AN INVESTIGATION OF SOIL ACTINIDE-HUMIC INTERACTIONS USING ELECTROPHORESIS

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DECLARATION

I hereby declare that this thesis has been composed by myself, and that the work described herein is my own.

ABSTRACT

Soils in the UK contain both naturally occurring and anthropogenic actinides. The latter originate mainly from (i) atmospheric deposition from weapons testing and, in coastal areas, (ii) onshore transfer of contaminated particulates arising from industrial discharges to marine systems. Following deposition, the speciation of these highly radiotoxic elements within soils is important with respect to subsequent transport and potential availability. This study focused on the associations of U and Pu with humic substances and the implications for migration in deciduous broadleaf and coniferous forest soils.

Humic substances were isolated from soil samples by (i) solvent extraction (0.045 M Tris-borate and 0.1 N NaOH) and (ii) novel gel electrophoretic extraction (pH 8.5; 0.045 M Tris-borate as running buffer). Gel electrophoresis was also used to extract humic substances from soil that had been 'pre-equilibrated' with a solution containing ^{236}U or ^{242}Pu (pH 4.2). The relative concentrations of humic substances in the solvent and electrophoretic extracts were obtained by UV-visible spectrophotometry ($A_{254\ nm}$) whilst the ^{238}U concentrations in the extracts were determined by inductively coupled plasma-mass spectrometry (ICP-MS). In addition, the molecular size and functional group content of the humic molecules in selected fractions of the gel electrophoretic extracts were investigated using gel filtration chromatography and FTIR spectroscopy, respectively.

The methodology developed in this study enabled identification of several operationally defined soil actinide pools: (i) labile (< 0.2 μm, at pH 4.2), (ii) hydrophilic-mobile (< 0.15 μm, negatively-charged at pH 8.5) and (iii) immobile (> 0.15 μm and/or uncharged at pH 8.5). The adopted procedures avoid the extremes of pH used in the traditional methods of extraction (pH 13) and fractionation (pH < 2) which may alter humic substances. It was found that the ^{238}U content of the hydrophilic-mobile pool (i.e. the gel electrophoretic extract) accounted for 14-41 % and 15-34 % of the total ^{238}U in the deciduous broadleaf and coniferous forest soils, respectively. In general, these values were greater than those obtained by 0.045 M Tris-borate solvent extraction but less than those obtained using 0.1 N NaOH as the solvent. A further advantage of the new methodology was that the distribution of ^{238}U within the humic extract could be investigated. Two patterns were observed for the distribution of ^{238}U amongst the gel electrophoretic fractions. The first was characterised by the association of ^{238}U with predominantly less mobile fractions and by a

strong correlation between ^{238}U content and humic concentration. In the second, ^{238}U was more evenly distributed amongst the fractions. In all cases, however, the largest ^{238}U pool was the immobile pool (67-81 %), i.e. the ^{238}U remaining in the soil residue after gel electrophoretic extraction. The results for humic concentration and associated ^{238}U of the solvent and electrophoretic extracts revealed that humic substances (preferentially the higher molecular weight fractions) and actinides were removed from the soil solution to a greater extent in the coniferous relative to the deciduous broadleaf soil profile. This was interpreted as due to a chemical difference in the humic ligands, as influenced by the lignin constituents of the two types of forest vegetation.

From the soil-spiking experiment, the percentage of the total added ^{236}U or ^{242}Pu in the hydrophilic-mobile pool generally decreased with increasing soil depth. The ^{236}U content decreased from 13 to 10 % and 16 to 5 % and the ^{242}Pu from 8 to 4 % and from 8 to 2 % in the deciduous broadleaf and coniferous soils, respectively. This was attributed to the decrease in concentration of extractable humic substances with increasing soil depth. Characterisation of the gel electrophoretic fractions revealed only the first pattern for the distribution of introduced actinides, i.e. association with the less mobile fractions. As for ^{238}U, however, the greatest proportion of added ^{236}U was found in the immobile pool (63-84 %). In contrast, ^{242}Pu was found to a greater extent in the labile pool (55-88 %), in accord with previous observation of the greater mobility of Pu relative to that of U in forest soils.

Characterisation of the less mobile molecules within the hydrophilic-mobile pool showed that they comprise a polydisperse component (with molecular weights in the 1000's-100,000's D range) of the total humic substances. Also identified were small, highly electrophoretically mobile molecules that are likely to be very mobile upon release to natural receiving waters. ^{238}U was associated with these molecules whilst added ^{236}U or ^{242}Pu was associated with the less mobile humic molecules.

PUBLICATIONS

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Vinogradoff, S.I., Abbott, A., Graham, M.C. and Farmer, J.G. 1998. The influence of microbial alteration of humic substances on humic-actinide binding: implications for actinide mobility in soils. In *ConSoil '98: the 6th International FZK/TNO Conference on Contaminated Soil.* pp. 931-932. Vol. 2. London: Thomas Telford. Edinburgh, Scotland, 17-21 May 1998.

Vinogradoff, S.I., Graham, M.C., Farmer, J.G. and Abbott, A. in press. Application of an electrophoretic isolation procedure to the investigation of actinide-humic complexes in soils. In *9th International Meeting of the International Humic Substance Society.* Adelaide, Australia, 20-25 September 1998.

COURSES ATTENDED (MSc ENVIRONMENTAL CHEMISTRY COURSES)

- Environmental risk assessment
- Contaminated land
- Environmental microbiology
- Pesticides in the environment
- Estuarine chemistry
- Industrial ecology
- Environmental modelling

CONFERENCES ATTENDED

- 9th International Meeting of the International Humic Substances Society, 1998
- ConSoil '98 Sixth International FZK/TNO conference
- RSC Radiochemical Methods Group Meeting '98
- SEGH '97 Joint Meeting of the Society of Environmental Geochemistry and Health and Royal Society of Chemistry Analytical Division (Scotland)
- COGER '97, '96 NERC Co-ordinating Group on Environmental Radioactivity
- RSC 2nd Annual Young Environmental Chemists Meeting '97

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1 INTRODUCTION

1.1 THE INVESTIGATION OF ACTINIDE INTERACTIONS WITH SOIL HUMIC SUBSTANCES: THE MODEL SYSTEM, OBJECTIVES AND 'TOOLS' REQUIRED

1.1.1 The model system

Forest soils (deciduous broadleaf and coniferous stands of the same forest) were chosen as the model system for the investigation of actinide interactions with soil humic substances. One of the five factors affecting soil horizon differentiation (pedogenesis) is vegetation. The major vegetative input to a forest soil is the tree leaves, the chemical composition of which differs between hardwood (angiosperm i.e. deciduous broadleaf trees) and softwood (gymnosperm i.e. coniferous trees) trees. Lignin is considered an important precursor material in the formation of humic substances, and thus, the difference in lignin aromatic subunit structure (aromatic substitution) between deciduous broadleaf and coniferous tree vegetation provides a possible comparison for the investigation of the effect of ligand (humic substance) structure on actinide association. A forest soil provides differentiated horizons consisting of altering mineral (primary and secondary minerals) and organic matter (in particular, humic substances of changing chemical composition and molecular size). Importantly, the differentiation of forest soil horizons produces zones of humic substances of different mean age, so that the influence of diagenetic alteration of humic substances on actinide association can be studied as a function of soil depth. The forest soil model, therefore, allows observation of the interaction of actinide elements with humic substances of changing structure and functional group content (functionality) in the context of the whole soil. The implications for the mobility of the actinide elements can be discussed in terms of the size of the humic substance ligands, their conformation, hydrophilic nature (hydrophilicity) and intermolecular associations (discrete or aggregated humic macromolecules; humic-humic, humic-metal-bridged-humic or humic-secondary mineral aggregates).

1.1.2 The objectives

This study involved the investigation of actinide-humic interactions in forest soils. A major objective was to test the hypothesis that actinides interact preferentially with only certain types of humic molecules and not with others; thus highlighting the need to characterise actinide interactions with components of humic substances rather than with a bulk humic extract. The aim was, therefore, to determine the distribution of actinide elements (U and Pu) amongst fractions of humic substances isolated from two differing forest soils (deciduous broadleaf and coniferous stands) and then to determine the potential environmental mobility of the humic substances which were found to be most important with respect to actinide binding. As most laboratory experimental work to characterise humic-metal interactions involves the addition of metal to humic materials, a further objective was to compare the distribution of experimentally 'added' ^{236}U with that of naturally occurring ^{238}U.

1.1.3 The tools required

The development of two analytical protocols was an integral part of this work. The first requirement was a method for the isolation and fractionation of soil humic substances in a 'near-native' state. To this end, mild solvent extraction and electrophoretic extraction and fractionation of humic substances were investigated and, on the basis of results obtained, a gel electrophoretic method was selected as the optimum approach. The second requirement was a method for the interaction of 'added' actinides with humic substances under environmentally relevant conditions. In particular, the prerequisites were:

- exposure of only environmentally accessible actinide binding sites present on the humic macromolecules, i.e. not those naturally involved in associations with other components of the soil
- addition of the actinide element in a speciation likely to occur in the soil solution.

These prerequisites were met by placing an actinide-containing solution (at the pH of the soil solution) directly in contact with the whole soil, rather than with a humic extract containing molecules with significantly different functionality and conformation to *in situ* soil humic molecules.

Experimental work thus involved gel electrophoretic extraction and fractionation of (naturally occurring and added) actinide-humic complexes from soil samples. Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) was the most applicable technique for actinide determination, given the large number of samples generated and the small concentrations of actinide element associated with individual fractions of humic substances. Characterisation of humic substances required application of a suite of techniques: size exclusion (gel) chromatography, UV-visible and Fourier Transform Infra-red (FTIR) spectroscopy, to identify key molecular properties (size and hydrophilicity).

1.2 FOREST SOILS

1.2.1 Definition of a soil

Soils are the interface between the lithosphere and the biosphere that originate from the weathering (physical, chemical and biochemical) of rock (Campbell, 1990; Faure, 1998). The soil matrix of the earth's surface may be described as that which mechanically supports vegetation and supplies nutrients, water and aeration for plant growth (Armson, 1979). Importantly, soils also support a diverse range of micro- and macro- fauna and flora (Armson, 1979; Campbell, 1990). In the organic litter, and underlying soil horizons (termed mineral horizons) of a forest, the flora include fungi, yeast, actinomycetes, bacteria and algae (microflora), and supported on the soil are plants (macroflora). The soil fauna include earthworms (macrofauna; > 10 mm) ants, mites (mesofauna; 0.2-10 mm) and protozoa (microfauna; < 0.2 mm) (Armson, 1979). The components (organic matter – living and non-living - and minerals) of this largely unconsolidated material are connected via a number of dynamic processes (Figure 1.1). For example, solar energy is used in the formation of organic matter by photosynthesizing plants; this organic matter enters the soil on death of the plant and may be mineralised (to CO_2) (Armson, 1979). Partial breakdown products may be assimilated by the soil biota, or may be involved in the chemical breakdown of mineral components of the soil (Brady and Weil, 1999). The soil experiences both seasonal (temperature, moisture and dissolved organic carbon) and successive variations (e.g. changes over years in amount and composition of litter fall) (Armson, 1979).

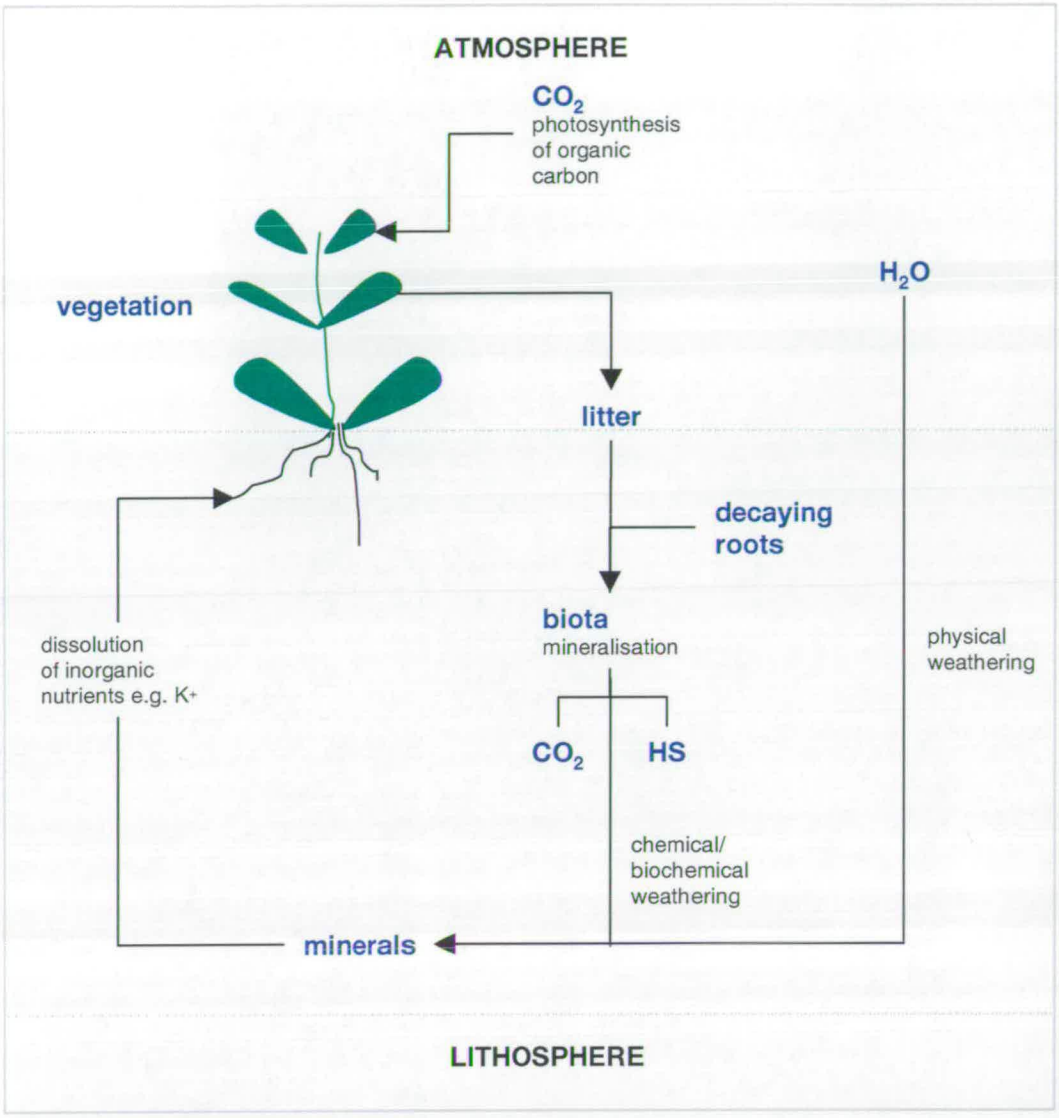


Figure 1.1: Diagrammatic representation of some of the processes that interconnect components of soil. (HS – humic substances).

1.2.2 Soil formation (pedogenesis) and mineral weathering processes

Pedogenesis is the collective term for the weathering processes leading to the formation of soil organic and mineral horizons from the underlying parent geological material. In the following paragraphs, the four soil-forming (pedogenic) processes and the five main factors influencing soil formation, are described. A simplified example of the formation of a soil is then presented. This is followed by a description of mineral weathering processes, i.e. the dissolution of fragments of primary minerals and the formation of intermediate products and secondary minerals.

The four general soil-forming processes are addition, transformation, translocation, and loss (Brady and Weil, 1999). Addition to the soil includes litter input, deposition of atmospheric dust, and inputs of soluble salts from groundwater. Transformation processes involve modification, destruction and synthesis of soil components, e.g. mineral weathering, organic matter breakdown and secondary mineral and humic substance synthesis. Translocation is the process of movement of soil components up and down the profile, predominantly by water (soluble and suspended materials) but also by soil fauna (pedoturbation) e.g. burrowing earthworms and construction of mineral mounds by ants (Armson, 1979; Brady and Weil, 1999). Finally, loss includes soil surface erosion and leaching of soluble soil components. It is the transformation and translocation processes that often result in the accumulation of materials, and thus lead to the differentiation of horizons.

The five factors that affect the soil pedogenic processes, and thus the soil formed, are climate, parent material (original geological material), biota, topography and time. Climate is the most influential factor in soil formation as it dictates the intensity of the percolating water, and thus weathering and translocation processes (Faure, 1998). The biota, i.e. natural vegetation and soil flora and fauna, influence the organic soil layers and the degree of mixing (pedoturbation) of the organic and mineral soil components. For example, a forest soil in which the major organic matter input is the leaf litter results in a forest floor (O-horizon) and thin A-horizon (mineral horizon with a high organic matter content; Section 1.2.3). In contrast, a grassland soil receives organic matter mainly from the plant root system and results in the formation of a thicker A-horizon (Brady and Weil, 1999). The topography influences the type of vegetation supported and the amount of percolating rainfall (effective precipitation). Time zero in soil formation is defined as the point at which the parent material's surface was first exposed to the weathering environment. The time will determine the maturity (differentiation) of the soil profile, although this in turn is influenced by the weatherability of the parent material and climate. The formation of an A-horizon can take as little as tens of years to form, whereas a B-horizon, with an accumulation of secondary clay minerals, can take thousands of years to form. A mature forest soil is likely to result from the order of 100,000 years

of formation, compared to the order of 10,000 years for the formation of a mature grassland soil. The depth of the soil profile is a reflection of the soil forming and soil erosion (by water and wind) processes (Brady and Weil, 1999; Faure, 1998).

In the simplest expression, the formation of a surficial soil may be described as beginning with the deposition of plant debris on the parent geological material (Brady and Weil, 1999). This organic matter is mixed with the mineral material by soil macrofauna and is mineralized by soil organisms. The combined processes of plant organic matter decomposition and synthesis of organic compounds by soil organisms results in the formation of soil humic substances. Thus a mixed organic-mineral material is commonly the first soil horizon to be differentiated from the geological material. Further horizon differentiation is effected during physical and chemical weathering of the mineral material.

Mineral weathering proceeds with physical weathering, which constitutes the disintegration of rocks into smaller fragments primarily by the expansion of freezing water in fissures, but also as a result of growth of plant roots (Campbell, 1990). The ensuing chemical weathering requires the presence of water in contact with primary mineral surfaces. These processes result in the alteration of primary minerals (e.g. unaltered feldspar, quartz) and the formation of secondary minerals. The latter differ structurally, or in chemical composition, from the primary minerals and are formed from the recombination of some or all of the dissolved primary mineral constituents (Sparks, 1995). The intermediate products of primary mineral weathering include residual minerals of low solubility (composed of Si and Al), silicic acid and alkali and alkali earth metal ions in solution (Faure, 1998). Solubilised primary mineral components are either leached, then immobilised at lower zones (translocated), or leached entirely from the profile. The formation of secondary minerals (clay minerals, metal (hydr)oxides, carbonates and sulphates) depends on the weatherability of the primary mineral and on the degree of weathering (amount of rainfall, efficiency of soil drainage, average daily temperature, presence of dissolved acids, e.g. from CO_2, low molecular weight organic acids and humic substances (Campbell, 1990)). Metal (hydr)oxide is a general term representing oxide,

oxyhydroxide, hydroxides and hydrous oxide minerals. In terms of susceptibility to weathering, for example, quartz is very resistant, whereas the feldspars range from easily weathered to moderately stable minerals (Alloway, 1995). An example of the effect of the degree of weathering on the particular secondary mineral formed is the weathering of feldspar. Under moderate conditions the formation of kaolinite (clay aluminosilicate) predominates whilst gibbsite (aluminium hydroxide) is the main product of intense weathering (Faure, 1998). Thus the intensity of the weathering is reflected in the composition of the secondary minerals. Under more intense conditions the secondary minerals will contain only the least soluble primary mineral constituents. Minerals may either be translocated to lower zones in colloidal suspension, or accumulate at the zone by *in situ* formation.

The soil profile does not reach a final equilibrium, as the individual soil properties approach steady state at different rates, while the soil as a whole, is the dynamic interaction of these soil-forming processes (Armson, 1979).

1.2.3 Forest soil horizons

This section describes the vertical sequence of differentiated forest soil horizons resulting from the soil formation processes. A description of the general classification of soil horizons is followed by a specific description of the forest floor (litter) horizons of deciduous broadleaf and coniferous forest soils. The definition and vertical profile of a Brown Earth soil, the soil type upon which the deciduous broadleaf and coniferous stands in this study were supported, is then given.

The main soil horizons (ordered from surface to depth) are designated O, A, B, C and R. A general set of possible soil horizons and subhorizons is given in Table 1.1.

Horizon label	Description
Olf	Slightly decomposed organic matter of identifiable origin
Olh	Partially decomposed organic matter, the origin of which is discernible with some difficulty
Ofh	Organic matter, the origin of which is unidentifiable
A	Mineral horizon, dark coloured, mixed with humus (eluvial)
Ah	Humus accumulation
Ah-E	Humus accumulation and eluviation
E	Maximum eluviation of e.g. silicate clays, Fe, Al (hydr)oxides
AB or EB	Gradual transition zone (more like A or E than B)
BE or BA	Gradual transition zone (more like B than A or E)
A/B	Interfingered horizons
B	Mineral horizon (illuvial)
Bt	Horizon of clay accumulation
Bfe	Horizon of iron enrichment
Bfeh	Horizon of humus and iron enrichment
Bh	Horizon of humus enrichment
Bs(cambic)	Horizon slightly altered by hydrolysis, oxidation, dissolution or all three (associated with a change in colour and/or structure)
C	Relatively unaltered unconsolidated geological materials. Zone of least weathering. Some possible characteristics are accumulation of carbonates (Ca, Mg) and soluble salts, temporary or permanent water saturation and cementation
R	Consolidated bedrock

Table 1.1: Description of soil horizons (ordered from surface to depth) present in aerobic soils. Adapted from: Armson, 1979; Brady and Weil, 1999.

The O-horizon is the surface organic layer (forest floor litter) and may be subdivided into lf, lh and fh layers. The subdivisions relate to the degree of decomposition of the accumulated forest floor litter and whether the origin of the litter is still recognisable (Table 1.1). The A-horizon is the mineral horizon near the soil surface and is classed as *eluvial*, i.e. the dominant soil processes result in a net loss of materials from the horizon in suspension or solution. The A-horizon shows a degree of accumulation of humified organic matter from the above litter horizon. Materials that may be leached from the A-horizon include clay minerals and metal (hydr)oxides in suspension. The A-horizon may therefore consist of coarser particles i.e. contain the more weather-resistant primary minerals such as quartz (Brady and Weil, 1999). The B-horizon occurs below the A-horizon and, or, the litter horizon, and is described as *illuvial*, i.e. shows zones of accumulation of materials precipitated from solution or deposited from suspension (Armson, 1979). Below this is the C-horizon, a mineral horizon which may be similar to the overlying mineral A- and, or, B-horizons. This horizon, however, shows the least weathering of mineral

materials and may show accumulation of carbonates and soluble salts (Brady and Weil, 1999). The C-horizon may also show evidence of cementation or evidence of permanent or temporary water saturation (Armson, 1979). Cemented or indurated soil is described as soil material cemented (by cementing substances such as humic substances, calcium carbonate and oxides of silicon, iron and aluminium) into a hard mass which does not soften on wetting (Brady and Weil, 1999). The R-horizon is the underlying bedrock (geological material). The set of eluvial and illuvial horizons in a profile is termed a *sequum*, i.e. A- and B- horizons and the term *solum* is used to describe the set of mineral horizons that are distinct from the underlying geology, i.e. A-, B- and C-horizons (Armson, 1979).

In this study the samples were from deciduous broadleaf and coniferous forest stands supported on Brown Earth soil. The forest floors of deciduous broadleaf and coniferous forest stands are described as mull and mor, respectively. A description of these forest floors and the typical soil profiles expected beneath deciduous broadleaf and coniferous forest vegetation follows below.

1.2.4 Vertical profile of a Brown Earth (Brown Forest soil)

A Brown Earth (Brown Forest) soil is defined as a soil characteristic of the mid-latitude world regions that were originally covered with deciduous broadleaf woodland (Oxford Reference Concise Science Dictionary, 1991). The classification, as used by the UK Forestry Commission, is set out in Table 1.2.

Characteristic	Description
Colour	Brownish-reddish soil
Acidity	Moderate-strong
Texture	Coarse-loamy
Stones	Stony
A horizon	Dark brown (incorporation of humified organic matter)
E horizon	Absent
B horizon	Richer brown than underlying C horizon due to weathering and residual accumulation of iron (hydr)oxides
C horizon	Unconsolidated and friable (easily crumbled) or very stony and merges into bedrock. Little alteration other than physical weathering

Table 1.2: Description of a Brown Earth soil profile, adapted from: Pyatt, 1970

1.2.5 The deciduous broadleaf forest floor: a mull

The mull forest floor consists of an Olf litter layer and an underlying Ah layer. The high rate of litter decomposition and seasonal broadleaf litter deposition (autumn in temperate world regions) means that at certain times of the year the Olf layer is absent, i.e. at the end of the growing season. The presence of burrowing fauna results in an Ah layer that consists of well mixed humified organic matter and mineral soil (Armson, 1979). Decomposition of the leaf litter takes place in the Ah layer (Stevenson, 1994).

1.2.6 The coniferous forest floor: a mor

The mor forest floor of a coniferous forest stand consists of Olf, Olh and Ofh layers. The transition from O- to A- horizon is very distinct (Armson, 1979). Decomposition of the pine needle litter takes place on the surface of the mineral soil (Stevenson, 1994).

1.3 FOREST SOIL SOLUTION pH

Soil solution pH is important with respect to the speciation of actinides in the solution and the implication of the solution actinide species with respect to subsequent interaction with humic substances and the mobility of actinide-humic complexes formed. This section describes firstly the measurement and meaning of soil solution pH. This is followed by a description of the inorganic and organic inputs and outputs of acidity to a soil system which define the pH. Specific forest soil horizon pH studies are then given which highlight the components and properties of the forest soil which influence the solution pH.

1.3.1 Measurement of soil solution pH

The pH is measured by suspension of soil in either deionised water or a dilute salt solution (e.g 0.01 M $CaCl_2$). In effect, what is measured is the pH of the soil solution (Rowell, 1996). The measured soil solution pH, relative to the pH of atmospheric carbon dioxide in equilibrium with carbonic acid in pure water (Box 1.1), indicates whether the soil is acting as an acid or a base. Thus a soil solution determined with pH greater than pH 5.6 indicates an alkaline soil, and a soil solution pH below pH 5.6

indicates an acid soil (Rowell, 1996). Sample size and the suspension solution used may influence the measured soil solution pH (Section 1.3.4).

1.3.2 Acidity: the balance between acidic inputs and outputs

The acidity of a soil depends on the relation of acid inputs and outputs (Rowell, 1996). The inputs may be classed as external (atmospheric, e.g. rain) and internal (a result of the soil processes). The internal production of acidity is difficult to measure, and the real system so complex, that quantitative chemical models are used to investigate the effects of input changes (Rowell, 1996; Tipping and Hurley, 1988). Soil processes producing acidity (H^+) include the production of organic acids: low molecular weight organic acids by microorganisms and plant roots and organic acids released from the decomposition of vegetation. Other bioprocesses contribute to soil acidity, e.g. respiration (CO_2, and thus carbonic acid, production). The role of mineral components in soil acidity includes hydrolysis of basic minerals with neutralisation of soil acidity, and the hydrolysis of aluminium ions producing acidity (Box 1.2). Most minerals of the Earth's crust are salts of weak acids and strong bases, e.g. carbonates and silicates, therefore hydrolysis of these basic rocks results in the formation of the weak acid and production of alkali (hydroxyl ions, OH^-) (Box 1.3). The outputs of acidity include consumption of acid in the weathering of minerals and leaching, whilst cation exchange processes allow storage of acidity (H^+, Al^{3+}) in the soil.

$CO_{2(aq)} + H_2O \leftrightarrow H_2CO_3$

Dissociation of carbonic acid:

$H_2CO_3 \leftrightarrow HCO_3^- + H^+ \quad K_a = 4.30 \times 10^{-7}, \quad pK_a = 6.37$

$HCO_3^- \leftrightarrow CO_3^{2-} + H^+ \quad K_a = 5.61 \times 10^{-11}, \quad pK_a = 10.25$

The pH of pure water in equilibrium with atmospheric CO_2 (0.03 % v/v) is 5.6

Box 1.1: The equilibrium of atmospheric carbon dioxide with carbonic acid in pure water. Adapted from: Lide, 1994; Rowell, 1996.

$Al^{3+} + H_2O \leftrightarrow AlOH^{2+} + H^+$

$AlOH^{2+} + H_2O \leftrightarrow Al(OH)_2^+ + H^+$

Box 1.2: Hydrolysis of aluminium ions in solution with production of acidity

$CaCO_3 \rightarrow Ca^{2+} + CO_3^{2-}$

$CO_3^{2-} + H_2O \leftrightarrow HCO_3^- + OH^-$

$HCO_3^- + H_2O \leftrightarrow H_2CO_3 + OH^-$

Box 1.3: An example of the dissociation and hydrolysis of a salt of a weak acid and strong base (calcite) and production of hydroxyl ions. Adapted from: Faure, 1998.

It is suggested, though no chemical justification is given, that greater leaching of basic cations (e.g. Ca^{2+}, Mg^{2+}) from the coniferous forest soils is reflected in the greater acidity of coniferous, compared to deciduous broadleaf, forest soil (Brady and Weil, 1999). However, high rainfall and formation of an acid soil is concomitant with low levels of calcium (through leaching of base cations) and higher levels of available aluminium (through dissolution and ion exchange with base cations) (Salisbury and Ross, 1985).

1.3.3 Vertical (soil profile) variation in solution pH

Rennie (1966) applied a micropedological (0.05-0.5 cm depth section) approach to investigating forest soil profile pH. The samples included soils of long established coniferous and deciduous broadleaf stands (50-100 years). Although hydrolysis of Al, Fe and Mn results in the formation of H^+, the soils investigated in this study contained low levels of clay mineral sources of these ions. This fact, and the presence of humified organic layers of the forest soils, suggested to the author that the pH of these soils would be a measure of acidity resulting from the dissociation of carboxylic and other organic acids. This is discussed further in Section 1.3.5. It was found that acidity increased descending the sequential organic litter horizons, then decreased descending the subsequent mineral soil horizons of the profiles. The coniferous forest soil surface horizons were more acidic than those of the deciduous broadleaf forest soil profiles. The pH of the litter layers decreased slightly with increasing depth toward the surface of the mineral soil, at which the pH was approximately pH 4, and then pH was observed to increase with increasing soil depth to approximately pH 6 by the C-horizon. Within the C-horizon the pH was approximately constant with depth. The magnitude of the change in pH with depth was suggested to be related to mineral component influences (e.g. neutralisation of

acidity by dissolution of calcium carbonate). The gradient of pH change with depth may also be partly influenced by the pedogenic processes affecting the depth of the profile. In conclusion to the study the importance of the micropedalogical approach to studying soil properties, which could change acutely with depth was stressed, as opposed to studies of complete horizons which may not be visually distinct, and further, which might mask the acute changes.

1.3.4 Seasonal changes in soil solution pH

Vézina (1965) studied the pH of mor forest soil organic layers (Olf and Olh), assessing the use of different suspension solutions (including water and 0.1 N KCl), small individual and large composite samples and the influence of seasonal changes on pH. Seasonal variations of pH were of the magnitude of 1.1 to 1.3 pH units. The lowest acidity was observed in mid-summer which increased towards winter. It was suggested that this might reflect decreasing soil temperature and thus decreasing chemical and biological activity. Decreasing biological activity would be expected to result in lower respired CO_2 levels and thus an increase in pH. The only chemical activity described was the release of H^+ and Al^{3+} from ion exchange sites as rainfall and soil moisture decreases, and thus a lowering of soil pH. The opposite conditions, however, would be expected towards the winter months. One factor not considered was the decomposition of litter and concomitant release of organic acids. The flushing of organic acids from litter with high effective precipitation is known to result in an increase, rather than a dilution, of dissolved organic carbon (Section 1.6.7). The acidity of the Olf layers was seen to be lower than that of the Olh layers, in agreement with Rennie (1966). The use of salt suspension solution (0.1 N KCl) resulted in less variable pH values whereas the water suspension values were subject to the greatest seasonal variations. It was suggested that the water pH values may be closest to the 'true' soil pH though it was stressed that the precise and real pH is not important, but rather the correlation between standardized pH measurements and observable soil phenomena. The variation of values obtained using a method was reduced when composite samples were used rather than smaller individual samples. It was commented that in comparison of pH between different soils, the actual pH values and ranges must be used and not pH unit differences, because of the

logarithmic scale of pH. The pH of the Olf and Olh layers, determined in water and salt suspension, are shown in Table 1.3.

Organic layer	pH (water)	pH (0.1 N KCl)
Olf	3.76	3.59
Olh	3.04	2.88

Table 1.3: pH values of the mor organic layers of a forest soil. Adapted from: Vézina, 1965.

1.3.5 Dissolved organic acids and soil cation exchange capacity (CEC)

Soil exchangeable cations are described as those cations held by electrostatic forces to negatively charged surfaces (e.g. humic substances and secondary minerals). The total charge represented by these cations is known as the cation exchange capacity (CEC - cmol per kg of negatively chaged surface) (Rowell, 1996). You *et al.* (1999) investigated soil pH, the partitioning of soil organic matter between solid and solution phases (K_d - ratio of solid to dissolved phase organic matter) and soil CEC. It was found that an increase in soil pH increased the concentration of solution phase organic matter. The pH also influenced the nature of the dissolved soil organic matter; as pH increased higher molecular weight organic matter was solubilised. The partition coefficient (K_d) was observed to correlate with the soil CEC at a fixed pH. After normalization with soil CEC, the normalized partition coefficient was negatively, linearly, correlated to pH, i.e. increase in solution phase organic matter was directly correlated to increase in pH. Vézina (1965) found that soil CEC was highest in the forest Olh layer and decreased with increasing soil depth. Whilst Dai *et al.* (1996) found that overall the organic anions of the dissolved organic carbon (DOC) contributed approximately one-third of the total anionic charge character of forest soil O-horizon leachates. Dissolved soil organic matter contributes to the negative charge (CEC) of the soil and therefore represents a component of the soil available for the complexation of actinides.

Dai *et al.* (1996) studied the relationship between DOC and soil solution acidity of forest floor O-horizons. This study compared the properties of the bulk soil solution with those of operationally defined fractions of DOC. Fractionation, using a sequence of ionic and non-ionic exchange resins (Section 1.12.2.4), yielded hydrophobic acids (representing dissolved humic substances) and hydrophilic acids

(less well characterised, but comprising smaller organic acids (Thurman, 1985) (Section 1.6.7). The hydrophobic and hydrophilic acids represented 68 and 22 % of the total DOC, respectively. Collectively then, the organic acids constitute 90 % of the total DOC. Although the hydrophobic acids had a lower carboxylic acid content than the hydrophilic acids (0.127 μeq (mol C)$^{-1}$ cf. 0.198 μeq (mol C)$^{-1}$, respectively), the hydrophobic acid titration curve showed greatest similarity to the bulk DOC titration curve (which gave a carboxylic acid content of 0.131 μeq (mol C)$^{-1}$, reflecting the predominance of the hydrophobic acid fraction. The pK_a of the hydrophobic acid fraction was 4.10. The pH of the forest floor (O-horizon) soil solution was 3.57 and 2.79, determined in water and 0.01 M $CaCl_2$ respectively. The dissociated carboxylic acid groups of humic substances are implicated in the complexation of actinides (Section 1.8.4).

1.3.6 Summary

The preceding paragraphs have highlighted the factors contributing to and affecting soil solution pH. It has been shown that forest soil pH reflects the dissociation of organic acids. The pH profile of a forest can be explained by the organic acid content of the soil DOC, the dominant fraction of which is humic substances (Section 1.6.7). The direct importance of pH, in the context of this study, is that this parameter will, in the first instance, influence the speciation of actinide elements in the soil solution and, thereafter, influence their propensity to interact with other components in the solution and solid phases. Indirectly, the influence of pH on the dissolution and extent of dissociation of organic acids, predominantly humic substances, is also important with respect to humic complexation of actinides and the subsequent mobility of the actinide-humic complexes.

1.4 FOREST SOIL MINERAL COMPONENTS

This section describes the physical (particle size) and chemical (common primary and secondary mineral) parameters of forest soil minerals. Soil minerals comprise the greatest volume of the soil solid phase. For example, generally in a silt loam soil the total volume of the soil consists of ~ 45 % minerals, ~ 5 % organic matter, ~ 20-30 % gases and ~ 20-30 % water (Sparks, 1995).

1.4.1 Particle size classes and soil texture

Particle size is a key factor in governing the chemical and physical properties of a soil. For instance coarse particles allow aeration of the soil whilst fine particles are essential for moisture and nutrient (inorganic and organic) retention (Campbell, 1990). The sizes of particles in soil are classified as sand (coarse and fine), silt and clay (Table 1.4). The chemical composition of the size classes may be generalized (Section 1.4.2). The texture of a soil is also classified according to the distribution of particle sizes present (Figure 1.2).

Particle Size Class	Particle Size (μm)
Coarse Sand	200-2000
Fine Sand	60-200
Silt	2-60
Clay	<2

Table 1.4: European system of classification of fine earth particle size (adapted from: Rowell, 1996). Particles > 2 mm are classed as stones.

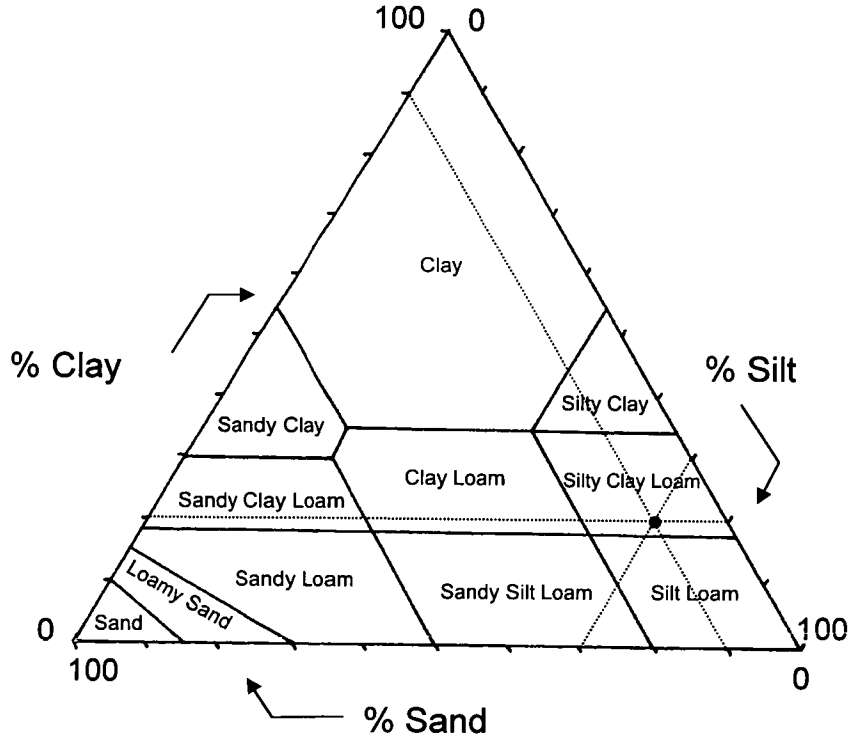


Figure 1.2: Triangular diagramatic representation of the UK system used to assign soil textural class (adapted from: Rowell, 1996). The point of intersection of the dashed lines for a soil with particle size class distribution of 20 % clay, 70 % silt and 10 % sand, demonstrated above, defines this example soil as a silty clay loam.

1.4.2 Common primary and secondary minerals in soils and their relevance to actinide binding and transport

The common primary minerals in soils, quartz and feldspar, occur predominantly in the sand and silt particle size classes (Sparks, 1995) (Table 1.5). Indeed sand and silt classes in temperate regions are predominantly quartz unless the soil has been formed on limestone in which instance the dominant minerals are likely to be calcite and dolomite (Ca, Mg carbonates) (Rowell, 1996).

	Mineral group	Specific minerals common to soil	Chemical description
Primary minerals	Quartz		Silica
	Feldspar		Silicates of Al
Secondary minerals	Clay silicate	Kaolinite	1:1 aluminosilicate
		Montmorillonite	2:1 aluminosilicate
	(Hydr)oxide	Gibbsite	Aluminium hydroxide
		Bohemite	Aluminium oxyhydroxide
		Goethite	Iron oxyhydroxide
		Haematite	Iron oxide
		Birnessite	Manganese oxide
	Carbonate	Calcite	Calcium carbonate
	Sulphate	Gypsum	Calcium sulphate

Table 1.5: The common minerals found in soils. Adapted from: Sparks, 1995.

Common secondary minerals in soil include the clay minerals kaolinite and montmorillonite, metal (hydr)oxides such as gibbsite and bohemite, goethite and haematite and birnessite (Sparks, 1995) (Table 1.5). Carbonate and sulphate minerals (the most common include calcite and gypsum) are also found in soils but as they are far more soluble compared to the silica minerals they are more common to arid and semiarid regions. The secondary minerals are typically found in the clay particle size fraction of soils but may also be found in the silt fraction (Sparks, 1995). The clay particle size class may be composed of clay minerals, metal (hydr)oxides, or these minerals in association with humic substances (Rowell, 1996).

Secondary minerals, as well as humic substances, are important with respect to the soil CEC, and are thus important to actinide binding (Section 1.9.1). The colloidal fraction of the clay particle size class, composed of secondary minerals, may be responsible for the transport of associated actinide ions. The size of colloids is often operationally defined by 1000 D ultrafilter or 0.45 μm filter cut-offs (Gustafsson and

Gschwend, 1997). Secondary minerals and humic substances may also coat primary mineral grains, and thus indirectly, primary minerals influence the behaviour of actinides associated with the immobilised secondary mineral and humic substance coatings (Section 1.6.7).

1.5 FOREST SOIL ORGANIC MATTER

This section describes the amounts, depth distribution and components of soil organic matter. This is then followed by the description of the chemistry of humic substances, which are the major fraction of soil organic matter (Section 1.6).

Soil organic matter generally comprises 0.5-5 % of the soil mass (Sparks, 1995), with the amount being controlled by the five factors influencing pedogenesis (Section 1.2.2). The most important factor is climate, then vegetation (Stevenson, 1994). In the two soils under study in this work (deciduous broadleaf and coniferous forest soil), a difference exists between the depth distributions of organic matter. The presence of earthworms and other soil fauna in the deciduous broadleaf forest soil (better drained and higher in Ca than the coniferous soil) results in the mixing of organic matter with the mineral horizon, whereas in the coniferous soil, organic matter is found at the very top of the mineral layer (Stevenson, 1994). In the near surface horizons, soil organic matter content consists of the following components: litter (macro-organic matter lying on top of the soil surface); light fraction (undegraded plant/animal material and early degradation products within the soil); biomass (living organisms in the soil); non-humic organic substances (primary plant/animal degradation products and soil microorganism generated biomolecules e.g. polysaccharides, sugars, proteins, amino acids, extracellular enzymes and lipids); and humic substances (Stevenson, 1994). In general, the organic matter content decreases down the soil profile (Table 1.6). Most components of fresh organic litter, which are input into the surface soils, are rapidly broken down and reassimilated by soil microorganisms (Brady and Weil, 1999; Thurman, 1985). With increasing depth, it is only the more stable components which remain (Section 1.6.6).

Soil Horizon	Percentage Soil Organic Carbon (%)
O	5-20
A	5-10
B	2-5
C	<2

Table 1.6: Soil horizon organic carbon content. Adapted from: Thurman, 1985.

1.6 FOREST SOIL HUMIC MATERIAL

This section begins with a general introduction to the role of humic substances in the environment. This is then followed by more detailed information on the chemistry of humic substances, the theories of the formation of these complex macromolecules and the chemical components of forest vegetation lignin which are probably an important source of precursors to humic materials. The chemical changes associated with the humification process, and diagenetic alteration subsequent to humic synthesis, are then described. These processes are important with respect to the effect on the humic actinide-binding sites. The vertical change in humic age with soil depth, which can be used as a measure of increasing humification and diagenesis, is then described. Finally, the mobility and geochemistry of soil humic substances are described. This includes a description of the dissolved organic carbon phase, the immobilisation of humic substances in the soil profile, and the fate of humic substances leached to natural waters.

1.6.1 General introduction

Humic substances are ubiquitous organic materials of terrestrial and aquatic environments. The amount of C found in humic substances on earth is greater than that found in living organisms (60×10^{11}t and 7×10^{11}t respectively) (Stevenson, 1994). Humic and fulvic acid fractions (Section 1.6.2 and Section 1.12.1) combined may represent as much as 70 % of soil organic carbon (Thurman, 1985). Collectively, humic substances may be referred to as the stabilised component of soil organic matter, i.e. they are relatively resistant to microbial decomposition. Humic substances are secondary synthesis products formed from plant and animal decay, and microbially generated products. There is much interest in humic substances as a material which has a broad spectrum of interactions (Davies and Ghabbour, 1999), especially with regard to their high affinity for anthropogenic species such as organic

pollutants and heavy metals, including the actinides. Although a small component of soil as a whole, the chemical properties of humic substances are such that they often dominate pollutant binding in soil. Despite the importance of humic substances, the complexity and heterogeneity of this material, its close association with secondary minerals, and multiple interactions with metal and organic species, are such that its structure and chemistry are still not well understood (Davies and Ghabbour, 1999; Sparks, 1995). For example, a recent description of humic substances was a series of polymers, ranging in size from several hundred to over 300,000 D, that exhibit a continuum of any given property (Stevenson, 1994).

1.6.2 Chemical properties, solvent fractions, structure and interactions

The major elements of humic substances are C, O, H and N. Other elements include P and S. The elemental composition of humic substances from different soils is very similar, typically 52-58 % C, 34-39 % O, 3.3-4.8 % H and 3.7-4.1 % N (Sparks, 1995). Chemically, humic substances may be described as a group of polydisperse, polyanionic organic molecules containing O functional groups, most importantly carboxylic acid groups (Stevenson, 1994). Other important functional groups are acidic (enol, phenol and quinone), neutral (alcohol, ether, ketone, aldehyde and ester) and basic groups (amine, amide) (Sparks, 1995). Two different types of carboxylate groups have been observed in humic substances by potentiometric titration, with apparent pK_a values of approximately 4 and 8 (Torres and Choppin, 1984). The total carboxylate group content is often used as an estimate of metal binding sites in the determination of humic-metal interaction constants (Section 1.8). Properties of humic substances include the observation of fluorescence and UV-visible light absorption. Use has been made of the ratio of absorption at 465 nm to 665 nm (called the E_4/E_6 ratio) to infer differences in humic molecular size and aromaticity between samples, i.e. the E_4/E_6 ratio decreases with increasing molecular weight and degree of aromaticity/condensation (Chen *et al.*, 1977; Davis *et al.*, 1999) (Section 1.13). Another property of humic substances is redox behaviour, which has been ascribed to free-radical quinone structures (Stevenson, 1994). Humic acid is observed to be an effective reducing agent for metals (Alberts *et al.*, 1974; Bondietti *et al.*, 1976; Szalay and Szilagyi, 1967). The total reducing capacity of humic acid has been shown to be greater than its proton equivalence, and oxidation of aliphatic

carbon atoms, possibly even aromatic carbon, has been suggested to account for the difference (Nash *et al.*, 1981).

Humic substances may be broadly fractionated into humin, humic acid and fulvic components according to their solubility in acid and alkali (Section 1.12.1). This methodology may not necessarily produce chemically distinct fractions of humic substances, and has been criticised for altering the nature of the humic substances (Section 1.11.3). Humic acid is the most abundant of the fractions of humic substances (Davies and Ghabbour, 1999). Characterisation has shown that humic acids have higher molecular weights than fulvic acids (HA ~3000-100,000 D, FA ~500-5000 D) (Stevenson, 1994). Fulvic acids have higher O (40-50 %) and lower C (41-51 %) contents than humic acids (O 33-38 % and C 54-59 %) (Sparks, 1995). Acid soil humic substances tend to have higher O contents than other soils (Schnitzer and Khan, 1972). The total acidity of humic acids is lower than that of fulvic acids and a greater proportion of the humic acid O content is contained in ether linkages (Stevenson, 1994). Fulvic acids have higher amounts of carboxylic acid, phenolic and carbonyl groups and smaller amounts of alcohol, quinone and ketone groups compared to humic acid, whilst humin has the highest alcohol group content (Sparks, 1995). Fulvic acid is more strongly reducing than humic acid (Thurman, 1985).

Research into the structure and composition of humic substances using both degradative (oxidative/reductive cleavage, pyrolysis) and non-degradative (NMR spectrometry, ESR, IR, UV-visible and fluorescence spectroscopy) techniques has been conducted (Norwood, 1988). Computational studies have also been used to construct a 3D structural representation of humic macromolecules using data from degradative studies (Schulten, 1995). There is continued debate as to whether humic molecules are small supramolecule-forming entities or covalently bonded polymers (Davies and Ghabbour, 1999). The presence of separate subgroups of humic substances within the complex mixture is still an open question. This is likely to be answered as isolation and analytical characterisation studies advance. Industries based on humic substance products, i.e. products for improving soil fertility, have

emerged and will also increase in number with advances in understanding of humic substance chemistry (Davies and Ghabbour, 1999).

Chemical interactions of humic substances within soil include: metal-ion complexation (non-actinide metals - (Dissanayake, 1991), actinide metals – (Ewart and Williams, 1986; Graham *et al.*, 1995a); mineral association (Stevenson, 1994); biosorption (Zhou, 1992); enzyme association (Ruggiero and Radogna, 1988); non-humic substance organic binding (e.g. binding of amino acids and sugars) (Cheshire *et al.*, 1992); and binding of anthropogenic organic pollutants (Szabo and Bulman, 1994).

1.6.3 The formation of humic substances

The biochemistry of the formation of humic substances is still not well understood (Stevenson, 1994). Essentially plant and animal remains are decomposed to simpler chemical constituents which then undergo synthesis to more complex substances. Plant materials are termed indirect sources for the formation of humic substances as their chemical constituents undergo complex changes during the humification process (Kononova, 1966). The types of precursors involved in the formation of humic substances are believed to be numerous and include plant lignin, cellulose and microbially synthesised sugars, amino acids and polyphenols (Stevenson, 1994). Microorganisms are thought to be responsible for alterations of precursor materials, such as oxidation of terminal side chain aliphatic groups to carboxylic acids and cleavage of aromatic substituent methoxyl groups to yield phenol groups (Stevenson, 1994). The four main pathways for the formation of humic substances are presented in Figure 1.3. The currently favoured pathway is via the condensation of phenolic and quinone structures (pathways 2 and 3; Figure 1.3) though all processes are considered possible in soils, the extent of each differing with the soil conditions. In forest soils synthesis from polyphenols leached from leaf litter is considered of major importance whereas in sediments the lignin pathway (pathway 4; Figure 1.3) may predominate (Stevenson, 1994). A factor complicating the interpretation of the formation processes is the number of precursor sources that may be involved (e.g. *in situ* and terrestrial inputs to sediments) (Stevenson, 1994). In this study a forest soil

environment was selected in which the major vegetative input to the soil is the leaf litter (Section 1.6.4).

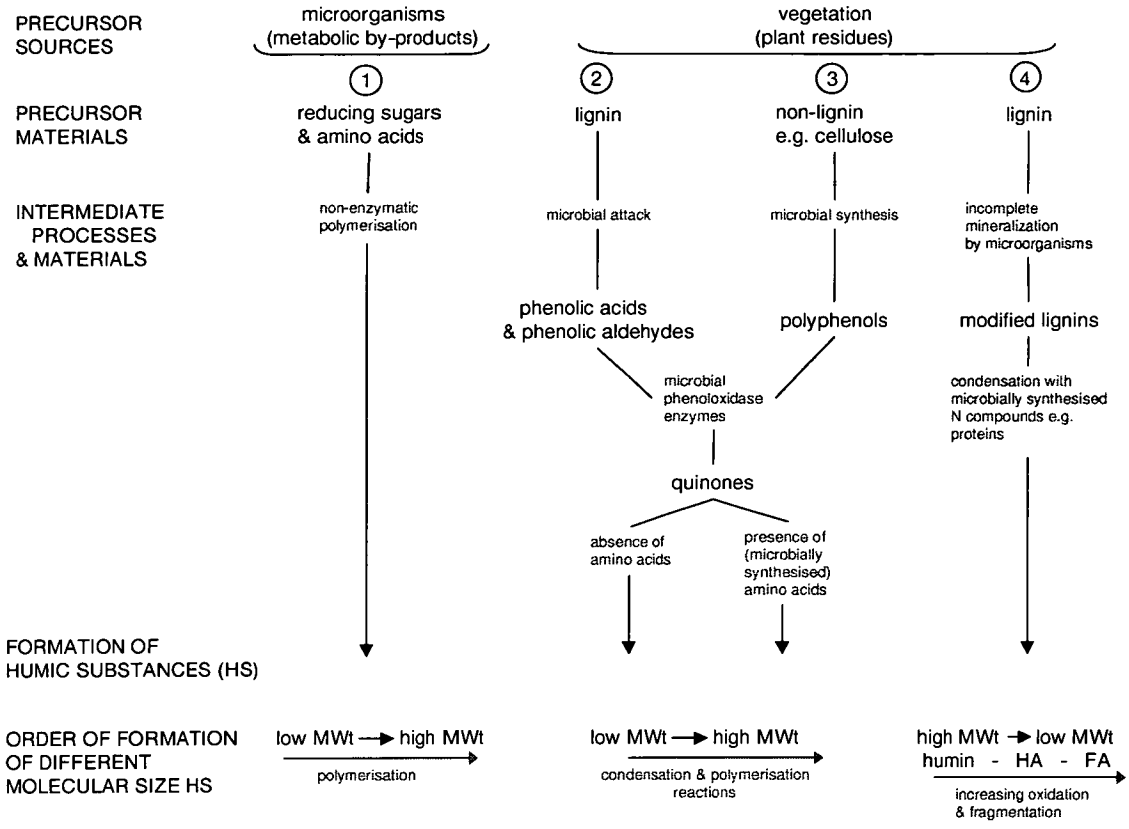


Figure 1.3: Synthesis of humic substances – precursor materials and sources, intermediate processes and materials formed and order of formation of different molecular size humic substances. Adapted from: Stevenson, 1994.

It has also been suggested that there is an infinite array of combinations possible between altered precursor materials resulting in the formation of heterogeneous humic substances, as although they may contain similar subunits, the precise structure of two compounds is thought unlikely to be the same (Stevenson, 1994). This concept has been challenged (Davies and Ghabbour, 1999) and it may be that further research highlights more specific pathways that favour particular types of linkages between altered precursors. 'Genetic' relationships between molecular size and age of humic substances are still unknown. For example, the relationship between humic precursor compounds (e.g. lignin) and fulvic acid, humic acid and humin are not yet known. The work of Ramunni *et al.* (1987) suggested that the order of formation of fulvic acid, humic acid and humin is not chronological but that

the decomposition of each single class results in the formation of the other two. The mechanism which produces different humic substances (e.g. humic acids and fulvic acids; high and low molecular weight humic substances respectively) may in fact be different (Stevenson, 1994). Suggestions have, however, been made for the order of formation of different size species by any one route (Figure 1.3). A clearer understanding of the formation of humic substances in diverse environments, and the order of formation and chemical difference between size species, would be invaluable for assessing the implications for actinide mobility in the environment of actinides associated with particular humic components. For example, it is possible that the hydrophilic component of humic substances are preferentially immobilised in the B-horizon of soils (Schnitzer *et al.*, 1959) (Section 1.6.7) and thus actinides associated with these humic substances would not be likely to be transported to receiving environments e.g. streams and rivers.

The progression of humification of organic matter, by whatever pathway, is associated with the following chemical changes to the humic substances:

- increase in COOH, phenolic OH, quinone and ketone C=O
- decrease in alcoholic OH
- decrease in molecular complexity

(Chernikov, 1992; Stevenson, 1994)

The alteration of humic substances subsequent to formation, i.e. diagenesis, is discussed in Section 1.6.5.

1.6.4 Forest vegetation lignin content and composition

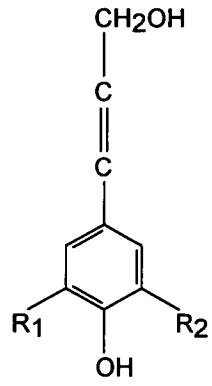
The strength of plant cell walls is provided by a composite of lignin, hemicellulose and cellulose. The lignin is interspersed with hemicellulose which in turn surrounds the cellulose microfibrils (Kirk and Farrell, 1987). The lignin content of tree leaves and wood is greater than that of grass leaf or stems (Table 1.7).

Deciduous broadleaf		Coniferous		Grass	
Leaf	Wood	Leaf	Wood	Leaf	Stem
30	17-26	23	23-30	14	11

Table 1.7: The lignin content (expressed as a percentage of the total organic components) of deciduous broadleaf and coniferous forest vegetation compared to grassland vegetation. Adapted from: Swift *et al.*, 1979.

Lignin is biosynthesised from three precursor alcohols; *p*-coumaryl, coniferyl and sinapyl alcohol. The constituent aromatic ring units of these alcohols are known as *p*-hydroxyphenyl, guaiacyl, and syringyl units, respectively. The structures of these precursor alcohols, and their simplified aromatic ring constituents, are presented in Figure 1.4. The composition of lignin of different plant species differs. Deciduous broadleaf tree lignin is composed predominantly of guaiacyl and syringyl units, coniferous tree lignin is composed predominantly of guaiacyl units and the lignin of grasses is composed of guaiacyl, syringyl and *p*-hydroxyphenyl units (Lewis and Yamamoto, 1990). There are exceptions to these generalizations and composition may vary with age of the plant and morphological structure (tissue) as well as plant species. The microbial degradation of macromolecular lignin (600-1000 kD) is carried out predominantly by fungi (Kirk and Farrell, 1987). Abiotic degradation of lignin with the release of small fragments is favoured by high temperature, acidic and alkaline environments (Kirk and Farrell, 1987). Brown-rot fungi, which preferentially degrade the cellulose component of cells leaving a brown lignin residue, achieves limited alteration of the lignin, most notably the demethylation of methoxyl groups. The white-rot fungi, which decompose lignin, leaving a white cellulose residue, cause more extensive alteration of the lignin. The changes include oxidation and progressive depolymerisation with the release of low molecular weight fragments, predominantly less than 1 kD in size (Kirk and Farrell, 1987). The conservation and distinction between the substituted phenol structures of deciduous broadleaf and coniferous leaf lignin is unambiguous such that these structures are classed as biomarkers in recent sediments (Killops and Killops, 1993). The difference in composition of the aromatic constituents of the refractory lignin component of different vegetation may be preserved in the synthesis of humic substances. Thus a study of humic substances formed under deciduous broadleaf and

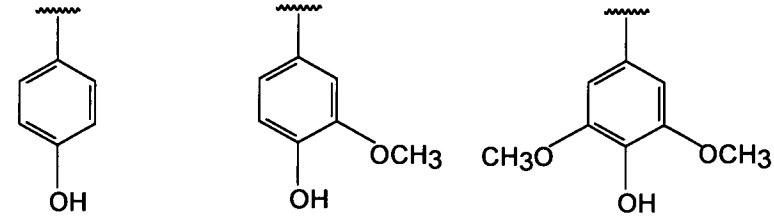
coniferous forest vegetation was chosen to investigate contrasting ligand and actinide binding characteristics.



Lignin precursor alcohols:

p-coumaryl R_1,R_2 - H,H
coniferyl R_1,R_2 - OCH_3,H
sinapyl R_1,R_2 - OCH_3,OCH_3

aromatic moiety constituents of lignin:



p-hydroxyphenyl guaiacyl syringyl

Figure 1.4: The alcohol precursor units of lignin and the corresponding aromatic constituents comprising the synthesised lignin. Adapted from: Lewis and Yamamoto, 1990.

1.6.5 Diagenetic alteration of humic substances

Following formation, humic substances are subject to microbial diagenetic alteration (carbonisation). The humic substances may be either completely biologically mineralised or altered to humin. Humin is not well characterised but it is possible that this fraction represents either highly diagenetically altered component of humic substances or that intimately bound to mineral matter (Stevenson, 1994). The chemical changes concomitant with diagenesis of humic substances are:

i) constituent losses

- decomposition of weakly bound non-humic organic components (e.g. polysaccharides and proteins)
- loss of peripheral side chains
- loss, in order, of O containing groups COOH, OCH_3 and C=O; the greatest loss of O content as COOH

ii) changes in elemental composition

- increase in C and decrease in O content, loss of N

iii) changes in solubility

- reduced solubility in alkali

(Killops and Killops, 1993; Stevenson, 1994)

Thus, humic substances reside in soils for long periods of time, but in this time they are subjected to diagenetic alteration which may influence the actinde-binding ability of the humic molecules.

1.6.6 Humic substance age as a function of soil depth

The average age of soil humic substances may be determined by ^{14}C-dating (Stevenson, 1994; Thurman, 1985). Soil humic matter is continually being synthesized and decomposed and thus the average age determined is termed the mean residence time (MRT) of the humic material present. Models proposed in the study of soil organic matter dynamics often create 'pools' which are organic matter MRT compartments of the soil. Three major divisions are:

- Active (MRT = 0.14 yr)
- Protected (MRT = 5 yr) and,
- Stable pools (MRT = 150 yr).

(Stevenson, 1994)

Stabilisation of humic substances in soil is, in major part, attributed to the formation of clay- and metal-humic complexes (Stevenson, 1994). In particular humic substances are resistant with respect to biological decomposition. The MRTs of soil humic substances have been found to vary from 250 to 1900 years (Stevenson, 1994). Soil humic and fulvic acid MRT values of 700 to 1400 years and 550 years, respectively, have been determined (Campbell *et al.*, 1967). Importantly to this study MRT increases with soil depth (Stevenson, 1994). Factors complicating

simple depth increases in MRT include transport of recent organic matter to depth via roots, percolating water, and burrowing fauna.

1.6.7 Humic substance mobility and geochemistry

This section describes the influence of size on humic substance mobility, the composition and depth changes of soil solution organic carbon and the immobilisation process (podzolisation). The leaching and fate of soil organic carbon to natural waters is then outlined, though it must be noted that the transport of organic carbon is not yet well understood.

Small molecular size humic substances are believed to be more mobile and bioavailable than the large molecular size substances (Nisbet *et al.*, 1993). The relative importance of the small molecular size species is greater in the soil solution than in the solid soil (Szabo *et al.*, 1997).

The dissolved organic carbon content of the soil solution is 2 to 30 mg l^{-1}. The low ionic strength and concentration of inorganic ions in the solution allows dissolution of soil organic matter and transport down the soil profile to the A- and B-horizons. The dissolved organic carbon content of the soil solution decreases down the profile as a function of decreasing soil organic carbon (Section 1.5) and as a result of the soil chemical (sorption) and biological (mineralisation) processes (Table 1.8). Dissolved organic carbon is highest after fresh litter fall followed by high precipitation and leaching of the leaf litter (Thurman, 1985).

Cronan and Aiken (1985) studied the dissolved organic carbon content (DOC) of forest soil horizon solutions and associated stream, groundwater, lake inlet and outlet waters (Table 1.8). Much of the DOC in the receiving aquatic environments originated from the leaching and decay of organic matter of the upper soil horizons. The quantity of DOC released was a function of vegetation type and season. Soil solution DOC content under mixed (deciduous broadleaf and coniferous) stand and coniferous stand forests was approximately twice that under the deciduous broadleaf stand. In all cases, the DOC contents of soil surface solutions were much reduced in winter compared with the summer growing season. The DOC was characterised and

was shown to be mainly organic acids; hydrophobic (fulvic) and hydrophilic (aliphatic) acids. The hydrophobic acid percent of the DOC decreased in the B-horizon soil solution whilst the hydrophilic acid percent increased. This change in the soil DOC organic acids with increasing soil depth was independent of vegetation (deciduous broadleaf or coniferous stand) type.

(Cronan and Aiken, 1985)		(Thurman, 1985)	
Solution	**DOC content (mg C l^{-1})**	**Solution**	**DOC content (mg C l^{-1})**
O/A soil horizon	21-32	O	22-36
		A	23
B soil horizon	5-7	B	10
Groundwater	2-4	Groundwater	7
Stream	6-8		
Lake inlet	3-8		
Lake outlet	2-7		

Table 1.8: Literature values of the dissolved organic carbon content of forest soil horizons and associated natural water systems. Adapted from: Cronan and Aiken, 1985; Thurman, 1985.

The amount of soil organic carbon leached to natural waters depends on the efficiency of organic matter mineralisation within the profile and sorption to mineral matter (clay minerals and metal (hydr)oxides in the B-horizon) (Thurman, 1985). In the pedogenic process of podzolisation Fe and Al are complexed in solution by dissolved organic matter which is then retained on secondary minerals in the B-horizon. Biochemical precipitation of ferrous ions by iron-reducing bacteria is also responsible for the iron oxide coatings on soil particles (Armson, 1979). Schnitzer *et al.* (1959) studied the organic matter of the A- and B-horizons of a forest soil and concluded that high molecular weight negatively charged material, readily water soluble when discrete, was immobilised on clay mineral and metal (hydr)oxide particles in the B-horizon. In the A-horizon the organic matter was observed to have a lower concentration of hydroxyl and carboxyl groups. It was therefore proposed that organic matter leached from the leaf litter, or from decomposing material in the A-horizon, is translocated to the B-horizon. At this depth interactions of the negatively-charged functional groups of the organic matter with metal ions, clay minerals and metal (hydr)oxides result in its complete immobilisation.

The organic matter in natural waters may be described in terms of two components; dissolved organic carbon (DOC) and suspended organic carbon (SOC) (Thurman, 1985). The dissolved organic carbon is the component of essentially dissolved species filtered through a 0.45 μm membrane. Humic substances comprise the major fraction of dissolved organic carbon (Table 1.9), and these aquatic humic acids have lower molecular weights than those of soil (Sparks, 1995). The suspended organic carbon component retained on the filter is predominantly plant and animal detritus and organic coatings on silt or clay sized particles. Suspended particles in natural waters may be as large as 10 μm in size (Gustafsson and Gschwend, 1997).

