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# Geochemical and Microbiological Controls on the Transport of Uranium through Soil

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# Abstract

Widespread use of depleted uranium (DU) in munitions around the world has raised questions about contamination of soils, water and vegetation with uranium (U). However, understanding of processes controlling the fate and behaviour of U in soils is poor. The aim of this research was to investigate the contributions of abiotic and biotic processes to U transport in soils, by measuring transport in well-controlled experimental systems, and comparing the results with predictions of models of solute transport and reaction.

Investigating the role of abiotic processes is challenging due to the complex speciation chemistry of U in soil solutions, sorption reactions with soil surfaces, and the kinetics of local equilibration with soil particles. To simplify the system, the self-diffusion of $^{235}U$ against $^{238}U$ isotopes was considered, such that speciation and sorption environments were constant. Rates of self-diffusion of these isotopes were measured in four contrasting soils, together with the components of the soil U diffusion coefficient. The results showed that U diffusion was controlled by sorption processes in all the soils, and that slow local-equilibration processes had a major effect. The concentration-distance profiles of U in the soils could not be explained with a simple model assuming instantaneous solid:solution equilibration, and some U spread far further than predicted for equilibrium sorption. Differences in U sorption between the soils were not simply related to differences in soil pH, clay content, CEC or mineralogy.

To investigate biotic effects, rates of bulk diffusion of U were measured in sterilised soil, and soil in which prokaryotes or eukaryotes were inhibited by biocides. Slow local-equilibration processes were again found to affect diffusion, but transport was also somewhat increased by biotic processes, hypothesised to be due to differences in $CO_2$ pressure arising from microbial activity and thereby U speciation. This has implications for the effects of perturbation on rates of U transport through soil.

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# Chapter 1: Introduction

Uranium (Atomic Mass 238.03, Atomic Number 92) was discovered by M.H. Klaproth in 1789. It is a silvery white, weakly radioactive, metallic element. It mainly occurs in nature as various oxides, particularly uraninite (largely $UO_{2,}$ also know as pitchblende or as secondary minerals including complex oxides, silicates, phosphates and vandates (Bleise *et al.*, 2003). There are three major naturally-occurring isotopes: $^{238}U$; $^{235}U$ and $^{234}U$ (Table 1.1). Half lives of these isotopes vary, the more active $^{234}U$ decaying almost a thousand times faster than $^{235}U$ and $^{238}U$ (Ragnarsdottir & Charlet, 2000). In addition to its weak radioactivity, U is categorized as a heavy metal with chemotoxic potential (Bleise *et al.*, 2003).

Table 1.1: Characteristics of uranium isotopes in natural uranium (Bleise *et al.*, 2003)

| **Isotope** | **Half-life (years)** | **Relative mass (%)** | **Specific activity (Bq $g^{-1}$)*** |
|---|---|---|---|
| $^{238}U$ | $4.47 \times 10^9$ | 99.275 | 12 455 |
| $^{235}U$ | $7.04 \times 10^8$ | 0.719 | 80 011 |
| $^{234}U$ | $2.46 \times 10^5$ | 0.0057 | $234 \times 10^6$ |

*one Bq (Becquerel) is the activity of a quantity of radioactive substance in which one nucleus decays per second

The U content of the Earth is 0.019 mg $kg^{-1}$ distributed unequally between layers (Figure 1.0.1).

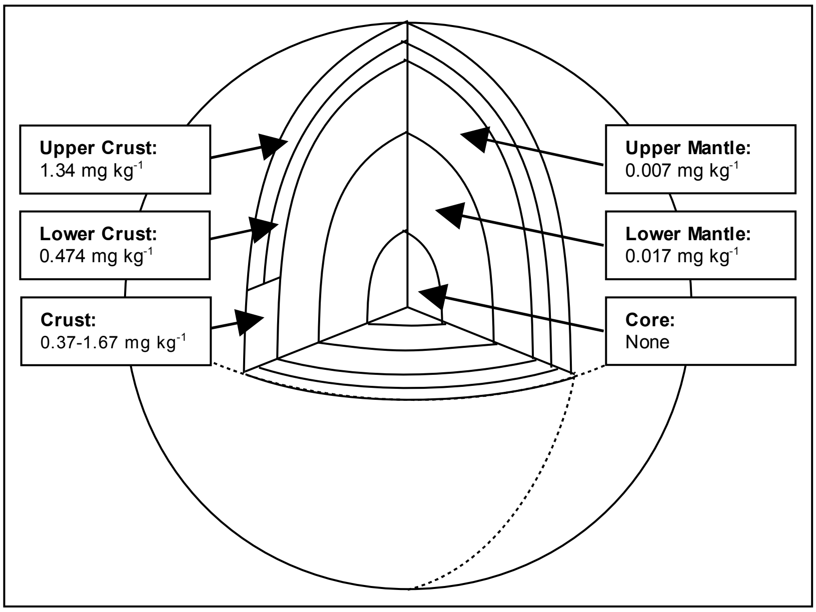


Figure 1.0.1: Distribution of uranium in the Earth (Ragnarsdottir & Charlet, 2000).

Rock, soil, water and biota naturally contain U at around 1 ppm or 1 mg kg$^{-1}$ (see also Table 1.2. and Figure 1.0.2) with a typical activity of 5-125 x 10$^{-3}$ Bq g$^{-1}$ (UNEP, 2003).

Table 1.2: Uranium values in environmental matrices (Bleise *et al.*, 2003)

| **Matrix** | **Typical concentration range** | **Reference** |
|---|---|---|
| Soil | 0.3 – 11.7 mg kg$^{-1}$ | UNSCEAR, 1993 |
| Air | 2.5 x 10$^{-8}$ – 10$^{-7}$ mg m$^{-3}$ | NCRP, 1999 |
| Surface water | 3x10$^{-2}$ – 2.1 µg L$^{-1}$ | WHO, 2001 |
| Ground water | 3x10$^{-3}$ – 2.0 µg L$^{-1}$ | WHO, 2001 |

The wide natural range of U concentration in soil over the whole planet (Table 1.2) is also found on a smaller scale (Table 1.2). Reported concentrations of U found in surface soils around the planet vary widely despite a generally low mean value.

Table 1.3: Natural levels of uranium found in surface soils of different countries (Kabata-Pendias, 2001)

| **Country** | **Range (mg kg$^{-1}$)** | **Mean (mg kg$^{-1}$)** |
|---|---|---|
| Canada | 0.72-2.05 | 1.22 |
| Great Britain | | 2.60 |
| Germany | 0.42-11.02 | |
| India | | 11.00 |
| Italy | 1.5-8 | 3.17 |
| Poland | 0.10-2.33 | 0.79 |
| U.S.A. | 0.30-10.70 | 3.70 |
| U.S.A. (Alaska) | <0.22-45 | 2.3 |
| Russia | | 3.8 |

Depleted uranium (DU) is the term used to describe uranium with a reduced proportion of the radioactive isotopes $^{235}$U and $^{234}$U (UNEP, 2003). With the dominance of the less active $^{238}$U isotope, depleted uranium is weaker radioactively (Table 1.4). Concentrations of the three major isotopes in depleted uranium are circa 99.8% $^{238}$U, 0.2% $^{235}$U and 0.0006% $^{234}$U and DU has about 60% of the radioactivity of non-depleted uranium (WHO, 2003).

Table 1.4: Radio-activities (Bq mg$^{-1}$) of natural uranium and DU (0.2% $^{235}$U) (Bleise *et al.*, 2003)

| **Isotope** | **Natural uranium** | **Depleted uranium** |
|---|---|---|
| $^{238}$U | 12 40 | 2.26 |
| $^{235}$U | 0.57 | 0.16 |
| $^{234}$U | 12.40 | 12.40 |
| Total | 25.28 | 14.80 |

Depleted uranium comes from a varied number of sources (Figure 1.0.2). However, most is produced as a by-product of uranium enrichment for the production of nuclear fuel. During enrichment the concentration of $^{235}$U is increased from 0.7% to around 4%. The process of enrichment produces a large amount of waste. This waste (depleted uranium) has a lower concentration of $^{235}$U (around 0.2-0.3%).

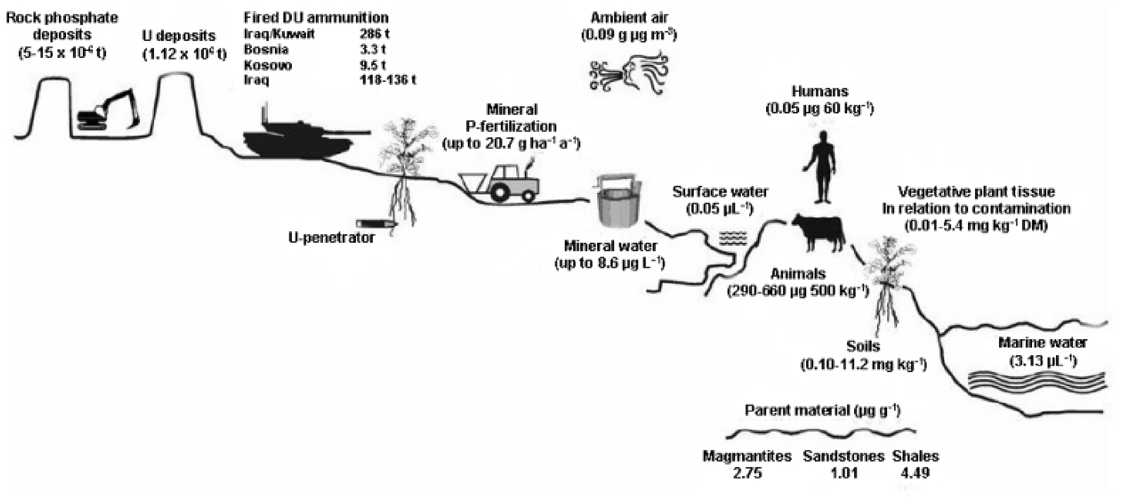


Figure 1.0.2: Natural uranium sources and anthropogenic input of uranium into the environment (Rivas, 2005)

Depleted uranium is strong, ductile and very dense (density = 19 g cm$^{-3}$, which is 65% denser than lead). It also has a tensile strength comparable to most steels (Bleise *et al.*, 2003). It is pyrophoric; derived from the Greek “fire-bearing”, (i.e. it can spontaneously ignite in air), and therefore shells and bullets can self-sharpen when a solid target is hit, and can burn through armour (Figure 1.0.3). These properties are considered effective by various militaries around the world for armour piecing kinetic energy munitions (Meyer *et al.,* 1998), and DU is used widely as an alloy coating with titanium on armour piercing shells and bullets. An estimated 286 t of DU was used in the Iraq Kuwait wars (1991), 3.3 t DU in Bosnia (1994-1995), 9.5 t DU in Kosovo (1999) and between 118 and 136 t DU in Iraq (2003) (Rivas, 2005 – from Brandt, 2004). Depleted uranium also has a number of peaceful applications, including counterweights for aircraft, radiation shielding and containers for transport of radioactive materials (WHO, 2003).

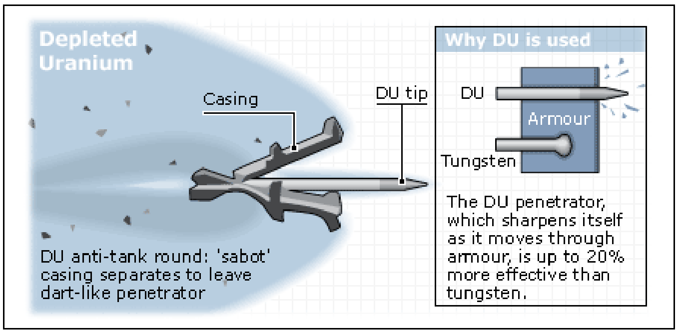


Figure 1.0.3: Depleted uranium munition (BBC, 2003)

It is not within the remit of this thesis to investigate the possible negative health effects associated with DU use. Due to the already complex nature of the system to be studied it was decided not to venture into this complicated and often contentious area. However, a short summary of the impact associated with uranium has been included below for the sake of completeness.

Though risk assessment for most radionuclides is based on the total dose to the organism of concern in the case of uranium there is also a possibility of greater risk arising from chemical toxicity (Sheppard *et al.*, 2005). If the radionuclide in question is not especially bio-accumulative and the radiological emissions do not have a relatively large biological impact chemical toxicity can exceed radiological toxicity (Sheppard *et al.*, 2005). In addition to this uranium has a long decay half life ($4.5 \times 10^9$) and a low specific activity which also contributes to the dominance of chemical toxicity when assessing risk.

In the environment various parameters affect the chemical toxicity of U including the species of uranium present (further detail of uranium complexes in solution will be covered later in this chapter). Lower bio-availability is found in the case of metallic U and particles of insoluble U compounds (Sheppard *et al.*, 2005).

Sheppard *et al.* (2005) lay out a series of non-human predicted no-effect concentrations (PNECs) for chemical toxicity of U:

- terrestrial plants: 250 mg U $kg^{-1}$ dry soil;
- other soil biota: 100 mg U $kg^{-1}$ dry soil;
- freshwater plants: 0.005 mg U $L^{-1}$ water;
- freshwater invertebrates: 0.005 mg U $L^{-1}$ water;
- freshwater benthos: 100 mg U $kg^{-1}$ dry sediment;

Depleted uranium is potentially toxic to humans both chemically and radiologically, both externally and internally if ingested. The degree of potential toxicity depends on the physical and chemical nature of the DU, and the level and duration of exposure (WHO, 2003). Exposure to uranium can result from a number of pathways including drinking contaminated water, eating contaminated food and breathing in dust particles or decay products (Ragnarsdottir & Charlet, 2000). Most uranium is eliminated from the body via the faeces (95%). Of that which is absorbed into the bloodstream 67% is excreted in urine within 24 h (after filtration by the kidneys – kidney problems can be linked to DU exposure). Absorbance from ingestion is also low (typically, between 0.2 and 2% is absorbed by the gastrointestinal tract) though soluble compounds are more readily absorbed than insoluble (WHO, 2003).

A comprehensive review of the risk assessment of uranium with regard to renal damage and body development thresholds can be found in Sheppard *et al.* (2005) and WHO (2003).

### *1.1 Soil contamination from depleted uranium munitions*

The two primary means of DU contamination linked to DU munitions are either dust generated through firing/impact or corrosion of intact/large fragments of projectile (Ringelberg *et al.*, 2004). Following ammunition impact, it is estimated that approximately 20% of the DU is converted to dust that can be inhaled (diameter ≤ 0.01 mm). This dust is estimated to only travel a few tens of meters from the impact site. Residual fragments or intact projectiles can penetrate to depths as great 7 m depending on soil type (Ringelberg *et al.*, 2004). Though conflict areas are likely to be heavily polluted with DU from bombardment by armour piercing munitions, there is another source of contamination, that of corrosion of stored munitions at non-conflict sites.

Depleted uranium corrodes in a similar fashion to metallic U. Solid U oxidises slowly in air at room temperature and turns yellow. Later, as oxidisation continues there is darkening and after three to four weeks the metal is black (UNEP, 2003). Finely divided DU corrodes more rapidly than large pieces, so fragments of shells produced by separation on impact or abrasion will have faster corrosion rates. In addition, it was found in Kosovo that ground impact causes fine cracks in penetrators, favouring corrosion (UNEP, 2003). Training and testing of DU rounds at firing sites in the US has led to contamination levels of over 10,000 mg kg$^{-1}$ in certain areas (Meyer *et al.*, 1998). Currently soil decontamination methods are favoured over *ex situ* methods due to their lower costs and reduced impact on the ecosystem. These methods include dilution, immobilisation or extraction (Rivas, 2005).

Studies in Kosovo (UNEP, 2003) found varying levels of DU contamination from penetrators at storage facilities after 7 years (natural U concentrations found to be 1.3 to 4.8 mg U kg$^{-1}$ soil). The condition of penetrators had an effect on DU transport (Table 1.5). A concentration of 3.8 mg DU kg$^{-1}$ soil was found on hard ground within 10-20 m of penetrator impact but no contamination was found further than 100 m from impact points. Figure 1.1.1 shows results from a case study at Han Pijesak's Artillery Storage and Barracks (UNEP, 2003).

Table 1.5: Contamination (g DU kg$^{-1}$ soil) around and beneath penetrators at storage facilities

| | **Penetrator without jacket** | **Penetrator with jacket** |
|---|---|---|
| Soil around munitions | 24 | 2.6 |
| Subsoil beneath munitions | 45 | 4.7 |

Decreasing concentrations of DU were found with increasing distance from the source of contamination. The penetrator without a jacket (Soil Series 1) showed a greater contamination of the surrounding soil than the penetrator with jacket remaining. The jacket refers to an aluminium casing which as well as helping the round to fly straight, protects munitions from corrosion. On impact with a hard object the penetrator proceeds without the jacket (Figure 1.0.3).

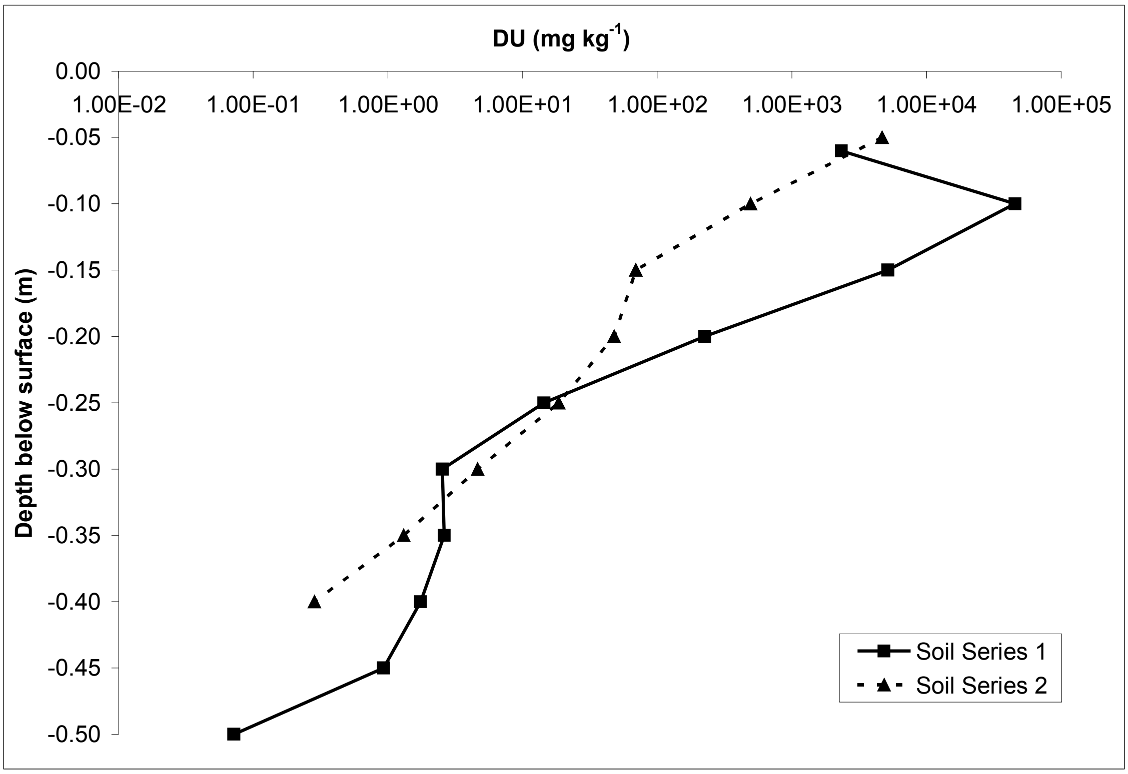


Figure 1.1.1: Concentration of DU in soil profiles from Han Pijesak artillery storage and barracks, 7 years after DU deposition. From UNEP (2003)

### 1.2 Behaviour of uranium in soil

Due to the environmental pollution risks posed by U, there is a wide variety of literature covering the behaviour of U in different media, whether natural or man-made. Taking into account that DU is similar in isotopic make-up to natural U with only a relatively small difference in $^{235}$U, assumptions on the behaviour of DU in soil have been drawn from published knowledge on natural U.

#### 1.2.1 Redox chemistry of uranium within soil

Uranium naturally occurs in five valency states: +2, +3, +4, +5 and +6. In aqueous solutions, for example soil solutions, U(III) and U(IV) can exist as the cations $U^{3+}$ and $U^{4+}$, or as U(V) and U(IV) oxyanions, depending on redox conditions and pH. The most common valency states in weathering systems are U(IV) and U(VI) (Ragnarsdottir & Charlet, 2000). In the natural environment U is most commonly found in its hexavalent form, U(VI). In aqueous solution U(VI) exists as complexes of the stable linear uranyl ion $UO_2^{2+}$ (Duff & Amrhein, 1996). Both $U_3O_8$ and hydrated $UO_3$ have been found along with $UO_2$ in corroded DU penetrators and DU particles (Fomina *et al.,* 2007). In soil, in the absence of high organic matter content U will be in the form of uranyl ion $UO_2^{2+}$. This ion is divalent, highly mobile, predominates at pH 5.0–5.5 and is most readily available to plants (Rivas, 2005).

Reduced tetravalent U (i.e. U(IV)) is less soluble than U(VI) and therefore less mobile (Mortvedt, 1994). Uranium can be reduced to U(IV) in soil profiles with reductive conditions.

### *1.2.2 Uranium species in soil*

Figure 1.2.1 shows the speciation of U(VI) in $CO_2$-free aqueous solution at different pHs. In the absence of $CO_2$, the hydroxyl species $(UO_2)_2(OH)_2^{2+}$ and $(UO_2)_3(OH)_5^+$ dominate, whereas in its presence at ambient atmospheric concentrations and neutral and alkaline pHs, carbonate complexes dominate (Waite *et al.,* 1994). Hence carbonate complexes of U(VI) are dominant in most natural waters.

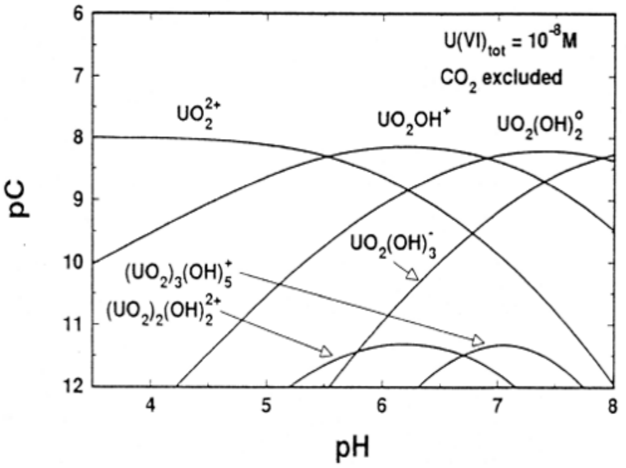


Figure 1.2.1: Distribution of major U(VI) species in the absence of $CO_2$ ($I$=0.1). pC (-log concentration) of species as a function of pH. Speciation of U(VI) at a total dissolved concentration of $10^{-8}$ M (from Waite *et al.,* 1994)

In acid or oxidising soil conditions, U moves through soil as $UO_2^{2+}$ or $UO_2(OH)^+$ cations, whereas in neutral soil solutions it moves as $UO_2(CO_3)_2^{2-}$ (Mortvedt, 1994). Stability constants for the range of complexes that the oxidised uranyl ion $UO_2^{2+}$ can potentially form in water are listed in Table 1.6. Above pH 8 $UO_2(CO_3)_3^{4-}$ is the dominant form in natural waters (Fetter, 1993). In excessively alkaline conditions such as an alkaline leach process, a soluble uranyl tricarbonate complex, $UO_2(CO_3)_4^{3-}$, is formed. This is a dominant U complex and has high mobility in calcareous conditions (Duff & Amrhein, 1996).

Table 1.6: Formation of U complexes in the U(VI)-$CO_2$-$H_2O$ system. Stability constants from Grenthe *et al.* (1992) except [b] from Tripathi (1983) (Waite *et al.,* 1994). Values are log*K* for the indicated equilibrium at infinite dilution and 25°C.

| **Equilibrium reaction** | **Log*K* (*I*=0)** |
|---|---|
| $UO_2^{2+} + OH^- = UO_2OH^+$ | 8.8 |
| $UO_2^{2+} + 2OH^- = UO_2(OH)_2^0$ | 16.0 |
| $UO_2^{2+} + 3OH^- = UO_2(OH)_3^-$ | 22.0 |
| $UO_2^{2+} + 4OH^- = UO_2(OH)_4^{2-}$ | 23.0 |
| $2UO_2^{2+} + OH^- = (UO_2)_2(OH)^{3+}$ | 11.2 |
| $2UO_2^{2+} + 2OH^- = (UO_2)_2(OH)_2^{2+}$ | 22.37 |
| $3UO_2^{2+} + 4OH^- = (UO_2)_3(OH)_4^{2+}$ | 44.1 |
| $3UO_2^{2+} + 5OH^- = (UO_2)_3(OH)_5^+$ | 54.44 |
| $3UO_2^{2+} + 7OH^- = (UO_2)_3(OH)_7^-$ | 67.0 |
| $4UO_2^{2+} + 7OH^- = (UO_2)_4(OH)_7^+$ | 76.1 |
| $UO_2^{2+} + CO_3^{2-} = UO_2CO_3^0$ | 9.7 |
| $UO_2^{2+} + 2CO_3^{2-} = UO_2(CO_3)_2^{2-}$ | 17.0 |
| $UO_2^{2+} + 3CO_3^{2-} = UO_2(CO_3)_3^{4-}$ | 21.63 |
| $2UO_2^{2+} + CO_3^{2-} + 30H^- = (UO_2)_2CO_3(OH)_3^-$ | 40.82[b] |
| $\beta\text{-}UO_2(OH)_2 = UO_2^{2+} + OH^-$ | -23.07 |

In typical groundwater conditions, uranium carbonate complexes are more common than uranium hydroxide complexes (Ragnarsdottir & Charlet, 2000). In calcareous soil where there is free carbonate present, the uranyl ion can complex with the carbonate to form anionic complexes which are very mobile (Shahandeh & Hossner, 2002). Reported U concentrations in shallow groundwaters are in the range of $\mu g\ L^{-1}$ to $mg\ L^{-1}$ dependant on conditions (Ragnarsdottir & Charlet, 2000). Under reducing conditions (where there is a potential for precipitation) and in the absence of ligands, concentrations of U can be below $\mu g\ L^{-1}$. Alternatively, concentrations can reach $mg\ L^{-1}$ with increasing Eh in solutions above pH 5 and up to thousands of $mg\ L^{-1}$ at pH 2.

The distribution of U in the lithosphere is strongly influenced by the oxidation state and the Eh-pH system. Actinides (including U) form strong complexes with oxygen ligands, speciation with inorganic ligands ($OH^-$, $CO_3^{2-}$, $HPO_4^{2-}$) is likely in both geological and environmental conditions (Kabata-Pendias, 2001). However, actinides also appear to be sorbed preferably by naturally occurring organic substances.

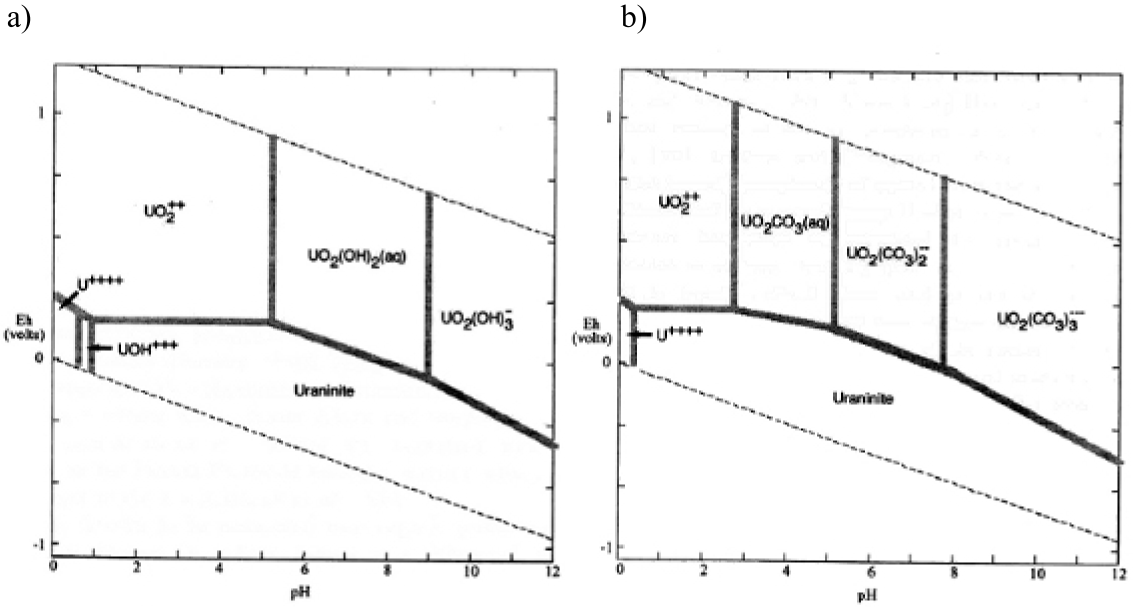


Figure 1.2.3: Eh-pH diagrams for aqueous species in the $UO_2$-$CO_2$-$H_2O$ system in pure water at 25°C, 1 bar total pressure and for total U = $10^{-8}$ M: (a)$P_{CO2} = 10^{-3.8}$ and (b) $P_{CO2} = 10^{-2.0}$ bar (Ragnarsdottir & Charlet, 2000).

Changes in pH within the soil environment affect the sorption of U(VI) and its consequent mobility. As pH rises, ionic U species change from the positively charged $UO_2^{2+}$ to the neutral $UO_2(OH)_2$, or $UO_2CO_3$ which are less strongly sorbed. But concomitantly the charge of variable-charge surfaces becomes more negative as pH increases, tending to increase sorption of cations but decrease sorption of anions. Precipitation reactions also increase with rises in pH (Mortvedt, 1994).

### *1.2.3 Uranium transport through soil*

Uranium held in the aqueous phase is able to move through soil through the processes of mass flow and/or diffusion. Mass flow, with ions carried along in water moving under gravitational or capillary forces was not studied in this thesis. Instead the diffusion of U through soil along concentration gradients was investigated.

#### Theory

If a concentration gradient of U exists across a particular region of soil, the various U species present in the soil solution will move by diffusion through the solution according to their individual concentration gradients. The resulting diffusive flux of each species is related to its concentration gradient by Fick's first law:

$$F = -D \, \mathrm{d}C/\mathrm{d}x \tag{1.1}$$

where $F$ is the flux, i.e. the amount of the species crossing unit section of soil in unit time; $\mathrm{d}C/\mathrm{d}x$ is the concentration gradient across the section; and $D$ is the diffusion coefficient of the species in the soil (Tinker & Nye, 2000). If the soil water (solution) is also moving, the U species are also carried by mass flow, and then an additional flux term needs to be added to Equation (1.1), equal to the product of the water flux and the species concentration in solution. This will only be significant if the solution concentration is large. If there is no water flux, the rate of change in the species concentration in the soil section is given by Fick’s second law:

$$\frac{\partial C}{\partial t} = \frac{\partial}{\partial x}\left( D \frac{\partial C}{\partial x} \right) \tag{1.2}$$

Where $t$ is time.

From the theory of solute diffusion in soil (Tinker & Nye, 2000), the diffusion coefficient is given by:

$$D = D_{\mathrm{L}} \theta_{\mathrm{L}} f_{\mathrm{L}} \frac{\mathrm{d}C_{\mathrm{L}}}{\mathrm{d}C} \tag{1.3}$$

where $D_{\mathrm{L}}$ = diffusion coefficient in free solution ($4.26 \times 10^{-6}$ cm$^2$ s$^{-1}$ for $UO_2^{2+}$ in water at 25°C), $\theta_{\mathrm{L}}$ = fraction of the soil volume occupied by solution, $f_{\mathrm{L}}$ = impedance factor for diffusion through the soil pore network, and $C_{\mathrm{L}}$ = amount of solute per unit volume of soil solution. These equations are for individual U species; the total diffusion of U is found from the sum of the fluxes of the individual species.

Different soils have intrinsically different properties which control the way ions diffuse through them. This is due to a number of physical and chemical factors, aside from the biological processes that also act on diffusing solutes (which will be considered later). They include the tortuosity of the soil pore network, exclusion of solutes from very narrow pores due to viscosity and electrostatic interactions, and sorption of solutes onto the soil solid.

The presence of solid particles in a porous system means that the diffusion path of a species is forced to deviate from straight lines (Shen & Chen, 2007), known as tortuosity. The impedance factor of the diffusion coefficient takes into account the tortuosity of the liquid phase (Olesen *et al.*, 2001). It increases as the diffusion pathway becomes less tortuous (tortuosity decreases) in line with increases in water content (Olesen *et al.*, 2001).

Diffusion is retarded to the extent that solutes are sorbed on the soil solid. This is accounted for in the derivative $\mathrm{d}C_\mathrm{L}/\mathrm{d}C$ in Equation (1.3). Sorption processes in soils are complex, notably so for U because of its complex speciation chemistry, and they vary greatly between soils and within the same soil as various interacting variables change.

The spatial organisation of the soil system is of paramount importance to the transport of elements within that system. The pore network provides a framework within most soil processes occur. The impedance of the soil to diffusion, governed by the geometry of the pore network and solute exclusion from narrow pores through the action of a double diffuse layer (DDL) around clay particles, is a characteristic of the soil at a given bulk density and moisture content. Double diffuse layers form due to boundary phenomenon between clay particles and water molecules (Mojid & Cho, 2006). When negatively charged clay surfaces adsorb water containing soluble cations these exchangeable cations then produce the diffuse double layer in the course of balancing the negative charge of the clay particle. These DDLs can be of varying thickness dependant on both the cations present in the soil water and the overall soil-water content, DDLs expand with higher soil-water content (Mojid & Cho, 2006). In extreme examples of high soil exchangeable sodium percentage (ESP) clay dispersion can occur as the thickness of the DDL increases and the forces holding clay particles together decrease (Menneer *et al.*, 2001). Dispersed clay particles can block water-conducting soil pores.

The difference in movement between dense compacted soil and soil with good porosity can be large. Structural properties affect transport processes for example if solutes or particulates are carried via preferential and bypass-flow channels of water through macropores.

Transport of otherwise soluble contaminants is affected by sorption onto soil or other sites or mineral precipitation. The retention of a contaminant by the soil system can be affected by soil type, the concentration and spatial arrangement of binding sites within the soil, the concentration of competition ions and the presence of colloids and various types of particulate matter (Johnson *et al.*, 2004). In addition transport of U is directly affected by speciation and sorption on mobile soil minerals or biological matter. As most uranium is transported as U(VI), or complexes thereof, it is important to look at the factors affecting U(VI) speciation and transformation. Uranium carbonate complexes as described in section 1.2.2 can be transported over long distances (Ragnarsdottir & Charlet, 2000) and are important as not only do they increase the solubility of U, they also limit U adsorption in oxidised waters (Langmuir, 1997).

U transport occurs in aqueous solutions of high Eh, the oxidising conditions ensuring that it remains in the mobile form U(VI) often as $UO_2^{2+}$ (Figure 1.2.3). Oxidised U(VI) has varied solubility depending on the physiochemical properties of the soil (Shahandeh & Hossner, 2002). With decreases in Eh, U tends to be either sorbed or precipitated as U(IV) species.

Though not generally saturated, local points of super saturation can allow precipitation (Stubbs *et al.*, 2006). If appropriate ligands are available, U can also precipitate to form stable minerals (Ragnarsdottir & Charlet, 2000). Reduction reactions can be catalysed by the presence of mineral surfaces, with deposition occurring when the carrying solution reaches sedimentary environments or barrier areas containing sulphide, organic matter or Fe(II)-bearing minerals. The presence of microbes (including a large variety of bacteria) is also known to catalyse redox reactions.

Soil properties change seasonally and are also affected by anthropogenic changes and other factors arising from outside forces. As such, the effects of measured soil properties on U transport (including organic matter, clay content, CEC and biotic systems) will be varied over a selected time period.

### Measurement of U movement in the literature

The range of rates of movement of U in different environments is large. Using data from field sites in Kosovo with a mobilisation of U to depths of 40 cm over 7 years (though horizontal mobilization was measured in metres), UNEP estimate the movement of U at ~500 mm $yr^{-1}$ (UNEP, 2003 – Appendix D). Other models have predicted 333 mm $yr^{-1}$ (20 m in 60 years, The Royal Society, 2002). In Kosovo, U was found to be mobilised to a depth of 400 mm but predominantly found (87%) within the top 100 mm of the soil profile (UNEP, 2003 – Appendix D).

Soil properties affect rates of U movement (preceding sections). Brown *et al.* (1998) calculated that the lateritic soils they studied would have a high U sorptive capacity (5.8 g U $kg^{-1}$ soil) and consequently any U would be retained in the surface layers of soil (calculated as top 10 mm). However, their study did not take into account mixing by soil fauna or redistribution by roots.

Johnson *et al.* (2004) found distinct layers of visible U in an alkaline arid soil taken from a test facility. Precipitation (the cause of the visible U) was possible due to the high concentrations of aqueous U and the rapid evaporation of water after rainfall events. The distinct layers found included a surface layer of corrosion products underneath the projectile. No precipitated (visible) U was found at depths shallower than 20 mm depth. In soil sampled between 20-40 mm depth large U particles were found and thought to be uranyl hydroxide minerals. Soil at depths of 40-80 mm contained calcium carbonate that cemented soil particles together and the U occurred within the cemented aggregates. Below 80 mm depth, adsorption rather than precipitation was the main factor. Lack of precipitation at depth may have been due to a lower concentration of U, or other conditions not conducive to precipitation. The U moved more than 80 mm in the time investigated (22 y).

### *1.2.4 Uranium sorption in soil*

Adsorption to iron oxides and oxyhydroxide mineral surfaces is influenced by pH and dissolved carbonate (Duff & Amrhein, 1996). This affects both speciation in solution and charge on surfaces.

With soil of intermediate pH (pH 6-8) sorption of U to organic matter and small minerals (less than 100 μm diameter) is important (Ragnarsdottir & Charlet, 2000). At intermediate pH in the presence of oxy(hydr)oxides or clay minerals the uranyl ions can be sorbed onto oxides and minerals (Ragnarsdottir & Charlet, 2000). Hydrolysis species dominate (e.g. $(UO_2)_2(OH)_2^{2+}$) in the absence of dissolved inorganic ligands such as carbonate, fluoride, sulphate, phosphate (Duff & Amrhein, 1996).

In acid soils the uranyl cation $UO_2^{2+}$ can be adsorbed onto the cation exchange sites of clay minerals and form oxide and hydroxide complexes with positively charged ions (Sheppard & Thibault, 1992). However, at low pH there is less sorption of the uranyl ion ($UO_2{}^{2+}$) on oxides and minerals with pH-dependent charge because their surfaces are more-positively charged at low pH (Ragnarsdottir & Charlet, 2000).

At neutral pH (above pH 6) U(VI) adsorption onto iron oxide and oxyhydroxide surfaces decreases sharply as pH increases. Adsorption of U(VI) species onto goethite (which can absorb both cationic and anionic solution species, depending on the pH) increased with increasing pH in carbonate-free solution across a large pH range (Duff & Amrhein, 1996).

At high pH, uranyl ions complex with carbonate ($CO_3^{2-}$) and hydroxide ($OH^-$) and cannot therefore sorb to negatively charged minerals (Langmuir, 1997, UNEP, 2003 – Appendix D).

### Sorption: Soil texture

Divalent cations are sorbed to soil clay particles through cation exchange mechanisms, and the extent of sorption tends to increase with increasing clay content (Mortvedt, 1994). Hence U mobility may be expected to decrease in fine-textured soils and increase in sandy soils if they have low organic matter content. The effects of soil texture on the movement of uranyl ions appears to be similar to its effects on the movement of the simple divalent $Ca^{2+}$ and $Mg^{2+}$ (Mortvedt, 1994). Shahandeh and Hossner (2002) found a significant portion of U contamination in soils exposed to vented air from U ore mine shafts was incorporated into the lattice of soil clay minerals. Johnson *et al.* (2004) found that soil clay content was the predominant factor controlling uranium sorption in alkaline arid soil samples.

### Sorption: Soil structure

Apart from differences in the density of sorption sites with texture, there may be differences in access to sorption sites within soil particles because of texture-related differences in aggregation and structure. It has been shown through mathematical modelling (Nye & Staunton, 1994; Ptashnyk *et al.*, 2009) that, for strongly-sorbed solutes such as orthophosphate anions, slow access to sorption sites within soil particles may indeed influence overall rates of diffusion in soil on time scales relevant to pollutant transfer to vegetation and water bodies (i.e. weeks to months following contamination).

### Sorption:Iron oxides

Iron oxides and oxyhydroxides are common and important sorbents for U. Examples include hematite ($Fe_2O_2$), goethite (ά-FeOOH), ferrihydrite and amorophous ferric oxyhydroxides (Stubbs *et al.* 2006).

Shahandeh and Hossner (2002), using a sequential fractionation method, found that in soils rich with the Fe and Mn oxides that were spiked with U(VI) as $UO_2(NO_3)_2 \cdot 6H_2O$ and analysed shortly afterwards, the dominant portion of U(VI) was found in association with the Fe-Mn oxide fraction of the soil.

Reed *et al.* (1993) working with sandstone columns found that adsorption was dominated by the presence of Fe oxide coatings surrounding the quartz grains. There was a strong affinity to natural mineral surfaces even under oxidising conditions. Brown *et al.* (1998) working on lateritic soils (enriched in iron and aluminium), red earths, yellow earths and siliceous sands found that the siliceous sands had a smaller surface area for adsorption. A substantial proportion of the total surface area of a soil is due to iron oxide and these sands with their reduced iron content were consequently able to adsorb less uranium.

### Sorption: Phosphates

Stubbs *et al.* (2006) studied the extent of U penetration into soil parent materials in contaminated soil and found U associated with phosphates, including discrete uranium phosphates. The binding of U and P to iron oxides may precede precipitation of uranium phosphates. The soils used in the study were acidic and it was assumed no carbonates were present. At low pH and in the presence of phosphates, the formation of ternary U-P surface complexes on iron oxides can occur. Uranium sorbed to iron oxides as coatings on shale chips and fractures were thought to be inaccessible to microbes, though as the U is still as the mobile U(VI) species, under different conditions it may desorb and become available to microbes.

In the presence of fungi, U sorption to phosphates and uranium phosphate precipitation within and without fungi cellular structures has been recorded (Fomina *et al.,* 2007). This will be further discussed in Section 1.3.1.

**Sorption: Recorded behaviour of DU in soils**

Sequential extraction procedures that are commonly used in soil chemistry remove different geochemical phases of U. Figure 1.1.4 gives an example. The phases extracted include exchangeable U species, U bound to carbonate and clay minerals, U bound to Fe$^{-}$and Mn oxides, U bound to organic matter, and residual fractions. Though there is overlap between these components, the proportions in each can help with interpretation of sorption mechanisms (Sheppard & Thibault, 1992). Binding mechanisms, or binding sites can be identified by observing the soil matrix removed with each extracted phase (Johnson *et al.*, 2004). However, it must be noted that the overlap between the fractions does result in operational bias.

Following research on Kosovo soils by UNEP (2003), it was found that in near surface horizons, readily available (or exchangeable U) was the most prevalent fraction (74% up to 40 mm, 35% between 40 and 100 mm). As depth increased, the U bound to carbonates increased (over 50% below 375 mm). The fraction associated with Fe/Mn oxides also increased below the surface layers, and U bound to organic matter increased between 40 mm and 100 mm but then remained steady with depth.

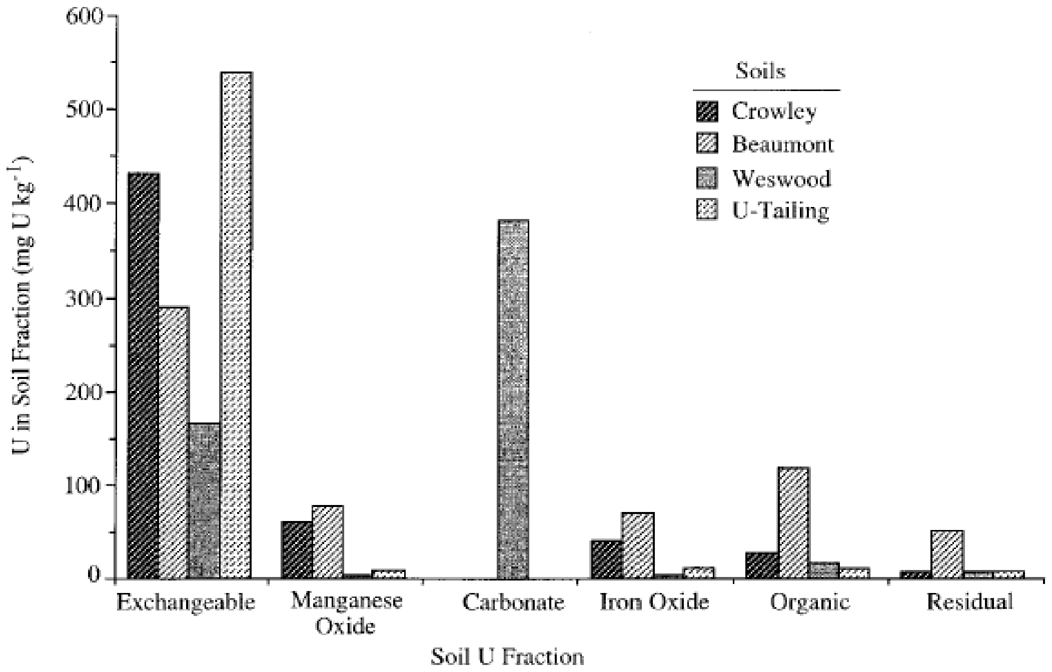


Figure 1.2.4: Example of the usefulness of the sequential fractionation method in determining the sorption behaviour of U in soil. From Shahandeh and Hossner (2002). Concentration of U associated with various soil fractions in 3 soils and a uranium tailing following 42 days of incubation. Soils were contaminated with 600 mg U kg$^{-1}$.

Shahandeh and Hossner (2002) performed sequential fractionation of U in acid and calcareous soils. In calcareous soil, more U was found to be bound to carbonates than in any of the other fractions. In a calcareous (pH 7.5) Westwood soil series (24% clay, 21% sand, classified as a silt loam), 69% of the U was bound to carbonate with only 27% in an exchangeable form. Beaumont soil series (63% clay, 16% sand, classified as a clay) had a significant proportion of U bound to Fe and Mn and organic complexes (50%) with 48% in an exchangeable, or readily available form. The importance of organic matter and oxides in this soil in the role of retaining U is clearly seen. In the Crowley soil series (only 24% clay, 57% sand, classified as a sandy clay loam) the exchangeable fraction accounted for most of the U recovered (91%). In U tailing soils (classified as a loam) exchangeable U accounted for most of the U recovered (74%).

Sheppard & Thilbaut (1992) performed sequential extraction on two soils; three horizons of a sandy soil and the gleyed clay subsoil of a sedge peat soil. The sandy soil used was an Aquic Udic Dystrochrept sandy soil profile including the O-A1, A2 Bir-Birg and Cgj horizons (Sheppard & Thilbaut, 1992).

The clay had a pH of 7.3, CEC of 22 cmol kg$^{-1}$ and 1.5% w/w organic C. Sequential extraction revealed that carbonates retained 35.9% of the uranium. The organic horizon of the sandy soil had the highest organic matter content (11.9% w/w organic C) and cation exchange capacity (81.2 cmol kg$^{-1}$). Both these parameters were lower in the other two horizons than the clay soil. The pH varied from 5.2 in the organic layer to 6.0 in the B horizon. In all four experiments the highest proportion of uranium was recovered from the oxides fraction. The lowest proportion was recovered in the exchangeable fraction (in keeping with strong sorption behaviour of U), except in the B horizon of the sandy soil. The proportion of U recovered in the carbonate fraction was greater in the B horizon of the sandy soil (pH 6) and in the clay soil (pH 7.3) than in the other two sandy soil horizons (pH ~ 5).

Choy *et al.* (2006) performed sequential extraction on the fine fraction (< 0.075 mm) of two soils contaminated by DU. Both soils were of a sand and silt mixture and of pH ~ 7. However, the two soils differed in distribution of DU. The first soil, a well graded sand, had most DU complexed with carbonates (41%) with 26% associated with oxides. The second soil, a sandy silt, only had 20% associated with carbonates, the majority of DU was recovered from the residual fraction. Explanations put forward for this large proportion of DU in the residual fraction included the development of silica coatings around DU particles and the possibility that these may have been over-heated by the explosions of DU munitions leading to DU incorporation into glass matrices. The Pyrophoric nature of DU coated shells (Section 1) may also be a factor.

### 1.2.5 Diffusion of uranium through crystalline structures

Alonso *et al.* (2004) investigated the heterogeneous diffusion of U through two crystalline rocks (Grimsel granite and Spanish granite). Spanish granite contains less Fe-bearing minerals on average, and fewer surface sites accessible to U. Heterogeneous sorption onto particular minerals seemed to be the initial step prior to solid-state diffusion. Though the work investigated rocks, it has some bearing on U diffusion through soil components.

