An Investigation of the Role of Soil Micro-organisms in Phosphorus Mobilisation.

A report submitted to fulfil the requirements of the degree of

Doctor of Philosophy.

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'To discern or create a symmetry, 'put something in its proper place,' is a mental adventure common to the poet and the scientist.'

Primo Levi.

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Abstract.

Over 35 years ago Hannapel *et al.* (1964a, b) published work that showed that soil micro-organisms mobilised phosphorus (P) in a calcareous soil. It is somewhat surprising to find that there appears to have been little appreciation of the significance of this result. The work carried out and reported upon in this thesis extends the work done earlier by exploring the relationship between soil micro-organisms and P mobilisation in an acid mineral soil and a sand.

The underlying rationale for this series of experiments was simple, the amount of P mobilised from biologically active soil samples (sterile, re-inoculated) were compared to the amounts mobilised from samples that were not biologically active (sterile). Water was the carrier material used to transport the mobilised P from the soil sample. The soils were sterilised using γ-radiation and sterility was maintained by leaching sets of sterile columns in a biological control cabinet. The forms of P that were mobile were characterised by physically and chemically fractionating the leachate from the columns.

The results of the experiments showed that micro-organisms mobilised P in both soil types. Furthermore, all of the P mobilised in this fashion was in the particulate fraction (>0.22 μm) of the leachate. SEM showed that the particulate material obtained from the acid mineral soil consisted of clay particles and microbial

mucilage, while the material from the sand consisted of fungal spores and microbial mucilage.

In the acid mineral soil no clear mechanism of mobilisation could be established. The two mechanisms postulated to account for this were that the mobilised P was associated with cellular debris generated by an active microbial population or that the P was associated with clay particles dispersed by soil micro-organisms. While in the sand the mobilised P was clearly associated with microbial debris generated by an active microbial population. The implications of the findings are then discussed.

Declaration.

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

I give consent to this copy of my thesis when deposited in the University Library, being available for loan and photocopying.

Kieran Coyle.

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Chapter 1.

General Introduction.

Many important biogeochemical processes are influenced by the activity of micro-organisms. There are several reasons why micro-organisms play such an important role in these cycles:

1. in the biosphere micro-organisms constitute the vast bulk of the mass and typically have growth rates several orders of magnitudes greater than those of higher organisms;
2. micro-organisms inhabit a wider range of environments than do plants or animals;
3. micro-organisms carry out many unique reactions of geochemical significance; and
4. micro-organisms have been present on the earth 4-5 times longer than higher organisms.

It is interesting to speculate that this close relationship between micro-organisms and element cycling may be in part at least, be a reflection of the long evolutionary heritage of micro-organisms (Trudinger *et al.* 1979).

The physical and chemical characteristics of the environment can be modified by organisms in numerous ways. The various chemical processes involved can be classified into two broad groups, those involving specific metabolic reactions, which are termed primary processes and those which are the result of biological activity, but

which aren't necessarily a part of the organisms physiology. These are termed secondary processes.

The accumulation of elements into the cellular structure of organisms and metabolically induced changes in the oxidation state of elements leading to their accumulation/mobilisation are two of the most important primary processes. A third process, biomethylation may be important in the mobilisation and transportation of certain elements.

Important secondary chemical processes include the following:

1. the production and consumption of acids and bases which may influence the pH of the environment;
2. the production and consumption of oxygen which controls the *Eh* of the environment; and
3. the production of organic compounds which may play a role in the mobilisation, transportation and immobilisation of certain elements and also provide energy for other biogeochemical processes.

The physical effects of organisms on the environment may be the result of a range of processes including bioturbation, stabilisation of land surfaces by plants, translocation of elements within higher plants and the transportation of elements in mobile organisms.

By definition, the movement of material is an integral part of any biogeochemical cycle. When discussing the role of transport processes in biogeochemical cycles it is useful to separate 'transport' into its three component phases: mobilisation, transportation *per se* and deposition. Mobilisation involves transforming an element from an immobile, fixed form to one that is mobile and readily moved by a carrier force. While there is widespread understanding of the role that micro-organisms play in the mobilisation of various elements including nitrogen (N), there is little appreciation of their role in the mobilisation of phosphorus (P). The original work that demonstrated that soil micro-organisms mobilised P in a calcareous soil was carried out by Hannapel *et al.* (1964a, b) over 35 years ago. The work reported upon in this thesis seeks to further explore the role that micro-organisms play in the mobilisation of P.

Chapter 2.

Literature Review.

2.1.0 Introduction

Phosphorus (P) belongs to Group 15 of the periodic table. This group which consists of nitrogen (N), phosphorus (P), arsenic (As), antimony (Sb), and bismuth (Bi) is termed the Pnictides or Pnicogens. Amongst these elements the chemistries of N, and P are by far the most important. The chemical behaviour of N is different to that of other members of the group. Even within the sub-group P, As, Sb and Bi there is a considerable range in chemical behaviour. For example P like N tends to form covalent bonds, while As, Sb and Bi exhibit increasing tendencies to form ionic bonds. Overall the chemical behaviour of P is most similar to As.

In P compounds bonds are predominantly covalent in nature. In most compounds P typically forms bonds to 3 or 4 atoms, but can in fact be bonded to between 1 and 6 atoms. The largest group of P compounds contains P bonded to either 3, 4, 5 or 6 atoms. The most important stereochemical configurations are pyramidal (3 bonds); tetrahedral (4 bonds); trigonal (5 bonds) and octahedral (6 bonds). There are six isotopes of P known, but only $_{15}P^{31}$ is stable. The electronic structure of P is shown below:

$1s^2 2s^2 2p^6 3s^2 3p^3$.

This element can be assigned a range of oxidation states, but in natural compounds the +5 oxidation state is almost universal.

In nature, P occurs most commonly as phosphate. Strictly speaking, phosphates may be defined as compounds, which contain P-O linkages. In these compounds three, four, five or six oxygen (O) atoms can be linked to a central P atom. However, the term phosphate is more commonly used to refer to compounds in which the P atoms are tetrahedrally co-ordinated or approximately so, by oxygen atoms. Compounds that contain discrete $(PO_4)^{3-}$ ions are called orthophosphates. Condensed phosphates are those compounds that contain PO_4 tetrahedra linked by sharing oxygen atoms. If the PO_4 tetrahedra are linked by sharing two oxygen atoms and form ring structures they are called metaphosphates (cyclic condensed phosphates). If the tetrahedra linked in this manner form an unbranched chain structure, the compound is classified as a polyphosphate (linear condensed phosphate). Finally, if some of the tetrahedra are linked by sharing three oxygen atoms then the compounds formed are called cross linked phosphates (ultra-polymeric phosphates). Detailed information on the chemical behaviour of this element and the classification of its compounds can be found in a review by van Wazer (1958). More detailed information on condensed phosphates is contained in a review by Thilo (1962).

P is a ubiquitous element being found through-out the biosphere and geosphere. In the biosphere P plays a central role in two important biochemical processes. It is an important component of deoxyribose and ribose-nucleic acid (DNA and RNA) and thus plays a role in the genetic regulation of organisms. It is also an important component of adenosine triphosphate (ATP) and its di- and mono-phosphate precursors, ADP and

AMP. ATP and ADP are molecules involved in energy transfer processes in organisms, while AMP controls the activity of various enzymes. In the geosphere P is found in a variety of minerals in both the regolith and mantle rock. Of the numerous phosphatic minerals that have been identified, the most commonly occurring is apatite (Norrish and Rosser, 1983). On average the concentration of P in the geosphere is 1180 ppm, which makes it the eleventh most frequently occurring element.

The behaviour and fate of P in the biosphere and geosphere is strongly influenced by its chemical behaviour and a variety of other biological, chemical and physical processes. The idea of a biogeochemical cycle is an elegant way of conceptualising this complex set of interacting processes. Pierzynski *et al.* (2000) defined a biogeochemical cycle as, 'a conceptual description of the mechanisms by which an element or compound is transformed within a system of interest including the means by which the various forms are interchanged between the solid, liquid and gaseous phases of that system.'

This definition like many in the literature stresses the important role that transformation processes play in biogeochemical cycles. Such definitions do however have a fundamental shortcoming. Namely, that they fail to highlight that the movement of material is a central component of any biogeochemical cycle.

In this context it is interesting to compare the definition provided by Odum (1971), with that provided by Pierzynski *et al.* (2000). According to Odum, 'The chemical elements, including all of the essential elements of the protoplasm tend to circulate in the

biosphere in characteristic paths from environment to organism back to environment. These more or less circular paths are known as biogeochemical cycles.' This definition highlights that the movement of material is fundamental to the concept of a biogeochemical cycle.

The underlying theme of this review, indeed of this thesis is that in relation to understanding the P cycle in soil, transformation processes have been stressed to the detriment of processes associated with movement. To place this general contention in context and to allow for an examination of its specific implications, it will be useful to broadly discuss transport processes that operate in the major biogeochemical cycles (i.e. carbon (C), sulphur (S), N and P).

2.2.0. Transport Processes in Biogeochemical Cycles.

Clearly with any cycle the transport of material must be a key process. When considering the transport of material in this context it is important to note the following points:

1. movement can occur over a wide range of spatial scales. In all cycles there are critical boundaries where the scale of movement can shift suddenly. An example is the transfer of material from the internal tissue of plants to their external surfaces and the subsequent movement of this material into the atmosphere. Awareness of these shifts in scale is important according to Reiners (1983) because they represent the interconnectedness of cycles of all scales; and

2. the rate of movement can also vary widely. That is both the actual distance an element may move in a given time period (km y^{-1}) and the time taken for an atom or molecule to complete one circuit of the cycle.

When discussing the role of transport processes in biogeochemical cycles Reiners (1983) separated 'transport' into three phases: mobilisation, transportation *per se* (movement) and deposition. This is a useful approach because it creates a framework for a process oriented understanding of transport or movement in biogeochemical cycles.

In this context mobilisation involves transforming an element from an immobile, fixed form to one that is mobile and readily moved by a carrier force. The transportation phase involves the physical movement of material from one place to another. Deposition is where the material being moved ceases to move. This includes immobilisation processes such as chemical precipitation and photosynthesis. Clearly the very general nature of these definitions means many processes will be involved in the transport of material. Reiners (1983) however produces a surprisingly small and relatively comprehensive list of the major processes involved in the mobilisation, transportation *per se* and deposition of material in biogeochemical cycles (refer to Table 2.1). At this stage a few pertinent comments on the assumptions that underlie the structure of this table are warranted. The focus is clearly on transport processes across the full scale range, from the very small to the global. The conceptual model that underlies the format of this table is that of a set of nested cycles. This means that in

some situations a process that is classified as a mobilisation process on one level can be classified as an immobilisation process on another. For example plant uptake can be classified as a mobilisation process when viewed on the organism level, yet when viewed on a catchment level it can be classified as an immobilisation process. This should be borne in mind during the following discussion.

2.2.1. Mobilisation Processes.

Mobilisation processes have been divided into three major categories - biological, physico-chemical and human induced. It should be noted that there are often interactions between the processes listed in these first two categories. The production of organic acids and the effects that these have on chemical weathering is an example of such an interaction.

Biological Processes.

Biological processes are further categorised into those processes associated with plants, animals and micro-organisms. There are three important observations relating to these processes worth noting:

1. the strength of these processes will be controlled by the supply of the rate limiting nutrient;
2. most of these processes are sensitive to pH; and
3. microbial processes often form mobile gaseous species of some elements that can become involved in long range transport.

Mobilisation Processes	Transport Processes and Range[1]	Deposition Processes
Biological Processes *Plant Processes* Mineralisation of C compounds by respiration. Productions of volatile organics (terpenes). Movement of chemical material from internal tissues to surfaces. Plant uptake - desorption. *Animal Processes* Mineralisation of organic materials. Incorporation of food and ions into mobile animals (animal uptake). *Microbial Processes.* Mineralisation/Fermentation. Nitrification. Denitrification. Sulphate (SO_4^{2-}) reduction. Organic C reduction. P reduction (Phosphine). Fe reduction. Microbial uptake - desorption. Grazing by amoeba and nematodes. **Physico-chemical Processes** Physical weathering. Chemical weathering/dissolution. *Soil Processes.* Cation exchange (NH_4^+). Anion exchange (SO_4^{2-}, $H_2PO_4^-$). Entrainment by wind and water. Ammonia volatilisation. Wetting and drying cycles. *Glacial acquisition.* *Marine aerosol formation.* *Volcanic exhalations.* *Atmospheric electrical discharge.* *Wildfires.* *Suspension of particles on leaf surfaces.* **Human Induced Processes** *Wood fuel combustion.* *Land management fires.* *Fossil fuel combustion.* *Agricultural land tillage.* *Food and fibre processing.* *Fertilizer manufacture.* *Mining.* *Smelting.*	**Biological Processes** *Plant Processes* Translocation within plants (very local). Canopy leaching and throughfall – stemflow (very local). Plant litter fall to ground (very local). *Animal Processes* Translocation within animals (very local). Animal movement (very local to continental). *Microbial Process* Translocation within organisms (very local). Movement of micro-organisms (very local). **Physico-chemical Processes** *Mass-wasting processes (local).* *Glacial movement (very local to continental).* *Fluvial Processes.* Ion diffusion in soil solution (very local). Saturated and unsaturated flow in soil (very local). Groundwater flow (very local to regional). Surface water flow (very local to continental). Ocean currents (hemispheric to global). *Atmospheric Processes* Saltation - particles (very local to mesoscale). Suspension (mesoscale to global). Solution - gases (mesoscale to global). **Human Related Processes** *Movement of food and fibre products, ores, industrial products (mesoscale to global).*	**Biological Processes** *From Plant Processes* Death and litter accumulation. Incorporation into tissue. *From Animal Processes* Incorporation into tissue. Excretion. Death of organism. *From Micro-organisms* Incorporation into tissue. Death of organism. **Physico-chemical** *From Mass-wasting Processes* Deposition at base of slope. *From Glacial Transport* Deposition as till, kames, moraines, outwash etc. *From Fluvial Transport* Uptake of dissolved substances by sessile organisms- e.g. aquatic plants and corals. Physical sedimentation. Chemical precipitation. Sorption reactions. Filtering by soil. *From Atmospheric Transport* Wet deposition. Dry Deposition. Sedimentation (large particles). Gaseous absorption. -Active: photosynthesis and N fixation. -Passive: absorption to chemical sinks e.g., foliage, soil and water. Inertial impaction (soil particles and aerosols >1μm). Molecular diffusion. **Human Related Processes** Fertiliser application. Disposal of wastes.

Table 2.1. Major processes involved in the transport of material in biogeochemical cycles (Adapted from Reiners (1983)[1].

[1] Scale of Movement: - v local, < 1km; local, 1-10 km; mesoscale, 10-100 km; regional, 100-1000 km; continental, 1000-3000km; hemispheric, 3000 km –40 000 km; global > 40 000km.

Reiners' (1983) list was relatively comprehensive but failed to include several processes that may mobilise elements in the soil. Firstly, microbial cell contents may be released by the grazing action of amoebas and nematodes, thus releasing P, S, N and C from the microbial biomass. Secondly, P is often associated with Fe in acid soils so it can be mobilised as an indirect consequence of Fe reduction.

Reiners (1983) had included plant uptake and translocation as a single transport process. Conceptually, it is perhaps better to separate these into a mobilisation phase that includes all of the different physico-chemical and biological processes by which plant roots mobilise anions and cations from various soil constituents and a transport phase that includes all of the relevant processes associated with translocation. In relation to P, at present there is a considerable degree of uncertainty surrounding the relative importance of the role different rhizosphere mechanisms play in mobilising it (Morel *et al.* 2000). However, the absorption of P by roots and the subsequent desorption from the surface of various soil constituents and diffusion along the concentration gradient established within the soil solution, is considered to be an important mechanism in transferring P from soil constituents to root surfaces. To simplify matters the mobilisation processes associated with plant uptake have simply been termed desorption, while ion diffusion has been included as a physico-chemical transport process. It should be noted that the incorporation of ions into plant tissue has been included as a deposition process.

In addition to plant uptake, microbial uptake of various ions also occurs. This is a complicated topic beyond the scope of this review, consequently the mobilisation processes associated with microbial uptake have simply been termed desorption. Clearly, in this context ion diffusion would be a transport process associated with microbial uptake. Translocation of ions within micro-organisms also occurs but the scale of movement is typically much smaller than in plants. In this context incorporation of P into microbial tissue would be an immobilisation process. Translocation of ions also occurs in animals and has been included in Table 2.1. In this context incorporation into animal tissue would be an immobilisation process. Processes associated with micro-organisms are most directly relevant to the work carried out for this thesis.

Physico-chemical Processes.

This category was further divided into mineral weathering, soil centred processes and other miscellaneous processes. Obviously processes listed in the first two categories are most directly relevant to this work. Clearly there is a degree of arbitrariness to this classification process, as chemical weathering and physical weathering are important processes that occur in the soil environment. Perhaps it would have been better to categorise these processes using the nature of each (i.e. physical or chemical) as the criterion for classification. The following points are of interest:

1. rates of mineral weathering vary enormously due mainly to variations in temperature and rainfall;

2. as noted earlier living organisms can contribute to both chemical weathering and physical weathering; and
3. entrainment is the process whereby loose material is picked up by wind or moving water. Typically, the material mobilised in this manner will come from the finer fraction of the soil and so will be enriched in C, N, S and P relative to the overall source soil.

Reiners' (1983) list of mobilisation processes was relatively comprehensive, but he failed to include anion/ligand exchange processes that may play a role in the mobilisation of P and S under certain circumstances. Also not on his list were soil wetting and drying cycles that may play a role in mobilising material in the soil environment. It should be noted that with cation exchange ammonium is the only cationic form of all four elements (C, N, S and P), so consequently only N can be mobilised in this fashion.

Human Induced Processes.

Table 2.1 includes a list of processes associated with human activities such as food and fibre production and processing. From an environmental point of view these activities are often seen in a negative light. However in general terms, the adverse effects of such activities result from the localised acceleration of natural processes. According to Reiners (1983) the major differences between human induced and natural mobilisation processes are the geographic distribution, extreme localisation, and very high intensity of the human caused processes.

2.2.2. Transport Processes.

These processes have been grouped into three broad categories: biological, physico-chemical and human related.

Biological Transport Processes.

In this instance Reiners' (1983) list was relatively comprehensive. However, he failed to make a clear distinction between animal mobilisation (uptake) and transport (movement) processes, as has been done in Table 2.1. In most cases the processes listed in this category will only be important in moving material over very short distances.

Physico-chemical Transport Processes.

Again Reiners' (1983) list seemed to be relatively comprehensive. From the point of view of P cycling fluvial processes play an important role. Saturated flow and unsaturated flow in the soil are the processes that have most relevance to the work undertaken for this thesis. The process of moving material through the soil profile in this manner is termed leaching. It should be noted that this process does not necessarily involve the loss of material from the soil profile. Rather it may simply lead to the translocation of material within the profile.

Human Related Transport Processes.

An increasingly important transport mechanism is the movement of materials by humans. Wide ranges of materials are transported over vast distances.

2.2.3. Deposition/Immobilisation Processes.

Reiners (1983) did not include adsorption/absorption reactions in his list of deposition processes associated with fluvial transport. Obviously in the soil environment these are important immobilisation processes. Also, the filtering effects of the soil could be important in terms of particulate and colloidal materials (McDowell-Boyer, 1986).

Also excretion and death had been included as transport processes associated with animals. These seem more like deposition processes and have been listed as such in this table. The author also had no deposition processes from plant transport systems. The most obvious would seem to be incorporation into plant tissue, plant death and litter accumulation. Similarly for micro-organisms, incorporation of ions into fresh tissue would be an immobilisation process. With all living organisms there are internal transport processes occurring which cease upon death. In this context it seemed appropriate to classify organism death as a deposition process.

Given that the underlying theme of this thesis is that the understanding of processes associated with P movement in the soil has been neglected. Then the general observations discussed above have to be placed into the appropriate context in relation to the P cycle. Thus, the next stage in the development of this theme is an examination of the P biogeochemical cycle.

2.3.0. Biogeochemical Cycling of P.

Two basic groups of biogeochemical cycles can be distinguished, those where the main element reservoir is the atmosphere or hydrosphere (gaseous types) and those where the earth's crust is the main reservoir (sedimentary types). The P cycle is characterised as a sedimentary type cycle. There are several unique features of the P biogeochemical cycle worth noting, the first is that the element does not undergo any valence changes during the cycle, the second being that there are no important gaseous components of this system.

A variety of models have been developed to describe P cycling at various scales. The main types in the literature are process, compartmental and budgeted flow. Obviously transport processes can best be examined in the context of a process model. Varying degrees of complexity can be incorporated into the conceptual model used to describe the P cycle. It is probably easiest to begin by briefly considering a simplified model of the global P cycle. This will help place the soil sub-cycle into the appropriate context.

2.3.1 Global P Cycle.

A simplified conceptual model of the global P cycle showing the main reservoirs and transfer processes is shown in Figure 2.1. Table 2.2 presents a summary of the estimates of the amounts of P held in each reservoir as well as the amounts that flux between them.

In this model there is a primary cycle and two sub-cycles. The primary cycle begins with the weathering of parent rock that releases P into the soil solution. The released P is eventually transferred to the marine environment. Almost all of the fluvial transport processes listed in Table 2.1 are involved in this transfer. Physical sedimentation (organic/inorganic) removes the P from the marine reservoir and diagenesis incorporates it back into the earth's crust. The turn-over rate of this cycle is very slow, with a complete circuit taking tens to hundreds of millions of years. So for all intents and purposes this cycle can be viewed as the unidirectional transport of P from rocks to marine sediments.

The two sub-cycles are terrestrial and aquatic based. The turn-over rate of the terrestrial cycle being measured in years, is much faster than the primary cycle. Uptake of P by plants and return of plant and animal residues to the soil are the processes that link the soil and terrestrial biota reservoirs. The turn-over rate of the aquatic cycle being measured in months, is faster again than the turn-over rate of the terrestrial cycle. Uptake of P by organisms and then its subsequent release by death and decay are the processes that link both biota reservoirs to the relevant aquatic reservoirs.

In the soil reservoir P is associated mainly with immobile, usually solid material. In this context plant uptake can be viewed as a mobilisation process and translocation can be viewed as a transport process (Refer Table 2.1). The situation in the freshwater and marine reservoirs is somewhat different. In these reservoirs P is associated with a liquid that may be mobile. In this way P can be transported by mass flow. In this context the

uptake of P from a medium (water) that is mobile by sessile organisms is more appropriately termed a deposition process (refer to Table 2.1). Although there will be internal transport processes occurring at the organism scale. The uptake of P by mobile aquatic organisms will not obviously alter the state of mobility of P but may well alter its form (i.e. dissolved to particulate).

The data presented in Table 2.2 show that the estimates of the magnitudes of P in the various reservoirs and of the fluxes between them are relatively consistent. However, it should be remembered that these numbers are not precise. This is because the calculations are based on estimates of various parameters. For more detailed information on the underlying assumptions and estimates used in these calculations the interested reader should refer to the various individual authors (refer to Table 2.2).

It is clear that the vast bulk of P is contained within the earth's crust (sediments and rocks). In comparison only minor amounts are found in the biosphere (terrestrial and aquatic) and the world's oceans. Most of the P contained within the earth's oceans is below the euphotic zone, while on land most of the P is either contained within the matrix of sparingly soluble minerals or strongly bound to the surface of others. Thus, little of the total P that is present is bio-available, that is in a form that can be readily taken up by living organisms. As discussed earlier P plays an important role in several critical biochemical processes. Consequently, in many eco-systems P is a limiting nutrient in terms of biomass production.

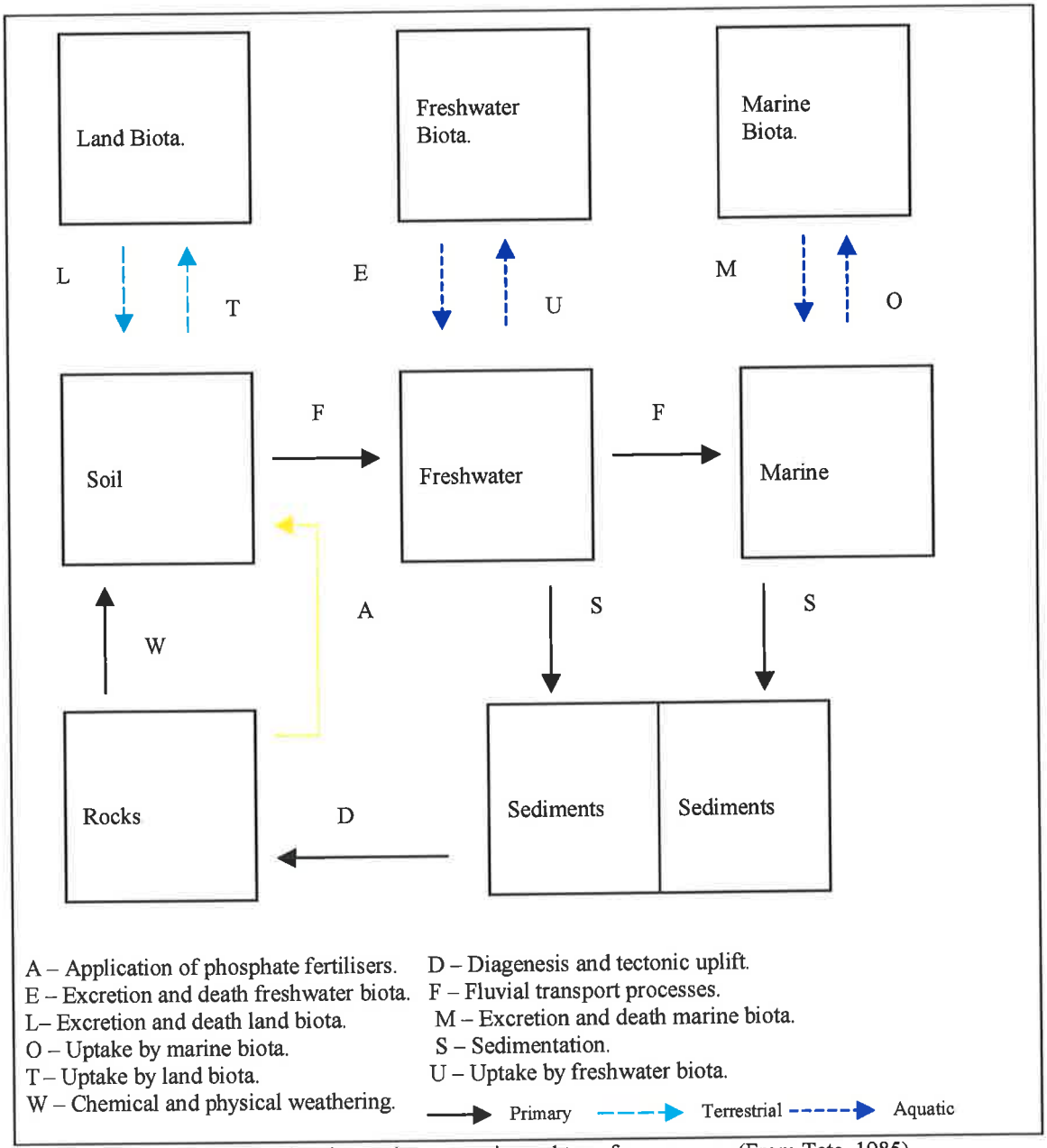

Figure 2.1. Global P cycle showing major reservoirs and transfer processes (From Tate, 1985).

The large scale mining of rock phosphate, its subsequent transformation into fertiliser and widespread application to soils is becoming an increasingly important part of this natural cycle. In many agricultural regimes P can move from the soil into surrounding water-ways and cause eutrophication. Pierzynski *et al.* (2000) cited enhanced algal growth, decreased dissolved oxygen and reduced water transparency as being the

primary effects associated with this phenomenon. Often however, the most serious water quality problems are secondary effects associated with these primary problems. For example in many water-ways extensive blooms of cyanobacteria occur. These blooms produce toxins and anoxic conditions that can kill fish and also render water unsafe for many uses.

Reservoirs (Tg P)	**Authors**				
	Stumm	*Lerman et al.*	*Pierrou*	*Richey*	*Tate*
	(1973)	(1975)	(1976)	(1983)	(1985)
Land biota	1950	3000	1850	2600	2000
Freshwater biota	-	-	-	-	10
Marine biota	124	138	128	50-120	128
Soil	-	200000	160000	96000-160000	160000
Freshwater	-	-	90	90	90
Sea	124000	92600	120-128000	81000	120000
Sediments	8×10^{8}	4×10^{8}	-	8×10^{8}	1×10^{6}
Rocks	-	-	-	-	1×10^{13}
Fluxes (Tg P/Yr)					
Rocks → Soil*	-	-	-	-	13
Rocks → Soil**	12.4	12.4	12.6	14	13
Soil ↔ Biota	229	63.6	136-237	200	200
Soil → Freshwater	-	-	2.5-12.3	4-7	12
Freshwater ↔ Biota	-	-	-	-	10
Freshwater → Sediment	-	-	-	-	1
Freshwater → Sea	1.9#	1.7#	17.4@	1.5-4#/17@	17
Sea ↔ Biota	961	992-1042	990-1300	600-1000	1000
Sea → Sediments	1.9	1.7	13	2-13	13
Sediments → Rocks	?	?	?	?	?

Table 2.2. Estimates of the amounts of P held in various reservoirs and of the magnitudes of the fluxes between them (Adapted from Richey, 1983). * Weathering/** Fertiliser application/ #Dissolved/@Particulate.

These blooms obviously increase the cost and difficulty of drinking water purification. Changes in dissolved oxygen levels and a reduction in water transparency can reduce biodiversity in water bodies. Thus, there can be major environmental and economic costs associated with the eutrophication of water bodies. This process and all of the

issues that surround it, including the movement of environmentally significant amounts of P from agricultural land are currently areas of very active research.

From our perspective the most important component of the global cycle is the terrestrial based sub-based cycle. The contention being developed for this thesis is that there are certain aspects of P movement in the soil that have been overlooked, specifically that there are aspects of P leaching that are not fully understood. P leaching is a complex process that has chemical, physical and biological components. All too often the role played by the chemical components of the system is stressed to the detriment of fully understanding the role that the other components of the system may play in the process. For example, it has been known for some time that preferential flow can move P through soil (Kanchanasut *et al.* 1978; Heckrath *et al.* 1997; Thomas *et al.* 1997). In effect the physics of the system can over-ride the chemical characteristics of the system. There is evidence in the literature to suggest that the biology of the system may also be able to over-ride at least to some extent, the chemistry of the system. Before reviewing this evidence it will be useful to develop a conceptual model of P cycling in the soil (refer to Figure 2.2).

Most models in the literature are focused on transformation processes that transfer P from one pool to another. In other words the focus is on intrasystem cycling. The focus of this work is on transformation (mobilisation) processes that are linked to transport processes that may remove P from the soil cycle. In this context the model and discussion are designed to provide a very concise formulation of our current understanding of P leaching.

2.3.2. Soil P Cycle.

The model developed for this purpose is shown in Figure 2.2. This is a relatively simple model having only a few pools of P. It should be noted that this is a model of an undisturbed eco-system. There are no inputs in terms of fertiliser or exports in the form of plant materials or animals. The pools and processes have been grouped into geochemical and biological sub-cycles. The transfer of reactive species through the soil solution links these sub-cycles.

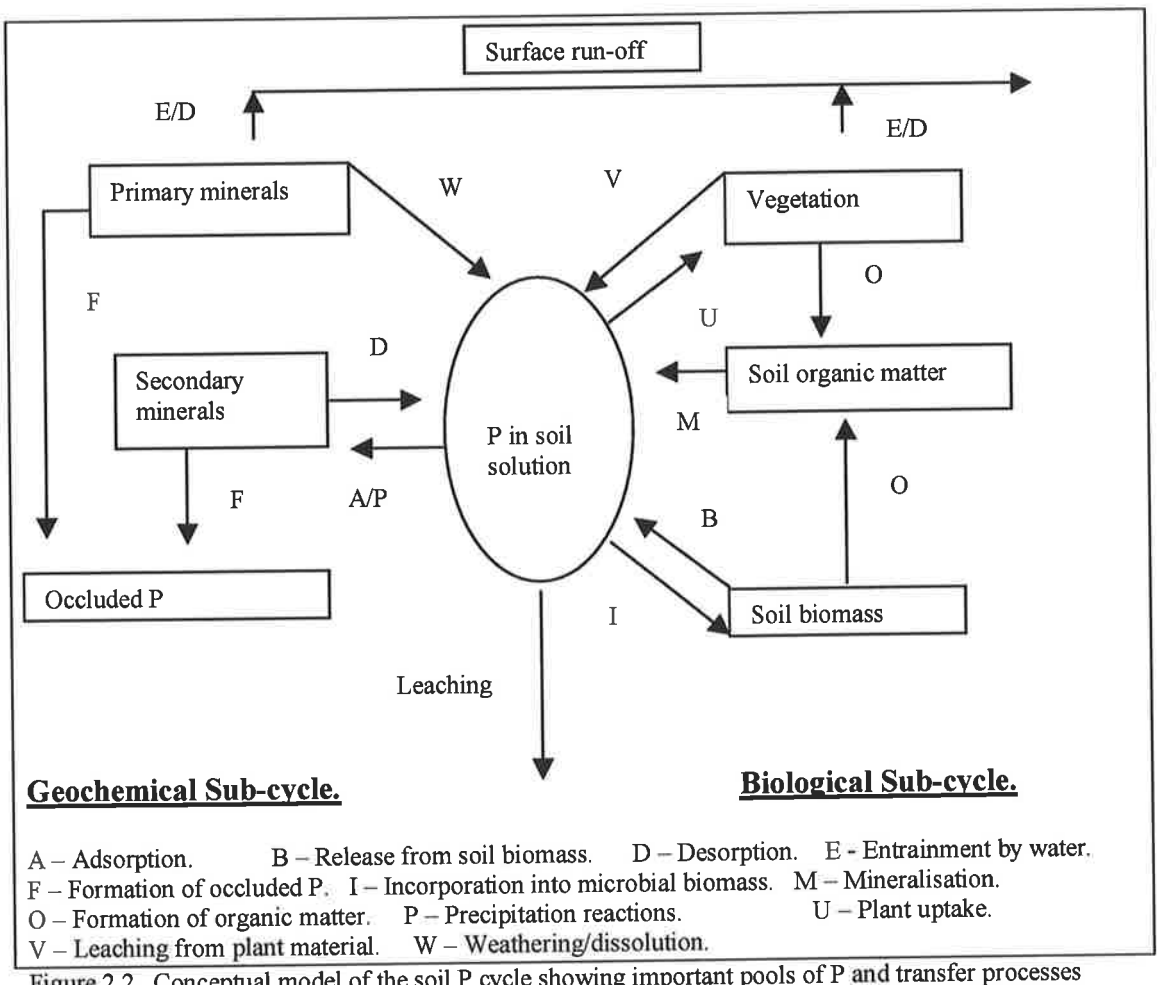

Figure 2.2. Conceptual model of the soil P cycle showing important pools of P and transfer processes (Adapted from Walbridge *et al.* 1991).

Geochemical Sub-cycle.

All soil P is ultimately derived from P containing minerals. The release of P from the matrix of primary minerals (i.e. apatite) occurs during weathering. This involves the formation of various secondary minerals, some of which may contain P, while others may be reactive towards orthophosphate. The P released in this manner can enter the soil solution or be encapsulated by Fe and Al oxides to form occluded P (Figure 2.2). Within a pedologic timeframe the end point of this sub-cycle is a sink, in the form of occluded P. Obviously within a geologic timeframe even this form of P must be mobilised. The P released into the soil solution can undergo a variety of reactions. Relatively insoluble salts can be formed with Ca, Fe or Al or it can be adsorbed by a variety of soil constituents.

Mineral	Formula
Apatites	
Fluorapatite	$Ca_5(PO_4)_3F$
Chlorapatite	$Ca_5(PO_4)_3Cl$
Hydroxapatite	$Ca_5(PO_4)_3OH$
Rare earths	
Monazite	$(Ce,La,Th)PO_4$
Xenotime	YPO_4
Plumbogummites	
Crandalite	$CaAl_3(PO_4)_2(OH)_5H_2O$
Goyazite	$SrAl_3(PO_4)_2(OH)_5H_2O$
Gorceixite	$BaAl_3(PO_4)_2(OH)_5H_2O$
Florencite	$CeAl_3(PO_4)_2(OH)_6$
Plumbogummite	$PbAl_3(PO_4)_2(OH)_5H_2O$
Hinsdalite	$(Pb,Sr)Al_3(PO_4)(SO_4)(OH)_6$
Others	
Vivianite	$Fe_3(PO_4)_28H_2O$
Wavellite	$Al_3(OH)_3(PO_4)_25H2O$
Perhamite	$3CaO_3.5Al_2O_3.3SiO_2.2P_2O_5.18H_2O$

Table 2.3. P minerals identified in soils.

It is now accepted that adsorption/desorption reactions play the most important role in buffering the concentration of P in the soil solution. In the soil, inorganic P occurs in

two major forms, either as sparingly soluble minerals or adsorbed on the surface of various soil components.

Table 2.3 lists the various minerals that have been positively identified in soils. With most of these minerals it is impossible to determine if they are residual (primary) or pedogenetic (secondary) in origin (Norrish and Rosser, 1983).

Biological Sub-cycle.

In terms of the number of reservoirs and related processes, the biological cycle is far more complex and dynamic than the geochemical cycle (Smeck, 1985). There are three main reservoirs in this model (Figure 2.2), vegetation, soil organic matter and soil biomass.

Vegetation.

Vegetation plays a major role in the soil P cycle. In undisturbed eco-systems P removed from the soil solution by plant uptake is usually returned in the form of plant residues. In agricultural regimes the export of plant (either directly or indirectly in the form of animals) material may remove significant amounts of P from the soil. P removed from the soil solution by plants is used in the production of biomass. In this way a variety of organic P compounds are synthesised including, nucleic acids, phospholipids and sugar phosphates. A proportion of total biomass is returned to the soil annually.

Soil Organic Matter.

This material is decomposed by soil micro-organisms resulting in the accumulation of soil organic matter. Many workers have argued that most of the organic P compounds found in soils are produced by microbial synthesis. The accumulation of organic P compounds from biomass residues is not considered to be important in this regard (Anderson, 1980; Brookes *et al.* 1984; McLaughlin *et al.* 1988). This argument is based on the observation that the proportions of P compounds in the soil are very different to those found in living organisms that make up the ecosystem biomass. Tate (1984) takes a different position and argues that a variety of inositol phosphates may be primarily of plant origin (decomposition), while Pant *et al.* (1994) suggest that plant root exudates may also be an important source.

Despite the advent and use of ^{31}P – nuclear magnetic resonance (NMR), the characterisation of the forms of organic P in soil remains one of the major challenges facing soil analytical chemistry. In general terms most authors classify soil organic P compounds into three main groups, namely, inositol phosphates, phospholipids and nucleic acids (Dalal, 1977; Anderson, 1980; Sanyal and Detta, 1991; Haygarth and Jarvis, 1999).

Inositol can form a series of phosphate esters ranging from monophosphates to hexaphosphates. Of these compounds myo-inositol hexaphosphate (phytic acid) occurs most commonly in soils. This compound reacts strongly with various soil constituents (e.g. clay compounds and sesquioxides). Anderson (1980) suggests that this reactivity

stabilises this compound in the soil. He also suggests that most of the phytic acid found in soils is probably synthesised *in situ* by micro-organisms.

Phosphoglycerides are the dominant form of phospholipids in the soil (Dalal, 1977). According to this author choline phosphoglyceride is the dominant soil phosphoglyceride, accounting for 40%. Phospholipids in soil may be sourced from plant debris, animal wastes and microbial biomass. Dalal (1977) notes that phospholipids make up the major part of total organic P in plant tissue but only a small amount of soil organic P. This suggests that their degradation occurs fairly rapidly in soils.

Nucleic acids are found in all living organisms and occur in two forms, ribonucleic acid (RNA) and deoxyribonucleic acid (DNA). These compounds are probably added to soil in greater quantities than most other phosphate esters, yet they comprise only a small proportion (3%) of the total soil organic P. This suggests that these compounds are either rapidly degraded or resynthesised.

According to Dalal (1977) the three groups of organic compounds discussed above only account for about half the amount of organic P in soil. It seems reasonable to suggest that micro-organisms are the most likely major source of much of this unidentified material. It is known that bacterial cell walls contain very stable phosphate esters that may be degraded very slowly in soil (Anderson, 1980).

Soil Biomass.

For the purposes of this discussion the soil biomass can be divided into three broad categories: the microbial biomass, plant roots and all other organisms. From our perspective the most important of these is the microbial biomass.

The microbial biomass is defined as the living part of the soil organic matter excluding plant roots and soil animals larger than 5×10^3 μm^3 (Tate, 1985). There are four major groups of micro-organisms that make up the microbial biomass, namely bacteria, actinomycetes, fungi, algae and protozoa. The following discussion is based on material from Alexander (1977).

In general terms bacteria are usually the most abundant group of organisms in the soil. Yet, because of their relatively small size they probably account for much less than half of the total microbiological cell-mass. Bacterial characterisation can be based upon morphology. Using this approach three major types can be identified. These are bacilli or rod shaped, cocci or spherical shaped and spirilla or spiral shaped. Spirilla do not commonly occur in soils. A variety of environmental factors including soil moisture content, aeration, temperature, pH and substrate availability will determine the size and composition of the bacterial community in the soil. For example highly acid or alkaline conditions tend to inhibit the growth of many common bacteria. It is thought that bacteria are rarely free in the soil solution, but are found either in colonies that develop on micro-sites associated with various soil components, or within the rhizosphere. They

are also known to produce organic compounds that are thought to play a variety of roles in the soil environment.