Fraction	**Percentage of the total dissolved organic carbon**
FA: 2nm diameter discrete dissolved ions 1000-2000 Da	50-75 %
HA: 2-50 nm diameter colloidal large aggregates of humic acids often associated with clay minerals/Al or Fe oxides 2000-100,000 Da	~ 10 %
Sugars, amino acids, fatty acids and hydroxy acids	10-20 %
Hydrophilic acids: continuum of humic substances; contain more carboxyl, hydroxyl and carbohydrate character	Remainder

Table 1.9: The fractions comprising natural water dissolved organic carbon. Adapted from: Thurman, 1985.

Soil particles may be physically transported to receiving natural waters. Though particles produced in soil horizons are often modified during transport (either wind-borne or in aqueous solution) a large proportion of the aggregates persist (Matthews, 1994). It is the fine soil particles (≤ 10 μm) which may be transported in very high precipitation/storm events from soils to receiving natural waters, though this field of work is recent and is currently receiving much attention. Particles of size range 0.5-1.5 μm have been observed to migrate from the upper horizons of forest soils (Wang and Benoit, 1996). The surfaces of soil particles may be coated with organic matter or metal (hydr)oxides. These coatings have been observed to confer a uniform charge to the natural water suspended particles, and are suggested to be of major importance to the chemistry of the particles rather than that of the underlying particles (Hunter and Liss, 1982; Hunter and Liss, 1979). Thus, substantial amounts of some trace metals are transported through estuaries within the particle coatings (Gibbs, 1977; Gibbs, 1973; Moore *et al.*, 1979). The residence times of the SOC in

river and estuarine sediments in its travel to the oceans (10s to 1000s of years depending on the size of the water system) imply that this organic matter will be decomposed before reaching the ocean (Thurman, 1985). Furthermore, a large proportion of organic matter in marine sediments is thought to be autochthonous (i.e. is formed *in situ*) because most of the remaining dissolved/colloidal terrestrial and freshwater organic matter is flocculated at high salinities in estuarine waters (Thurman, 1985).

1.7 ACTINIDE ELEMENTS

The actinide series comprises the fifteen elements from atomic number 89 to 103 (Ac-Lr). All of these elements have more than one known isotope, and all isotopes are radioactive. The stability of the actinides decreases across the series (with increasing atomic number) as half-lives shorten and the tendency toward spontaneous fission increases (Choppin *et al.*, 1995). A small number are naturally occurring, i.e. mainly isotopes of Th and U, but also traces of certain isotopes of Ac, Pa, Pu and Np. Of these, only ^{232}Th, ^{235}U, ^{238}U (and possibly ^{244}Pu) are primordial, i.e. formed by nucleosynthesis and present since the formation of the Earth 4.5×10^9 years ago (Greenwood and Earnshaw, 1997; MacKenzie, 1998). The other naturally occurring nuclides, e.g. ^{234}U, ^{231}Pa and ^{227}Ac are produced as a result of the natural radioactive decay of ^{238}U and ^{235}U. Although ^{239}Pu, ^{244}Pu and ^{237}Np have been detected on earth these elements are not thought to be primordial but result from either neutron capture and beta decay reactions in minerals containing U and Th (^{239}Pu, ^{237}Np), or as contaminants from more recent supernova events (^{244}Pu) (Choppin *et al.*, 1995). The artificial actinide isotopes have been produced by bombardment of ^{238}U and the lighter transuranium elements, with neutron, deuterium and light element (helium, boron, nitrogen, oxygen and neon) particles.

1.7.1 Naturally occurring actinide elements

U is widely distributed in nature and is present at a mean concentration of 2.3 ppm in the earth's crust. Th is also widely distributed in nature and, at mean crustal concentration of 8.1 ppm, is more abundant than U. Ac, Pa, Pu and Np are much less abundant than both U and Th (Greenwood and Earnshaw, 1997).

U tends to be found in faults of older rocks as a consequence of crystallisation late in the formation of igneous rocks (Greenwood and Earnshaw, 1997) but may also be found, often at higher concentrations (as 'ore bodies'), in younger rocks (Choppin *et al.*, 1995). Minerals containing U include uraninite (UO_{2+x}, where x = 0.01-0.25) and carnotite ($K_2(UO_2)_2(VO_4)_2.3H_2O$) (Choppin *et al.*, 1995; Greenwood and Earnshaw, 1997). Uraninite, the main oxide of U, can be either a primary or a secondary mineral. Large secondary uraninite deposits are thought to have formed as a result of leaching and reprecipitation processes. For example, ore bodies have formed downstream of mountain ranges comprising granitic rocks (U ~ 2.3 ppm). It is suggested that U in the rock has been oxidised (U (IV) to U (VI)), dissolved in groundwaters (e.g. as $UO_2(CO_3)_n^{2-2n}$ or $UO_2(SO_4)_n^{2-2n}$) and then transported and redeposited (via reduction and precipitation) (Choppin *et al.*, 1995). The deposited U (U (IV)) is, however, often found in association with other minerals, e.g. iron sulphides, and thus many such ores are only about 0.1 % U (Greenwood and Earnshaw, 1997). U also occurs in non-granitic rocks such as sedimentary sandstone (0.45 ppm) and limestone (2.2 ppm) rocks (Bowen, 1979). Weathering of rock matrices containing U is the main process accounting for a mean soil concentration of 1 ppm (Alloway, 1995).

U has seventeen isotopes in total ranging from atomic mass 225 to 242. Two of the three naturally occurring isotopes of U (^{235}U and ^{238}U) are primordial and the third (^{234}U) is the daughter of ^{234}Pa in the ^{238}U decay series (Greenwood and Earnshaw, 1997). The natural abundances of ^{234}U, ^{235}U and ^{238}U are 0.0055 atom %, 0.7200 atom % and 99.2745 atom %, respectively. All three isotopes are alpha-emitters and their associated half-lives are 2.45×10^5, 7.04×10^8 and 4.468×10^9 years, respectively (Choppin *et al.*, 1995).

1.7.2 Anthropogenic actinide elements

Anthropogenic isotopes of the actinide elements have been released into the environment by (i) disposal of authorised wastes from the nuclear fuel cycle, (ii) nuclear weapons testing, (iii) nuclear accidents and (iv) the use of nuclear weapons. For example, ^{237}Np, $^{238\text{-}241}Pu$, ^{241}Am and $^{242\text{-}244}Cm$ have been released into aquatic environments from nuclear fuel reprocessing plants (e.g. BNFL, Sellafield,

W. Cumbria, UK). Atmospheric weapons testing (banned in 1992) has also released $^{239\text{-}241}Pu$ and $^{243\text{-}244}Cm$ into the environment (mainly during the period 1959-1963). A further source of ^{239}Np and $^{238\text{-}241}Pu$ was the Chernobyl nuclear reactor accident (April 26, 1986). The largest contributions to the total inventory of actinides introduced to the environment have been from the use of nuclear weapons at (Hiroshima and Nagasaki in 1945) and from nuclear weapons testing (Choppin *et al.*, 1995). Table 1.10 shows the relative amounts of actinides released into the environment from some of the above sources.

Source	Nuclides	Associated activity (PBq)
Weapons testing	$^{238\text{-}241}Pu$, $^{243\text{-}244}Cm$	430
Chernobyl accident	^{239}Np, $^{238\text{-}240}Pu$	970
BNFL Sellafield fuel reprocessing plant	^{237}Np, $^{238\text{-}241}Pu$, ^{241}Am, $^{242\text{-}244}Cm$	5

Table 1.10: Activities of actinides released to the environment from different sources. Adapted from: Choppin *et al.*, 1995. (Note – weapons testing is not inclusive of the Hiroshima and Nagasaki bomb releases of actinides).

Soils in the UK have been contaminated with artificial actinides via (i) atmospheric deposition (weapons testing plus satellite accidents) and (ii) marine inundation of coastal soils (e.g. from BNFL Sellafield marine discharges) and (iii) deposition of windborne particles, such as seaspray or dry surface sediments, also contaminated by BNFL Sellafield marine discharges (Bulman *et al.*, 1992; Hursthouse and Livens, 1993; Livens and Baxter, 1988; MacKenzie and Scott, 1993; MacKenzie *et al.*, 1994). These sources contribute to differing extents to the total activity of actinides in UK soils. For example, the specific activity of Pu in soils of northern England from weapons testing is approximately 2 Bq kg^{-1} whereas the surface layers of coastal soils regularly inundated with contaminated marine particles contain much higher levels of Pu (11,000 Bq kg^{-1}) and forest soils further inland contain levels of Pu approximating 11 Bq kg^{-1} in the surface layers (Livens and Baxter, 1988). Weapons testing represents a diffuse source of actinides introduced via atmospheric deposition to UK soils over relatively short time periods. The introduction, via controlled releases, of actinides from the BNFL Sellafield nuclear fuel reprocessing plant is an example of a localised source.

Future risks from artificial actinides are likely to be posed by the waste disposal of spent nuclear fuel. The elements within the actinide series exhibit a range of chemical properties and the assessment of their environmental behaviour, and routes of these radiotoxic elements to man, is an area of continued research. The relative contributions of the above anthropogenic sources and natural radioactivity sources to the total environmental radioactivity, and the radiotoxicity of actinides, is discussed below in Section 1.7.3.

1.7.3 Radiotoxicity of the actinide elements

The actinides, and in particular, the transuranium (TRU) elements, are important with regards to human health because of their long half-lives (e.g. ^{237}Np - 2.14×10^6 years, ^{239}Pu - 2.41×10^4 years) and high radiotoxicity. The actinide elements are the predominant radiotoxic elements of spent nuclear fuel after the first 400 years of decay (Choppin *et al.*, 1995). Radiotoxicity is the hazard a substance presents within the body, after ingestion or inhalation, because of its radioactivity. This toxicity is a function of parameters of concentration and retention within the body, and the type of ionizing radiation of the substance. Generally, this may be represented by the effective half-life of the substance, which is a function of the time the substance is present in the body before excretion and the radioactive half-life of the substance (Equation 1.1).

$$\frac{1}{t_{eff}} = \frac{1}{t_{biol}} + \frac{1}{t_{1/2}} \qquad \text{Equation 1.1}$$

The major component of the collective effective dose to the public from the nuclear fuel cycle (uranium mineral mining through to nuclear waste disposal) is the mining/milling stage. This takes the form of gaseous radon and radon daughters released from the decay of ^{238}U (Choppin *et al.*, 1995).

The contribution of anthropogenic and natural sources of ionizing radiation to the average annual dose per person places the hazard posed by artificial actinides to human health in context (Table 1.11). It can be seen that the radioactivity released to the environment from the nuclear fuel cycle is comparatively very small.

Source	Percentage of average annual dose (to 3 d.p.)
Natural background	70.336
Medical diagnostics	29.307
Nuclear weapons tests	0.293
Occupational	0.059
Nuclear fuel cycle	0.006

Table 1.11: The contribution to the average annual dose per person presented by different sources of radioactivity. Adapted from: Choppin *et al.*, 1995.

The risk posed by actinide radionuclides is not simply a function of their concentration in the environment and the toxicity of these elements, but also the pathway from the source to the target (human population) (Choppin *et al.*, 1995). It is the understanding of radioecology, the environmental behaviour of the actinide elements and routes to man, which is pivotal in assessing the potential hazards to human health presented by radioactive waste from the nuclear fuel cycle. The behaviour of the actinides released to the environment from nuclear bombs and weapons testing may be investigated *in situ* and interpreted with the aid of relevant laboratory studies. Relevant laboratory studies must investigate the species of actinides that are important to radioecology. For example, it has been suggested that the behaviour of Pu species generated in the nuclear bomb explosions, soluble on return to the earth from the stratosphere (unlike the high-fired oxides of unexploded Pu), will resemble that of Pu released to the environment from nuclear reprocessing plants or waste repositories (Choppin *et al.*, 1995). Actinide speciation in the environment is discussed further in Section 1.7.5.

1.7.4 Properties of the actinide elements

The precursor element of the actinide series is $_{89}$Ac, the atomic configuration of which is $5f^0 6d^1 7s^2$. The main feature across the actinide series is the successive filling of the 5f orbitals. Irregularity to this feature is attributed to the increase in stability of the half-filled $5f^7$ configuration and the decreased stability of the 6d orbitals with increasing atomic number. The outer shell, 5f, 6d and 7s, orbitals are nearly degenerate, and the energies of these orbitals are closest for the elements of atomic number 90 to 94 ($_{90}$Th-$_{94}$Pu). It is also possible that the f electrons may be shielded more strongly (i.e. are of lower energy) in some elements than in others, but

it is certain that f electrons are present in the actinide series after $_{91}$Pa (Choppin *et al.*, 1995).

There is slight contraction (actinide contraction) of atomic and ionic radii across the actinide series. The actinide elements can form compounds and complexes in high oxidation states because of the near-degeneracy of the 7s, 6d and 5f orbitals and the resultingly low ionisation energies (Greenwood and Earnshaw, 1997). This has important consequences for the stable oxidation states of the actinides. The most stable oxidation state of the actinides may change from one compound, or complex, to another. Thus, actinide solution chemistry is sensitive to the ligands present. The most stable oxidation states, in acidic aqueous solution in the absence of complexing anions, varies for the different actinides from III to VI (Table 1.12). The pentavalent oxidation state is less stable than other oxidation states, except for Pa and Np. Of the available oxidation states for Pu (III-VI), the lower oxidation states (III, IV) are more stable in acid conditions, and the higher oxidation states (V, VI) are more stable under alkaline conditions. In acid solution, at concentrations of greater than 10^{-8}M, Pu disproportionates, and all available oxidation state species are represented. The chemical properties of different actinide elements in the same oxidation state resemble one another closely but differ when the actinides are in dissimilar oxidation states (Choppin *et al.*, 1995).

Actinide	Oxidation state
$_{90}$Th	IV
$_{91}$Pa	V
$_{92}$U	VI
$_{93}$Np	V
$_{94}$Pu	IV
$_{89}$Ac, $_{95}$Am-$_{103}$Lr (except $_{102}$No)	III
$_{102}$No	II

Table 1.12: The most stable oxidation states of the actinides in acidic aqueous solution, in the absence of complexing anions. Adapted from: Choppin *et al.*, 1995.

As ionic radius, in a given oxidation state, decreases with increasing atomic number across the actinide series, so the charge density increases, and the probability of hydrolysis and complexation increases with increasing atomic number (Choppin *et al.*, 1995). The f-element cations are hard acids, which show preferentially stronger

ionic interactions with ligands containing hard base anions (e.g. oxygen) (Choppin, 1991). The stability of complexes of actinides in the IV and VI oxidation states follows the order:

$$Th^{4+} < U^{4+} < Np^{4+} < Pu^{4+} \quad \text{(oxidation state IV)}$$

$$UO_2^{2+} < NpO_2^{2+} < PuO_2^{2+} \quad \text{(oxidation state VI).}$$

The order of stability for a given actinide element in different oxidation states follows the order:

$$\underset{IV}{M^{4+}} \geq \underset{VI}{MO_2^{2+}} > \underset{III}{M^{3+}} \geq \underset{V}{MO_2^{+}}$$

The stability of the hexavalent actinide oxidation state is greater than that of the trivalent state because the effective charge of linear dioxo actinyl cation, MO_2^{2+}, is greater than the formal charge of trivalent cation M^{3+} (Section 1.8.1). This is because of the poor shielding of the cationic charge of the actinide ion by the O atoms in the dioxo actinyl ion (Choppin *et al.*, 1995). Similarly, the effective charge of the pentavalent actinide ion, MO_2^{+}, is greater than the formal charge of + 1 (+ 2.2 ± 0.1).

1.7.5 Speciation and mobility of actinides in the environment

The actinide elements of U, Np and Pu have a more diverse environmental behaviour than other members of the series, reflecting the greater number of oxidation states available to these elements (Choppin, 1991). The oxidation states of a number of actinides in natural water and rock systems are presented in Table 1.13.

Actinide	Oxidation state
Th	IV
U	IV, VI
Np	IV, V
Pu	III, IV, V, VI
Am	III

Table 1.13: Important oxidation states of a selection of actinides in natural water and rock systems. Adapted from: Langmuir, 1997.

Naturally occurring U and Th can be used as analogs for predicting the migration of actinides from nuclear waste repositories. Similarly, the study of the migration of lanthanides (Ln^{3+}) in the environment can be used to understand the long term environmental behaviour of trivalent actinides (Choppin, 1991; Hooker, 1991; Petit, 1991). To aid interpretation of the chemistry, and environmental behaviour, of the actinides Eh-pH and speciation diagrams, constructed from thermodynamic constants for actinide oxidation, hydrolysis and complexation reactions, are employed to demonstrate predominant actinide oxidation states and species present for a given set of environmental conditions (Section 1.8.8). For example, from the Eh-pH diagram of Np solution species (Figure 1.5) it can be seen that the most stable species in oxic conditions, at pH < 10, is NpO_2^+. Also, the dominant U species in natural waters, below pH 8, has been suggested to be U-humate (Figure 1.6). The redox potential, Eh, is the hypothetical measure of the concentration of electrons in a system. The redox potential is proportional to the negative logarithm of the concentration of electrons. Therefore, a system with a high, positive Eh has a tendency to oxidise aqueous species whereas a system with a high, negative Eh has a tendency to reduce aqueous species.

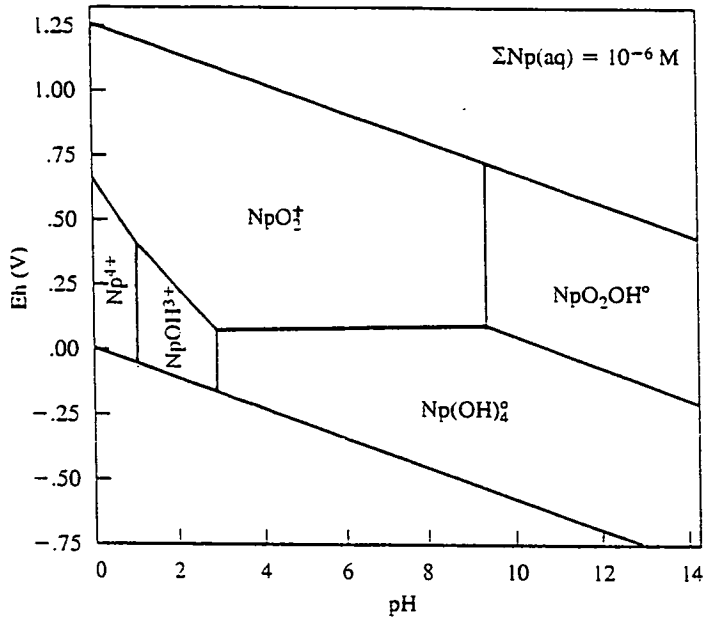


Figure 1.5: Eh-pH diagram for the aqueous Np system, Np-O_2-H_2O, at 25 °C and 1 atm and total Np concentration equal to 10^{-6} M. From: Langmuir, 1997. (Eh – redox potential).

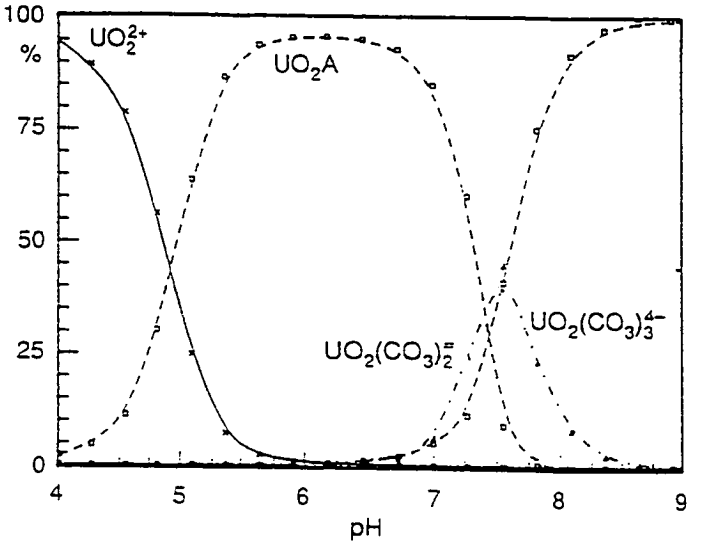


Figure 1.6: U species percentage distribution in the pH range 4-9 for the system U(VI)-OH-CO_3-humic substances, in the presence of competing cations Ca^{2+} and Al^{3+}. From: Moulin *et al.*, 1992. (A = humic ligand).

A seminal paper, discussing the important oxidation states and species of actinides in the environment, is that of Bondietti (1982). The factors governing migration of the actinides in the environment, and the species of actinides observed in different environments, as discussed in this paper, are summarised below.

The migration of the actinides in the environment will result from chemical and, or physical modes of transport. The three solution phase modes of chemical transport are:

i) transport of hydrated actinide anions,
ii) displacement of actinide ions from the solid phase by competition or exchange processes, and
iii) transport by a solute carrier i.e. complexes of actinide elements (subject to inorganic/organic complex partitioning between solid and solution phases.

Airborne and waterborne physical transport is responsible for relocating contaminated sediment or soil particles.

The findings of investigation of the solution phase (mobile) species of the U, Np, Pu, Am and Cm actinide species, in different environments, are summarised below. The environments studied were a coral soil and associated lagoon water (which had received actinides from weapons tests), and a groundwater and pond water which had received actinides from storage of nuclear fuel waste. Also investigated was the adsorption of actinides to shale from the nuclear waste storage site.

In the coral soil at Enewetok Atoll, Marshall Islands, contaminated by bomb test fallout, most of the Pu was found in the surface 0 cm to 10 cm, predominantly in the tetravalent state. In the subsequent 20 cm to 50 cm, where there was very little organic matter present, 5 % to 20 % of the Pu was in the pentavalent and hexavalent states. In the associated lagoon water, soluble pentavalent and hexavalent Pu species were reported to have been present.

Sample	**Plutonium oxidation state**
0-10 cm coral soil associated Pu	Pu (IV)
20-50 cm coral soil associated Pu (v. little organic matter present)	Pu (V, VI) - 5-20 % of the total plutonium
Lagoon water Pu	Pu (V, VI) have been reported

Table 1.14: The oxidation state of coral soil and associated lagoon water Pu at the nuclear test site of Enewetok Atoll, Marshall Islands. Adapted from: Bondietti, 1982.

In the groundwater study (pH 7.2-9.1), a small mobile (soluble) fraction of Pu was observed. This mobile Pu fraction was observed to be in oxidation states III to VI. The tetravalent Pu was suggested to be associated with high molecular weight (500 Da ultrafilter retention of 40 % of the total mobile Pu), probably organic, materials (yellowish colour retained by ultrafilter). By comparison, mobile groundwater Am and Cm species were similarly retained by ultrafiltration (45 % and 41 % respectively). The mobile groundwater U, however, was all ultrafilterable and the speciation was considered to most probably be carbonate. The groundwater was thus concluded to be sufficiently reducing for the presence of soluble forms of actinides in the lower oxidation states.

Mobile actinide oxidation state	Suggested speciation
U (VI)	Carbonate
Pu (III, IV, V, VI)	Pu (IV) associated with high MWt organics
Am (III)	Associated with high MWt organics
Cm (III)	"

Table 1.15: Mobile groundwater (pH 7.2-9.1) actinide species. The groundwater was from a shallow observation well downslope of an intermediate level radioactive waste trench (natural acidic shale trench with alkaline dolomitic infill). Adapted from: Bondietti, 1982.

In the pond water (pH 8.8-9.3) study, the soluble species of actinides were found to be U (VI), Np (V) and Pu (V). The species were anionic, with the U (VI) species demonstrating greater anionic character than Np (V) or Pu (V), observed by the interaction of the species with ion exchange resins. The percentage of the actinide species in solution, in the presence of different solid phase adsorbents was assessed to provide further information regarding the soluble species. It was found that the amount of actinides removed from solution followed the order:

Ca-humate (soil origin)>sediment>dolomitic rock>dolomitic rock (in 1 mM $NaHCO_3$ solution).

On addition of the soil Ca-humate adsorbant, the soluble species of U (VI) and Pu (V) were reduced to 65 % and 23 % of the original species concentration, respectively. In all adsorbent experiments, the percentage of hexavalent U remaining in solution was greater than the percentages of pentavalent Np or Pu. This was suggested to be because of the stronger uranyl carbonate complex formed with U. The source of the Pu (V) soluble species was suggested to be either from dissolution of the pond sediment Pu (V) (though Pu (V) probably accounted for less than 1 % of the total sediment Pu), or from the oxidation of a small fraction of the total sediment Pu (IV).

In investigations of mobile species of actinides in the presence of shale, with changing pH conditions, it was shown that increasing pH reduced sorption to the shale for U (VI), increased sorption in the case of Np (V) and had no effect on sorption of Pu (IV). The pH effect was attributed to competition between the solution phase carbonate complexation, and solid phase sorption reactions.

In conclusion, the factors affecting the mobility of actinides in the environment were considered to be the redox potential of the environment (e.g. in the case of the reducing groundwater, low oxidation state soluble Pu species were observed), the presence of ligands (organic and carbonate) and solid phase adsorbants (humics and minerals), the residence time (with respect to equilibration between species) and solution pH.

1.8 HUMIC-ACTINIDE INTERACTIONS

This section describes the interaction of humic substances with actinide elements and is subdivided into the characteristics of the actinide-humic interaction, the actinide-humic interaction constants, and the mobility of actinide-humic complexes. The section on actinide-humic interactions includes the importance of actinide cation charge density, actinide reduction, binding competition between cations, the humic binding sites, chelation, and the effect of polyelectrolyte conformation on binding. The importance of measurements of interaction constants, with respect to environmental actinide speciation is then described, including a brief outline of methods used in their determination and the limitations of interaction constants. Lastly, the speciation of actinides, in the presence of humic substances, and the mobility of actinide-humic species in the environment, is discussed.

1.8.1 Charge density of actinyl ions experienced by humic ligands

The effective charge of the dioxo actinyl cations (AnO_2^{n+}) is more likely to reflect the actinide charge experienced by complexed ligands rather than the formal cationic charge (Choppin and Rao, 1984). Rao *et al.* (1994) described the charge density of the uranyl ion (UO_2^{2+}) bound to a specific site, and bound as a condensed ion, to humic macromolecules as approximately +3.3 and +2, respectively. Estimated effective charges of U, Np and Pu are shown in Table 1.16.

Formal charge (net cationic charge)	Actinide oxidation state	U	Np	Pu
+1	V	-	+2.2	-
+2	VI	+3.1	+3.0	+2.9

Table 1.16: Estimated effective dioxo actinyl (AnO_2^{n+}: n=1, n=2 for oxidation states V and VI) cationic charge. Adapted from: Choppin and Rao, 1984.

The structure of chelated U with humic acid (solid phase, acid pH) was investigated using EXAFS by (Denecke *et al.*, 1997), and was interpreted as five equatorial O ligand donor atoms surrounding the uranyl (UO_2^{2+}) ion. Two of the five donor atoms were suggested to be O atoms neutralizing the formal charge (2+) of the uranyl ion, and the remainder neutral ligands. The interaction was interpreted as direct, predominantly monodentate, uranyl-humate complexation rather than physisorption. The greater effective charge experienced by the binding of the linear dioxo uranyl ion, through its equatorial plane, to a humic macromolecule will result in a stronger interaction than the physisorption interaction of the lower uranyl ion formal charge density with the charged humic macromolecular surface.

1.8.2 Reduction of actinides chelated by humic substances

Interaction of actinides with humic substances has been observed to be concomitant with lowering of the oxidation state of the actinide ion (Choppin and Allard, 1985; Choppin *et al.*, 1986). Humic acid was observed to reduce Np (VI) to Np (V), and Pu (VI) to Pu (IV), although the reduction of Np was much faster than that of Pu (2 hours compared with 24 hours) (Nash *et al.*, 1981). Humic acid also complexed U, but did not reduce the hexavalent uranyl ion, UO_2^{2+}, ion within a 2 month experiment contact time (Nash *et al.*, 1981). Also, the interaction constants (Section 1.8.7) of humic substances with U (IV) and U (VI) have not been found to be different (Li *et al.*, 1980). In a separate study, the reduction step of Pu (VI) to Pu (V) was observed to be very fast, whilst reduction of Pu (V) to Pu (IV) was much slower (Tan *et al.*, 1993). Overall reduction of Pu may thus be considered as the reduction of Pu (V) to Pu (IV and III) (Tan *et al.*, 1993). The mechanism for the reduction of Pu by humic acid was suggested as complexation of Pu (VI), followed by reduction of Pu by the humic ligands.

1.8.3 Cation competition effects of actinide-humic binding

Competition between cations for complexation sites on humic molecules is an important consideration for the stability of actinide-humic complexes in the environment. Competition between cations will only occur if the complexation sites are the same for the competing actinide ions (Moulin *et al.*, 1992). A number of

literature studies suggest that different complexation sites exist for different metal cations (Bidoglio *et al.*, 1991; Hering and Morel, 1988a; Hering and Morel, 1988b).

1.8.4 Functional groups implicated in actinide (hard acid) - humic (hard base) binding

According to the Irving and Williams's classification of metals and ligand donor atoms (Greenwood and Earnshaw, 1997), the functional groups of the humic substances responsible for the interaction with the hard acid actinides will be hard bases. In particular, the donor atoms suggested to be responsible for the interaction are the oxygen lone pair donor atoms of the carboxylic acid functional group. Humic acids of different origin (soil and aquifer) have been observed to show similar complexation behaviour toward the trivalent lanthanides and actinides (Shin *et al.*, 1995), i.e. coordination of the central actinide ion by similar number, and type, of ligand groups (carboxylate) and water molecules. In part, the similarity of complexation behaviour is suggested to reflect the strong preference of the large lanthanide and actinide cations for the carboxylate functional groups inherent in all humic acids. The complexity of functional groups, and the conformation of the humic macromolecules, provide a heterogeneity of anionic binding sites (Giesy *et al.*, 1986). Spectrophotometric observation of the complexation of Pu and Np by humic acids, in individual experiments, in the pH range 3-11, suggested that Pu (VI) formed at least two types of humate complex, whilst Np (V) formed a single complex (Nash *et al.*, 1981). The pH at which the complexes were observed to form suggested that Pu (VI) was complexed by carboxylate groups at low pH, and phenolic acid groups at higher pH (intrinsic $pK_a = 9$ for phenolic groups), whilst Np (V) was complexed by phenolic acid groups only.

1.8.5 Forms of humic-actinide binding: monodentate and chelating ligands

There are different interpretations and suggestions for the form of the actinide-humic complex. These include monodentate, bidentate and bridging (monodentate) actinide-humic interactions. Even tridentate complexation has been suggested (Kim *et al.*, 1989). The three important modes of coordination of the uranyl ion by a carboxylate functional group are monodentate (anti or syn, according to which oxygen electron pair is involved in the coordination), monodentate bridging

(symmetric or asymmetric depending on whether the equatorial U-O bond lengths are the same or not) and bidentate (Denecke *et al.*, 1997). The uranyl ion favours bidentate binding by carboxylate ions (Carrell *et al.*, 1988), though the study of Denecke *et al.* (1997) suggested that the number of monodentate and monodentate bridging coordination complexes exceeded that of bidentate complexes.

1.8.6 Effects of polyelectrolyte ligand conformation: strong or weak actinide-humic interactions

Actinide- and lanthanide-humic (U and Eu) complexes were classed as strong and weak according to observations of cation exchange between solution phase actinide/lanthanide-humic complexes and a cation exchange resin (Rao *et al.*, 1994). The strong and weak interactions were suggested to be reversible and in equilibrium, and result from changes in humic macromolecular conformation with increasing metal ion complexation. This interpretation was based on known changes in polyelectrolyte conformation with metal loading (Nishide *et al.*, 1982), and agrees with the theory of increased stability of chelate and macrocyclic metal complexes (Greenwood and Earnshaw, 1997) compared with monodentate metal-ligand complexes. In nature, preformed holes or chelating ring structures of biological molecules have evolved that are selective for binding particular metal ions (Frausto da Silva and Williams, 1991). It is possible that selective chelate sites exist within the humic macromolecular structure for the binding of actinide elements.

1.8.7 Actinide-humic interaction constants

Many works have derived experimental interaction constants for actinide-humic complexes. The experimental methods used to measure the interaction constants are varied and include solvent extraction (Mahajan *et al.*, 1989), ion exchange (Tao and Du, 1994), ultrafiltration (Kim *et al.*, 1993), dialysis (Kribek and Podlaha, 1980; Li *et al.*, 1980; Shanbhag and Choppin, 1981) and electrofocusing (Marquard *et al.*, 1992). Even in situations in which humic substance concentration may be low (e.g. nuclear repository systems) the actinide-humic interaction constants are sufficiently large to suggest complexation by humic substances will be significant (Minai and Choppin, 1991). Accordingly, trivalent Am-humate complexes may be the dominant americium groundwater species (Torres and Choppin, 1984). The interaction

constants are determined for solution phase humic-actinide interactions, and as such, have been suggested to be of little relevance to humic macromolecules sorbed to soil phases, e.g. clay (Mahajan *et al.*, 1989). Shin *et al.* (1995) also commented that the interaction constant is an average value of many similar humic acid intrinsic site constants. Maes *et al.* (1991) commented that understanding of the interaction of actinides with humic substances requires much more research into the complexation mechanism, and consequently, elucidation of the nature and concentration of the reacting humic functional groups.

1.8.8 Mobility of humic-actinide species in the environment

Speciation calculations, in conditions of competitive hydrolysis and carbonate and humic complexation of actinides, have been conducted to predict dominant environmental actinide species. In natural waters the strength of the interaction of actinides with humic substances is great enough that actinide-humic complexes were the dominant actinide species in natural waters in the pH range 4-7, (Moulin *et al.*, 1992). The authors, however, stated that there were too few studies of the interaction of humic substances with actinides at neutral or alkaline pH for actinide-humic interactions above pH 7 to be conclusively dismissed. Nash *et al.* (1981) studied the complexation and reduction of hexavalent ^{237}Np and ^{242}Pu and U with humic acids in alkaline solution (pH 8 to 11) with the presence of carbonate and bicarbonate ions. It was concluded that humic acids complexed tetra-, penta- and hexavalent oxidation states of actinides strongly enough to prevent carbonate complexation.

The bioavailability and mobility of radionuclides in soils is influenced by their chemical speciation and association with different phases (Desmet *et al.*, 1991). It has been suggested that interaction of metals with low molecular weight humic macromolecules is likely to promote solution phase speciation and transport of metals (Section 1.6.7). To an extent, the migration and toxicity of U to organisms is controlled by the actinide-humic acid or actinide-fulvic acid complex stability (Boggs Jr. *et al.*, 1985; Carlsen, 1989; Choppin, 1988). Nash *et al.* (1981) suggested that mobility of the actinides (in oxidation states IV, V and VI) bound to humic acids would be governed by the mobility of the humic acid. As humic acids are often strongly bound to clays the authors suggested that, subject to experimental

verification, bound actinides would therefore probably be of low mobility. The oxidation state of the actinides is the most significant variable in determining actinide solubility, as it influences the susceptibility of the actinides towards hydrolysis, complexation and sorption reactions (Choppin and Wong, 1998). Thus, oxidation state, is very important with regard to the mobility of actinides in the environment.

In summary, the interaction of actinides with humic substances may dominate speciation in a wide range of natural water pH. The mobility of these species in the environment is dependent on the size of the humic substances and the adsorption of the humic substances to solid phases. However, the structure of humic macromolecule ligands is still not well characterised. Further information regarding the structure, stereochemistry, nature and number of functional groups would provide a greater understanding of metal-humate interactions (Denecke *et al.*, 1997). Characterisation of humic macromolecular structure and size, and the investigation of the interaction of actinides with the full range of humic substances, will add new insights into the environmental behaviour of the toxic actinide elements. The study of environmentally important actinide species (taking into account the oxidation states of the actinides), and investigation of actinide-humic interactions using humic substances of near-native structure, is imperative.

1.9 METAL DISTRIBUTION IN FOREST SOIL PROFILES

This section firstly describes the pedogenic processes and soil components which influence metal distribution in a soil profile. This is then followed by examples of actinide distributions in forest soil profiles as influenced by immobilisation and leaching processes. Finally, examples of the distribution of other metallic elements in forest soils are described for their insights into the influence of forest soil humic substances on soil metal speciation and mobility.

1.9.1 Soil components influencing metal profile distribution

In general, the distribution of a metal within a soil profile will depend on the translocation and accumulation processes, and the time since introduction of the metal to the soil. For example, metals concentrated in the upper organic layer of the

soil result from associations with organic matter, incorporation (and thus cycling) in plants, or from the recent introduction of metals by atmospheric deposition. Those metals concentrated in the lower mineral horizons may result from association with translocated secondary minerals (clays and (hydr)oxides) (Alloway, 1995).

The translocation and accumulation of soil components, which have the ability to bind metals, will be important with respect to the behaviour of actinides in soil (Alloway, 1995). Soil components that have a non-zero CEC have the ability to bind actinides. The CEC of a soil changes with depth, reflecting the horizon composition of clay minerals, metal (hydr)oxides and humic substances (Faure, 1998). Humic substances, of course, may also bind actinides by complexation. The CEC is pH dependent as a result of the pH dependence of the number of negatively-charged sites of metal (hydr)oxides and humic substances and edge binding sites of clay minerals. A component of the total soil CEC is pH independent, this is associated with pH independent (resulting from lattice substitutions) permanent surface negative charge of clay minerals. The specific surface areas (SSA - $m^2\ g^{-1}$) and CECs of humic substances are generally higher than those of clay minerals (Brady and Weil, 1999), and the greater part of the soil CEC is attributed to the soil organic matter (Sparks, 1995).

1.9.2 Actinide distributions in forest soil profiles

Fujikawa *et al.* (1999) investigated soil Pu and U in a forest soil at Nagasaki, Japan. The ecosystem had recovered after the area was destroyed by the fire of the Pu bomb (1945) and was now covered by an Oak Forest (soil pH 4.9 to 5.1). A percentage of the Pu had high mobility; 3 % of the total Pu had travelled a distance of 4 m down the soil profile, compared to ^{137}Cs which had migrated less than 0.4 m down the profile. The authors investigated the association of fallout Pu, ^{137}Cs and natural U with humic substances in the soil in order to explain the high mobility Pu species. Cs was not observed to associate with the humic substances, whereas Pu and U showed associations with humic substances accounting for 1 to 9 % and 1 % of the total soil Pu and U, respectively (Table 1.17).

Soil depth (cm)	% of total soil Pu		% of total soil U		% L.O.I.
	HA	FA	HA	FA	
0-4	9.2	1.0	1.1	0.0	33.6-22.7
4-10	4.0	1.0	0.7	1.3	11.8

Table 1.17: Percentage of total soil 'bomb' $^{239+240}$Pu and natural U associated with humic acid (HA) and fulvic acid (FA). The undecomposed litter was removed from the soil and thus soil depth 0 cm represents the beginning of the A horizon. The organic matter content as approximated by loss on ignition (L.O.I.), is also given. Adapted from: Fujikawa *et al.*, 1999.

The authors suggested that the fulvic acid component was responsible for the observed high mobility of Pu in the soil profile, and that this Pu component originated from the weathering of the unexpended bomb high-fired Pu oxides. Usually, however, the high-fired Pu is interpreted as refractory. Approximately one-third of the bomb Pu is formed into high-fired oxides in the bomb explosion. The other two-thirds of bomb Pu is generated from the ^{238}U by neutron capture and β-decay (^{238}U(n, γ) → ^{239}U(2β$^-$) → ^{239}Pu), and it is this component of bomb fallout Pu which is generally accepted to be more reactive (Choppin, 1991; Choppin and Wong, 1998). The actinide association with the humic substances was suggested to be very strong as the actinides had not been removed in the mineral acid rinse step (0.1 M HCl + 0.3 M HF) of the humic isolation procedure.

Agapkina *et al.* (1995) studied Chernobyl fall-out $^{239+240}$Pu, ^{241}Am and fission product ^{137}Cs and ^{90}Sr in the soil solution associated with a mixed forest soil (80 % pine, 20 % birch; age ~ 60 years). Polyacrylamide gel filtration chromatography was used to obtain four soil organic matter fractions (≤ 2000, 1300-1000, 800 and 400 D). Pu and Am of the litter horizons were found predominantly in the high molecular weight fraction (≤ 2000 D), and to a lesser extent in the 1300-1000 D fraction. In the A-horizon soil solution, Am was also found in the lower molecular weight fractions. Cs was found in the 400 D fraction of the uppermost litter layer (Olf) and as an inorganic species (< 400 D) in the lower litter layer (Olh) and mineral A-horizon soil solutions. Sr was associated with the 800 D fraction in the upper litter layer (Olf) and associated with all organic and inorganic fractions in the lower Olh and A-horizon soil solutions. The authors suggested that the different distributions of radionuclides between the organic fractions demonstrated that the nature of the

organic matter was different in each soil horizon. Of the radionuclides studied, Cs was most affected by changes in the nature of the organic matter (and presence of inorganic matter) and Pu was least affected.

Porcelli *et al.* (1997) studied the aqueous speciation of U in Kalix River mire (peat), head- and mouthwater, and Baltic Sea, i.e. the sequential movement of U from peat to river to ocean environments. The authors determined that solution phase U increased, from the headwater (underlying Caledonian rocks) to the mouthwater, by a factor of three, in contrast to other major cations (Na, K, Mg, Ca), which demonstrated conservative behaviour. Of the total solute U, 30-90 % was found in a colloid fraction (> 10 kD; ultrafiltration). This colloid fraction was suggested to be humic acid. Approximately half of the solution U was removed at low salinities in the Baltic waters, a fraction that was equivalent to the colloid-bound U, and it was suggested that flocculation of the colloids had occurred. There remained a small fraction of colloid-bound U in higher salinity waters of the Baltic Sea. The colloid fraction of U was therefore suggested to be important in the behaviour of U in estuaries. The source of the U was indicated to be from bedrock groundwater, and the relatively constant concentration in the river water throughout the year was attributed to accumulation of spring meltwater by the mire.

Livens and Baxter (1988) investigated the associations of Pu in a mixed woodland Brown Earth soil which was located on the bank of the Esk Estuary, approximately 5 km south of the BNFL reprocessing plant at Sellafield, W. Cumbria, UK. The presence of $^{238\text{-}240}$Pu was attributed to low-level sea and atmospheric discharges from the reprocessing plant. The soil content of Pu decreased with increasing soil depth, from 9 Bq kg^{-1} to 0.1 Bq kg^{-1} at soil depth intervals of 0-5 cm and 10-15 cm, respectively. The predominant fraction (53 % at the 0-5 cm soil depth interval) of the total soil Pu was in a non-exchangeable organic speciation. This fraction had been extracted using 0.1 M sodium pyrophosphate after pretreatment of the soil to remove exchangeable Pu. This organic phase of the soil, acting as a sink for Pu, was suggested to be inert and the mobility of the associated Pu was considered to be low.

1.9.3 Examples of factors influencing the distributions of other heavy metals in forest soils

Below are described the profile of other metals in soils. The examples are for Pb, Au and Cu, i.e. soft acids. Therefore, the interaction of these metals with humic substances in the soil profiles may be expected to show preferential binding to soft base donor atom ligands (e.g. I, S) of the humic molecules.

Wang and Benoit (1996) showed that approximately 50 % of total filtrate (< 0.45 μm) Pb in a forest soil (pH < 4.5, sandy loam soil) was in a colloidal form (> 3000 D) beneath the litter O-horizon. In the B_s-horizon the colloidal Pb was reduced to 10 % of the A-horizon concentration. Organically complexed Pb accounted for less than 10 % of the truly dissolved Pb (< 3000 D). A positive correlation between soil solution Pb and DOC was demonstrated, and though partly attributed to Pb-organic colloids, was suggested to be due to the independent retention of organic carbon and Pb during transport down the profile. Truly dissolved Pb (<3000 D, but not complexed to organic molecules) was dominated by Pb^{2+}, whilst complexes with Cl^-, F^-, SO_4^{2-}, OH^- and HCO_3^- species were negligible. Increasing acidity was suggested to be likely to result in Pb desorption from soil solid phases, though the mobility of this Pb was not expected to be high because of reduced dissolved ligand concentrations at lower pH. Instead the Pb was expected to be redeposited further down the profile by adsorption of Pb^{2+} or adsorption of colloidal Pb in association with colloidal organic material, to soil solid phases. In conclusion, the mobile Pb from the forest litter layer was expected to be immobilised in lower mineral horizons before reaching streams. Johnson *et al.* (1995) reported that, although the mineral horizons of forest soils minimise losses of Pb to drainage water by immobilisation processes, soil and aquatic sediments are not as efficient at accumulating Pb as some literature sources had suggested. Thus the authors stated that the potential for pollution of drainage waters with Pb by release of Pb from forest soils may be greater than previously anticipated. One explanation for the difference between literature experimental findings and also between experimental results and modelled metal behaviour, may be the collection of metal species using

filter membranes. It is important that all size species are studied and also that the experimental methods used to separate different size species are reliable.

Bowell *et al.* (1993) investigated the weathering of Au by fulvic acids in a tropical rain forest. Sulphur and nitrogen donor atoms of the fulvic acids were demonstrated to be important to Au-fulvate complexation. It was found that fulvic acid composition influenced the amount of Au that could be dissolved from the soil minerals. Fulvic acids with higher sulphur content dissolved more Au than those with higher N contents. The Au associated with fulvic acid was reduced (oxidation state lowered from I to 0). The low level of Au in the forest soil solution (0.01-84.4 $\mu g\ l^{-1}$) was attributed to a low availability of Au and not to the stability of the Au-fulvate complexes.

Kuiters and Mulder (1993) performed gel filtration chromatography experiments to investigate the association of added Cu with soil solution organic species from deciduous broadleaf and coniferous forest soil litter and mineral horizons. The gel used was Sephadex G-25, the exclusion limit of which is defined as 1,000-5,000 D and 100-5,000 D for globular proteins and dextrans, respectively (Pharmacia Biotech, 1994) (Section 1.12.2.1). It was found that the highest levels of Cu were complexed by the organic species of the deciduous broadleaf forest soils. Cu was predominantly associated (64-94 % of the organic associated Cu species) with the lower molecular weight soil solution organic species, i.e. those which passed through the gel pore spaces. A fulvic acid extract of the coniferous forest soil also demonstrated predominant association (71 % of the total Cu associated with the fulvic acid) with the lower molecular weight organic species. The higher molecular weight soil solution organic species of the coniferous forest soil solution were shown to be removed from the soil percolating water with increasing soil depth. A comparison with the deciduous broadleaf soil was not made. The molecular size distribution of Cu complexes formed with organic species of the soil solution, and of a water extract of soil, was different for the deciduous broadleaf and coniferous forest soils. The distribution of Cu with different size organic species for the deciduous broadleaf soil was the same for the soil solution and the soil extract

species, i.e. association was approximately 35 % and 65 % with the gel excluded (high MWt) and included (low MWt) organic fractions, respectively. For the coniferous soil, however, the association of Cu shifted toward higher molecular weight organic species from the soil solution (10 % and 90 % high and low MWt associations, respectively) to the soil extract (60 % and 40 % high and low MWt associations, respectively). It would therefore appear that the higher molecular weight Cu-binding organic species are less extractable with increasing soil depth than those of the deciduous broadleaf forest soil.

1.10 INDUCTIVELY COUPLED MASS SPECTROMETRY (ICP-MS)

Analytical technologies available for the quantification of α-emitting actinide isotopes include α-spectrometry, solid state nuclear track detection (SSNTD) and inductively-coupled plasma mass spectrometry (ICP-MS) (Choppin *et al.*, 1995; Hill, 1999). Alpha-spectrometry requires the preparation of uniform thin films of the sample (to eliminate absorption of the ionizing radiation by the sample itself). Biogeochemical samples containing U and Th require separation of these radionuclides from each other prior to analysis because these nuclides have similar, and thus interfering, ionizing radiation properties. In the SSNTD technique absorption of the ionizing radiation by the photographic film activates AgBr crystals for the chemical reduction of silver. The reduced silver grains can be viewed by microscope (tracks are of the order of μm). The track length, number of grains per track length (grain density) and the gap between grains is characteristic of the ionizing radiation and its kinetic energy. This technique is less well suited to quantitative actinide element analysis. With the advent of ICP-MS instruments multi-element determination of environmental levels of actinides by mass spectrometry became achievable. The detection limit of the most sensitive ICP-MS instruments is of the order of parts per trillion (ng kg^{-1}) for ^{238}U (Hill, 1999). For the actinide elements the limiting factor in the sensitivity that can be achieved will most likely result from the procedural blanks (Hill, 1999). Isobaric interference is possible between ^{238}U and ^{237}Np or ^{239}Pu when ^{238}U concentration in the sample is comparatively high; the tail end of the ^{238}U peak overlaps with the ^{237}Np and ^{239}Pu peaks (Moreno *et al.*, 1997). The ratio of the concentration of actinides analysed in

this study was not expected to be of an order for such interference to occur. The straightforward sample preparation (acid digestion and filtration), auto-sampling accessory and automated detection of the ICP-MS made this the appropriate choice for actinide analysis of the large number of samples generated in this study.

1.11 ISOLATION OF HUMIC SUBSTANCES FROM SOILS

This section describes the procedures used in the isolation of humic substances from soil. These procedures all involve solvent extraction and thus the solvent types employed and the mechanisms of solvation are detailed. Purification of the samples, prior to, or subsequent to the dissolution of humic substances, is often employed to remove non-humic organic and inorganic contaminants. These methods are also described. The isolation and purification procedures are then discussed with respect to their effect on both the associated metal content and the structure of the isolated humic extracts, relative to the *in situ* soil form. The ability of the current isolation procedures to meet the prerequisites of this study is assessed, and the rationale for a new procedure highlighted.

1.11.1 Solvent extraction, extraction yield and extraction mechanisms

The solvents used in the extraction of humic substances from soil include aqueous and organic solvents, aqueous-organic mixtures, and the use of chelating agents and derivitisation of functional groups to aid dissolution. Examples of the solvents used are given in Table 1.18.

Aqueous
Basic
NaOH
$NaOH/SnCl_2$
KOH
NH_4OH
$NaHCO_3+Na_2CO_3$
Acidic
HCl
Neutral
KCl
Chelating Inorganic
$Na_4P_2O_7$
Chelating Organic
Tris-borate EDTA
NTHA
DTPA

Organic
Protic Organic
Ethanol
HCOOH+LiBr
Aprotic Organic
Dioxane
DMF
Acetone
Derivitisation-Aprotic Organic
TMCS:DMF
TECS:DMF

Aqueous-Organic Mixtures
Acidic-Organic (Aprotic) Mixtures
$DMSO-H_2O-HCl$
$DMF-H_2O-HCl$
$Acetone-H_2O-HCl$
Basic-Organic (Protic) Mixtures
$NH_4OH/MeOH$

Table 1.18: Examples of aqueous, organic and aqueous-organic mixture solvents used in the isolation of humic substances from soils. Fuller details of extraction methods can be found in Table 8.56 of the Appendix.

Extraction yields, expressed as the percentage of the total soil humic substances extracted using different solvents, are given in Table 1.19. Table 1.19 shows that extraction yield cannot be easily correlated to solvent type. It is clear, however, that aqueous NaOH (the International Humic Substance Society standard solvent: www.gatech.edu) achieves the highest yield of humic substances from soil.

Solvent (Eloff and Pauli, 1975)	Extraction Yield (%)
$NaOH/SnCl_2$	80
Tris-borate EDTA	30-40
HCOOH+LiBr	1-4
Acetone	0.1-0.5
Dioxane	"
Ethanol	"

Solvent (Piccolo and Mirabella, 1987; Piccolo *et al.*, 1989)	Extraction Yield (%)
NaOH	30-50
$Na_4P_2O_7$	10-20
$DMSO-H_2O-HCl$	4-6
$DMF-H_2O-HCl$	"
$Acetone-H_2O-HCl$	"

Solvent (Stevenson, 1994)	Extraction Yield (%)
NaOH	≤80
$HCOOH+LiBr/LiF/HBF_4$	≤55
Na_2CO_3	≤30
$Na_4P_2O_7$	≤30
$Acetone-H_2O-HCl$	≤20
HCl	(very little)

Table 1.19: Comparison of percentage yields of humic substances obtained from soil using a range of different solvents. Percentage values were calculated from relative yield data of Eloff and Pauli (1975) assuming that the NaOH extract represented an 80 % extraction yield.

Solvation of humic substances targets the intermolecular and intramolecular humic bonds (e.g. hydrogen bonds and divalent/polyvalent metal-ion bridge bonds) and the interactions between humic substances and other soil components (clay

minerals/metal (hydr)oxides/non-humic organic compounds) which render humic substances insoluble (Figure 1.7).

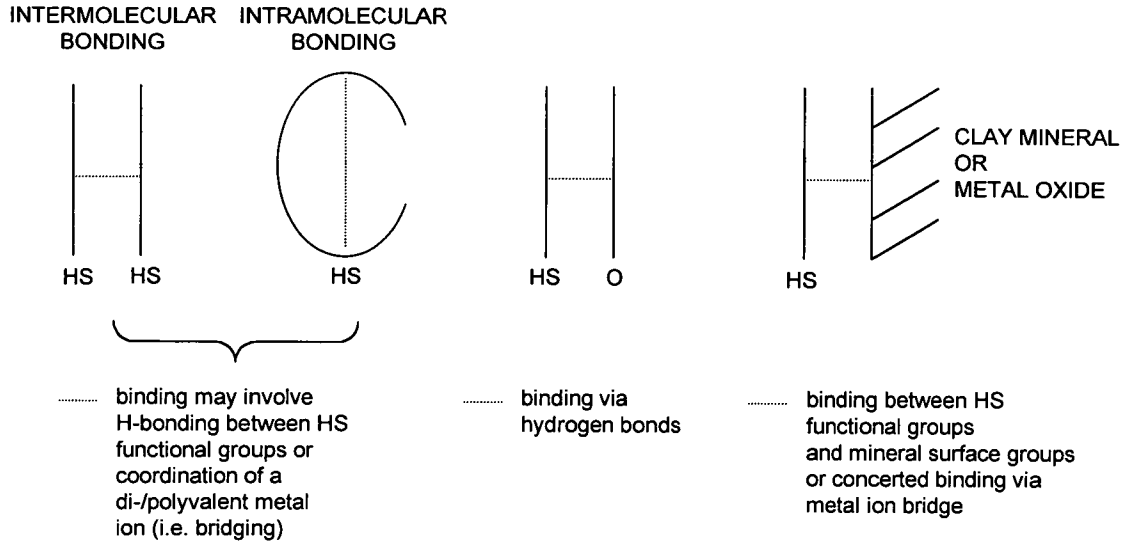


Figure 1.7: Simple pictorial representation of some chemical interactions of soil humic substances (HS) which may be responsible for their reduced solubility. Solubility is reduced as the area of the humic substance molecule accessible to solvent decreases and as RMM of the humic substance molecule increases. O - soil non-humic organic molecule e.g. lipid/resin.

A description of the mechanisms of solvation of humic substances using the solvent types shown in Table 1.18 now follows.

The mechanism of alkali extraction has been described by different authors as i) ionisation of the acidic groups of humic substances, subsequent repulsion between the charged groups and opening of the molecular conformation (Piccolo *et al.*, 1990; Piccolo and Mirabella, 1987), ii) ionisation of acidic humic substance functional groups followed by solvation, then disruption of inter-/intramolecular hydrogen bonds as solvation increases (Hayes, 1990), iii) exchange of divalent/polyvalent metal ions with monovalent metal ions of solvent to form soluble acid salts of humic substance and also release of humic substances from inorganic soil components by bond disruption (Stevenson, 1994). Ionisation of acid groups is thus the basis of alkali dissolution (Equation 1.2). The higher yields observed with the stronger bases (NaOH cf. Na_2CO_3, Table 1.19) reflects the greater proportion of dissociated acid groups (pK_a of acid groups of humic substance ranges from strongly acidic

carboxylic acid groups through phenolic hydroxyl groups to weakly acidic enolic groups) (Hayes, 1990).

$$\underset{\text{(insoluble)}}{\text{Humic substance-H}} + \text{NaOH} \rightarrow \underset{\text{(soluble)}}{[\text{Humic substance}]^- \text{Na}^+} + \text{H}_2\text{O} \qquad \text{Equation 1.2}$$

The use of strong bases for this project may not be appropriate because of possible alteration of humic substances under the conditions of high pH (Section 1.11.3).

The yields using mineral acids are reported to be very low unless they are used in combination with hydrofluoric acid (HF). The mechanism of solvation using HF is described as via release of humic substances bound to mineral components by dissolution of the minerals (Stevenson, 1994). A mechanism for dilute mineral acid solvation in the absence of HF was not given. Protonation may displace weakly associated metal ions from the humic substances but it is not certain whether or not this increases the solubility of the humic substances. Protonated humic acid groups can be involved in inter-/intra-molecular H-bonding which reduces solubility whilst metal ions associated with humic substances also reduce the solubility of humic macromolecules by cation bridges between molecules or with other soil components (Hayes, 1990). The low pH conditions may alter the natural state of the humic macromolecules (Section 1.11.3) and thus limit the applicability of these solvents for the isolation of humic substances in this project.

Solvation of humic substances in aqueous neutral solutions will only extract the more hydrophilic molecules by hydrogen bond formation and solvation in water. Though the yield would be expected to be low, water extracts may be important for elucidation of a small component of the total soil humic substances in near-native state. The KCl solution used by Szabo *et al.* (1991) was observed to yield a colourless solution. Ion exchange between K^+ ions and labile divalent/polvalent metals associated with the humic macromolecules would have been expected, followed by dissolution of the hydrophilic humic macromolecules in the aqueous solution.

The inorganic chelating agent, sodium pyrophosphate, solvates humic substances by complexation of divalent/polyvalent metal ions to the pyrophosphate group allowing solvation of the freed anionic humic macromolecule functional groups, with the charge being balanced by the solvent sodium ions (Hayes, 1990). The sodium pyrophosphate solution is also alkaline and thus dissociation of acidic functional groups will increase the solubility of the humic substances. Piccolo *et al.* (1990) also describe the mechanism as ligand exchange of the divalent/polyvalent metal ions from humic substances to insoluble metal-pyrophosphate complexes. Similarly organic chelating agents are believed to solvate humic substances by complexation of divalent/polyvalent metal ions. For example, Eloff and Pauli (1975) proposed that solvation using Tris-borate EDTA was most likely via the mechanism shown below in Equation 1.3. The Tris-borate was responsible for buffering the solution at pH 8.9.

$$\underset{\text{(insoluble)}}{2\ \text{Ca-humate} + \text{Na}_4\text{-EDTA}} \rightarrow \underset{\text{(soluble)}}{2\ \text{Na}_2\text{-humate} + \text{Ca}_2\text{-EDTA}} \qquad \text{Equation 1.3}$$

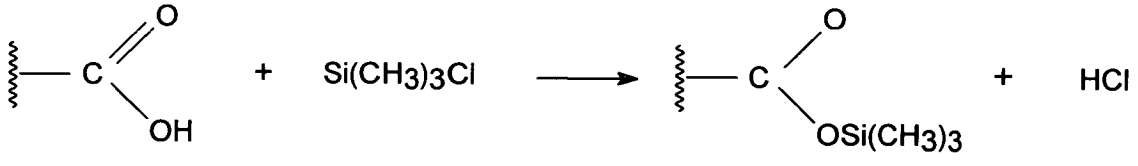
The chelating aqueous solvents are relatively effective at solvating soil humic substances (from Table 1.19: $Na_4P_2O_7$ ≤30% cf. NaOH ≤80%; Tris-borate EDTA yield is one half to one third that of $NaOH/SnCl_2$). However, their use with respect to this project needs to be carefully considered because the extraction mechanism involves release of associated metal ions, which in turn influences the conformation and ligand characteristics of the humic macromolecules (Section 1.8.6).

Hayes (1990) described solvation of humic substances by organic solvents, such as dimethylsulphoxide (DMSO), as the result of inter- and intra-molecular hydrogen bond disruption when the solvent interacts with the functional groups involved in the bonding, e.g. carboxylic and phenolic groups. At the same time the hydrophobic methyl groups of DMSO can interact with hydrophobic groups on the humic macromolecule and thus enhance dissolution. The definition of a good organic solvent for humic substances is a solvent that has a high electrostatic factor, i.e. a high dielectric constant and dipole moment (Hayes, 1990). The dielectric constant is a measure of a solvent's ability to separate a cation from its anionic counterion, and

the dipole moment is the degree of polarisation of electron density between two bonded elements e.g. a high dipole moment in the solvent molecule allows strong hydrogen bonding to the solute. Extraction with anhydrous methanoic acid (e.g. HCOOH+LiBr mixtures) has been briefly described as a hydrogen bond disruption, and metal ion complexation, process (Stevenson, 1994). Extraction by ethanol will be via hydrogen bonding interactions with humic substances.

Dissolution in aqueous-acidic-aprotic organic solvent mixtures involves breaking divalent/polyvalent metal ion interactions between humic substances and mineral colloids and solvation of the then accessible humic substance sorption sites by the organic solvent (Stevenson, 1994). The work of Piccolo *et al.* (1990) described solvation by this solvent class as intermolecular humic substance hydrogen bond disruption. As regards the aqueous-acidic-organic solvent mixtures their use may be limited as they necessarily involve conditions of low pH (pH 1 to 2) which may be detrimental to the native humic macromolecular structure (Section 1.11.3). The same argument applies to the use of aqueous-basic-protic organic solvent mixtures where conditions of high pH are incurred.

In situ derivitisation of the ionisable groups of humic substances (i.e. carboxylic, phenolic, alcoholic and possibly amino acid groups) with silyl groups and dissolution in aprotic organic solvents has been reported (Bulman *et al.*, 1990, 1992; Szabo *et al.*, 1991) (Equation 1.4). Silylation increases the hydrophobicity of the humic macromolecules, which should then increase their solubility towards the organic solvents.



Equation 1.4: Example of extraction by derivitisation/dissolution of humic substances: derivitisation with TMCS (trimethylchlorosilane)

Derivitisation will necessarily alter the intramolecular bonding and conformation of the humic macromolecules both by changing the nature of the functional groups and by steric hindrance effects of the bulky trimethyl (TMCS) or triethyl (TECS) groups. Szabo *et al.* (1991) investigated this extraction procedure because of its implications for humic-metal complexation studies, i.e. that the extraction would not release humic-associated metals.

1.11.1.1 Summary of solvent extraction

The ideal extraction procedure is one which is applicable to all soil types and isolates representative, unaltered humic substances which are free of contaminants. No procedure yet realises this ideal (Stevenson, 1994). The highest yield of humic substances from soil, and thus likely to be the most representative extract, is achieved using strong bases. In contrast water will extract a select component of hydrophilic humic substances in near-native state. As a humic extract may not be representative of the total soil humic substances then the character of the extract must be analysed. Once the nature of the humic extract is characterised then the potential mobility of metals associated with the humic substances can be assessed.

Knowledge of the extraction mechanism is essential in the assessment of a procedure to establish whether it will meet the criteria of the subsequent investigations of the humic substances. For instance, in this study it was the environmental behaviour of actinide elements towards humic substances which was to be investigated. It was therefore deemed inappropriate to study the actinide binding characteristics of humic extracts obtained by a procedure which would expose functional groups not available *in situ*, i.e. groups previously bound to mineral phases. The solvents that employ mineral dissolution as the mechanism of humic substance solvation are thus not appropriate to this study (see Table 1.20). Solvation procedures that release metals associated with the humic substances are also not appropriate for this work (see Table 1.20). This is because quantification of naturally occurring ^{238}U bound to humic substances *in situ* was required. The pH of the solvent is also important with respect to the possible alteration of the native state of the humic substances. This is discussed in Section 1.11.3.

Solvent group	Dissolution mechanism
Alkali	Ionisation of acidic groups Exchange of di-/polyvalent, with monovalent, metal ions
Mineral acid-HF	Dissolution of minerals
Chelating agents	Complexation of di-/polyvalent metal ions
Organic	Inter-/intramolecular H-bond disruption H-bonding to solvent
Acidic or Basic aqueous-organic mixtures	Dissolution of minerals H-bond disruption Ionisation of acidic groups H-bond disruption
Derivitisation	Silylation of H-bonding groups Increased steric hindrance and greater solute-solvent hydrophobic interactions

Table 1.20: Summary of solvent extraction mechanisms

1.11.2 Purification of humic extracts

Solvent extracted soil humic substances will contain contaminating soil mineral components, and soil organic components other than humic substances. Procedures to remove contaminants are discussed below.

1.11.2.1 Removal of inorganic contaminants

The simplest, non-chemical, reduction of mineral matter is achieved by high speed centrifugation of the extract and retrieval of a purer humic extract by decanting and filtering the resulting supernatant (Stevenson, 1994). Washing extracts with mineral acid has been effective in mineral matter reduction (Piccolo *et al.*, 1989). Pretreatment of soil with mineral acids is seen to improve extraction yield by decomposing carbonates and removing polyvalent cations (Hayes, 1990; Stevenson, 1994). Removal of polyvalent cations increases the solubility of humic substances. The apparent improvement in humic yield observed by removal of carbonates most likely reflects the greater proportion of humic substances in the extract when mineral matter is reduced. Magi *et al.* (1995) proposed the use of a milder acid pretreatment, i.e. ethanoic acid, to lessen metal mobilisation from humic substances. Comparable extraction yield was demonstrated using either CH_3COOH or HCl pretreatment, whilst a higher metal content (Cr, Zn, Ni, Mn and Cu) was retained in the CH_3COOH pretreated extract. Treatment with mineral acid mixtures containing HF is thought to remove mineral matter by dissolution of clay minerals, giving soluble fluorosilicic compounds (Stevenson, 1994). It has been shown that HCl-HF treatment of extracts

lowered mineral matter content but was accompanied by changes in the extract character, i.e. FT-IR spectra, ^{13}C-NMR spectra and thermogravimetric profiles compared to the original untreated extract (Piccolo *et al.*, 1989). For example, the ^{13}C-NMR spectra demonstrated loss of aliphatic, carbohydrate and amino acid structures. Weight loss of humic extracts may be observed alongside mineral content reduction. This may be the result of solvation of previously mineral bound humic substances (Stevenson, 1994).

1.11.2.2 Removal of organic contaminants

Lipids can be removed from humic extracts by solvent extraction. Eloff and Pauli (1975) pretreated soils with tetrachloromethane or ether extraction to remove lipids. Grimalt *et al.* (1989) removed lipids, and residual lipids from humic acid by ethanol, then methylene chloride/methanol extraction, respectively.