The calculated diffusion coefficients of U in the rocks were similar to expected uranium diffusion in crystalline rocks at $10^{-13}$ to $10^{-4}$ m$^2$ s$^{-1}$). Detection of uranium dropped off at 0.2 μm depth, even when left to diffuse for the longer time of 24 h. The use of μ-Particle Induced X-ray Emission (μPIXE) allowed the determination of Si, K, Fe and U on an area of the granite. U was associated mainly with Fe in the different minerals and greater penetration of the U was seen in the Gimsel Granite than the Spanish, attributed to the lower Fe content of the latter.

### 1.2.6 Indications of possible U transport behaviour from investigation of other radionuclides

It has been shown that clay content is the most important determinant of background levels of $^{40}$K, $^{226}$Ra, and $^{232}$Th in soils (van den Bygaart *et al.,* 1999). The distribution of these radionuclides through soil profiles is further influenced by pedogenic processes, particularly carbonate leaching from the solum and clay illuviation from Ae to Bt horizons. The lateral and vertical distribution of bomb-fallout $^{137}$Cs is influenced by soil management such as tillage, biopedoturbation by soil animals such as earthworms and groundhogs, soil erosion and soil organic matter cycling. Multiple regression analysis showed that clay content, sand content, percent $CaCO_3$, pH and organic carbon content had variable influences on each of the radionuclide contents in the soil.

## 1.3 Uranium and soil biology

Soils are rarely if ever sterile in the natural environment and it is important to consider the part that soil biota play in the movement of elements and molecules through a soil system. The diversity of micro-organisms in soil by far surpasses that found in other ecosystems, it has been estimated that soil samples may contain as many as 13,000-30,000 different species, though disturbed arable soils or those affected by heavy metal pollution are found to be much lower (Torsvik & Øvreås, 2007). A few grams of soil can contain billions of bacteria, hundreds of kilometres of fungal hyphae, tens of thousands of protozoa and thousands of nematodes, 45 tonnes ha$^{-1}$ (fw) of organisms beneath a temperate grassland (Ritz *et al.,* 2004).

Microbial diversity within soil is integral to soil ecosystem function (Torsvik & Øvreås, 2007). Organic matter decomposition and element cycling are only two examples of soil functions regulated by complex interactions between the physical-chemical world and micro-organisms in soil. These interactions create variations in spatial and temporal habitats in a cycle of positive feedback to underpin the microbial diversity that supports the functional diversity of soil (Torsvik & Øvreås, 2007). Micro organisms have a close relationship with their surrounding environment based on their high surface area to volume ratio (Rivas, 2005). The complex and dynamic nature of natural microbial communities has a profound effect on the soil medium. These effects can include impacts on the geochemical behaviour of metallic elements including uranium (Fomina *et al.*, 2007).

The microbial community is vast; bacteria can occur at concentrations in the range of $10^7$-$10^{10}$ cells $g^{-1}$ dry soil (Van Elsas *et al.,* 2007). Prokaryotes can be considered especially important as they represent the largest phylogenetic diversity of any grouping on Earth and are involved in all biogeochemical cycling (Torsvik & Øvreås, 2007). Fungi impact upon the structural dynamics of soil therefore any transport process that are affected by soil structure are also likely to be affected by the effect of fungi on soil architecture (Ritz, 2006). Due to their filamentous branching growth habit and frequent exopolymer production they have a role in the maintenance of soil structure (Gadd 2007). Fungi, a major component of soil biota, can be tolerant to toxic metal and under certain environmental conditions (low pH, pronounced toxic metal pollution) can become the dominant microbial group (Fomina *et al.,* 2007).

Microbial communities exert a profound influence on element cycling in the biosphere (Haas *et al.*, 1998). The microscopic fraction of the soil biota has an ability to adapt to most environmental changes and extreme environments. There are direct effects, for example secretions from bacteria and fungi both during a normal life cycle and as a response to stress may affect metal solubility and transport. Indirect effects are also present, including processes involving organic matter within soil, organic acids increasing the solubility of U in soils (Kabata-Pendias, 2001), and the effects of the dissolution of $CO_2$ released in respiration.

Other components of the soil biological system such as plant roots also have a role to play. With root hairs behaving in a similar manner to AMF in stimulating P and U uptake by plant roots (Chen *et al.,* 2005) as well as effects on the physical soil structure and element cycling plants have a role to play in U transport. However, as the intent of the research was to look at uranium transport within a controlled microcosm this was not investigated further.

### *1.3.1 Direct effects of microbial populations on uranium transport*

Direct effects are taken to mean those effects which come about due to direct contact between an element (in this case uranium) and living microbes or their exudates. This includes the adsorption of U to living material (sequestration), alteration of U species both within microbes and as an effect of exudates within soil and the transport of U through fungal hyphae outside of the sphere of reactions with soil surfaces.

The concentration of metals out of dilute aqueous solution by micro-organisms is a potential source of immobilisation. Both bacteria and fungi concentrate metals out of solution (Milodowski *et al.,* 1990). Such microbes both immobilise and complex metals by mechanisms such as volatilisation, extra-cellular complexing, intracellular accumulation and cell surface binding (Ragnarsdottir & Charlet, 2000). Bio accumulation by bacteria or fungi plays a vital role in both cycling and dispersal. Fungi that both accumulate radionuclides and are also able to form mycorrhizal links to vascular plants and thus enhance radionuclide uptake by vascular plant hosts (Haas *et al.*, 1998). Chen *et al.* (2005) found that AMF increased U uptake by roots along with increased P uptake from contaminated environments, though innoculation by AMF decreased the translocation of U from root to shoots. Transfer between trophic levels by the grazing of bio-accumulating lichens can also widen distribution of toxic metals (Haas *et al.*, 1998).

In an investigation into U uptake from aqueous solution by 83 species of micro-organisms, high uranium-absorbing ability was found in *Pseudomonas stutzeri*, *Neurospora sitophila*, *Streptomyces albus* and *Streptomyces viridochromogenes* (Nakajima and Sakaguchi, 1986) U uptake in *Streptomyces* sp was between 2-14 % dry weight (Gadd, 1992). In this work, actinomycetes and fungi differed in the selective accumulation of uranium and mercury. The removal of U from solution by soil biology and its consequent inability to sorb to soil particle surfaces (which can be defined as a constant calculated from shaken suspension experiments and thus modelled) brings an additional variable to the modelling of U movement through soil. As this variable would be linked to the presence or absence of microbes with a specific function it is likely to differ widely between soils of seemingly similar composition, without any identifiable reasoning. As such the separate direct effects of the different components of a soil microbial community will be assessed individually.

### Direct effects of bacteria on uranium transport: Reduction

Bacteria produce a range of chemical species (Valsami-Jones & McEldowney, 2000) and excrete a diverse range of compounds into their environment (Table 1.7).

Heterotrophic bacteria effectively act as organic ligands by accumulating cations from solution. They also produce a range of organic compounds outside the cell (e.g. polysaccharide exopolymers and organic acid waste products), which may act as ligands for cations. The characteristics of exo-products will vary with the species involved and their growth stage and physiological status. There are major differences in exo-products between aerobic and anaerobic metabolism. Other factors include a variety of environmental effects, for example a high carbon environment has been linked to the production of exo-polysaccarides. In environments of low iron, bacteria can secrete siderophores, small molecules with high affinity for ferric ion, which can also bind to other divalent/trivalent metal ions (Valsami-Jones & McEldowney, 2000).

Table 1.7: Chemical species formed by bacteria and possible consequences to the transport of U in soil (summarised from Valsami-Jones & McEldowney, 2000)

| **Type** | **Details** | **Consequences** |
|---|---|---|
| Inorganic acids | Carbonic, sulphuric, nitric and inorganic bases. | Localised Ph effects, U[$CO_2$] complexes. |
| Organic acids | Acetic, citric, oxalic produced by catabolism. | Act as ligands of cations. |
| Siderophores | Iron chelating compounds, bind to $Fe^{3+}$ thus allowing take up by active transport processes. | Generally only produced in iron deficient environments to acquire the necessary micro-nutrient. |
| Exo-polysaccharides | External secretions from cells as a method of cell protection. May be linked to growth phases. | Act as ligands of cations. |
| Exo-enzymes | Enzyme excreted from cell to act extracellularly. | Bind to active mineral sites acting as dissolution inhibitors. |

Bacteria can reduce uranium both directly and indirectly. Bio-reduction of soluble U(VI) to U(IV) removes the immobilised U out of aqueous solution (Spear *et al.*, 2000). The direct reduction of U occurs as bacteria catalyse the reduction reaction of U(VI) to U(IV) whilst at the same time the oxidation of an organic substrate occurs. Indirect reduction of uranium occurs when bacteria reduce Fe(III) oxyhydroxides and the resulting Fe(II) reduces U(VI). Examples include: *Shewanella putrefaciens*, *Alteromonas putrefaciens* and *Delsulfibrio desulfuricans* (Ragnarsdottir & Charlet, 2000). Some bacteria can "respire" U for example the direct enzymatic U(VI) reduction coupled to anaerobic oxidation of acetate to $CO_2$ (Lovley *et al.* 1992).

Bacteria facilitate the precipitation of solid U phases directly from solution, through adsorption of aqueous U onto bacterial cells walls and subsequent nucleation of hydrous uranyl phases (Fomina *et al.,* 2007). U adsorption followed by reduction of $UO_2^{2+}$ or U(VI) to relatively insoluble $U^{4+}$ or U(IV) occurs at the cell wall interface (Haas *et al.,* 1998). Common examples of this are sulphate reducing bacteria (SRBs) which transfer the electron produced during respiration to sulphate, producing hydrogen sulphide. Uranium reduction has been hypothesized to occur in the periplasmic space (outside of the cytoplasmic membrane) (Valsami-Jones & McEldowney, (2000). There are also iron reducing species, Caccavo *et al.* (1992) found that BrY (*Shewanella alga* strain BrY) provided a model for enzymatic metal reduction by respiratory metal-reducing microorganisms indicating the potential to contribute to the mobilization of iron and trace metals and to the immobilization of uranium in sediments in the Great Bay Estuary.

Others working with SRBs have discovered other species able to reduce uranium and additional details about the processes involved. Spear *et al.* (2000) found a number of other bacterial species capable of reducing U(VI) to U(IV), including *Geobacter metallireducens*, *Shewanella putrefaciens*, and *Shewanella alga* strain BrY (BrY, or *Shewanella halotolerans* strain BrY). Lovley and Phillips (1992), working on the potential of *D. desulfuricans* to reduce uranium, demonstrated that cytochrome c3 was an essential component of uranium reduction.

Macaskie *et al.* (2000), working with a *Citrobacter* sp. reported the accumulation of the uranyl ion ($UO_2^{2+}$) in association with a phosphate ligand. The species of bacteria, originally isolated from metal polluted soil over produces a phosphatase which mediates metal uptake and results in precipitation of the U as U-phosphate. The rate of U accumulation varied with cellular phosphatase activity and the precipitate was seen along cell peripheries.

Actinomycetes, like many bacteria have electronegative cell walls (Milodowski, 1990). These are able to sequester cations (such as $UO_2^{2+}$) out of solution. In a case study in Scotland (Needles Eye on the north coast of the Solway Firth), it was found that ground water flowing through fractures in the cliffs leached uranium and re-deposited it in organic rich mudflats (peat bog and flood plain silts). The site was studied by the British Geological Survey, and Milodowski (1990) published SEM micrographs showing filaments of biogenic origin within hydrocarbons, with the appearance of the filaments and fruiting bodies of actinomycetes. There were distinctive spatial patterns seen, with bismuth or bismuth sulphide mineralisation within the "core" regions of the filaments and the wall regions enriched or mineralised by a calcium uranium mineral. Where cell structure could be recognised the calcium uranium enrichment could be seen to be occurring within cell walls. The metal enrichment observed could not be definitely attributed to active accumulation by living micro-organisms, but could have been post mortem accumulation. Examining this work, it appears that the actinomycete mediated reduction of uranium reduced mobility for thousands of years. Actinomycetes are grazed, but not apparently in this case. The calcium uranium mineral deposited in cell walls may have restricted such grazing.

### Direct effects of bacteria on uranium transport: Adsorption and internal accumulation

Bacterial surfaces generally have an overall negative charge (Valsami-Jones & McEldowney, 2000) and are therefore available to be sorption sites for cations, such as $UO_2^{2+}$. External environmental conditions such as temperature can directly affect attachment. Indirect effects such as nutritional restrictions can affect the growth of the cell and thus may affect cell surfaces and ability to bond. This can also relate to species differences and bacterial cell surfaces will differ between species. Binding of metals to bacterial surfaces is likely to be a local phenomenon and reversible. Binding occurs in favourable conditions and when such conditions change the uranium is freed back into solution. Both sets of conditions can occur within a small volume due to micro-scale heterogeneity within soil.

Site selectivity in relation to metal uptake in bacteria is related to ion-exchange or complexation between metal ions and active functional groups such as amine, phosphate, phosphodiester and carboxyl groups in polymers comprising cell walls (Milodowski, 1990). Fein *et al.* (1997) worked on metal adsorption onto the cell wall surfaces of *Bacillus subtilis*. They attempted to determine site-specific stability constants for the important metal-bacteria surface complexes (Cd, Cu, Pb, and Al). Results indicated that both carboxyl and phosphate sites contribute to metal uptake and that these metal bacterial interactions were stable enough to affect the mobility of such metals in many systems.

Adsoprtion also intrinsically linked to reduction as stated above with the adsorption of aqueous U onto bacterial cells walls and subsequent nucleation of hydrous uranyl phases (Fomina *et al.,* 2007).

Micro-organisms accumulate heavy metals and radionuclides from their external environment and these metals are involved in all aspects of microbial metabolism, growth and differentiation (Gadd, 1992). The amounts of uranium accumulated by bacteria can be large, though bio-adsorption may be limited by complexation of uranyl in solution to carbonate and hydroxyl ions (Ragnarsdottir & Charlet, 2000).

### Direct effects of fungi on uranium transport: Reduction to U[IV]

Fungi decompose substrates more usually as extracellular processes, rather than internally. The breakdown products are usually smaller and more mobile. Protons and organic acids released from fungi contribute towards extracellular precipitation of metals It is also possible for some metal elements to bind externally to fungi (Ritz, 2006).

Fungi produce extra cellular organic acids, some of which have metal complexing properties (citric and oxalic acid) with consequent effects on metal speciation and mobility in the environment (Gadd, 2007). In the process of heterotrophic leaching, organic acids provide both protons and metal-complexing organic acid anions (Gadd 2000). Elements can be mobilised into forms available for cellular uptake or leaching from the soil (to reduce stress). Organic acids with two or more electron donor groups (e.g. citric and oxalic acids) can form metal chelates (ring like structures). Oxalic acid is widely found and produced by plants and microbes. Oxalic acids can solublise U by protonation of anion species (acidolysis). Oxalate ion ($C_2O_4^{2-}$) is a bidente ligand (forms complexes with metals when more than one oxalate ion is present). Citric acid is able to form mononuclear, binuclear or polynuclear complexes depending on the metal and these complex formations affect metal mobility. Depending on the complex formed there can either be increased recalcitrance of a metal citric complex, or increased mobility, some metal-citrate complexes are highly mobile and not readily degraded by micro-organisms.

Oxalic acid can mediate the reduction iron by redoxolysis (Fe(III) to Fe(II)) (Bosshard *et al*., 1996). The resulting Fe(II) may be available in the soil to reduce U(VI) to U(IV) as occurs in some bacterial reduction. It must be noted that the release of organic acids from fungi is not a single injection into the system but a continuous release over time and as such cumulative effects produced can be large in relation to that intrinsic to the soil chemistry alone. In work on fungi interactions with other metals, it has been found that amounts of aluminium and phosphorus released are proportional to the cumulative oxalate loading rate suggesting that the continuous release of even small amounts of organic anions could solubilize large amounts of P and Al on an annual basis. Fomina *et al.* (2007) found oxalic acid to be the most significant mechanism of uranium oxide solubilisation. Although organic acids may accelerate dissolution by lowering pH, this may only be significant below ~pH 5.

### Direct effects of fungi on U transport: Adsorption and internal accumulation

Fungal effects on heavy metals in soil solution can cause immobilisation. Both free-living and mycorrhizal fungi can be efficient bio-geochemical agents and bio-accumulators of soluble and particulate forms of metals (Fomina *et al.,* 2007). Once elements are incorporated into fungal biomass, by whatever means, they will no longer be able to undertake further transport. Fungal decomposition or grazing by other soil fauna may release these elements again (Ritz, 2006).

Bio-accumulation includes both the processes involved in the uptake of bio-available U and intracellular accumulation including bio-precipitation mechanisms (Finlay, 2007). Heavy metals can undergo sorption onto cell components; immobilisation by bio sorption (Fomina *et al.,* 2007), or the removal or recovery of free metal ions from solution by prokaryotic and/or eurcaryotic bio-sorbent (Finlay, 2007). Chitin is an effective radionuclide bio-sorbent and the phenolic polymers and melanins of fungi present a number of potential sites for binding metal (Finlay, 2007). Metal accumulation is a method of toxic metal tolerance in fungi (Gadd, 2007). In bio-sorption fungi bind toxic metal ions through both chemical and physical means. Fungi do not behave as mono-functional ion-exchange resins (Finlay, 2007), instead providing a varied selection of function sites. Fungi cells walls provide a long list of components with negative charge to act in cation exchange. Sites include carboxyl, imidazole, sulphuydryl, amino, phosphate, sulphate, thioether, phenol, carbonyl, amide and hydroxyl moieties (Finlay, 2007).

Fungi can perform both intra- and extra-cellular sequestration and both promote precipitation of metals. In the presence of high metal concentrations the formation of insoluble metal oxalates (e.g. Cu) may ensure fungal survival. Fomina *et al.* (2007) investigated the potential for fungi to transform oxides of uranium using a complex methodological approach (not *in vivo*). The study revealed fungi to exhibit a high uranium oxide tolerance, posses the ability to solubilise $UO_3$ and $U_3O_8$ and to accumulate U within mycelia. Accumulation exceeded 80 mg (g dry wt)$^{-1}$. In most fungi this uranium was co-ordinated to phosphate ligands, but in ectomycorrhizal fungi a mixture of phosphate and carboxylate co-ordination was observed. X-ray absorption spectroscopy indicated uranium phosphate minerals encrusted the hyphae.

Fungi have been investigated for potential as bio-sorbents of uranium (Haas *et al.* 1998). A fungal by-product of industrial fermentation was discovered to have a uranium uptake capacity of >180 mg (g$^{-1}$ dry weight). This species, *Rhizopus arrhizus*, is also reusable as the uranium can be eluted from the biomass using a bicarbonate solution. Mechanisms identified for uptake include: Co-ordination with the amine nitrogen of the chitin component of the cell walls, complexed uranium acting as a nucleation site for accumulation of additional uranium and hydrolysis and subsequent precipitation of uranyl hydroxide on the cell wall.

*Saccharomyces cerevisiae* and *Psuedomonas aeruginosa* can accumulate 10-15% of their dry weight in uranium (Ragnarsdottir & Charlet, 2000). However, the mechanism of reduction differs between micro organisms. *Saccharomyces cerevisiae* accumulate uranium on the cell surface whereas *Psuedomonas aeruginosa* accumulate uranium internally but do not require a metabolic reaction to move uranium across the cell membrane. Accumulation of uranium by basidiomycetes was examined by Nakajima and Sakaguchi (1993). 46 species of fungi were tested and all basidiomycetes tested took up uranium far more readily than other heavy metals from a solution containing seven metals. Extremely high abilities to accumulate uranium were found in *Favolus arcularis*, *Inonotus mikadoi* and *Tricholoma conglobatum*. Fomina *et al.* (2007) showed that fungal cultures were able to transform uranium solids, with solubilisation, accumulated U in biomass and biomineralised uranyl-phosphate complexes were detected.

### Direct effects of fungi on uranium transport: Increased solubility

Mineral solubilisation by fungi occurs as a result of either acidification (protonation), complexation (chelation) or metal accumulation by biomass (Fomina *et al.,* 2007). All three were seen in the study by Fomina *et al.* (2007) on fungi and uranium.

*Glomus intraradices* an arbuscular mycorrhizal fungus can translocate U towards the roots of host plants (Rufyikiri *et al.,* 2003). However, the role of fungi in uranium solid transformation has remained unclear (Fomina *et al.,* 2007). This will be covered in more detail below in the section on indirect effects of fungi on U transport.

### Direct effects of other parts of the soil community on uranium transport: lichens

Lichens, or members of the Lichenes are a group of organisms consisting of fungi and algae growing together symbiotically (McGraw-Hill, 1984). These, being long lived are likely to bio-accumulate a greater amount of U than shorter lived species. Short term cation-uptake is an abiotic process where aqueous cations complex with exposed functional groups on the lichen biomass surface or where there is precipitation onto cell walls (Haas *et al.*, 1998). These processes tend to be rapid and this was seen in the work of Haas *et al.* (1998) where uptake was measured over 24 h. Haas *et al.* (1998) used the lichen *Peltigera membranacea* to measure bio-accumulation of aqueous uranium. The strongest U sorption was found between pH 4 and pH 5 and averaged ~42 000 mg kg$^{-1}$. Estimation of the distribution of aqueous species as a function of pH was carried out and the species dominating at this pH range were thought to be mixed cationic and neutral uranyl-hydroxide complexes. Electron probe microanalysis revealed that the U uptake was spatially heterogeneous. It was discovered that the U uptake was not only achieved through surface complexation reactions (TEM investigation) but that precipitation of solid phases also occurred.

### Direct effects of other parts of the soil community on uranium transport: Earthworms

Earthworms are capable of accumulating pollutants and can be used to measure the biologically availability within a terrestrial ecosystem. They are known to take up many inorganic and organic soil contaminants (Di Lella *et al.*, 2005). The availability of contaminants for uptake from the soil is controlled by the soil characteristics. However, the availability of contaminants from plant litter in varying degrees of decomposition is a rather complex and poorly understood topic (Di Lella *et al.*, 2005). Factors that influence the concentration of pollutants accumulated include chemical concentration, characteristics and properties plus the size and physiology of the accumulating species.

In the study carried out by Di Lella *et al.* (2005) on soil samples taken from a conflict site in Kosovo three species of earthworms were used. *Allolobophora rosea*, (epigeal species) which lacks cutaneous pigment and spends most of its life near the soil surface, in the organic layer, feeding on well-decomposed plant material. *Nicodrilus caliginosus* is similar to *A. rosea* in lifestyle and feeding habits. Both species ingest large quantities of soil while feeding (geophagous species). *Lumbricus terrestris* L. (anecic species) mainly lives in a deeper soil environment and migrates to the surface, usually at night or when the soil becomes very wet (Di Lella *et al.,* 2005). Earthworm concentrations of uranium did not differ from concentrations in individuals collected in an uncontaminated area Accumulation did not differ in line with increasing concentrations of U in soil. *L. terrestris* had the highest total U concentrations. Juveniles tended to accumulate more than adults, probably due to age related metabolism differences.

### *1.3.2 Indirect effects of microbial populations on uranium transport*

Indirect effects are taken within this section to mean those effects which come about due to processes occurring between uranium and both the live components of a microbial community and any associated organic matter. Included are any other effects and interactions arising from the presence of a microbial community within soil. Though individual species may have a direct effect on uranium within the soil the varied functions of a diverse soil community may produce greater indirect effects through the side effects of other unrelated functions.

Respiring organisms within soil produce $CO_2$. The weak carbonic acid produced when this respired $CO_2$ dissolves in the aqueous phase can have a minor dissolution effect on minerals. However, of more interest is the potential for a local decrease in pH. $CO_2$ production can reduce the pH in the surface of concrete from 12.5 to 8.5 (Valsami-Jones & McEldowney, 2000). With the effects on the speciation of U by both pH and $CO_2$ partial pressure and the consequent alteration of sorption potential and mobility (section 1.2.2 and 1.2.4) respiration has the potential to locally affect U transport on a large scale.

### Indirect effects: soil organic matter

Soil organic matter consists of all organic material that has previously been alive, this includes partly degraded material. Most soils contain at least a small amount of organic matter. The main point of difference between organic matter and live material is that live organisms can react and adapt to changes in the environment, organic matter is merely present.

Soil organic matter (SOM) provides a variety of charged surfaces for uranium to sorb to. With the ubiquitous nature of SOM it is important to asses the behaviour of uranium in relation to it. The effect of SOM presence varies. Organic matter can act as a potential sorption surface for an element of interest. However, complexation by organic ligands (increased mobility) and sorption and reduction reactions (reduced mobility) are all possible in the presence of organic matter (Ragnarsdottir & Charlet, 2000). In addition the degradation (chemical or microbiological) of organic materials in close proximity to uranium produces water soluble species with the potential to alter uranium mobility (Read *et al.*, 1998).

Uranium complexed to organic matter has reduced mobility through soil and uranium remains in the top layers of soil where there is a high level of organic matter. Mortvedt (1994) divided soil into two fractions, one with high organic matter and the other with low organic matter content. It was found that after spiking, the uranium concentration was higher in the fraction containing the high organic matter content. In Kosovo, the highest SOM content was found in the top layer of soil (150 mm) which is consistent with the depth (100 mm) with the highest U concentration (UNEP, 2003).

However, examples of increased mobility are also found. In acid soils, SOM can increase the mobility of U through the soil profile by forming humic acid complexes (UNEP, 2003 – Appendix D). Uranyl is known to be associated with organic matter constituents such as humic and fulvic acids in groundwater (Ragnarsdottir & Charlet, 2000). The presence of humic acids provides a wide range of functional groups (–COOH, –OH, – $NH_2$, etc.) which can form chelates with metal ions (Lubal *et al.*, 2000). This can lead to increased biological availability of some metal ions.

Soil contains a variety of organic materials that as they degrade, provide a pool of substances able to increase U mobility. Products of degraded cellulosic materials (oxidised glucose derivatives) have been shown to increase the solubility of radionuclides. Read *et al.* (1998) used ethylenediaminetetraacetic acid (EDTA) as an example of a waste-derived organic ligand and found increased solubility of uranium with reduced affinity to natural mineral surfaces. The EDTA was co injected with saccharic acid, and various complexes were formed, actively promoting the migration of U. Under the acidic pH 5.5 and oxidising atmospheric conditions of the column experiments, uranium was present almost entirely as the $UO_2H$ $EDTA^-$ complex. Similar calculations for saccharic acid indicated U dominantly complexed as $UO_2HSacc^+$. The formation of these species promoted the migration of U through the sandstone columns, reducing the time to peak breakthrough by factors of ~6 and ~10, respectively. Sheppard and Thilbaut (1992) also used EDTA to extract uranium (along with other elements) from a calcareous clay soil and three horizons of an acidic sandy soil. The EDTA extraction was effective for U, extracting 85% in the clay soil and 97% within the B horizon of the sandy soil. It was not as effective in the upper horizons of the sandy soil indicating that here the U was perhaps not as dominantly complexed with organic matter.

### Indirect effects: bacteria

Indirect effects of bacteria on the transport of U in soil are not easy to predict. With the exudation of secondary metabolites bacteria have the potential to create soil conditions that can both immobilise or mobilise minerals depending on species and environmental conditions. With the complexity of soil microbial communities and the associated massive number of interactions and functions predicting effects on U transport with confidence is difficult. Though research has taken place into the effects of specialised bacteria (e.g. sulphur reducing bacteria) in relation to uranium precipitation, interactions between microbes are more likely to produce the effects seen in the field.

Shahandeh and Hossner (2002) reported that there had to date been no reports of research designed to evaluate the effects of soil properties on U bioavailability. Though not a recommended technique the effects of pH, texture and organic matter on the bio-availability of uranium may possibly be deduced from papers on plant uptake experiments where soil properties are recorded thus making use of the assumption that soluble ions are most readily sorbed by plants (Mortvedt, 1994). Shahandeh and Hossner (2002) found that the solubility and availability of U to plants were the limiting processes in the phytoextraction of U. It is important to note that differences in plant uptake can also be linked to indirect effects, for example the higher levels of Ca and Mg that result at increased pH (Mortvedt, 1994).

**Indirect effects: fungi**

Fungi as degraders of naturally occurring complex molecules in soil indirectly affect geochemical cycling, their actions as organic matter decomposers releasing previously held U back into the soil system (Gadd, 2007). Fungi also provide surfaces for bacterial growth and the production of biomass by fungal species over time adds to the organic matter content of soil.

The production of secondary metabolites by fungi are another source of indirect effects. As seen in bacteria exo-polysaccharides can both inhibit mineral dissolution and accelerated it depending on the chemicals in question (Gadd, 2007). In addition exo-polysaccharides provide nutrition for bacteria, thereby supporting additional functional populations within the soil. Such provision of food may also allow a switch in growth stage of bacterial populations with the consequent release of different secondary metabolites.

A living fungal network affects the chemistry of the soil around it by the release of enzymes and metabolites including protons, carbon dioxide and organic acids. Microbe mediated pH changes and their effects on U transport have been discussed earlier in this section.

### *1.3.3 Effects of uranium pollution on the microbial community*

It was not within the remit of this project to look at the effect of DU on microbial communities but rather the effects of such communities on DU transport through soil. For this reason the work assumes the impact of a projectile into a soil community as yet unaffected by U pollution. However, to provide context, the effects of DU pollution on soil microbial function are briefly summarised below.

Changes in microbial populations provide an early indicator of soil improvement or degradation, changes in communities or activity can preceded detectable changes in soil chemical and physical properties (Rivas, 2005). Meyer *et al.* (1998) used a calcareous fine loam soil (pH 7.27) and added 50, 500, 5000, 10 000, 25 000 mg DU $kg^{-1}$ to soil microcosms (U concentrations at USA training grounds can reach 10 000 mg kg$^{-1}$). A significant decrease in overall activity calculated from measurements of soil respiration was found in soil contaminated with more than 500 mg DU kg$^{-1}$. At extreme levels of contamination (25000 mg DU kg$^{-1}$) there was a decrease in the decomposition of added organic matter. This decrease was dependant on litter type, with a greater effect seen on poorer quality litter consisting of cellulose and wood. There were also changes in Biolog data with increasing contamination, related to decreases in soil respiration. Biolog is a method of comparing soil function as an outcome of microbial community diversity between different soils using substrate induced respiration (SIR). It is however restricted in its ability to represent the complete soil community as it only measures plate cultured micro-organisms.

Soil enzymes activities show a rapid response to changes in soil and are sensitive to both natural and anthropogenic alterations. Enzyme activities can be considered as effective indicators changes in soil quality resulting from environmental stress (Rivas, 2005).

Rivas (2005) showed that the addition of U to a soil/plant microcosm reduced the micro-organism populations (colony forming units method). In grassland soil actinomycetes were reduced at the higher levels of contamination (above 357 mg kg$^{-1}$), anaerobic heterotrophic bacteria at all levels. The fungi count only showed a significant decrease at the 357 mg kg$^{-1}$, yet not at the higher level of 652 mg kg$^{-1}$. In comparison, within the forest soil actinomycetes were reduced at only the highest level of contamination (652 mg kg$^{-1}$). Anaerobic heterotrophic bacteria were shown to be significantly more populous at the middle levels of contamination (357 mg kg$^{-1}$ and 170 mg kg$^{-1}$).

### *1.4 Summary, Aims and Objectives*

The fate and transport of U contamination from DU munitions in soil is controlled by complex interactions between U, soil constituents and soil biota. Application of models to real soils is complicated by the complexity of such soils, as models tend to assume homogeneity. The work focuses on two main areas affecting the transportation of uranium in soil, the effects of soil heterogeneity and the effects of biological processes. Due to the difficulty of studying the fate and transport of uranium *in situ*, most work is carried out *in vitro*. However, it must be noted that research carried out *in* *vitro* can never fully mimic the actual diversity of the soil biological segment of the puzzle. Consequently, though research has returned results and knowledge regarding the behaviour of uranium in soil, these results may not be robust when tested against a more realistic soil microcosm which is the approach taken here.

At present, it is unclear if effects of soil microbiology on U transport need to be allowed for in addition to the abiotic processes governing transport and sorption on and in soil constituents. By characterising U sorption and transport in abiotic soils, and then comparing this with transport in real, biologically active soils, the importance of biotic processes can be assessed. The broad aim of this research was to investigate the contributions of biotic and abiotic processes to U transport through soil. The broad approach was to measure rates of transport in well-controlled experimental systems, and to compare the results with predictions based on the standard theory of solute transport through soil.

Accordingly the work has two main objectives:

1. To understand and quantify abiotic processes controlling U transport and sorption in soils, including the effects of soil micro-heterogeneity and restricted access to sorption sites. It was intended to explore the extent to which U transport and sorption in abiotic soils differ from that predicted with simple models of diffusion and sorption.

2. To understand the effects of biological processes on U transport in soil. It was hypothesised that the presence of a diverse soil microbiological community will affect transport, for example through excretion of solubilising or immobilising agents and ingestion and translocation of U. By examining transport through soils with different, manipulated communities, a clearer picture of these effects should be obtained.

The following hypotheses are tested:

1. In the absence of biological effects, rates of diffusion of U through soil can be predicted from independently-measured soil parameters, allowing for the effects of soil impedance, surface sorption reactions and restricted access to sorption sites.

2. Uranium transport rates are greater in biologically-active systems than in sterile systems.
3. Uranium transport rates differ between fungal and bacterial dominated systems.

# Chapter 2: General methodology

This chapter provides an overview of the materials and methods used in the thesis. It includes any method common to more than one experiment. Method development is described as appropriate.

## *2.1 Experimental soils*

### *2.1.1 Selection of soils*

To investigate the effects of soil properties on uranium transport, four soils differing in texture, pH and soil microbial communities were selected. Using the National Soil Resources Institute (NSRI) soil map of the region (1:25,000 scale) the area around Silsoe, Bedfordshire, was assessed for potential soils. Four soils were selected with widely differing parameters; a riverside site, an arable field in the valley, a site up on the Greensand Ridge and one within Shuttleworth Agricultural College. However, before sampling could begin the potential of soils held within the NSRI Soil Archive was highlighted. These were known to be varied and already well characterised by the department. It was decided to select soils from amongst these at a range of pH from acidic to alkaline and textural variation from sandy to clay soils.

The soils used were selected from 288 soils collected from Bedfordshire for a previous project at NSRI: *Towards a general method to 'scale up' process models in the arable landscape* (BBSRC BB/C506813/1, in collaboration with Rothamsted Research). The information available to select soils consisted of hand texturing data and soil parent material (Table 2.1).

Table 2.1: The original four soils selected. The soil ID numbers refer to their locations in the sampling scheme of the original project (Appendix 1).

| Soil ID | Ordinance Survey | Description | Parent Material | Hand Texture |
|---|---|---|---|---|
| 9G | | Non-calcareous sandy soil | Sand | Sandy Silt Loam |
| 13A | 5,15,194<br>2,40,990 | Non-calcareous clay loam | Sand | Clay Loam |
| 23E | 5,13,247<br>2,34,520 | Calcareous clay loam | Chalk | Clay Loam |
| 25C | 5,13,251<br>2,30,976 | Very calcareous chalky soil | Chalk | Sandy Silt Loam |

Having selected the soils by texture and parent material their pHs were determined. It was discovered that the pH of neither of the non-calcareous soils was below 7. As the intention was to compare soils of acidic and alkaline pH a further survey was carried out in order to find a soil within the collection available that had a pH of below 7. Only one soil was found that matched the parameters needed (Table 2.2).

Table 2.2: The final four soils selected

| Soil ID | Ordinance Survey | Description | Parent Material | Hand Texture |
|---|---|---|---|---|
| 11B | 5,10,977<br>2,40,938 | Non-calcareous sandy soil | Till/Sand | Loamy Sand |
| 13A | 5,15,194<br>2,40,990 | Non-calcareous clay loam | Sand | Clay Loam |
| 23E | 5,13,247<br>2,34,520 | Calcareous clay loam | Chalk | Clay Loam |
| 25C | 5,13,251<br>2,30,976 | very calcareous chalky soil | Chalk | Sandy Silt Loam |

### *2.1.2 Field sampling of soils*

The soils had been collected from the field the previous year. The recorded method of sampling was to extract 10 kg of soil from each sampling site by use of a spade, discarding the top 10 cm (Corstanje *et al.*, 2008). These soils were air dried and then ground and sieved to 0.5 mm. Sieved soils were stored in the NSRI soil archive until used. The 0.5 mm sieved soils were used in the work laid out in Chapters 3 and 4.

Fresh soil was collected for the experiments laid out in Chapter 5 as this work required field fresh soil with a a representative microbiological community. The original sampling sites were re-located using the Ordinance Survey references and a GPS device in the field. The precise location of the site sampled was confirmed using the original field descriptions. Soils were sampled using a Dutch augur. The top 30 cm of soil were sampled in a W pattern across the original site with 3 kg of soil removed and placed into a refrigerated container for the journey back to the laboratory.

### *2.1.3 Preparation of soils for analysis*

The soils collected by Corstanje *et al.* (2008) were washed with 0.01 M $CaCl_2$ after being ground and sieved to 0.5 mm in order to remove any ions left over from recent fertilisation (principally ammonium and nitrate ions) and to ensure there were no arbitrary differences between the soils. Soil (1 kg) was washed with 5 $dm^3$ of 0.01 M $CaCl_2$ solution. Five washes were carried out; each time the soil was left in the $CaCl_2$

solution for one hour, except for the third wash where the soil was left overnight. Between washings the $CaCl_2$ solution was siphoned off and replaced with fresh 0.01 M $CaCl_2$ solution. After washing soils were air dried and sieved to 0.5 mm before being stored until needed.

The fresh field soil collected for the experiments in Chapter 5 was placed in a refrigerated container immediately after sampling in the field. The soils were kept at the original moisture content, sieved to 2 mm and stored at 5ºC until required (further details can be found in Chapter 5).

## 2.2 Soil parameters

Soil pH was determined in 0.01 M $CaCl_2$ following the Cranfield University Soil Laboratory SOP (Appendix 2.1). Five replicates of each soil (5 g) were shaken for 1 h with 0.01 M $CaCl_2$. Soil parameters are collected in Table 2.3.

Table 2.3: Soil parameters

| Soil ID | Hand Texture | Parent material | $pH_{CaCl2}$ | CEC[†] ( $mol_c\ kg^{-1}$) |
|---|---|---|---|---|
| 11B | Loamy Sand | Till/Sand | 6.28 | 0.062 |
| 13A | Clay loam | Sand | 7.55 | 0.165 |
| 23E | Clay loam | Chalk | 7.60 | 0.212 |
| 25C | Sandy Silt Loam | Chalk | 7.58 | 0.067 |

[†] CEC of soils was determined by an MSc student in Reading.

Soil texture was calculated from particle size determination following the Cranfield University Soil Laboratory SOP (Appendix 2.2) by the pipette method (Table 2.4).

Table 2.4: Particle size results

| Soil ID | Sand % | Silt % | Clay % | Classification |
|---|---|---|---|---|
| 11B | 81 | 11 | 8 | Loamy Sand |
| 13A | 36 | 36 | 29 | Clay Loam |
| 23E | 11 | 50 | 40 | Silty Clay |
| 25C | 14 | 55 | 31 | Silty Clay Loam |

From this point on, soils will be referred to as their particle size classification in place of their original field identifier for clarity.

## 2.3 Experimental Unit

### 2.3.1 Packing soil into collars

For the measurements of transport through the soils, soil was packed uniformly into 'collars' cut from 3.9-cm internal diameter acrylic piping supplied by Engineering & Design Plastics Ltd, Cambridge. Each collar was around 3 cm high (Figure 2.3.1). The internal volume of the collars was determined by measuring the base and top diameter (using callipers) and the height. The average volume was 36.59 cm$^3$.

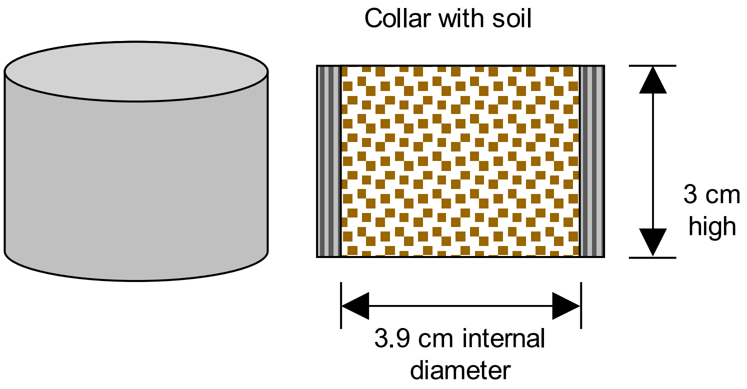


Figure 2.3.1: Collar, and collar packed with soil.

A weighed amount of soil was packed into the collars. A regular volume (one small spatula) was placed in the collar, evenly spread using a small paint brush, and then packed down with a plunger of the same diameter as the collar by sequential tamping until the desired bulk density was achieved. This process was repeated until the collar was full, containing all of the pre-weighed mass of soil.

### 2.3.2 Obtaining required bulk density and water content

It was originally planned to use a range of water contents and bulk densities for each soil, but this turned out to be impracticable. The experimental design was therefore altered to aim for a similar bulk density and water content in all four soils. The method development is summarised below for the silty clay loam soil:

**A.** First an attempt was made to determine how to pack the soil at a constant bulk density ($\rho$) and also how much liquid could be adsorbed by the soil column. Soil was packed whilst air dry and wetted up from below (to prevent air bubbles being trapped within the column). The soils were packed to $\rho$ = 1.16, 1.10 and 1.13 g cm$^{-3}$, with a gravimetric water content ($\theta_g$) of 250 mg g$^{-1}$.

Problems were found when packing air dry soil, as large bulk densities were needed to prevent the soil falling out of the collar. At $\theta_g$ < 200 mg g$^{-1}$ the soil was not equally wetted.

**B.** Second, soil was packed wet at $\theta_g$ = 200 mg g$^{-1}$. The soil and water were mixed in a tray before packing. It was assumed that this would allow packing to a range of bulk densities as the soil should no longer fall out of the column. There was an attempt to pack soil to two bulk densities and add further water to achieve a range of water contents. The soils were packed to $\rho$ = 1.1 and 1.2 g cm$^{-3}$.

Further moistening of the soil at $\rho$ = 1.1 g cm$^{-3}$ was found to be impractical as the soil then fell out of the collar. It was possible to moisten the collar at $\rho$ = 1.2 g cm$^{-3}$ to $\theta_g$ = 250 mg g$^{-1}$. But no more liquid could be absorbed.

**C.** Third, an attempt was made to achieve higher water contents. As the soil was unable to absorb more water when packed at an initial $\theta_g$ = 200 mg g$^{-1}$ it was decided to moisten to a higher water content before packing (thus achieving a lower bulk density when packed). The soil was moistened to $\theta_g$ = 350 mg g$^{-1}$, but became too wet to handle. It was then dried overnight to 320 mg g$^{-1}$. But this was still too wet to handle and the soil oozed out of the collar on application of pressure.

**D.** After collating data from previous trials it was assumed that the soil could potentially be used with $\theta_g$ = 200 to 250 mg g$^{-1}$. An attempt was then made to produce a wide range of bulk densities: $\rho$ = 1.0, 1.2 , 1.5 and 1.6 g cm$^{-3}$. It was found to be difficult to achieve consistency at $\rho$ = 1.2 g cm$^{-3}$.

**E.** The water contents achievable at these new densities were tested. At $\rho$ = 1.2, g cm$^{-3}$, $\theta_g$ values achieved were: 200, 220 mg g$^{-1}$ (at 250 mg g$^{-1}$ liquid dripped from the collar). At $\rho$ = 1.4, g cm$^{-3}$, $\theta_g$ values achieved were: 200, 230 mg g$^{-1}$ (attempt for 250 mg g$^{-1}$ failed as the column couldn't absorb any more liquid).

It was concluded that practice was needed in order to ensure that replication collars could be packed to consistent bulk densities. Bulk densities of 1.1 to 1.6 g $cm^{-3}$ were possible but it was only practical to produce water contents of between 200 to 250 mg $g^{-1}$.

Having produced a parameter range for the silty clay loam, attention was turned to the clay loam (soil 9G – one of the original soils selected before being rejected due to its pH). The tests carried out were reduced from the list above as prior experimental results had narrowed the field of enquiry.

**F.** Soil was packed whilst air dry and continuously wetted up to determine maximum water content. Bulk density was 1.26 g $cm^{-3}$ (this soil packed for low density better when air dry as opposed to the silty clay loam). Water content achieved was 184 mg $g^{-1}$.

**G.** Soil was packed at a water content of 100 mg $g^{-1}$ in an attempt to achieve a range of bulk densities. Packed collars were then further wetted to achieve a range of water contents. Bulk densities achieved were: 1.38 and 1.71 g $cm^{-3}$.

With $\rho$ = 1.38 g $cm^{-3}$, the highest $\theta_g$ achieved was 150 mg $g^{-1}$, but and the soil fell from the collar. With $\rho$ = 1.71 g $cm^{-3}$ the highest $\theta_g$ achieved was 190 mg $g^{-1}$ but no more liquid could be absorbed.

It was possible to pack the clay loam to a higher bulk density than was possible for the silty clay loam. This was a positive in terms of achieving a range of bulk densities, but once it was decided to attempt to pack all soils to the same bulk density it was no longer considered. The two soils did not appear to have much of a crossover in the range of water contents available for use. However, it was planned to use a water content of 200 g $cm^{-3}$ in further experimental work and attempt to produce this with similar bulk densities for the soils. The silty clay loam would need to be packed at a slightly higher bulk density than the clay loam in order that this water content (200 mg $g^{-1}$) would be enough to wet the entire collar.

Following these tests, it was determined that bulk densities of 1.1 to 1.6 g $cm^{-3}$ were possible and water contents of 200 to 250 mg $g^{-1}$. Principle obstacles included too loosely packed soil that could not be transported as the packed unit fell from the collars, and the impossibility of achieving high bulk density with the tools used. A bulk density of 1.3 g cm$^{-3}$ was decided upon as standard.

The method of moistening collars to the required water content was to wet up soils from below to prevent air bubble formation. Collars were placed in Petri dishes and a predetermined amount of solution (water or 0.01 M $CaCl_2$) was pippetted into the dish. The collars were then left in a water-saturated atmosphere (a desiccator with deionised water in the base) to take up the solution. Collars were equilibrated in the water-saturated atmosphere for 24 hours before being used.

### *2.4 Microtome apparatus used in soil slicing*

A microtome was used to take thin slices of soil perpendicular to the axis of diffusion in order to measure concentration-distance profiles. A hand microtome (Griffin and George, type DIEH 600-B) was connected to a Perspex barrel holding the soil collar (Figure 2.4.1). Twenty five turns of the microtome screw lifted the soil approximately 0.5 mm proud of the collar. The extruded soil was then sliced off using a ‘cut-throat’ or ‘straight’ stainless steel razor. 12 turns of the screw were used to produce 0.25 mm slices when needed. In this way the top 20 mm of soil in each collar was sliced. Accuracy of slicing improved with experience. Slice thickness was determined from slice weight and the average bulk density of the soil column. Slice thickness could only be assumed rather than measured as in every case it was assumed that bulk density was constant throughout volume of soil in collar and this could not be determined.

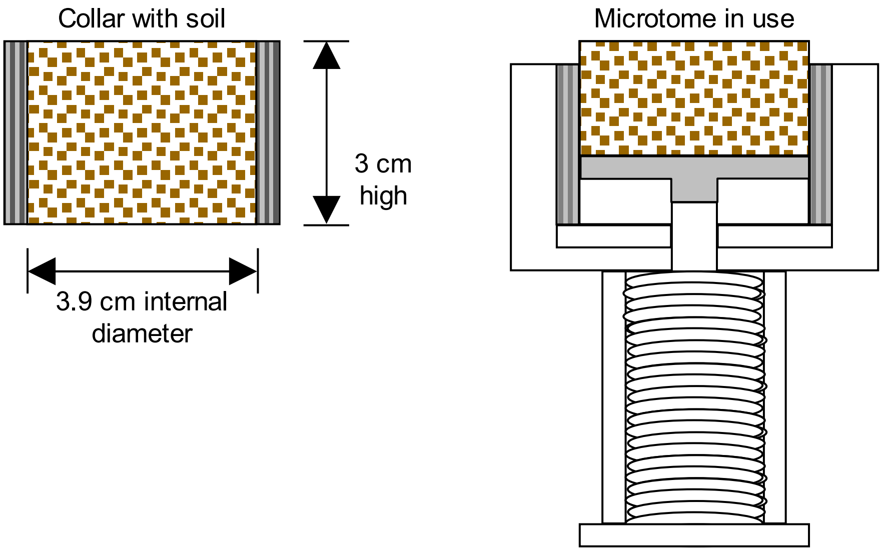


Figure 2.4.1: Experimental apparatus: collar packed with soil and microtome

### 2.5 Pulse application

In the experiments in Chapters 3, 4 and 5, rates of diffusion in soil collars were measured after a pulse application of the solute of interest (either U species or non-adsorbed $Br^-$ ions to measure $f_L$) on the soil surface. A pulse application was used because this allows analysis of the results using a particularly simple solution of the relevant diffusion equations (see Chapter 3). The precise details of pulse application were specific to the individual experiments. But the overall method was the same.