Actinomycetes can be thought of as being a transition group between simple bacteria and fungi. Strictly speaking they are classified as bacteria, but they typically produce slender, branched filaments similar to fungi. In terms of abundance in soil they are probably only second to bacteria. In high pH environments they can make-up a large proportion of the total community and as a group are not particularly tolerant of low pH (< 5.0). These organisms do not tolerate anaerobic conditions. They are better able to withstand dry conditions than are true bacteria. When organic matter is added to soils, bacterial and fungal flora will generally proliferate initially while actinomycetes tend to predominate during the latter stages of decomposition.

Fungi because of the relatively large diameter and extensive network of their filaments tend to make up a significant part of the microbial biomass in soils. They tend to be the major agents of decay in the organic layer of forest and woodland soils in temperate climates. In the grassland soils of these zones Clarke and Paul (1970) found that they constituted the majority of the microbial biomass. These organisms can tolerate a wide pH range, from highly acid (pH < 3.0) to extremely alkaline (pH> 9.0). In acid environments fungi tend to dominate the microbial community because bacteria and actinomycetes do not flourish under these conditions. Fungi can persist and even remain metabolically active under relatively dry conditions. They are however strictly aerobes and do not grow in waterlogged soils.

Algae generally make up only a small portion of the soil biomass. They may be unicellular or occur in short filaments. Along with a few genera of bacteria these are the only photosynthetic micro-organisms found in soil. The need for light means that these organisms are restricted to the upper few centimetres of the soil profile. In general terms algae do not play a major role in the various biochemical processes that occur in soils. However, because they can generate organic matter from inorganic substrates they probably play a major role in colonising denuded or barren land surfaces. They may also play a role in stabilising soil surfaces by forming crusts.

Protozoa are the simplest form of animal life and are often found in soils. These organisms are generally most abundant in the surface horizons of the soil profile. Protozoa consume soil bacteria and it is thought that in this way they serve to regulate the size of the bacterial population. This action may also serve to enhance the cycling of various nutrients. This topic will be elaborated upon further in the discussion below.

According to Tate (1985) a range of P compounds can be found in the cell contents of micro-organisms. These include RNA (30-50%), DNA (5-10%), acid soluble inorganic and organic P (15-20%) and phospholipids (10%). P excess to needs may accumulate as inorganic polyphosphates inside bacterial and fungal cells (Harold, 1966).

Soil Cycle – P Loss.

P can be lost from the cycle in two ways, either through surface run-off or leaching. It is generally accepted that in mineral soils reactions between orthophosphate and various

soil components, notably hydrous oxides of Fe and Al in acid soils and carbonates in calcareous soils mean that little P is lost through leaching. However it has been known for some time that leaching losses can be significant in sandy (Neller, 1947; Ozanne *et al.* 1961; Diggle and Bell, 1984) and organic soils (Larsen et al. 1958; Fox and Kamprath, 1971; Cogger and Duxbury, 1984). In agricultural regimes surface flow is thought to be the major pathway of loss from most soil types (Committee on Long-Range Soil and Water Conservation, 1993; Council for Agricultural Technology, 1993). Some workers (Schoenau and Bettany, 1987; Frossard *et al.* 1989; Donald *et al.* 1993) have suggested that in temperate forest and grassland soils of North America, leaching of P rich organics may be an important export mechanism. This body of work will be reviewed in some detail later. The focus of this thesis is not on surface run-off as mechanism of P loss but on leaching. It should be stressed that the scope of this work is not restricted to just sandy and organic soils. P in surface run-off is a complicated topic and beyond the scope of this review.

Leaching is a transport process and as such can be separated into the three stages suggested by Reiners (1983): mobilisation, transportation *per se* and deposition/immobilisation. It should be stressed that this approach does not view leaching as a sequential process, but rather as a series of inter-related and overlapping processes that can be classified into three broad groups.

Geochemical/Physical Processes	Biological Processes
Mobilisation Processes. Weathering/Dissolution. Desorption. Release from microbial biomass- Seasonal variation causing cell rupture. *Immobilisation Processes.* Adsorption. Precipitation reactions. *Transport Processes.* Saturated and unsaturated flow.	*Mobilisation Processes.* Mineralisation. Release from microbial biomass - grazing by amoeba and nematodes. Fe reduction. *Immobilisation.* Incorporation into microbial biomass. Plant uptake. *Transport Processes.* Translocation – plant and micro-organisms.

Table 2.4. Processes involved in the leaching of P from the soil.

In Table 2.4 the processes that transfer P between the various pools and the soil solution have been classified as being either geochemical or biological in nature. The processes in each of these categories have been further sub-divided according to the role that they play in P leaching.

The soil solution plays a central role in the leaching process the composition of which will be determined by the interplay between the various mobilisation and immobilisation processes listed in Table 2.4. It should be noted that the work carried out for this thesis focused on soil micro-organisms rather than plants, consequently plant uptake as an immobilisation process and leaching from plant material as a mobilisation process will not be discussed. Similarly, because this was a mobilisation study, transport processes *per se* will not be discussed either.

2.4.0. Soil Solution – Processes Controlling Composition.

The following discussion will initially centre on those processes that control the equilibrium concentration of P in the soil solution. A review of the forms of P found in soil solution will then be provided; this will be followed by some general comments on P leaching. The evidence from the literature that supports the underlying contention of this thesis will then be reviewed.

2.4.1. Geochemical /Physical Mobilisation Processes.

Weathering

Weathering as a process can be classified as being either physical or chemical in nature. Physical weathering includes all those processes such as freezing and thawing cycles that mechanically reduce the particle size of minerals, while chemical weathering involves the chemical transformation of mineral phases. In simple terms, chemical weathering reactions can be divided into five broad classes: solution, carbonation, hydration, hydrolysis and redox reactions (Ross, 1989).

In relation to P minerals, hydrolysis type reactions are likely to be the most important. The dissolution of a mineral such as fluorapatite can be represented in the following manner (Sanyal and Detta, 1991):

$$Ca_{10}(PO_4)_6F_{2\,(s)} + 12H^+_{(aq)} = 10Ca^{2+}_{(aq)} + 6H_2PO_4^-{}_{(aq)} + 2F^-_{(aq)}.$$

Obviously the dissolution of the solid phase will be favoured by high concentrations of H^+ or low concentrations of $H_2PO_4^-$.

As noted earlier soil micro-organisms can play a role in the chemical weathering process through the production of various organic acids. These materials can be classified into two groups on the basis of chemical reactivity; those whose weathering effect is a direct acidic (H^+) effect and those that also have an additional interaction (electrostatic, chelation or water bridging) effect (Tan, 1986). Thus, P may be released from apatite by chelation of Ca^{2+} or from Al and Fe phosphates by chelation of Fe^{3+} and Al^{3+}. Given the energy requirements of most micro-organisms Tan (1998) suggested that the rhizosphere is the most likely habitat for micro-organisms that act as agents for mineral weathering.

In relation to P bound as salts in the soil, solution type reactions will be the most important in terms of mobilisation. Redox reactions can also play a role in P mobilisation. This topic will be discussed under biological mobilisation processes. It should be noted that hydration and hydrolysis reactions play another important indirect role in the P cycle. Reactions of this type produce a variety of secondary mineral phases such as kaolinite and goethite that are highly reactive toward orthophosphate.

Desorption.

The transfer of ions from the surface of soil constituents to the soil solution is termed desorption. In this context the orthophosphate buffering capacity of a soil is an

important property. In simple terms this is the ability of P associated with solid soil components to move into solution in response to a decrease in the concentration of orthophosphate in solution. Thus, the response of a soil to decreases in orthophosphate ion concentration in solution as a result of absorption by either plant roots or micro-organisms will largely be determined by its buffering capacity.

Direct anion exchange may also be important in terms of P desorption. The conjugated anions of low molecular weight organic acids such as citric, tartaric, oxalic and malic which are found in root exudates, have been reported as being effective in desorbing P from soil components (Nagarajah *et al.* 1968; Lopez-Hernandez *et al.* 1979). Inorganic anions such as HCO_3^- and OH^- may also displace adsorbed orthophosphate from the surface of soil components. It should be noted that various soil constituents can also adsorb organic forms of P. Lan *et al.* (1995) reported that organic anions such as malonate, malate and tartarate were effective in mobilising organic P compounds from various soils. The authors did not speculate on the mechanisms responsible for this release, but it does not seem unreasonable to suggest that organic anion exchange may have played a role.

Release from the Microbial Biomass – Seasonal Variation.

Cyclic variations in various environmental parameters (i.e. soil moisture, temperature etc) may play an important role in releasing P from the microbial biomass. In this context most attention has been focused on the impact that wetting/drying and freeze/thawing cycles may have.

Sparling *et al.* (1985b) suggested that seasonal fluctuations in soluble P levels might be related to microbial death and turn-over linked to soil wetting and drying cycles. The argument being put is that soil desiccation will cause the death of micro-organisms thus releasing their contents into the soil environment. According to Bartlett and Jones (1980) as desiccation proceeds, the surface tension of surface oriented water may tear apart molecular and cell structures as contracting stress forces parallel to the surface reach thousands of atmospheres. Brookes *et al.* (1982) reported that desiccation of soil released considerable amounts of both organic and inorganic P from the microbial biomass. Grierson *et al.* (1998) found that there was an initial flush of P from a surface sample of a Spodosol after re-wetting. These authors noted that wetting and drying cycles could also influence P mineralisation kinetics and adsorption/desorption reactions, as well as releasing the contents of soil micro-organisms. In many soils it may prove to be difficult to differentiate between these effects and determine their contribution to the observed flushes of P upon re-wetting. It should also be borne in mind that in climatic regimes characterised by cyclic periods of wet and dry, micro-organisms may have evolved that are relatively resistant to desiccation. Under these circumstances only limited amounts of P and other nutrients would be released as a result of these periodic fluctuations in soil moisture.

There are climatic regimes in which soils are subject to episodes of freezing and thawing. It could be assumed that freezing would have similar biocidal effects as desiccation on soil micro-organisms. However, according to Ron Vaz *et al.* (1994) the available information is very limited and often contradictory. They found that freezing

substantially increased the concentration of P in the soil solution from both a mineral soil and a peat soil. They attributed this increase in part to the release of P from the microbial biomass and also from freezing causing direct solubilization of organic compounds. On the other hand Schmidt *et al.* (1999) who examined mineralisation and immobilisation of N and P in arctic soils could not find any strong evidence to support the argument that there was high microbial mortality during winter and subsequent release of P and N from the biomass upon thawing. They concluded that high winter nutrient release is not a general feature across all arctic ecosystems. Again it should be remembered that micro-organisms may have adapted to freezing/thawing cycles where they are an integral part of the climatic regime. This would minimise the effectiveness of these processes as a mechanism for release of P from the microbial biomass.

2.4.2. Geochemical/Physical Immobilisation Processes.

Adsorption.

A great deal of work has been done on P adsorption by soils. Much of this effort has been expended because this process renders much of the P applied to soils unavailable to plants. In the context of the model depicted in Figure 2.2 this process is important because it removes P from the soil solution and associates it with a variety of secondary minerals. In most mineral soils it is this process that renders orthophosphate species immobile. It has also been recognised for some time that various organic P compounds are also adsorbed by various soil components (Anderson and Arlidge, 1962; Ognalaga *et al.* 1994).

Various studies have highlighted the importance of hydrous metal oxides of Al and Fe in sorbing P. However, comparison of the results reported in the literature is difficult because of the wide variations in the conditions under which the experiments were conducted. Nevertheless the following general conclusions can be drawn (Sanyal and De Datta, 1994). Amorphous components (Fe and Al oxide gels) are more efficient at sorbing P than their crystalline counterparts and crystalline hydrous metal oxides are more efficient than layer alumino-silicates and pure calcium carbonate has relatively little ability to sorb P. It has also been reported that poorly crystalline alumino-silicates materials have a large sorption capacity (Cloos *et al.* 1968) while Holford and Mattingly (1975) suggested that with naturally occurring calcium carbonate, hydrous ferric oxide impurities may be responsible for sorbing considerable amounts of P.

P sorption on the surface of hydrous Fe oxides is thought to involve ligand exchange between water or hydroxyl groups present on the surface of the gel (Parfitt *et al.* 1975; Ryden *et al.* 1977). According to Sample *et al.* (1980) similar mechanisms are thought to be involved with the sorption of P on the surface of hydrous Al oxides. These workers also suggest that in calcareous soils ligand exchange mechanisms involving water, bicarbonate or hydroxyl ions are important.

Various workers have reported that the application of organic matter to soils tends to decrease P adsorption (Abbot and Tucker, 1973; Reddy *et al.* 1980). Competition between orthophosphate and various organic anions for binding sites is thought to be responsible for this effect (Hue, 1991). Organic matter can also inhibit Al and Fe oxide

crystallization according to Walbridge *et al.* (1991). They suggested that in forest soils rich in Fe and Al the regular input of organic matter leads to high concentrations of non-crystalline Fe and Al materials in the surface layer thus enhancing P adsorption.

It has long been recognised that complexation reactions can occur between organic matter, various metal ions and P in soils. However the isolation of these compounds is difficult because of their low amounts in soil and the dual nature (organic/inorganic) of the P (Levesque, 1969). Levesque and Schnitzer (1967) prepared model organo-metallic complexes and reacted these with orthophosphate. The characteristics (chemical, spectroscopic and thermogravimetric) of these materials were then compared with those of organo-metallic complexes extracted from the soil. The results indicated that similar compounds to the model ones exist in the soil, but only in limited amounts. In a similar vein Sinha (1971a, b) prepared and studied the properties of various organo-metallic – P complexes. Gerke (1992) found evidence of humic-Fe/Al-P complexes in soil solutions obtained from acidic sandy soils. This work suggests that P complexed in this manner might be an important form in various soil types.

As noted earlier losses of applied P through leaching can be significant in sandy and organic soils. This occurs because these soils lack inorganic components that are highly reactive towards orthophosphate.

Precipitation Reactions.

According to Larsen (1967) the concentration of P in soil solutions ranges from 0.1 to 1 mg/kg. Given this, it is highly unlikely under most circumstances that precipitation reactions are going to be important immobilisation reactions. However, this process may be important in solutions surrounding dissolving fertiliser pellets (Sample *et al.* 1980). The formation of occluded P is a process associated with precipitation reactions where P becomes physically encapsulated by minerals that do not contain P as a structural component. It should also be noted that drying and freezing episodes might generate soil solutions with a high ion concentration. Under these conditions a variety of localized precipitation reactions may occur.

2.4.3. Biological Mobilisation Processes.

Mineralisation.

Mineralisation is the term used to describe the production of inorganic ions by the microbially mediated oxidation of organic compounds. McGill and Cole (1981) suggested that mineralisation of organic P can occur independently of carbon oxidation. The mechanism they proposed involved the hydrolysis of phosphate esters being catalysed by phosphohydrolase. They postulated that plant roots and micro-organisms release the enzyme in response to the need for P. The authors used the term biochemical mineralisation to distinguish this process from that process associated with the oxidation of C.

Mineralisation is a critical process because it transforms organic P compounds from various residues back into orthophosphate. The general consensus seems to be that this is the most important form of bio-available P in the soil solution. In general terms the rate of mineralisation will be controlled by those factors that control the population of soil micro-organisms. These include temperature, moisture status, aeration and soil pH (Dalal, 1977; Harrison, 1982; Tate, 1985). There have been several reports that wetting and drying cycles can enhance the rate of organic P mineralisation (Birch and Friend, 1961; Grierson *et al.* 1998). Because of adsorption and or precipitation reactions, measurement of P mineralisation rates is difficult. However, Frossard *et al.* (2000) suggest that recent work has demonstrated that isotope exchange kinetics can be used to determine gross organic P mineralisation in soil.

Release from Soil Biomass - Grazing by Amoeba and Nematodes.

While it is thought that soil animals (protozoa and nematodes) make a significant contribution to the cycling of nutrients through their interactions with soil micro-flora (Srivastava, 1992), little work appears to have been carried out in this area. An exception is the elegant experiment conducted by Cole *et al.* (1978) who devised a microcosm study using combinations of bacterial, amoebal and nematode populations. They found that while an active bacterial population rapidly converted labile inorganic P to microbial P, most of this was converted back to inorganic P by the actions of amoeba. Perrot *et al.* (1990) postulated that this might have been one of the mechanisms responsible for the seasonal changes observed in the levels of microbial P in a New Zealand pastoral soil.

Fe Reduction.

While P is not directly involved in any redox reactions, it is often closely associated with Fe (III) oxides that are involved in redox reactions. During the reduction of an acid soil these oxides are subject to progressive reductive dissolution that will release sorbed and co-precipitated P (Willet, 1991). There may be an indirect pH effect associated with the reductive dissolution of ferric hydrous oxide. This process causes the soil pH to rise which favours P desorption from materials that have variable charge characteristic.

2.4.4. Biological Immobilisation Processes.

Incorporation into microbial biomass.

As far as can be ascertained, only a limited amount of work has been carried out on the actual mechanisms of P uptake by an active microbial population, although Stewart and McKercher (1982) quote Beever and Burns (1976) and Burns and Beever (1977) as sources on this topic. In general terms micro-organisms may take up P as a result of changes in substrate availability or in response to changes in physiology caused by moisture fluctuation or other disturbances (Frossard *et al.* 2000).

Addition of organic residues to soil can either lead to net immobilisation or mineralisation of P depending on the C:P ratio of the residues. Alexander, (1961) (as quoted in Dalal, 1977) and Singh and Jones (1976) both reported that when residues containing 0.2% P were added to soil immobilisation occurred, while mineralisation occurred when residues containing 0.2% P were added to soil.

2.5.0. Soil Solution – Forms of P.

The preceding discussion highlighted the role that a variety of processes play in controlling the equilibrium concentration of P in the soil solution. Both mobilisation and immobilisation processes are involved. A closely related issue is the question of which forms of P are found in the soil solution.

Despite the vast amount of work carried out on P chemistry in the soil, relatively little has been directed towards characterising the forms of P in the soil solution. This is a consequence of the difficulties involved with sampling soil solution, limitations in the volumes obtained and the low concentrations of P in most soil solutions (Shand *et al.* 1994). Another complicating factor is that the soil solution is a complex mixture and the separation, identification and quantification of the components of such a mixture is inherently difficult. The complexity of the mixture is based not only on the variety of chemical forms present, but also on the fact that the compounds may occur in various physical forms. Given this, then it is hardly surprising that a variety of techniques have been used in an attempt to distinguish between the various forms of P. It should be remembered that there are no techniques that will discriminate perfectly between the various forms; the best that can be achieved is operationally or analytically determined fractions, which may contain a variety of P compounds.

It should be noted that from a limnological and oceanographical viewpoint much has been done to characterise the forms of P that occur in natural waters. In the context of the analysis of P in natural solutions these two disciplines have much in common with

soil science. Therefore anyone with an interest in characterising P in soil solutions should keep abreast of developments in these other two disciplines. There are a number of excellent reviews on P in natural waters to which the reader is referred (Olsen, 1967; Burton, 1973; Broberg and Pettersson, 1988; Broberg and Persson, 1988; Holtan *et al.* 1988; Gibson, 1997).

2.5.1. Characterisation.

Almost all characterisation procedures begin with a physical fractionation step. Single step filtration is the procedure most commonly used to make the distinction between particulate and dissolved material. It should be noted that because particle size distribution of material in soil solutions is continuous and no separation procedure exists that will produce a well-defined particle size cut off limit, these terms are being used to designate a particle size range. Unfortunately, there is no consistent usage of these terms in the literature. The standard separation procedure is filtration through a 0.45 μm pore diameter membrane filter (American Public Health Association, 1995); the P in the material retained by the filter is termed particulate while P in the filtrate is termed dissolved.

It should be remembered that the dissolved fraction contains particles in suspension that are small enough to pass through the filter membrane. These materials are termed colloids, thus creating a third analytical fraction.

Particulate P.

Broberg and Persson (1988) suggested that in relation to natural waters there are six main sources of P containing particles. These are:

1. biologically produced cells;
2. weathering products such as primary or secondary minerals;
3. direct precipitation of inorganic P (authigenic mineral formation) or sorption to other precipitates;
4. degradation of cells producing detritus;
5. flocculation of organic macromolecules to produce larger aggregates; and
6. formation of organic/inorganic co-precipitates or inclusion of P in organic aggregates by metal (Fe, Al) binding.

Thus, in this way particulate P can be characterised by its origin or source.

In terms of soil solution biologically produced material is likely to be an important source of P containing particles. In relation to P minerals while they may be mobile in the soil environment, P is more likely to be adsorbed on to the surface of any mobile secondary minerals such as kaolinite and goethite. Given the low concentration of P found in most soil solutions the direct precipitation of inorganic P minerals is not likely to be a major source of particulate P. However, P adsorbed onto other precipitates that are mobile may be an important source. In relation to the soil solution a variety of factors will determine the relative importance of these six sources at any given time. Factors that may play a role include climatic regime and seasonal variation.

Dissolved P.

As noted earlier, little work has been directed towards identifying specific P compounds in the filtrate. The usual procedure is to chemically fractionate the 'dissolved' P by reacting the filtrate with an acidified molybdate solution. The P that reacts to form a phospho-molybdate complex is termed molybdate-reactive (MRP) while the P that does not is termed unreactive (URP). URP is determined as the difference between total dissolved P and MRP. This procedure is based on the analytical technique first developed by Murphy and Riley (1962).

It should be noted that a variety of separation techniques and ^{31}P-NMR spectroscopy (Newman and Tate, 1980; Tate and Newman, 1982; Guggenberger *et al.* 1996) have been used with some success to characterise the forms of P found in various soil extracts. The separation procedures used include anion exchange (Cosgrove, 1980; Sibbesen *et al.* 1994), gel filtration (Moyer and Thomas, 1970; Veinot and Thomas, 1972; Tate, 1979; Condron and Goh, 1989), high pressure liquid chromatography (Gerritse, 1978) and ultrafiltration (Gerke and Jungk, 1991). There is considerable potential for these procedures to be adapted for use in characterising P in the filtrate. The low concentration of P in this fraction would probably present the biggest problem in adapting these procedures. In this context it is interesting to note that Shand *et al.* (1996) (as quoted by Chapman *et al.* 1997) reported that an attempt to use solid-state ^{31}P-NMR spectroscopy to elucidate the nature of the chemically defined fractions of a soil solution, failed.

As long ago as 1927 Pierre and Parker reported that the amount of organic P in soil solution is greater than the amount of inorganic P. Numerous other studies have reinforced this observation (Timmons *et al.* 1977, Kelly *et al.* 1983). There have been only a few studies that have attempted to positively identify specific organic P compounds in soil solution. Rogers *et al.* (1941) while not identifying any specifically did find that the organic P compounds present in water extracts of soil were not plant available. This suggests that the compounds were not lecithin, nucleic acids, nucleotides or glycerophosphate. Martin (1970) found evidence of P esters in cold water extracts of soil.

Pant *et al.* (1994) took a slightly different approach to characterising organic forms of P in soil solution. These workers used enzymatic hydrolysis to classify the compounds into broad groups. Using this approach they found that there were major differences in organic P extracted from four different soils. They also fractionated the organic material in solution according to molecular size by gel filtration and measured the amount of MRP and URP in each fraction. They found that on average 30% of the total P in each fraction was molybdate reactive. However, in the lower molecular weight fractions reactive P accounted for almost 80% of the total P present. It is worth noting that prior to fractionation the water extracts were concentrated using a rotary evaporator. This raises the question of whether or not this process would change the forms or distribution of P across the various fractions. As far as can be ascertained no work has been done to clarify this issue.

Orthophosphates are considered to be the main forms of inorganic P in soil solutions. Polyphosphates have been reported as occurring but are not considered to be important. The forms of orthophosphate in solution are determined by protonation reactions and complex formation (Larsen, 1967). Phosphoric acid (H_3PO_4) is a tribasic acid that dissociates to give the following ions, $H_2PO_4^-$, HPO_4^{2-}, PO_4^{3-}. In the pH range 4-6 $H_2PO_4^-$ is the predominant species, consequently it should be the most common ion in most soil solutions. Soil solutions will usually contain various metallic cations that are able to complex with $H_2PO_4^-$ and under some circumstances HPO_4^-.

In the laboratory White *et al.* (1976) found evidence of the presence of soluble polymeric complexes of Al and orthophosphate in dilute solutions. From this evidence they suggested that these compounds might also occur in soil solutions while Gerke (1992) found evidence of humic-Fe/Al-P complexes in soil solutions obtained from acidic sandy soils. In one particular instance these complexes accounted for more than 50% of the total organic P in solution.

Colloidal P.

Colloids are commonly defined as small particles with dimensions between 1 nm and 0.45 μm. These particles have several unique properties worth noting. Firstly, they have very large specific surface areas (>10 m^2/g), which means that they may be enriched with surface reactive species (including P) relative to larger particles. Secondly, they can remain in suspension for long periods of time thus facilitating transport by either mass flow or diffusion.

According to Kretzschmar *et al.* (1999) there are four potential sources of mobile colloidal particles. These are:

1. *in situ* mobilization of colloidal particles that are naturally present;
2. formation of particles by precipitation from saturated solutions;
3. mobilization of 'biocolloids' such as viruses or bacteria; and
4. introduction from external sources, (e.g. from waste disposal practices).

Given the fact that most P in soil is associated with material in the finer size fraction, then *in situ* mobilisation could be an important source of mobile colloidal P. In certain circumstances the mobilisation of biocolloids could also be another important source of these materials.

A variety of soil materials can be mobilised as colloidal particles including aluminosilicate minerals; oxides and oxyhydroxides of Fe, Al and Mn; silica; carbonates and organic matter (including viruses and bacteria). It should be noted that in the soil environment these materials usually occur as mixtures or aggregates, which behave differently to the pure components. For example, it is well known that organo-clay complexes exhibit much higher colloidal stability than do comparable reference clays that are not coated with organic matter (Heil and Sposito, 1993; Kretzschmar *et al.* 1993).

Ultrafiltration involving sequential size fractionation and dialysis are the two techniques most commonly used to investigate and characterise the colloidal materials found in

natural waters. The difficulties associated with using these techniques mean that little work has been done in characterising colloidal P in soil solutions. However, Shand *et al.* (2000) examined the distribution of P between the different size fractions of a soil solution. They divided the total P in solution into three procedurally defined components: molybdate reactive P (MRP), organic P (OP) and condensed P (CP). The amounts of these components in the following size fractions were then determined: particles > 1.2 μm; 0.22 μm < colloids < 1.2 μm; molecules < 0.22μm. They found that for MRP 55% was associated with particles, 23% with colloids and 22% with molecules, while for OP 34% was associated with particles, 46% with colloids and 20% with molecules. CP behaved differently with none being associated with particles, 54% being associated with colloids and 46% with molecules. The distribution of MRP, OP and CP in different molecular sized fractions of 0.45 μm filtered soil solution were determined using 100, 10 and 1 kD ultrafilters. This process showed that 32% of MRP, 95% of OP and 90% of CP was in the >10 kD size fraction.

In a slightly different vein Mayer and Jarrell (1995) assessed the amounts of colloidal (0.05-1.0 μm) P and Fe that were present in the streams of the Tualatin River Basin. They found that colloidal P and Fe accounted for between 0 - 48% and 2 - 77% of the total P and Fe, respectively. In addition to Fe and P the colloids also contained Si, Al and Ca. The authors suggested that the colloids formed as groundwater or sediment released Fe(II) is oxidised to Fe(III). The Fe associates with P, either as surface coatings on clays or organics or as homogeneous particles. Clearly, these workers did not see surface run-off as the major source of colloids.

These two studies suggest that colloidal P can play an important role in P transport. This has important implications for the management of P movement within landscapes.

As the discussion above illustrates ultrafiltration, and to some extent gel filtration have been used by various workers to concentrate and molecular size fractionate the P present in a variety of natural waters including soil solutions (Pant *et al.* 1994 (refer to pp 46); Donald *et al.* 1993 (refer to pp 53); Nanny *et al.* 1994; White and Payne, 1980; Lean, 1973). These procedures again are based on a set of operationally or analytically determined fractions, which may contain P in a variety of chemical and physical forms.

2.6.0. P Leaching General Considerations.

When strictly defined, leaching refers to the translocation of soluble ions along with percolating soil water during drainage (Ross, 1989; Haygarth and Sharpley 2000). When defined in this manner leaching can be described using a model that has two phases. These are an immobile solid phase (soil matrix) and a mobile aqueous phase (soil solution). With this definition it is implicitly assumed that for solid material to be mobilised it must dissolve. However, this view ignores the fact that particulate material and colloids may be mobile in the soil environment. Particulates and colloids can sorb various organic and inorganic materials thus stabilising them in the aqueous phase and increasing their mobility. When discussing the subsurface transport of contaminants McCarthy and Zachara (1989) noted that particles and colloids could also be mobile in this environment. They argued that using a model to describe aquifer flow that

consisted of a mobile aqueous phase and an immobile solid phase did not adequately address the complexity of this natural system. A similar argument can be made in relation to leaching.

These authors suggested that modelling aquifer flow using a three-phase system in which the particulate and colloidal materials are the third phase is one way of addressing this problem. Obviously such an approach can also be adopted when modelling the leaching process. To do so would be useful because it shifts the focus onto processes that generate particulate and colloidal material in the soil environment. However, the terminology used by these authors to describe what is happening is confusing. The word phase is being used here to both describe the physical state of material (i.e. solid, liquid or gas) as well as its state of mobility (i.e. mobile/immobile).

Clearly both of these definitions have shortcomings. Obviously with leaching, there must be a liquid carrier material (usually water) and an immobile solid porous matrix of material that the liquid can move through. In this context the relevant question is what processes are involved in associating parts of the solid matrix with the carrier liquid? In other words what processes transform the material from an immobile fixed state to one that is mobile and readily moved by the carrier material, irrespective of its physical state? Thus it is probably best to use a model that consists of an aqueous phase and a solid phase without making any assumptions about the state of mobility of these two phases. Parts of the solid matrix are potentially mobile (components that readily

dissolve, particles/colloids that readily disperse) and parts of the aqueous phase are potentially immobile (liquid in micro-pores).

Part of the argument being developed here is that to fully understand the leaching of P, it is necessary to adopt a relatively flexible definition of the process. Heal (1979, as quoted in Ross, 1989) included the removal of fine particulate organic matter in his definition of leaching. The definition of leaching adopted for this work includes the translocation of soluble ions along with dispersed organic and inorganic particles and colloids. The discussion in Section 2.4 highlights that our current understanding is based on the definition put forward by Ross (1989) and others. All of the processes discussed centre around the transformation of orthophosphate. The chemical behaviour of this species in the soil environment is well understood. A tremendous effort has been put into understanding the inorganic components of the system, while scant attention has been paid to the biological components and the role that they may play in P leaching. There is however a small body of work in the literature that suggests a re-examination of this position is warranted. Thus the underlying theme of this thesis can be focused more sharply. The argument being developed is that if the definition of leaching is expanded to include particulate and colloidal material, then there is evidence to suggest that the biological components of the soil system can play an important role in P leaching.

2.7.0. P Leaching - Biological Aspects.

Schoenau and Bettany (1987) examined the role that organic matter leaching played in nutrient cycling. To do this they sampled three soil types (Typic Cryboralf, Aridic Haploboroll and an Argiaquoll) by genetic horizon at upper, mid and lower slope positions. They extracted (NaOH + acidification) humic (HA) and fulvic (FA) acid constituents from the soil samples.

They reported that the HA/FA ratios for C, N, P and S decreased with increasing profile depth. They argued that this shift in nutrients from the humic to the fulvic acid fraction was consistent with FA being preferentially leached from the upper horizons and accumulating in the lower. In all the horizons they studied the HA P/FA P ratios were very low indicating that there was a high proportion of P in the FA fraction as compared to the other elements. The P/N and P/S ratios all increased with depth. This meant that the FA in the lower horizons was enriched in P relative to N and S. The authors argued that this suggested that organic matter that was leached from the upper horizons had a higher P content than the organic matter that was not leached.

Donald *et al.* (1993) examined the role that dissolved organic carbon (DOC) played in P movement in a forest soil. As part of their study they collected soil solutions using ceramic cup lysimeters. The molecular weight distribution and the C and P contents of each fraction generated were determined. They found that the hydrophobic neutral fraction of DOC contained approximately 65% of total P in solution. This fraction had the highest number- average weight (894 daltons) of all the fractions. The authors

concluded that the leaching of high molecular weight hydrophobic neutral compounds might be responsible for the movement and redistribution of P in the soils studied.

The argument that organic P compounds may play an important role in the leaching process, is further strengthened by the fact that several workers have reported that organic P compounds are more mobile in the soil environment than inorganic P species (Pinck *et al.* 1941; Rolston *et al.* 1975; Castro and Rolston, 1977; Hoffman and Rolston, 1980). Ron Vaz *et al.* (1993) found evidence that suggested that dissolved organic forms of P were mobile down the soil profile thus making them more susceptible to deep leaching. While Chapman *et al.* (1997) concluded that the supply of dissolved organic P appeared to be related to microbial activity rather than being linked to the solubilization of soil organic P compounds.

In a slightly different vein numerous workers have examined the transformation and redistribution of P during pedogenesis. The focus of the work carried out by Walker and Syers (1976) was on using changes in the amounts, forms and distributions of P in soils as a dating tool. According to Day *et al.* (1987) P distributions have also been used for a variety of other purposes including identifying paleosols, developing landscape models and distinguishing between forest derived and prairie derived soils. From the perspective of the work carried out for this thesis, more important are the studies that have examined P losses from the soil profile.

Frossard *et al.* (1989) measured P losses from the profiles of three soil types: a Calcareous Brown (Grassland soil - Aridic Haploboroll), Orthic Black (Grassland soil - Udic Haploboroll) and an Orthic Grey (Forest soil - Typic Cryoboralf). They calculated that both of the grassland soils had lost 20% (550 kg/ha and 500 kg/ha respectively) of their initial P content over a 10-15 000 year time period. While over the same length of time the forest soil had lost 41% (3020 kg/ha). They noted that these losses could not be due to orthophosphate species being leached because the B horizons in all three soils had a high P sorption capacity and would act as a sink for this element. Furthermore, they found that all B horizons had suffered a net loss of P rather than any gain. They also found that in these soils a variety of organic P compounds (glucose 6-phosphate, choline phosphate and adenosine triphosphate) were more mobile than orthophosphate species. They also noted the predominance of organic P in water extracts from these soils. From these observations they concluded that the loss of P from these soils was due at least in part to the leaching of organic P compounds.

Xiao *et al.* (1991) examined the effect that pedogenetic processes had on the distribution of P, Ca and Mg in Gray Luvisols. These soils had 40% less P in their A and B horizons than was calculated to have been present at the start of soil formation. The authors noted that little of the P that had been lost could be accounted for by secondary accumulation in the deep sub-soil. According to their calculations a total of 2000 kg ha^{-1} of P had been lost from the system over a 10 000 year period. This represented a yearly loss of 0.2 kg ha^{-1}. Leaching of P from the soil to the ground water was the mechanism proposed by the authors to account for this loss.

Letkeman *et al.* (1996) examined the redistribution and loss of P from three Canadian soils (Ardill Association, Brown Chernozemic, semiarid short grass prairie; Weyburn Association, Dark Brown Chernozemic, sub-humid mixed prairie/aspen parkland transition; Waiteville Association, Gray Luvisolic soil zone, sub-humid aspen forest). They found that two (Brown Chernozem and Gray Luvisol) of the three profiles examined had lost P and that in all three profiles there had been downward movement of P into the subsoil. They argued that the leaching of stable soluble inorganic forms of P was responsible for the observed translocation of P. However, they did not dismiss entirely the possibility that the leaching of organic P compounds may have also played a role.

This body of work effectively does two things. Firstly, it clearly supports the argument that the biological components of the soil may play an important role in P leaching. Secondly, it highlights how the issue of P mobility has a temporal aspect to it. Losses of P from an ecosystem that are small on an annual basis can over time become quite significant. Thus, while P can be considered to be immobile in the 'short run', in the 'long run' or using a pedogenetic time frame it can be considered to be a relatively mobile element. This aspect of P leaching should be borne in mind when evaluating the arguments put forward to support and elaborate the contention that underlies this thesis.

In terms of the role that the biological constituents of the soil may play in P leaching it was Hannapel *et al.* (1964a, b) who first suggested that soil micro-organisms may play a direct role in the leaching of P from mineral soils.

2.7.1. Soil Micro-organisms - P Mobilisation.

In their first study Hannapel *et al.* (1964a, b) worked on the hypothesis that in calcareous soils it was organic forms of P that were mobile. In order to test this an experiment was carried out to examine the effects that various organic matter treatments had on the magnitude and forms of P moving through a calcareous sandy loam (refer to Table 2.5). ^{32}P labelled plant residues were used in this study.

The amounts of total P and inorganic P mobilised with each treatment were measured using the method developed by Pons and Guthrie (1946). Organic P was calculated as the difference between total P and inorganic P. The results are presented in Table 2.6.

These results can be summarised in the following manner. Compared to the control the addition of barley and bean residues (10 t/acre) and sucrose increased the total amount of P leached from this soil. In all three cases the increase was due to an increase in the amount of organic P that moved. The ^{32}P tracer studies showed that only 25% of the organic P in the leachate came from the added residues, while the remaining 75% came from the native soil fraction. Clearly, the addition of an energy source was more important in increasing P movement in this soil than was the addition of P, in either form. During the experiment the authors noted that there seemed to be a close

relationship between the amount of P in the leachate and the turbidity of the displaced solution. This suggested to them that the P might have been moving through the soil sequestered in microbial cells or cellular debris. The authors also noted that the addition of barley and bean residues (10t/acre) and sucrose also mobilised significant amounts of Ca.

Material Added	*Amount added / column*		*Total P added*	*Total ^{32}P added*
	(tons/acre)	(g/column)	(mg/column)	(cpm × 10^{-6}/column)
^{32}P (control)	-	-	-	104.10
Barley residues*	2	5.48	10.1	8.58
Barley residues*	10	27.40	50.7	42.88
Bean residues*	2	5.48	12.6	17.18
Bean residues*	10	27.40	63.0	85.90
H_3PO_4 + ^{32}P	P≈ to 10 T bean residue	0.233	63.0	104.10
K_2HPO_4 + ^{32}P	P≈ to 10 T bean residue	0.354	63.0	104.10
Sucrose +	C≈ to 10 T bean residue	27.03	-	104.10
NH_4NO_3 + ^{32}P	N≈ to 10 T bean residue	1.38	-	-

Table 2.5. Description of soil treatments. * Residues from plants grown in ^{32}P labelled solutions (from Hannapel *et al.* (1964a, b).

Material Added	*Amount added*	*Cumulative P*				
		Total P	Or P	Ino P	Org ^{32}P	Ino ^{32}P
	(tons/acre)	----------(μg)----------			(cpm ×10^{-4})	
^{32}P (control)	-	153a	79a	74c	0.0a	0.2a
Barley residues	2	201a	148a	54ab	0.5a	0.4a
Barley residues	10	740b	662b	78bc	12.6a	3.2b
Bean residues	2	218a	155a	63ab	0.2a	0.5a
Bean residues	10	887c	789b	98cd	28.2a	3.7b
H_3PO_4 + ^{32}P	P≈ to 10 T bean residue	157a	95a	62ab	0.7a	0.6a
K_2HPO_4 + ^{32}P	P≈ to 10 T bean residue	157a	116a	41a	0.5a	0.4a
Sucrose +	C≈ to 10 T bean residue	1197d	1077c	120d	849.5b	14.4c
NH_4NO_3 + ^{32}P	N≈ to 10 T bean residue					

Table 2.6. Cumulative P displaced from soil columns by water. The same letter following any two values indicates that they belong to the same population at the 0.05 level according to the Duncan Multiple Range Test (from Hannapel *et al.* (1964a, b).

In light of these results a second experiment was carried out to determine whether or not an active microbial population had an effect on the magnitude and forms of P that were mobile. In this experiment comparisons were made between a control and two treatments (refer to Table 2.7).

Sucrose was added to stimulate microbial activity in treatment one, while in treatment two formaldehyde was used as a soil sterilant. The leachate was physically fractionated and then both fractions were chemically characterised so that inorganic and organic P could be determined. A total of six displacements were collected.

Over the course of the experiment 2092 μg of P were leached with treatment one and 686 μg with treatment two. But only 55 μg were leached from the soil in the control column. The addition of sucrose produced a 38 fold increase in the total amount of P that moved. With treatment two there was also a marked increase in the amount of P that moved, but substantially less than that with treatment one. The authors believed that the increase observed in treatment two was due to incomplete suppression of the microbial population by formaldehyde. The fractionation of the leachates showed that almost all the extra P mobilised with treatment one was particulate in nature that is, associated with material retained by a 0.45μm membrane filter. Most of the P in this fraction was organic.

Treatments
No 1. Soil + sucrose + NH_4NO_3 equivalent to 10 t/acre of plant residue (40% C).
No 2. Soil + sucrose + NH_4NO_3 equivalent to 10t/acre of plant residues (40% C) + formaldehyde.
No 3. Soil (control).

Table 2.7. Description of treatments used in second experiment (from Hannapel *et al.* (1964a, b).

The most important finding of this work was that micro-organisms played a key role in leaching P from a calcareous soil. In light of this it is possible to refine the underlying contention of this thesis, to generate several hypotheses and to develop an experimental procedure to test them.

2.8.0. Hypotheses

If leaching is conceptually separated into its three component phases of mobilisation, transportation *per se* and deposition, then the work of Hannapel *et al.* (1964a, b) suggests that micro-organisms can play a hitherto unrecognised role in the mobilisation phase. None of the currently recognised biological mobilisation processes listed in Table 2.4 can adequately account for the results reported by these workers, primarily because all of these processes are concerned with transformations that involve orthophosphate, while the work of Hannapel *et al.* (1964a, b) suggests that particulate organic forms of P may be important in the context of mobilisation.

