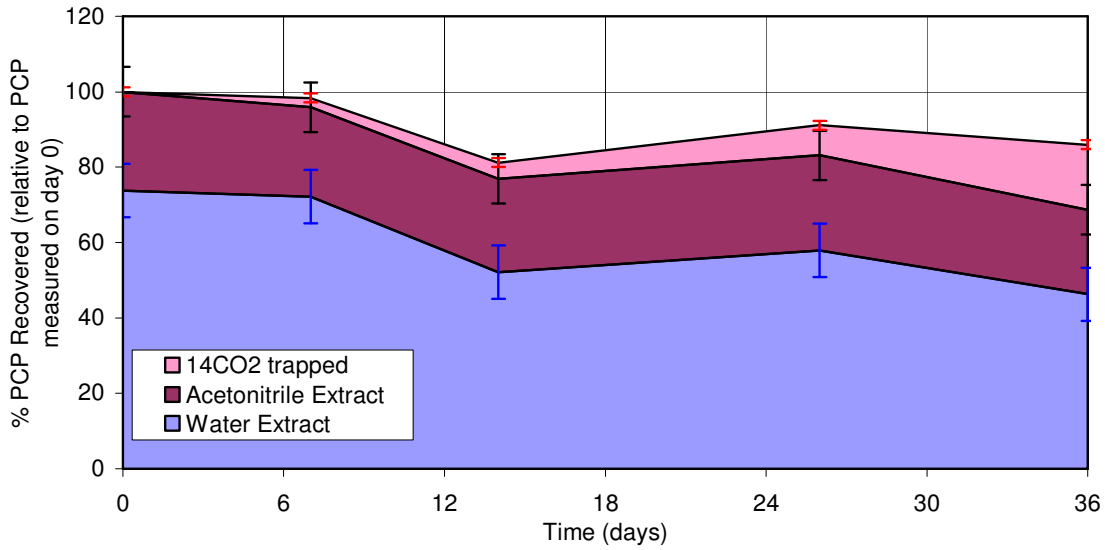

Figure 4-94: PCP recovery vs. time from control specimens (experiment PI_16)

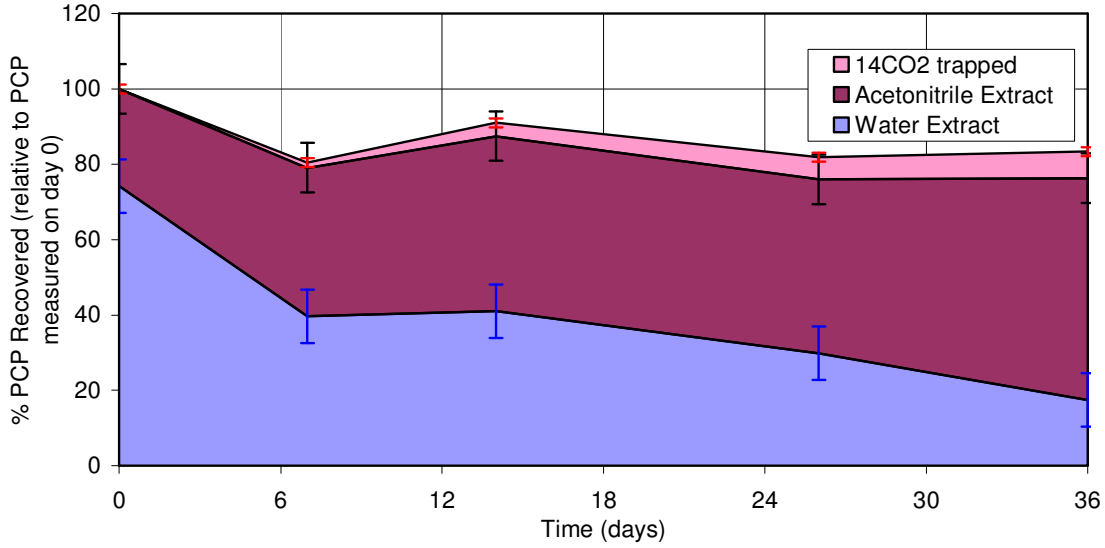

Figure 4-95: PCP recovery vs. time from electrokinetic specimens (experiment PI_16)

Experiments with a constantly applied electric field (and radiolabelled PCP) have several points in common, therefore. A significant decrease in water-extractable ('bioavailable') PCP occurred with an applied electric field, along with a much-reduced output of mineralised PCP as carbon dioxide.

4.8.3. Pulsed and Low Current Experiments

The reduced power input to the experiments presented here is shown in the reduced effect of the electric field – there were found to be few differences between electrokinetic and control specimens.

Figure 4-96 shows that by the end of experiment PI_14 there was very little difference between electrokinetic and control specimens, in either total recovery or soil recovery. The only significant differences came at day 7 (total recovery, p=0.004) and days 7 and 14 (soil recovery, p=0.004 and p=0.047 respectively), but there are no obvious explanations for these.

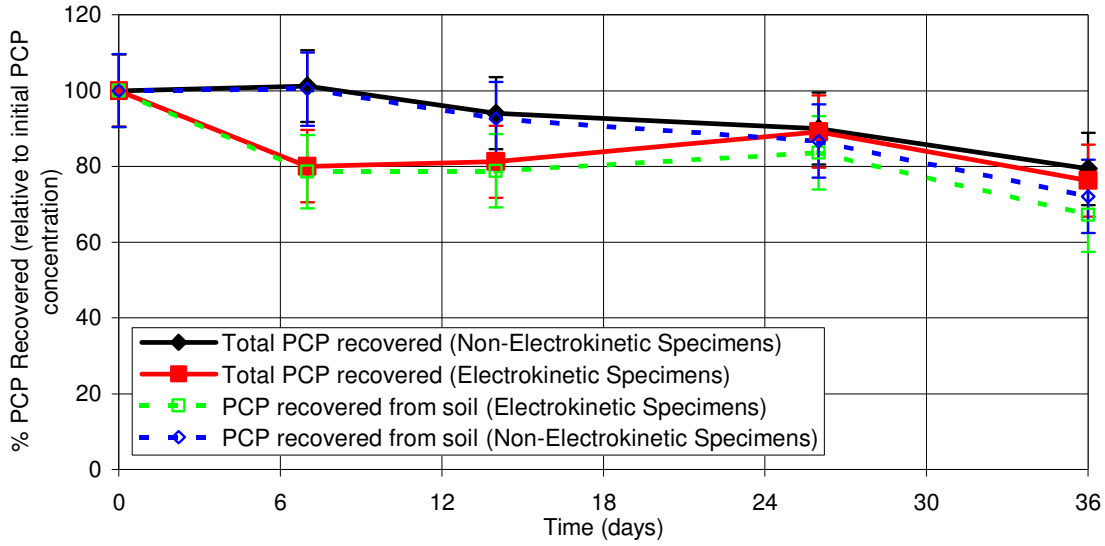

Figure 4-96: Total and soil PCP recoveries vs. time (experiment PI_14)

If the differences at day 7 are ignored, it is difficult to see a difference between controls and electrokinetic specimens. In contrast to experiments with a constant current of 10mA presented earlier, the water-extractable fraction of PCP remains at approximately 70% throughout testing (Figure 4-97 and Figure 4-98).

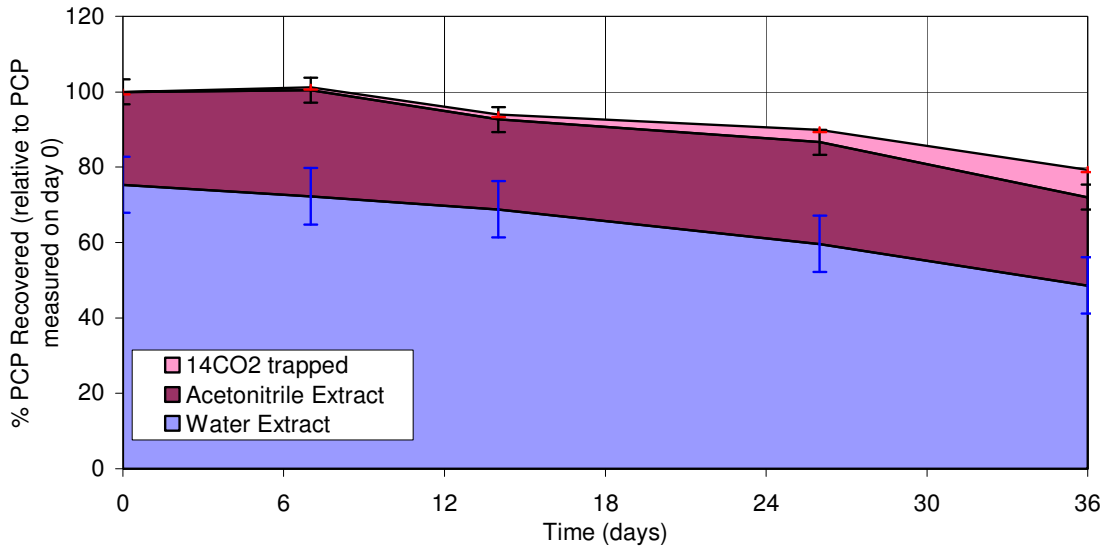

Figure 4-97: PCP recovery vs. time - control specimens (experiment PI_14)

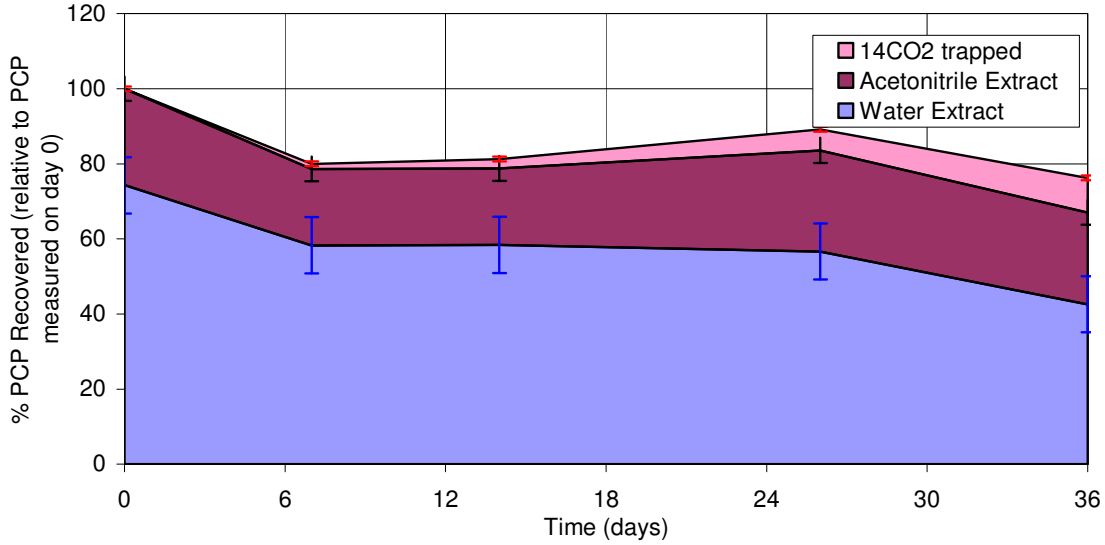

Figure 4-98: PCP recovery vs. time - electrokinetic specimens (experiment PI_14)

Experiment PI_17 was run with a constant current of 1mA, in an effort to get constant current effects with the same overall level of power input as would be experienced in pulsed current experiments. It was run in tandem with experiment PI_16 and the same controls were used for both experiments.

Radiolabelled CO_2 given off in this experiment is plotted in Figure 4-99, and shows a great deal more similarity between electrokinetic and control specimens than has been seen previously. Again, however, the electrokinetic specimens do mineralise less carbon dioxide, with the difference becoming significant ($p \leq 0.05$) at 25 days.

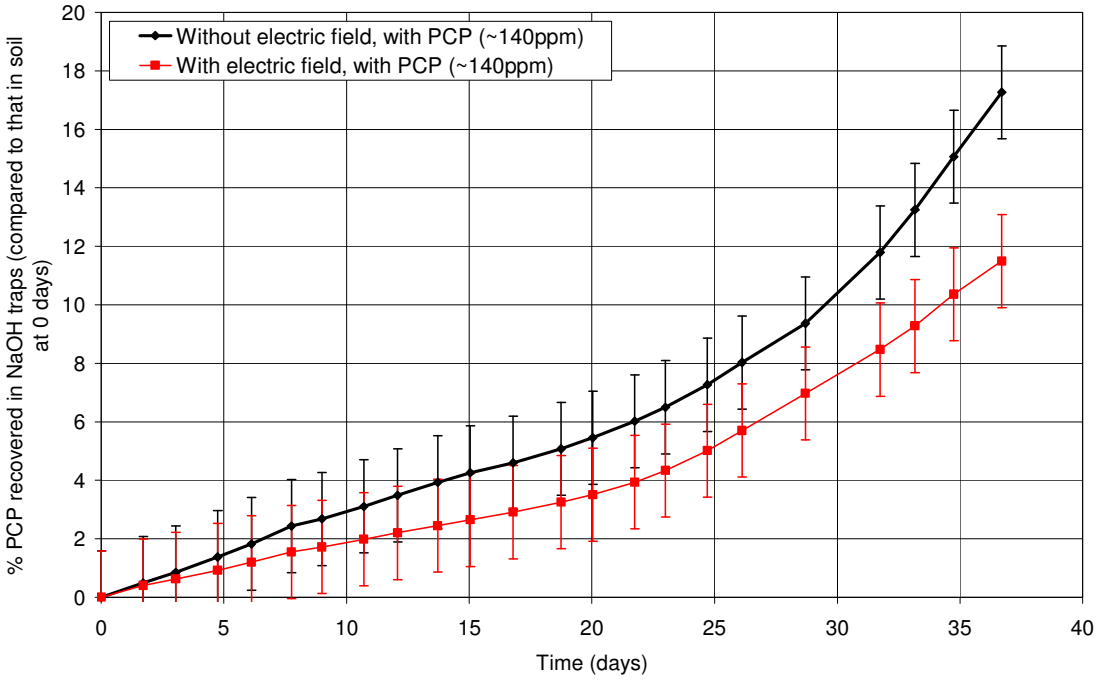

Figure 4-99: Mineralised PCP trapped as CO_2 versus time (experiment PI_17)

As in PI_16 (Figure 4-93), there are differences in recovered PCP at days 7 and 14, which are hard to explain. Apart from this, Figure 4-100 shows little difference between controls and electrokinetic specimens. There were no significant differences by the end of the test.

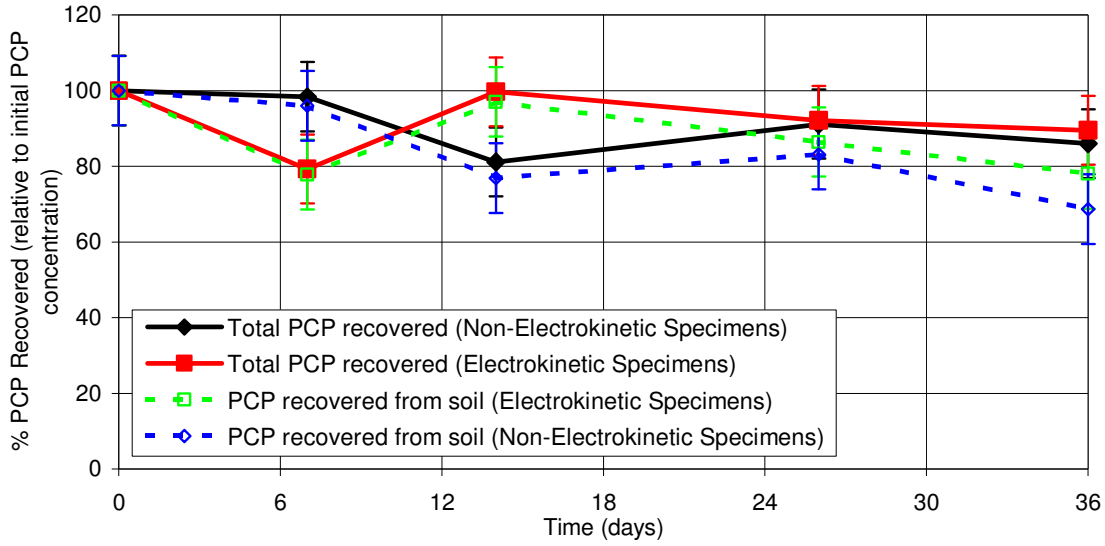

Figure 4-100: Total and soil PCP recoveries vs. time (experiment PI_17)

Recovery profiles in Figure 4-101 and Figure 4-102 show that there was little difference in how the PCP was extracted – a slight increase in acetonitrile extractable PCP was seen with an electric field. This may be because the constantly applied current would not have given conditions in the soil the chance to recover, as a short period of applied electric field (as occurred in PI_14 and PI_5) would have done.

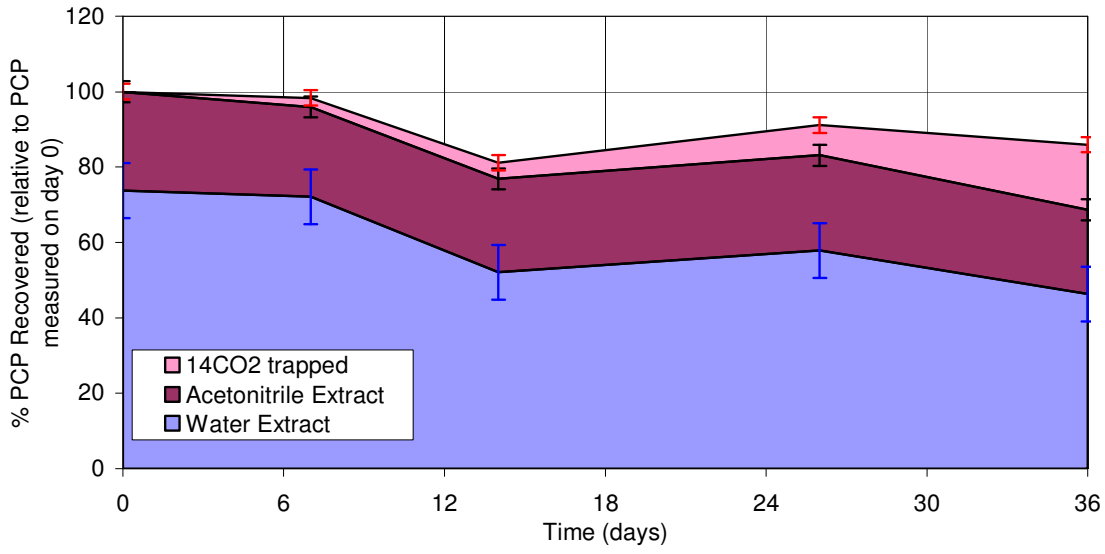

Figure 4-101: PCP recovery vs. time - control specimens (experiment PI_17)

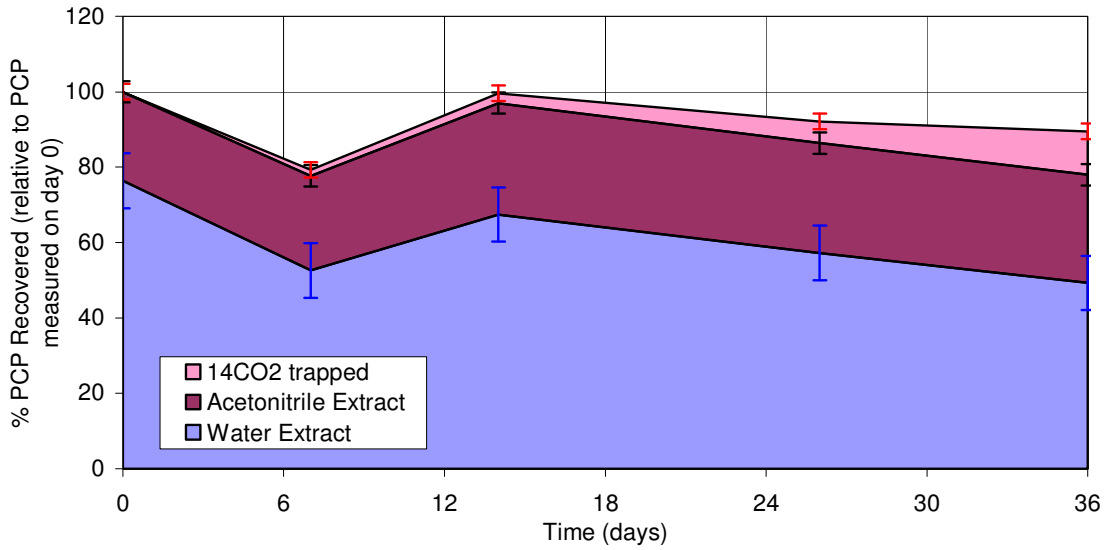

Figure 4-102: PCP recovery vs. time - electrokinetic specimens (experiment PI_17)

4.8.4. Effect of pH control at both electrodes

Control of pH at the anode as well as the cathode led to changes in the way constant current experiments behaved. Both the tests presented in this section had 10mA constant currents, although one had a 2 month period prior to applying the electric field during which degradation occurred.

The ability of electrokinetics to enhance the degradation of PCP some time after contamination was investigated in experiment PI_18. Control and electrokinetic specimens were set up as in previous experiments, but no electric field was applied until 66 days had passed. The effect on degradation was then investigated. Also in this experiment, pH control was applied to both electrolyte fluids.

Figure 4-103 illustrates the effect of application of an electric field. For the first 66 days, conditions were identical and both sets of specimens behaved similarly. There was no

significant difference between the two, although $^{14}CO_2$ recovery from specimens designated to receive an electric field later was slightly higher on average. However, once the electric field was applied at 66 days, the rate of CO_2 production in electrokinetic specimens gradually decreased until very little further CO_2 was being produced, whilst control specimens continued at the same rate. Total carbon dioxide produced was significantly larger ($p<0.05$) in control specimens from 91 days onwards.

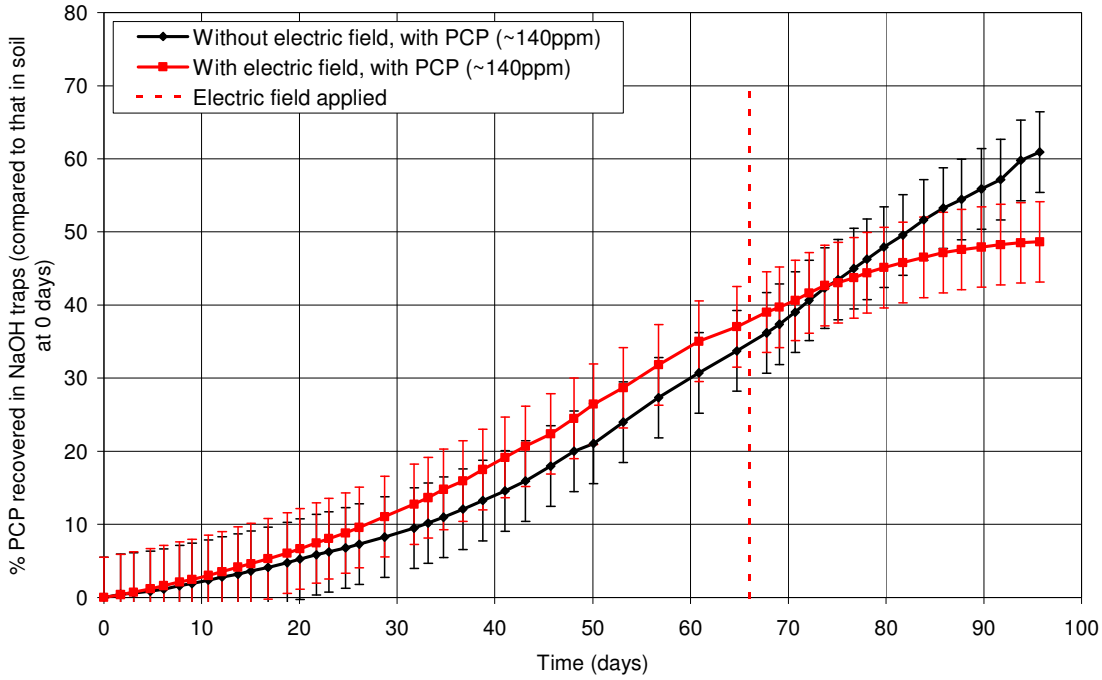

Figure 4-103: Mineralised PCP trapped as CO2 versus time (experiment PI_18)

Specimens with an applied electric field gave consistently lower average PCP recoveries (both total and from soil), which were significantly lower at 73 days and 95 days than those in controls (Figure 4-104, Figure 4-105 and Figure 4-106). These results do not correlate well with those from carbon dioxide traps, which show a decreasing amount of carbon dioxide given off with the applied field. This may be due to movement of PCP out of the electrokinetic specimens into electrolyte fluids, although no evidence for such an effect was seen in this particular experiment. A greater proportion of acetonitrile-extractable PCP was seen at the end of testing in electrokinetics specimens.