A solution (0.3 ml) containing the solute of interest was applied by pipette to a piece of filter paper cut to match the internal diameter of the collar. The filter paper was then held in contact with the soil surface for a length of time sufficient to ensure transfer of measurable amounts of solute into the soil, but short enough that it was negligible in comparison with the total run time. The filter paper was removed using tweezers to prevent any damage to the soil surface, and placed in a small glass bottle which was sealed and stored for later analysis.

#### 2.5.1 Method development for bromide pulsing

Preliminary experiments were made to develop method for pulsing the soil with the solute of interest, in order to ensure that sufficient quantities could be recovered at distances through the soil to determine concentration-distance profiles. A first consideration is that not so much salt is applied that salt diffusion occurs. But sufficient must be added to meet detection requirements. At the very small concentrations of $Br^-$ that were used, avoiding background contamination was a major consideration. All glassware had to be acid-washed and only ultra-pure water used to make up reagents. Great care had to be taken to avoid any contamination of glassware once cleaned.

The recovery of the Br applied was also a problem at low concentrations. Bromide was analysed by ICP-MS. A set of experiments was carried out to optimise the concentration and volume of applied bromide, application time and diffusion period. These are summarised in Table 2.5. The procedures were tested on all four soils.

Table 2.5: Method development for measuring diffusion of a $Br^-$ pulse

| | Concentration Applied | Volume Applied | Length of time left on soil | Diffusion Period | Notes |
|---|---|---|---|---|---|
| Silty Clay Loam | 0.01 μg $ml^{-1}$ | 0.1 ml | 4 h | 20 h | No diffusion profile seen |
| Silty Clay Loam | 0.01 μg $ml^{-1}$ | 0.1 ml | 5 sec | 5 h | No diffusion profile seen |
| Clay Loam | 0.01 μg $ml^{-1}$ | 0.1 ml | 5 sec | 5 h | No diffusion profile seen |
| Increased decontamination of glassware (acid washing) | | | | | |
| Clay Loam | 0.01 μg $ml^{-1}$ | 0.1 ml | 5 sec | 2 h | No diffusion profile seen |
| Silty Clay Loam | 0.1 μg $ml^{-1}$ | 0.1 ml | 5 sec | 2 h | No diffusion profile seen |
| Silty Clay | 0.1 μg $ml^{-1}$ | 0.1 ml | 5 sec | 2 h | No diffusion profile seen |
| Method altered to use filter paper as an application technique instead of anion membrane | | | | | |
| Clay Loam | 0.1 μg $ml^{-1}$ | 0.1 ml | 5 min | 2 h | No diffusion profile seen |
| Sandy Loam | 0.1 μg $ml^{-1}$ | 0.1 ml | 5 min | 2 h | Slight diffusion profile seen |
| Sandy Loam | 1 μg $ml^{-1}$ | 0.3 ml | 5 min | 2 h | Diffusion profile seen |

### *2.5.2 Method development for uranium pulsing*

The pulse of U applied to be applied needed to be sufficiently large that the resulting concentration-distance profiles in the soil were measurable, but not so large that it induced other changes in soil chemistry. Particularly, because of solubility considerations, commercially available sources of U are in dilute nitric acid, it was important to ensure that the resulting addition of $H^+$ to the soil induced no significant pH gradient. A solution of 1000 μg $ml^{-1}$ uranium in 1% (wt.) nitric acid was purchased (Sigma Chemicals; details in Chapter 4). This was applied to the soil without dilution. In the self diffusion experiments the pulsing period was 15 minutes and the diffusion period was 28 days.

### 2.6 Analytical methods

To determine concentration-distance profiles, the element of interest had to be extracted from soil slices and its concentration measured. Slice fresh weight was taken immediately after the soil was separated from the main collar (4 dp balance). Samples were then taken for analysis. The extraction methods differed for each element.

#### 2.6.1 Bromide extraction using $CaCl_2$

In the determination of $f_L$, slices were immediately extracted to remove the bromide following the method of Júniora *et al.* (2004). The slices were shaken for 1 h in 10 ml of 0.01 M $CaCl_2$ solution in acid-washed glass bottles. The resultant suspension was centrifuged and the $Br^-$ concentration in the supernatant determined by ICP-MS.

The initial $Br^-$ concentration in the soils was determined by shaking 1 g samples of soil (5 replicates) in 10 $cm^3$ of 0.01 M $CaCl_2$ for 1 h. Five blank $CaCl_2$ solutions without soil were also shaken. The resulting suspensions were filtered through Whatman No. 542 filter papers and analysed for $Br^-$. The measured $Br^-$ concentrations were 0.09 ± 0.003, 0.10 ± 0.011 and 0.08 ± 0.007 $\mu g\ g^{-1}$ (soil dry wt) in Soils 9G, 13A and 23E respectively, and 0.06 ± 0.005 $\mu g\ g^{-1}$ (equivalent soil dry wt) in the extractants without soil.

To test the recovery of $Br^-$ added to the soil, 1 g of air-dry soil was weighed into glass bottles (in triplicate) and 0.1, 0.5 or 1 $cm^3$ of 100 $\mu g\ ml^{-1}$ $Br^-$ solution were added (i.e. 0.127, 0.633 and 1.266 nmol $Br^-$ $g^{-1}$ soil, respectively). After a few minutes of equilibration, the samples were shaken in 10 $cm^3$ of 0.01 M $CaCl_2$ for 1 h, the resulting suspensions filtered and the filtrates analysed for $Br^-$. The recovery efficiencies were in the range 75 to 85%.

#### 2.6.2 Analysis of Br by ICP-MS

Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) allows the determination of trace and ultra-trace element concentrations in environmental samples, with rapid simultaneous multi element capabilities (Becker & Deitze, 2000; Yamasaki, 2000). The instrument used in this work was a PerkinElmer Elan 9000.

ICP-MS works by passing a sample solution through a nebuliser to create a stream of small droplets which are carried through a plasma torch (3000ºC). The element of interest is decomposed into its atomic constituents and, after ionization, positively-charged ions are pulled into the high vacuum of the mass spectrometer via an interface. The flow of ions passes through two focussing cones and then into a quadrupole mass spectrometer where the ions are separated by mass before being assayed on one of two detectors (pulse for low concentrations and analogue for higher concentrations). Repeated sweeps are made per reading and readings per replicate. The average of the data collected is calculated with associated relative standard deviation (RSD). Every new element analysed needs to have a specific method developed for it.

Samples were diluted and an internal standard added before being introduced to the ICP-MS (Figure 2.6.1). Samples were diluted for two reasons. Firstly it was important not to overwhelm the ICP-MS pulse detector, which was used as it reads the difference between samples of low concentration more precisely. Secondly when dealing with soil extracts, it is vital to ensure no particulate matter is introduced to the nebuliser of the ICP-MS as this can restrict the flow of solution through into the plasma torch.

Standards were made up to calibrate the ICP-MS before reading and were re-read every 20 samples. These standards were made up in the extraction media (0.01 M $CaCl_2$) as ICP-MS is susceptible to matrix effects (Ting *et al.,* 1996). Ions present in the carrying media affect the ability of the detector to measure the element of interest and thus must be present in the standard solutions at the same concentration as in the analysed samples.

The already low concentration of bromide in the extracts meant that further dilution was not practicable. Consequently the solutions were carefully filtered to reduce the possibility of nebuliser-clogging particles remaining in solution. Filtrate (8 $cm^3$) was combined with 2 $cm^3$ of a solution containing 10 $\mu g\ l^{-1}$ of Rh. To reduce contamination, all dilutions were made in Ultra-Pure water and all glassware was acid-washed prior to use.

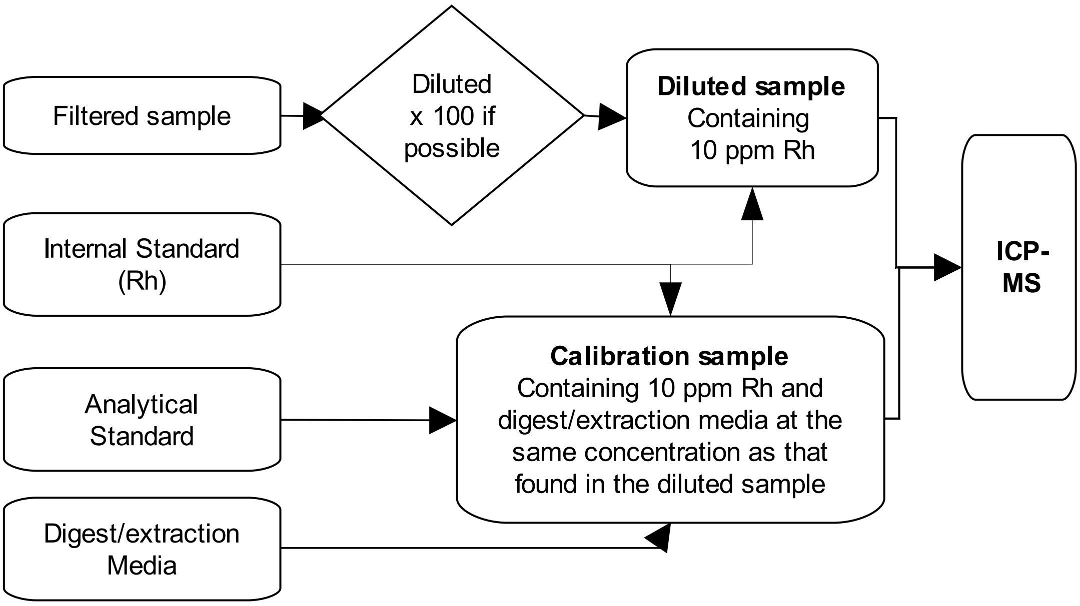


Figure 2.6.1: Mixing solutions for ICP-MS analysis

A bromide method was developed for the ICP-MS. It was built up from previous methods where $Br^-$ was extracted from soil in 0.01 M $CaCl_2$. The bromide concentration was measured by high performance liquid chromatography (HPLC) with UV detection at 210 nm (Smelt *et al.,* 2003). The extraction efficiency ranged from 90 to 110% and the limit of quantification (LOQ) for bromide in soil was 0.3 mg $kg^{-1}$ (Júniora *et al*., 2004). In other methods, $Br^-$ was extracted with deionized water at 1:1 field-moist soil to water (Paramasivam *et al.,* 2002). In examples where soil moisture data was collected and the soil samples were dried, the water used for the extraction was increased to 30 ml. The samples were shaken for 30 minutes in a 50 ml centrifuge tube, centrifuged for 10 minutes at 3270 x g followed by the supernatant being filtered through a Whatman (Maidston, UK) 42 filter paper. In this example bromide analysis was performed by colorimetric analysis on a continuous flow auto analyzer (Stevens *et al.,* 2005).

Detection limits for an Elan DRC II (Quartz Concentric Nebuliser and spray chamber, standard mode) are in the range of 1 $\mu g\ L^{-1}$ for both bromate and bromide (Perrone *et al.,* 2005). Using the standard mode of analysis, comparable results can be expected from the Elan 9000. Using a standard range of bromide concentrations the replication within the method was reduced as far as possible in order to speed up analysis time

(Table 2.6). At the same time the length of wash was adjusted until it was certain all previous sample had been expelled through the system.

Table 2.6 ICP-MS set-up for the determination of Br extracted by $CaCl_2$ from soil slices.

| **Method parameter** | |
|---|---|
| RF Power | 1500 W |
| Detector used | Dual |
| Dwell time | 50 ms |
| Integration time | 3000 ms |
| Scan mode | Peak hopping |
| Autosampler | Cetac ASX-500 |
| Sweeps per reading | 20 |
| Readings per replicate | 3 |
| Replicates per sample | 3 |
| Wash time between samples | 45 seconds at 48 rpm |
| Number of samples between standards | 20 |

### *2.6.3 Method development for soil U extraction*

The extraction of uranium from soil was carried out by acid digest. Several alternative acid-digest methods are available (Boulyga *et al.,* 2001, 2002 and Boulyga and Sabine Becker 2002). Three were compared to select the most appropriate.

It is widely agreed that soil needs to be dried, generally to constant weight at 105 ˚C for 24 hours (Boulyga *et al.,* 2001, 2002 and Boulyga and Sabine Becker 2002). After the fresh weight of soil slices were taken, each slice was oven dried for at least 24 h (105°C) and dry weights recorded. The dry soil material was then transferred to acid-washed glass bottles for storage. During this process any large particles of soil were broken up.

#### **Testing digest methods on reference soils**

Three different digest methods were tested for efficiency at removing elements from soil material. Four different soils were subjected to aqua regia digest, a $HNO_3$ digest and a peroxide digest. These were:

Commission of the European Communities BCR Reference Material 141: Calcareous Loam Soil

Commission of the European Communities BCR Reference Material 143: Sewage Sludge Amended Soil

National Research Centre for Certified Reference Materials (Beijing, China) GBW 07402 (Soil)

ICP-MS Internal Reference Sample (1997): 5

The aqua regia method used was the Cranfield University Soil Laboratory SOP (Appendix 2.3). Samples (1.000 ± 0.001 g) were weighed into digestion tubes. Concentrated HCl (22.5 ml) and concentrated $HNO_3$ (7.5 ml) were added to the tubes and left over night. The mixture was then boiled for 2 h and allowed to cool. The resulting solution was filtered into 100 ml flasks and topped up with 6% $HNO_3$.

The nitric acid digest used was developed from the aqua regia method, due to reported ICP-MS problems with $Cl^-$ ions in analyte solution. Samples (1.000 ± 0.001 g) were weighed into digestion tubes and 30 ml concentrated (69%) $HNO_3$ added. The tubes were heated to 130ºC for 2 h, the solutions filtered into 100 ml volumetric flasks and made up to 100 ml with 6% $HNO_3$.

The peroxide method was developed from an acid and peroxide method in the NIOSH Manual of Analytical Methods (1994). Samples (1.000 ± 0.001 g) were weighed into glass beakers and 10 ml concentrated (69%) $HNO_3$ was added. The beakers were heated to dryness (< 0.5 ml remaining) on a hotplate (170ºC). This was repeated twice. Ten ml $H_2O_2$ was added to the beakers, washed down the beaker walls to collect any residue. The beakers were returned to the hotplate and evaporated to dryness. Any residue was dissolved in 5 ml concentrated $HNO_3$ and filtered into 100 ml volumetric flasks which were made up to 100 ml with 6% $HNO_3$.

The nitric acid and peroxide digest did not produce the satisfactory results. Therefore another $HNO_3$–$H_2O_2$ digest method was tested. Samples (1.000 ± 0.001 g) were weighed into 100 ml glass beakers and anti-bumping granules added. Ten ml concentrated (69%) $HNO_3$ was added to the beaker and after covering with a watch glass this was heated for 30 min at 130˚C on a hot plate. Five ml of $H_2O_2$ was added to the cooled beaker and the solution swirled to clean the sides. The beaker was heated for

1 h at 130°C on a hot plate. The ensuing solution was filtered through Whatman No 542 filter paper into 100 ml volumetric flasks. The filter paper was washed with 6% $HNO_3$ and the flasks made to 100 ml with same solution.

All digest solutions were analysed for element concentration using ICP-MS. Using the recorded concentrations for the soils (Appendix 3) extraction efficiencies were calculated by comparing concentrations extracted by the digest methods to those recorded as being present within the individual soils (Table 2.7).

Standards were made up in 50 ml centrifuge tubes using 1% media, 10 $\mu g\ l^{-1}$ Rh and ICP-MS quality control solution 1 (QC1) which contained all the relevant metals in solution. Digested solutions were diluted ten-fold to ensure that the particulate matter was at a low enough level for the ICP-MS to function.

Table 2.7 Extraction efficiencies of recorded element content from three standard soils (sources described in the text) by the use of three different digest methods. Means of three replicates shown with SEM in brackets.

| | Element | Aqua Regia Digest Extracted (%) | Nitric Acid Digest Extracted (%) | Peroxide Digest Extracted (%) |
|---|---|---|---|---|
| Soil A | Cd (114) | 91.05 (1.07) | 78.34 (7.95) | 105.15 (2.62) |
| Soil B | Cd (114) | 147.58 (1.66) | | |
| Soil C | Cd (114) | 366.04 (21.56) | 89.16 (12.41) | -187.55 (30.73) |
| Soil A | Cr | 89.85 (0.99) | 70.54 (7.79) | 66.80 (0.8) |
| Soil B | Cr | 115.68 (4.07) | | |
| Soil C | Cr | 72.31 (3.67) | 76.18 (1.09) | 75.94 (1.9) |
| Soil A | Cu (63) | 87.33 (0.50) | 157.93 (79.91) | 88.07 (5.61) |
| Soil B | Cu (63) | 82.20 (2.12) | | |
| Soil C | Cu (63) | 73.59 (3.53) | 87.90 (1.16) | 92.50 (1.85) |
| Soil A | Ni | 90.40 (1.00) | 74.82 (8.28) | 74.30 (1.68) |
| Soil B | Ni | 102.58 (1.41) | | |
| Soil C | Ni | 80.72 (4.53) | 86.75 (1.04) | 86.90 (2.26) |
| Soil A | Pb (208) | 91.67 (0.42) | 79.70 (9.11) | 88.58 (1.92) |
| Soil B | Pb (208) | 82.46 (2.41) | | |
| Soil C | Pb (208) | 54.43 (2.85) | 60.82 (1.32) | 62.81 (4.86) |
| Soil A | Zn (66) | 75.50 (0.72) | 55.17 (5.15) | 60.91 (0.94) |
| Soil B | Zn (66) | 131.22 (44.99) | | |
| Soil C | Zn (66) | 107.12 (3.42) | 96.39 (0.19) | 70.06 (1.33) |

It was decided not to use the peroxide method as the errors of the method were higher than the others. In practical terms it was also over-complicated as a method and there was a high probability of operator error. The standard aqua regia method performed best in terms of producing results that did not indicate an extraction efficiency that was not practically possible. It was revealed in all three methods that a complicated analysis method incorporating an ICP-MS required further experience on the part of the operator before acceptable results could be produced. Bearing in mind this inexperience, and its effect on the outcome of the analysis, both the nitric and standard aqua regia methods were taken forward for further testing.

**Testing uranium extraction efficiency**

The four experimental soils (loamy sand, clay loam, silty clay and silty clay loam) were contaminated with known amounts of U by adding solutions of appropriate concentrations. These soils had previously been dried, ground and sieved to 0.5 mm. Sub-samples (10 g) were weighed into glass bottles and mixed with 2.5 ml of 0.5, 5 or 50 ppm U solution. The tubes were shaken for 1 h on a side to side shaker and then left for one week. The soil and bottles were oven dried for 24 h. Half of the bottles were sub-sampled and ashed. Sub-samples (1 g) were weighed into digestion tubes and digested by one of two methods:

3. Nitric acid: The dried sample was heated under reflux for 3 h, then centrifuged and the supernatant removed. More acid was added and the remainder was again heated under reflux for 2 h and filtered.
4. The dried sample was extracted with a hydrochloric/nitric acid mixture by standing for 16 h at room temperature, followed by boiling under reflux for 2 h. The extract was then clarified and made up to volume with nitric acid.

Table 2.8: Results of the comparison between two acid digest methods (df 14)

| **$HNO_3$** | **U extracted (mg $g^{-1}$ dry soil)** | | **Efficiency of extraction** |
|---|---|---|---|
| | Blank | 5 µg $ml^{-1}$ contamination | **(extracted - blanks) %** |
| Loamy Sand | 0.00071 | 0.00209 | 104.87 |
| Clay Loam | 0.00023 | 0.00166 | 114.28 |
| Silty Clay | 0.00072 | 0.00183 | 88.46 |
| Silty Clay Loam | 0.00084 | 0.00229 | 114.43 |
| F | | 109.08 | 3.08 |
| p | | <0.01 | <0.1 |

Table 2.8 (cont): Results of the comparison between two acid digest methods (df 14)

| **$HNO_3$ Ashed** | **U extracted (mg $g^{-1}$ dry soil)** | | **Efficiency of extraction** |
|---|---|---|---|
| | Blank | 5 µg $ml^{-1}$ contamination | **(extracted - blanks) %** |
| Loamy Sand | 0.00068 | 0.00175 | 86.68 |
| Clay Loam | 0.00025 | 0.00181 | 123.33 |
| Silty Clay | 0.00071 | 0.00165 | 75.06 |
| Silty Clay Loam* | 0.00097 | 0.00205 | 81.8 |
| * One sample read only. | | | |
| F | | 92.95 | 21.44 |
| p | | <0.01 | <0.01 |

| **Aqua regia** | **U extracted (mg $g^{-1}$ dry soil)** | | **Efficiency of extraction** |
|---|---|---|---|
| | Blank | 5 µg $ml^{-1}$ contamination | **(extracted - blanks) %** |
| Loamy Sand | 0.00067 | 0.00238 | 136.50 |
| Clay Loam | 0.00013 | 0.00144 | 104.32 |
| Silty Clay | 0.00077 | 0.00262 | 150.06 |
| Silty Clay Loam* | 0.00087 | 0.00267 | 156.87 |
| F | | 77.18 | 2.75 |
| p | | <0.01 | <0.5 |

| **Aqua regia Ashed** | **U extracted (mg $g^{-1}$ dry soil)** | | **Efficiency of extraction** |
|---|---|---|---|
| | Blank | 5 µg $ml^{-1}$ contamination | **(extracted - blanks) %** |
| Loamy Sand | 0.00063 | 0.00146 | 67.96 |
| Clay Loam | 0.00019 | 0.00119 | 79.98 |
| Silty Clay | 0.00053 | 0.00140 | 72.55 |
| Silty Clay Loam* | 0.00064 | 0.00185 | 96.02 |
| F | | 146.51 | 4.77 |
| p | | <0.01 | <0.05 |

The results for 0.5 and 50 ppm U solutions are included in the microwave digestion method development section (Table 2.10).

### *2.6.4 Uranium extraction by acid digest: microwave using $HNO_3$*

Microwave digestion is carried out in closed vessels at high temperature and pressure resulting in a more efficient extraction. Following the previous method development on acid digest methods a microwave digester (Multiwave 3000, Anton Paar) was purchased and consequently a microwave method was developed.

Calcium carbonate content (Table 2.9) was determined for calcareous soils (silty clay and silty clay loam) as problems had been experienced in test digestions with foaming and loss of material.

Table 2.9: Calcium carbonate concentration of the two calcareous soils as determined by calcimeter on 2 g soil. Mean of two samples.

| **Soil** | **$CaCO_3$ (g $kg^{-1}$)** |
|---|---|
| Silty Clay | 198.2 |
| Silty Clay Loam | 700.8 |

Soil slices were transferred to microwave digestion vessels. Any samples from silty clay loam soils were pre-digested in conical flasks in a fume hood overnight in 10 ml concentrated $HNO_3$. The soil-acid solution slurry was washed into the digestion tubes with the volume of acid allotted to that digest. Concentrated $HNO_3$ (10 ml) was added to the vessels. They were then sealed and placed into the microwave rotation apparatus and the programme started (Appendix 2.4).

Following digestion, tubes were allowed to cool and filtered through Whatman No. 542 filter papers into 50 ml centrifugation tubes for storage. Samples were diluted with 10 ml Ultra pure $H_2O$ before storage at 5°C until reading by ICP-MS.

Table 2.10: Extraction efficiency of two $HNO_3$ digest methods (block digest and microwave digest). U concentration read by ICP-MS on digest liquid samples (df 14)

| **Soil** | **Concentration of U solution 2.5 ml applied to 10 g soil** | **U extracted by different digest methods mg g$^{-1}$ dry soil** | | **Efficiency of extraction (extracted - blanks) %** | |
|---|---|---|---|---|---|
| | | Block | Microwave | Block | Microwave |
| Loamy Sand | Blank | 0.000644 | 0.000691 | | |
| Loamy Sand | 0.5 mg L$^{-1}$ | 0.000727 | 0.000772 | 67.11 | 65.51 |
| Loamy Sand | 5 mg L$^{-1}$ | 0.001885 | 0.002055 | 102.24 | 112.64 |
| Loamy Sand | 50 mg L$^{-1}$ | 0.012706 | 0.013232 | 99.03 | 102.93 |
| F | | 1755.924 | | 1.658 | |
| p | | <0.001 | | <0.5 | |
| Clay Loam | Blank | 0.000756 | 0.000664 | | |
| Clay Loam | 0.5 mg L$^{-1}$ | 0.000925 | 0.00072 | 142.04 | 46.99 |
| Clay Loam | 5 mg L$^{-1}$ | 0.002304 | 0.002122 | 128.90 | 121.24 |
| Clay Loam | 50 mg L$^{-1}$ | 0.014268 | 0.012547 | 113.73 | 99.98 |
| F | | 100.739 | | 5.027 | |
| p | | <0.001 | | <0.05 | |
| Silty Clay | Blank | 0.000743 | 0.000687 | | |
| Silty Clay | 0.5 mg L$^{-1}$ | 0.000929 | 0.000941 | 149.28 | 205.22 |
| Silty Clay | 5 mg L$^{-1}$ | 0.002171 | 0.002034 | 117.14 | 110.55 |
| Silty Clay | 50 mg L$^{-1}$ | 0.01467 | 0.015573 | 113.98 | 122.09 |
| F | | 191.557 | | 12.098 | |
| p | | <0.001 | | <0.001 | |
| Silty Clay Loam | Blank | 0.00019 | 0.00021 | | |
| Silty Clay Loam | 0.5 mg L$^{-1}$ | 0.00029 | 0.00031 | 84.17 | 81.93 |
| Silty Clay Loam | 5 mg L$^{-1}$ | 0.00146 | 0.00141 | 104.33 | 98.39 |
| Silty Clay Loam | 50 mg L$^{-1}$ | 0.01123 | 0.01162 | 92.22 | 95.19 |
| F | | 178.137 | | 3.485 | |
| p | | <0.001 | | <0.05 | |

Though extraction at low concentrations (0.5 ppm) was below 80%, other extraction efficiencies using the microwave extraction method were acceptable.

### 2.6.5 Uranium extraction by acid digest (total) by hot plate (BGS) using $HNO_3/HF/HClO_4/H_2O_2$

The experiments on self diffusion of U isotopes (Chapter 4) were carried out at the Environmental Materials Facility at the British Geological Survey, Keyworth, Nottingham. Digestion of soil samples followed the BGS standard procedure of total digestion by hot plate using a series of acids including HF (Appendix 2.5).

Dried soil slices were sub-sampled (0.2500 g ± 0.0025 g). Samples were pre-digested with diluted $HNO_3$ and then attacked with a series of acids:

1. By heating to dryness on a hotplate with concentrated $HNO_3$, concentrated HF and $HClO_4$.
2. By reconstituting with diluted $HNO_3$ and then heating with $H_2O_2$.

The remaining solution was diluted with MilliQ water and stored before analysis by ICP-MS. Two reference materials were used to determine extraction efficiency: one an internal BGS soil and the second Reference Material JR2 (Tables 2.11 and 2.12)

Table 2.11: Extraction efficiency of Reference material JR2 over six individual digests. Two samples of JR2 were included in each digest run except for digest 6 where a reduced number of samples underwent digest

| | **$^{238}U$ (mg kg$^{-1}$)** | |
|---|---|---|
| Digest 1 | 12.2 | 10.8 |
| Digest 2 | 12.3 | 11.8 |
| Digest 3 | 12.7 | 11.7 |
| Digest 4 | 12.8 | 12.1 |
| Digest 5 | 11.7 | 13.5 |
| Digest 6 | 12.9 | |
| Mean ± SD | 12.2 ± 0.7 | |
| RSD% | 5.9 | |
| **Expected result from BGS (% Recovery)** | **11.6 (105.5)** | |
| **Official concentration (% Recovery)** | **10.5 (116.5)** | |

Table 2.12: Extraction efficiency of individual isotopes in reference material JR2 over six digests

| | **Mean $^{238}U$ /$^{234}U$** | **Mean $^{238}U$ /$^{235}U$** |
|---|---|---|
| Digest 1 | 15265 | 136.3 |
| Digest 2 | 17450 | 137.0 |
| Digest 3 | 18553 | 137.3 |
| Digest 4 | 15265 | 136.3 |
| Digest 5 | 17829 | 137.1 |
| Digest 6 | 17024 | 135.5 |
| Mean ± SD | 16898 ± 1361 | 136.6 ± 0.7 |
| RSD% | 8 | 0.5 |
| Expected (% Recovery) | **18225 (93)** | **137.9 (99)** |

### *2.6.7 Analysis of U by ICP-MS*

A method had to be developed for the ICP-MS for determining uranium concentrations. The length of time between samples when dilute acid was flushed through the sample tubing and nebuliser (wash time) was important, because the uranium was not as easy as the bromide to wash out of the tubing system between the auto-sampler and the nebuliser. Tests were run to calculate how long a wash was needed to ensure the concentration on the detector dropped three orders of magnitude before the next sample was introduced. Due to the dominance of $^{238}U$ isotope in the solution measured, the length of time the detector was programmed to spend collecting data at a particular mass (dwell time) was increased when it was counting $^{235}U$ and $^{234}U$.

Table 2.13: ICP-MS set-up for the determination of U in digested soil samples

| **Method parameter** | **$^{234}U$** | **$^{235}U$** | **$^{238}U$** |
|---|---|---|---|
| RF Power | 1500 W | | |
| Detector used | Dual | | |
| Dwell time | 150 ms | 75 ms | 50 ms |
| Integration time | 18000 ms | 9000 ms | 6000 ms |
| Scan mode | Peak hopping | | |
| Autosampler | Cetac ASX-500 | | |
| Sweeps per reading | 20 | | |
| Readings per replicate | 5 | | |
| Replicates per sample | 3 | | |
| Wash time between samples | 60 seconds at 48 rpm | | |
| Number of samples between standards | 20 | | |

Once a method had been established, digested samples were analysed. Throughout the intensive sample processing, the method was monitored to ensure it remained fit for purpose. Standards were made up from he U standard (Sigma Aldrich) and calibration curves were checked before the ICP-MS was cleared to begin processing samples, with associated maintenance of ICP-MS components should the calibration not prove satisfactory. Samples were ordered before analysis from predicted low to high concentration in order to avoid the flooding of the detector. By altering the dilution factor of samples predicted to contain a high concentration of U, it was possible to ensure they fell within the range measurable on the pulse detector.

## *2.7 Washing of glassware to reduce contamination*

All glassware and equipment was washed in Detcon, rinsed in deionised water and then placed in an acid bath for at least 8 h. The acid bath contained 5 % $HNO_3$ (Aristar grade) made up with ultra pure $H_2O$ (ELGA Purelab Ultra). Upon removal from the acid bath, the items were rinsed twice in ultra pure $H_2O$ and allowed to dry in the ICP-MS clean room.

A similar procedure was in place at the laboratories used at BGS. The standard method was to wash in Detcon, rinse in MilliQ $H_2O$, stand in an aqua regia solution (Aristar grade acid and MilliQ), triple rinse in MilliQ and then dry in a fume cupboard within a clean room.

### 2.8 Soil sterilisation

Soil sterilisation was carried out by gamma irradiation at Isotron PLC (Swindon, Wilts). Samples were given a dose of 25-40 kGy. Sterile soils were packed in sealed containers before transport to Swindon for sterilisation and stored in the same sealed containers at 5°C after sterilisation. Further details regarding the maintenance of sterility and the checks undertaken to ensure this are given in Chapter 5.

# Chapter 3: Diffusion of a non-sorbed solute

In a portion of soil in which there exists a concentration gradient of a particular solute, the solute's diffusion coefficient, $D$, is defined by the relation

$$F = -D\,\mathrm{d}C/\mathrm{d}x \tag{3.1}$$

where $F$ is the solute flux – i.e. the amount crossing unit section of the soil in unit time – and $\mathrm{d}C/\mathrm{d}x$ is the concentration gradient across the section. Equation (3.1) is an expression of Fick's first law.

For solutes that are substantially adsorbed on the soil solid, such as $UO_2^{2+}$ and other U species, quantifying $D$ is complicated by the need to measure the distribution of the solute between the soil solid and solution, $\mathrm{d}C_L/\mathrm{d}C$, under the conditions of the diffusion system being investigated. Often $\mathrm{d}C_L/\mathrm{d}C$ for a particular solute species varies sensitively with $C$, pH, redox, and other factors.

With reference to equation 3.1, if diffusion on the soil solid is unimportant, as in general it will be for most solutes (Nye, 1979; Tinker & Nye, 2000), then

$$D = D_L\,\theta_L f_L\,d\mathrm{C}_L/d\mathrm{C} \tag{3.2}$$

where: $D_L$ is the diffusion coefficient of the solute in free solution,
$\theta_L$ is the fraction of the soil volume occupied by water,
$f_L$ is the impedance factor,
$C_L$ is the concentration of the solute in the soil solution, and
$C$ is the concentration in the whole soil.

For a non-adsorbed solute, $C = \theta_L C_L$ and hence $\theta_L\mathrm{d}C_L/\mathrm{d}C = 1$, and so $D = D_L f_L$, and the problem is greatly simplified. Thus the diffusion of a non-adsorbed solute with a known $D_L$ through a soil can be used to determine the impedance factor ($f_L$) for that particular soil. Once $f_L$ is determined for a particular soil it can be applied to the diffusion of any solute through that soil. Consequently the first step in determining the diffusion of uranium species through different soil types was to measure the diffusion impedance factor.

### *3.1 Method*

The method for measuring diffusion impedance was developed from that of Pinner & Nye (1982). The basic principle is to apply a pulse of a non-adsorbed solute on the surface of a moist block of the soil of interest, allow diffusion to occur, and then extract the tracer from thin slices of the soil taken perpendicular to the axis of diffusion. This allows the determination of the profile of tracer concentration versus distance diffused through the soil.

The basis of Pinner & Nye (1982)'s method is that the distribution at time $t$ of a non-adsorbed solute deposited instantaneously on the planar surface of a semi-infinite soil column is given by the equation

$$\ln \frac{C}{C_0} = - \frac{x^2}{4D_{\mathrm{L}} f_{\mathrm{L}}\, t} \tag{3.3}$$

where $C$ is the concentration at distance $x$ from the surface and $C_0$ is the concentration at the surface. Thus a graph of ln $C$ against $x^2/(4D_{\mathrm{L}}t)$ should have slope $-1/f_{\mathrm{L}}$ and intercept ln $C_0$.

The development of the method is described in the following sections. In the original method, Pinner & Nye (1982) used the radioactive tracer $^{36}\mathrm{Cl}^-$ self-diffusing against $^{35}\mathrm{Cl}^-$ in the soil. In the present work, to avoid using $^{36}\mathrm{Cl}$, trace concentrations of $\mathrm{Br}^-$ were used, counter-diffusing against $\mathrm{Cl}^-$ in the soil. It was considered this would satisfy the requirements of the theory if sufficiently small concentrations of $\mathrm{Br}^-$ were used, as there would then be no complications of salt diffusion. Using an ICP-MS allowed the detection of Br at concentrations of ng $\mathrm{l}^{-1}$.

A number of small experiments were made to determine the optimal soil bulk densities and water contents for the impedance measurements.

#### *3.1.1 Diffusion of bromide against chloride ions in soil*

Four soils were assessed for diffusion impedance properties using a bromide pulse. It was decided to use 3 cm diameter collars packed with soil and sliced using a miniature microtome capable of producing slices of less than 0.5 mm. Following on from pilot studies to establish the best technique for packing the soil collars (Chapter 2), method development was undertaken to determine the most effective Br tracer addition and recovery technique. A number of tests were run with different amounts of tracer and diffusion periods (Table 3.1).

Table 3.1: Summarised method development carried out to determine the best method of applying Br to the soil collars in order to trace diffusion. Variables investigated included the amount of tracer to use in the pulse, the amount of time to leave the filter-paper containing the tracer in contact with the soil (pulse period) and the length of diffusion period after the pulse was applied. Some tests were only carried out on a single collar for time saving purposes, other experiments carried out on three replicates are accompanied by SEM values for bulk density and water content in brackets.

| **Soil Type** | **Bulk Density (g cm$^{-3}$)** | **Water content (mg g$^{-1}$)** | **Tracer added*** | **Pulse period** | **Diffusion period** |
|---|---|---|---|---|---|
| Silty Clay Loam | 1.31 | 230 | 0.1 ml of 10 $\mu$g L$^{-1}$ | 4 hrs | 20 hrs |
| Silty Clay Loam | 1.49 | 200 | 0.1 ml of 10 $\mu$g L$^{-1}$ | 5 mins | 5 hrs |
| Sandy Silt Loam† | 1.78 | 110 | 0.1 ml of 10 $\mu$g L$^{-1}$ | 5 mins | 5 hrs |
| Sandy Silt Loam† | 1.91 (0.03) | 104 (0.4) | 0.1 ml of 10 mg L$^{-1}$ | 5 sec | 2 hr |
| Silty Clay Loam | 1.64 (0.02) | 231 (0.07) | 0.1 ml of 100 mg L$^{-1}$ | 5 sec | 2 hr |
| Silty Clay | *Not recorded* | | 0.1 ml of 100 mg L$^{-1}$ | 5 sec | 2 hr |
| Clay Loam | 1.44 (0.01) | 239 (0.63) | 0.1 ml of 100 mg L$^{-1}$* | 5 mins | 2 hrs |
| Loamy Sand | 1.67 (0.08) | 191 (0.79) | 0.1 ml of 100 mg L$^{-1}$ | 5 mins | 4 hrs‡ |
| Loamy Sand | 1.58 (0.03) | 209 (1.34) | 0.3 ml of 1000 mg L$^{-1}$ | 5 mins | 4 hrs‡ |
| Clay Loam | 1.30 | 250 | 0.3 ml of 1000 mg L$^{-1}$ | 5 mins | 2 hrs‡ |
| Loamy Sand | 1.55 | 210 | 0.3 ml of 1000 mg L$^{-1}$ | 5 mins | 2 hrs‡ |
| Silty Clay | 1.18 | 270 | 0.3 ml of 1000 mg L$^{-1}$ | 5 mins | 2 hrs‡ |

* From this example on, filter paper was used to apply the Br to the collar.
† Hand texture only, no particle size analysis carried out.
‡ A clear decrease in bromide away from the source was seen.

The amount of $Br^-$ in the pulse needed to be sufficient to provide a measurable $Br^-$ concentration profile through the soil, but not so large that it significantly increased the salt concentration in the soil solution near the labelled surface, thereby inducing salt diffusion of $Br^-$ into the soil, rather than counter-diffusion against $Cl^-$ with no significant movement of cations. After trying 0.1 ml of a 10 µg $L^{-1}$ solution of calcium bromide, and it was concluded that 0.3 ml of 1000 mg $L^{-1}$ bromine solution was needed to ensure a clear diffusion profile away from the source. The maximum $Br^-$ concentration found in soil in the penultimate test of the method on three soils (Table 3.1) was 25 µg $g^{-1}$. With a bulk density of 1.0 g $cm^{-3}$ and volumetric water content ($\theta_L$) of 0.33 $cm^3$ $cm^{-3}$, and atomic weight of bromine = 79.9 g $mol^{-1}$, this is equivalent to

$$[Br^-_L] = 25 \times 1.0 / (79.9 \times 0.33) \approx 1 \text{ mM}$$

This is less than the concentration of chloride ions in the soil solution ($[CaCl_2]$ = 10 mM) by at least a factor of 10:

$$[Cl^-_L] \approx 20 \text{ mM}$$

So the conditions required for simple counter-diffusion are not violated.

Different combinations of pulse and time allowed for the tracer to diffuse were tried (Table 3.1). Diffusion theory states the spread varies approximately with the square root of time, so calculating from these results a run of 2 h was predicted to produce a profile within the zone sectioned. It was therefore decided to select a period of 2 h as this allowed more collars to be processed in a 24 h period and was therefore more practical.

Collars of the loamy sand (LS), clay loam (CL) and silty clay (SC) were packed to a target bulk density of 1.4 g $cm^{-3}$. The silty clay loam (SCL) was packed to a target bulk density of 1.1 g $cm^{-3}$ as it was impossible to pack this soil any more compactly when dry. The base of the collars was covered with parafilm to prevent soil falling out during transportation, and pierced four times in quarters to allow liquid movement. The collars were then moistened from below to 200 mg $g^{-1}$ water content using 0.01M $CaCl_2$ (made with Ultra-Pure $H_2O$) and then left overnight in a water-saturated atmosphere. Additional $CaCl_2$ solution was applied to the silty clay loam (400 mg $g^{-1}$) as this was needed to ensure the moisture reached the soil surface.

A pulse of 0.3 ml of a 1000 ppm $Br^-$ solution was applied to a piece of filter paper cut to the diameter of the soil surface and held in contact with the soil surface for 5 minutes. On removal, the filter paper was weighed and shaken in a glass bottle with 10 ml of 0.01 M $CaCl_2$ for 1 h. The pulsed soil collars were placed in a water-saturated atmosphere for 2 h to allow diffusion of $Br^-$ into the soil.

Using the microtome, each collar was sliced into 20 slices of c. 0.5 mm thickness. Immediately after cutting the mass of slices was recorded and they were placed in glass bottles containing 10 ml of 0.01M $CaCl_2$. These were then shaken on a side to side shaker for 1 h, before being centrifuged and the supernatant filtered through Whatman 542 filter paper. This filtrate was refrigerated and later analysed for Br concentration by ICP-MS.

The mass of the soil remaining in the collar was measured. This soil was then oven dried for 24 h and the mass measured again in order to determine water content.

In order to draw up a budget of Br within the collar, the remaining soil was shaken with 250 ml 0.01 M $CaCl_2$ for 1 h, centrifuged and filtered in the same way as the slices. Samples were made up into solutions analysis by ICP-MS (Table 3.2).

Table 3.2: Dilution of filtrate for ICP-MS analysis: br extracted from soil slices and the filter-paper used to apply the pulse to the soil.

| | **Sample volume (ml)** | **Volume of 1000 ppb solution of Rh internal standard (ml)** | **Volume of digestion media (ml)** | **Consequent range of bromide concentration (mg $L^{-1}$)** |
|---|---|---|---|---|
| Slice extract | 1 | 0.1 | 0.1 | 0.01 – 0.5 |
| Filter paper extract | 0.1 | 0.1 | 0.1 | 0.1-0.15 |

### *3.2 Results*

Average water contents and bulk densities in the four experimental soils are given in Table 3.3. The differences are due to the different packing properties of the soils.

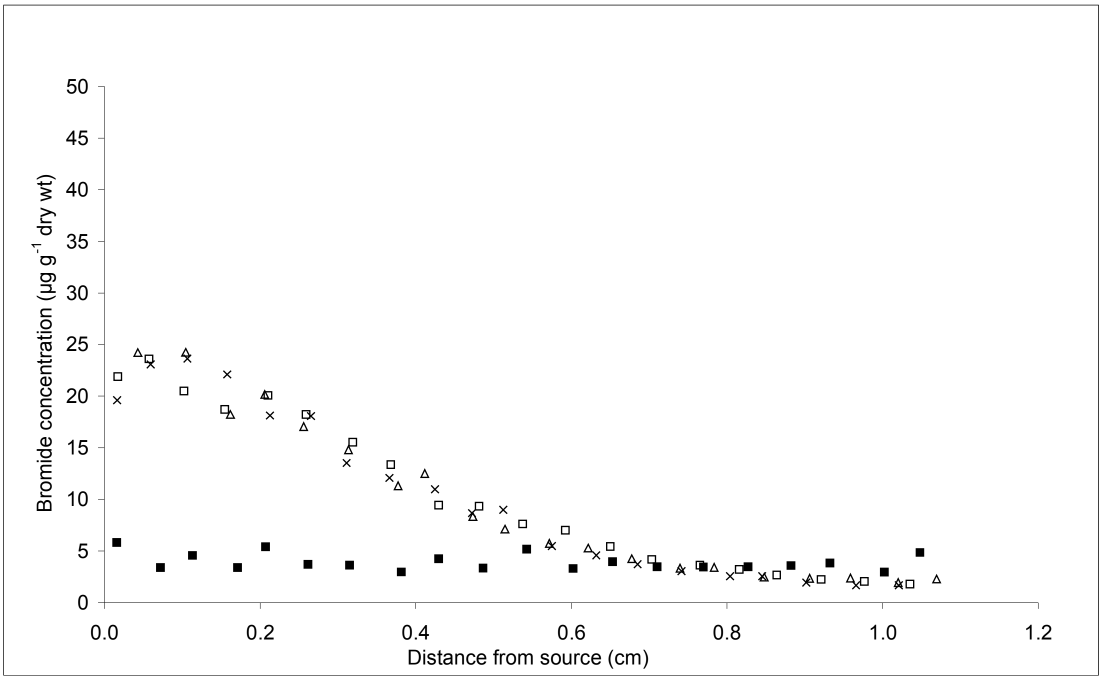
Table 3.3: Bulk densities and water contents (gravimetric and volumetric) in packed soil collars for the impedance experiments. SEM values included in brackets (df=3)

| **Soil** | **Bulk density ($\rho$) (g cm$^{-3}$)** | **Water content ($\theta$) (cm$^3$ g$^{-1}$)** | **Volumetric water content ($\theta_L$) (cm$^3$ cm$^{-3}$)** |
|---|---|---|---|
| Loamy Sand | 1.30 (0.01) | 0.19 (0.01) | 0.25 |
| Clay Loam | 1.27 (0.01) | 0.21 (0.01) | 0.27 |
| Silty Clay | 1.22 (0.01) | 0.25 (0.01) | 0.31 |
| Silty Clay Loam | 0.97 (0.00) | 0.31 (0.00) | 0.30 |
| F | 308.18 | 1778.56 | |
| p | <0.001 | <0.001 | |

Following analysis of Br in the soil slices, Br concentrations were plotted against depth for each soil (Figures 3.2.1, 3.2.2). There was clear evidence that Br$^-$ was transferred from the pulse on the filter paper to the soil surface and diffused down through the soil profile. All soils showed Br$^-$ diffusion over 2 h to a depth of around 1 cm.

The total quantity of Br$^-$ recovered from the soil was calculated from the sum of the amounts in the individual soils slices. This was compared to the amount added in the original pulse (minus the amount remaining on the filter paper that had been used to apply the pulse). A similar amount was found on the filter paper as in the soil sliced and this was approximately two thirds of the amount added in the original pulse. The remainder evidently diffused into the portion of the soil core not sliced.