Thus the contention that forms the basis of this thesis can be concisely stated in the following manner:

> *'That soil micro-organisms play a hitherto unrecognised role in mobilising P in various soil types, and that processes associated with this role coupled with water flow may over time lead to the redistribution within or export of P from the soil profile.'*

The nature of the work carried out for this thesis was to be largely laboratory based; hence the main focus was on mobilisation processes rather than the long-term redistribution of P. With this in mind it was possible to develop and test two

hypotheses. This process was designed to help establish a valid experimental basis for the contention stated above.

2.8.1. Hypothesis One.

The mobility of P in a soil system will be determined by the interaction of the various geochemical and biological mobilisation and immobilisation processes. In mineral soils the mobility of P is relatively low in the short run because of the strong immobilisation processes, both geochemical and biological that operate. The work of Hannapel *et al.* (1964a, b) suggests that in a calcareous soil a microbially mediated process or group of processes occur that over-ride the operation of the very strong geochemical and biological immobilisation processes. In fact, their data suggest that the process/processes is/are so predominant that more P is mobilised in a biologically active system than in a sterile one. In other words, that there would be net mobilisation of P in a biologically active system. This is a somewhat surprising finding given the nature of the geochemical and biological immobilisation processes that operate in calcareous soils.

To explain this finding, the authors essentially postulated that an active microbial population would generate a considerable amount of organic debris that contained P. This material could be carried through the soil by moving water. The P sequestered within this material would be chemically inert towards reactive soil components. Presumably, the organic material in question would be removed from the soil system

before mineralisation occurred and the P was incorporated back into the microbial biomass or sorbed by inorganic soil components.

From a geochemical point of view if this process operates in calcareous soils where Ca controls the mobility of P then it may also operate in acid mineral soils where Fe and Al control the mobility of P. From a biological viewpoint there does not seem to be any reason why an active microbial population would not produce organic debris in an acid mineral soil.

Thus hypothesis one is:

> *'that soil micro-organisms mobilise P in an acid mineral soil and that the mobilised P is contained within cells or cellular debris.'*

2.8.2. Hypothesis Two.

As noted earlier the reason for developing and testing these hypotheses was to establish a valid experimental base for the general contention that forms the basis of this thesis. So far, it has been established from a review of the literature that in calcareous soil types micro-organisms appear to mobilise P. The first hypothesis and associated experimental procedure was designed to determine if this process also occurred in an acid mineral soil. The next logical step seemed to be to determine if this process also occurred in sandy soil types.

In relation to P mobility, sandy soils behave differently to mineral soils because they lack inorganic components that are reactive towards orthophosphate. Therefore in contrast to mineral soils where geochemical immobilisation processes are very important in terms of P mobility, in these soils types these processes are relatively unimportant. So, much of the P retained by these soils must be either bound up with soil organic matter or contained within the microbial biomass. This means the relative importance of the microbial biomass in terms of P retention has changed, with it becoming far more important.

In this context it is pertinent to ask whether soil micro-organisms would mobilise or immobilise P. *Prima facie* the work of Hannapel *et al.* (1964a, b) suggests that they should mobilise P because there appear to be no reasons why P sequestered in microbial cells or cellular debris could not be transported through a sandy soil.

Thus hypothesis two is:

> *' that soil micro-organisms mobilise P in sandy soils and that the mobilised P is contained within microbial cells or cellular debris.'*

2.9.0. Experimental Approach.

This was not a classic leaching study but rather a mobilisation study. In terms of P mobilisation there are many difficulties associated with the collection and interpretation of data from *in situ* soil ecosystems. These difficulties arise from the dynamic nature and the complexity of the interactions between the processes that mobilise P in the soil

and numerous other environmental variables. Compounding this already difficult situation are the potential interactions between various environmental variables and the sampling equipment. Because of this it was decided to study a simplified system in the laboratory, using packed soil columns. The use of these simplified systems to study more complex natural processes is justified provided the models have been constructed so they 'capture' or mimic the relevant properties of the more complex processes.

The basis of the experimental work carried out for this thesis was a series of mobilisation studies using repacked soil columns. The underlying rationale for this work was: to compare the amount of P mobilised from biologically active soil samples (sterile, re-inoculated) with the amounts mobilised from samples that were not biologically active (sterile). Water was the carrier material used to transport the mobilised P from the soil sample. This is a very simple model that focuses exclusively on the question of whether or not soil micro-organisms mobilise P. If they do mobilise P then greater amounts should be measured in the leachate obtained from the re-inoculated soil.

It does not seem unreasonable to suggest that this model, that is the movement of water through packed soil columns 'captures' enough of the more complicated leaching process that occurs in the field particularly the mobilisation phase, to make it a relevant experimental procedure. By manipulating the conditions (i.e. sterile/non-sterile) under which this process occurs it was possible to determine if soil micro-organisms played a role in mobilising P. Conceptually it is difficult to see just how this issue could have

been appropriately examined in the field. Of course when interpreting the results obtained from these experiments due regard must be paid to their inherent limitations.

One of the major problems is that the structure of the soil packed in the columns does not duplicate the actual soil structure in the field. This means that the flow regime within the columns can be very different to the regime in the field. This in turn can dramatically alter the movement of material. This was graphically illustrated by Smith *et al.* (1985) who showed that between 22-79% of *E coli* added to intact soil cores were carried to a depth of 28 cm, while only 0.2-7% were carried to the same depth in repacked soil columns.

2.9.1. Reporting Details.

Chapter 3 provides details of the specific methods used in the work carried out for this thesis. Chapter 4 reports on the experiments designed to test hypothesis one. Chapter 5 reports on the work carried out to test hypothesis two, while Chapter 6 draws together the experimental results and considers their broader implications.

Chapter 3.

Materials and Methods.

3.1.0. Columns used in Mobilisation Studies.

The columns used in this work were constructed from high pressure 40 mm polyvinyl chloride (PVC) pipe cut to the desired length (10 cm). These lengths of pipe were acid washed prior to use. A small circular piece of gauze was glued across one end of the tube. This material provided support to the soil that was packed into the column. Before being glued onto the pipe the gauze was carefully washed in DI water and then dried in an oven. Once the gauze had been glued across the bottom of the pipe, the entire fixture was soaked in DI water for 12 h.

Drainage at the bottom of the column was provided in the following manner. A 12.5 mm hole was drilled into the bottom of a Class 18 PVC end cap. A 5 cm piece of rigid clear plastic tubing was then glued into the hole. The end caps and plastic tubing were all acid washed prior to use. After the plastic tubing had been glued into the end-cap the entire fixture was acid washed.

A small piece of gauze was placed in the bottom of the end caps that were then packed with approximately 1 cm of acid washed sand. This ensured that there was no gap between the soil packed in the column and the bottom of the cap. Soil was then carefully packed into the prepared PVC columns. The packed columns were then pushed into the caps until the gauze was flush with the acid washed sand. Selleys

Silicone Roof and Gutter Sealant was then applied around the exterior join between the PVC pipe and the end cap.

3.2.0. Materials used in the Fractionation Procedure.

3.2.1. Filter Membranes.

Millipore mixed cellulose acetate and nitrate membrane filters with a mean pore size of 0.22 μm were used (catalogue No: GSW 025 00). The membranes were washed before use to minimise contamination of the samples. The procedure followed is set out in the Standard Methods for Examination of Water and Wastewater (19th Ed. 1995).

3.2.2. Collection Containers.

There is evidence to suggest that the containers used to collect samples may influence the concentration of various P ions and complexes in the collected solution (Murphy and Riley, 1956; Heron, 1963; Haygarth *et al.* 1995).

Hassenteufel *et al.* (1963) examined the adsorption of phosphate ions onto a variety of plastic and glass containers. They found that adsorption was prevented by treating glassware with 0.5-1.0% HF in either 2M HCl or deionised (DI) water. These authors recommended the use of Pyrex glassware to collect samples containing P ions. In light of this work it was decided that leachate was to be collected in Pyrex bottles (100 ml) that had been treated in a solution of 1% HF in 2M HCl for two days.

3.3.0. Procedure used to Fractionate Leachate Samples.

The leachate collected was fractionated using the scheme outlined in Figure 3.1. This is a relatively simple scheme that uses a filtration step with a 0.22 μm membrane filter to create two P fractions, particulate P (Pp) and dissolved (Pd). P in the material retained by the filter is termed particulate while P in the filtrate is termed dissolved. P in the filtrate (Pd) was chemically fractionated into molybdate-reactive (MRP) and molybdate-unreactive (URP) forms. MRP was determined using the method of Murphy and Riley (1965), whilst unreactive P was determined as the difference between Pd and MRP.

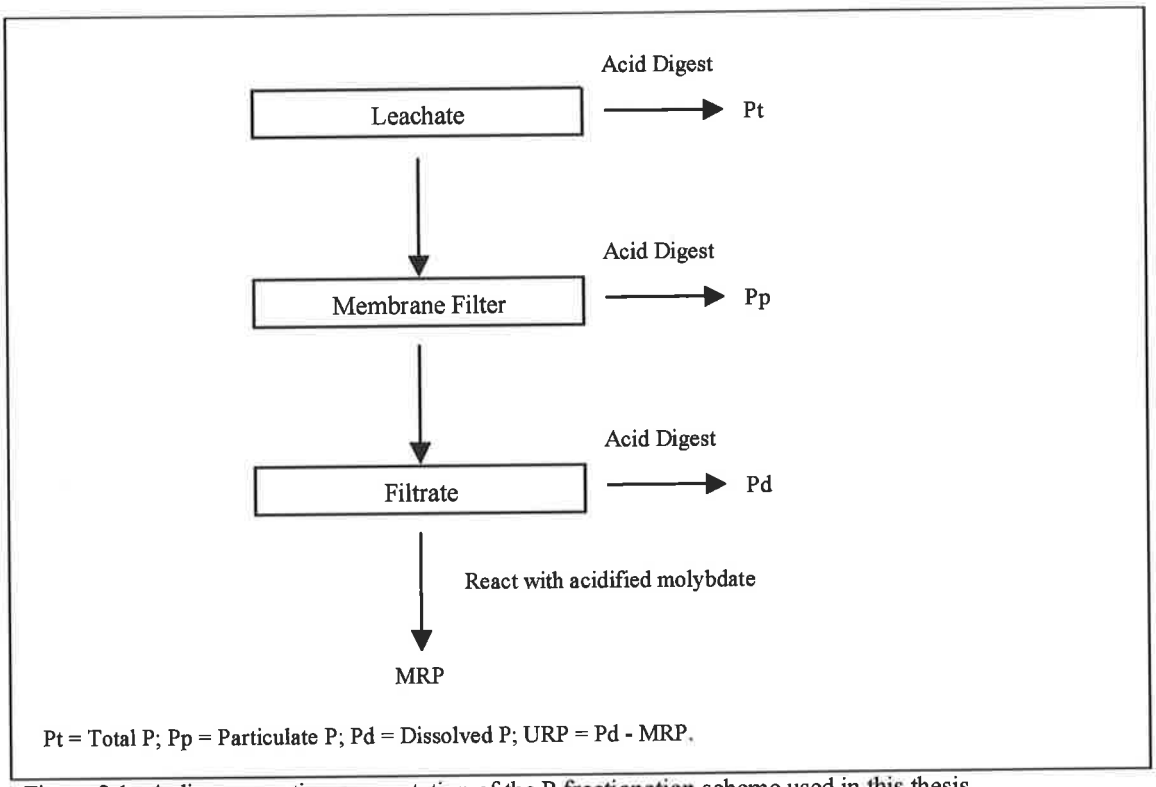

Figure 3.1. A diagrammatic representation of the P fractionation scheme used in this thesis.

An aliquot of unfiltered leachate was taken and acid digested ($HNO_3/HClO_4$) prior to being analysed for P. This value was termed total P (Pt). As a data quality monitoring procedure, for each sample this value was compared with the sum of the two fractions (Pp +Pd). Generally the variance between the values was less than 10%. This ensured that the results obtained from the fractionation procedure were consistent. The amounts of Al, Fe, Ca, Na, Mg and S were also measured in each of the fractions. In this way these elements were also partitioned into particulate and dissolved forms.

3.4.0. Storage of Samples.

During the storage of samples prior to analysis a variety of processes may alter the distribution of P forms in the sample. Adsorption by container walls and biological action are the two main processes that alter the distribution of P forms in the sample.

For this series of experiments it was important that biological activity was suppressed whilst minimising cell rupture and release of contents. The data of Fitzgerald and Faust (1967) and Haygarth *et al.* (1995) showed that refrigeration at 3-5°C was an appropriate storage environment for waters containing P. In order to minimise the impact of sample storage and preservation on the distribution of P forms in solution, the leachate was filtered as soon as possible after collection. All samples were stored in HF treated Pyrex containers at 4°C.

3.5.0. Chemical Methods.

3.5.1. Determination of MRP.

Molybdate reactive phosphorus (MRP) was determined using a modification of the original Murphy and Riley (1965) procedure. Most of the leachates were strongly coloured with an absorption maximum at approximately 290 nm, however some absorption was also recorded at 882 nm. This was compensated for by subtracting the absorbance of coloured blanks at 882 nm for each sample. Taking a portion of the sample and reacting it with a modified reagent prepared the blanks. The modified reagent contained no ammonium molybdate consequently the phospho-molybdate complex could not form.

All measurements were made with a double beam uv/visible spectrophotometer (GBC 918) using either 10 mm or 40 mm quartz cells (matched pair).

3.5.2. Digestion of Samples.

Both leachate and filter membranes (including particulate material) samples were digested using a $HNO_3/HClO_4$ mixture. O'Connor and Syers (1975) recommended $HClO_4$ digestion of samples when determining total P in waters containing particulate material. The samples were digested using Pyrex digestion tubes and a digestion block (Tecator Digestion System 40, Tecator, Sweden). The procedure followed is set out below.

Membranes or 20 ml of leachate were placed into digestion tubes along with a few drops of DI H_2O (filter membranes only). Then 5 ml of concentrated HNO_3 were added and the tubes placed into a digestion block and the following temperature program (refer to Table 3.1) was run.

Temperature Program.
100°C for 9h
115°C for 5h
130° for 2h

Table 3.1. Temperature program for digestion procedure.

The temperature was then increased to 160°C until the volume was reduced to 1 ml. Once the desired volume had been reached the tubes were allowed to cool. Then 1 ml of concentrated $HClO_4$ was added to each tube. The tubes were returned to the digestion block and heated at 230°C until dense white fumes were given off (20-30 min). Once cool they were diluted to the required volume (20 ml) with DI H_2O. Samples of leachate that had been spiked with known amounts of orthophosphate were processed as outlined above and the recovery values were satisfactory.

3.5.3. Analysis of Acid Digest Solutions – Determination of P, Al, Fe, Ca, Na, Mg and S Concentrations.

Inductively Coupled Plasma Atomic Emission Spectrometry (ICPAES) [Spectro Flame Modula (Spectro-GMB. Kleve, Germany)] was used to measure element concentrations in the acid digest solutions. A V groove nebuliser used in conjunction with a cyclonic cloud chamber produced consistent results.

3.5.4. Dissolved Organic Carbon (DOC) Analysis.

Dissolved organic carbon concentrations in the leachate were measured using a Dohrmann Total Organic Carbon Analyser, DC-180, (Rosemount Analytical Inc). This system is based on the UV promoted persulphate oxidation of organic compounds and the infrared detection of the CO_2 product.

3.5.5. pH and EC Measurements.

pH measurements were made with a pH meter: Orion model 940, fitted with an Orion glass electrode.

EC measurements were made with an EC meter: Orion model 160.

3.6.0. Scanning Electron Microscopy and EDS Analysis.

The particulate material retained by the filter membranes was examined using a JOEL 6300 Scanning Electron Microscope using the secondary electron detector (15 kV accelerating current). The material was chemically characterised using energy dispersive spectroscopy (EDS).

Chapter 4.

Acid Mineral Soil – Effects of Soil Microbial Activity on P Mobility.

4.1.0. Introduction.

This chapter reports on the results of two experiments. Experiment one was designed to test the first hypothesis developed from the review of the literature. Namely, that soil micro-organisms mobilise P in an acid mineral soil and that the mobilised P is contained within cells or cellular debris.

The second experiment had two objectives. The first was to demonstrate the general repeatability of the initial experiment. The second objective was to explore the relationship between microbial substrate availability and P mobility. This topic will be discussed in full in Section 4.3.0. The results from both of these experiments along with the comparison between them will be presented and described first. The synthesis and analysis of the data will then be presented.

As noted in Chapter 3 the concentration of various other elements (Fe, Al, Ca, Na, Mg, S and C) were measured along with the concentration of P in the leachate. It was felt that a better understanding of the behaviour of P would be gained when the behaviour of the other elements was taken into account.

The results presented in this chapter fall into two categories. Firstly, as tabulated analytical results showing how the various elements were partitioned between dissolved

and particulate forms along with pH and EC values of the leachate. Secondly, as images obtained using an SEM. This allowed a visual characterisation of the particulate material to be made. Energy dispersive spectroscopy (EDS) was used to chemically characterise this material. In the second experiment the flow characteristics of sets of sterile and re-inoculated columns were investigated using Cl break-through curves (BTC).

The soil used in both experiments was from the A horizon (0-10 cm) of a sandy loam (Typic Haploxeralf). Selected chemical and physical properties are presented in Table 4.1. Before proceeding, details are provided of some preliminary investigations that were conducted.

4.1.1. Preliminary Investigations.

ICP Analysis of Leachate.

As discussed in Chapter 2 ICP was the method used to determine the concentration of P and various other elements in the leachate. According to McKelvie *et al.* (1993) the use of ICP to analyse water samples obviates the need for acid digestion of the samples prior to analysis. If this contention were true it would mean that analysis of leachate would be a relatively simple procedure. To test the validity of this contention the following procedure was carried out.

A 110 ml sample of leachate was filtered using a 0.22 μm membrane filter. The filtrate was mixed and ten, 10 ml samples were taken, five of which were acid digested

($HNO_3/HClO_4$). After digestion these samples were made up to a final volume of 10 ml. The remaining five samples were left undigested. All samples were then analysed using ICP (V groove nebuliser + cyclonic cloud chamber). The results of the analyses are presented in Table 4.2.

Property Determined	*Value*
pH (1:5 soil:H_2O)	4.9
EC (1:5 soil:H_2O) dS/m	0.08
Total C (Leco) %	4.6
Total N (Leco) %	0.38
Available P (HCO_3) (mg/kg)	17
CEC (cmol(+)/kg (NH_4)	10.6
Exchangeable cations (cmol(+)/kg)	
Ca	2.77
Mg	1.72
K	0.43
Na	0.16
Trace elements (mg/kg) (DTPA ext)	
Fe	175
Mn	2.0
Zn	1.4
Cu	0.9
Texture	
Sand %	68
Silt %	21
Clay %	11

Table 4.1. Selected chemical and physical properties of Mt Bold sandy loam.

Sample No	*Treatment*	*P (mg/l)*
1	Digested	0.172
2	Digested	0.176
3	Digested	0.167
4	Digested	0.170
5	Digested	0.170
6	Undigested	0.071
7	Undigested	0.073
8	Undigested	0.072
9	Undigested	0.069
10	Undigested	0.070

Table 4.2. Results of P analysis using ICP comparing digested with undigested samples of leachate.

The amount of P measured in the undigested samples was approximately only 40% of that determined in the digested samples (blanks subtracted). This indicates that the use of ICP does not remove that need to predigest samples. It could not be determined why

the undigested samples gave lower values. There are several ways in which such a discrepancy could arise. It may be that colloidal P settles out in the sampling tubes and so a representative sample is not introduced into the nebuliser. Alternatively, a representative sample may be introduced into the nebuliser, but the architecture of the system may cause colloids or higher molecular weight dissolved species to be preferentially removed from solution and thus not be fed into the plasma. Given these results it was decided that all leachate samples were to be acid digested ($HNO_3/HClO_4$) prior to analysis.

Use of Formaldehyde as a Biocide.

The aim of this experiment was to examine whether or not it was possible to use a dilute formaldehyde solution to suppress biological activity in packed soil columns without greatly changing the nature of the soil constituents.

Four columns were prepared from lengths of PVC as outlined in Section 3.1.0. Each column was packed with approximately 236 g of soil (refer to Tables 4.1 and 4.3). Two of these columns were leached with a 1% solution of formaldehyde while the remaining two were leached with deionised (DI) water (controls). The leaching solutions were pumped onto the top of the columns at a rate of 10 ml/h using a small peristaltic pump. 50 ml of leachate were collected from each column, an aliquot of which was acid digested ($HNO_3/HClO_4$) and analysed.

The data from this comparison are shown in Table 4.4. These data show that the use of formaldehyde as a soil sterilant in this situation is unacceptable because it enhanced the mobility of numerous elements.

Columns	*Wt Soil*	*Bulk Density*
	(g)	(g/cm^3)
Control 1	236.7	0.996
Control2	237.2	0.998
Treatment 1	236.5	0.995
Treatment 2	235.8	0.992

Table 4.3. Amounts of soil used and bulk densities of prepared columns.

	Al	*Ca*	*Fe*	*K*	*P*	*S*
	mg/50 ml					
Control	0.109	0.109	0.042	0.675	0.010	0.283
Formaldehyde	0.509	7.920	0.390	2.260	0.060	0.327

Table 4.4. Concentration of selected elements in the leachate.

Low Temperature Inhibition of Soil Microbial Activity.

In the context of a mobilisation study with packed soil columns, the use of low temperature to inhibit microbial activity is a somewhat novel approach. Although McCalla (1950) used this technique to examine the effect that micro-organisms had on the rate of water flow through columns. To ascertain whether or not this particular procedure could be used to further explore the relationship between soil microbial activity and P mobility, a set of four columns were packed with approximately 236 g of air-dry soil (Table 4.1). The actual amounts of soil used and the bulk densities of the prepared columns are provided in Table 4.5. Two of these columns were leached with DI water in the laboratory at 20°C. The remaining columns were set-up and leached in a cold-room at 4°C. The DI water was pumped onto the top of the columns at a rate of 10 ml/h using a small peristaltic pump.

Columns	*Wt Soil*	*Bulk Density*
	(g)	(g/cm^3)
Column 1at 20°C	236.9	0.997
Control 2 at 20°C	235.7	0.992
Column 1 at 4°C	237.4	0.999
Column 2at 4°C	236.2	0.994

Table 4.5. Amounts of soil used and bulk densities of prepared columns.

The minimal impact that this method has on the chemical nature of the various soil components is its main advantage. However, a low temperature may reduce the reaction rates of abiotic chemical processes. The significance if any, of this rather subtle change was difficult to gauge. In practice, the impact of low temperature on the flow regime within the columns turned out to be more important. The viscosity of the water increased to such an extent that its capacity to move freely through the packed columns was severely reduced. The difficulties associated with ensuring an even flow through the columns maintained at 4°C, meant that little progress could be made using this procedure. The length of columns (20 cm) added to these difficulties, it was decided that in future experiments shorter columns (10 cm) would be used. Given the results obtained from these preliminary experiments it was decided to use γ-radiation to sterilise the soil columns and a biological control cabinet to maintain sterility during the leaching process.

4.2.0. Experiment No 1.

To test the first hypothesis the following experiment was carried out. A set of sterile soil columns (irradiated) was leached under sterile conditions in a biological control cabinet. A second non-sterile (irradiated, re-inoculated) set was leached in the laboratory under non-sterile conditions. The contention that underlay the design of this experiment was very simple. If micro-organisms mobilised P in the soil then there

should have been more P in the leachate from the re-inoculated soil than in the leachate from the sterile soil. The forms of P that were mobilised by micro-organisms were characterised by physically and chemically fractionating the leachate.

The design of this experiment took the effect of γ-radiation into account by ensuring both sets of columns had been irradiated. One set of which was re-inoculated and hence was biologically active. It would however have been useful to have some knowledge of the effect that the irradiation step had on the mobility of elements in the soil used in this work. To explore this, a further set of four columns was packed with soil and leached in the laboratory under identical conditions to the other two sets. The leachate obtained was fractionated and the results of the analysis were compared with those obtained from the sterile leachings. In this manner a direct comparison was made between soil that had been exposed to γ-radiation and soil that had not.

4.2.1. Materials and Methods.

Twelve columns were prepared from 10 cm lengths of PVC (Section 3.1.0) and packed to a depth of 8 cm with approximately 125 g of soil (refer Table 4.1). The actual amounts of soil used and the bulk densities of the prepared columns are shown in Table 4.6. Eight columns were then sterilised using γ-radiation (50 kGy) from a ^{60}Co source.

Under sterile conditions (biological control cabinet) four sterile columns were attached (vertically) to a wire frame held upright by a clamp and retort stand (refer to Figure 4.1). The columns were capped with Al foil.

Sterile DI water (autoclaved at 121°C for 20 min) was added to the sterile columns in the following manner. A small peristaltic pump was set up directly beside the control cabinet. A sterile circuit of tubing was used to pump sterile DI water from flasks in the bottom of the control cabinet onto the sterile soil in the columns. A sterile circuit was made by attaching appropriate lengths of medical grade vinyl tubing (ID 1.2 mm, OD 1.7 mm) to both ends of a flow measured pump tube (orange/orange, ID 0.035 mm). The join between the two pieces of tubing was sealed (air-tight) using a small strip of Parafilm®. Each length of tubing was sealed into individual plastic bags and sterilised using γ-radiation (50 kGy).

Each bag was opened in the control cabinet and the sterile tubing removed. One end of the tube was then placed in the top of a sterile column of soil and the other end was inserted into a flask of sterile DI water. The section of tubing containing the pump tube was then carefully removed from the control cabinet and attached to the pump. In this manner, a sterile circuit that could be used to pump sterile water onto the top of a sterile column was created.

Figure 4.1. Leaching of columns under sterile conditions.

Figure 4.2. Leaching of columns under non-sterile conditions.

Figure 4.3. Sterile and non-sterile leachate plated out and incubated.

Columns	*Wt Soil*	*Bulk Density*
	(g)	(g/cm^3)
Sterile 1	126.5	1.18
Sterile 2	126.6	1.18
Sterile 3	125.9	1.17
Sterile 4	126.4	1.18
Re-inoculated 1	127.0	1.18
Re-inoculated 2	126.0	1.17
Re-inoculated 3	125.6	1.17
Re-inoculated 4	126.4	1.18
Non-irradiated 1	125.9	1.18
Non-irradiated 2	125.8	1.18
Non-irradiated 3	125.7	1.18
Non-irradiated 4	125.6	1.18

Table 4.6. Amounts of soil used and bulk densities of prepared columns.

Another set of four sterile columns was set up in a similar manner but under non-sterile conditions in the laboratory (refer to Figure 4.2). These columns were re-inoculated in the following manner. The columns were wetted up with 20 ml of DI water and 5 ml of inoculum. The inoculum was prepared by wetting unsterilised soil to –33 kPa water potential and incubating for 2 days at 25°C. Then 1 g of this incubated soil was mixed with 50 ml of DI water.

Procedure	*Sterile Treatment*		*Re-inoculated Treatment*		*No Radiation Treatment*	
	Time (h)	Flow rate (ml/h)	Time (h)	Flow rate (ml/h)	Time (h)	Flow rate (ml/h)
(A)dding DI H_2O	1.5		1.5		1.5	
(I)ncubation +A	168		168		168	
(S)aturation + I	48		48		48	
(L)eaching	5	12	5	12	5	12
I	72		72		72	
L	5	12	4.5	13.3	5	12
I	72		72		72	
L	5.5	11	5	12	4.5	13.3
I	72		72		72	
L	6	10	5	12	4.5	13.3
I	72		72		72	
L	6	10	4.5	13.3	4.5	13.3
	Total= 533 Av leaching = 5.5	Av rate=11	Total= 529.5 Av leaching = 4.8	Av rate=12.5	Total= 529 Av leaching = 4.7	Av Rate=12.8

Table 4.7. Details of the procedure followed during experiment 1.

To each sterile column, 25 ml of sterile DI water was added and then the columns were incubated at 25 °C under sterile conditions for seven days. The non-sterile columns were incubated under non-sterile conditions for seven days. In both instances the water was added to the top of the columns under unsaturated conditions. During this period of incubation the columns were not completely saturated. In fact only a little over ½ a

pore volume was added to each. On every second day during the incubation period, 2ml of DI water (sterile/non-sterile as appropriate) were added to each column.

On day seven, enough DI water (sterile/non-sterile) was added to saturate the columns. This was accomplished by adding water to the top of the columns until drainage water began to flow from the bottom of the columns under the force of gravity. Once 'saturated' in this manner the columns were left to incubate for a further 2 days. Leaching was begun on day ten when enough DI water (sterile/non-sterile) was added to displace one pore volume (60 ml). This was accomplished by adding enough water to the top of the columns to ensure that one pore volume of drainage water could be collected from the bottom. Once drainage water had ceased flowing the columns were allowed to stand and incubate for a further 3 days. Then after every third day, enough DI water (sterile/non-sterile) was added to displace one pore volume, for a total of five displacements. Each time this was accomplished by adding enough water to the top of the columns to ensure that one pore volume of drainage water could be collected from the bottom. In all cases the water flowed from the columns under the force of gravity. A detailed description of the actual procedures followed is given in Table 4.7.

To ensure the integrity of the sterile system, samples of leachate from each column were plated out on nutrient agar and 2% potato dextrose agar and incubated for a week (refer to Figure 4.3).

All leachates obtained were fractionated using the scheme outlined in Section 3.3.0. The results of the chemical analyses of the various fractions were analysed using

SigmaStat statistical software, with treatments being compared using an unpaired t-Test. In certain instances it was necessary to transform the data prior to analysis. Data that could not be transformed into a suitable parametric form were analysed using the Wilcoxon Two Sample Test. Results that were below the detection limit were assigned a zero value. It should be noted that in analysing the data the following treatments were compared, non-irradiated versus irradiated (sterile) and sterile versus re-inoculated (irradiated, re-inoculated). The non-irradiated and re-inoculated treatments were not compared because in this case there were two variables that could influence the mobility of elements including P in the soil: γ-radiation and microbial activity. These data could not be analysed using two-way analysis of variance (ANOVA) because of an incomplete data set and interactions between the treatments. In this situation no definitive conclusions could be drawn, thus it would be only possible to speculate on the causes of any differences between these treatments.

The particulate material retained by the filter membranes was examined using an SEM. EDS was also used to chemically characterise this material. Given the nature of the samples it was not possible to quantify the amount of each element present using this technique. However, the presence or absence of certain elements including P could be determined.

After the completion of this work, the four columns that had not been irradiated were set-up and leached under identical conditions to the other eight columns. However, P in the leachate from these columns was not fractionated into MRP and URP because of

technical difficulties with the procedure. Ideally, these columns should have been leached at the same time as the others. However, equipment and space constraints made this impossible. The results were compared with those obtained from the sterile leaching using the same statistical procedure described above.

4.2.2. Results.

For ease and clarity, the comparison between the non-irradiated and sterile soils will be discussed first. The comparison between the re-inoculated and sterile soils will then be discussed.

Tabulated Analytical Results.

Phosphorus.

Non-irradiated Treatment vs Sterile Treatment.

These data show that the use of γ-radiation to sterilise this soil mobilised significant amounts of P. Essentially all of the P mobilised in this manner was in the dissolved fraction of the leachate and was flushed from the soil during the first two displacements. In contrast approximately 25% of the P mobilised in the non-irradiated soil was in the particulate fraction of the leachate, although the absolute amounts involved were relatively small. Consequently, in total across the five displacements more particulate P was mobilised in the non-irradiated soil than in the sterile soil.

Soil Treatment	*Total (P+D)*$^{+}$	*Particulate (P)*$^{+}$	*Dissolved (D)*$^{+}$	*% Particulate*
	µg/60 ml^{+}			
D1-Re-inoculated	192*	129$^{\beta}$	63*	67*
D1-Sterile	109	2	107	2
D1-Non-irradiated	19$^{\#}$	5$^{\chi}$	14$^{\#}$	26$^{\#}$
D2-Re-inoculated	76*	51*	21	72$^{\beta}$
D2-Sterile	41	1	40	1
D2-Non-irradiated	15$^{\#}$	3$^{\#}$	12$^{\#}$	24$^{\#}$
D3-Re-inoculated	44*	26*	18	58*
D3-Sterile	23	1	22	1
D3-Non-irradiated	16	5$^{\#}$	11$^{\#}$	33$^{\#}$
D4-Re-inoculated	28*	14*	14	49*
D4-Sterile	14	1	13	1
D4-Non-irradiated	12	3$^{\#}$	9	28$^{\chi}$
D5-Re-inoculated	16	7*	9	43*
D5-Sterile	15	1	14	1
D5-Non-irradiated	11	4$^{\#}$	7$^{\#}$	35$^{\#}$
Σ-Re-inoculated$^{@}$	358*	233*	125*	-
Σ-Sterile$^{@}$	201	5	196	-
Σ-Non-irradiated$^{@}$	74$^{\#}$	21$^{\#}$	53$^{\#}$	-
$^{@}$µg/300 ml				

Table 4.8. Amount of P mobilised and its distribution between particulate and dissolved fractions.

For each displacement the only comparisons made were re-inoculated vs sterile and non-irradiated vs sterile soils. For the re-inoculated vs sterile comparison, * indicates that the mean values are significantly different ($p \leq 0.05$). The treatments were compared using an unpaired t-Test. For the non-parametric data, β indicates that the mean values are significantly different ($\alpha \leq 0.05$). The treatments were compared using the Wilcoxon Two Sample Test. For the sterile vs non-irradiated comparisons, # indicates that the mean values are significantly different ($p \leq 0.05$). The treatments were compared using an unpaired t-Test. For non-parametric data, χ indicates that the mean values are significantly different ($\alpha \leq 0.05$). The treatments were compared using the Wilcoxon Two Sample Test. In the treatment column the letter D followed by a number denotes a particular displacement, while Σ denotes the total amounts of P displaced over the course of the experiment (five displacements).

Re-inoculated Treatment vs Sterile Treatment.

It is clear from these data that appreciably more P was mobilised in the re-inoculated soil than in the sterile soil. In the leachate from the re-inoculated soil, approximately 50% of the mobilised P was in the particulate fraction. In contrast, in the leachate from the sterile soil essentially all (99%) of the mobilised P was in the dissolved fraction. In fact over the five displacements, 45 times more particulate P was mobilised in the re-inoculated soil than in the sterile soil. There were no significant differences in the

amounts of P in the dissolved fraction of the leachates from the sterile and re-inoculated soils except in the first displacement, where there was less P in the leachate from the re-inoculated soil. With the re-inoculated soil the percentage of total P in the particulate fraction of the leachate fell from 67% in the first displacement to 43% in the fifth. With the sterile soil the percentage of total P in the particulate fraction remained constant at around 1-2%.

The data in Table 4.9 show the amounts of MRP and URP in the filtrate. These data show that with either treatment only a small portion (5-10%) of dissolved P was molybdate reactive. There were no significant differences between the treatments in terms of the amounts of MRP in the filtrate. The only significant difference between the treatments in terms of the amounts of URP in the filtrate occurred in the first displacement, where there was significantly less in the filtrate from the re-inoculated soil.

Soil treatment	*MRP*$^{+}$	*URP*$^{+}$	*% MRP*
	µg/60 ml^{+}		
D1-Re-inoculated	5	58*	18
D1-Sterile	5	102	7
D2-Re-inoculated	2	19	10
D2-Sterile	3	37	8
D3-Re-inoculated	2	16	11
D3-Sterile	3	19	10
D4-Re-inoculated	2	12	8
D4-Sterile	1	12	8
D5-Re-inoculated	1	8	12
D5-Sterile	1	13	8

Table 4.9. Concentration of MRP and URP in the filtrate.

Treatments were compared using an unpaired t- Test. * indicates that the mean values are significantly different ($p \leq 0.05$).

Iron.

Soil Treatment	*Total (P+D)*$^{+}$	*Particulate (P)*$^{+}$	*Dissolved (D)*$^{+}$	*% Particulate*
	µg/60 ml^{+}			
D1-Re-inoculated	208$^{\beta}$	219*	61	78*
D1-Sterile	137	40	96	30
D1-Non-irradiated	97$^{\#}$	42	55$^{\#}$	43$^{\#}$
D2-Re-inoculated	101	66*	35*	65*
D2-Sterile	100	24	76	24
D2-Non-irradiated	137	71$^{\#}$	66	51$^{\#}$
D3-Re-inoculated	109	43	66	39
D3-Sterile	109	29	80	27
D3-Non-irradiated	105	38	67	35
D4-Re-inoculated	159*	37	122*	23
D4-Sterile	93	26	67	28
D4-Non-irradiated	92	22	70	23
D5-Re-inoculated	355*	147*	208*	39
D5-sterile	76	19	57	25
D5-Non-irradiated	79	15	64	19
Σ-Re-inoculated$^{@}$	1003*	512*	491	-
Σ-Sterile$^{@}$	514	138	376	-
Σ-Non-irradiated$^{@}$	509	187$^{\#}$	322	-
$^{@}$µg/300 ml				

Table 4.10. Amount of Fe mobilised and its distribution between particulate and dissolved fractions.

For each displacement the only comparisons made were re-inoculated vs sterile and non-irradiated vs sterile soils. For the re-inoculated vs sterile comparison, * indicates that the mean values are significantly different ($p \leq 0.05$). The treatments were compared using an unpaired t-Test. For the non-parametric data, β indicates that the mean values are significantly different ($\alpha \leq 0.05$). The treatments were compared using the Wilcoxon Two Sample Test. For the sterile vs non-irradiated comparisons, # indicates that the mean values are significantly different ($p \leq 0.05$). The treatments were compared using an unpaired t-Test. For non-parametric data, χ indicates that the mean values are significantly different ($\alpha \leq 0.05$). The treatments were compared using the Wilcoxon Two Sample Test. In the treatment column the letter D followed by a number denotes a particular displacement, while Σ denotes the total amounts of Fe displaced over the course of the experiment (five displacements).

Non-irradiated Treatment vs Sterile Treatment.

In overall terms these data suggest that γ-radiation had little effect on the mobility of Fe in this soil (Table 4.10). However, this result conceals the fact that there were some significant differences between the treatments in some of the individual displacements. During the initial stages of the experiment there was a tendency for there to be more dissolved Fe in the leachate from the sterile soil than in the leachate from the soil that

had not been irradiated. During the latter stages of the experiment the situation was reversed and there tended to be more dissolved Fe in the leachate from the soil that had not been irradiated than in the leachate from the soil that had. In terms of particulate Fe the only significant difference between the treatments occurred in the second displacement where significantly more was mobilised in the non-irradiated soil than in the irradiated soil.

Another feature of these data is the decrease in the percentage of total Fe in the particulate fraction of the leachate from the non-irradiated soil. The percentage fell from 43% in the first displacement to 19% in the fifth. No such trend was evident in the data from the sterile soil, where the percentage of total Fe in the particulate fraction remained relatively constant at around 27%.

Re-inoculated Treatment vs Sterile Treatment.

Microbial activity had a major impact on the mobility of Fe in this soil. These data show that there were two distinct episodes of mobilisation. The first occurred during displacements one and two. In these displacements there was significantly ($p \leq 0.05$) more particulate Fe in the leachate from the re-inoculated soil than in the leachate from the sterile soil. In both of these displacements there tended to be less dissolved Fe in the leachate from the re-inoculated soil than in the leachate from the sterile soil.

The second significant mobilisation of Fe occurred during the fourth and fifth displacements. In both of these displacements there was significantly more Fe in the dissolved fraction of the leachate from the re-inoculated soil than in this fraction of the

leachate from the sterile soil. Also, in displacement five significantly more particulate Fe was mobilised in the re-inoculated soil than in the sterile soil.

There was a general trend over the five displacements for the amount of Fe in the particulate fraction of the leachate from the re-inoculated soil to fall. The amount fell from 77% of the total in the first displacement to 39% in the fifth. As discussed earlier no such trend was evident in the data from the sterile soil.

Aluminium

Non-irradiated Treatment vs Sterile Treatment.

These data (Table 4.11) show that in overall terms γ -radiation mobilised significant amounts of Al. Both particulate and dissolved forms were mobilised. In both cases almost half of the material mobilised was flushed from the soil during the first displacement.

The percentages of total Al in the particulate fraction of the leachate from the soil that had not been irradiated fell from 48% in the first displacement to 20% in the fifth. In contrast, the percentage of total Al in the particulate fraction of the leachate from the irradiated soil remained relatively constant at around 30% over the course of the experiment. Similar trends were noted in the data for P and Fe.

Soil Treatment	*Total (P+D)*$^{+}$	*Particulate (P)*$^{+}$	*Dissolved (D)*	*% Particulate*
	µg/60 ml^{+}			
D1-Re-inoculated	1410	891*	508	64*
D1-Sterile	1563	503	1060	32
D1-Non-irradiated	323$^{\#}$	149$^{\#}$	174$^{\#}$	48$^{\#}$
D2-Re-inoculated	351*	240	112*	68*
D2-Sterile	598	220	378	35
D2-Non-irradiated	478	263	215	55$^{\#}$
D3-Re-inoculated	323	183	140*	56
D3-Sterile	471	158	313	33
D3-Non-irradiated	363$^{\#}$	129	234$^{\#}$	36
D4-Re-inoculated	304	109	195	36
D4-Sterile	352	136	216	38
D4-Non-irradiated	283	74	209	26$^{\#}$
D5-Re-inoculated	130*	32*	98*	24
D5-sterile	266	74	192	28
D5-Non-irradiated	242	45	197	20
Σ-Re-inoculated$^{@}$	2500	1450	1050*	-
Σ-Sterile$^{@}$	3250	1090	2160	-
Σ-Non-irradiated$^{@}$	1691$^{\#}$	661$^{\#}$	1030$^{\#}$	-
$^{@}$µg/300 ml				

Table 4.11. Amount of Al mobilised and its distribution between particulate and dissolved fractions.