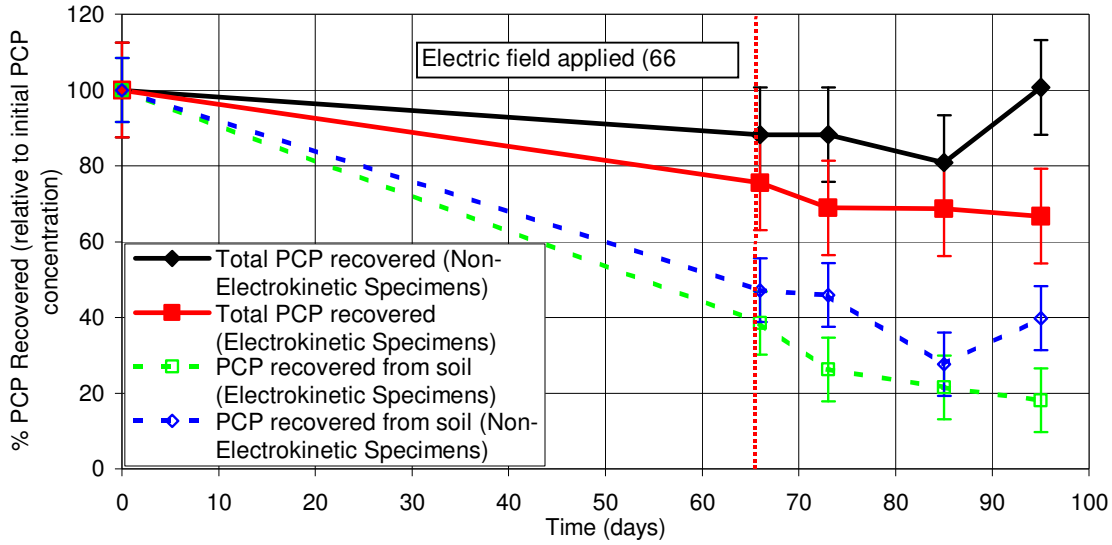

Figure 4-104: Total and soil PCP recoveries vs. time (experiment PI_18)

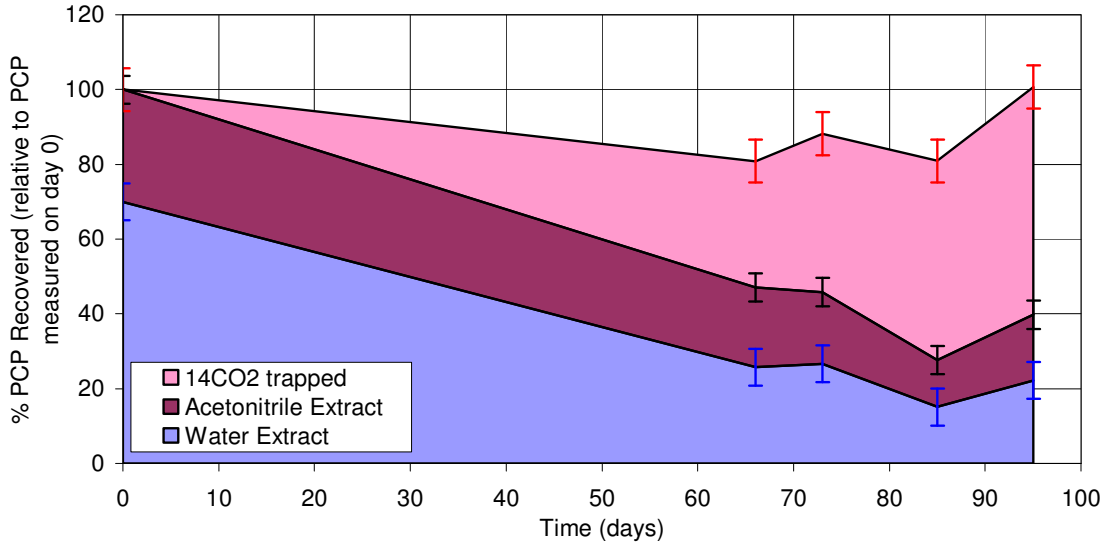

Figure 4-105: PCP recovery vs. time - control specimens (experiment PI_18)

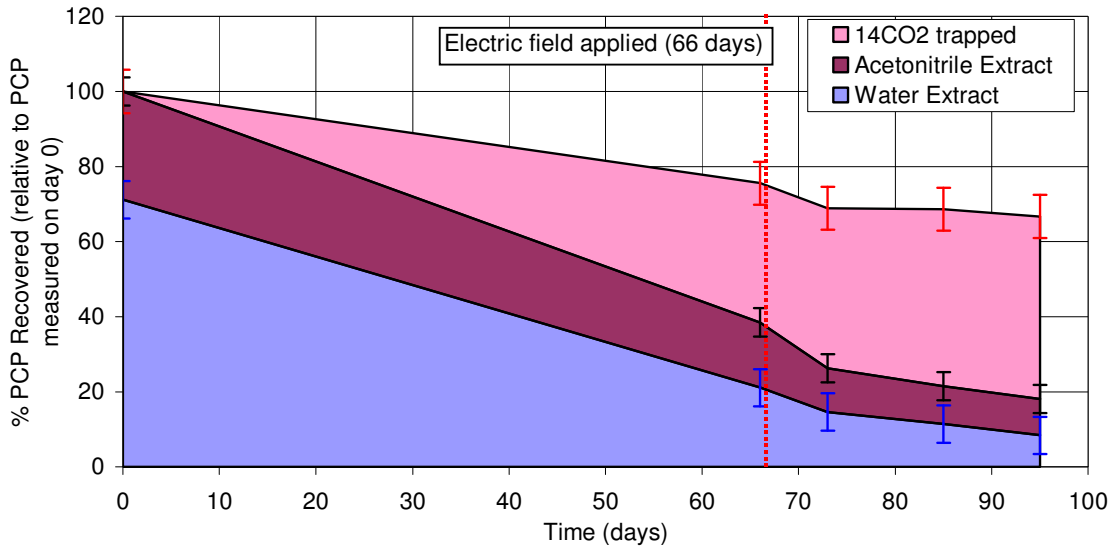

Figure 4-106: PCP recovery vs. time - electrokinetic specimens (experiment PI_18)

Experiment PI_20 was performed in the same way as experiment PI_18, except that it lacked the initial 2-month degradation period. Control of the pH was again employed at both electrodes.

No significant differences in $^{14}CO_2$ production could be determined until 17 days, at which point the total output of the control specimens became significantly larger than that of the electrokinetic specimens ($p<0.05$). These two curves can be seen to diverge at this point (Figure 4-107). This is a similar effect to that seen in constant current experiments with pH control at only the cathode.

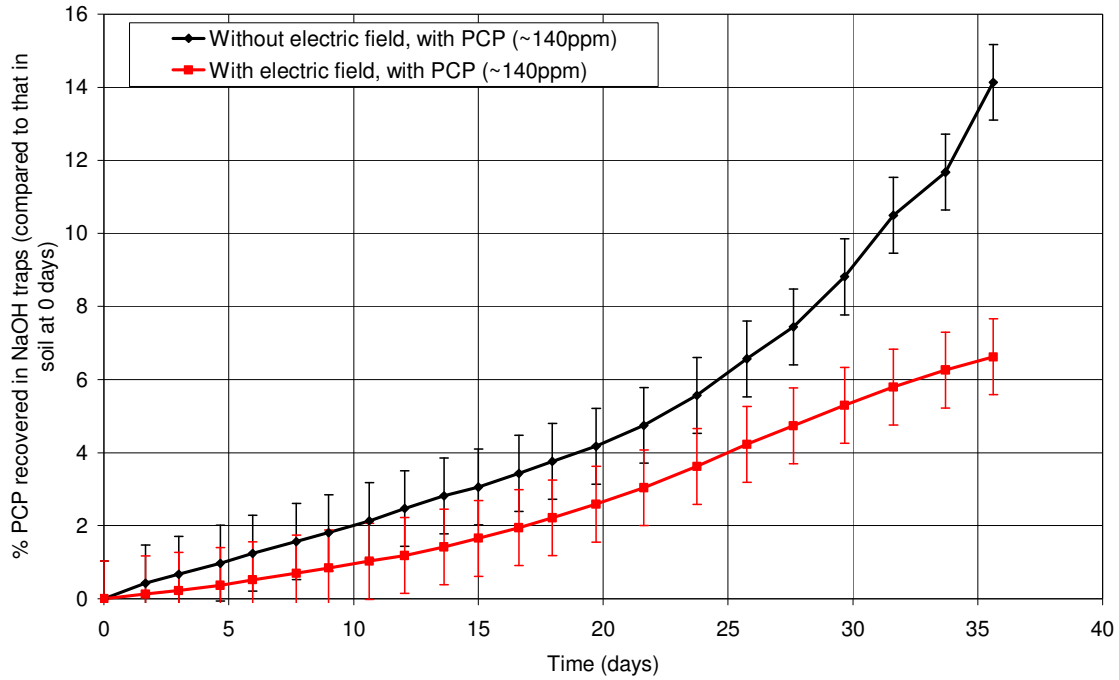

Figure 4-107: Mineralised PCP trapped as CO2 versus time (experiment PI_20)

It may be seen in Figure 4-108 that whilst total recovery of PCP from control specimens remained more or less constant, with an electric field the recovery dropped substantially, with less than 40% of the original total accounted for after 36 days. It is thought that movement of the PCP into membranes and electrolyte fluids may account for a large proportion of this loss (although none was discovered here, possibly for the same reasons as given at the start of this section). The lower recovery of $^{14}CO_2$ in specimens with the electric field would support this argument.

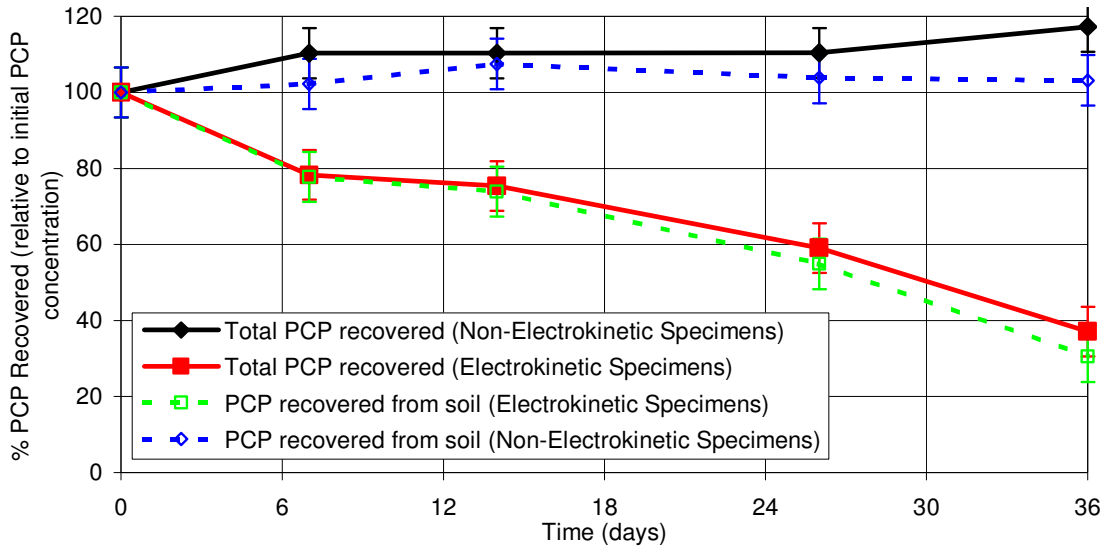

Figure 4-108: Total and soil PCP recoveries vs. time (experiment PI_20)

As was seen with experiment PI_18, a slight increase in acetonitrile-extractable PCP was noted after 36 days with an electric field (comparing Figure 4-109 to Figure 4-110).

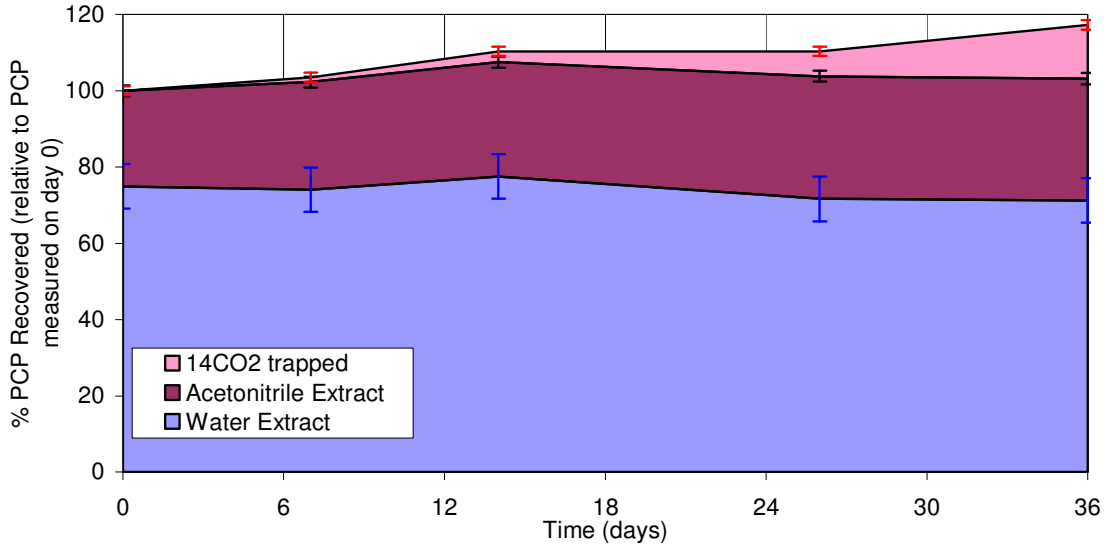

Figure 4-109: PCP recovery vs. time - control specimens (experiment PI_20)

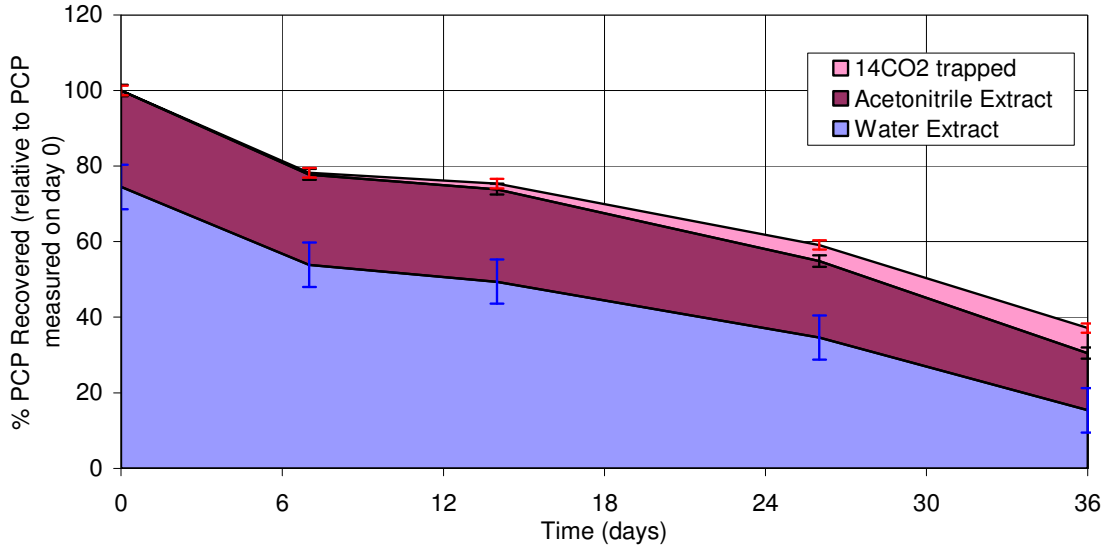

Figure 4-110: PCP recovery vs. time - electrokinetic specimens (experiment PI_20)

4.8.5. Control of Moisture Content and pH

In this experiment, the term 'anode end' refers to the end of the soil at which the anode was located at the start of the experiment.

Chemical or physical changes in the soil have characterised earlier experiments, with either large changes in pH, moisture content or both seen. This experiment (experiment PI_22) was designed to overcome these problems that were thought to hinder PCP degradation by making conditions unsuitable for bacterial survival, amongst other effects. Reversing the current every 24 hours would allow pH changes in the soil to be naturally controlled, as hydrogen ions produced in one 24 hour period would largely be neutralised by hydroxyl ions produced in the next. Simply by not employing external electrolyte fluids, the possibility of drastically increasing the moisture content was removed. Electrodes placed directly in contact with the soil were found to provide sufficient current, although it was necessary to periodically wet the soil near these electrodes as

water was transported away from them, presumably by electroosmotic flow or heating effects.

It can be seen in Figure 4-111 that PCP was mineralised (and trapped as $^{14}CO_2$) at a more substantial rate *with* an electric field than without. Towards the end of the experiment, however, the electrokinetic curve tailed off and the control curve caught up. Nevertheless, between 31 and 53 days the total PCP output is significantly larger with an electric field ($p<0.01$).

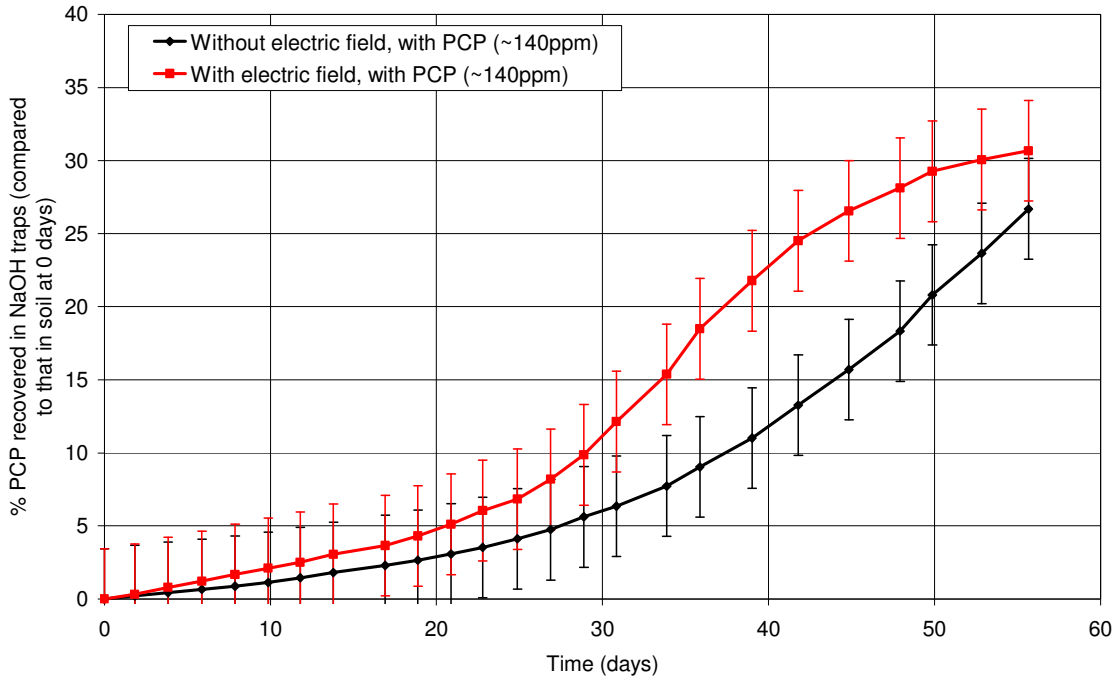

Figure 4-111: Mineralised PCP trapped as CO_2 versus time (experiment PI_22)

Similar trends are evident in both sets of specimens (Figure 4-112), with large decreases in recovered PCP (both total and in soil) with an electric field by the end of the experiment. Total recovery in soil was significantly lower in electrokinetic specimens ($p=0.035$) at day 56. There was no possibility for PCP to have moved out of the soil as was thought to happen in experiments PI_18 and PI_20, as there were no Daramic

membranes or electrolyte fluids employed in this experiment. It is therefore thought that all non-recoverable PCP (apart from a small percentage remaining in the soil, as indicated by combustion in experiments PI_13i and PI_13u) was mineralised but not trapped.

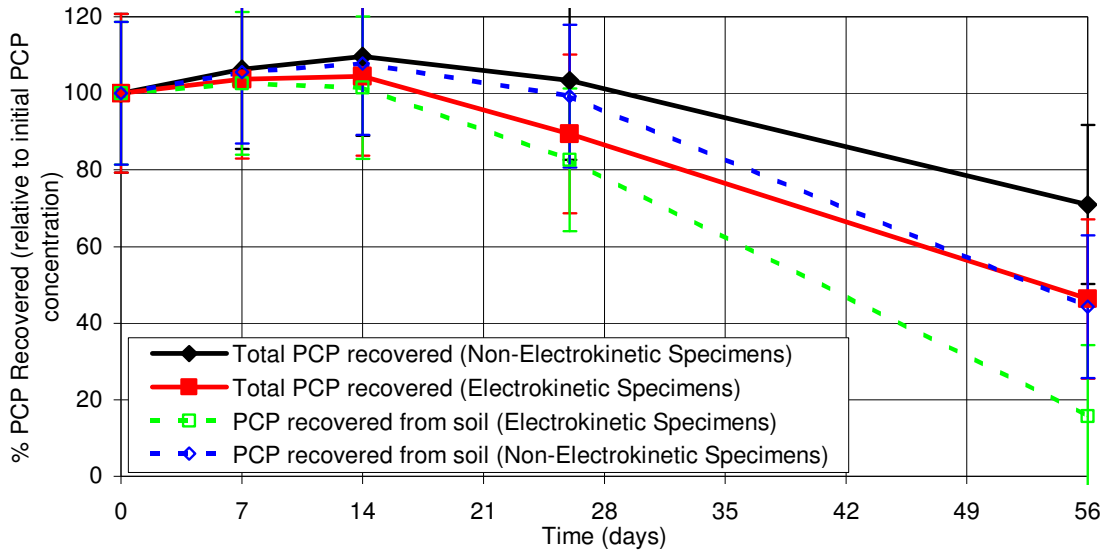

Figure 4-112: Total and soil PCP recoveries vs. time (experiment PI_22)

Figure 4-113 and Figure 4-114 show the differences in extractable fractions. Once more, an increase in acetonitrile-extractable PCP was found with an electric field. In this case, it may be that this is due to mineralisation of the more accessible water-extractable fraction, leaving the more strongly sorbed remainder (although redistribution would be expected if some PCP were removed). At day 56, there is on average approximately 40% of the initial PCP remaining in the soil in control specimens, whilst with an electric field this value is approximately 15%.