(a)



(b)

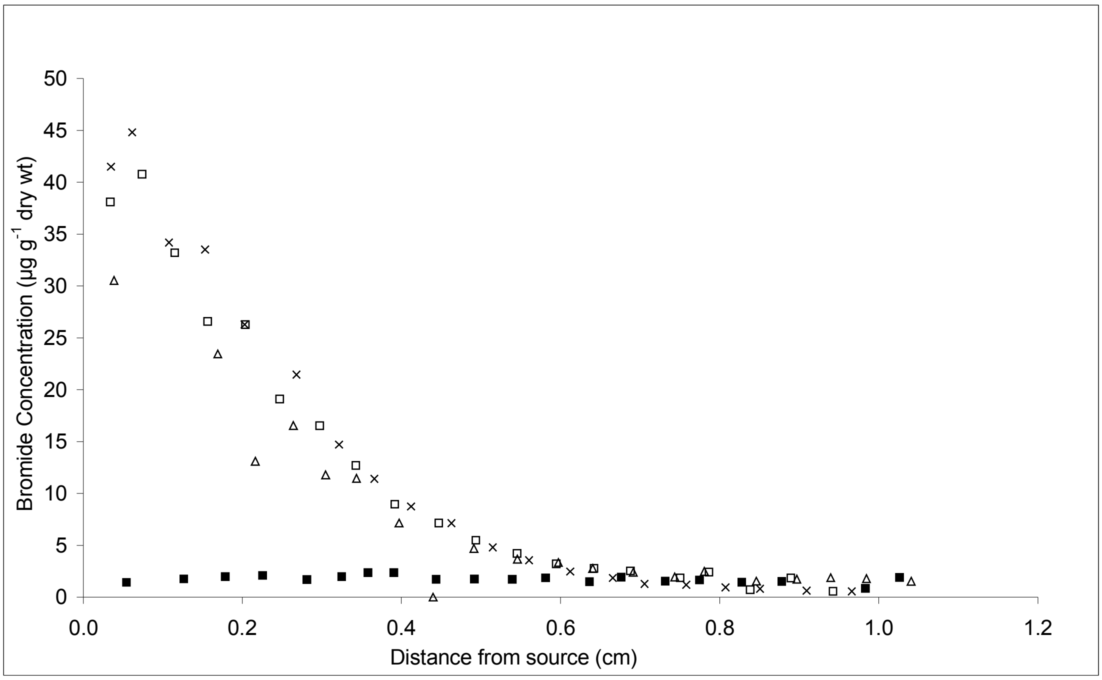
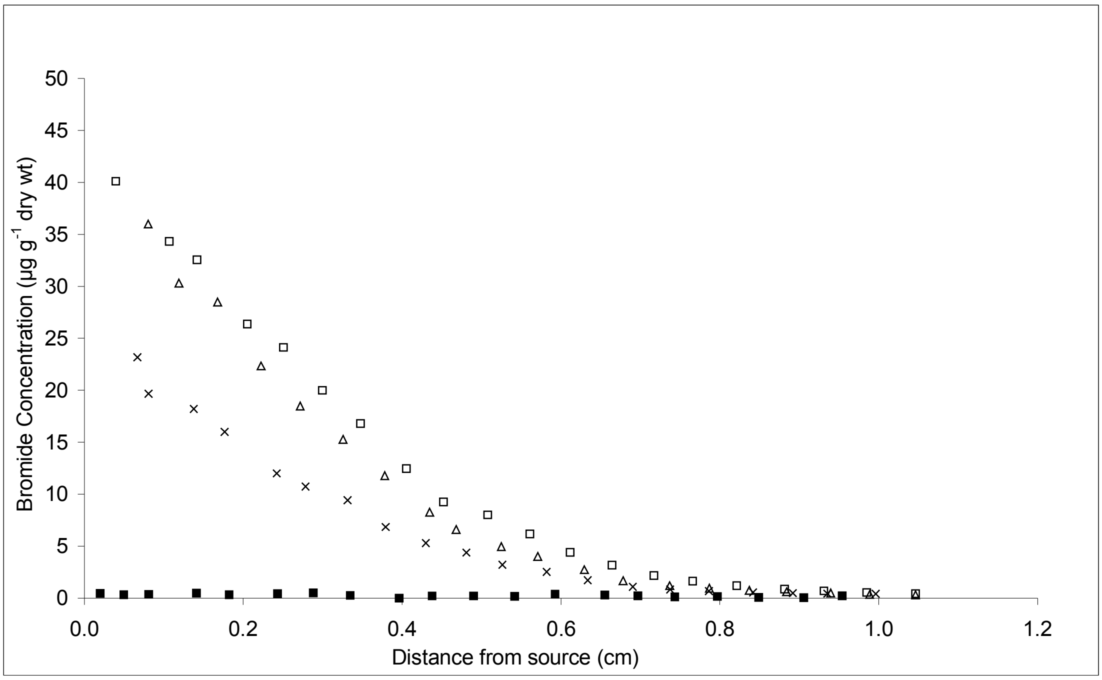


Figure 3.2.1: Bromide concentration distance profiles for two soils (a) Sandy loam (b) Clay loam. Under standard conditions, *t* = 2 hours. For other parameters see Table 3.3 (symbols: □, Δ, x three replicate collars pulsed with Br, ■ collar pulsed with $H_2O$)

(a)



(b)

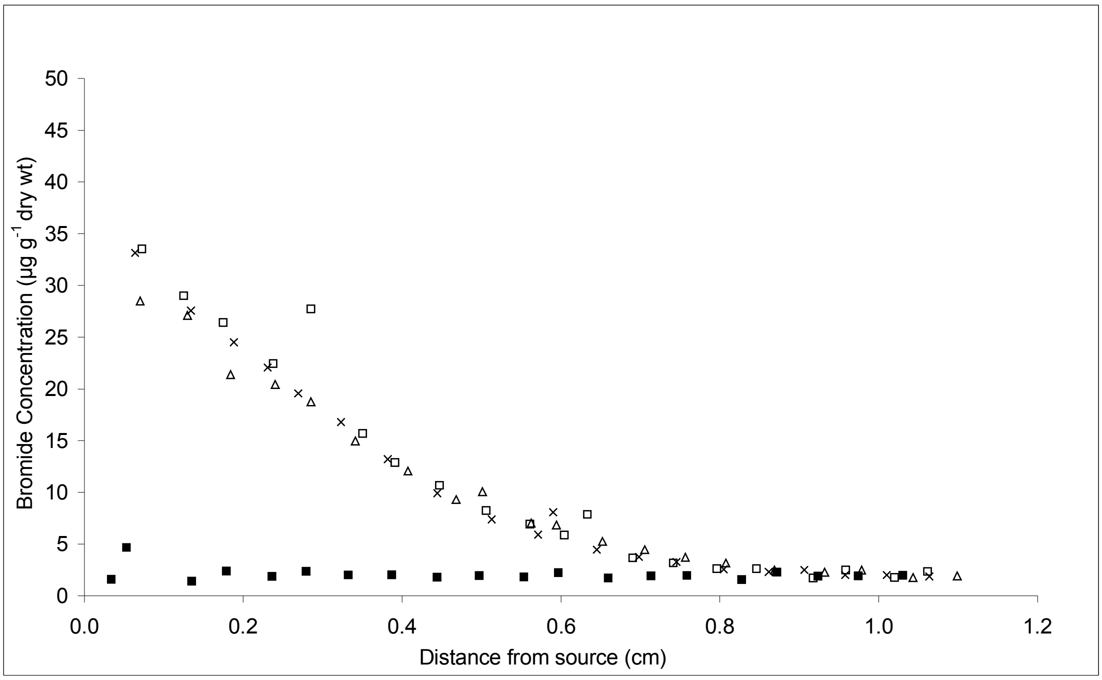


Figure 3.2.2: Concentration distance profiles for two soils (a) Silty Clay (b) Silty Clay Loam. Under standard conditions, *t* = 2 hours. For other parameters see Table 3.3 (symbols: □, Δ, x three replicate collars pulsed with Br, ■ collar pulsed with $H_2O$)

The concentration profiles were used to determine $f_L$. The average concentrations of Br at distance $x$ in non-pulsed soil (blanks) was subtracted from concentrations within the soil profiles. These adjusted values were used to calculate $f_L$ in accordance with Equation 3.3, re-arranged to:

$$\ln C = \ln C_0 - \frac{1}{f_L} \times \frac{x^2}{4D_L t} \qquad (3.4)$$

i.e. a plot of ln$C$ against $x_2/4D_Lt$ should produce a straight line of gradient $1/f_L$ (Figure 3.2.3). Where C is the concentration of diffusing substance and $x$ is the space coordinate measured normal to the section.

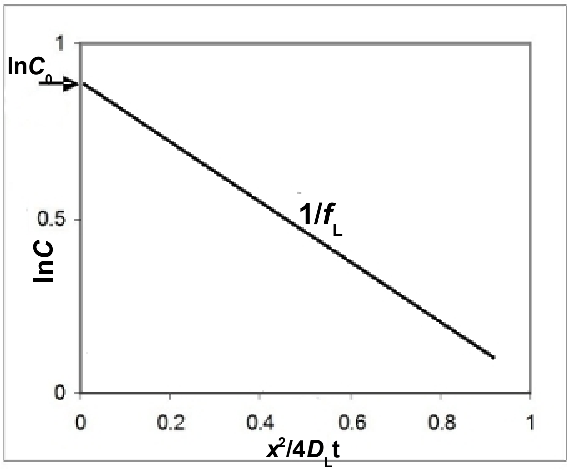


Figure 3.2.3 Representation of the method to calculate $f_L$ using ln$C$ and $x_2/4D_Lt$

The value of $D_L$ used was that for $Br^-$ counter-diffusing against $Cl^-$ under the conditions of the experiments (2.08 x $10^{-5}$ $cm^2$ $s^{-1}$). Due to the electric potential between the two diffusing ions, the faster one tends to be slowed down by the slower and vice versa, so that the flux of each ion is the sum of the diffusion due to its own concentration gradient and that due to the gradient of the diffusion potential arising from differences in the mobilities of the ions.

Equation (3.1 relating to Flux) is therefore modified (Nye, 1979):

$$F_{LA} = -D_{LA}\frac{dC_{LA}}{dx} + Z_A C_{LA} D_{LA} \frac{\sum D_{Li}\, dC_{Li}/dx}{\sum Z_i^2 D_{Li} C_{Li}}\,. \qquad (3.5)$$

where subscripts $_A$ and $_i$ refer to the ion of interest and all diffusing ions respectively, and $Z$ represents the ion's charge. Equation (3.5) shows that the effect is small for ions whose concentrations are small compared with the total solution concentration. Hence the counter-diffusion coefficient of $Br^-$ and $Cl^-$ in the microcosm used here should be close to that of the minor component, $Br^-$, which is $2.08 \times 10^{-5}$ $cm^2$ $s^{-1}$. The plots used in the calculation of $f_L$ are shown in Figure 3.2.4.

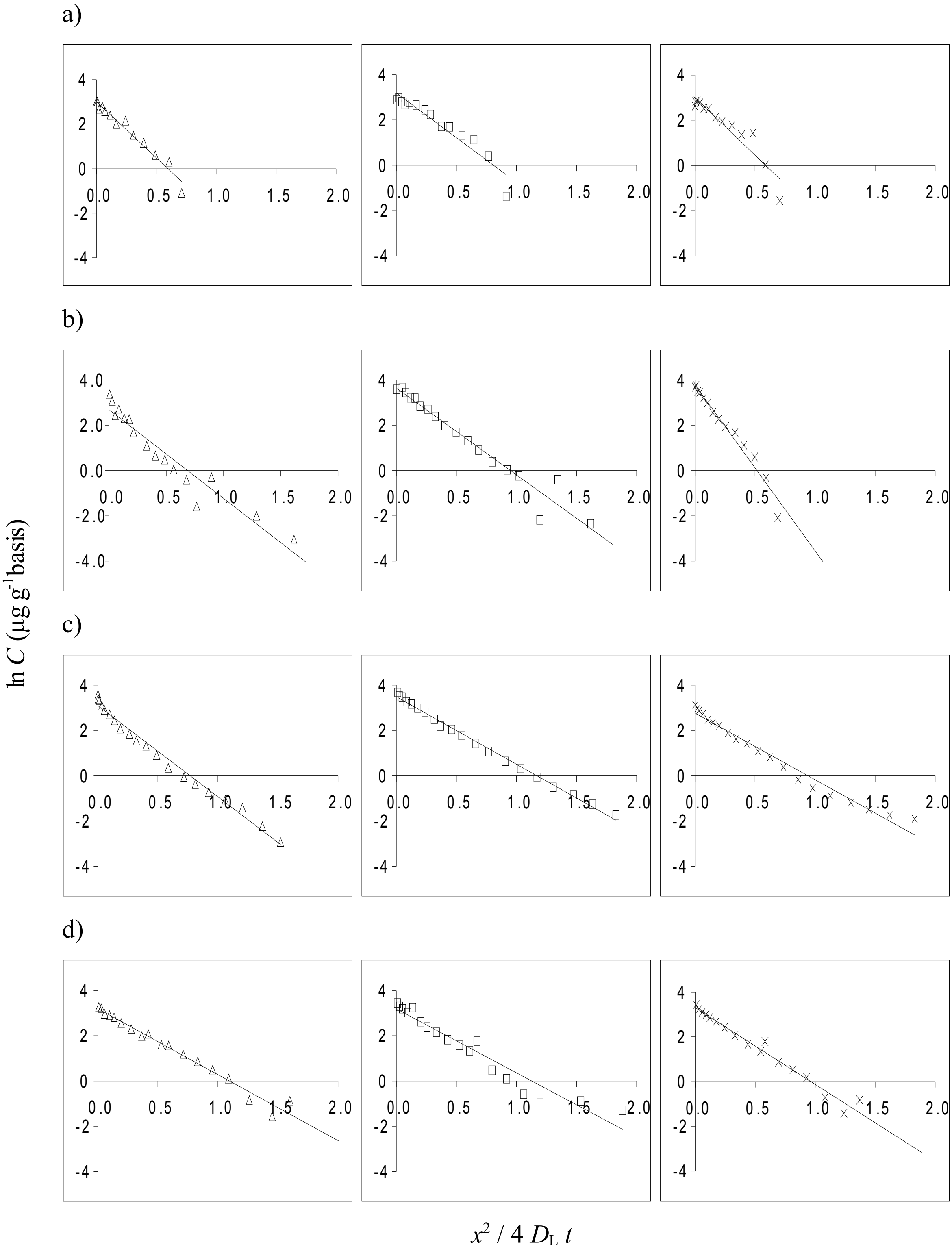


Figure 3.2.4: Plots of ln$C$ against $x^2/4D_Lt$ using data from Figures 3.2.1 and 3.2.2 used in the calculation of $f_L$ a) Sandy loam, b) Clay loam, c) Silty clay, d) Silty clay loam

The gradients differed between the soils (Figure 3.2.5). The calcareous soils (silty clay and silty clay loam) had a less steep gradients than the non-calcareous soils (loamy sand and clay loam). The soil with lowest clay content (sandy loam) had the steepest gradient.

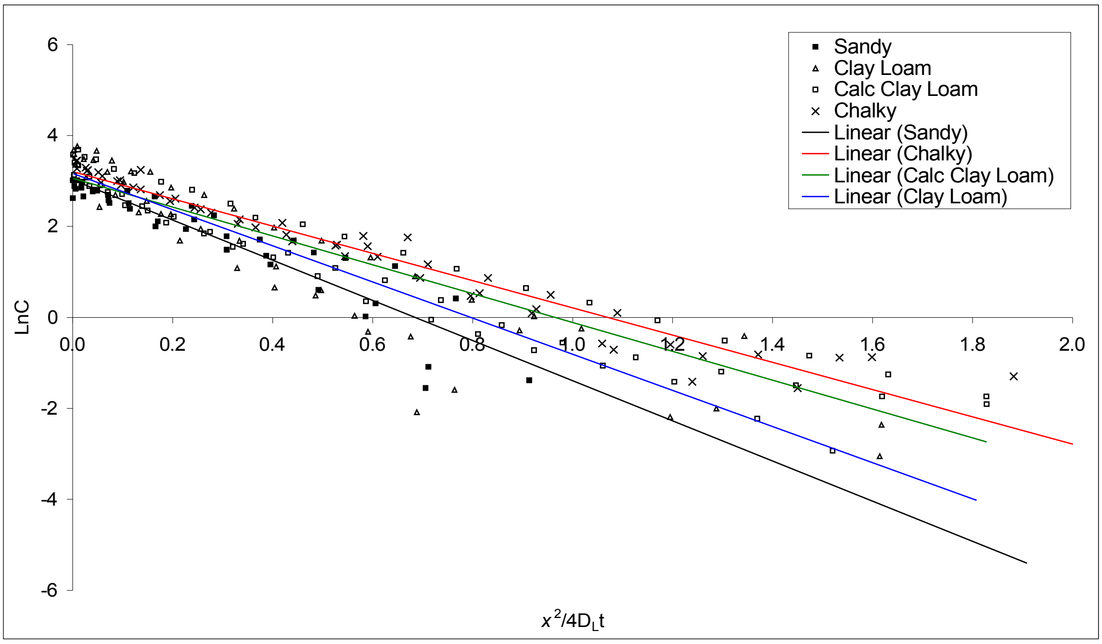


Figure 3.2.5: Plots of ln$C$ against $x^2/4D_Lt$ with linear fit ($1/f_L$) calculated from the amalgamated data from three replicates.

The different gradients for each replication were averaged and the $R^2$ values for the slope were also averaged. Table 3.4 also includes bulk density and water content from table 3.3 as both these variables have an effect on diffusion.

Table 3.4: Calculated $f_L$ and for the four soils under specific bulk density and water content conditions

| | **Bulk Density ($g\ cm^{-3}$)** | **Water Content ($mg\ g^{-1}$)** | **Gradient ($1/f_L$)** | $f_L$ | **Variance** | **$R^2$** |
|---|---|---|---|---|---|---|
| Loamy Sand | 1.30 | 190 | 4.8220 | 0.2074 | 0.38 | 0.92 |
| Clay Loam | 1.27 | 210 | 4.8345 | 0.2068 | 3.70 | 0.95 |
| Silty Clay | 1.22 | 250 | 3.1914 | 0.3133 | 0.35 | 0.98 |
| Silty Clay Loam | 0.97 | 310 | 2.9422 | 0.3399 | 0.10 | 0.96 |

Comparing the different soils, the impedance factor tended to increase (i.e. diffusion less impeded) as the volumetric water content increased (sandy loam versus silty clay).

At a given volumetric water content, it tended to increase as the bulk density decreased (silty clay versus silty clay loam). These broad trends are in agreement with published findings for other soils (Nye, 1979; So & Nye, 1989; Olesen *et al.*, 2001; Kirk *et al.,* 2003).

### 3.3 Discussion

The impedance factor accounts primarily for the geometry of the soil pore network but also for ion exclusion from narrow pores by negative adsorption and for the increased viscosity of water near charged surfaces (Nye, 1979). The latter effects are likely to be most important at small water contents and in soils with large CECs. Hence the smaller $f_L$ in the Silty Clay compared with the Silty Clay Loam at similar $\theta_L$, may in part reflect the Silty Clay's much larger CEC (21.2 versus 6.7 $\mathrm{cmol_c\ kg^{-1}}$). However the difference also reflects the greater bulk density of the Silty Clay.

At a given volumetric water content, $f_L$ tends to be smaller in clay soils than in sandy soils because a greater proportion of the soil water is in fine pores (Nye, 1979). But at a given water potential, $f_L$ is larger in clayey than sandy soils because they hold more water (So & Nye, 1989). These effects may account for the similar $f_L$ values in the sandy loam and the clay loam at similar $\rho$ and $\theta_L$, in spite of their differing textures.

Anion exclusion (Nye 1979) may also be a factor. Due to electrostatic repulsion from negatively charged solid surfaces, anions are displaced away from pore edges. Hence the cross-sectional area for diffusion is decreased. This is known as anion exclusion (Gvirtzman & Gorelick, 1991) and may account for the lower impedance seen in the calcareous soils (higher $f_L$ means diffusion less impeded).

### 3.4 Conclusion

The methods developed here for measuring diffusion impedance are satisfactory and the results obtained for the four experimental soils are in broad agreement with theoretical expectations and findings for other soils. The experimental soils are therefore suitable for experiments on the details of uranium diffusion in soil using related methods.

# Chapter 4: Diffusion of uranium in soil

The determination of the diffusion impedance factor ($f_L$) of the experimental soils by measuring the counter diffusion of bromide ions against chloride ions was described in Chapter 3. As stated in Chapter 3 because chloride and bromide ions are largely not adsorbed on soil surfaces, the impedance to their diffusion through the soil pore network can be calculated from diffusion measurements using simple diffusion equations. But for ions that are sorbed on soil surfaces, as uranium ions are, the situation is more complicated. This chapter deals with the additional complications arising from this.

There are several complications:

1. Unlike bromide and chloride, uranium is present in the soil solution as several species, particularly as complexes with hydroxyl and carbonate ions. Depending on the pH of the soil solution and the partial pressure of $CO_2$ in the soil air, the dominant species may be positively charged (e.g. $UO_2^{2+}$), neutral (e.g. $UO_2(OH)_2$, $UO_2CO_3$) or negatively charged (e.g. $UO_2(OH)^{3-}$, $UO_2(CO_3)_2^{2-}$) (Figure 4.0.1).

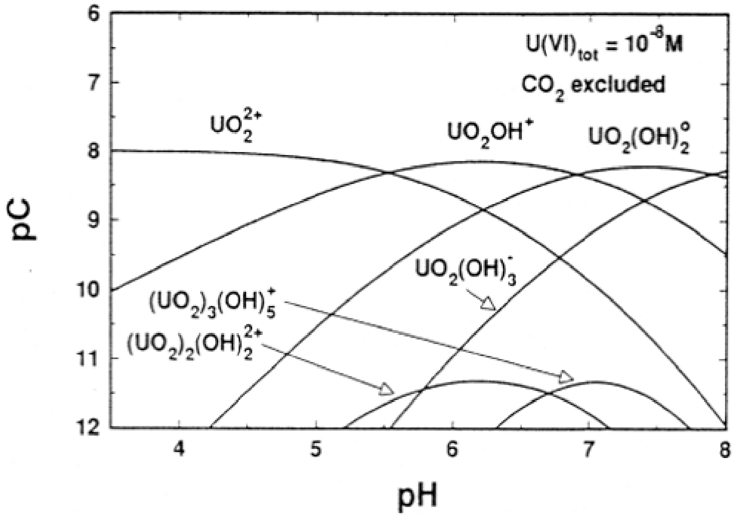


Figure 4.0.1: Distribution of major U(VI) species in the absence of $CO_2$ ($I = 0.1$). pC (-log concentration) of species as a function of pH. Speciation of U(VI) at a total dissolved concentration of $10^{-8}$ M (from Waite *et al.*, 1994)

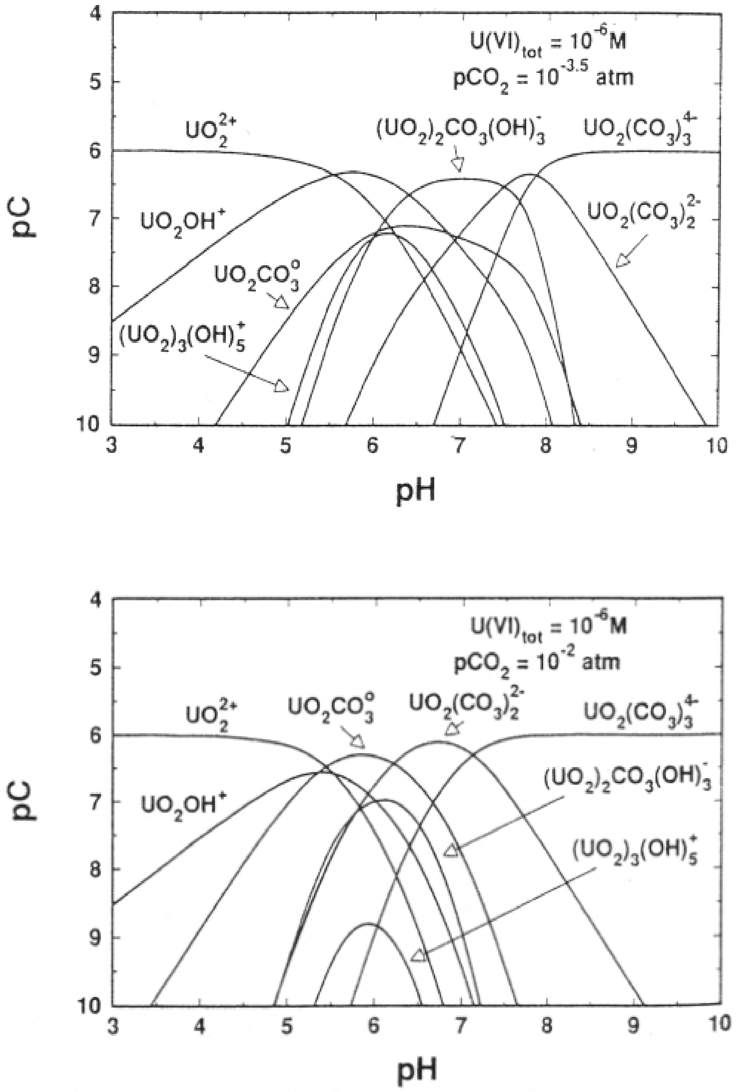


Figure 4.0.2: Dissolved speciation of U(VI) at a total concentration of $10^{-6}$ in an open system equilibrated with (a) a partial pressure of $CO_2$ of $10^{-3.5}$ atm or (b) a partial pressure of $CO_2$ of $10^{-2}$ atm. Ionic strength = 0.1. pC –log concentration) of species as a function of pH (from Waite *et al*., 1994)

These different uranium species interact in different ways with soil surfaces, and the interactions also depend on the pH and pressure of $CO_2$ because they affect the charge on soil surfaces.

2. While simple ion exchange reactions are very rapid in comparison with diffusion through the soil, and so can be considered effectively instantaneous and at equilibrium on the timescale of diffusion, some types of reaction are much slower, particularly those involving strongly-sorbed solutes, such as uranium (Sposito, 2008). This means it may

be necessary to include additional terms in the diffusion equations as in Equation (3.5) in Chapter 3.

3. The access of uranium to sorption sites on surfaces within soil particles by diffusion through narrow pores may also be slow in comparison with diffusion through the main soil pore network. Such access effects will differ between ions. For example some exchangeable cations apparently are mobile on soil surfaces, so equilibrium between intra- and extra-particle pores is likely to be enhanced compared to immobile ions (Nye and Staunton 1994). Also ion exclusion by repulsion from negatively charged surfaces will be less important for cations than anions. Again these effects would mean more complicated diffusion equations would be required.

A way of studying such slow micro-scale equilibration processes is to follow the self diffusion and exchange of isotopes in soil (Pinner & Nye, 1982; Nye & Staunton, 1994). With self diffusion there is no net exchange of sorbed species and so the sorption reactions are necessarily linear. The varying concentration-, pH- and $CO_2$-dependence of sorption inherent in bulk diffusion and net exchange are thereby avoided. The results can be analysed with simple solutions of the diffusion equations to test for slow equilibration processes. As discussed in Chapter 1, three isotopes of uranium occur naturally: $^{238}U$ (99.28%), $^{235}U$ (0.71%) and $^{234}U$ (0.006%). It is possible to obtain sources of uranium with manipulated isotopic ratios and consequently it is possible to set up a system to follow the self diffusion of uranium isotopes against each other.

This chapter will describe experiments on the self diffusion of $^{235}U$ against $^{238}U$ in soil columns to assess the importance of the slow equilibration processes listed above and also measurements of uranium sorption under the conditions of the self-diffusion experiments. Data from the uranium sorption experiments will be used to interpret the self diffusion results. Finally the chapter will describe experiments on the effects of $CO_2$ pressure on uranium sorption and discuss the consequences for diffusion.

It was hypothesised that the rates of diffusion of uranium through the four experimental soils would be measurably different and that the diffusion measured would be explainable from separately measured parameters of the soil uranium diffusion coefficient. The basis of the experiments is that for a pulse application of a solute on a column of soil (as for the experiments on $Br^-$ diffusion in Chapter 3), if local equilibration with the soil is rapid, a plot of ln $C$ against $x^2/t$ (where $C$ is concentration, $x$ is distance perpendicular to the application surface and $t$ is time) should be linear, but if equilibration is slow the plot should be curved (Pinner & Nye, 1982).

In order to independently measure the solid : solution distribution of U in the experimental soils under conditions similar to those in the self-diffusion experiments, experiments in shaken soil suspensions were made.

To probe the effects of soil microbes on the solid : solution distribution of U via their effects on local $CO_2$ production and therefore pressure, a further set of experiments was made in shaken suspensions with different $CO_2$ pressures. It was hypothesised that, aside from the reduced sorption of neutral carbonate species of U compared with the divalent cation $UO_2^{2+}$, higher $CO_2$ pressures could cause some local acidification of the soil. The possible effects of this include the alteration of U species in solution and a change in soil surface charge (an increase in $H^+$ ions would compete with the uranium for sorption sites), both of which would alter U sorption. There was also additional interest into whether such localised effects would vary between the soils as a result of differences in such variables as pH and organic matter, clay and carbonate contents.

## *4.1 Methods*

### *4.1.1 Self diffusion of $^{235}U$ against $^{238}U$*

An experiment was carried out to determine the self diffusion of $^{235}U$ against $^{238}U$ in the sandy loam, clay loam, silty clay and silty clay loam soils used in Chapter 3. The work was carried out at the British Geological Survey at Keyworth, Nottinghamshire. In brief, portions of the four experimental soils were equilibrated with non-enriched uranium by a process of soil washing. They were then packed into columns and sterilised to avoid complications of biological activity. These microcosms were then pulsed with $^{235}U$ enriched uranium at one end. After 28 d the soil was sectioned parallel to the application surface and the uranium concentrations ($^{235}U$ and $^{238}U$) in each slice measured by digestion and ICP-MS. The details follow.

A solution of slightly depleted uranium (95.440% $^{238}U$, 4.5167% $^{235}U$ and 0.0369% $^{234}U$) 1000 ppm in 2 % $HNO_3$ was purchased from Sigma Chemicals UK, Poole Dorset. The uranium source was diluted to 300 ppm with ultra pure $H_2O$ and the pH adjusted to pH 4 with NaOH solution.

The soils used had previously been air dried and sieved to 0.5 mm. Portions (150 g) of soil were weighed (2 dp) into 1 L conical flasks. This soil was mixed with 375 ml of the 300 ppm uranium solution (the silty clay loam soil was mixed with 400 ml of the solution to make up for its calculated lower sorption). The soil:solution mixture (uranium concentration c. 3.15 μmol $g^{-1}$ or 750 μg $g^{-1}$ soil) was shaken on a horizontal shaker for 24 h. The resulting slurry was filtered through Whatman No. 4 filter papers. The soil and filter papers were air dried, the soil removed from the filter paper and passed through a 0.5 mm sieve. The filtrate was kept and its uranium concentration determined.

The amount of uranium added to the soil in the washings prior to the diffusion experiments, estimated from the total volume and concentration of the wash solution, was 714 μg $g^{-1}$ or 3.0 μmol $g^{-1}$. The actual concentrations of $^{238}U$ measured by digestion of the washed soils are given in Table 4.1. Due to the small amount of uranium added to the system in the pulse application it was assumed that washed soil $U_{TOT}$ was likely to be equivalent to that of the $^{238}U$ in the pulsed soils.

Table 4.1: Total U concentrations in the soils measured by digestion

| **Soil** | **$U_{TOT}$ in washed soil (μmol $g^{-1}$)** |
|---|---|
| Loamy Sand | 3.41 |
| Clay Loam | 3.45 |
| Silty Clay | 2.69 |
| Silty Clay Loam | 2.88 |

The air dried and sieved soils were packed into soil collars to a depth of 1.5 cm at a bulk density of 1.3 g $cm^{-3}$ and moistened to a water content of 0.3 g $cm^{-3}$ as described in Chapter 2. Following packing, the soil microcosms were sterilised by gamma irradiation (as described in Chapter 2) to prevent any complications due to soil microbes. After sterilisation the microcosms were stored at 5ºC until taken to BGS for the self diffusion experiments 5 months later. Great care was taken to ensure the maintenance of sterility throughout the experiment with the use of laminar flow cabinets and sterile technique whenever soils were removed from their sterile experimental units and exposed to the environment. Sterilisation was confirmed by visual analysis only, at soil slicing, as the entirety of the soil microcosm was taken for digest analysis.

The soil microcosms were pulsed with a solution of uranium enriched in $^{235}$U (0.0369% $^{234}$U, 4.5167% $^{235}$U, 0.0069% $^{236}$U, 95.440% $^{238}$U). The original source (IRMM 187 0.004 mol U in 5 ml concentrated $HNO_3$) was diluted with utrapure $H_2O$ to 900 ppm and sufficient $CaCl_2$ was added to provide a solution of 10 mM $CaCl_2$. The pH was adjusted to pH 4 by drop-wise addition of 0.1 M NaOH.

Following the application method described in Chapter 2 soils were pulsed with 0.3 ml of the diluted uranium solution added to a filter paper and placed on the soil surface for 15 minutes. After pulsing, the columns were left for 28 days in a moisture saturated environment. At the end of the diffusion period soils were sliced perpendicular to the axis of diffusion into approximately 0.5 mm thick slices (c. 1 g soil) to a depth of 10 mm. Immediately following slicing the wet weights of slices were measured. The slices were then dried for 2 days (monitored until there was no change in measured weight) and dry weight taken. They were then digested and $^{238}$U concentrations and $^{235}$U/$^{238}$ U ratios measured in the digests as described in Chapter 2

### 4.1.2 Sorption of uranium by the soils

Soil (1.000 g) was weighed into 25 ml acid washed glass bottles and 3 ml of solution containing U concentrations of 0, 25, 50, 75, 150 300 and 600 ppm added (Table 4.2). These solutions were prepared from a 1000 ppm U standard in 2% $HNO_3$ diluted with 0.01 M $CaCl_2$ and adjusted to pH 4 by addition of NaOH. At this pH solutions were clear, but at higher pH they took on a yellow colour indicating U precipitation. The resulting additions of $H^+$ ions to the soil (0.3 µmol $H^+$ $g^{-1}$ soil) did not produce significant changes in the soil pH after shaking.

Five replicates were made for each U concentration. The soil and solution mixtures were shaken on a side to side shaker for 24 h, centrifuged at 9,000 ***g*** (2000 rev $min^{-1}$ or approx. 700 RCF) for 10 minutes (Falcon 6/300 Refrigerated Centrifuge) and the uranium concentration determined in 1 ml of the supernatant taken by pipette. Sub-samples of each flask containing dilution were taken and concentration measured by ICP-MS.

Table 4.2: Solutions used to measure U sorption in shaken suspensions. The original 1000 ppm solution is recorded as being 0.62% $HNO_3$ (70%) or 0.1 M.

| Uranium concentration in solution (ppm) | Volume of U stock solution (0.1 M $HNO_3$) (ml) | Volume of $CaCl_2$ solution (0.01 M) (ml) | Volume of NaOH solution (0.1 M) (ml) | $[NO_3^-]+[Cl^-]$ (mol $l^{-1}$) |
|---|---|---|---|---|
| 600 | 40 | 22.00 | 4.00 | 0.190 |
| 300 | 20 | 44.00 | 2.00 | 0.103 |
| 150 | 10 | 55.00 | 1.00 | 0.063 |
| 75 | 5 | 60.50 | 0.50 | 0.040 |
| 50 | 3.33 | 62.33 | 0.33 | 0.033 |
| 25 | 1.67 | 64.17 | 0.17 | 0.027 |
| 0 | 0 | 66.00 | 0.00 | 0.020 |

By subtracting the amount of uranium left in solution after the 24 h shake from the amount originally added to the soil, the amount sorbed to soil particles could be calculated, i.e.

$$[U_S]' = [U_{added}]' - R\,[U_L] \qquad 4.1$$

Where $R$ is the solution:soil ratio (i.e. 3 $cm^3\ g^{-1}$)

$[U_S]'$ the concentration of sorbed U (μmol $g^{-1}$)

$[U_{added}]'$ the concentration added (μmol $g^{-1}$)

$[U_L]$ the concentration of U in solution after shaking (μmol $cm^{-3}$).

Note that the superscripts (') indicate concentrations on a per unit soil mass basis. These were converted to a per unit soil volume basis by multiplying by the bulk density of the collars packed for the self diffusion experiment to get values in μmol $cm^{-3}$. The values of $[U_S]$ and $[U_L]$ are not independent of each other because one is calculated from the other, not from a total digest of the suspension. But the resulting bias is insignificant if most of the element is sorbed by the soil (Barrow, 2008).

The soil uranium diffusion coefficient is (from Equation 3.7):

$$D = \frac{D_L \theta_L f_L}{d[U]/d[U_L]} \qquad (4.2)$$

where [U] is the concentration of diffusing U in the soil (sorbed and in solution, mol $cm^{-3}$ soil) and $[U_L]$ the concentration in solution (μmol $cm^{-3}$) given by:

$$d[U]/d[U_L] = \theta_L + \rho\, d[U_S]/ d[U_L] \tag{4.3}$$

Therefore:

$$D = \frac{D_L \theta_L f_L}{\theta_L + \rho\, d[U_S]/d[U_L]} \tag{4.4}$$

As it had been previously determined to apply the information gathered about the buffering power of the four particular soils investigated to the self diffusion experiments (Section 4.1.1) equations 4.3 and 4.4 were not used. In self diffusion, the diffusing isotope is distributed between the soil solution and rapidly-equilibrating sorbed froms in the soil solid so that the specific activity is the same in both. Hence:

$$\frac{[U]}{[U_L]} = \frac{[^{235}U]}{[^{235}U_L]} = \frac{[^{238}U]}{[^{238}U_L]} \tag{4.5}$$

and

$$\frac{d[U]}{d[U_L]} = \frac{d[^{235}U]}{d[^{235}U_L]} = \frac{[U]}{[U_L]} \tag{4.6}$$

Therefore the self diffusion coefficient of U is

$$D_{self} = \frac{D_L \theta_L f_L}{[U]/[U_L]} \tag{4.7}$$

Hence $D_{self}$ can be calculated from a knowledge of $[U]/[U_L]$ (and $D_L$, $\theta_L$, $f_L$ obtained as in Chapter 3). With reference to the known possibility of slow equilibrium reactions within soil it must be noted that [U] is the concentration of U that is in rapid equilibrium with the U in soil solution. This is not necessarily the same as the total U concentration in the soil. In Section 4.2.2 values of $[U]/[U_L]$ are inferred from the self diffusion results and compared with the values measured in shaken suspensions.

### 4.1.3 Effect of $CO_2$ on sorption

The same method of determining uranium sorption by shaken suspension experiments (Section 4.1.2) was used with different $CO_2$ pressures in the sealed bottles containing the soil/solution mix. Two treatments were applied. An environment of decreased $CO_2$ pressure compared to ambient was produced by filling the bottles with nitrogen gas, forcing out the ambient air mix before sealing and shaking. An environment of increased $CO_2$ levels compared to ambient was produced by placing opened bottles inside a MACS cabinet in a controlled air mix of 1 % $CO_2$ before sealing them within the cabinet and removing for shaking. To determine whether the higher $CO_2$ levels affected the pHs of the suspensions, the pH was measured after shaking and found not to differ from that in the ambient treatment.

## *4.2 Results*

### *4.2.1 Self diffusion of $^{235}U$ against $^{238}U$*

Recorded water contents within soil collars ranged from 1.14 to 1.22 cm$^3$ cm$^{-3}$ (Table 4.3). Statistical analysis indicated statistical differences in water content between replicates (Table 4,4), though not in bulk density. The effects of these differences, if any, will be discussed in Section 4.3.1.

Table 4.3: Bulk densities ($\rho$) and volumetric water contents ($\theta_L$) of soil collars (df = 3, df effect = 16)

| **Soil** | **$\rho$** **(g cm$^{-3}$)** | $\theta_L$ **(cm$^3$ cm$^{-3}$)** |
|---|---|---|
| Loamy sand | 1.204 | 0.316 |
| Clay Loam | 1.167 | 0.393 |
| Silty Clay | 1.148 | 0.393 |
| Silty Clay Loam | 1.225 | 0.357 |
| F | 1.949 | 12.963 |
| p | N.S. | $p<0.05$ |

Table 4.4: Variation in $\theta_L$ between replicates in the four soils. ** significant at $p< 0.05$, *** significant at $p< 0.01$

| **Soil** | Loamy Sand | Clay Loam | Silty Clay | Silty Clay Loam |
|---|---|---|---|---|
| Loamy sand | — | *** | ** | ** |
| Clay Loam | *** | — | N.S. | ** |
| Silty Clay | *** | N.S. | — | ** |
| Silty Clay Loam | ** | ** | ** | — |

### Mass balance of U in the soil columns

Concentration/distance profiles of $^{238}$U in the U pulsed soil columns are plotted in Figure 4.2.1 and the corresponding bulk densities and water contents are given in Table 4.5. Distances from the $^{235}$U source were calculated from the individual slice weights and the mean bulk density of the soil collar. The profiles were examined for evidence of non uniform distribution of U. The plots were examined for anomalous points, and two points in a replicate of the silty clay loam (Figure 4.2.1 d) were deleted. No gradients of $^{238}$U were found except in the loamy sand where significant redistribution of U was evident. It is hypothesised that this was caused by separation and re-settling of fine dust from the soil during packing into the collars. Because a large proportion of the U is associated with fine clay particles in the loamy sand, any redistribution of this fraction would result in a large redistribution of U. The packing was done in stages, a small portion of soil being added and then packed down, so this may explain the slight banding apparent in the U profiles. Whatever the cause, the redistribution meant these profiles could not be analysed using simple diffusion equations so these data were not analysed further.

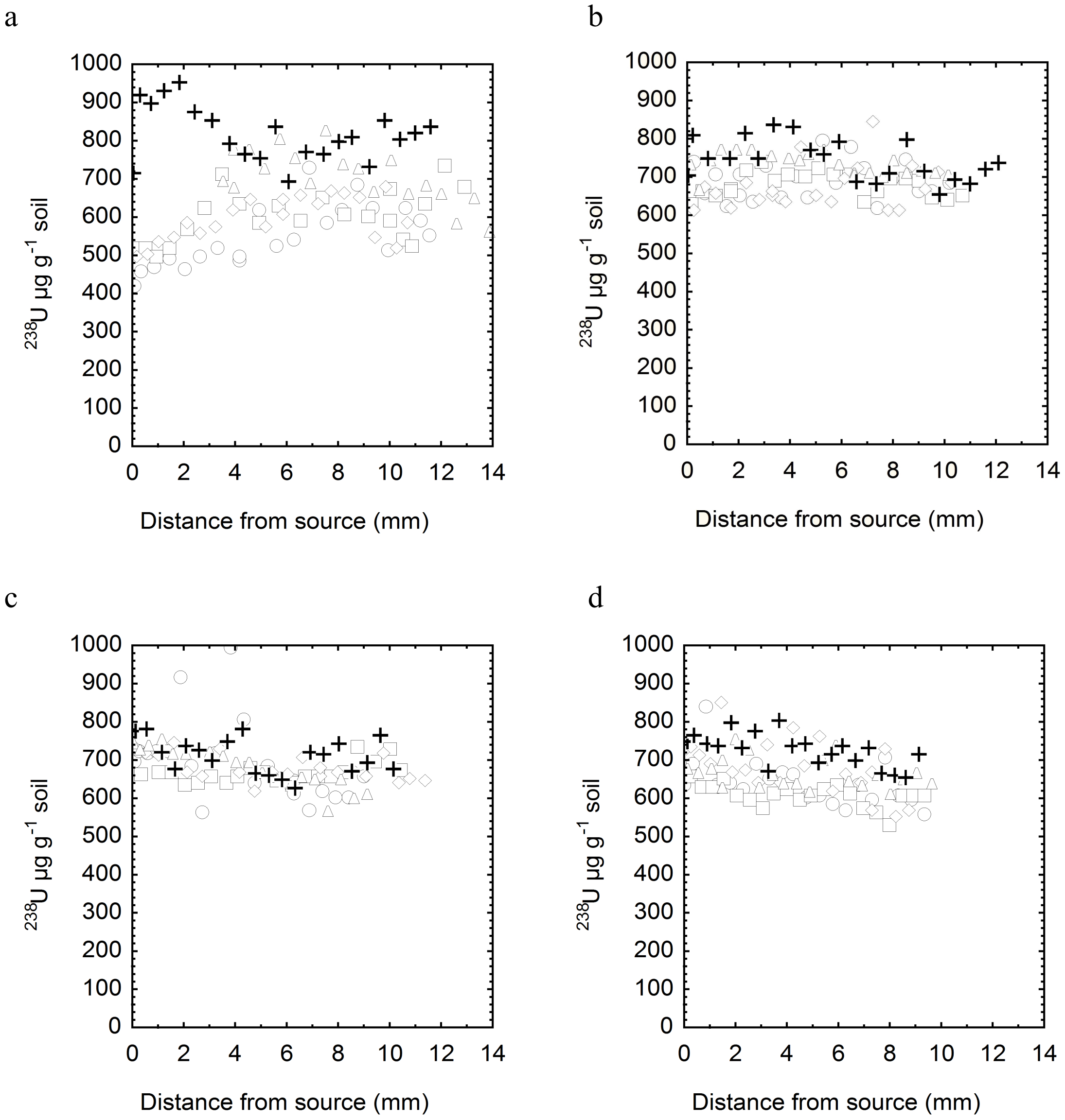


Figure 4.2.1: Concentration-distance profiles of $^{238}$U in the four experimental soils: a) loamy sand, b) clay loam, c) silty clay, d) silty clay loam. The profiles were measured 28 d after pulsing the soil with $^{235}$U. The different symbols indicate the five replicates for each soil as shown in Table 4.5.

Table 4.5: Bulk densities and water contents of replicates shown in Figure 4.2.1

| Rep | Loamy sand | | Clay Loam | | Silty Clay | | Silty Clay Loam | |
|---|---|---|---|---|---|---|---|---|
| | $\rho$ g cm$^{-3}$ | $\theta_L$ cm$^3$ cm$^{-3}$ | $\rho$ g cm$^{-3}$ | $\theta_L$ cm$^3$ cm$^{-3}$ | $\rho$ g cm$^{-3}$ | $\theta_L$ cm$^3$ cm$^{-3}$ | $\rho$ g cm$^{-3}$ | $\theta_L$ cm$^3$ cm$^{-3}$ |
| 1 [○] | 1.244 | 0.274 | 1.154 | 0.405 | 1.121 | 0.386 | 1.198 | 0.365 |
| 2 [□] | 1.130 | 0.335 | 1.174 | 0.394 | 1.165 | 0.393 | 1.178 | 0.364 |
| 3 [◇] | 1.187 | 0.344 | 1.216 | 0.340 | 1.062 | 0.401 | 1.162 | 0.342 |
| 4 [△] | 1.224 | 0.321 | 1.144 | 0.413 | 1.212 | 0.383 | 1.229 | 0.379 |
| 5 [✚] | 1.235 | 0.305 | 1.147 | 0.413 | 1.178 | 0.402 | 1.359 | 0.337 |

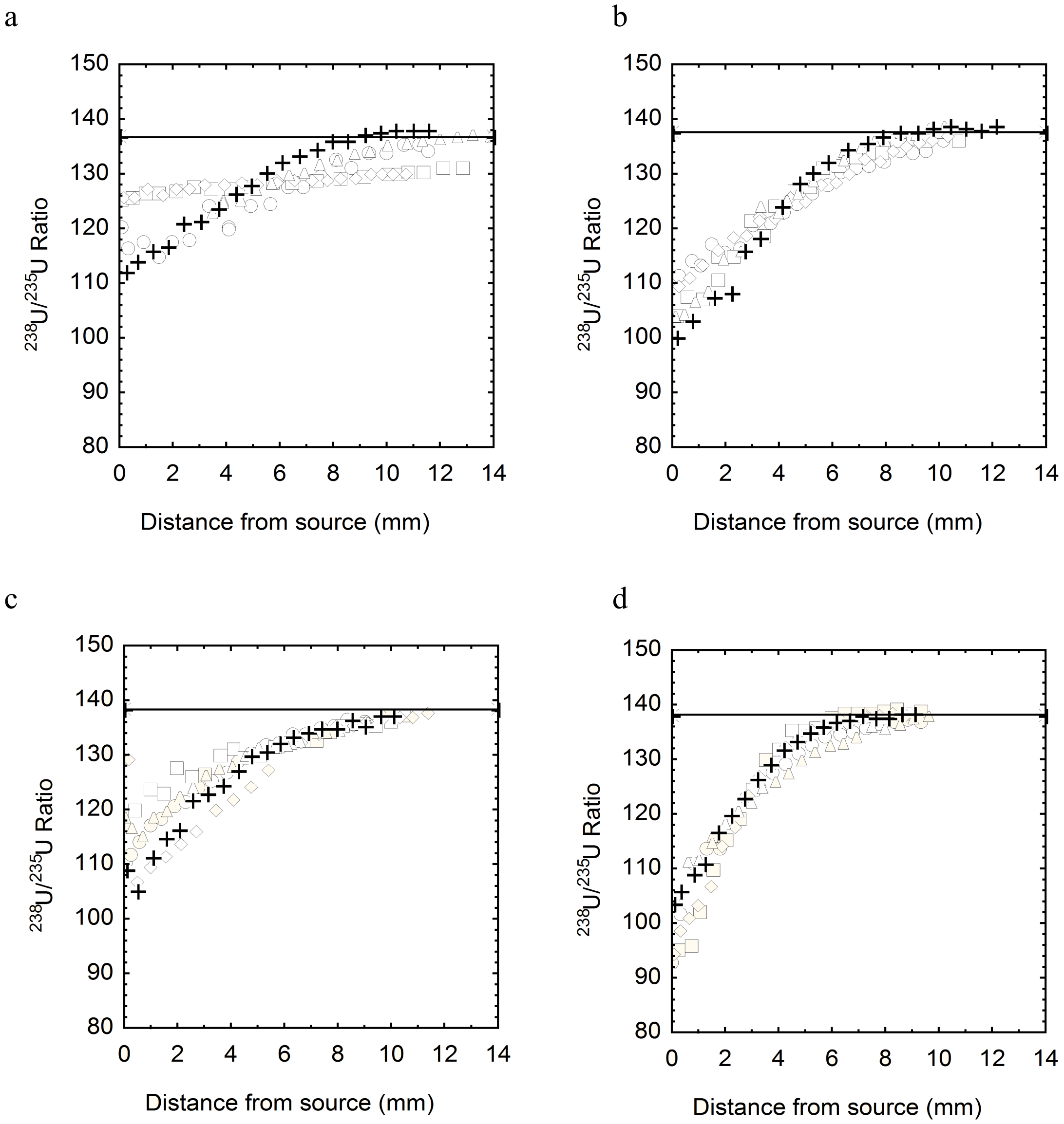


Figure 4.2.2: Measured $^{238}$U/$^{235}$U ratios plotted against distance from the application surface. a) loamy sand, b) clay loam, c) silty clay, d) silty clay loam. The horizontal line in each panel indicates the ratio in the original soil. Symbols for the replicates as in Table 4.5.

**Concentration-distance profiles**

Figure 4.2.2 shows the ratio $^{235}$U/$^{238}$U plotted against distance from the $^{235}$U source for the four soils. The ratio increased with distance from the source as $^{235}$U decreased. The ratio increased to that of the initial soil (represented by the horizontal line in Figure 4.2.2) at between 6 and 10 mm from the source, depending on the soil: 6 mm in the silty clay loam, 8 mm in the clay loam, and 10 mm in the silty clay. The results for the sandy loam are compromised by the redistribution problem discussed in the previous section, and show the greatest variation between replicates. Two of the sandy loam replicates were very wet and in these $^{235}$U diffused throughout the soil collar giving almost horizontal profiles. The three drier replicates have less diffuse concentration–distance profiles as expected.