For each displacement the only comparisons made were re-inoculated vs sterile and non-irradiated vs sterile soils. For the re-inoculated vs sterile comparison, * indicates that the mean values are significantly different ($p \leq 0.05$). The treatments were compared using an unpaired t-Test. For the non-parametric data, β indicates that the mean values are significantly different ($\alpha \leq 0.05$). The treatments were compared using the Wilcoxon Two Sample Test. For the sterile vs non-irradiated comparisons, # indicates that the mean values are significantly different ($p \leq 0.05$). The treatments were compared using an unpaired t-Test. For non-parametric data, χ indicates that the mean values are significantly different ($\alpha \leq 0.05$). The treatments were compared using the Wilcoxon Two Sample Test. In the treatment column the letter D followed by a number denotes a particular displacement, while Σ denotes the total amounts of Al displaced over the course of the experiment (five displacements).

Re-inoculated Treatment vs Sterile Treatment.

There are several observations that can be made about the data presented in Table 4.11. Firstly, in the initial displacement there was significantly more particulate Al mobilised in the non-sterile soil than in the sterile soil. Secondly, across the five displacements there tended to be less Al in the dissolved fraction of the leachate from the re-inoculated than in that fraction of the leachate from the sterile soil. Thirdly, by the fifth displacement significantly less dissolved and particulate Al was being mobilised in the

re-inoculated soil than in the sterile soil. Finally, the percentages of total Al in the particulate fraction of the leachate from the re-inoculated soil fell from 69% in the first displacement to 24% in the last. As discussed earlier no such trend was evident in the leachate from the sterile soil. Fe and P behaved in a similar manner.

It should be noted that in some cases (e.g. displacement one – dissolved fraction) it was not possible to establish statistically significant differences between values because of variability between replicates.

Calcium.

Non-irradiated Treatment vs Sterile Treatment.

The data in Table 4.12 show that the vast bulk of Ca mobilised with either treatment was in the dissolved fraction of the leachate. Negligible amounts of this element were mobilised in the soil that had not been irradiated after the first displacement. But, in the soil that had been irradiated significant amounts were mobilised in the second, third and fourth displacements. Clearly then, γ-radiation mobilised significant amounts of Ca in this soil. These data also show that a small, but nevertheless statistically significant amount of particulate Ca was mobilised by this treatment as well. The vast bulk of this material was flushed from the soil in the first displacement.

Re-inoculated Treatment vs Sterile Treatment.

It is clear from the discussion above that a substantial amount of Ca was mobilised in this soil as a result of the γ-radiation treatment. However, re-inoculating the soil prior

to leaching lead to a substantial drop in the amount of Ca in the leachate. Essentially all of the Ca that was mobilised and leached from the re-inoculated soil was in the dissolved fraction of the leachate. In the last displacement little if any Ca was mobilised by either treatment.

Soil Treatment	*Total (P+D)*$^{+}$	*Particulate (P)*$^{+}$	*Dissolved (D)*$^{+}$	*% Particulate*
	µg/60 ml^{+}			
D1-Re-inoculated	937*	17	920*	2
D1-Sterile	2403	43	2360	2
D1-Non-irradiated	757$^{\#}$	7$^{\#}$	750$^{\#}$	1
D2-Re-inoculated	117*	1	116*	1
D2-Sterile	489	8	481	2
D2-Non-irradiated	0	0	0	0
D3-Re-inoculated	132*	3	129*	2
D3-Sterile	221	3	218	1
D3-Non-irradiated	0	0	0	0
D4-Re-inoculated	106	2	104	2
D4-Sterile	118	1	117	1
D4-Non-irradiated	0	0	0	0
D5-Re-inoculated	0	0	0	0
D5-sterile	0	0	0	0
D5-Non-irradiated	0	0	0	0
Σ-Re-inoculated$^{@}$	1290*	20	1270*	-
Σ-Sterile$^{@}$	3230	60	3170	-
Σ-Non-irradiated$^{@}$	758$^{\#}$	7$^{\#}$	751$^{\#}$	-
$^{@}$µg/300 ml				

Table 4.12. Amount of Ca mobilised and its distribution between particulate and dissolved fractions.

For each displacement the only comparisons made were re-inoculated vs sterile and non-irradiated vs sterile soils. For the re-inoculated vs sterile comparison, * indicates that the mean values are significantly different ($p \leq 0.05$). The treatments were compared using an unpaired t-Test. For the non-parametric data, β indicates that the mean values are significantly different ($\alpha \leq 0.05$). The treatments were compared using the Wilcoxon Two Sample Test. For the sterile vs non-irradiated comparisons, # indicates that the mean values are significantly different ($p \leq 0.05$). The treatments were compared using an unpaired t-Test. For non-parametric data, χ indicates that the mean values are significantly different ($\alpha \leq 0.05$). The treatments were compared using the Wilcoxon Two Sample Test. In the treatment column the letter D followed by a number denotes a particular displacement, while Σ denotes the total amounts of Ca displaced over the course of the experiment (five displacements).

Sodium.

Soil Treatment	*Total (P+D)*$^{+}$	*Particulate (P)*$^{+}$	*Dissolved (D)*$^{+}$	*% Particulate*
	µg/60 ml^{+}			
D1-Re-inoculated	2022*	32	1990*	2
D1-Sterile	3190	40	3150	2
D1-Non-irradiated	1210$^{\#}$	20	1190$^{\#}$	1
D2-Re-inoculated	796*	14	782*	2
D2-Sterile	1280	20	1260	2
D2-Non-irradiated	703$^{\#}$	9	694$^{\#}$	1
D3-Re-inoculated	409	6	403	2
D3-Sterile	454	6	448	1
D3-Non-irradiated	540	7	533	1
D4-Re-inoculated	402	10	392	1
D4-Sterile	254	2	252	2
D4-Non-irradiated	387$^{\#}$	6	381$^{\chi}$	2
D5-Re-inoculated	131	3	128	1
D5-sterile	127	2	125	2
D5-Non-irradiated	313$^{\#}$	5	308$^{\#}$	2
Σ-Re-inoculated$^{@}$	3766*	66	3700*	-
Σ-Sterile$^{@}$	5297	67	5230	-
Σ-Non-irradiated$^{@}$	3150$^{\#}$	45	3105$^{\#}$	-
$^{@}$µg/300 ml				

Table 4.13. Amount of Na mobilised and its distribution between particulate and dissolved fractions.

For each displacement the only comparisons made were re-inoculated vs sterile and non-irradiated vs sterile soils. For the re-inoculated vs sterile comparison, * indicates that the mean values are significantly different ($p \leq 0.05$). The treatments were compared using an unpaired t-Test. For the non-parametric data, β indicates that the mean values are significantly different ($\alpha \leq 0.05$). The treatments were compared using the Wilcoxon Two Sample Test. For the sterile vs non-irradiated comparisons, # indicates that the mean values are significantly different ($p \leq 0.05$). The treatments were compared using an unpaired t-Test. For non-parametric data, χ indicates that the mean values are significantly different ($\alpha \leq 0.05$). The treatments were compared using the Wilcoxon Two Sample Test. In the treatment column the letter D followed by a number denotes a particular displacement, while Σ denotes the total amounts of Na displaced over the course of the experiment (five displacements).

Non-irradiated Treatment vs Irradiated Treatment.

These data (Table 4.13) show that very little particulate Na was mobilised by either treatment. It is clear that significant amounts of this element were mobilised by γ-radiation, most of which was flushed from the soil in the first two displacements. In

fact, by the fourth and fifth displacements significantly more Na was being mobilised in the non-irradiated soil than in the irradiated soil.

Re-inoculated Treatment vs Sterile Treatment.

As noted above substantial amounts of Na were mobilised in this soil by γ- radiation. These data show that re-inoculating the soil prior to leaching substantially decreased the amount of Na in the leachate. As in the sterile soil, the vast bulk of the Na mobilised in the re-inoculated soil was in the dissolved fraction of the leachate.

Magnesium.

Non-irradiated Treatment vs Sterile Treatment.

These data show that essentially all of the Mg mobilised by either treatment was in the dissolved fraction of the leachate. In the soil that had not been irradiated very little Mg was mobilised after the first displacement. However, in the soil that had been irradiated substantial amounts were mobilised during the first three displacements. Clearly then, γ-radiation mobilised significant amounts of this element.

Re-inoculated Treatment vs Sterile Treatment.

The preceding discussion shows γ-radiation mobilised significant amounts of Mg in this soil. These data show that re-inoculating the soil prior to leaching substantially decreased the amount of Mg in the leachate. In fact, little if any Mg was measured in the leachate from the re-inoculated soil after the second displacement. Essentially all of

the Mg that was mobilised and leached from the re-inoculated soil was in the dissolved fraction of the leachate.

Soil Treatment	*Total (P+D)*$^{+}$	*Particulate (P)*$^{+}$	*Dissolved (D)*$^{+}$	*% Particulate*
	µg/60 ml^{+}			
D1-Re-inoculated	775*	15	759*	2
D1-Sterile	2330	20	2310	1
D1-Non-irradiated	753$^{\#}$	15	738$^{\#}$	2
D2-Re-inoculated	181*	3	179*	2
D2-Sterile	847	12	835	1
D2-Non-irradiated	0	0	0	0
D3-Re-inoculated	0	0	0	0
D3-Sterile	142	3	139	2
D3-Non-irradiated	0	0	0	0
D4-Re-inoculated	0	0	0	0
D4-Sterile	0	0	0	0
D4-Non-irradiated	0	0	0	0
D5-Re-inoculated	0	0	0	0
D5-sterile	0	0	0	0
D5-Non-irradiated	0	0	0	0
Σ-Re-inoculated$^{@}$	956*	20	936*	-
Σ-Sterile$^{@}$	3320	40	3280	-
Σ-Non-irradiated$^{@}$	754$^{\#}$	16	738$^{\#}$	-
$^{@}$µg/300 ml				

Table 4.14. Amount of Mg mobilised and its distribution between particulate and dissolved fractions.

For each displacement the only comparisons made were re-inoculated vs sterile and non-irradiated vs sterile soils. For the re-inoculated vs sterile comparison, * indicates that the mean values are significantly different ($p \leq 0.05$). The treatments were compared using an unpaired t-Test. For the non-parametric data, β indicates that the mean values are significantly different ($\alpha \leq 0.05$). The treatments were compared using the Wilcoxon Two Sample Test. For the sterile vs non-irradiated comparisons, # indicates that the mean values are significantly different ($p \leq 0.05$). The treatments were compared using an unpaired t-Test. For non-parametric data, χ indicates that the mean values are significantly different ($\alpha \leq 0.05$). The treatments were compared using the Wilcoxon Two Sample Test. In the treatment column the letter D followed by a number denotes a particular displacement, while Σ denotes the total amounts of Mg displaced over the course of the experiment (five displacements).

Sulphur

Soil Treatment	*Total (P+D)*$^{+}$	*Particulate (P)*$^{+}$	*Dissolved (D)*$^{+}$	*% Particulate*
	µg/60 ml^{+}			
D1-Re-inoculated	1477*	132*	1344*	9*
D1-Sterile	2117	32	2084	2
D1-Non-irradiated	721$^{\#}$	12	709$^{\#}$	2
D2-Re-inoculated	547*	10	538$^{\beta}$	2
D2-Sterile	710	12	698	2
D2-Non-irradiated	192$^{\#}$	3$^{\#}$	189$^{\#}$	2
D3-Re-inoculated	529*	9	522*	2
D3-Sterile	365	5	360	2
D3-Non-irradiated	115$^{\#}$	1	114$^{\#}$	2
D4-Re-inoculated	284*	3	281*	1
D4-Sterile	65	1	64	1
D4-Non-irradiated	53	1	52	1
D5-Re-inoculated	107*	1	106*	1
D5-sterile	43	1	42	1
D5-Non-irradiated	25	1	24	2
Σ-Re-inoculated$^{@}$	2945	155*	2790*	-
Σ-Sterile$^{@}$	3292	52	3240	-
Σ-Non-irradiated$^{@}$	1110$^{\#}$	20$^{\chi}$	1090$^{\#}$	-
$^{@}$µg/300 ml				

Table 4.15. Amount of S mobilised and its distribution between particulate and dissolved fractions.

For each displacement the only comparisons made were re-inoculated vs sterile and non-irradiated vs sterile soils. For the re-inoculated vs sterile comparison, * indicates that the mean values are significantly different ($p \leq 0.05$). The treatments were compared using an unpaired t-Test. For the non-parametric data, β indicates that the mean values are significantly different ($\alpha \leq 0.05$). The treatments were compared using the Wilcoxon Two Sample Test. For the sterile vs non-irradiated comparisons, # indicates that the mean values are significantly different ($p \leq 0.05$). The treatments were compared using an unpaired t-Test. For non-parametric data, χ indicates that the mean values are significantly different ($\alpha \leq 0.05$). The treatments were compared using the Wilcoxon Two Sample Test. In the treatment column the letter D followed by a number denotes a particular displacement, while Σ denotes the total amounts of S displaced over the course of the experiment (five displacements).

Non-irradiated Treatment vs Irradiated Treatment.

These data (Table 4.15) show that treating this soil with γ-radiation mobilised significant amounts of this element. The majority of S mobilised in this manner was in the dissolved fraction of the leachate, although small amounts were also mobilised in the particulate fraction. Most of this material was flushed from the soil in the first two

displacements. Essentially the entire amount of S mobilised and leached from the soil that had not been irradiated was dissolved in nature.

Re-inoculated Treatment vs Sterile Treatment.

These data show that re-inoculating the soil prior to leaching had a variety of effects on the behaviour of this element. In the first two displacements the presence of micro-organisms in the soil decreased the amount of S in the dissolved fraction of the leachate. But in the final three displacement the presence of micro-organisms tended to elevate the amount of S in this fraction of the leachate. These data also show that micro-organisms mobilised a small but statistically significant amount of particulate S in the first displacement. So in general terms micro-organisms decreased the amount of S in the leachate during the initial stages of the experiment but increased the amount during the latter stages. The former effect was stronger than the latter, so in overall terms less S was mobilised and leached from the re-inoculated soil than from the sterile soil.

Carbon

In this initial experiment only the total amount of C in each sample was determined.

Non-irradiated Treatment vs SterileTreatment.

The data in Table 4.16 clearly show that γ-radiation mobilised a significant amount of C in this soil. Approximately 4 times as much C was mobilised and leached from the soil that had been irradiated than from the soil that had not.

Re-inoculated Treatment vs Sterile Treatment.

It is clear from the discussion above that γ-radiation mobilised significant amounts of C in this soil. These data show that re-inoculating the soil prior to leaching decreased the amount of C in the leachate. The difference between treatments was most pronounced during the initial stages of the experiment.

Soil Treatment	*Total*
	mg/60 ml
D1-Re-inoculated	28*
D1-Sterile	81
D1-Non-irradiated	11#
D2-Re-inoculated	11*
D2-Sterile	41
D2-Non-irradiated	9#
D3-Re-inoculated	10*
D3-Sterile	21
D3-Non-irradiated	9#
D4-Re-inoculated	11
D4-Sterile	17
D4-Non-irradiated	8#
D5-Re-inoculated	6
D5-sterile	16
D5-Non-irradiated	9
Σ-Re-inoculated@	62*
Σ-Sterile@	175
Σ-Non-irradiated@	47#
@mg/300 ml	

Table 4.16. Total amounts of C mobilised.

For each displacement the only comparisons made were, re-inoculated vs sterile and non-irradiated vs sterile soils. For the re-inoculated vs sterile comparison, * indicates that the mean values are significantly different ($p \leq 0.05$). The treatments were compared using an unpaired t-Test. For the sterile vs non-irradiated comparisons, # indicates that the mean values are significantly different ($p \leq 0.05$). The treatments were compared using an unpaired t-Test. In the treatment column the letter D followed by a number denotes a particular displacement, while Σ denotes the total amounts of C displaced over the course of the experiment (five displacements).

pH and EC Data

Non-irradiated Treatment vs SterileTreatment.

The data in Table 4.17 show that the pH of the leachates obtained from the irradiated soil were substantially lower than those of the leachates obtained from the soil that had not been irradiated. The difference between treatments was maintained across all five displacements. In displacement one the difference was 0.71 of a pH unit, but from the second displacement onwards the difference was over 1 pH unit. The trend over the course of the experiment was for the pH values of the leachates from both sets of columns to rise. In all displacements the EC values of the leachate obtained from the irradiated soil were higher than those of the leachate from the soil that had not been irradiated. Over the course of the experiment the trend was for the EC values of the leachate from both sets of columns to fall.

Re-inoculated Treatment vs SterileTreatment.

These data show that the pH of the leachates from the re-inoculated soil were higher than those of the leachates from the sterile soil. In the first displacement the difference was 0.18 of a pH unit. In the remaining four displacements the average difference was 0.61 of a pH unit. The trend over the course of the experiment was for the pH values of the leachate from the re-inoculated soil to rise. As noted earlier the pH values of the sterile leachate also rose over the course of the experiment.

In general the EC values of the leachate from the re-inoculated soil were lower than those of the leachate from the sterile soil. Both sets of values tended to decline over the course of the experiment.

Soil Treatment	*pH*	*EC*
		mS/cm
D1-Re-inoculated	4.46*	0.523*
D1-Sterile	4.28	0.787
D1-Non-irradiated	4.99#	0.513#
D2-Re-inoculated	5.06β	0.205*
D2-Sterile	4.52	0.400
D2-Non-irradiated	5.67#	0.113#
D3-Re-inoculated	5.31*	0.142*
D3-Sterile	4.71	0.181
D3-Non-irradiated	5.76#	0.100#
D4-Re-inoculated	5.51β	0.118
D4-Sterile	4.86	0.120
D4-Non-irradiated	5.91#	0.081χ
D5-Re-inoculated	5.57*	0.063*
D5-sterile	4.90	0.106
D5-Non-irradiated	6.02#	0.081#

Table 4.17. pH and EC values of the leachate.

For each displacement the only comparisons made were re-inoculated vs sterile and non-irradiated vs sterile soils. For the re-inoculated vs sterile comparison, * indicates that the mean values are significantly different ($p \leq 0.05$). The treatments were compared using an unpaired t-Test. For the non-parametric data, β indicates that the mean values are significantly different ($\alpha \leq 0.05$). The treatments were compared using the Wilcoxon Two Sample Test. For the sterile vs non-irradiated comparisons, # indicates that the mean values are significantly different ($p \leq 0.05$). The treatments were compared using an unpaired t-Test. For non-parametric data, χ indicates that the mean values are significantly different ($\alpha \leq 0.05$). The treatments were compared using the Wilcoxon Two Sample Test. In the treatment column the letter D followed by a number denotes a particular displacement.

SEM Data.

For these results it is easiest to discuss the non-irradiated, irradiated and reinoculated treatments separately.

Non-irradiated Treatment

Figures 4.4 to 4.6 are scanning electron micrographs of the surface of filter membranes that were used to physically fractionate the leachate (first displacement). Figure 4.4 shows the typical features of a membrane surface at low magnification. There were numerous relatively large particles (10-100 μm) scattered across the surface that was covered in part, by a dark coloured material. The typical morphology and structure of the large particles is shown in Figure 4.5. There were two types of particle present, those that were aggregates of smaller ones and those that consisted of a single grain. There were also numerous small particles (< 10 μm) scattered across the membrane surface. Few of the particles, large or small exhibited anything other than an irregular morphology. As noted above the membrane surfaces were also partly covered by a dark coloured amorphous material (Figure 4.6). Examination of the membranes from the other four displacements revealed little change in the nature of the particulate material, although there tended to be fewer large particles isolated from the leachate obtained from each successive displacement.

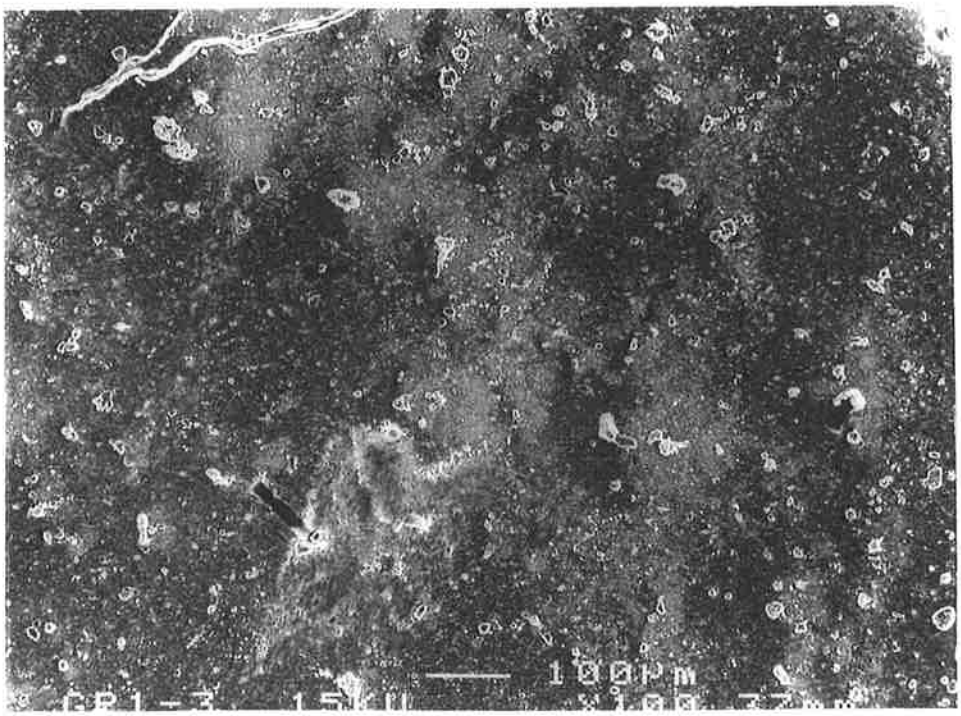

Figure 4.4. Scanning electron micrograph showing the surface of the filter membrane at low magnification (non-irradiated treatment).

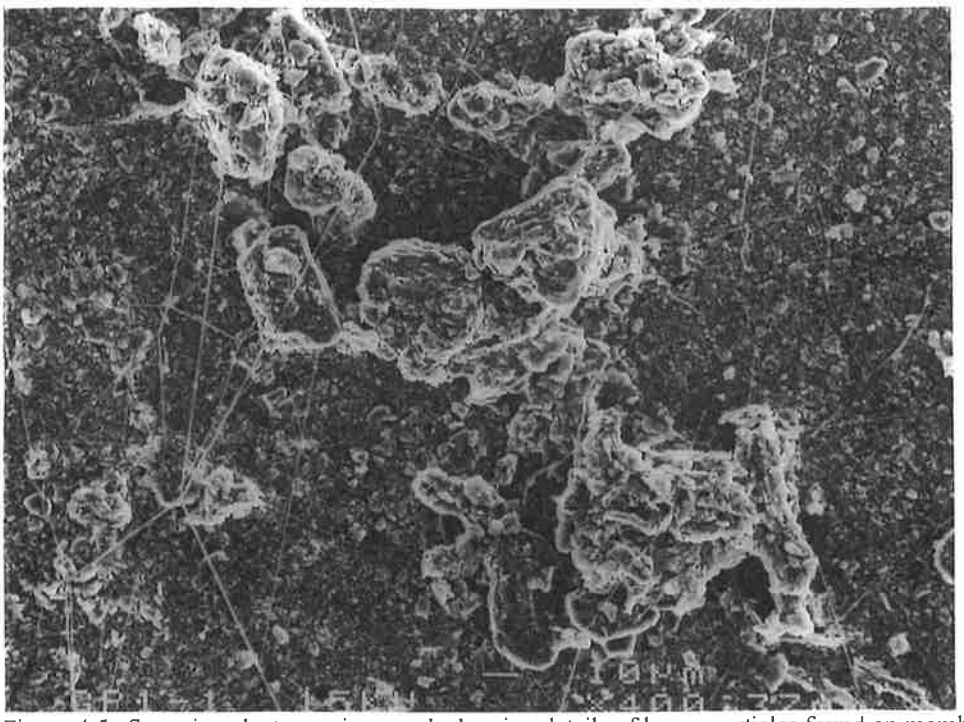

Figure 4.5. Scanning electron micrograph showing details of larger particles found on membrane surface (non-irradiated treatment).

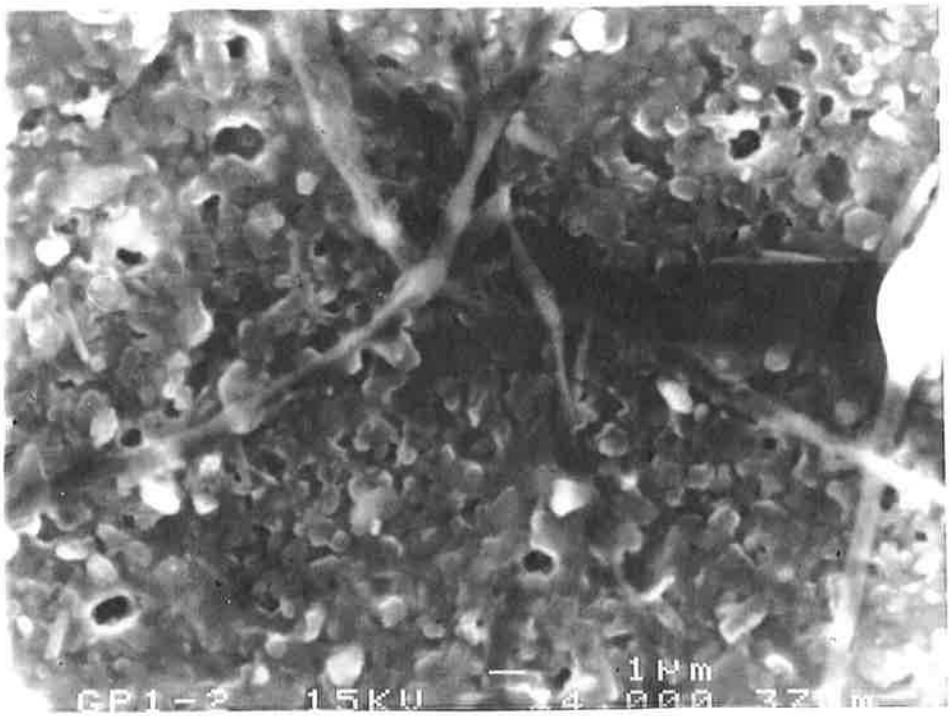

Figure 4.6. Scanning electron micrograph showing the dark coloured material that partly covered the membrane (non-irradiated treatment).

Sterile Treatment.

Figures 4.7 to 4.9 are scanning electron micrographs of the material that was retained by the filter membranes when the leachate was physically fractionated (first displacement). The material was very similar to that which was retained by the membranes used to filter leachate from soil that had not been irradiated. The large particles were either single grains or aggregates of smaller ones. Scattered across the surface were numerous sub-micron to micron-sized particles (Figure 4.9). One difference in the material from the sterile leachate was that it contained very little of the dark coloured amorphous compound that was found in the particulate matter isolated from leachate from the non-irradiated soil.

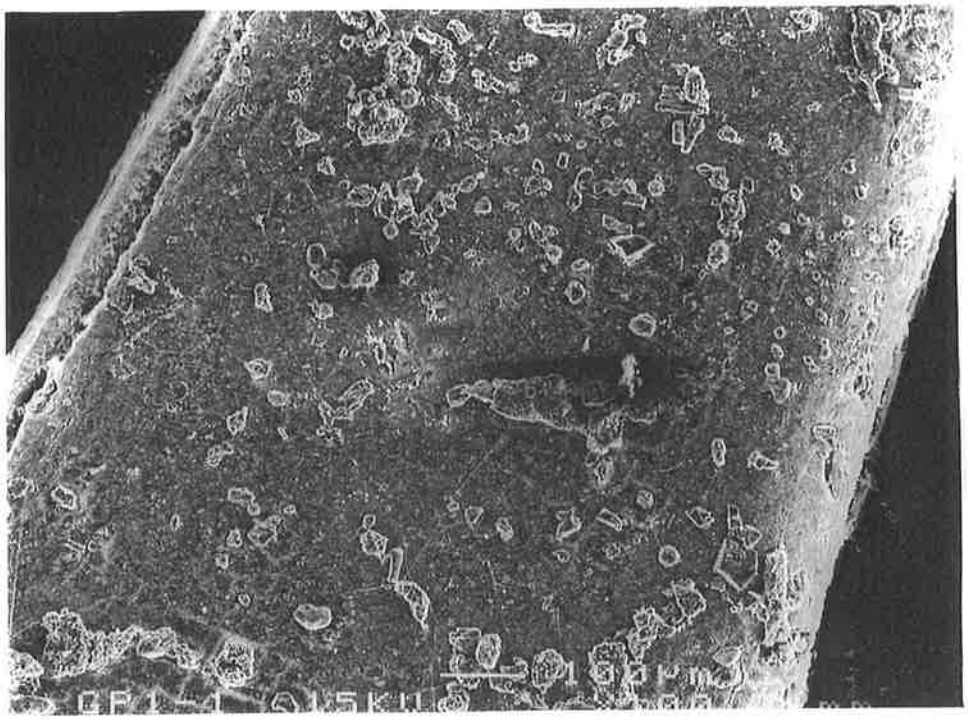

Figure 4.7. Scanning electron micrograph showing the surface of the filter membrane at low magnification (sterile treatment).

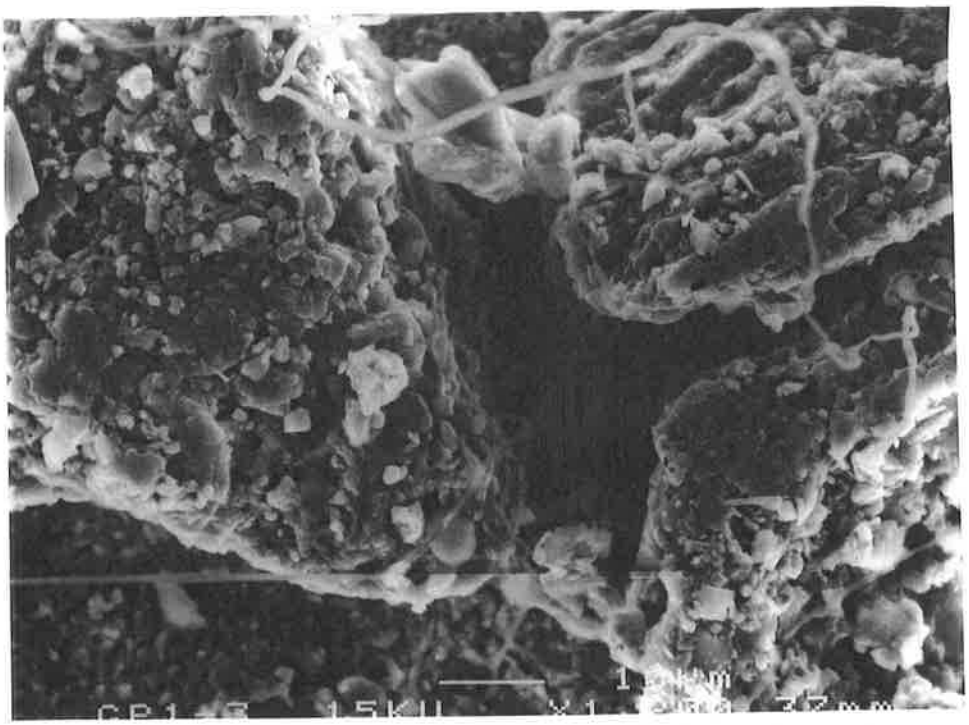

Figure 4.8. Scanning electron micrograph of the large particles found on the membrane surface (sterile treatment).

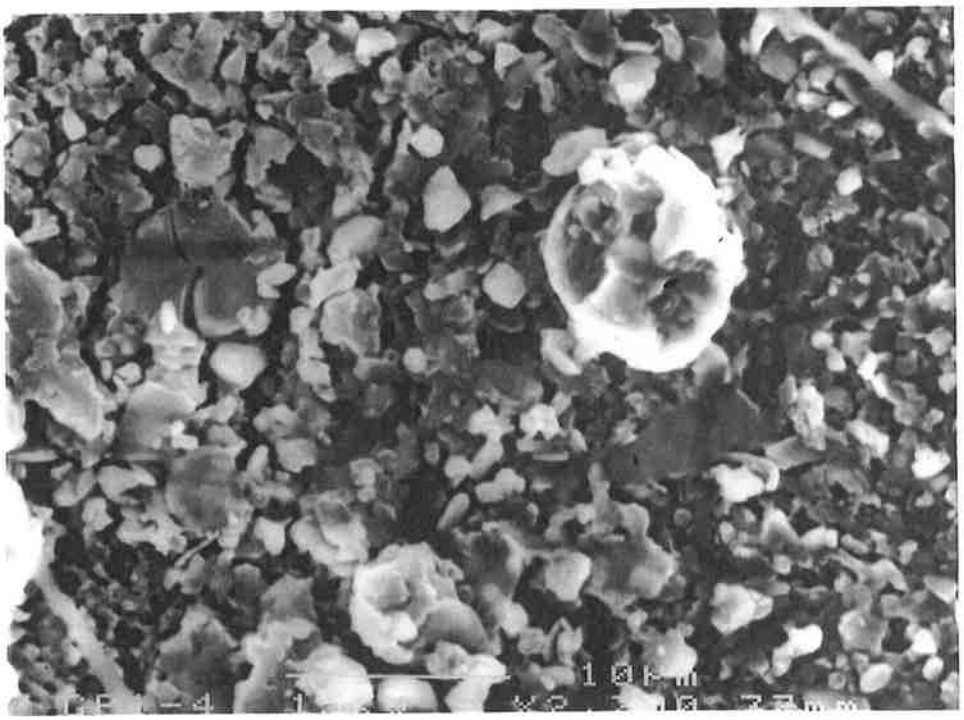

Figure 4.9. Scanning electron micrograph showing the mass of smaller particles that cover the membrane surface (sterile treatment).

Over the course of the experiment the nature of the material retained by the membranes did not change greatly, however with each successive displacement there tended to be fewer large particles mobilised.

Re-inoculated Treatment.

Figures 4.10 to 4.14 are scanning electron micrographs of the material retained by the filter membranes when the leachate from the first displacement was physically fractionated. Figure 4.10 shows a membrane after 8 ml of leachate had been filtered. When this leachate was being filtered only approximately 8 ml would pass through before the membrane became clogged. It took three membranes to filter 25 ml of this leachate compared to one for the sterile leachate. It is clear from Figure 4.10 why this occurred. A layer of material, in which visible cracks appear to have developed on drying, covers the surface of the membrane. This suggests that the material covers the

membrane to a considerable depth. The material consisted of numerous large particles (Figure 4.11) embedded in a matrix of material (Figure 4.12). As was the case with the non-irradiated and sterile treatments there were two types of large particle, those that were aggregates and those that were simply a single grain of material.

An examination of the matrix material at higher magnification (Figures 4.13 and 4.14) showed that it consisted of numerous small particles embedded in a mass of dark coloured amorphous material. In appearance this was very similar to the material that covered part of the membrane used to filter the leachate from the non-irradiated soil. Examination of the membranes from the other four displacements revealed little change in the nature of the particulate material, although there tended to be fewer particles (both large and small) in the material isolated from the leachate obtained from each successive displacement.

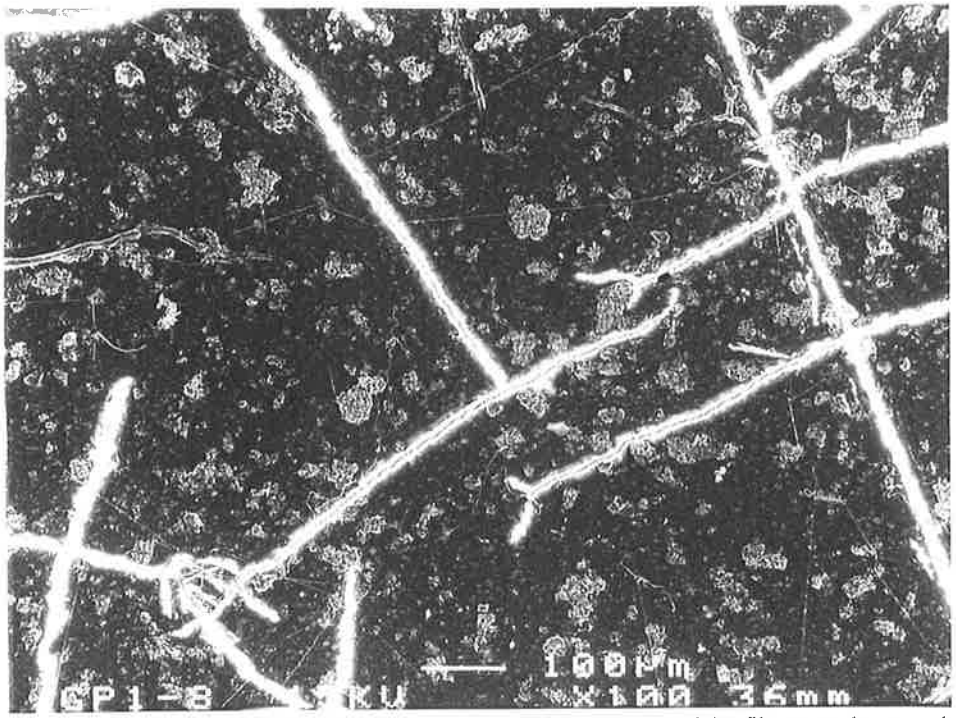

Figure 4.10. Scanning electron micrograph showing the surface of the filter membrane at low magnification (re-inoculated treatment).

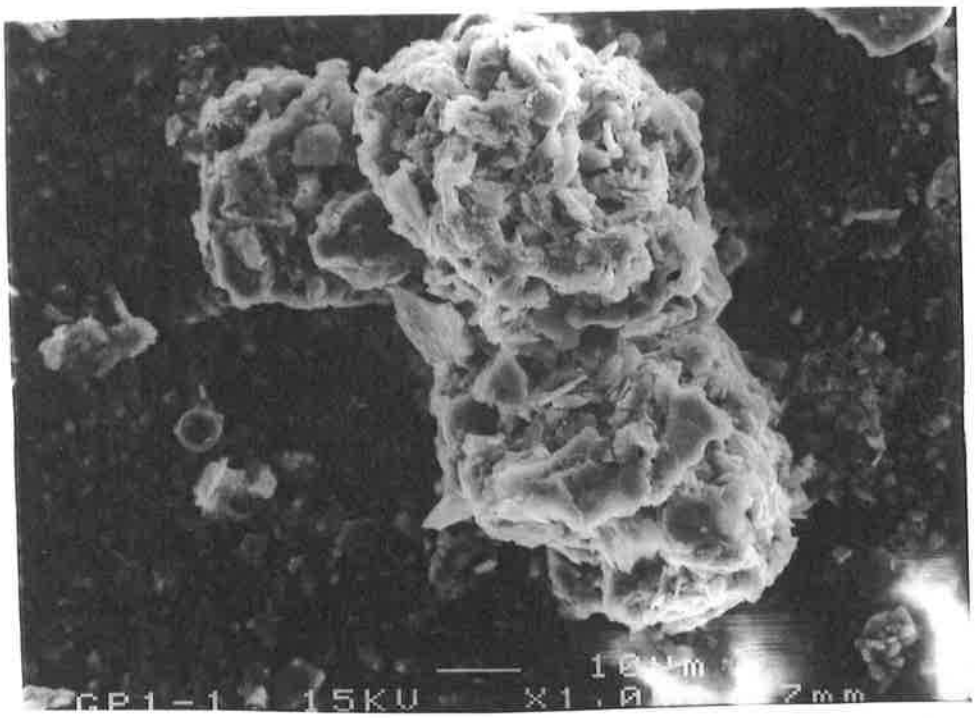

Figure 4.11. Scanning electron micrograph of a typical large particle found on the membrane surface (re-inoculated treatment).

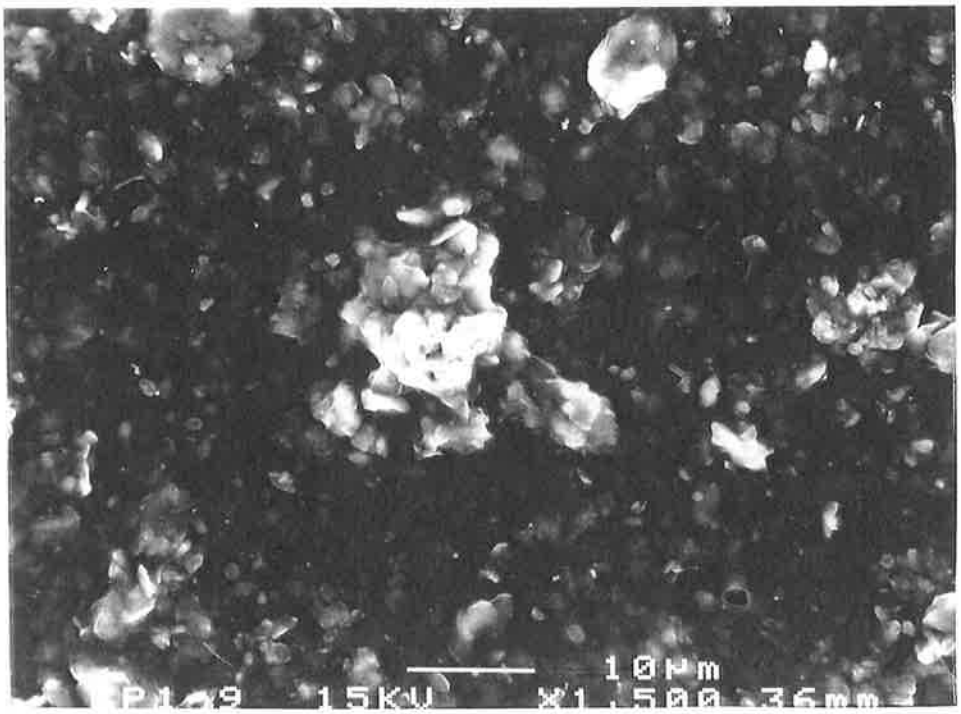

Figure 4.12. Scanning electron micrograph showing the matrix of material that covers the membrane surface (re-inoculated treatment).