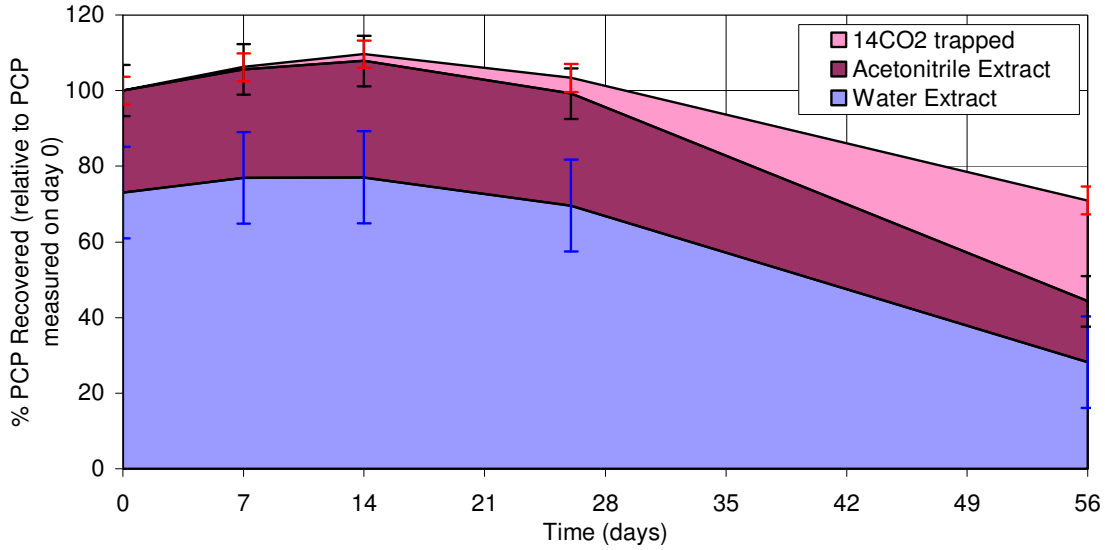

Figure 4-113: PCP recovery vs. time - control specimens (experiment PI_22)

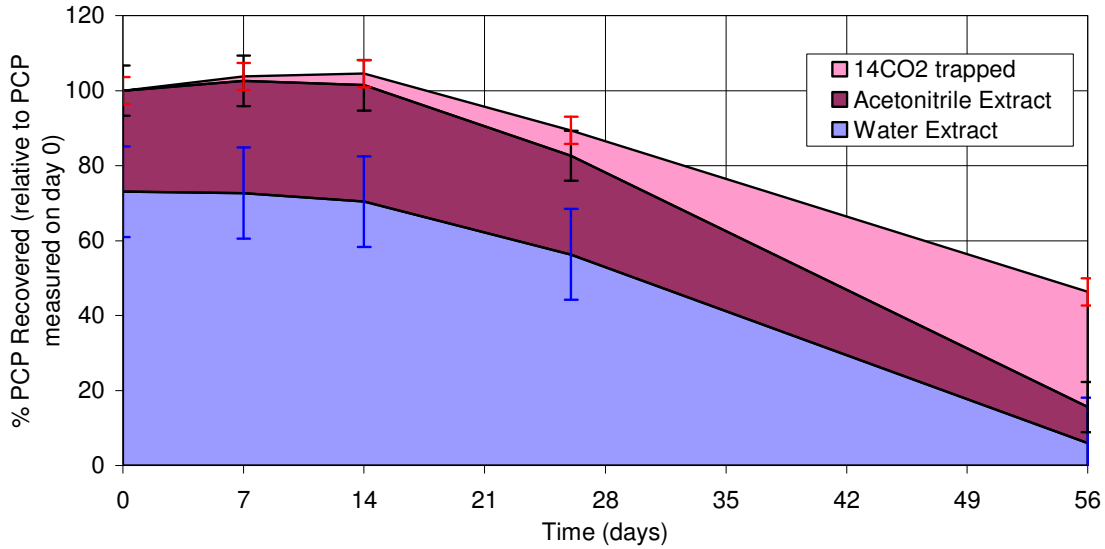

Figure 4-114: PCP recovery vs. time - electrokinetic specimens (experiment PI_22)

4.9. Effect of Ageing of the Contaminant in Soil

At the start of experiment API_1 it was discovered that two of the cartridges used had suffered substantial PCP loss whilst in storage, despite not being inoculated with and being isolated from *Sphingobium* sp. UG30. One had virtually no PCP remaining, whilst in the other, there was no PCP in one end of the sample. It was decided to continue the experiment but with only four cartridges, two as controls, two with an applied field.

The plot of mass balance versus time (Figure 4-115) shows similar average PCP recovery with and without a pulsed current. There is certainly no significant difference between the two at any point (due in part to the uncertainty involved in only having one degree of freedom for a statistical analysis). However, the similarity between the two is consistent with the results obtained for other pulsed power experiments (e.g. PI_5, PI_14) which also show little difference between electrokinetic and control specimens.

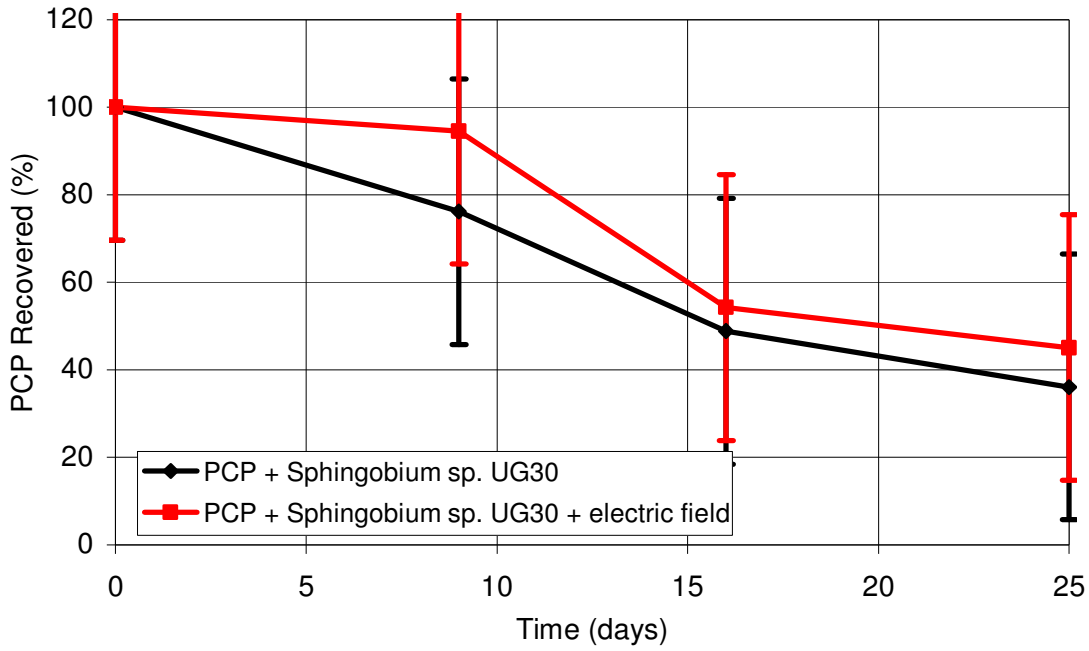

Figure 4-115: Mass balance (experiment API_1)

In experiment API_21, specimens contaminated with radiolabelled PCP and stored for ten months (whilst being kept isolated from degrading bacteria at all points) suffered the same fate as specimens from experiment API_1a. The PCP level remaining in the cells before the application of the electric field was found to be extremely variable and much lower than expected (between 6 and 53% remaining). The conductivity of the specimens was also higher than was seen in other tests (by a factor of 3, although in electrokinetic specimens after testing this rose to a factor of 20).

The results presented in Figure 4-116 and Figure 4-117 show pH change effect (notably at the anode) with a large reduction in water-extractable PCP / increase in acetonitrile-extractable PCP.

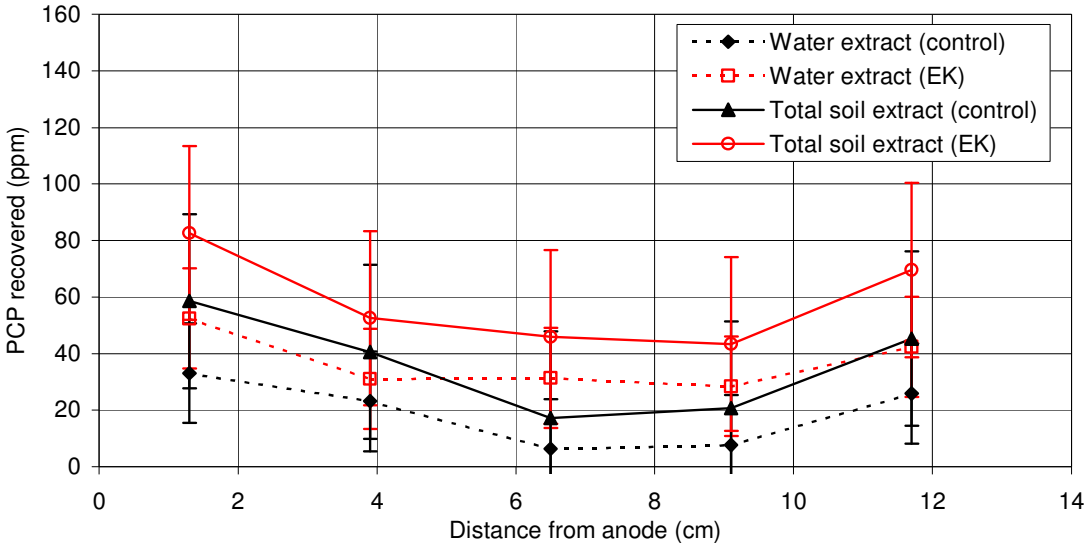

Figure 4-116: Spatial PCP distribution - day 0 (experiment API_21)

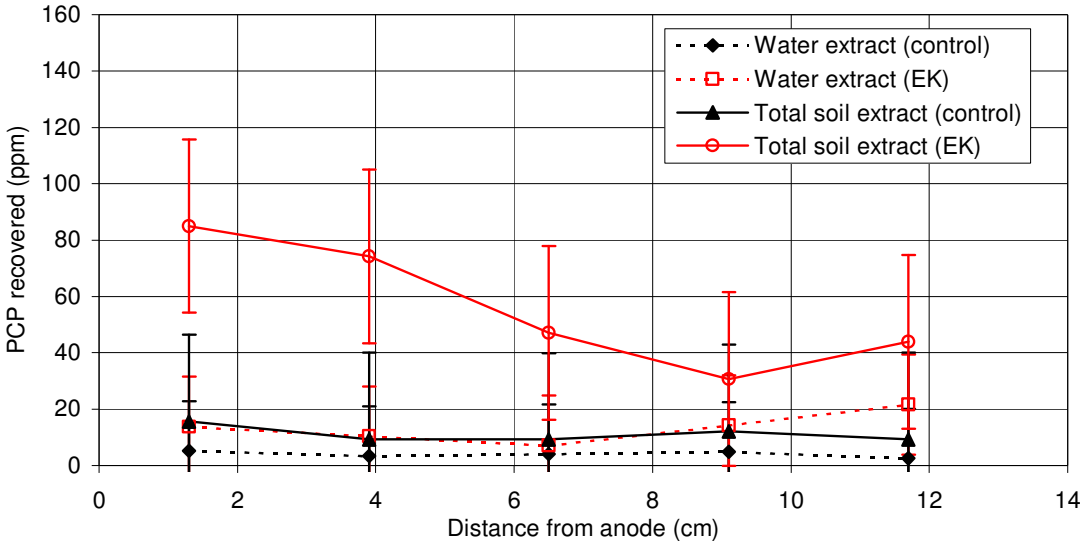

Figure 4-117: Spatial PCP distribution - day 42 (experiment API_21)

5. Mathematical Modelling

The effect of an electric field on several aspects of the soil specimens, as well as PCP and its transport, has been modelled, in order to assess the validity of hypotheses for phenomena seen in experiments. The limited nature of the modelling meant that only physical behaviour was investigated. Changes in pH in the electrode chambers and soil due to water electrolysis, and transport and state of the PCP were the major factors modelled. The first aim was to compare the theoretical progression of an acid front into the soil with that seen in numerous experiments. The movement of PCP by electromigration and electroosmosis was also determined theoretically, based in part on the pH results. Comparison was made with experimental results, with particular attention paid to rate of transport and sorption. The hypothesis that a decrease in pH increased the protonation of the PCP and thereby increased sorption to soil was investigated. The majority of this work has been performed in Microsoft® Excel. Although several of the equations used to model transport of chemical species have been taken from other sources, the majority of the model has been developed specifically for this project, with many relationships being determined empirically.

5.1. Design of Method

5.1.1. General Description

A simple finite difference method has been used to solve a model of contaminant transport and fate in a replica of the experimental system. This model was constructed in two 'layers', the first of which describes the creation, transport and fate of hydrogen and hydroxyl ions due to the electrolysis of water at the electrodes. This therefore describes

the pH profile across the soil at any time. The second 'layer' was very similar to the first in terms of the mathematics involved, although it dealt with the state, transport and fate of pentachlorophenol instead of H^+ and OH^- ions. It used the pH conditions calculated in the first 'layer' to determine the state (protonated or non-protonated) of the PCP molecules. Conditions at both electrodes as well as five equally spaced points within the soil were modelled, as indicated in Figure 5-1. The five points where calculations take place in the model were in identical locations to sampling positions in the actual apparatus, allowing direct comparisons to be made.

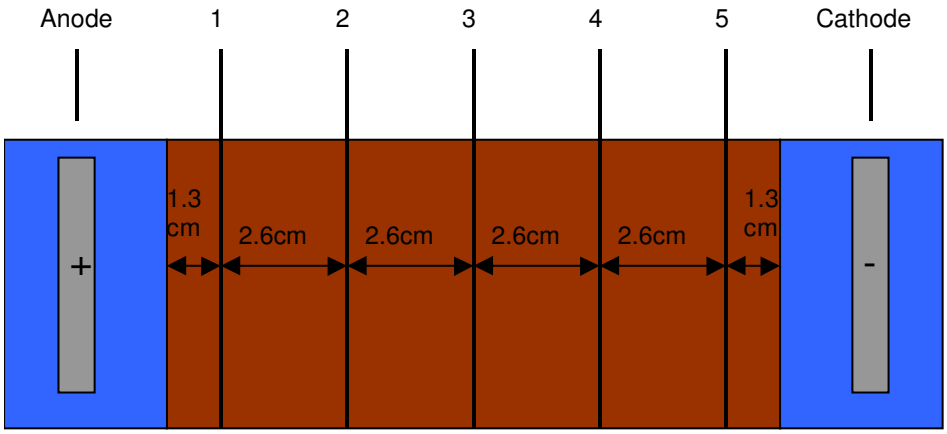

Figure 5-1: Idealized apparatus layout for mathematical model

5.1.2. Equations

The creation of hydroxyl and hydrogen ions in the system is due to the electrolysis of water at the electrodes. The equations below describe the chemical reactions:

$$\text{Anode: } 2H_2O - 4e^- \rightarrow O_2 + 4H^+$$

$$\text{Cathode: } 2H_2O + 2e^- \rightarrow H_2 + 2OH^-$$

In each case, the number of electrons generated or consumed is equal to the number of either H^+ or OH^- ions produced. The number of electrons flowing per second is described

by the current, and so the amount of ions produced can be directly calculated from this using the following equation:

$$Number\ of\ ions\ created\ in\ unit\ time\ (moles) = \frac{I}{c_e n_a}$$

(I – current; c_e – charge on an electron ; n_a – Avogadro number)

The creation of H^+ and OH^- ions at the respective electrodes leads to changes of pH in these electrode chambers. Due to the processes of diffusion, electromigration and electroosmosis, however, these ions are redistributed through the soil cartridge, leading to pH gradients within the soil itself. The basic equations used to describe these different methods of transport are taken from Açar and Alshawabkeh (1993), and are presented below. These equations are general, and apply to PCP molecules in the soil as well as hydrogen and hydroxyl ions.

Diffusive Mass Flux: $J_j^d = D_j \tau n \nabla(-c_j)$

Electromigrational Mass Flux: $J_j^m = \frac{D_j \tau n z_j F}{RT} c_j \nabla(-E)$

Electroosmotic Mass Flux: $J_j^e = c_j * \left(\frac{\varepsilon \zeta n}{\eta}\right) \nabla(-E)$

Terms used (a table of values used for constants is presented below):

J - mass flux

D - diffusion coefficient

τ - tortuosity

n - porosity

c - concentration

z - ionic charge on the j^{th} species

F - Faraday's Constant

E - Potential Gradient

c_w - molar concentration of water

ε - Permittivity of the medium

ζ - Zeta Potential

η - viscosity of medium

As the model specimen has been assumed to be one dimensional (from anode to cathode), then the ∇ function ($\nabla = \mathbf{i}\frac{\partial}{\partial x} + \mathbf{j}\frac{\partial}{\partial y} + \mathbf{k}\frac{\partial}{\partial z}$) can be reduced to the term $\frac{\partial}{\partial x}$.

At each location in the spreadsheet (i.e. the centre of each segment), the amount of H^+, OH^-, PCP^0 (protonated PCP) and PCP^- (ionic PCP) entering and leaving, both to left and right, was calculated using these formulae. The total increase or decrease of that species at that location was used to amend the original concentration.

Constants used in the above equations, and elsewhere in the model, are given in Table 5-1 and Table 5-2:

Initial pH	7.57
Faraday's Constant, F (C/mol)	96485
Molar Gas Constant, R (J/(K mol))	8.3144
Temperature (K)	298
Permittivity of pore water (F/m)	7.1719×10^{-10}
Viscosity of pore water (Pa s m)	1.00
Porosity of soil specimens, n (-)	0.62
Tortuosity of soil specimens, τ (-)	0.57

Initial Conductivity of pore water (S/m)	0.262
Initial Saturation (%)	70
Soil specimen length (m)	0.13
Soil specimen cross-sectional area (m^2)	3.186×10^{-3}
Electrode chamber volume (m^3)	1.593×10^{-4}
Mass of dry soil in cartridge (g)	420

Table 5-1: Table of constants (general)

Chemical Species:	H^+	OH^-	PCP^0	PCP^-
Diffusion coefficient (m^2/s)	9.31×10^{-9}	5.30×10^{-9}	6.10×10^{-10}	6.10×10^{-10}
Dissociation Constant (-)	1×10^{-14} $H_2O \Leftrightarrow H^+ + OH^-$		4.47×10^{-5} $PCP^0 \Leftrightarrow PCP^- + H^+$	

Table 5-2: Table of constants (species-specific)

5.1.2.1. Electrical Conductivity

The variation in electrical conductivity during experiments has been modelled using a slightly amended form of the Nernst-Einstein relationship, taken from Atkins (1990). The outcome of the equation below is added to the initial electrical conductivity of the pore fluid (section 4.1.11); initially, the result of this equation is negligible, but this changes as concentrations of H^+ and OH^- ions vary.

$$\kappa = \left(\frac{z^2 F^2}{RT} \right) \left(c^{H+} D^{H+} + c^{OH-} D^{OH-} \right)$$

5.1.2.2. Zeta Potential

The effect of pH on the zeta potential has been analysed, as described in sections 3.2.9 and 4.1.9. A curve has been fitted to the data in Figure 5-2:

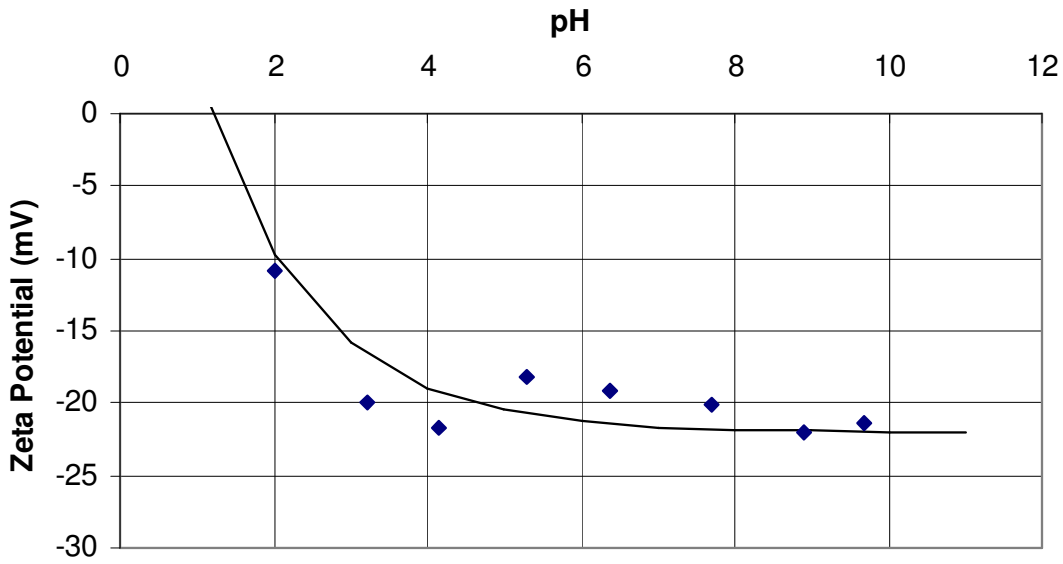

Figure 5-2: Variation in zeta potential with pH (curve fit to data)

The form of the equation used to model this behaviour has been based on that described by Eykholt and Daniel (1994) (after Lorenz (1969)). This is shown below (the numerical coefficients are changed to relate to the behaviour seen here).

$$\frac{\zeta}{\zeta_{pH=7}} = \alpha + \beta * (e^{\chi * pH}) \quad \text{(where } \alpha = 1.017; \beta = -2.312; \chi = -0.7\text{)}$$

5.1.2.3. Buffering of pH changes

The behaviour of the Wytham soil used in the current project has been investigated with respect to buffering of hydrogen and hydroxyl ions (sections 3.2.6 and 4.1.6). A curve fit is presented for both of these ions (Figure 5-3 and Figure 5-4), with the blue line and points showing actual measured data, while the red line is the relevant fitted curve, the

equations for which are given below. Despite the problems in obtaining a curve for OH^- ions, an equation is presented using the same format as for H^+ ions, to fit the data as well as possible. These equations are used in the model to determine the proportion of hydrogen and hydroxyl ions in the pore fluid itself, which in turn affect the state of the PCP. The equation used to model hydrogen ion buffering is:

$$x = 1.67 * 10^{-4} \left(1 - \exp\left(-1000 * \left(c - 10^{-7}\right)\right)\right)^{0.35}$$

That used to model buffering of hydroxyl ions is:

$$x = 3.8 * 10^{-4} \left(1 - \exp\left(-3000 * \left(c - 10^{-7}\right)\right)\right)^{0.6}$$

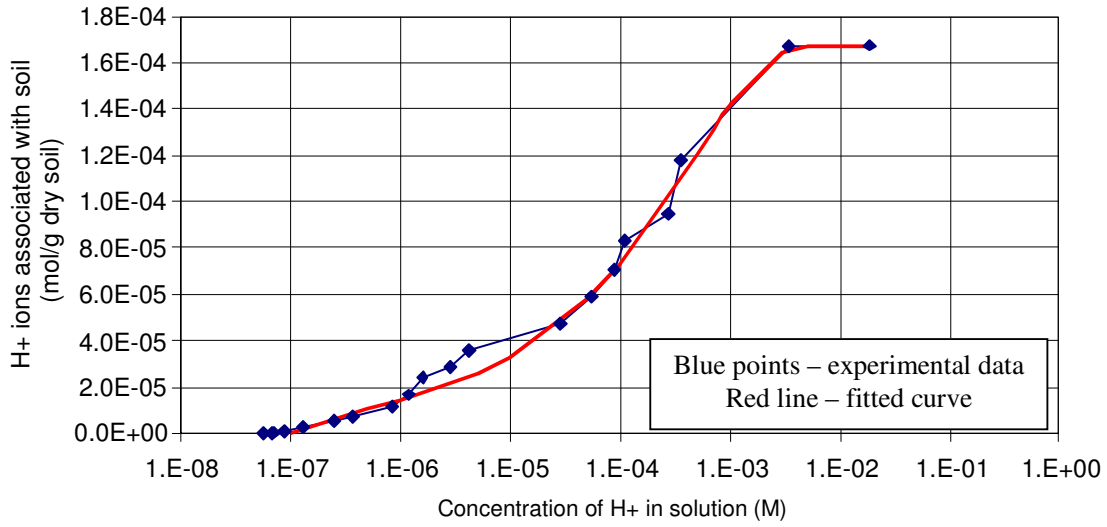

Figure 5-3: H+ buffering - data fit

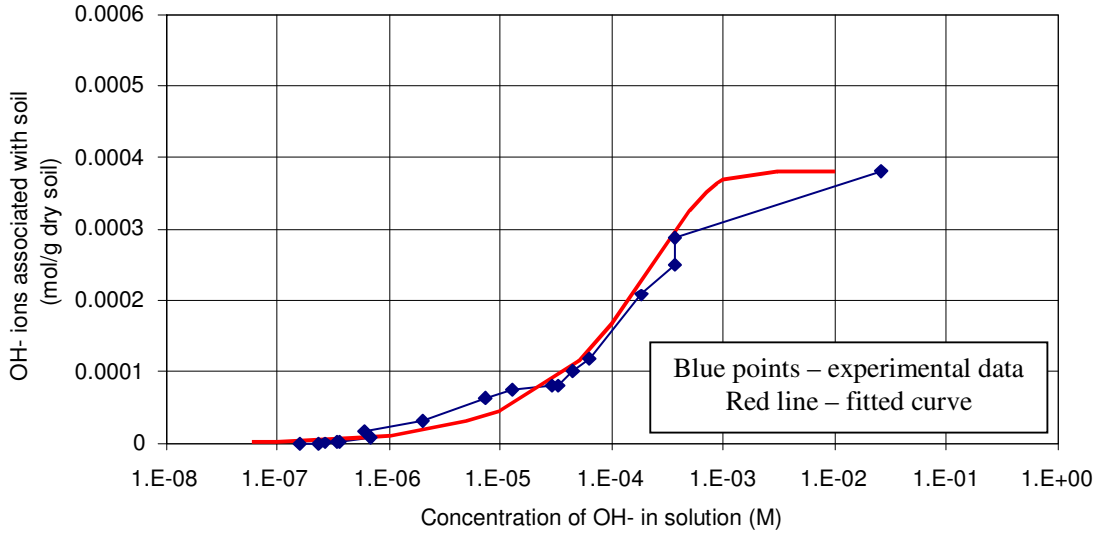

Figure 5-4: OH- buffering - data fit

The concentration of hydrogen and hydroxyl ions in soil was calculated by a three-stage process. Firstly, it was assumed that all ions were in the pore water, and recombination of the two to form water was modelled. The amount of sorption and reaction with the soil matter was then calculated for the remaining ions, followed by a further calculation similar to the first, ensuring that the relative concentrations of the two ions were in equilibrium.