Plots of ln$C$ against $x^2/t$ to test for slow local equilibration processes were prepared from the self diffusion results as follows. The relevant solution of the equation for this experimental systems is:

$$\ln\left(\frac{[\mathrm{U}]-[\mathrm{U}]_\mathrm{i}}{[\mathrm{U}]_0-[\mathrm{U}]_\mathrm{i}}\right)=-\frac{x^2}{4D_\mathrm{self}t} \qquad (4.7)$$

where $[\mathrm{U}]_0$ is the ratio of $^{235}$U/$^{238}$U at $x = 0$, $[\mathrm{U}]_\mathrm{i}$ is the initial ratio in the soil and $D_\mathrm{self}$ is the self-diffusion coefficient of uranium in the soil, given by

$$D_\mathrm{self} = D_\mathrm{L}\, \theta_\mathrm{L} f_\mathrm{L}\, [\mathrm{U_L}]/[\mathrm{U}] \qquad (4.8)$$

where $D_\mathrm{L}$ (diffusion coefficient of U in water) is the same for all soils ($4.26 \times 10^{-6}$ cm$^2$ s$^{-1}$). Values for $\theta_\mathrm{L}$ were calculated for each collar from the water content and these values carried forward through all calculations for that replicate for that individual soil.

Hence, we can allow for differences in moisture content and bulk density between the replicates by plotting ln$C$ against $x^2/(4D_\mathrm{L}\theta_\mathrm{L}f_\mathrm{L}t)$ using the known values of $D_\mathrm{L}$, $\theta_\mathrm{L}$ and $f_\mathrm{L}$ (values for the latter being 0.207, 0.313 and 0.340 in the clay loam, silty clay and silty clay loam respectively). If local equilibration is instantaneous the slope of these plots will be $-1/(C_\mathrm{L}/C)$. The apparent solution : solid ratio of U species ($C_\mathrm{L}/C$) is measured independently in Section 4.2.2.

To prepare the plots of ln $C$ against $x^2/t$ the data was transformed as follows:

The $^{235}$U concentrations were not measured directly because the low concentrations of $^{235}$U in the soil digests meant that for accurate measurement by ICP-MS the sample solutions would have to be concentrated to reach the minimum counts/min acceptable for the machine. This would have entailed the removal of other elements using ion exchange resins in order to prevent matrix interference effects and possibly damage to the ICP-MS. The additional cost in both time and money ruled this option out. However, the ICP-MS can read a $^{238}$U:$^{235}$U isotope ratio to an acceptable resolution even at small $^{235}$U concentrations.

In principle it should then be possible to calculate $^{235}$U concentration from the $^{238}$U:$^{235}$U ratio and the $^{238}$U concentration. However, due to the extent of variation in the $^{238}$U concentration data and the variation this introduced to the calculated $^{235}$U concentration, it was decided to use the $^{235}$U:$^{238}$U ratio as the working concentration variable. Allowance was made for the initial $^{35}$U:$^{238}$U ratio in the soil before addition of the pulse by subtracting it from the measured ratio (as in Equation 4.7).

Therefore the actual working concentration variable is

$$\ln\left({}^{235}\mathrm{U}/{}^{238}\mathrm{U}\right)_{\text{measured}} - \left({}^{235}\mathrm{U}/{}^{238}\mathrm{U}\right)_{\text{initial}} \qquad (4.9)$$

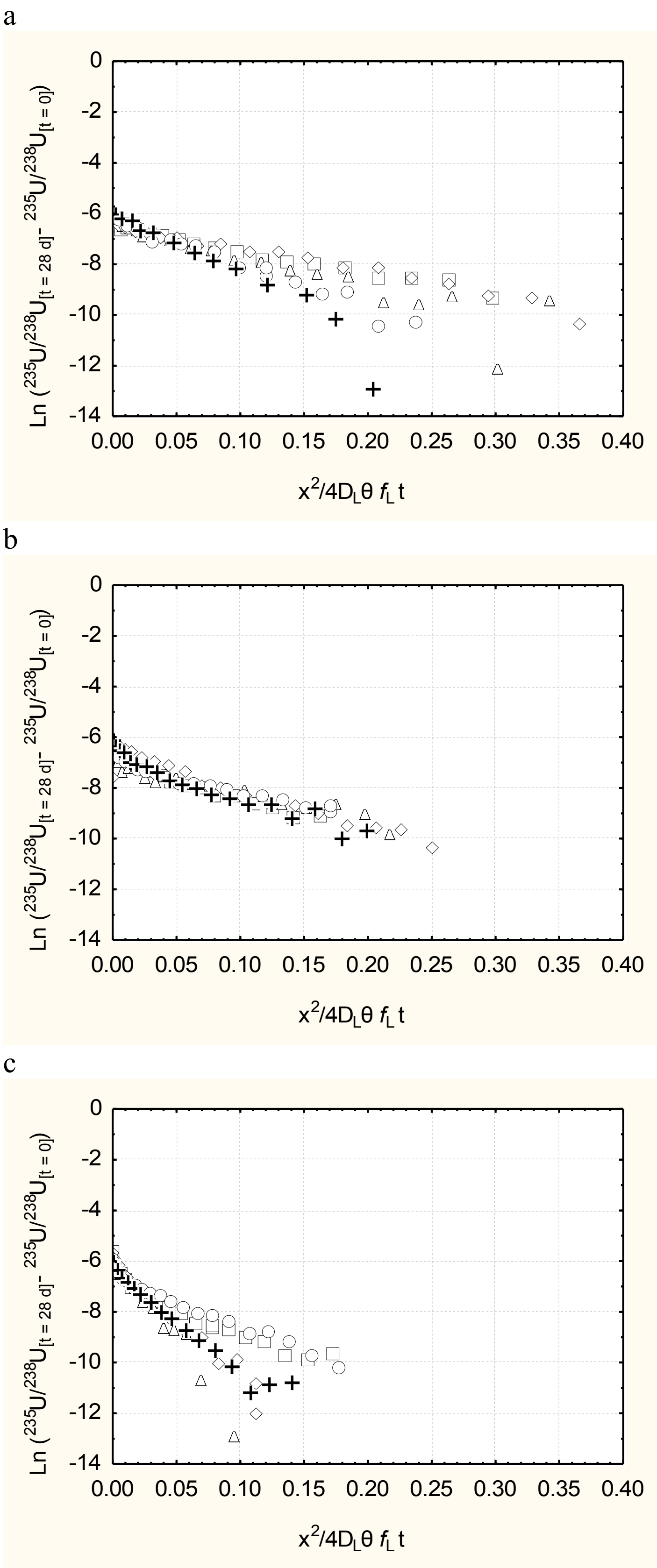


Figure 4.2.3: Plots of ln$C$ against $x^2/4D_L\theta f_L t$ for the results of the diffusion experiments in a) clay loam soil, b) silty clay soil, c) silty clay loam soil. Symbols for the replicates as in Table 4.5.

The plots were curved for all the soils, indicating slow local equilibration. The slopes and curvature differed between the soils indicating differences in $[U]/[U_L]$ equilibrium and approach to equilibrium on the time scale of diffusion through the soil bulk. The steepest gradient is seen in the silty clay loam soil. The uranium has moved the furthest in the clay loam soil and the gradient of the plot is similar to the silty clay. The variation seen in the silty clay is much smaller than in the other soils, the clay loam and the silty clay loam both presenting a range of relationships of uranium movement consistent with the variation in $\theta$ and $\rho$ between the replicates.

It is possible to fit single straight lines to the data in each graph with apparently acceptable $R^2$ values. But there is clearly curvature in the data, particularly at small values of the $x$ coordinate which is where the data is most reliable because $^{238}U:^{235}U_{measured}$ is largest and most different from $^{238}U:^{235}U_{initial}$.

### *4.2.2 Sorption of uranium in soil: sorption isotherms*

The water content of the soil was determined from a sub-sample taken and oven dried at 105 °C for 24 h and used to determine the exact weight of dry soil interacting with the uranium solution. pH was measured in the soil : solution mix after the 24 h shake and found not to be affected by the pH of the solution added. The concentration of uranium sorbed to soil particle surfaces $[U_S]$ and in solution $[U_L]$ was transformed into mol U and plotted to reveal the relationship between the two and any concentration-dependant effects. Figure 4.2.4 gives the measured sorption isotherms for the four soils at their original pHs and ambient $CO_2$ pressure.

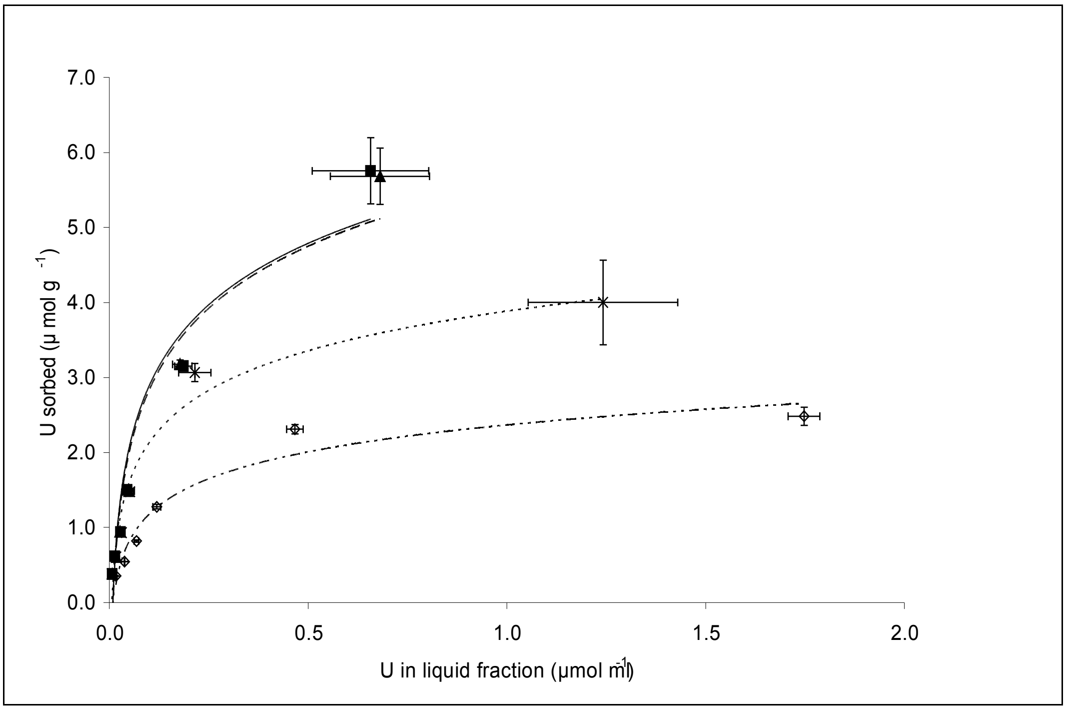


Figure 4.2.4: Distribution of uranium between sorbed and liquid fractions. Ambient $CO_2$ and room temperature (20°C) ■ : loamy sand, ▲ : clay loam + : silty clay ○ : silty clay loam. Logarithmic curves fitted.

As expected the uranium was strongly adsorbed onto soil particle surfaces and this was seen across all the soil types. The lines in Figure 4.2.4 are sorption isotherms fitted to the data: Freundlich ($U_S = a[U_L]^b$) for the loamy sand, clay loam and silty clay; but Langmuir:

$$([U_S] = \frac{a[U_L]}{b + [U_L]})$$

for the silty clay loam because a Freundlich equation could not be fitted over the whole concentration range. The coefficients of the sorption curves are given in Table 4.6.

Table 4.6: Coefficients for the fit of $U_S = a[U_L]b$ to the data in Figure 4.2.4 (using a non-linear estimation method in Statistica).

| **Soil** | |
|---|---|
| Loamy sand | $U_S = 7.02\ [U_L]^{0.505}$ |
| Clay Loam | $U_S = 6.90\ [U_L]^{0.506}$ |
| Silty Clay | $U_S = 3.90\ [U_L]^{0.341}$ |
| Silty Clay Loam | $U_S = 2.28\ [U_L]^{0.325}$ |
| Silty Clay Loam* | $U_S = 3.48\ [U_L]^{0.524}$ |
| Silty Clay Loam** | $[U_S] = \frac{2.79[U_L]}{0.140 + [U_L]}$ |

*Top concentration removed and Freundlich fitted. **Langmuir fitted

The amount sorbed increased with increasing total concentrations of uranium in the shaken suspensions, but the slope of the sorption curve (i.e. the buffer power $d[U_S]/d[U_L]$) decreased with increasing U concentration. The buffer powers of the sandy loam and the clay loam over the measured concentration range are almost identical under these conditions. Both have more uranium associated with the solid phase than the silty clay and the silty clay loam. The silty clay loam shows the least capacity for sorption with almost 50% of the uranium in the liquid phase at U additions more than 300 ppm or 1.26 μmol $ml^{-1}$.

**Analysis of concentration distance profiles**

The sorption data was used to analyse the concentration-distance profiles measured in the soil collars pulsed with $^{235}U$. To evaluate the diffusion coefficient $D_{\text{self}}$, and thereby the expected slope of the plots in Figure 4.2.3 if the distribution of the diffusing isotope between the soil solid and solution is at equilibrium, values of $[U]/[U_L]$ in Equation (4.8) are required. These were calculated from the sorption curves for each soil over the range of U additions by assuming [U] = [U] added. The results are given in Figure 4.2.5. The values of $[U]'/[U_L]'$ varied with log [U]' as shown in the figure and Table 4.7. The silty clay loam stands out as having the lowest amount of uranium sorbed to soil particles at the whole concentration range measured.

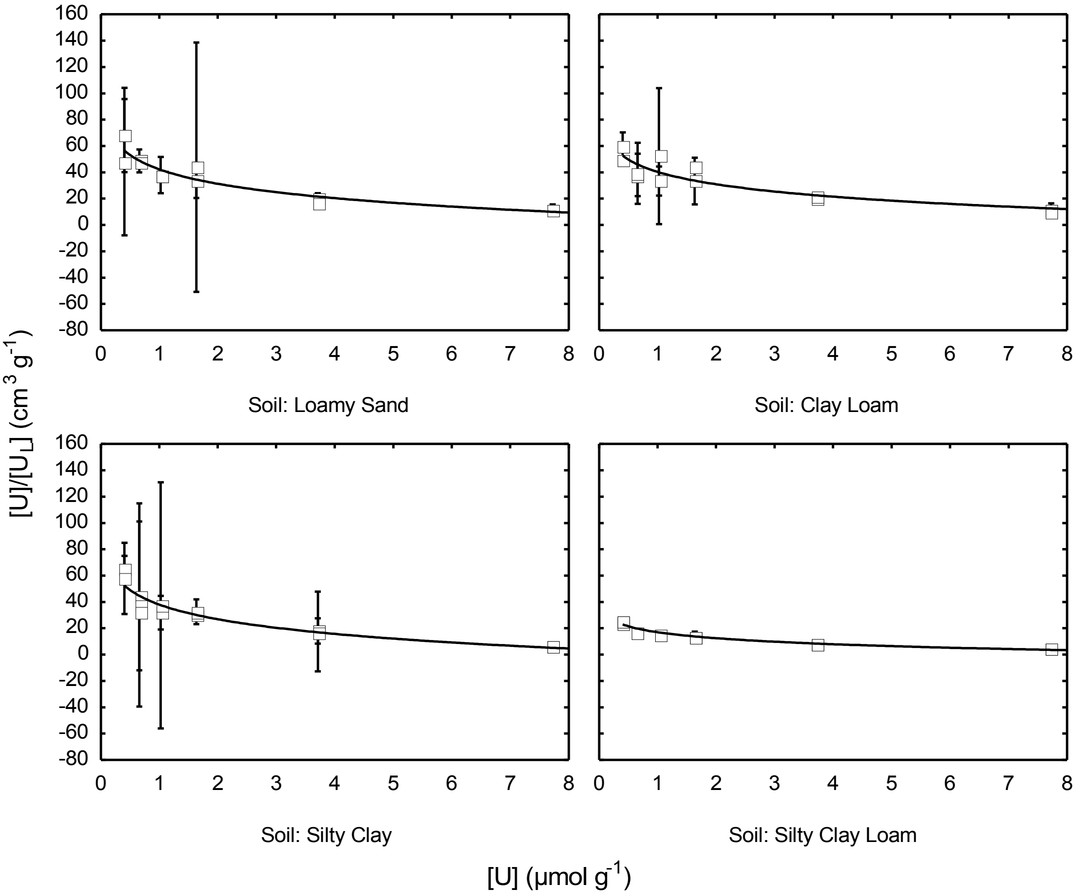


Figure 4.2.5: Plots of [U]'/[U$_L$] versus [U]' for the sorption isotherms. Data are means and 95% CI and fits to the relationship [U]'/[U$_L$] = $a - c$ log [U]. The value of the coefficients $a$ and $c$ are given in Table 4.7.

Table 4.7: Fits of the data in Figure 4.2.5 to the relationship [U]'/[U$_L$] = $a - c$ log [U].

| **Soil** | ***a*** | ***c*** | **R$^2$** |
|---|---|---|---|
| Loamy Sand | 42.836 | 36.412 | 0.823 |
| Clay Loam | 40.305 | 32.637 | 0.769 |
| Silty Clay | 39.130 | 38.665 | 0.862 |
| Silty Clay Loam | 16.697 | 14.791 | 0.941 |

The relationship [U]'/[U$_L$] = $a - c$ log [U] was used to calculate the distribution of uranium between the soil solid and solution in the diffusion experiments. The concentration of U that was equilibrating with the diffusing isotope ([U]) is likely to have been less than the total concentration of U in the soil due to both slow sorption and precipitation reactions over the months that the soil was stored between the addition of

U to the soil and the later diffusion measurements. However, the value of [U] can be inferred from the diffusion data as follows:

In the region of the diffusion plots close to $x = 0$ the isotope pulse was in contact with the soil for the longest period and thus any slow equilibration processes are expected be most advanced here. The slope of the ln$C$ versus $x^2/4D_L\theta f_L t$ plot in this region should best approximate to [U]/[$U_L$] (see Equation 4.7 and associated explanatory paragraphs). Comparisons to values of [U]/[$U_L$] taken from the measurements of sorption in the shaken suspensions (where slow equilibration processes are accelerated by disaggregation of soil particles and disruption of intra-particle diffusion) should indicate values of [U]. Accordingly Figure 4.2.6 compares the measured diffusion data with the slopes predicted from the shaken suspension data for different values of [U].

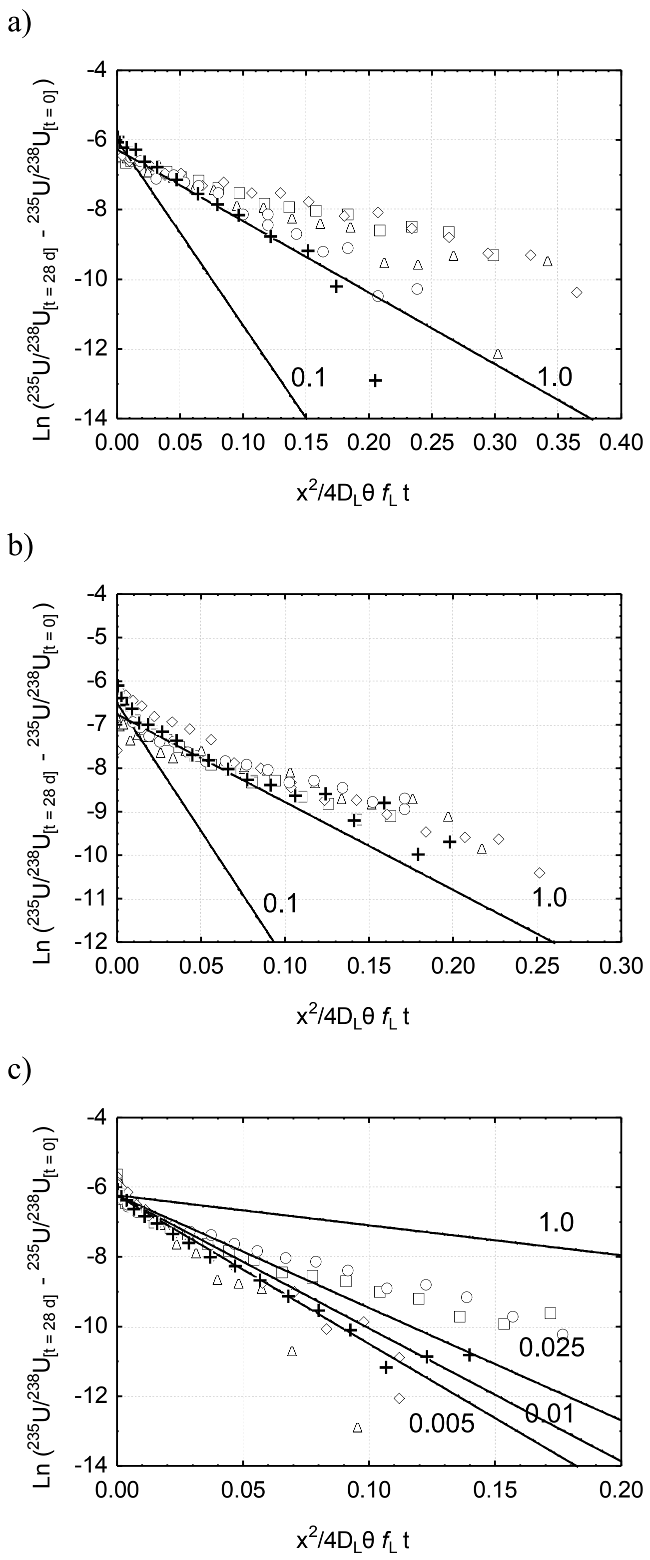


Figure 4.2.6: The results of the diffusion experiments as in Figure 4.2.5 with lines indicating the values of [U]/[$U_L$] predicted from the shaken suspension experiments for different values of [U]. Numbers on curves are values of [U]/[$U_{TOT}$] used to calculate [U]/[$U_L$]. a) Clay loam ([$U_{TOT}$] = 4.03 µmol cm$^{-3}$), b) Silty clay ([$U_{TOT}$] = 3.09 µmol cm$^{-3}$), c) Silty Clay Loam ([$U_{TOT}$] = 3.53 µmol cm$^{-3}$)

As clearly shown in Figure 4.2.6, in all cases the initial slope is steeper than that predicted for $[U] = [U_{TOT}]$ (i.e. $[U]/[U_{TOT}] = 1$), though to differing effect in the different soils. The discrepancy is least in the clay loam and greatest in the silty clay loam, indicating differences in the extent of slow sorption and precipitation reactions in the time since the U was added to soils (some weeks before the diffusion experiments). This would imply that a large proportion of the uranium added has moved into small pore spaces over the time the soil collars were stored before pulsing and is not available for equilibrating.

### *4.2.3 Effect of $CO_2$ on sorption*

The concentration of uranium sorbed to soil particle surfaces $[U_S]$ and in solution $[U_L]$ at different $CO_2$ pressures was transformed into mol U and sorption isotherms plotted to reveal the relationship between the two and any concentration-dependant effects (Figure 4.2.7).

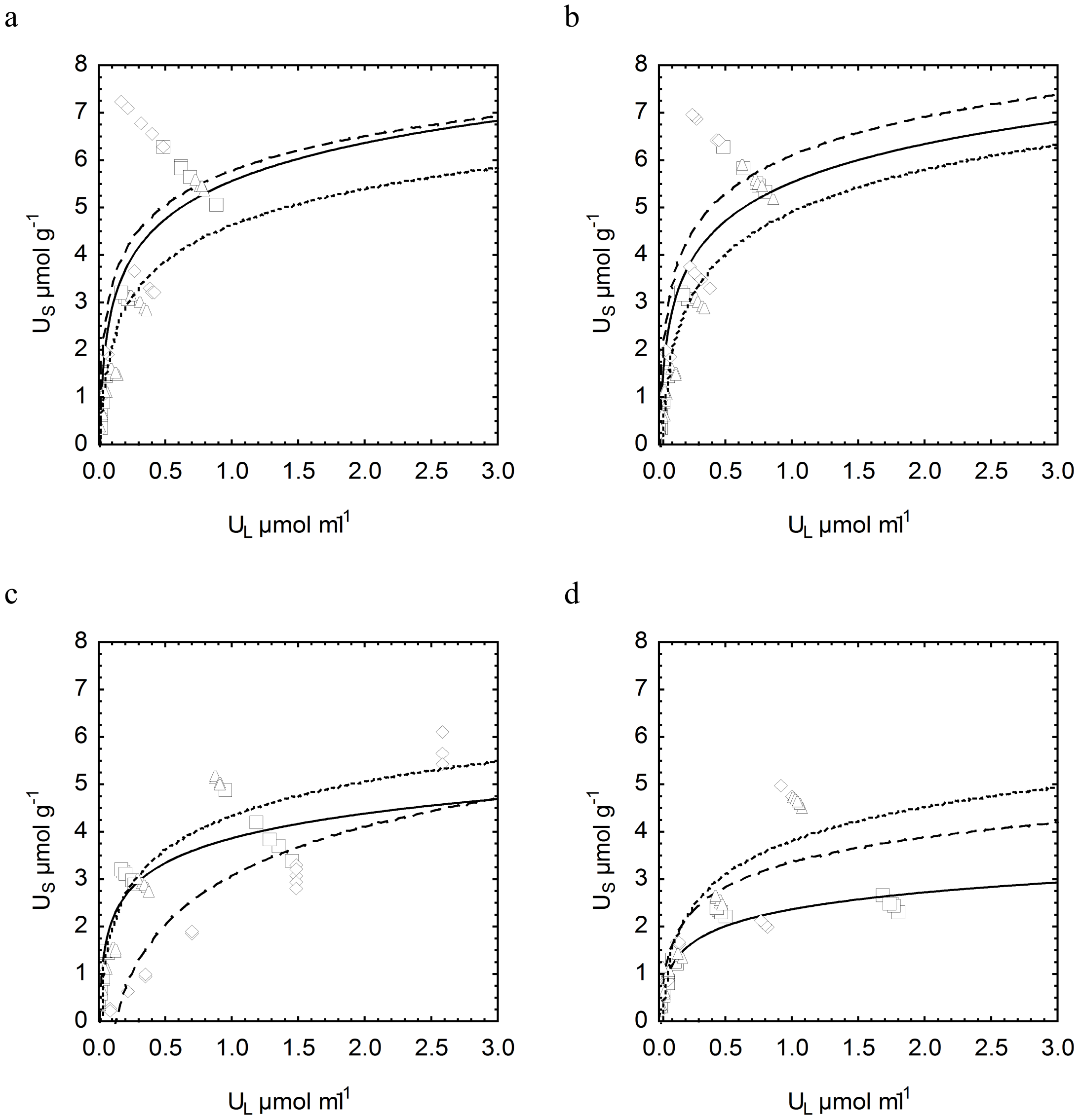


Figure 4.2.7: The effect of $CO_2$ pressure on U sorption in the four soils. Sorption isotherms presented for a) sandy loam, b) clay loam, c) silty clay, d) silty clay loam. □, (—) Ambient, ◇, (- -) Low $CO_2$, △, (···) High $CO_2$.

The lines in Figure 4.2.7 are sorption isotherms fitted to the data, Freundlich ($U_S = a[U_L]^b$) for the sandy loam, clay loam and silty clay but Langmuir:

$$([U_S] = \frac{a[U_L]}{b + [U_L]})$$

for the silty clay loam. The coefficients of the sorption curves are given in Table 4.8.

Table 4.8: Coefficients for the fit of $U_S = a[U_L]^b$ to the data in Figure 4.2.7. using a non-linear estimation method provided by the statistica programme.

| **Soil** | **Ambient** | **Low $CO_2$** | **High $CO_2$** |
|---|---|---|---|
| Sandy Loam | $U_S = 7.02 [U_L]^{0.505}$ | $U_S = 7.64 [U_L]^{0.438}$ | $U_S = 6.38 [U_L]^{0.670}$ |
| Clay Loam | $U_S = 6.90 [U_L]^{0.506}$ | $U_S = 9.57 [U_L]^{0.556}$ | $U_S = 6.60 [U_L]^{0.698}$ |
| Silty Clay | $U_S = 3.90 [U_L]^{0.341}$ | $U_S = 2.36 [U_L]^{0.901}$ | $U_S = 5.35 [U_L]^{0.599}$ |
| Silty Clay Loam | $U_S = 2.28 [U_L]^{0.325}$ | $U_S = 3.81 [U_L]^{0.560}$ | $U_S = 4.42 [U_L]^{0.639}$ |

The results show that sorption tended to decrease as $CO_2$ pressure increased, except in the silty clay loam where it increased both with increase and decrease of $CO_2$ pressure compared with ambient.

The sandy loam and clay loam show similar differences between ambient and low $CO_2$. Both follow the same gradient at low concentrations but show greater sorption at high concentrations in reduced $CO_2$ manipulations. The data from the silty clay shows more uranium present in the liquid phase at reduced $CO_2$. The variation between soils is smaller in the high $CO_2$ manipulation.

In the silty clay loam more sorption is seen at high concentrations of uranium in the high $CO_2$ manipulations than in the ambient. However, the silty clay loam soil occupies a similar position under both $CO_2$ conditions. The extensive curvature of the relationship seen in the ambient data is no longer present at reduced $CO_2$ and the Freundlich relationship fits the data much better.

Figure 4.2.8 and Figure 4.2.9 show the relationship between $[U]/[U_L]$ and $[U]$ calculated from the results in Figure 4.2.7 for the low and high $CO_2$ treatments respectively.

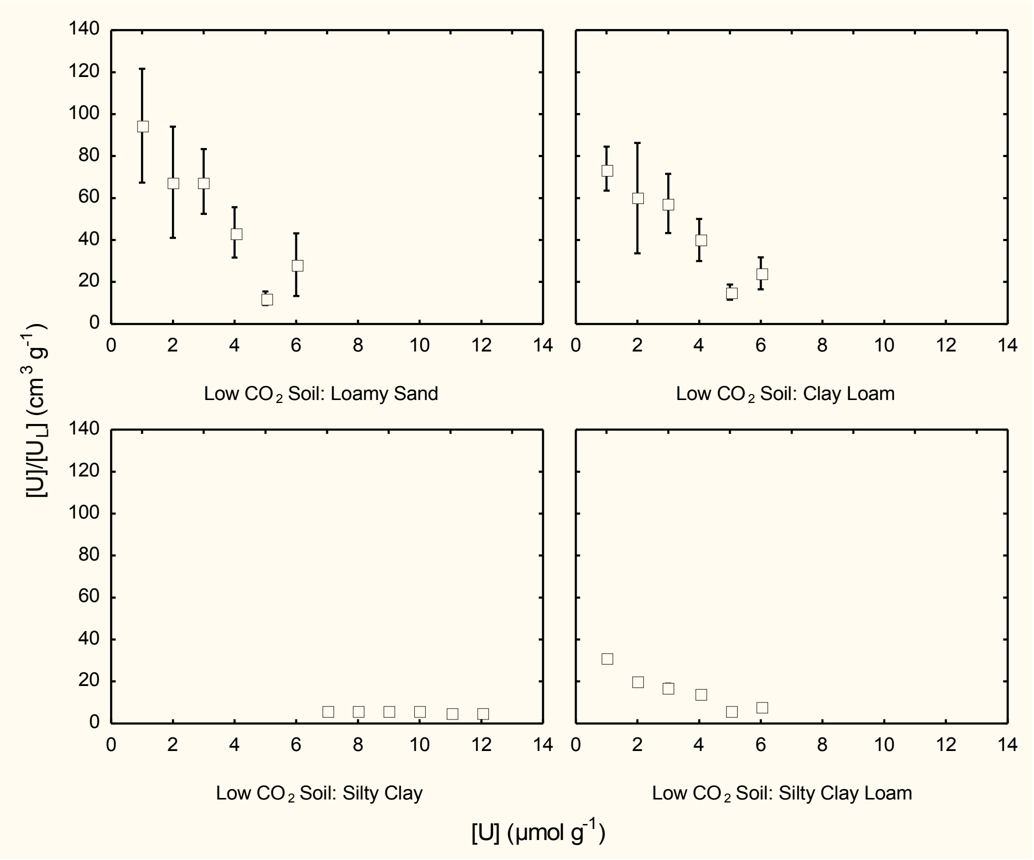


Figure 4.2.8 Plots of [U]/[U$_L$] versus [U] for the low CO$_2$ data in Figure 4.2.7. Data are means and 95% CI.

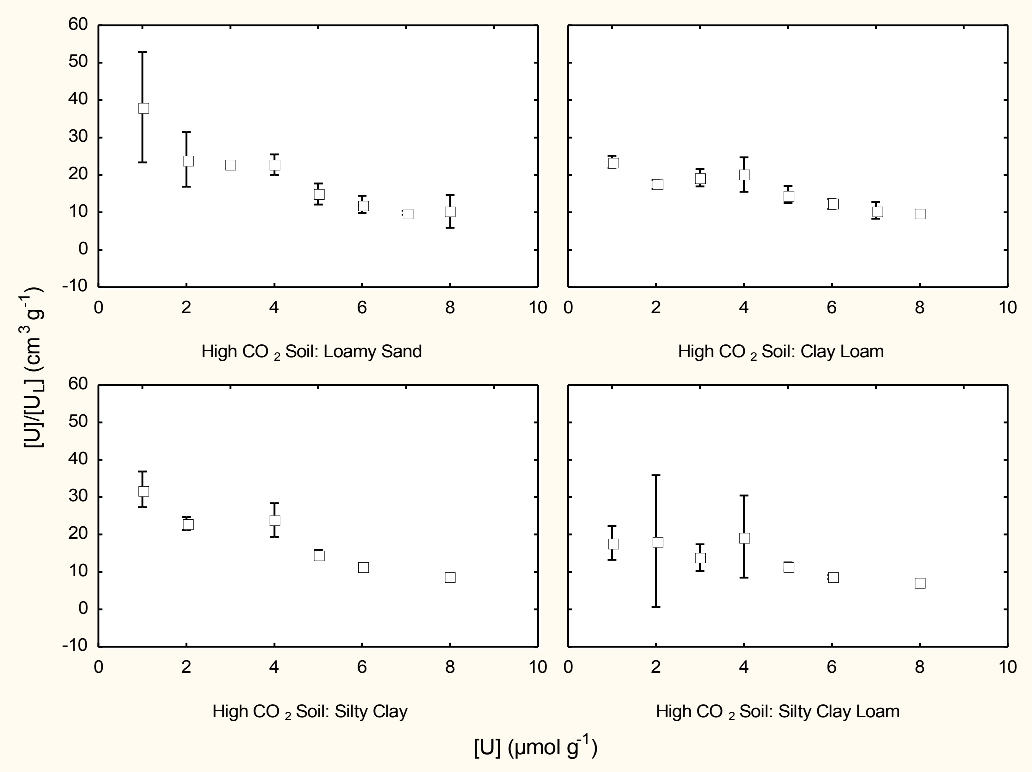


Figure 4.2.9: Plots of [U]/[U$_L$] versus [U] for the high CO$_2$ data in Figure 4.2.7. Data are means and 95% CI.

The [U]/[$U_L$] plots show the reduced sorption at high $CO_2$ pressure seen in the sorption curves in all four soils.

## 4.3 Discussion

### 4.3.1 Self diffusion of $^{235}U$ against $^{238}U$

The experiments were successful and showed that uranium does move in real soils and this movement can be measured over a one month period at a millimetre scale. The spread of uranium though the soils was slower than that of the bromide ions measured in Chapter 3, reflecting the effects of sorption and the resulting much smaller diffusion coefficients. But also, the curvature of the ln$C$ vs $x^2/4t$ plots indicates that U sorption processes were time-dependent, and that some part of the U diffused through the soil bulk faster than expected for rapid local solid:solution equilibration. This is important because it means that, although most of the U from a source of contamination will spread only very slowly into soil, some part may spread much faster and thereby reach absorbing plant roots or soil drainage channels. It is therefore important to understand such phenomena in some detail.

As discussed in the Introduction, measuring self-diffusion of ions in soil is useful for studying diffusion and reaction processes because the complicating effects of heterogeneous reactions between multiple ion species are removed. This is particularly important for an element such as uranium with complicated speciation chemistry. Though the individual diffusion coefficients of an ion's isotopes may differ slightly because of their different masses, and there may also be small differences in bonding energies and other determinants of reaction kinetics and equilibria (Weiss *et al.*, 2008), these differences will have negligible effects on overall rates of diffusion and reaction.

The result of most interest is that the plots of ln$C$ vs $x^2/4t$ were, to a varying extent, curved with long tails, indicating slow equilibration processes were at work. The curves seen in these plots appear to be a conglomeration of at least two slopes of differing gradient. The slope is steepest close to $x = 0$, indicating greater sorption of the uranium on and in soil particles. Then, as the front of the isotope pulse moves through the soil (non-adsorbed U diffusing through the liquid phase), the sorbed U is released from soil particles through the processes of localised de-sorption and re-sorption. At the leading edge of the pulse, high U concentrations mean most of the U pulse will be sorbed on soil particle surfaces. However, once the pulse has passed on, U will be released back into the liquid phase in accordance with solid-solution equilibria. However adsorption and desorption processes are not necessarily reversible on the time-scale of diffusion though the soil solution, and sorbing particles may remove and relinquish U only slowly. This process whereby the sorption sites are first exposed to an increasing concentration of $^{235}$U, and then to a decreasing one as the peak of the $^{235}$U pulse passes, is different to the continuing slow reaction of U uniformly mixed into the soil and then left to react.

In comparison, a simple linear relationship between ln $C$ and $x^2/t$ was found for $Br^-$ counter diffusion against $Cl^-$ in Chapter 3. Bromide, a non-adsorbed ion, is not expected to behave in the same manner as uranium species. Nye and colleagues have found linear plots of ln $C$ against $x^2/t$ for diffusion of non-adsorbed ions in soil following pulse applications (Pinner & Nye, 1982; So & Nye, 1989; Kirk *et al.*, 2003). However, they also found approximately linear plots for the self diffusion of the sorbed exchangeable cations $Na^+$, $Ca^{2+}$, $Rb^+$ and $Cs^+$ (references in Nye & Staunton, 1994), contrary to that seen from the results here for U species. But similar to the results here, Staunton & Nye (1989) and Moritsuka *et al.* (2009) found plots for the self diffusion of $H_2PO_4^-$ anions, which are strongly-sorbed by soils, were strongly curved, indicating non-instantaneous equilibration.

Non-instantaneous equilibration processes include slow penetration of U species into soil particles through narrow access pores, as well as slow chemical reactions of U species with soil surfaces outside and inside particles. By comparing the measured self-diffusion coefficients of exchangeable cations obtained from linear ln$C$ against $x^2/t$ plots with those expected for diffusion in the liquid phase alone, Nye & Staunton (1994 and references therein) concluded the cations were mobile in the sorbed state on soil surfaces as well as in solution, and that this contributed between 27 and 97% of the overall diffusion coefficient depending on the cation. Further, being positively charged, cations are not excluded from very narrow pores by electrostatic repulsion, unlike negatively-charged anions. Hence equilibration between intra- and extra-particle pores is likely to be faster than for strongly-sorbed anions such as phosphate, which are essentially immobile on soil surfaces.

If the U species present under the conditions of the present experiments behave in a similar way to the exchangeable cations studied by Nye and Staunton, then the U in the soil at the start of the diffusion experiment and that added in the pulse would have been freely mobile on soil particle surfaces and there should have been rapid equilibration between intra and extra particle pores. Over the period of months between the addition of un-enriched U to the soil and the application of the $^{235}$U-enriched pulse, slow equilibrium processes should have had sufficient time to run their course. Consequently the sorbed U equilibrating with diffusing $^{235}$U would have included both U sorbed on external soil surfaces and available for instantaneous equilibrium reactions, and also that present within particles and potentially subject to slower equilibration processes.

The speciation diagram in Figure 4.0.1 indicates that at the pHs of the three soils (all approx. pH 7.6) and typical $CO_2$ pressures in soil atmospheres, the dominant uranium species in solution would have been anionic, probably mainly $UO_2(CO_3)_2^{2-}$. Specific sorption reactions of $UO_2(CO_3)_2^{2-}$ on soil surfaces (Sherman *et al.*, 2008) could presumably lead to similar behaviour to strongly sorbed $H_2PO_4^-$ ions, i.e. slow access to intra-particle reaction sites, as in Staunton & Nye's (1989) results.

Further evidence of the presence of slow equilibrium and precipitation reactions was discovered when values for $[U]/[U_L]$ from sorption isotherms created from shaken suspension experiments were used to model $[U_{TOT}]$ in the self diffusion soil microcosms. This will be discussed further in section 4.3.2.

The self diffusion results also revealed differences between the experimental soils. The differences between the soils reflect their different physico-chemical characteristics, particularly clay content and mineralogy and organic matter content, but not pH.

There was no significant difference in bulk density between the different soil microcosms, despite the small differences measured between "replicates". There were also no significant difference in volumetric water content between the soils, though the silty clay loam was slightly dryer. However, the soils were all sufficiently moist that intra-particle pores will have been largely water filled. So small differences in water content are unlikely to have affected the solid-solution equilibration processes.

### 4.3.2 Sorption of uranium in soil

A high proportion of uranium was found to be associated with the solid phase in all the soils in the shaken suspension experiments. This was expected from previous studies on U sorption in soils (Chapter 1). The curved shape of the U sorption isotherms, with decreasing slope as concentration increased, was also expected from previous studies.

Over the range of U added (0-600 ppm in the initial solution) the ionic strength of the solution varied from 0.02 to 0.2 M (Table 4.2). This might be expected to affect U speciation and soil surface charge and therefore sorption, confounding the direct effect of U concentration on sorption. However, according to Waite *et al.* (1994) U sorption is independent of ionic strength except at high pH (pH > 8-9). As the soils tested were all less alkaline than this it was not taken to be a problem.

The experimental soils differed in several variables expected to influence U sorption kinetics and equilibria, including clay content, pH, CEC and mineralogy. However, there were no clear trends in U sorption with any of these. Sorption in the soil with the highest clay content (silty clay, clay content = 0.04 g $kg^{-1}$) was intermediate between that in the clay loam and silty clay loam (both with clay content ≈ 0.03 g $kg^{-1}$). As well as affecting the total density of surface sorption sites, clay content affects soil aggregation and the possible disaggregation during shaking in the sorption experiments affected access to intra-particle sorption sites.

There were also no clear trends in sorption with soil pH. The two soils with the greatest difference in soil pH (the loamy sand, $pH_{CaCl2}$ = 6.28, and clay loam, $pH_{CaCl2}$ = 7.60) produced very similar Freundlich sorption isotherms. At a given $CO_2$ pressure, pH is expected to affect U sorption both through changes in U speciation in solution and changes in surface charge (Sherman *et al.*, 2008). However the soils also differed in clay content and other variables affecting U sorption.

There were also no clear trends in U sorption with soil CEC. The two soils with the most similar CEC (loamy sand and silty clay loam, CEC = 0.062 and 0.067 $mol_c$ $kg^{-1}$, respectively) differed widely in sorption isotherms. The soil with the highest CEC (silty clay, CEC = 0.212 $mol_c$ $kg^{-1}$) showed less sorption than the clay loam (CEC = 0.165 $mol_c$ $kg^{-1}$).

The low U sorption capacity of the silty clay loam across the concentration range studied may be related to its high carbonate content ($CaCO_3$ = 0.07 g $kg^{-1}$). This soil had the same clay content and pH as the clay loam, but only a fraction of its sorption capacity.

Comparison of the values of the apparent soil U buffer power, [U]/ [$U_L$], obtained from the shaken suspension experiments (measuring the behaviour of U over 24 h) with those obtained from the self-diffusion experiments (behaviour of U over 28 d) resulted in some interesting conclusions. In the self-diffusion experiments, the apparent [U]/ [$U_L$] values at distances close to $x$ = 0 were greater than those obtained from the shaken suspension experiments, whereas at large distances the apparent [U]/ [$U_L$] values are smaller than or similar to those from the shaken suspension experiments. By calculating at what [$U_{TOT}$] such a value of soil buffer power would be found in the specific soils tested it was possible to compare this to the U concentration known to be present in the said soils measured by total digest and ICP-MS. As the sorption isotherms showed, the ability of all the soils to sorb U was greater at lower concentrations of [$U_{TOT}$] and consequently the high levels of sorption seen in the surface slices of the self diffusion experiments corresponded to a lower $U_{TOT}$] estimated than was recovered experimentally.

This is understandable in terms of the different lengths of time the diffusing $^{235}$U at different distances down the soil column has been in contact with soil reaction sites, and the time-dependency of reaction. Close to $x$ = 0, the diffusing $^{235}$U has been in contact with reaction sites for longest, and it will therefore have exchanged with a greater proportion of the soil U participating in the diffusion and reaction process. By comparing the apparent [U]/[$U_L$] values with the [U]/[$U_L$] versus [$U_{TOT}$] relations obtained from the shaken suspensions experiments, it was possible to estimate the proportion of the soil U participating in diffusion and reaction.

From these calculations the importance slow equilibration reactions was clear. In all the soils, a large proportion of the soil U was unavailable for instantaneous equilibrium reactions, so the effective [$U_{TOT}$] in the soil was much smaller. From the large difference between [$U_{TOT}$]$_{effective}$ and [$U_{TOT}$]$_{actual}$ it is apparent that slow equilibrium processes had taken place over the 5 months the soil had been stored between the first U washing and the self diffusion experiments. The outcome of these processes would be to transform the U into forms that equilibrate with the diffusing $^{235}U$ only very slowly. These forms might include U in precipitates, or U at sorption sites that are only accessible by very slow diffusion through narrow pores or clay inter-layers.

Shaken suspensions remove “constraints” to transport within small pores, allowing access to both the external sorption sites and internal ones, so short-circuiting long-term equilibrium processes. In the soil diffusion columns, the U at large distances from the source hadn't been in contact with soil particle surfaces long enough to diffuse into small narrow access areas by slow processes. Therefore there was a smaller surface available for sorption (limited access to reaction sites), with associated reduction in the amount of U sorbed, producing the less steep concentration-distance gradients seen at greater distances from the source.

Modelling studies by Nye & Staunton (1994) and Ptashnyk *et al.* (2009) show that slow access to sorption sites has the effect of decreasing the adsorption of a solute into soil from a source or of desorption from soil to a sink. Hence, the rate of movement of U away from a corroding source of DU – for example to an absorbing plant root or a drainage channel – will be faster than that expected from a simple model of diffusion through the soil assuming instantaneous equilibration with intra- and extra-particle sorption sites. Failure to allow for slow access to reaction sites can lead to seriously erroneous results.

### 4.3.3 Sorption in manipulated $CO_2$ environments

The effects of $CO_2$ pressure on U sorption varied between the soils. In the loamy sand and clay loam, sorption tended to decrease with increasing $CO_2$ pressure, whereas in the silty clay it increased with increasing $CO_2$ pressure, and in the silty clay loam it increased with both increase and decrease relative to ambient atmospheric $CO_2$. These differences are in part explainable in terms of the effects of $CO_2$ pressure and pH on U speciation in solution shown in Figure 4.0.2.

In the loamy sand and clay loam, over the relevant pH range (pH 6.3 and 7.5 respectively) increasing $CO_2$ pressure will mean an increasing proportion of the U is present as carboxy anions (especially $UO_2(CO_3)_2^{2-}$), which may be less strongly sorbed than $UO_2^{2+}$ and $UOH^+$ cations, which dominate at lower $CO_2$ pressure. This is therefore consistent with the observed trends for these soils. However $CO_2$ pressure will also affect sorption through its effects on soil surface charge. Though there were no changes in soil pH with $CO_2$ pressure in the experiments, changes in carbonate equilibria in the soil will have altered surface charge and hence sorption in ways that depend on other soil variables. So given the differences in mineralogy and clay content between the soils, the mixed trends in the effects of $CO_2$ pressure are credible.

These results are used in Chapter 5 to help understand the effects of biological activity on U transport.

## *4.4 Conclusions*

The self diffusion of U through soil microcosms could not be explained with a simple model assuming instantaneous equilibration of U between the soil solid and solution. Slow equilibration processes were evidently involved. Therefore it was not possible to predict the diffusion of U using simple sorption isotherms measured in shaken soil suspensions. Slow equilibration has the effect of decreasing the net adsorption of U into soil from a contaminant source, but it also means that a small proportion of the U may diffuse rapidly through the soil.

Differences in U sorption between soils measured in shaken suspensions were not simply related to differences in soil pH, clay content, CEC or mineralogy. Likewise the effect of $CO_2$ pressure on U sorption differed between soils, and was not explicable in terms of differences in solution speciation alone.

# Chapter 5: Soil biological effects on diffusion of uranium

The biological component of soil is of importance in relation to the transport of nutrients and elements through soils, and possibly of equal importance as the chemical and physical components. Though a specific "microbial effect" is not measurable the presence of a microbial community within a soil will have a number of effects on soil processes and thereby on elemental transport rates, including those of U through the soil system. These include direct effects, for example translocation within fungal hyphae, or via secretions from bacteria and fungi both during a normal life cycle and as a response to stress affecting metal solubility and transport, or the possibility of uptake or immobilisation of uranium by microbes. There are also indirect effects including processes involving organic matter dynamics within the soil. Organic acids may increase the solubility of U in soils (Kabata-Pendias, 2001), and the effects of the dissolution of $CO_2$ released in respiration on pH and partial pressure of $CO_2$ in pore spaces affects sorption (e.g. Section 4.3.3).