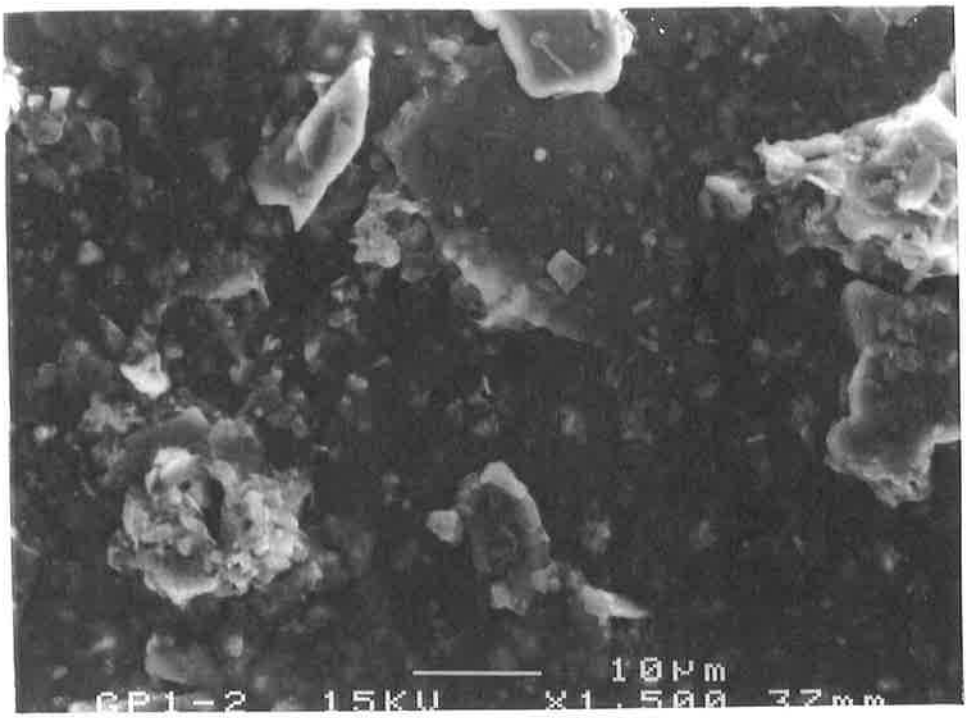

Figure 4.13. Scanning electron micrograph showing fine detail of the matrix material (re-inoculated treatment).

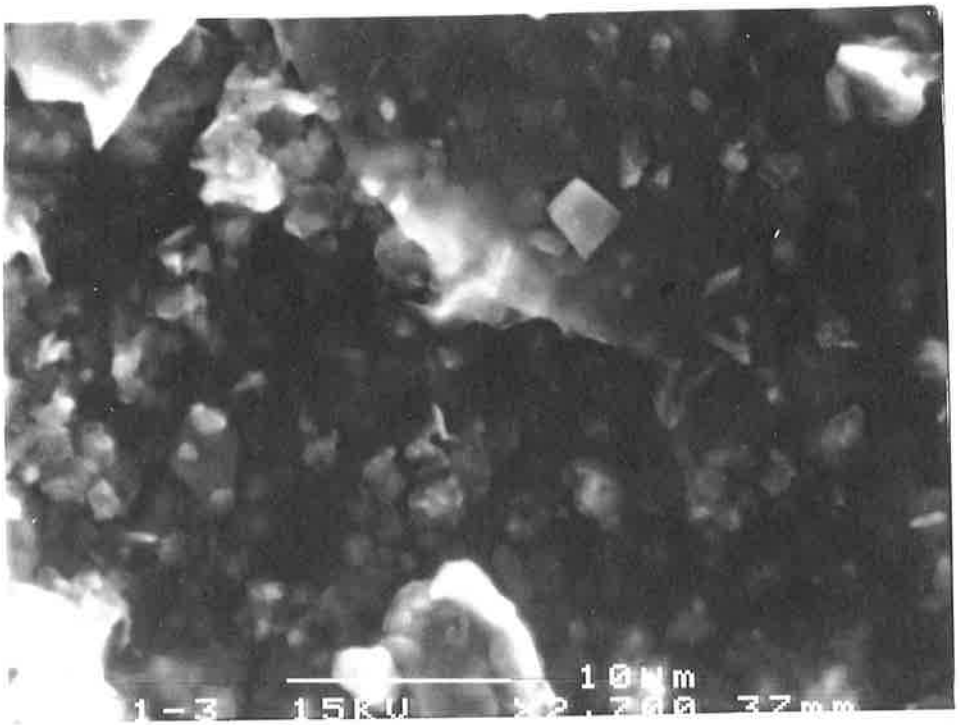

Figure 4.14. Scanning electron micrograph showing matrix material at high magnification (re-inoculated treatment).

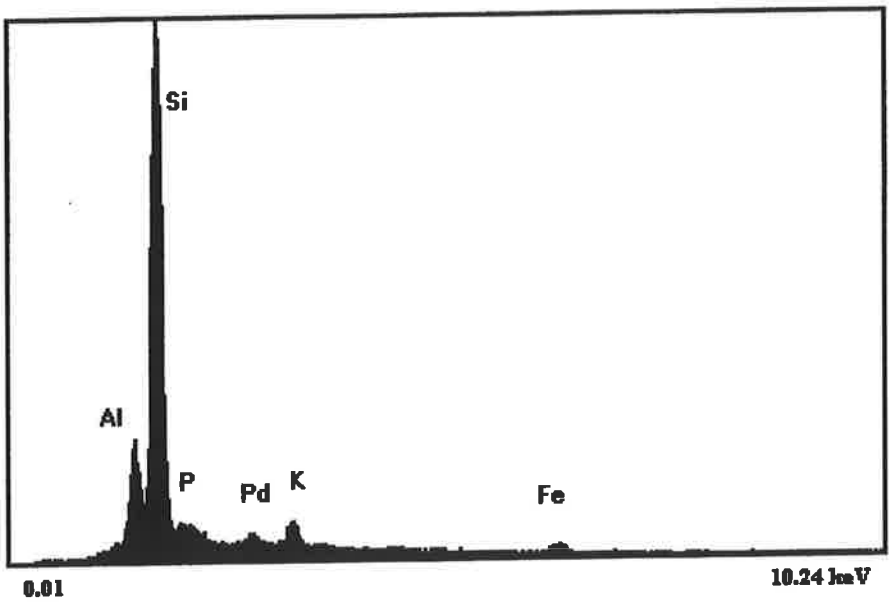

Figure 4.15. EDS spectrum – particle analysis 1.

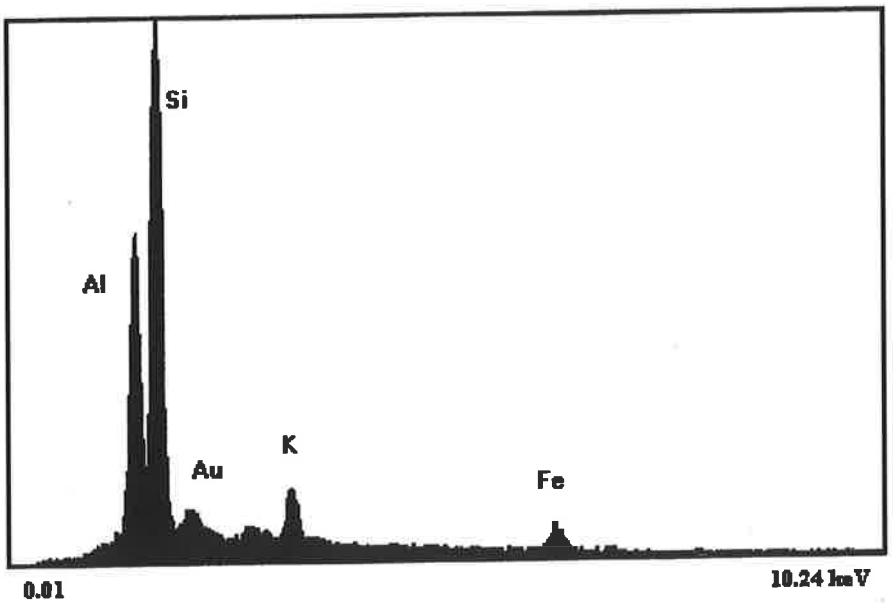

Figure 4.16. EDS spectrum – particle analysis 2.

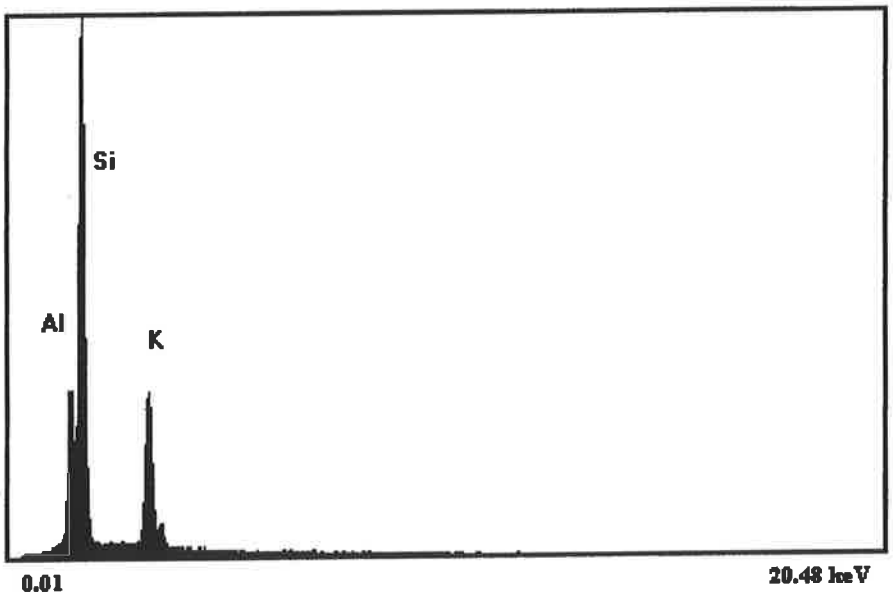

Figure 4.17. EDS spectrum – particle analysis 3.

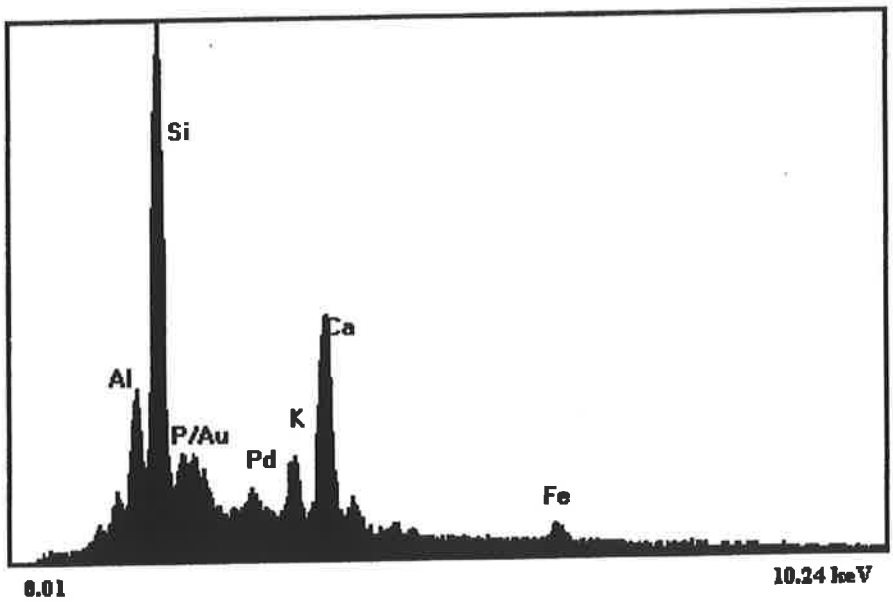

Figure 4.18. EDS spectrum – particle analysis 4.

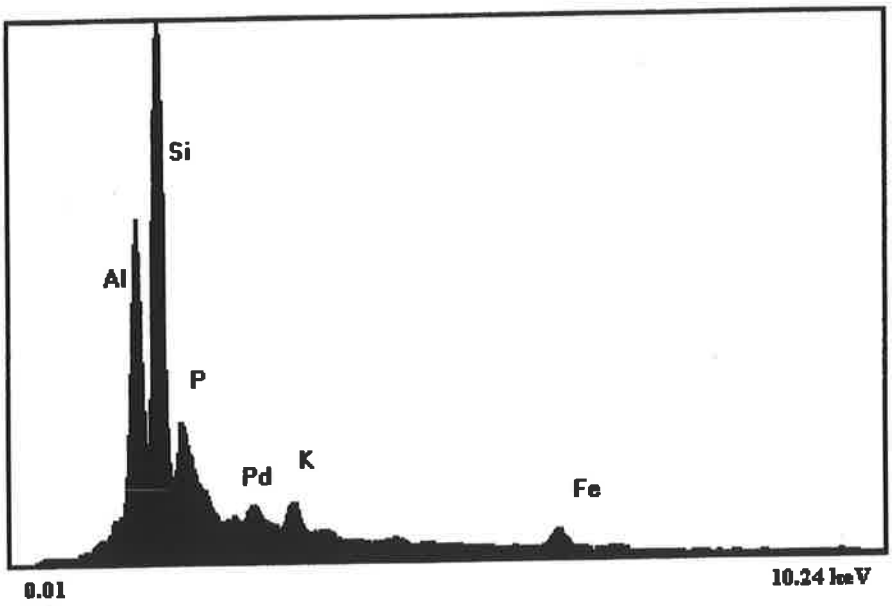

Figure 4.19. EDS spectrum – matrix analysis 1.

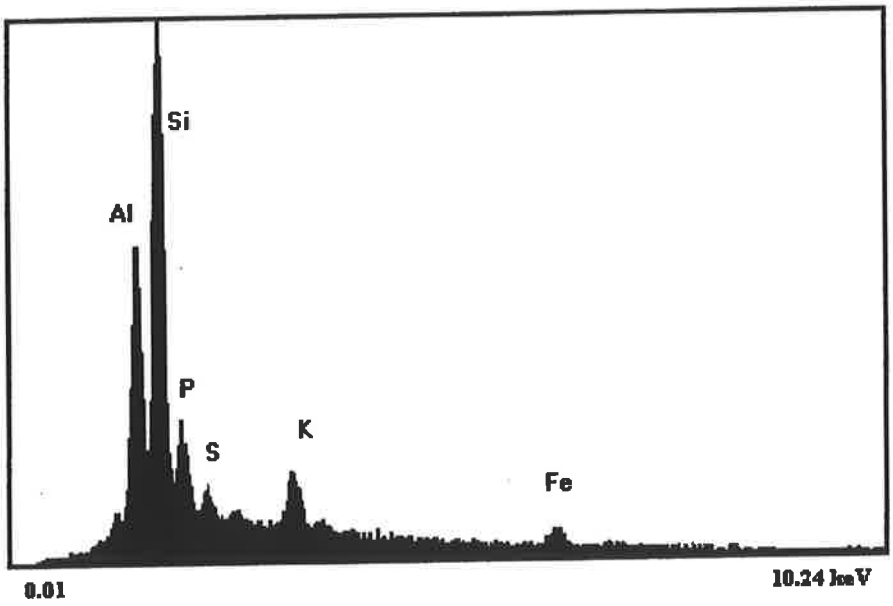

Figure 4.20. EDS spectrum – matrix analysis 2.

As noted earlier, given the nature of the samples it was not possible to quantatively determine the chemical composition of the material analysed. However, the presence or absence of elements whose atomic mass was greater than that of Na could be determined.

Particle Analysis.

The minimum sized particle that could be analysed was approximately 5 μm. It should be noted that these samples were C coated and it appears that during this process some of the samples were contaminated with Au and Pd. Unfortunately in the spectrum P and Au have peaks that are very close together which means that considerable care has to be taken when interpreting these data.

Typical analyses of aggregate particles are shown in Figures 4.15 and 4.16. These analyses showed that these were alumino-silicate materials that contained trace amounts of K, Fe and in some cases P. The particles that were single grains had two quite distinct compositions. The first (Figure 4.17) was an alumino-silicate material that contained considerable amounts of K but no Fe or P. This compositional analysis suggests the presence of illite. The second distinct composition (Figure 4.18) was another alumino-silicate phase that contained considerable amounts of Ca along with minor amounts of K, P and Fe. This composition is consistent with the presence of a feldspar related material. It should be noted that these particles were relatively rare.

Matrix Analysis - Re-inoculated Treatment.

Figures 4.19 and 4.20 are typical analyses of the matrix material. This material contained considerable amounts of Al and Si, probably in the form of alumino-silicate clays, along with moderate amounts of P and K and trace amounts of S and Fe. The S peak in Figure 4.19 was probably obscured by the presence of the Au peak. In fact it appears that in this spectrum the three separate peaks have merged to form a single large broad one.

4.3.0. Experiment No 2.

As discussed earlier this experiment was conducted with two compatible objectives in mind. The first was to demonstrate the general repeatability of the initial experiment and to confirm the results obtained. The second objective was to test a hypothesis that was developed from the following observations.

The work conducted by Hannapel *et al.* (1964a, b) suggested that one of the most important factors in mobilising P in a calcareous soil was the addition of a microbial energy source. It was observed in experiment one that the percentage of total P and Al in the particulate fraction of the leachate from the re-inoculated soil tended to decline over the course of the experiment. A similar tend was evident with Fe during the first four displacements. One possible explanation for the relative decline in the amount of these particulate forms being mobilised over time is that most of the particulate material was mobilised during the initial stages of the experiment when microbial activity would

be at its most intense. Given these two pieces of information the following hypothesis was formulated:

'Given two biologically active soils most particulate P will be mobilised in that soil which contains the greatest amount of readily available microbial substrate'

The contention that underlies this hypothesis is that the mobilisation, redistribution within and export of P from the soil profile will be controlled by the availability of organic C.

This hypothesis was tested by conducting a second experiment that was identical to the first except for the following modifications. It was clear from the initial experiment that treating the soil with γ-radiation solubilised a considerable amount of C. After the soil was re-inoculated there should have been a rapid expansion in the microbial population as the substrate made available by γ-radiation was utilized. Now, when the soil was wetted up and allowed to incubate for a week the columns were not initially saturated. In fact only a little over ½ of a pore volume was added to each column. This meant that a variable portion of each was dry and not accessible to micro-organisms. With the first experiment after the initial incubation period the columns were saturated and incubated for 48 h. With the second experiment the columns were saturated and then one pore volume was immediately displaced (refer Table 4.19). This should have meant that much of the soluble C released from the newly wetted up sections of the columns was immediately removed in the second experiment and so could not be utilised by micro-organisms. The incubation period between each subsequent displacement was lowered from 72 h in experiment 1 to 48 h in experiment 2. As a consequence of these changes

there should have been substantially more substrate available to micro-organisms in experiment 1 than in experiment 2. Therefore if the hypothesis was correct then more particulate P should have been mobilised with experiment 1 than with experiment 2.

4.3.1. Materials and Methods.

The columns used in this experiment were prepared, packed and sterilised exactly as described in Section 4.2.1. The bulk densities of the prepared columns are shown in Table 4.18.

Columns	*Wt Soil*	*Bulk Density*
	(g)	(g/cm^3)
Sterile 1	125.1	1.18
Sterile 2	125.1	1.18
Sterile 3	125.8	1.19
Sterile 4	125.2	1.18
Re-inoculated 1	125.6	1.19
Re-inoculated 2	125.1	1.18
Re-inoculated 3	125.5	1.19
Re-inoculated 4	125.7	1.19

Table 4.18. Amounts of soil used and bulk densities of prepared columns.

The sets of sterile and non-sterile columns were set-up and then incubated for seven days as in experiment one (Section 4.2.1). On day seven, enough DI water (sterile/non-sterile as appropriate) was added to saturate the columns and then to displace one pore volume (60 ml). Then after every second day, enough DI water (sterile/non-sterile) was added to displace one pore volume, for a total of five displacements. A detailed description of the actual procedures followed is set out in Table 4.19.

After each displacement samples of leachate from each sterile column were plated out on nutrient agar and 2% potato dextrose agar and incubated for one week. This

procedure was carried out to ensure that sterile conditions were maintained over the course of the experiment.

The leachates were fractionated using the procedure set out in Section 3.3.0. However, dissolved P was not fractionated into MRP and URP. The first experiment demonstrated that very little of the dissolved P in the leachate from either treatment was molybdate-reactive and little additional information would have been gained by conducting this procedure with experiment 2.

In this experiment unlike the first, C in the leachates was fractionated into dissolved (C_D) and particulate (C_P) forms. The fractionation procedure for this element was different from the one used for the others. In this procedure a total (C_T) value was determined on each sample using the procedure outlined in section 3.5.4. The samples were then filtered and the C in the filtrate was determined using the same method as for total C. The C in the filtrate was termed dissolved. Particulate C was determined as the difference between total and dissolved C ($C_P = C_T - C_D$).

Scanning electron microscopy was used to characterise the particulate material obtained from the leachate.

Following the fifth displacement Cl^- breakthrough curves (frontal displacement) were obtained for three sterile and three non-sterile columns in the following manner. Two pore volumes (120 ml) of a 100-ppm Cl^- solution ($CaCl_2$) were leached through the

columns, 5 ml fractions were collected and analysed for Cl^-. Cl^- was determined using an automated colorimetric method as set out in Rayment and Higginson (1992).

The results from this section were analysed using SigmaStat statistical software as described in Section 4.2.1.

PROCEDURE	*STERILE TREATMENT*		*RE-INOCULATED TREATMENT*	
	Time (h)	Flow rate (ml/h)	Time (h)	Flow rate (ml/h)
(A)dding DI H_2O	1.5		1.5	
(I)ncubation +A	168.0		168.0	
(S)aturation + L	7.0	12.0	7.0	12.0
I	48.0		48.0	
(L)eaching	4.0	15.0	4.0	15.0
I	48.0		48.0	
L	5.0	12.0	5.0	12.0
I	48.0		48.0	
L	6.0	10.0	5.0	12.0
I	48.0		72.0	
L	6.0	10.0	5.0	12.0
	Total= 389.5 Av leaching = 5.2	Av rate=11.8	Total= 387.5 Av leaching= 4.8	Av rate=12.6

Table 4.19. Details of the procedure followed during experiment 2.

4.3.2. Results.

Tabulated Analytical Results

Phosphorus.

The data in Table 4.20 show that micro-organisms mobilised significant amounts of P in this soil. All of the P mobilised in this way was in the particulate fraction of the leachate. In fact, over the course of the experiment approximately 80 times as much particulate P was mobilised in the re-inoculated soil than in the sterile soil. In general,

there tended to be slightly less dissolved P in the leachate from the re-inoculated soil than the leachate from the sterile soil. With the re-inoculated soil, over the course of the five displacements, the percentage of total P in the particulate fraction fell from a high of 80% in the second to 28% in the fifth. In contrast, with the sterile soil the percentage in the particulate fraction remained relatively constant at around 1%.

Soil Treatment	*Total (P+D)*$^{+}$	*Particulate (P)*$^{+}$	*Dissolved (D)*$^{+}$	*% Particulate*
	µg/60 ml^{+}			
D1-Re-inoculated	251*	170$^{\beta}$	81	68$^{\beta}$
D1-Sterile	83	1	82	1
D2-Re-inoculated	88*	70$^{\beta}$	18*	80*
D2-Sterile	25	1	25	1
D3-Re-inoculated	59*	43$^{\beta}$	16	73*
D3-Sterile	19	1	19	2
D4-Re-inoculated	52*	22*	30	42$^{\beta}$
D4-Sterile	23	1	22	2
D5-Re-inoculated	21	6*	15*	28*
D5-sterile	21	1	20	1
Σ-Re-inoculated$^{@}$	473*	313*	160	-
Σ-Sterile$^{@}$	171	4	167	-
$^{@}$µg/300 ml				

Table 4.20. Amounts of P mobilised and its distribution between dissolved and particulate forms.

For the re-inoculated vs sterile comparison, * indicates that the mean values are significantly different ($p \leq 0.05$). The treatments were compared using an unpaired t-Test. For the non-parametric data, β indicates that the mean values are significantly different ($\alpha \leq 0.05$). The treatments were compared using the Wilcoxon Two Sample Test. In the treatment column the letter D followed by a number denotes a particular displacement, while Σ denotes the total amounts of P displaced over the course of the experiment (five displacements).

Iron.

These data (Table 4.21) show that significant ($p \leq 0.05$) amounts of both particulate and dissolved Fe were mobilised in the re-inoculated soil. There appears to have been two distinct episodes of mobilisation. The first was in displacements one and two when large amounts of particulate Fe were mobilised. In these displacements there tended to

be less dissolved Fe in the leachate from the re-inoculated soil than in the leachate from the sterile soil. The second mobilisation event occurred during displacements three, four and five. In these displacements significantly more particulate and dissolved Fe was mobilised in the re-inoculated soil than in the sterile soil. With the re-inoculated soil the percentage of total Fe in the particulate fraction of the leachate fell from 76% in the first to 16% in the last. In the sterile soil the decline was not as great, falling from 54% in the first to 23% in the fifth.

Soil Treatment	*Total (P+D)*$^{+}$	*Particulate (P)*$^{+}$	*Dissolved (D)*$^{+}$	*% Particulate*
	μg/60 ml^{+}			
D1-Re-inoculated	341*	261*	80	76*
D1-Sterile	220	120	100	54
D2-Re-inoculated	99	45*	54	45*
D2-Sterile	106	29	77	27
D3-Re-inoculated	328*	95*	233*	28
D3-Sterile	105	31	74	29
D4-Re-inoculated	347*	103*	244*	30
D4-Sterile	91	26	65	28
D5-Re-inoculated	266*	44*	222*	16
D5-sterile	81	19	61	23
Σ-Re-inoculated$^{@}$	1382*	549*	833*	-
Σ-Sterile$^{@}$	603	226	373	-
$^{@}$μg/300 ml				

Table 4.21. Amounts of Fe mobilised and its distribution between dissolved and particulate forms.

For the re-inoculated vs sterile comparison, * indicates that the mean values are significantly different ($p \leq 0.05$). The treatments were compared using an unpaired t-Test. For the non-parametric data, β indicates that the mean values are significantly different ($\alpha \leq 0.05$). The treatments were compared using the Wilcoxon Two Sample Test. In the treatment column the letter D followed by a number denotes a particular displacement, while Σ denotes the total amounts of Fe displaced over the course of the experiment (five displacements).

Aluminium.

Soil Treatment	*Total (P+D)*$^{+}$	*Particulate (P)*$^{+}$	*Dissolved (D)*$^{+}$	*% Particulate*
	µg/60 ml^{+}			
D1-Re-inoculated	1162*	830*	332*	71*
D1-Sterile	894	418	476	47
D2-Re-inoculated	383	154	229*	40*
D2-Sterile	456	131	325	29
D3-Re-inoculated	522	199	323	38
D3-Sterile	444	144	300	32
D4-Re-inoculated	353*	98	255	28
D4-Sterile	393	113	280	29
D5-Re-inoculated	229	55	174*	24
D5-sterile	353	89	264	25
Σ-Re-inoculated$^{@}$	2645	1333*	1312*	-
Σ-Sterile$^{@}$	2545	895	1650	-
$^{@}$µg/300 ml				

Table 4.22. Amounts of Al mobilised and its distribution between dissolved and particulate forms.

For the re-inoculated vs sterile comparison, * indicates that the mean values are significantly different ($p \leq 0.05$). The treatments were compared using an unpaired t-Test. For the non-parametric data, β indicates that the mean values are significantly different ($\alpha \leq 0.05$). The treatments were compared using the Wilcoxon two sample test. In the treatment column the letter D followed by a number denotes a particular displacement, while Σ denotes the total amounts of Al displaced over the course of the experiment (five displacements).

The data in Table 4.22 show that soil micro-organisms mobilised a significant amount of particulate Al, most of which was flushed from the soil during the first displacement. In general there tended to be significantly less dissolved Al in the leachate from the re-inoculated soil than in the leachate from the sterile soil. With the re-inoculated soil the percentage of total Al in the particulate fraction declined across the five displacements, dropping from 71% in the first to 24% in the fifth. With the sterile soil the decline was not as pronounced going from 47% in the first to 25% in the last displacement.

Calcium.

Soil Treatment	*Total (P+D)*$^{+}$	*Particulate (P)*$^{+}$	*Dissolved (D)*$^{+}$	*% Particulate*
	µg/60 ml^{+}			
D1-Re-inoculated	1197*	17	1180*	2
D1-Sterile	1810	40	1770	2
D2-Re-inoculated	129*	1	128*	1
D2-Sterile	268	6	262	2
D3-Re-inoculated	111	2	109	2
D3-Sterile	121	2	119	2
D4-Re-inoculated	0	0	0	0
D4-Sterile	0	0	0	0
D5-Re-inoculated	0	0	0	0
D5-sterile	0	0	0	0
Σ-Re-inoculated$^{@}$	1432*	22	1410*	-
Σ-Sterile$^{@}$	2198	48	2150	-
$^{@}$µg/300 ml				

Table 4.23. Amounts of Ca mobilised and its distribution between dissolved and particulate forms.

For the re-inoculated vs sterile comparison, * indicates that the mean values are significantly different ($p \leq 0.05$). The treatments were compared using an unpaired t-Test. For the non-parametric data, β indicates that the mean values are significantly different ($\alpha \leq 0.05$). The treatments were compared using the Wilcoxon Two Sample Test. In the treatment column the letter D followed by a number denotes a particular displacement, while Σ denotes the total amounts of Ca displaced over the course of the experiment (five displacements).

It is clear from the data (Table 4.23) that re-inoculating the soil prior to leaching substantially decreased the amount of Ca in the leachate. The vast majority of Ca mobilised by either treatment was in the dissolved fraction of the leachate and was flushed from the soil during the initial stages of the experiment.

Sodium.

Soil Treatment	*Total (P+D)*$^{+}$	*Particulate (P)*$^{+}$	*Dissolved (D)*$^{+}$	*% Particulate*
	µg/60 ml^{+}			
D1-Re-inoculated	2197*	27	2170*	1
D1-Sterile	2885	35	2850	1
D2-Re-inoculated	675	8	667	1
D2-Sterile	721	15	706	2
D3-Re-inoculated	350	6	344	2
D3-Sterile	319	3	315	1
D4-Re-inoculated	202	3	199	1
D4-Sterile	158	2	156	1
D5-Re-inoculated	113	2	112	2
D5-sterile	115	2	113	2
Σ-Re-inoculated$^{@}$	3535*	47	3488*	-
Σ-Sterile$^{@}$	4198	58	4140	-
$^{@}$µg/300 ml				

Table 4.24. Amounts of Na mobilised and its distribution between dissolved and particulate forms.

For the re-inoculated vs sterile comparison, * indicates that the mean values are significantly different ($p \leq 0.05$). The treatments were compared using an unpaired t-Test. For the non-parametric data, β indicates that the mean values are significantly different ($\alpha \leq 0.05$). The treatments were compared using the Wilcoxon two sample test. In the treatment column the letter D followed by a number denotes a particular displacement, while Σ denotes the total amounts of Na displaced over the course of the experiment (five displacements).

These data (Table 4.24) show that re-inoculating this soil decreased the amount of Na in the leachate. This effect was most pronounced in the first displacement, over the remaining four displacements there were no significant differences between the amounts of Na mobilised by either treatment. The vast bulk of this element mobilised with either treatment was dissolved in nature.

Magnesium.

Soil Treatment	*Total (P+D)*$^{+}$	*Particulate (P)*$^{+}$	*Dissolved (D)*$^{+}$	*% Particulate*
	µg/60 ml^{+}			
D1-Re-inoculated	1082*	12	1070*	1
D1-Sterile	1597	27	1570	2
D2-Re-inoculated	104*	2	102*	2
D2-Sterile	201	2	199	1
D3-Re-inoculated	0	0	0	0
D3-Sterile	0	0	0	0
D4-Re-inoculated	0	0	0	0
D4-Sterile	0	0	0	0
D5-Re-inoculated	0	0	0	0
D5-sterile	0	0	0	0
Σ-Re-inoculated$^{@}$	1180*	10	1170*	-
Σ-Sterile$^{@}$	1790	30	1760	-
$^{@}$µg/300 ml				

Table 4.25. Amounts of Mg mobilised and its distribution between dissolved and particulate forms.

For the re-inoculated vs sterile comparison, * indicates that the mean values are significantly different ($p \leq 0.05$). The treatments were compared using an unpaired t-Test. For the non-parametric data, β indicates that the mean values are significantly different ($\alpha \leq 0.05$). The treatments were compared using the Wilcoxon Two Sample Test. In the treatment column the letter D followed by a number denotes a particular displacement, while Σ denotes the total amounts of Mg displaced over the course of the experiment (five displacements).

These data (Table 4.25) show that re-inoculating the soil prior to leaching significantly decreased the amount of Mg in the leachate. Only minor amounts of particulate Mg were mobilised with either treatment with the vast bulk being in the dissolved fraction of the leachate. This element behaved most similarly to Ca in that little if any was mobilised and leached from either soil during the latter stages of the experiment.

Sulphur.

Soil Treatment	*Total (P+D)*$^{+}$	*Particulate (P)*$^{+}$	*Dissolved (D)*$^{+}$	*% Particulate*
	µg/60 ml^{+}			
D1-Re-inoculated	1332	21	1311	2
D1-Sterile	1515	30	1485	2
D2-Re-inoculated	340	8	332	2
D2-Sterile	362	5	357	1
D3-Re-inoculated	258*	4	254*	2
D3-Sterile	171	2	169	1
D4-Re-inoculated	373*	6	367*	2
D4-Sterile	186	2	184	1
D5-Re-inoculated	158*	3	155*	2
D5-sterile	109	1	108	1
Σ-Re-inoculated$^{@}$	2465	42	2423	-
Σ-Sterile$^{@}$	2341	41	2300	-
$^{@}$µg/300 ml				

Table 4.26. Amounts of S mobilised and its distribution between dissolved and particulate forms.

For the re-inoculated vs sterile comparison, * indicates that the mean values are significantly different ($p \leq 0.05$). The treatments were compared using an unpaired t-Test. For the non-parametric data, β indicates that the mean values are significantly different ($\alpha \leq 0.05$). The treatments were compared using the Wilcoxon Two Sample Test. In the treatment column the letter D followed by a number denotes a particular displacement, while Σ denotes the total amounts of S displaced over the course of the experiment (five displacements).

These data (Table 4.26) show that re-inoculating this soil had a variety of effects upon the concentration of S in the leachate. During the initial stages of the experiment there tended to be to be slightly less S in the leachate from the re-inoculated soil than in the leachate from the sterile soil. During the latter stages of the experiment however, there was significantly more S in the leachate from the re-inoculated soil than in the leachate from the sterile soil. This meant that overall there was no statistically significant difference between the treatments in terms of the amounts of S mobilised. Only relatively minor amounts of particulate S were mobilised by either treatment.

Carbon.

Soil Treatment	*Total (P+D)*$^{+}$	*Particulate (P)*$^{+}$	*Dissolved (D)*$^{+}$	*% Particulate*
	mg/60 ml^{+}			
D1-Re-inoculated	74$^{\beta}$	6	68*	8
D1-Sterile	100	3	97	3
D2-Re-inoculated	19*	2	17*	10
D2-Sterile	30	1	29	3
D3-Re-inoculated	18	2	16	11
D3-Sterile	19	1	18	5
D4-Re-inoculated	23	3	16	13
D4-Sterile	19	1	18	5
D5-Re-inoculated	16	1	15	6
D5-sterile	17	1	16	6
Σ-Re-inoculated$^{@}$	151*	13	138*	-
Σ-Sterile$^{@}$	183	6	177	-
$^{@}$mg/300 ml				

Table 4.27. Amounts of C mobilised and its distribution between dissolved and particulate forms.

For the re-inoculated vs sterile comparison, * indicates that the mean values are significantly different ($p \leq 0.05$). The treatments were compared using an unpaired t-Test. For the non-parametric data, β indicates that the mean values are significantly different ($\alpha \leq 0.05$). The treatments were compared using the Wilcoxon Two Sample Test. In the treatment column the letter D followed by a number denotes a particular displacement, while Σ denotes the total amounts of C displaced over the course of the experiment (five displacements).

Re-inoculating this soil prior to leaching significantly decreased the concentration of C in the leachate obtained during displacements one and two. For each of the subsequent displacements essentially the same amounts of C were mobilised with either treatment. These data show that only a small amount of this element was particulate in nature, with the majority being in the dissolved fraction of the leachate. During the initial stages of the experiment there was a tendency for more particulate C to be mobilised in the re-inoculated soil than in the sterile soil, although the amounts involved were minor.

pH and EC values.

Soil Treatment	*pH*	*EC*
		mS/cm
D1-Re-inoculated	4.54	0.535*
D1-Sterile	4.41	0.710
D2-Re-inoculated	5.01*	0.183
D2-Sterile	4.75	0.213
D3-Re-inoculated	5.60β	0.140
D3-Sterile	4.89	0.120
D4-Re-inoculated	5.66*	0.160*
D4-Sterile	4.89	0.125
D5-Re-inoculated	6.04*	0.127*
D5-sterile	4.96	0.103

Table 4.28. pH and EC values of the leachate.

In this table * indicates that the mean values are significantly different ($p \leq 0.05$). The treatments were compared using an unpaired t-Test. For the non-parametric data, β indicates that the mean values are significantly different ($\alpha \leq 0.05$). The treatments were compared using the Wilcoxon Two Sample Test.

In all of the displacements except the first, the pH of the leachate from the re-inoculated soil was significantly higher than that of the leachate from the sterile soil. In the first displacement the pH of the leachate from the re-inoculated soil was 0.13 of a unit higher than the pH of the leachate from the sterile soil. By the fifth displacement the difference had increased to over 1 pH unit.

In the first displacement the EC value of the leachate from the sterile soil was significantly higher than that of the leachate from the re-inoculated soil. By the fourth and fifth displacements the situation had reversed with the EC values of the leachate from the re-inoculated soil being higher than those from the sterile soil.

SEM Data.

Examination of the particulate material retained by the membranes used to filter the leachates form the re-inoculated soil confirmed the findings of the first experiment. The particulate material consisted of clay particles and a dark coloured amorphous material (data not presented).

Cl Breakthrough Curves.

It should be noted that while there were four sterile and non-sterile columns leached only three from each set were used for this work because one of the sterile columns had ceased to flow during the final displacement. Another of these columns ceased to flow after approximately 1.3 of a pore volume had been collected. Hence an incomplete data set was obtained for this column

Sterile Columns.

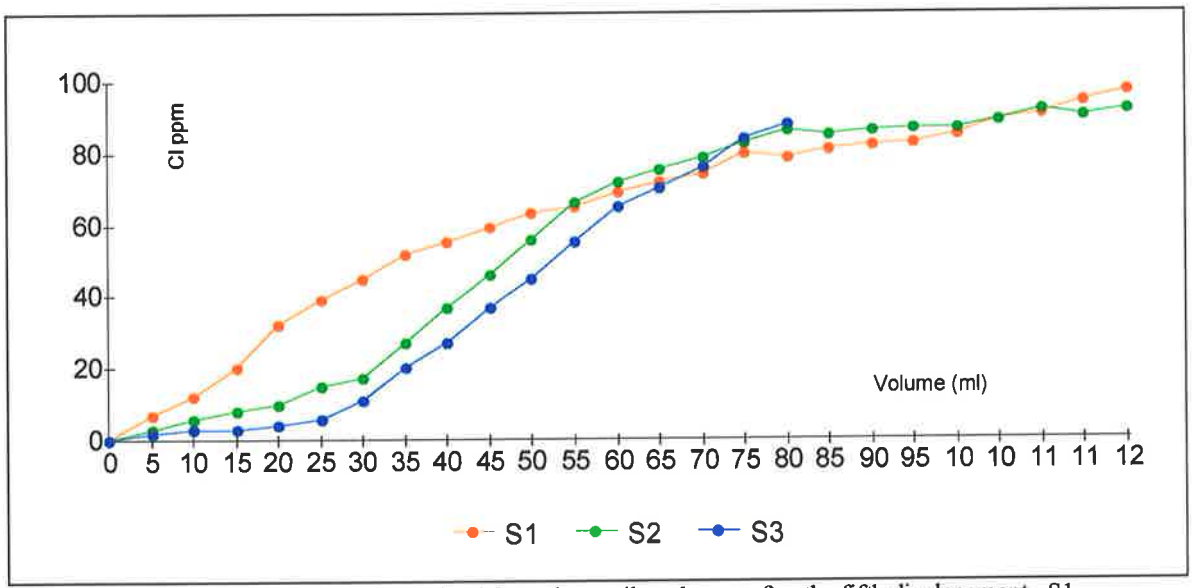

Figure 4.21. Cl breakthrough curves obtained from the sterile columns after the fifth displacement. S1, S2 and S3 denote the individual columns.

Clearly these three columns exhibited a reasonable degree of uniformity in terms of their flow regimes. In general the elution curves are spread or displaced both forward and backwards from the position of an idealized curve. The concentration of Cl^- in the eluent tended to be fairly low until approximately ½ of a pore volume had been displaced, then there was a gradual increase until the concentration reached approximately 100-ppm by the time 2 pore volumes had been displaced.

Non-sterile Columns.