5.1.2.4. Behaviour of PCP in Soil

Factors such as the state of PCP and its sorption to soil have been modelled using simple techniques. The state of PCP (protonated or non-protonated (charged)) is important in terms of whether electromigration acts, as well as how much sorption occurs. The proportion of PCP present in either state is determined by the dissociation constant (pK_a = 4.35), and the following equations:

$$pK_a = -\log_{10}(K_a), \text{ where } K_a = \frac{[H^+][PCP^-]}{[PCP^0]}$$

The concentration of hydrogen ions is known from pH calculations, and the total number of PCP molecules is known (PCP^- + PCP^0). Therefore, the proportion of PCP in each state can be calculated.

Sorption of PCP in either state was determined using adsorption isotherms (section 4.4). To model the sorption of protonated PCP, the isotherm for PCP with 100µl acid was used, as the majority of the PCP would have been in the protonated form. For the non-protonated form, the isotherm for PCP with 100µl alkali was used. In both cases, the equations given on Figure 4-36 were used to derive the proportions of PCP sorbing and remaining in solution. The equations used are given below:

$$PCP\ (protonated\ \ form): \frac{x}{m} = \frac{c_e^{\ 0.801}}{93.4}$$

$$PCP\ (non-protonated\ \ form): \frac{x}{m} = \frac{c_e^{\ 0.651}}{437.2}$$

In both cases, x/m has the units 'grams of PCP per gram dry soil', and c_e has the units 'moles per litre'.

The fate of PCP at each time-step was calculated using a similar three-stage process to that used with hydrogen and hydroxyl ions, described earlier (calculating proportions of protonated / non-protonated, followed by sorption, followed by a repeat of the first calculation).

5.2. Model Data

The model developed as described above is limited in that it only concerns changes in pH, state of the PCP (protonated or not) and movement of the PCP. However, data produced in this model are useful in confirming reasons for several of the phenomena seen in the

experimental results. Changes in pH in the electrode chambers (with constant 10mA current, without pH control) have been modelled, and compared to experiment P_1 in Figure 5-5. It may be seen that the long-term behaviour of the experiment was in very good agreement with the model, but this stable state was reached more slowly than was expected by calculation.

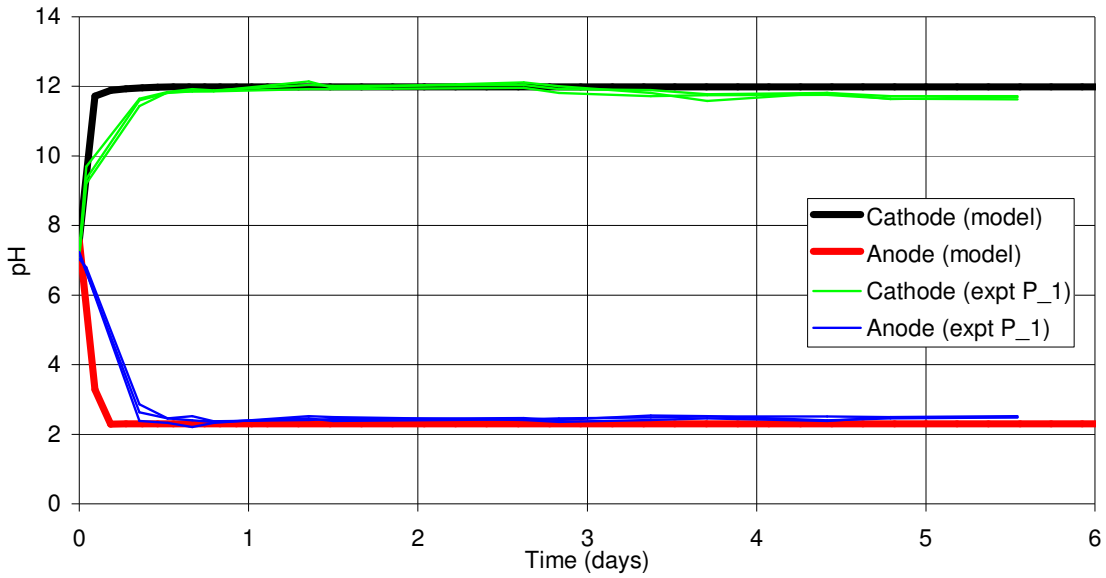

Figure 5-5: Electrode chamber pH - comparison between model and results from experiment P_1

The development of a pH profile within the soil was not measured in this case, but the mathematical model was used to indicate the pH changes that might be expected within the soil mass (Figure 5-6). It can be seen that significant pH changes might be expected, and that hydrogen ions travel faster, due to their greater mobility, than hydroxyl ions (the central point, 3, decreases in pH after 30 days, whilst at approximately the same time, the pH at point 4 is only just beginning to increase).

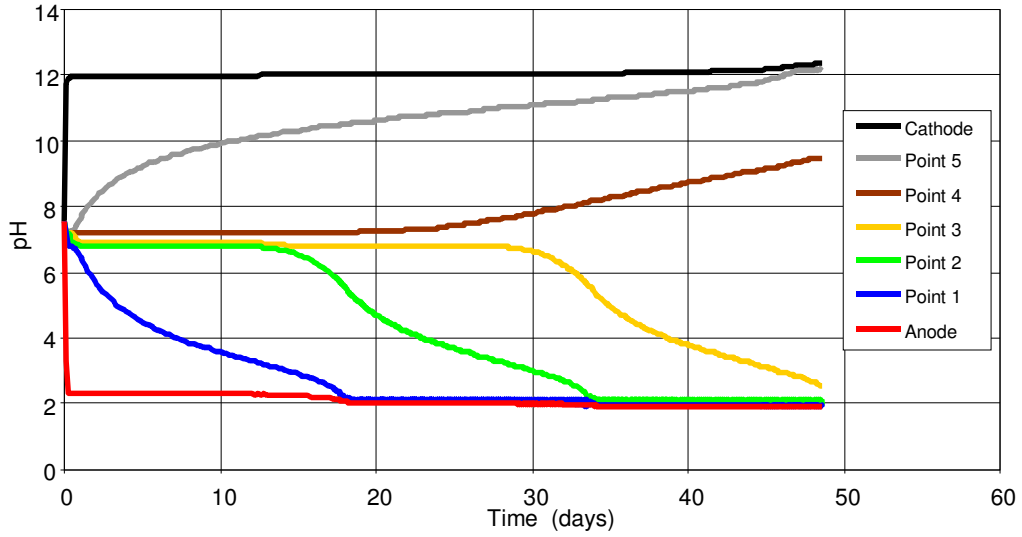

Figure 5-6: Calculated distribution of pH throughout soil specimen without pH control

Changes in pH were measured within the soil mass in later experiments (Figure 5-7) and this has been modelled (Figure 5-8). The trends evident in both graphs are in excellent agreement, although the values calculated in the model were slightly lower than those measured. This may be due to the dilution of samples used for actual measurement, which was not taken into account in the mathematics.

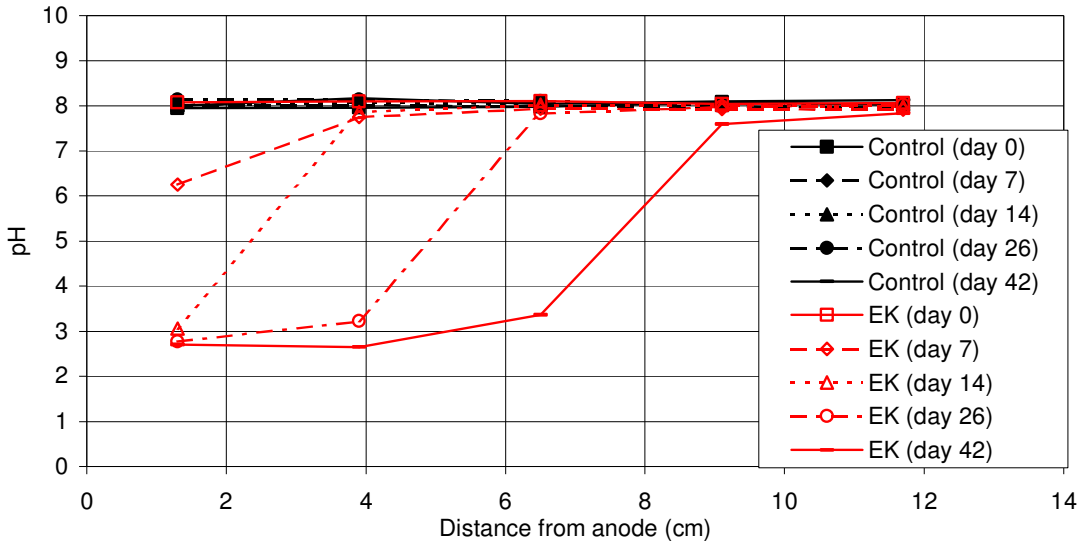

Figure 5-7: pH changes in soil for experiment API_21 (experimental)

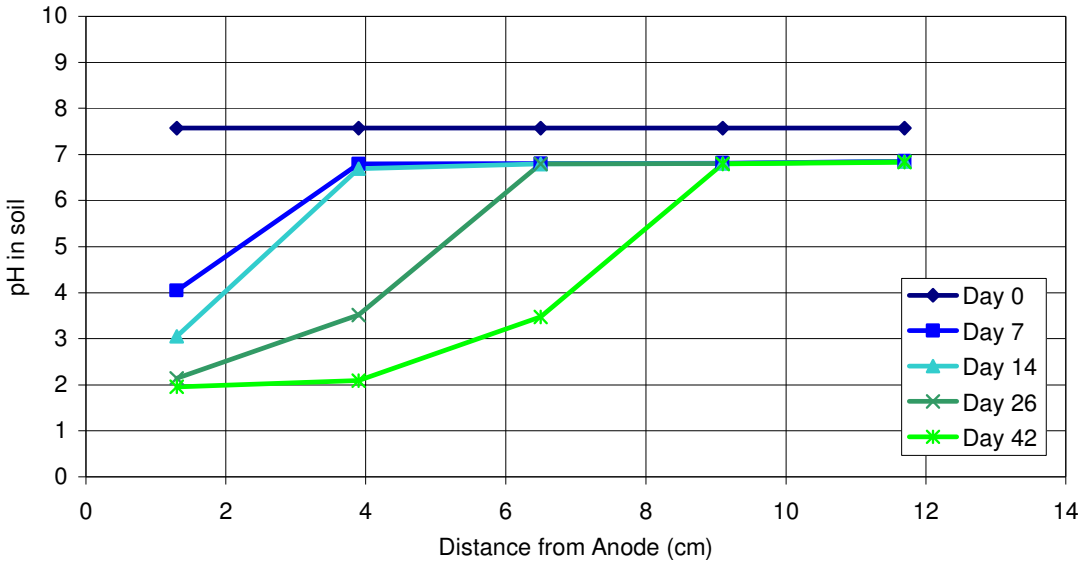

Figure 5-8: pH changes in soil for experiment API_21 (modelled)

The calculated movement of PCP in the soil again has similar trends to that seen in experiments. However, in this case there are greater differences, with PCP seen to move much more slowly than would be expected from calculations. In addition, the effect of pH changes is more marked in the model. For example, Figure 5-9 shows the change in dissolved PCP (a good approximation to the water-extractable fraction) with time in a model with a constantly applied 10mA current and pH control at the cathode only (to model experiment PI_13u). Whilst the initial amount of PCP in solution is very similar to that recovered in several experiments (approximately 70%), the reduction in this fraction is much larger than that seen experimentally.

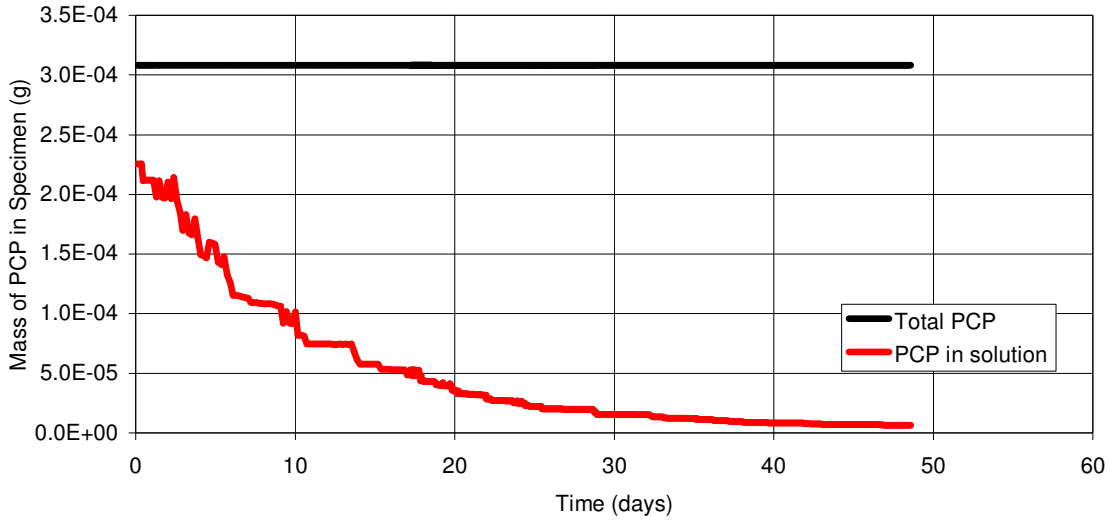

Figure 5-9: Water-extractable PCP fraction versus time (modelled, 10mA constant current)

Actual results from experiment PI_13u are compared with calculated results for similar times (day 0 and day 36) on Figure 5-10 and Figure 5-11. It may be seen that the large reduction in water-extractable fraction (dissolved PCP) seen in the experiment is recreated in the model, as is the movement from cathode to anode (largely by electromigration in the model). However, the changes seen by day 36 in this model are much greater than in the experiment. It is easier to see similarities between day 36 (experiment) and day 7 (model), as shown on Figure 5-12. It would appear that in much of the modelled data (PCP movement and some pH changes) there is a tendency for developments to occur more quickly in the mathematical model. It has not been determined why this is the case, although sorption is thought to be important – increasing the level of sorption (of both PCP and H^+ ions) led to results which more accurately described behaviour seen experimentally. It may be that a proportion of PCP transport is partially due to sorption to organic matter, which may itself be transported *via* electromigration, but which was not included in this model. However, the general trend of PCP movement towards the anode *via* electromigration has been successfully

modelled, as has pH development through the movement of an acid front into the soil, giving confidence in the model.

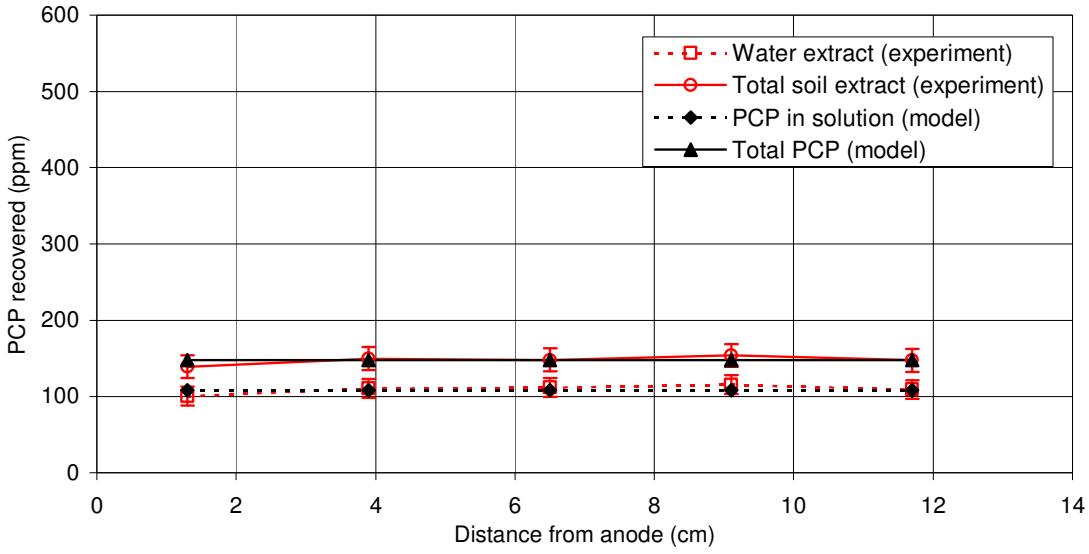

Figure 5-10: PCP distribution comparison between PI13u and model (day 0)

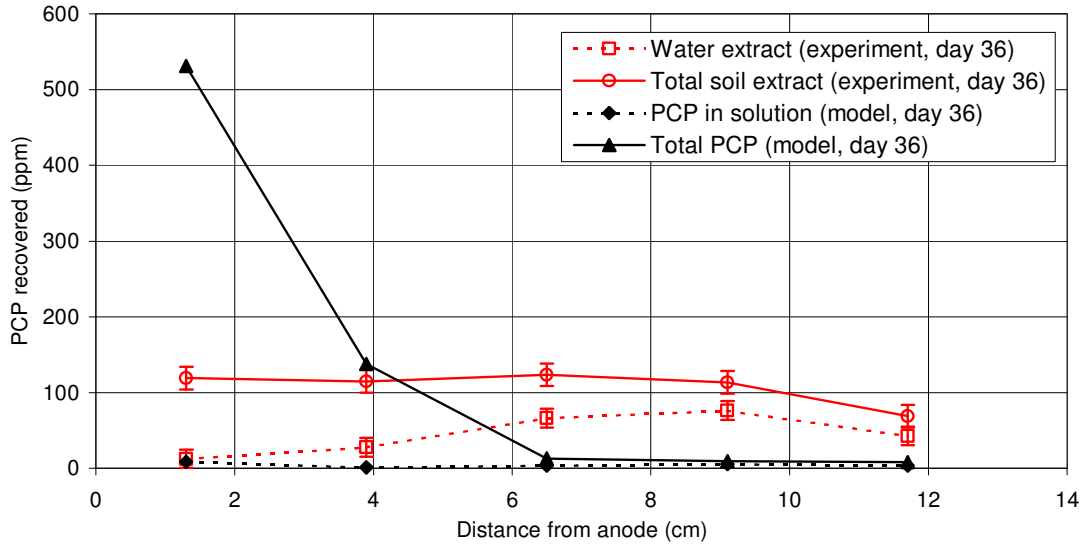

Figure 5-11: PCP distribution comparison between PI13u and model (day 36)

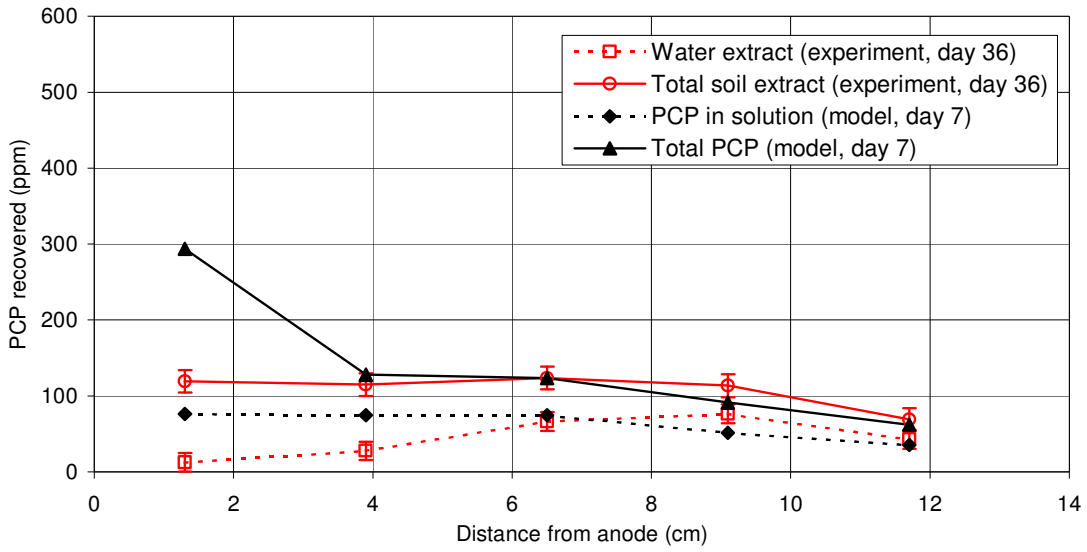

Figure 5-12: PCP distribution comparison between experiment PI13u (day 36) and model (day 7)

The behaviour that was seen in experiment PI_20 (disappearance of PCP near the anode) was investigated using the model (Figure 5-13), and can be compared to the model without anodic pH control (shown on Figure 5-11). Although the presence of degrading bacteria was not included, the implementation of pH control at both electrodes led to a significant increase in the removal of PCP to the anolyte fluid. In the model of PI_13u (no pH control at anode), approximately 2% of the total PCP was found to be in the anolyte after almost 50 days. In the PI_20 model (pH control at the anode as well as the cathode), this was found to be approximately 37% of the total PCP present.

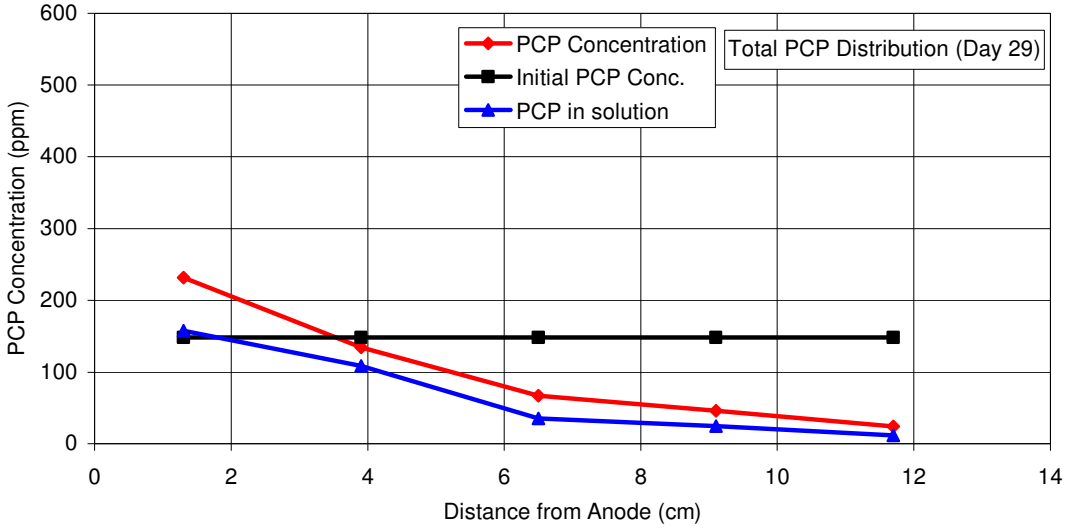

Figure 5-13: PCP distribution (day 36) in experiment PI_20 (modelled)

The graph showing change of PCP distribution with time (Figure 5-14) shows in more detail the movement of PCP due to the electric field. Movement of PCP towards the anode and a build-up at point 1 was seen, although after 20 days, the concentration at this position decreased slightly, whilst that at point 2 began to increase. This was due to protonation of PCP at point 1, and its subsequent diffusion towards the cathode, as well as further electromigration from the cathode end towards the anode.