Removal of carbohydrate and protein has usually involved harsh chemical and or thermal conditions (Stevenson, 1994). Preston and Schnitzer (1984) described the alteration of humic extracts after 24-hour acid hydrolysis (100° C, 6M HCl). The extract ^{13}C-NMR spectrum after acid treatment was obviously simplified. There was a reported loss of diversity of structures; all amino acid and amino sugar structures were removed and about 90 % (by weight) of carbohydrate structures were lost. It was, however, suggested that gross structural change to the humic substance backbone had not been incurred. The criticism of this harsh acid hydrolysis treatment is that large weight loss of the extract (including possible leaching of acid soluble humic substances) and chemical changes to the humic substance structure, greater than the intended desorption of contaminating organic compounds, does occur (Stevenson, 1994).

Unbound organic contaminants present in the humic extracts have been removed by reversible adsorption of humic substances onto non-ionic exchange resins (Stevenson, 1994), though protonation of the extract for adsorption, and alkaline conditions for desorption, is usually required.

1.11.2.3 Trace metal contamination from solvents

The possibility of contamination of humic extracts with trace metal ions of the solvent has been investigated by Randle and Hartmann (1985). Contamination was not observed, but the amount of metal associated with the humic substances was seen to decrease drastically after sequential extractions with sodium pyrophosphate; the metal content (including Fe, La, Ce, Eu and U) after nine sequential extractions was reduced to 18% of the metal content after a single extraction. The greatest reduction was observed for the most abundant humic-associated metal, Fe. As detailed in Section 1.11.1, sodium pyrophosphate solvates humic substances by replacing humic-associated divalent and polyvalent cations with Na^+ ions. The observed reduction of humic-associated metal by repeated extraction is therefore not surprising. The authors commented on the importance of initial characterisation of humic-associated metal content in the interpretation of metal complexation studies. Complexation will be affected by competition between humic-bound and introduced metal ions for humic binding sites. This is important because traditionally the humic acid fraction of humic substances (Section 1.12.1) is repeatedly dissolved (in alkali) and reprecipitated (in acid) as a purification step. Thus the resulting metal content of the purified humic acid will differ from that of the *in situ* soil humic substance content. In addition, interactions of metals (e.g. added during laboratory experiments) with the extract will differ from those with *in situ* soil humic substances.

1.11.2.4 Summary of purification methods

In general, the purification of humic substances involves harsh conditions of temperature and also pH extremes. The conditions of purification are detrimental to the structure of the humic substances and also result in the release of humic-associated metals. It has also been highlighted that one of the traditional methods of preparation of humic acids, dissolution in sodium pyrophosphate and precipitation in acid, results in a large reduction of humic-associated metals. This is due to both complexation of metals by the inorganic chelating agent (sodium pyrophosphate) and the acid pH conditions of the fractionation step.

1.11.3 Problems associated with extraction and purification pH conditions

The formation of artefacts as a result of autoxidation and base catalysed reactions under alkaline conditions have been criticisms of the extraction of soil humic substances using strong bases (Hayes, 1990; Stevenson, 1994). To minimise autoxidation, extraction under a nitrogen atmosphere is recommended (Stevenson, 1994). Alternatively extraction with non-alkaline solvents or organic solvents (that do not form chemical bonds with the sample but interact via weak electrostatic interaction) is recommended (Hayes, 1990). Some investigations, however, have found no evidence of humic substance alteration. For example, Tan *et al.* (1991) characterised humic samples that had been extracted with sodium hydroxide either under a nitrogen atmosphere or an air atmosphere. The samples were characterised for elemental composition (C, H, O, N and S), total acidity, carboxyl and phenolic group content, ^{13}C-NMR- and IR-spectroscopy. No significant differences were observed between the character of the air and nitrogen atmosphere extracts. It may, however, have been more appropriate to measure oxygen uptake, or sample molecular weight distribution data to deduce alteration by autoxidation. Bremner (1949) investigated the volume of oxygen uptake in extraction medium during a seven hour extraction period for various basic solvents. It was found that oxygen uptake increased with pH of the solvent, and became significant above pH 8, concluding that increasing pH and time of exposure to alkaline conditions increased the extent of chemical alteration. Overall, the potential introduction of artefacts as a result of alkaline extraction is still a topic receiving considerable attention. This was discussed at the 9th International Meeting of the International Humic Substance Society. The use of novel studies in which humic substances are characterised *in situ* (i.e. unextracted soil samples (Tremblay, in press)) were considered an advance in understanding the environmental behaviour of humic substances.

In addition to the concerns about alkaline extraction, solvent fractionation of humic extracts by acid precipitation of humic acids leaving fulvic acids in solution (Section 1.12.1) is questioned because of chemical alteration of the humic macromolecules themselves, and also release of associated metal ions under acid pH conditions. In a study of non-precipitated humic substances (alkaline extraction), humic acid, and

combined humic and fulvic acid fractions, it was found that acid precipitation changed the molecular weight distribution of the extract and released a proportion of the associated metals (Graham, *pers. com.*, 1996). Extrapolation of metal-humic interactions, as revealed by laboratory studies of humic and fulvic acids, to environmental soil humic substance behaviour were therefore questioned.

Ramunni and Palmieri (1985) investigated the possibility of reducing extraction time (and thus chemical alteration) by the use of ultrasound treatment (1h) rather than the traditional mechanical shaking (24h) of the extraction medium. Extracts prepared by mechanical shaking and ultrasound treatment were compared by characterisation of extraction yield, mineral content, C, H, N, S and O elemental analysis, total acidity, carboxyl and phenol group content, cation exchange capacity, UV-visible- and IR-spectroscopy, and sequential gel permeation chromatography. Soil was extracted using either sodium pyrophosphate or sodium hydroxide. Yield in a given solvent was found to be comparable for both the mechanical shaking and ultrasound treatments. Differences in the character of the mechanical shaking and ultrasound extracts were, however, observed. Whether the differences in character were attributable to ultrasound wave action on the soil or a result of reduced extraction time could not be concluded but the extent of the differences was less than that observed between the two different solvent extracts.

1.11.3.1 Summary of effects of extraction and purification pH on humic substances

It is believed that strongly alkaline extraction conditions alter the structure of humic substances, though further evidence is required to support this. Acidic conditions, as employed in purification and solvent fractionation procedures, alter the structure of humic substances and release metals associated with humic substances.

1.11.4 Requirements of a new isolation procedure

This study is concerned with the investigation of the *in situ* association of naturally occurring ^{238}U with soil humic substances, and the characterisation of the humic substances with affinity for the actinides (^{238}U and added artificial actinides ^{236}U and ^{242}Pu). The conditions of extraction of humic substances from soil must avoid the

extremes of pH that would either induce changes to the humic substances, and, or release associated actinides. Electrophoretic extraction of soil was therefore highlighted as a procedure that would isolate humic substances from bulk soil, by virtue of the net negative charge of these macromolecules, under buffered pH conditions (Tris-borate, pH 8.5).

1.12 FRACTIONATION OF HUMIC SUBSTANCES

Fractionation of humic substances is necessary both to deduce the properties of specific components of the complex mixture and to obtain samples of sufficient homogeneity for analysis. Fractionation has traditionally involved separation of the humic substances into broad groups according to their solubility in different reagents. The modern approach uses separation technologies to fractionate humic macromolecules based on various molecular properties of the sample. A discussion of the fractionation methods and their applications now follows.

1.12.1 Solvent fractionation

Some of the traditional solvent fractionations of humic substances are summarised in Figure 1.8. The broad fractionation of humic substances (extracted in strong base) into alkali-soluble/acid-insoluble (humic acid) and acid-soluble (fulvic acid) components is still the commonest method, despite growing acknowledgement that the properties of such extracts and fractions may not best represent those of humic substances in natural environments (Sections 1.11.2 and 1.11.3). There is sometimes confusion in the use of the terms humic and fulvic acids in the literature. These terms are not always used simply to refer to the preparation of the samples. Fulvic acids are supposed to represent the low molecular weight, hydrophilic component of humic substances, and thus this term is sometimes used in the discussion of the highly mobile component of humic substances. The actual preparation of the samples has to be checked to verify whether the term has been applied precisely (as to extraction and fractionation method) or generally for discussion purposes.

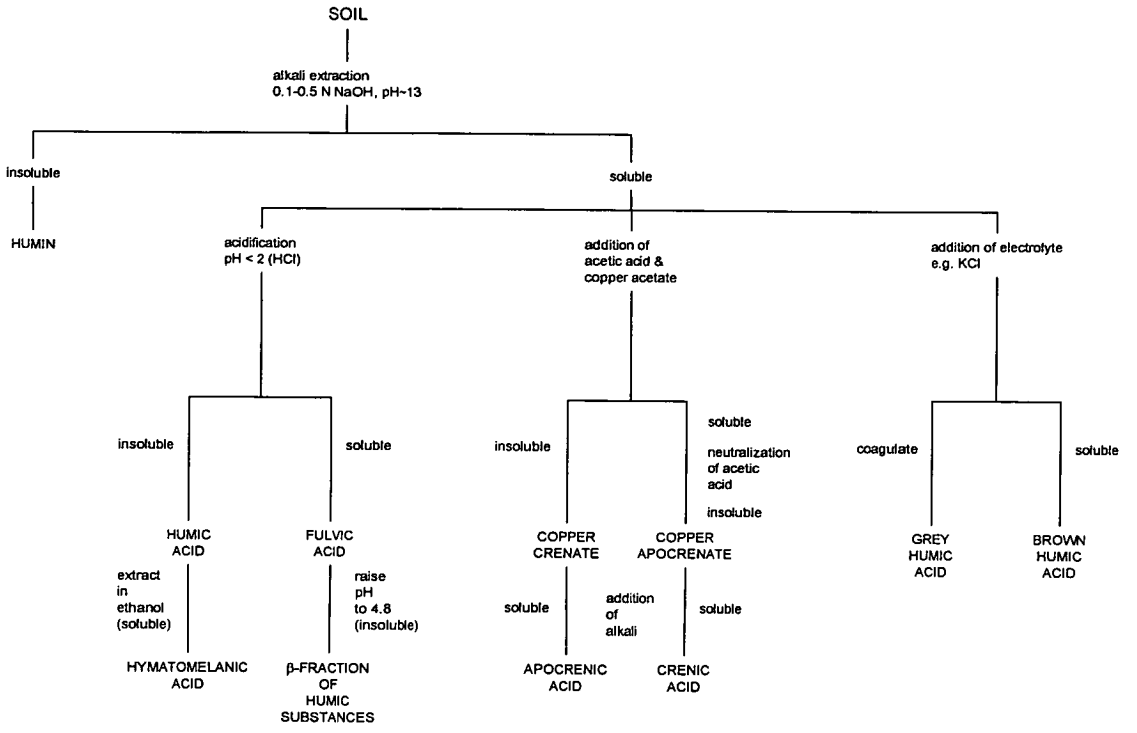


Figure 1.8: Schematic of classification of humic substance related to chemical extraction method. This is a compilation and adaptation of data from: Stevenson, 1994.

1.12.2 Separation techniques

Alternatively, with advances in separation technologies, humic extracts may be fractionated using ultrafiltration, gel filtration chromatography (GFC), high performance size exclusion chromatography (HPSEC), electrophoresis and exchange resins (ionic and non-ionic) (Table 1.21). The application of these technologies to the separation of soil humic substance samples now follows.

Separation Technique	Fractionation Principle(s)
Ultrafiltration	Size and shape (filter of nominal MWt cut-off)
Gel filtration chromatography (GFC)	Size and shape (range of fractions within gel pore size)
High performance size exclusion chromatography (HPSEC)	Size and shape (range of fractions within gel pore size)
Electrophoresis	Size, shape and anionic charge (range of fractions)
Exchange resins (ionic) " (non-ionic)	Strength of electrostatic interaction with resin Strength of hydrophobic interaction with resin

Table 1.21: A summary of separation techniques applied to the fractionation of humic substances and the molecular properties upon which the separation is based.

1.12.2.1 Gel filtration chromatography (GFC)

Gel filtration is an example of size exclusion chromatography (SEC). Fractionation by gel filtration chromatography (GFC) is based on the degree of diffusion of solute from the solution phase to the gel pore spaces. There is no interaction between the solute and the gel. Fractionation is based solely on the size and shape of the solute molecules. The gel pore size determines the molecular size of solute which can pass through the gel. Solute molecules too large to pass through the gel pores are carried down the chromatography column in the solution phase solely, while the smaller solute molecules are able to diffuse into the gel pore spaces and thus their transport is delayed. The order in which solute is eluted from the column is from large to small molecules. Solute molecules larger than the pore size of the gel are eluted first. The volume of eluent containing these excluded solute molecules is called the void volume. The eluent volume which corresponds to the lower limit of the size fractionation range of the gel is called the total column volume (Pharmacia Biotech, 1994).

In the application of GFC to the fractionation of humic substances, the experimental parameters of pH, ionic strength and buffer composition have been investigated to ensure that there is no gel-solute interaction and thus that separation is based on molecular size. Examples of these studies are: (Shaw *et al.*, 1994), pH; (Berden and Berggren, 1990), pH and ionic strength; (Hine and Bursill, 1984), optimum buffer system; and (Blondeau, 1986) (buffer composition and effect of salt). The use of a 0.1 M strength salt solution is recommended to ensure that there are no gel-solute interactions. Estimation of the molecular weight of humic substances separated by GFC has been attempted using calibration standards of: proteins (Town and Powell, 1992); and polystyrenesulphonates (Berden and Berggren, 1990). However, the conformation of humic substances in solution is still not known and thus the applicability of such standards to assigning molecular sizes is uncertain. Sequential gel filtration chromatography uses a number of gels with different pore sizes to collect fractions with definite molecular size ranges (Kawahigashi *et al.*, 1995; Tao, 1994) (Section 1.13.2). Considering the wide span of molecular sizes in a humic sample, application of a number of gels covering a large range of pore sizes would

seem most appropriate for collection of molecular size fractions. Gel filtration chromatography has been applied to study the interaction of humic substance with metals (Kuiters and Mulder, 1993) and radionuclides (Graham *et al.*, 1995b; Graham *et al.*, 1993a; Graham *et al.*, 1993b) to interpret molecular size fractions of humic macromolecules with preferential metal binding ability (Section 1.13.2). Gel filtration chromatography has been demonstrated to be effective at fractionating humic substances into different size fractions. Though standards may be used to calibrate a column, until there is a better understanding of the conformation of humic substances in solution then molecular size assignments are arbitrary.

Chin and Gschwend (1991) used HPSEC with a column consisting of modified silica to investigate the ionic strength parameter for interpretation of the true molecular weight distribution of sediment porewater humic/fulvic acids. It was concluded that the ionic strength of the sample and eluent should be matched so that conformational changes in the sample are not induced. Proteins of folded globular structure, and polystyrene sulphonates with a random coil conformation, probably less branched and cross-linked than humic substances, were used as upper and lower molecular weight limits in estimating the molecular weight of humic substances. It was demonstrated that humic colloids accumulated with depth of the sediment profile and were depleted near the sediment/water interface. The organic macromolecules lost near the surface of the sediment were suggested to be likely to play an important role in the cycling of surface-reactive chemicals. The increase in organic macromolecules with increasing sediment depth was suggested to be the result of condensation reactions or incomplete mineralisation of non-labile organic matter. The organic macromolecules comprised as much as 50 % of the interstitial water at depth and were suggested to be likely to be important to the carbon cycle of sediments.

1.12.2.2 Electrophoresis

Electrophoresis separates molecules which have a net charge, by their movement through a medium under an applied electric field. The migration velocity of the molecules is determined by the magnitude of the net charge, size and shape of the molecule, electric field strength and electrophoresis medium. Electrophoretic

mobility (μ) is proportional to the net charge on the molecule (Q) and the electric field strength (E), and inversely proportional to the radius of the molecule (r) (molecular shape is also important) and the viscosity of the medium (η) (Equation 1.4).

$$\mu \propto \frac{Q \cdot E}{r \cdot \eta} \qquad \text{Equation 1.5}$$

The application of an electric field and the net negative charge on the humic substance molecules will effect the acceleration of humic macromolecules towards the anode, whilst viscous drag of the movement of the humic macromolecules through the electrophoresis medium will oppose this motion. A constant migration velocity is reached, and is known as the electrophoretic effect (Atkins, 1994).

Electrophoresis has been highly developed as a separation technique. The types of electrophoresis which have been applied to fractionation of humic substances have included: cellulose column-, polyacrylamide gel (PAGE)-, disc and capillary electrophoresis, isoelectric focusing (IEF), combined isoelectric focusing and linear concentration gradient PAGE (an example of a 2D separation technique) and isotachophoresis. A description of each electrophoresis technique and examples of their application to the study of humic substances follows.

In PAGE electrophoresis the size of the pore spaces created in the 3D cross-linked network of the polymer can be adjusted to set the frictional resistance to electrophoretic mobility. The fractionation of the sample is based on size, shape and charge of the sample molecules. Trubetskoj *et al.* (1991) used PAGE (polyacrylamide gel electrophoresis) to fractionate humic extracts and observed a brown migration band. When denaturing agents, 7M urea and SDS, were incorporated in the sample and electrophoresis buffer, humic substance extracts were separated into four bands. Trubetskoj *et al.* (1992) continued the above work and used preparative PAGE, with denaturing agents, to fractionate humic substance extracts into five bands. Studying the isolated fractions by UV-visible spectrophotometry revealed that E_4/E_6 ratios (the ratio of absorbance at 465 to

665 nm) for the fractions increased in order of increasing electrophoretic mobility. Increasing E_4/E_6 ratios reflect decreasing molecular size (Chen *et al.*, 1977) and therefore PAGE fractionation of humic substances using denaturing agents was considered to be based on molecular size. This was further tested by denaturing agent-PAGE of the fractions of a humic substance extract which had firstly been collected by gel filtration chromatography (GFC). Electrophoretic bands appeared in the order of increasing electrophoretic mobility with increasing GFC elution time, thus confirming molecular size/shape fractionation.

Disc electrophoresis consists of a series of two polyacrylamide gels, an upper gel with large pore sizes and effectively no molecular sieving property, and a lower gel with smaller pore sizes, molecular sieving ability and a buffer medium of higher pH and ionic strength than the upper gel medium. The effect of this arrangement of gels is to minimise diffusion of separated bands by concentrating the sample before application on the lower gel. The higher ionic strength and smaller pore spaces retards the bands on entrance to the lower gel. De Gonzalez *et al.* (1981) showed that disc electrophoresis fractionated humic substance into three bands, or five bands when urea was incorporated in the sample buffer.

In isoelectric focusing a stable, linear pH gradient is set up between cathode and anode so that electrophoresis of the sample molecules proceeds until the molecules reach the pH at which their net charge is negated (isoelectric point). Fractionation is thus based on the acidity, i.e. pK_a of the sample molecule. The fractionation of humic substances by isoelectric focusing (IEF) is difficult to interpret, and possible pseudo-separation by complexation of humic substance with the ampholyte molecules of the pH gradient has been investigated (de Nobili *et al.*, 1990; Kutsch and Schumacher, 1994). Both studies found that humic substance samples were fractionated into three main pH regions. de Nobili (1988) demonstrated that a large contribution to the neutral region (pH 6.5) IEF bands was composed of negatively charged iron and aluminium humic substance complexes. By using 2D electrophoreis Zhang and Lu (1987) found that humic and fulvic acid samples were separated into a dozen bands. The 2D electrophoresis fractionation using combined

electrophoresis and isoelectric focusing involves firstly the separation of the sample by isoelectric focusing. The separated fractions are then applied to sodium dodecylsulphate (SDS)-linear concentration gradient PAGE which is used to separate molecules solely according to molecular size difference. Linear concentration gradient polyacrylamide gel consists of a gradation of pore size spaces. SDS serves to promote random coil molecular conformation by hydrophobic bonding to the humic molecule once intramolecular bonds have been disrupted. The steric bulk of the bound SDS groups prevents reformation of intramolecular bonds and refolding of the humic macromolecule. However, unlike proteins bound with SDS, the charge-to-size ratio of the all humic-SDS molecules will not be identical because humic molecules are unlikely to have evenly distributed SDS binding sites.

In isotachophoresis the molecules of the sample are separated stacked one behind one another. Amino acids and organic acids are used as spacers to attain the stacking effect. Isotachophoresis of humic and fulvic acid without ampholyte molecules (pH 6.7) separated the samples into five and three bands, respectively, while presence of ampholytes (pH 3-10) separated the samples into thirteen and ten bands, respectively (Curvetto, 1974).

Capillary electrophoresis exploits the electroosmotic flow induced in a silica capillary tube at the solid/liquid interface under an applied electric field (capillary wall/buffer) to effect the transport and separation of negatively charged molecules in the direction of anode to cathode. Separation is based on electrophoretic mobility relative to electroosmotic flow. Capillary zone electrophoresis (CZE) has been used to characterise humic substance samples. Optimisation of buffer system, sample volume, voltage and UV light detection wavelength parameters for CZE has been investigated (Garrison *et al.*, 1995; Rigol *et al.*, 1994). Properties studied by CZE have included metal complexation (Scmitt *et al.*, 1996), molecular size fractions prepared by ultrafiltration (Ciavatta *et al.*, 1995) and comparison of young and old groundwater fulvic acids, which showed different fingerprint electrophoresis absorbance patterns (polydisperse and monodisperse patterns, respectively) (Garrison *et al.*, 1995).

1.12.2.3 Ultrafiltration

MacFarlane (1978) used sequential ultrafiltration (300,000 D, 100,000 D, 50,000 D, 1,000 D and 500 D molecular size cut-off filters) to determine the molecular weight distribution of a sediment humic substances. It was found that the major component of humic acids was of molecular size > 300,000 D, whilst for fulvic acids the major components were <500 D and 100,000-300,000 D in molecular size. It was also shown that in natural waters of increased salinity preferential flocculation of higher molecular weight humic acids had occurred, i.e. the ratio of humic acids to fulvic acids in the underlying sediment was higher. Tanaka and Senoo (1995) studied the preferential binding of added ^{60}Co and ^{241}Am to different size fractions of a commercial humic acid (Section 1.13.2). Leonard *et al.* (1994) investigated applicability of ultrafiltration to environmental radionuclide-humic complexes. It was commented that conclusions, with regard to radionuclide speciation, were limited, because association data obtained was for broad groups and not specific fractions of humic substances.

1.12.2.4 Exchange resins (ionic and non-ionic)

The application of non-ionic and ionic exchange resins, used in sequence, to the fractionation of humic substances has been investigated (Yonebayashi and Hattori, 1990; Zhou and Banks, 1990). The humic sample is applied, in turn, to non-ionic exchange resin (e.g. Amberlite XAD-8), then cation exchange resin (e.g. Bio-Rad AG-MP-50), and finally, to anion exchange resin (e.g. Duolite A-7): Hydrophobic and hydrophilic acidic, basic and neutral fractions of the original humic sample are collected. The extremes of pH required for this fractionation (i.e. protonation of sample prior to application to the non-ionic resin, followed by elution in alkali) make it inappropriate for the preparation of fractions of humic substances in near-native state.

1.12.2.5 Summary of fractionation

Size exclusion chromatography and ultrafiltration have been shown to be effective for fractionating humic substances according to molecular size. In sequential gel filtration chromatography, the fractions obtained may be either broad fractions with lower and upper limits defined by exclusion from sequential gel columns of different

pore size, or a range of fractions eluted at different times from a gel column after percolation through the pore spaces. Sequential ultrafiltration is similarly useful for providing humic fractions with lower and upper molecular weight cut-off limits. Though many electrophoresis techniques have demonstrated fractionation of humic substances, according to size, charge and shape, a number of the techniques employ pH conditions regarded to be detrimental to the structure of humic substances. This includes the techniques of isoelectric focusing (IEF), 2D IEF, isotachophoresis using ampholytes, and PAGE using denaturing agents (urea and SDS). However, electrophoresis has the potential for obtaining fractions of humic substances of greater chemical distinction than those obtained by the techniques which are based on molecular size separation alone, i.e. GFC. The use of ion exchange and non-ionic exchange resins to provide separation of humic substances is not appropriate for this study because of the pH conditions employed.

1.13 CHARACTERISATION OF HUMIC SUBSTANCES

1.13.1 UV-visible spectrophotometry

Qualitative and quantitative use has been made of the UV-visible light absorption of humic substances. Qualitative applications include the observation of humic fractions during elution from separation columns (e.g. GFC and capillary electrophoresis columns; Section 1.12.2.2) and characterisation of molecular size and aromaticity using the E_4/E_6 ratio (Chen *et al.*, 1977; Davis *et al.*, 1999). Absorption (254 nm) by humic substances has also been used quantitatively to determine the recovery of humic samples after application and elution on GFC columns (Shaw *et al.*, 1994).

1.13.2 Fourier Transform Infra-red spectroscopy (FTIR)

Fourier Transform Infra-red spectroscopy (FTIR) has been used to characterise the functional group content of both bulk and fractionated humic substances. Important qualitative applications, discussed in further detail below, have included the observation of changes in spectra associated with:

- molecular size fractions (de Gonzalez *et al.*, 1981; Francioso *et al.*, 1996; Kawahigashi *et al.*, 1996; Tomikawa and Oba, 1991)
- soil depth (and thus humification and diagenesis) (Graham, 1993a)
- humification (Kumada, 1958; Tomikawa and Oba, 1991)
- heavy metal (Francioso *et al.*, 1996) and actinide ion interactions (Tanaka and Senoo, 1995)

De Gonzalez *et al.* (1981) used gel filtration chromatography for the separation of humic acids and FTIR for characterisation of the fractions. The higher molecular weight fractions were observed to show greater condensation and aromatic character whereas the lower molecular weight fraction substances were thought to be bound tightly with inorganic material (the 1050 cm^{-1} absorption band was interpreted as belonging to silicate Si-O absorption, though this is close to the C-O stretch of C-OH; 1098 cm^{-1}). In contrast, Tomikawa and Oba (1991) found that higher molecular size substances, obtained by gel filtration chromatography, contained strong absorptions suggesting protein, polysaccharide, aliphatic and quinone constituents, whilst the lower molecular size substances had stronger carboxylic group and aromatic constituents. Of the other fractions, the intensity of absorptions from protein and polysaccharide constituents increased toward the upper end of the intermediate molecular size range, and CH_2 and CH_3 content was greater toward the lower end of the small molecular size range. Kawahigashi *et al.* (1996) found that decreasing molecular size was associated with a decrease in aliphatic and amide band absorptions and an increase in carbonyl band absorption. Unique to the smallest molecular size fractions was carboxylate band absorption whereas alcohol and polysaccharide absorptions were singular to the higher molecular size fractions. The authors suggested that humic acids are a homologous series in which aliphatic chain length and methoxyl group bonds increase with increasing molecular size. Kumada (1958) suggested that increasing humification was associated with the increase of absorptions of O containing functional groups and aromaticity.

Francioso *et al.* (1996) found that lower molecular weight humic acids (ultrafiltration fractions) contained a greater number of oxygenated groups and suggested that the higher concentration of metals in these fractions was attributable to the greater concentration of carboxylic and phenolic functional groups. The lower molecular weight fractions were also observed to have a lower aromatic character. Different metals were found associated with different size fractions of the humic acid: Fe with higher molecular weight fractions, and Cu and Zn with lower molecular weight fractions. The authors concluded that very different O ligand structures therefore existed in the different size fractions. Tanaka and Senoo (1995) demonstrated a difference in the predominance of functional groups with humic acid fraction molecular size (Table 1.22). It was found that preferential binding of ^{60}Co and ^{241}Am occurred with the humic acid fraction dominated with aromatic COOH and phenolic OH groups (intermediate size fraction). Binding of ^{60}Co to the smallest humic acids, dominated with aromatic COOH groups, was also observed whilst neither ^{60}Co nor ^{241}Am was bound to the largest molecular size fraction. The difference in binding was interpreted as bidentate binding of the metal ions by adjacent aromatic substituent groups. This would result in metal chelate 7 and 6 membered rings for bidentate carboxyl, carboxyl and carboxyl, phenol interactions, respectively. This would fit with the observation of binding of ^{60}Co only with the smallest humic fraction. A 7-membered chelate ring with the larger hydrated ^{241}Am ion would show greater ring strain.

Molecular size fractrion (D)	Dominant functional groups(s)
> 100,000	Aliphatic groups
30,000-100,000	Aromatic COOH and phenolic OH
5,000-30,000	Aromatic COOH

Table 1.22: The dominant functional groups associated with different humic acid molecular sizes, as observed in FTIR spectra. Adapted from: Tanaka and Senoo, 1995.

Graham *et al.* (1993a) demonstrated that O containing functional groups (including free carboxyl and carboxylate groups) decreased with soil depth. U was shown to be associated with discrete size humic fractions.

FTIR Spectra have also been used quantitatively, for purposes including the determination of humic substance total carboxylic acid group content (Celi *et al.*, 1997), the C to O relationship and degree of aromaticity of humic substances (Davis *et al.*, 1999) and quantification of organic matter content in whole sediment depth samples without extraction (Tremblay *et al., in press*). For example, Davis *et al.* (1999) used FTIR quantitatively, with potassium thiocyanate (single absorption at 2050 cm^{-1}) as internal standard, to compare the functional group content of various humic substances (commercial, soil sediment, river and lake). It was demonstrated that the O/C atomic ratio of the humic substances correlated with peak height of the absorption band at 1700 cm^{-1} thereby indicating the importance of the carboxylic acid functional groups in determining the overall O/C ratio of the humic substances. An inverse correlation of the E_4/E_6 ratio (the ratio of UV-visible light absorption at 465 nm to 665 nm) with peak height of the absorption band at 1600 cm^{-1} indicated that the E_4/E_6 ratio is strongly influenced by the aromatic C content of the humic substances.

A summary of FTIR absorptions by humic samples from the literature and the functional groups assigned to these absorptions is presented in Table 8.55 of the Appendix.

1.14 SUMMARY

Actinide elements have been released into the environment by the use of nuclear weapons and weapons testing, nuclear accidents and by the authorised discharges of wastes from the nuclear fuel cycle. Humic substances are the major component of soil organic matter, and though they constitute a small proportion of the soil as a whole, the properties of humic substances are such that they often dominate pollutant (including actinide), as well as nutrient, binding in soil. There is still much debate as to the formation, structure and size of, and genetic relationship between, fractions of soil humic substances. Further insights into the nature of humic substances are expected to follow advances in isolation, separation and characterisation techniques. Further information will be invaluable in assessing the potential mobility of actinide-humic complexes in the environment, e.g. in assessing how diagenetic alteration will

affect the stability of the complexes, the mean residence time of actinide-humic complexes immobilised in soil, and the fate of complexes transported to receiving natural waters as hydrophilic discrete complexes or humic-mineral aggregates (fine particles or colloids) or humic-humic colloids. In forest soils the major vegetative input to the soil is the tree leaves. It is possible that the differences between the composition of the refractory lignin constituent of deciduous broadleaf and coniferous forest vegetation may provide different humic substance ligands for the binding of actinides. Thus in this study the behaviour of actinides towards the humic substances of a deciduous broadleaf and a coniferous forest soil was contrasted. With the progression of humification there is an increase in O containing functional groups which have the ability to bind actinides, whereas diagenetic alteration results in the gradual loss of these groups. The average age of humic substances increases with increasing soil depth and thus the influence of diagenetic alteration on actinide binding may be observed as a function of soil depth. Humic substances which are immobilised in the soil profile have residence times of the order of several hundred to a couple of thousand years, whilst the amount of humic substances leached from the soil profile will be determined by the efficiency of soil mineralisation and sorption (immobilisation) processes. The actinide elements U, Np and Pu have a more diverse environmental behaviour than other members of the actinide series because of the greater number of oxidation states available to these elements. For this reason, and also because of the high radiotoxicity of the actinides, the behaviour of U and Pu in two soil profiles was investigated to provide further insight into the speciation and potential mobility of these complex elements in the environment. It has been shown that actinides interact preferentially with fractions of the total soil humic substances. Thus methodology is required to isolate components of the total mixture so that the optimum humic ligands can be identified, allowing assessment of the potential mobility of bound actinides in the environment. It has been highlighted that procedures for the isolation of humic substances must provide near-native state humic molecules so that actinide binding to these ligands is representative of the environmental behaviour of actinides. Equally importantly, the conditions under which the actinides are added to the soil, or soil humic substances, must be relevant to environmental conditions. Thus in this study electrophoresis was highlighted as a

'soft' method for the isolation of near-native state fractions of humic substances in buffered pH conditions, whilst actinides were added to whole soil samples in a solution buffered at the approximate pH of the soil solutions. The interaction of added ^{236}U with the soil was compared to that of naturally occurring ^{238}U to assess this new methodology.

1.15 THESIS LAYOUT

The thesis is presented in order of experimental methods, results, discussion and finally conclusions. The experimental methods chapter is succeeded by a method development chapter. This latter chapter documents the development of the novel experiments of the electrophoretic humic substances isolation procedure and the addition and 'equilibration' of artificial actinides (^{236}U, ^{242}Pu) with whole soil samples.

2 EXPERIMENTAL METHODS

2.1 DECIDUOUS BROADLEAF AND CONIFEROUS FOREST SOIL SAMPLING

The importance of composition of humic substances with respect to actinide binding and potential mobility was of prime importance in this study. Resistant organic components derived from lignin will make a significant contribution to humic substances formed in a soil environment. The structure of lignin of deciduous broadleaf trees leaves differs from that of coniferous trees pine needles in the subunit aromatic substitutions (Section 1.6.4). Thus deciduous broadleaf and coniferous forest soils were examined in this work. Soil humic substances are subject to diagenetic alteration with time from formation. The result of alteration is the loss of functional groups. This has implications for the actinide binding capability of the humic material and so vertical variations (analogous to age and degree of alteration of the humic substances) in humic composition and actinide binding were also investigated.

2.1.1 Site location and description

Sampling sites at Grizedale Forest, Cumbria, N. W. England, were selected beneath adjacent deciduous broadleaf and coniferous tree canopies of similar age on the same face of a moderate slope (Figure 2.1). The tree species of the deciduous broadleaf forest stand were European beech (*Fagus sylvatica*) and European birch (*Betula pendula*) planted in 1950 amongst an existing stand of English oak (*Quercus robur*), planted in 1870. The coniferous forest stand tree species, Sitka spruce (*Picea sitchensis*), was planted in 1950. The grid references of the deciduous broadleaf and coniferous soil sites are given in Table 2.1. The coniferous and deciduous broadleaf stands were separated by a track; the coniferous stand located uphill of the deciduous broadleaf stand.

Forest Soil Stand	Grid Reference (m)
Deciduous broadleaf (Beech/Birch/Oak)	334,850 , 492,180
Coniferous (Sitka Spruce)	334,670 , 492,130

Table 2.1: Full coordinate grid references of the deciduous broadleaf and coniferous forest soil sampling sites in the SD 100 000 m British National Grid square

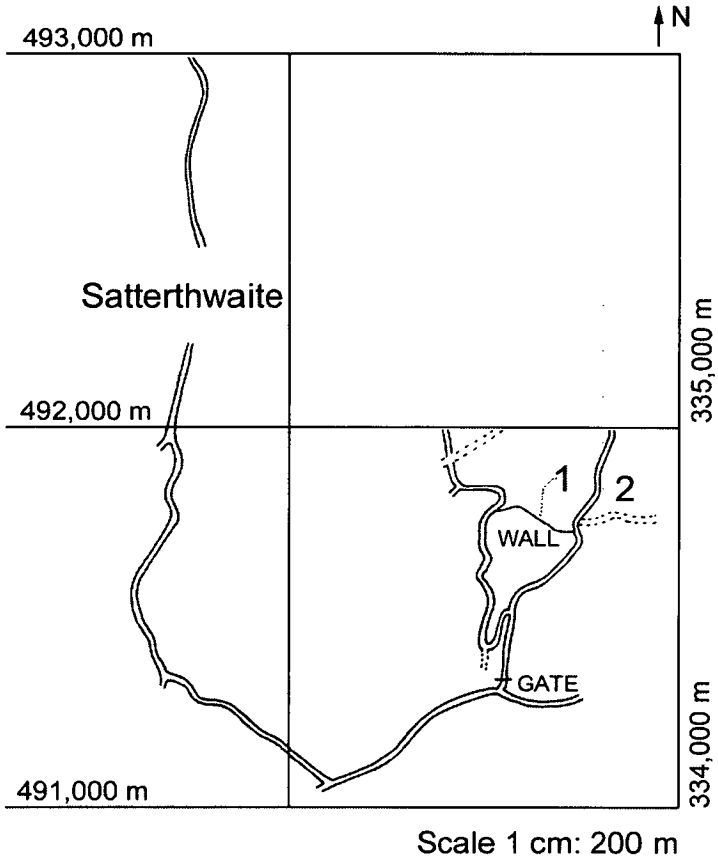


Figure 2.1: Map of Grizedale Forest sampling sites. **1** – the coniferous soil site, **2** – the deciduous broadleaf soil site. Adapted from Grizedale 2 Forest Enterprise map (map details: Section 8.1.1).

The specific location (Figure 2.1, site **1**) of sampling at the deciduous broadleaf soil site was on a level area. This area was chosen for sampling because it was probable that the underlying soil layers were more horizontally uniform to the surface than would be found in a sloping area. This attribute would facilitate depth sectioning of the sample into layers relative to soil horizons. There were no oak trees in the immediate vicinity of the sample location (the majority of trees in the stand were beech) though oak leaves were visible in the litter layer. Thinning of the trees in the area was evident from tree stumps. There was no underlying flora besides the litter: the canopy cover was too dense to allow the growth of bracken or grass observed in other parts of Grizedale Forest.

The specific location (Figure 2.1, site **2**) of sampling at the coniferous site was on a terrace part way up the slope. At the edge of the terrace this stand dropped away

sharply toward the main track below. As well as providing a sample amenable to the proposed horizontal sectioning, this site afforded a sample of sufficient depth for the study. The accumulation of soil and litter was greater on the terrace than the shallow soil developed on the rocky slope that comprised most of this stand. Again thinning of the trees was apparent by tree stumps. Fungal fruiting bodies were observed on the tree stumps and other wood debris in the area. There was some moss growing amongst the litter, and some grass in a more exposed area leading toward the terrace, otherwise there was no underlying flora.

2.1.2 Underlying geology of the study samples – the Windermere Group

The soils of Grizedale Forest, Cumbria, N. W. England, which were sampled for this study, reside on the Windermere Group of sedimentary rocks. Along with the Skiddaw Group (sedimentary rock, northern and western Lake District) and Borrowdale Volcanic Group (central Lake District) these rock groups comprise the major groups of the Lake District, eroded remnants of the Caledonian mountain range (Moseley, 1978; Smith, 1992). These groups were formed in the Ordovician (510-450 Ma) and Silurian (450-405 Ma) geological periods of the Lower Palaeozoic era (600-405 Ma) (Figure 2.2). The American and European tectonic plates are believed to have converged with the result of uplift and erosion of the Skiddaw Group and Borrowdale Volcanic Group rocks. The lowest sedimentary layer of the Windermere Group (Dent Subgroup) was deposited on the volcanic rocks. This was followed by continuous sedimentary deposition throughout the Silurian period of the Windermere Group rocks (Smith, 1992).

The rock groups underlying the sampling sites were the Silurian Bannisdale Slate and Coniston Grit rocks of the Windermere Group (Moseley, 1978; Smith, 1992). The Coniston Grits are sandstones and mudstones while the Bannisdale Slates are mudstone and siltstone rocks (Moseley, 1990; Moseley, 1978).

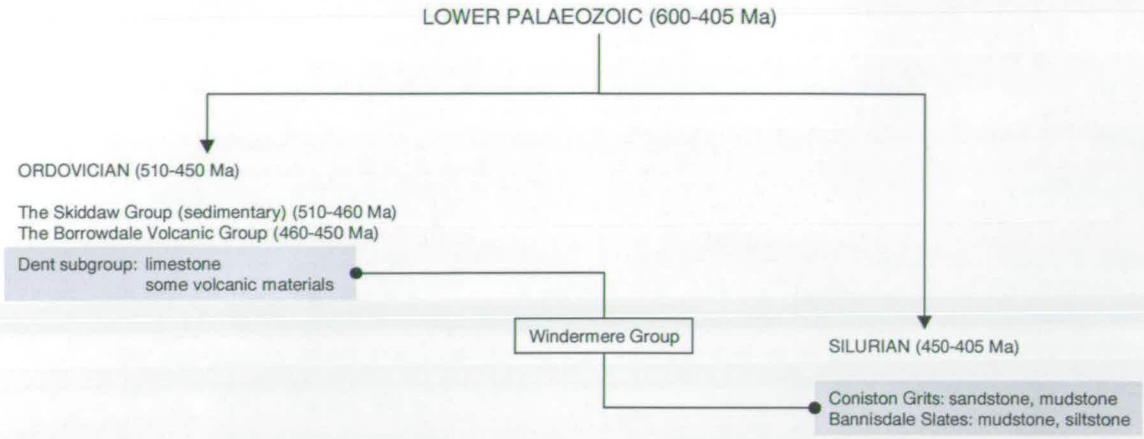


Figure 2.2: Geologic timescale of the formation of the major rock groups in the Lake District. Adapted from: Smith, 1992.

2.1.3 Excavation of forest soil sample blocks

Stainless steel pins were pushed into the ground to mark the corners of squares (approximately 30 cm × 30 cm). Trenches were dug around the squares to enable excavation of soil sample blocks (Figure 2.3).

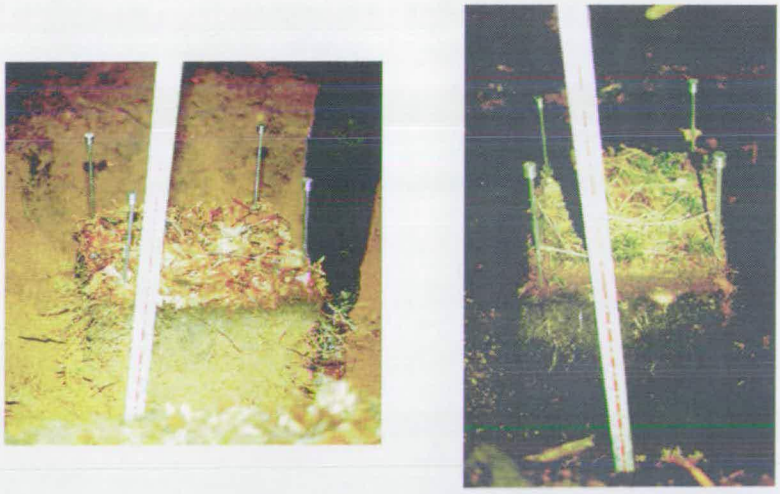


Figure 2.3: Excavation of deciduous broadleaf (left) and coniferous (right) sample soil blocks. The blocks were approximately 30 cm × 30 cm square.

Two blocks were excavated from both the deciduous broadleaf canopy site (labelled B1D and B2D – i.e. deciduous broadleaf soil blocks 1 and 2) and the coniferous canopy site (labelled B1C and B2C – i.e. coniferous soil blocks 1 and 2). The depth of the blocks was restricted by the emergence of large rocks/debris at approximately 25 cm depth and 30 cm depth for the deciduous broadleaf and coniferous canopy

sites, respectively. The base of the deciduous broadleaf soil solum was characterised by large, light grey rocks and debris whereas the coniferous soil solum base was a white solid, possibly a carbonate cementation, which was wet and crumbly on the surface. The blocks were lifted onto large plastic bags, placed in trays and then secured by wrapping in further plastic bags. On return to the laboratory, the samples were stored in the dark in a cold room at 4 °C. The soil at the sample sites, as classified by the Forestry Commission, is a Brown Earth. A mor forest floor was developed on the original Brown Earth at the coniferous stand site. The gradation from A- to B-horizons was not visibly apparent in the coniferous soil.

2.2 PREPARATION OF EXCAVATED DECIDUOUS BROADLEAF AND CONIFEROUS FOREST SOIL BLOCKS INTO SOIL PROFILE DEPTH SECTION SAMPLES

The outer five centimetres of each side of the blocks, likely to have been disturbed by sampling, was cut away using a scalpel/knife and discarded. The trimmed blocks (Figure 2.4) were laid on their sides on top of a plastic-covered piece of card marked with centimetre line divisions. The top of the blocks was aligned parallel to one of the marked lines. One centimetre depth intervals from the top of the upper side of the block were marked with incisions made by scalpel or by using cocktail sticks as markers. A ruler was then used to join the centimetre depth marks on the upper and under sides of the blocks, and centimetre depth sections horizontal to the soil surface were cut using a scalpel/knife (Figure 2.5).

Figure 2.4: Trimmed deciduous broadleaf block 1(B1D) (left) and trimmed coniferous block 2 (B2C) (right). The blocks were approximately 20 cm × 20 cm square after trimming.

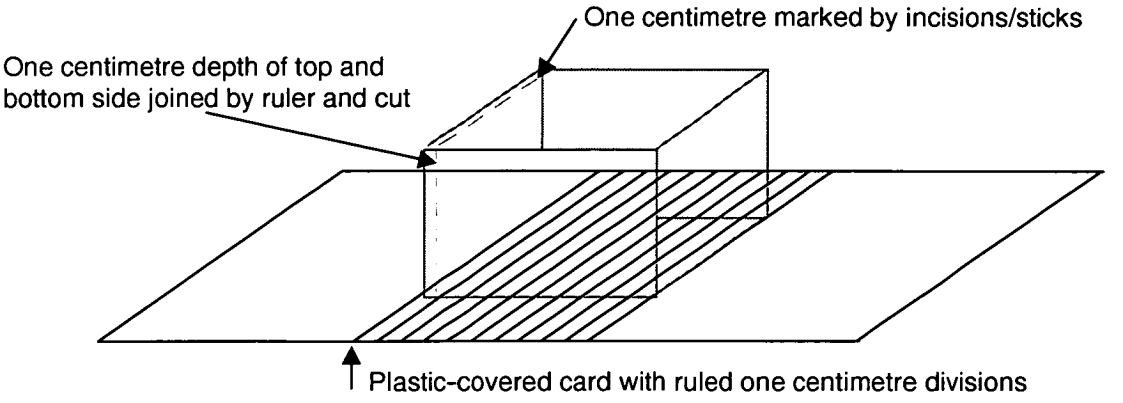


Figure 2.5: Depiction of soil block sectioning method

The general observations of soil horizon colour and thickness for the soil block samples were as follows. Approximately the first three centimetres of the deciduous broadleaf stand mull consisted of broadleaf litter and some twigs. The subsequent three centimetres was dark-brown soil, the next centimetre was brown soil, and the soil from six centimetres beneath the litter surface and down to and amongst the rock and debris at the base of the soil was a sandy orange-brown colour. The layers of the deciduous broadleaf soil samples were by no means uniform, for example, the dark-brown layer contained some, if only a little, of the brown and sand-coloured soil. The uppermost centimetre of the litter of the coniferous soil sample consisted of moss, twigs and pine needles. The next two centimetres of litter were compacted pine needles followed by a highly degraded brown-coloured litter layer of approximately three centimetres depth. The soil beneath this mor layer was dark-brown to black in colour, from six centimetres beneath the litter surface of the sample and down to the white solid base. Some charcoal was also observed amongst the soil, in agreement with the site being close to the old bridleway and an area of remnant charcoal pitsteads (Gregory, *pers. com.*, 1998). This generalised visual description of the deciduous broadleaf and coniferous soil samples is summarised in Table 2.2.

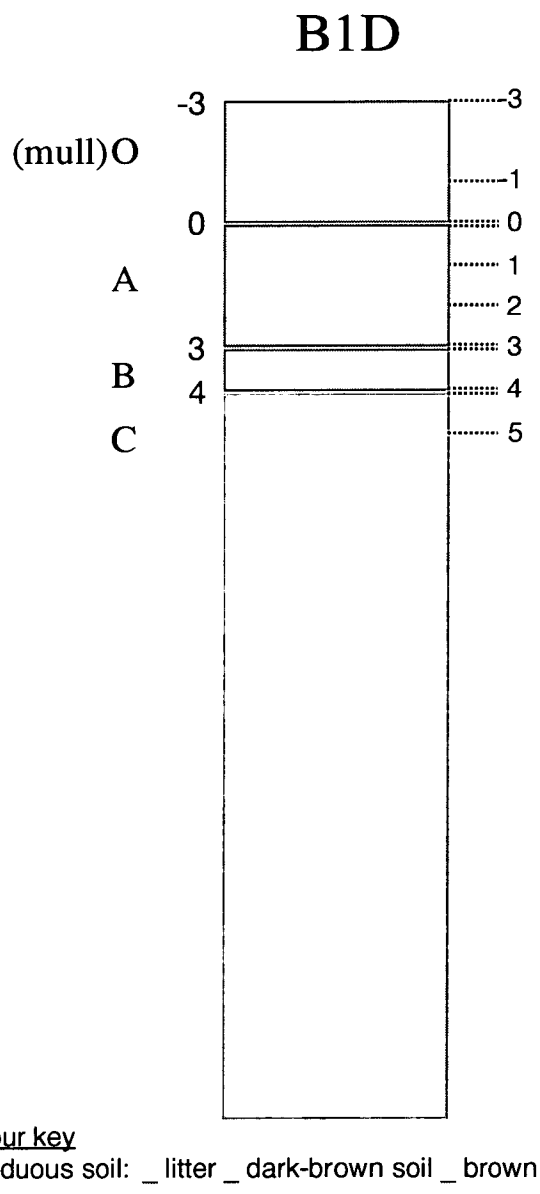
A note was made of the soil flora observed during the soil sample sectioning. Some white mould was observed on the broadleaf litter of the deciduous broadleaf soil samples. Amongst the deciduous broadleaf leaf litter, white fungal hyphae were also

seen. In the coniferous soil samples, in addition to the fungal fruiting bodies observed above ground (Section 2.1.1), white fungal hyphae were observed on the fine root in the leaf litter. Bright yellow fungal biomass was also observed on the root in the litter. There was also evidence of the presence of brown rot fungi, from the observation of buried and decaying branches/roots which were orange-brown in colour and sawdust-like in texture.

Deciduous Broadleaf Soil Sample		**Coniferous Soil Sample**	
Depth from surface of litter layer (cm)	*Generalised visual description of layer*	*Depth from surface of litter layer (cm)*	*Generalised visual description of layer*
0-3	Broadleaf, mostly beech, and twigs	0-1	Moss, twigs and pine needles
3-6	Dark-brown soil	1-3	Compacted pine needles
5-6	Brown soil	3-6	Brown, highly degraded litter
6-25	Sandy orange-brown soil	6-30	Dark-brown to black soil. Some evidence of charcoal
25	Large light-grey rock and debris	30	White solid, wet and crumbly on the surface

Table 2.2: A generalised description of the observed layers of the deciduous broadleaf and coniferous soil samples

In sectioning the two deciduous broadleaf soil blocks, five 1 cm depth samples of each block were obtained. Eighteen and fourteen approximately 1 cm depth samples were obtained from sectioning of the coniferous soil blocks B1C and B2C, respectively. These section samples are also shown schematically in Figure 2.6 and Figure 2.7 for the deciduous broadleaf and coniferous soil samples, respectively. Greater detail of the section samples is given in Section 8.1.2. Soil depth was conventionally designated zero centimetres at the interface of the base of the litter and the underlying mineral soil horizons (Armson, 1979). The organic litter layers were therefore assigned negative values to distinguish them from the underlying mineral soil layers.



Colour key
Deciduous soil: _ litter _ dark-brown soil _ brown soil _ sandy orange-brown soil

Figure 2.6: Schematic representation of the layers of the deciduous broadleaf soil block B1D. Depth values and letter labels to the left of the block indicate the positions of the soil layers/horizons while values to the right indicate the sample intervals into which the block was sectioned. Deciduous broadleaf soil block B2D was similarly sectioned, details are given in Section 8.1.2.

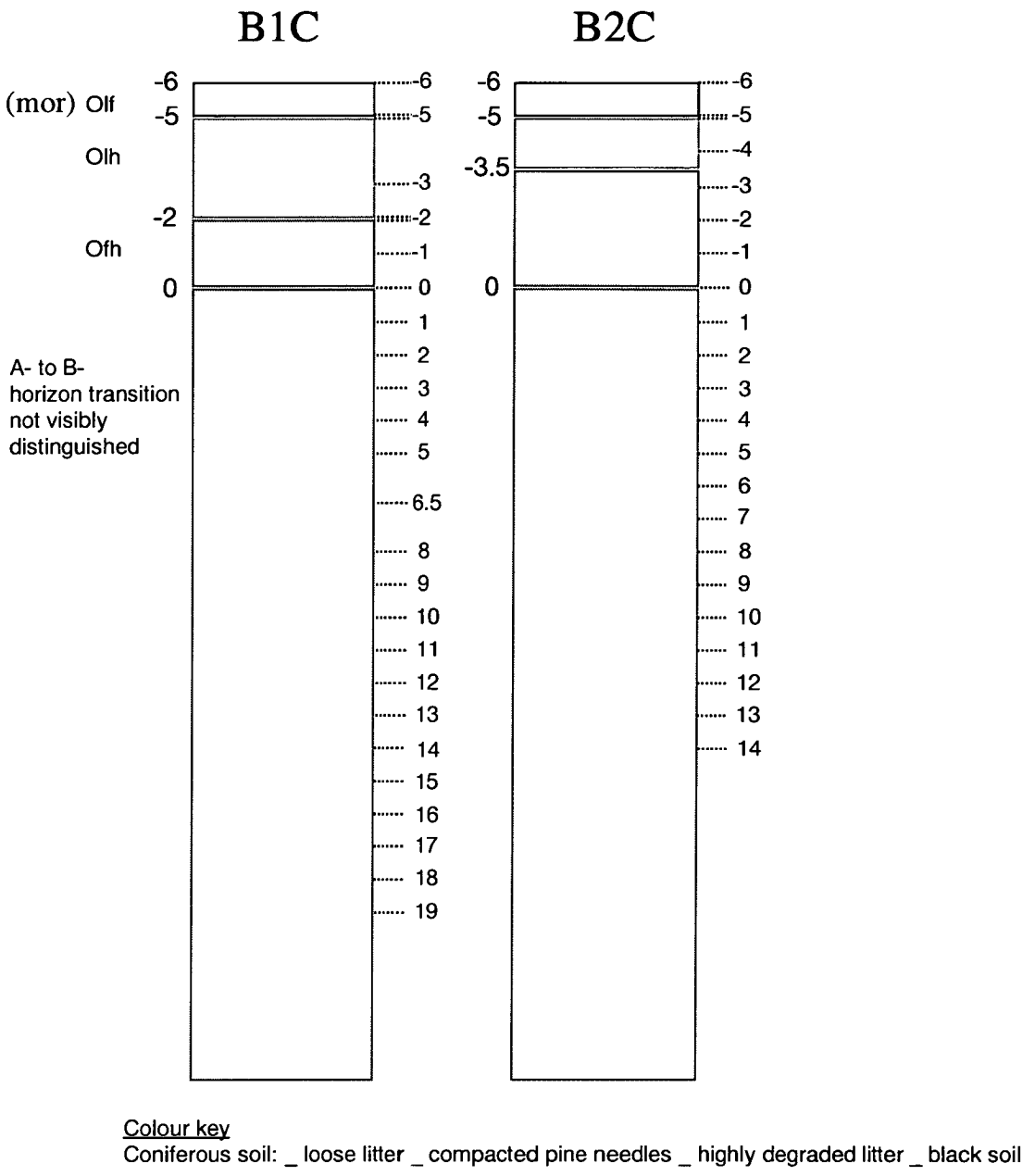


Figure 2.7: Schematic representation of the layers of the coniferous soil blocks B1C and B2C. Depth values to the left of the blocks indicate the positions of the soil layers/horizons while values to the right indicate the sample intervals into which the blocks were sectioned.

The sectioned soil samples were freeze-dried and sieved through consecutive 2, 1 and 0.5 mm sieves to remove stones and root/leaf. Soil clumps were crumbled by hand so that most of the sample passed through the sieves to give a representative sample with particle size nominally less than 0.5 mm. The resulting samples were kept within two layers of sealed plastic bags (air expelled) and stored under cool/dark conditions.

Determination of soil pH was carried out on samples prior to freeze-drying and sieving so that all components (including litter vegetation and roots) of the soil were present for this measurement. For all further experiments the freeze-dried and sieved (< 0.5 mm) soil section samples were used. The soil section samples were labelled according to soil block and average soil depth, i.e. B1D 0.5 was the < 0.5 mm sample obtained from the freeze-dried and sieved 0-1 centimetre soil depth section of deciduous broadleaf soil block 1.

2.3 AN OVERVIEW OF THE EXPERIMENTS AND THE SAMPLES SELECTED FOR STUDY

An overview of the experiments conducted, in relation to whole soil and soil humic extracts, is shown schematically in Figure 2.8. The whole soils were characterised for soil solution pH, loss on ignition, particle size and ^{238}U content prior to in-depth analysis of humic extracts of the soils.

The samples selected for each experiment, and the number of replicates in each experiment, are summarised in Table 2.3. In one experiment only, measurement of soil solution pH, single subsample analysis was conducted on every depth section sample. The soil solution pH was determined for all depth section samples (including the litter horizons) for both deciduous broadleaf (B1D, B2D) and coniferous (B1C, B2C) forest sample blocks. It was considered more appropriate to measure pH of each single subsample of soil depth sections and to compare pH values obtained for the duplicate soil blocks than to measure replicates of single blocks. With one exception, all other experiments were conducted on a selection of five depth section samples of one deciduous broadleaf and one coniferous soil block. The samples selected were deciduous broadleaf soil block B1D – centimetre sections 0.5, 1.5, 2.5, 3.5 and 4.5 cm – and coniferous soil block B2C – centimetre sections

0.5, 3.5, 6.5, 9.5 and 12.5 cm. The deciduous broadleaf soil samples were adjacent one-centimetre depth section samples of the A- and B- and B/C- horizons. The depth of the coniferous soil sequum (the set of A- and B-horizons) was greater than that of the deciduous broadleaf soil (24 cm *cf.* 4 cm). Thus to compare depth changes of the coniferous soil mineral horizons with those of the deciduous broadleaf soil, one-centimetre depth sections, separated by intervals of two centimetres, were selected for study. The electrophoretic extraction of soil, after addition of ^{242}Pu, was conducted on two selected soil depth section samples of the deciduous broadleaf and coniferous soil blocks B1D and B2C.

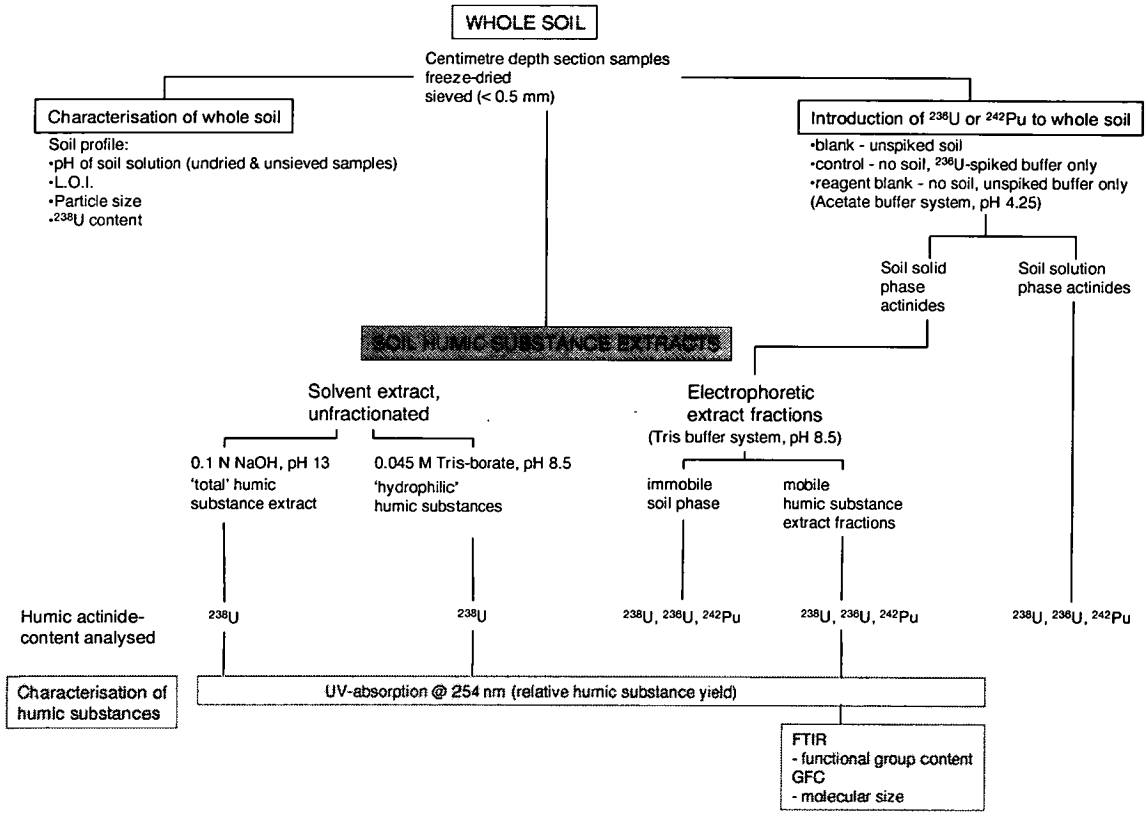


Figure 2.8: Schematic overview of all the experiments, shown in relation to whole soils and soil humic extracts and extract fractions.

Experiment	Soil Blocks		Depth Section Samples	Replicates
	Deciduous broadleaf	Coniferous		
pH	B1D, B2D	B1C, B2C	All	Single
L.O.I.	B1D	B2C	Selected five	Triplicate
Particle Size	"	"	"	Single 2 measurements
^{238}U	"	"	"	Triplicate
Solvent Extraction	"	"	"	Triplicate
Electrophoretic Extraction	"	"	"	Triplicate
Electrophoretic Extraction after addition of ^{236}U	"	"	"	Duplicate
Electrophoretic Extraction after addition of ^{242}Pu	"	"	Two of the selected five	Duplicate

Table 2.3: Overview of the samples, and number of replicates, in each experiment. The five selected deciduous broadleaf soil B1D depth section samples were 0.5, 1.5, 2.5, 3.5 and 4.5 cm. The five selected coniferous soil B2C depth section samples were 0.5, 3.5, 6.5, 9.5 and 12.5 cm. The samples selected for the ^{242}Pu experiment were B1D 0.5 and 2.5 cm and B2C 0.5 and 12.5 cm.

2.4 LABORATORY CHARACTERISATION OF THE DECIDUOUS BROADLEAF AND CONIFEROUS FOREST SOIL DEPTH SECTION SAMPLES

The whole soil depth section samples were characterised in the laboratory for soil solution pH, soil loss on ignition, particle size and ^{238}U content (Figure 2.8 and Table 2.3).

2.4.1 Measurement of forest soil solution pH

Soil solution pH of individual sections of deciduous broadleaf and coniferous soil samples were measured primarily to determine the pH environment in which the humic substances resided. Single subsamples of all soil depth section samples (including the litter horizons) for both soil blocks of each the deciduous broadleaf (B1D, B2D) and coniferous (B1C, B2C) soils were analysed (Figure 2.8 and Table 2.3). The standardised method involved shaking approximately 10 ml of soil with 25 ml 0.01 M $CaCl_2$ for 15 minutes (Rowell, 1996). A pH electrode was placed in the soil/dilute electrolyte solution and a pH reading taken after 30-second equilibration of the electrode in the solution.

Additionally, the pH of samples of the deciduous broadleaf soil block (B2D) were determined in 25 ml deionised water to allow comparison of values obtained in water and dilute salt solution measurements.

2.4.2 Loss on ignition (L.O.I.): approximation of forest soil organic matter content

The approximate organic matter content of the whole soil samples was characterised by loss on ignition. Loss on ignition was determined, in triplicate, for subsamples of the five selected depth section samples of the deciduous broadleaf soil, B1D (0.5, 1.5, 2.5, 3.5 and 4.5 cm) and coniferous soil, B2C (0.5, 3.5, 6.5, 9.5 and 12.5 cm) (Figure 2.8 and Table 2.3).

Soil (0.1 g) was accurately weighed into beakers containing a known amount (0.01 g) of potassium nitrate. In the blank experiment only 0.01 g of potassium nitrate was added to the beakers. The beakers were covered with watchglasses and placed in an ashing furnace (Carbolite Furnace, type ESF 12/23, Sheffield, England). The furnace was programmed (Eurotherm 808P) to heat the samples at a rate of 1 °C/min to a final temperature of 450 °C, which was maintained for 24 hours. Once the beakers had cooled to room temperature the beakers, containing the ash residue, were re-weighed.

The ashed samples were later digested in preparation for the determination of 'pseudo' total soil ^{238}U content. Potassium nitrate was therefore added to all samples prior to ashing with this objective in view (Section 2.4.4).

2.4.3 Forest soil particle size determination: range and distribution of sample particle sizes, and classification of soil texture

Particle size was determined using a laser diffraction instrument (Mastersizer, Malvern Instruments) in order to assign a textural class (Section 1.4.1) to the soil samples. Measurement of single subsamples of the five selected soil depth section samples of the deciduous broadleaf (B1D 0.5, 1.5, 2.5, 3.5 and 4.5 cm) and coniferous (B2C 0.5, 3.5, 6.5, 9.5 and 12.5 cm) forest soils was repeated twice to observe the consistency of the measurement (Figure 2.8 and Table 2.3). The

detection limits of the instrument, using the 300 RF lens to focus the forward-scattered light onto the detector, was 0.05-900 μm. The measurements were made for samples treated, and untreated, for the destruction of soil organic matter. Decomposition of organic matter was necessary for obtaining a sample consisting only of mineral matter, as soil textural class is based on the distribution of mineral particle size (absolute particle size). Particle size measurement of untreated soil was carried out to observe the natural *in situ* size distribution of soil particles (effective particle size). The shift in particle size distribution of absolute and effective soil particle size reflects the influence of organic matter on aggregation of soil mineral particles.