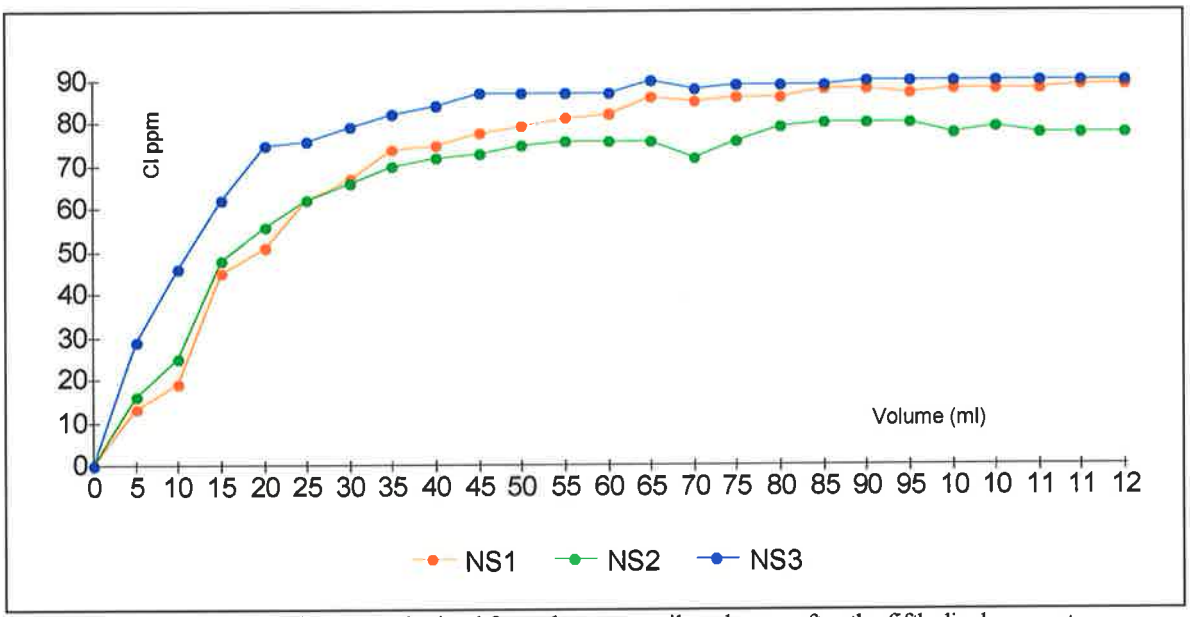

Figure 4.22. Cl breakthrough curves obtained from the non-sterile columns after the fifth displacement. NS1, NS2 and NS3 denote the individual columns.

It is clear from these data that these three columns also exhibited a fair degree of uniformity in terms of their flow regimes. The elution curves are displaced forward from the position of an idealized curve. The concentration of Cl^- in the eluent increased very rapidly during the initial stages of the experiment, but plateaued after approximately ½ of a pore volume had been displaced. The concentration of Cl^- in the eluent then only very slowly approached the concentration of the input solution.

4.4.0. Experiment 1 vs Experiment 2.

4.4.1. Sterile Treatment.

Treatment - Sterile	*Total (D+P)*	*Particulate*	*Dissolved*
Experiment 1-P	201*	5*	196*
Experiment 2-P	171	4	167
Experiment 1-Fe	514	138*	376
Experiment 2-Fe	599	226	377
Experiment 1-Al	3250*	1090	2160*
Experiment 2-Al	2545	895	1650
Experiment 1-Ca	3230*	60	3170*
Experiment 2-Ca	2198	48	2150
Experiment 1-Na	5297*	67	5230*
Experiment 2-Na	4198	58	4140
Experiment 1-Mg	3320*	40	3280*
Experiment 2-Mg	1790	30	1760
Experiment 1-S	3292*	52	3240*
Experiment 2-S	2341	41	2300
Experiment 1-C#	175	-	-
Experiment 2-C#	183	-	-
		-	-
Experiment 1-H^{+}@	38*	-	-
Experiment 2-H^{+}@	28	-	-
#mg/300ml/@ng/300ml			

Table 4.29. Comparison between experiments 1 and 2 – sterile treatment.

In this table * indicates that the mean values are significantly different ($p \leq 0.05$). The treatments were compared using an unpaired t-Test.

These data (Table 4.29) show that for all of the elements except Fe and C, significantly greater amounts were mobilised and leached from the soil with experiment one than with experiment two. Approximately the same amount of C was mobilised with either experiment, as was the case with Fe. However there was significantly less particulate Fe mobilised with experiment 1 than with experiment 2.

4.4.2. Re-inoculated Treatment.

Treatment – Re-inoculated	*Total (D+P)*	*Particulate*	*Dissolved*
Experiment 1-P	358*	233β	125
Experiment 2-P	473	313	160
Experiment 1-Fe	1000*	512	491*
Experiment 2-Fe	1382	549	833
Experiment 1-Al	2500	1450	1050
Experiment 2-Al	2645	1333	1312
Experiment 1-Ca	1292	22	1270
Experiment 2-Ca	1432	22	1410
Experiment 1-Na	3766	66	3700
Experiment 2-Na	3535	47	3488
Experiment 1-Mg	955	19	936
Experiment 2-Mg	1180	10	1170
Experiment 1-S	2945*	155*	2790*
Experiment 2-S	2465	42	2423
Experiment 1-C#	62*	-	-
Experiment 2-C#	151	-	-
Experiment 1-H^{+}@	16	-	-
Experiment 2-H^{+}@	15	-	-
# mg/300ml/@ng/300ml			

Table 4.30. Comparison between experiments 1 and 2 – re-inoculated treatment.

In this table * indicates that the mean values are significantly different ($p \leq 0.05$). The treatments were compared using an unpaired t-Test. For the non-parametric data, β indicates that the mean values are significantly different ($\alpha \leq 0.05$). The treatments were compared using the Wilcoxon Two Sample Test.

With the exception of P, S, C and Fe, approximately the same amounts of each element were mobilised with either treatment. Significantly greater amounts of P and C were mobilised with experiment 2 than with experiment 1. The reverse situation occurred with S where significantly less was mobilised with experiment 2 than with experiment 1. It is interesting to note that with micro-organisms present P joins Fe and Al in having relatively large amounts of the total mobilised in the particulate fraction of the leachate. Whereas under sterile conditions Fe and Al were the only elements where there were relatively large amounts of the total mobilised in the particulate fraction of the leachate.

4.5.0. Discussion and Analysis.

This section will begin with some general observations, these will be followed by an analysis of the results obtained by comparing soil that had been irradiated with soil that had not been (effects of γ-radiation). Then the results of the comparison between the sterile and re-inoculated soils will be discussed. Finally, there will be further discussion of the results of the comparison between the two experiments.

The first point to note is that the second experiment demonstrated the repeatability of the first and broadly confirmed the general conclusions drawn from it. The changes made to the leaching regime meant that it was valid to compare the general behaviour of the elements. This topic will be elaborated upon further when the results of the two experiments are compared. It was felt that given the somewhat novel approach taken with this work and the results obtained with experiment 1, it was essential from a scientific point of view to have this work verified.

In terms of the procedure used to fractionate the leachate a brief comment on the choice of the 0.22 μm membrane filter is warranted. Rigler (1964) has shown that the concentration of P in the filtrate is strongly influenced by the procedure that is used to separate out the particulate material. His results showed, not surprisingly that the amount of P in the filtrate decreased progressively with the pore size of the membrane used to filter the solution. According to the Standard Methods for Examination of Water and Wastewater (19th Ed, 1995) filtration through a 0.45 μm pore diameter membrane filter is used to make the distinction between dissolved and particulate forms

of P. However, for this work it was thought that the particulate fraction might be strongly influenced by microbial activity and consequently, removal of as much particulate material as possible from the solution was desirable. Of course selection of a filter membrane with too small a pore size would lead to long filtering times while sequential filtering would complicate the processing of the samples. The use of a 0.22 μm pore diameter membrane filter seemed an appropriate compromise given the nature of the competing considerations.

In both experiments the leachates from the sterile soil contained very little suspended material, and as a consequence, the solutions were not turbid. In contrast to this, the leachates from the re-inoculated soil were highly turbid, containing a considerable amount of suspended material that tended to settle out upon standing. The difference between the leachates is clearly shown in Figure 4.23. As discussed earlier the material in the leachate from the re-inoculated soil tended to clog the filter membranes during the filtering step. It took three membranes to filter 25 ml of this leachate compared with one for the sterile leachate (refer to Figure 4.24). The leachates from the soil that had not been irradiated were also turbid. These observations suggest that soil microbes mobilised particulate material in the soil. It is interesting to note that Hannapel *et al.* (1964a, b) reported obtaining turbid leachate from columns of soil that had received organic residues or sucrose. Such treatments were designed to stimulate microbial activity.

Figure 4.23. Leachate from sterile (R) and re-inoculated (L) soil.

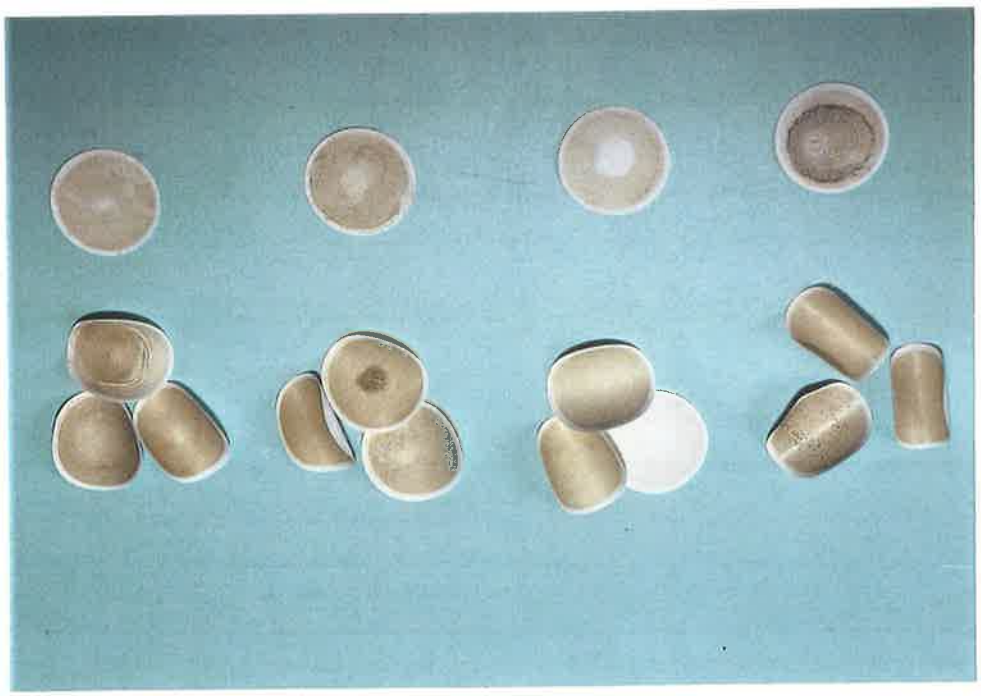

Figure 4.24. Membranes after filtering step.

With both experiments, during the initial stages little difference could be discerned between the flow rates of the sterile and non-sterile columns. But during the latter stages of the experiments the flow rates of the columns containing sterilised soil tended to be lower than those containing re-inoculated soil.

4.5.1. Non-irradiated Treatment vs Sterile Treatment - (Effects of γ-radiation).

There were two main effects associated with treating this soil with γ-radiation. The first was the mobilisation of significant amounts of all the elements of interest. The second was to lower the pH of the soil.

Mobilisation of Elements.

The data show that treating this soil with γ-radiation mobilised considerable amounts of C, P, Ca, Na, Mg, S, Fe and Al. According to Cawse (1975) increases in the extractability of various elements from soil have been reported following treatment with γ-radiation (refer to Table 4.31). It is clear from these data that there is a considerable degree of variability in the response of soils to doses of γ-radiation. The response is likely to be a function of the dose as well as a variety of other factors including the chemical properties of the soil and its water content at the time of treatment. Cawse (1975) suggests that the majority of C and other biologically important elements released from soils as a result of γ-radiation treatment are derived from cell lysis. He does however acknowledge that γ-radiation can induce chemical changes in soil organic matter that may enhance its solubility.

Soil	Dose (kGy)	Elements showing increase[a]	Elements showing no increase[a]
Organic clay loam	0.015	NO_3 (k)	
Organic clay loam	2.0	NH_4(k), NO_3(k), Org-C (s), Org- P(s)	Inorg-P(s)
Light and heavy loams	2.0	NH_4(k)	
Organic clay loams	6.0	NH_4(k), NO_2(w), NO_3(k)	
Peat	20.0	N(k), P(a)	Ca(a), K(a), Mg(a)
Loam	20.0	Cu(a), Mn(a)	K(a), Zn(a)
Loam	20.0	NH_4(k), NO_3(k)	S(k)
Sandy loam	25.0		Mo(s)
Clay	30.0	B(w), Ca(w), Cu(w), Mg(w), Mn(w)	K(w), Na(w)
Muck	30.0	S(m)	
Clay loam	50.0	Org-C(s), Cu(s), Org-Mg(s), Mn(s), Org-N(s), NH_4(s), Org-P(s)	Ca(s), Cl(s), Fe(s), Na(s), Zn(s)
Clay loam	120.0	NO_2(w)	
Sandy and clay loams	600.0	NH_4(k)	

Table 4.31. Increases in extractable ions in irradiated soils (from Cawse, 1975).

[a]Extractants used: (a) ammonium acetate solution, (k) potassium chloride solution, (m) Morgan's solution, (s) soil solution, and (w) water.

Powlson and Jenkinson (1976) also reported that irradiation mobilised a considerable amount of organic C in the soil. However, their results suggested that the majority of the C mobilised in this manner came from 'the biologically resistant fractions of the soil organic matter, rather than from the ... material released from lysed cells'. The results from experiments 1 and 2 support the finding that treating soil with γ-radiation mobilises C. It was not possible however to draw any conclusions as to the source of the mobilised material.

While C is the dominant element in organic material a variety of other biologically important elements will also be contained within this material. Hence, when C in this material is mobilised by γ-radiation the elements associated with it should also be mobilised. This process probably accounts for some of the P, Ca, Na, Mg and S mobilised by γ-radiation in this soil. Of course cell lysis would also release some of

these elements as well. It is postulated that for the elements listed above these two processes were responsible for the observed mobilisation that occurred after the soil had been irradiated.

This argument is further supported by the fact that the leaching behaviour of all these elements was essentially the same in the sterile soil. The vast bulk of all these elements were found in the dissolved fraction of the leachate. While C was not partitioned into dissolved and particulate forms in the first experiment the data from the second confirms that there was mainly dissolved C in the leachate from the sterile soil. This behaviour is consistent with the mechanisms proposed to account for the mobilisation of these elements by γ-radiation. If these elements were mobilised along with C or released by cell lysis then they should have been predominantly in the dissolved fraction of the leachate from the sterile soil.

Small amounts of particulate Ca and S were mobilised in the sterile soil. This observation is not necessarily inconsistent with the mechanisms proposed above, as they could easily have generated a small amount of organic debris. Much of this material would probably have been flushed from the soil during the initial stages of the experiment. It was observed that most of the particulate Ca and S was removed from this soil with the first displacement. While the mechanisms discussed above can adequately explain the observed behaviour of C, P, Ca, Na, Mg and S they cannot explain the behaviour of Fe and Al. The behaviour of these elements was most likely linked to changes in soil pH that γ-radiation induced.

Soil pH.

Given the experimental design it was not possible to make direct measurements of the soil pH. However, the pH of the leachates was measured. For each displacement the sterile leachate consistently had a lower pH than the leachate from the soil that had not been irradiated. It is reasonable to infer from this observation that the sterile soil had a lower pH than the non-irradiated soil. The fact that it was not possible to make direct measurements meant that the difference between the soils could not be quantified. The finding that irradiation decreased the soil pH is consistent with the results reported by Boyer *et al.* (1966) (as quoted by Cawse, 1975). They measured decreases of 0.25-0.6 units in three soils exposed to 40 kGy. Although Cawse (1975) also reports on several studies that found that irradiation of soils up to 30 kGy caused no change in pH. There do not appear to have been any comprehensive and systematic studies on the relationship between radiation dose and soil pH change. This is an area that warrants further investigation given that irradiation of soil is used extensively as a cold sterilisation technique.

The inference that irradiation lowered the pH of the soil used in this experiment can be used to help explain the observed behaviour of Al and Fe in this experiment. Below a pH of 5.5 alumino-silicates clays and Al hydroxide minerals begin to dissolve releasing Al – hydroxy cations and Al^{3+} into solution. Below a pH of approximately 4.0 the solubility of various Fe mineral species increases considerably (Ross, 1989). The pH of the leachates suggests that the soil pH was probably below the upper range limit for the solubility of various Al mineral species and at or near the upper limit for various Fe

mineral species. This suggests that the decrease in soil pH and subsequent increase in the solubility of various mineral phases probably accounts for a considerable portion of the Al and Fe mobilised by γ-radiation

There are several additional factors that need to be considered. Firstly, it is known that organic chelating agents enhance the solubility of Fe and Al compounds in the soil (Stevenson and Fitch, 1986; Tan, 1998). Considerable amounts of organic C were mobilised by the irradiation process, some of which may have had chelating properties. Secondly, both Fe and Al can be bound to soil organic matter. Hence, some Fe and Al may have been mobilised along with the C. Additionally, because Fe is a trace element a small amount of the mobilised material may have been derived from killed microbial cells (Sparling and Berrow, 1985a).

These processes can account for the material mobilised in the dissolved fraction of the leachate. But, in the first displacement a significant amount of particulate Al was mobilised in the sterile soil, while in the second there was a significant mobilisation of particulate Fe. It is difficult to provide any systematic explanations for these events. It was felt that they may have been isolated one-off incidences.

It is clear from this experiment that irradiating the soil generated a considerable number of H^+ ions (protons). Several mechanisms can be postulated to explain this. Firstly, some of these may have been associated with the release of contents from ruptured cells. The fact that most of the C mobilised was in the dissolved fraction of the leachate

suggests that the irradiation process may have actually solubilised soil organic matter. It is possible that organic acids could be produced as a by-product of this process. In general the application of high-energy ionising radiation to a complex system such as a soil would be expected to produce a variety of ions including H^+. Given the ubiquitous nature of hydrogen, it does not seem unreasonable to suggest that many of the protons in the sterile soil were generated in this manner.

4.5.2. Re-inoculated Treatment vs Sterile Treatment – Effects of Microbial Activity on P Mobility.

This experiment showed that in overall terms significantly more P was mobilised in the re-inoculated soil than in the sterile soil. The easiest way to attempt to understand this result is to examine the behaviour of the particulate and dissolved forms of this element separately.

Dissolved P.

To attempt to understand the behaviour of P in this fraction of the leachate careful consideration needs to be given to the details of the experimental procedure. Before proceeding however, it should be noted that the chemical partitioning of this element into MRP and URP forms, showed that little of the dissolved P was molybdate reactive.

Ideally, the MRP fraction should have consisted solely of dissolved orthophosphate; any colloidal or organic P species should have been contained in the URP fraction. However, there is evidence to suggest that molybdenum blue methods induce acid

hydrolysis of organic P compounds (Olsen, 1966) and colloid degradation (Stainton, 1980). Clearly then this scheme will tend to overestimate the amount of orthophosphate in solution and underestimate the amount of colloidal and organic P. A range of techniques has been developed in an attempt to overcome these problems. Chamberlin and Shapiro (1968) shortened the reaction time with molybdate, while Dick and Tabatabai (1977) used arsenite to complex the excess molybdate.

Thus it can be concluded from these data (Table 4.9) that very little of the dissolved P was present as orthophosphate. This is not unexpected in a leachate from an acid mineral soil containing moderate amounts of Fe and clay. Little can be concluded about the exact chemical nature of the material in the URP fraction except to note that it probably consisted of colloidal and organic P compounds that were relatively resistant to acid hydrolysis.

It is clear from the preceding discussion that the irradiation process generated a considerable amount of water soluble C. The data also show that substantial amounts of all the other elements were also mobilised by this process. After re-inoculation the population of soil micro-organisms would have rapidly expanded as the substrates made available by γ-radiation were utilised. Consequently P and other nutrients solubilised by γ-radiation would have been incorporated into fresh microbial tissue. This process probably accounts for the substantial drop in the concentration of dissolved P, Ca, Na, Mg and S observed in the leachates from the re-inoculated soil, although it is not clear

why in the second experiment the drop in the concentration of P in the leachate was not as marked as in the first.

A point of interest is that the data suggest that most of the P incorporated into microbial tissue came from the fraction that was unreactive towards molybdate. As discussed earlier this material was not readily converted to orthophosphate by acid hydrolysis, yet soil micro-organisms appear to have been able to utilise it.

The situation with C was a little more complicated. According to Picek *et al.* (2000) the four possible fates of organic substrates consumed by micro-organisms are:

1. mineralised to CO_2 in aerobic conditions or metabolised to CO_2 and other volatile or soluble compounds in anaerobic conditions;
2. used for biosynthesis and incorporated into new biomass;
3. stored unchanged in cells for future use; and
4. used for the synthesis of reserve polymers and then stored in cells.

From our perspective the distinction between the final three fates is somewhat academic, given that the substrates are all effectively incorporated into the microbial biomass. Hence the drop in the concentration of C in the leachate from the re-inoculated soil can be attributed to two processes. Firstly, a substantial amount would have been lost from the system, as CO_2 while the remainder would have been immobilised by being incorporated into microbial tissue.

While these mechanisms can effectively account for the observed behaviour of P, Ca, Na, Mg, S and C in the dissolved fraction of the leachate, they cannot account for the behaviour of Al and Fe.

With both experiments there was substantially less Al in the leachate from the re-inoculated soil than in the leachate from the sterile soil. This is most likely the result of the inferred (from leachate data) rise in soil pH that accompanied re-inoculation and the associated decrease in the solubility of various Al compounds in the soil. Why re-inoculating the soil should have had this effect on soil pH will be discussed shortly.

The behaviour of Fe in this fraction of the leachate was a little more complicated than Al. During the initial stages of both experiments there tended to be less Fe in the leachate from the re-inoculated soil than in the leachate from the sterile soil. The increase in the soil pH and associated decrease in the solubility of various Fe compounds probably accounts for most of this decrease. However as noted earlier Fe is a trace element and a relatively small amount would have been immobilised by being incorporated into the microbial biomass.

During the latter stages of both experiments substantial amounts of dissolved Fe were mobilised in the re-inoculated soil. The most obvious interpretation of these data is that over time microbial activity lowered the pE of the soil in the columns producing a redox induced mobilisation of this element. No measurements (*Eh*) were made so this can

only remain a postulated mechanism. However, this argument is further supported by the S data.

S is another redox sensitive element, significant amounts of which were mobilised during the latter stages of both experiments in the re-inoculated soil. After the completion of each experiment the soil was removed from each column. The characteristic odour of hydrogen sulphide gas (H_2S) was detected in the re-inoculated soil. According to Willett (1983) there is a well-defined sequence of reduction of inorganic elements in the soil. The sequence of reduction reactions is provided in Table 4.32. The fact that the presence of H_2S was detected strongly suggests that SO_4^{2-} was being biologically reduced to H_2S in the re-inoculated soil. The sequence of reduction reactions means that Fe^{3+} should have been reduced prior to SO_4^{2-} being reduced. Although it has been reported that if microbial activity is intense then multiple reactions can occur at the same time (Picek *et al.* 2000). Either way the S data strongly supports the argument the soil pE influenced the mobility of Fe in this system.

Reduction half-reaction	E_o(V)
$O_2 + 4H^+ + 4e^- = 2H_2O$	0.814
$2NO_3^- + 12H^+ + 2e^- = N_2 + 8H_2O$	0.741
$MnO_2 + 4H^+ + 2e^- = Mn^{2+} + 2H_2O$	0.401
$Fe(OH)_3 + 3H^+ + e^- = Fe^{2+} + 3H_2O$	-0.185
$SO4_2^- + 10H^+ + 8e^- = H_2S + 4H_2O$	-0.214
$CO_2 + 8H^+ + 8e^- = CH_4 + 2H_2O$	-0.244
$2H^+ + 2e^- = H_2$	-0.413

Table 4.32. The sequence of reduction reactions in soils (from Willett, 1983).

Microbial Activity and H^+ Concentration.

The data show that microbial activity in the soil substantially decreased the concentration of H^+ ions in the leachate. This means that microbially mediated processes must have occurred that consumed protons. As is clear from Table 4.33 there

are a variety of processes that involve proton transfer either from micro-organisms to environment or vice versa. After re-inoculation the oxidation of C (respiration) would have occurred, but this process should not have directly affected the concentration of protons in the leachate. If anything the CO_2 production associated with respiration should have increased the concentration of H^+ in the leachate through the formation of carbonic acid. Processes such as the mineralization of organic N, uptake of orthophosphate, sulphate and nitrate may all have played a role in decreasing the concentration of H^+ ions in the leachate. It should be noted that the greatest difference between the treatments occurred during the latter stages of the experiment. It was during this period that redox reactions (Fe^{3+}, SO_4^{2}) would have occurred that consume protons. Such processes may also have contributed to the difference in pH between the sterile and re-inoculated soils.

Process from l to r	Reaction equation		Process from r to l
	H^+ - indifferent processes.		
	Biota↔atmosphere		
Photosynthesis	$CO_2 + H_2O$	$=CH_2O + O_2$	Respiration
N_2-fixation	$N_2 + H_2O + 2R.OH$	$=2.R.NH_2 + +3/2O_2$	
NH_3-uptake	$NH3 + R.OH$	$=R.NH_2 + H_2O$	Volatilisation of NH_3
H_2S-uptake	$H_2S + R.OH$	$=R.SH + H_2O$	Volatilisation of H_2S
H^+ - source	*H^+ - transfer*		*H^+ - sink*
	Biota↔solution		
Uptake of cations	$M^+ + ROOH$	$=ROOM + H^+$	Mineralisation of M^+
Uptake of NH_4^+	$NH_4 + R.OH$	$=R.NH_2 + H_2O + H^+$	Mineralisation of org N
Mineralisation + nitrification of org N	$R.NH_2 + 2O_2$	$=2.OH + NO_3^- + H^+$	Uptake of NO_3^-
Mineralisation + oxidation of organic S	$R.SH + 3/2\,H_2O + 7/4\,O_2$	$=R.OH + SO_4^{2-} + 2H^+$	Uptake of SO_4^{2-}
Mineralisation of P	$R.H_2PO_4 + H_2O$	$=R.OH + H_2PO_4^- + H^+$	Uptake of P
	Solution or solution↔atmosphere		
Dissociation of H_2O	$2H_2O$	$=OH^- + H^+$	Protonation of OH^-
Dissociation of CO_2	$CO_2 + H_2O$	$=CO_3^- + H^+$	Protonation of HCO_3^-
Dissociation of org acids	$ROOH$	$=ROO^- + H^+$	Protonation of org anions
Complexation of metal ions (L=org ligand or OH-)	$HL + M^+$	$=ML + H^+$	Decomplexation of metal ions
Oxidation of H_2S	$H_2S + 2O_2$	$=SO_4^{2-} + 2H^+$	Sulphate reduction
Oxidation of SO_2	$SO_2 + 1/2O_2 + H_2O$	$=SO_4^{2-} + 2H^+$	
Nitrification of NH_4^+	$NH_4^+ + 2O_2$	$NO_3^- + H_2O + 2H^+$	
Nitrification of N_2	$N_2 + 5/2O_2 + H_2O$	$=2NO_3^- + 2H^+$	Denitrificaton
	Solids↔solution		
Reverse weathering	$M^{n+} + n/2H_2O$	$=n/2M_{2/n}O + nH^+$	Weathering
M^{n+}/H^+ exchange	$M^{n+} + nH.exch$	$=M.exch + nH^+$	H^+/M^{n+} exchange
Oxidation of Fe^{2+}	$Fe^{2+} + 1/4\,O_2 + 5/2H_2O$	$=Fe(OH)_3 + 2H^+$	Reduction of $Fe(OH)_3$
Oxidation of FeS	$FeS + 9/2O_2 + 5/2H_2O$	$=Fe(OH)_3 + SO_4^{2-} + 2H^+$	Reduction of $Fe(OH)_3$ and SO_4^{2-}
Desorption of SO_4^{2-}	Exch $SO_4^{2-} + 2H_2O$	exch $(OH)_2 + SO_4^{2-} + 2H^+$	Adsorption of SO_4^{2-}

Table 4.33. H^+ transfer processes that involve biota (From Mulder and Cresser, 1994).

Particulate P.

The data from both experiments show that all of the P mobilised by microbial activity was in the particulate fraction of the leachate. Several hypotheses can be postulated to explain these data:

1. the P was mobilised as a secondary effect of the reductive dissolution of Fe compounds;
2. the mobile P was contained within microbial cells or cellular debris; and
3. the mobile P was associated with dispersed clay materials.

The first mechanism can be discounted on the following grounds. If the P had been mobilised in this manner most of it should have been in the dissolved fraction of the leachate, rather than in the particulate fraction. Also there should have been a close correlation between the concentration of dissolved Fe and P. Yet in the latter stages of the experiment when there was a mobilisation of dissolved Fe there was no associated mobilisation of P. It could be argued that the reductive dissolution of Fe destabilised soil particles, either through dissolution of binding cement or altering surface charge and that these materials dispersed into the soil solution. In this way P containing particulate material could be mobilised as a secondary effect of the reductive dissolution process. During the latter stages of both experiments significant amounts of both dissolved and particulate Fe were mobilised in the re-inoculated soil. It is postulated that this mobilisation of particulate Fe was indeed a result of the reductive dissolution process. However, in both experiments the trend with particulate Fe over the five displacements was for the amount mobilised with each displacement to initially decline and then to increase again during the latter stages of the experiment. The trend for dissolved Fe followed a similar pattern. Yet the trend with P (both dissolved and particulate) was for the amount mobilised with each displacement to decrease over the course of the experiment. This suggests that in this soil at least, P was not closely

associated with Fe compounds and was not mobilised as an indirect consequence of the reductive dissolution of these materials.

The second mechanism was first postulated by Hannapel *et al.* (1964a, b) to account for the movement of P through a calcareous sandy loam. The SEM data from this experiment show that the P mobilised was not contained within whole cells or easily recognisable cell fragments. The particulate material isolated from the leachates collected from the re-inoculated soil consisted of numerus clay particles and a dark coloured amorphous material. As will be discussed below it could not be determined whether the P in this mixture was associated with clay particles or the amorphous material. It is clear that substantially more of this mixture was flushed from the re-inoculated soil than from the sterile soil. This observation supports the argument that microbial activity mobilised significant amounts of clay material.

The operation of this mechanism is consistent with the observed behaviour of Fe and Al. During the initial stages of both experiments significant amounts of particulate Al were mobilised in the re-inoculated soil. The mobilisation of various alumino-silicate clay minerals could account for this. EDS analyses of various particles confirmed that many of the particles (> 5 μm) were indeed alumino-silicate clay minerals.
In the soil the surfaces of clay minerals are often coated with Fe compounds, hence, the mobilisation of significant amounts of clay material coated with Fe could account for the initial mobilisation of particulate Fe. This supports the argument that there were two distinct episodes of Fe mobilisation. The first occurred during the initial stages of

the experiments and was associated with the microbially mediated mobilisation of clay material. Only particulate Fe was mobilised by this process. The second occurred during the latter stages of the experiment and was associated with the reductive dissolution of Fe compounds. Both forms were mobilised by this process.

It is tempting to conclude from these observations that microbial activity mobilised P rich clay minerals. EDS analysis showed that most of the P in the particulate material was associated with the matrix of small particles and amorphous material rather than with larger (> 5μm) particles. However as noted earlier, it could not be established whether the P in this mixture was actually associated with the small clay particles or the amorphous material.

In soils P is usually associated with material from the finer size fraction. This suggests that the P in this material may well have been associated with the small clay particles. However, the possibility that the dark coloured amorphous material was composed of P rich organic compounds cannot be ruled out. The EDS analyses (Figures 4.19 and 4.20) showed that the matrix of material contained Al, Si, P, S, K and Fe. The clay particles in the mixture probably account for the presence of Si, Al, K and Fe, while the presence an organic material would account for the P and S. Thus, it is postulated that the dark coloured amorphous material was in fact microbial mucilage. This argument is further supported by the observation that material of a similar nature was found on the surface of the membranes used to filter the leachate from the soil that had not been irradiated. Yet little if any was found on the surface of the membrane used to filter the sterile

leachate. This suggests that there was a close relationship between microbial activity and the production of this dark coloured amorphous material. If the P was associated with the amorphous material and this was microbial mucilage then it could be argued that the mobilised P was associated with microbial debris. Thus, on the available evidence it was not possible to determine which of the two remaining mechanisms was responsible for the observed mobilisation of P.

The observation that soil microbes mobilised clay material in this soil is supported by the different flow regimes observed in the re-inoculated and sterile columns. As noted earlier the Cl^- breakthrough curves obtained from the sterile columns were spread or displaced both forward and backwards from the position of an idealized curve. The operation of hydrodynamic dispersion and anion exclusion produces these effects on the elution curve (Ross, 1989). With the non-sterile columns the concentration of Cl^- in the eluent increased very rapidly during the initial stages of the experiment. This very rapid increase in the concentration of Cl^- in the eluent only after a relatively small fraction of a pore volume had eluted indicates that preferential flow was occurring (Kanchanasut *et al.* 1978). Therefore these data show that there was a stronger preferential component to the flow regime in the re-inoculated columns than in the sterile columns. This observation is consistent with an increase in soil porosity caused by the mobilisation and subsequent translocation of clay through the columns.

How might soil micro-organisms mobilise/disperse clay particles/colloids? According to Tan (1998) there is evidence that clays and organic compounds can form complexes

that are mobile in the soil environment. It has also long been known that organic acids can act like anionic surfactants, causing changes in surface charge leading to the release of colloidal particles in porous media (Kretzchmar *et al.* 1999). Several authors have shown that carboxylic acids and higher molecular weight dissolved organic carbon can disperse soil aggregates (Shanmuganathan and Oades, 1983; Durgin and Chaney, 1984). According to Kretzchmar *et al.* (1999) organic compounds such as these are constantly produced in soils by plant roots, soil fauna and micro-organisms. Thus, it is postulated that organic compounds produced by soil micro-organisms were responsible for dispersing the clay particles/colloids.

4.5.3. Experiment 1 vs Experiment 2.

From the preceding discussion it is clear that the first objective for conducting the second experiment was accomplished. The same clear broad patterns were evident in both experiments. Namely, that micro-organisms mobilised P in this soil and that all of the P mobilised in this fashion was in the particulate fraction of the leachate. Substantial amounts of particulate Fe and Al were also mobilised. There appeared to have been two quite distinct episodes of Fe mobilisation, one during the early stages and one during the latter stages of the experiments. Finally, micro-organisms mobilised considerable amounts of clay material. Therefore the broad results from the first experiment have been reinforced and the repeatability of the experimental procedure has been demonstrated.

The second objective for carrying out this experiment was to test the hypothesis that:

'Given two biologically active soils, most P will be mobilised and leached from that soil which contains the greatest amount of readily available microbial substrate.'

In experiment 1 the data in Table 4.29 show for each column on average approximately 175 mg of C were solubilised by γ-radiation, of which approximately 113 mg (refer to Table 4.30) were utilized by soil micro-organisms. In the second experiment approximately 183 mg (refer to Table 4.29) of C was solubilised but only 32 mg (refer to Table 4.30) were utilised by micro-organisms. Thus, if there was a positive relationship between the amount of available substrate and the mobility of particulate P then more should have been mobilised with experiment 1 than with experiment 2. However the data (Table 4.30) show that significantly more particulate P was mobilised with experiment 2 than with experiment 1. Given this result little evidence can be found to support the contention that the mobilisation, redistribution within and export of P from the soil profile is controlled by the availability of organic C.

There are two further points about the data in Tables 4.29 and 4.30 that warrant further discussion. Firstly, the data show that in the sterile columns with the exception of C and Fe significantly greater amounts of each element were mobilised with the first experiment than with the second. In the sterile system the concentration of any given element in the soil solution will be controlled by a variety of abiotic processes (e.g. dissolution, desorption, exchange etc). A crucial difference between the two experiments was a shortening in the length of time the solution was allowed to remain

in contact with the solid components of the soil. This meant that in terms of the concentration of any element in the soil solution the system in experiment 2 had less time to equilibrate than the system in experiment 1.

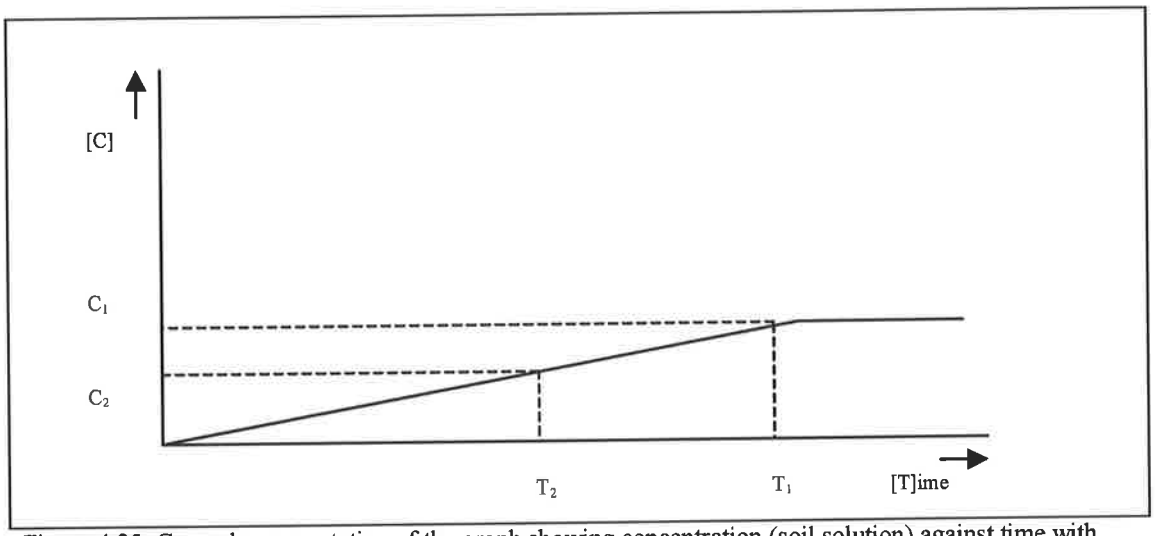

Figure 4.25. General representation of the graph showing concentration (soil solution) against time with slow equilibration processes.

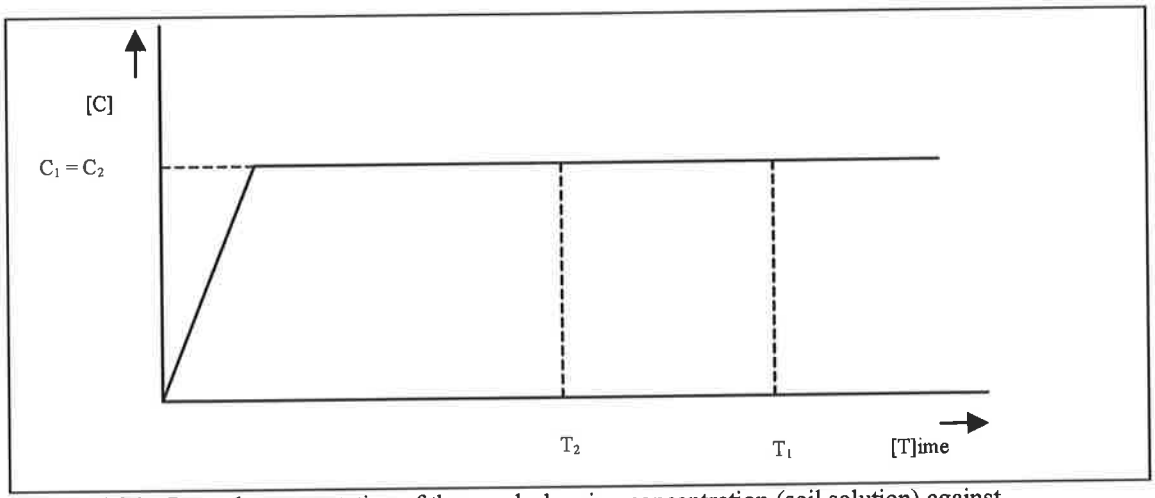

Figure 4.26. General representation of the graph showing concentration (soil solution) against time with rapid equilibration processes.

How this change affected the amounts of various elements leached can best be understood with reference to Figure 4.25. For most elements a plot of concentration [c] against time [t] should take the general form shown in this figure.

Now with experiment 1 after the initial week-long incubation period the columns were saturated and allowed to incubate (equilibrate) for a further 48 h (T_1). This would have generated a concentration (of any element) in the soil solution of C_1. With the second experiment the columns were saturated and then the solution was displaced immediately. This meant that the components of the system were in contact for a very much shorter time period (time taken to displace one pore volume T_2 = 5 h). This would have generated a lower concentration C_2 in the soil solution. This means that in the first pore volume (60 ml) displaced, larger amounts of the various elements would have been mobilised with experiment 1 than with experiment 2. Exactly the same argument applies to each of the subsequent displacements. The data in Table 4.35 supports this argument in so much as there tended to be greater amounts of the elements mobilised with experiment 1 than with experiment 2. Although not shown the data for the other elements follows a similar pattern. Consequently over the five displacements significantly greater amounts of the various elements were mobilised with experiment 1 than with experiment 2. This does not however explain the behaviour of Fe and C.

Displacement	*P*[#]	*Fe*[#]	*Ca*[#]	*S*[#]	*C*[^]	*pH*
	Total Values - [#] *µg/60 ml /* [^] *mg/60 ml*					
Displacement 1						
Ex1-S	109	137	2403	2117	81	4.28*
Ex2-S	83	220	1810	1515	100	4.41
Ex1-NS	192*	208	937*	1477	28*	4.46
Ex2-NS	251	341	1197	1332	74	4.54
Displacement 2						
Ex1-S	41*	100	489*	710*	41*	4.52*
Ex2-S	25	106	268	362	30	4.75
Ex1-NS	76	101	117	547*	11β	5.06
Ex2-NS	88	99	129	340	19	5.01
Displacement 3						
Ex1-S	23	109	221*	365*	21	4.71*
Ex2-S	19	105	121	171	18	4.89
Ex1-NS	44	109β	132	529*	10	5.31*
Ex-2-NS	59	328	111	258	19	5.60
Displacement 4						
Ex1-S	14*	93	118	65*	17	4.86
Ex2-S	23	91	0	186	19	4.89
Ex1-NS	28*	159*	106	284	11*	5.51
Ex2-NS	52	347	0	373	23	5.66
Displacement 5						
Ex1-S	15*	76	0	43*	16	4.90
Ex2-S	21	81	0	109	17	4.96
Ex1-NS	16*	355	0	107	6*	5.57*
Ex2-NS	21	266	0	158	16	6.04

Table 4.34. A comparison between experiments 1 and 2 of the total amounts of selected elements mobilised during each displacement.