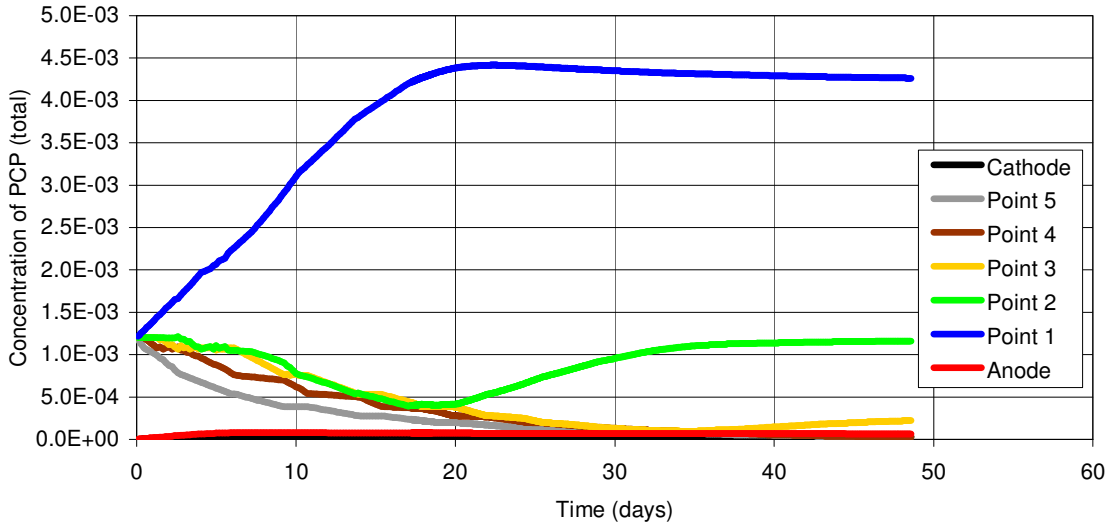

Figure 5-14: Distribution of PCP with time (experiment PI13u, modelled)

5.3. Conclusions

In both the modelling of pH changes and PCP behaviour, trends similar to those seen in experiments were evident. The calculated pH changes in electrode chambers and soil matched test data well, with very similar values arising at consistent times. The behaviour of PCP was less well modelled, however, although the trends were replicated. The proportion of protonated to non-protonated PCP increased with decreasing pH in both cases. In addition, PCP movement was seen towards the anode *via* electromigration, with electroosmotic water flow having little effect (similar to effects seen in experiments). However, the rate of movement of PCP was substantially greater in the model, with movement after 7 days being comparable to that after 36 days in experiments.

The model therefore backs up the experimental results in terms of pH change and contaminant behaviour, with the hypothesis of pH affecting the state of PCP (and therefore its sorption) being confirmed.

6. Analysis of Results

6.1. Critical Analysis of Experimental Performance

Preparation of samples for experiments was consistent throughout the project. The contamination procedure worked well, and generally satisfied the required criteria, in that it gave a sufficiently uniform distribution of PCP whilst allowing contamination of a pre-prepared soil specimen. The use of the sodium salt of PCP was justified in that it allowed the uniform contamination of ready-prepared samples in this way. Protonated PCP could also not be dissolved sufficiently to allow a high enough level of contamination (20ppm could be achieved, instead of the required 100ppm or thereabouts – results not presented). Inoculation of bacteria proved to be more problematic, with the number inoculated being more variable than hoped. In one or two cases, the number was much less than required. The target level of inoculation for earlier tests (experiments PI_2, PI_3, PI_4, PI_5 and API_1) was $1x10^6$ cfu / g dry soil. However, experiments with this number of bacteria did not always show degradation, possibly due to low survivability in some cases, so it was decided that this number was too low. Experiment PI_7 showed that there was little degradation of PCP in cartridges or flasks under these conditions, whilst the bacteria themselves died off within three weeks. From experiments PI_13i onwards, the target was $1x10^8$ cfu / g dry soil and degradation was seen in each of the later tests (although inoculated numbers were still variable).

The initial (large) apparatus design was used for experiments A_4 and PCP_1 to PCP_5. Several problems were encountered in the operation of these tests that made design changes necessary. The major design flaw was the difficulty in sealing the soil chamber from the external electrolyte fluid. As the cartridge of soil effectively sat in a tank of this

fluid, it was found that, despite efforts to seal this chamber (apart from allowing water flow *via* the membranes themselves), maintaining a seal was impossible. Consequently, these experiments were performed at a saturation level much above 70%. These experiments, however, did not involve inoculated (aerobic) degrading bacteria, and so there was no hindrance of any of the effects. The increase in moisture content and consequent increase in water-filled pathways may well have improved the transport of PCP in these experiments. The membrane used in these tests (Vyon) possibly allowed too much flow once saturated, and exacerbated the wetting of the soil.

The redesigned apparatus used from experiment P_1 onwards proved to be substantially more robust. As the electrode chambers were self-contained, water was retained within them and did not permeate the soil very easily, apart from through the membrane itself (the Vyon membranes were replaced with Daramic). On one or two occasions, a high pH in an electrode chamber led to the membrane stiffening and pulling away from the perspex face to which it was affixed. This allowed water to seep out, either into the soil or out of the apparatus entirely, but these leaks were noticed quickly and were easily fixed. No experiments were affected by this behaviour. Other than this, there were no major problems experienced with this apparatus design.

Control of pH was applied to the electrolyte fluids in many experiments. Initially, this was in the form of manual addition of suitable chemicals. This, however, proved unsatisfactory, as the pH rapidly returned to its former levels (a prime example is given in Figure 4-11). Automatic control was introduced, with fluid pumped into a control chamber before flowing back to the electrodes under gravity. This was initially unsuccessful, as small diameter tubing was employed, which had a high resistance to flow under gravity due to surface tension, and there would be a cyclical effect of fluid

building up before allowing flow. This was corrected with larger diameter tubing and there were no further problems. Control of the pH at both electrodes implemented by mixing of anolyte and catholyte (experiments PI_18 and PI_20, as in Lee *et al* (2000)) were successful in these terms, with little change in pH noted over the duration of testing (Figure 4-14). Similarly, a regularly reversed current used to prevent pH changes was successful, as shown in experiment PI_22 (Figure 4-15).

6.2. Variability of Data

The majority of the data presented in Chapter 4 (Experimental Programme and Results) are in good agreement with the overall patterns that are described in the remainder of this chapter. There are, however, several examples where presented results are not in accordance with this, and are otherwise difficult to explain. In all aspects of this work, care was taken to ensure that consistency was maintained in areas such as sample preparation and contamination (as discussed in Chapter 3 - Experimental Apparatus, Materials and Methods). Because the majority of experiments involved the use of living organisms in soil, it was accepted that there would be an unavoidable degree of variability in results. Each experiment was therefore performed in triplicate, with accompanying statistical analysis. The chances of variability were therefore minimised.

6.3. Effects of Electrokinetics on Soil

The application of an electric field to soil has had several major effects.

6.3.1. pH

As explained in section 2.1, the application of an electric field across a soil specimen leads to electrolysis of water at both electrodes, creating a current. The production of acid at the anode and hydroxyl ions at the cathode leads to a pH gradient across the soil as

these ions migrate into the soil mass (Açar and Alshawabkeh, 1993; Eykholt and Daniel, 1994). In the current project, typical values obtained in the electrode chambers (without pH control) were pH2 at the anode and pH12 at the cathode, as seen in experiment P_1 (Figure 4-10), amongst others.

Experiment PCP_1 showed that the control of pH in the cathode chamber allowed electroosmotic flow to persist for a greater length of time than would otherwise have been possible (Figure 4-20). It has been previously noted (Probstein and Hicks, 1993; Eykholt and Daniel, 1994) that precipitation of metals in a region close to the cathode was possible due to the formation of insoluble metal hydroxides in the high pH zone. In PCP_1, a white powdery substance was noted on and around the membrane at the cathode end, indicating that a similar process may be occurring in this case. It may be that a significant amount of such a precipitate would lead to blocked pores and a certain level of cementation of soil particles, thereby reducing fluid flow through the soil. The apparent removal of the problem with the addition of acid lends further weight to this argument. In order to prolong electroosmotic flow, and therefore maintain this force on the soil constituents for as long as possible, pH control was employed at the cathode in many experiments.

Three plots are presented (Figure 6-1, Figure 6-2 and Figure 6-3) showing changes in pH in the soil with time in a number of experiments. Data from control soil specimens has been subtracted from that from electrokinetic specimens, giving an indication of the effect of the electric field on a single line. Each graph represents a separate point along the specimens (anode end, centre and cathode end). These pH values in soil were measured in water extracts and so whilst they did not give the actual pH value in the soil pore fluid, they provided an indication of changes.

These plots are a comparison between different types of experiment, and show the different effects of pH control and electric field. Whilst they are plotted against time, it is the behaviour of the pH that is important, rather than at what time changes occurred. A good example is experiment PI_18, in which pH changes were only seen after the electric field was switched on, after 66 days, but in which the changes seen can be compared to those that occurred at an earlier time in other experiments. Therefore, experiments are grouped and colour-coded according to the type of testing regime. Experiments with a 10mA constant current applied and no pH control at the anode (PI_13u, PI_13i and API21) are represented by black lines. Experiments with pH control at both electrodes (due to electrolyte mixing) are shown as blue lines, and the experiment with pH controlled by a reversing current (PI_22) is shown as a green line.

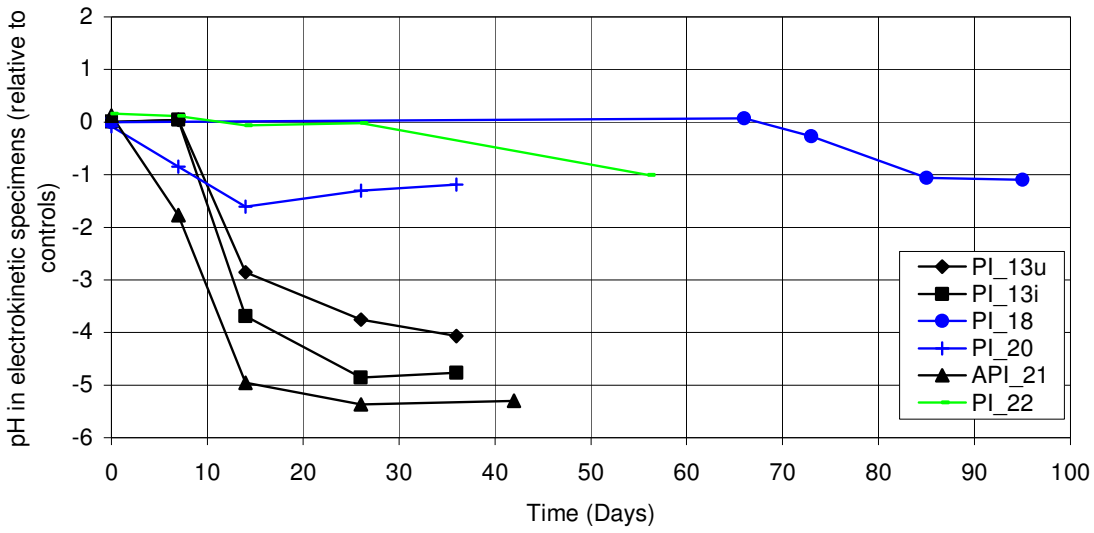

Figure 6-1: Variation of pH with time at position 1 (anode end)

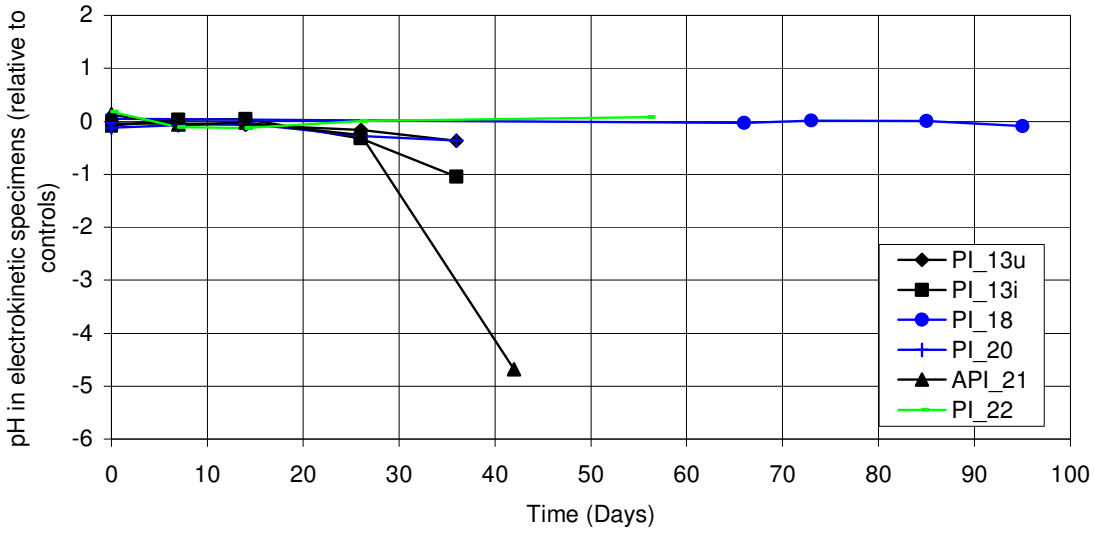

Figure 6-2: Variation of pH with time at position 3 (centre)

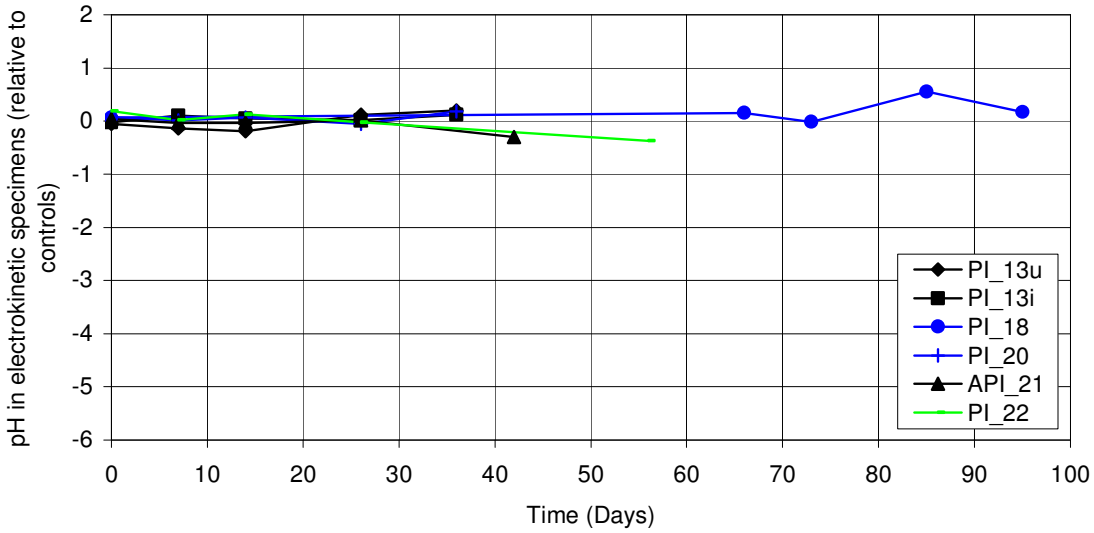

Figure 6-3: Variation of pH with time at position 5 (cathode end)

Constant current experiments show consistent behaviour at all locations, with large decreases in pH (of up to 5 pH units) at the anode and no effect at the cathode. The front

of hydrogen ions moving into the soil can be seen to have reached the centre of the soil specimens by around 30 days in these tests (Figure 6-2), as a pH decrease occurs.

Control of pH by electrolyte mixing was successful in both cases as a 10mA constant current only produced decreases in pH (relative to controls) of around one pH unit at the anode end, compared to five units without this control. No change was seen at the cathode end. Control of pH by current showed similar behaviour.

Whilst pH changes were again seen in the electrode chambers when a pulsed or low current was applied (experiments PI_5, PI_14 etc), evidence is presented later to show that these changes were unlikely to have been transmitted to the soil in the same way as experiment PI_13u, for example.

The mobility of hydrogen ions (speed in a field of unit strength) is 3.63×10^{-7} $m^2 s^{-1} V^{-1}$ (Atkins, 1990). With a typical voltage across the soil of 15 Volts, a field of 115V/m was applied, which should have led to a velocity of 4.2×10^{-5} m/s, or 3.6 metres per day (far greater than that seen in any test). It can therefore be concluded that passage into and through the soil is hindered by the soil itself (possible factors being tortuosity, sorption or chemical buffering) or by the membrane between electrolyte and soil. The reaction of soil to the presence of acid is shown in Figure 4-4, and indicates that approximately 1.7×10^{-4} moles of H^+ ions would be buffered by a dry gram of soil. At a current of 10mA typically used in these experiments, approximately 1.04×10^{-7} moles of H^+ ions are produced each second (enough to saturate two grams of soil per hour). This would lead to complete acidification of the soil in less than ten days; therefore, there must be a further sink for hydrogen ions. It is known that there is a certain level of chemical buffering in the soil, which may account for a large proportion of this.

6.3.2. Moisture Content

Moisture content profiles measured at the end of testing in a number of experiments are presented on Figure 6-4 (showing the difference between electrokinetic and control specimens, as with pH in the previous section). The same colour coding for different groups of experiments is employed here as was for changes in pH in section 6.3.1, but with one new group. Experiments with a constant 10mA current and cathodic pH control exhibited either no change or a decrease in moisture content across the specimens. Pulsed or low current tests (for example experiments PI_14 and PI_17, represented here by red lines) showed a significant increase in moisture content. Constant current tests with pH control at both electrodes resulted in a large, significant increase in moisture content. Experiment PI_22 showed no change in moisture content.

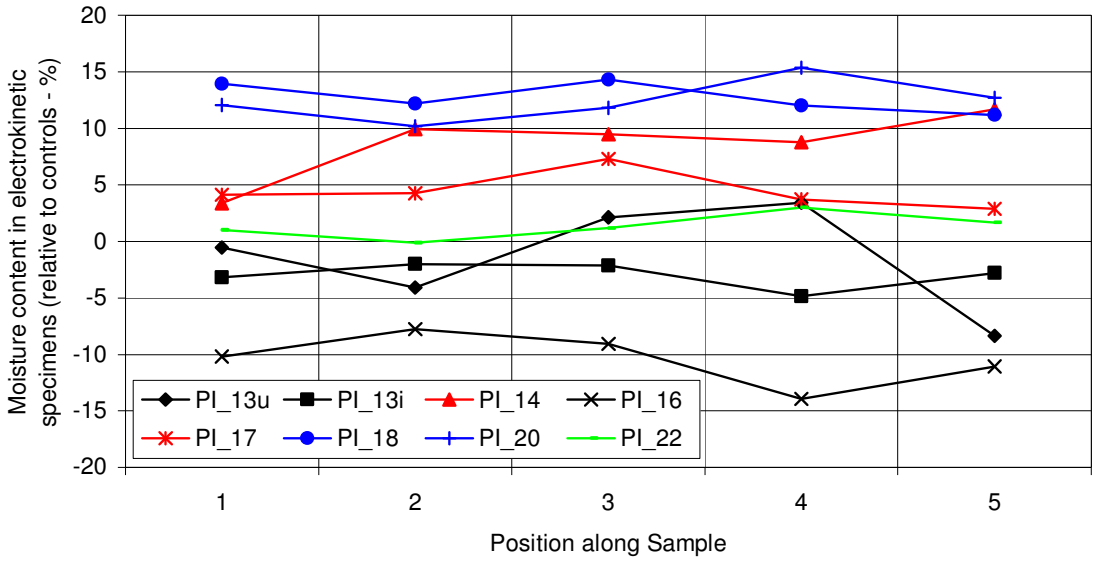

Figure 6-4: Variation in moisture content across soil specimens at end of testing

It was expected that the presence of electroosmotic flow in the unsaturated soil specimens used would lead to an increase in the moisture content, due to the suction exerted by the soil in this state. However, this eventuality was only seen in a few experiments, especially PI_18 and PI_20. In both cases, the soil was found to be near-saturated, with

increases of 10 to 15% in moisture content measured across the specimens. It is thought that the low permeability, hydrophobic properties of the Daramic membranes used in this work would have allowed electroosmotic flow to be drawn out of one chamber, but would not have permitted it into the other as there was a preferential flow path by simply leaving the apparatus (the soil specimens were not sealed to keep water in). It is therefore suggested that water entered the soil *via* electroosmosis, but did not exit the soil in the same way. Eventual saturation led to large amounts of fluid being collected beneath the electrokinetic specimens in both these experiments.

Experiments PI_14 (pulsed current) and PI_17 (low current) had a similar total charge passed through them. In both cases, the moisture content with an electric field was increased over that in control samples, significantly so in the case of PI_14. Again, it is thought that this is due to electroosmotic flow wetting the soil. Increases were not as great as those seen in PI_18 and PI_20, being of the order of 5-10% greater in electrokinetic specimens, presumably because of the lower power input and therefore lower electroosmotic flow.

In constant current tests (experiments PI_13u, PI_13i and PI_16), the expected increase in moisture content (due to soil wetting by electroosmotic or diffusion effects) was not seen. In PI_13i and PI_16 there were decreases (significant, in the latter case) whilst PI_13u showed little change relative to controls. The major difference between these experiments and those discussed previously was the large pH changes that occurred within the soil (especially at the anode end). As is shown in Figure 4-6, the electrical potential around clay particles (measured as the zeta potential) can increase as the pH drops, possibly changing from negative to positive at very low pH. The electroosmotic flow will then reverse, and flow towards the anode (see section 2.1). If the pH is

sufficiently low due to an applied electric field, then this effect may be seen in the region of soil closest to the anode once sufficient acidity has built up in this region. The soil specimen would then be divided into two segments, one with flow towards the anode, the other with flow towards the cathode. No water flow would then enter the soil, and water from the soil would be moved *via* electroosmosis towards the electrodes, drying the bulk of the soil, as shown in Figure 6-5. A drying effect such as this may explain in part the cessation of electroosmotic flow seen in early experiments that could not be overcome with cathode pH control. A higher suction induced by the removal of water would mean that less water would flow, until the force imparted by electroosmosis would be negated entirely by that of suction.

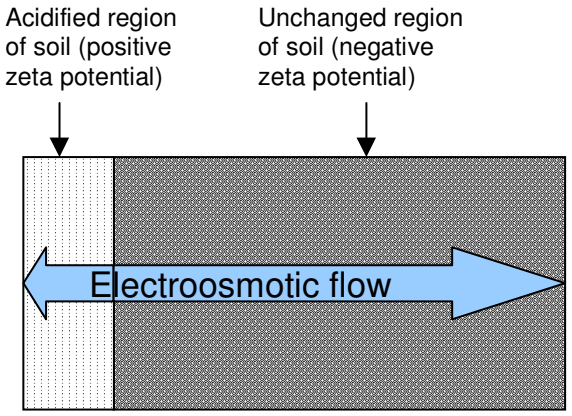

Figure 6-5: Possible schematic of electroosmotic water flow in acidified soil

An undergraduate project performed at the same time as this work has shown further evidence for this to be the case (Tate, 2003), with pre-acidification of the soil at the anode used to prevent water flowing into the soil whilst an acidic region was created. The moisture content increases noted in other experiments where pH was not expected to be important also show that this pH change is a dominant effect.

Analysis of Results

Experiment PI_22 was designed to prevent pH build up (by reversing the current regularly) and to remove the possibility of moisture content changes (by having no external fluid systems). No moisture content changes were seen over the testing period.

6.4. Interaction of Model Contaminant with Soil

Results of adsorption isotherm experiments (section 4.4) describe the properties of PCP in soil under a range of conditions. The removal of organic matter from soil resulted in a large reduction in the proportion of PCP associated with the soil (see Figure 4-35 and Figure 4-36). For example, with an initial PCP solution concentration of 700µm, 65% (±4% (1 s.d.)) sorbed to the soil with organic matter present. When the organic matter was removed, this percentage was reduced to 25% (±1% (1 s.d.)). This trend was repeated at all other concentrations of PCP solution. This strongly suggests that PCP will preferentially sorb to organic matter rather than soil particles themselves. The technique used to remove organic matter (hydrogen peroxide) is known to be less than 100% efficient (Head, 1980b) and so it is possible that if all organic matter were removed then more PCP would remain in solution.