The procedure used to determine effective particle size of the soil subsamples was as follows. A large spatula of soil was added to a 100 ml conical flask. Approximately 20 ml of deionised water was added and the sample was stirred with a magnetic follower. Approximately 1 l of deionised water was added to the instrument sample unit and the pump and stirrer instrument options set to maximum to ensure a representative suspension sample was achieved. The laser was aligned and a background blank, with deionised water in the sample unit, was measured. Aliquots of the stirring soil suspension were pipetted (from sample mid-height) to the sample unit until an obscuration value of about 20 % was attained. This indicated that the sample concentration was amenable to measurement – sufficient sample for measurable intensity of forward-scattered light but not so much sample that light would be principally back-scattered and omitted from the detector. The particle size of the sample was then measured.

The method used for removal of organic matter and subsequent dispersion of the soil mineral matter in solution was based on that of Rowell (1996). Approximately 10 g of soil was weighed to a tared 600 ml beaker. Deionised water (200 ml) was added and watchglass covers placed on the beakers. The beakers were then placed on a hot plate to heat the contents gently. Aliquots (increasing volume of 1, 5 then 10 ml as the reaction became less vigorous) of 30 % w/v hydrogen peroxide were added to the soil solution. Vigorous effervescence/frothing was observed. A total of 125 ml of

30 % w/v hydrogen peroxide was added to each beaker by which time more gentle bubbling, attributed solely to thermal decomposition of hydrogen peroxide, was observed. The soil solution was refluxed for a further hour then allowed to cool. An aliquot (20 ml) of Calgon dispersion solution (Section 8.7) was added and the beaker placed in an ultrasonic bath for 15 minutes to further disperse the soil mineral material. The beaker was removed from the bath and immediately stirred with a magnetic follower, and aliquots of digested soil suspension were pipetted (from sample mid-height) to the sample unit of the particle sizer and particle size measured.

2.4.4 Preparation of forest soil depth sections for determination of 'pseudo-total' soil ^{238}U content

The ash residues of soil samples (Section 2.4.2) were acid digested (Section 2.9.3) to determine 'pseudo-total' ^{238}U contents. A 'pseudo-total' soil ^{238}U concentration was required so that the association of ^{238}U with humic substances could be viewed in the context of the whole soil, i.e. taking account of all competing actinide-binding soil components. The ashed samples were an obvious choice for this measurement as organic matter, detrimental to ICP-MS analysis, had already been eliminated in the ashing process. Potassium nitrate, used to act as a carrier for the ^{238}U in the acid digestion step (Zhang *et al.*, 1997), was incorporated from the initial ashing step. The labelling scheme used for these samples is shown in Table 2.4.

Sample type	Label suffix	Label prefix
Soil ash residues	Sx	Soil block, soil depth section (cm) i.e. B1D 0.5
Soil ash residue blanks	Sbx	

Table 2.4: Labelling scheme for the soil (and blank) ash residue samples. x – replicate number.

2.5 ISOLATION AND FRACTIONATION OF HUMIC SUBSTANCES FROM FOREST SOIL DEPTH SECTION SAMPLES: SOLVENT AND ELECTROPHORETIC EXTRACTION

Subsamples of each of the five selected depth section samples of the deciduous broadleaf (B1D 0.5, 1.5, 2.5, 3.5 and 4.5 cm) and coniferous (B2C 0.5, 3.5, 6.5, 9.5 and 12.5 cm) forest soils were extracted for humic substances (Figure 2.8). Each method, solvent and electrophoretic extraction, was used to extract triplicate subsamples of each soil depth section (Table 2.3). Two different solutions were used in the solvent extraction of humic substances – sodium hydroxide and Tris-borate.

The following sections describe the extraction procedures in detail. The method development, which led to the optimised electrophoretic extraction protocol, is detailed in Chapter 3.

2.5.1 Solvent extraction using aqueous protic solvents: 0.1 N NaOH (pH ~13) and 0.045 M Tris-borate buffer, $(CH_2OH)_3CNH_3^+$:$H_4BO_4^-$ (pH 8.5)

Separate sub-samples (~ 0.1 g; in triplicate) of each soil depth section were accurately weighed into conical flasks and extracted with three 10 ml aliquots of solvent. The solvents were 0.045 M Tris-borate buffer (Section 8.4.4) and 0.1 N NaOH. In each of the three consecutive extractions the soil/solvent mixtures were shaken on an orbital shaker (Luckham Rotatest Shaker, Model R100/TW, speed 4) for a period of 1 hour. The contents of the flask were then poured into a 50 ml centrifuge tube. The sample was then centrifuged (Sanyo Gallenkamp MSE Mistral 2000) at 3,500 rpm (2199 × g) for 5 minutes to separate solution and solid phases. The extract was pipetted to a second centrifuge tube and the centrifuge step repeated; the resulting supernatant was pipetted to a third centrifuge tube. The soil pellets of the first and second centrifuge tubes were transferred back to the conical flask, using the second aliquot of solvent to resuspend them, and the second extraction step commenced. The extract supernatants from the three consecutive solvent aliquot extractions were combined and filtered through 0.2 μm polyethersulphone membranes (Whatman Puradisc 25 AS). The labelling scheme used for these samples is shown in Table 2.5.

Sample type	Label suffix	Label prefix
0.1 N NaOH soil humic extracts	Nx	Soil block, soil depth section (cm) i.e. B1D 0.5
0.1 N NaOH solvent blanks	Nbx	
0.045 M Tris-borate soil humic extracts	TBx	
0.045 M Tris-borate solvent blanks	TBbx	

Table 2.5: Labelling scheme for the soil (and solvent blank) solvent humic extracts. x – replicate number.

2.5.2 The optimised electrophoretic extraction and fractionation protocol

Electrophoretic extraction of the five selected (Figure 2.8 and Table 2.3) depth section samples of both the deciduous broadleaf (B1D) and coniferous (B2C) forest soils was conducted according to the protocol described below.

2.5.2.1 The apparatus

The electrophoresis equipment comprised a horizontal tank (Maxi Gel Tank and Tray, Kramel Biotech, Northumberland) and a power pack (BIO RAD PowerPac 3000). The distance between the electrodes in the tank was 35.5 cm and the width of the tank (length of the electrodes) was 20 cm. The gel casting tray dimensions were 20 cm (width) by 20.5 cm (length) with well positions at 0.7-1.0 cm and 10.7-11.0 cm along the length of the tray. Customised (single tooth) Perspex combs (18.8 cm × 1 cm × 0.3 cm) were produced in the Mechanical Workshop, Department of Chemistry, University of Edinburgh.

2.5.2.2 Preparation of 1% w/w agarose gel

A 1% w/w agarose gel (pore size 150 nm: Serwer (1980)) was prepared by addition of 200 g of 0.045 M Tris-borate buffer (pH 8.5) to an accurately weighed amount of agarose (~ 2 g). The gel was formed by heating this solution gently, and with occasional agitation, using a Bunsen flame. The steps of accurately weighing the agarose and weighing of the buffer solution were undertaken to ensure uniformity between gel experiments. The gel was poured into a casting tray containing two combs (at the front cathode-end and middle of the tray; to provide moulds for the soil wells) and left to set at room temperature for approximately 30 minutes. The depth of the gel was approximately 0.5 cm. The casting tray and set gel was then placed in the electrophoresis tank. An aliquot (1400.0 g) of 0.045 M Tris-borate, pH 8.5, was added between the electrode compartments of the tank. The level of this mass of running buffer reached, but did not flow over, the surface of the gel.

2.5.2.3 'Equilibration' of whole soil in an acetate/acetic acid buffer

Soil (0.1 g) was accurately weighed to a silylised (Section 2.9.1) 100 ml glass beaker. A 25 ml aliquot of buffer (blank or with added artificial actinides ^{236}U or ^{242}Pu, Section 2.8) was added to the beaker using a bulb pipette. The soil solution was stirred overnight for 14 hours using a magnetic follower.

The 'equilibrated' soil solution was poured into a 50 ml centrifuge tube. The beaker was rinsed with deionised water and the washings added to the tube. The sample was centrifuged at 3,500 rpm (2,199 × g) for 5 minutes to separate solution and solid phases. The supernatant was transferred by pipette into a plastic 125 ml reagent bottle. The soil pellet was then re-suspended in about 10 ml deionised water and re-centrifuged as above. This rinse step was repeated a second time. The rinse supernatants were combined with the initial buffer supernatant. This buffer solution was required for mass balance of the ^{238}U. In preparation for ICP-MS analysis of ^{238}U the soil buffer solution was syringe-filtered through a 0.2 μm polyethersulphone membrane (Whatman Puradisc 25 AS) to separate any solid material disturbed and transferred by pipette.

Removal of the solution phase was required so that complete exchange of the 'equilibration' (acetate/acetic acid) buffer and the electrophoresis loading buffer (Tris-borate) was achieved, otherwise the pH of the soil/loading buffer suspension would have been lower than desired. The rinse steps were included to ensure removal of solution phase actinides.

2.5.2.4 Loading of the soil solid phase sample into the electrophoresis well

The soil pellet was re-suspended in 2 ml loading buffer (0.05 M Tris-HCl, pH 8.5) (Section 8.4.5) and loaded in the well of a 1 % agarose gel. Electrophoretic extraction at a fixed current (10 mA) for a total of 3 hours was commenced. The electrophoretic extraction conditions are summarised in Table 2.6. A schematic diagram of the electrophoretic experiment is shown in Figure 2.9 below.

Soil	0.1 g per well
Agarose gel	1 % w/w (agarose/0.045 M Tris-borate, pH 8.5)
Loading buffer	2 ml per well 0.05 M Tris-HCl, pH 8.5
Running buffer	1400.0 g 0.045 M Tris-HCl, pH 8.5
Fixed current (mA)	10
Typical voltage (V)	62-80
Duration of extraction (min)	180

Table 2.6: Electrophoretic extraction conditions

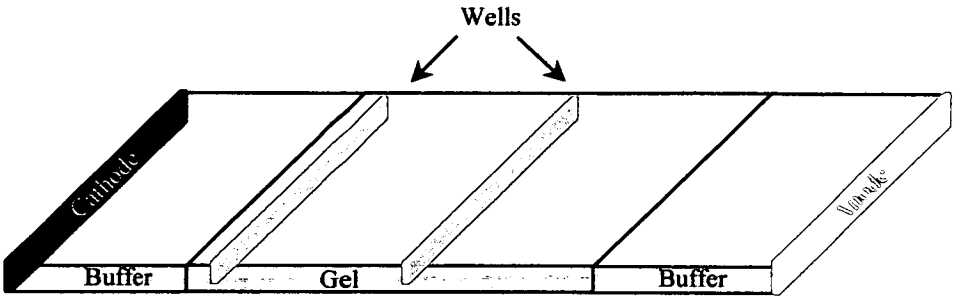


Figure 2.9: Schematic representation of the electrophoretic extraction experiment.

2.5.2.5 Sampling of the electrode compartment buffer

At the end of extraction aliquots of buffer were sampled from the cathode and anode buffer compartments of the tank. Triplicate 75 ml aliquots of cathode and anode end buffer were analysed by ICP-MS for actinide content. The pH of aliquots of the electrode compartment buffer was measured to see if any discernible pH change had occurred during the electrophoretic experiment – no change was found.

2.5.2.6 Excision of the electrophoretic extract fractions

The casting tray on which the gel was supported was removed from the tank. The gel itself was carefully lifted from the tray and laid on a piece of Benchcote™ on a casting tray platform. The sides of the platform were adjusted to hold the gel in place as it was cut with the gel cutter. A customised gel cutter had been designed to cut the gel into strips of fractionated extract. The gel cutter (Figure 2.10) was made from two 3 mm threaded stainless steel rods, with washers and nuts separating razor blades (excepting the first pair) at 1 cm distances along the rods. The first pair of razor blades was separated such that they would cut out the width of the well. Each gel was thus cut into a soil well residue and ten approximately 1 cm gel extract fractions. The gel extract fractions were labelled F1 to F10 in the direction of mobility from cathode to anode, i.e. F10 was the most mobile anionic extract fraction.

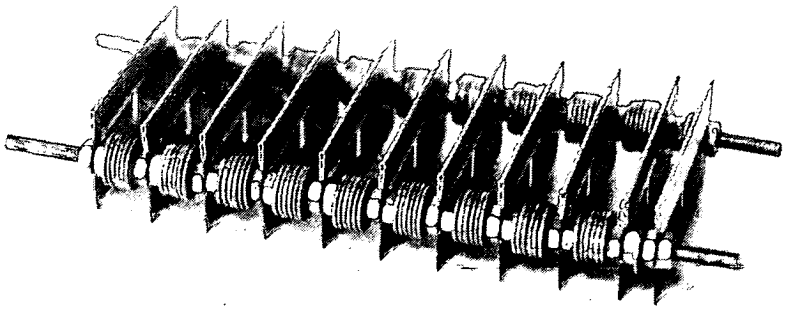


Figure 2.10: Photo of gel cutter

2.6 PREPARATION OF SOLVENT AND ELECTROPHORETIC HUMIC SUBSTANCE EXTRACT FOR CHARACTERISATION

The solvent humic extracts of soil were prepared for spectrophotometric analysis (absorption at 254 nm) to determine yield. The electrophoretic humic extract fractions of soil were eluted from the agarose gel and similarly analysed for yield. Selected electrophoretic humic extract fractions were purified for further structural analyses: GFC and FTIR.

2.6.1 Preparation of solvent humic extracts

The solvent extracts of all five depth section samples of both the deciduous broadleaf (B1D) and coniferous (B2C) forest soils were prepared for humic yield analysis. The extract solutions were made up to a known volume (deciduous broadleaf soil extract volumes of 50 ml) or a known mass (coniferous soil extract masses of 75 g) with deionised water. The reason for the difference in method was that the extraction of deciduous broadleaf and coniferous soil samples was conducted on different occasions. The volume of the deciduous broadleaf soil solvent extracts was determined using plastic disposable pipettes, a choice which reflected the large number of samples involved and the necessity for equipment to be free of contaminating trace metals. The technical experience gained by the later date at which the coniferous soil samples were extracted suggested that the method of making the samples up to a known mass was a simpler, more economical and more precise method.

2.6.2 Preparation of electrophoretic humic extract fractions

The ten electrophoretic extract fractions of the two selected soil depth section samples of the deciduous broadleaf (B1D) and coniferous (B2C) forest soil blocks were freeze-thaw eluted from the agarose gel. The samples chosen were deciduous broadleaf forest soil samples B1D 0.5 and 2.5 cm and coniferous soil B2C 0.5 and 12.5 cm. The eluted extract fractions were analysed using UV-visible spectrophotometry to determine the relative total extract, and individual extract fraction, humic substance yields. The brown and UV-fluorescent bands (Sections 3.1.1 and 3.1.4) of these two depth section samples of each the deciduous broadleaf (B1D) and coniferous (B2C) forest soil extracts were selected for purification prior to further investigation of the structure of the extract humic substances. The details of the samples selected are given in Table 2.7 (Section 2.6.2.2).

2.6.2.1 Freeze-thaw elution of humic substances from the agarose gel

Each of the ten gel fractions was cut into pieces with a razor blade. The pieces were wrapped in clingfilm, placed within plastic bags, and frozen overnight. The samples were then placed in 50 ml centrifuge tubes and allowed to thaw at room temperature for approximately 2 hours. Once thawed the solutions were decanted to a second set of 50 ml centrifuge tubes. The residual gel pieces were re-frozen and thawed. The freeze-thaw procedure was conducted a total of three times. Final elution was achieved by a centrifuge step. The gel pieces were placed in 30 ml plastic disposable tubes which had conical skirted bases. Holes made at the bottom of the tubes with a needle were covered with silylised (Section 2.9.1) glass wool. The tubes were placed within 50 ml centrifuge tubes and the residual gel fraction pieces were thus centrifuged (Sanyo Gallenkamp MSE Mistral 2000 centrifuge) at 3,500 rpm (2,198 × g) for 5 minutes. The skirting of the 30 ml inner tubes acted to support the inner tube above the base of the centrifuge tube, and thus allowed solution to flow easily into the centrifuge tube. The eluted solutions were decanted and added to the previously freeze-thaw eluted solutions. The centrifuge elution step was conducted a total of three times. The eluted extract fraction solutions were filtered through 0.2 μm polyethersulphone membranes (Whatman Puradisc 25 AS) to tared 50 ml centrifuge tubes and made up to a known mass (~ 40 g) with deionised water.

2.6.2.2 Purification of the electrophoretic humic extract fractions using ultrafiltration and GFC

The forest soil electrophoretic extract fractions selected for purification and further characterisation are detailed in Table 2.7. It was necessary to purify the samples from Tris-borate buffer, as this organic basic buffer would otherwise interfere with the structural analyses, in particular the FTIR analysis.

Soil block sample	Depth section sample (cm)	Electrophoretic extract band	Electrophoretic fraction label	Characterisation
B1D	0.5, 2.5	Brown	F4	FTIR
"	"	UV-fluorescent	F6	FTIR
B2C	0.5, 12.5	Brown	F3	GFC, FTIR
"	"	UV-fluorescent	F6	FTIR

Table 2.7: Forest soil electrophoretic extract fraction samples selected for purification and structural characterisation

Approximately 15 g of each eluted extract fraction solution was weighed into the sample receiver of an ultrafilter (Omega Macrosep 1K Centrifugal Ultrafilters). The samples were centrifuged at maximum speed (3,632 × g) for 3 hours. The filtrates were decanted, and pre-weighed concentrate cups were attached to the filter units. The retentate was transferred from the sample receiver to the concentrate cup by centrifuging at maximum speed for 3 minutes. A further 15 g of each eluted extract fraction solution was weighed to the ultrafilter units and ultrafiltration repeated. A longer filtration time was required for the re-use of the filters, typically 4.5 hours, to obtain the same retentate volume (approximately 1.5 ml). The concentrate cups containing the sample retentates were frozen overnight, then freeze-dried for 24 hours.

Some white salt (Tris-borate) was observed in the freeze-dried extract fractions. A GFC (desalting) column containing Sephadex G-10 gel (Pharmacia Biotech) was designed to further purify the samples. This method was more appropriate than further ultrafiltration, because a shorter purification time could be achieved using GFC with the small sample volumes that had been produced by the initial ultrafiltration step.

Figure 2.11: Sephadex G-10 desalting column.

A column (Figure 2.11) was made from a 50 ml plastic syringe barrel (cut down to the 32 ml line). Glass fibre filters (Whatman GF/A; 42.5 mm) were cut to size to fit the top and bottom of the gel bed. The lower filter was used to prevent elution of gel and the upper filter to prevent disruption of the gel bed on application of eluant or sample solution. A cut-down pipette tip was added to the bottom of the column to support the eluent tube. Sephadex G-10 gel, prepared in deionised water, was poured into the column as an approximately 75 % gel/water suspension. Gel was added until a settled volume of 27-28 ml of gel was obtained. The seal to the top of the column was made from a rubber bung. Deionised water eluant was pumped through the column (Pharmacia Biotech Peristaltic pump; pump setting 3.6×10) until the gel bed was packed to the 25 ml column volume line.

The exclusion and total column volumes of the designed column were characterised by separate elution and analysis of Blue dextran and acetone, respectively. An aliquot (1 ml) of a 0.2 mg ml^{-1} solution of Blue dextran in deionised water was applied to the column and eluted. Fractions (1 ml) of eluent were collected using a fraction collector (FRAC 100, Pharmacia Biotech) and Blue dextran detected spectrophotometrically by absorption at 260 nm. An aliquot (1 ml) of a 0.2 % v/v acetone/deionised water solution was applied to the column and eluted. Fractions

(1 ml) of eluent were collected and acetone detected spectrophotometrically by absorption at 266 nm.

Initially a volatile buffer, 0.04 M ammonia/ammonium acetate (pH 8.4) (Section 8.4.7), was investigated, as well as deionised water, for use as eluant. The hypothesis was that the buffer would volatilise in the next step of purification, i.e. freeze-drying. However, residual solid of the buffer was observed in the freeze-dried fractions of eluent in which no sample had been added. Therefore, deionised water was selected as eluant.

The ultrafiltered, freeze-dried electrophoretic extract fractions were re-dissolved in 1 ml deionised water, applied to the desalting column and eluted in deionised water. Eluted fractions (1 ml) were collected. Deionised water was pumped through the column between sample applications for a minimum of 75 minutes to elute the buffer and sample salts. The eluent fractions containing the purified extract (elution volume 8-12 ml following sample application) were combined, frozen overnight then freeze-dried for 24 hours.

2.7 CHARACTERISATION OF THE SOLVENT AND ELECTROPHORETIC HUMIC EXTRACTS

The relative humic substance yield of the solvent and electrophoretic extracts of the forest soil samples was determined using spectrophotometry (absorption at 254 nm). Selected electrophoretic extract fractions (Table 2.7) were further structurally characterised using Fourier Transform Infra-red (FTIR) spectroscopy and gel filtration chromatography (GFC).

2.7.1 UV-visible spectrophotometry: relative yield of the solvent and electrophoretic humic extracts

The absorption of UV-light by forest soil solvent extracts (0.1 N NaOH and 0.045 M Tris-borate) and eluted electrophoretic extract fractions (individual fractions F1 to F10) was measured at 254 nm. The samples were diluted, where necessary, to obtain absorbance values between 0.2 and 1.0 a.u. (a.u. - absorbance units). The solvents were used for background blank absorbance.

2.7.2 FTIR spectroscopy: functional groups of the electrophoretic humic extract fractions

The selected, purified electrophoretic extract fractions (brown and UV-fluorescent bands), dissolved in 0.1 M sodium perchlorate, were spotted onto IR cards (Polyethylene substrate, Qualitative Mid-IR, Type 61 Disposable IR Cards, 3M) using a pipette. Nine applications of each fraction were dried onto the cards, except for sample B2C 0.5 F3, of which six applications were dried on an IR card only because of the greater concentration of this extract. The Infra-red spectra of the samples were recorded on a Perkin Elmer Paragon 1000 FTIR spectrometer. The samples were scanned four times with a resolution of 4.0 cm^{-1}. The recorded spectra were smoothed to a resolution of 32 cm^{-1}.

2.7.3 GFC: molecular size of the electrophoretic humic extract fractions

Only the purified electrophoretic extract fraction F3 (brown band) of the coniferous forest soil sample B2C 0.5 cm was intense enough in colour for its elution on a gel column to be followed visually. The gel filtration of this sample was compared to that of electrophoretic extract brown, and UV-fluorescent band fractions of an organic-rich soil sample (Graham *et al.*, *in press*).

Columns were prepared from 2 ml glass Pasteur pipettes (Figure 2.12). Sephadex G-200 gel (Pharmacia Biotech) was prepared in deionised water. Once the gel was hydrated the water was exchanged for ammonium acetate/acetic acid/0.1 M sodium perchlorate buffer (pH 4.2) (Section 8.4.3). This was achieved by repeated additions of buffer, gentle swirling, and decanting of the solution from the gel. Silylised (Section 2.9.1) glass wool was placed at the bottom of the pipettes. The gel was then poured into the column and packed by addition and elution of buffer through the column under gravity. A glass fibre filter (Whatman GF/A; 42.5 mm), cut to size, was added to the top of the gel bed. An aliquot (20 µl) of the purified electrophoretic extract gel fractions, dissolved in 0.1 M sodium perchlorate, was applied to the column. Eluent was collected in a tared plastic tube from the time of sample application until the sample was visually observed to elute from the column. The tube was then re-weighed. The experiment was performed in duplicate. The

exclusion volume, characterised by elution of a Blue dextran solution, was similarly determined by the mass of eluent collected before elution of the Blue dextran.

Figure 2.12: Sephadex G-200 gel chromatography column.

2.8 'EQUILIBRATION' OF ADDED ARTIFICIAL ACTINIDES (^{236}U OR ^{242}PU) WITH WHOLE FOREST SOIL SAMPLES

The selected whole soil depth section samples (0.1 g) (Table 2.7) were 'equilibrated' with spiked (^{236}U or ^{242}Pu) acetate/acetic acid buffer for 14 hours. The soil solution phase was then separated from the soil solid phase and the spiked soil pellet electrophoretically extracted according to the previously described method (Section 2.5.2). One sample, coniferous forest soil sample B2C 0.5 cm, was selected for investigation of the effect of increased 'equilibration' time. Separate subsamples of this soil were stirred in both blank and ^{236}U-spiked acetate/acetic acid buffer for 86 hours prior to electrophoretic extraction. The samples generated by the 'equilibration' and electrophoretic extraction experiments, which were analysed for actinide content, are summarised in Figure 2.13 and Table 2.8.

Actinide tracer solutions (^{236}U and ^{242}Pu) were purchased from Isotrak, QSA Amersham International; prepared and characterised at AEA Technology UK

(Section 8.3). Spiked (^{236}U or ^{242}Pu) acetate/acetic acid buffers were prepared for the addition and 'equilibration' of artificial actinides with whole soil subsamples prior to the electrophoretic extraction of humic substances. Sections 2.8.1 to 2.8.4 detail the preparation of the buffers.

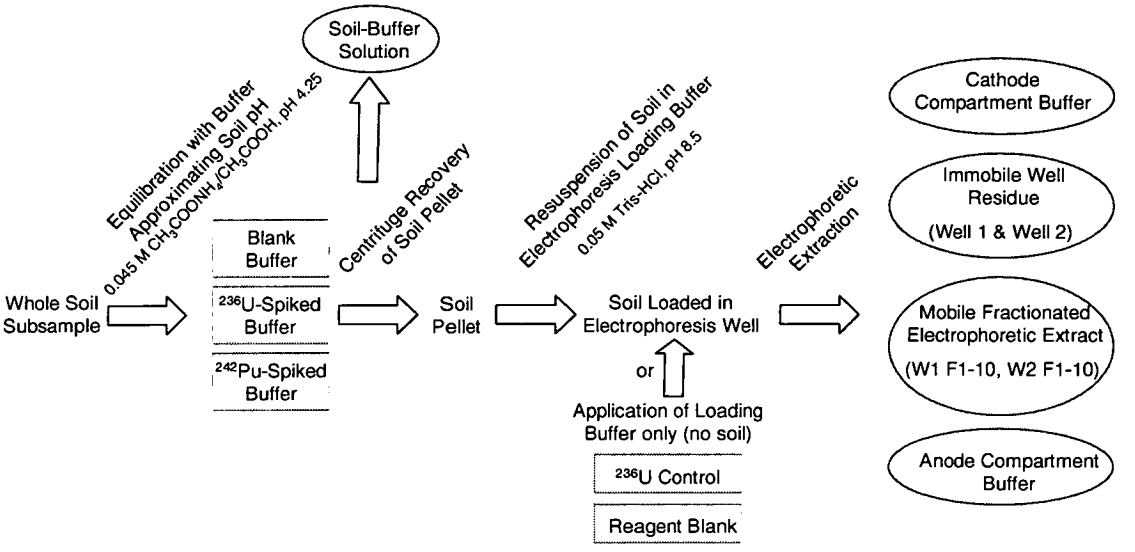


Figure 2.13: Schematic of the samples generated in the soil 'equilibration' and electrophoretic extraction experiments

Sample type	Label suffix	Label sub-prefix	Label prefix
Soil buffer solution	SBS Well 1 or SBS Well 2	B, S, Pu, CON-U, BLANK, (86hr) – representing unspiked, ^{236}U-spiked, ^{242}Pu-spiked whole soil, ^{236}U control, reagent blank and 86-hour equilibration experiments respectively	Soil block, soil depth section (cm) i.e. B1D 0.5 An example of the full label for the Well 1 immobile soil residue from the electrophoretic extraction of ^{236}U-spiked soil is: B1D 0.5 S Well 1
Electrophoretic extract fractions	W1 F1 (to F10) or W2 F1 (to F10)		
Immobile soil well residue	Well 1 or Well 2		
Electrode compartment buffer	CATHODEx ANODEx		

Table 2.8: Labelling scheme of the samples generated in the soil-actinide 'equilibration' and electrophoretic extraction experiments. x – aliquot number.

2.8.1 Preparation of the ^{236}U-spiked 0.045 M ammonium acetate/acetic acid buffer ($CH_3COO^-NH_4^+$:CH_3COOH), pH 4.2

A 0.045 M ammonium acetate/acetic acid buffer (pH 4.2), containing 100 pmoles ^{236}U was prepared as follows. A 0.1 μM ^{236}U solution, in 2 % v/v HNO_3, was prepared from the tracer solution (Section 8.3.2). An aliquot (1 ml) of the 0.1 μM ^{236}U solution was added to a plastic reagent bottle containing 80 ml stock

ammonium acetate/acetic acid buffer (Section 8.4.1) and 2 ml 2M HNO_3. The total volume of the solution was made up to 100 ml with addition of deionised water. Thus each 25 ml aliquot of spiked buffer contained 100 pmoles ^{236}U in 0.045 M ammonium acetate/acetic acid solution (pH 4.2).

2.8.2 Preparation of ^{242}Pu-spiked 0.045 M ammonium acetate/acetic acid buffer ($CH_3COO^-NH_4^+$:CH_3COOH), pH 4.2

An additional consideration in the preparation of this spiked buffer was the oxidation state of the Pu. The oxidation state of Pu uncomplexed by organic ligands in soil is Pu (V, VI), predominantly $Pu(V)O_2^+$ (Bondietti, 1982; Choppin *et al.*, 1995). Therefore the oxidation state of the plutonium was raised, prior to preparation of the spiked buffer, according to the method used by Zhang *et al.* (1997). This involved heating the required aliquot of ^{242}Pu with hydrogen peroxide, allowing the solution to cool, then decomposing the excess peroxide by heating with nitric acid (see below for fuller details). The quantities of acid present in both the aliquot of diluted ^{242}Pu-HNO_3 tracer solution, and the HNO_3 used to decompose the excess peroxide were considered in the preparation of an acetate/acetic acid buffer which would have a buffered pH of 4.2.

A 0.045 M ammonium acetate/acetic acid buffer (pH 4.2) containing 100 pmoles ^{242}Pu was prepared as follows. A 0.1 μM ^{242}Pu solution was prepared in 2 % HNO_3 (Sections 8.3.7). An aliquot (1 ml) of the 0.1 μM ^{242}Pu solution was pipetted to a glass beaker and treated to raise the oxidation state of the ^{242}Pu. An aliquot (50 μl) of 30 % w/v hydrogen peroxide was added to the beaker, a watchglass cover added, and the beaker heated on a hot plate for 20 minutes. The beaker was then removed from the hot plate and allowed to cool for 10 minutes. An aliquot (0.5 ml) of 2 M HNO_3 was then added. The watchglass was replaced and the beaker was reheated for 20 minutes, then allowed to cool for 10 minutes. The required aliquots of stock acetate/acetic acid buffer solution (80 ml), 2 M HNO_3 (1.5 ml) and deionised water (14 ml) were added to a plastic reagent bottle. The raised oxidation state ^{242}Pu solution was thoroughly mixed with this buffer solution by several decants back and forth between beaker and reagent bottle. Aliquots (25 ml) of the

^{242}Pu-spiked buffer thus contained 100 pmoles ^{242}Pu (V) in 0.045 M ammonium acetate/acetic acid solution (pH 4.2).

2.8.3 Electrophoretic extraction ^{236}U-Control

A 'control' electrophoretic extraction experiment was performed whereby 100 pmoles ^{236}U in 0.1 M Tris-HCl (pH 8.5) (Section 8.4.6) was loaded in the wells of the agarose gel to investigate the electrophoretic migration of U which was not associated with soil components.

2.8.4 Electrophoretic extraction reagent blank

A reagent blank electrophoretic extraction experiment was performed in which only 0.5 M Tris-HCl loading buffer (pH 8.5) was loaded in the wells of the agarose gel.

2.9 ICP-MS ACTINIDE ELEMENT (^{238}U, ^{236}U AND ^{242}Pu) ANALYSIS OF WHOLE SOIL, AND SOIL SOLVENT AND ELECTROPHORETIC HUMIC EXTRACTS

The preparation of the whole soils (soil ash residues; Section 2.4.2), soil solvent extracts and samples generated in the soil 'equilibration' and electrophoretic extraction experiments for ICP-MS actinide content analysis included hot acid/peroxide digestion, sample dilution and addition of internal standard (^{209}Bi). Sets of actinide (^{238}U, ^{236}U and ^{242}Pu) calibration standards were prepared which also contained internal standard (^{209}Bi). The methods of sample preparation, the ICP-MS instrument, quality assurance of ICP-MS instrument determinations, and calculation of sample actinide contents, are described below.

2.9.1 Preparation of experimental glassware: alkaline detergent pre-soak, hot acid wash and silylisation methods

Glassware used in all experiments was pre-soaked in 5 % Decon-90 alkaline detergent, then thoroughly rinsed with deionised water and washed in hot acid to remove trace metal contaminants. The acid baths contained approximately 5 M HNO_3. The glassware was heated in the baths on a hot plate for 3 hours. Once cooled the glassware was removed from the acid bath and thoroughly rinsed with deionised water. The plastic electrophoresis tank and casting trays were soaked in 5 % Decon 90 detergent only, and thoroughly rinsed with deionised water.

The beakers in which whole soil was 'equilibrated' with the acetate/acetic acid buffer (Sections 2.5.2.3 and 2.8) were treated with a silylating agent, Repelcote™ (VS), to minimise interaction of the actinides and humic substances with the beaker walls. The glass-wool used as plugs in the centrifuge elution tubes, the Sephadex G-10 desalting column (Section 2.6.2), and the GFC column (Section 2.7.3) was also silylised using Repelcote™ to minimise interaction between the humic substances and the glass-wool.

2.9.2 ^{209}Bi internal standard

The element ^{209}Bi was chosen as internal standard, having a relative atomic mass close to that of the actinide elements of interest. All sample solutions and calibration standards contained 0.2 ppb ^{209}Bi.

^{209}Bi is present in soil as a trace element, and so it was necessary for some samples (whole soil ash and soil solvent extract digests) to be prepared with and without internal standard. Thus the ^{209}Bi content of the sample could be determined, and by subtraction, the value of the internal standard could be calculated.

2.9.3 Acid digestion and dilution of samples for ICP-MS analysis

All samples for ICP-MS actinide analysis were subjected to a hot acid digestion. The samples were placed in beakers containing 0.01 g potassium nitrate as carrier (Zhang *et al.*, 1997). Aliquots of approximately 8 M HNO_3 (30 ml) and 30 % w/v H_2O_2 (5 ml) were added to the samples. Watchglass covers were added to the beakers which were then placed on a hot plate for 2 hours. Nitric acid and hydrogen peroxide were chosen to dissolve the actinides and decompose organic material, respectively, as digestion reagents which were suitable for the subsequent ICP-MS analysis (Arunachalam *et al.*, 1996). After digestion the watchglass covers were removed from the beakers and the samples allowed to dry down to approximately 1 ml solution volume. The acid-digested whole soil ash samples were filtered through Whatman 40 Ashless filter papers to remove the solid residue. All samples were filtered through Whatman 40 Ashless filter papers into volumetric flasks. The solutions were then made up to volume with 2 % v/v HNO_3. These were the stock digest sample solutions. The overall dilution of the sample required was based on

the concentration of dissolved solids, which were to be less than 0.1 % for ICP-MS analysis. Aliquots of the stock sample solutions were pipetted to 10 ml volumetric flasks containing 0.1 ml 20 ppb ^{209}Bi. The solutions were made up to volume with 2 % v/v HNO_3.

Aliquots of blank and spiked (^{236}U or ^{242}Pu) acetate/acetic acid buffer solutions, used in the soil 'equilibration' experiment, were digested to determine the 100 % spike actinide contents. The labelling scheme for these samples is given in Table 2.9.

Buffer type	Label
0.045 M CH_3COONH_4/CH_3COOH, pH 4.25	BUFF
^{236}U spiked-0.045 M CH_3COONH_4/CH_3COOH, pH 4.25	BUFF 236/x
^{242}Pu spiked-0.045 M CH_3COONH_4/CH_3COOH, pH 4.25	BUFF 242/x
0.1 M Tris-HCl buffer, pH 8.5	CON-U BUFF x
^{236}U-spiked 0.1 M Tris-HCl buffer, pH 8.5	CON-U BUFF 236/x

Table 2.9: Labelling scheme for the 'equilibration' (reagent blank and spiked) and electrophoretic extraction experiment buffers used in the quantification of the 100 % actinide (^{236}U and ^{242}Pu) spikes

2.9.4 Preparation of ^{238}U, ^{236}U and ^{242}Pu calibration standards

Calibration standards covering the expected concentration range of the samples were prepared for each actinide element of interest. The five ^{238}U calibration standards prepared contained 10, 5, 1, 0.5 and 0.1 ppb ^{238}U (Section 8.5.2). The five ^{236}U calibration standards prepared contained 1.18, 0.472, 0.236, 0.118 and 0.0236 ppb ^{236}U (Section 8.5.3), and the four ^{242}Pu calibration standards prepared contained 0.521, 0.260, 0.130 and 0.026 ppb ^{242}Pu (Section 8.5.4). All standards contained 0.2 ppb ^{209}Bi internal standard.

2.9.5 ICP-MS analysis instrument settings

Actinide concentrations were determined by ICP-MS analysis (VG Plasmaquad 3; actinide detection limit 0.1-1.0 ppt) run in peak-jumping mode (150 s uptake; 30 s acquisition; 3 repeats per sample or standard; 120 s wash). The sets of calibration standards run at the beginning and end of sample runs, and interspersed after approximately every twentieth sample, were followed by an acid rinse step (2 % v/v HNO_3). One standard of each actinide calibration set (1.0 ppb ^{238}U, 0.236 ppb ^{236}U

and 0.130 ppb ^{242}Pu) was interspersed after approximately every eighth sample and was also followed by an acid (2 % v/v HNO_3) rinse step.

2.9.6 The ICP-MS instrument

The sensitivity of the VG Plasmaquad 3 (VG Elemental) ICP-MS instrument used in this study was 0.1-1 ppt for actinide elements. The ICP-MS instrument consists of a nebulizer, an ICP torch, three vacuum chambers (of decreasing pressure), in the last of which is situated the quadrupole mass filter, and finally an electron multiplier ion detector. The nebulizer converts the aqueous sample into a spray of fine droplets. The finest droplets (2-5 μm) are selected in the water-cooled spray chamber for passage onto the ICP torch. Energy is transferred from an electromagnetic coil (load coil) into the argon gas/sample aerosol to create plasma. In the plasma the sample is desolvated, atomised then ionized. The energy of the plasma is sufficient for the first ionisation energy to be realised at a distance 12-14 mm (T ~ 7500 K) from the load coil. The sampling cone is therefore positioned at this distance in the torch to select singly-ionised sample ions. The three consecutive vacuum chambers are at pressures of 2×10^{-3} bar (expansion stage), less than 1×10^{-7} bar (intermediate stage) and 5×10^{-9} bar. At these pressures the sample is i) isolated from the argon gas, ii) the sample ions are separated from other gas species (electrons and neutrals) and iii) the sample ions are separated according to their mass/charge ratio. The sample ions are accelerated through the vacuum chambers towards the quadrupole by ion lenses (metal rings with electric potentials applied to them). The quadrupole is a set of four poles. An oscillating electric field is generated by applying a voltage across the poles. The effect of the oscillating electric field is to send ions in changing directions, and because the speed with which the ions are accelerated towards the electrodes is inversely proportional to the mass and directly proportional to the charge of the ion, the ions are thus separated by their mass/charge ratio prior to detection (VG Elemental, 1997; Hywel Evans *et al.*, 1995).

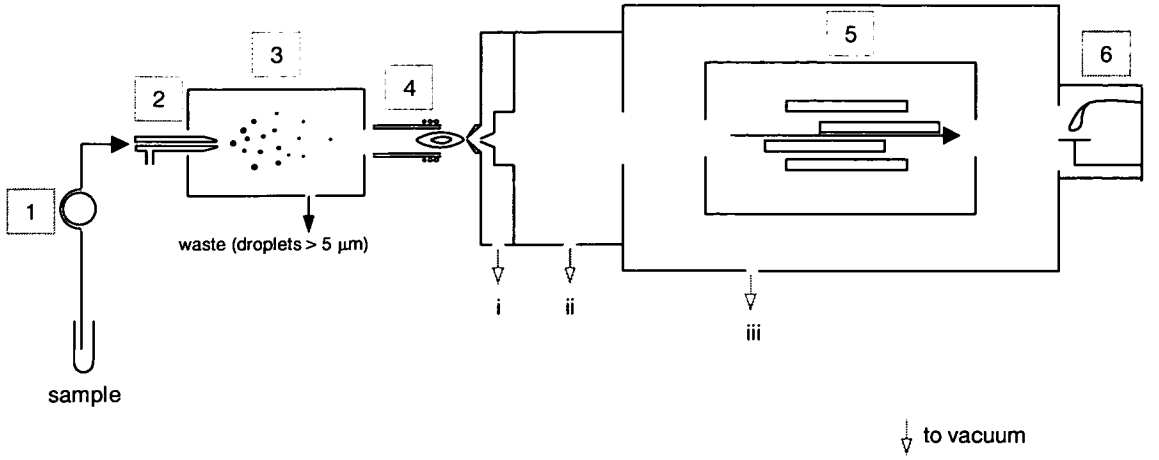


Figure 2.14: Schematic representation of an inductively-coupled plasma mass spectrometer. Components are: 1 - peristaltic pump 2 - nebulizer 3 - water-cooled spray chamber 4 - ICP torch 5 - quadrupole mass filter 6 - electron multiplier. The three consecutive vacuum chambers are i - expansion stage (2×10^{-3} bar) ii - intermediate stage (1×10^{-7} bar) iii - (5×10^{-9} bar) analyser stage. Adapted from: Hywel Evans *et al.* (1995).

2.9.7 Quality assurance of ICP-MS instrument determinations

VG Elemental ran the calibration standard solutions alongside in-house actinide standard solutions and confirmed the accuracy of these solutions.

A plot of calibration standard actinide content versus ^{209}Bi content, for calibrations performed on several separate occasions, was drawn to establish the relationship between instrument detection of actinide elements and the ^{209}Bi internal standard element (Figure 2.15). The actinide and ^{209}Bi corrected counts were normalised to counts per ppb actinide and ^{209}Bi element, respectively. It can be seen that all actinides (^{238}U, ^{236}U and ^{242}Pu), at all standard solution concentrations, fitted an imposed one-to-one trendline of actinide-to-^{209}Bi normalised counts. This demonstrated that there is a linear relationship of instrument response between the actinides and ^{209}Bi, thereby validating ^{209}Bi as choice of internal standard. All sample and calibration standard solution actinide counts were corrected for small shifts in instrument sensitivity during operation using the shift observed of internal standard counts from that of the first sample/standard solution introduced to the instrument.

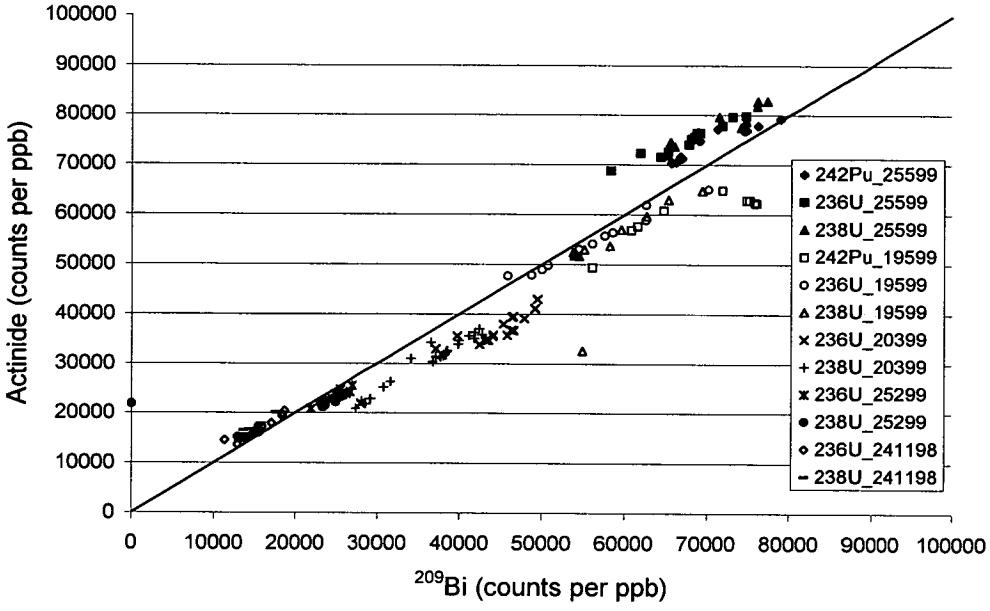


Figure 2.15: Detected calibration standard actinide versus internal standard ^{209}Bi element counts, normalised to an element solution concentration of 1 ppb actinide and ^{209}Bi

The linear regression plots of all actinide calibration sets (corrected counts *vs.* ppb), on all analysis dates, produced regression coefficient values of 0.99-1.00 (to 2 d.p.).

The standard deviation of triplicate uptake and analysis of individual standards and samples (raw counts) was typically less than 3 %. The standard deviation of corrected actinide counts for the interspersed calibration standard solutions (1.0 ppb ^{238}U, 0.236 ppb ^{236}U and 0.130 ppb ^{242}Pu) throughout instrument operation was calculated. Typically standard deviation of each of these standards throughout a run was less than 3 %.

Reproducibility of ICP-MS actinide determinations was observed by the re-run analysis of samples on separate analysis dates (Table 2.10).

Sample	^{238}U (ppb)		^{236}U (ppb)		^{242}Pu (ppb)	
B1D 0.5 B W1F4	0.006	0.006	-	-	-	-
B1D 0.5 Pu W2F5	0.008	0.007	-	-	0.001	0.001
B2C 12.5 S W1F3	0.006	0.005	0.002	0.002	-	-
B1D 0.5 Pu Well 1	0.325	0.320	-	-	0.030	0.029
B1D 2.5 S Well 1	0.454	0.451	0.200	0.198	-	-
B2C 12.5 B Well 1	0.488	0.486	-	-	-	-
B2C 0.5 B W1 SBS	0.003	0.003	-	-	-	-
B2C 0.5(86hr) S W2 SBS	-	-	0.002	0.001	0.004	0.004
B2C 0.5 Pu W1 SBS	0.013	0.013	-	-	0.018	0.018
B1D 0.5 S1	0.643	0.646	-	-	-	-
B2C 9.5 S2	0.302	0.297	-	-	-	-
B1D 0.5 TB1	0.033	0.032	-	-	-	-
B2C 0.5 TB2	0.035	0.034	-	-	-	-
B1D 2.5 N1	0.049	0.049	-	-	-	-
B2C 12.5 N1	0.104	0.104	-	-	-	-

Table 2.10: Reproducibility of sample analysis.

2.9.8 Calculation of sample actinide content: conversion of detected counts to μg per kg of soil

All raw actinide data, in counts, were corrected for small shifts in instrument sensitivity using the fractional shift observed for the ^{209}Bi internal standard from that of the first introduced sample/standard ^{209}Bi counts (Equation 2.1 and 2.2):

$$\text{Fractional sensitivity change} = \frac{\text{sample/standard internal standard } ^{209}\text{Bi (cts)}}{\text{first sample/standard internal standard } ^{209}\text{Bi (cts)}} \quad \text{Equation 2.1}$$

$$\text{Sample actinide content (corrected cts)} = \frac{\text{sample (cts)}}{\text{fractional sensitivity change}} \quad \text{Equation 2.2}$$

All ICP-MS data were then converted from corrected actinide counts to actinide concentration (ppb), using the average interspersed standard counts-to-ppb ratio (Equation 2.3):

$$\text{Sample actinide content (ppb)} = \frac{\text{sample (corrected cts)} \times \text{standard (ppb)}}{\text{average standard (cts)}} \quad \text{Equation 2.3}$$

Sample actinide content, in micrograms, was then calculated using the final sample volume and incorporating the fraction of sample that had been digested for analysis

(Equation 2.4). The final sample volume considered the stock digest solution volume, the aliquot of the digest solution used in the preparation of the analysis solution and the analysis solution total volume.

$$\text{Sample actinide content } (\mu g) = \frac{\text{sample (ppb : } \mu g\, l^{-1}) \times \text{final sample volume (l)}}{\text{fraction digested}} \qquad \text{Equation 2.4}$$

When a comparison of results between different experiments was required the actinide content of samples were normalised for the amount of soil used in each experiment (Equation 2.5):

$$\text{Sample actinide content } (\mu g\ kg^{-1}) = \frac{\text{sample } (\mu g)}{\text{soil mass (kg)}} \qquad \text{Equation 2.5}$$

The average, standard deviation and 95 % confidence limit of sample replicates were determined.

3 METHOD DEVELOPMENT

This chapter details the development of the novel methods of (i) electrophoretic extraction and fractionation of soil humic substances and (ii) addition of artificial actinides to whole soil samples which led to the final procedures documented in Sections 2.5.2 and 2.8, respectively.

3.1 ELECTROPHORETIC EXTRACTION AND FRACTIONATION OF SOIL HUMIC SUBSTANCES

Following an exploratory experiment, which demonstrated that electrophoresis could be applied to the isolation of humic substances from bulk soil, a number of parameters were investigated in order to optimise both extraction and fractionation. The exploratory experiment is described in Section 3.1.1. The requirements which had to be met by the optimal procedure, and the parameters investigated to achieve optimisation, are described in Section 3.1.2. This is followed by the results of the individual investigations (Sections 3.1.3 to 3.1.7). Finally, the electrophoretic extraction of a solvent humic extract was compared to that of soil to observe any differences in the direct electrophoretic extraction and fractionation of humic substances from soil (Section 3.1.8).

3.1.1 Exploratory experiment: the application of electrophoresis to the isolation of humic substances from bulk soil

In an exploratory electrophoresis experiment, soil was loaded in the wells of an agarose gel. On application of an electric field extraction of two main bands of material of differing electrophoretic mobility were visually observed to migrate from the soil well into the gel. Both bands were composed of material migrating towards the anode, i.e. the bands were composed of negatively-charged materials. The more mobile band was dark-brown in colour, whilst the less mobile band was brown, less intense than the preceding band, and tailed back towards the well. A third band, which had the greatest anionic electrophoretic mobility, was visible when viewed in UV-light. The extract material of this band was visually observed to emit light when the gel was stimulated by UV-light (302 nm). The result of electrophoretic

extraction of soil was similar to the paper electrophoresis of solvent extracts of soil humic and fulvic acids observed by Kononova (1966):

'three zones appeared in the electrophoretograms: A, a zone remaining on the starting line; B, a negatively charged zone (moving towards the anode); and C, the most labile zone, which was fluorescent'.

The exploratory electrophoretic soil extraction experiment therefore showed application to the direct extraction and simultaneous fractionation of humic substances from soil into three regions: UV-fluorescent, brown and tailing brown bands.

3.1.2 The requirements for method optimisation and parameters investigated

The aim of the method development was to devise a protocol for the isolation of humic substances from bulk soil in which both the concentration and fractionation of isolated humic substances were optimised. The concentration of humic substances extracted into the agarose gel could be increased by increasing the mass of soil loaded in the well compartment up to a point where overloading effects became apparent. Overloading negates fractionation because the higher concentrations of humic substances cannot be focused prior to fractionation. Humic substances with different electrophoretic mobilities then appear in the same region of the gel. Fractionation is optimised when the starting point of migration is as narrow as possible. Factors investigated for their effect on the extraction and fractionation of humic substances were:

- Buffer composition, including presence of metal-ion chelating agent (EDTA)
- Continuous/discontinuous loading and running buffer systems*
- Freeze-drying preparation of the soil and soil rehydration time prior to extraction
- Mass of soil loaded and the soil/loading buffer ratio

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* The loading and running buffers are those in which soil is suspended and loaded in the well and those which are used to prepare the agarose gel and contact the gel with the electrodes, respectively.

A final study was included to compare the electrophoretic extraction of a Tris-HCl (i.e. loading buffer) solvent extract with the electrophoretic extraction of soil to see if there were any obvious differences in the extract obtained by direct soil extraction. The initial method development was conducted on a sample of the surface layer (0-5 cm) of a floodplain soil. The site location (near the Esk Estuary, W. Cumbria, UK), description and sampling details are given in Section 8.2.

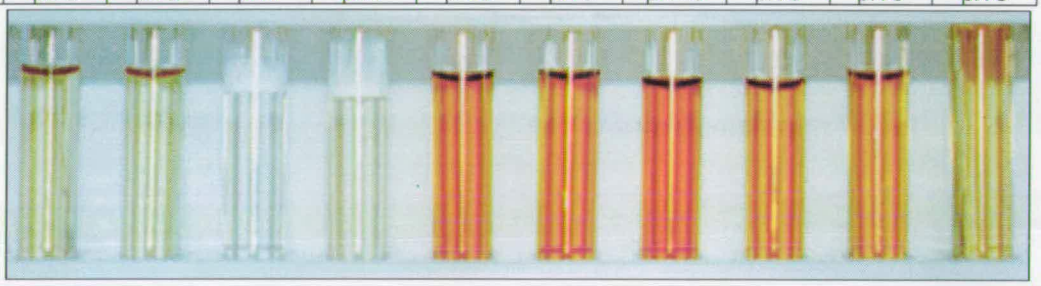
3.1.3 Relation of buffer composition to humic solvent extraction efficiency

This study compared the solvent humic extraction efficiency of different Tris buffers in order to select an appropriate loading buffer. The influence of Tris buffer counter-anion, solvent pH and presence of a metal chelating agent (EDTA; ethylenediamine tetraacetic acid) were investigated. The influence of freeze-drying preparation of the soil prior to extraction compared to the extraction of unprepared moist soil was included in the investigations.

In the investigation of buffer composition, Tris base ($(CH_2OH)_3CNH_2$), in conjunction with three different anions, was studied to identify which buffer had the greatest solvent extraction efficiency for humic substances from soil. The influence of metal-chelating agent (EDTA) was studied by inclusion in the Tris buffer composition. Extraction was conducted on freeze-dried soil subsamples, except in the study of freeze-drying preparation on extraction efficiency. The Tris buffers were: Tris-acetate, Tris-acetate-EDTA, Tris-HCl, Tris-HCl-EDTA, Tris-borate, Tris-borate-EDTA and Tris base alone (Figure 3.1). The buffers all contained 0.045 mM Tris, and where present, 1 mM EDTA. The buffer pH in all experiments was approximately pH 8, except for the Tris base solution without added acid, which was pH 10. An experiment with Tris-HCl (with and without EDTA) was studied at two different pHs; pH 7 and pH 8. Each extraction was of 4 g soil (wet or freeze-dried) in 9 ml of Tris buffer (except in the Tris-HCl pH 7 experiment in which the solvent volume was 8 ml). Soil was extracted in test tubes, placed horizontally on an orbital shaker, for 14 hours (except for the pH 7 Tris-HCl experiment, in which extraction was 24 hours). The solvent humic extracts of soil were separated from the soil residue by centrifuging the suspension at 10,000 rpm for 10 minutes, followed by syringe-filtering of the supernatant (Gelman 32 mm diameter, 0.2 μm pore size

polyethersulfone filter membranes). The volume of the supernatant of moist soil was greater than that of the freeze-dried soil extracts: 5.5 ml compared to 4.5 ml, respectively. The absorbance of the extract solutions, diluted so that absorbance was in the range 0.2 to 1.0 absorbance units (a.u.), was measured at 254 nm to determine the relative yields of the Tris buffer extracts. The results are presented below in Figure 3.1. The absorbance values have been divided by the dilution factors (i.e. the dilution required for absorbance to lie in the range 0.2-1.0 a.u.) and normalised to the extraction of 1 g freeze-dried weight of soil (using a correction for the wet-weight of soil with a moisture content of 18.3 %, Section 8.2) and to an extract volume of 5.5 ml. The raw data are presented in Section 8.12.

Soil	freeze-dried	freeze-dried	freeze-dried	freeze-dried	freeze-dried	freeze-dried	freeze-dried	freeze-dried	freeze-dried	undried
Tris buffer approx. pH	Tris acetate pH 8	Tris acetate EDTA pH 8	Tris HCl pH 7	Tris HCl EDTA pH 7	Tris HCl pH 8	Tris HCl EDTA pH 8	Tris base pH 10	Tris borate pH 8	Tris borate EDTA pH 8	Tris borate pH 8



$A_{254\ nm}$ (normalised)	1.28	1.60	nd	nd	4.81	4.83	5.43	3.87	3.93	2.03

Figure 3.1: Photograph of Tris buffer humic substance extracts of floodplain soil. Extraction details and absorbance values of the humic substance extracts at 254 nm (corrected for dilution and normalised to absorbance per g dry-weight of soil and an extract volume of 5.5 ml) are presented above and below the photograph, respectively. Solvent extraction was for a period of 14 hours with a soil/solvent ratio of 4 g/9 ml, except for extraction in Tris-HCl, pH 7 solvents, which was 24-hour and employed a soil/solvent ratio of 4 g/8 ml. (nd - not determined).

Outcomes of the Tris buffer solvent extract experiment, with reference to Figure 3.1, were:

- Increasing pH was observed to increase relative humic substance yield. This was demonstrated in the visual distinction between the Tris-HCl and Tris-HCl-EDTA extracts at pH 7 and pH 8, regardless of the longer extraction time for the pH 7

experiment (24-hour *cf.* 14-hour). Also the greatest extract absorbance value was observed for the solution of highest pH, i.e. Tris base solution, pH 10.

- Presence of metal chelating agent, EDTA, increased relative humic substance yield. The effect was greatest when overall extraction was low, as in the extraction with Tris-acetate ($\Delta_{A\ 254\ nm.}$ = 0.32 a.u.). When overall extraction was higher, as in the Tris-HCl and Tris-borate extracts, then the effect of EDTA was much lower ($\Delta_{A\ 254\ nm}$ = 0.02 and 0.06 a.u., respectively).
- The order of increasing humic substance solvent extraction efficiency of Tris buffers was shown to be:

Tris-HCl > Tris-borate > Tris-acetate

- It was observed that in the freeze-dried *vs.* wet soil extraction experiment the yield of humic substances was greatest for the freeze-dried soil. The absorbance of the extract of the freeze-dried soil was almost twice that of the wet soil (1.91-fold to 2 d.p.). This would appear to show that freeze-drying preparation increases humic substance yield. This could be effected by an increase in surface area of the sample, as a result of the freeze-drying and re-hydration processes.

The conclusion of the investigations of Tris buffer composition, with respect to humic extraction efficiency, was that Tris-HCl was the optimum extraction buffer. Fifty percent or greater of the buffering capacity (a quantitative measure of the buffering ability of a solution) of a monoacidic base is realized in the pH range $pK_a \pm 0.75$ (Perrin and Dempsey, 1974). The pK_a of Tris is pH 8.1 (Lide, 1994). A Tris solution of pH 8.5 was used in all subsequent solvent and electrophoretic extraction experiments. This pH was within the Tris buffering capacity pH range (pH 7.35-8.85) and toward the upper end of the range, thereby increasing extraction efficiency. The use of chelating agent, EDTA, had only a small influence on extraction efficiency at this pH. The effect of EDTA on the electrophoretic fractionation of soil humic substances was also investigated (Section 3.1.5).

3.1.4 Effect of continuous and discontinuous buffer systems on electrophoretic fractionation

The Tris buffer system of the electrophoresis experiment, as either a continuous or discontinuous buffer system (i.e. loading and running buffers identical or dissimilar, respectively) was studied to observe the effect on the fractionation of humic substances. The continuous buffer system was that in which 0.045 M Tris-borate (pH 8.5) was used as both loading and running buffer. In the discontinuous buffer system 0.05 M Tris-HCl (pH 8.5) was used as loading buffer, and 0.045 M Tris-borate (pH 8.5) was used as running buffer. It was found that extraction in the discontinous buffer system produced a focused band of extract that entered the gel. In the continous buffer system a diffuse extract emerged from the well. The discontinuous buffer system demonstrated the moving boundary effect (Atkins, 1994), focusing the humic substances into a concentrated band prior to electrophoretic fractionation. The discontinuous buffer system was therefore used in all subsequent experiments as it achieved the aims of optimising concentration and fractionation of the soil extract humic substances. A fluorescence-scanning line graph of the length of the electrophoretic extract of floodplain soil, using the discontinuous buffer system, is shown below in Figure 3.2.

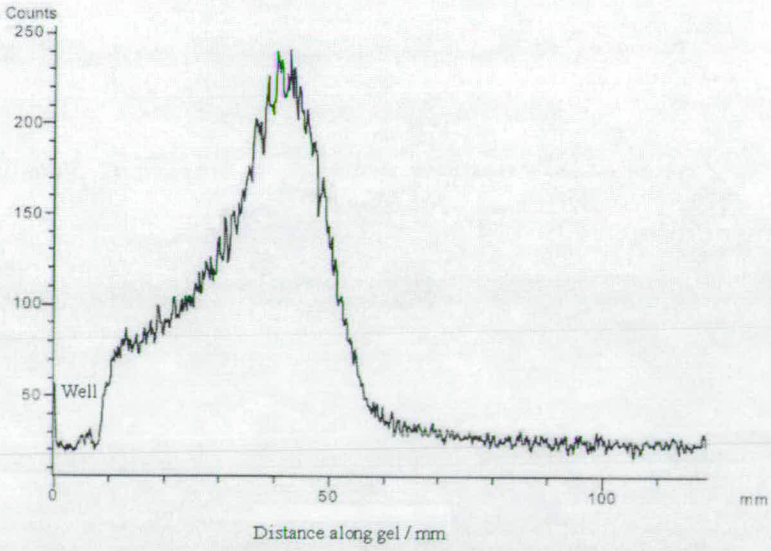


Figure 3.2: Fluorescent emission (520 nm, blue/green visible light) line graph (along the length of the extract gel) of the electrophoretic humic extract of floodplain soil excited by light of wavelength 450 nm (purple/blue visible light). Line graph recorded on STORM 840 Scanning Fluorimeter (Molecular Dynamics). The position of the well is indicated. The direction of electrophoretic mobility of the anionic humic extract is from left to right. The maximum coincides with the end of the brown band whilst the end of the UV-fluorescent band coincides with the beginning of the lower plateau (right hand side).

3.1.5 Effect of the presence of metal chelating agent (EDTA) on electrophoretic fractionation

The metal chelating agent, EDTA, was further investigated for its effect on electrophoretic fractionation of soil humic substances. Figure 3.3 shows the UV-fluorescent image of the electrophoresis gel of extracted floodplain soil, obtained using loading and running buffers in which EDTA was either present or absent. The effect observed was that the brown band migrated further in the buffer system which contained EDTA. In the presence of EDTA the brown band migrated a distance of 2.9 cm from the well, compared to a migration distance of 1.9 cm in the buffer system in which EDTA was absent. Also, the total length of the extract was greater in the EDTA buffer system. This suggests that the humic substances extracted in the presence of EDTA had a greater mass-to-charge distribution, shifted towards smaller and more highly-charged materials. The influence of EDTA on extraction is through chelation of multi-valent metal ions (Section 1.11.1), and as such the observation of smaller humic materials in the presence of EDTA might suggest that chelated metal ions were involved in *in situ* aggregation of a proportion of the humic substances. A chelating agent, which has the potential for removing metals, was not used in this study as quantification of the ^{238}U content of the soil humic substances was desired.

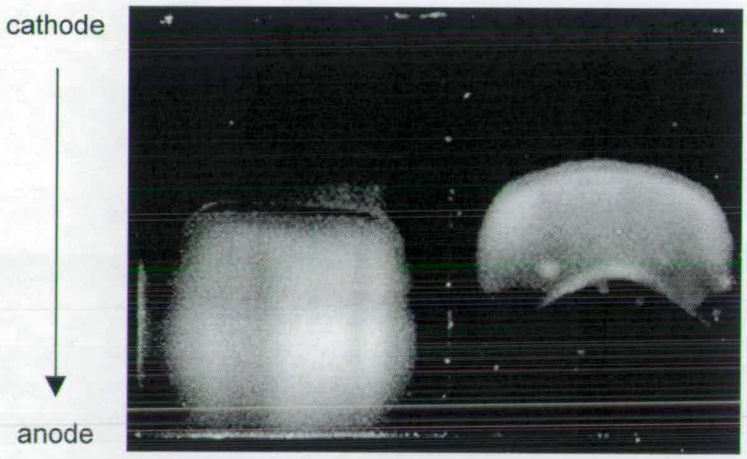


Figure 3.3: Photograph of the electrophoretic extract of floodplain soil in the presence (left) and absence (right) of EDTA in the buffer system

3.1.6 Effect of time of rehydration of freeze-dried soil in loading buffer prior to electrophoretic extraction

Rehydration time of the freeze-dried floodplain soil in loading buffer prior to extraction was studied for influence on electrophoretic extraction of humic substances. An aliquot (1.65 ml) of 0.05 M Tris-HCl was added to each of four 0.2 g subsamples of freeze-dried soil. The aliquots were added to the soil subsamples at different times, and the soil-solution mixtures placed on an orbital shaker, so that when the shaker was stopped the four subsamples had been shaken for 0, 1, 1.5 and 2.5 hours. The soil slurries were then pipetted into four wells in an agarose gel and electrophoretic extraction commenced. The result, as observed by viewing the UV-fluorescent image of the gel extract, was that there was no discernible difference for soil rehydration times of 0 to 2.5 hours. Therefore in the optimised protocol freeze-dried soil was mixed with loading buffer and immediately loaded in the agarose gel wells for extraction.

3.1.7 Optimal mass of soil and soil/loading buffer ratio

The maximum mass of soil which could be re-suspended in 2 ml of loading buffer whilst minimising overloading effect was investigated. Overloading may be observed as the continual emergence of extract, either across the whole width of the gel (excess soil) or in streaks (point excess; high humic substance concentration resulting from natural inhomogeneity of the soil sample), rather than a focused band. Soil-to-loading buffer ratios of 1:1, 1:2 and 1:20 were investigated. At a ratio of 1:1 the extract intensity of the tailing brown band was almost indistinguishable from the brown band. A soil-to-loading buffer ratio of 1:2, suitable for extraction of subsurface soil samples (i.e. those with lower humic substance content), showed streaks of overloading in the extracts of surface soils. An optimal soil/loading buffer ratio of 1:20 was concluded.

3.1.8 Electrophoretic fractionation of a solvent humic extract vs. direct electrophoretic extraction of soil

The electrophoretic fractionation of a solvent (0.5 M Tris-HCl, pH 8.5) humic extract of soil, and electrophoretic extraction of whole soil, were compared to see if there was any difference in directly extracting humic substances from soil. The two

electrophoretic extracts appeared to be identical when viewed in visible light (Figure 3.4). When viewed fluorescing under UV-light (Figure 3.5) strong fluorescence was observed for a tailing band of the whole soil extract but not for the solvent extract. Thus, after migration of the focused soil humic extract, further humic substances were extracted from the soil well.