In this table * indicates that the mean values are significantly different ($p \leq 0.05$). The treatments were compared using an unpaired t-Test. For the non-parametric data, β indicates that the mean values are significantly different ($\alpha \leq 0.05$). The treatments were compared using the Wilcoxon Two Sample Test.

The data for these elements suggest that the abiotic processes that controlled the equilibrium concentration of these elements in the soil solution were relatively rapid. In this case a plot of concentration against time should take the general form shown in Figure 4.26. The same arguments outlined above apply in this case except that the

difference between equilibration times (T_1, T_2) does not generate a difference in the concentration (C_1=C_2) of these elements in the soil solution and hence the amounts leached.

Secondly, it is difficult to provide any systematic explanations for the behaviour of the elements in the biologically active system. The exception is of course C and it has already been explained why more of this element was mobilised with the second experiment than with the first. The behaviour of these elements is perhaps a reflection of the fact that living systems are inherently complex and variable.

4.6.0. Conclusions.

4.6.1. Non-irradiated Treatment vs SterileTreatment – Effects of γ-radiation.

The data from the first experiment show that γ-radiation had two main effects on this soil. The first was to mobilise significant amounts of P, Ca, Na, Mg, S, C , Al, and Fe. Secondly it lowered the pH of the soil. It was postulated that P, Ca, Na, Mg, S and C were mobilised either as a result of cell lysis or the solubilization of soil organic matter. The mobilisation of Al and Fe was thought to be a pH related effect.

4.6.2. Re-inoculated Treatment vs Sterile Treatment – Effects of Microbial Activity on P Mobility.

The results from this work clearly support the hypothesis that soil micro-organisms mobilise P in an acid mineral soil. All of the P mobilised in this fashion was in the

particulate fraction. This finding is in agreement with that reported by Hanapel *et al.* (1964a, b). However, no unequivocal evidence could be found to support the contention that the P mobilised in this manner was sequestered in cells or cellular debris. In fact there was some evidence to suggests that the P was mobilised in association with clay particles. However the exact nature of this relationship could not be elucidated. Furthermore, no experimental evidence could be found to support the contention that the mobilisation, redistribution within and export of P from the soil profile is controlled by the availability of organic C.

Chapter 5.

Sandy Soil – Effect of Soil Microbial Activity on P Mobility.

5.1.0. Introduction.

This chapter reports on the work carried out to test the second hypothesis developed from the review of the literature. The results from the initial set of experiments demonstrated that in an acid mineral soil, P was mobilised by micro-organisms. If it can be shown that microbial activity also mobilised P in a sandy soil then this effect will have been demonstrated to occur in two important soil types. The experiments conducted by Hannapel *et al.* (1964a, b) strongly suggest that a similar process also occurs in calcareous soils, a third major soil type. This body of work would then establish a strong experimental foundation for the contention that forms the basis of this thesis.

Selected physical and chemical properties of the soil (Willow Creek Sand - Haplorthod) chosen for this experiment are provided in Table 5.1. A visual examination of the soil showed that it consisted of a mixture of sand grains that were probably quartz and organic matter. This soil was chosen because there should have been minimal geochemical control on the mobility of P. The majority of P retained by this soil would have to be either associated with the soil organic matter or contained within the soil biomass. Thus using this soil and the procedure developed for the initial set of experiments it would be possible to test the second hypothesis.

A comparison in terms of the amount of P mobilised was made between soil that was biologically active (irradiated + re-inoculated) and soil that was not (irradiated). If soil micro-organisms mobilised P then there should have been significantly more P mobilised in the re-inoculated soil than in the sterile soil. As in the first experiment the effect that γ-radiation had on this soil was examined by leaching a set of columns that contained soil that had not been irradiated. The results obtained were compared with those obtained from the sterile leaching.

5.2.0. Experiment No 3.

5.2.1. Materials and Methods.

Twelve columns were prepared from 10 cm lengths of PVC as described in Section 3.1.0. The columns were then packed to a depth of 6 cm with approximately 125 g of air-dry soil (Willow Creek, A horizon, 0-10 cm). The actual amounts of soil used and the bulk densities of the prepared columns are shown in Table 5.2.

The columns were sterilised and set-up as described in Section 4.2.1. The four sterile columns that were set-up under non-sterile conditions were re-inoculated by wetting up the columns with 10 ml of DI water and 5 ml of non-sterile inoculum. The inoculum was prepared as described in Section 3.2.1. Then 15 ml of sterile DI water were added to each sterile column. Both sets of columns were incubated at 25°C (under sterile/non-sterile conditions as appropriate) for seven days. On every second day during this period 1.2 ml of DI water (sterile/non-sterile) were added to each column.

Property Determined	*Value*
pH (1:5 soil:H_2O)	6.00
EC (1:5 soil:H_2O) dS/m	0.18
Total C (Leco) %	2.80
Total N (Leco) %	0.24
Available P (HCO_3) (mg/kg)	56.00
CEC (cmol(+)/kg (NH_4)	6.80
Exchangeable cations (cmol(+)/kg)	
Ca	4.06
Mg	1.85
K	0.55
Na	0.07
Trace elements (mg/kg) (DTPA)	
Fe	39.00
Mn	10.80
Zn	7.50
Cu	0.60
Texture	
Sand %	92.00
Silt %	4.00
Clay %	4.00

Table 5.1. Chemical and physical properties of Willow Creek sand.

Columns	*Wt Soil*	*Bulk Density*
	(g)	(g/cm^3)
Sterile 1	125.4	1.53
Sterile 2	125.9	1.54
Sterile 3	125.6	1.53
Sterile 4	125.8	1.54
Re-inoculated 1	125.8	1.54
Re-inoculated 2	125.7	1.53
Re-inoculated 3	125.3	1.53
Re-inoculated 4	125.6	1.53
Non-irradiated 1	125.9	1.54
Non-irradiated 2	125.6	1.53
Non-irradiated 3	125.8	1.54
Non-irradiated 4	125.8	1.54

Table 5.2. Amounts of soil used and bulk densities of prepared columns.

On the eighth day enough DI water (sterile/non-sterile) was added to saturate the columns. Both sets of columns were then left to incubate for a further two days. On the tenth day leaching was begun when enough DI water (sterile/non-sterile) was added to displace one pore volume (35 ml). Both sets of columns were then allowed to incubate

for a further 3 days, after which enough DI water (sterile/non-sterile) was added to displace one pore volume. This procedure was followed until a total of five pore volumes (175 ml) had been displaced. The details of the procedure followed are set out in Table 5.3.

Procedure	*Sterile Treatment*		*Re-inoculated Treatment*		*No Radiation Treatment*	
	Time (h)	Flow rate (ml/h)	Time (h)	Flow rate (ml/h)	Time (h)	Flow rate (ml/h)
(A)dding DI H_2O	1.0		1.0		1.0	
(I)ncubation +A	168		168		168	
(S)aturation + I	48		48		48	
(L)eaching	1.5	23.3	1.5	23.3	1.5	23.3
I	72		72		72	
L	1.5	23.3	2.0	17.5	1.5	23.3
I	72		72		72	
L	1.5	23.3	2.5	14.0	2.0	17.5
I	72		72		72	
L	2.0	17.5	2.0	17.5	2.0	17.5
I	72		72		72	
L	2.0	17.5	2.5	14.0	2.0	17.5
	Total= 513.5	Av rate=21	Total= 515.5	Av rate=17.3	Total=	Av Rate=19.8
	Av leaching = 1.7		Av leaching= 2.1		514.0 Av leaching = 1.8	

Table 5.3. Details of the procedure followed during experiment 3.

To ensure the integrity of the sterile system, samples of leachate from each column were plated out on nutrient agar and 2% potato dextrose agar and incubated for a week.

The leachates were fractionated using the scheme outlined in Section 3.3.0. The results of the chemical analyses of the various fractions were analysed using SigmaStat statistical software as described in Section 4.2.1. SEM was used to characterise the particulate material obtained from the leachate.

After the completion of this work, the four columns that had not been irradiated were set-up and leached under non-sterile conditions in the laboratory. The procedures followed were the same as those described above. The conditions under which the leachings were conducted were also identical to those under which the first leachings were conducted. The results were compared to those obtained from the irradiated soil. In this manner a direct comparison could be made between soil samples that had been irradiated and between those that had not.

5.2.2. Results.

Tabulated Analytical Results.

Phosphorus.

Non-irradiated Treatment vs Sterile Treatment.

The data in Table 5.4 show that most of the P mobilised by either treatment was in the dissolved fraction of the leachate. Although a small amount of particulate P was mobilised in the non-irradiated soil during the final displacement. These results show that significant amounts of P were mobilised in this soil by γ-radiation. The data in Table 5.5 show the amounts of MRP and URP found in the dissolved fraction of the leachate. Clearly, with both treatments most of the P in this fraction was molybdate-reactive.

Soil Treatment	*Total (P+D)*$^{+}$	*Particulate (P)*$^{+}$	*Dissolved (D)*$^{+}$	*% Particulate*
	µg/35 ml^{+}			
D1-Re-inoculated	1685*	177*	1508*	10*
D1-Sterile	2130	20	2110	1
D1-Non-irradiated	1315$^{\#}$	22	1293$^{\#}$	2
D2-Re-inoculated	1205	73*	1132	6*
D2-Sterile	1135	20	1115	2
D2-Non-irradiated	641$^{\#}$	14	627$^{\#}$	2
D3-Re-inoculated	877*	44*	832*	5*
D3-Sterile	562	7	555	1
D3-Non-irradiated	500	14	486	3
D4-Re-inoculated	621*	123*	498	20*
D4-Sterile	491	6	485	2
D4-Non-irradiated	424$^{\#}$	6	417$^{\#}$	2
D5-Re-inoculated	550*	62*	488	11*
D5-Sterile	442	7	434	2
D5-Non-irradiated	257$^{\#}$	26$^{\#}$	231$^{\#}$	10
Σ-Re-inoculated$^{@}$	4940*	480*	4460	-
Σ-Sterile$^{@}$	4761	62	4699	-
Σ-Non-irradiated$^{@}$	3143$^{\#}$	83	3060$^{\#}$	-
$^{@}$µg/175 ml				

Table 5.4. Amount of P mobilised and its distribution between particulate and dissolved fractions.

For each displacement the only comparisons made were re-inoculated vs sterile and non-irradiated vs sterile soils. For the re-inoculated vs sterile comparison, * indicates that the mean values are significantly different ($p \leq 0.05$). The treatments were compared using an unpaired t-Test. For the non-parametric data, β indicates that the mean values are significantly different ($\alpha \leq 0.05$). The treatments were compared using the Wilcoxon Two Sample Test. For the sterile vs non-irradiated comparisons, # indicates that the mean values are significantly different ($p \leq 0.05$). The treatments were compared using an unpaired t-Test. For non-parametric data, χ indicates that the mean values are significantly different ($\alpha \leq 0.05$). The treatments were compared using the Wilcoxon Two Sample Test. In the treatment column the letter D followed by a number denotes a particular displacement, while Σ denotes the total amounts of P displaced over the course of the experiment (five displacements).

Re-inoculated Treatment vs Sterile Treatment.

These data show that in overall terms more P was mobilised in the re-inoculated soil than in the sterile soil. An examination of the data for individual displacements reveals that in all five, significantly more particulate P was mobilised in the re-inoculated soil than in the sterile soil. In the first displacement even though a great deal more particulate P was mobilised in the re-inoculated soil than in the sterile soil, the large

drop in the amount of P in the dissolved fraction of the leachate from the re-inoculated soil meant that in total significantly less P was mobilised in the re-inoculated soil than in the sterile soil. In the third displacement there was significantly more particulate and dissolved P mobilised in the re-inoculated soil than in the sterile.

Soil treatment	*MRP*	*URP*	*% MRP*
	µg/35 ml		
D1-Re-inoculated	1453*	55	97
D1-Sterile	2083	27	99
D1-No radiation	1273#	20	98
D2-Re-inoculated	1090	42	96
D2-Sterile	1095	20	98
D2-No radiation	620#	7	97
D3-Re-inoculated	817*	15	98
D3-Sterile	537	18	97
D3-No radiation	474	11	97
D4-Re-inoculated	464	34	93
D4-Sterile	472	13	97
D4-No radiation	399#	18	95
D5-Re-inoculated	466	22	95
D5-Sterile	417	17	96
D5-No radiation	224#	7	97

Table 5.5. Concentration of MRP and URP in the filtrate.

For each displacement the only comparisons made were re-inoculated vs sterile and non-irradiated vs sterile soils. For the re-inoculated vs sterile comparison, * indicates that the mean values are significantly different ($p \leq 0.05$). The treatments were compared using an unpaired t-Test. For the sterile vs non-irradiated comparisons, # indicates that the mean values are significantly different ($p \leq 0.05$). The treatments were compared using an unpaired t-Test. In the treatment column the letter D followed by a number denotes a particular displacement.

In displacements two, four and five no statistically significant difference could be established between the amounts of dissolved P mobilised with either treatment. However, in displacements four and five because significant amounts of particulate P were mobilised in the re-inoculated soil, in total significantly more P was mobilised in the re-inoculated soil than in the sterile soil. Clearly, the majority of the P mobilised by micro-organisms was particulate in nature. It is worth noting however, that this form of P made up only a small fraction (up to a maximum of 20%) of the total amount

mobilised and leached from this soil. The data in Table 5.5 show that the majority of P in the dissolved fraction of the leachates obtained with either treatment was molybdate reactive.

Iron.

Soil Treatment	*Total (P+D)*$^{+}$	*Particulate (P)*$^{+}$	*Dissolved (D)*$^{+}$	*% Particulate*
	µg/35 ml^{+}			
D1-Re-inoculated	49	17*	32*	34*
D1-Sterile	48	9	39	19
D1-Non-irradiated	23$^{\#}$	11	12$^{\#}$	48$^{\#}$
D2-Re-inoculated	37*	8	29*	21
D2-Sterile	52	10	42	19
D2-Non-irradiated	24$^{\#}$	8	16$^{\#}$	33$^{\#}$
D3-Re-inoculated	32	4	28	14
D3-Sterile	35	4	31	13
D3-Non-irradiated	0	0	0	0
D4-Re-inoculated	0	0	0	0
D4-Sterile	40	6	34	14
D4-Non-irradiated	0	0	0	0
D5-Re-inoculated	0	0	0	0
D5-Sterile	37	4	33	10
D5-Non-irradiated	0	0	0	0
Σ-Re-inoculated$^{@}$	118*	26	92*	-
Σ-Sterile$^{@}$	211	32	179	-
Σ-Non-irradiated$^{@}$	45$^{\#}$	18$^{\#}$	27$^{\#}$	-
$^{@}$µg/175 ml				

Table 5.6. Amount of Fe mobilised and its distribution between particulate and dissolved fractions.

For each displacement the only comparisons made were re-inoculated vs sterile and non-irradiated vs sterile soils. For the re-inoculated vs sterile comparison, * indicates that the mean values are significantly different ($p \leq 0.05$). The treatments were compared using an unpaired t-Test. For the non-parametric data, β indicates that the mean values are significantly different ($\alpha \leq 0.05$). The treatments were compared using the Wilcoxon Two Sample Test. For the sterile vs non-irradiated comparisons, # indicates that the mean values are significantly different ($p \leq 0.05$). The treatments were compared using an unpaired t-Test. For non-parametric data, χ indicates that the mean values are significantly different ($\alpha \leq 0.05$). The treatments were compared using the Wilcoxon Two Sample Test. In the treatment column the letter D followed by a number denotes a particular displacement, while Σ denotes the total amounts of Fe displaced over the course of the experiment (five displacements).

Non-Irradiated Treatment vs Sterile Treatment.

Compared to most of the other elements only minor amounts of Fe were mobilised and leached from this soil with either treatment. Little if any Fe was mobilised in the non-irradiated soil after the second displacement. However, significant amounts were mobilised in the sterile soil during the final three displacements. Thus, the data show that γ-radiation mobilised significant amounts of this element. Most of the Fe mobilised in this manner was in the dissolved fraction of the leachate, although small amounts of particulate Fe were also mobilised across the five displacements. In the first two displacements where Fe was mobilised in the non-irradiated soil, there was a significantly higher percentage of the total in the particulate fraction of the leachate from this soil than in that fraction of the leachate from the sterile soil.

Re-inoculated Treatment vs Irradiated Treatment.

These data show that in total, significantly less Fe was mobilised in the re-inoculated soil than in the sterile soil. In fact, in the re-inoculated soil little of this element was mobilised during the latter stages of the experiment. It should be noted that in the first displacement a small but nevertheless statistically significant amount of particulate Fe was mobilised in the re-inoculated soil.

Aluminium.

Non-irradiated Treatment vs Sterile Treatment.

Like Fe, the amounts of Al mobilised and leached from this soil were relatively modest compared to the other elements (Table 5.7). All of the Al mobilised in the non-

irradiated soil was flushed from the system during the initial two displacements. In contrast small amounts of both particulate and dissolved Al continued to leach from the sterile soil during the final three displacements. As a consequence the data show that in total significantly more Al was mobilised in the sterile soil than in the non-irradiated soil. There appears to have been a significant mobilisation of particulate Al in the non-irradiated soil during displacement two.

Soil Treatment	*Total (P+D)*$^{+}$	*Particulate (P)*$^{+}$	*Dissolved (D)*$^{+}$	*% Particulate*
	µg/35 ml^{+}			
D1-Re-inoculated	51*	32*	19	63
D1-Sterile	80	48	32	61
D1-Non-irradiated	75	66	9$^{\beta}$	88$^{\#}$
D2-Re-inoculated	50	18	32	36*
D2-Sterile	51	25	26	49
D2-Non-irradiated	79$^{\#}$	53$^{\#}$	26	68$^{\#}$
D3-Re-inoculated	23*	3*	20*	12*
D3-Sterile	45	34	11	75
D3-Non-irradiated	0	0	0	0
D4-Re-inoculated	0	0	0	0
D4-Sterile	40	17	23	43
D4-Non-irradiated	0	0	0	0
D5-Re-inoculated	0	0	0	0
D5-Sterile	35	11	24	32
D5-Non-irradiated	0	0	0	0
Σ-Re-inoculated$^{@}$	123*	53$^{\beta}$	70*	-
Σ-Sterile$^{@}$	253	136	117	-
Σ-Non-irradiated$^{@}$	154$^{\#}$	119	35$^{\#}$	-
$^{@}$µg/175 ml				

Table 5.7. Amount of Al mobilised and its distribution between particulate and dissolved fractions.

For each displacement the only comparisons made were re-inoculated vs sterile and non-irradiated vs sterile soils. For the re-inoculated vs sterile comparison, * indicates that the mean values are significantly different ($p \leq 0.05$). The treatments were compared using an unpaired t-Test. For the non-parametric data, β indicates that the mean values are significantly different ($\alpha \leq 0.05$). The treatments were compared using the Wilcoxon Two Sample Test. For the sterile vs non-irradiated comparisons, # indicates that the mean values are significantly different ($p \leq 0.05$). The treatments were compared using an unpaired t-Test. For non-parametric data, χ indicates that the mean values are significantly different ($\alpha \leq 0.05$). The treatments were compared using the Wilcoxon Two Sample Test. In the treatment column the letter D followed by a number denotes a particular displacement, while Σ denotes the total amounts of Al displaced over the course of the experiment (five displacements).

Re-inoculated Treatment vs Sterile Treatment.

These data show that re-inoculating the soil decreased the amount of Al in the leachate. This decrease occurred across both fractions of the leachate that is, particulate and dissolved. The vast bulk of Al mobilised in the re-inoculated soil was flushed from the system during the initial stages of the experiment. During the final two displacements there were negligible amounts of Al in the leachate from this soil. With both treatments the percentage of total Al in the particulate fraction tended to fall over the course of the experiment

Calcium.

Non-irradiated Treatment vs Sterile Treatment.

The data in Table 5.8 show that essentially all of the Ca mobilised with either treatment was in the dissolved fraction of the leachate. With both treatments most of the Ca mobilised was flushed from the soil during the first displacement. It is clear from this data that γ-radiation mobilised significant amounts of Ca in this soil.

Re-inoculated Treatment vs Sterile Treatment.

These data show that microbial activity had a variety of effects on the mobility of Ca. Initially in the re-inoculated soil, there was a significant decrease in the amount of dissolved Ca in the leachate coupled with a significant increase in the amount of particulate Ca. During the latter stages of the experiment however, significant amounts of both dissolved and particulate Ca were mobilised in the re-inoculated soil. So in overall terms there was significantly less dissolved Ca in the leachate from the re-

inoculated soil than in the leachate from the sterile soil, while there was significantly more particulate Ca in the leachate from the former soil than in the leachate from the latter soil.

Soil Treatment	*Total (P+D)*$^{+}$	*Particulate (P)*$^{+}$	*Dissolved (D)*$^{+}$	*% Particulate*
	µg/35 ml^{+}			
D1-Re-inoculated	2010*	330*	1680*	17$^{\beta}$
D1-Sterile	5690	72	5620	1
D1-Non-irradiated	3840$^{\#}$	70	3770$^{\chi}$	2
D2-Re-inoculated	1020	7	1010	1
D2-Sterile	1040	12	1030	1
D2-Non-irradiated	253$^{\#}$	5	248*	2
D3-Re-inoculated	431	8	423	2
D3-Sterile	380	5	375	1
D3-Non-irradiated	143$^{\#}$	3	140$^{\#}$	2
D4-Re-inoculated	511$^{\beta}$	45*	465$^{\beta}$	9
D4-Sterile	343	5	338	2
D4-Non-irradiated	197$^{\chi}$	3	194$^{\chi}$	2
D5-Re-inoculated	617*	17*	600*	3
D5-Sterile	449	5	444	1
D5-Non-irradiated	134$^{\#}$	3	131$^{\#}$	2
Σ-Re-inoculated$^{@}$	4590*	410*	4180*	-
Σ-Sterile$^{@}$	7900	100	7800	-
Σ-Non-irradiated$^{@}$	4570$^{\#}$	84	4490$^{\chi}$	-
$^{@}$µg/175 ml				

Table 5.8. Amount of Ca mobilised and its distribution between particulate and dissolved fractions.

For each displacement the only comparisons made were re-inoculated vs sterile and non-irradiated vs sterile soils. For the re-inoculated vs sterile comparison, * indicates that the mean values are significantly different ($p \leq 0.05$). The treatments were compared using an unpaired t-Test. For the non-parametric data, β indicates that the mean values are significantly different ($\alpha \leq 0.05$). The treatments were compared using the Wilcoxon Two Sample Test. For the sterile vs non-irradiated comparisons, # indicates that the mean values are significantly different ($p \leq 0.05$). The treatments were compared using an unpaired t-Test. For non-parametric data, χ indicates that the mean values are significantly different ($\alpha \leq 0.05$). The treatments were compared using the Wilcoxon Two Sample Test. In the treatment column the letter D followed by a number denotes a particular displacement, while Σ denotes the total amounts of Ca displaced over the course of the experiment (five displacements).

Sodium.

Soil Treatment	*Total (P+D)*$^{+}$	*Particulate (P)*$^{+}$	*Dissolved (D)*$^{+}$	*% Particulate*
	µg/35 ml^{+}			
D1-Re-inoculated	1400*	27	1370*	2
D1-Sterile	2010	30	1980	2
D1-Non-irradiated	984$^{\#}$	17	967$^{\#}$	2
D2-Re-inoculated	537*	7	530*	1
D2-Sterile	375	4	371	1
D2-Non-irradiated	325	3	322	1
D3-Re-inoculated	300*	6	294*	2
D3-Sterile	105	2	103	2
D3-Non-irradiated	165$^{\#}$	2	163$^{\chi}$	2
D4-Re-inoculated	281*	4	277*	1
D4-Sterile	175	4	171	2
D4-Non-irradiated	210$^{\#}$	2	208$^{\#}$	1
D5-Re-inoculated	268*	3	265*	1
D5-Sterile	179	3	176	1
D5-Non-irradiated	116$^{\#}$	1	115$^{\#}$	1
Σ-Re-inoculated$^{@}$	2780	46	2730	-
Σ-Sterile$^{@}$	2840	42	2800	-
Σ-Non-irradiated$^{@}$	1800$^{\#}$	26	1770$^{\#}$	-
$^{@}$µg/175 ml				

Table 5.9. Amount of Na mobilised and its distribution between particulate and dissolved fractions.

For each displacement the only comparisons made were re-inoculated vs sterile and non-irradiated vs sterile soils. For the re-inoculated vs sterile comparison, * indicates that the mean values are significantly different ($p \leq 0.05$). The treatments were compared using an unpaired t-Test. For the non-parametric data, β indicates that the mean values are significantly different ($\alpha \leq 0.05$). The treatments were compared using the Wilcoxon Two Sample Test. For the sterile vs non-irradiated comparisons, # indicates that the mean values are significantly different ($p \leq 0.05$). The treatments were compared using an unpaired t-Test. For non-parametric data, χ indicates that the mean values are significantly different ($\alpha \leq 0.05$). The treatments were compared using the Wilcoxon Two Sample Test. In the treatment column the letter D followed by a number denotes a particular displacement, while Σ denotes the total amounts of Na displaced over the course of the experiment (five displacements).

Non-irradiated Treatment vs Sterile Treatment.

These data show that essentially all of the Na mobilised and leached with either treatment was in the dissolved fraction of the leachate. During the initial two displacements there was a significant mobilisation of Na in the sterile soil. However, during displacements three and four the situation was reversed and more of this element

was mobilised in the soil that had not been irradiated than in the soil that had been. During the fifth displacement the situation had changed yet again and more Na was being mobilised in the sterile soil than in the non-irradiated soil. It is clear from these data that γ-radiation mobilised significant amounts of Na. The vast bulk of which was flushed from the soil during the initial displacement.

Re-inoculated Treatment vs Sterile Treatment.

These data show that re-inoculating this soil did not significantly alter the total amount of Na mobilised and leached over the five displacements. However, in the first displacement re-inoculating the soil decreased the amount of Na in the dissolved fraction of the leachate. This decrease was off-set by the increase in the amount of Na in the dissolved fraction of the leachate in the remaining four displacements. Only minor amounts of this element were mobilised in the particulate form.

Magnesium.

Non-irradiated Treatment vs Sterile Treatment.

It is clear from the data in Table 5.10 that γ-radiation mobilised significant amounts of Mg in this soil. Most of the Mg mobilised in this fashion was in the dissolved fraction of the leachate, although a small amount of particulate Mg appears to have been mobilised during the first displacement. All of the Mg mobilised in the non-irradiated soil was flushed from the system during the first displacement, with the majority being in the dissolved fraction of the leachate.

Soil Treatment	*Total (P+D)*$^{+}$	*Particulate (P)*$^{+}$	*Dissolved (D)*$^{+}$	*% Particulate*
	µg/35 ml^{+}			
D1-Re-inoculated	997*	99	897*	10*
D1-Sterile	3310	60	3250	2
D1-Non-irradiated	2650$^{\#}$	20	2630$^{\#}$	1
D2-Re-inoculated	480	12	468	3
D2-Sterile	473	9	464	2
D2-Non-irradiated	0	0	0	0
D3-Re-inoculated	228*	2	226*	1
D3-Sterile	123	3	120	3
D3-Non-irradiated	0	0	0	0
D4-Re-inoculated	361*	25*	336*	7
D4-Sterile	218	4	214	2
D4-Non-irradiated	0	0	0	0
D5-Re-inoculated	441*	11$^{\beta}$	430*	2
D5-Sterile	290	5	285	2
D5-Non-irradiated	0	0	0	0
Σ-Re-inoculated$^{@}$	2510$^{\beta}$	147$^{\beta}$	2360$^{\beta}$	-
Σ-Sterile$^{@}$	4410	80	4330	-
Σ-Non-irradiated$^{@}$	2650$^{\chi}$	20$^{\#}$	2630$^{\chi}$	-
$^{@}$µg/175 ml				

Table 5.10. Amount of Mg mobilised and its distribution between particulate and dissolved fractions.

For each displacement the only comparisons made were re-inoculated vs sterile and non-irradiated vs sterile soils. For the re-inoculated vs sterile comparison, * indicates that the mean values are significantly different ($p \leq 0.05$). The treatments were compared using an unpaired t-Test. For the non-parametric data, β indicates that the mean values are significantly different ($\alpha \leq 0.05$). The treatments were compared using the Wilcoxon Two Sample Test. For the sterile vs non-irradiated comparisons, # indicates that the mean values are significantly different ($p \leq 0.05$). The treatments were compared using an unpaired t-Test. For non-parametric data, χ indicates that the mean values are significantly different ($\alpha \leq 0.05$). The treatments were compared using the Wilcoxon Two Sample Test. In the treatment column the letter D followed by a number denotes a particular displacement, while Σ denotes the total amounts of Mg displaced over the course of the experiment (five displacements).

Re-inoculated Treatment vs Sterile Treatment.

Re-inoculating this soil had a variety of effects upon the concentration and forms of Mg in the leachate. By far the most important was the decrease in the amount of dissolved Mg in the leachate from this soil that occurred during the first displacement. This decrease was so dramatic it ensured that in total substantially less Mg was mobilised in the re-inoculated soil than in the sterile soil, even though significantly greater amounts

of dissolved Mg were mobilised in the re-inoculated soil than in the sterile soil during the latter stages of the experiment. During displacements four and five significantly more particulate Mg was mobilised in the re-inoculated soil than in the sterile soil.

Sulphur.

Non-irradiated Treatment vs Sterile Treatment.

The data in Table 5.11 show that significant amounts of this element were mobilised by γ-radiation. Essentially all of the S mobilised in either soil was dissolved in nature, although a small amount of particulate S appears to have been mobilised in the sterile soil during the first displacement. With both treatments most of the S mobilised was flushed from the soil during initial stages of the experiment.

In the sterile soil the trend over the five displacements was for the amount of S mobilised to fall during the initial stages of the experiment and then to increase again during the final stages of the experiment. In the soil that had not been irradiated after the initial displacement the amount mobilised tended to remain relatively constant.

Re-inoculated Treatment vs Sterile Treatment.

These data show that re-inoculating this soil had a variety of effects on the mobility of this element. In total significantly greater amounts of particulate S were mobilised in the re-inoculated soil than in the sterile soil. The vast bulk of which was flushed from the soil in the first displacement, although small amounts were also mobilised during the fourth and fifth displacements. In terms of dissolved S the only significant differences between the treatments were in the third and fifth displacements.

Soil Treatment	*Total (P+D)*$^{+}$	*Particulate (P)*$^{+}$	*Dissolved (D)*$^{+}$	*% Particulate*
	µg/35 ml^{+}			
D1-Re-inoculated	1675	225*	1450	1
D1-Sterile	1630	22	1610	1
D1-Non-irradiated	599$^{\#}$	8	591$^{\#}$	1
D2-Re-inoculated	419*	16*	403	4
D2-Sterile	350	3	347	1
D2-Non-irradiated	32$^{\#}$	1	31$^{\#}$	4
D3-Re-inoculated	198*	3	195*	1
D3-Sterile	105	1	104	1
D3-Non-irradiated	28$^{\#}$	0	28$^{\#}$	1
D4-Re-inoculated	209$^{\beta}$	25*	184	12*
D4-Sterile	191	4	187	2
D4-Non-irradiated	37$^{\#}$	0	37$^{\#}$	1
D5-Re-inoculated	219	14*	205*	6*
D5-Sterile	239	5	234	2
D5-Non-irradiated	35$^{\#}$	0	35$^{\#}$	1
Σ-Re-inoculated$^{@}$	2730*	282*	2450	-
Σ-Sterile$^{@}$	2515	35	2480	-
Σ-Non-irradiated$^{@}$	732$^{\#}$	11$^{\#}$	721$^{\#}$	-
$^{@}$µg/175 ml				

Table 5.11. Amount of S mobilised and its distribution between particulate and dissolved fractions.

For each displacement the only comparisons made were re-inoculated vs sterile and non-irradiated vs sterile soils. For the re-inoculated vs sterile comparison, * indicates that the mean values are significantly different ($p \leq 0.05$). The treatments were compared using an unpaired t-Test. For the non-parametric data, β indicates that the mean values are significantly different ($\alpha \leq 0.05$). The treatments were compared using the Wilcoxon Two Sample Test. For the sterile vs non-irradiated comparisons, # indicates that the mean values are significantly different ($p \leq 0.05$). The treatments were compared using an unpaired t-Test. For non-parametric data, χ indicates that the mean values are significantly different ($\alpha \leq 0.05$). The treatments were compared using the Wilcoxon Two Sample Test. In the treatment column the letter D followed by a number denotes a particular displacement, while Σ denotes the total amounts of S displaced over the course of the experiment (five displacements).

In the third displacement significantly more S was mobilised in the re-inoculated soil than in the sterile soil. While in the fifth displacement the reverse situation occurred and more S was mobilised in the sterile soil than in the re-inoculated soil. In the first displacement while there was less dissolved S in the leachate from the re-inoculated soil than in the leachate from the sterile soil, the difference was not statistically significant.

In total there was no statistical difference between the treatments in terms of the amount of dissolved S mobilised. However, because of the extra particulate S mobilised in the re-inoculated soil over the course of the experiment, significantly more S in total was mobilised in the re-inoculated soil than in the sterile soil.

Carbon

Soil Treatment	*Total (P+D)*$^{+}$	*Particulate (P)*$^{+}$	*Dissolved (D)*$^{+}$	*% Particulate*
	mg/35 ml^{+}			
D1-Re-inoculated	35*	4	31*	11
D1-Sterile	66	0	66	0
D1-Non-irradiated	7$^{\#}$	1	6$^{\#}$	14
D2-Re-inoculated	35*	3	32*	8
D2-Sterile	54	0	54	0
D2-Non-irradiated	8$^{\#}$	0	8$^{\#}$	0
D3-Re-inoculated	29*	3	26*	10
D3-Sterile	47	1	46	2
D3-Non-irradiated	7$^{\chi}$	0	7$^{\chi}$	0
D4-Re-inoculated	-	-	-	-
D4-Sterile	-	-	-	-
D4-Non-irradiated	-	-	-	-
D5-Re-inoculated	-	-	-	-
D5-Sterile	-	-	-	-
D5-Non-irradiated	-	-	-	-
Σ-Re-inoculated$^{@}$	98*	8	90*	-
Σ-Sterile$^{@}$	168	2	166	-
Σ-Non-irradiated$^{@}$	21$^{\chi}$	1	20$^{\chi}$	-
$^{@}$mg/105 ml				

Table 5.12. Amount of C mobilised and its distribution between particulate and dissolved fractions.

For each displacement the only comparisons made were re-inoculated vs sterile and non-irradiated vs sterile soils. For the re-inoculated vs sterile comparison, * indicates that the mean values are significantly different ($p \leq 0.05$). The treatments were compared using an unpaired t-Test. For the non-parametric data, β indicates that the mean values are significantly different ($\alpha \leq 0.05$). The treatments were compared using the Wilcoxon Two Sample Test. For the sterile vs non-irradiated comparisons, # indicates that the mean values are significantly different ($p \leq 0.05$). The treatments were compared using an unpaired t-Test. For non-parametric data, χ indicates that the mean values are significantly different ($\alpha \leq 0.05$). The treatments were compared using the Wilcoxon Two Sample Test. In the treatment column the letter D followed by a number denotes a particular displacement, while Σ denotes the total amounts of C displaced over the course of the experiment (five displacements).

Non-irradiated Treatment vs Sterile Treatment.

Data for this element could only be obtained for the first three displacements because of equipment failure. However, it is clear that γ-radiation mobilised a considerable amount of C, mostly in the dissolved form. A modest amount (14% of the total) of particulate C was mobilised in the non-irradiated soil during the first displacement. However, given the data set it is difficult to draw any conclusions from this observation.

Re-inoculated Treatment vs Sterile Treatment.

These data show that re-inoculating this soil significantly decreased the amount of C in the leachate. There also tended to be more particulate C in the leachate from the re-inoculated soil than in the leachate from the sterile soil, although it is difficult to draw any conclusions because the amounts involved were relatively small.

pH and EC Data.

Non-irradiated Treatment vs Sterile Treatment.

These data (Table 5.13) show that for each of the five displacements the pH of the leachate from the irradiated soil was significantly lower than the pH of the leachate from the soil that had not been irradiated. The trend behaviour of these data over the course of the experiment is not clear-cut. Certainly during the first three displacements the trend with both treatments, was for the pH of the leachates to rise. In the final two displacements the pH of the leachate from the non-irradiated soil tended to remain relatively constant, while that of the leachate from the sterile soil rose then fell. In all

five displacements the EC of the leachates obtained from the sterile soil were higher than those of the leachates from the non-irradiated soil.

Soil Treatment	*pH*	*EC*
		mS/cm
D1-Re-inoculated	7.05$^{\beta}$	1.77*
D1-Sterile	5.29	2.96
D1-Non-irradiated	5.57$^{\#}$	2.25$^{\#}$
D2-Re-inoculated	7.81*	0.960*
D2-Sterile	5.93	0.688
D2-Non-irradiated	6.90$^{\#}$	0.240$^{\#}$
D3-Re-inoculated	7.49*	0.670*
D3-Sterile	6.29	0.360
D3-Non-irradiated	7.18$^{\chi}$	0.200$^{\#}$
D4-Re-inoculated	7.61*	0.460*
D4-Sterile	6.42	0.290
D4-Non-irradiated	7.11$^{\#}$	0.160$^{\#}$
D5-Re-inoculated	7.37*	0.580*
D5-Sterile	6.28	0.330
D5-Non-irradiated	7.21$^{\#}$	0.140$^{\#}$

Table 5.13. pH and EC values of the leachate.

For each displacement the only comparisons made were re-inoculated vs sterile and non-irradiated vs sterile soils. For the re-inoculated vs sterile comparison, * indicates that the mean values are significantly different ($p \leq 0.05$). The treatments were compared using an unpaired t-Test. For the non-parametric data, β indicates that the mean values are significantly different ($\alpha \leq 0.05$). The treatments were compared using the Wilcoxon Two Sample Test. For the sterile vs non-irradiated comparisons, # indicates that the mean values are significantly different ($p \leq 0.05$). The treatments were compared using an unpaired t-Test. For non-parametric data, χ indicates that the mean values are significantly different ($\alpha \leq 0.05$). The treatments were compared using the Wilcoxon Two Sample Test.

Re-inoculated Treatment vs Sterile Treatment.

In all five displacements the pH of the leachates from the re-inoculated soil were higher than those of the leachates from the sterile soil. In all cases the pH of the non-sterile leachate was 1-1.5 pH units higher. The trend with the re-inoculated treatment was for the pH of the leachate to rise during the initial stages of the experiment then to fall during the latter stages.

In the first displacement the EC value of the leachate obtained from the re-inoculated soil was significantly lower than that of the leachate obtained from the sterile soil. In the remaining four displacements the EC values of the leachate from the re-inoculated soil were significantly higher than those of the leachate from the sterile soil.

SEM Data.

As in the previous chapter it will be easiest to discuss the non-irradiated, irradiated and re-inoculated soils separately.

Non-irradiated Soil.

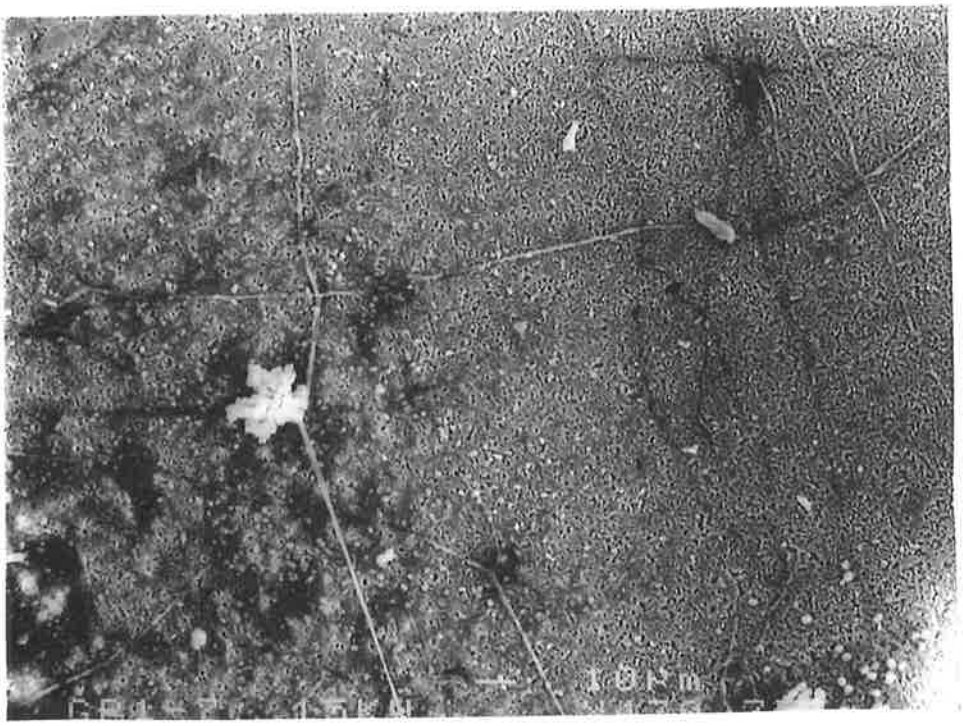

Figure 5.1. Scanning electron micrograph of the membrane surface at low magnification (non- irradiated soil).