The previous chapters have considered the processes by which U is transported through soils. Uranium sorption to soil surfaces was shown to dominate the pathway by which diffusion of U moved away from a pulse application The effect of soil texture on U sorption was as hypothesised, the number of sorption sites related to the different clay contents of individual soils. However, the presence of an active soil community within soil is likely to have both direct and indirect effects on the processes of U movement and hence is necessary to consider. The active component of soil organic matter comprises 10 to 20% of the total (Brady & Weil, 2002). In an arable soil supporting circa 200 $\mu g\ g^{-1}$ microbial biomass this would indicate a total soil organic matter content of up to 10 times of that found in the passive fraction – including the colloidal fractions (humin and some humic acids). The potential for complexation by organic matter leading to immobilisation and/or increased mobility through the action of organic acids produces a situation whereby it can be hypothesised that the presence of a soil community may both accelerate or retard U transport. It is not clear which outcome (immobilisation/increased mobility) will be affected by particular biotic actions or interactions. Different parts of the soil microbial community may affect transport in different ways and come to dominate under different conditions or circumstances, for example in relation to abiotic stresses.

Previously much of the work carried out on U and DU behaviour has been carried out *in vitro*, with monotonic microbial cultures within the controlled environment of laboratory Petri dishes (e.g. Lovley and Phillips, 1992; Spear *et al.*, 2000; Fomina *et al.*, 2007) from which it is very difficult to extrapolate to field conditions. Since it is the intention of this project to investigate DU behaviour in a more realistic environment, the effects of soil biota upon U transport in soil microcosm systems were used in experimentation rather than monitoring single species' interactions on plates or in solution.

Two main hypotheses were developed from a review of the literature in this field and in related fields regarding the likely soil microbial community interaction with U:

1. The presence of a living soil microbial community within a soil matrix will result in a measurable difference in the rate of U transport.
2. The differing effects of components of the soil community on the transport of uranium, will result in a measurable difference in the rate of U transport between soil microcosms manipulated to produce a fungal-dominated versus bacterial-dominated soil.

A preliminary study (Appendix 4) to determine the difference between a live and a sterile soil in terms of U diffusion over a two-week period revealed no difference between live and sterilised soil. The diffusion period was extended to 28 d and the original hypothesis adapted to take into account the possibility that the effects of the different components of the soil community may have been cancelling each other out. An experiment was then designed to determine the diffusion of U through soils with manipulated communities.

As described in Section 4, U is present in the soil solution as several species, particularly as complexes with hydroxyl and carbonate ions. Depending on the pH of the soil solution and the partial pressure of $CO_2$ in the soil air, the dominant species may be positively charged (e.g. $UO_2^{2+}$), neutral (e.g. $UO_2(OH)_2$, $UO_2CO_3$) or negatively charged (e.g. $UO_2(OH)^{3-}$, $UO_2(CO_3)_2^{2-}$). These different uranium species interact in different ways with soil particle surfaces, and the interactions also depend on the pH and pressure of $CO_2$ because they affect the charge on soil particle surfaces.

In an attempt to determine the potential effect of soil microbial community respiration on U transport (effects including localised acidification and/or the changing of $UO_2$ bonds from hydroxyl to carbonate with associated decreased charge and potential increased mobility) $CO_2$ was measured in identical soil microcosms.

In order to test the possibility that the process of manipulating the soil community had in some way affected the availability of sorption sites, an experiment was also devised to determine the soil sorption of U in the treated soils.

### *5.1 Methods*

The methods used in the investigation of U transport in biologically active soils can be split into three main sections:

1. The set-up of soil microcosms containing four different soil community structures and the procedures carried out to establish the community composition (Section 5.1.1-5.1.4). The four soil treatments consisted of:
    a. Sterile
    b. Benchmark soil ('live' from field)
    c. Prokaryote-inhibited (notionally fungal dominated)
    d. Eukaryote inhibited (notionally bacteria dominated)
2. Application and recovery of a U pulse and consequent calculation of U movement through the aforementioned soil microcosms.
3. Investigation of two factors affecting U movement:
    a. $CO_2$ produced from soil microcosms
    b. Soil community treatment effects on U sorption.

#### *5.1.1 Soil*

The clay loam used in earlier experiments was selected from the four soils available. This soil was chosen as U would travel the slowest through it, allowing time to determine the transport through the measurable area with adequate resolution. As a representative microbial community was needed, soil was freshly collected from the recorded original sample site (Section 2.1). This soil was stored without further modification at 5ºC until needed.

### *5.1.2 Determination of the effects of $HNO_3$ application*

The uranium source was provided to the soil as soluble U in a solution of concentrated nitric acid. Before the U pulse could be applied to the soil microcosm it was necessary to ensure that no part of the application process (including the pH of the pulse applied) would adversely affect the soil community and thus introduce additional variables. Consequently a set of experiments was carried out to determine the soil capacity to buffer $HNO_3$. Using the pH determination technique (Section 2.2) the pH of the clay loam soil was determined to be 5.2.

In order to ensure that the soil could buffer the acidity of the added uranium formulation, the pH change in the top 5 mm of the soil in a microcosm following addition of $HNO_3$ was determined. A 0.3 M solution of $HNO_3$ was neutralised by addition of NaOH. A 0.3 ml aliquot of $HNO_3$ solution at pH 2.926 and pH 4.052 was placed onto a 35 mm diameter cut circle of filter paper and applied to the soil surface for 15 minutes. This process replicated that which was planned for use in the uranium pulse application (Section 2.5). Slices were taken as in Section 2.4, and the pH of slices to a depth of 1 cm determined.

### *5.1.3 Manipulation of soil biological community*

The soil was sieved to 2 mm and prepared by the addition of Tryptone soy broth (0.0987 g added to 31.2 g dry soil giving c. 8 mg dextrose, c. 56 mg casein and c. 10 mg soy peptone) in powder form to the soil before packing. This additional nutritional input was applied to boost the microbial population already within the soil before treatments were applied. It was not thought that this addition would affect U sorption and transport as it was assumed the soil microbes would act in the pre-incubation period to feed on and transform the added substrate. After packing to a bulk density of 1.3 g $cm^3$, following the method laid out in Section 2.3.1, collars were wet up from below to achieve the required moisture content of 0.25 ml $g^{-1}$ dry soil dissolving the broth and allowing it to percolate throughout the soil collar.

Four treatments were established

1. **Sterilised**: Sterilisation by gamma irradiation. Soil was not treated with the Typtone soy broth prior to packing into collars. Once packed, collars were sent to Isotron plc (Swindon, UK) for sterilisation at 25-40 kGy and stored at 5ºC on return.

2. **Live**: Tryptone soy broth was added to soil before packing and packed collars were incubated for 10 d at 25°C. No further modification was carried out.

3. **Eukaryote-inhibited**: Tryptone soy broth was added to soil before packing and packed collars were incubated for 10 d at 25°C. A eukaryotic inhibitor was applied to collars 24 h before they were pulsed with U.

4. **Prokaryote-inhibited**: Tryptone soy broth was added to soil before packing and packed collars were incubated for 10 d at 25°C. A prokaryotic inhibitor was applied to collars 24 h before they were pulsed with U.

A practical method of applying inhibitors to soil microcosms was adapted from the literature. The cyclohexaminde application at 2 mg ml$^{-1}$ soil solution was taken from Velvis (1997) who found over application (more than 5 mg ml$^{-1}$ with cyclohexamide and 10 mg ml$^{-1}$ with streptomycin) led to non target-specific inhibition and overlap, but a mixture of 2 mg ml$^{-1}$ soil solution cycloheximide and 10 mg ml$^{-1}$ streptomycin was effective. Though Velvis (1997) used only a small amount of streptomycin (4 mg g$^{-1}$ soil) to avoid non-specific inhibition other studies have used higher amounts for example Alphei *et al.* (1997) used 16 mg g$^{-1}$, Ananyva *et al.* (2006) used 20 mg g$^{-1}$ on arable soils of 2% soil organic matter, Imberger and Chiu (2001) used 62 mg g$^{-1}$. In other work, Bailey *et al.* (2003) did not find streptomycin to be a very effective inhibitor, but there were problems with non target inhibition and overlap with the most effective bactericide: bronopol. As the aim of the inhibition was to create an environment where the selected subset of the soil community would predominate it was decided to apply concentrations of inhibitors which would impair rather than potentially eradicate the target groups, to prevent non-target specific inhibition.

The method of prokaryotic and eukaryotic inhibition was tested before being applied to the packed soil microcosms. One g of soil was weighed into glass bottles (3 replicates per soil). The selected inhibitors were then added to the soil.

Cycloheximide was tested at a concentration to produce 2 mg ml$^{-1}$ soil solution, the amount of solution added was that needed to bring the soil to 100% WHC. The cycloheximide solution was made with ethanol instead of water and it was assumed that the ethanol would evaporate from the soil leaving the overall moisture content of the microcosms unaffected by the application of the inhibitor. Once the cycloheximide had been added the bottles containing the soil were left for 24 h. Following this sterile water (1 ml) was added to the soil to create a slurry. A 100 µl aliquot of this slurry was pipetted onto a plate of malt extract agar (MEA) in a laminar flow cabinet. Plates were incubated for 5 days at 29ºC and then examined for evidence of fungi survival. When the cyclohexamide was applied to the operational soil microcosms there was an error and the solution was actually applied at 0.2 mg ml$^{-1}$ soil solution (i.e. 10% of target).

Streptomycin was applied at 10 mg g$^{-1}$ to the clay loam soil and left for 24 h. Sterile water (1 ml) was added to the soil to create a slurry. An 100 µl aliquot of this slurry was pipetted onto plates of nutrient agar in a laminar flow cabinet. Plates were incubated for 3 days at 24ºC and examined for evidence of bacterial survival. When applying the inhibitor to the soil microcosms an attempt was made not to change the water content of the packed collars too greatly once they had been moistened and incubated. Therefore the streptomycin was added in concentrated solution (0.2857 g in 2 ml to 31.2 g dry soil).

### *5.1.4 Characterisation of microbial communities*

In order to determine if the treatments had produced the required effect, respiration measurements and phenotypic profiling by PLFA of the microbial community structure were carried out.

Respiration measurements were carried out to observe any changes in the activity of the soil from the 4 treatments. The soil microcosms containing the sterile soil were subsampled at the end of the U diffusion period (28 d). Five replicates of 1 g soil were taken and respired $CO_2$ measured using a Rapid Automated Bacterial Impedance Technique (RABIT), as described in Ritz *et al.* (2006). This technique measures the change in conductivity of an alkaline gel as a result of respired $CO_2$ dissolving within it. Conductivity measurements were made every 6 minutes for 16 h, and converted to respiration rate using conversion factors provided in Ritz *et al.* (2006).

The soil microcosms used in the U movement measurements and those used in the respiration measurements were both sub-sampled for PLFA. Only the live, eukaryote inhibited and prokaryote inhibited soils were sub-sampled, the sterilised soil collars were not sub-sampled for PLFA analysis as the soil was required for the respiration test above.

At the end of the 28 d experimental period soil was removed from collars and placed into plastic bags (in the case of collars used for U movement measurements, this soil was the remaining 10 mm). The soil was frozen ($^-$80°C) and then freeze-dried. Freeze-dried samples were homogeneously mixed before five replicates of c.10 g samples were taken and placed in glass bottles. PLFA profiles were determined using a modification of the method described by Frostegard *et al.* (1991), as based on the method described by Bligh and Dyer (1959) and White *et al.* (1979).

In brief, soil in glass bottles underwent lipid extraction using a 0.8:1:2 solution of citrate buffer: chloroform: methanol: (v:v:v). Soil/solvent solutions were placed in an ultrasonic bath (30 min), and then centrifuged at 2000 rev min$^{-1}$ (or approx. 700 RCF) for 10 minutes using the Falcon 6/300 Refrigerated Centrifuge. The organic layer was removed into a clean glass media bottle and separated into two phases by the addition of 4 ml of chloroform and 4 ml citrate buffer. The separated layers were left overnight to allow a clear interface between the two phases. The aqueous layer was then discarded and the organic lower layer dried under $N_2$ in a heating block (40°C). The constant stream of nitrogen prevents the organic layer from coming in contact with oxygen which would react with double bonds, breaking down the unsaturated fatty acids.

The lipid extract was separated into the lipid classes of neutral lipids, glycol-lipids and polar lipids using fractionation. Fractionation was carried out done using the method of Solid Phase Extraction, (also known as Silic acid column chromatography). Silic acid is slightly acidic precipitated silica. Silanols (active sites on the silic acid granules) contain hydroxyl (OH) groups directly bound to the silicon atom. The silanols interact with the polar groups of the lipid classes (the non-polar end of the lipid molecule contributes little to separation). As the polarity of the solvents increases, the lipid classes are selectively eluted from the silanols, thereby effecting separation. Commercially prepared SPE columns (3ml/ 500 mg silica Sep-pak Vac™ from Waters Chromatography) were used for this procedure. The Sep-pak SPE columns have a polar sorbent to absorb analytes from non-polar solvents. The SPE cartridges were prepared by adding 0.5 g sodium sulphate to the top of the SPE cartridge and then the silica washed with 2 ml of methanol, acetone and then chloroform. Cartridges were then conditioned by the addition of 2 ml chloroform. The lipid extract was re-suspended by the addition of 1 ml of chloroform (washing down the side of the vessel) and loaded onto the conditioned SPE cartridge. Neutral lipids were eluted with 5 ml chloroform, glycol lipids were eluted with 12 ml acetone. A fresh glass media bottle was placed beneath the cartridges and polar lipids were eluted with 8 ml methanol into this bottle and dried under $N_2$ in a heating block (40°C).

The phospholipid fraction was then methylated by mild alkaline methanolysis (Dowling *et al.*, 1986). This procedure cleaves the fatty acid from the phospholipid glycerol backbone and replaces the glycerol bonds with a methyl group thereby creating fatty acid methyl esters (FAMEs) which can subsequently be analysed by gas chromatography. The polar lipid fraction was reconstituted the using 1 ml of 1:1 toluene: methanol. 1 ml of 0.2 M methanolic potassium hydroxide was added to hydrolyse the lipids. The solution was incubated for 30 minutes (37ºC) before the reaction was stopped by the addition of 0.25 ml of 1 M acetic acid. 5 ml of 4:1 v/v hexane:chloroform solution and 3 ml of deionised water were added and the ensuing solutions were placed in an ultrasonic bath (30 min), and then centrifuged at 2000 rev $min^{-1}$ (or approx. 700 RCF) for 10 minutes using the Falcon 6/300 Refrigerated Centrifuge. The aqueous lower layer was removed and 3ml of 0.3 M sodium hydroxide was added as a base wash to clean the sample and remove any underrivatised fatty acids or other acidic components. The top layer was then filtered through sodium sulphate (using Whatman No.4 filter paper) to a clean glass media bottle by careful removal using a Pasteur pipette. The ensuing liquid was dried under $N_2$ in a heating block (20-25°C). The fatty acid methyl esters (FAMEs) were stored in a freezer at <-20ºC under nitrogen

The ester linked fatty acid methyl esters (EL-FAMEs) were separated by capillary gas chromatography (G.C.) due to their molecular weight and boiling point. FAMES are identified by their retention times. The G.C. retention time of the resulting fatty acid methyl esters depends on the length of the fatty acid chain and the presence and position of double bonds, *iso* and *ante-iso* branching of the fatty acid C-chain, and hydroxyl fatty acids. Short chain fatty acids (lower molecular weight) come off the G.C. column first. The dried sample was defrosted and reconstituted with 200 µl of hexane before being transferred into a GC vial.

The G.C. used was an Agilent Technologies 6890N. Software used was Agilent G2070 ChemStation for G.C. systems. The G.C. was fitted with a split/splitless injector and a HP-5 (Agilent Technologies) capillary column (30 m length, 0.32 mm ID, 0.25 µm film) which is 5% phenylmethyl siloxane. Helium was used as the carrier gas (1 ml min per min) and the FAMEs separated by using a temperature program, starting at 50°C for 1 min (splitless hold time), increasing at 25°C per min to 160°C followed by 2°C per min to 240°C and 25°C per min until reaching 310°C. Samples were injected (1 µl) using an autosampler (injector temperature of 310°C) and FAMEs detected using a FID operating at 320°C. Standard mixtures of known PLFAs (SUPELCO) were used to identify the main PLFAs in the soil samples and determine (by mass comparison) the relative concentrations of each PLFA.

### 5.1.5 Measurement of uranium movement in clay loam soil

Following the method laid out in Section 2.5 collars were pulsed with a 3 µl ampoule of U solution at c. 900 ppm adjusted to pH 4. Collars were then placed in incubation (25ºC) and left to diffuse for 28 days. This period was calculated from the assumed speed of uranium movement in the preliminary experiment (Appendix 4).

Collars were sliced using the technique laid out in Section 2.4, but at 0.5 mm intervals instead of 1 mm. This method produced thinner slices, with associated reduced soil volume for analysis. Thinner slices were used as the uranium was not expected to move far and resolution of the early phases was expected to give a more accurate measurement of uranium movement. It was calculated that slices of 0.5 mm would produce 7 data points of increased U concentration per collar above background levels over the distance sampled.

Immediately following slicing the wet weights of slices were measured. The slices were then dried for 24 h at 105℃ and dry weight taken. These measurements were used to determine water content of slices as an indicator of homogeneity between replicate collars both in terms of bulk density and water content.

The number of soil slices collected during the experiment was too large to analyse in the time available. Consequently only slices in the sequence of 1, 3, 5, 10, 15, 20 were digested. These are equivalent to distances of 0.5, 1.5, 2.5, 5, 7.5 and 10 mm from the source of U applied. This selection was deemed to contain the possible range of movement, but reduced enough to allow an increased number of replicates.

Uranium concentration in slices was determined by microwave digestion and ICP-MS analysis (Section 2.5.4).

### Transformation of data

Plots of ln*C* against $x^2/t$ were prepared from the slice concentrations of U as follows. The relevant solution of the equation for this experimental systems is:

$$\ln(U_i - U_0) = -\frac{x^2}{4D_{\mathrm{self}}t} \qquad 5.1$$

where $[U]_0$ is the concentration of U at x = 0, $[U]_i$ is the concentration in the soil slices and $D_U$ diffusion coefficient of uranium in the soil, given by

$$D_{\mathrm{U}} = D_{\mathrm{L}}\ \theta_{\mathrm{L}} f_{\mathrm{L}}\ [\mathrm{U_L}]/[\mathrm{U}] \qquad 5.2$$

Where $D_L$ (diffusion through liquid) is the same for all soils ($4.26 \times 10^{-6}$ cm$^2$ s$^{-1}$) and $\theta_L$ (volumetric water content – thus taking into account bulk density) does differ between soils. Values for $\theta_L$ were calculated for each collar from the water content measurements taken and these values carried forward through all calculations for that replicate for that individual soil.

Hence we can allow for differences in moisture content and bulk density between the replicates by plotting ln*C* against $x^2/(4D_L\theta_L f_L t)$ using the known values of $D_L$, $\theta_L$ and $f_L$ (Section 3.2., specifically Table 3.4) Curves were fitted to the plots using the model ($y = a + ce^{(bx)}$).

A model was fitted to the data for statistical analysis between treatments. Saturation curves (Mead *et al.*, 2003) were fitted through each treatment using the equation:

$$Y = Y_0 + Y_{\text{final}} * X / (X + K) \qquad 5.3$$

where

$Y_0$ is the estimated $\ln C$ where X=0

Though all $Y_0$ values were expected to be similar this was not the case. In light of this the Y-axis was expressed as $\ln(C/C_0)$. All the Y-values were transformed by subtracting $\ln C_{0\text{estimated}}$ from individual $\ln C$ values (calculated from original U concentration data measured by ICP-MS in soil slices).

$Y_{\text{final}}$ is a negative value, giving $\ln C$ for $x_{\text{infinity}}$

Final $Y'_{\text{final}}$ values were by definition produced by subtracting $\ln C_{0\text{estimated}}$ from calculated $Y_{\text{final}}$ values. Recalibration of $Y'_{\text{final}}$ after transformation gives, by definition, $Y'_{\text{final}} = Y_{\text{final}} - \ln C_{0\text{estimted}}$

K is the value of X where Y = average of $Y'_0$ and $Y'_{\text{final}}$

Because $Y'_0$ now equals zero by definition, $K = 0.5 \times Y'_{\text{final}}$.

This parameter, representative of the rate at which $Y_{\text{final}}$ was approached, is the parameter of interest, describing as it does the movement of U through the soil microcosms tested. K was unaffected by the transformation of the y-axis.

This produced a model where $Y = Y'_{\text{final}} * X / (X + K)$ as $Y'_0 = 0$ ($\ln C - \ln C_0 = 0$ if $\ln C = \ln C_0$). $Y'_0 = 0$, since $Y'_0 = Y_0 - \text{Ln}C_{0\text{estimted}}$. This model has only two parameters, *viz.* $Y_{\text{final}}$ and K.

### 5.1.6 Soil respiration responses

The $CO_2$ respired from soil collars was measured in the live soil, eukaryote inhibited and prokaryote inhibited treatments. The sterile soil was not included in this test as the soil would have to be freshly sterilised and a lack of respiration had already been measured in the test used to determine that sterility had been maintained (section 5.1.4).

The $CO_2$ respired from the soil collars was measured over 28 days using a sodium hydroxide solution titration method (BS ISO 16072:2002: Section 5.3: *Determination of $CO_2$ release by titration in a static system*). Each treatment was measured over 5 replicates and five blanks were also used. The sodium hydroxide solution was exchanged for fresh every 3 days except in soils treated with eukaryote inhibitor where the trap was replaced every 24 h.

### 5.1.7 Sorption in a clay loam with manipulated soil community measured by shaken suspension

The method laid out in section 4.1.2 was followed to test the ability of the clay loam soil to buffer U. The treated soil microcosm used in the measurement of respired $CO_2$ were subsampled and shaken with solutions of measured U concentration as laid out in section 4.1.2. U sorbed to soil and free in solution was calculated.

### 5.1.8 Statistical analysis

One-way ANOVA of parameters was conducted. Principal component analysis (PCA) was used to analyse PLFA profiles. The statistical software programme used was Statistica (Statsoft Inc 1984-2008, STATISTICA8)

## 5.2 Results

### 5.2.2 Determination of the effects of $HNO_3$ application

Unaltered soil pH was measured in five replicate samples (following the method in section 2.2) as 5.2. The average pH measured after an application of $HNO_3$ at pH 3 and pH 4 to the soil surface of a packed collar was 5.1. Neither collar showed a gradient of pH change down through the slices taken (Table 5.1).

Table 5.1: pH of slices taken at increasing distance from an application of $HNO_3$ at the surface of a soil microcosm. Means of two replicates shown for $HNO_3$ applied at pH 3, only one collar was tested with an application of $HNO_3$ at pH 4.

| **Slice No. (from surface)** | **pH of slice taken (pH of $HNO_3$ applied: 2.926)** | **pH of slice taken (pH of $HNO_3$ applied: 4.052)** |
|---|---|---|
| | pH | pH |
| 1 | 5.13 | 5.1 |
| 2 | 5.15 | 5.26 |
| 3 | 5.13 | 5.11 |
| 4 | 5.12 | 5.09 |
| 5 | 5.12 | 5.04 |
| 6 | 5.1 | 5.04 |
| 7 | 5.08 | 5.06 |
| 8 | 5.11 | 5.05 |
| 9 | 5.08 | 5.15 |
| 10 | 5.11 | 5.23 |
| | | |
| Mean | 5.11 | 5.11 |
| St Dev | 0.020 | 0.076 |

### *5.2.3 Manipulation of soil biological community*

After 28 days incubation, prolific fungal mycelia were visible on the surface of the prokaryote-inhibited soils (Figure 5.2.1 b). Such material was absent in the eukaryote-inhibited cores (Figure 5.2.1 a) or in the non-treated collars (not pictured). No visible signs of contamination were seen in the sterile collars.

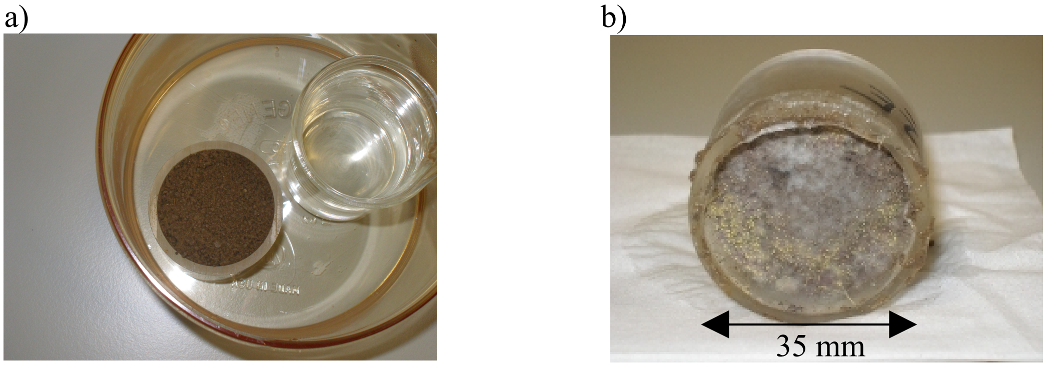


Figure 5.2.1: Representative examples of antibiotic-treated soil cores after incubation for 28 days. (a) eukaryote-inhibited; (b) prokaryote-inhibited.

Soil respiration was measured in sterile soils. Comparison of respiration measured by the RABIT system between empty blanks, sterile soils with water added and sterile soils that had been treated with a glucose solution immediately before being placed within the RABIT module indicated that sterility had been maintained. $CO_2$ evolution between soil-only and soil plus glucose treatments were not significantly different (238 and 171 mg $L^{-1}$ between $T_2$-$T_6$ respectively, $p>0.05$). A small amount of $CO_2$ evolution is generally picked up by the RABIT module both from the $CO_2$ present within the tube that holds the soil for respiration measurement and from inorganic sources.

### *5.2.4 Characterisation of microbial communities*

Following PLFA analysis FAMEs were identified from their retention times (incorporating molecular weight and boiling point) by comparison with tables of previously identified PLFA markers for taxonomic groups. Unidentified FAMES were rejected.

Principal component analysis produced values for PC1 and 2 that accounted for 40% and 30% of the variance respectively. Control soils were shown to cluster in the same area of the axis as prokaryote-inhibited treatments. There was no apparent effect of U on the PLFA profiles (Figure 5.2.2) with both U treated and non-U treated soils clustering in the same area. The treatments were not significantly discriminated by PC1, but PC2 separated the eukaryote-inhibited soils from the prokaryote-inhibited treatments (Figure 5.2.2a). This separation was predominantly driven by the proportion of FAME 16ω1:7t (Figure 5.2.2 b), where the proportion of this marker was significantly lower in eukaryote-inhibited soils (Figure 5.2.3). 16:1 ω 7t is used in the calculation of stress indicators and associated with gram negative bacteria (Table 5.2).

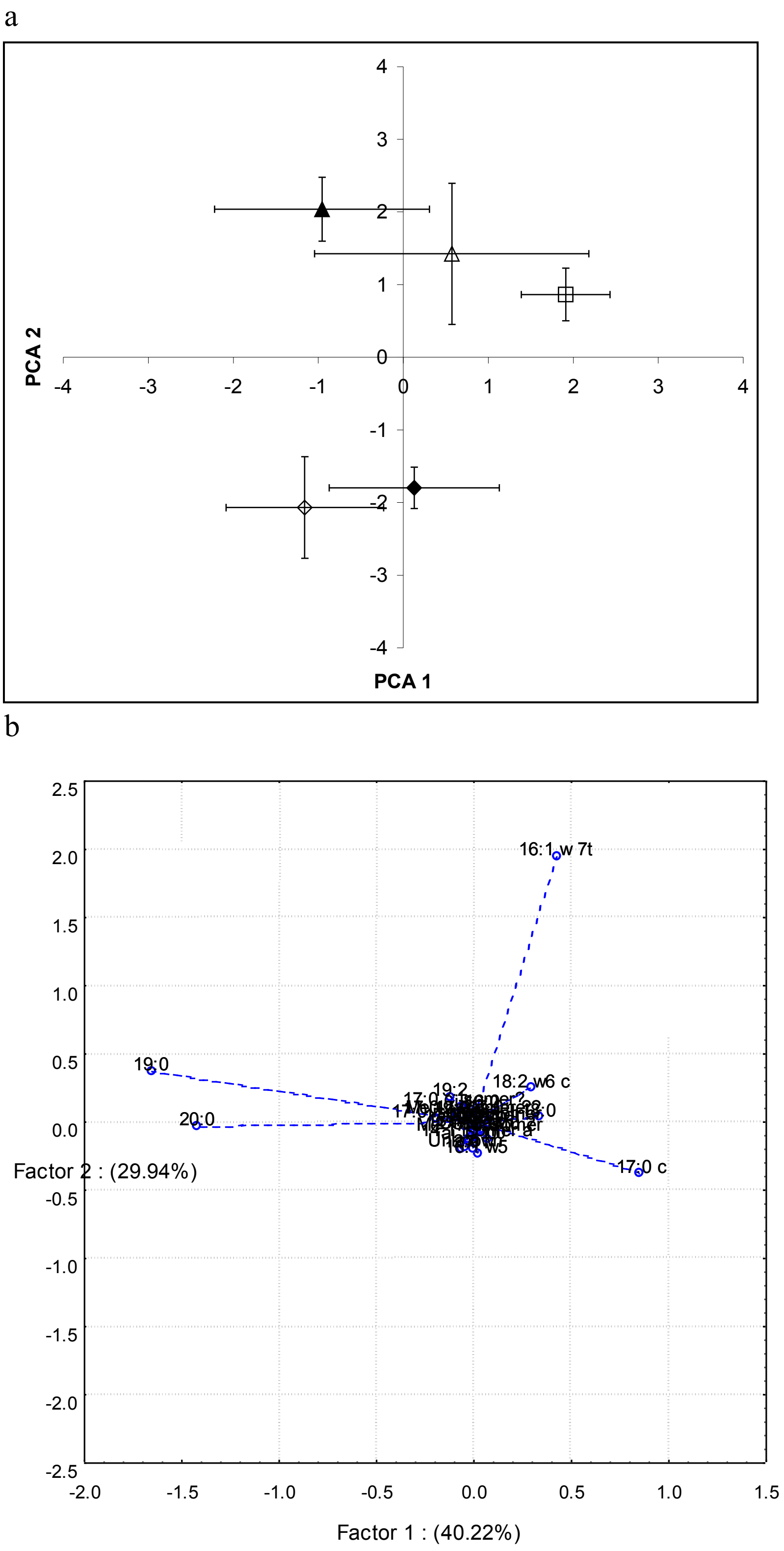


Figure: 5.2.2: (a) First and second principal components of PLFA profiles from treated soils: □: Un manipulated, △: prokaryote inhibited, ◇: eukaryote inhibited . Filled data points relate to microcosms treated with uranium prior to PLFA analysis. Points show means (n=5) bars show SE. (b) Loadings associated with principal components.

Table 5.2: Prominent PLFAs identified from FAME retention time and associated taxonomic groups.

| **PLFA** | **Type** |
|---|---|
| 16.1 ω 7t | Prokaryote, Bacterial, Gram negative |
| 17.0 c | Prokaryote, Bacterial |
| 18.2 ω 6 c | |
| 19.0 | (19:0cy) Prokaryote, Bacterial, Gram negative |
| 20.0 | Eukaryote |

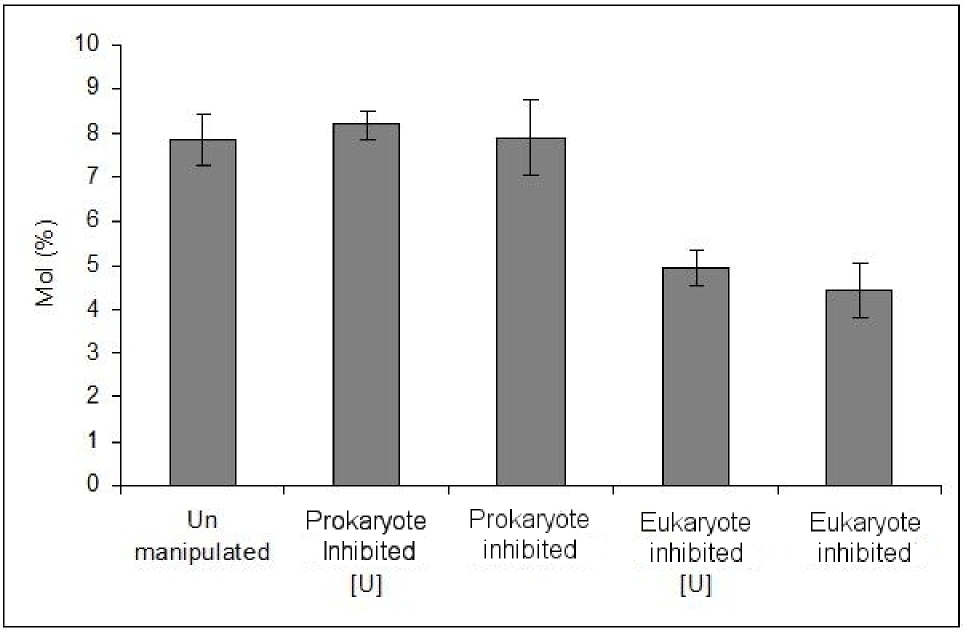


Figure: 5.2.3: Measured incidence of FAME 16:1 ω 7t in soil microcosms that underwent treatment to manipulate soil community. U denotes microcosms treated with uranium prior to PLFA analysis. Means and s.e, ($p<0.05$).

It was also noted that the 17:0 c 19.0 and 20.0 peaks contributed significantly to the weighting in PCA Factor 1. Specifically, 17:0 c peak is related to gram negative bacteria associated with sulphur reducing gram positive bacteria. Though the FAME did not contribute to the differentiation between treatments, its presence was noted for future discussion (Section 5.3.4) in relation to the known potential for such bacteria to reduce uranium, either directly or indirectly by affecting redox conditions in soil.

### *5.2.5 Measurement of uranium movement in clay loam soil*

Measured moisture contents within slices taken for U concentration determination differed within the collars, oscillating around the mean moisture calculated for that individual soil microcosm (Figure 5.2.4).

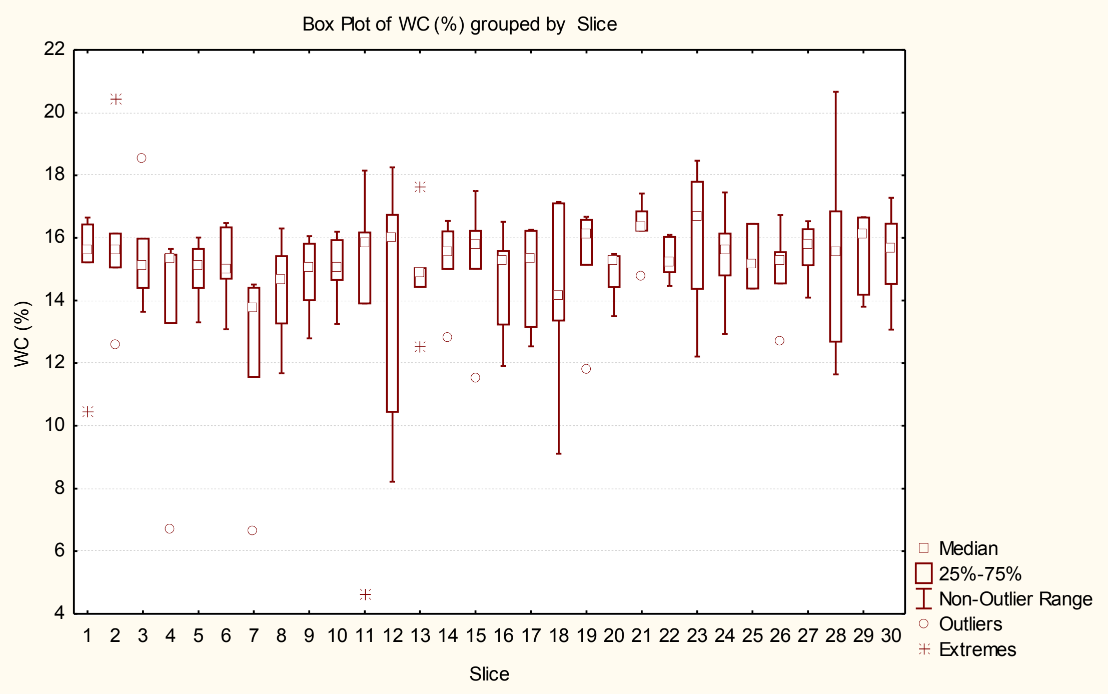


Figure 5.2.4: Representative example of moisture contents measured in the six sterile soil microcosms. Moisture content (g g$^{-1}$) of all 30 slices taken for U concentration measurement down to a depth of 2 cm is shown.

Despite these small differences no trend was found with depth. Mean moisture content did not different statistically between collars or treatments despite the soil community manipulation treatments being applied in solution (Table 5.4).

Table 5.3: Mean moisture content of the soil microcosms measured after 28 d. ANOVA revealed no significant difference at $p<0.05$ (degrees of freedom:17)

| | Water content (g cm$^{-3}$) | | | |
|---|---|---|---|---|
| Treatment | Calculated from slice weights | | Calculated from cell weights | |
| | mean | St. Err | Mean | St. Err |
| Sterile | 0.22 | 0.010 | 0.25 | 0.010 |
| Live | 0.27 | 0.011 | 0.27 | 0.009 |
| Eukaryotic inhibitor | 0.26 | 0.014 | 0.28 | 0.012 |
| Prokaryotic inhibitor | 0.24 | 0.024 | 0.28 | 0.024 |
| F | 1.75 | | 1.13 | |
| p | NS | | NS | |

U concentration:distance profiles were plotted for individual treatments (Figure 5.2.5). The distance travelled by the pulse was minimal, ranging from 1 to 2 mm, though concentrations or U above those measured in blank soils were recorded to 10 mm depth. It must be noted that distance travelled from source was calculated from the dry weight of individual slices and the bulk density of the collar as a whole. Consequently these are assumed values for distance, not measured.

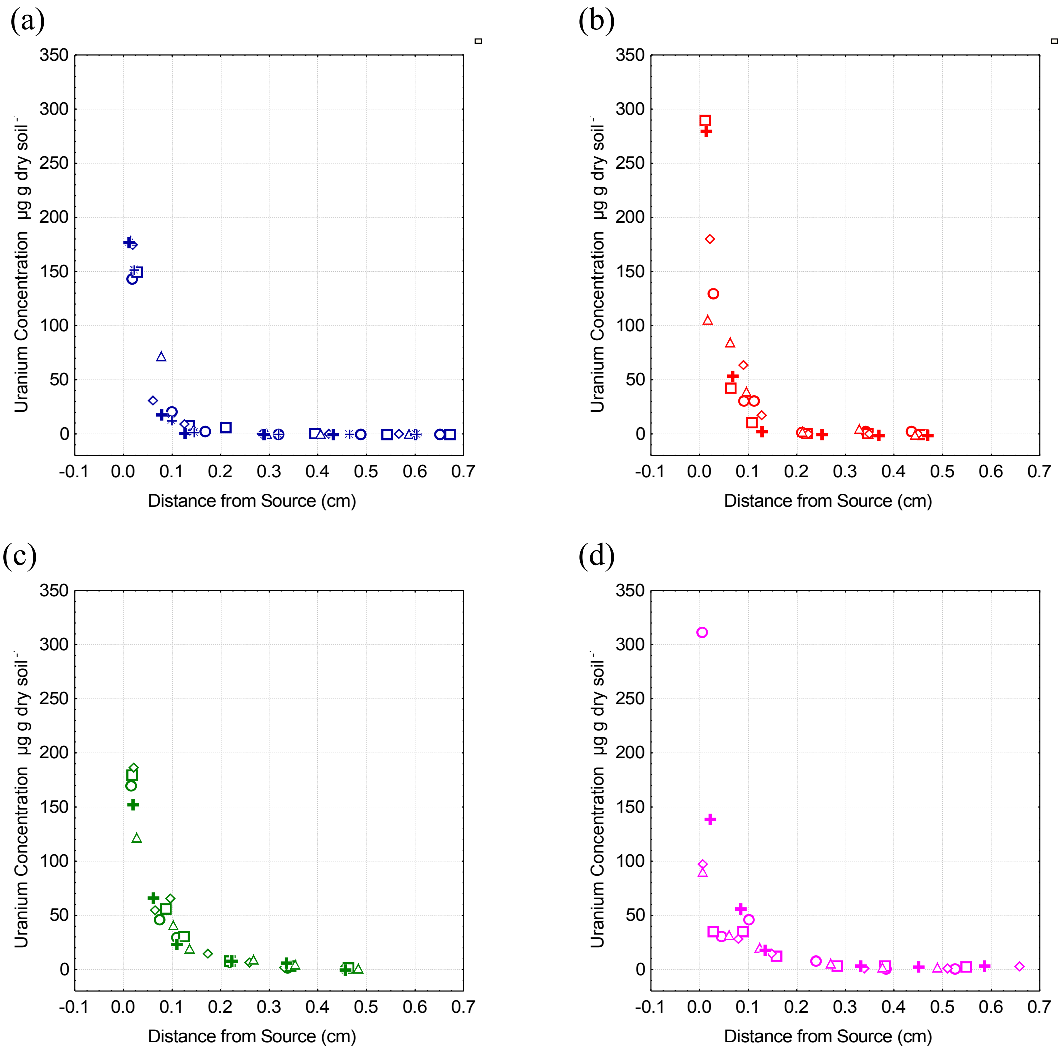


Figure 5.2.5: Concentration of U in relation to distance from pulsed-source in soil microcosms of different biotic status: (a) sterile; (b) non-sterile; (c) prokaryote-inhibited; (d) eukaryote-inhibited. Symbols denote replicates (n = 6 for sterile treatment, n=5 for others).

The concentration:distance profiles were distinctly non-linear. Most of the slices sampled contained uranium concentrations around the measured concentration found in the uncontaminated soils (blanks). However, high U concentrations were found in surface soils and a certain amount of the U applied in a pulse at the soil surface did undergo diffusion, moving throughout the measured distance.

The amount of uranium present in the surface slice varied between treatments and, in the non-manipulated and prokaryote-inhibited treatments, replicates. The difference was between 200 – 300 µg $g^{-1}$. This difference (indicating a possible technical problem with the contact between the soil surface and the U application through filter paper) was removed by the transformation of the data that took place before plots of lnC vs *x*/4*D*t were produced (Figure 5.2.6). These plots take account of the gradient of the slope rather than the intercept with the y axis.

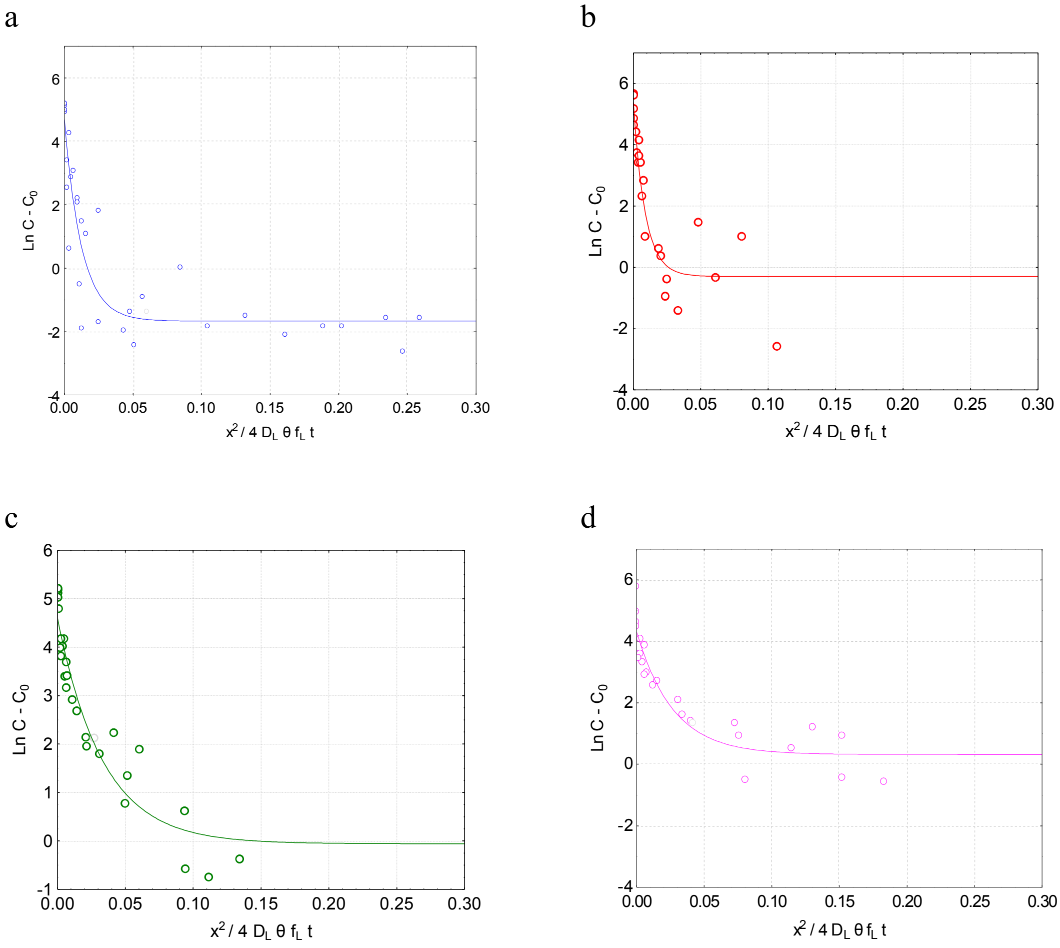


Figure 5.2.6: U transport through soil microcosms, concentration transformed to ln*C* and depth (x) transformed incorporating water content and bulk density (equation fitted is $y = a+ b*\exp^{(c*x)}$). Four treatments represented a) Sterile, b) non manipulated, c) eukaryote inhibited d) prokaryote inhibited.

Comparing U movement in soil between the different treatments it becomes clear that the plots are non-linear for all four treatments, indicating slow local equilibria. The slopes and curvature differ between treatments. The curvature was more prominent in the manipulated treatments while the sterile and un-manipulated treatments show a more acute asymptote.

The variation seen in the un-manipulated treatment is greater than any of the others. The variation indicates heterogeneity inherent in the system within this treatment. In order to compare the different treatments, the four modelled relationships were plotted on one figure (Figure 5.2.7).

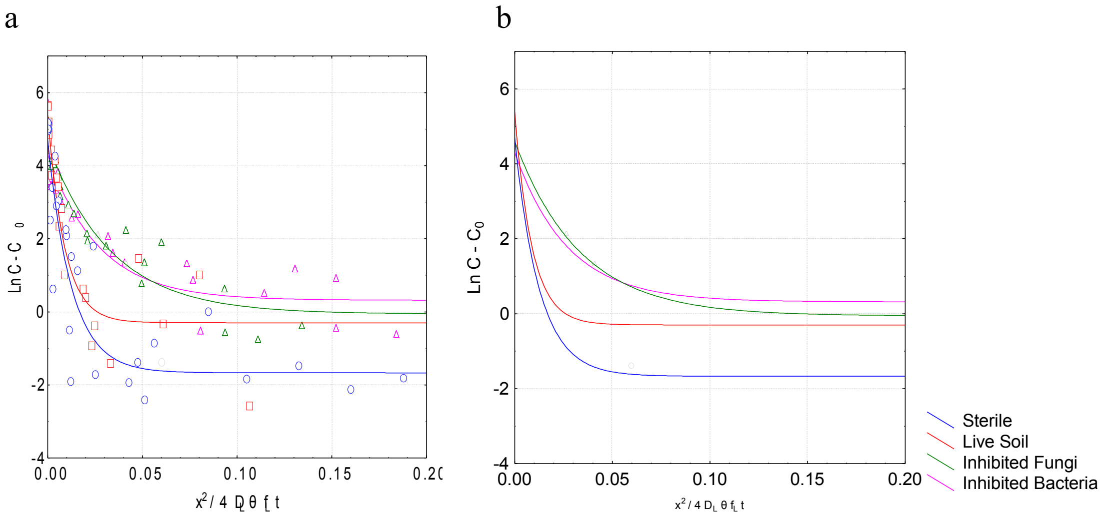


Figure: 5.2.7: Aggregated plot of movement of U pulse through soil microcosms for all treatments. Concentration (*C*) at individual depth transformed into ln*C*-*C*$_0$. Depth (x) transformed by incorporation of parameters that differed between microcosms i.e. bulk density and diffusion time. Plotted lines of model y = a+ b exp$^{(cx)}$. Both plots a) and b) show the same model, but individual data points have been removed in b) to show the differences between the lines more clearly.

The steep gradient to the left of the figure is an indication of the expected high sorption of U in this soil preventing U transport down through the soil column by holding it tightly to soil particles. As all treatments were applied to the same clay loam and this soil was assumed to be the same in terms of intrinsic ability to sorb uranium as determined in Section 4.1.2 (all treatments were subsampled from the same original field sampling) this effect can be related to biological driven differences in soils.