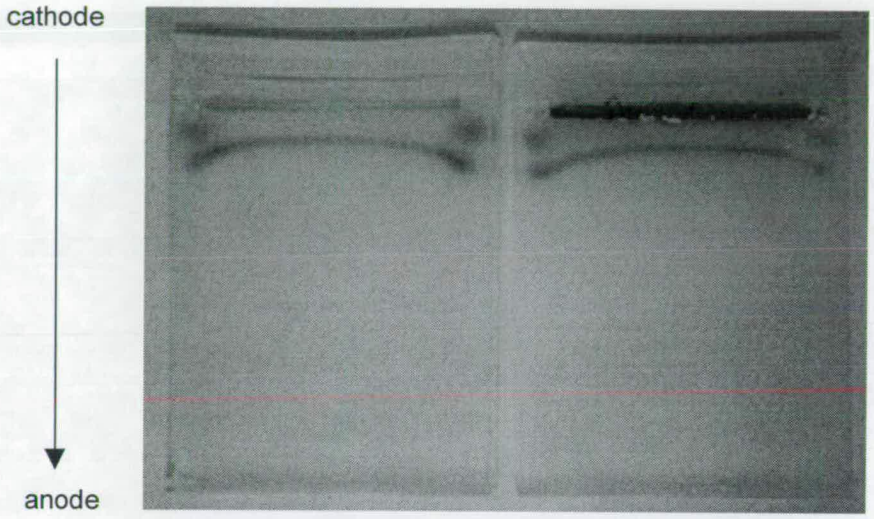


Figure 3.4: Photograph of electrophoretic extracts of soil (right) and solvent humic extract (left) in visible light

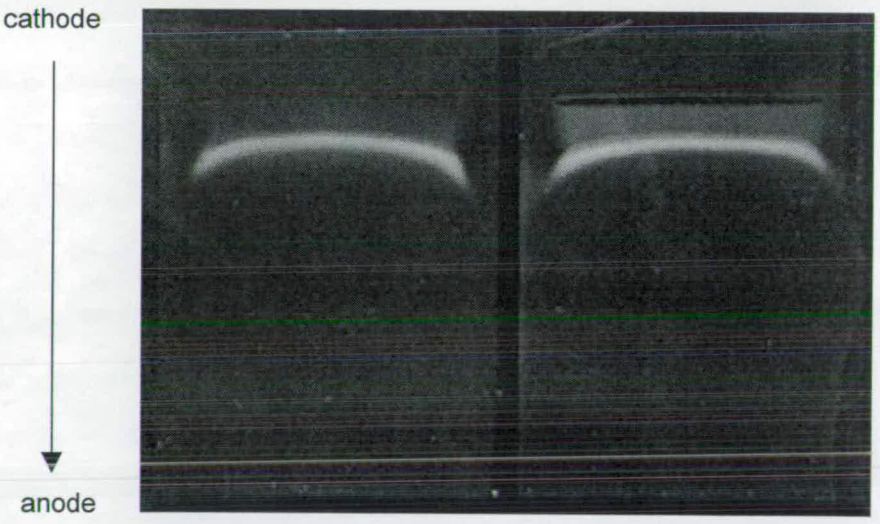


Figure 3.5 Photograph of electrophoretic extracts of soil (right) and humic solvent extract (left) fluorescing under UV light

3.2 ADDITION OF ARTIFICAL ACTINIDES (^{236}U AND ^{242}Pu) TO WHOLE SOIL

Spiking of the whole soil allows investigation of the association of introduced actinides with humic substances in the natural soil matrix. This has the advantage that the introduced actinides are added to the humic substances which are both in native conformation and *in situ* association with other soil components, in contrast to a humic extract which may have been altered by the extraction method. Also, competition between all soil components, for the binding of the actinides, is enabled.

3.2.1 Choice of buffer for the 'equilibration' of added artificial actinides with whole soil samples

A solution was required to buffer the soil samples at approximately the forest soil solution pH. The buffer chosen was 0.045 M ammonium acetate/acetic acid ($CH_3COO^-NH_4^+$:CH_3COOH) (pH 4.2) (Section 8.4.2).

Sodium acetate (0.01 M)/acetic acid/0.1 M sodium perchlorate buffer, pH 4.2, has been used previously in actinide-humic binding experiments (Rao *et al.*, 1994). It was found that complexation of U by the acetate ion was less than 5 %, and the degree of ionisation of the lake sediment humic acid sample was about 40 % at pH 4.2 (Rao *et al.*, 1994). Therefore, the soil humic substances of this study, in the acetate/acetic acid buffer, are expected to be partially ionised and thus in a form to interact with the actinide elements, whilst the acetate ion is not expected to exclude this interaction. The sodium acetate salt was not an appropriate choice for this study, with respect to ICP-MS analysis, as high levels of sodium cause ionisation suppression in the plasma (Arunachalam *et al.*, 1996). Thus alternatively ammonium acetate buffer was used in this study.

4 RESULTS

This chapter presents the results of whole soil characterisation (soil solution pH, organic matter content and soil particle size), solvent (NaOH, Tris-borate buffer) and electrophoretic (Tris-borate/Tris-HCl buffer) extraction of humic substances, and humic substance characterisation (yield, functional groups and actinide binding) for samples from a deciduous broadleaf and a coniferous forest soil. Section 4.1 describes the initial characterisation of samples selected from the vertical profiles of these two soils. This was undertaken to provide background information on the soil solution pH, organic matter content, soil texture and presence of organic matter in soil aggregates. Section 4.2 then details the observations and yields of humic extracts obtained by traditional solvent, and the novel electrophoretic, extraction methods. The results of further characterisation of electrophoretic humic extracts are contained in Section 4.3. Thereafter, the remaining section, Section 4.4, contains the results of ICP-MS actinide analyses. Sections 4.4.1 and 4.4.2 contain the results of naturally occurring actinide (^{238}U) concentrations in whole soil, and solvent and electrophoretic humic extracts of the forest soil samples, respectively. Actinide speciation is then more specifically detailed following subdivision of soil actinides into three main, operationally-defined, pools – labile, hydrophilic-mobile and immobile soil actinide pools (Section 4.4.3). These pools represent the portions of actinide in the 'equilibration' buffer solution, associated with the electrophoretic humic extract, and remaining in the electrophoresis well after extraction, respectively. The different patterns of actinide content with the individual electrophoretic extract fractions are then presented in relation to the distributions of actinides between the soil actinide pools and in relation to soil depth (Section 4.4.4).

4.1 CHARACTERISATION OF THE DECIDUOUS BROADLEAF AND CONIFEROUS FOREST SOILS

The samples chosen for characterisation of the forest soils were summarised in Section 2.3. The determination of soil solution pH, at each soil depth, was important with respect to interpreting the speciation of uncomplexed actinides in the soil solution. Organic matter content was determined by loss on ignition (L.O.I.). The effective (organo-mineral) and absolute (mineral) size of the aggregated and

disaggregated soil particles, respectively, was determined to allow classification of the soil texture of the samples, observation of the *in situ* soil particle size distribution, and the influence of organic matter in the aggregation of the soil mineral particles.

4.1.1 Soil solution pH

The soil solution pH (in weak electrolyte, 0.01 M $CaCl_2$) was determined for each depth of the duplicate deciduous broadleaf (B1D, B2D) and coniferous (B1C, B2C) forest soil blocks (Table 4.1 and Table 4.2). The range of values obtained for samples from both the deciduous broadleaf soil blocks was 3.0-4.1. The maximum value of 4.1 corresponded to the surface of the litter layer of B1D. Minimum values of 3.3 (B1D) and 3.0 (B2D) at a depth of 0.5 cm, the interface between the litter and mineral soil, were obtained for both depth profiles. Below this depth, there was a small increase to values of 3.5 (B1D) and 3.4 (B2D) (Figure 4.1). The range of soil pH values obtained for the coniferous soil blocks (2.8-3.6) was not as wide as for the deciduous soil blocks, and corresponded to greater acidity in the near surface. The trend with increasing depth was, however, similar to that observed for the deciduous soil. For both blocks (B1C and B2C), pH decreased (from 3.3 and 3.4, respectively) through the litter layer to a minimum value (2.8 and 2.9, respectively) again close to the interface between the litter layer and the mineral soil. Below this depth, there was also an increase in pH (2.8-3.6 and 2.9-3.6 for B1D and B2C, respectively) (Figure 4.2). The apparent difference in the magnitude of increase (0.2 cf. 0.8) below the litter/mineral soil interface between the two types of soil can be attributed to the difference in the depth ranges under consideration. For example, changes in pH between 0.5 and 4.5 cm for deciduous broadleaf blocks are 0.2 and 0.4 units for B1D and B2D, respectively (Table 4.3). Comparison over the same depth interval (0.5-4.5 cm) for the coniferous blocks reveals a change of only 0.1 and 0.2 units, respectively (Table 4.3). Therefore, the rate of increase of pH with increasing soil depth below the litter/mineral soil interface is similar for each soil. This is also observed when the data are converted to proton activities (where $a_{H^+} = 10^{-pH}$) (Table 4.4). For example, the average decrease in proton activity for the deciduous broadleaf and coniferous soils for the interval 0.5-4.5 cm is 0.43×10^{-3} and

0.41×10^{-3}, respectively. There is also very little difference between average values for blocks (excluding the litter layers) from the same forest soil and between the two types of forest soil (Table 4.3).

B1D		B2D	
Average soil depth (cm)	**Soil solution pH (weak electrolyte)**	**Average soil depth (cm)**	**Soil solution pH (weak electrolyte)**
-2.0	4.1	-	-
-0.5	3.3	-1.25	3.2
0.5	3.3	0.5	3.0
1.5	3.3	1.5	3.1
2.5	3.4	2.5	3.2
3.5	3.4	3.5	3.3
4.5	3.5	4.5	3.4

Table 4.1: Soil solution pH of whole soil depth section samples of the deciduous broadleaf forest soil blocks B1D and B2D as measured in weak electrolyte (0.01 M $CaCl_2$). (- no equivalent depth sample available).

B1C		B2C	
Average soil depth (cm)	**Soil solution pH (weak electrolyte)**	**Average soil depth (cm)**	**Soil solution pH (weak electrolyte)**
-5.5	3.3	-5.5	3.4
-	-	-4.5	3.2
-4.0	2.9	-3.5	3.0
-2.5	2.9	-2.5	2.9
-1.5	2.9	-1.5	2.8
-0.5	2.8	-0.5	2.8
0.5	2.8	0.5	2.9
1.5	2.8	1.5	3.0
2.5	2.9	2.5	3.0
3.5	2.9	3.5	3.1
4.5	2.9	4.5	3.1
5.75	3.0	5.5	3.2
7.25	3.0	6.5	3.2
8.5	3.0	7.5	3.3
9.5	3.2	8.5	3.3
10.5	3.3	9.5	3.3
11.5	3.3	10.5	3.4
12.5	3.4	11.5	3.4
13.5	3.5	12.5	3.6
14.5	3.5	13.5	3.6
15.5	3.5	-	-
16.5	*nd*	-	-
17.5	3.6	-	-
18.5	3.6	-	-

Table 4.2: Soil solution pH of whole soil depth section samples of the coniferous forest soil blocks B1C and B2C as measured in weak electrolyte (0.01 M $CaCl_2$). (*nd* – not determined). (- no equivalent depth sample available).

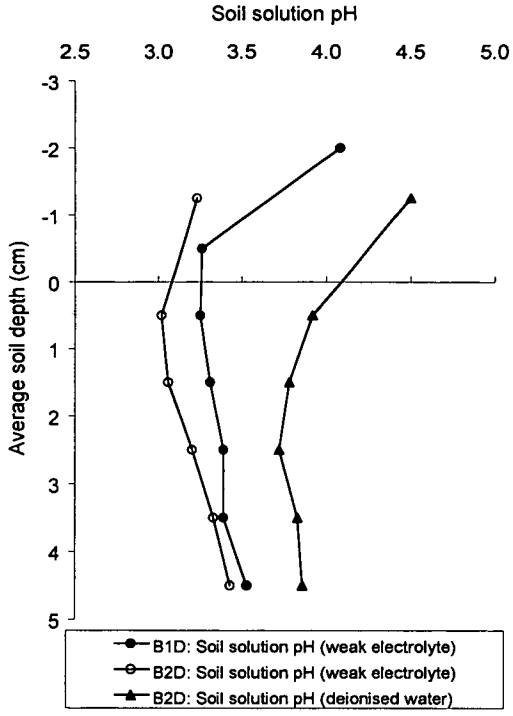


Figure 4.1: Variation of soil solution pH (as measured in weak electrolyte, 0.01 M $CaCl_2$, and deionised water) of the deciduous broadleaf forest soil profiles (B1D and B2D). The x-axis intercepts the y-axis at zero centimetre soil depth – the litter/mineral soil interface.

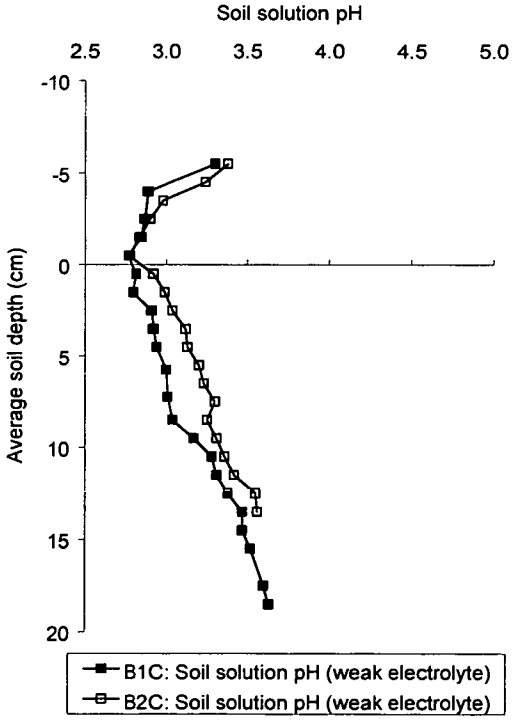


Figure 4.2: Variation of soil solution pH (as measured in weak electrolyte, 0.01 M $CaCl_2$) of the coniferous forest soil profiles (B1C and B2C). The x-axis intercepts the y-axis at zero centimetre soil depth – the litter/mineral soil interface.

	B1D	B2D	B1C	B2C
pH range (litter layers only)	4.1 - 3.3	3.2	3.3 - 2.8	3.4 - 2.8
pH range (excluding litter)	3.3 - 3.5	3.0 – 3.4	2.8 – 3.6 (0.8)	2.9 – 3.6 (0.7)
pH range (excluding litter) (soil depth 0-5 cm)	3.3 – 3.5 (0.2)	3.0 – 3.4 (0.4)	2.8 – 2.9 (0.1)	2.9 – 3.1 (0.2)
Average pH (excluding litter)	3.4	3.2	3.2	3.2

Table 4.3: The range and average of soil solution pH values (measured in weak electrolyte, 0.01 M $CaCl_2$) for the deciduous broadleaf (B1D, B2D) and coniferous (B1C, B2C) forest soil samples. The magnitude of increases in pH descending the mineral soil profiles is indicated in parentheses.

Deciduous broadleaf soil solutions		Coniferous soil solutions	
Average soil depth (cm)	**Average proton activity ($\times 10^{-3}$)**	**Average soil depth (cm)**	**Average proton activity ($\times 10^{-3}$)**
0.5	0.76	0.5	1.36
1.5	0.68	3.5	0.98
2.5	0.52	6.5	0.80
3.5	0.44	9.5	0.62
4.5	0.33	12.5	0.40

Table 4.4: Average soil solution proton activities for the deciduous broadleaf (B1D, B2D) and coniferous (B1C, B2C) forest soils calculated from the measured soil solution pH values ($a_{H^+} = 10^{-pH}$).

Measurement of pH in deionised water was also conducted separately for the deciduous broadleaf soil block, B2D. The soil solution values obtained by this method were all greater than the respective values obtained by measurement in weak electrolyte (0.01 M $CaCl_2$) (Table 4.5). The mineral soil solution pH range encountered, descending the profile, was smaller as measured in deionised water (pH 3.7 to 3.9) compared to measurement in weak electrolyte (pH 3.0 to 3.4) (Table 4.6). The average mineral soil solution pH was higher measured in deionised water (pH 3.8) compared with measurement in weak electrolyte (pH 3.2). The soil solution pH, measured in deionised water, decreased from the litter, to a minimum at 2.5 cm soil depth, then increased with subsequent increases in depth. This trend was unlike that observed by measurement in weak electrolyte for all forest soil (deciduous broadleaf and coniferous) profiles, in which a minimal soil solution pH coincided with the litter/mineral soil interface. The magnitude of the depression of soil solution pH

measured in weak electrolyte, compared to deionised water, was greatest in the surface litter sample and decreased with depth (Figure 4.3). Two slopes were observed for the rate of change of pH depression with soil depth. Between the litter layer and 2.5 cm depth samples the rate of change was a depression of 1 pH unit per 20 cm depth increase while the slope between 2.5 cm and 4.5 cm depth samples showed a rate of change of 1 pH unit per 5 cm depth increase.

Average soil depth (cm)	Soil solution pH (weak electrolyte)	Soil solution pH (deionised water)	ΔpH
-1.25	3.2	4.5	-1.3
0.5	3.0	3.9	-0.9
1.5	3.1	3.8	-0.7
2.5	3.2	3.7	-0.5
3.5	3.3	3.8	-0.5
4.5	3.4	3.9	-0.4

Table 4.5 Soil solution pH of whole soil depth section samples of the deciduous broadleaf forest soil block B2D as measured in weak electrolyte (0.01 M $CaCl_2$) and deionised water. The soil solution pH depression (ΔpH) observed from measurement in deionised water compared to weak electrolyte is also given.

	weak electrolyte (0.01M $CaCl_2$)	deionised water
pH range (litter layers only)	3.2	4.5
pH range (excluding litter)	3.0 – 3.4 (0.4)	3.7 – 3.9 (0.2)
Average pH (excluding litter)	3.2	3.8

Table 4.6: The range and average of soil solution pH values (measured in weak electrolyte, 0.01 M $CaCl_2$, and deionised water) for the deciduous broadleaf forest soil B1D samples. The magnitude of increases in pH descending the mineral soil profiles is indicated in parentheses.

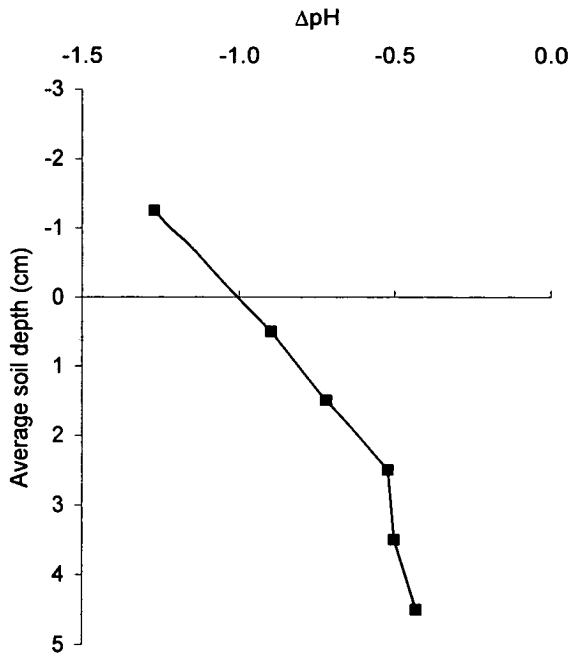


Figure 4.3: Variation of depression of soil solution pH (ΔpH) of the deciduous broadleaf forest soil profile B2D measured in weak electrolyte (0.01 M $CaCl_2$) compared to measurement in deionised water.

4.1.2 Organic matter content: loss on ignition (L.O.I.)

Organic matter content was determined by loss on ignition (L.O.I.) (450 °C for 24 hours). The data (expressed as a percentage of the original mass of freeze-dried soil which was lost upon ignition) for the triplicate analyses of soil samples (not including litter samples) are contained in Section 8.10. The average L.O.I. values (± 95 % confidence limit values, Section 8.9) are presented in Table 4.7 (B1D) and Table 4.8 (B2C) and are plotted as a function of soil depth in Figure 4.4.

The range of values obtained for B1D and B2C was 20.7±0.9 - 35.3±0.6 % and 23.4±0.3 - 45.8±0.6 %, respectively. Comparing near surface soil samples, B2C has a higher organic content than B1D. In both cases, the rate of decrease of organic matter content decreases with increasing soil depth. Soil solution pH and organic matter content show an inverse relationship with increasing mineral soil depth.

Average soil depth (cm)	Average % L.O.I. (g)	95 % confidence limit
0.5	35.3	0.9
1.5	30.9	0.6
2.5	25.1	0.2
3.5	23.1	0.3
4.5	20.7	0.9

Table 4.7: The average percentage L.O.I. values of the triplicate deciduous broadleaf forest soil B1D depth section subsamples and the respective 95 % confidence limit values

Average soil depth (cm)	Average % L.O.I. (g)	95 % confidence limit
0.5	45.8	0.6
3.5	32.3	0.5
6.5	31.0	0.4
9.5	27.0	0.8
12.5	23.4	0.3

Table 4.8: The average percentage L.O.I. values of the triplicate coniferous forest soil B2C depth section subsamples and the respective 95 % confidence limit values

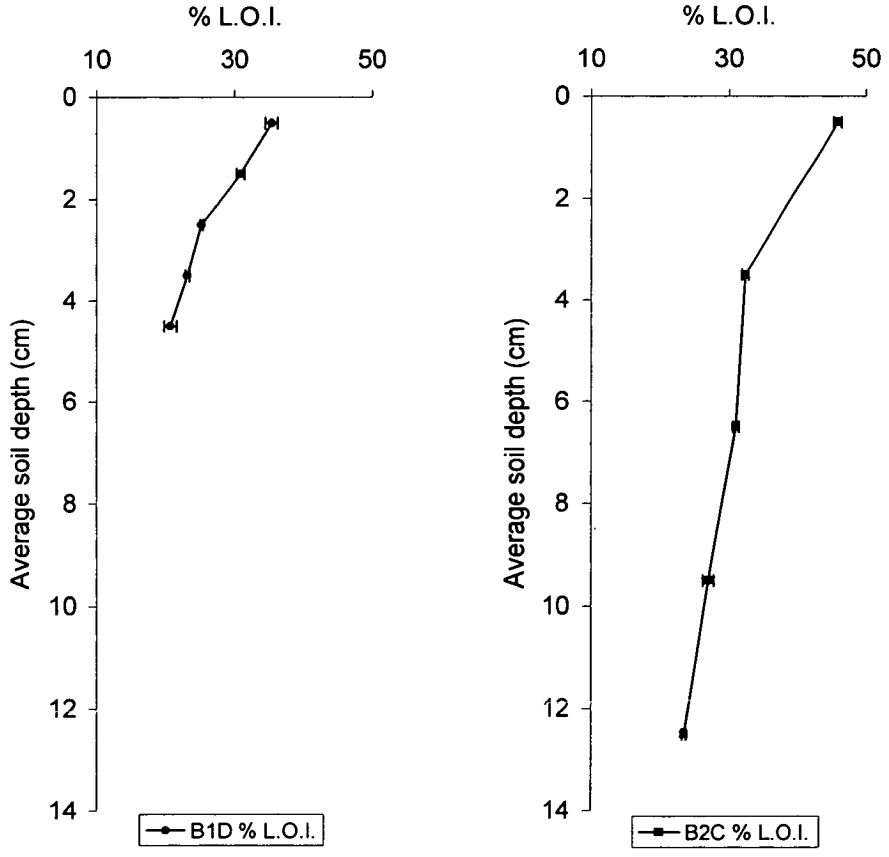


Figure 4.4: Variation of % L.O.I. of the deciduous broadleaf forest soil (B1D) and coniferous forest soil (B2C) samples with depth. Error bars of the 95 % confidence limit from the mean of the triplicate subsample analyses are shown.

4.1.3 Effective (in situ organo-mineral aggregate) and absolute (disaggregated mineral) soil particle size

The rationale for particle size determination was to assign a textural class to the deciduous broadleaf (B1D) and coniferous (B2C) forest soils and to investigate the involvement and influence of organic matter in soil aggregate formation and soil particle size distribution. The term absolute, with respect to particle size distribution, refers to the distribution of mineral particle size, i.e. after treatment of the soil to remove organic matter, whilst the term effective refers to the distribution of particle size in the soil environment, i.e. no treatment to break down aggregates. The data are obtained in the form of cumulative percentages of the total sample volume for 65 given particle sizes, ranging from diameters of 0 to 900 μm.

The absolute particle size data obtained for each soil subsample (duplicate measurement of single subsamples) were converted to a percentage of the total sample volume within the particle size classes of clay, silt and sand, according to the European system of particle size classification (Section 1.4). The absolute particle size data for the deciduous broadleaf and coniferous forest soils are presented in Section 8.11.

The ranges of percentage clay, silt and sand particle size classes found for all the pretreated, duplicate deciduous broadleaf and coniferous forest, soil samples are summarised in Table 4.9. It was found that all soil depth samples of both the deciduous broadleaf and coniferous forest soils corresponded to a soil textural class of silty clay loam (Table 4.10).

B1D		B2C	
Particle size class	**Range (%)**	**Particle size class**	**Range (%)**
Clay	22-29	Clay	19-30
Silt	60-65	Silt	59-69
Sand	6-17	Sand	8-14

Table 4.9: The ranges of percent particle size classes clay, silt and sand found in the deciduous broadleaf (B1D) and coniferous (B2C) forest soil samples (absolute particle size data)

Particle size class	Range (%)
Clay	18-35
Silt	46-82
Sand	0-20

Table 4.10: The ranges of percent particle size classes clay, silt and sand that correspond to the soil textural class of silty clay loam (based on the UK triangular diagram, Section 1.4)

Particle size (effective and absolute) distributions, typical of the deciduous broadleaf and coniferous forest soil depth section samples, are presented as histograms in Figure 4.5 and Figure 4.6, respectively. The effective particle size distributions for the deciduous broadleaf and coniferous forest soils show two peaks centred around ~ 0.3 μm and ~ 200-300 μm (skewed toward ~ 60-70 μm) particle size diameters. The peak at ~ 0.3 μm represents a total sample volume of less than 5 %, whilst the peak at ~ 200-300 μm represents approximately 95 % of the total sample volume. There is a small shift in the broad peak at ~ 200-300 μm, toward particle size of ~ 60-70 μm, with increasing soil depth. The absolute particle size distributions for both the deciduous broadleaf and coniferous forest soils show four peaks centred around ~ 0.2-0.3 μm, ~ 20-30 μm, ~ 3 μm and ~100-200 μm particle size diameters. The peaks at ~ 0.2-0.3 and ~ 100-200 μm both represent approximately 10 % of the total sample volume, whilst the peaks at ~ 3 μm and ~ 20-30 μm each represent approximately 40 % of the total sample volume. There is a small shift in the peak at ~ 20-30 μm, towards that at ~3 μm, with increasing soil depth.

The effective and absolute particle size distribution ranges, representing 80 % of the total sample volume (about the median volume), are presented in Table 4.11 (B1D) and Table 4.12 (B2C). The duplicate data the mean ranges are based on are presented in Section 8.11. The effective particle size distribution for the deciduous broadleaf soil (B1D) generally decreased with depth from 24.2±4.2 - 410±51 μm to 12.2±2.6 - 334±110 μm (0.5-4.5 cm depth). The absolute particle size distribution for B1D also generally decreased with depth from 0.60±0.03 - 109±71 μm to 0.51±0.01 - 55±4 μm (0.5 to 4.5 cm depth). The effective and absolute particle size distributions for the coniferous soil (B2C) were similar to those for the deciduous

broadleaf soils, at 21.1±6.2 - 405±67 μm to 10.8±1.7 - 344±37 μm (effective) and 0.60±0.02 - 68±10 μm to 0.43±0.02 - 54±14 μm (absolute) for soil depths 0.5-12.5 cm. Both the deciduous broadleaf and coniferous forest soil effective and absolute particle size distributions showed small shifts toward decreases in particle size with increasing depth, i.e. a decrease in mineral particle and aggregate size with depth.

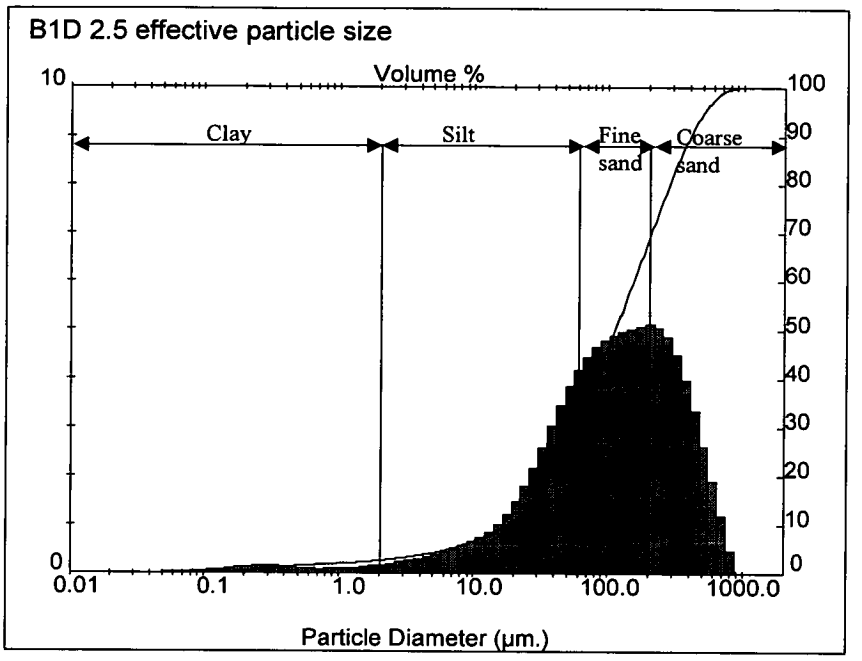


Figure 4.5: Histogram of effective particle size distribution of the deciduous broadleaf forest soil B1D 2.5 cm sample, as typical of all depth samples of both the deciduous broadleaf and coniferous forest soil samples. The cumulative percent total sample volume is represented by the superimposed line graph.

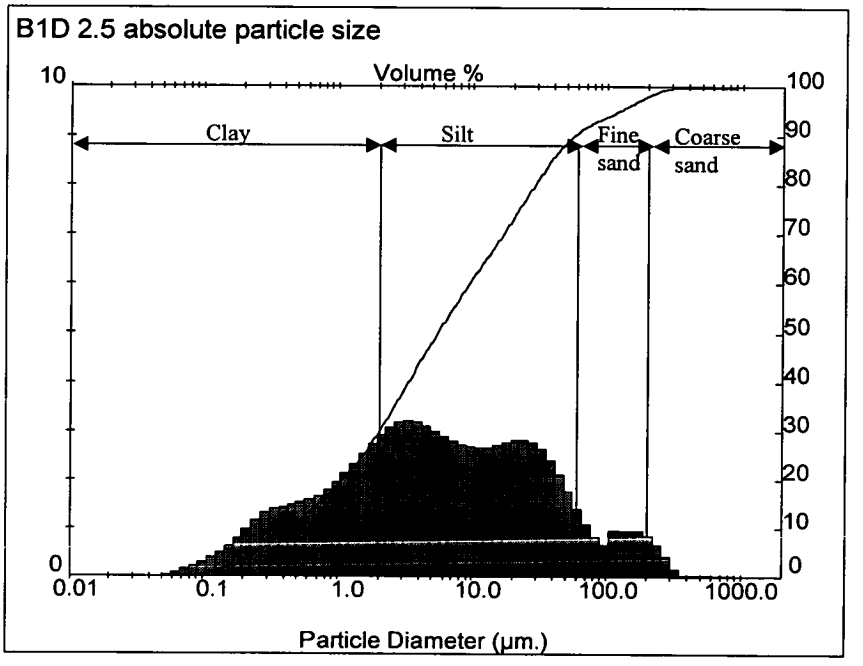


Figure 4.6: Histogram of absolute particle size distribution of the deciduous broadleaf forest soil B1D 2.5 cm sample, as typical of all depth samples of both the deciduous broadleaf and coniferous forest soil samples. The cumulative percent total sample volume is represented by the superimposed line graph.

Average soil depth (cm)	Absolute particle size distribution (μm)		Effective particle size distribution (μm)	
	90 % sample volume particle size	10 % sample volume particle size	90 % sample volume particle size	10 % sample volume particle size
0.5	0.60 ± 0.03	109 ± 71	24.2 ± 4.2	413 ± 51
1.5	0.50 ± 0.02	120 ± 12	19.2 ± 4.3	347 ± 97
2.5	0.47 ± 0.04	61 ± 9	15.6 ± 4.0	337 ± 126
3.5	0.50 ± 0.01	63 ± 5	14.2 ± 4.6	353 ± 126
4.5	0.51 ± 0.01	55 ± 4	12.2 ± 2.6	334 ± 110

Table 4.11: The distribution (90 % to 10 % of the total sample volume with particle sizes above the given values) of particle sizes (effective and absolute) of the deciduous broadleaf soil block B1D depth section samples and respective 95 % confidence limit values for the duplicate measurements of these single subsamples.

Average soil depth (cm)	Absolute particle size distribution (μm)		Effective particle size distribution (μm)	
	90 % sample volume particle size	10 % sample volume particle size	90 % sample volume particle size	10 % sample volume particle size
0.5	0.60 ± 0.02	68 ± 10	21.1 ± 6.2	405 ± 67
3.5	0.44 ± 0.02	59 ± 5	9.7 ± 1.2	366 ± 74
6.5	0.41 ± 0.04	61 ± 1	10.2 ± 2.1	387 ± 90
9.5	0.43 ± 0.02	60 ± 1	13.1 ± 1.0	364 ± 14
12.5	0.43 ± 0.02	54 ± 14	10.8 ± 1.7	344 ± 37

Table 4.12: The distribution (90 % to 10 % of the total sample volume with particle sizes above the given values) of particle sizes (effective and absolute) of the coniferous soil block B2C depth section samples and respective 95 % confidence limit values for the duplicate measurements of these single subsamples.

4.2 VISUAL DESCRIPTION AND HUMIC CONCENTRATION OF FOREST SOIL SOLVENT AND ELECTROPHORETIC EXTRACTS

This section describes the characteristics of the humic extracts obtained from the deciduous broadleaf (B1D) and coniferous (B2C) forest soils by the traditional solvent and novel electrophoretic extraction methods. Traditional solvent extraction of soil using 0.1 N NaOH was used to obtain 'total' soil humic substances, whilst 0.045 M Tris-borate was used to obtain a humic extract for direct comparison with the electrophoretic humic extract (0.045 M Tris-borate running buffer). Comparisons of the three types of extracts were made on the basis of extract colour, concentration of humic substances (UV-visible absorbance index), and of associated ^{238}U (Section 4.4).

4.2.1 Description of the solvent humic extracts of forest soils

The 0.1 N sodium hydroxide solvent extracts of the deciduous broadleaf soil block (B1D) were gold/yellow in colour, with a slight green hue. The intensity of the colour of the extracts decreased with soil sample depth. By comparison the sodium hydroxide extracts of the coniferous (B2C) soil block were all dark brown and a difference between samples of differing soil depth could not be discerned.

The 0.045 M tris-borate extracts of the deciduous broadleaf soil samples ranged in colour from gold for the subsurface soil B1D 0.5 cm, then decreased in intensity with depth, to light gold for sample B1D 4.5 cm. The colour intensity of the Tris-borate extracts of the coniferous soil, in contrast to that of the deciduous broadleaf extracts, decreased obviously with depth: orange-brown, orange, yellow, light yellow and pale yellow for samples B2C 0.5, 3.5, 6.5, 9.5 and 12.5 cm, respectively.

4.2.2 Description of the electrophoretic humic extracts of forest soils

During electrophoretic extraction migration of a 'focused' brown band of extract in the direction of the anode was observed. The focused band was only observed when a discontinuous buffer system was used (Section 3.1.4). In this study the discontinuity was between the loading buffer (Tris-HCl) and the agarose gel and running buffers (Tris-borate) of the electrophoretic system. Pigmented (brown-yellow) extract was observed to extend back, from behind the brown band, to the well. Pigmented extract was typically observed in the 1 cm fractions F1 to F6, and possibly extended into F7. Upon viewing the fractionated extract under UV light, fluorescent material was observed in fractions F6 and F7. The intensity was greatest in fraction F6. A general description of the electrophoretic extract fractions is given in Table 4.13. A colour photograph of the electrophoretic extract of coniferous soil sample B2C 0.5 cm, and a black and white photograph of the same extract fluorescing under UV light, is shown in Figure 4.7.

B1D	
Fraction number	**Description of Electrophoretic Extract**
	Average soil depths (cm): 0.5-4.5
F1-F6/F7	Extent of visually observed pale brown colouration of gel
F4	Darkest colouration (brown focused band fraction)
F6	Brightest UV-fluorescent fraction (focused band)
F7	Fluorescence only observed
F8-F10	No visible brown colouration. Possibly weak UV-flourescence

B2C	
Fraction number	**Description of Electrophoretic Extract**
	Average soil depth (cm): 0.5
F1-F6/F7	Extent of visually observed deep orange-brown colouration of gel
F3	Darkest colouration (brown focused band fraction)
F6	Brightest UV-fluorescent fraction (focused band)
F7	Fluorescence only observed
F8-F10	No visible brown colouration. Possibly weak UV-fluorescence
	Average soil depths (cm): 3.5-12.5
F1-F10	As for above except that the extract was pale brown in colour and the position of the brown band was ambiguous because of the low visually observed sample colour intensity

Table 4.13: General description of electrophoretic extracts of deciduous (B1D) and coniferous (B2C) forest soils

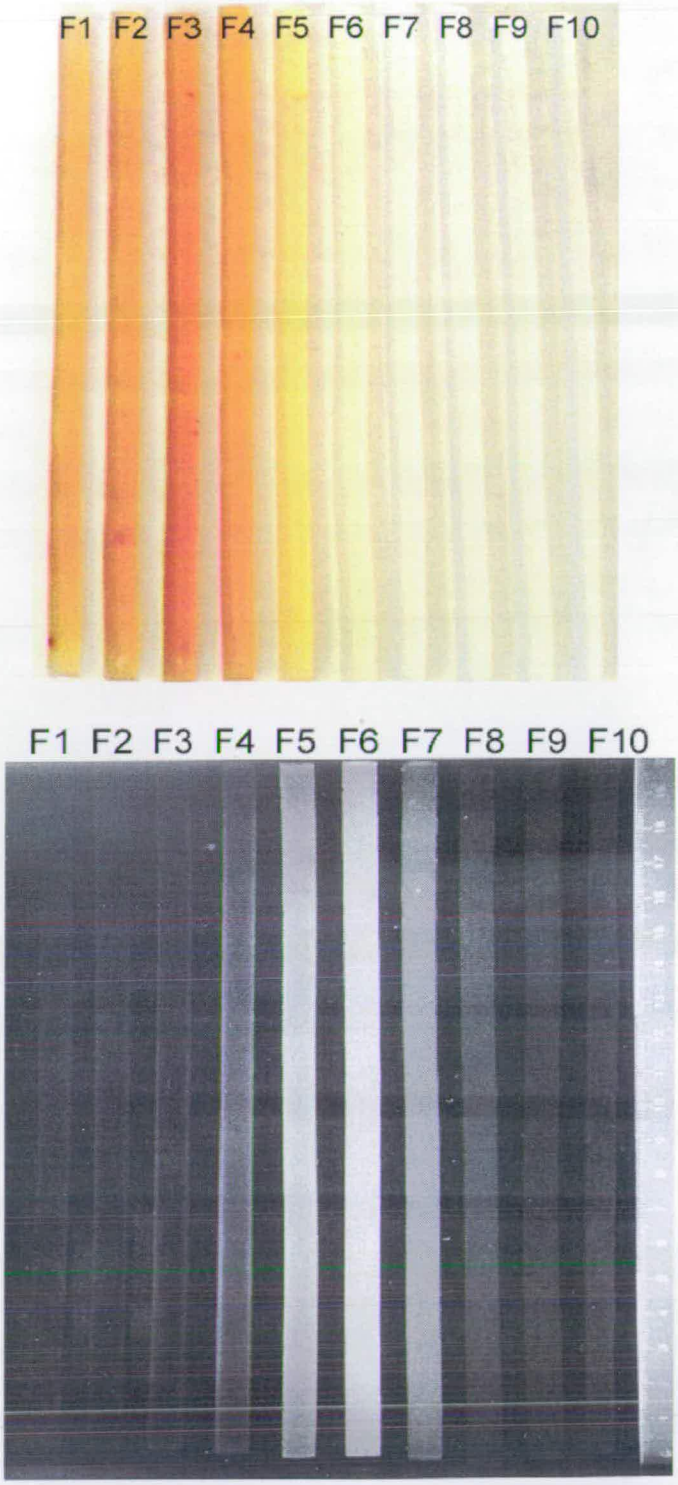


Figure 4.7: Photographs of electrophoretic extract of coniferous soil B2C 0.5 cm in visible (top) and UV (bottom) light.

4.2.3 Absorbance index ($A_{254\ nm}$) values of the forest soil solvent and electrophoretic humic extracts

Absorbance of extracts at 254 nm ($A_{254\ nm}$) was used as a measure of the concentration of humic substances. The absorbance data were converted to an absorbance index (A.I. = $A_{254\ nm}$ (normalised)) in which the data were corrected for dilution of the extract prior to measurement, and normalised for mass of soil extracted and the final extract volume according to Equations 4.1 and 4.2. Absorbance data were normalised for the extraction of 1 g of soil and a final extract volume of 100 ml. The raw data are presented in Section 8.14.

$$A_{254\,nm}\ (\text{corrected}) = \frac{A_{254\,nm}\ (\text{a.u.})}{\text{dilution factor}} \qquad \text{Equation 4.1}$$

$$\text{A.I.} = A_{254\,nm}\ (\text{normalised}) = \frac{A_{254\,nm}\ (\text{corrected}) \times 100\ \text{ml}}{\text{mass of soil (g)} \times \text{final extract volume (ml)}} \qquad \text{Equation 4.2}$$

The absorbance index values of the humic extracts obtained by solvent (0.1 N NaOH and 0.045 M Tris-borate) and electrophoretic extraction of the deciduous broadleaf (B1D) and coniferous (B2C) forest soils are shown in Table 4.14 and Table 4.15. The humic substance yield of extracts, obtained by all methods, for both the deciduous broadleaf and coniferous forest soils, decreased with increasing soil depth. All Tris-borate humic substance yields were lower than those obtained by solvent extraction using sodium hydroxide extracts. The electrophoretic extract yields (sum of the individual extract fraction, F1-F10, absorbance indices) of humic substances showed greatest similarity to the Tris-borate solvent extract yields. The electrophoretic extract yields of the deciduous broadleaf 0.5 cm and 2.5 cm soil depth samples were slightly lower than those obtained by Tris-borate solvent extraction (electrophoresis, A.I. 20 and 17 *cf.* Tris-borate, A.I. 38 and 34; soil depths 0.5 cm and 2.5 cm, respectively). Conversely the electrophoretic extract yields of the coniferous 0.5 cm and 12.5 cm soil depth samples were slightly higher than those obtained by Tris-borate solvent extraction (electrophoresis, A.I. 74 and 6 *cf.* Tris-borate, A.I. 58 and 5; soil depths 0.5 cm and 12.5 cm, respectively). The depth trends of humic substance yield using the solvent and electrophoretic extraction

methods are reported in Sections 4.2.3.1 and 4.2.3.2, respectively. The patterns of humic substance concentration with the individual electrophoretic extract fractions are presented in Section 4.2.3.3. Finally, the extractability of humic substances with increasing soil depth using the different extraction methods is described in Section 4.2.3.4.

Average soil depth (cm)	0.1 N NaOH solvent extract	0.045 M Tris-borate solvent extract	Electrophoretic extract
0.5	105.6	38.4	20.1
1.5	103.6	42.5	n.d.
2.5	75.4	33.9	17.2
3.5	63.1	25.8	n.d.
4.5	57.1	20.5	n.d.

Table 4.14: Absorbance Index (254 nm) of electrophoretic, 0.1 N NaOH and 0.045 M Tris-borate solvent extracts of deciduous broadleaf (B1D) soil samples. (n.d. – not determined).

Average soil depth (cm)	0.1 N NaOH solvent extract	0.045 M Tris-borate solvent extract	Electrophoretic extract
0.5	117.1	58.1	74.4
3.5	90.1	30.8	n.d.
6.5	80.2	14.0	n.d.
9.5	72.8	8.7	n.d.
12.5	61.0	4.7	5.7

Table 4.15: Absorbance Index (254 nm) of electrophoretic, 0.1 N NaOH and 0.045 M Tris-borate solvent extracts of coniferous soil (B2C) samples. (n.d. – not determined).

4.2.3.1 Absorbance of the solvent humic extracts of the forest soils

The depth trends of the humic extract concentration of the deciduous broadleaf and coniferous soils were different. However, the trend for either the deciduous broadleaf or coniferous soil was similar regardless of the solvent, 0.045 M Tris-borate or 0.1 N NaOH. The absorbance index values of the deciduous broadleaf (B1D) soil solvent extracts using both 0.1 N NaOH and 0.045 M Tris-borate show a decrease from the 1.5 cm sample downward (Figure 4.8). The absorbance index values of the coniferous (B2C) soil showed a decrease with depth from the surface (0.5 cm) soil downward (Figure 4.8).

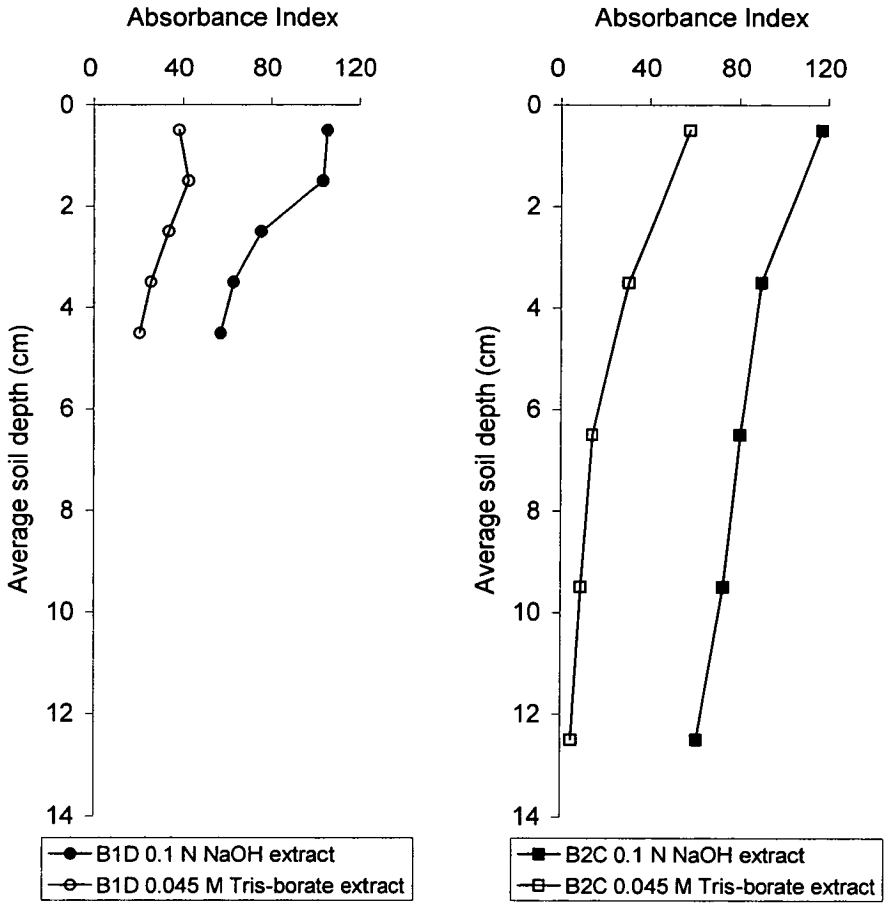


Figure 4.8: Variation of absorbance index values of the 0.1 N NaOH and 0.045 M Tris-borate solvent humic extracts of the deciduous broadleaf soil block B1D and coniferous soil block B2C

4.2.3.2 Absorbance patterns of the electrophoretic humic extracts of the forest soils

The electrophoretic extract fraction absorbance patterns of deciduous broadleaf (B1D 0.5 and 2.5 cm) and coniferous (B2C 0.5 and 12.5 cm) samples are shown in Figure 4.9 to Figure 4.12. Only small differences in total extract absorbance index (14 % reduction Table 4.14) and minor differences in the pattern across the electrophoresis gel were observed for the two deciduous samples, 0.5 cm (Figure 4.9) and 2.5 cm (Figure 4.10). The absorbance index values increased across the length of the gel, to a maximum at fraction F4 that corresponded to ~21% of the total extract humic concentration. Fractions F3 to F5 had similar absorbance index values, each representing approximately 20-21% of the total extract humic concentration. After fraction F5, the absorbance index decreased sharply toward fractions F8 to F10. The

changes, over the greater depth range presented by the coniferous soil samples, were much more apparent. A 92 % reduction in total extract absorbance index was observed between the 0.5 cm and 12.5 cm samples as well as distinct changes in the absorbance index pattern across the gel extracts of these two soil samples. Of particular importance was the shift in the maximum absorption from F3 (0.5 cm) to F5 (12.5 cm). This suggested a decrease in molecular mass or a decrease in mass/charge ratio of the humic macromolecules with increasing soil depth.

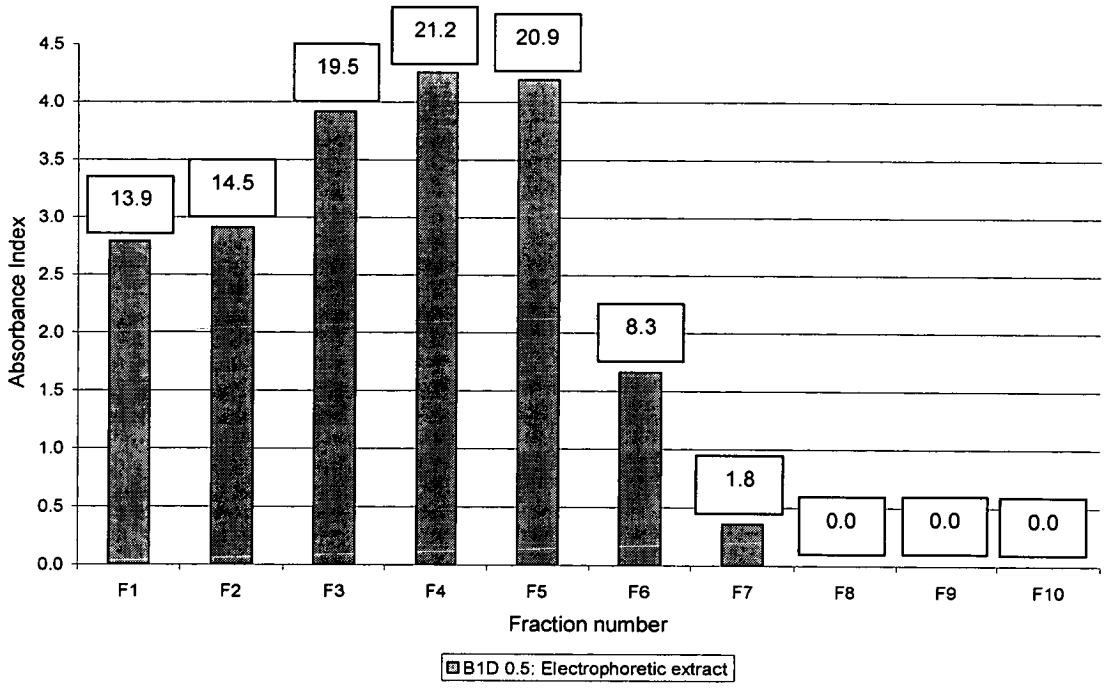


Figure 4.9: Absorbance index values of the individual electrophoretic humic extract fractions of deciduous broadleaf soil sample B1D 0.5 cm. The values in the boxes indicate the percentage of the total extract humic concentration contained within each of the individual extract fractions.

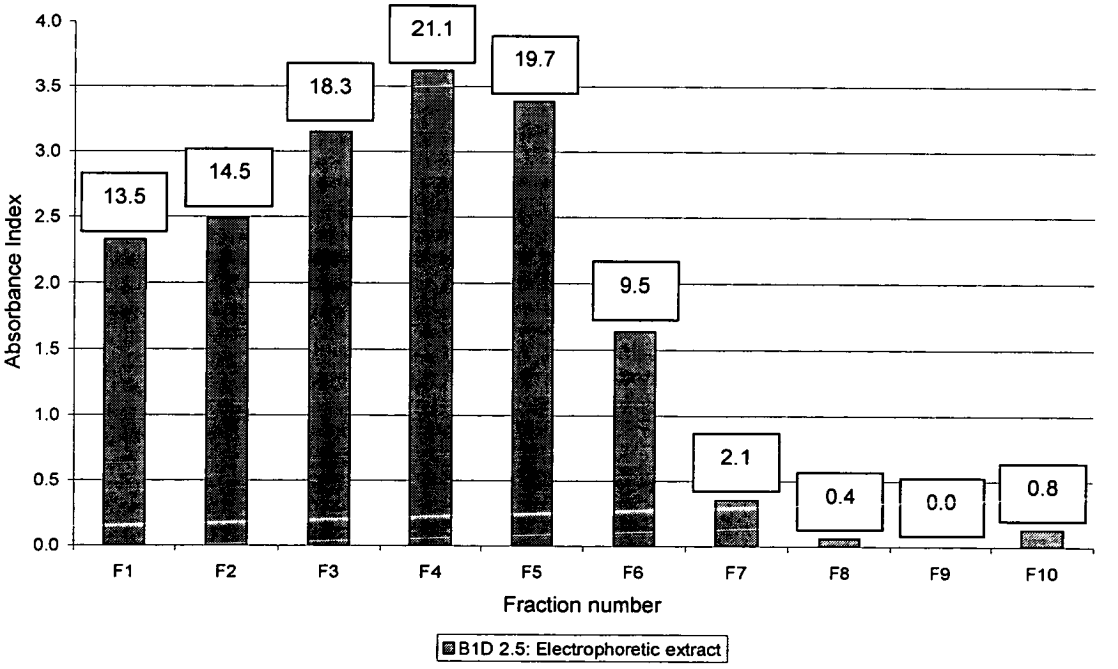


Figure 4.10: Absorbance index values of the individual electrophoretic humic extract fractions of deciduous broadleaf soil sample B1D 2.5 cm. The values in the boxes indicate the percentage of the total extract humic concentration contained within each of the individual extract fractions.

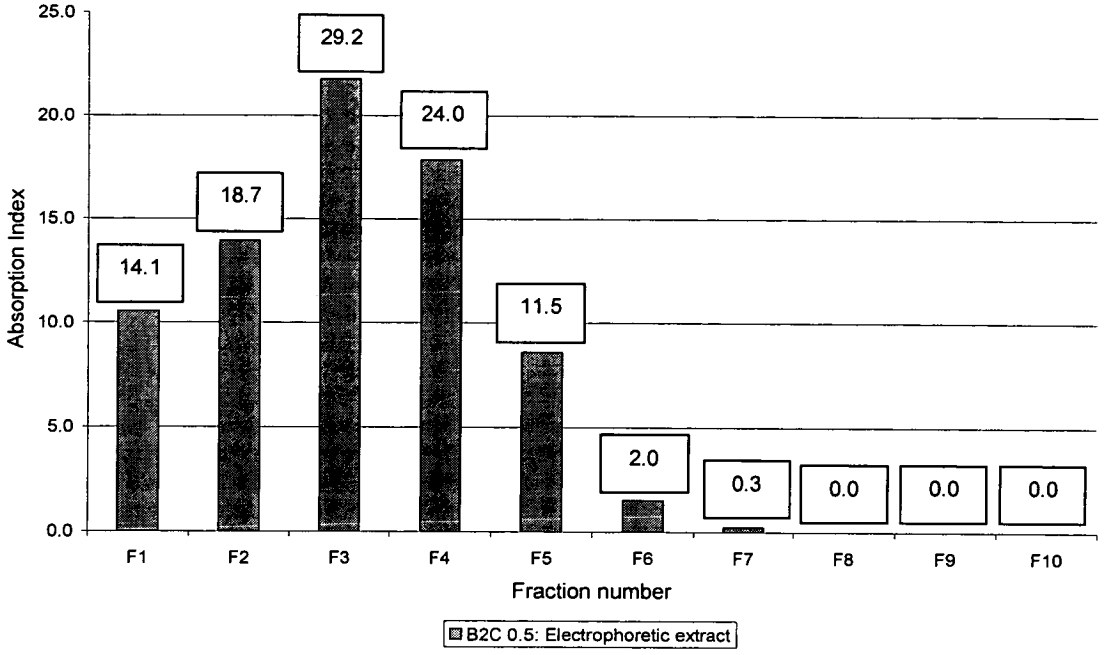


Figure 4.11: Absorbance index values of the individual electrophoretic humic extract fractions of coniferous soil sample B2C 0.5 cm. The values in the boxes indicate the percentage of the total extract humic concentration contained within each of the individual extract fractions.

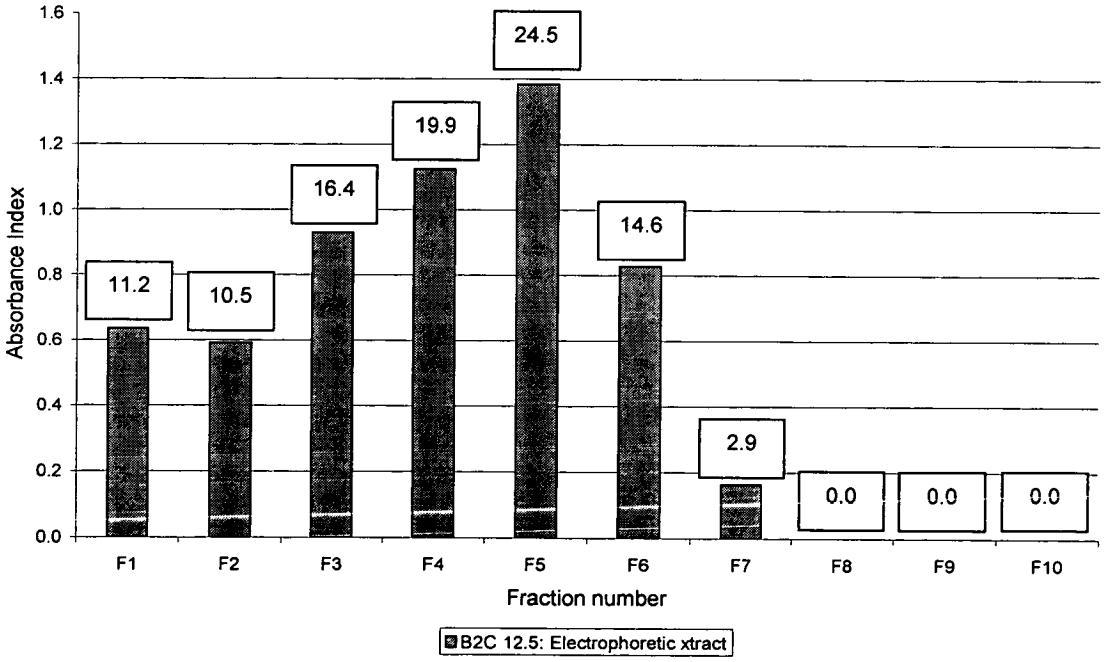


Figure 4.12: Absorbance index values of the individual electrophoretic humic extract fractions of coniferous soil sample B2C 12.5 cm. The values in the boxes indicate the percentage of the total extract humic concentration contained within each of the individual extract fractions.

4.2.3.3 Relative efficiency of the solvent and electrophoretic extraction methods

Based on absorption index, the concentration of humic substances extracted using 0.045 M Tris-borate solvent, and electrophoretic extraction, was compared with that obtained by the traditional method of 0.1 N NaOH solvent extraction (Table 4.16 and Table 4.17, respectively). Direct comparison of 0.045 M Tris-borate as a solvent per se and electrophoretic extraction (using the same solvent as running buffer) was also made (Table 4.16 and Table 4.17).

Average soil depth (cm)	Tris-borate /NaOH	Electrophoresis /NaOH	Electrophoresis /Tris-borate
0.5	36.4	19.0	52.3
1.5	41.1	n.d.	n.d.
2.5	44.9	22.8	50.6
3.5	40.8	n.d.	n.d.
4.5	36.0	n.d.	n.d.

Table 4.16: The relative efficiency of the solvent (0.1 N NaOH and 0.045 M Tris-borate) and electrophoretic methods for the extraction of humic substances from deciduous broadleaf forest soil B1D. (n.d. not determined).

Average soil depth (cm)	Tris-borate /NaOH	Electrophoresis /NaOH	Electrophoresis /Tris-borate
0.5	49.6	63.5	128.1
3.5	34.2	n.d.	n.d.
6.5	17.5	n.d.	n.d.
9.5	12.0	n.d.	n.d.
12.5	7.7	9.3	121.3

Table 4.17: The relative efficiency of the solvent (0.1 N NaOH and 0.045 M Tris-borate) and electrophoretic methods for the extraction of humic substances from coniferous forest soil B2C. (n.d. not determined).

For the deciduous broadleaf soil samples 0.045 M Tris-borate solvent extraction of humic substances produced an extract with yield 36-45 % of that obtained by solvent extraction with 0.1 N NaOH ('total' humic substances). The percentage of the 'total' humic substances extracted by 0.045 M Tris-borate from the deciduous broadleaf soil samples was similar for each soil depth section sample. This reflected the individual trends of the 0.045 M Tris-borate and 0.1 N NaOH extract yields, which demonstrated similar trends of decreasing humic concentration with increasing soil depth (Figure 4.8). Although there was a similar trend with increasing depth in the coniferous forest soil (Figure 4.8), the relative efficiency of 0.045 M Tris-borate

extraction relative to 0.1 N NaOH decreased significantly with depth, from ~ 50 % (0.5 cm) to ~ 8 % (12.5 cm) (Table 4.17).

Comparison of electrophoretic extraction with 0.1 N NaOH extraction revealed two contrasting situations. For the deciduous broadleaf forest soil, the absorbance index value of the electrophoretic extract was only ~ 19-23 % of that of the 0.1 N NaOH extract, whereas, for the coniferous forest soil, values of ~ 64 % (0.5 cm) and ~ 9 % (12.5 cm) were obtained. As illustrated directly by the relative efficiency of electrophoretic and Tris-borate extraction, electrophoresis appears to be significantly less efficient (~ 51-52 %) for the deciduous broadleaf soil but slightly more efficient (~ 121-128 %) for the coniferous soil.

4.2.3.4 Extractability of humic substances with increasing soil depth

The extractability of humic substances with increasing soil depth, relative to the surface 0.5 cm soil, was calculated using the absorbance index values. Table 4.18 shows that the extractability of humic substances (both solvent methods) from the deciduous broadleaf soil (B1D) decreased with increasing soil depth, reaching a final humic concentration of ~ 54 % of that of the surface 0.5 cm soil. The final humic concentration for the coniferous soil obtained using the 0.1 N NaOH extraction was also similar, at ~ 52 % of that of the surface 0.5 cm soil (Table 4.19). A larger decrease, however, was observed for both the 0.045 M Tris-borate and electrophoretic extraction methods (both ~ 7-8 % of the surface 0.5 cm soil) (Table 4.19).

Ratio of average soil depths (cm:cm)	NaOH	Tris-borate	Electrophoresis
1.5:0.5	98.1	110.7	n.d.
2.5:0.5	71.5	88.2	85.4
3.5:0.5	59.8	67.1	n.d.
4.5:0.5	54.1	53.4	n.d.

Table 4.18: Extractability of humic substances (using solvent and electrophoretic methods) from deciduous broadleaf soil B1D with increasing soil depth. (n.d. – not determined).

Ratio of average soil depths (cm:cm)	NaOH	Tris-borate	Electrophoresis
3.5:0.5	76.9	53.0	n.d.
6.5:0.5	68.5	24.1	n.d.
9.5:0.5	62.2	15.1	n.d.
12.5:0.5	52.1	8.0	7.6

Table 4.19: Extractability of humic substances (using solvent and electrophoretic methods) from coniferous soil B2C with increasing soil depth. (n.d. – not determined).

4.3 CHARACTERISATION OF THE FOREST SOIL ELECTROPHORETIC HUMIC EXTRACTS

Selected electrophoretic extracts were analysed by gel filtration chromatography (GFC) to determine the relative molecular size of the brown and UV-fluorescent band components. This was in order to establish the basis of the electrophoretic fractionation of soil humic substances. The functional group character of the brown and UV-fluorescent band components was investigated using Fourier Transform Infra-red (FTIR) spectroscopy.

4.3.1 Gel filtration chromatography (GFC) of electrophoretic humic extract fractions

The eluent masses of electrophoretic extract fractions eluted on the Sephadex G200 column are presented in Table 4.20. Sample ED1M-fluorescent was a UV-fluorescent fraction of an electrophoretically extracted organic-rich soil (Graham *et al.*, *in press*). The eluent mass of ED1M-fluorescent determined in this study was in good agreement with previous quadruplicate experiment results (Graham *et al.*, *in press*). The collected eluent mass which represented the exclusion volume of the column was 0.59 g. The eluent mass of the coniferous soil electrophoretic brown band fraction, B2C 0.5 cm F3, was in good agreement with that for the organic-rich soil brown band fraction. Furthermore, this fraction was observed to extend over the full length of the column during elution, with darkest pigmentation of the separated extract observed at the top end of the column. This result suggests that the brown band consists of a mixture of humic molecules with a broad range of sizes, extending from the exclusion volume (high molecular weight molecules) and skewed towards the much later eluting low molecular weight molecules. The UV-fluorescent band of the organic-rich soil extract was not as complex a mixture; a band rather than an

extended separated sample was observed to elute down the gel column, consisting of lower molecular weight humic molecules.