The addition of acid to PCP solution and soil led to a greater proportion of PCP associated with the soil. Again comparing conditions with 700µm PCP samples, an average of 72% (±1% (1 s.d.)) of the PCP sorbed to soil with 50µl acid per sample, rising to 78% (±2% (1 s.d.)) with 100µl (compared to the 65% (±4% (1 s.d.)) without added acid). This may be explained with the help of the following equation, showing the equilibrium between dissociated and undissociated PCP.

$$C_6Cl_5OH \Leftrightarrow C_6Cl_5O^- + H^+$$

Analysis of Results

As the concentration of hydrogen ions is increased with the addition of acid, the equilibrium will shift to the left with the creation of more undissociated, largely insoluble PCP. This has been seen to preferentially associate with soil matter rather than remain in solution. The addition of alkaline sodium hydroxide solution to the samples led to a large reduction in sorbed PCP (47% (±4% (1 s.d.)) sorbed to soil, compared to 65% (±4% (1 s.d.)) without sodium hydroxide). This reduced the concentration of hydrogen ions through combination of OH^- and H^+ to form water. The equilibrium above then would have shifted to the right, with the subsequent creation of the more soluble non-protonated PCP.

Figure 6-6 clearly shows the effect of the addition of acid or alkali, with increasing sorption as the concentration of hydrogen ions increases. These data are for a PCP solution of 700μM. The current work is concerned with the bioavailability of this particular contaminant, which is clearly affected by changes in pH. A more strongly sorbed contaminant will be less accessible to degrading organisms than one in the pore fluid. Therefore, a decrease in pH would have the effect of reducing the bioavailability of the PCP (as well as increasing the toxicity of the soil environment to the soil micro-organisms). This has important consequences for many of the experiments presented here.

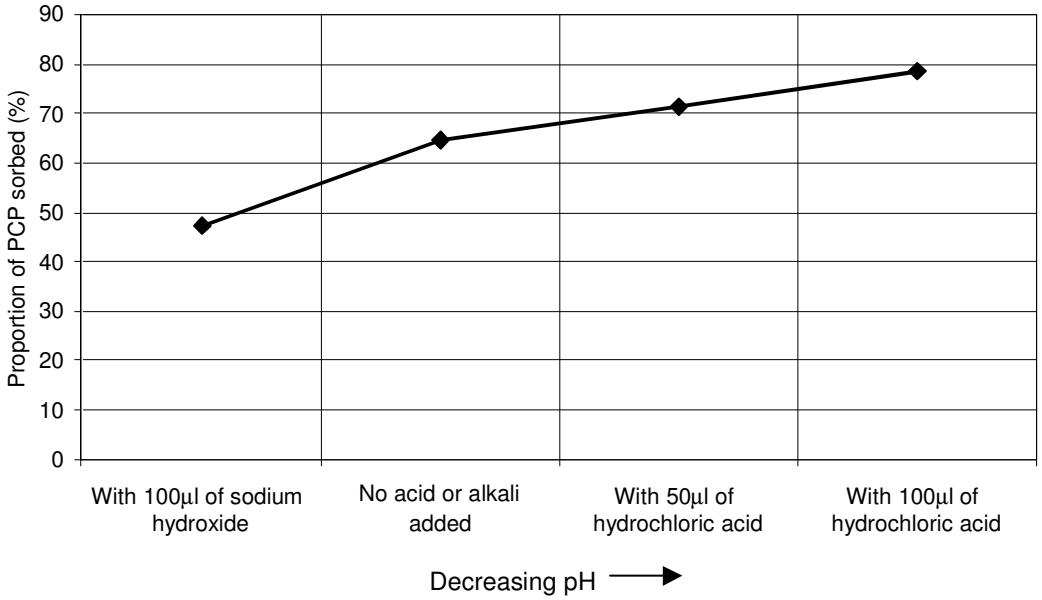

Figure 6-6: Effect of pH changes on PCP sorption

Sorption and desorption kinetics were also investigated using similar techniques. It is shown (Figure 4-37) that the sorption of PCP to soil is a rapid process. Within one hour, 40% (±1% (1 s.d.)) of the total PCP (approximately 60% of total sorbed PCP) had associated with the soil matter. With the addition of acid (Figure 4-38), sorption was speeded up significantly, with all sorption occurring in the few minutes before the first reading could be taken. Desorption kinetics (shown in Figure 4-39) are again rapid, with almost all desorbing PCP doing so within the first hour.

6.5. Interaction between model contaminant, degrading bacteria and soil

Experiment PI_7 was an investigation into the survivability of *Sphingobium* sp. UG30 in soil, with and without the presence of PCP. In general, the effect of PCP was to reduce the numbers of these bacteria found in soil (Figure 4-41), relative to uncontaminated specimens. Both flasks and cartridges containing 10^6 cfu / g dry soil (initially) had few culturable cells remaining after 22 days testing. This goes some way to explaining the

lack of PCP degradation seen in these tests (Figure 4-40). A significant decrease in culturable cells was also found with an initial density of 10^8 cfu / g dry soil in flasks, although in this case there was still a large number of cells remaining in the soil at the end of testing. PCP was degraded quickly in this latter experiment (approximately 80% was removed in two days), such that there was little remaining by the end of testing (approximately 10%). A comparison has been drawn up between cartridges and flasks containing 10^8 cfu / g dry soil by taking results from this experiment (in flasks) and also from control data from experiment PI_22 (section 4.8 – in cartridges), which had approximately $1.9x10^8$ cfu / g dry soil. Recoverable PCP data is presented in Figure 6-7. The difference is clear; in flasks, there was only approximately 20% of the PCP remaining after 5 days, whereas after 56 days in cartridges there was approximately 40% left. The effect of a more naturally structured soil (in cartridges) was to slow degradation, compared to flasks where soil, degrading bacteria and contaminant were homogenously mixed. Point inoculation into cartridges is likely to have been a major cause also, with a large proportion of the soil initially not containing inoculated bacteria. The unsaturated nature of the soil would have provided barriers to unconfined movement and growth of bacteria, as well as to any movement of PCP.

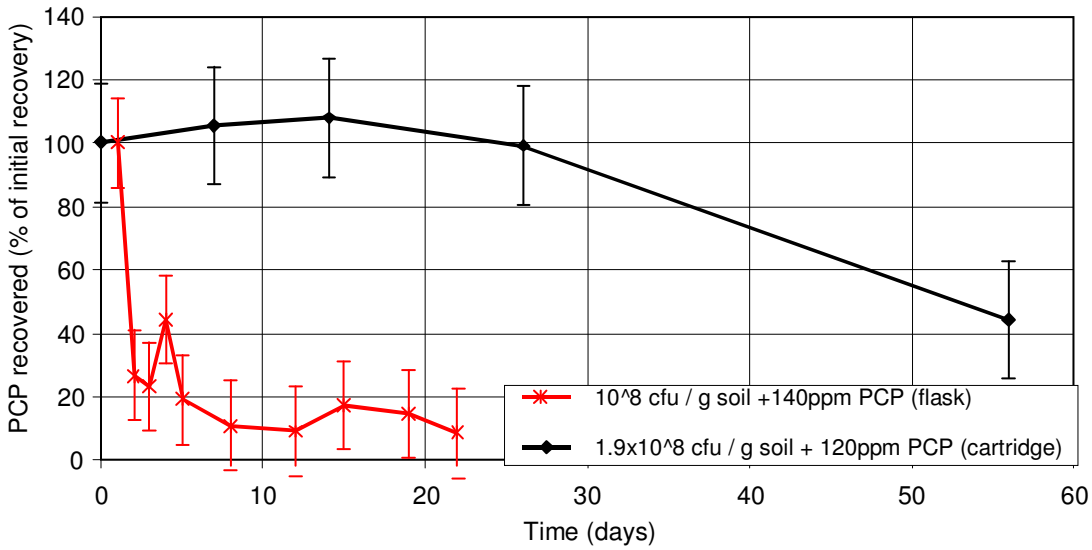

Figure 6-7: Comparison of PCP degradation in flasks and cartridge

6.6. Effect of Electric Field on Soil Microbial Populations

Experiment I_9 was performed specifically to investigate the effect of a constant current electric field (and consequent changes in soil chemistry) on naturally occurring microbes in the soil.

Physical effects on the soil in electrokinetic specimens included a change in pH similar to that seen in other experiments (with a decrease in pH at the anode, and little change at the cathode (due to pH control at this electrode)). Conductivity in the soil was also seen to increase, due at least in part to the migration of hydrogen and hydroxyl ions into the soil. The moisture content was not found to change over the 27 days of the experiment with an electric field; water amended samples did, however, show a slight increase.

Analysis of Results

Respiration of organisms in the soil, measured by carbon dioxide emission, is presented in Figure 4-44 and Figure 4-45 (day 7 and day 27 respectively). As the graphs show, it is difficult to draw any conclusions from this data; no trends are evident.

Bacterial and fungal counts are presented as log-transformed data ($\log_{10}$ (count+2)), which allows easier interpretation of results by reducing scatter. Effectively no changes in fungal counts were noted with time. However, over 27 days, bacteria at the anode end of the electrokinetic specimens reduced in number when compared to the controls. As the rest of the sample was unchanged, the prime candidate for this effect is the change in pH at this point. The smaller decrease in pH across the rest of the sample may not have been sufficient to affect the bacteria to an observable degree. However, these differences are small and overall there was little change with the application of the field.

Acidification of the soil or increased ionic concentration have been suggested as possible causes of further changes in bacterial community structure in this experiment (Lear, 2004). For example, species from the genera *Arthrobacter* and *Microbacterium* were found only in electrokinetic samples after the end of testing, whereas *Bacillus* and *Pseudomonas* were more common in control samples.

6.6.1. Dehydrogenase Testing

Dehydrogenase activity was analysed in experiments PI_13i, PI_14, PI_18, PI_20 and PI_22. Substantial differences between electrokinetic and control specimens were noted in most experiments. These can be seen on Figure 6-8, Figure 6-9 and Figure 6-10, which show the changes of activity in electrokinetic specimens (relative to controls) at the anode and cathode ends of the soil as well as at the centre. The terms 'anode' and 'cathode' do not apply particularly to experiment PI_22, due to the regular current

reversal. However, they have been employed here in order to differentiate one end from the other – the anode end is that where the anode was located at the start of the experiment. Colour coding is the same as for previous parameters (moisture content and pH), i.e. black lines – 10mA constant current tests; red lines – low power / pulsed current tests; blue lines – pH control at both electrodes; and green line – pH and moisture content control.

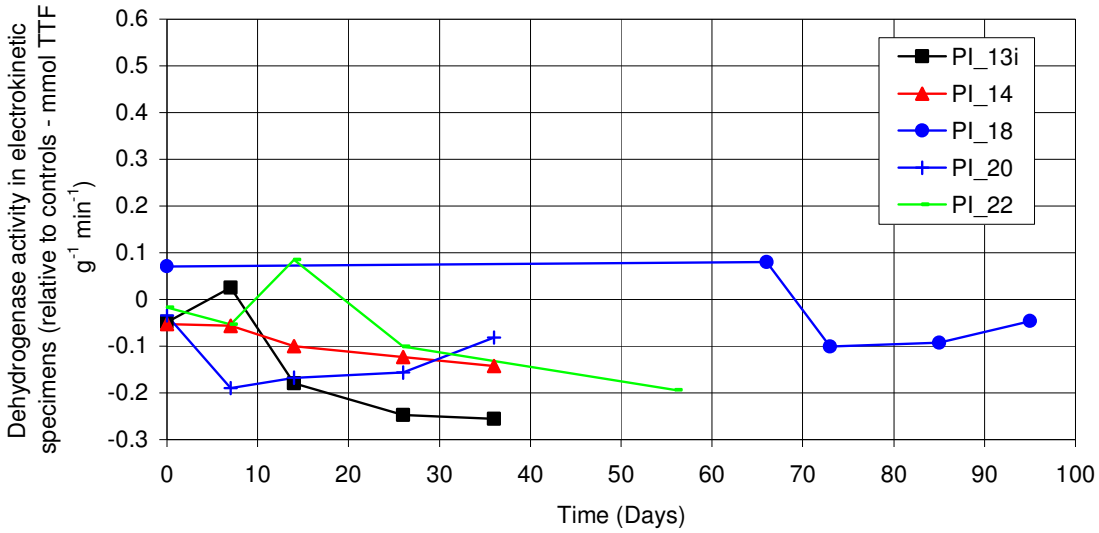

Figure 6-8: Dehydrogenase activity versus time at position 1 (anode end)

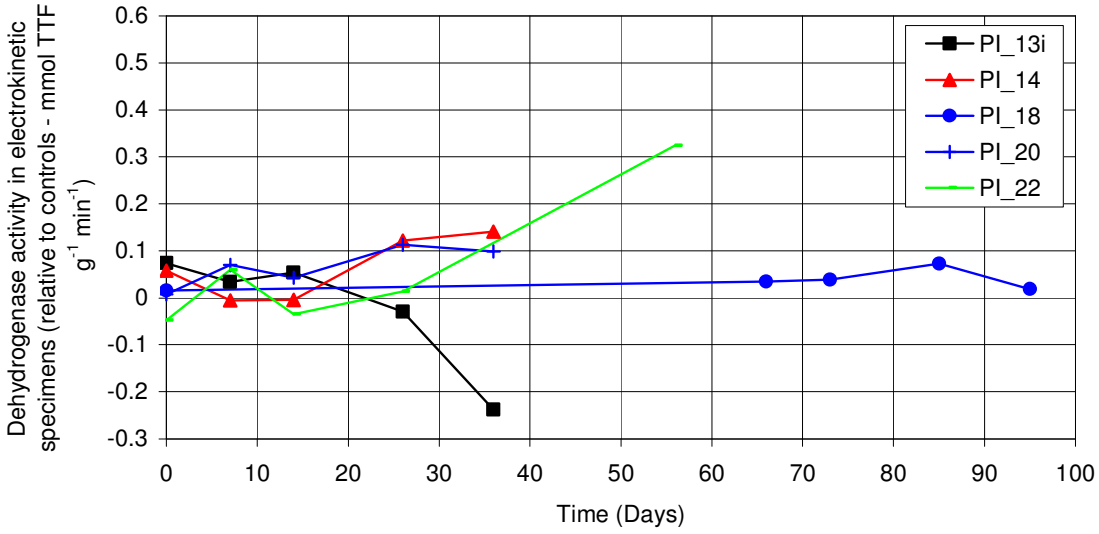

Figure 6-9: Dehydrogenase activity versus time at position 3 (centre)

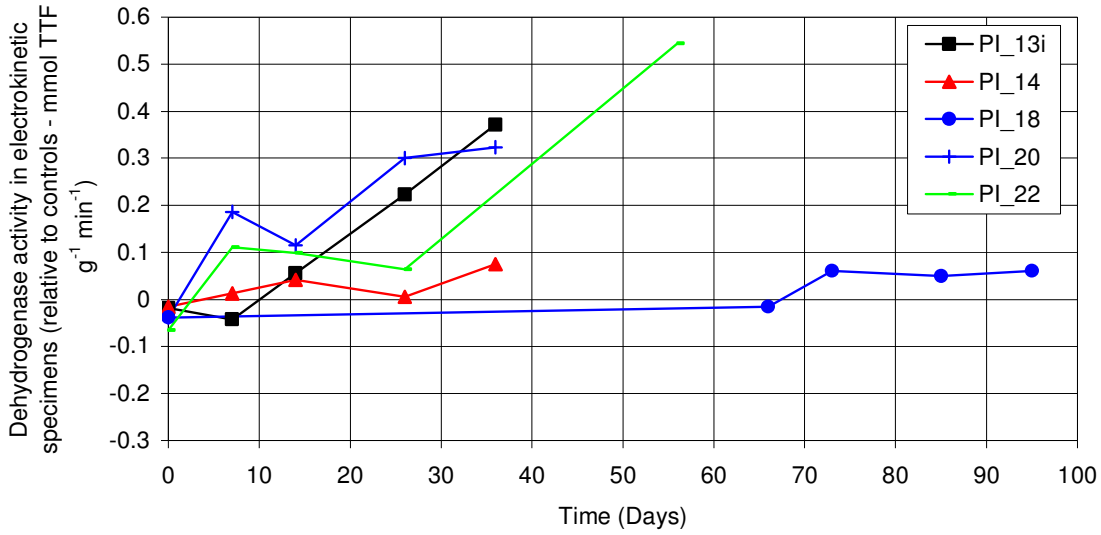

Figure 6-10: Dehydrogenase activity versus time at position 5 (cathode end)

A general decrease in dehydrogenase activity was noted in control specimens at all locations during the testing. It is expected that this was due to the toxic effects of the contaminant.

All experiments showed decreases in enzyme activity (relative to controls) at the anode end of the soil once an electric field was applied (at 8 days for PI_13i and 66 days for PI_18, otherwise from the start of testing). Enzymatic activity is known to be pH-dependent (Prescott *et al*, 1999), but in several cases (notably PI_18 and PI_20), little pH change was found at this location. It may be, however, that sufficient pH decrease occurred to cause these changes. The increase (in all tests) in activity at the cathode end of the soil due to an electric field is more difficult to explain. No large pH increases were measured at this location (due to pH control) in any of these tests. A range of different effects was noted at the centre of the electrokinetic specimens. Experiment PI_22 had a large increase in activity, whilst that in PI_13i decreased substantially. Only slight increases were noted in the remainder of the tests. The acid front moving through the soil

in PI_13i (illustrated in Figure 6-1 and Figure 6-2) was thought to account for the decrease in activity at the anode and centre positions, as there was a good agreement between their timing. However, pH was not thought to have played a role in the increases in activity at other points and in other tests. Large increases were noted at the cathode in PI_13i and PI_20, and at the centre and the cathode in PI22. At all these points, a large decrease in PCP concentration was noted with an electric field (due to either movement or degradation), and so it is thought that the subsequent reduction in toxicity may be important. However, such an effect was not seen at the anode in PI_22, where there was little pH change and little PCP remaining after testing – possibly even a small pH decrease could mask the reduction in PCP toxicity. In addition, PI_18 had very little change in pH, and a large reduction in PCP by the time the field was applied, but only a small increase in activity at the cathode was seen. It may be that the effect of the electric field, when not masked by changes in pH, is to somehow increase the accessibility of the PCP. As the current was reversed regularly in experiment PI_22, it might be expected that similar conditions would be seen at either end. This was clearly not the case, as the cathode and central activity at the end of testing were significantly higher than at the anode. This may be because, over the first day, the pH would decrease at the anode and increase at the cathode. Over the second day, these would be expected to change back to around pH7 due to the reversed current. On the third day, however, anode pH would again decrease and cathode pH increase. This process would be repeated until the end of testing, and so the average pH at the anode over the whole experiment would be below pH7, whilst that at the cathode it would be above pH7. It may therefore be that a more rigorous pH control (changing more frequently) would achieve a better result in terms of maintaining a constant pH, and possibly in terms of overall conditions for degradation.

Figure 6-11 displays dehydrogenase activity versus pH for all points from the displayed experiments. It shows that there is no distinct correlation between these parameters, apart from a low pH always corresponding to a low enzyme activity. There was found to be no correlation between PCP concentration and dehydrogenase activity (r^2=0.034), and only slightly greater with PCP water extract ('bioavailable' fraction - r^2=0.102).

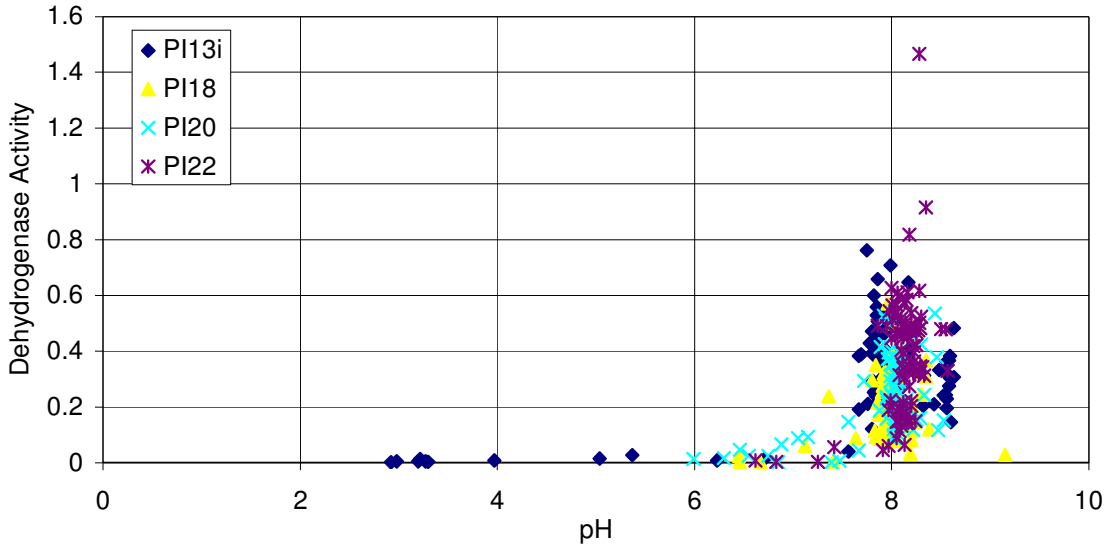

Figure 6-11: Variation of dehydrogenase activity with pH

6.7. Behaviour of PCP Under an Electric Field

Significant transport of PCP in soil has been achieved under a variety of conditions.

It should be noted that in no experiment was any significant amount of PCP discovered in any of the external fluid systems. In most tests the anode chamber became very dark with organic matter, and so testing for PCP was not possible using the techniques available (either scintillation or HPLC) as PCP would have been masked in both cases.

The effects of the electric field and biodegradation on the PCP within the soil can be seen on Figure 6-12, Figure 6-13 and Figure 6-14. Categories have been defined in the same

colours as used in previous sections of this chapter. The variation of total soil PCP concentration (relative to controls) for all radiolabelled PCP experiments is shown versus time, for three positions within the soil specimens (anode and cathode ends, and the centre). At the anode (Figure 6-12), there were few discernible trends in the behaviour of PCP with and without an electric field in the majority of experiments. Experiments PI_13i and PI_16 were both inoculated with degrading bacteria, and both exhibited an increased PCP concentration at this point over that in control specimens. At this location, a significant decrease in pH occurred, which would have been toxic to micro-organisms and also have led to reduced bioavailability of the contaminant (as explained in section 6.4). More degradation of the contaminant would therefore have been expected at this location in control specimens than with the field, as no pH change occurred in controls, and PCP movement towards the anode may have exacerbated this. Experiment PI_13u, similar to the previous two experiments but without inoculated bacteria, did not show significant degradation in either set of soil specimens. Whilst a decrease in the pH at the anode would have reduced the bioavailability of the contaminant in the same way, there was little degradation actually occurring and so the difference would not have been detected in this way. There appears to have been little PCP influx due to electromigration either. Constant current experiments (including PI_20) showed a gradual decrease in PCP with time at the cathode end of the soil. This was thought to be mostly due to movement of PCP towards the anode *via* electromigration, as there was no change in pH in this region in any test. Similar transport effects were also seen in non-radiolabelled constant current and constant voltage experiments (P_1, PI_2 and PI_3), and appeared in the mathematical model of these experiments (section 5.2). It is not known to what extent PCP transportation occurred due to association with organic matter a proportion of which was moved by the field, but it is not thought to be large as organic matter

movement was noted in pulsed current tests, which did not have evidence of PCP movement.

Little difference was observed between different positions in experiment PI_18. Despite the presence of a constant current, no evidence was seen of movement, most likely due to the low level of PCP remaining following the initial 2-month degradation period. In PI_20, the large reduction in PCP content at the anode and (later in the test) at the central location was thought to be due to movement of the contaminant from the soil. PCP may have remained primarily non-protonated, as the pH was controlled at anode as well as cathode, and therefore would have been more mobile and easier to extract into the electrolyte fluid. However, it is unknown why the removal of the PCP commenced at the anode rather than the cathode, if the contaminant was moving towards the anode. No PCP was recovered from the electrolyte fluid, but this was thought to be due to the presence of organic matter preventing PCP from being picked up with the optical techniques used. The mathematical model (Figure 5-13) agreed with the experimental data in that a significant proportion of the PCP was moved from the soil (and found in the anode chamber). This suggested that in the experiment, the PCP was in the electrolyte fluid but could not be detected. However, the movement of PCP was different in the model, with the lowest proportion of PCP remaining at the cathode end, rather than the anode as seen in the experiment.