Using the modelling technique described in Section 5.1.5. an attempt was made to differentiate between the different treatments. The complicated nature of the curves meant that a simple comparison was impossible. Instead two parameters were selected that both described the relationship and had some biological significance rather than simple mathematical purpose. By using K (the mid point of projected concentrations from the surface of the microcosm to infinity) and $Y_{final}$ (the projected concentration at an infinite depth) a comparison between treatments was undertaken. The 95% confidence intervals of K and $Y_{final}$ were computed simultaneously (Figure 5.2.8) revealing that if K was considered in isolation none of the treatments differed significantly. Considering $Y_{final}$ alone there was only a difference between the sterile and prokaryote inhibited treatment.

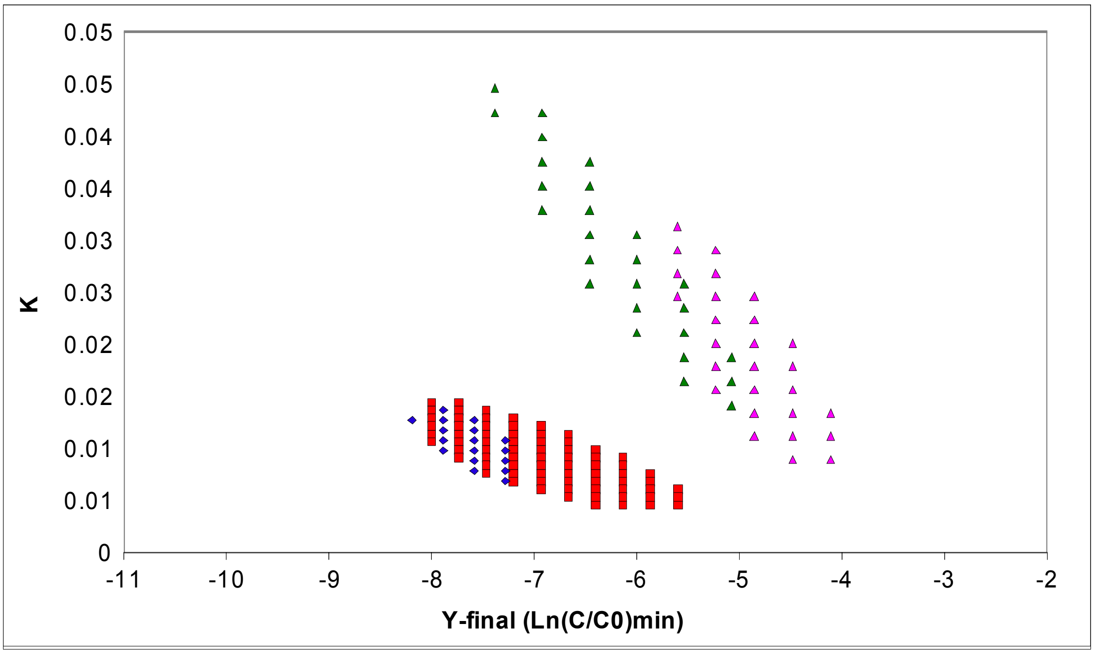


Figure 5.2.8: model returned 95% confidence intervals for $Y_{final}$ and K calculated from the movement of U through a soil microcosm. ◆ Sterilised soil, ■ non-manipulated soil, ▲ eukaryote inhibited treatment, ▲ prokaryote inhibited treatment.

When both parameters were considered simultaneously, it was seen that as shown in Figure 5.2.8 there was a difference between the treatments that had undergone manipulation with bactericide or fungicide and the treatments that had not.

From the confidence intervals produced by the model, 95% confidence intervals of the four regression lines were calculated by producing alternative regression lines for each of the treatments, using the full factorial matrix of significant K and $Y_{final}$ values (Figure

5.2.8). Taking the min and max Y values for each value of X, these were plotted as boundary lines (Figure 5.2.9).

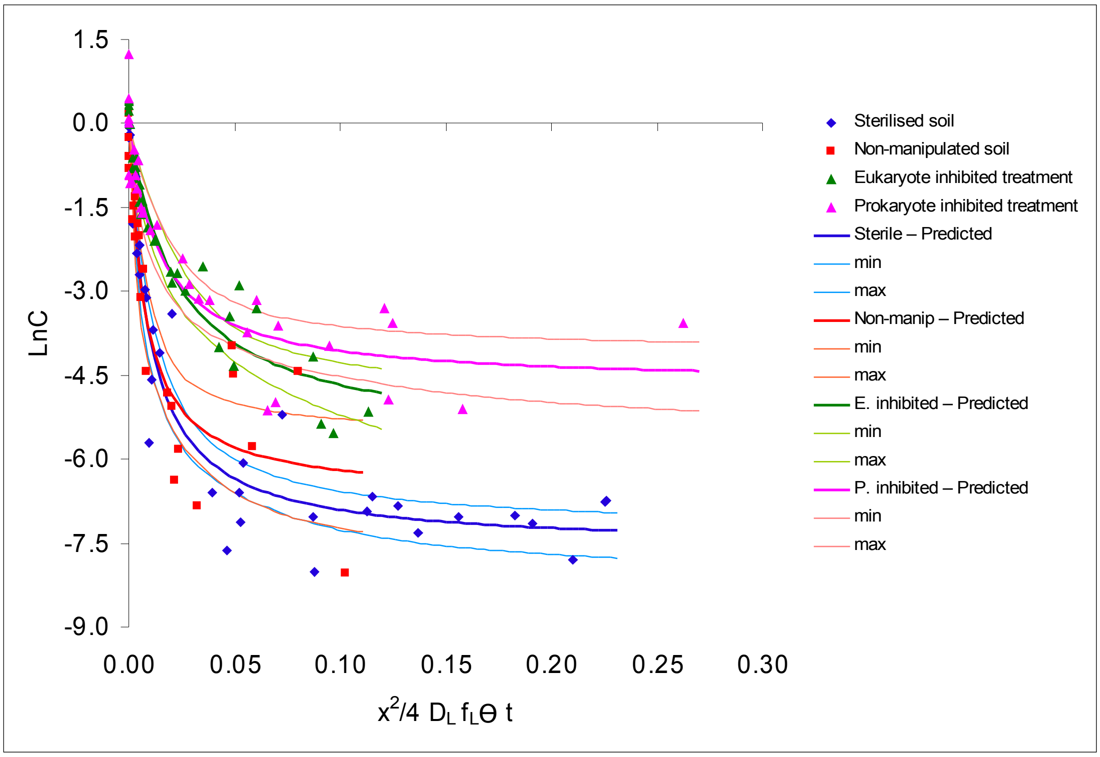


Figure 5.2.9: Measured U in soil microcosms with predicted U calculated from model with min and max values plotted as boundary lines. The coefficient of determination is the ratio of Explained variation v. Total variation, i.e. (1-Residual SS)/(Total SS). Coefficients of determination: Sterilised soil = 0.84, non-manipulated soil =0.86, eukaryote inhibited treatment =0.88, prokaryote inhibited treatment =0.88.

### *5.2.6 Soil respiration responses*

Measured $CO_2$ released from soil (both from respiration and/or chemical breakdown) was plotted over 28 d (Figure 5.2.10).

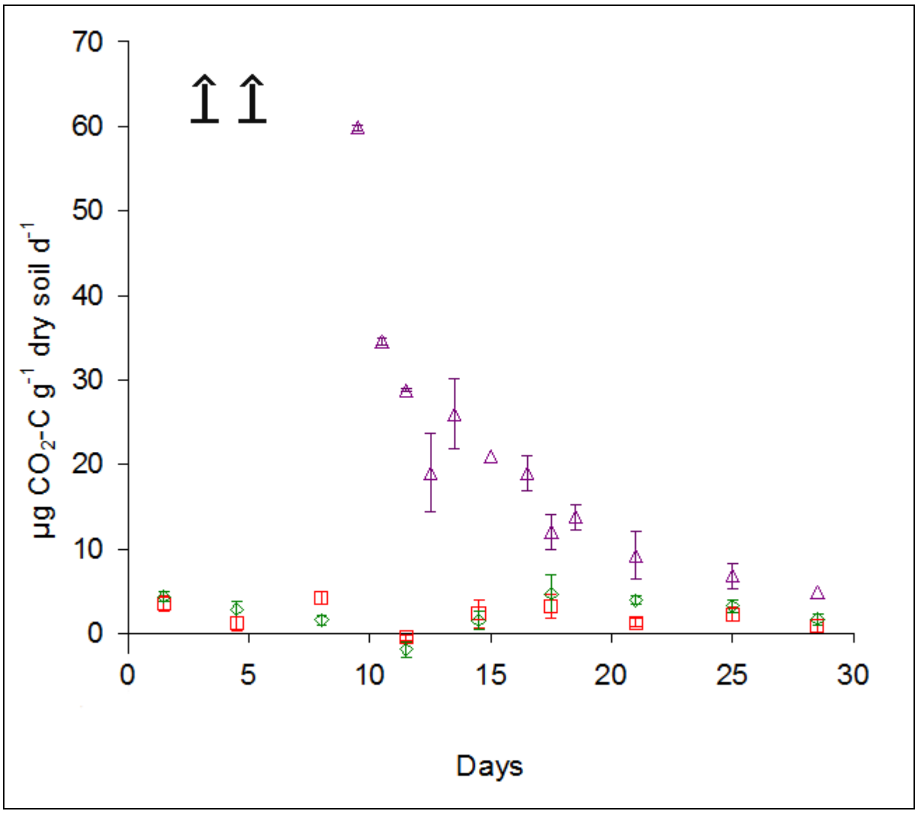


Figure 5.2.10: $CO_2$ released from soil microcosms over a 28 d period (mean and s.e. for five replicates). Amounts of $CO_2$ measured in blanks was subtracted from data shown. □ live soil, ◇ eukaryote-inhibited, **Δ** prokaryote-inhibited. Arrows denote values above the limit of detection.

Neither the live or eukaryote-inhibited soil microcosms showed an increase in $CO_2$ release over the period measured (Figure 5.2.10). However, considerably higher levels of $CO_2$ respiration were measured in the prokaryote-inhibited soils, particularly immediately after application. The $CO_2$ measured from these soils decreased over the time period producing a curve similar to that seen in instances where a disturbed community produces a flush of respired $CO_2$ after a major stress of input of readily-assimilable substrate (Park & *et al.,* 2008; Xinag *et al.,* 2009).

### *5.2.5 Sorption in a clay loam with manipulated soil community measured by shaken suspension*

The concentrations of U in liquid and sorbed to soil in the shaken suspension experiments were plotted on log log axes. As expected the uranium was strongly adsorbed to soil with little of the U found in the liquid phase. Sorption seen was greater in all treatments than in previously tested soil at a smaller sieve size (Figure 5.2.11).

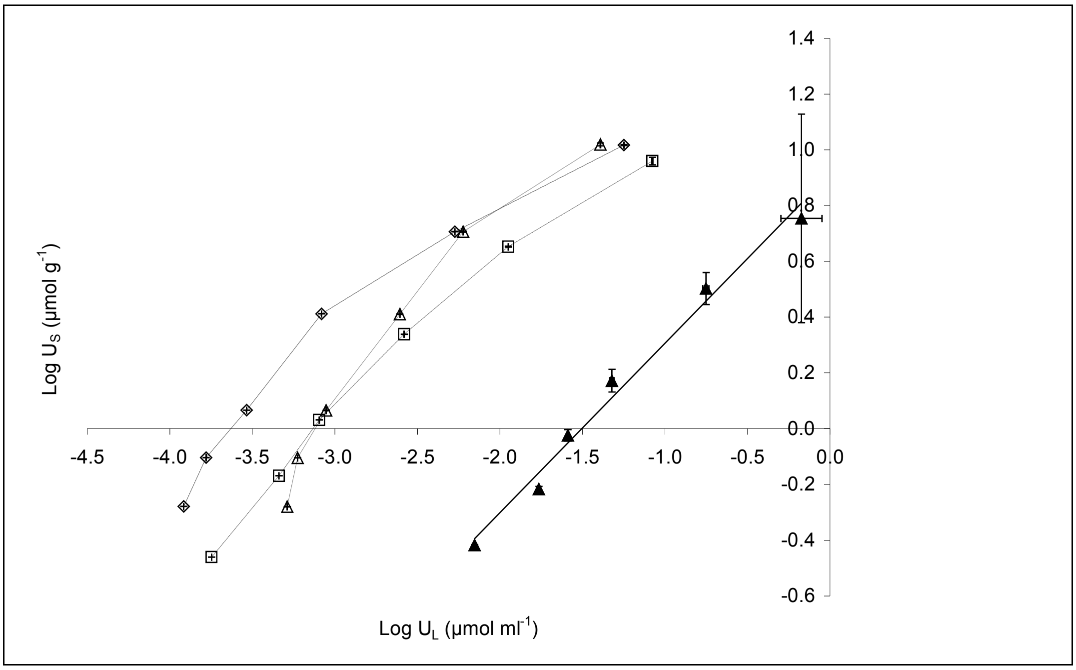


Figure 5.2.11: Distribution of uranium between sorbed ($U_S$) and liquid ($U_L$) phases at six concentrations of U after shaken suspension experiments in a clay soil under manipulations of community. ◇: live soil, □: Prokaryote inhibited, △: Eukaryote inhibited, ▲: clay soil (ground and 0.5 mm sieve (assumed dormant))

As it is generally accepted that cation buffering follows a Freundlich relationship, linear regression lines were fitted and analysed (Table 5.5) in order to allow the calculation of $U/U_L$ or buffering power (Section 4.1.2). Though $R^2$ values were within acceptable limits it was noted for discussion that curvature could be seen in the plots with the naked eye.

Table 5.4: Parameters of the general linear model applied to the distribution of U between sorbed ($U_S$) and liquid ($U_L$) phases in a clay soil after shaken suspension experiments (Figure 5.2.11).

| | **Constant a** | **Constant c** | **$R^2$** |
|---|---|---|---|
| Live | 0.469 | 1.70 | 0.946 |
| Eukaryote inhibited | 0.655 | 2.03 | 0.968 |
| Prokaryote inhibited | 0.526 | 1.61 | 0.968 |
| F | 10.7 | 2350 | |
| p | <0.001 | <0.001 | |

The live soil showed the greatest sorption at low concentrations of U. The manipulated soils shared a similar, if less strong ability to sorb U. At high U concentrations the live and eukaryote inhibited soils were similar in their ability to sorb U, with the soil from the prokaryote inhibited microcosms having more U found in the liquid phase. Overall buffering was less in the prokaryote inhibited soils (more associated with the liquid phase) than in the live soils. Decreased sorption onto mineral matter is correlated to higher levels of $CO_2$ within soil (*c.f.* Chapter 4). However it was found that the ability of the soil to sorb U in live and eukaryote inhibited treatments was not associated with a similar difference in measured $CO_2$ release from these microcosms.

## 5.3 Discussion

### 5.3.2 Determination of the extent of negative effects from $HNO_3$ application

It was determined that by altering the pH of the uranium solution to pH 4 before application, no consequent effect on the soil community should occur.

### 5.3.3 Manipulation of soil biological community

Respiration tests confirmed that the sterile soils were not contaminated. The $CO_2$ evolution data indicated that sterility was retained in the sterile treatments throughout the experimental period.

### 5.3.4 Characterisation of microbial communities

Phenotypic structure of the communities in antibiotic treated soils (as indicated by PLFA analysis) were significantly different between pro- and eukaryotic inhibitors. This indicates that the communities in these soils after manipulation were therefore distinct and contrasting, as was intended by the application of the different antibiotic treatments. However the phenotypic profile of the prokaryote inhibited soils did not show a large eukaryote peak (18:2ω6, Klamer and Baath, 2004) as had been predicted, despite visual inspection revealing-prolific fungal mycelia visible on the surface of the soil microcosms. In addition there was not a significant difference between the community indicated as present in non-manipulated soils from that indicated as present in prokaryote inhibited though on visual inspection no fungal mycelia were seen on the non-manipulated soils.

The spectra produced by PLFA analysis were compared to that produced by research staff from a standard qualitative bacterial acid methyl ester mix (Supelco) (Pawlett, 2003). After it became clear that there was no 18:2ω6 peak the spectra were re-examined with close attention being paid to the area around the retention time for this peak. No evidence was found that the retention time had shifted in either direction and so the result stands.

In regard to the success of the community manipulation, from the visual inspection during the checks undertaken as the experiment was ongoing it was concluded that bacteria had been killed in the prokaryote inhibited soils in comparison to the non-manipulated microcosms thus removing inter-fraction competition (as evidenced by the increased mycelial growth). The increased respiration from prokaryote inhibited soil microcosms adds further evidence to this conclusion.

Though the results of the PLFA analysis were not in agreement with the visual and respiration results, this is no reason to label the manipulation a failure. The PLFA analysis did separate the prokaryotic and eukaryotic manipulations and with visual observations and differences in respiration measured between the treated soils it is evident some the proportions of the eukaryotic/prokaryotic fractions were altered by the treatments. Some antibiotic resistance was expected as it is unrealistic to expect to remove all of the many and varied bacteria present in a soil by one application of a prokaryote inhibitor. These results suggest that a wider range of independent measures of community composition should be included when assessing affects of antibiotic treatment. For example, the use of an ergosterol determination would be beneficial towards clarifying the situation with respect to fungi (Montgomery *et al.*, 2000).

Peaks associated with sulphur reducing bacteria were found. Sulphur reducing bacteria are associated with indirect effects on uranium reduction by altering the redox potential of soils at the micro scale. The FAME 17:0 c was shown to be dominant in Factor 1 of the PCA. However Factor 1 was found not to be able to differentiate significantly between the treatments, this instead falling to Factor 2 in which FAME 17:0 c was not nearly so active. Though this FAME is associated with sulphur reducing bacteria (Kaur *et al.,* 2005) its inability to contribute to the separation of the treatments means it will not be discussed further.

In PCA Factor 2 the FAME 16.1 ω 7t was shown to differentiate significantly between the treatments. The change of *cis* unsaturated fatty acids (16.1 ω 7c) to *trans* unsaturated fatty acids (16.1 ω 7t) is an adaptation mechanism induced by environmental stress (Kaur *et al.,* 2005). The conversion from *cis* to *trans* reduces membrane fluidity thus counteracting the stress. This isomerisation is generally not activated in the presence of a stress that reduces the ability of the micro-organisms to grow. It is associated instead with stresses that specifically result in an increase in membrane fluidity hand has been documented in response to varied stress factors including heavy metal toxicity (Kaur *et al.,* 2005).

### *5.3.5 Measurement of uranium movement in clay loam soil*

The range of measured water contents within individual slices fell within acceptable levels. Although the method of packing was monitored to ensure a consistent bulk density throughout each collar an oscillating trend was apparent in the data. However, this oscillation, when viewed in a coarser scale is merely variation around the mean water content. Though the additions of inhibitors to the manipulated community treatments were associated with liquid additions, there was no overall effect. With no statistical differences in moisture content between collars it can be assumed that any differences in diffusion of U through said soil collars was caused by the treatment applied and not due to the dominance of the parameter $D_L$ (diffusion coefficient in water).

Having characterised U diffusion through the clay loam soil in Chapters 3 and 4, this chapter intends to examine the effects of the presence of a soil biological community on U diffusion. As was seen in the self diffusion experiments, the distance U diffuses through soil is shorter than seen in the diffusion of $Br^-$ due to strong sorption onto soil particles. Though uranium above blank concentrations was recovered to the full depth sampled, reasonable concentrations were only measured in the top 3 mm. This slow rate of movement, i.e. less than 5 mm over 28 d, is in line with the high levels of sorption seen in the shaken suspension experiments to determine buffer power in chapter 4.

As seen in Chapter 4, a curved rather than linear relationship was seen in the lnC vs $x^2/D_Ut$ plots, indicating that slow equilibrium reactions were affecting soil buffer power for U. The U is extremely strongly sorbed to soil surfaces at the site of pulse application. The concentration already within soil is low (at around 2 ppm) thus causing little competition for soil sorption sites. The amount of U added in the pulse was more than capable of being sorbed to soil surfaces in the first 0.5 mm of soil. However, it is interesting to note that some U remained in solution and continued diffusing. The U found in slices at depth was evidence for U movement to depth and may still have been moving away from the source.

The amount of uranium present in the surface slice varied between treatments and in the case of both non manipulated and prokaryote inhibited treatments, between replicates. The difference (between 200 – 300 $\mu g\ g^{-1}$) can be explained by a number of factors both practical and theoretical. Due to the method of U application the amount originally transferred to the soil surface differs each time. However, this amount of difference between replicates was not expected as operator skill improved with repetition of the method (and by this point a number of applications of pulse to soil surface had been carried out). It was noted that the two treatments which show this variation are those with fungal communities present. The indeterminate nature of fungal mycelia means that in some circumstances their community dynamics are inconsistent (e.g. White *et al.,* 1998), and this may explain the increased variability seen here. This variability was curtailed by data transformation before comparing treatments.

The differences in rates of U moment through the soil microcosms found between soil treatments were small where they were found. Following application of the model, differences in predicted movement were found between two groups, a) the sterile and non-manipulated soils and b) the pro- and eukaryote inhibited soils. Bearing in mind that impedance did not differ between treatments (clay loam impedance calculated in Chapter 3) and any moisture content differences between the collars were judged statistically irrelevant the only parameter left to differ between treatments is $[U]/[U_L]$ or the soil buffer power for U. This was expected, the hypothesis being that the presence of soil biology would increase the number of sorption sites. Instead it was seen that the manipulation of the soil community reduced the capacity of the soil to sorb U with higher concentrations at depth indicating that the U had diffused further. This was also seen in the un-manipulated soil where despite a similar response to U addition in surface slices, higher concentrations of U at depth were measured.

In surface slices, where sorption is strongest and the difference between U concentrations highest, when compared to that in uncontaminated samples, the sterile and non-manipulated soils behaved in a similar manner indicating the dominance of soil sorption sites over biological effects. With depth U sorption behaviour in the non manipulated treatment switches from behaving like a sterile soil (indicating that the biology was not having a dominant effect on U diffusion) to showing increased U mobility. This may indicate that despite the dominance of non-biological effects in areas of high concentration, biological effects non-the-less have a part to play at lower concentrations of U contamination.

Reduced sorption in the presence of a biological community is not completely unheard of, from the literature there was evidence that both reduced (Valsami-Jones & McEldowney, 2000 and Fomina *et al.,* 2007) and increased (Ragnarsdottir & Charlet, 2000 Spear *et al.*, 2000 and Valsami-Jones & McEldowney, 2000) sorption in the presence of a biological community could be expected. However, both fungi and bacteria can adsorb, immobilise and ingest uranium, preventing diffusion (Ragnarsdottir & Charlet, 2000 and Formina *et al.,* 2007) and consequently it was thought (though not assumed) that the effect of an active soil community would be to retard the diffusion of U through soil.

From the data presented here it appears the mobilising effects of the soil community are more dominant under the conditions that transport was measured. There are a number of mobilising pathways, fungi can mediate mineral solubilisation by acidification (protonation), complexation (chelation) or metal accumulation by biomass (Formina *et al* 2007). In a similar fashion bacteria can indirectly produce an environment that increases the mobility of a contaminant through the release of secondary metabolites (Valsami-Jones & McEldowney, 2000). These include organic acids and exo-polysaccharides that can act as ligands to cations, keeping them from sorbing to soil particle surfaces in some cases.

The potential of the biocides used to inhibit components of the soil biological community to affect the transport of uranium through the soil collars cannot be completely refuted by the controls used in this experiment. Both streptomycin and cycloheximide (applied in ethanol) could potentially have increased the number of ligands within the soil. This was investigated further in Section 5.2.5 with regard to measured sorption in the different manipulated microsoms by shaken suspension.

With regard to the additional nutrition arising from the streptomycin application, this is small when compared to the amount of dead bacteria within the soil. With an application rate of 10 mg $g^{-1}$ and a total weight of soil within the collars of c. 36.7 g there is a total 367 mg of streptomycin potentially available to the microbial community. The molecular weight of streptomycin is 581.5 of which 43% is carbon and 17% nitrogen so there would be 4.3 mg C $g^{-1}$ soil and 1.7 mg N $g^{-1}$ soil. The cycloheximide applied was even less in terms of nutritional provision. At 0.2 mg $ml^{-1}$ soil solution c. 20 mg were added to 36.7 g of soil. Assuming 40% of this was carbon this would have produced a meal of 0.2 mg C $g^{-1}$ soil. As the purpose of the biocide application was to increase the activity of a selected component of the microbial community, neither of these added nutrients was considered to effect the resultant manipulation. A further experiment where additional controls of gamma irradiated soil were treated with the biocides used here would be needed to completely prove the streptomycin and cyclohexamide had no effect.

The trend of reduced U sorption and associated increased U mobility was seen more strongly in the microcosms with manipulated communities and this brings to light the concept that the potential impact of perturbation on a stable community previously assumed to be modelled in terms of U diffusion cannot be underestimated. If perturbation negatively affects sorption during the community's return to equilibrium as indicated by the increased U transport in the prokaryote inhibited soil microcosm with associated respiration data, any assumptions with regard to U diffusion through a soil previously modelled may become inaccurate the moment perturbation occurs. With the known sources of DU including penetrator corrosion within soil, and the location of DU pollution within war zones perturbation from shell impact, tank/vehicle manoeuvres and cleaning up upon return of the civilian population is quite likely with regard to this pollutant.

Another way to look at the difference between the treatments would be to state that by removing part of the soil biological community a vital function was affected i.e. the ability to sorb U. Soil communities are complex and expressed functionality is built upon a myriad of interwoven relationships (Ritz *et al.*, 2004). By removing either the bacterial or fungal component, this aspect of soil function was apparently reduced.

The similarity between the diffusion of U between prokaryote inhibited and eukaryote inhibited treatments was not hypothesised. Within the complexity of a diverse soil community it was expected that the effects of the fungal or bacterial components would cancel each other out. Thus, when altering the soil community by removing one or the other component, individual effects were expected to result in increased or decreased rates of U movement in soil measured. However, increased mobility was seen in both manipulated treatments suggesting that the intrinsic effects of each microbial component were cancelled out by another mobilising effect when either fungi or bacteria were allowed to dominate. Another option is that the mobilising effects seen in the pro- and eukaryote inhibited treatments were suppressed in the non-manipulated soil community with its associated inter-specific population pressures.

In respect to the first option, if there was an over-riding mobilising effect that came into play when the antibiotics were applied to the soil microcosms, this would hide any immobilising effects intrinsic to the fungi or bacteria. This theory formed the basis of the additional experimentation with regard to respired $CO_2$ and determination of sorption by shaken suspension and will be discussed further in Section 5.3.4 and 5.3.5.

In respect to the second option, it can be theorised that the complexity of a soil community can result in a soil function that is in direct opposition to the function of individual species within that community. This warrants further investigation.

### *5.3.4 Soil respiration responses*

Examining the difference in U transport between microcosms manipulated by antibiotic application and those manipulated by sterilisation or not manipulated at all it was hypothesised that population changes following community manipulation (the removal of competition and provision of a food source) had led to an increase in population size of either the bacterial or fungal component depending on antibiotic applied. With reference to the effects of $CO_2$ on U sorption (an increase in carboxyl U species with consequent decreased charge, *c.f.* Chapter 4) it was hypothesised that an increasing population associated with increasing respiration would have an over-arching effect on U transport. This was addressed by the soil respiration measurements.

The increased $CO_2$ respiration in the prokaryote inhibited treatment can be explained by fungal community growth in the removal of inter-specific competition from bacteria followed by population decline as intra-specific competition becomes a factor. The similar levels of respiration in the eukaryote-inhibited and non-manipulated treatments may be attributed to the relatively low concentration of cycloheximide added in the former treatment. Thus the hypothesis that increased $CO_2$ concentrations from increases in soil respiration can explain the reduced sorption of U in the manipulated microcosms cannot be accepted or refuted by the data presented.

If high levels of respired $CO_2$ explain the decrease in sorption seen in the prokaryote inhibited microcosm then another factor is needed to explain the decrease in sorption seen in the eukaryote inhibited community. If high levels of $CO_2$ have no effect on sorption of U within soils, as may be indicated by the eukaryote inhibited soil, then some other factor is needed to explain the decrease in sorption seen in both manipulated soils.

In either case there is at least one other, as yet unidentified mechanism strongly controlling U sorption and by association, U transport through soil not identified in these studies.

### 5.3.5 Sorption in a clay loam with manipulated soil community

In order to further clarify the reasons for the difference in U transport between microcosms manipulated by antibiotic application and those manipulated by sterilisation or not manipulated at all, the soil microcosms were assessed for differences in soil buffering capacity, or the intrinsic ability of the soil to sorb U. In light of the potential increase in the number of ligands available for uranium complexation in the microcosms treated with biocides a difference between the non-manipulated microcosms and those that underwent community manipulation was investigated.

In terms of soil buffer power the mere presence of a microbial community appeared to be more important than whether that community was dominated by fungi or bacteria. The increased U sorption in the soils with an active community compared to soil ground and sieved to 0.5 mm (with the assumption that such procedures have rendered the soil community dormant) indicated that the presence of an active microbial community within soil increased U sorption as hypothesised. This is, however, incongruent with the concentration:distance profiles for U found in actual soil collars where there were similar levels of U sorption seen in surface slices of live soil and sterile soil when a pulse of U was applied and allowed to diffuse.

The two systems are not directly comparable. Gamma irradiation of a soil with a well established community microbial present cannot be said to be equal to the effects of aggressive mechanical breakdown of a soil with associated wetting and drying cycles. In addition there is a difference between U sorption in a shaken suspension and U sorption in a soil packed into a column with associated air spaces.

The curvature of the sorption isotherms on the log-log plots where a linear Freundlich isotherm was expected, revealed further information about the effects of a microbial community on the soil buffer power with the increased number of sorption surfaces, both on inert organic matter and on live microbe surfaces. The curvature indicates that these sorption sites are affected by the concentration of the U contamination. At higher concentrations sorption is less than the model would predict, indicating that sorption sites saturate more quickly than expected.

Comparing the similarity of $CO_2$ respired in non manipulated and eukaryote inhibited soils and the difference in soil buffer power between the same soils throws up an interesting conundrum. If the reduced U sorption in the prokaryote inhibited microcosms is explained by an increase in respired $CO_2$, it could be assumed that eukaryote inhibited soil would show a balancing reduction in soil buffer power when compared to prokaryote inhibited soil. If this were found it would explain the eukaryote inhibited soil's reduced ability to sorb U when a pulse is applied and allowed to diffuse. However, the differences in sorption between the three treatments were low (though statistically different) and the prokaryote inhibited manipulation showed the least ability to sorb. This would indicate that it is not the fungi-inhibited soil's intrinsic inability to sorb U that has produced the level of U diffusion seen in the soil microcosms, but instead another, unknown factor.

### *5.4 Conclusions*

Uranium movement through representative soil microcosms was found to be very slow and dominated by sorption. The curvature of the plots indicated slow local equilibrium was a factor in the diffusion of U through soil. The manipulation of the soil community to create a prokaryote or eukaryote inhibited system did increase the rate of U diffusion, but neither manipulation was found to differ from the other.

There was an increase in $CO_2$ measured in prokaryote inhibited soil microcosms, but this could not be correlated with U rates of movement as both manipulated microcosms contained the same rates of diffusion, but only one treatment presented with increased $CO_2$ respiration.

Taking the opposing conclusions from the $CO_2$ experimentation and the shaken suspension buffer power experiments it appears there is at least one other, as yet unidentified mechanism controlling U sorption and by association, U transport through soil.

# Chapter 6: Conclusions: Modelling the movement of U through soil

The mechanisms of transport of uranium through soil are complicated with many different processes interacting to affect the outcome. This work purposefully set out to investigate the effects of both chemical and biological subsystems upon such phenomena, with the aim of elucidating which, if any, were more important when modelling the movement of U within soil. It was intended that from the data collected on U movement under different conditions a clear picture would emerge as to which variables most strongly affected transport both in terms of mobilisation and immobilisation of the element. If such dominant variables were identified, then they could be used to construct a simple model for U transport in real soil systems.

This chapter will address the dominant factors found through experimental work on real soil and what this might mean in terms of modelling U movement. Unsurprisingly, different conclusions about U movement were arrived at from work done on live compared to more simple, manipulated, soil systems.

## *6.1 The sorption parameter and possible affecting factors*

Examining the parameters associated with a prescribed diffusion coefficient equation, it became clear that sorption (or parameter *b* or $d[U_S]/d[U_L]$) would be important. The rates of diffusion of U(VI) species (the $UO_2^{2+}$ cation and its charged and uncharged hydroxyl and carbonate relatives) are strongly affected by sorption of these species on the surfaces of soil particles and other components of the soil, including biological fractions. The other parameters of the diffusion equation could be calculated from simple measurements (bulk density, water content) or were unchanged by the treatments applied and thus could be calculated *a priori*. The diffusion impedance factor was measured in the experimental soils using the counter-diffusion of bromide and chloride ions. The diffusion coefficient of U species in water was taken from other work. Sorption under a range of conditions was predicted from simple shaken suspension experiments and checked against observed values to test the model.

### *6.1.1 Potential model assumptions with regard to the rapid equilibrium of a U pulse.*

From the results of the self-diffusion experiments and the U sorption measurements in shaken soil suspensions, it was apparent that only part of the total U present in the soil exchanged with the U isotope pulse as it diffused through the soil. The soil buffer power for U obtained from 24 h shaken suspension experiments was at least a factor of 10 greater than the apparent values in the self-diffusion experiments. That this was found in all cases, independent of soil type indicated that slow equilibrium processes were operating in all the soils tested. Thus over shorter time periods (such as the 28 d in this work) the process of reaching equilibrium within the soil between $[U_S]$ and $[U_L]$ is carried out with a fraction of the total U and is correspondingly biased toward U sorption with associated lower mobility than modelled.

This evidence that the pulse of U isotope diffusing through the soil only exchanged with part of the U in the soil ($[U_{TOT}]$ measured by total digest) is important information for modelling U movement in soil. The relative simplicity of measuring $[U_{TOT}]$ lends this parameter an advantage in the selection of factors to use when modelling U diffusion. However, the discovery that sorption is time dependent impacts on the method of modelling U movement through soil. Though transport modelling would incorporate mass flow and perturbation events as well as diffusion of the element in question, a potential inaccuracy in predicting U sorption has implications for risk assessment.

A usable model would need to contain a subset of terms to transform $[U_{TOT}]$ into $[U_{TOT}]_{effective}$, (the proportion of $[U_{TOT}]$ in soil that is involved in exchanging with the U isotope as the pulse diffuses) taking into account parameters affecting slow equilibrating processes. These terms for slow equilibration could possibly be obtained in a time-series of measurements in shaken soil suspensions. In the absence of information on slow equilibration, conclusions about U movement from $[U_{TOT}]$, measurements cannot be made with any confidence. In case studies on the fate of DU in soil after long exposure, such as those based in Bosnia and Herzegovina where contamination was measured 7 y after the original conflict (UNEP, 2003) it is less problematical to assume that equilibrium had been reached. But this assumption should not be made for shorter term measurements of DU movement and its potential entry into vegetation and drainage waters.

### *6.1.2 Potential model assumptions with regard to soil type.*

By using four different soil types, it was possible to broadly determine the effects of soil properties on U diffusion. This is not a completely novel area of research but this present study goes beyond simple comparison of diffusion of U through, for example, clays versus loams or sands, and this was not the only intended outcome of this experimentation. There is a separation in the literature between the highly mathematical modelling of U diffusion through adequately described matrices and the action of individual micro-organisms, or carefully separated fractions of the soil community on U within soil. It was thought that a comparison between the magnitude of the two different effects on U diffusion might reveal whether one had more of an impact on the rate of movement of U through soil. Should a clear principal mechanism emerge then the process of modelling U movement through soil could safely concentrate on explaining this whilst minimising the effect of other parameters (though with a clear rider that other parameters were present). On the other hand, should no principal mechanism emerge, the process of modelling U movement using only the simplest parameters would have to incorporate the more uncertain world of soil biology.

From shaken soil suspension experiments, it became clear that at low concentrations of U, the different soils sorbed similar amounts of U, but at higher concentrations there were substantial differences between soils. As the range at which the sorption potential was tested was below that found in the vicinity of a penetrator (UNEP, 2003, UNEP, 2001) due to health and safety constraints associated with the handling of uranium, these differences in soil type can be assumed to be a factor in differences in rate of diffusing U in real situations.

The reduced ability of the calcareous soils to sorb U at high concentration was expected. A report published by the Royal Society (2002) concluded that in semi-arid chalky soils mobility of DU would be greater, but that due to low rainfall this would not be a problem. However, it must be noted that chalky soils are not only found in semi-arid areas. The very low sorption found in the silty clay loam soil (collected from calcareous grassland such as can be found over limestone in many parts of Europe) would indicate a potential source of U contamination of water sources or other areas by wind erosion.

Clay content was not as important a factor in sorption as expected. The presence of carbonate in the calcareous soils was more important. The process of shaking the soil suspensions was assumed to break down aggregates and expose clay surfaces, and this may have diminished differences between the soils. In light of this, despite differing clay contents the clay loam and loamy sand were similar in their sorption isotherms. This drawback in the method of measuring sorption potential of such soils should be addressed in future investigations of this nature.

In light of the discovery that slow local-equilibration processes have a major effect on the diffusion of U into soil suggests soil structure and micro-aggregation are likely to be important. Soil texture is not only important for the number of soil particle surfaces it provides, but for the effect it has on slowly diffusing U and its ability to lock away U and decrease the concentration available for rapid equilibrium. Further discussion of the comparative importance of the soil biological component can be found in Section 6.3.

### *6.1.3 Potential model assumptions with regard to pH.*

It is well known that U sorption is sensitive to soil pH. Further, U sorption might itself alter soil pH if the sorbed U species displace $H^+$ or $OH^-$ ions from soil surfaces. However the measured pH's in the shaken soil suspensions here indicated there was little if any pH change with sorption over the U concentration range considered. As concentrations around DU penetrators are higher than used in the shaken suspensions here, and also other components dissolved from penetrators may cause pH changes, this conclusion is provisional. The effects of pH on U speciation is well known, from the simplistic changes from $UO_2^{2+}$ to ionic U bonded to carbonate or hydroxyl at higher pH with associated minor charge. Shaken suspensions carried out using fresh field soil brought further evidence of the greater sorption at lower pH. The clay loam soil collected from the field was found to be of lower pH than the archived material. Associated with this lowered pH was an increase in sorption.

Monitored pH in shaken suspensions with adjusted $CO_2$ concentrations indicated there was no pH effect of the altered carbon dioxide levels and consequently any changes in sorption seen could not be attributed to this parameter.

These conclusions about pH effects on U movement through soil are all based on the shaken suspension experiments; there were necessarily no pH changes in the self-diffusion experiments. Further work would be required to determine if slow equilibration processes are affected by pH changes.

### 6.1.4 Potential model assumptions with regard to $CO_2$ partial pressure.

The investigation of the effects of $CO_2$ partial pressure within soils arose following observations on the respiration data derived from soil microcosms. Element sorption under different $CO_2$ conditions is a standard investigative path for the characterisation of an element, but in this case it was again useful in the comparison between the relative importance of soil biology in terms of U diffusion.

The main effects seen from alterations in the $CO_2$ levels in soil were related to U speciation under different conditions. The alteration of U species from $UO_2^{2+}$ to [U, $CO_3^{2-}$] species with associated reduced sorption was expected and seen in the clay loam and loamy sand soils. As with pH effects, speciation changes with altered $CO_2$ partial pressure can be calculated using standard equilibrium thermodynamic data.

The ability of oxidised uranyl ions to complex with carbonate, the associated increase in solubility and related change in risk when assessing uranium in the environment is already well known (Sheppard *et al.*, 2005). Testing this in the four soils under investigation confirmed that the presence of carbonate, a potential solubilising complex for oxidised U(IV) ions remains as expected an important factor when modelling U transport for risk assessment.

## 6.2 Soil biology and U diffusion in soils with a diverse microbial community.

The effects on U diffusion from the presence of a diverse soil community found were not in accord with the original hypotheses. It was postulated that one of two effects would be seen: either decreased mobility due to the actions of fungi and bacteria to concentrate the U out of solution by such methods as extra-cellular complexing, intra-cellular accumulation and cell surface binding (Ragnarsdottir and Charlet, 2000); or increased mobility due to the actions of micro-organisms including the actions of fungi to solubilise mineral through protonation or chelation (Fomina *et al.,* 2007).

Measurements of the rate of U diffusion through soil microcosms revealed no difference between soils sterilised by gamma irradiation and soils that had undergone no manipulation to the soil community collected fresh from the field. This third outcome was unexpected. The first conclusion that might be drawn from this data is that there really is no difference between live and sterile soil in terms of U diffusion. This leads to the suggestion that there is therefore no need to investigate the movement of U through live soil and no further work on soil microcosms needs to be carried out.

However, it is apparent that there was some difference between the soils manipulated by biocides and associated controls. The rate of U movement in the prokaryote- and eukaryote-dominated soils was quicker than that in the sterile and non-manipulated soils. As the same soil source was used in all the microcosms the only difference between the soils must therefore be biological in nature. The precise nature of this effect could not be determined from the experiments carried out, but that there were effects is certainly evident.

Two explanations were investigated: (i) that respiration differed between microcosms and was thus linked to $CO_2$ affected sorption; (ii) that sorption itself differed intrinsically between the treatments for some reason. Both of these were found to have some effect, but neither could explain fully the difference between the treatments seen. Respiration could be correlated with decreased sorption in the prokaryote-inhibited treatment, but not with the eukaryote-inhibited. In turn sorption calculated from shaken suspensions on a sub-sample of the soil microcosms explained the increased mobility in the eukaryote-inhibited treatment, but also implied that such an increased mobility should have been seen in the non-manipulated soil also. Barrow (2008) states that with cation buffering in soil, high concentrations are affected by a buildup in $H^+$ ions and consequent pH effects. With the non-linear sorption isotherms in soils with active communities more pronounced than in soils that that were not treated in a manner beneficial to community recovery, it could be concluded that pH is affecting sorption at higher U concentrations in live soils, but these affected concentrations are lower than in non-live soil. The reduced effectiveness of buffering at high concentration in the eukaryote-inhibited soil indicates an increase of $H^+$ in solution over and above that the relationship would predict compared to that seen in the live soil.

Despite the lack of a clear explanation for the difference in U mobility some conclusions can be drawn with reference to the effects of biological perturbation. If the treatments are assumed to represent the recovery of soil from perturbation, then any scenario that impinges on the soil microbial community and causes it to have to recover towards stability may have a mobilising effect on U movement. Bearing in mind that the impact of a shell is in itself a perturbation effect this is may be of real significance, notwithstanding the problems that may arise when civilians return to an area of conflict and begin rebuilding operations.

Investigating the effects of individual micro-organism species with regard to the adsorption, ingestion or other immobilisation of U in a Petri-dish or soil slurry is not an adequate basis for modelling U behaviour as the complexity of a diverse soil community does not replicate these effects when tested. Evidence of this can be found in the PLFA results. The presence of sulphur reducing bacteria was indicated and these have been linked to the reduction of U and associated immobilisation. But in the complex soil community in the soil microcosms these bacteria were swamped in the wide range of functions present and were not associated with increased immobility in the eukaryote inhibited treatments. The relative size of the population of sulphur reducing bacteria is small relative to the entire biomass. Despite evidence that in isolation reducing bacteria can have a disproportionate but significant effect on the diffusion rates of U (Ragnarsdottir & Charlet, 2000, Valsami-Jones & McEldowney, 2000) extrapolating such conclusions to soil with a live and complex soil community is not straightforward should only be carried out with caution.

### *6.3 Comparative importance of identified factors affecting the movement of uranium*

Having set out to investigate both the chemical and biological effects on diffusion with the aim of comparing the strength of these effects, it is apparent that direct comparison was more complicated than initially supposed. With the interrelated effects of both chemistry and biology, a clear picture is difficult to arrive at. There are not clear systems within soil biological and chemical compartments each affecting U movement in separate and clearly defined ways, they clearly interact and feedback.

In terms of slow equilibration reactions the discovery that the parameter $[U_{TOT}]_{effective}$ could not measured within the context of the work complicated the comparison greatly.

Though pH and $CO_2$ effects with regard to U speciation and associated sorption potential were found to be within expected patterns, the inability to calculate $d[U_S]/d[U_L]$ without further data on the time-period over which slow equilibration reactions are affecting diffusion makes designing a model problematic. How long the U originally in the soil has been there (a factor in a war zone where repeated DU impact events are likely to occur), and how long the new arrival of U has been equilibrating with the soil will both have a large effect on the potential of the soil to sorb and thus immobilise the U.

However, the potential impact from increased soil microbial community respiration after a perturbation effect indicates increased mobility linked to the presence of a soil community.

In terms of the biological effects, the surprising result of no difference between sterilised and non-manipulated soil did not support hypotheses regarding the effects of biology on diffusion, both through impacts on sorption and other routes. This would by definition indicate that the chemical effects were greater. Effects related to the intrinsic soil were greater in terms of U travelled such as that seen in the self-diffusion exp.

However, the potential impact from increased soil microbial community respiration after a perturbation effect and associated increased mobility should still be discussed. The manipulated soils did show increased mobility and one of the major implications of an effect linked to biology is that this can keep acting on U diffusion, it is not a finite process. In addition such effects are difficult to measure and can be expected to differ from point to point within an area otherwise identical in terms of soil type. In terms of modelling U movement in soils it is perhaps the most worrying fact that has come to light. In terms of actually producing a usable model of U movement within soil however, a good picture can be achieved (taking into account slow reactions) from chemical parameters measured in soil alone.

## *6.4 Conclusion and further work*

In light of the inability to model U diffusion though soil based on measurements of equilibrium sorption in shaken soil suspensions (Section 6.1.1 and 6.3), a more intensive investigation into the effect of slow equilibrium processes in soil is essential. Potential inaccuracies in the current assessment of risk with regard to uranium in the environment are implied from the results described in chapter 4 that indicate time dependant sorption of U in soil. In order to correct these inaccuracies a clear picture of the sorption behaviour of U in soil over medium to long time periods is needed. If the possible factors producing such a low $[U_{TOT}]_{effective}$ with regard to calculating soil buffer power for U could be mapped out in more detail, this could then be taken into account when modelling U movement in soil and thus removed as a problem variable. The methods laid out in Section 6.1.1 with regard to a series of shaken suspension experiments over varied time periods would produce a rapid idea of the factors involved. However, a longer term investigation of sorption without recourse to shaking would also be recommended in light of the similar results produced by a clay loam and a loamy sand in shaken suspension tests due to the exposure of the clay particles in a manner unlikely to be replicated in field situations.

There was an intention to investigate U movement through soils that had been sterilised and then re-inoculated with either the fungi or bacterial fraction of the soil community (using soil slurries). This was never realised. In light of the similarities in U movement seen in microcosms treated with biocides this investigation becomes even more important. Though after consideration it was assumed that there was no effect on the transport of U by the lingering presence of either biocide, an experiment measuring U behaviour in re-inoculated soils would add further confidence to the results presented here. Should such a study echo the results presented here (as is expected) the potential impact on modelling of U behaviour in field soils would be significant. Not only would such conclusions highlight the need to maintain a representative microbial soil community in any experimental methods when drawing conclusions about the possible behaviour of U in soil under different conditions (such as is not seen in many laboratory based research e.g. Fomina *et al.*, 2007) but would herald the need to investigate the effects of perturbation on U movement, especially important in the case of DU release from impacting shells.