Sample	Eluent Mass (g)
ED1M fluorescent	1.48
B2C 0.5 F3	0.61
Blue dextran	0.59

Table 4.20: The eluent masses collected immediately prior to sample elution on a Sephadex G200 column (~ 10 cm x 0.5 cm diameter).

4.3.2 Fourier Transform Infra-red spectroscopy of electrophoretic humic extract fractions

A typical FTIR spectrum of the electrophoretic brown and UV-fluorescent band humic substances is shown in Figure 4.13. The absorption bands observed and assignments of organic molecule bond vibrations are detailed in Section 8.14. The principal regions of absorption of the electrophoretic extract fraction FTIR spectra were 3650-3200 cm^{-1} (peaks at ~3600, 3424 and 3275 cm^{-1}), 1720-1550 cm^{-1} (two sharp peaks near 1635 cm^{-1}) and 1210-990 cm^{-1} (two peaks at ~ 1135 and 1100 cm^{-1}). The functional groups assigned to the electrophoretic extract fraction absorption bands, based on the literature assignments of organic molecule bond vibrations (Table 8.54, Section 8.14) and humic substance FTIR studies (Table 8.55, Section 8.14), are summarised in Table 4.21.

Absorption band(s) (cm^{-1})	Functional group(s) assigned
~ 3600	Free OH
3424	H-bonded OH, or NH
3275	OH of COOH or COH (phenols and alcohols), or NH
1635, ~ 1270	COO^- (ionic coordination with $^*Na^+$)
1135	CO (aliphatic ethers)
1100	CO (polysaccharides, ethers) or OH (1° alcohols or polysaccharides)

Table 4.21: Principal Infra-red absorption bands and assigned functional groups of the electrophoretic humic extracts of the forest soils (deciduous broadleaf and coniferous). ($^*Na$ from sample preparation, Section 2.7.2).

The electrophoretic humic extract FTIR spectra show similarity to Type III humic spectra (Stevenson, 1994). Type III humic FTIR spectra are distinguished from other humic spectra by the presence of bands that indicate carbohydrate and protein structures. Type III spectra are typical of lake humic acids.

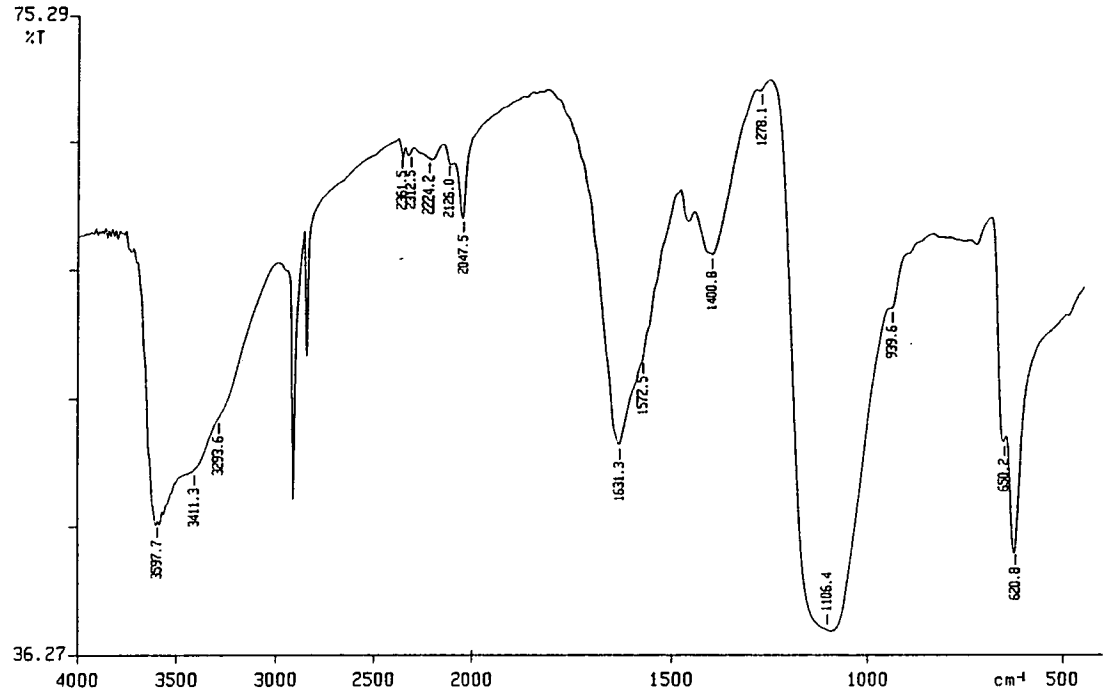


Figure 4.13: An FTIR spectrum typical of the electrophoretic extract brown and UV-fluorescent band components - coniferous soil brown band fraction B2C 0.5 cm F3

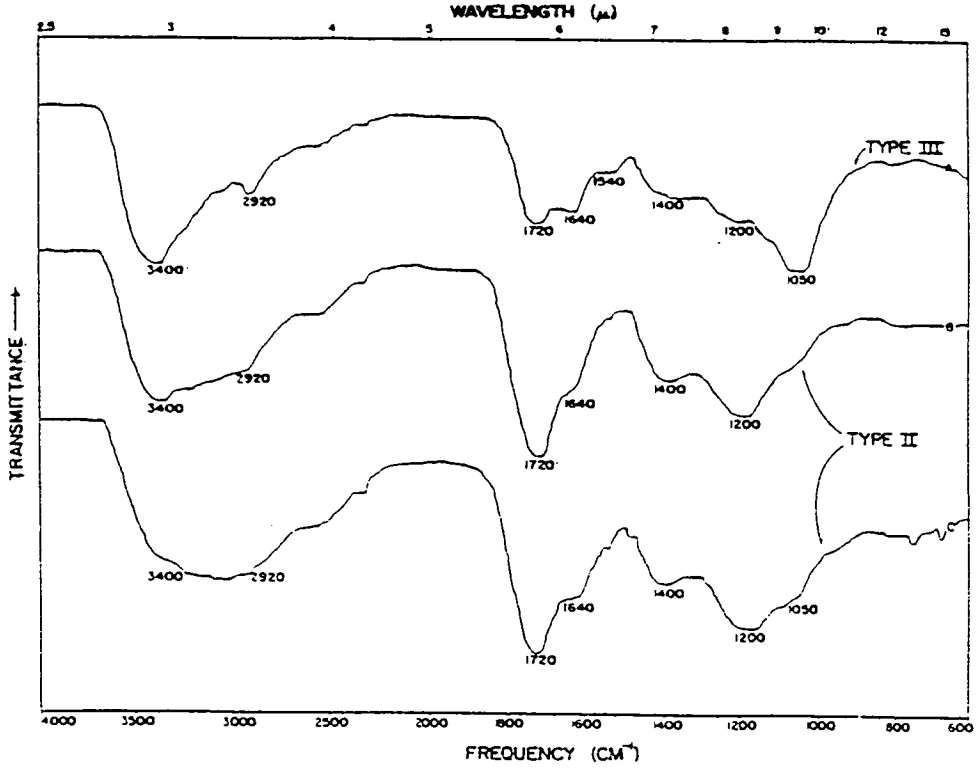


Figure 4.14: Type III humic substance Infra-red spectrum (a fulvic acid from a prairie grassland soil). From: Stevenson (1994).

4.4 ACTINIDE DISTRIBUTIONS AND ASSOCIATIONS IN DECIDUOUS (B1D) AND CONIFEROUS (B2C) FOREST SOILS

The 'pseudo-total' concentrations of ^{238}U, ^{237}Np, $^{238-240}Pu$ in soil from each of five depths of the two forest soil blocks, B1D and B2C, were first established. This was to determine the vertical distribution of ^{238}U (the most abundant naturally occurring U isotope; 99.2745 atomic %) in the soils and the extent of contamination of the soils with radionuclides from artificial sources (e.g. Np and Pu – fallout from nuclear weapons testing). Initial results showed that there was no detectable concentration of Np and Pu in any of the soil samples, and so only the results for ^{238}U are described in Section 4.4.1. The absence of detectable fallout Np and Pu suggests that these soils do not readily immobilise actinides. Following this, Section 4.4.2 illustrates the importance of humic substances with respect to complexation of ^{238}U as determined by analysis of extracts of 'total' humic substances (0.1 N NaOH extracts). This section also contains the results for 0.045 M Tris-borate and electrophoretic extraction of ^{238}U-humic complexes. The distributions of actinides, ^{238}U and added ^{236}U and ^{242}Pu, between the operationally defined soil actinide pools (immobile, hydrophilic-mobile and labile) are described Section 4.4.3. The associations of actinides (^{238}U, ^{236}U and ^{242}Pu) with the individual fractions of the hydrophilic-mobile (electrophoretic extract) are presented in Section 4.4.4. Finally, the recoveries of the actinides studied in the soil 'equilibration' and electrophoretic extraction experiments are reported in Section 4.4.5.

4.4.1 'Pseudo-total' concentrations of ^{238}U in deciduous broadleaf (B1D) and coniferous (B2C) forest soils

The average 'pseudo-total' concentrations (± 95 % confidence limit) of ^{238}U of each soil sample (triplicate analyses) from both B1D and B2C are presented in Table 4.22. The range of values was 407±14 - 497±133 μg ^{238}U (kg soil)$^{-1}$ and 513±75 - 611±36 μg ^{238}U (kg soil)$^{-1}$ for B1D and B2C, respectively. In general, the ^{238}U content of the coniferous soil depth section samples were greater than those of the deciduous broadleaf soil samples. Figure 4.15 shows the variation in ^{238}U concentration with increasing depth for both B1D and B2C. There was an apparent decrease in concentration of ^{238}U from 497±133 μg (kg soil)$^{-1}$ at 0.5 cm to 491±18 μg (kg soil)$^{-1}$

at 1.5 cm of the deciduous broadleaf soil but the large error on the average value for the 0.5 cm sample meant that the 95 % confidence limits for the two samples overlapped. Below 2.5 cm there was a decrease in concentration of ^{238}U to a minimum value of 407±14 μg (kg soil)$^{-1}$ at 4.5 cm. In contrast with the deciduous broadleaf soil, the ^{238}U concentration of the coniferous soil samples increased with increasing soil depth. For both soils, the variation in ^{238}U concentration in triplicate subsamples of soil was greatest in the surface section (0.5 cm).

B1D		B2C	
Average soil depth (cm)	**^{238}U (μg (kg soil)$^{-1}$)**	**Average soil depth (cm)**	**^{238}U (μg (kg soil)$^{-1}$)**
0.5	497 ± 133	0.5	513 ± 75
1.5	491 ± 18	3.5	527 ± 21
2.5	486 ± 28	6.5	549 ± 19
3.5	459 ± 16	9.5	574 ± 20
4.5	407 ± 14	12.5	611 ± 36

Table 4.22: The mean concentrations (and 95 % confidence limit) of ^{238}U (μg (kg soil)$^{-1}$) obtained by acid/peroxide digestion of ashed triplicate subsamples of soil from the deciduous broadleaf (B1D) and coniferous (B2C) forest soils

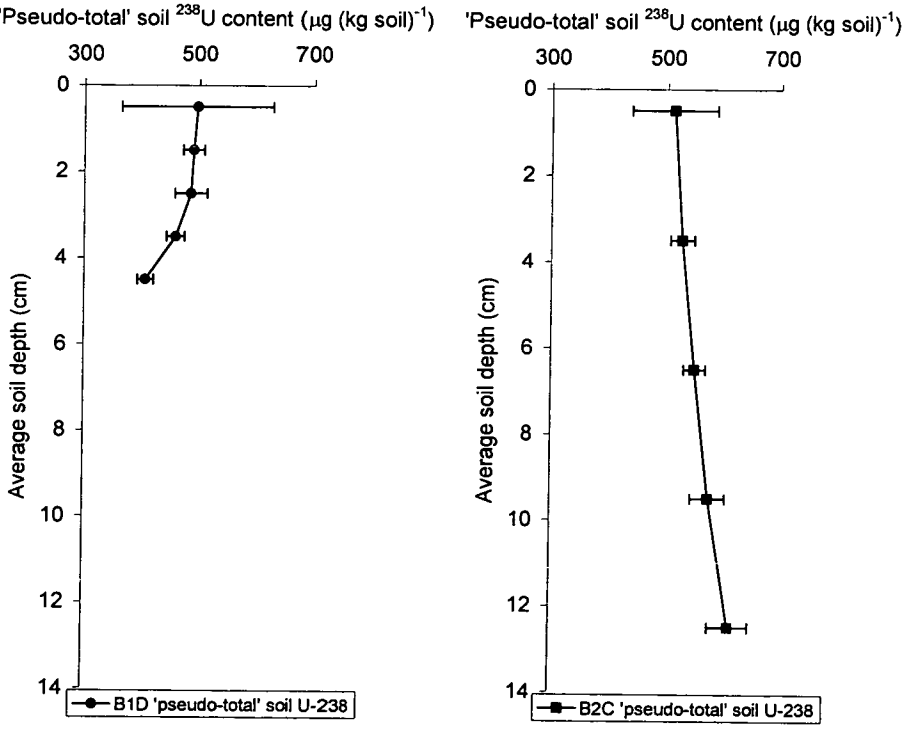


Figure 4.15: Variation of the mean 'pseudo-total' ^{238}U concentration (μg (kg soil)$^{-1}$) with increasing soil depth for the deciduous broadleaf (B1D) and coniferous (B2C) forest soils. Error bars represent the 95 % confidence limits about the mean value obtained from triplicate analyses.

4.4.2 Concentration of ^{238}U associated with the humic extracts of the deciduous broadleaf (B1D) and coniferous (B2C) forest soils

4.4.2.1 Association of ^{238}U with the 0.1 N NaOH humic extracts

The average concentrations of humic-associated ^{238}U extracted by 0.1 N NaOH from soil samples from both B1D and B2C are presented in Table 4.23 and Table 4.24. The range of values for B1D was 116±10 - 222±5 μg ^{238}U (kg soil)$^{-1}$, which was generally lower than that for B2C (257±10 - 354±27 μg ^{238}U (kg soil)$^{-1}$ ^{238}U). Comparison of the vertical profiles of humic-associated ^{238}U obtained by this extraction method further revealed differences between the two soils (Figure 4.16). For the deciduous broadleaf soil, a slight sub-surface maximum was observed at 1.5 cm, whilst for the coniferous soil there was a sub-surface minimum at 3.5-6.5 cm, and thereafter an increase in concentration with increasing soil depth. These trends are also observed in the vertical profiles where 0.1 N NaOH-extracted ^{238}U is expressed as a percentage of 'pseudo-total' ^{238}U (Table 4.23 and Table 4.24).

Average soil depth (cm)	^{238}U (μg (kg soil)$^{-1}$)	% of 'pseudo-total' soil ^{238}U
0.5	187 ± 30	38 ± 6
1.5	222 ± 5	45 ± 1
2.5	170 ± 2	35 ± 1
3.5	137 ± 13	30 ± 3
4.5	116 ± 10	29 ± 2

Table 4.23: The ^{238}U content (μg (kg soil)$^{-1}$ and % of the 'pseudo-total' soil ^{238}U) of the 0.1 N NaOH solvent extracts of the deciduous broadleaf (B1D) forest soil samples

Average soil depth (cm)	^{238}U (μg (kg soil)$^{-1}$)	% of 'pseudo-total' soil ^{238}U
0.5	333 ± 21	65 ± 4
3.5	257 ± 10	49 ± 2
6.5	258 ± 22	47 ± 4
9.5	310 ± 4	54 ± 1
12.5	354 ± 27	58 ± 4

Table 4.24: The ^{238}U content (μg (kg soil)$^{-1}$ and % of the 'pseudo-total' soil ^{238}U) of the 0.1 N NaOH solvent extracts of the coniferous (B2C) forest soil samples

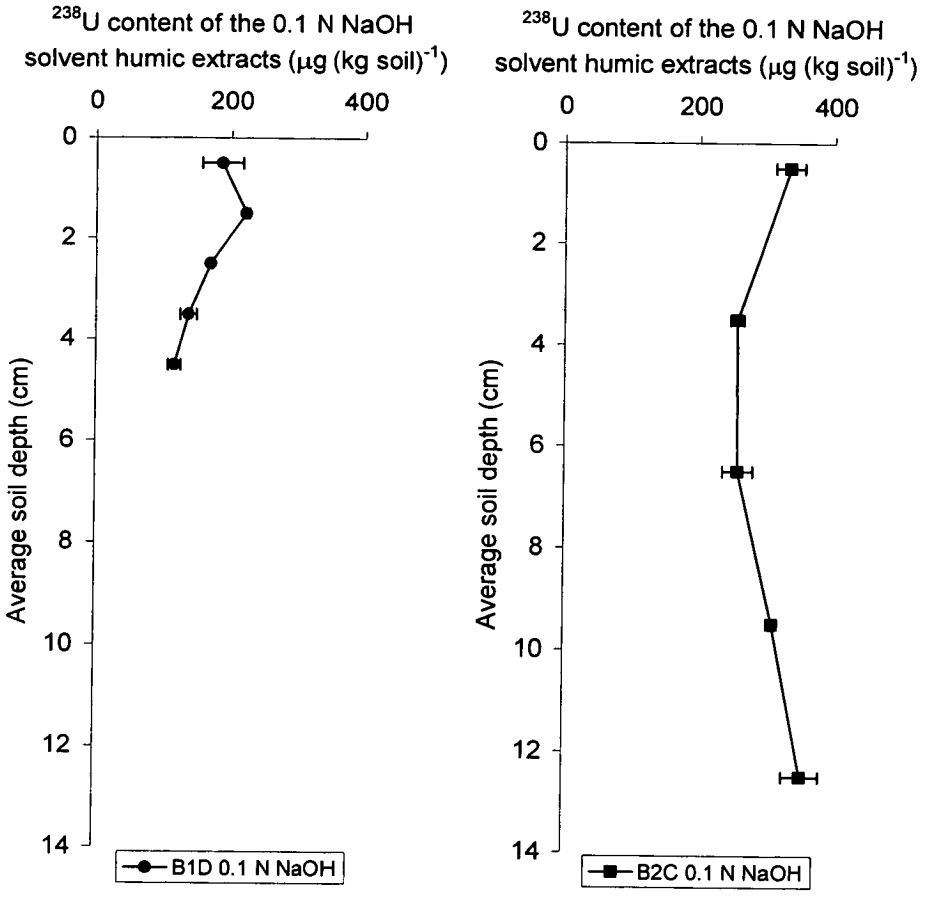


Figure 4.16: Variation in the ^{238}U content (µg (kg soil)$^{-1}$) of the 0.1 N NaOH solvent humic extracts of the deciduous broadleaf (B1D) and coniferous (B2C) forest soil samples

4.4.2.2 Association of ^{238}U with the 0.045 M Tris-borate humic extracts

The average concentrations of humic-associated ^{238}U extracted by 0.045 M Tris-borate from both B1D and B2C are presented in Table 4.25 and Table 4.26. The range of values for B1D was 72±11 - 95±15 µg ^{238}U (kg soil)$^{-1}$, whilst that for B2C was slightly wider at 26±1 - 90±8 µg ^{238}U (kg soil)$^{-1}$. These data contrast with the 0.1 N NaOH data, in that a greater proportion of 'pseudo-total' ^{238}U was extracted from B1D than from B2C. The distribution of humic-associated ^{238}U showed very little change with depth in B1D, but a sharp decrease from surface with depth in B2C (Figure 4.17).

Average soil depth (cm)	^{238}U (μg (kg soil)$^{-1}$)	% of 'pseudo-total' soil ^{238}U
0.5	90 ± 16	18 ± 3
1.5	81 ± 3	17 ± 1
2.5	95 ± 15	20 ± 3
3.5	75 ± 6	16 ± 1
4.5	72 ± 11	18 ± 3

Table 4.25: The ^{238}U content (μg (kg soil)$^{-1}$ and % of the 'pseudo-total' soil ^{238}U) of the 0.045 M Tris-borate solvent extracts of the deciduous broadleaf (B1D) forest soil samples

Average soil depth (cm)	^{238}U (μg (kg soil)$^{-1}$)	% of 'pseudo-total' soil ^{238}U
0.5	90 ± 8	17 ± 2
3.5	61 ± 3	12 ± 1
6.5	41 ± 1	7 ± 0
9.5	35 ± 1	6 ± 0
12.5	26 ± 1	4 ± 0

Table 4.26: The ^{238}U content (μg (kg soil)$^{-1}$ and % of the 'pseudo-total' soil ^{238}U) of the 0.045 M Tris-borate solvent extracts of the coniferous (B2C) forest soil samples

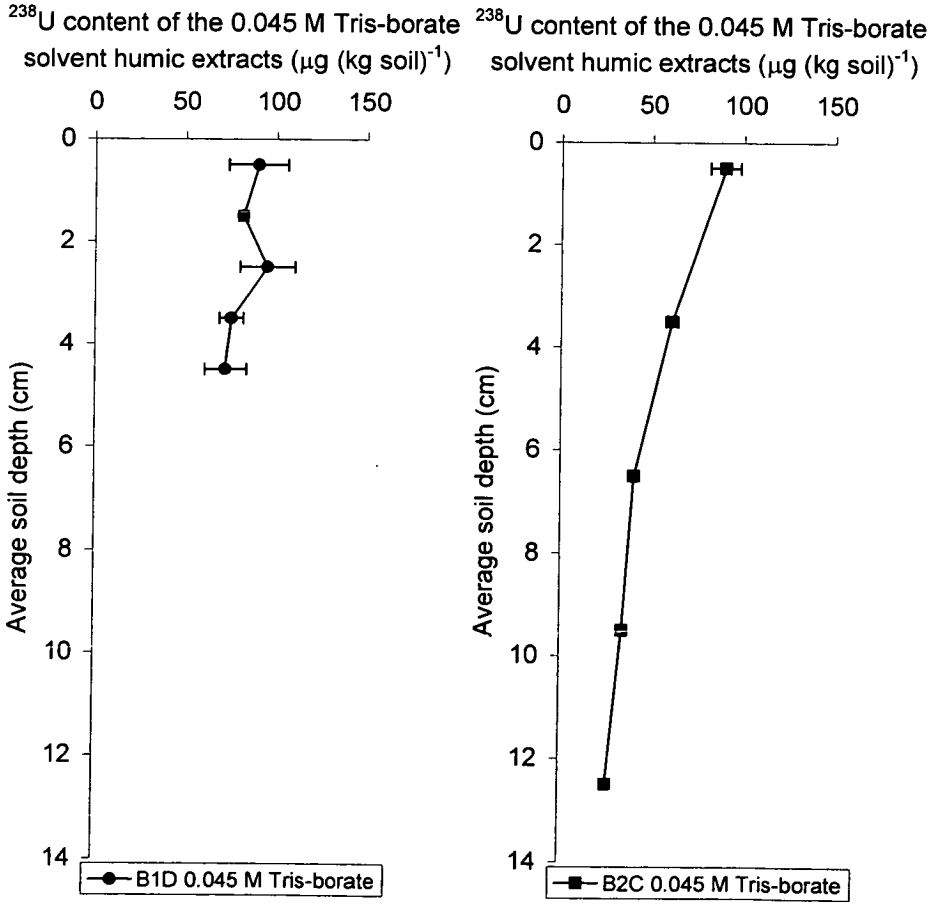


Figure 4.17: Variation in the ^{238}U content (μg (kg soil)$^{-1}$) of the 0.045 M Tris-borate solvent humic extracts of the deciduous broadleaf (B1D) and coniferous (B2C) forest soil samples

4.4.2.3 Association of ^{238}U with the electrophoretic humic extracts

The results for electrophoretic extraction of humic-associated ^{238}U are presented in Table 4.27 and Table 4.28. The range of values for B1D was 39±24-181±118 μg (kg soil)$^{-1}$, similar to the range of values for B2C (79±67-178±8 μg (kg soil)$^{-1}$. Heterogeneity in the composition of the soil, and the small sample size used, may have contributed to the relatively large 95 % confidence limit values. These values were larger for B1D than for B2C. For B2C, the percentage of 'pseudo-total' soil ^{238}U extracted by electrophoresis was intermediate between the percentages extracted by 0.1 N NaOH and 0.045 M Tris-borate. For B1D, however, the electrophoretic extract values were much closer to those obtained using 0.1 N NaOH than those for 0.045 M Tris-borate. The vertical profiles of electrophoretically extracted ^{238}U for B1D and B2C show very little change with depth, except that the surface B1D sample (0.5 cm) had a much lower electrophoretic extract concentration of ^{238}U than all subsequent B1D soil depth samples (Figure 4.18).

Average soil depth (cm)	^{238}U (μg (kg soil)$^{-1}$)	% of 'pseudo-total' soil ^{238}U
0.5	39 ± 24	13 ± 4
1.5	181 ± 118	37 ± 24
2.5	125 ± 95	29 ± 15
3.5	159 ± 20	35 ± 4
4.5	167 ± 2	41 ± 1

Table 4.27: The ^{238}U content (μg (kg soil)$^{-1}$ and % of the 'pseudo-total' soil ^{238}U) of the electrophoretic extracts of the deciduous broadleaf (B1D) forest soil samples. Error is presented as 95 % confidence limit values about the mean values for quintuplet (0.5 cm and 2.5 cm) and triplicate (1.5 cm, 3.5 cm and 4.5 cm) experiments.

Average soil depth (cm)	^{238}U (μg (kg soil)$^{-1}$)	% of 'pseudo-total' soil ^{238}U
0.5	99 ± 65	20 ± 12
3.5	178 ± 8	34 ± 2
6.5	136 ± 35	25 ± 6
9.5	176 ± 25	31 ± 4
12.5	79 ± 67	15 ± 9

Table 4.28: The ^{238}U content (μg (kg soil)$^{-1}$ and % of the 'pseudo-total' soil ^{238}U) of the electrophoretic extracts of the coniferous (B2C) forest soil samples. Error is presented as 95 % confidence limit values about the mean values for quintuplet (0.5 cm and 12.5 cm) and triplicate (3.5 cm, 6.5 cm and 9.5 cm) experiments.

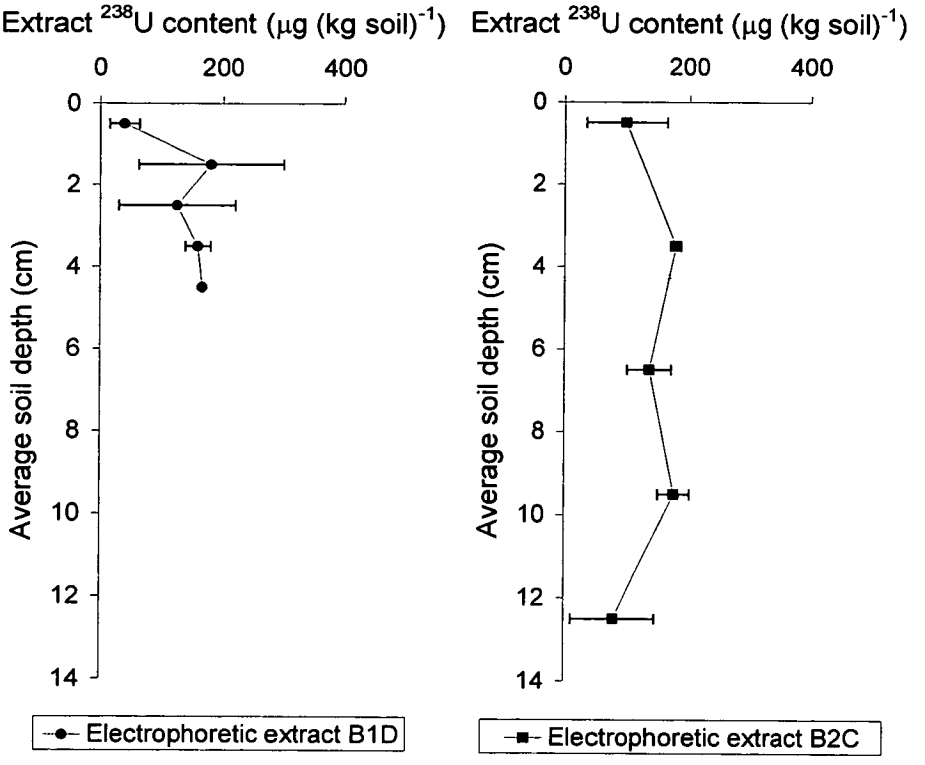


Figure 4.18: Variation in the ^{238}U content (μg (kg soil)$^{-1}$) of the electrophoretic humic extracts of the deciduous broadleaf (B1D) and coniferous (B2C) forest soil samples

4.4.3 Distribution of actinides between operationally defined soil actinide pools

Artificial actinides (^{236}U or ^{242}Pu) were 'equilibrated' with the forest soil samples by suspension of the soils in a spiked, simulated soil solution (0.045 M ammonium acetate/acetic acid, pH 4.2). The pH of the solution was chosen to approximate that of the forest soil, so that the artificial actinides were introduced to the soil in an aqueous uncomplexed speciation likely to occur in a forest soil environment. The distribution of naturally occurring (^{238}U) and artificial (^{236}U or ^{242}Pu) actinides between operationally defined soil actinide pools, generated by the soil 'equilibration' and electrophoretic extraction experiments, was investigated (Figure 4.19). The soil 'equilibration' experiment generated soil solution and solid phases between which the naturally occurring (^{238}U) and added (^{236}U, or ^{242}Pu) actinides could be distributed. The two phases were separated by repeated centrifuging, decanting and rinsing of the soil pellet. The supernatant was collected, filtered through a 0.45 μm filter, and has been termed by its operational definition as the

'labile' pool of soil actinides. The soil pellet was electrophoretically extracted and generated samples of electrophoretically mobile humic substances, an immobile soil well residue and anode and cathode compartment buffer solution. No actinides (^{238}U, ^{236}U or ^{242}Pu) were detected in the cathode and anode compartment running buffer and so only results of actinide distribution between the electrophoresis experiment samples of soil extract and well residue are presented. The further pools of actinides generated in the electrophoresis experiment were termed 'hydrophilic-mobile' (extract) and 'immobile' (well residue). Samples which were selected for the investigation of actinides between the soil actinide pools were the deciduous broadleaf (B1D) 0.5 cm and 2.5 cm depth samples, and the coniferous (B2C) 0.5 cm and 12.5 cm depth samples. The standard experiment was an 'equilibration' of the soil samples with the simulated soil solution (unspiked/spiked) for 14 hours, followed by electrophoretic extraction of the soil solid phase (centrifuge pellet). The distribution of actinides (^{238}U and ^{236}U) for one soil sample (B2C 0.5 cm) was also investigated following an extended 'equilibration' time of 86 hours.

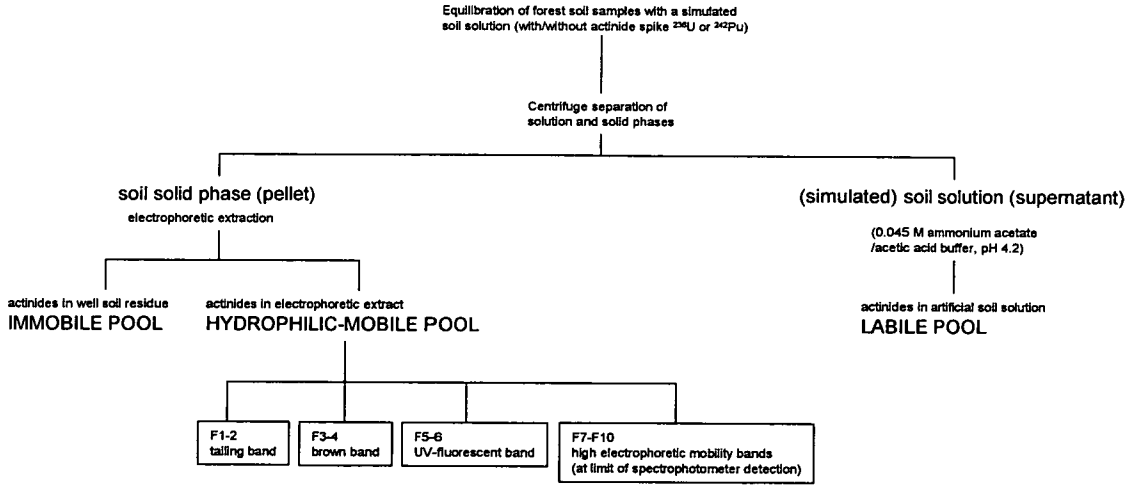


Figure 4.19: Schematic diagram showing the operationally defined soil actinide pools

The results of distribution of each actinide between the soil actinide pools, expressed as mean percentages of the total recovered actinide concentration associated with each pool, are presented in Section 4.4.3.1 (^{238}U), Section 4.4.3.2 (^{236}U), Section 4.4.3.3 (^{238}U and ^{236}U of the longer, 86-hour, 'equilibration' experiment) and Section 4.4.3.4 (^{242}Pu) below.

4.4.3.1 Distribution of naturally occurring ^{238}U between the soil actinide pools

The results of the quadruplicate 'equilibration' and electrophoretic extraction experiments showed that the distribution of ^{238}U between the soil actinide pools, for the deciduous broadleaf soil (B1D) samples (Table 4.29), in order of decreasing mean percentage of the total ^{238}U, was:

^{238}U (B1D): 67-77 % immobile, 13-21 % hydrophilic-mobile, 9-12 % labile

Comparing the soil depths of 0.5 cm and 2.5 cm (B1D), a small shift in distribution of ^{238}U between the soil actinide pools was observed. The mean percentage of ^{238}U in the immobile pool decreased (~ -10 %), whilst ^{238}U in the hydrophilic-mobile (~ +8 %) and labile (~ +3 %) pools increased with increasing soil depth (Table 4.29).

B1D				B2C			
Average soil depth (cm)	**Immobile**	**Hydrophilic -mobile**	**Labile**	**Average soil depth (cm)**	**Immobile**	**Hydrophilic -mobile**	**Labile**
0.5	77 ± 7	13 ± 4	9 ± 5	0.5	78 ± 9	19 ± 11	3 ± 4
2.5	67 ± 2	21 ± 8	12 ± 7	12.5	81 ± 6	16 ± 9	4 ± 3

Table 4.29: Distribution (%) of ^{238}U between the immobile, hydrophilic-mobile and labile soil actinide pools for the deciduous broadleaf (B1D) and coniferous (B2C) forest soils. Error is presented as the 95 % confidence limit for the quadruplicate results about the mean value.

The order of the distribution of ^{238}U between the soil actinide pools for the coniferous soil samples (B2C) was found to be the same as for B1D (Table 4.29). However, a slightly higher mean percentage of ^{238}U association with the immobile pool, and a slightly lower mean percentage association with the labile pool was observed for B2C:

^{238}U (B2C): 78-81 % immobile, 16-19 % hydrophilic-mobile, 3-4 % labile

Comparison of the soil depths 0.5 cm and 12.5 cm (B2C) showed a slight increase in immobile (~ +3 %), and a slight decrease in hydrophilic-mobile (~ -3 %) pools of ^{238}U with increasing soil depth (Table 4.29).

4.4.3.2 Distribution of added ^{236}U between the soil actinide pools

The results of the distribution of added ^{236}U between the soil actinide pools are presented in Table 4.30. The 95 % confidence limit values for mean ^{236}U distributions were observed to be much lower than those for the naturally occurring soil ^{238}U, demonstrating both reproducibility of the spiking experiment and the heterogeneity of soil and thus ^{238}U, respectively. The distribution of ^{236}U between the soil actinide pools, for the deciduous broadleaf soil (B1D), in the order of decreasing mean percentage ^{236}U was:

^{236}U (B1D): 63-64 % immobile, 19-25 % labile, 12-17 % hydrophilic-mobile

Comparison of the soil depths of 0.5 cm and 2.5 cm (B1D) showed a slight decrease in immobile (~ -1 %), a decrease in hydrophilic-mobile (~ -5 %) and an increase in labile (~ +6 %) pools of ^{236}U with increasing soil depth. Compared with the distribution of ^{238}U, the hydrophilic-mobile (^{238}U, 13-21 %; ^{236}U, 12-17 %) and labile (^{238}U, 9-12 %; ^{236}U, 19-25 %) actinide pools were reversed in importance for the distribution of ^{236}U for B1D (Table 4.29 and Table 4.30).

B1D				B2C			
Average soil depth (cm)	**Immobile**	**Hydrophilic -mobile**	**Labile**	**Average soil depth (cm)**	**Immobile**	**Hydrophilic -mobile**	**Labile**
0.5	64 ± 4	17 ± 2	19 ± 2	0.5	78 ± 1	19 ± 1	3[a]
2.5	63 ± 2	12 ± 1	25 ± 1	12.5	84[a]	7[a]	10[a]

Table 4.30: Distribution (%) of ^{236}U between the immobile, hydrophilic-mobile and labile soil pools for the deciduous broadleaf (B1D) and coniferous (B2C) forest soils. Error is presented as the 95 % confidence limit for the duplicate results about the mean value. ([a] – error below 0.5 %).

For the coniferous soil (B2C), the distribution of ^{236}U between the soil actinide pools, in order of decreasing mean percentage ^{236}U, was:

^{236}U (B2C): 78-84 % immobile, 7-19 % hydrophilic-mobile, 3-10 % labile

Comparison of soil depths 0.5 cm and 12.5 cm (B2C) showed an increase in immobile (~ +6 %) and labile (~ +7 %), and a decrease in hydrophilic-mobile

(~ -12 %) pools of ^{236}U with increasing soil depth (Table 4.30). The distributions of both ^{236}U and ^{238}U between the soil actinide pools for B2C were similar (Table 4.29 and Table 4.30).

4.4.3.3 Distribution of naturally occurring ^{238}U and added ^{236}U between the soil actinide pools following the extended (86 hr) 'equilibration' experiment (B2C 0.5 cm)

The association of ^{238}U with the electrophoretic humic extract of the coniferous (B2C) 0.5 cm soil sample was observed to be lower following the extended 'equilibration' experiment time (86 hours *vs.* 14 hours). Following the 86 hour 'equilibration' experiment, 29±6 μg (kg soil)$^{-1}$ of ^{238}U was associated with the electrophoretic humic extract (7±2 % of the 'pseudo-total' soil ^{238}U), whilst the association of ^{238}U with the extract following 14 hour 'equilibration' was 99±65 μg (kg soil)$^{-1}$ of ^{238}U (20±12 % of the 'pseudo-total' soil ^{238}U) (Table 4.29 and Table 4.31). Also, in the longer 'equilibration' experiment the 95 % confidence limit values for the distribution of ^{238}U were lower than for the 14 hour experiment (Table 4.31). The distribution of ^{238}U between the soil actinide pools, in the order of decreasing mean percentage ^{238}U, was:

^{238}U (B2C 0.5 cm, 86 hr): 93 % immobile, 7 % hydrophilic-mobile, 0 % labile

The mean percentage of ^{238}U associated with the immobile pool was higher (~ +15 %) for the 86 hour experiment, relative to the 14 hour experiment, whilst the mean percentages of ^{238}U associated with the hydrophilic-mobile and labile pools were lower (~ -12 % and ~ -3 %, respectively) (Table 4.29 and Table 4.31).

The order of the distribution of ^{236}U between the soil pools following the longer 'equilibration' experiment was the same as for the ^{238}U distribution:

^{236}U (B2C 0.5 cm, 86 hr): 82 % immobile, 15 % hydrophilic-mobile, 3 % labile

The percentage of ^{236}U, compared with the percentage of ^{238}U, was lower in the immobile pool (~ -11 %), higher in the hydrophilic-mobile pool (~ +8 %) and slightly higher in the labile pool (~ +3 %) (Table 4.31). The association of ^{236}U with the electrophoretic extract following the 86 hour 'equilibration' experiment was slightly lower than that observed following the 14 hour experiment (~ 13 % *cf.* ~ 16 % of the total added ^{236}U, respectively) (Table 4.38 and Table 4.42). By comparison, the distribution of ^{236}U, following the 86 hour 'equilibration', showed a slightly higher immobile pool (~ +4 %), and a slightly lower hydrophilic-mobile pool (~ -4 %) of ^{236}U than was observed following the 14 hour 'equilibration' experiment (Table 4.30 and Table 4.31).

^{238}U			^{236}U		
Immobile	**Hydrophilic -mobile**	**Labile**	**Immobile**	**Hydrophilic -mobile**	**Labile**
93 ± 2	7 ± 2	0[a]	82 ± 2	15 ± 2	3 ± 1

Table 4.31: Distribution (%) of ^{238}U and ^{236}U between the immobile, hydrophilic-mobile and labile soil actinide pools for the coniferous (B2C) forest soil 0.5 cm sample 'equilibrated' for 86 hours. Error is presented as the 95 % confidence limit for the duplicate results about the mean value. ([a] – error below 0.5 %).

4.4.3.4 Distribution of added ^{242}Pu between the soil actinide pools

The 95 % confidence limit values for the distributions of added ^{242}Pu between the soil actinide pools of the deciduous broadleaf (B1D) and coniferous (B2C) soil samples, similarly to the added ^{236}U results, were much lower than those for the naturally occurring soil ^{238}U. This again demonstrated both the reproducibility of the spiking experiment and the heterogeneity of soil, respectively. The distribution of added ^{242}Pu between the soil actinide pools, for the deciduous broadleaf (B1D) soil, in the order of decreasing mean percentage ^{242}Pu was (Table 4.32):

^{242}Pu (B1D): 79-88 % labile, 9-14 % immobile, 4-7 % hydrophilic-mobile

Comparison of the soil depths 0.5 cm and 2.5 cm showed a decrease in immobile (~ -5 %), a slight decrease in hydrophilic-mobile (~ -3 %), and an increase in labile (~ +9 %) pools of ^{242}Pu with increasing soil depth (Table 4.32). In contrast to the distributions of naturally occurring ^{238}U, and introduced ^{236}U the immobile (^{238}U,

67-77 %; ^{236}U, 63-64 %; ^{242}Pu, 9-14 %) and labile (^{238}U, 9-12 %; ^{236}U, 19-25 %; ^{242}Pu, 79-88 %) pools were reversed in importance for Pu (Table 4.29, Table 4.30 and Table 4.32). Also, the size of the ^{242}Pu hydrophilic-mobile pool (4-7 %) was smaller than that of either ^{238}U (13-21 %) or ^{236}U (12-17 %) for B1D.

B1D				B2C			
Average soil depth (cm)	**Immobile**	**Hydrophilic -mobile**	**Labile**	**Average soil depth (cm)**	**Immobile**	**Hydrophilic -mobile**	**Labile**
0.5	14 ± 1	7[a]	79 ± 1	0.5	36 ± 1	9[a]	55 ± 1
2.5	9[a]	4[a]	88 ± 1	12.5	33 ± 1	1[a]	65 ± 1

Table 4.32: Distribution (%) of ^{242}Pu between the immobile, hydrophilic-mobile and labile soil pools for the deciduous broadleaf (B1D) and coniferous (B2C) forest soils. Error is presented as the 95 % confidence limit for the duplicate results about the mean value. ([a] – error below 0.5 %).

For the coniferous soil (B2C), the distribution of ^{242}Pu between the soil actinide pools, in order of decreasing mean percentage ^{242}Pu, was:

^{242}Pu (B2C): 55-65 % labile, 33-36 % immobile, 1-9 % hydrophilic-mobile

Comparison of the soil depths of 0.5 cm and 12.5 cm (B2C) showed a slight decrease in immobile (~ -3 %), a decrease in the hydrophilic-mobile (~ -8 %) and an increase in the labile (~ +10 %) pools of ^{242}Pu with increasing soil depth (Table 4.32). Compared with the distribution of ^{242}Pu for the deciduous broadleaf soil (B1D), the mean percentage of ^{242}Pu associated with the labile pool was lower (B1D; 79-88 %, B2C 55-65 %), and the mean percentage of ^{242}Pu in the immobile pool was higher (B1D; 9-14 %, B2C 33-36 %), for the coniferous soil (B2C). As for B1D, comparison of the mean percentage distributions of ^{242}Pu, ^{238}U and ^{236}U, showed that the immobile (^{238}U; 78-81 %, ^{236}U; 78-84 %, ^{242}Pu; 33-36 %) and labile (^{238}U; 3-4 %, ^{236}U; 3-10 %, ^{242}Pu; 55-65 %) pools were reversed in importance for the elements Pu and U for B2C (Table 4.29, Table 4.30 and Table 4.32). Also, as for B1D the mean percentage of ^{242}Pu in the hydrophilic-mobile pool (1-9 %) was lower than that for either ^{238}U (16-19 %) or ^{236}U (7-19 %) for B2C (Table 4.29, Table 4.30 and Table 4.32).

4.4.4 Actinide association patterns of the fractions of the electrophoretic extract (hydrophilic-mobile pool)

This section presents the association of naturally occurring ^{238}U, or introduced ^{236}U or ^{242}Pu, with the individual fractions of the hydrophilic-mobile soil pool (i.e. the electrophoretic extract). The distribution of ^{238}U, ^{236}U and ^{242}Pu amongst gel electrophoretic fractions is expressed as percentages of the total electrophoretic extract (all fractions). Appendix 8.13.3 contains the content of ^{238}U, ^{236}U and ^{242}Pu in each gel fraction of the electrophoretic humic extracts of the deciduous broadleaf and coniferous forest soil samples (B1D and B2C).

4.4.4.1 Pattern of association of naturally occurring ^{238}U with the hydrophilic-mobile pool

For the deciduous broadleaf (B1D) forest soil it was shown that the largest ^{238}U soil actinide pool, for the studied depths of 0.5 cm and 2.5 cm, was the immobile pool (67-77 % of the total soil ^{238}U) (Section 4.4.3.1). With increasing soil depth (0.5 cm cf. 2.5 cm), the lability of the ^{238}U appeared to increase, i.e. there was an approximate shift of 10 % of the total soil ^{238}U from the immobile to the hydrophilic-mobile and labile pools (Section 4.4.3.1). The vertical profile of the association of ^{238}U with the hydrophilic-mobile pool showed very little change with depth for the deciduous broadleaf soil (B1D) (Section 4.4.2.3, Figure 4.18), except that the surface 0.5 cm soil sample showed much lower association of ^{238}U than all subsequent depth samples (0.5 cm, 13± 4 % *vs.* 1.5-4.5 cm, 29 ± 15 – 41 ± 1 % of the total soil ^{238}U). The replicate experiment data for the association of the total soil ^{238}U with the hydrophilic-mobile pool, upon which this trend was based, are presented in Table 4.33.

Average soil depth (cm)	% of 'pseudo-total' soil ^{238}U				
	BW1	SW1	SW2	PuW1	PuW2
0.5	5	15	13	16	16
1.5	51	47	12	n.d.	n.d.
2.5	41	37	48	10	11
3.5	33	32	39	n.d.	n.d.
4.5	41	41	n.d.	n.d.	n.d.

Table 4.33: Percentage of 'pseudo-total' soil ^{238}U content of the electrophoretic humic extract of the deciduous broadleaf (B1D) soil samples. (BW1 – unspiked soil, SW1, SW2 – ^{236}U-spiked soil and PuW1, PuW2 – Pu-^{242}spiked soil replicate experiments).

The variation in ^{238}U association with the hydrophilic-mobile pool was attributed to soil heterogeneity (Section 4.4.2.3). Variation attributable to heterogeneity of soil was also seen in the underlying association of ^{238}U with the individual fractions of the hydrophilic-mobile pool (Figure 4.20). However, trends observed include a small decrease in association of ^{238}U with the greater electrophoretic mobility fractions, F7 to F10, with increasing soil depth (e.g. BW1 1.5-4.5 cm replicate results, Figure 4.20). Also, in the examples of low association of ^{238}U with the hydrophilic-mobile pool (0.5 cm, all replicates; 1.5 cm, SW2; 2.5 cm, PuW1 and PuW2) (Table 4.33), association of ^{238}U was mainly observed with the lower electrophoretic mobility fractions F1 to F5. In observing only fractions that demonstrated greater than or equal to 15 % association of the total hydrophilic-mobile pool ^{238}U (Table 4.34), the trends of association of ^{238}U with the individual fractions, and observation of fractions which showed maximal association of the extract ^{238}U, were clarified. Thus, in the examples of low association of ^{238}U with the hydrophilic mobile pool, association of ^{238}U was, typically, mainly with fractions F2-F4, and was maximal in fraction F3 (Table 4.34). The actinide fraction patterns corresponding to low association of the total soil ^{238}U with the hydrophilic-mobile pool are similar to those observed for the concentration of humic substances in the electrophoretic extract of the deciduous broadleaf (B1D) soil (Table 4.35). Humic concentration was greater than or equal to 15 % of the total humic extract concentration in fractions F3-F5, and was maximal in fraction F4, for B1D.

	Fractions showing ≥ 15 % association of the total extract ^{238}U				
	BW1	SW1	SW2	PuW1	PuW2
0.5 cm	3-5 (4)	3-4 (4)	3 7 9 (9)	2-4 (3)	2-4 (3)
1.5 cm	*	*	1-3 10 (10)	n.d.	n.d.
2.5 cm	*	*	*	2-4 (3)	2-4 (3)
3.5 cm	*	*	*	n.d.	n.d.
4.5 cm	*	*	*	n.d.	n.d.

Table 4.34: Hydrophilic-mobile pool fractions showing ≥ 15 % association of the total extract ^{238}U. Fraction numbers in parentheses signify the fraction(s) with maximal extract ^{238}U association. (* - no fraction displayed ≥ 15 % of the hydrophilic-mobile pool ^{238}U association – ^{238}U was associated with all ten fractions). (BW1 – unspiked soil, SW1, SW2 – ^{236}U-spiked soil and PuW1, PuW2 – Pu-^{242}spiked soil replicate experiments). (n.d. – not determined).

Fractions showing ≥ 15 % of the total humic extract concentration			
B1D		B2C	
0.5 cm	3-5 (4)	0.5 cm	2-4 (3)
2.5 cm	3-5 (4)	12.5 cm	3-5 (5)

Table 4.35: Fractions of the electrophoretic extracts of deciduous broadleaf (B1D) and coniferous (B2C) forest soils containing ≥ 15 % of the total humic extract concentration (Section 4.2.3.2, page 144). Fraction numbers in parentheses signify the fraction(s) with maximal humic concentration.

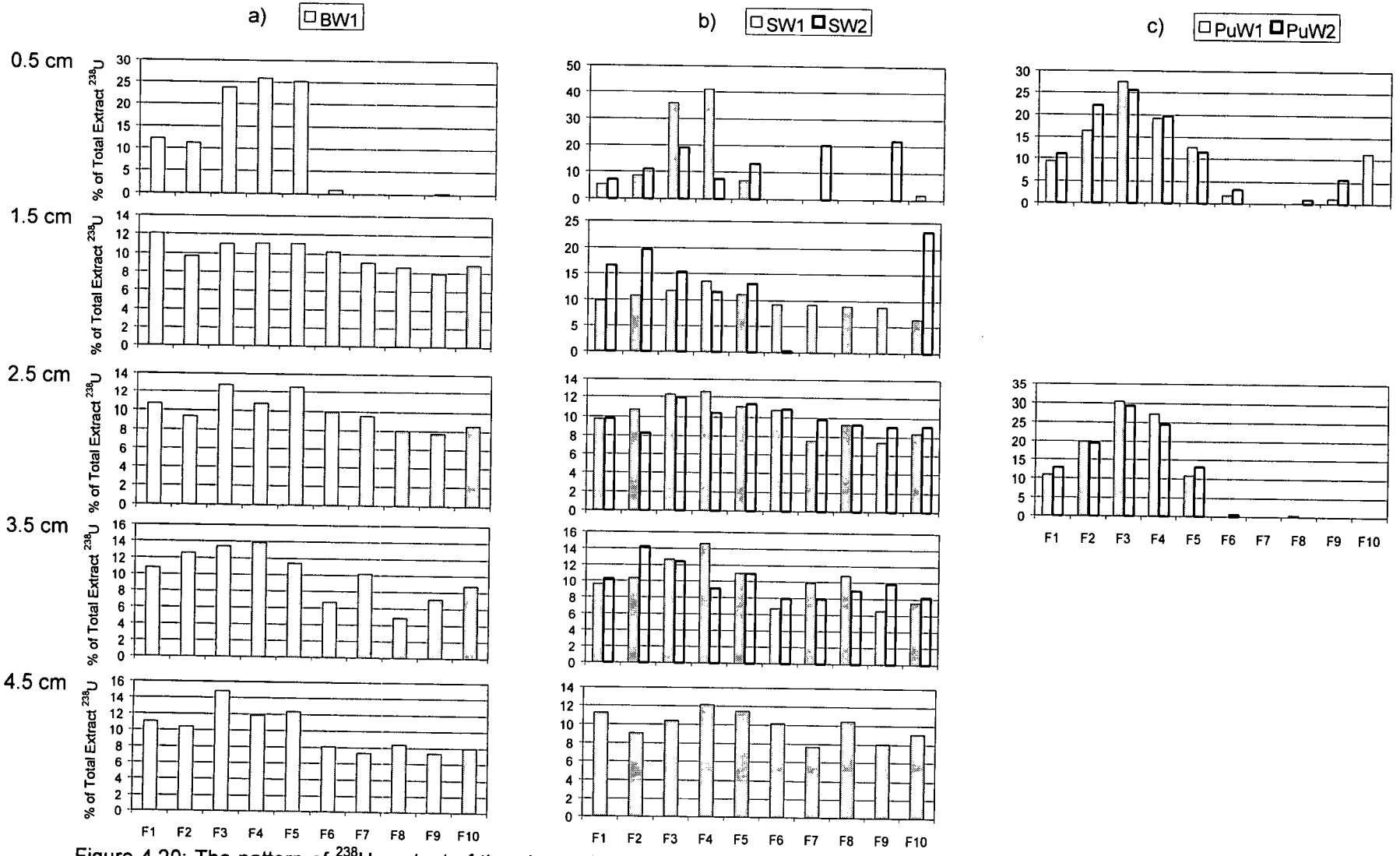


Figure 4.20: The pattern of ^{238}U content of the electrophoretic extract fractions (F1 to F10) of the deciduous broadleaf soil (B1D) 0.5-4.5 cm samples: a) unspiked soil b) ^{236}U-spiked soil and c) ^{242}Pu-spiked soil. (^{238}U content of the individual fractions expressed as a percentage of the total extract ^{238}U content).

For the coniferous (B2C) forest soil, the association of ^{238}U with the soil actinide pools was shown to be similar to that for the deciduous broadleaf (B1D) forest soil, except that the association with the immobile and labile pools were slightly higher and lower, respectively, than those for B1D (Section 4.4.3.1). Again, the largest soil ^{238}U pool was the immobile pool (78-81 % of the total soil ^{238}U) (Section 4.4.3.1). With increasing soil depth (0.5 cm *cf.* 12.5 cm), the immobility of the ^{238}U appeared to increase, i.e. a small shift of ~ 3 % of the total soil ^{238}U from the hydrophilic-mobile to the immobile pool was observed with increasing soil depth (Section 4.4.3.1). The vertical profile of the association of ^{238}U with the hydrophilic-mobile pool showed very little change with depth (Section 4.4.2.3, Figure 4.18). The association of ^{238}U with the hydrophilic-mobile pool for B2C was slightly lower than for B1D (15±9 - 34±2 % and 13±4 - 41±1 % of the total soil ^{238}U, respectively) (Section 4.3). The replicate experiment data for association of ^{238}U with the hydrophilic-mobile pool are presented in Table 4.36.

Average soil depth (cm)	% of pseudo total soil ^{238}U				
	BW1	SW1	SW2	PuW1	PuW2
0.5	26	33	30	5	5
3.5	35	33	33	n.d.	n.d.
6.5	19	30	26	n.d.	n.d.
9.5	32	34	26	n.d.	n.d.
12.5	17	17	30	4	6

Table 4.36: Percentage of 'pseudo-total' soil ^{238}U content of the electrophoretic humic extract of the coniferous (B2C) soil samples. (BW1 – unspiked soil, SW1, SW2 – ^{236}U-spiked soil and PuW1, PuW2 – Pu-^{242}spiked soil replicate experiments).

As for the deciduous broadleaf soil (B1D), the variation in ^{238}U association with the hydrophilic-mobile pool of the coniferous soil (B2C) was attributed to soil heterogeneity (Section 4.4.2.3) and was also observed in the underlying association of ^{238}U with the individual fractions of the hydrophilic-mobile pool (Figure 4.21). The variation in fraction pattern with increasing soil depth was more obvious for B2C than for B1D. This was observed as a loss of association of ^{238}U with entire fractions of the hydrophilic-mobile pool, rather than the smaller decreases in association with the higher mobility fractions that was observed for B1D. These losses were particularly obvious in the replicate experiments that demonstrated low overall ^{238}U association (e.g. BW1 6.5 cm and 12.5 cm replicate results), but were

also apparent when association was relatively high (e.g. SW1 and SW2 6.5 cm replicate results) (Figure 4.21 and Table 4.36). When association of ^{238}U with the hydrophilic-mobile pool was very low (e.g. PuW1 and PuW2 0.5 cm and 12.5 cm replicate results), ^{238}U was mainly associated with the lower mobility fractions F1 to F5. As for the deciduous broadleaf soil (B1D), the association in these examples was typically greater than or equal to 15 % of the total extract ^{238}U for fractions F2-F4, and was maximal in fraction F3, showing similarity to the humic extract concentration patterns (Table 4.35 and Table 4.37). The humic concentration pattern for B2C at 0.5 cm and 12.5 cm showed greater than or equal to 15 % of the total concentration in fractions F2-F4 and F3-F5, and was maximal in fractions F3 and F5, respectively. A shift in maximal ^{238}U association toward the maximal humic concentration fraction, F5, with increasing soil depth was not, however, clearly demonstrated.

	Fractions showing ≥ 15 % association of the total extract ^{238}U				
	BW1	SW1	SW2	PuW1	PuW2
0.5 cm	3 (3)	*	*	2-4 (3)	2-4 (3)
3.5 cm	*	*	*	n.d.	n.d.
6.5 cm	3-4 7-8 10 (3)	2-3 (2)	*	n.d.	n.d.
9.5 cm	*	*	*	n.d.	n.d.
12.5 cm	2 5-6 (2)	4 7-9 (8)	*	2-3 7 (2)	2-4 10 (3)

Table 4.37: Hydrophilic-mobile pool fractions of the coniferous (B2C) soil showing ≥ 15 % association of the total extract ^{238}U. Fraction numbers in parentheses signify the fraction(s) with maximal extract ^{238}U association. (* - no fraction displayed ≥ 15 % of the hydrophilic-mobile pool ^{238}U association – ^{238}U was associated with all ten fractions). (BW1 – unspiked soil, SW1, SW2 – ^{236}U-spiked soil and PuW1, PuW2 – Pu-^{242}spiked soil replicate experiments). (n.d. – not determined).

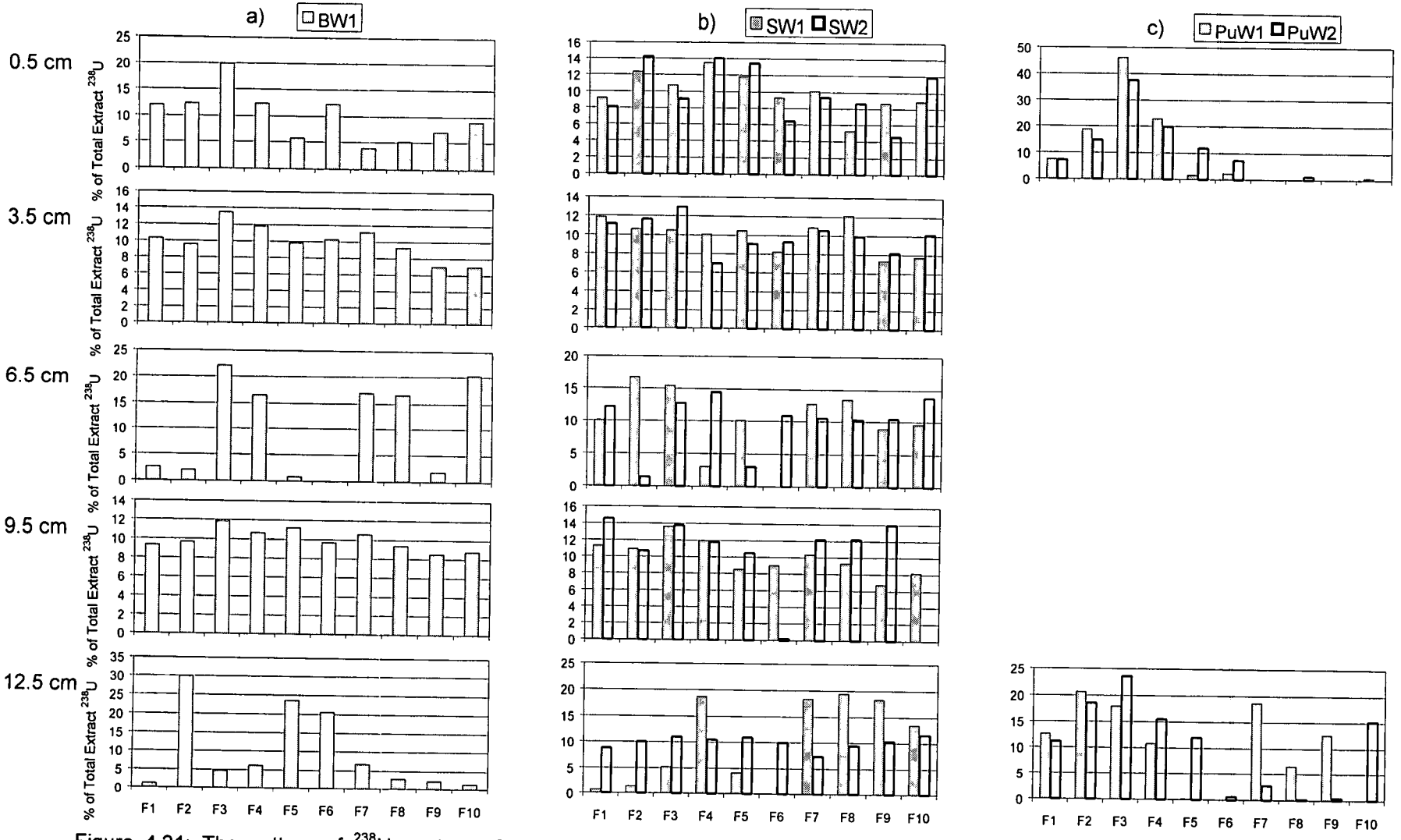


Figure 4.21: The pattern of ^{238}U content of the electrophoretic extract fractions (F1 to F10) of the coniferous soil (B2C) 0.5-12.5 cm samples: a) unspiked soil b) ^{236}U-spiked soil and c) ^{242}Pu-spiked soil. (^{238}U content of the individual fractions expressed as a percentage of the total extract ^{238}U content).

4.4.4.2 Pattern of association of added ^{236}U with the hydrophilic-mobile pool

For the deciduous broadleaf (B1D) soil, the largest soil actinide pool for ^{236}U, as for ^{238}U, was the immobile pool (63-64 % of the total soil ^{238}U) (Section 4.4.3.2). With increasing soil depth, for the studied soil depths of 0.5 cm and 2.5 cm, there was an increase in the lability of the added ^{236}U (Section 4.4.3.2). This was observed as a shift in the distribution of added ^{236}U from the immobile and hydrophilic-mobile pools (~ -9 % and ~ -3 %, respectively) to the labile pool (~ +12 %). Thus, the hydrophilic-mobile pool was slightly smaller for added ^{236}U than for naturally occurring ^{238}U (12-17 % ^{236}U *cf.* 13-21 % ^{238}U). The replicate experiment data of association of the added ^{236}U with the hydrophilic-mobile pool are presented in Table 4.38. The association of added ^{236}U with the hydrophilic-mobile pool decreased slightly with increasing depth (Table 4.38). With increasing soil depth, for the studied depths of 0.5 cm and 2.5 cm, the lability of added ^{236}U increased slightly (Section 4.4.3.2). This was observed as a shift of added ^{236}U from the immobile and hydrophilic-mobile pools (~ -1 % and ~ -5 %, respectively) to the labile pool (~ + 6 %).

B1D			B2C		
Average soil depth (cm)	% of total ^{236}U		Average soil depth (cm)	% of total ^{236}U	
	SW1	SW2		SW1	SW2
0.5	13	12	0.5	16	16
1.5	13	13	3.5	8	8
2.5	10	10	6.5	9	9
3.5	10	10	9.5	5	5
4.5	10	n.d.	12.5	6	6

Table 4.38: Percentage of the total introduced ^{236}U associated with the electrophoretic extract of the deciduous broadleaf (B1D) and coniferous (B2C) forest soil samples. (SW1, SW2 – ^{236}U-spiked soil replicate experiments).

The reproducibility of the association of added ^{236}U with the hydrophilic-mobile pool (Section 4.4.3.2) was also seen in the underlying association of ^{236}U with the individual fractions of the hydrophilic-mobile pool (Figure 4.22). The association of ^{236}U was mainly with the lower electrophoretic mobility fractions, F1 to F5, and was maximal in fraction F4/F5 (Table 4.39 and Figure 4.22). With increasing soil depth, though the patterns were similar, the percentage of ^{236}U in fraction F4 increased slightly relative to fraction F5. The ^{236}U association pattern was similar to that of the

humic concentration of the electrophoretic extract – humic concentration was associated mainly with fractions F3-F5 and was maximal in fraction F4 (Table 4.35). Thus added ^{236}U was observed to be associated with a wider spread of extract fractions, and was skewed toward lower molecular weight humic molecules than was observed for the association of ^{238}U (^{238}U, F2-F4, maximum F3 *cf.* ^{236}U, F1-F5 maximum F4/F5).

Fractions showing ≥ 15 % association of the total extract ^{236}U					
B1D			B2C		
	SW1	SW2		SW1	SW2
0.5 cm	3-5 (4)	3-5 (5)	0.5 cm	2-5 (4)	2-5 (4)
1.5 cm	3-5 (5)	1-5 (5)	3.5 cm	1-5 (3-4)	1-5 (1)
2.5 cm	3-5 (4)	3-5 (5)	6.5 cm	1 3-5 (4)	1-3 5 (5)
3.5 cm	2-5 (4)	3-5 (4)	9.5 cm	2 4-5 (4-5)	1-5 (4)
4.5 cm	3-5 (4)	n.d.	12.5 cm	2-5 (4)	3-5 (5)

Table 4.39: Hydrophilic-mobile pool fractions of the deciduous broadleaf (B1D) and coniferous (B2C) soils showing ≥ 15 % association of the total extract ^{236}U. Fraction numbers in parentheses signify the fraction(s) with maximal extract ^{236}U association. (SW1, SW2 – ^{236}U-spiked soil replicate experiments). (n.d. – not determined).