The particle material isolated from the leachate from the non-irradiated soil consisted primarily of a dark coloured amorphous material and numerous small light coloured spherical particles (refer to Figure 5.1). There were also a few larger (≈ 100 μm) sized mineral particles scattered across the membrane surface. The composition of the particulate material changed little over the course of the experiment, although there tended to be fewer of the spherical particles present in the material obtained during the latter stages.

Sterile Soil.

The sterile leachate contained only a small amount of particulate material that was similar in composition to the material isolated from the leachate from the soil that had not been irradiated (refer to Figure 5.2).

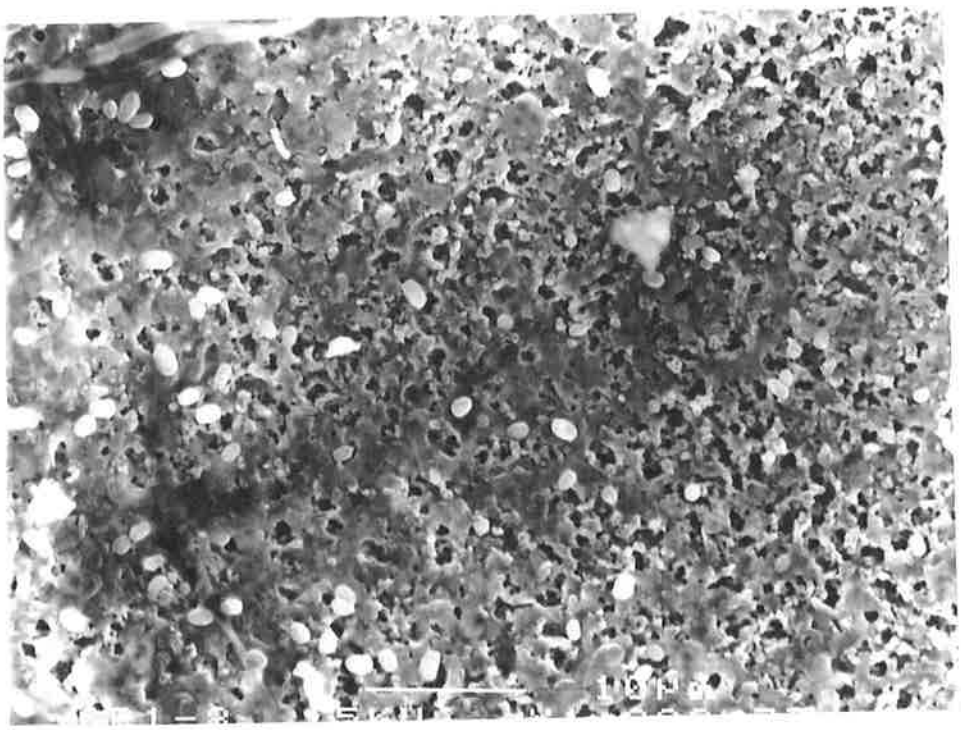

Figure 5.2. Scanning electron micrograph of membrane surface at high magnification (sterile soil).

Re-inoculated Soil.

In contrast to the other two treatments a substantial amount of particulate material was isolated from the leachate from the re-inoculated soil. Figures 5.3 to 5.6 are scanning electron micrographs of the membrane surface used to fractionate from the first displacement. The entire surface was covered to a considerable depth by a mixture of materials. There were a few large discrete mineral grains embedded in this covering matrix (refer to Figures 5.3 and 5.4). EDS analysis showed that these were predominantly quartz grains (refer to Figure 5.7) with a very minor amount of what was probably a feldspar related mineral phase (refer to Figure 5.8). The thin threads visible in Figures 5.3 and 5.4 were fungal hyphae that had obviously grown after the filter membrane had been mounted on the stub.

A closer examination of the matrix (refer to Figures 5.5 and 5.6) revealed that it consisted of numerous small ($\approx$ 1.5 μm) spherical to oblong shaped particles embedded in a dark coloured amorphous material. The high degree of regularity shown by these particles in terms of morphology and size strongly suggests that they were biological in nature. It is postulated that they were fungal spores and that the amorphous material was microbial mucilage. EDS analysis of the matrix material (refer to Figures 5.9 and 5.10) revealed that it contained a range of elements including Si, P, S, Cl and K along with relatively minor amounts of Na and Mg. The presence of P, S, K and Mg support the contention that most of the particulate material was biological in nature. The presence of Si in the samples suggests that small amounts of inorganic materials were

also present, which is not unexpected given that nature of the soil. It is difficult to account for the presence of Cl given that the sampling site was inland which means this element could not have been deposited by sea spray drift. It should be noted that the number of fungal spores isolated from the leachate declined noticeably during the latter stages of the experiment.

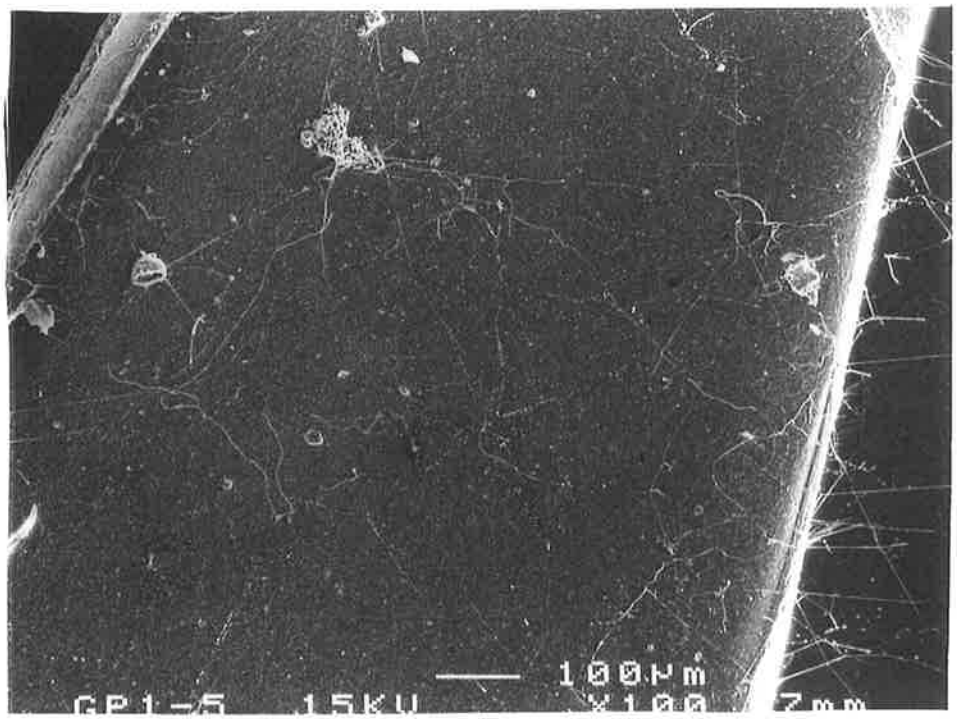

Figure 5.3. Scanning electron micrograph showing membrane surface at low magnification (re- inoculated soil).

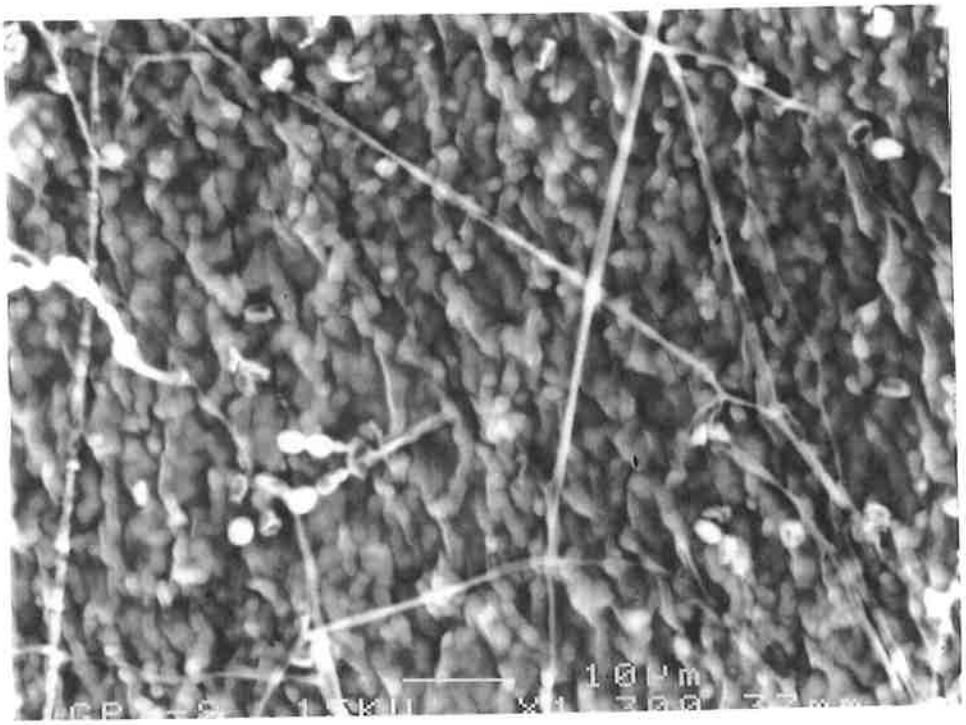

Figure 5.4. Scanning electron micrograph showing the matrix of material that covers the membrane surface (re-inoculated soil).

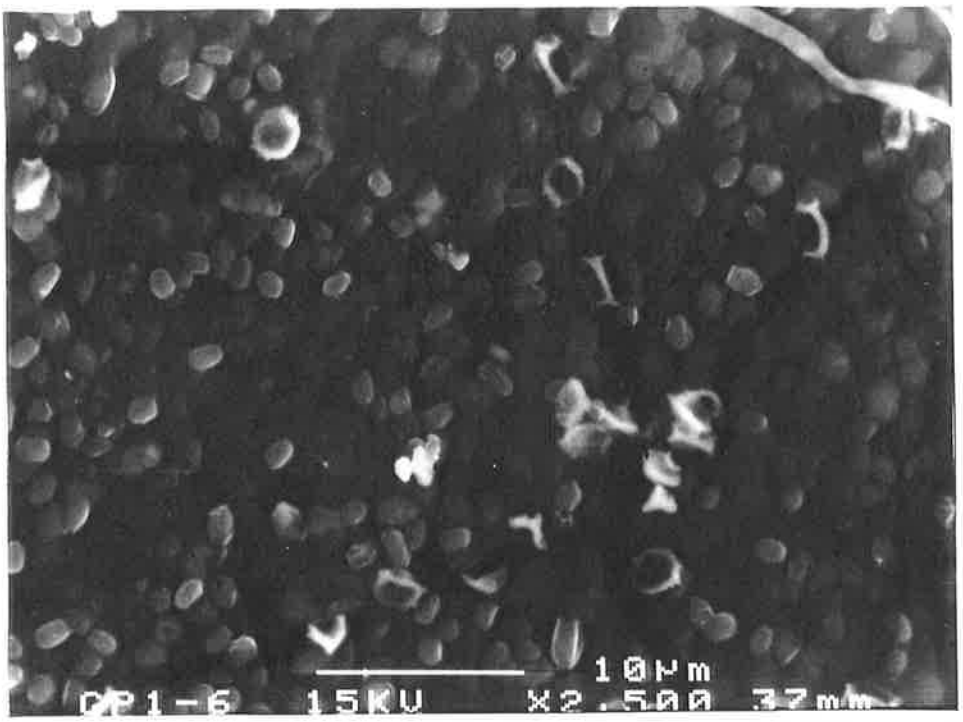

Figure 5.5. Scanning electron micrograph showing details of matrix material at high magnification (re-inoculated soil).

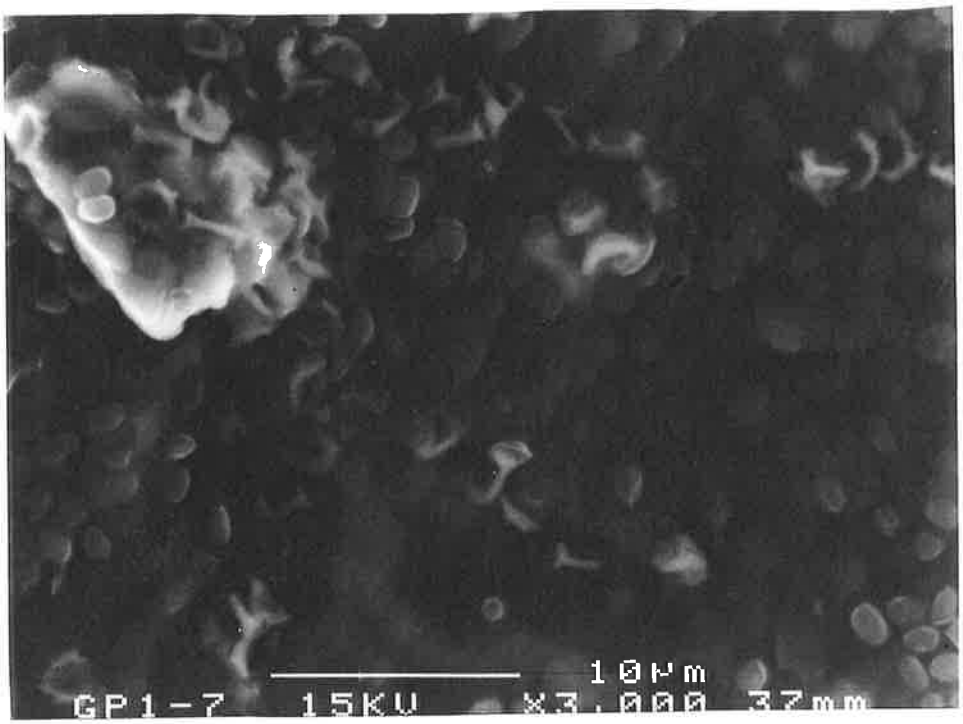

Figure 5.6. Scanning electron micrograph showing fine detail of the matrix material (re-inoculated soil).

EDS Analysis.

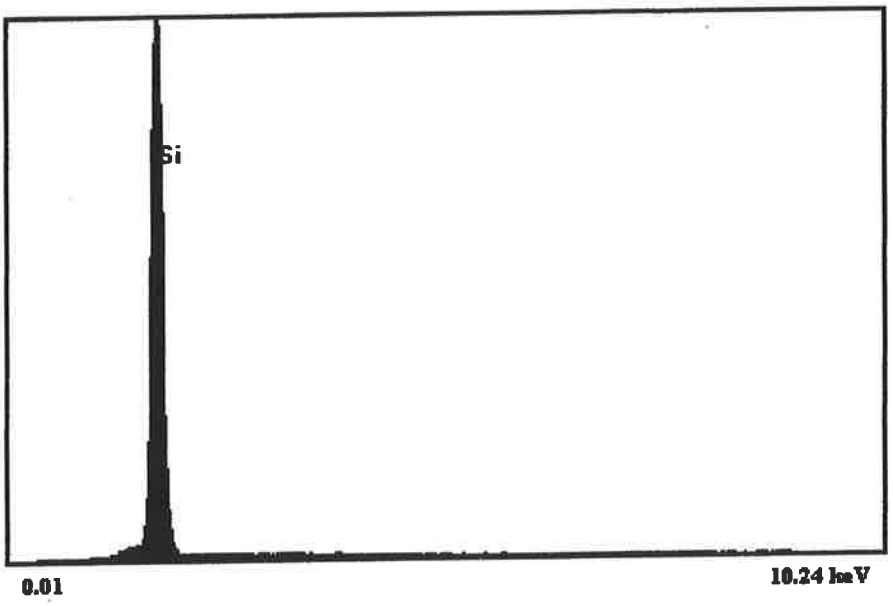

Figure 5.7. EDS spectrum - particle analysis 1.

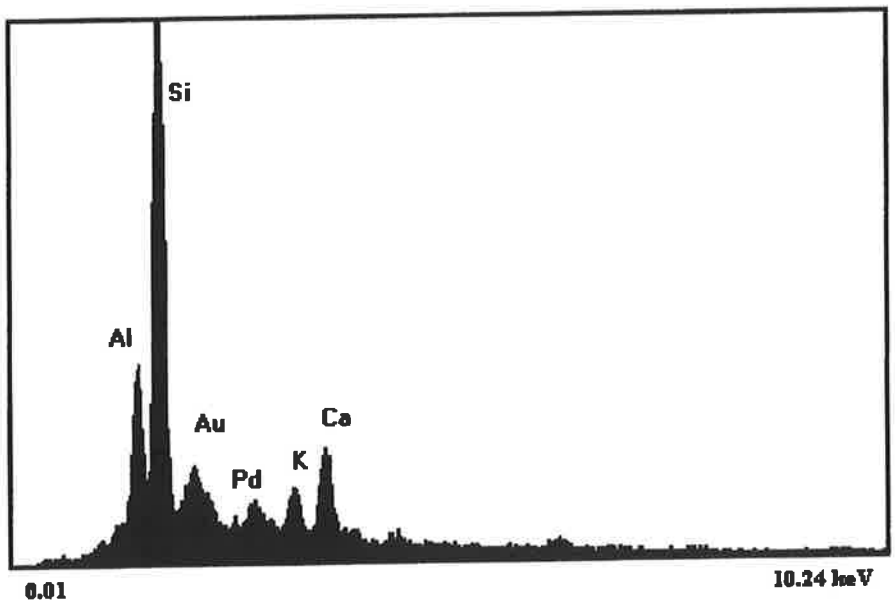

Figure 5.8. EDS spectrum - particle analysis 2.

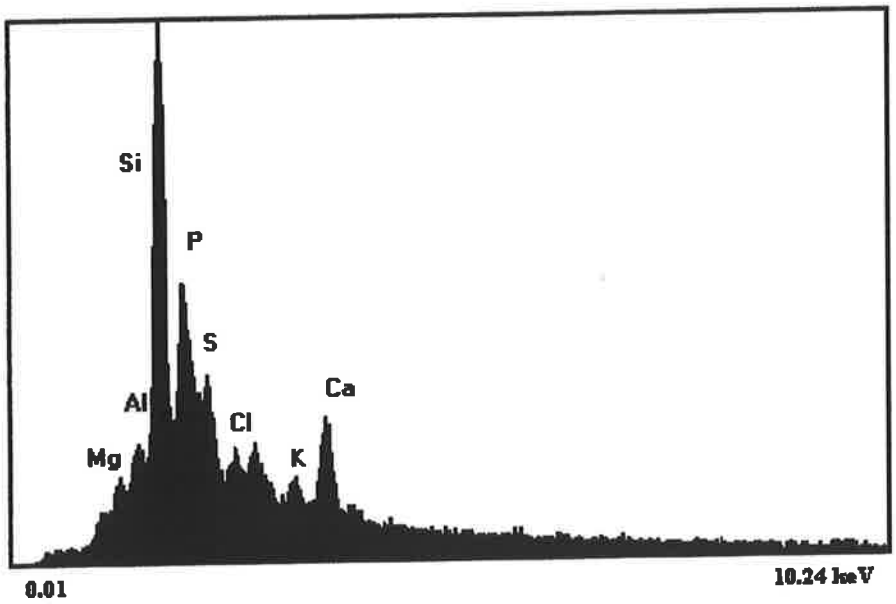

Figure 5.9. EDS spectrum – matrix analysis 1.

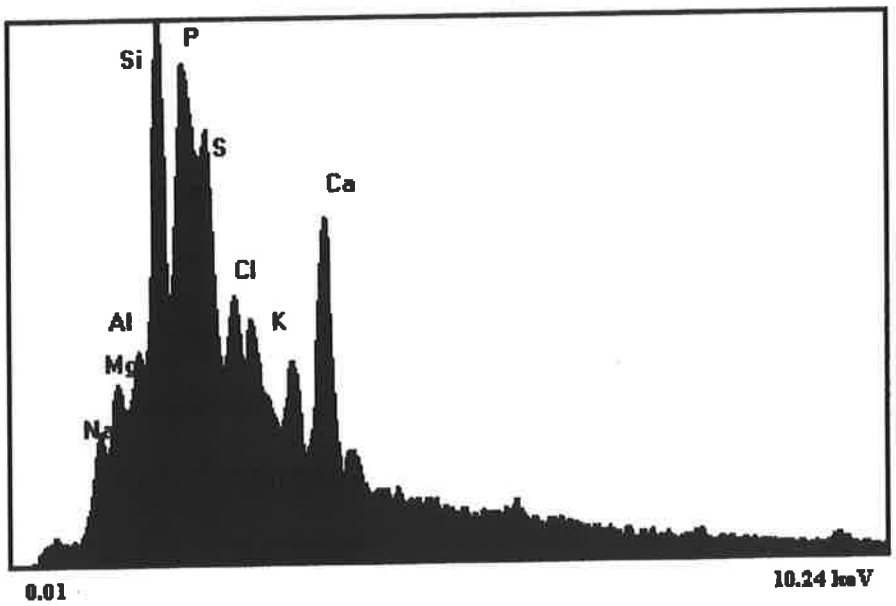

Figure 5.10. EDS spectrum – matrix analysis 2.

5.3.0. Discussion and Analysis.

This section begins with an analysis of the results of the comparison between the non-irradiated soil and the irradiated soil (effects of γ-radiation) and will conclude with an analysis of the results of the comparison between the sterile and re-inoculated soil (effects of microbial activity on P mobility). Before proceeding with this the following points should be noted.

Typically with all treatments, the flow rates during the leachings tended to fall as the experiment proceeded (Table 5.3). During each displacement, the flow rates of the sterile columns tended to be highest and those of the re-inoculated columns the lowest. The flow rates of the columns that had not been irradiated were between those of the other two groups. The leachate obtained from all three groups of columns was coloured a deep reddish brown. The leachates obtained from the soil that had been re-inoculated contained a considerable amount of suspended material and were very turbid. The leachates from the sterile soil contained little suspended material.

5.3.1. Non-irradiated Treatment vs Sterile Treatment - Effects of γ-radiation.

In general terms γ-radiation had a similar effect on this soil as it did on the soil used in the first set of experiments. The process mobilised considerable amounts of P along with substantial amounts of Ca, Na, Mg, S and C as well as relatively minor amounts of Al and Fe. It also lowered the pH of the soil. As in the first set of experiments, this second effect was inferred from the pH data from the leachates, as no direct soil measurements were made.

Mobilisation of Elements.

It is postulated that the two mechanisms proposed to account for the mobilisation of P, Ca, Na, Mg, S and C observed in the soil used in the first set of experiments also partly explains the mobilisation of these elements in this soil. The two mechanisms proposed earlier were the solubilization of soil organic matter with the release of C and the other biologically important elements listed above, as well as the release or leakage of compounds from lysed or damaged cells. In general the leaching behaviour of these elements supports this argument. As would be expected the bulk of material mobilised was in the dissolved fraction of the leachate, with the majority being flushed from the soil during the initial stages of the experiment. There is a third process associated with the drop in soil pH that may also have played a secondary role in mobilising these elements. This process will be discussed in the section below.

With P, after the initial flush there was a gradual decline in the amounts mobilised during each of the subsequent displacements. With the other elements (Ca, Na, &S) however, while there was a decline following the initial flush during the later stages of the experiment the amounts mobilised increased again. Unfortunately because of the incomplete data set associated with C it was impossible to know whether or not this element behaved in a similar manner to the others. Certainly no such trend was evident with these elements in the leachate from the soil that had not been irradiated. One possible interpretation of these data is that the γ-radiation created two 'pools' of mobile material. The first consisted of material that was easily solubilised by water and hence removed from the soil during the initial stages of the experiment. The second was a

pool of material that was considerably less soluble and only came into solution after prolonged contact with water. The first pool could have contained material mainly from lysed cells while the second may have contained material derived from soil organic matter that had been chemically altered by exposure to γ-radiation. Unfortunately this idea does not provide an adequate explanation of why P should behave differently to the other elements.

Soil pH.

As noted above it can be inferred from the leachate data that the irradiation process lowered the pH of this soil. The same mechanisms can be postulated to explain the production of H^+ ions by γ-radiation in this soil as in the acid mineral soil. These were, release from lysed cells, the production of organic acids as a by-product of the solubilization of organic matter and finally simply the application of high-energy radiation ionising hydrogen from numerous organic and inorganic sources.

As noted earlier the soil used in this experiment was essentially a mixture of quartz sand and organic matter. EDS analysis supports this observation showing that almost all of the mineral grains retained by the filter membranes were indeed quartz. It is postulated that in a soil such as this there would only be minor amounts of mineral colloids and as such most of the cation exchange capacity (CEC) would be associated with organic colloids. Thus a relatively large proportion of the total CEC would come from materials that exhibit pH dependent charge characteristics. In this context an important effect of the decrease in pH would have been the increase in positive charge associated

with these materials. It does not seem unreasonable to suggest that this process may have played a role in mobilising various cations in this soil including Ca^{2+}, Na^{+} and Mg^{2+}. This process would tend to immobilise any anionic forms of P and S present in the system.

There were only minor amounts of Al and relatively modest amounts of Fe mobilised and leached from this soil. This is consistent with the argument that there were only small amounts of alumino-silicate clay minerals present in the soil. Examination of the particulate material retained by the filter membranes using SEM and EDS showed that not very much of the material was made up of alumino-silicate mineral phases. Thus it is highly unlikely that the mobilisation of Al was associated with a pH-induced solubilization of clay minerals. The change in pH dependent charge associated with the drop in pH is the most likely process to have caused the mobilisation of this element. This process may have also been responsible for mobilising Fe, although because Fe is a trace element some of the material mobilised may have been released from lysed cells.

The trend behaviour of the soil pH over the course of the experiment is interesting. It rose over the first four displacements then fell during the fifth. It would have been useful to have additional data to see if the fall in pH continued or was just a one-off in an otherwise upward trend, as appears to have been the case with the soil that had not been irradiated. Given this, it is difficult to provide an explanation as to why this occurred. It is possible that there was a link between this mobilisation event (H^{+} ions mobilised) and the mobilisation of the other elements (Ca, Na, Mg & S) during the

latter stages of the experiment. However, with most of the other elements the upward trend in concentration began with the fourth displacement while with H^+ the increase in concentration only occurred during the fifth displacement. The EC data show a falling trend across the first four displacements and then an increase during the fifth that corresponds to the fall in pH. All of this suggests that there were abiotic mobilisation processes occurring during the latter stages of the experiment. From the available data it was not possible to provide a coherent mechanistic explanation for this.

5.3.2. Re-inoculated vs Sterile – Effect of Microbial Activity on P Mobility.

These data show that over the course of the experiment significantly more P was mobilised in the re-inoculated soil than in the sterile soil. As in the preceding chapter the easiest way to understand this result and to place it into the appropriate context, is to separately examine the behaviour of the dissolved and particulate forms of this element.

Dissolved P.

In this soil as in the first set of experiments, the irradiation processes made available a considerable amount of microbial substrate. Under these conditions the population of soil micro-organisms would have expanded rapidly after re-inoculation. As a result an unknown amount of the P, Ca, Na, Mg and S mobilised by γ-radiation would have been immobilised by being incorporated into the microbial biomass. This process probably accounts for the significant decrease in the concentration of these elements observed in the leachate from the re-inoculated soil during the initial stages of the experiment.

It is difficult to offer any reason why significantly more dissolved P should have been mobilised in the re-inoculated soil than in the sterile soil during the third displacement.

The chemical characterization of the P in this fraction of the leachate revealed that the vast majority was reactive towards molybdate. The data show this to be true irrespective of the soil treatment. As discussed in the previous chapter the MRP fraction should consist of dissolved orthophosphate and any organic and colloidal P compounds readily converted to orthophosphate by acid hydrolysis. Clearly these data represent maximum values for orthophosphate. Given the nature of this soil it is hardly surprising that the majority of P in the dissolved fraction was molybdate-reactive.

As in the first set of experiments, re-inoculating this soil also significantly lowered the concentration of C in the leachate. The same two mechanisms postulated to account for this in the first set of experiments also probably account for it in this experiment as well. The two mechanisms were the incorporation of C into the microbial biomass as the population expanded and the loss of C from the system in the form of CO_2 as a result of the oxidation of organic substrates. Given the nature of this soil it is possible that during the latter stages of the experiment fermentation processes may have become increasingly important. In fermentation processes, organic compounds act as both electron donors and acceptors. This topic will be discussed further in the text below.

While there was an initial decline in the concentration of P, Ca, Na, Mg and S in the leachate following re-inoculation during the latter stages of the experiment the

concentration of Ca, Mg and S in the leachate began to rise again. This mobilisation may account for the fact that during the latter stages of the experiment, the EC values of the leachate from the re-inoculated soil were higher than those of the leachate from the sterile soil. As discussed earlier similar behaviour was observed in the sterile system. This observation perhaps suggests that this was an effect of the irradiation process. In this instance the concentration of P and Na in the leachate fell over the course of the experiment. Again it is difficult to provide a coherent explanation for these behaviours.

Microbial Activity – pH Effect.

It can be inferred from the leachate data that re-inoculating this soil increased its pH. A similar effect was noted in the soil used in the first set of experiments. Before proceeding to discuss this effect, the following points should be noted.

As discussed earlier, given the nature of this soil it was likely that a relatively large proportion of its total CEC came from materials that exhibited pH dependent charge characteristics. An increase in soil pH would have produced an increase in negative charge associated with these materials thus creating additional binding sites for various cations. This mechanism probably accounts for the drop in the amount of Fe and Al found in the dissolved fraction of the leachate following re-inoculation. Why there should have been a decrease in the amount of particulate Al in the leachate following re-inoculation is harder to explain.

An interesting feature of the data from the re-inoculated soil is the variability in pH. The trend could best be characterised as a rapid increase followed by a somewhat spasmodic decline. The initial rise may have been related to the rapid increase in the microbial population that would have accompanied re-inoculation. Processes such as the mineralisation of organic N, uptake of sulphate, orthophosphate and nitrate would have been occurring, all of which consume protons. A possible explanation for the decline over the latter part of the experiment was the production of organic acids as an end product of the fermentation process. Ethanol, formate, acetate, lactate, propionate, butyrate, molecular hydrogen and carbon dioxide are the main end products of fermentation by bacteria (Yoshida, 1975). According to Picek *et al.* (2000) fermentation processes predominate in environments that lack inorganic oxidizing agents (i.e. electron acceptors).

It is postulated that during the initial stages of the experiment aerobic respiration was the dominant microbiological process occurring in the columns. Over the course of the experiment reducing conditions developed in the columns and fermentation became the dominant microbiological process, because of the lack of suitable inorganic oxidizing agents in this soil. What evidence is there to suggest that this change occurred? Again, because no direct measurements of soil *Eh* were made only circumstantial evidence can be provided that fermentation processes occurred in this soil. The data show that there were only modest amounts of Fe in the soil and that unlike in the first set of experiments there was no evidence to suggest that this element was mobilised in the re-inoculated soil. In essence the argument is that there simply wasn't enough Fe^{3+} present

in this soil for it to be an important electron acceptor. The S data show that while there were substantial amounts present in this soil there was no marked mobilisation in the re-inoculated soil during the latter stages of the experiment. A possible explanation for this is that most of the S was present in the reduced organic form. These data suggest that this element did not play a major role as an electron acceptor during the oxidation of C.

After the fifth displacement the soil was carefully removed from all of the columns and examined. There was a very strong and pronounced odour associated with the soil from the columns that had been re-inoculated. For the reasons discussed above it was thought that this odour was not simply H_2S produced as a result of the reduction of SO_4^{2-} but rather, was produced as a result of the fermentation of nitrogenous compounds (putrefaction). This process produces a variety of compounds including ammonium, amines, indole, skatole, mercaptans, hydrogen sulphide, carbon dioxide, hydrogen as well as organic acids and alcohols (Yoshida, 1975). Certainly the production of some of these compounds would account for the strong odour associated with this soil.

Particulate P.

The data show that all of the extra P mobilised by soil micro-organisms was particulate in nature. This finding is in agreement with the results obtained from the first set of experiments and with those reported by Hannapel *et al.* (1964a, b).

This suggests that the mobile P was either associated with clay minerals or contained within microbial cells or cellular debris. The SEM data showed that the particulate material consisted mainly of what was thought to be a mixture of fungal spores and microbial mucilage. This suggests that most of the particulate P mobilised in this soil was contained within material that had a microbial origin.

5.4.0. Conclusions.

5.4.1. Non-irradiated Treatment vs Sterile Treatment- Effects of γ-radiation.

The data show that the effect of γ-radiation on this soil were similar to those that were observed on the soil used in the first set of experiments, firstly considerable amounts of P, C, Ca, Na, Mg and S were mobilised and secondly the pH of the soil was lowered.

5.4.2. Re-inoculated Treatment vs Sterile Treatment – Effects of Microbial Activity on P Mobility.

The results from this experiment showed that soil micro-organisms mobilised P in a sandy soil. All of the P mobilised in this manner was in the particulate fraction of the leachate. SEM revealed that this P was associated with organic material (debris) generated by an active microbial population. This finding is consistent with those reported by Hanapel *et al.* (1964a, b) and supports the hypothesis that :

> *'soil micro-organisms mobilise P in sandy soils and that the mobilised P is contained within cells or cellular debris.'*

Chapter 6.

General Discussion and Conclusions.

6.1.0. Introduction.

As discussed earlier the purpose of the work described in the preceding chapters, that is formulating the hypotheses, developing and executing an experimental procedure to test them and interpreting the results, was to establish a valid experimental basis for the following contention:

> *'That soil micro-organisms play a hitherto unrecognised role in mobilising P in various soil types and that processes associated with this role, coupled with water flow may over time lead to the re-distribution within or export of P from the soil profile.'*

As the discussion below will demonstrate by and large this goal has been effectively achieved. A review of the literature (Chapter 2) established that there was evidence to support the argument that in the long run P is mobile in the soil environment. The general consensus was that given the chemical nature of orthophosphate, in most soil types the organic components of the soil system coupled with water flow probably played the most important role in this process. However, the exact nature of this role had never been clearly elucidated.

Having established that there was evidence to support the second part of this contention it was then necessary to do the same for the first part, that is that soil micro-organisms play a hitherto unknown role in mobilising P in various soil types. It was this part of the contention that was most amenable to examination under carefully controlled experimental conditions in the laboratory.

The work carried out in this manner can be characterised as a microcosm laboratory study that focused on the mobilisation of P. In general terms there were three important results to come from this work. These were:

1. that soil micro-organisms mobilised P in a variety of soil types;
2. that an active microbial population may be a powerful generator of mobile particles /colloids in the soil environment; and
3. that γ-radiation had a variety of effects on the chemical properties of soil.

6.2.0. Mobilisation of P.

The work carried out and reported upon in Chapter 4 demonstrated that in an acid mineral soil, micro-organisms mobilised P. In this instance the nature of the mobilisation process could not be clearly determined. However, it was clear that all of the P mobilised in this fashion was in the particulate fraction (> 0.22 μm) of the leachate and that this material consisted of clay particles and an amorphous material that was probably organic in nature (i.e. microbial mucilage). The P could have been associated with one or both components of the mixture. If the P was associated with the amorphous material and this was microbial mucilage then it could be argued that the

mobilised P was associated with microbial debris. This mechanism was first proposed by Hannapel *et al.* (1964a, b) over 35 years ago, yet there is still no recognition that this may be an important biological mobilisation process in many soil types. On the other hand if the mobilised P was associated with the clay particles then a completely new microbially mediated process must have been responsible. The fact that clay particles were mobilised by soil micro-organisms is a very important point that will be elaborated upon further, in the discussion below.

The work carried out and reported upon in Chapter 5 showed that in a sandy soil P was also mobilised by soil micro-organisms. Again, all of the P mobilised in this manner was in the particulate fraction (> 0.22 μm) of the leachate. In this instance the particulate material was a mixture of what was probably fungal spores and microbial mucilage. In this soil the mobilised P appears to have been associated with debris from the active population of micro-organisms. This finding highlights the importance of the concepts first formulated by Hannapel *et al.* (1964a, b).

Thus, it has been established experimentally that micro-organisms mobilised P in two different soil types. From the evidence it is clear that none of the biologically mediated processes listed in Table 2.2, that is mineralisation, release from the soil biomass by grazing and Fe reduction could have caused the observed mobilisation of this element. It is postulated that two other processes account for this phenomenon, namely that the mobilised P was associated with microbial debris generated by an active microbial population or that the P was associated with clay particles dispersed by soil micro-

organisms. The soils used in these experiments and that used by Hannapel *et al.* (1964a, b) exhibited a variety of chemical and physical properties, ranging from calcareous to acid in nature and from a loam to a sand. Therefore, the results from this work coupled with those of Hannapel *et al.* (1964a, b), experimentally demonstrate that soil micro-organisms play a hitherto unknown role in mobilising P in various soil types. Thus the basic objectives for carrying out this work have been accomplished.

The limitation that the experimental approach adopted places upon the extrapolation of these results to the broader soil environment have been discussed elsewhere. Nevertheless an interesting feature of the results from the soils used in this work is the similarity of the findings to those reported by Hannapel *et al.* (1964a, b). The soils used in this work were Australian while Hannapel *et al.* (1964a, b) used North American soils. This suggests that the processes in question may occur across a range of climatic regimes and may be important processes in the soil environment. This is perhaps not surprising given the general nature of the processes involved. For example, the production of organic debris from an active microbial population is unlikely to be limited to a very specific set of circumstances in the soil environment. Similarly, the mobilisation of clay particles could occur in any mineral soil. Therefore, it does not seem unreasonable to suggest that the microbially mediated mobilisation of P coupled with water flow may account for the losses of P from the soil profiles reported by Frossard *et al.* (1989) and Xiao *et al.* (1991).

In conclusion this work supports the argument that mineralisation, release from the soil biomass by grazing action and Fe reduction (Table 2.2) are not the only important microbially mediated processes that mobilise P in the soil environment. In this context two other processes need to be considered, the production of organic debris by an active microbial population and the mobilisation of clay particles by micro-organisms.

6.3.0. Production of Mobile Particles and Colloids.

Little if any work appears to have been done on the role that soil micro-organisms play in the production of mobile particles and colloids in the soil environment. The results from the work carried out for this thesis strongly suggest that they play an important role in this process. Obviously there are two ways in which this may occur, firstly as organic particles produced as a by-product of an active microbial population and secondly through the microbially mediated dispersion of inorganic clay particles. Thus, a variety of materials, both organic and inorganic can be 'mobilised' by micro-organisms in the soil environment. Such materials would possess a range of chemical and physical properties. These materials could play an important role in transporting a variety of elements and compounds through the soil. For example, with the dispersion of clay particles, compounds that are strongly sorbed onto these materials and hence immobilised in the soil, would be mobilised and stabilised in the mobile phase.

If it can be shown that dispersion of clay particles by soil micro-organisms occurs in other mineral soils, then it does not seem unreasonable to suggest that this could be a

very important pedogenetic process. For example, in the formation of soils with a texturally-differentiated profile.

In humid temperate regions this could be an important process associated with podzolisation. In very simple terms the formative processes associated with podsol profiles are divided into translocation (eluviation) and deposition (illuviation). According to Ross (1989) translocation consists of three component processes:

1) leaching: the translocation of soluble salts
2) cheluviation: the translocation of organo-metallic complexes
3) lessivage: the translocation of colloidal clay

In such soils there is clear evidence that clay is mobilised in the A horizon, transported down the profile and deposited in the B horizon. This raises the question of what processes are involved in clay transport, particularly what processes are responsible for the mobilisation of clay? Few if any workers appear to have considered soil micro-organism important in controlling clay translocation. The evidence from the work described in this thesis strongly suggests that this ought now be given serious consideration.

Another interesting feature of the data associated with the dispersion of clay in the Mt Bold soil was the change in the flow regime that it generated within the columns. The Cl^- breakthrough curves strongly suggest that the micro-organisms altered the physical

properties (i.e. pore geometry) of the packed soil and hence the flow regimes of the columns. Whilst acknowledging the dangers of extrapolating the findings of carefully controlled laboratory experiments to the broader soil environment, it is nonetheless fruitful to speculate that this phenomenon may not just be restricted to the laboratory. For example, the magnitude of the three component processes listed above will be ultimately controlled by the flow of water through the soil profile. If soil micro-organisms alter the physical properties of the upper horizons and so influence the regime that controls water flow, then they may play a crucial role in profile development.

These results suggest that serious consideration now needs to be given to the role that soil micro-organisms play in some important pedogenetic processes.

6.4.0. Effects of γ-radiation.

When faced with an experimental procedure that requires sterilised soil the researcher has only a limited range of options. The main procedures used are heat treatments (autoclaving), chemical biocides and γ-radiation. While each of these procedures may be relatively efficient at suppressing biological activity, the cost incurred, in terms of changes to the chemical and physical properties of the soil constituents can be high.

It has been reported (Xie and McKenzie, 1991) that heating changes the sorption capacity of a soil by converting highly reactive gels of Fe and Al oxides to less reactive crystalline forms. While some preliminary investigations in this laboratory revealed

that certain chemical biocides mobilised various elements. Given these findings, it should perhaps come as no surprise that γ-radiation also had a variety of effects on the chemical properties of soils.

Firstly, there was the mobilisation of a suite of elements including C. Data from the literature suggests that at least some of the C mobilised by γ-radiation comes from the biologically resistant fractions of the soil organic matter. This implies that once a soil is re-inoculated the basic dynamics of soil organic matter decomposition will be altered. The other significant effect was to lower the pH of the soil. As discussed earlier this effect had to be inferred indirectly as no direct measurements could be made. As a result the change in pH could not be quantified. Nevertheless, in mineral soils such a change could alter the solubility of various mineral species. Also, in soils that have a high level of pH –dependent surface charge such a change will alter the exchange capacity of the soil. This work suggests that a more systematic investigation should be made into the effects of γ-radiation on the chemical properties of soils.

These results show that irradiation like other sterilisation techniques, changes the chemical properties of soils. When faced with a choice of which technique to use the researcher should make a decision informed by a full understanding of the effects associated with each technique.

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