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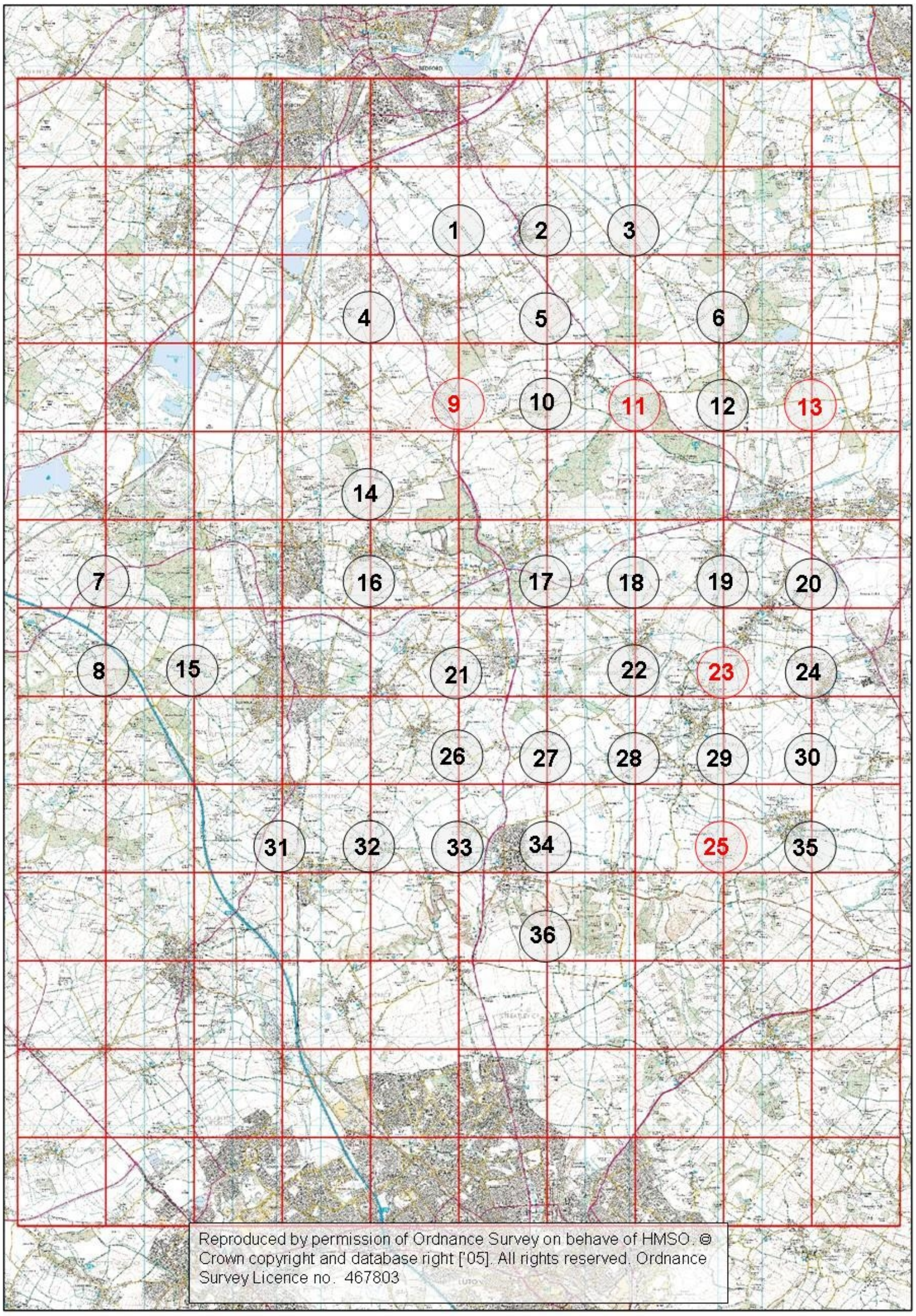
# Appendix 1: Soil Map

A grid was laid out over the Bedfordshire countryside between Bedford and Luton and bordered to the west and east by the M1 and the A1 respectively (*Towards a general method to 'scale up' process models in the arable landscape* (BBSRC BB/C506813/1, in collaboration with Rothamsted Research)).



# Appendix 2: Laboratory SOPs

## *2.1: Determination of soil pH NR-SAS / SOP 6 / Version 1*

### *2.1.1. Source*

This SOP is based on the British Standard BS ISO 10390:2005 *Determination of pH*.

### *2.1.2. Scope*

This SOP identifies an instrumental method for the routine determination of pH using a glass electrode in a 1:5 (volume fraction) suspension of soil in water (pH in $H_2O$), in a solution of 1 mol/l potassium chloride (pH in KCl) or in a solution of 0.01 mol/l calcium chloride (pH in $CaCl_2$).

This SOP is applicable to all types of air-dried soil samples.

### *2.1.3. Principle*

A suspension of soil is made up in five times its volume of one of the following:

water;
1 mol/l solution of potassium chloride (KCl) in water;
0.01 mol/l solution of calcium chloride ($CaCl_2$) in water.

### *2.1.4. Laboratory sample*

Use air-dried soil samples, for example samples pre-treated according to NR-SAS / SOP 1.

### *2.1.5. Reagents*

1 mol/l potassium chloride (KCl) solution in water (RPU 2)
0.01 mol/l calcium chloride ($CaCl_2$) solution in water (RPU 3)

### *2.1.6. Calibration check*

Ensure water dispenser gives volume of 50ml ±2ml
Ensure potassium chloride dispenser gives volume of 50ml ±2ml
Ensure calcium chloride dispenser gives volume 50ml ±2ml

### *2.1.7. Procedure for preparation of the suspension*

1. Measure 10ml of sample into a labelled 100ml glass bottle, using the 10ml brass scoop, struck off level without tapping.
2. Add 50ml, by dispenser, of either water, 1 mol/l potassium chloride solution, or 0.01 mol/l calcium chloride solution.
3. Shake on the side-to-side shaker (set at 300 $\text{min}^{-1}$) for 60 minutes ± 10 minutes.
4. The sample should then be left to stand for 1 hour but not longer than three hours and the pH then measured as below.

### *2.1.8. Measurement of the pH*

1. Calibrate the pH meter using pH 4.0, pH 7.0 and pH 10.0 buffers according to the manufacturer's instructions.
2. Immediately before measuring the pH of the sample, shake the suspension thoroughly and the measure the pH in the settling suspension after stabilisation of the value is reached. The reading can be considered stable for example when the pH measured over a period of five seconds varies by not more than 0.02 pH units.
3. Thoroughly rinse the pH probe with demineralised water and then carefully dry, between samples.
4. After use, rinse the electrode and immerse tip in the storage solution.

### *2.1.9. Expression of results*

The pH reading of the sample is recorded to three decimal places.
The result for pH is reported to one decimal place.

### ***2.2: Particle size distribution NR-SAS / SOP 5 / Version 1***

#### ***2.2.1. Source***

This SOP is based on the British Standard BS 7755 Section 5.4:1998 *Determination of particle size distribution in mineral soil material – Method by sieving and sedimentation* which is identical to ISO 11277:1998.

#### ***2.2.2. Scope***

This SOP specifies a basic method of determining particle size distribution (PSD) applicable to a wide range of mineral soil materials, including the mineral fraction of organic soils.

This SOP does not apply to the determination of the PSD of the organic components of soil, i.e. the more or less fragile, partially decomposed, remains of plants and animals.

It should also be realised that the chemical pretreatments and mechanical handling stages in this SOP could cause disintegration of weakly cohesive particles that, from field inspection, might be regarded as primary particles, even though such primary particles could be better described as aggregates.

#### ***2.2.3. Principle***

Organic matter in the soil is destroyed with hydrogen peroxide. The resulting slurry is dispersed with buffered sodium hexametaphosphate solution, and the various particle size fractions are determined by a combination of sieving and sedimentation. The latter makes use of the pipette method.

#### ***2.2.4. Laboratory sample***

Use air-dried soil samples, for example samples pre-treated according to NR-SAS / SOP 1.

#### ***2.2.5. Apparatus***

Numbered, 250ml capacity polycarbonate centrifuge bottles, complete with leak-proof caps. Check the bottles for cracks before use. Those which are badly cracked or leak must be discarded.

Hotplate set at 100°C.

Numbered towers of wire-mesh, brass or stainless-steel sieves. Unless otherwise specified, these will consist, in descending order, of sieves with the following apertures: 0.6mm, 0.212mm and 0.063mm. At the base of the tower place is a receiver. Fewer sieves may be required on occasion, as may be others of different apertures. Record changes in sieve sizes and sieve identification numbers on the record sheet. Whatever is used, the principle is the same - coarsest at the top. The sieve sequence must always be recorded on each study. Check the fit between sieves at regular intervals. If they become ill-fitting, mark the tower 'DO NOT USE' and report the defect to laboratory management.

### 2.2.6. Reagents

100 vol hydrogen peroxide solution - this solution looks harmless but is extremely corrosive. It causes severe burns to the skin and will destroy eyesight within seconds. Whenever handling this solution you must wear undamaged gloves and a face-mask. This solution must always be taken from a container to which a dispenser has been fitted. Never attempt to pour this solution from one container to another. Always wash out these containers prior to disposal. If any of this solution is spilt, use copious quantities of water to dilute it before any attempt is made to mop it up.
buffered sodium hexametaphosphate dispersing solution (RPU 1).
octan-2-ol.

### 2.2.7. Calibration check

Ensure buffered sodium hexametaphosphate dispenser gives 20ml ±2ml

### 2.2.8. Sedimentation times

The sedimentation time at 25°C and at a sampling depth of 9cm is as follows.

| | |
|---|---|
| 0.002mm | $6^{\text{hours}}\ 23^{\text{minutes}}$ |

### 2.2.9. Procedure for removal of organic matter

1. Place approximately 10ml of air-dry, <2mm soil in a labelled polycarbonate bottle, using the specially made 10ml brass scoop.

2. Add, by measuring cylinder, 30ml ±1ml of water to each soil sample bottle, and 25ml ±2.5ml, by dispenser, of 100 vol hydrogen peroxide solution. Point the bottle away from yourself and others as spectacular frothing may occur.
3. Gently swirl to mix the contents. Place the bottle on a cold hotplate in a fume cupboard. Keep a careful eye on the bottle for the next few hours. If the contents show signs of vigorous frothing, add a few drops of octan-2-ol by means of a Pasteur pipette. Leave the bottle on the cold hotplate overnight.
4. Switch on the hotplate and raise the temperature to 100°C ±2°C. Leave the bottle at this temperature for at least 2 hours. Control any frothing with a few drops of octan-2-ol. Do not allow the contents of the bottle to dry out, add more water if necessary.
5. If there appears to be incomplete decomposition of the organic matter, remove the bottle from the hotplate, allow to cool, add another 25ml ±2.5ml, of peroxide and replace on the hotplate. For most soils, one treatment should be sufficient. Do not allow the contents of the bottle to dry out, add more water if necessary. When the decomposition appears to be complete, remove the bottle from the hotplate, and allow to cool.

### *2.2.10. Procedure for dispersal and wet sieving*

1. Balance bottle to 200g ±1g by adding demineralised water. Put on the screw cap, and shake the contents of the bottle vigorously. Inspect for leaks. If there are any, transfer the contents of the bottle to a new one without visible loss of sediment. Centrifuge the bottle and contents at 2000rpm ±100rpm, for at least 20min and discard the supernatant.
2. Add, by dispenser, 20ml ±2ml of buffered sodium hexametaphosphate dispersing solution to each bottle. Add, by measuring cylinder, 150ml ±2ml of water, cap and shake thoroughly.
3. Place the bottles on the end-over-end shaker overnight (18 hours). Remember to adjust the timer if the bottles are to be shaken over the weekend, so that the total shaking time does not exceed 18 hours.
4. Add, by dispenser, 20ml ±2ml of buffered sodium hexametaphosphate dispersing solution into a weighed, to 4 d.p., glass bottle. Place the bottle and contents in the

oven set at 105°C and dry overnight. Allow to cool in the desiccator and reweigh (*d*)

5. Place a large plastic funnel into one of the 500ml measuring cylinders. Place a 0.063mm sieve in the funnel. Choose the appropriately numbered bottle for the cylinder, and pour the contents of the bottle onto the sieve. Wash all the material out of the bottle and cap onto the sieve, and wash the material on the sieve. The gentle use of a rubber policeman can be used to keep the contents of the sieve moving. The amount of water used must not come above the cylinder graduation.
6. Very carefully wash any residue on the sieve into the appropriately numbered drying tin and dry in an oven set at 105°C ±2°C for a minimum of four hours.

### 2.2.11. Procedure for dry sieving the sand fraction

1. Sieve the contents of each beaker in turn through a nest of sieves on the sieve shaker for a minimum of 15 minutes.
2. Record the mass, to 4 d.p., of each full sieve and sample and then just the sieve on the results form.
3. The contents of the receiver should be returned to the cylinder for that sample, and the volume made up to 500ml with demineralised water.

### 2.2.12. Determination one silt and one clay fraction by pipette extraction

1. Place the cylinders in a water bath. The water bath and cylinders need to be equilibrated to 25°C overnight, before sampling is to take place. Record this temperature using a thermometer.
2. Weigh, to 4 d.p., the masses of two sets of glass bottles according to the following scheme on the record form:
   One set of bottles in the spaces opposite the 0.002mm-0.063mm;
   One set of bottles in the spaces opposite the <0.002mm space.
3. Stir the cylinder for approximately 30 seconds to thoroughly mix the contents, avoiding a vigorous action which might introduce air (the stirrer should not go above the level of the liquid). At the end of stirring begin timing. Immediately pipette a 25ml aliquot into the appropriate 0.002mm-0.063mm bottle at a depth of 10cm from the surface of the liquid - do not lower the pipette during sampling. ***This portion of sample contains silt plus clay.*** After the sedimentation time for a 0.002mm particle has elapsed ($6^{\text{hours}}$ $23^{\text{minutes}}$), pipette another 25ml aliquot into the

appropriate 0.002mm bottle, at a depth of 9cm from the new surface of the liquid - do not lower the pipette during sampling. ***This portion of sample contains only clay.***

4. When the second round of sampling is complete, dry all the sample bottles by placing in an oven set at 105°C ±2°C for a minimum of twenty four hours.
5. Remove the dried bottles from the oven and cool in a desiccator. Weigh each bottle in turn, and record the weight in the appropriate place on the form.

### 2.2.13. Expression of results

The Dispersant Factor ($D$) is calculated and recorded to four decimal places.
The Factor ($F$) is calculated and recorded to four decimal places.
The particle size fractions are calculated and recorded to two decimal places.
The results for particle size distribution are reported to two decimal places.

### 2.2.14. Calculation of PSD for one silt and one clay fraction

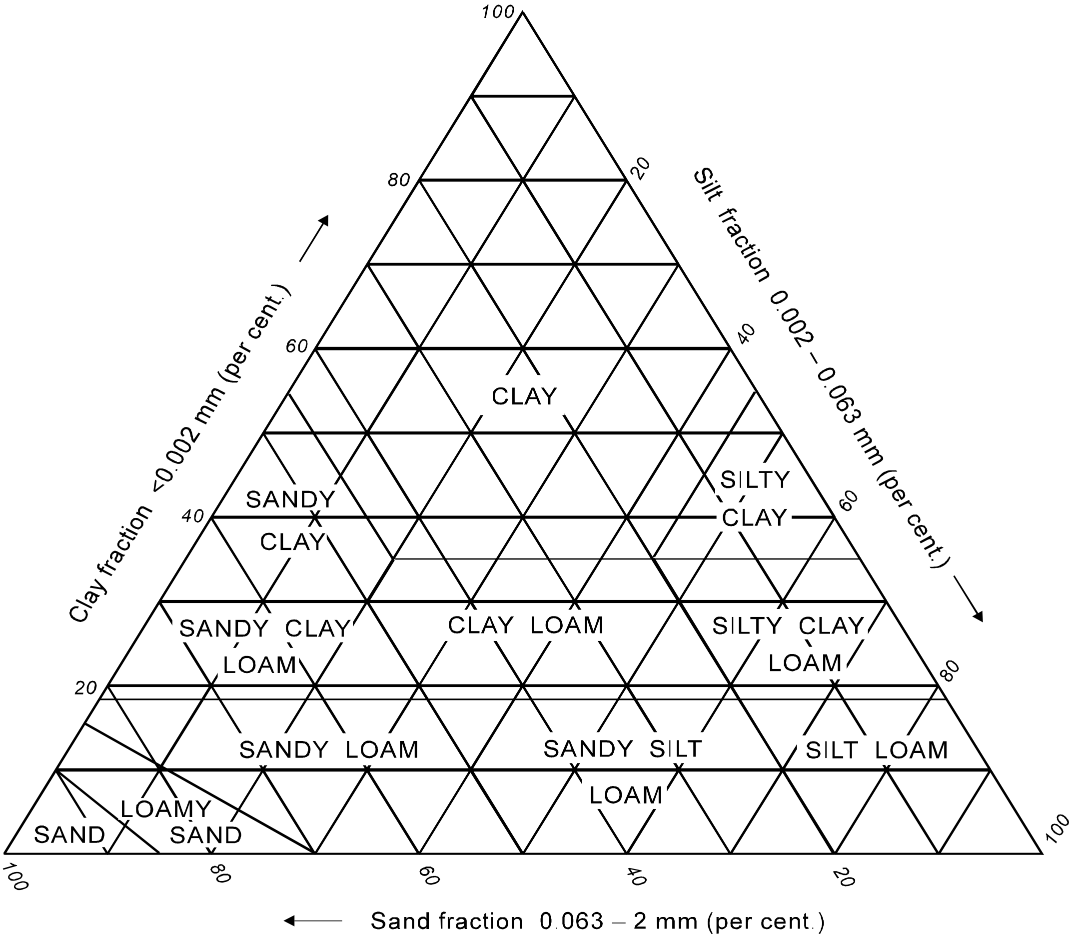
$d$ = oven-dry mass of sodium hexametaphosphate dispersing solution (g)
$Z$ = mass of 0.002mm-0.063mm (pipetted sample (**Silt + Clay**);
$C$ = mass of <0.002mm pipetted sample (**Clay**);
$S$ = Total mass of **SAND** (may be **one** or **several** fractions);
Dispersant Factor ($D$) = $d/20$
Factor ($F$) = $S + ((Z-D) \text{ x } 20)$
The following stage is repeated for each separate sand fraction:

$$\% \text{ Sand} = \frac{\text{Mass of Particular Sand Fraction}}{F} \text{x100}$$

$$\% \text{ 0.002mm-0.063mm} = \frac{(Z-C) \text{ x } 20}{F} \text{x100}$$

$$\% <\text{0.002mm} = \frac{(C-D) \text{ x } 20}{F} \text{x100}$$

Addition of all percentages should give 100% ± 0.2% If it doesn't, check your arithmetic. If it still doesn't, then check all your weighings. If the error is still too great, inform laboratory management. DO NOT THROW ANYTHING AWAY AS YOUR PROBLEM MIGHT IDENTIFY A MORE SERIOUS ONE.


100
80
60
40
20
Clay fraction <0.002 mm (per cent.)
20
40
60
80
100
Silt fraction 0.002 – 0.063 mm (per cent.)
100
80
60
40
20
Sand fraction 0.063 – 2 mm (per cent.)
CLAY
SANDY CLAY
SILTY CLAY
SANDY CLAY LOAM
CLAY LOAM
SILTY CLAY LOAM
SANDY LOAM
SANDY SILT LOAM
SILT LOAM
LOAMY SAND
SAND

### *A.2.3 Determination of elements soluble in aqua regia (Block) NR-SAS / SOP 17 / Version 1*

#### *2.3.1. Source*

This SOP is based on British Standard BS 7755: Section 3.9:1995 which is identical to ISO 11466:1995 and British Standard BS 7755: Section 3.13:1998 which is identical to ISO 11047:1998.

#### *2.3.2. Scope*

This SOP describes a method for the determination of trace elements soluble in aqua regia. This SOP is applicable to all types of air-dried soil samples.

#### *2.3.3. Principle*

The sample is extracted with a hydrochloric/nitric acid mixture by standing overnight at room temperature, followed by boiling under reflux for two hours. The extract is then clarified and made up to volume with nitric acid. The trace metal content of the extract is then determined by atomic absorption or emission.

#### *2.3.4. Laboratory sample*

Use air-dried soil samples, for example sample pre-treated according to NSRI / SOP 1.

#### *2.3.5. Apparatus*

Tecator digestion block and scrubber unit (NSRI / SOP E6).
100ml digestion tubes - kept specifically for trace element determinations.
100ml volumetric flasks - kept specifically for trace element determinations.

#### *2.3.6. Reagents*

Hydrochloric acid (1.18 specific gravity)
Nitric acid (1.42 specific gravity)
6% v/v nitric acid (RPU 37)
Lanthanum chloride solution (RPU 38)
Cadmium working standard solutions (RPU 39)
Chromium working standard solutions (RPU 40)
Copper working standard solutions (RPU 41)

Lead working standard solutions (RPU 42)

Nickel working standard solutions (RPU 43)

Zinc working standard solutions (RPU 44)

Cobalt working standard solutions (RPU 45)

Molybdenum working standard solutions (RPU 46)

## *7. Calibration check*

Ensure hydrochloric acid dispenser gives volume of 22.5ml ±2ml

Ensure nitric acid dispenser gives volume of 7.5ml ±0.5ml

## *8. Preparation of soil digests*

1. Weigh 3g ±0.001g of sample into a labelled digestion tube.
2. Add 22.5ml ±0.1ml of hydrochloric acid (1.18 specific gravity) using a dispenser, and 7.5ml ±0.1ml of nitric acid (1.42 specific gravity) using another dispenser. Swirl the tube to mix the contents. Leave to digest at room temperature overnight.
3. Place the exhaust manifold onto the digestion tubes, insert into the heating block and turn on the scrubber unit.

- **The operating procedure (NSRI / SOP E6) for the digestion block and scrubber unit must be followed carefully.**

4. The contents of the tube must be boiled under reflux for two hours. This means heating them to approximately 115°C. The speed at which this temperature is reached will vary on the type of soil. With organic soils, the temperature should be increased by approximately 10°C every thirty minutes. With inorganic soils, the temperature can be increased by approximately 10°C every ten minutes. The following shows how a heating cycle with a ten minute delay is programmed into the **Controller**.

- After turning on the power to the digestion block the first step is displayed.
- Press and hold ***TEMP***, press ▲▼ to 40°C.
- Press and hold ***TIME***, press ▲▼ to 00hours:10minutes.
- Press ▲▼ to move to step two.
- Press and hold ***TEMP***, press ▲▼ to 50°C.
- Press and hold ***TIME***, press ▲▼ to 00hours:10minutes.

- Press ▲▼ to move to step three.
- Press and hold ***TEMP***, press ▲▼ to 60°C.
- Press and hold ***TIME***, press ▲▼ to 00hours:10minutes.
- Press ▲▼ to move to step four.
- Press and hold ***TEMP***, press ▲▼ to 70°C.
- Press and hold ***TIME***, press ▲▼ to 00hours:10minutes.
- Press ▲▼ to move to step five.
- Press and hold ***TEMP***, press ▲▼ to 80°C.
- Press and hold ***TIME***, press ▲▼ to 00hours:10minutes.
- Press ▲▼ to move to step six.
- Press and hold ***TEMP***, press ▲▼ to 90°C.
- Press and hold ***TIME***, press ▲▼ to 00hours:10minutes.
- Press ▲▼ to move to step seven.
- Press and hold ***TEMP***, press ▲▼ to 100°C.
- Press and hold ***TIME***, press ▲▼ to 00hours:10minutes.
- Press ▲▼ to move to step eight.
- Press and hold ***TEMP***, press ▲▼ to 115°C.
- Press and hold ***TIME***, press ▲▼ to 02hours:00minutes.
- Press ▲▼ to move to step nine.
- Press and hold ***TEMP***, press ▲▼ to 10°C.
- Press and hold ***TIME***, press ▲▼ to 00hours:00minutes. This final step effectively turns the heating block off.
- Press the ***RUN/STOP*** key to save the programmed cycle. Press ▲▼ to return the display to the first step.

5. Press ***RUN/STOP*** tostart the heating cycle. Pressing the same button will stop the cycle at any point.
6. Allow the block to cool, lift the tubes and exhaust manifold clear. The scrubber unit must be left running until the tubes have reached room temperature.
7. Filter the contents of the tubes through a Whatman No. 542 filter paper into labelled 100ml volumetric flasks. Rinse the filter and residue with 6% v/v nitric acid several times. Finally make up to volume with 6% v/v nitric acid.

8. Carry out a blank digest.

### 8. Expression of results

1. Aqua regia soluble cadmium and molybdenum are calculated and recorded to two decimal places, the other elements are calculated and recorded to one decimal place.
2. Aqua regia soluble cadmium and molybdenum are reported to two decimal places, the other elements are reported to one decimal place.

### 9. Determination and calculation of cadmium by electrothermal atomic absorption

1. Set the AAS according to the instruction handbook (NSRI / SOP E1). Prepare a calibration curve for cadmium using the appropriate standard working solutions.
2. Pipette an aliquot (***v*** ml), of the soil extract into a 25ml volumetric flask and make up to volume with 6% v/v nitric acid. **NOTE: initially run through the undiluted extract, in which case the aliquot used is 25ml.**
3. Measure the concentrations of cadmium in the extracts.

$$\text{mg/kg cadmium} = \frac{(Cd_s - Cd_b) \times (2500/\mathbf{v})}{m_1}$$

where

- $Cd_s$ is the concentration, in µg/ml, of cadmium in the sample extract;
- $Cd_b$ is the concentration, in µg/ml, of cadmium in the blank extract;
- **v** is the aliquot, in ml, used;
- $m_1$ is the mass, in grams, of sample taken for digestion.

### 10. Determination and calculation of chromium by flame atomic absorption

1. Set the AAS according to the instruction handbook (NSRI / SOP E1). Prepare a calibration curve for chromium using the appropriate standard working solutions.
2. Pipette an aliquot (***v*** ml, but not exceeding 20ml), of the soil extract into a 25ml volumetric flask. Add 2.5ml of the lanthanum chloride solution and make up to volume with 6% v/v nitric acid.

3. Measure the concentrations of chromium in the extracts.

$$\text{mg/kg chromium} = \frac{(Cr_s - Cr_b) \times (2500/\mathbf{v})}{m_1}$$

where

- $Cr_s$ is the concentration, in µg/ml, of chromium in the sample extract;
- $Cr_b$ is the concentration, in µg/ml, of chromium in the blank extract;
- **v** is the aliquot, in ml, used;
- $m_1$ is the mass, in grams, of sample taken for digestion.

## *11. Determination and calculation of copper by flame atomic absorption*

1. Set the AAS according to the instruction handbook (NSRI / SOP E1). Prepare a calibration curve for copper using the appropriate standard working solutions.
2. Pipette an aliquot (***v*** ml), of the soil extract into a 25ml volumetric flask and make up to volume with 6% v/v nitric acid. **NOTE: initially run through the undiluted extract, in which case the aliquot used is 25ml.**
3. Measure the concentrations of copper in the extracts.

$$\text{mg/kg copper} = \frac{(Cu_s - Cu_b) \times (2500/\mathbf{v})}{m_1}$$

where

- $Cu_s$ is the concentration, in µg/ml, of copper in the sample extract;
- $Cu_b$ is the concentration, in µg/ml, of copper in the blank extract;
- **v** is the aliquot, in ml, used;
- $m_1$ is the mass, in grams, of sample taken for digestion.

## *12. Determination and calculation of lead by flame atomic absorption*

1. Set the AAS according to the instruction handbook (NSRI / SOP E1). Prepare a calibration curve for lead using the appropriate standard working solutions.
2. Pipette an aliquot (***v*** ml), of the soil extract into a 25ml volumetric flask and make up to volume with 6% v/v nitric acid. **NOTE: initially run through the undiluted extract, in which case the aliquot used is 25ml.**
3. Measure the concentrations of lead in the extracts.

$$\text{mg/kg lead} = \frac{(Pb_s - Pb_b) \times (2500/\mathbf{v})}{m_l}$$

where

- $Pb_s$ is the concentration, in µg/ml, of lead in the sample extract;
- $Pb_b$ is the concentration, in µg/ml, of lead in the blank extract;
- **v** is the aliquot, in ml, used;
- $m_l$ is the mass, in grams, of sample taken for digestion.

### *13. Determination and calculation of nickel by flame atomic absorption*

1. Set the AAS according to the instruction handbook (NSRI / SOP E1). Prepare a calibration curve for nickel using the appropriate standard working solutions.
2. Pipette an aliquot (***v*** ml), of the soil extract into a 25ml volumetric flask and make up to volume with 6% v/v nitric acid. **NOTE: initially run through the undiluted extract, in which case the aliquot used is 25ml.**
3. Measure the concentrations of nickel in the extracts.

$$\text{mg/kg nickel} = \frac{(Ni_s - Ni_b) \times (2500/\mathbf{v})}{m_l}$$

where

- $Ni_s$ is the concentration, in µg/ml, of nickel in the sample extract;
- $Ni_b$ is the concentration, in µg/ml, of nickel in the blank extract;
- **v** is the aliquot, in ml, used;
- $m_l$ is the mass, in grams, of sample taken for digestion.

### *14. Determination and calculation of zinc by flame atomic absorption*

1. Set the AAS according to the instruction handbook (NSRI / SOP E1). Prepare a calibration curve for zinc using the appropriate standard working solutions.
2. Pipette an aliquot (***v*** ml), of the soil extract into a 25ml volumetric flask and make up to volume with 6% v/v nitric acid. **NOTE: initially run through the undiluted extract, in which case the aliquot used is 25ml.**
3. Measure the concentrations of zinc in the extracts.

$$\text{mg/kg zinc} = \frac{(Zn_s - Zn_b) \times (2500/\mathbf{v})}{m_l}$$

where

- $Zn_s$ is the concentration, in µg/ml, of zinc in the sample extract;
- $Zn_b$ is the concentration, in µg/ml, of zinc in the blank extract;
- **v** is the aliquot, in ml, used;
- $m_1$ is the mass, in grams, of sample taken for digestion.

## *15. Determination and calculation of cobalt by flame atomic absorption*

1. Set the AAS according to the instruction handbook (NSRI / SOP E1). Prepare a calibration curve for cobalt using the appropriate standard working solutions.
2. Pipette an aliquot (***v*** ml), of the soil extract into a 25ml volumetric flask and make up to volume with 6% v/v nitric acid. **NOTE: initially run through the undiluted extract, in which case the aliquot used is 25ml.**
3. Measure the concentrations of cobalt in the extracts.

$$\text{mg/kg cobalt} = \frac{(Co_s - Co_b) \times (2500/\mathbf{v})}{m_1}$$

where

- $Co_s$ is the concentration, in µg/ml, of cobalt in the sample extract;
- $Co_b$ is the concentration, in µg/ml, of cobalt in the blank extract;
- **v** is the aliquot, in ml, used;
- $m_1$ is the mass, in grams, of sample taken for digestion.

## *16. Determination and calculation of molybdenum by electrothermal atomic absorption*

1. Set the AAS according to the instruction handbook (NSRI / SOP E1). Prepare a calibration curve for molybdenum using the appropriate standard working solutions.
2. Pipette an aliquot (***v*** ml), of the soil extract into a 25ml volumetric flask and make up to volume with 6% v/v nitric acid. **NOTE: initially run through the undiluted extract, in which case the aliquot used is 25ml.**
3. Measure the concentrations of molybdenum in the extracts.

$$\text{mg/kg molybdenum} = \frac{(Mo_s - Mo_b) \times (2500/\mathbf{v})}{m_1}$$

where

- $Mo_s$ is the concentration, in µg/ml, of molybdenum in the sample extract;
- $Mo_b$ is the concentration, in µg/ml, of molybdenum in the blank extract;
- **v** is the aliquot, in ml, used;
- $m_1$ is the mass, in grams, of sample taken for digestion.

### *A.2.4 Determination of elements soluble in aqua regia NR-SAS / SOP 17 / Version 1*

#### *2.4.1. Source*

This SOP is based on US EPA Method 3051 and British Standard BS 7755: Section 3.13:1998 which is identical to ISO 11047:1998.

#### *2.4.2. Scope*

This SOP describes a method for the determination of trace elements soluble in aqua regia. This SOP is applicable to all types of air-dried soil samples.

#### *2.4.3. Principle*

The sample is extracted with a hydrochloric/nitric acid mixture using a microwave digestion system. The extract is then clarified and made up to volume with water. The trace metal content of the extract is then determined by atomic absorption or emission. The phosphorus content is then determined by a spectrometric measurement in solution.

#### *2.4.4. Laboratory sample*

Use air-dried soil samples, for example sample pre-treated according to NR-SAS / SOP 1.

#### *2.4.5. Apparatus*

Microwave digestion unit with associated digestion liner.
100ml volumetric flasks - kept specifically for trace element determinations.

#### *2.4.6. Reagents*

1. Hydrochloric acid (1.18 specific gravity)
2. Nitric acid (1.42 specific gravity)
3. 10% m/vol lanthanum chloride solution
4. 0.15% m/v ammonium molybdate solution (RPU 19)
5. 1.5% m/v ascorbic acid solution (RPU 20)
6. Phosphorus working standards (RPU 106)
7. Cadmium working standard solutions (RPU 39)
8. Chromium working standard solutions (RPU 40)

9. Copper working standard solutions (RPU 41)
10. Lead working standard solutions (RPU 42)
11. Nickel working standard solutions (RPU 43)
12. Zinc working standard solutions (RPU 44)
13. Cobalt working standard solutions (RPU 45)
14. Molybdenum working standard solutions (RPU 46)

### *2.4.7. Preparation of soil digests*

1. Weigh 0.5g ±0.001g of sample into a labelled microwave digestion liner.
2. Add 6ml ±0.1ml of hydrochloric acid (1.18 specific gravity), and 2ml ±0.1ml of nitric acid (1.42 specific gravity) using another dispenser. Swirl the liner to mix the contents and if necessary wait for pre-reactions such as gas formation to subside.
3. Insert the liner into the pressure vessel and close the screw cap hand-tight.
4. Carry out a blank digest.
5. One of the sample vessels must be sealed using the p/T Sensor Accessory (this **must** be an actual sample not a blank). Expand the seal using the seal forming device and then screw the cap hand-tight in a clockwise direction until you reach the stop position. For correct pressure measurement, this sensor vessel has to be re-opened from the stop position by approximately 60°.
6. Place the sensor vessel in rotor position 1. Place the remaining vessels in the appropriate rotor positions (this is not number order, vessels **must** be spaced evenly within the rotor).
7. Put the lid on the rotor and then place it into the microwave. Ensure that the fume hood is on.
8. On the microwave menu start the method titled “NR-SAS SOP 17”.
9. At the end of digestion, place the rotor in the fume hood.
10. Vent each reaction vessel very carefully with the vent tube pointing away from you. Filter (using a Whatman 542 or equivalent) the contents of each liner into a separate 100ml volumetric flask. Rinse the liner and seals with demineralised water, adding this to the appropriate flask. Make up to volume with demineralised water.

### 2.4.8. Determination and calculation of cadmium or molybdenum by electrothermal atomic absorption

1. Prepare a calibration curve for cadmium or molybdenum using the appropriate standard working solutions.
2. Measure the concentrations of cadmium or molybdenum in the extracts.

$$\text{mg/kg metal}^x = \frac{(M^x_s - M^x_b) \times 100 \times D}{m_1}$$

where

$M^x_s$ is the concentration, in µg/ml, of metal$^x$ in the sample extract;

$M^x_b$ is the concentration, in µg/ml, of metal$^x$ in the blank extract;

D is a dilution factor;

$m_1$ is the mass, in grams, of sample taken for digestion.

### 2.4.9. Determination and calculation of chromium by flame atomic absorption

4. Prepare a calibration curve for chromium using the appropriate standard working solutions.
5. Pipette an aliquot (***v*** ml, but not exceeding 20ml), of the soil extract into a 25ml volumetric flask. Add 2.5ml of the lanthanum chloride solution and make up to volume with demineralised water.
6. Measure the concentrations of chromium in the extracts.

$$\text{mg/kg chromium} = \frac{(Cr_s - Cr_b) \times (2500/\mathbf{v})}{m_1}$$

where

$Cr_s$ is the concentration, in µg/ml, of chromium in the sample extract;

$Cr_b$ is the concentration, in µg/ml, of chromium in the blank extract;

**v** is the aliquot, in ml, used;

$m_1$ is the mass, in grams, of sample taken for digestion.

### 2.4.10. Determination and calculation of other metals by flame atomic absorption

1. Prepare a calibration curve for metal$^x$ using the appropriate standard working solutions.
2. Measure the concentrations of metal$^x$ in the extracts.

$$\text{mg/kg metal}^x = \frac{(M^x_s - M^x_b) \times 100 \times D}{m_1}$$

where

$M^x_s$ is the concentration, in µg/ml, of metal$^x$ in the sample extract;

$M^x_b$ is the concentration, in µg/ml, of metal$^x$ in the blank extract;

D is a dilution factor;

$m_1$ is the mass, in grams, of sample taken for digestion.

### *2.4.11 Determination of phosphorus*

*Preparation of standard graph*

1. Pipette 5ml of each phosphorus working standard into a labelled 100ml beaker.
2. Add, by dispenser, 20ml of 0.15% m/v ammonium molybdate reagent, and, by dispenser, 5ml of ascorbic acid solution. Swirl and allow colour to develop for 30 minutes but not more than 35 minutes.
3. Measure the absorbance at 880nm in the spectrophotometer.
4. Construct a graph relating the absorbance to µg of phosphorus present. [**NOTE**: not plotted against µg/ml]
5. The absorbance value of the top standard (35µg of P) should read approximately 0.8. An absorbance of less that 0.7 or greater than 0.9 indicates an error and must be reported to senior laboratory staff and analysis of samples not continued.

*Determination of phosphorus*

1. Pipette 5ml of each sample digest into labelled 100ml beaker.
2. Add, by dispenser, 20ml of 0.15% m/v ammonium molybdate reagent, and 5ml of ascorbic acid solution. Swirl and allow colour to develop for 30 minutes but not more than 35 minutes.
3. Measure the absorbance at 880nm in the spectrophotometer.
4. If the absorbance is higher than the top standard, repeat the determination using a diluted sample, calling the dilution factor *d*.

*Basis of the calculation*

The results are expressed as phosphorus. From the standard graph calculate mathematically the amount of P corresponding to the absorbance. Call the sample value $V_s$, and the value of the blank, $V_b$.

*m* g of sample digested to 100ml;

5ml aliquot from 100ml;

measurement gives µg P.

then:

5ml to 100ml gives dilution of 20.

so:

$\mu g\ P\ \text{in sample} = 20(V_s - V_b)$

To convert µg to mg - multiply by $10^{-3}$; to get from *m* g to 1kg, multiply by 1000/*m*

thus:

$$\text{mg/kg} = 20(V_s - V_b) \times 10^{-3} \times 1000/m$$

$$= 20(V_s - V_b)/m$$

*Calculation*

From the standard graph calculate mathematically (absorbance divided by the gradient of the line) the number of µg of phosphorus equivalent to the absorbances of the sample and blank determinations.

$$\text{mg/kg of Phosphorus}_{\text{total}} = \frac{20(V_s - V_b) \times d}{m}$$

where

$V_s$ is the µg equivalents of sample;

$V_b$ is the µg equivalents of blank;

*m* is the mass, in grams, of sample taken for digestion;

*d* is the dilution factor (if necessary).

### *A.2.5 AGN 2.2.2 Digestion of solid materials by hot block mixed acid attack*

Dried soil slices were subsampled. The amount taken for digestion was weighed on a 4dp place balance. The amount digested was 0.2500 g ± 0.0025 g. The digestion method followed was that developed by the British Geological Survey for the total digestion of material (AGN 2.2.2 Digestion of solid materials by hot block mixed acid attack). Digestions were carried out using a programmable hot block, consisting of a custom-made polytetrafluoroethylene (PTFE or Teflon)-coated graphite block, with spaces for up to 24 Perfluoroalkoxy (PFA) vials, resting on a PTFE coated graphite hot-plate.

To prevent interference from carbonate, samples were pre-digested with 3 ml of 5% $HNO_3$ (made with concentrated nitric acid ($HNO_3$) – 69% (density 1.42 g $ml^{-1}$), Aristar grade and Ultra pure 18 MΩ deionised water [known as MilliQ]). 1 ml of the acid was added three times with autopipette and any effervescence allowed to subside between additions. A further 3 ml of 50% $HNO_3$ was added and the vials left on the hot block under a fume hood to heat at 80°C overnight.

A volume of 2 ml $HNO_3$ (concentrated nitric acid – 69% (density 1.42 g $ml^{-1}$), Aristar grade), 2.5 ± 0.25 ml HF (Concentrated hydrofluroric acid – 48% (density 1.15 g $ml^{-1}$), Aristar grade) and 1 ml $HClO_4$ (Concentrated perchorlic acid – 70% (density 1.70 g $ml^{-1}$), Aristar grade) was added to each PFA vial. The vials were swirled and replaced in the hot block. Hot block heating programme 3 was started which runs for 8 h at 80°C, 2 h at 100°C, 1 h at 120°C, 3 h at 140°C and 4 h at 160°C.

When the programme was finished, the block was allowed to cool to 50°C. 2.5 ml of 50% v/v $HNO_3$ was added to the cooled vials and they were swirled before being returned to the hot block and heated at 50°C for 30 minutes.

Vials were allowed to cool again. 2.5 ml $H_2O_2$ (Concentrated hydrogen peroxide – 30%, Aristar grade) was added and they were heated on the hot block at 30°C for 15 minutes.

Once the programme of digestion was finished, 10 ml of MilliQ water was added to the vials by autopipette and the contents of the vials poured out into labelled containers. The vials were rinsed with a further 10 ml of MilliQ water which was added to the containers. The caps were left loose for 24 h before the containers were capped securely and placed into storage before being analysed by ICP-MS.

## Appendix 3: Standard Soils

BCR Reference Material 143: Sewage Sludge Amended Soil (Soil A). *The certification of the contents of Cadmium, Copper, Mercury, Nickel, Lead, and Zinc in Sewage Sludge Amended Soil.* Commission of the European Communities. Report EUR 8835 (1983)

| **Element** | **ug/g** | **95% confidence interval (±)** |
|---|---|---|
| Cadmium (Cd) | 31.1 | 1.2 |
| Chromium (Cr) | 208 | 20 |
| Copper (Cu) | 236.5 | 8.2 |
| Nickel (Ni) | 99.5 | 5.5 |
| Lead (Pb) | 1333 | 39 |
| Zinc (Zn) | 1272 | 30 |

BCR Reference Material 141: Calcareous Loam Soil (Soil B). *The certification of the contents of Cadmium, Copper, Mercury, Lead, and Zinc in a Calcareous Loam Soil.* Commission of the European Communities. Report EUR 8833 (1983)

| **Element** | **ug/g** | **95% confidence interval (±)** |
|---|---|---|
| Cadmium (Cd) | 0.36 | 0.10 |
| Chromium (Cr) | 53 | 9 |
| Copper (Cu) | 32.6 | 1.4 |
| Nickel (Ni) | 28.0 | 4.9 |
| Lead (Pb) | 29.4 | 2.6 |
| Zinc (Zn) | 81.3 | 3.7 |

(grey shading = info from that found by aqua regia in other labs)

National Research Centre for Certified Reference Materials (Beijing, China) GBW 07402 (Soil) (Soil C)

| **Element** | **ug/g** | **±** |
|---|---|---|
| Cadmium (Cd) | 0.071 | 0.009 |
| Chromium (Cr) | 47 | 2 |
| Copper (Cu) | 16.3 | 0.4 |
| Nickel (Ni) | 19.4 | 0.5 |
| Lead (Pb) | 20.2 | 1.0 |
| Zinc (Zn) | 42.3 | 1.2 |

# Appendix 4: Pre-experiments related to Chapter 5

## *A.4.1. Preliminary measurement of Uranium movement in soil*

From a review of the literature in this field and in related fields a hypothesis was developed that the presence of a living soil microbial community within a soil matrix will result in a measurable difference in the rate of U transport though sterile versus non-sterile soil. In order to test this, an experiment was derived to measure U transport through soil microcosms comparing the rate of movement between sterilised and non sterilised treatments.

### *4.1.1. Methods*

The four soil types used in the determination of impedance (Section 3) were involved in the experimentation. Using the Ordinance Survey Locations recorded when the soils were originally sampled field fresh soils were collected.

Following the methods laid out in Section 5.1 soil was mixed with typtone soy broth (Section 5.1.3) packed into containing collars, sterilised and then pulsed with U (Section 5.1.5) before being left for a diffusion period of 14 d. Microcosms were then sliced perpendicular to the plane of diffusion and U concentration in slices was determined by microwave digestion and ICP-MS analysis (section 2.5.4).

There was a small occasion of operator error during the application of the uranium pulse. In both the sterile treatments of the Silty Clay and the Silty Clay Loam the concentration of U applied was lower than intended.

### *4.1.2. Results*

Concentration:distance profiles were plotted for the four soils (figure A1.1). Variation between replicates was found to be greater than that between treatments and the distance travelled by the U through the soil microcosms was minimal.

The lower application of U in the Silty Clay and the Silty Clay Loam soils resulted in a recovered value of uranium from soil slices that barely registered when data was plotted.

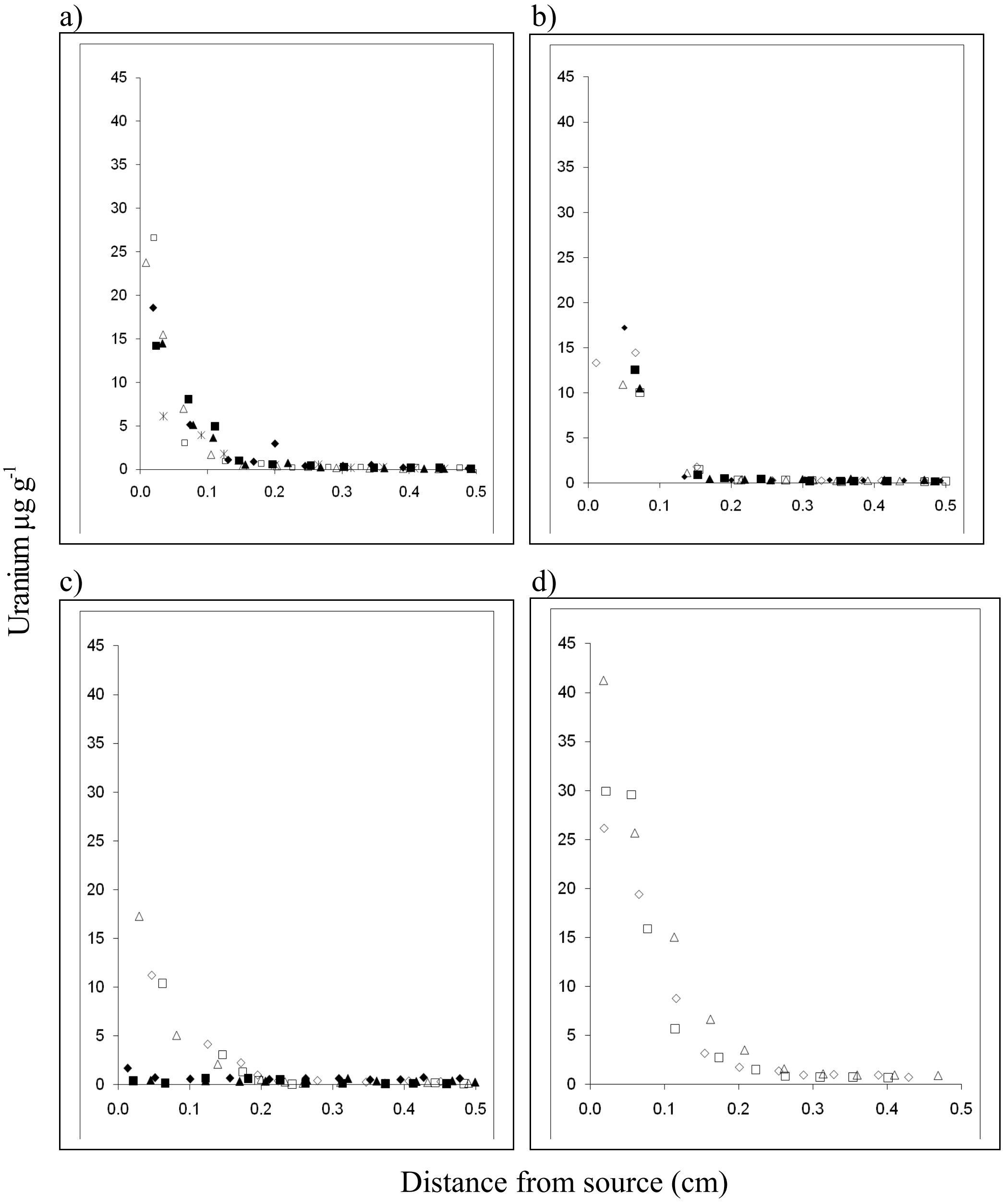


Figure A1.1: Concentration:distance profiles. Concentration of U minus blank, distance from source was calculated from slice weight and bulk density values, not directly measured. Three replicates are shown, sterile as closed [icons] a) Sandy Loam b) Clay Loam, c) Silty Clay, d) Silty Clay Loam

Uranium concentrations above those of blank samples was not found below 2 mm down the soil profile. Indications were present that the rate of U movement was faster in the silty clay loam soil and slowest in the clay loam soil.

### 4.1.3. Discussion

As the potential for drawing any conclusions from the movement of uranium in systems with a live soil biological component from this experiment was impossible a new experiment was devised. This incorporated a longer diffusion period and a more complicated set of treatments to investigate further the effects of soil community on U transport. This is laid out in Section 4.

### A.4.2 Using $d^2$ = Dt to calculate the predicted distance of uranium travel and consequent slice depth needed.

Prior experiments on the sandy loam soil had measured uranium movement over 2.5 mm and this was used to calculate the most practical diffusion period and associated number of slices needed to best capture the U movement in a more complicated experiment.

The equation:

$$d = \sqrt{Dt} \qquad \text{(A 1.2.1)}$$

Was rearranged to give:

$$d^2 = Dt \qquad \text{(A 1.2.2)}$$

Where $d$ is the distance moved by the element in question, $D$ is the diffusion coefficient and $t$ is the diffusion period

At 14 d ($t$ = 1209600 seconds) measured $d$ (metres) was 0.0025, giving $d^2$ 6.3 x $10^{-6}$. Calculating $d$ with an increasing diffusion period resulted with a practical time period of 28 d (2419200 seconds) with an estimate of distance travelled of 3.5 mm.

In light of this, slice depth was reduced to 0.25 mm in order to capture more of the concentration:distance profile ensuring that at least the top 15 slices should contain a measurable amount of the U pulse.