For the coniferous (B2C) soil the distribution of introduced ^{236}U, investigated for the two soil depths 0.5 cm and 12.5 cm, between the soil actinide pools showed that the order and size of the pools for ^{236}U were similar to those for naturally occurring ^{238}U (Sections 4.4.3.1 and 4.4.3.2). The percentage association of ^{236}U with the hydrophilic-mobile pool of each experiment is presented in Table 4.38. The association of ^{236}U with the hydrophilic-mobile pool, greater for the surface B2C sample compared to that of B1D, decreased more sharply with increasing depth than was observed for B1D (Table 4.38). With increasing soil depth, a shift in the distribution of added ^{236}U between the soil actinide pools occurred (Section 4.4.3.2). The net result did not affect the lability of the added ^{236}U (shift in immobile pool ~ +6 % ≈ shift in labile pool ~ + 7 %). As for B1D, the reproducibility of the association of added ^{236}U with the hydrophilic-mobile pool was seen in the underlying association of ^{236}U with the individual fractions of the hydrophilic-mobile pool for B2C (Figure 4.22). The association of ^{236}U was mainly with the lower electrophoretic mobility fractions, F1 to F5, and was typically maximal in fraction F4/F5 (Table 4.39 and Figure 4.22). The ^{236}U fraction pattern for B2C showed greater variation with increasing soil depth than was observed for B1D. At 0.5 cm,

the ^{236}U content was maximal in fraction F4/F5. At soil depths 3.5 cm, 6.5 cm and 9.5 cm, the ^{236}U association between fractions F1 to F5 was more even, whilst at soil depth 12.5 cm, ^{236}U association peaked again in fractions F4/F5. As for B1D, addition of ^{236}U to the coniferous (B2C) soil was observed to be associated with a wider group of extract fractions, and was skewed toward lower molecular weight humic molecules than was observed for the association of ^{238}U (^{238}U, F2-F4 maximum F3 *cf.* ^{236}U, F1-F5 maximum F4/F5). As for B1D, the ^{236}U association pattern for B2C was similar to that of the humic concentration of the electrophoretic extract. The humic concentration pattern for B2C at 0.5 cm and 12.5 cm showed greater than or equal to 15 % in fractions F2-F4 and F3-F5, and was maximal in fractions F3 and F5, respectively (Table 4.35).

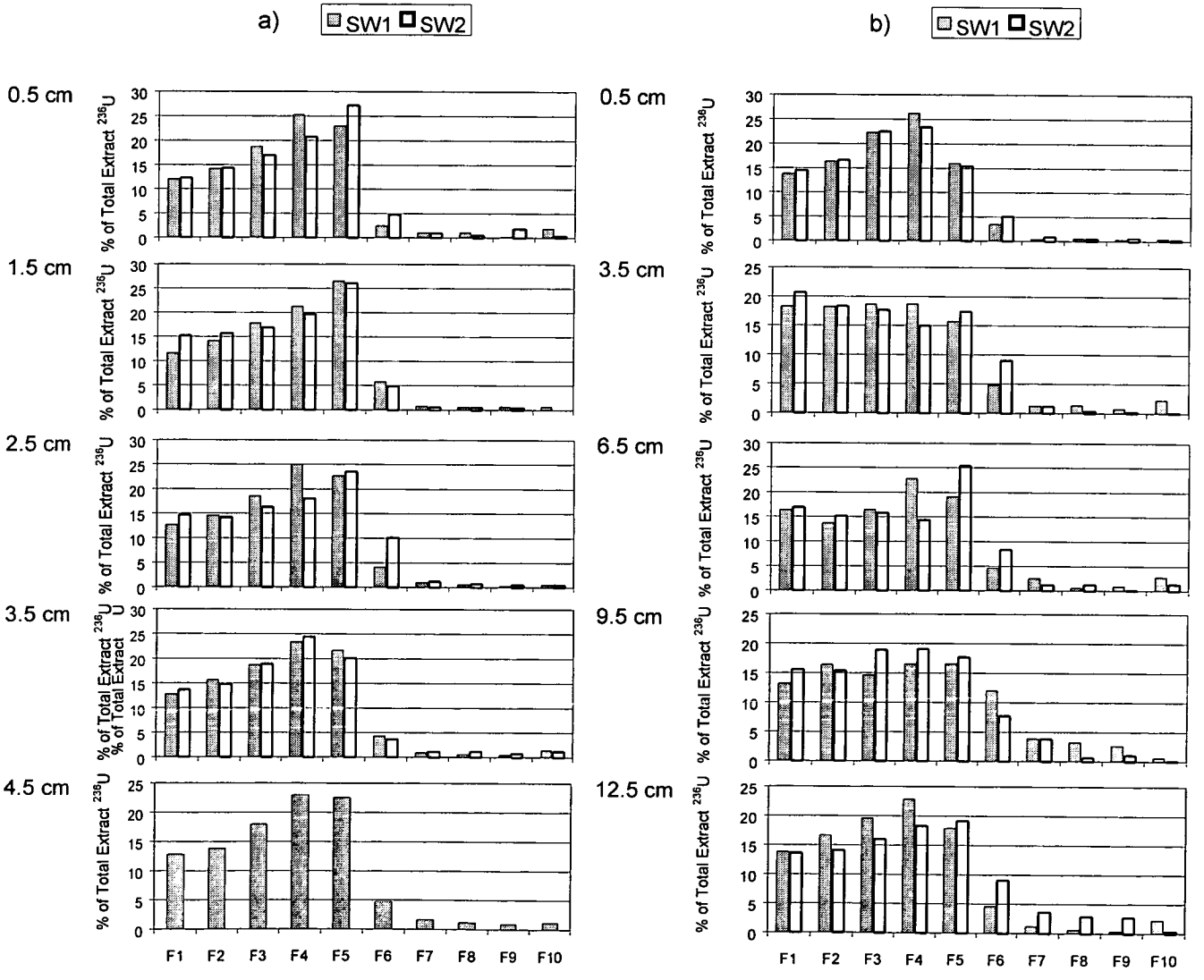


Figure 4.22: The pattern of ^{236}U content of the electrophoretic extract fractions (F1 to F10) of a) the deciduous broadleaf (B1D) 0.5-4.5 cm soil samples and b) the coniferous (B2C) 0.5-12.5 cm soil samples. (^{236}U content of the individual fractions expressed as a percentage of the total extract ^{236}U content).

4.4.4.3 Pattern of association of naturally occurring ^{238}U and added ^{236}U with the hydrophilic-mobile pool following the extended (86 hr) 'equilibration' experiment

In the longer, 86 hour, 'equilibration' experiment (B2C 0.5 cm sample) the order of distribution of ^{238}U between the soil actinide pools was the same as for the 14 hour 'equilibration' experiment (Sections 4.4.3.2 and 4.4.3.3). The immobile pool was again the largest soil actinide pool for ^{238}U. The distribution of ^{238}U in the longer (86 hr) 'equilibration' experiment, however, showed greater immobility compared to the shorter (14 hr) 'equilibration' experiment. This was seen as a shift of ^{238}U from the hydrophilic-mobile and labile pools (~ -12 % and ~ -3 %, respectively) to the immobile pool (~ + 15 %). The association of ^{238}U with the hydrophilic-mobile pool was thus lower in the extended 'equilibration' experiment (86 hr, 7 ± 2 % ^{238}U *cf.* 14 hr, 20 ± 12 % ^{238}U) (Table 4.28 and Table 4.40). The association of ^{238}U with the hydrophilic-mobile pool in the longer experiment showed lower variation usually attributed to soil heterogeneity. The reproducibility of the experiment was also observed in the pattern of hydrophilic-mobile ^{238}U with the individual fractions (Figure 4.23). The association of ^{238}U was mainly with the lower electrophoretic mobility fractions, F1-F4, and was maximal in fraction F3 (Table 4.41 and Figure 4.23).

% of pseudo total soil ^{238}U		
BW1	SW1	SW2
5	7	5

Table 4.40: Percentage of 'pseudo-total' soil ^{238}U content of the electrophoretic humic extract of the coniferous (B2C) 0.5 cm soil sample following 86 hour 'equilibration'. (BW1 – unspiked soil, SW1, SW2 – ^{236}U-spiked soil replicate experiments).

Fractions showing ≥ 15 % association of the total extract ^{238}U or ^{236}U				
^{238}U			^{236}U	
BW1	SW1	SW2	SW1	SW2
2-5 (3)	1-4 (3)	1-4 (3)	2-5 (4)	1-5 (3)

Table 4.41: Hydrophilic-mobile pool fractions of the coniferous (B2C) soil 0.5 cm sample showing ≥ 15 % association of the total extract ^{238}U and ^{236}U following the extended 'equilibration' (86 hr) experiment. Fraction numbers in parentheses signify the fraction(s) with maximal extract ^{238}U and ^{236}U association. (BW1 – unspiked soil, SW1, SW2 – ^{236}U-spiked soil replicate experiments). (n.d. – not determined).

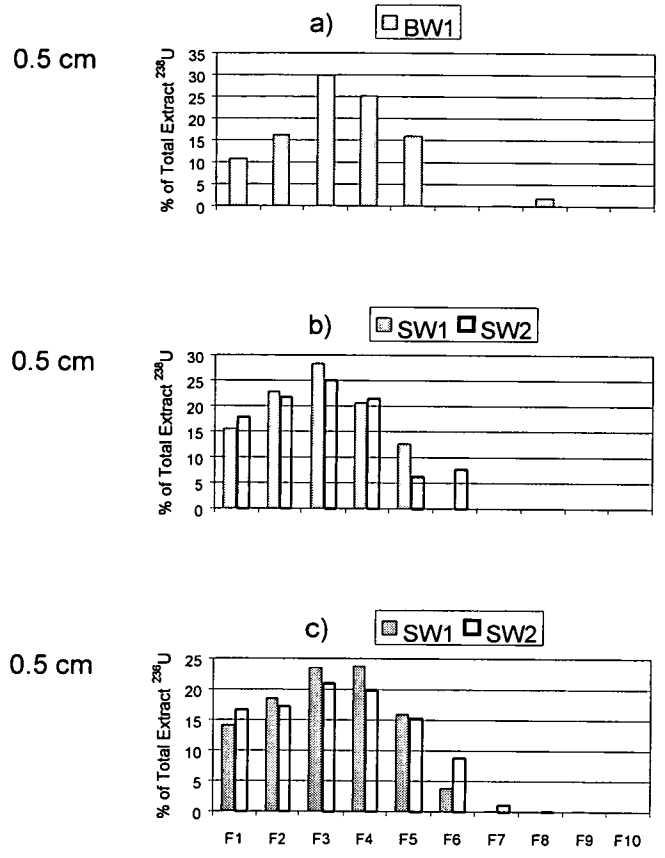


Figure 4.23: Electrophoretic extract a) ^{238}U pattern of unspiked soil, b) ^{238}U pattern of ^{236}U-spiked soil and c) ^{236}U pattern of the ^{236}U-spiked soil coniferous (B2C) 0.5 cm soil sample.

The distribution of added ^{236}U between the soil actinide pools following the extended (86 hr)'equilibration' experiment was similar to that following the 14 hour 'equilibration' experiment (Sections 4.4.3.2 and 4.4.3.3). The immobile pool was the largest soil actinide pool for ^{236}U. The immobility of the added ^{236}U had, however, increased slightly after the extended 'equilibration' period. This was observed as a shift of added ^{236}U from the hydrophilic-mobile pool (~ - 4 %) to the immobile pool (~ + 4 %). The association of ^{236}U following the extended 'equilibration' period was thus lowered (86 hr, ~ 13 % ^{236}U *cf.* 14 hr, ~ 16 % ^{236}U) (Table 4.38 and Table 4.42). The reproducibility of the association of added ^{236}U with the hydrophilic-mobile pool was also observed in the underlying association of ^{236}U with the individual fractions of the hydrophilic-mobile pool (Figure 4.23). The association of added ^{236}U was mainly with the lower electrophoretic mobility fractions, F1-F5, and was maximal in fractions F3/F4 (Table 4.41). Again, the ^{236}U actinide pattern was similar to that of

humic concentration in the electrophoretic extract. The concentration of the humic extract of the B2C 0.5 cm sample was associated mainly with fractions F2-F4 and was maximal in fraction F3 (Table 4.35).

% of total ^{236}U	
SW1	SW2
13	12

Table 4.42: Percentage of the total introduced ^{236}U associated with the electrophoretic extract of the coniferous (B2C) 0.5 cm soil sample following 86 hour 'equilibration'. (SW1, SW2 – ^{236}U-spiked soil replicate experiments).

4.4.4.4 Pattern of ^{236}U in the electrophoretic gel of the ^{236}U control experiment

In the ^{236}U control experiment, approximately 95 % of the ^{236}U loaded in the well was associated with the electrophoretic extract. The distribution of ^{236}U in the electrophoretic extract, unlike the ^{236}U spiked soil experiments, showed a single peak in fraction F3, representing approximately 67 % of the total extract ^{236}U (~ 64 % of the total loaded ^{236}U) (Figure 4.24).

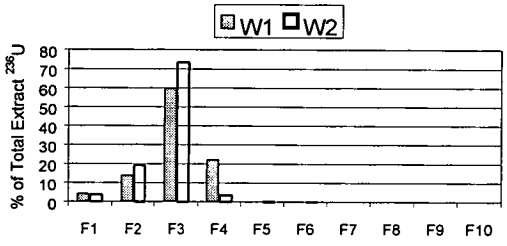


Figure 4.24: The pattern of ^{236}U content of the electrophoretic fractions (F1 to F10) in the control experiment. (^{236}U content of the individual fractions expressed as a percentage of the total ^{236}U spike).

4.4.4.5 Pattern of association of added ^{242}Pu with the hydrophilic-mobile pool

For the deciduous broadleaf (B1D) soil, the largest pool for ^{242}Pu was the labile pool (79-88 % of the added ^{242}Pu) (Section 4.4.3.4). With increasing soil depth, for the studied depths of 0.5 cm and 12.5 cm, the lability of the added ^{242}Pu increased. This was seen as a shift in added ^{242}Pu from the immobile and hydrophilic-mobile pools (~ -5 % and ~ - 3 %, respectively) to the labile pool (~ + 9 %). The replicate experiment data for association of added ^{242}Pu with the hydrophilic-mobile pool are presented in Table 4.43. The association of added ^{242}Pu with the hydrophilic-mobile pool decreased with increasing soil depth.

B1D			B2C		
Average soil depth (cm)	% of total ^{242}Pu		Average soil depth (cm)	% of total ^{242}Pu	
	PuW1	PuW2		PuW1	PuW2
0.5	8	7	0.5	8	8
1.5	n.d.	n.d.	3.5	n.d.	n.d.
2.5	4	4	6.5	n.d.	n.d.
3.5	n.d.	n.d.	9.5	n.d.	n.d.
4.5	n.d.	n.d.	12.5	1	2

Table 4.43: Percentage of the total introduced ^{242}Pu associated with the electrophoretic extract of the deciduous broadleaf (B1D) and coniferous (B2C) soil samples. (PuW1, PuW2 – Pu-^{242}spiked soil replicate experiments).

The reproducibility of the association of added ^{242}Pu with the hydrophilic mobile pool was also seen in the underlying association of ^{242}Pu with the individual fractions of the hydrophilic-mobile pool (Figure 4.25). The association of added ^{242}Pu was mainly with the lower electrophoretic mobility fractions, F2-F4, and was typically maximal in fraction F3 (Table 4.1 and Figure 4.25). The association of ^{242}Pu with fraction F4 appeared to increase relative to fraction F3 with increasing soil depth. Added ^{242}Pu was therefore associated with a narrower group of the extract fractions, and showed association with higher molecular weight humic molecules than was observed for added ^{236}U (^{242}Pu, F2-F4, maximum F3 *cf.* ^{236}U, F1-F5 maximum F4/F5). The ^{242}Pu association pattern was similar to the humic concentration pattern. The humic concentration was mainly associated with fractions F3-F5 and was maximal in fraction F4 for B1D (Table 4.35).

Fractions showing ≥ 15 % association of the total extract ^{242}Pu					
B1D			B2C		
	PuW1	PuW2		PuW1	PuW2
0.5 cm	2-4 (3)	2-4 (3)	0.5 cm	2-4 (3)	2-4 (3)
2.5 cm	2-4 (4)	2-4 (3)	12.5 cm	2-4 (3)	1-4 (3)

Table 4.44: Hydrophilic-mobile pool fractions of the deciduous broadleaf (B1D) and coniferous (B2C) soils showing ≥ 15 % association of the total extract ^{242}Pu. Fraction numbers in parentheses signify the fraction(s) with maximal extract ^{242}Pu association. (PuW1, PuW2 – ^{242}Pu-spiked soil replicate experiments). (n.d. – not determined).

For the coniferous (B2C) soil, the largest actinide pool for ^{242}Pu was the labile pool, which was, however, smaller than for the deciduous broadleaf (B1D) soil (55-65 % and 79-88 % of the total added ^{242}Pu, respectively) (Section 4.4.3.4). As for B1D,

with increasing soil depth, for the studied depths of 0.5 cm and 12.5 cm, the lability of the added ^{242}Pu increased for B2C. This was observed as a shift in added ^{242}Pu from the immobile and hydrophilic-mobile pools (~ -3 % and ~ - 8 %, respectively) to the labile pool (~ + 10 %) (Section 4.4.3.4). The replicate experiment data for association of added ^{242}Pu with the hydrophilic-mobile pool are presented in Table 4.43. The association of added ^{242}Pu with the hydrophilic-mobile pool decreased with increasing soil depth. The reproducibility of the association of added ^{242}Pu with the hydrophilic mobile pool was also seen in the underlying association of ^{242}Pu with the individual fractions of the hydrophilic-mobile pool (Figure 4.25). The association of added ^{242}Pu, similarly to B1D, was mainly with the lower electrophoretic mobility fractions, F1-F4, and was typically maximal in fraction F3 (Table 4.1 and Figure 4.25). As for B1D, the association of ^{242}Pu with fraction F4 appeared to increase relative to fraction F3 with increasing soil depth for B2C. As for B1D, addition of ^{242}Pu to the coniferous (B2C) soil was observed to be associated with a narrower group of the extract fractions, and showed association with higher molecular weight humic molecules than was observed for added ^{236}U (^{242}Pu, F2-F4 maximum F3 *cf.* ^{236}U, F1-F5 maximum F4/F5). The ^{242}Pu association pattern was most similar to the humic concentration pattern observed for the 0.5 cm soil sample. The concentration of the humic extract of the B2C 0.5 cm sample was associated mainly with fractions F2-F4 and was maximal in fraction F3 (Table 4.35).

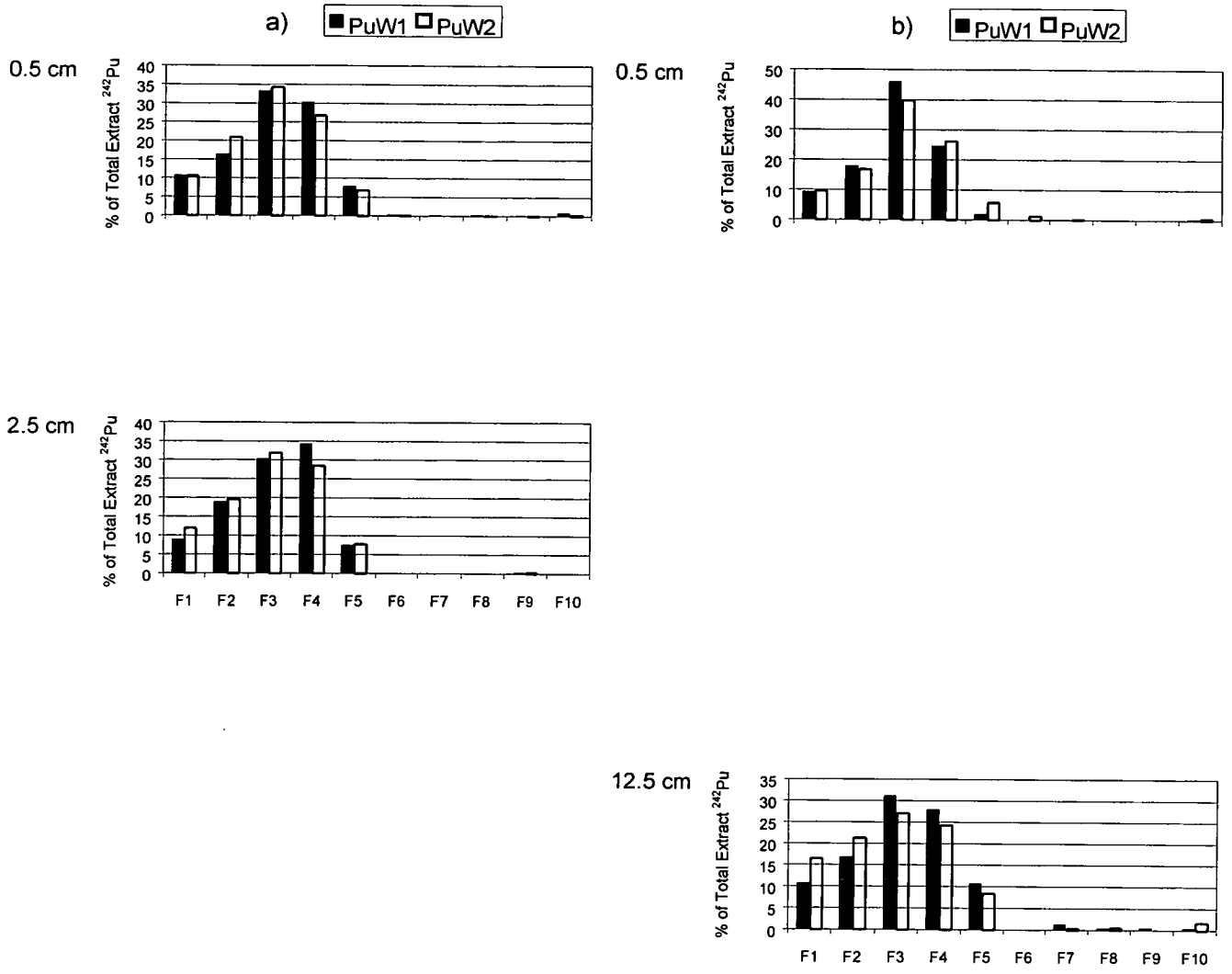


Figure 4.25: The pattern of ^{242}Pu content of the electrophoretic extract fractions (F1 to F10) of a) the deciduous broadleaf (B1D) 0.5 and 2.5 cm soil samples and b) the coniferous (B2C) 0.5 and 12.5 cm soil samples. (^{242}Pu content of the individual fractions expressed as a percentage of the total extract ^{242}Pu content).

4.4.5 Actinide recovery for the combined soil 'equilibration' and electrophoretic extraction experiments

Percentage recoveries of naturally occurring ^{238}U and of added nuclides, ^{236}U or ^{242}Pu were determined for the experiments involving 'equilibration' of ^{236}U or ^{242}Pu with soil prior to electrophoretic extraction. For each nuclide, the concentrations in the 'equilibration' buffer, electrophoresis well residue and in all electrophoretic extract fractions were added together and the total compared with the 'pseudo-total' ^{238}U concentration of the soil sample or with total added ^{236}U or ^{242}Pu. This mass balance calculation was undertaken for i) two subsamples from each soil profile

(B1D, 0.5 cm and 2.5 cm; B2C, 0.5 cm and 12.5 cm), ii) one subsample where a longer 'equilibration' time prior to extraction had been used (B2C 0.5 cm, 86 hours) and iii) for a control involving addition of ^{236}U directly in the electrophoresis well. The raw data are presented in Section 8.13.3.

	Sample	^{238}U (%) (S)	^{238}U (%) (Pu)	^{236}U (%)	^{242}Pu (%)
i)	B1D 0.5 cm	90	109	73	101
	B1D 2.5 cm	150	97	89	104
	B2C 0.5 cm	105	96	86	92
	B2C 12.5 cm	97	76	86	111
ii)	B2C 0.5 cm (86 hr)	86	n.d.	81	n.d.
iii)	^{236}U Control	-	-	96	-

Table 4.45: Percentage recovery for the soil 'equilibration' and electrophoretic extraction system of naturally occurring ^{238}U and of added nuclides, ^{236}U or ^{242}Pu for i) 14 hour 'equilibration', ii) 86 hour 'equilibration', and iii) ^{236}U control experiments. (S – ^{236}U-spiked soil and Pu – Pu-^{242}spiked soil duplicate experiments). (n.d. – not determined).

With the exception of one outlier (150%), the average recovery for all nuclides (^{238}U, ^{236}U and ^{242}Pu) in experiments (i) and (ii) was 93±11 %. These high recoveries, each based on the sum of twelve measurements, indicate that overall losses for the experiments involving real soil samples are small. Deviations from 100% recovery are attributed in part to natural soil heterogeneity (^{238}U) and errors associated with the summation of several small numbers (^{238}U, ^{236}U and ^{242}Pu). It should be noted that, in the control experiment, where the ^{236}U was present almost entirely in only two of the ten gel electrophoretic fractions, a high recovery (96 %) was achieved.

5 DISCUSSION

5.1 FOREST SOIL SOLUTION pH

5.1.1 Variations in forest soil solution pH

Typical of forest soils, both the deciduous broadleaf (B1D and B2D) and coniferous (B1C and B2C) forest soils were found to be acidic (i.e. pH < 5.6, Section 1.3.1) and the ranges of soil solution pH were pH 3.0-4.1 and pH 2.8-3.6, respectively. As found in other studies (Brady and Weil, 1999; Rennie, 1966; Vézina, 1965), the near surface sections of the coniferous forest soil were more acidic than those of the deciduous broadleaf soil (e.g. 0.5 cm: B1C and B2C, pH 2.8 and 2.9, respectively; B1D and B2D, pH 3.0 and 3.3, respectively). The average values of soil solution pH in the underlying mineral layers were, however, very similar (B1D and B2D; pH 3.4, pH 3.2, respectively, B1C and B2C; both pH 3.2). The general shape of the vertical profiles of soil solution pH for the deciduous broadleaf and coniferous forest soils (Section 4.1.1) were also similar and were typical of those found in the literature (Rennie, 1966) (Section 1.3.3). Soil solution pH decreased downward through the litter layer to a minimum at approximately the litter/mineral soil interface, then increased through the mineral soil sequum (A- and B-horizons). The rate of increase of pH, with increasing soil depth below the litter, was similar for both types of soil. This similarity was corroborated by calculation of the change in proton activity for the soil depth interval 0.5-4.5 cm. The average decreases in proton activity for the deciduous broadleaf and coniferous forest soils, for the soil depth interval 0.5-4.5 cm, were 0.43×10^{-3} and 0.41×10^{-3}, respectively.

5.1.2 Proton activity and concentration of dissolved humic substances

The pH of forest soil solutions is a measure of the acidity resulting from the dissociation of carboxylic and other organic acids (Rennie, 1966), of which humic substances represent the greatest component (Cronan and Aiken, 1985; Dai *et al.*, 1996). Thus, the vertical pH profile of a forest soil may be indicative of the changing concentration of dissolved humic substances relative to soil depth. From the average proton activity of the soil solutions, the concentration of dissolved humic

substances of the surface (0.5 cm) sample of the coniferous forest soils (1.36×10^{-3}) was almost twice that of the surface deciduous broadleaf forest soils (0.76×10^{-3}). This is in agreement with other studies which found that the dissolved organic carbon content of forest soil solutions under coniferous stands was approximately twice that under deciduous broadleaf stands (Cronan and Aiken, 1985). The results for this study also suggested that the concentration of dissolved humic substances then decreased through the mineral layers to a similar final concentration for both soil types.

5.1.3 Vertical variation of CEC of dissolved humic substances

At the pH of most forest soils, in the near surface, O-horizons, dissolved organic acids, and in particular humic substances, contribute approximately one-third of the total CEC charge (Dai *et al.*, 1996). The total CEC has also been shown to decrease with increasing soil depth (Vézina, 1965). In this study, it was also found that the concentration of dissolved humic substances had an influence on the soil CEC. The extent of soil solution pH depression, on measurement in weak electrolyte compared with measurement in deionised water, was determined for the deciduous broadleaf soil (B2D). The suspension of soil in weak electrolytes such as 0.01 M $CaCl_2$ causes displacement of H^+ and Al^{3+} from soil cation exchange sites into solution (Rowell, 1996), and a depression in soil solution pH when compared with the deionised water value is indicative of a soil with a net negative charge (Rowell, 1996). The results in this study revealed that the magnitude of the depression was greatest in the organic-rich A-horizon and then decreased with increasing soil depth. The magnitude of the depression was smaller in the more mineral-rich B-horizon. Thus, qualitatively, humic substances in the deciduous broadleaf soil (B2D) were shown to contribute to the soil CEC.

5.1.4 Importance of soil solution pH with respect to the actinide binding ability of humic substances

The importance of pH, with respect to the actinide binding ability of humic substances, is in its influence on the degree of dissociation of humic binding groups which in turn also influences the conformation and the molecular size of the dissolved humic polymer. At pH 4.2 (sodium acetate/acetic acid buffer) a lake

sediment humic acid was approximately 40 % dissociated (Rao *et al.*, 1994). Increasing the pH to 5.6 increased the dissociation to 70 %. The greater negative charge, and more open conformation of the humic polymer at the higher pH is expected to allow faster interaction of the polymer with actinide ions.

5.1.5 Importance of soil solution pH with respect to actinide speciation prior to humic complexation

The soil solution pH is also important because it determines the speciation of the actinides prior to interaction with the humic substances. In this study, artificial isotopes of U (^{236}U) or Pu (^{242}Pu) were added to the soil in the same speciation as predicted by soil solution pH. An acetate buffer (pH 4.2) was used to approximate soil solution conditions. From typical speciation diagrams the speciation of U (UO_2^{2+}) or Pu (PuO_2^{+}) under the pH conditions of the acetate buffer (pH 4.2) should not differ significantly from that under the range of the forest soil pHs (pH 2.8-4.5) (Choppin *et al.*, 1995).

5.2 CONCENTRATION OF ORGANIC MATTER IN FOREST SOILS

5.2.1 Vertical variations in soil organic matter content

The organic matter content of the deciduous broadleaf (B1D) and coniferous (B2C) soils in this study was found to decrease with increasing soil depth. This decrease is typically a result of mineralisation, leaching and sorption processes (Thurman, 1985). The amount of soil organic matter in the coniferous surface (0.5 cm) soil sample was greater than that observed for the deciduous broadleaf surface (0.5 cm) sample (46 % L.O.I. cf. 35 % L.O.I., Section 4.1.2). The rate of decrease in soil organic matter, for both soil types, decreased with increasing soil depth. This is generally a reflection of different rates of mineralisation of organic matter at different soil depths. Fresh inputs of organic material are subject to rapid microbial breakdown of the most easily degraded components (Brady and Weil, 1999). Humic substances, formed from partial degradation products, are however, relatively recalcitrant with respect to degradation by microorganisms, and are further protected from mineralisation in the soil B-horizon by association with mineral phases. Sorption to mineral phases is

suggested to be partly responsible for the long mean residence time of the humin pool of humic substances (Stevenson, 1994).

The soil profile of dissolved organic matter is related to the amount of soil organic matter (Thurman, 1985). Accordingly, a correlation between the proton activity of samples of the deciduous broadleaf ($r^2 = 0.93$) and coniferous ($r^2 = 0.98$) forest soils and their respective soil organic matter contents (% L.O.I. values) was found.

5.2.2 Soil textural class for the two forest soils

The deciduous broadleaf (B1D) and coniferous (B2C) soil textures were classed as silty clay loam in this study. This was less coarse than the texture suggested by the definition of a Brown Earth soil used by the Forestry Commission (coarse-loamy (Pyatt, 1970)), but probably reflects differences in both the sample preparation and the measurement technique used. This study used the modern technique of laser diffraction to determine particle sizes whereas soil texture has previously been assigned using either a field assessment or laboratory characterisation. The field assessment would have involved discerning the feel of moist soil rubbed between finger and thumb whilst the laboratory method would have used sieves and sedimentation followed by weighing of the coarse sand, fine sand, silt and clay classes to classify texture. In this study, the soil had also been sieved (< 0.5 mm) prior to determination of size distribution, and this may be another reason that the soil texture determined in this study was less coarse than that reported in the literature.

5.2.3 Influence of organic matter on soil particle size in forest soils

Comparison of chemically treated (removal of organic matter) and non-treated soil samples, using the laser diffraction method for determination of size distribution, revealed the influence of organic matter on particle size distribution. Disaggregation of organo-mineral aggregates for both soil types resulted in the observation of a decrease in size of the largest diameter peak from ~200-300 μm to ~ 100 μm, the appearance of peaks centred at ~ 3 μm diameter and ~20-30 μm diameter, and an increase in the population of the very fine particle size peak (0.2-0.3 μm diameter). The very small shifts in the population of both effective and absolute particle size

distribution peaks, with increasing soil depth, in both the deciduous broadleaf and coniferous soils may also indicate a slight increase in mineral particle weathering.

5.2.4 Effective and absolute soil particle size distribution in forest soils

The underlying geology of the adjacent deciduous broadleaf and coniferous forest stands studied, and thus the mineralogy of the two soil types, was the same. Also, the effective and absolute particle size distributions for all soil depth samples in both the deciduous broadleaf (B1D) and coniferous (B2C) soils were very similar. Thus, soil particle size distribution, and in particular, the proportion of fine particles with high specific surface area and with high cation exchange character, was not expected to be a variable in (i) the interaction of the actinides with the soil components from different vertical depths and (ii) the interaction of actinides with soil components in the two different soil types.

5.3 CONCENTRATIONS OF HUMIC SUBSTANCES IN FOREST SOILS OBTAINED BY SOLVENT EXTRACTION

5.3.1 Vertical variations in the concentration of humic substances obtained by solvent extraction

The humic substance concentration in the solvent extracts, for both types of soil, and regardless of the solvent (0.1 N NaOH or 0.045 M Tris-borate), decreased with increasing soil depth. The specific nature of the variations with increasing depth, however, differed for the two types of soil. For the deciduous broadleaf soil (B1D), regardless of solvent, concentrations of humic substances in the first two sections (0.5 cm and 1.5 cm) were similar, but thereafter there was a decrease downwards to 4.5 cm. For the coniferous soil (B2C), again regardless of solvent, the humic concentration decreased from the surface (0.5 cm) downward. This probably reflected the difference in the mixing of litter with mineral soil by soil organisms for the two types of soil and the soil level at which the litter was decomposed. For deciduous broadleaf forest soils, litter is mixed with the mineral soil by burrowing organisms and thus is decomposed in the Ah layer (humic-enriched mineral soil horizon, Section 1.2.6 and 1.2.3). For coniferous forest soils, decomposition of litter takes place on the surface of the mineral soil.

Extraction of 'total' soil humic substances was achieved using 0.1 N NaOH, which releases up to 80 % of the soil humic substances (Stevenson, 1994). The 'total' concentration of soil humic substances was shown to be greater in the surface of the coniferous forest soil (B2C) than in the deciduous broadleaf forest soil (B1D) (absorption indices of 117.1 and 105.6, respectively). At the greatest soil depths studied (B1D, 4.5 cm; B2C, 12.5 cm), however, the concentrations of 'total' soil humic substances for the two types of soil were similar (absorption indices of 57.1 and 61.0 for B1D and B2C, respectively).

For the deciduous broadleaf soil (B1D), the 0.045 M Tris-borate solvent yielded humic extracts that represented 36-45 % of the concentration of the 'total' soil humic substances. This was similar to the finding of Eloff and Pauli (1975), who showed that Tris-borate-EDTA (0.5 M Tris-borate, 0.21 M EDTA, pH 8.9) extracted 33-50 % of the total (0.1 N NaOH/3 g l^{-1} $SnCl_2$ extract) humic substances from various soils.

5.3.2 Factors influencing the extractability of certain components of humic substances with increasing soil depth

Comparison of the concentration of humic substances extracted by both solvents (0.1 N NaOH and 0.045 M Tris-borate) with increasing soil depth revealed differences in the extractability of certain components of humic substances from the two soils. For the 0.1 N NaOH extracts of B1D and B2C, and the 0.045 M Tris-borate extract of B1D, extractability of humic substances decreased with increasing soil depth to final depth extracts that represented approximately 53 % of the respective surface (0.5 cm) extract humic concentrations. For the 0.045 M Tris-borate extract of B2C, however, the extractability of humic substances decreased much more sharply with increasing soil depth, and the final depth extract represented only approximately 8 % of the surface extract humic concentration. From other studies, it is well known that the concentration of dissolved organic matter decreases down the soil profile as a function of the decreasing soil organic carbon and as a result of the soil sorption and mineralisation processes (Thurman, 1985). Studies have also shown that humic substances are removed from solution with increasing depth through the B-horizon (Cronan and Aiken, 1985; Schnitzer *et al.*, 1959). As a

result, the humic substances removed from the solution in the B-horizon may in fact be more hydrophilic (contain a higher concentration of hydrophilic hydroxyl and carboxyl groups) than the humic substances of the A-horizon (Schnitzer *et al.*, 1959). Alternatively, the greater hydrophilicity may be a result of increasing humification, which increases the number of functional groups, such as carboxyl, phenol, quinone and ketone groups (Section 1.6.3). In explanation of the former, removal of hydrophilic humic substances from the soil solution results from the lowering of the net negative charge of the humic molecules due to increasing complexation of metals, and/or, to sorption onto secondary minerals (i.e. clays and metal hydr(oxide)s) (Schnitzer *et al.*, 1959). The difference in extractability of the component of 'total' soil humic substances represented by the 0.045 M Tris-borate extract for B2C suggests that this component is strongly affected by removal processes operating in the coniferous soil. These processes must be more effective in the coniferous soil than in the deciduous soil. Characterisation of the changes in composition of DOC has shown that it was the higher molecular weight components which were removed from percolating soil water with increasing soil depth for a coniferous forest soil (Kuiters and Mulder, 1993). A direct comparison with DOC composition for a deciduous broadleaf soil was, however, not made. Nevertheless, the higher molecular weight, Cu-binding, aqueous organic species in the same study appeared to be differentially removed from solution in the coniferous soil but not in the deciduous broadleaf soil. Thus, the sharply decreasing extractability of the component of the 'total' humic substances represented by the 0.045 M Tris-borate extract may be explained by a sorption of these humic substances to the soil solid phase which could not be disrupted by 0.045 M Tris-borate solvation. This component of the 'total' humic substances may be important with respect to actinide binding, and as important consequently, to the mobility of the complexed actinides by removal from the soil solution.

5.4 CONCENTRATION OF U IN THE DECIDUOUS BROADLEAF AND CONIFEROUS FOREST SOILS AND IN SOLVENT HUMIC EXTRACTS

5.4.1 'Pseudo-total' ^{238}U content of the deciduous broadleaf (B1D) and coniferous (B2C) forest soils

The 'pseudo-total' soil ^{238}U content was in the range of 407±14 - 497±133 μg (kg soil)$^{-1}$ (0.41-0.50 ppm) for the deciduous broadleaf soil (B1D) and 513±75 - 611±36 μg (kg soil)$^{-1}$ (0.51-0.61 ppm) for coniferous soil (B2C). These values are slightly lower than average soil ^{238}U concentrations (1 ppm, Alloway (1995)) but are close to the average concentration of ^{238}U in the underlying sedimentary rock (0.45 ppm, Bowen (1979)) which is expected to be the main source of the soil ^{238}U.

5.4.2 ^{238}U content of solvent-soluble forest soil humic extracts

The ^{238}U content of the 0.1 N NaOH extracts for the deciduous broadleaf soil (B1D) was 116±10 - 222±5 μg (kg soil)$^{-1}$. This corresponded to 29-45 % of the 'pseudo-total' soil ^{238}U content. For the coniferous soil (B2C), the ^{238}U content of the 0.1 N NaOH extracts was 257±10 - 354±27 μg (kg soil)$^{-1}$. These values corresponded to 47-65 % of the 'pseudo-total' soil ^{238}U. Although there was a slightly greater 'pseudo-total' ^{238}U content and a greater organic matter content, particularly in the near surface sections, the amount of humic material extracted by 0.1 N NaOH did not differ significantly from that extracted from the deciduous broadleaf soil. These results strongly suggest differences in the composition and indeed the U binding ability of the humic material from the two soil types. In addition, the concentration of humic-associated ^{238}U was linearly correlated with concentration of 'total' humic substances for the deciduous broadleaf soil ($r^2 = 0.88$) but not for the coniferous soil. This suggests that, for the coniferous soil there may also be changes in the nature or binding by humic substances with increasing soil depth.

The ^{238}U content of the 0.045 M Tris-borate humic extracts of the deciduous broadleaf soil (B1D) samples was 72±11 - 95±15 μg (kg soil)$^{-1}$. This represented 16-20 % of the 'pseudo-total' soil ^{238}U content, and 37-62 % of the 'total' humic substance ^{238}U content. The ^{238}U content of the coniferous soil (B2C) 0.045 M Tris-borate humic extracts was 26±1 - 90±8 μg (kg soil)$^{-1}$. These values corresponded to

4-17 % of the 'pseudo-total' soil ^{238}U content, and to 7-27 % of the 'total' humic substance ^{238}U content. For the near surface section (0.5 cm), the proportion of the 'pseudo-total' soil ^{238}U contained in the 0.045 M Tris-borate extract was similar for both soil types. This, however, represented a smaller fraction of the ^{238}U associated with the 'total' soil humic substances in the coniferous soil than in the deciduous broadleaf surface soil. Other differences between the soils were also apparent. For the deciduous broadleaf soil, there was only a slight change with depth in the concentration of ^{238}U in the 0.045 M Tris-borate extract. For the coniferous soil, there was a sharp decrease in ^{238}U concentration which coincided with the sharp decrease in concentration of the 0.045 M Tris-borate extractable humic substances. The concentration of ^{238}U associated with the 0.045 M Tris-borate extractable humic substances was correlated to the concentration of extracted humic substances for the coniferous soil (r^2 = 1.00). There was no strong correlation between these parameters for the deciduous broadleaf soil.

The molecular size of humic substances extracted is known to increase with increasing solution pH (You *et al.*, 1999). Therefore, the 0.045 M Tris-borate (pH 8.5) extract will contain the predominantly lower molecular size fraction of the wide range of humic molecules in the 0.1 N NaOH (pH 13) extract. Smaller humic molecules are likely to be the most mobile (Nisbet *et al.*, 1993) component of the total humic substances. Moving down the soil profile, however, the rapid decrease in concentration of such molecules relative to the total humic substances in the coniferous soil suggest that these smaller molecules are being efficiently immobilised. It should be noted that although there is no evidence for this in the 0-5 cm of the deciduous broadleaf soil, this may also have occurred at greater depth simply as a result of the increased mineral surface area presented by the larger volume of soil.

5.5 ELECTROPHORETIC EXTRACTION

5.5.1 Extraction efficiency for the deciduous broadleaf and coniferous forest soil humic substances

In comparison with 0.045 M Tris-borate solvent extraction, the electrophoretic extraction of humic substances (in 0.045 M Tris-borate running buffer) was less efficient for the deciduous broadleaf soil (B1D, comparative yield ~ 52 %), and more efficient for the coniferous soil (B2C, comparative yield ~ 125 %). The conditions used in the electrophoretic extraction were similar to those used in solvent extraction, both with respect to the buffer system used (0.045 M Tris-borate, pH 8.5) and with respect to the extraction period (3 hours). Differences included the soil-solvent ratio (electrophoretic extraction: 0.1 g soil/1.5 ml of loading buffer; solvent extraction: 0.1 g soil/30 ml solvent), pre-equilibration with a simulated soil solution prior to extraction (electrophoresis only), and the applied electric field (electrophoresis only). The differences in soil-to-solvent ratio were a consequence of (i) the small size of the electrophoresis gel well and (ii) the need to maximise solvent extraction of humic material (3 × 10 solvent ml) within the time period of 3 hours. The differences likely to result from pre-equilibration with a simulated soil solution are that very low molecular weight humic substances (soluble at pH 4.2) would have been removed from the total soil humic substances and therefore would not be extracted by electrophoresis. A further difference between the electrophoretic and solvent extraction is the effect of the applied electric field on the electrophoretic extraction efficiency. Some interactions (e.g. H-bonding and electrostatic interaction) between humic substances and other soil components (i.e. humic-humic or humic-secondary mineral interactions) may be disrupted by the electric field thus enabling extraction of this component of the total soil humic substances. From the above considerations, therefore, the lower extraction efficiency of electrophoresis for the deciduous broadleaf soil, compared to the 0.045 M Tris-borate solvent extraction, is most probably attributable to the removal of very low molecular weight humic substances soluble at the approximate soil solution pH of 4.2. The higher extraction efficiency of electrophoresis toward the coniferous soil, compared to the Tris-borate solvent extraction, is most probably attributable to the disruption of weak interactions previously immobilising humic substances.

5.5.2 Molecular size difference between the extract fractions and depth trends

Gel filtration chromatography, using a Sephadex G-200 column (size separation 1000-600,000 Da for standard biomacromolecules, (Pharmacia Biotech, 1994), showed that the electrophoretic extract brown band (B2C 0.5 cm fraction F3) was a mixture of molecules, with a distribution of large to small molecular sizes which was skewed towards the lower molecular sizes. A UV-fluorescent extract band of a forest soil, by the same analysis, revealed that this band was a less complex mixture (Graham *et al.*, in press). The band of applied sample did not extend the length of the gel column, as the brown band material had, on elution through the column. The UV-fluorescent band contained molecules of small molecular size. A fraction of the tailing brown band of a forest soil electrophoretic extract eluted close to the exclusion limit of the column (Graham *et al.*, in press).

The focused brown band necessarily contained humic substances of similar electrophoretic mobility, though this extract band consisted of a spectrum of molecular sizes of humic molecules. Similarity in electrophoretic mobility between molecules of different molecular mass arises from differences in the charge, size and shape characteristics of the molecules i.e. small molecules of low charge or more elongated shape may have the same electrophoretic mobility as large molecules of high charge or more spherical shape. However, the similarity in electrophoretic mobility of the mixture of humic molecules of the brown band infers similar hydrophilicity of these molecules, which as a group, were less hydrophilic than the UV-fluorescent band humic molecules. The tailing band represents the largest and least hydrophilic humic molecules of the extract.

The total electrophoretic humic extract concentration and the fraction concentration patterns of the deciduous broadleaf (B1D) and coniferous (B2C) forest soils demonstrated differences for the two soil types. There was only a small reduction in the electrophoretic humic extract concentration (14 %) for the deciduous broadleaf soil and very little difference in the fraction concentration pattern with increasing soil depth (0.5 cm – 2.5 cm). In contrast, the coniferous soil showed a large decrease in

electrophoretic extract concentration (92 % reduction) with increasing soil depth, and a shift from large to small molecular size humic molecules. Therefore, it would appear that the larger humic molecules of the electrophoretic humic extract are differentially immobilised in the coniferous soil. This is similar to the result of (Kuiters and Mulder, 1993), who found that the higher molecular weight component of DOC was removed from the soil percolating water with increasing soil depth for a coniferous forest soil.

5.5.3 FTIR and other investigations of the chemical nature of the electrophoretic humic extract fractions

The FTIR spectra of the electrophoretic extract fractions demonstrated that these humic substances comprised oxygen containing functional groups: carboxylic acid, phenol, alcohol, ether and possibly amide groups. It is the oxygen-containing functional groups (i.e. hard base donor atom ligands), in particular the carboxylic acid groups, that are implicated in the binding of actinides (hard acid). Also, the presence of the oxygen-containing functional groups contributes to the hydrophilicity of the humic macromolecules. The FTIR spectra were observed to be similar to the literature classified Type III humic acids. The similarity suggests that the electrophoretic extract humic molecules contain carbohydrate and protein structures. Type III humic acid spectra are typical of lake humic acids (and soil fulvic acid, as the example in Figure 4.1.4, Section 4.3.2 depicts). This suggests similarity in the molecular size and hydrophilicity of the electrophoretic extract humic molecules and lake humic acids or soil fulvic acids. Traditionally, the hydrophilic component of humic substances, fulvic acid, is obtained by alkali extraction (pH ~ 13) followed by acid precipitation (pH < 2) (Section 1.12.1). Electrophoresis in buffered solution (0.045 M Tris-borate, pH 8.5), which extracts and fractionates humic substances on the basis of the mobility of the anionic humic molecules under an applied electric field, avoids possible chemical alteration induced by extremes of pH.

Further analyses (E_4/E_6 ratio, elemental CHN analysis and acid-base titration) in conjunction with the GFC characterisation work have demonstrated that electrophoretic extraction and fractionation of humic substances from forest soil is

based on chemical and size differences between the humic molecules (Graham *et al.*, in press).

5.5.4 Association of ^{238}U with the total electrophoretic extract

The mean association of ^{238}U with the deciduous broadleaf soil (B1D) electrophoretic extracts, except for the surface (0.5 cm) sample, was similar to that associated with the 'total' soil humic substances (0.1 N NaOH extract) (Table 5.1). For the surface (0.5 cm) deciduous soil sample, however, the concentration of ^{238}U associated with the electrophoretic extract (39±24 μg (kg soil)$^{-1}$) was lower than that observed with the 0.045 M Tris-borate or 0.1 N NaOH extracts (90±16 μg (kg soil)$^{-1}$ and 187±30 μg (kg soil)$^{-1}$, respectively). This lower association could reflect a low degree of functionality of humic substances recently formed at this soil depth. For a deciduous broadleaf soil, litter decomposition and formation of humic substances occurs in the Ah mineral horizon, rather than the surface of the soil as for coniferous soils, because of the influence of pedoturbation (Armson, 1979). Humification is associated with an increase in carboxylic acid, phenol, quinone and ketone groups (Stevenson, 1994), i.e. oxygen-containing functional groups which may bind actinides. The 0.045 M Tris-borate humic extract did not show a surface minimum of ^{238}U association and this difference probably reflected the incorporation in this extract, but not in the electrophoretic extract, of humic substances (and associated ^{238}U) soluble at pH 4.2. For the coniferous soil, at all soil depths, the concentration of ^{238}U associated with the electrophoretic extract was intermediate between the concentration of ^{238}U observed associated with the 0.045 M Tris-borate and 0.1 N NaOH extracts. The coniferous soil had, however, demonstrated a higher percentage association of the 'pseudo-total' ^{238}U with the 'total' soil humic substances than was observed for the deciduous broadleaf soil. Thus the lower association of the 'total' humic-associated ^{238}U with the electrophoretic humic component suggests that a greater proportion of the ^{238}U-humate complexes are immobilised in the soil solid phase by relatively strong interaction with other soil components. The assumption is made that the high association of the 'pseudo-total' soil ^{238}U with the 'total' humic substances is observed because the greater ionisation and solvation of humic substances at pH 13 (0.1 N NaOH) disrupts the immobilising interactions.

	B1D	B2C
0.1 N NaOH	116±10 - 222± 5	257±10 - 354±27
Electrophoresis	125±95 - 181±118	79±67 - 178± 8
0.045 M Tris-borate	72±11 - 95± 15	26± 1 - 90± 8

Table 5.1: The range of concentrations (and 95 % confidence limits) of ^{238}U (μg (kg soil)$^{-1}$) in the 0.1 N NaOH and electrophoretic and 0.045 M Tris-borate extracts of the deciduous broadleaf (B1D, excluding the 0.5 cm sample) and coniferous (B2C) forest soil

Investigation of the association of ^{238}U with the focused electrophoretic extract brown and UV-fluorescent bands (fraction F3-F6), rather than the total electrophoretic extract, demonstrates a better correlation between electrophoretic and 0.045 M Tris-borate solvent extract ^{238}U content (Figure 5.1 and Figure 5.2).

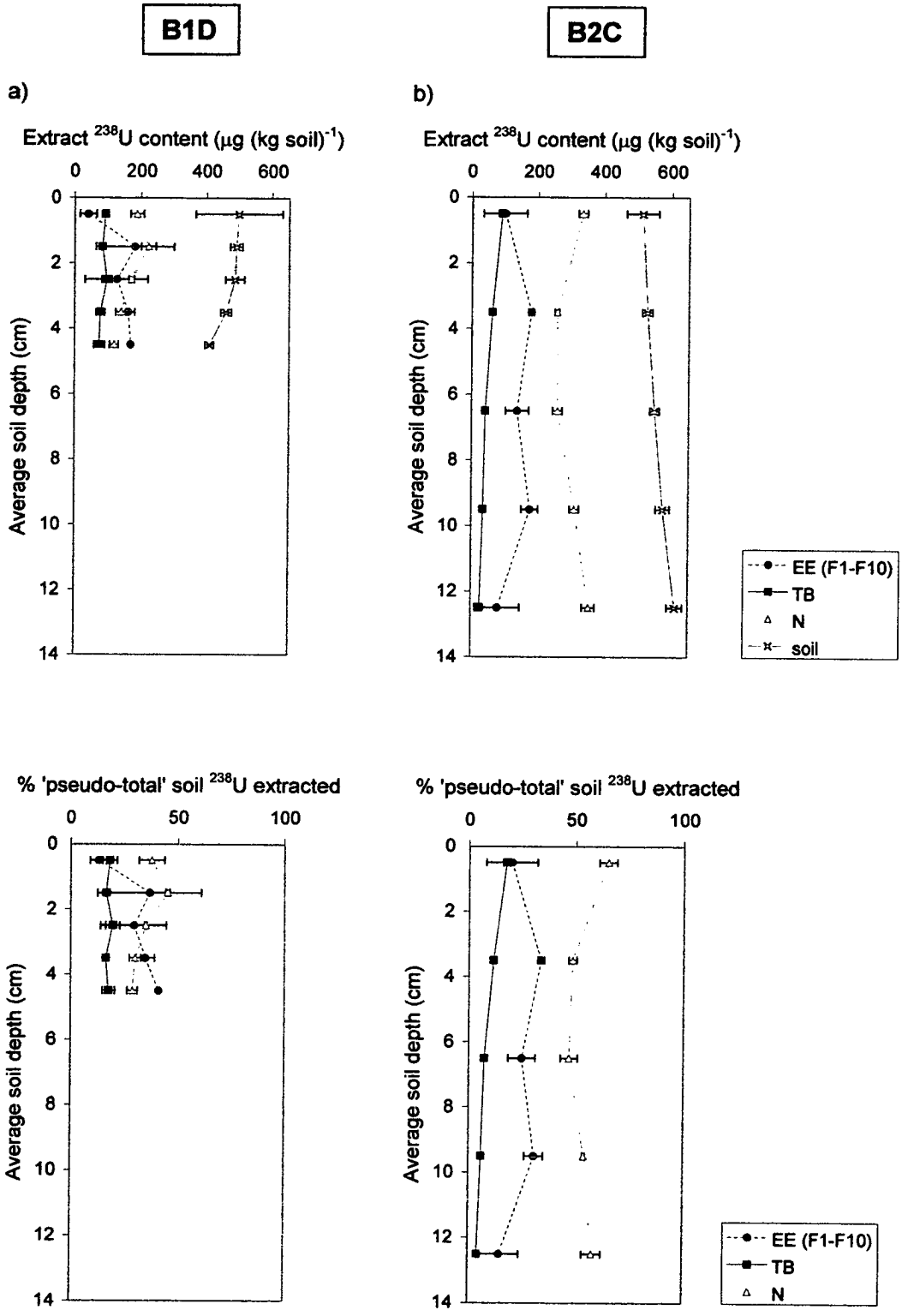


Figure 5.1: Vertical profiles of 'pseudo-total' soil and soil humic extract ^{238}U contents (µg (kg soil)$^{-1}$) (a and b), and profiles of the percentage of the 'pseudo-total' soil ^{238}U associated with the humic extracts (c and d), for the deciduous broadleaf (B1D) and coniferous (B2C) forest soils. (soil – 'pseudo-total' soil ^{238}U, N – 0.1 N NaOH extract, TB – 0.045 M Tris-borate extract, EE – electrophoretic extract).

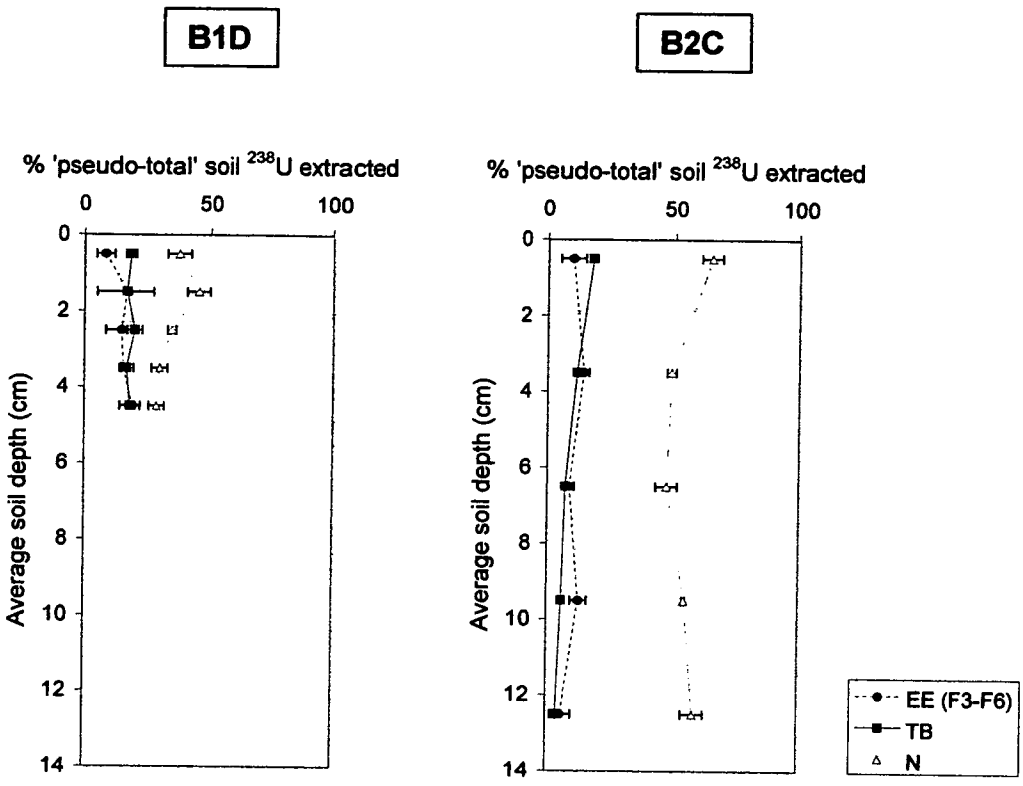


Figure 5.2: Comparison of the profile of association of ^{238}U with the focused (brown and UV-fluorescent) electrophoretic humic extract and the 0.045 M Tris-borate and 'total' soil (0.1 N NaOH) humic extracts for the deciduous broadleaf (B1D) and coniferous (B2C) forest soils. ^{238}U association is expressed as a percentage of the 'pseudo-total' soil ^{238}U. (EE (F3-F6) – focused electrophoretic extract brown and UV-fluorescent bands, TB – 0.045 M Tris-borate extract, N – 0.1 N NaOH extract).

The humic molecules of the focused electrophoretic extract bands therefore show greatest similarity to the 0.045 M Tris-borate humic extract, with respect to ^{238}U association. Thus, the extract fractions F1-F2 and F7-F10 may represent humic molecules immobilised in soil by weak interactions to other soil components. Alternatively, the very large molecular size humic substances of fractions F1-F2, which are extracted after separation of the focused brown band and UV-fluorescent band, may also in part result from a greater solvation efficiency of the electrophoresis experiment. The solvent extraction, using 0.045 M Tris-borate, possibly solvates only the more hydrophilic humic substances. Electrophoresis, however, may extract the very high molecular weight humic substances by virtue of efficient removal of the focused band humic substances, allowing the less soluble humic substances to equilibrate between the solid/solution phases. The electric field

may also contribute to the separation of the very high molecular weight humics form the bulk soil.

5.5.5 Definition of the humic substances of the soil actinide pools and implications for the fate of associated actinides

A definition of the humic substances of the soil actinide pools obtained by the 'equilibration' and electrophoretic extraction experiments, based on the discussion of the electrophoretic humic extract concentration, character and association of ^{238}U, compared with the 0.045 M Tris-borate humic extracts, is presented in Table 5.2.

Soil actinide pool	Humic fraction	Character and mobility
Labile		<0.2 μm, soluble at pH 4.2 lowest molecular size, most hydrophilic component of HS
Hydrophilic-mobile		all fractions <0.15 μm, negatively-charged at pH 8.5
	F1-F2	highest molecular size, least hydrophilic component of HS either supramolecules or polymers extracted by efficiency of removal of humic substances from bulk soil and the influence of the electric field or by disruption of weak immobilising interactions
	F3-F4	polydisperse - intermediate to small molecular sizes
	F5-F6	small molecular size
	(F3-F6)	immobilisation by weak interactions probably increases with increasing soil depth, the degree of which is greater for the coniferous cf. the deciduous broadleaf soil
	F7-F10	highly electrophoretically mobile fractions, probably very small molecular size immobilised in soil by weak interactions
Immobile		strongly immobilised/highly hydrophobic HS

Table 5.2: Definitions of the mobility and character of humic substances belonging to the different operationally defined soil actinide pools

A discussion of the implications of the character and mobility of the humic substances of the different soil actinide pools now follows. The labile pool humic substances are likely to be leached through the soil profile to receiving waters. The hydrophilic-mobile pool humic substances, which are not soluble at the soil solution

pH, are most likely to be transported in the form of humic colloids or organo-mineral aggregates (≤ 10 μm). The transport of these colloids/aggregates will be effected during events of either high precipitation or flooding. Once transported to receiving natural waters of higher pH, a proportion of the humic substances may be released as discrete humic substances. The very high molecular weight, less hydrophilic humic substances (fractions F1-F2) are most likely to remain in their soil form in the receiving waters and thus may deposit and decompose in the receiving water sediment. The discrete proportion of the high molecular size, hydrophilic humic substances (F3-F6) will be mobile in the receiving waters but may be removed from solution under conditions such as increasing salinity, i.e. in estuarine waters. The discrete proportion of the smallest molecular size, most hydrophilic humic substances (F7-F10) are likely to travel the furthest distances in the receiving waters. The humic substances associated with the immobile pool will remain in the soil profile. Strong interaction with the soil solid phases suggests mean residence times of these humic substances of the order of several hundred to a couple of thousand years (Stevenson, 1994).

5.6 ACTINIDE DISTRIBUTION BETWEEN SOIL POOLS

5.6.1 The immobile pool

The ^{238}U immobile pool represents ^{238}U associated with strongly immobilised/ highly hydrophobic humic substances, or ^{238}U strongly bound to mineral soil components.

For naturally occurring ^{238}U, the largest soil actinide pool was observed to be the immobile pool. This pool was larger for the coniferous soil (B2C; 78-81 %) than for the deciduous broadleaf soil (B1D; 67-77%).

The immobile pool was also shown to represent the largest soil actinide pool for the added ^{236}U. This pool was slightly lower in magnitude for the deciduous broadleaf soil (B1D) than was observed for the ^{238}U association, but was similar for both ^{238}U and ^{236}U association for the coniferous soil (B2C).

The longer 'equilibration' experiment (B2C 0.5 cm) demonstrated an increase in the immobility of both naturally occurring ^{238}U and added ^{236}U. Thus, by increasing the time in which U in the solution phase was in contact, with stirring, with the solid phase, an immobilisation of a proportion of the solution phase U had occurred.

5.6.2 The labile pool

For ^{242}Pu, the largest soil actinide pool was observed to be the labile pool. This pool was larger for the deciduous broadleaf soil (B1D, 79-88 %) than for the coniferous soil (55-65 %). The immobile pool of the coniferous soil (B2C, 33-36 %) was much larger than for the deciduous broadleaf soil (9-14 %) whilst the hydrophilic-mobile pool was the smallest ^{242}Pu pool for both soil types (B1D, 4-7 %; B2C, 1-9 %). Thus, added ^{242}Pu showed less labile associations with the coniferous soil than for the deciduous broadleaf soil.

The labile pool represents the smallest molecular size, most hydrophilic, humic substances. The high association of ^{242}Pu with this pool either represents ^{242}Pu complexed to this component of the total humic substances or uncomplexed Pu (PuO_2^+). Complexation of Pu by acetate may be discounted because U, which has a higher acetate complex stability constant than Pu, was not observed to such a large extent in this pool (available data: UO_2^{2+}, log K 2.61; PuO_2^{2+}, log K 2.31 at 20 °C (Martell and Smith, 1977). ^{242}Pu may have been observed to be more labile than U because of a difference in the kinetics of complexation to the immobile and hydrophilic-mobile pool humic substances. On thermodynamic considerations, ^{242}Pu would be expected to show greater complex stability with humic substances than UO_2^{2+}, if the PuO_2^+ was reduced after complexation by the humic ligand (to Pu^{4+}) as is reported in the literature (Nash et al. 1981; Tan *et al.*, 1993). If reduction is slow then in the competition between UO_2^{2+} and PuO_2^+, complexation of the UO_2^{2+} ion would be favoured because of the higher effective charge density of this ion (Choppin and Rao, 1984). Tipping (1993) found that the actinide humic substance interaction constant for metal proton exchange was greater for the hexavalent uranyl ion (UO_2^{2+}), than for the pentavalent plutonium ion (PuO_2^+), by approximately one order of magnitude.

5.7 ACTINIDE PATTERNS OF THE HYDROPHILIC-MOBILE POOL

5.7.1 Pattern of ^{238}U association

The naturally occurring ^{238}U was observed in association with all ten electrophoretic extract fractions for both soil types. A pattern of high association of ^{238}U for both soil types (> 15 % of the total extract ^{238}U) with fractions F2-F4, typically maximal in fraction F3, and often, but not exclusively, observed when overall extraction was low, was similar to the extract concentration of humic substances. With increasing soil depth for the deciduous broadleaf soil (B1D), there was a slight loss of ^{238}U association with higher mobility extract fractions F7-F10. For the coniferous soil (B2C), variable and much higher losses in association with individual fractions occurred with depth changes. The variability in the extract fraction patterns of both soils may be attributed to the heterogeneity of the soil. Instances of absence of ^{238}U association in fractions F7-F10 suggests absence of the low molecular weight, highly charged soil components.