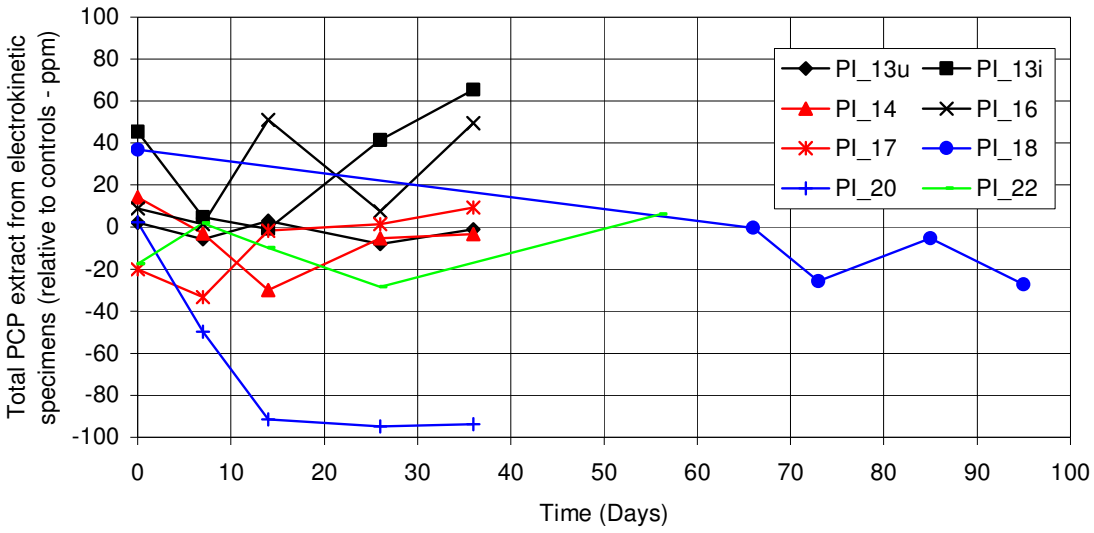

Figure 6-12: Total PCP extract versus time from position 1 (anode end)

Very little difference between electrokinetic and control specimens was observed in experiments with lower power consumption (red lines). This agrees well with information from other sources (e.g. production of $^{14}CO_2$ and dehydrogenase activity).

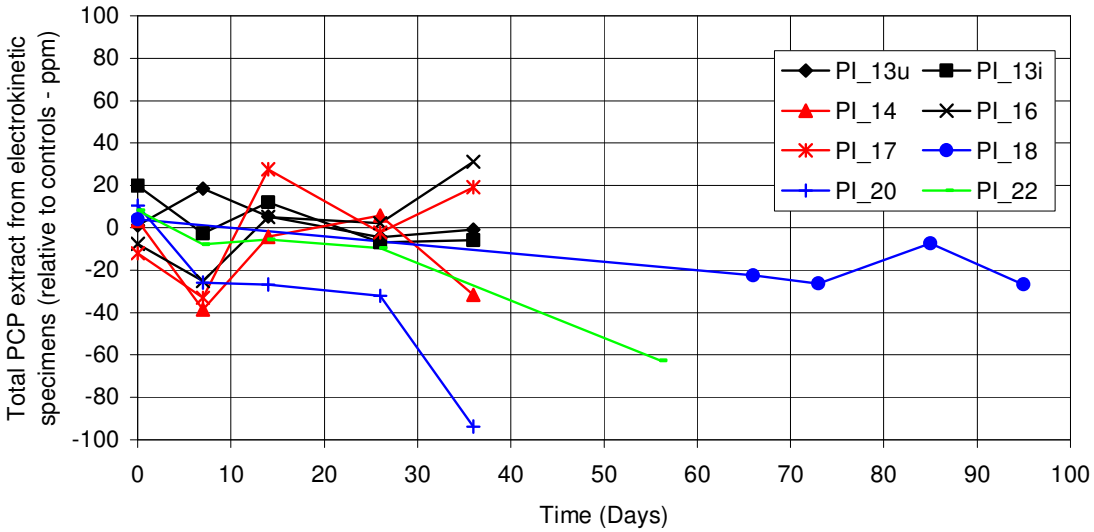

Figure 6-13: Total PCP extract versus time from position 3 (centre)

Analysis of Results

By the end of testing, there was a decrease in PCP concentration at the central location in experiment PI_22, when an electric field was applied. This was due to degradation of the contaminant – this effect was less marked at the electrodes, as most of the increased level of degradation took place away from the ends. For this experiment, it can clearly be seen in Figure 4-78 to Figure 4-82 that there is little apparent movement of the contaminant, and that changes in PCP concentration are not due to transport. Also, there was nowhere for the contaminant to be moved to, as there were no membranes or electrolyte fluids involved in this test. At the cathode end of electrokinetic specimens on day 26, there was an apparent decrease in PCP content, most likely due to a small amount of movement of the contaminant prior to this sampling time.

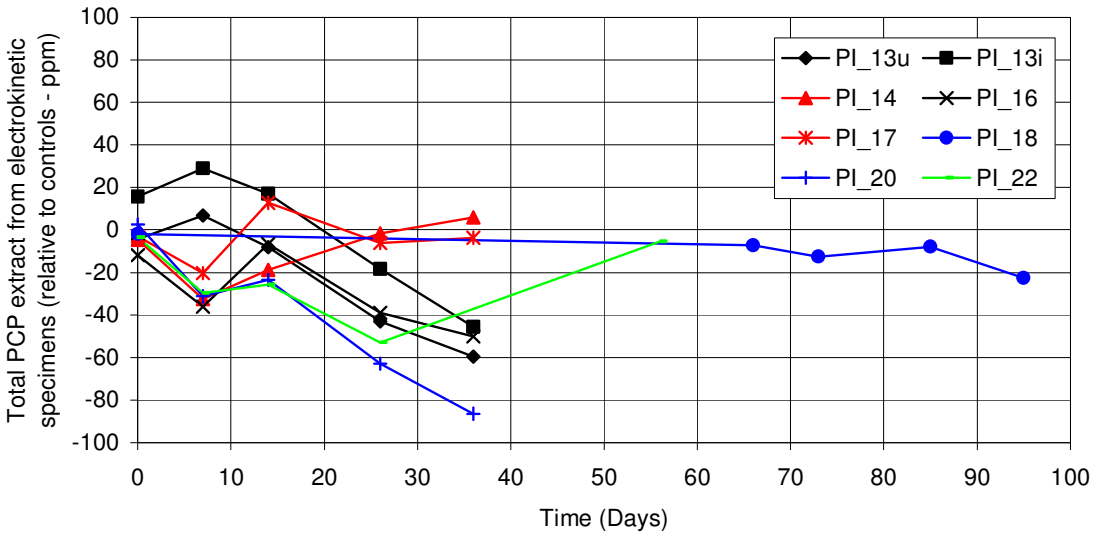

Figure 6-14: Total PCP extract versus time from position 5 (cathode end)

Further information on the fate of PCP and its chemical state within the soil has been obtained from sequential extractions with water and acetonitrile. Figure 6-15, Figure 6-16 and Figure 6-17 present PCP extracted with water, investigating the fate of the more accessible PCP fraction (that which is more likely to be available to degrading

organisms). The water extract from constant current experiments (black lines) and PI_20 showed a similar trend to the total extract at the cathode end, with a gradual decrease over time, most likely due to movement of the PCP *via* electromigration. However, the water-extractable fraction at the anode end quickly decreased in constant current experiments due to the influx of hydrogen ions and the subsequent formation of hydrophobic protonated PCP, which was much more strongly associated with the soil. This effect was also produced in the mathematical model, and evidence for this is presented in section 5.2.

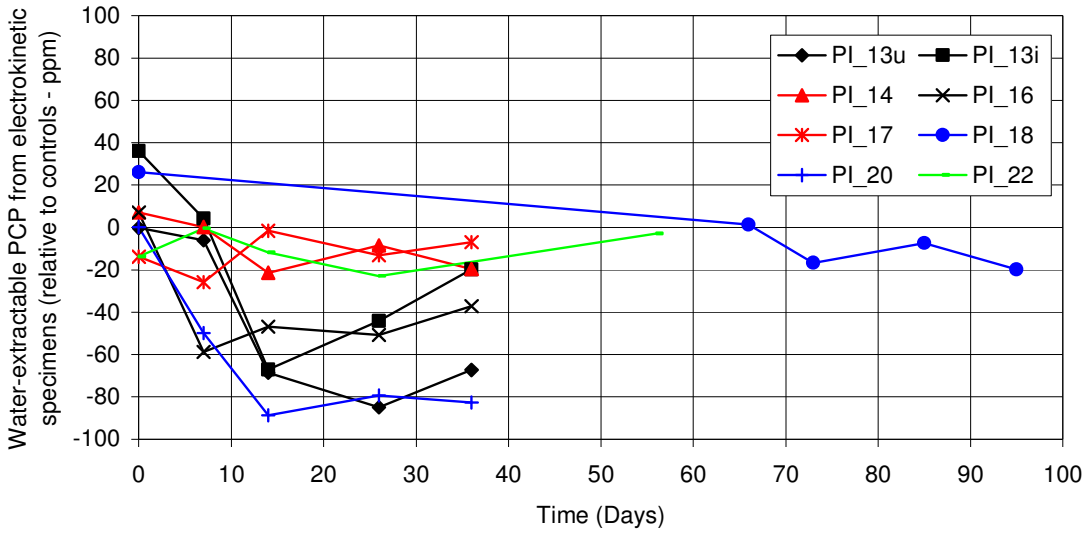

Figure 6-15: PCP water extract versus time at position 1 (anode end)

Other than the effects described above, observed trends are similar to those seen with the total PCP extract, as the water-extractable fraction is the most likely to be moved or to degrade. Therefore, any effects seen in the total extract are more likely to be due to transport or degradation of the water-extractable fraction. Low-power experiments again showed only small differences with and without an electric field, as did PI_18, possibly due to the reduced amount of PCP present by the time the field was applied. The

movement of PCP from the soil in PI_20 can also clearly be seen. Once more, PCP reduction seen in PI_22 cannot easily be explained by movement of the contaminant, as the distribution remained symmetrical across the soil samples between the electrodes.

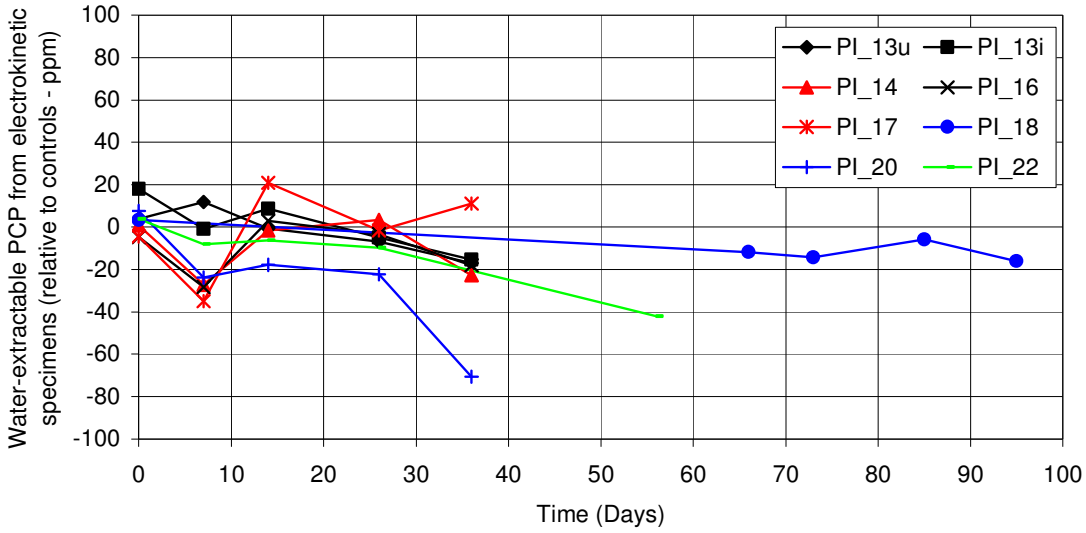

Figure 6-16: PCP water extract versus time at position 3 (centre)

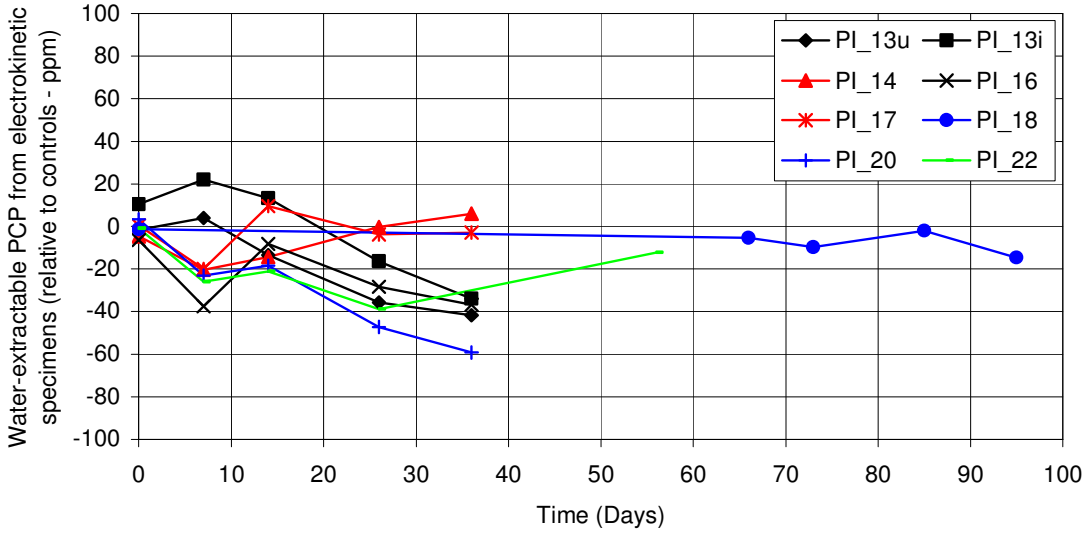

Figure 6-17: PCP water extract versus time at position 5 (cathode end)

Analysis of Results

Acetonitrile extractions (Figure 6-18, Figure 6-19 and Figure 6-20) represented the more recalcitrant fraction of PCP within the soil. The technique used was harsh enough to extract the majority of the PCP remaining after the water extraction. It was shown (in experiments PI_13u and PI_13i; Figure 4-87, Figure 4-88, Figure 4-91 and Figure 4-92) that only a small amount of PCP was not removed by these sequential extractions. Therefore, results from these extractions from all experiments are expected to provide an accurate picture of PCP distribution within the soil.

The only major difference between electrokinetic and control specimens in terms of the acetonitrile extract was seen in constant current experiments (black lines). With the electric field applied, a large increase in acetonitrile-extractable PCP was seen at the anode end and to a certain extent at the centre (by the end of testing, as the acid front reached this location). This coincided with the decrease in water-extractable PCP in the same experiments at the same locations, giving further evidence for the change of state of PCP from non-protonated to protonated in this region.

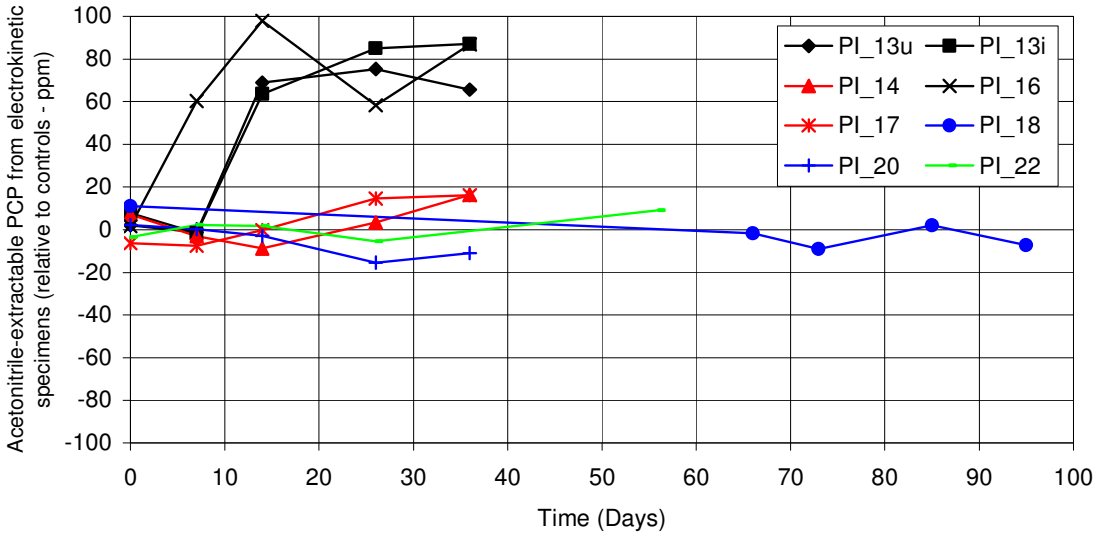

Figure 6-18: PCP acetonitrile extract versus time at position 1 (anode end)

In all other experiments, no significant change was recorded between control and electrokinetic specimens. This is further evidence that the behaviour of water-extractable PCP accounts for any changes seen in the total extractions, and that the acetonitrile-extractable fraction remains relatively stable (unless pH changes occur).

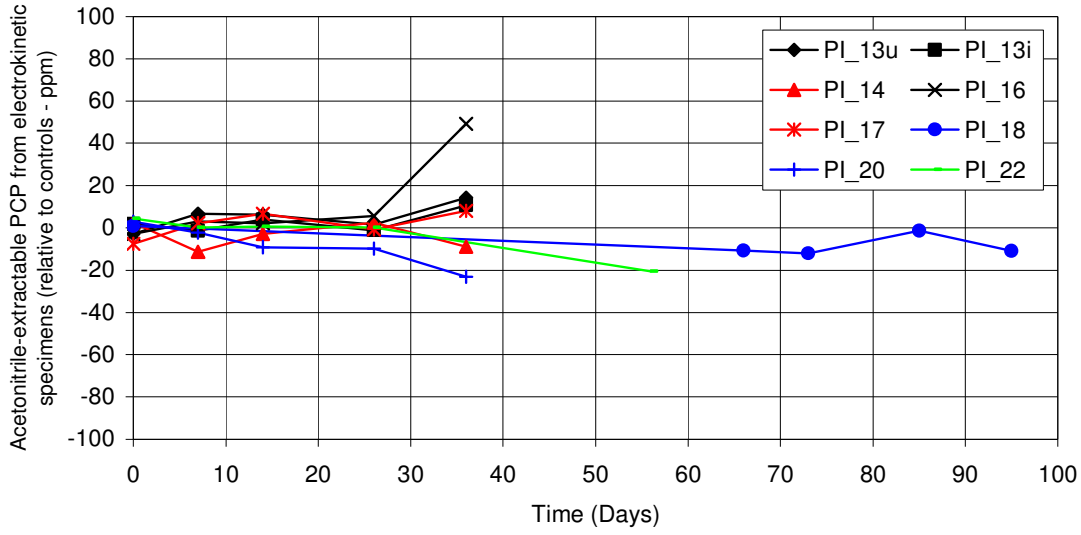

Figure 6-19: PCP acetonitrile extract versus time at position 3 (centre)

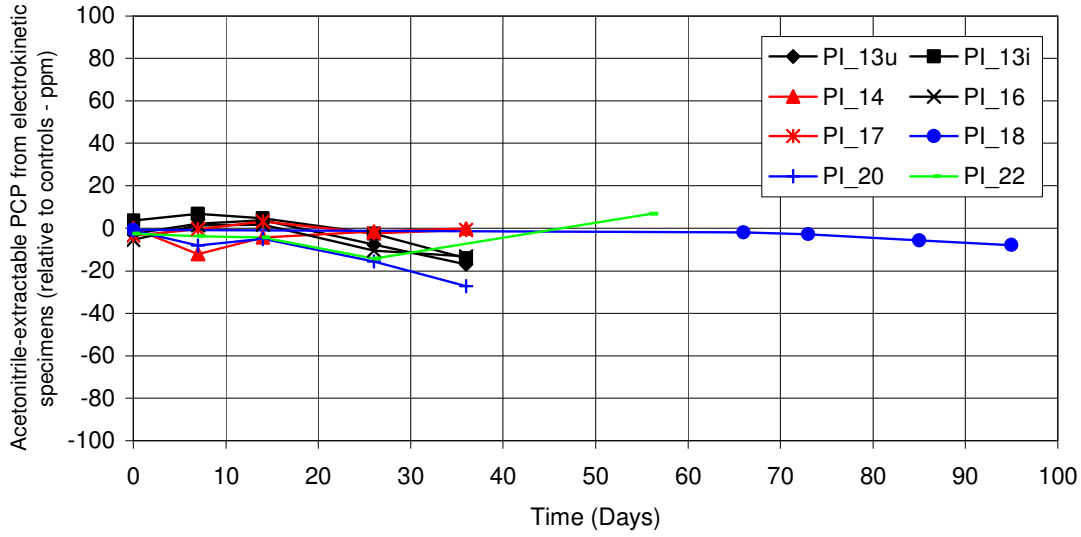

Figure 6-20: PCP acetonitrile extract versus time at position 5 (cathode end)

Figure 6-21 summarises the effect of pH on the distribution of PCP between water- and acetonitrile-extractable fractions. It can be seen that a decreasing pH will lead to a lower ratio of water- to acetonitrile-extractable contaminant.

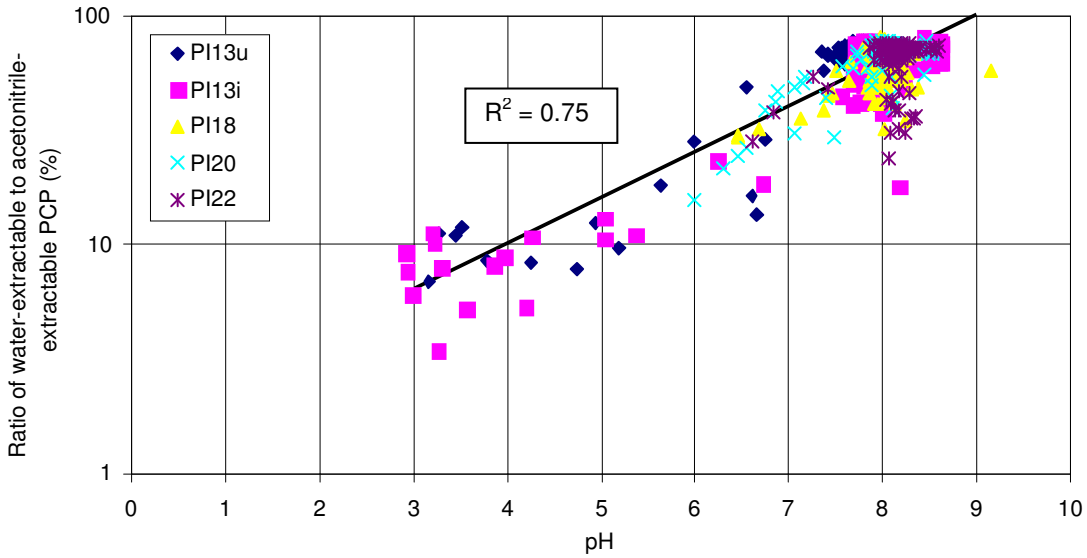

Figure 6-21: Variation of PCP extraction ratio (water to acetonitrile) with pH

6.8. Effect of Electric Field on PCP Degradation

Experiments with radiolabelled PCP (PI_13u to PI_22) provide much of the evidence for the conclusions drawn in this chapter, due to the relative ease of monitoring the fate of the PCP in these cases. Earlier tests without this facility provide useful backup evidence.

The best indication of the amount of biodegraded PCP is the trapped radiolabelled carbon dioxide (summarised on Figure 6-22), as this cannot arise in any way other than PCP degradation. The data on this graph are presented as in the previous section, with different coloured lines representing different groups of experiments. Each line shows the difference between the percentage of radiolabelled PCP recovered in electrokinetic

specimens and that recovered in control specimens. It is not expected that all of the CO_2 was trapped, as the soil and apparatus will all have acted as sinks to a certain extent. However, the proportion of PCP trapped in both electrokinetic and control samples will be largely the same, although a slightly lower recovery in electrokinetic samples might be expected due to electrolyte fluids acting as sinks. Therefore, electrokinetic results may be conservative.

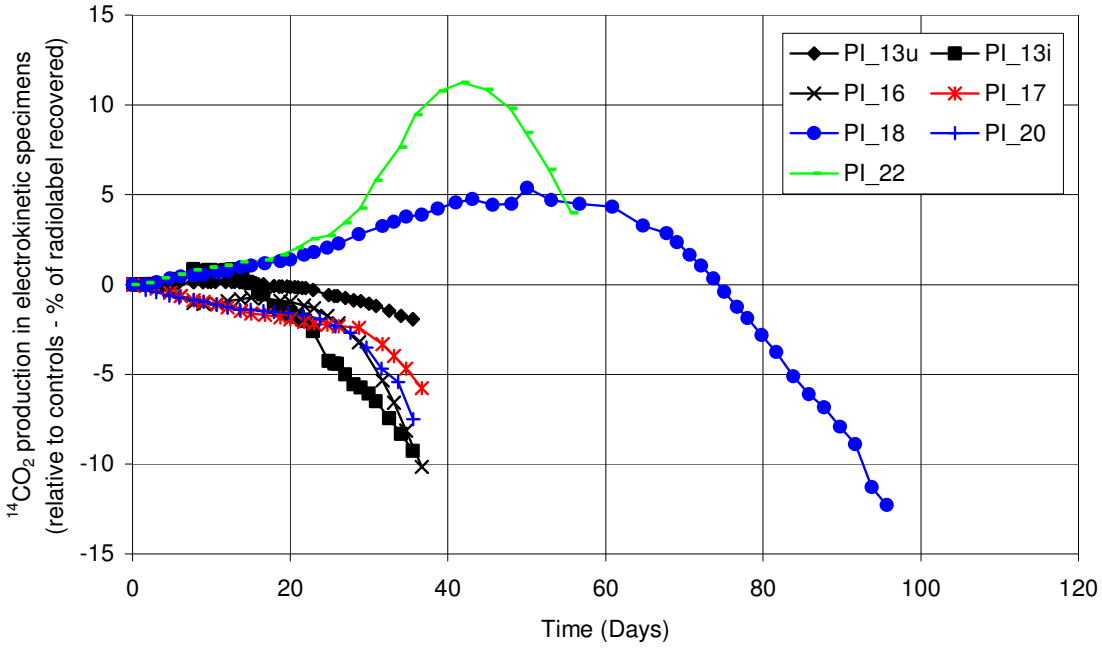

Figure 6-22: Cumulative $^{14}CO_2$ production versus time

The majority of experiments for which $^{14}CO_2$ was monitored showed less PCP mineralisation with an electric field rather than without, indicated by the downward curves on Figure 6-22. This was thought to be mainly because of chemical and physical changes within the soil affecting both the contaminant and the degrading organisms. The output of electrokinetic specimens in experiments with a constant current of 10mA and inoculated bacteria (black lines) was lower than the relevant control specimens by approximately 10% (of the total PCP input) by the end of testing. With a lower applied

current (PI_17), there was less difference (approximately 5%). Even without inoculated bacteria, there was still a greater level of mineralisation of PCP without the field. It is thought likely that there was a degree of natural attenuation, with background soil micro-organisms consuming the contaminant, but degradation in this case was approximately an order of magnitude smaller than with inoculated bacteria. In experiment PI_18, there was again an obvious reduction in the mineralisation of PCP when the field was applied from 66 days onwards. It is difficult to explain the difference in radiolabelled carbon dioxide output between the groups of specimens prior to the field being switched on, when the state of all soil specimens was considered identical. However, this difference is small when compared to the total output. The only experiment where an increased degree of PCP mineralisation was seen with the application of an electric field was PI_22. At its peak, electrokinetic specimens had seen degradation (and recovery) of approximately 11% more of the total PCP present than had control specimens. After this peak, which occurred at approximately 42 days, the reduction in the difference between the two sets of specimens was due to the low concentration of PCP remaining in electrokinetic specimens.

Figure 6-23, Figure 6-24 and Figure 6-25 show, respectively, summaries of total PCP, water-extractable PCP and acetonitrile extractable PCP recoveries for whole specimens versus time. All are presented as PCP recovery relative to that from control samples, as in previous graphs. These show similar trends to those seen in the previous section at individual sampling locations, but as they are taken over the whole soil specimens, then they may be compared directly with radiolabelled carbon dioxide emissions.

Total extracts (Figure 6-23) from 10mA constant current experiments (black lines) do not show a great deal of difference between electrokinetic and control specimens. However,

with an electric field, water extracts (Figure 6-24) can be seen to decrease and acetonitrile extracts (Figure 6-25) increase by a similar amount (thereby cancelling one another out in each case). These changes correlate well with the drop in pH at the anode that occurred at the same time – the resulting higher degree of protonated PCP is more strongly associated with the soil, leading to less coming off with the weaker water extraction. The pH decrease would therefore have had the dual effect of making the PCP less available to degrading bacteria, whilst making the soil conditions more toxic to the bacteria themselves. It is likely that these effects are the major causes of the reduced $^{14}CO_2$ output seen in these experiments (Figure 6-22).

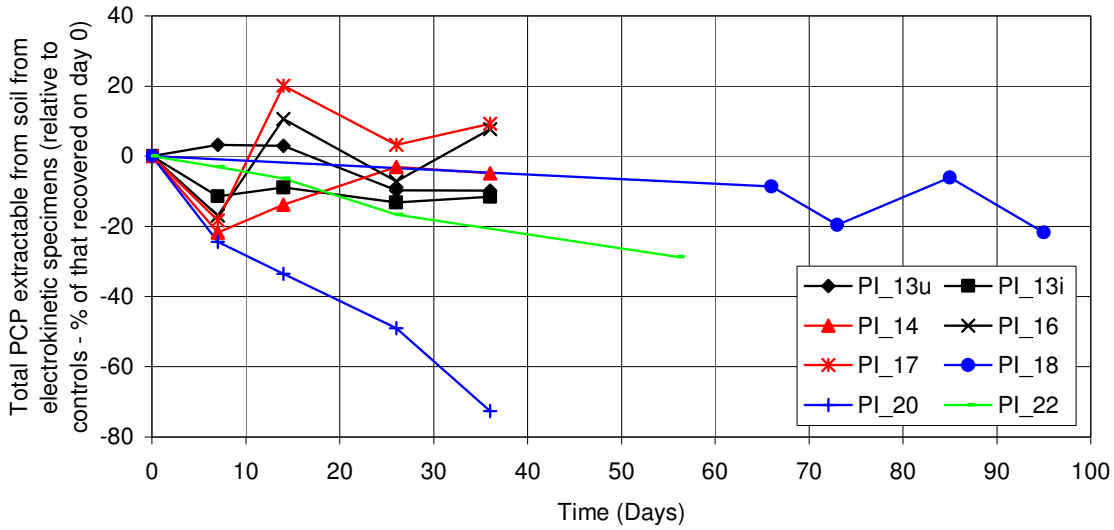

Figure 6-23: Total PCP extraction versus time

By the end of testing, both experiments that incorporated a lower power input (pulsed current or low current, denoted by red lines) showed very little difference between specimens with and without the field. There was a degree of initial variability, especially in the water-extractable fraction, and this is difficult to explain. However, it is apparent that a power input approximately ten percent of that seen in constant current experiments

did not have any significant effect on the degradation of PCP. The production of $^{14}CO_2$ in experiment PI_17 was slightly hindered by the electric field (Figure 6-22 and Figure 4-99), but not to any great extent. The lack of significant pH changes (as indicated by the relatively constant ratio of water- to acetonitrile-extractable PCP) would have been an important factor in preventing any major effects from occurring.

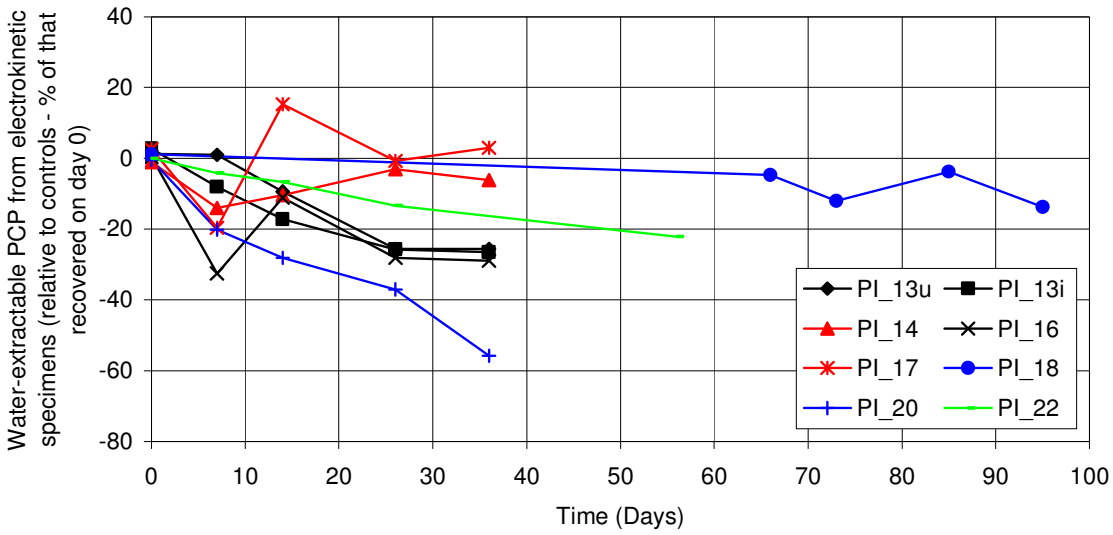

Figure 6-24: PCP water extraction versus time

An electric field also had very little noticeable effect on PCP extracted from soil in experiment PI_18. This is thought to be mainly due to a large proportion of the PCP having already been degraded before the application of the field. However, from the plots of trapped $^{14}CO_2$ (Figure 6-22 and Figure 4-103) it can be seen that the application of an electric current (at 66 days) led to a swift deceleration in PCP mineralisation. $^{14}CO_2$ emission from electrokinetic samples dropped to almost zero. As there was little change in pH at any point in the soil, then it may be that the large increase in moisture content caused this effect, as the soil quickly became heavily saturated. Aerobic degradation of the PCP would then have been hindered. In experiment PI_20, the removal of PCP from

electrokinetic specimens can clearly be seen once more, both in water- and acetonitrile extracts, and consequently in the total PCP recovery. Production of $^{14}CO_2$ with an electric field was significantly lower than in control specimens in this test, which indicates that the disappearing PCP was not being degraded, and was therefore being removed. It is difficult to say whether the moisture content increase had an effect or not, or whether it is simply the removal of the PCP that leads to less degradation.

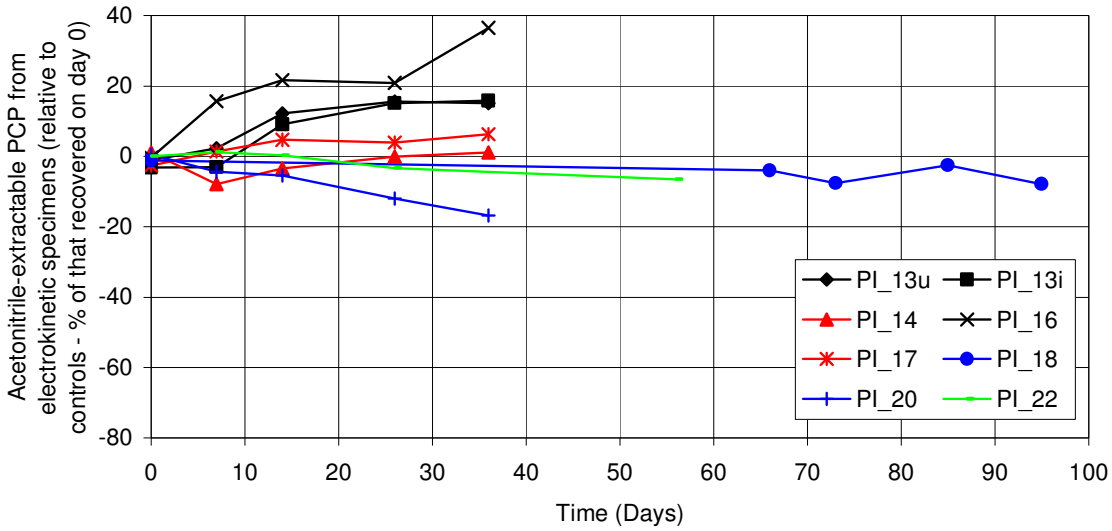

Figure 6-25: PCP acetonitrile extraction versus time

Experiment PI_22 was designed such that changes in pH and moisture content, as well as PCP reduction through movement, could be avoided. This was achieved, with very little change noted for either parameter. There was no electrolyte fluid system in this test, and so PCP that did not associate with the apparatus itself (a very small proportion) would either be remaining in the soil or have been degraded. As it has been shown (section 4.8.2) that a large proportion of PCP remaining in the soil was extracted using the techniques applied, then it is expected in this case that the majority of PCP not recovered was degraded. $^{14}CO_2$ recovery accounted for approximately 40% of the loss of PCP from

the soil in both control and electrokinetic specimens by the end of testing (Figure 4-113 and Figure 4-114), with the rest expected to have been lost to the atmosphere or trapped by other sinks in the apparatus. Both $^{14}CO_2$ recovery (Figure 4-111) and PCP recovery from soil (Figure 4-112) showed a significant improvement with an applied electric field, although there was substantial variability in the latter. The significant differences noted in $^{14}CO_2$ recovery between days 30 and 50 would be expected to correlate with a greater difference between the PCP in electrokinetic and control specimens at this time. By the end of testing (day 56), total $^{14}CO_2$ recovery was similar in both cases. It is apparent that a combination of pH and moisture content control and a reversing current field augmented the conditions needed for degradation in the soil to the extent that degradation was at times twice as effective (in terms of total PCP degraded) with an electric field than it was without.

6.9. Effect of Contaminant Ageing

PCP ageing was investigated in three experiments. PI_4 (Figure 4-42) was monitored continuously over a period of 104 days, and showed that over this period, PCP was entirely removed in samples with and without inoculated bacteria, although more slowly without UG30.

Experiment API_1 (Figure 4-115) showed similar behaviour; four soil cartridges remained 'intact' (i.e. with almost all PCP remaining) and so an experiment could still take place, although uncertainty in this test was higher than in other tests due to the loss of replicates. A large drop of 60% in recovered PCP was noted over the course of the experiment, both with and without pulsed current electric field. It is thought that this loss was due to PCP degradation by inoculated bacteria. There was no discernible difference

when the pulsed electric field was applied, indicating that the field under these conditions had little or no effect. This result can be compared to the changes observed in experiments PI_14 and PI_17, where a similar lower power was applied. In each of these cases, there was no significant difference between controls and electrokinetic specimens, in terms of either degradation or movement.

Results obtained from experiment API_21 (Figure 4-116 and Figure 4-117) were substantially affected by PCP disappearance whilst in storage (despite no inoculated degrading bacteria being present at this time). At the start of electrokinetic testing, there was a range of different PCP concentrations, all of which were substantially lower than expected. No effect of an electric field could be discerned in this case, apart from an increase in the proportion of acetonitrile-extractable PCP as noted in other specimens with an electric field (see section 4.7.2). Few comparisons were possible due to the differences in the low initial PCP levels.

It is therefore, unfortunately, difficult to draw any conclusions as to the effect of ageing on degradation of PCP, and to the effect of an electric field in such a case.

7. Conclusions

The use of electrokinetics to manipulate the behaviour of an organic contaminant in a fine-grained soil has been described. Substantial changes in soil properties have been induced which have had a significant bearing on contaminant properties. The main conclusions from this work, based on the initial aims and objectives in chapter 1, are summarised:

- The first objective of developing a simple, robust and effective set of apparatus was achieved, with the 'small' apparatus providing a high-quality platform upon which the majority of experiments were performed.
- The behaviour of the contaminant pentachlorophenol in soil has been characterised, with the pH of the soil pore water and the presence of organic matter found to have an important effect. A decrease in pH at the anode end of the electrokinetic soil specimens was seen in numerous experiments. This had the effect of making the contaminant less extractable by chemical means, because the ionic PCP was converted to protonated PCP, which was more hydrophobic and strongly sorbed to soil matter.
- Electromigration of ionic PCP on a large scale was seen towards the anode in many experiments. No evidence was seen for transport of the contaminant in electroosmotic flow, as protonated PCP would have been strongly sorbed to soil whilst non-protonated PCP would have been more strongly affected by electromigration. The successful movement of PCP was a major step towards bioavailability improvement, as the accessibility of the contaminant could be controlled to a certain extent. The satisfaction of this goal led to studies combining bacteria, contaminant and electric field.

Conclusions

- Differences between electrokinetic and control specimens were mostly seen when a constant current of 10mA was employed. In pulsed current and low power experiments, there was little change noted between the two.
- The efficiency of biodegradation of PCP was hindered by changes in several physical parameters during testing. Changes in pH were most commonly found to cause problems, especially where an increase in acidity led to PCP becoming more strongly sorbed to the soil as well as reducing the survivability of degrading bacteria. Large changes in moisture content were also expected to have affected the efforts of degraders.
- The primary objective of improving bioavailability through the application of an electric field to a contaminated and inoculated soil has been achieved. By controlling those factors that hindered bioremediation (namely changes in pH and moisture content), it was possible to enhance the degradation of the organic contaminant with the application of an electric field.

The movement of pentachlorophenol in soil has been shown, with electromigration being the dominant force seen. This behaviour only occurred when a constant current of 10mA was used. Pulsed current and low power experiments (such as PI_14 and PI_17) showed little movement of the chemical within the soil. The constant current experiments (e.g. PI_13u, PI_13i, PI_16) presented all showed a decrease in PCP concentration at the cathode end of the soil, with in some cases a build-up at the anode end, indicating the movement of PCP towards the anode. Evidence for the effect of electroosmosis was not seen in the experiments presented here, although this may be because the electric current applied was rather low, at a maximum of 10mA, and any effects were dwarfed by the effects of sorption or electromigration. Also, it may be that the high sorptive capacity of the soil used here (CEC was 22 meq/100g dry soil) led to more resistance to electro-

osmotic transport than would be seen in lower sorptive capacity soils such as kaolinite, which was used in many previous studies. The mathematical model developed as part of this work produced similar effects to those described here, with electromigration towards the anode only with a 10mA constant current, and with electroosmosis contributing only a very small amount to overall transport.

Changes in pH have been shown to strongly affect the PCP, with the chemical becoming more recalcitrant to extraction at lower pH due to protonation and a subsequent increase in hydrophobicity. This was a major factor in constant current experiments where anode pH was not controlled, as the pH in the soil at the anode end of the electrokinetic specimens became very acidic (approximately pH2 in several cases). The proportion of water-extractable PCP (measured in experiments with radiolabelled PCP only) was found to decrease significantly from 70% of the total in the specimen to as little as 25%, as a result of this effect.

The effect of electrokinetics on the microflora and microfauna in soil has also been investigated. An electric current in itself does not appear to have a significant effect on numbers of bacteria or fungi in this system, but the pH changes induced by an electric field may do. A reduction in the numbers of bacteria at the anode may be due to a low pH, induced by electrokinetics. Changes in bacterial community structure also can be attributed to this effect. The lack of effect seen in the remainder of the soil is likely to be due to the protecting effect of the soil matrix itself and the relatively low current used.

Degradation of PCP occurs readily in the soil, as long as the density of degrading bacteria inoculated is of the order of 10^8 cfu / g dry soil (or, presumably, higher). However, any effects of an electric field in increasing bioavailability can be masked, notably by the

creation of pH changes at the electrodes and subsequent migration of pH fronts into the soil. This has the effect of reducing the efficiency of the bioremediation process (the opposite of the intended use) by increasing toxicity levels for soil micro-organisms and increasing contaminant hydrophobicity and sorption. Low or intermittently applied currents did not have any discernible effect, possibly due to insufficient force on the contaminants involved to move them sufficiently. However, when a higher current was coupled with control of pH and moisture content (in experiment PI_22), a significant improvement in the bioremediation process was noted, which shows that electrokinetic phenomena can be used in the way intended to enhance the degradation of this particular organic contaminant in soil. A further advantage is the simplicity of the apparatus required in this case – no pH control system was required due to current reversal. The lack of physical changes in the soil would mean such a technique would be considered generally sustainable, as there might be few effects other than the removal of the contaminant. This work was done without the addition of any nutrients or other chemicals, showing that it was the electric field itself that provided the enhancement. However, it is unknown to what extent it is the movement and redistribution of chemicals that is the cause, or whether there is an effect on the micro-organisms themselves.

The technique employed in experiment PI_22 was seen to require a voltage drop across the soil of approximately ten volts throughout the experiment (results not shown). At a current of 10mA, this was a power consumption of 0.1 Watts. The approximate soil volume was $4.5 \times 10^{-4} m^3$, and so the consumption required under these conditions per cubic metre was approximately 220 Watts. If it were assumed that it would take 56 days to remediate the soil (similar to the experiment), and that the cost of electricity were £0.08 per kilowatt-hour (a typical UK domestic electricity charge), then the remediation cost would be approximately £24/m^3. Remediating a one hundred metre square site to a

depth of one metre would cost £240,000. This cost would compare favourably to other remediation technologies, although this only entails the cost of electricity. Many other project expenses would be accrued to increase the overall cost per cubic metre, but it might be expected that the technology could be made more efficient than found here, perhaps through the use of sustainable energy sources such as wind or solar power.

The experiments were performed at laboratory scale, and as such, there might be expected to be significant differences between these experiments and conditions on a typical contaminated site. In this work, factors such as the homogeneity of the soil, temperature, uniform distribution of contaminant and degrading organism and electric field distribution could be controlled rather better than might be expected at field scale. For example, many contaminated sites can have a significant amount of made ground present, with extreme heterogeneity in soil structure. The presence of metallic objects buried in the soil would be a significant hindrance to electrokinetic remediation, as the electric current would travel preferentially along these rather than through pore water. Despite this, the electric current used in the majority of tests (10mA) was low, and therefore scaling up of the process might not be as difficult as if a higher current was employed. The principle of electrokinetic remediation will still hold at larger scale, and due to phenomena such as electromigration and electroosmosis, fine-grained soils at field scale are expected to show an improvement in bioremediation efficiency as was seen in these laboratory experiments.

8. Future Work

The work described in this thesis has identified a particular method that enhances the degradation of a model contaminant (pentachlorophenol) in a fine-grained soil. However, substantial further work is necessary in order to investigate whether this is suitable for scale-up for field operation, and how robust it is in such situations.

Studies into whether the electric field primarily moves the chemicals or whether it increases bacterial activity would be of use in further understanding the phenomenon. It may be possible to improve the conditions under which the increased degradation occurred. The control of pH was not perfect, as a current reversal only once every 24 hours would have meant significant variations in pH at either end. A higher frequency of reversal might be more effective, possibly even as a sinusoidal rather than a step waveform, although the effects of higher frequency alternating current are unknown in this case.

An important step forwards would be the testing of the efficacy of this process with other contaminants and soils. With the knowledge gained from this project, it should be possible to determine a method of remediating a range of organic contaminants, given an understanding of the behaviour of these chemicals in soil. It may be that small changes in pH or moisture content could be implemented to great effect in order to best present a contaminant to degrading bacteria, and this could easily be performed using the techniques developed. It might also be of use to investigate the role of organic matter in this experimental system, in terms of sequestration of the contaminant, as well as transporting it (charged organic matter particles can be moved by electrokinetics). This could then be taken further, with the use of 'real' contaminated soils, possibly

incorporating a 'cocktail' of different chemicals, both organics and metals. The optimum conditions for remediating such a mixture would be difficult to determine, as these two types of contaminant will behave very differently, and other factors such as heterogeneity and ageing will arise, but it may be that a combination of movement of metals and degradation of organic chemicals can be used.

Further testing without inoculated bacteria would be useful, to determine whether electrokinetics can enhance natural attenuation significantly. This would be an important step forwards as it can be difficult to ensure a good distribution of degrading bacteria (in the laboratory, let alone the field), and if existing soil organisms could be used then this would remove this problem.

Ultimately, a successful laboratory method would be scaled up as a prototype method in the field. Many problems would have already been overcome when testing real contaminated soils in the laboratory, but on a much larger scale effects such as heterogeneity will become extremely important. Factors such as electrode placement will significantly affect results; in this work, a uniform current density has been used, but in the field, this may be impracticable, as large sites would require large numbers of electrodes. Also, distance between electrodes is important, in order to ensure that the required power is not too large, but that it would not take too long to remediate a sizeable site (Ho *et al*, 1999a; Ho *et al*, 1999b; Maini *et al*, 2000a).

It is expected that the techniques developed in this work can successfully be applied to real contaminated sites, with appropriate modifications, but it is necessary to perform a great deal of refinement and investigation prior to this being possible.

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