5.7.2 Pattern of ^{236}U association

The added ^{236}U fraction patterns for both soil types were reproducible, and association of ^{236}U was observed with fractions F1-F5, and was maximal in fraction F4/F5. The percentage of the total added ^{236}U associated with the hydrophilic-mobile pool was lower than the association of ^{238}U (B1D, 13±4 - 41±1 %; B2C, 15±9 - 34±2 %), and also showed a decrease with increasing soil depth (B1D, 13-10 %, 0.5 cm-4.5 cm; B2C, 16-6 %, 0.5 cm-12.5 cm). The decrease in association with increasing depth was greater for the coniferous soil than for the deciduous broadleaf soil. This was probably due to the greater immobilisation of humic substances in the coniferous soil with increasing soil depth. Added ^{236}U was not observed in association with the high mobility fractions F7-F10. It is possible that either the interaction of ^{236}U with these ligands was not kinetically permissible (within 14 hours) or that the binding sites were fully occupied. Slow interaction with actinides may result from the conformation of the ligands. Humic substances with a high loading of metal are more folded in conformation. The more folded conformation means that the diffusion and interaction of actinide metals to and from the humic ligand is slower (Rao *et al.*, 1994). The association of ^{236}U with the electrophoretic

humic extract was similar to the pattern of humic concentration. Thus, with increasing soil depth the interaction of ^{236}U decreased with decreasing humic concentration.

5.7.3 Pattern of ^{238}U and ^{236}U association of the extended 'equilibration' experiment

In the increased 'equilibration' time experiment the distribution of ^{238}U and ^{236}U between the soil actinide pool shifted slightly to an increase in the immobile pool. The effect was greater for the naturally occurring ^{238}U than for the added ^{236}U. The increase in immobility of ^{238}U coincided with a loss of ^{238}U association with the smaller molecular size fractions F7-F10. Thus, increased contact time may correspond to an immobilisation of low molecular size electrophoretic extract fractions.

5.7.4 Pattern of electrophoretic mobility of the control ^{236}U

The ^{236}U control electrophoretic extract pattern was different to both the naturally occurring ^{238}U and the added ^{236}U soil patterns. The electrophoretic mobility of the ^{236}U was centred at fraction F3. This pattern represents the electrophoretic mobility of the dominant U species at pH 8.5; $UO_2(CO_3)_2^{2-}$ (Choppin *et al.*, 1995). This U species was not expected to occur in the electrophoretic extract of soil, as the sample loaded in the electrophoresis wells represented the phase of the soil insoluble at pH 4.2, and thus naturally occurring ^{238}U, or added ^{236}U, of the soil pellet was bound to soil components.

5.7.5 Pattern of association of ^{242}Pu

The added ^{242}Pu fraction patterns for both soil types were reproducible, and association of ^{242}Pu was observed with fractions F2-F4, and was maximal in fraction F3. Thus, ^{242}Pu was observed in maximal association with larger molecular size humic molecules than was observed for ^{236}U (maximal association with fraction F4/F5). The percentage of the total added ^{242}Pu associated with the hydrophilic-mobile pool was lower than the association of added ^{236}U (B1D, 13-10 %, 0.5 cm-4.5 cm; B2C, 16-6 %, 0.5 cm-12.5 cm) or naturally occurring ^{238}U (B1D, 13±4 - 41±1 %; B2C, 15±9 - 34±2 %). As for added ^{236}U, added ^{242}Pu showed a depth trend

of decreasing association with increasing soil depth (B1D, 8-4 %, 0.5 cm-4.5 cm; B2C, 8-1 %, 0.5 cm-12.5 cm). Again, the decrease in association with increasing depth was greater for the coniferous soil than for the deciduous broadleaf soil. Added ^{242}Pu, as for added ^{236}U, was not observed in association with the high mobility fractions F7-F10.

5.8 ACTINIDE SPECIATION AND MOBILITY IN SOILS

This section compares the results of the few studies of actinide behaviour in forest soils with the findings of this study.

5.8.1 Soil solution U

Porcelli *et al.* (1997) found that 30-90% of the solute U of a peat mire was in the colloidal fraction (>10 000D), which was suggested to be humic acid. The solute U represented approximately 0.01 % of the solid phase peat mire uranium. Though dissimilar in magnitude, the proportion of the 'pseudo-total' soil ^{238}U in the solution phase (labile pool) in this study was also small (3-12 %) compared to the proportion in the solid phase, i.e. the combined pools of hydrophilic-mobile and immobile pool (88-97 % combined).

5.8.2 Soil solution Pu

In an investigation using gel filtration chromatography, Agapkina *et al.* (1995) found that of the total forest (mixed deciduous and coniferous stand, 60 years of age) soil solution $^{238\text{-}240}$Pu (from the Chernobyl incident), 70-86 % was associated with the largest studied organic matter fraction (>2000 D), 2-16 % was associated with the 1000-1300 D organic matter fraction, and less than 5 % was in an inorganic form. Nisbet *et al.* (1993) also found that in a loam soil (pH 7.1) artificially contaminated with ^{239}Pu approximately 7 years earlier, 61 % of the soil solution ^{239}Pu was associated with the colloidal and high molecular weight fractions (41 % ^{239}Pu > 3 kD, 20 % ^{239}Pu > 10 kD) which were suggested to be humic material and polysilicates. The high percentage of the total added ^{242}Pu associated with the labile soil actinide pool in this study may be partly associated with soil solution phase humic substances.

5.8.3 Soil U

Of the total soil ^{238}U, only 0-1 % was associated with the soil humic and fulvic acids (Fujikawa *et al.*, 1999). In this study the percentage of 'pseudo-total' soil ^{238}U associated with the 'total' soil humic substances was much higher than this (B1D, 29±2 - 45±1 %; B2C, 47±4 - 65±4 %) and probably reflected the difference in extraction procedure. The humic acid of the literature study had been obtained by extraction in 0.5 % NaOH at 85 °C for 30 minutes under a nitrogen atmosphere, and fractionation by precipitation at pH 1 (HCl). The precipitated humic acid was rinsed with mineral acid (0.1 M HCl + 0.3 M HF). Thus, only the most stable actinide-humic complexes were suggested by the authors to have been observed, i.e. less strongly associated ^{238}U would have been released from the humic substances under these acid conditions.

5.8.4 Soil Pu

Greater than 90 % of the total soil ^{239}Pu activity of an artificially contaminated loam soil was associated with organic species less susceptible to dissolution i.e. extracted by trimethylchlorosilane at 60° C (the activity of the 0.3 M NaOH extract obtained at 60° C was much lower, at less than 20 % of the total ^{239}Pu activity) (Szabo *et al.*, 1997). On further investigation this activity was found to be retained by an ultrafilter pore size of 100,000 D. Similarly, in this study ^{242}Pu was found in maximal association with high molecular weight humic fractions of the hydrophilic-mobile pool.

In a study of Pu released to a forest soil by the use of nuclear weapons, it was found that 1-9 % of the total soil Pu was associated with humic and fulvic acids (Fujikawa *et al.*, 1999). The order of magnitude of the Pu association with the soil humic substances was of the order of that observed with the hydrophilic mobile pool (1-8 %) in this study. However, the method of humic substance extraction was markedly different (Section 5.8.4). The association of Pu with the fulvic acid soil component was suggested to explain the observed migration of Pu down the forest soil profile, 3 % of the soil $^{239+240}Pu$ had travelled a distance of 4 m down the profile.

The association of $^{238\text{-}240}Pu$ in a mixed woodland soil (pH 6.9), derived mainly from low-level discharges from the BNF nuclear fuel reprocessing plant at Sellafield (N.W. England) which were maximal in the 1970s, was predominantly with soil organic matter (53 % of the total soil Pu, at 0-5 cm soil depth) (Livens and Baxter, 1988). The Pu was preferentially, but not exclusively, associated with the high molecular weight fractions of humic substances. Though it was suggested that the association might be an overestimate because the 0.1 M sodium pyrophosphate extraction solvent might also extract clays, the association was slightly higher than that observed for ^{242}Pu with the hydrophilic-mobile and immobile soil pools in this study (B1D, 13-21 %; B2C, 34-45 %, for the combined hydrophilic-mobile and immobile pools). Also, a much smaller association was observed in the literature study with extracts that might be supposed to contain the soil solution phase humic substances (0.05 M $CaCl_2$ and 0.5 M CH_3COOH; 3.5 %) compared to the association with the labile pool in this study (B1D, 79-88 %; B2C, 55-65 %). The difference in the proportions of Pu between pools for the two studies might reflect the source of the Pu and/ or the 'equilibration' time. In the literature study, the source of Pu was transported and redeposited contaminated marine sediment, whereas in this study it was the added solution phase Pu species, PuO_2^+. The samples in the literature study were collected in 1982, approximately 10 years after the maximal discharges from the reprocessing plant (although maximum concentration of Pu from transported and redeposited marine sediment in coastal soils may occur several years after maximum release from the plant (MacKenzie *et al.*, 1994)), whereas 'equilibration' in this study was 14 hours.

6 CONCLUSIONS

6.1 PROPERTIES (ORGANIC MATTER CONTENT, CONCENTRATION OF HUMIC SUBSTANCES AND PARTICLE SIZE) OF THE DECIDUOUS BROADLEAF AND CONIFEROUS FOREST SOILS

- For both soil types, soil organic matter content (L.O.I.) and proton activity (reflecting dissolved humic substances) decreased with increasing soil depth.
- Although for both soil types the concentration of total soil humic substances (0.1 N NaOH extract) also decreased with increasing depth, the precise trends reflected differences in the mixing of the litter and mineral soil horizons, and the depth at which litter was decomposed. In a deciduous broadleaf forest, the leaf litter is mixed with the surface mineral soil and is decomposed in the Ah horizon (a humic-enriched horizon). A sharp decrease, significantly greater than that observed in the total organic matter content profile, was observed from 1.5 cm downwards. In a coniferous forest soil, decomposition of litter occurs on the surface of the soil and humic concentration of the coniferous soil in this study was observed to decrease from the surface downwards. In addition, the change in concentration of total humic substances resembled more closely that of total organic matter for the coniferous rather than for the deciduous broadleaf soil.
- From the particle size data, there was no significant difference in the proportion of very fine/fine particles either with increasing soil depth or between soil types. The underlying bedrock was the same and thus mineral composition as well as particle size variations were discounted as possible variables in the interaction of actinides with soil components.

6.2 ELECTROPHORETIC EXTRACTION AND FRACTIONATION COMPARED WITH TRADITIONAL SOLVENT EXTRACTION AND FRACTIONATION

- The 0.045 M Tris-borate solvent extraction yielded ~36-45 % of the 'total' soil humic substances (0.1 N NaOH extract), similar to results reported in the literature. A comparison of the extractability of humic substances with increasing depth revealed that the hydrophilic soil humic substances (represented

by the 0.045 M Tris-borate, pH 8.5 solvent extract) were more efficiently sorbed to other soil components and immobilised in the lower horizons of the coniferous than in the deciduous broadleaf soil. It was also shown in the electrophoretic extraction experiment that higher molecular weight humic substances were preferentially sorbed, in agreement with literature results.

- Electrophoretic extraction and fractionation of humic substances from soil is based on the anionic mobility of humic substances (buffered solution, pH 8.5) in an applied electric field. This new alternative method avoids possible chemical alteration of humic substances resulting from exposure to the extremes of pH conditions used in the traditional methods of extraction (pH 13) and fractionation (pH < 2).

- Initial comparison of electrophoretic humic extract concentration with that of the 0.045 M Tris borate and 0.1 N NaOH ('total') solvent extracts revealed that, for the coniferous soil samples, the electrophoretic method extracted a slightly greater proportion of the 'total' humic substances than had been obtained using 0.045 M Tris-borate *per se.* In contrast, electrophoretic extraction was much less efficient than 0.045 M Tris-borate for the deciduous soil samples. This was attributed to the removal of humic substances in the simulated soil solution.

- Further characterisation revealed that, independent of extraction efficiency (based on UV absorbance at 254 nm), electrophoresis extracted a polydisperse component of the 'total' soil humic substances, i.e. molecular size of 1,000s to 100,000s D, and was thus capable of extracting humic molecules with a wide range of potential environmental mobilities.

- An important finding based on the ICP-MS results for ^{238}U was that, again independent of extraction efficiency, significantly more humic-associated ^{238}U was extracted during electrophoresis than by 0.045 M Tris-borate solvent extraction. This was attributed to differences in the composition of the organic material in the extracts obtained by these two methods.

- Clearly, the composition of extracts obtained by the 0.045 M Tris-borate solvent and electrophoretic extraction methods were not identical and it is contended that the focused brown and fluorescent bands (F3-F6) obtained by electrophoretic

extraction and fractionation most closely resemble the 0.045 M Tris-borate solvent extract. This is supported by early method development work involving electrophoretic fractionation of the 0.045 M Tris-borate humic extracts which revealed only brown and fluorescent bands. No ^{238}U data are available for this part of the work.

- The greater efficiency of electrophoretic extraction of humic-associated ^{238}U is therefore suggested to be the result of the following.

  1. Extraction of humic substances immobilised by weak interactions (e.g. H-bonding and weak electrostatic interaction) with other soil components (F7-F10).

  2. Extraction of less hydrophilic, high molecular weight humic substances by efficient removal of initially extracted humic substances from bulk soil (F1-F2).

- Further work involving gel chromatographic fractionation of individual electrophoretic fractions showed that fractionation based on size differences between the humic substances had also occurred during the electrophoretic extraction procedure.

  1. The tailing brown band (fraction F1/F2) contained the highest molecular weight, least hydrophilic component of the electrophoretic humic extract.

  2. The focused brown band (fraction F3/F4) also contained very large humic molecules but had a significant spread of humic molecular sizes (skewed towards low molecular size).

  3. The focused UV-fluorescent band (fraction F5/F6) contained significantly smaller humic molecules.

  4. The most mobile material (fractions F7-F10), as yet uncharacterised due to the small amount isolated, probably contained highly hydrophilic, low molecular size organic molecules. These molecules may not necessarily be humic in nature.

- From the series of results of (i) molecular size of the electrophoretic extract fractions, (ii) humic-associated ^{238}U data for electrophoretic and 0.045 M Tris-

borate soil extract and (iii) comparison of light absorption and fluorescence by an electrophoretically fractionated 0.045 M Tris-borate solvent extract and by soil directly electrophoretically extracted and fractionated, the electrophoretic extraction procedure was shown to result in an extract which had a greater polydispersity than that obtained by 0.045 M Tris-borate solvent extraction.

6.3 CLASSIFICATION OF SOIL POOLS CONTAINING U AND Pu AND IMPLICATIONS FOR MOBILITY OF U AND PU IN FOREST SOILS

- The labile pool comprises $U(VI)O_2^{2+}$ or $Pu(V)O_2^{+}$, and U and Pu associated with buffer-extractable (pH 4.2) organic ligands. The organic ligands in this pool, some of which could be a component of total humic substances, were not further characterised. Since the pH of the simulated soil solution was close to that of the measured pH values, the actinides either uncomplexed or associated with these organic ligands will be mobile in the environment and will be transported to receiving natural waters.

- The hydrophilic-mobile pool comprises U and Pu associated with humic substances, which are not soluble at the soil solution pH but which can be extracted electrophoretically at pH 8.5. These humic substances are either insoluble at pH 4.2 because of high molecular size or weak interactions with other soil components. Mobilisation of a proportion of these humic substances which are associated with fine or very fine soil particles (≤ 10 μm) may occur during very high precipitation/flooding events. This may result in the transport of humic-associated U and Pu down the soil profile and to receiving natural waters.

- It is contended, however, that the relative environmental mobility, of the proportion of different components of the electrophoretic extract released to and solubilised at the higher pHs of the receiving waters will vary. On the basis of their large molecular size, the humic molecules present in fractions F1-F2 will be most readily removed from the solution. Those present in fractions F3-F6 exhibit a range of sizes but are more soluble in aqueous solution (*cf.* similarity to humic substances extracted by 0.045 M Tris-borate) and may therefore remain in the solution for considerably longer time periods. The proportion of very

hydrophilic, small molecular size fractions F7-F10, appear to be held by interactions with the solid phases of the soil (*cf.* not extracted by 0.045 M Tris-borate). Upon transport to receiving waters, however, these fractions may be transported over long distances in the solution.

- The immobile pool includes U and Pu associated with humic substances which either have a predominantly hydrophobic character or which are immobilised by strong interactions with other soil components, as well as U and Pu associated with inorganic phases of the soil. These forms of U and Pu are most likely responsible for long-term retention of actinides within the soil profile.

6.4 INTRODUCTION OF ARTIFICIAL ACTINIDES TO WHOLE SOIL SAMPLES

- This novel laboratory experiment allowed investigation of actinide (added in the form predicted on the basis of soil solution pH) interactions with all components of the soil matrix, at different soil depths, in each of the forest soil types.
- The *in situ* conformation and dissociation character of the humic ligands, ensured by simulation of the soil solution pH, means that the actinide associations with soil humic substances in this study are relevant to the environmental behaviour of actinides.
- A measure of the effectiveness of this experiment was the high reproducibility of (i) the distribution of added actinides (^{236}U or ^{242}Pu) between the soil actinide pools, and (ii) the pattern of association with the individual fractions of the electrophoretic extracts.

6.5 MOBILITY OF U AND Pu IN THE DECIDUOUS BROADLEAF AND CONIFEROUS FOREST SOILS

6.5.1 Forest soil 'pseudo-total' ^{238}U concentration and concentration of ^{238}U in association with humic substances

- The 'pseudo-total' soil ^{238}U concentrations were slightly, but not significantly, higher in the coniferous compared with the deciduous broadleaf forest soil (B1D, 407±14 - 497±133 μg (kg soil)$^{-1}$, i.e. 0.41-0.50 ppm; B2C, 513±75 - 611±36 μg (kg soil)$^{-1}$, i.e. 0.51-0.61 ppm). These values were lower than average soil

^{238}U concentrations (1 ppm) but close to the typical concentration of ^{238}U in the underlying rock material (0.45 ppm), which is the main source of the ^{238}U to the soil.

- A higher proportion of the 'pseudo-total' soil ^{238}U was associated with the 'total' soil humic substances (0.1 N NaOH extract) in the coniferous compared with the deciduous broadleaf soil (B2C, 47-65 %; B1D, 29-45 %). This was attributed to differences in composition of the total humic substances in each of these soil types since the concentrations of extracted humic substances did not differ significantly.

- Electrophoretic extraction yielded 15±9 - 34±2 % (B2C) and 13±4 - 41±1 % (B1D) of the 'pseudo-total' soil ^{238}U in association with humic substances. For all depths of the coniferous soil (B2C), the values were intermediate between that for ^{238}U in the 0.1 N NaOH humic extract and in the 0.045 M Tris-borate humic extract. In the deciduous soil (B1D), excluding the surface (0.5 cm) section, the electrophoretic extract ^{238}U concentration was similar to that obtained for the 0.1 N NaOH extract. The lower association of ^{238}U with the coniferous soil electrophoretic extract, relative to the association with the 'total' soil humic substances in this soil, probably reflected the immobilisation of a greater proportion of the humic substances in the coniferous soil. It is assumed that the 0.1 N NaOH extract, however, contains the hydrophilic humic substances immobilised by interactions with other soil components.

6.5.2 Distribution of naturally occurring ^{238}U between the soil actinide pools and within the hydrophilic-mobile pool fractions

- There was a greater immobilisation of ^{238}U in the coniferous than in the deciduous broadleaf forest soil. This was observed as a greater immobile pool (B2C, 78-81 %; B1D, 67-77 %), and a smaller labile pool (B2C, 3-4 %; B1D, 9-12 %) of ^{238}U for the coniferous soil.

- The association of ^{238}U with the electrophoretic extract (hydrophilic mobile pool) of the deciduous broadleaf forest soil was significantly lower in the surface (0.5 cm) section, and possibly reflected the low degree of functionality of

recently formed humic substances at this depth. For the remainder of the deciduous broadleaf forest soil depth samples the concentration of ^{238}U associated with the electrophoretic extract did not vary. In addition, association of ^{238}U was generally observed with all fractions of the electrophoretic extract. Only small changes in the distribution of ^{238}U amongst these fractions were observed with increasing depth. Specifically, there was a slight decrease in association with fractions (F7-F10) of higher electrophoretic mobility.

- For the coniferous soil, there was no significant change in extracted ^{238}U with increasing depth. There was, however, evidence of loss of affinity for ^{238}U, particularly from individual fractions of extracts, at greater soil depth. There was no reproducible pattern of loss of binding capability highlighting the heterogeneity of humic substances extracted from different sub-samples of the coniferous soil.

- In some instances, often, but not exclusively, reflecting low association of ^{238}U with the total electrophoretic extract, there was a loss of association of ^{238}U with the higher electrophoretic mobility fractions. This was attributed to heterogeneity of soil, leading to the absence of these ligands. In these cases, the pattern of association of ^{238}U was similar to that of humic concentration in the extract; association of ^{238}U was mainly with fractions F2-F4 and was maximal for fraction F3.

6.5.3 Distribution of added ^{236}U between the soil actinide pools and association with the hydrophilic-mobile pool fractions

- There was a greater immobilisation of ^{236}U in the coniferous soil than in the deciduous broadleaf forest soil. This was observed as a greater immobile pool of ^{236}U (B2C, 78-84 %; B1D, 63-64 %), and a smaller ^{236}U labile pool (B2C, 3-10 %; B1D, 19-25 %) for the coniferous soil. Added ^{236}U showed immobilisation in the two soils similar to that observed for the naturally occurring ^{238}U.

- The lower association of ^{236}U with the electrophoretic extract, compared with that of ^{238}U, was coincident with the absence of ^{236}U from the higher

electrophoretic mobility fractions F7-F10. The association pattern of ^{236}U with the electrophoretic extract was similar to the humic concentration pattern. ^{236}U was associated with the lower electrophoretic mobility fractions F1-F5, and was maximal with the focused brown/UV-fluorescent fractions F4/F5. Further work is required to observe whether 'added' actinides will show interaction with the higher mobility fractions F7-F10 after longer 'equilibration' or whether the binding sites of these fractions are occupied and thus unavailable.

- Association of the total added ^{236}U with the electrophoretic extract was higher in the surface of the coniferous forest soil (~ 16 %) than in the deciduous broadleaf forest soil (~ 13 %). The decreasing association with increasing soil depth, more pronounced for the coniferous than for the deciduous broadleaf forest soil, was attributed to the decrease in extractable humic substances as sorption and immobilisation of humic substances increased.

6.5.4 Distribution and association of ^{238}U and ^{236}U following the extended (86 hour) 'equilibration' experiment (B2C 0.5 cm)

- Following extended 'equilibration' of the surface (0.5 cm) coniferous soil sample with the simulated soil solution, the immobility of the naturally occurring ^{238}U and added ^{236}U was observed to increase. This effect was greater for the naturally occurring ^{238}U than for the added ^{236}U. The increased immobility of the ^{238}U was concomitant with a loss of association of ^{238}U with the higher electrophoretic mobility fractions F7-F10. Thus, increased contact between the soil solution and solid phases resulted in an immobilisation of a proportion of the humic substances.

6.5.5 Distribution of added ^{242}Pu between the soil actinide pools and association with the hydrophilic-mobile pool fractions

- The association of the total added ^{242}Pu with the electrophoretic extract in the surface soil samples of both soil types was similar (B1D and B2C, ~ 8 %). The decreasing association of ^{242}Pu with increasing soil depth, as for the added ^{236}U, was greater for the coniferous than for the deciduous broadleaf soil, and was

attributed to the decrease in extractable humic substances as sorption and immobilisation increased.

- The association pattern of ^{242}Pu with the electrophoretic extract, as for the added ^{236}U, was similar to the humic concentration pattern. ^{242}Pu was associated with the lower electrophoretic mobility fractions F2-F4, and was maximal with the focused brown band fraction F3. Thus, ^{242}Pu was associated with higher molecular weight humic molecules than was the added ^{236}U. The few literature examples of the association of Pu with different size fractions of humic substances have also shown that Pu interacts preferentially, but not exclusively, with the higher molecular size fractions in both soil solution and solid phases.
- However, the most important soil actinide pool for ^{242}Pu was the labile pool, which was larger for the coniferous than for the deciduous broadleaf soil. This may have been a kinetic effect of slow reduction of Pu following complexation to the humic ligands of the hydrophilic-mobile and immobile soil pools rather than the ligands of the labile pool.
- For all actinides studied, ^{238}U, ^{236}U and ^{242}Pu, greater immobilisation was observed in the coniferous soil compared with the deciduous broadleaf soil. This may be a consequence of a difference in the nature of the humic substance ligands, as influenced by the different composition of the lignin of the vegetation of the two soils, which results in their greater sorption to other soil components. Alternatively, the greater depth of the coniferous soil may provide a greater volume and surface of secondary minerals for sorption of the humic substances.

6.6 FUTURE WORK

- Electrophoretic extraction with and without the preceding 'equilibration' experiment to investigate whether the highly electrophoretically mobile fractions (F7-F10) of the hydrophilic-mobile pool are distinct from the humic substances of the labile pool.
- Longer 'equilibration' experiments to investigate whether the 'added' actinides are able to associate with the higher electrophoretic mobility fractions (F7-F10)

and whether the highly labile Pu increases in association with the humic substances of the hydrophilic-mobile and immobile soil pools.

- Excision of electrophoretic extract fractions and re-application and electrophoretic extraction to verify the electrophoretic mobility of the fractions.
- Comparison of the humic-associated ^{238}U pattern of electrophoretic fractions of a 0.045 M Tris-borate solvent extract of soil compared with the patterns obtained by direct electrophoretic extraction and fractionation of soil to provide further information on the difference in composition of the humic extract obtained by electrophoresis.
- Scanning electron microscopy-energy dispersive X-ray analysis (SEM-EDX) to identify whether there are any very fine (< 0.15 μm) humic-coated mineral particles present in the electrophoretic extract of soil.

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8 APPENDIX

8.1 FOREST SOILS SAMPLE INFORMATION

8.1.1 Forest Enterprise map details

Information relating to the map used to survey and select sampling sites is presented below in Table 8.1.

Forest District:	Lakes
Survey Area:	Grizedale 2
Type of Map:	Stock
Scale:	1:10,000
Date of Survey:	1985
Revised on:	June 1987
Issued by the Forest Enterprise Regional Drawing Office, York	

Table 8.1: Forest Enterprise map details

8.1.2 Soil samples

The following tables contain the details of the samples sectioned from the deciduous broadleaf (B1D and B2D; Table 8.2 and Table 8.3, respectively) and coniferous (B1C and B2C; Table 8.4 and Table 8.5, respectively) forest soil sample blocks. The details include the soil depth intervals, the average soil depth of the samples, and a visual description of the samples.

Soil Depth Interval (cm)	Average Soil Depth (cm)	Description
-3 to –1	-2.00	broadleaf litter
-1 to 0	-0.50	compacted leaf litter
0-1	0.50	dark brown soil
1-2	1.50	
2-3	2.50	
3-4	3.50	brown soil
4-5	4.50	sandy orange-brown soil

Table 8.2: Soil depth interval, average soil depth, and visual description of the sectioned samples obtained from the deciduous broadleaf soil block B1D. The bold line indicates the approximate soil profile datum zero (boundary of the organic litter layer and mineral soil horizons) (Armson, 1979).

Soil Depth Interval (cm)	Average Soil Depth (cm)	Description
-2.5 to 0	-1.25	broadleaf litter, very small amount of moss
0 to 1	0.50	fine root (some compacted litter), dark brown soil
1-2	1.50	fine root (less than above), dark brown soil (small amount of brown and sandy soil)
2-3	2.50	as above, less root
3-4	3.50	brown to sandy-brown soil, small amount of dark brown soil, few roots
4-5	4.50	mix of soil, mostly sandy soil

Table 8.3: Soil depth interval, average soil depth, and visual description of the sectioned samples obtained from the deciduous broadleaf soil block B2D. The bold line indicates the approximate soil profile datum zero (boundary of the organic litter layer and mineral soil horizons) (Armson, 1979).

Soil Depth Interval (cm)	Average Soil Depth (cm)	Description
-6 to -5	-5.50	moss, few pine needles
-5 to -3	-4.00	compacted pine needles
-3 to -2	-2.50	
-2 to -1	-1.50	overlap of pine and dark-brown highly degraded litter at ~ -2cm
-1 to 0	-0.50	litter, root and brown-black highly degraded litter
0-1	0.50	black soil
1-2	1.50	(contained a yellow fungal biomass)
2-3	2.50	
3-4	3.50	
4-5	4.50	
5-6.5	5.75	
6.5-8	7.25	
8-9	8.50	
9-10	9.50	
10-11	10.50	
11-12	11.50	
12-13	12.50	
13-14	13.50	
14-15	14.50	
15-16	15.50	
16-17	16.50	
17-18	17.50	
18-19	18.50	

Table 8.4: Soil depth interval, average soil depth, and visual description of the sectioned samples obtained from the coniferous soil block B1C. The bold line indicates the approximate soil profile datum zero (boundary of the organic litter layer and mineral soil horizons) (Armson, 1979).

Soil Depth Interval (cm)	Average Soil Depth (cm)	Description
-6 to -5	-5.50	loose pine needles, branches, a few broadleaves, cones and moss
-5 to -4	-4.50	compacted pine needles dark brown highly degraded litter from ~ -3.5 cm
-4 to -3	-3.50	
-3 to -2	-2.50	
-2 to -1	-1.50	
-1 to 0	-0.50	
0-1	0.50	black soil
1-2	1.50	
2-3	2.50	
3-4	3.50	
4-5	4.50	
5-6	5.50	
6-7	6.50	
7-8	7.50	
8-9	8.50	
9-10	9.50	
10-11	10.50	
11-12	11.50	
12-13	12.50	
13-14	13.50	

Table 8.5: Soil depth interval, average soil depth, and visual description of the sectioned samples obtained from the coniferous soil block B2C. The bold line indicates the approximate soil profile datum zero (boundary of the organic litter layer and mineral soil horizons) (Armson, 1979).

8.2 FLOODPLAIN SOIL SAMPLE INFORMATION

The map of the sampling site, sampling site location and description details of the floodplain soil are presented in Figure 8.1 and Table 8.6. The dry weight determination details for the floodplain soil are given in Table 8.7.

Flora:	Mainly grass, with a sparse covering of clover and buttercups
Fauna:	A few earthworms in each 20 cm square section
Obvious Stones/Minerals	Stones appeared at an approximate depth of 16 cm
Mode of Collection	Soil sections were placed in a black plastic bag. Condensation on the inner surface of the bag was noted back at the laboratory, i.e. some moisture was lost during transport from the site.
Storage	The sample was stored in a cold room (+ 4° C).

Table 8.6: Description of the floodplain soil, sampling and storage conditions

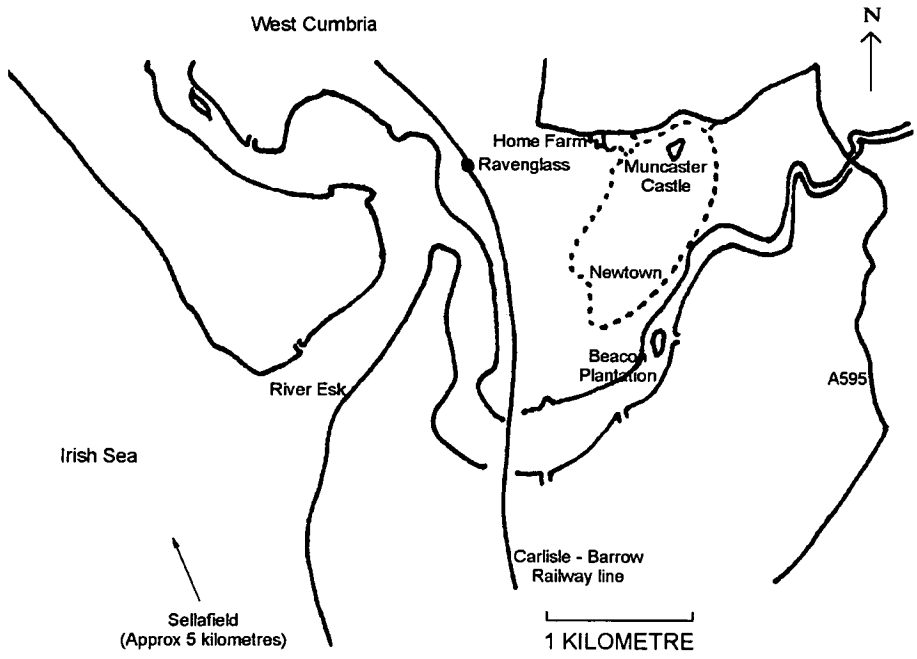

Figure 8.1: A map of the floodplain soil sample site (grid reference SD 095 951)

Mass of empty dish (g)	Mass of dish plus soil (g)	Mass of dish and freeze-dried soil (g)	Mass of freeze-dried soil (g)	% Moisture
88.69	98.69	96.79	8.10	19.0
89.55	99.55	97.78	8.23	17.7
92.82	102.82	101.01	8.19	18.1
				Average 18.3

Table 8.7: Dry weight measurements in the freeze-drying of three 10 g subsamples of the floodplain soil and calculated percentage moisture values

8.3 TRACER SOLUTIONS

Actinide tracer solutions (^{236}U and ^{242}Pu) were purchased from Isotrak, QSA Amersham International; prepared and characterised at AEA Technology UK.

8.3.1 ^{236}U Tracer Solution

Actinide ^{236}U tracer solution information is given in Table 8.8 below.

Reference date:	02 March 1992
Radioactive concentration:	7.227 Bq per g solution 7.694 Bq per ml solution
Mass of solution:	5.35 g
Volume of solution:	5.03 ml; 2 M HNO_3
Total activity:	38.7 Bq
Overall uncertainty in the radioactive concentration:	± 1.3% (~ 38.7 ± 0.5 Bq)

Table 8.8: ^{236}U Tracer solution details

The number of moles of ^{236}U in the tracer solution (68.5 nmoles to 1 d.p.) was calculated using Equation 8.2 (rearranged from Equation 8.1), and using solution data from Table 8.8 and half-life data from Choppin *et al.* (1995).

$$\text{Activity (A) (Bq; disintegrations s}^{-1}) = \frac{\text{no. of moles} \times N_A\,(\text{mol}^{-1}) \times \ln 2}{t_{1/2}\,(\text{s})} \qquad \text{Equation 8.1}$$

$$\text{no. of moles} = \frac{A\,(\text{s}^{-1}) \times t_{1/2}\,(\text{s})}{N_A\,(\text{mol}^{-1}) \times \ln 2} \qquad \text{Equation 8.2}$$

where, ^{236}U: A = 38.7 Bq ± 0.5; $t_{½}$ = 2.342 × 10^7 yr (converted to seconds by multiplication with 3.2 × 10^7 (s yr^{-1}) - [365.25 (days yr^{-1}) × 24 (hours day^{-1}) × (60 minutes hr^{-1}) × (60 s min^{-1})]); N_A, Avogadro constant = 6.022 × 10^{23} mol^{-1}; ln 2 = 0.693

8.3.2 Preparation of 0.1 μM (100 pmole per ml) ^{236}U solution in 2 % v/v HNO_3

The aliquot of tracer solution (68.5 nmoles ^{236}U in 5.03 ml, Section 8.3.1) required to prepare a solution of total volume 50 ml (in 2 % v/v HNO_3) and concentration of 0.1 μM ^{236}U was calculated as 0.367 ml, as shown below.

$$\text{no. of moles } ^{236}\text{U required (0.1 } \mu\text{M } ^{236}\text{U in 50 ml total volume)} = \frac{0.1\times10^{-6}\text{ (moles)}\times 50\text{ (ml)}}{1000\text{ (ml)}}$$

$$= 5\times10^{-10}\text{ moles } ^{236}\text{U}$$

$$\text{volume of tracer solution required (ml)} = \frac{5\times10^{-10}\text{ (moles)}}{68.5\times10^{-9}\text{ (moles)}}\times 5.03\text{ (ml)} = 0.367\text{ ml (to 3 d.p.)}$$

8.3.3 Dilution of ^{236}U Tracer solution

The tracer solution remaining after preparation of calibration standard stock solution and ^{236}U soil spike solution was made up to a larger total volume for ease of further handling and accuracy of preparation of solutions. The calculated remaining tracer solution volume was 3.86 ml. The tracer solution was made up to a total volume of 10 ml in a volumetric flask using 2 M HNO_3. The number of moles of ^{236}U in the diluted tracer solution was calculated as 52.6 nmoles, as shown below.

$$\text{moles of } ^{236}\text{U remaining} = \frac{3.86\text{ (ml)}}{5.03\text{ (ml)}}\times 68.5\times10^{-9}\text{ moles} = 52.6\times10^{-9}\text{ moles (to 1 d.p.)}$$

8.3.4 Preparation of 1 μM (100 pmoles per 0.1 ml) ^{236}U solution in 2M HNO_3

A 2 ml aliquot of the diluted ^{236}U tracer (52.6 nmoles ^{236}U in 10 ml) solution was made up to a total volume of 10 ml in a volumetric flask using 2 M HNO_3. The solution concentration was 105 pmoles per 0.1 ml ^{236}U, calculated as shown below.

$$\text{concentration of } ^{236}\text{U} = \frac{2\text{ (ml)}}{10\text{ (ml)}}\times 52.6\times10^{-9}\text{ (moles)}\times\frac{1}{10\text{ (ml)}} = 1.05\times10^{-9}\text{ mol ml}^{-1}\text{ (to 2 d.p.)}$$

$$= 105\text{ pmoles } ^{236}\text{U per 0.1 ml}$$

8.3.5 ^{242}Pu Tracer Solution

Actinide ^{242}Pu tracer solution information is given in Table 8.9.

Reference date:	26 November 1997
Radioactive concentration:	1,012 Bq per g solution 1,070 Bq per ml solution
Mass of solution:	5.329 g
Volume of solution:	5.037 ml; 2 M HNO_3
Total activity:	5,390 Bq
Overall uncertainty in the radioactive concentration:	± 1.6 % (~ 5,390 ± 86 Bq)

Table 8.9: ^{242}Pu Tracer solution details

The number of moles of ^{242}Pu in the tracer solution was calculated as 153.3 nmoles (to 1 d.p.), using Equation 8.2, certificated data from Table 8.9, and half-life data ($t_{½}$ = 3.76×10^5 y) from Choppin *et al.* (1995).

8.3.6 Dilution of ^{242}Pu Tracer solution

The tracer solution was made up to a total volume of 100 ml in 2 M HNO_3 for ease of handling.

8.3.7 Preparation of 0.1 μM (100 pmole per ml) ^{242}Pu solution in 2 % v/v HNO_3

An aliquot (7.3968 g) of diluted tracer (153.3 nmoles ^{242}Pu in 100 ml 2M HNO_3) solution was accurately weighed into a 100 ml volumetric flask and made up to volume with 2% HNO_3. The spike solution contained 108 pmole per ml ^{242}Pu in ~ 2 % v/v HNO_3, calculated as shown below.

$$\text{volume of diluted tracer solution added (ml)} = \frac{7.3968\ (\text{g})}{1.054\ (\text{g ml}^{-1})\ 2\,\text{M HNO (Section 8.6.3.)}}$$

$$= 7.018\ \text{ml (to 3 d.p.)}$$

$$\text{concentration of } ^{242}\text{Pu} = \frac{7.018\ (\text{ml})}{100(\text{ml})} \times \frac{153.3 \times 10^{-9}\ (\text{moles})}{100(\text{ml})} = 1.08 \times 10^{-10}\ \text{mol ml}^{-1}\ \text{(to 2 d.p.)}$$

$$= 108\ \text{pmoles } ^{242}\text{Pu per ml}$$

8.4 BUFFER SOLUTIONS

8.4.1 Stock solution ammonium acetate/acetic acid, pH 4.9

Acetic acid: pKa (at 25 °C) = 4.75 (Lide, 1994)

9.3460 g ammonium acetate
3.6 ml acetic acid

The above quantities of chemicals were dissolved, and the solution made up to volume in a 1 l volumetric flask using deionised water.

8.4.2 Working solution 0.045 M ammonium acetate/acetic acid, pH 4.2

Composition of 1 l solution: 800 ml stock solution
20 ml 2 M nitric acid
40 ml 2 % v/v HNO_3
Made up to volume with deionised water

8.4.3 Working solution 0.045 M ammonium acetate/acetic acid/0.1 M sodium perchlorate, pH 4.2

Composition of 1 l solution: 800 ml stock solution
14.046 g $NaClO_4$
20 ml 2 M nitric acid
40 ml 2 % v/v HNO_3
Made up to volume with deionised water

8.4.4 Working solution 0.045 M Tris-borate, pH 8.5

Tris: pKa (at 25 °C) = 8.1

Boric acid: pKa (at 20 °C), $pKa_1 = 9.14$, $pKa_2 = 12.74$, $pKa_3 = 13.80$ (Lide, 1994)

54 g Tris
27.5 g Orthoboric acid

The above quantities of chemicals were dissolved and the solution made up to volume in a 1 l volumetric flask. This solution was transferred to a 10 l plastic aspirator and diluted by addition of 9 l deionised water.

8.4.5 Working solution 0.05 M Tris-HCl, pH 8.5

0.31 ml concentrated HCl was added to deionised water and made up to volume in a 100 ml volumetric flask. 1.2114 g Tris was dissolved in deionised water and made up to volume in a 100 ml volumetric flask. The prepared HCl and Tris solutions were transferred to a reagent bottle and shaken.

8.4.6 Preparation of Control ^{236}U-spiked 0.10 M Tris-HCl buffer, pH 8.5

1.57 ml concentrated HCl was added to deionised water and made up to volume in a 100 ml volumetric flask. 5 g of Tris was dissolved in deionised water. A 1 ml aliquot of the prepared HCl solution was added to the Tris solution, and the solution was then made up to volume in a 100 ml volumetric flask using deionised water.

8.4.7 Working solution 0.04 M ammonia/ammonium acetate, pH 8.4

Ammonia: pK_b (at 25 °C) = 4.75 (Lide, 1994)

A 0.1 M ammonia solution was prepared by addition of 7.5 ml concentrated ammonia to a 1 l volumetric flask, made up to volume with deionised water.

3.854 g ammonium acetate
65 ml 0.1 M ammonia

The above quantity of ammonium acetate was dissolved in deionised water and transferred to a 1 l volumetric flask. The above aliquot of ammonia solution was then added and the solution made up to volume with deionised water.

8.5 ICP-MS INTERNAL AND CALIBRATION STANDARD SOLUTIONS

8.5.1 ^{209}Bi internal standard solution

Standard solutions (100 ppm, 1 ppm and 20 ppb) of ^{209}Bi in 2 % v/v HNO_3 were prepared by serial dilution of a certificated plasma standard ^{209}Bi solution (10,000 ±

30 μg ml^{-1} ^{209}Bi in 5% HNO_3). The 100 ppm ^{209}Bi solution was prepared from a 1 ml aliquot of the plasma standard solution made up to volume with 2 % HNO_3 in a 100 ml volumetric flask. The 1 ppm ^{209}Bi solution was prepared from a 1 ml aliquot of the 100 ppm ^{209}Bi standard solution made up to volume with 2 % HNO_3 in a 100 ml volumetric flask. Similarly, the 20 ppb ^{209}Bi solution was prepared from a 2 ml aliquot of the 1 ppm ^{209}Bi standard solution made up to volume with 2 % HNO_3 in a 100 ml volumetric flask.

8.5.2 ^{238}U calibration standards

Standard solutions (10 ppm, 100 ppb and 10 ppb) of ^{238}U in 2 % v/v HNO_3 were prepared by serial dilution of a certificated plasma standard ^{238}U solution (1,000 ± 3 μg ml^{-1} ^{238}U). The 10 ppm ^{238}U solution was prepared from a 1 ml aliquot of the plasma standard solution made up to volume with 2 % HNO_3 in a 100 ml volumetric flask. The 100 ppb ^{238}U solution was prepared from a 1 ml aliquot of the 10 ppm ^{238}U standard solution made up to volume with 2 % HNO_3 in a 100 ml volumetric flask. Similarly, the 10 ppb ^{238}U solution was prepared from a 10 ml aliquot of the 100 ppb ^{238}U standard solution made up to volume with 2 % HNO_3 in a 100 ml volumetric flask.

Five calibration standards were prepared as shown in Table 8.10 below. Each standard contained 0.2 ppb ^{209}Bi internal standard, and was made up to a final volume of 10 ml in a volumetric flask using 2 % HNO_3.

	^{238}U Calibration Standard				
	10 ppb	5 ppb	1 ppb	0.5 ppb	0.1 ppb
Volume of 100 ppb ^{238}U added (ml)	1	0.5	0.1	-	-
Volume of 10 ppb ^{238}U added (ml)	-	-	-	0.5	0.1
Volume of 20 ppb ^{209}Bi added (ml)	0.1	0.1	0.1	0.1	0.1

Table 8.10: Preparation of ^{238}U calibration standards.

8.5.3 ^{236}U calibration standards

A 0.01 μM ^{236}U solution, in 2 % v/v HNO_3 was prepared from which the calibration standards were then made. An aliquot (0.734 ml) of tracer solution was pipetted into

a 1 l plastic reagent bottle containing 1 l 2 M HNO_3. The pipette setting required had been determined gravimetrically (see Section 8.8).

The five calibration standards were prepared as described in Table 8.11 below. Each standard contained 0.2 ppb ^{209}Bi internal standard and was made up to a final volume of 10 ml in a volumetric flask with 2 % v/v HNO_3.

	^{236}U Calibration Standard				
	1.18 ppb	0.472 ppb	0.236 ppb	0.118 ppb	0.0236 ppb
Volume of 0.01 μM ^{236}U added (ml)	5	2	1	0.5	0.1
Volume of 20 ppb ^{209}Bi added (ml)	0.1	0.1	0.1	0.1	0.1

Table 8.11: Preparation of ^{236}U calibration standards.

8.5.4 ^{242}Pu calibration standards

A 0.01 μM ^{242}Pu solution, in 2 % v/v HNO_3 was prepared from which the calibration standards were then made. A 10 ml aliquot of the 0.1 μM ^{242}Pu solution was pipetted to a 100 ml volumetric flask and the solution was made up to volume with 2 % v/v HNO_3.

The four calibration standards were prepared as shown in Table 8.12 below. Each standard contained 0.2 ppb ^{209}Bi internal standard and was made up to a total volume of 10 ml in a volumetric flask using 2 % v/v HNO_3.

	^{242}Pu Calibration Standard			
	1.302 ppb	0.260 ppb	0.130 ppb	0.026 ppb
Volume of 0.01 μM ^{242}Pu added (ml)	5	1	0.5	0.1
Volume of 20 ppb ^{209}Bi added (ml)	0.1	0.1	0.1	0.1

Table 8.12: Preparation of ^{242}Pu calibration standards

8.6 PREPARATION OF NITRIC ACID SOLUTIONS

8.6.1 2 % v/v HNO_3

A 20 ml aliquot of concentrated HNO_3 was made up to volume in a 1 l volumetric flask with deionised water. The molarity of 2 % v/v HNO_3 solution is 0.311 M (to 3 d.p.).

8.6.2 2 M HNO_3

A 128 ml aliquot of concentrated HNO_3 was made up to volume in a 1 l volumetric flask with deionised water.

8.6.3 Specific gravity of HNO_3 solutions

Concentrated HNO_3 has a specific gravity of about 1.42 g ml^{-1} (at room temperature) and contains 1.56×10^{-2} (to 2 d.p.) moles per ml HNO_3. Deionised water has a specific gravity of 1.00 g per ml (at room temperature). Equation 8.5, which was used to calculate the specific gravity of different molarity HNO_3 solutions, was derived as shown below. A linear relationship between specific gravity of a nitric acid solution and moles nitric acid was assumed.

$y = mx + c$ Equation 8.3

where, y = specific gravity (g ml^{-1}), x = concentration of HNO_3 (mol ml^{-1})
For deionised water, y = 1.0 g ml^{-1} and x = 0 mol ml^{-1} HNO_3.
Substituting into Equation 8.3 $\rightarrow$ c = 1

$\therefore y = mx + 1$ Equation 8.4

For concentrated HNO_3, y = 1.42 g ml^{-1}, x = 1.56×10^{-2} mol ml^{-1} HNO_3
Substituting into Equation 8.4 $\rightarrow$ m = 27.01 g mol^{-1} (to 2 d.p.)

$\therefore y = (27.01x) + 1$ Equation 8.5

The specific gravities of nitric acid solutions were calculated and are presented in Table 8.13 below.

Nitric acid solution (M)	Specific gravity (g ml^{-1})
15.550 (concentrated)	1.420
2.0	1.054
0.311 (2 % v/v)	1.008

Table 8.13: Specific gravity of nitric acid solutions.

8.7 PREPARATION OF PARTICLE SIZE DISPERSION SOLUTION

'Calgon' dispersion solution was prepared by dissolution of 50 g sodium hexametaphosphate and 7 g sodium carbonate in deionised water, made up to a final solution volume of 1 l in a volumetric flask.

8.8 GRAVIMETRIC DETERMINATION OF PIPETTE SETTINGS

The pipette settings required for preparation of uranium solutions were determined by weighing the amount of deionised water dispensed by the pipettes.

8.8.1 Pipette: Gilson P200 (used in the preparation of the 0.1 μM ^{236}U solution)

The pipette setting determined for dispensing the aliquot of ^{236}U tracer in the preparation of the 0.1 μM ^{236}U solution was 0.1880 ml. This setting delivered an average volume of 0.1833 ml deionised water at room temperature (Table 8.14).

Pipette setting (ml)	Mass of deionised water dispensed (g)
0.1880	0.1835
"	0.1830
"	0.1842
"	0.1830
"	0.1830
Average	*0.1833 (to 4 d.p.)*

Table 8.14: Mass of deionised water dispensed by Gilson P200 pipette on setting 0.1880 ml at room temperature

8.8.2 Pipette: Gilson P1000 (used in the preparation of the 0.01 μM ^{236}U solution)

The pipette setting determined for dispensing the aliquot of ^{236}U tracer in the preparation of the 0.01 μM ^{236}U solution was 0.750 ml. This setting delivered an average volume of 0.7284 ml deionised water at room temperature (Table 8.15).

Pipette setting (ml)	Mass of deionised water dispensed (g)
0.750	0.7358
"	0.7225
"	0.7291
"	0.7255
"	0.7290
Average	*0.7284 (to 4 d.p.)*

Table 8.15: Mass of deionised water dispensed by Gilson P1000 pipette on setting 0.750 ml at room temperature

8.9 95 % CONFIDENCE LIMIT DETERMINATION

Equation 8.6 was used to determine the 95 % confidence limits of experimental data. Standard deviation, which was required for calculation of the confidence limits as

well as the population number (i.e. number of sample replicates) and population mean, was determined using Equation 8.7.

95 % confidence limit = $\bar{x}$ ±1.96 ($\sigma \div \sqrt{n}$) Equation 8.6

where $\bar{x}$ is the average of the triplicate sample values, σ is the standard deviation of the triplicate values from the average, n is the number of sample replicates and 1.96 corresponds to the 95 % area under a standard normal curve with mean $\bar{x}$, standard deviation σ and population n.

Standard deviation, $\sigma = \sqrt{\{(n\Sigma x^2-(\Sigma x)^2) \div (n(n-1))\}}$ Equation 8.7

where n is the number of sample replicates and x is the sample value

8.10 L.O.I. RAW DATA AND CALCULATIONS

The data for the loss on ignition blank experiments are presented in Table 8.16 to Table 8.17. The blank loss on ignition (L.O.I.) values were calculated according to Equation 8.8.

L.O.I. (g) = (mass of beaker + KNO_3 added)g – (mass of beaker + ash)g Equation 8.8

Sample label	Mass of beaker (g)	Mass of KNO_3 added (g)	Mass of beaker and ash (g)	L.O.I. (g)
B1D Sb1	51.1386	0.0100	51.1463	0.0023
B1D Sb2	46.7861	0.0109	46.7944	0.0026
B1D Sb3	45.5839	0.0099	45.5907	0.0031

Table 8.16: The masses of beakers, potassium nitrate added, ash and calculated L.O.I. in the blank experiment for the deciduous broadleaf forest soil B1D

Sample label	Mass of beaker (g)	Mass of KNO_3 added (g)	Mass of beaker and ash (g)	L.O.I. (g)
B2C Sb1	49.6670	0.0104	49.6775	-0.0001
B2C Sb2	49.7777	0.0105	49.7875	0.0007
B2C Sb3	51.2928	0.0106	51.3029	0.0005

Table 8.17: The masses of beakers, potassium nitrate added, ash and calculated L.O.I. in the blank experiment for the coniferous forest soil B2C

The loss on ignition of potassium nitrate, per gram of potassium nitrate, was calculated according to Equation 8.9.

L.O.I.(KNO_3)(g per g KNO_3) = L.O.I.(KNO_3)(g) ÷ mass of KNO_3 added(g) Equation 8.9

The average of the triplicate blank values for loss on ignition of one gram of potassium nitrate was calculated. The standard deviation of the triplicate values from the average value was determined and the 95 % confidence limit of the average was then calculated. The average and 95 % confidence limit values for the blank experiments are given in Table 8.18.

Sample label	Average L.O.I. (g per g)	95 % confidence limit
B1D Sb	0.2606	0.0517
B2C Sb	0.0347	0.0448

Table 8.18: The average, standard deviation and 95 % confidence limit values for the triplicate blank L.O.I. experiments for the deciduous broadleaf (B1D) and coniferous (B2C) forest soils

The data of loss on ignition for the deciduous broadleaf and coniferous forest soil sample experiments are given in Table 8.19 and Table 8.20. The soil loss on ignition (L.O.I.) values were calculated according to Equation 8.10.

L.O.I. (g) = (mass of beaker + soil added)g – (mass of beaker + ash)g Equation 8.10

Sample label	Mass of beaker (g)	Mass of KNO_3 added (g)	Mass of soil added (g)	Mass of beaker and ash (g)	L.O.I. (g)
B1D 0.5 S1	51.2776	0.0129	0.1028	51.3540	0.0393
B1D 0.5 S2	46.1494	0.0106	0.1016	46.2236	0.0380
B1D 0.5 S3	49.2778	0.0112	0.1007	49.3504	0.0393
B1D 1.5 S1	44.3006	0.0101	0.1007	44.3780	0.0334
B1D 1.5 S2	45.9744	0.0101	0.1011	46.0519	0.0337
B1D 1.5 S3	44.4765	0.0129	0.1022	44.5560	0.0356
B1D 2.5 S1	51.1373	0.0106	0.1003	51.2201	0.0281
B1D 2.5 S2	45.5833	0.0118	0.1003	45.6672	0.0282
B1D 2.5 S3	46.7851	0.0103	0.1004	46.8681	0.0277
B1D 3.5 S1	49.2769	0.0110	0.1000	49.3622	0.0257
B1D 3.5 S2	46.1484	0.0122	0.1014	46.2354	0.0266
B1D 3.5 S3	51.1061	0.0102	0.1011	51.1911	0.0263
B1D 4.5 S1	44.3001	0.0101	0.1005	44.3877	0.0230
B1D 4.5 S2	44.4759	0.0118	0.1000	44.5643	0.0234
B1D 4.5 S3	44.6190	0.0112	0.1007	44.7062	0.0247

Table 8.19: The masses of beakers, potassium nitrate and soil added, ash and calculated L.O.I. values for the deciduous broadleaf forest soil B1D depth section triplicate subsamples

Sample label	Mass of beaker (g)	Mass of KNO_3 added (g)	Mass of soil added (g)	Mass of beaker and ash (g)	L.O.I. (g)
B2C 0.5 S1	44.7274	0.0106	0.1008	44.7927	0.0461
B2C 0.5 S2	49.4852	0.0104	0.1022	49.5500	0.0478
B2C 0.5 S3	49.3651	0.0106	0.1015	49.4306	0.0466
B2C 3.5 S1	49.9481	0.0110	0.1005	50.0268	0.0328
B2C 3.5 S2	51.3651	0.0111	0.1001	51.4441	0.0322
B2C 3.5 S3	50.0733	0.0102	0.1006	50.1508	0.0333
B2C 6.5 S1	49.6535	0.0100	0.1008	49.7331	0.0312
B2C 6.5 S2	49.0897	0.0102	0.1019	49.1699	0.0319
B2C 6.5 S3	49.9313	0.0104	0.1014	50.0110	0.0321
B2C 9.5 S1	49.8514	0.0101	0.1015	49.9361	0.0269
B2C 9.5 S2	49.6547	0.0106	0.1018	49.7390	0.0281
B2C 9.5 S3	49.6361	0.0111	0.1015	49.7204	0.0283
B2C 12.5 S1	50.2415	0.0106	0.1021	50.3298	0.0244
B2C 12.5 S2	49.1553	0.0109	0.1021	49.2438	0.0245
B2C 12.5 S3	45.6423	0.0110	0.1007	45.7303	0.0237

Table 8.20: The masses of beakers, potassium nitrate and soil added, ash and calculated L.O.I. values for the coniferous forest soil B2C depth section triplicate subsamples

The mean percentage L.O.I. values, and 95 % confidence limit values, for the deciduous broadleaf (B1D) and coniferous (B2C) forest soil samples, as presented in Chapter 4 (Results), were calculated as follows. The soil sample loss on ignition (g) values were corrected for the contribution of ignition of potassium nitrate to the total ignition mass loss according to Equation 8.11. The corrected soil loss on ignition values were then normalised for loss on ignition of one gram of soil according to Equation 8.12. The percentage soil loss on ignition values were then calculated according to Equation 8.13. Percentage soil loss on ignition is the same as mass loss in grams per one-hundred grams ignited soil.

$$\text{L.O.I.(sample)(g)} = \text{average L.O.I.}(KNO_3)(g) \times \text{mass of } KNO_3 \text{ in sample(g)} \quad \text{Equation 8.11}$$

$$\text{L.O.I.(sample)(g per g of soil)} = \text{L.O.I.(sample)(g)} \div \text{mass of soil in sample(g)} \quad \text{Equation 8.12}$$

$$\%\ \text{L.O.I.} = \text{L.O.I.(sample)(g per g of soil)} \times 100 \quad \text{Equation 8.13}$$

8.11 PARTICLE SIZE RAW DATA

The raw data used in the determination of soil textural class for the deciduous broadleaf and coniferous forest soil samples are presented below in Table 8.21 and Table 8.22, respectively.

Sample label	% Clay	% Silt	% Fine Sand	% Coarse Sand	% Sand	Textural Class
B1D 0.5	22	62	9	8	17	Silty clay loam
B1D 0.5	23	64	8	4	12	"
B1D 1.5	24	61	8	7	15	"
B1D 1.5	23	60	10	7	16	"
B1D 2.5	26	62	8	3	12	"
B1D 2.5	29	61	8	2	10	"
B1D 3.5	24	65	8	3	11	"
B1D 3.5	24	64	8	4	12	"
B1D 4.5	25	66	8	2	9	"
B1D 4.5	25	64	8	2	10	"

Table 8.21: Absolute particle size data for deciduous broadleaf soil block B1D depth section samples: the percentage of the sample total volume in a given particle size class and the assigned soil textural class.

Sample label	% Clay	% Silt	% Fine Sand	% Coarse Sand	% Sand	Textural Class
B2C 0.5	19	67	12	2.06	14	Silty clay loam
B2C 0.5	20	69	10	1.47	12	"
B2C 3.5	27	62	8	3.28	11	"
B2C 3.5	26	64	8	1.73	10	"
B2C 6.5	30	59	9	2.32	11	"
B2C 6.5	27	62	9	2.24	11	"
B2C 9.5	29	60	9	1.84	11	"
B2C 9.5	28	61	9	2.35	11	"
B2C 12.5	30	63	7	0.51	8	"
B2C 12.5	27	62	9	1.64	11	"

Table 8.22: Absolute particle size data for coniferous soil block B2C depth section samples: the percentage of the sample total volume in a given particle size class and the assigned soil textural class.

The raw data used in the determination of the effective and absolute particle size distribution ranges for the deciduous broadleaf and coniferous forest soils are presented below in Table 8.23 to Table 8.26.

Sample label	90 % of sample volume with particle size > (μm)	10 % of sample volume with particle size > (μm)
B1D 0.5	26.3	439
B1D 0.5	22.0	386
B1D 1.5	21.4	396
B1D 1.5	17.0	297
B1D 2.5	17.6	401
B1D 2.5	13.5	273
B1D 3.5	16.5	417
B1D 3.5	11.9	289
B1D 4.5	13.5	390
B1D 4.5	10.8	278
B1D 5.5	14.6	310
B1D 5.5	8.8	211

Table 8.23: Distribution (10 % and 90 % of total sample volume with particle size above given value) of effective particle sizes for deciduous broadleaf soil block B1D depth section samples

Sample label	90 % of sample volume with particle size > (μm)	10 % of sample volume with particle size > (μm)
B1D 0.5	0.61	145
B1D 0.5	0.58	73
B1D 1.5	0.49	114
B1D 1.5	0.51	126
B1D 2.5	0.49	65
B1D 2.5	0.45	56
B1D 3.5	0.50	61
B1D 3.5	0.49	66
B1D 4.5	0.51	53
B1D 4.5	0.50	57

Table 8.24: Distribution (10 % and 90 % of total sample volume with particle size above given value) of absolute particle sizes for deciduous broadleaf soil block B1D depth section samples

Sample label	90 % of sample volume with particle size > (µm)	10 % of sample volume with particle size > (µm)
B2C 0.5	24.3	440
B2C 0.5	18.0	371
B2C 3.5	10.3	403
B2C 3.5	9.1	328
B2C 6.5	11.2	433
B2C 6.5	9.1	340
B2C 9.5	13.6	357
B2C 9.5	12.6	371
B2C 12.5	11.6	363
B2C 12.5	9.9	326

Table 8.25: Distribution (10 % and 90 % of total sample volume with particle size above given value) of effective particle sizes for coniferous soil block B2C depth section samples

Sample label	90 % of sample volume with particle size > (µm)	10 % of sample volume with particle size > (µm)
B2C 0.5	0.61	73
B2C 0.5	0.59	62
B2C 3.5	0.43	62
B2C 3.5	0.45	57
B2C 6.5	0.39	62
B2C 6.5	0.43	61
B2C 9.5	0.42	60
B2C 9.5	0.44	61
B2C 12.5	0.42	47
B2C 12.5	0.44	61

Table 8.26: Distribution (10 % and 90 % of total sample volume with particle size above given value) of absolute particle sizes for coniferous soil block B2C depth section samples

8.12 ABSORBANCE INDEX RAW DATA

The average and 95 % confidence limit values calculated from the raw data for the floodplain soil are presented in Table 8.27. The absorbance index raw data of the deciduous braodleaf and coniferous soil solvent and electrophoretic humic extracts are presented in Table 8.28 to Table 8.30. The absorbance index raw data of the Tris solvent extracts of the floodplain soil are presented in Table 8.31.

Sample	Average A.I. (254 nm)
TA	1.28 ± 0.05
TAE	1.60 ± 0.09
TH	4.81 ± 0.13
THE	4.83 ± 0.21
TRIS	5.43 ± 0.18
TB	*3.87
TBE	3.93 ± 0.16
TB W	2.03 ± 0.09

Table 8.27: Average absorbance index values and 95 % confidence limits for the Tris solvent extracts of the floodplain soil. (*single replicate value). (TA – Tris acetate, TAE – Tris acetate EDTA, TH – Tris HCl, THE – Tris HCl EDTA, Tris – Tris base, TB – Tris-borate, TBE – Tris-borate EDTA). Raw data are presented in Table 8.31.

Sample label	Dilution (fraction)	$A_{254\ nm}$ (a.u.)	$A_{254\ nm}$ (a.u.)(undiluted)	Mass of soil (g)	$A_{254\ nm}$ (a.u. per g of soil)	Volume/Mass of extract solution (ml or g)	$A_{254\ nm}$ (a.u. per g of soil extracted in a final solution volume of 100 ml)
B1D 0.5 N	0.10	0.541	5.41	0.1025	52.79	50 ml	105.6
B1D 1.5 N	0.10	0.522	5.22	0.1007	51.79	50 ml	103.6
B1D 2.5 N	0.10	0.382	3.82	0.1012	37.72	50 ml	75.4
B1D 3.5 N	0.10	0.320	3.20	0.1013	31.56	50 ml	63.1
B1D 4.5 N	0.20	0.579	2.90	0.1015	28.54	50 ml	57.1
B1D 0.5 TB	0.25	0.486	1.95	0.1013	19.21	50 ml	38.4
B1D 1.5 TB	0.25	0.535	2.14	0.1007	21.26	50 ml	42.5
B1D 2.5 TB	0.25	0.425	1.70	0.1002	16.95	50 ml	33.9
B1D 3.5 TB	0.50	0.668	1.34	0.1037	12.89	50 ml	25.8
B1D 4.5 TB	0.50	0.530	1.06	0.1032	10.26	50 ml	20.5
B2C 0.5 N	0.05	0.444	8.88	0.1011	87.84	75.0020 g	117.1
B2C 3.5 N	0.05	0.345	6.90	0.1021	67.57	75.0205 g	90.1
B2C 6.5 N	0.05	0.301	6.02	0.1000	60.17	75.0319 g	80.2
B2C 9.5 N	0.05	0.277	5.54	0.1013	54.71	75.1101 g	72.8
B2C 12.5 N	0.10	0.469	4.69	0.1024	45.79	75.0251 g	61.0
B2C 0.5 TB	0.10	0.443	4.43	0.1017	43.57	75.0386 g	58.1
B2C 3.5 TB	0.20	0.470	2.35	0.1017	23.08	75.0278 g	30.8
B2C 6.5 TB	0.50	0.532	1.06	0.1013	10.50	75.0261 g	14.0
B2C 9.5 TB	0.50	0.332	0.66	0.1013	6.56	75.0673 g	8.7
B2C 12.5 TB	1.00	0.356	0.36	0.1016	3.51	75.0255 g	4.7

Table 8.28: Raw data for calculation of normalised absorbance (a.u. per g extracted soil, total extract volume 100 ml) for 0.1 N NaOH (N) and 0.045 M Tris-borate (TB) solvent extracts of the deciduous broadleaf (B1D) and coniferous (B2C) forest soil samples

Sample label	Dilution (fraction)	$A_{254\ nm}$ (a.u.)	$A_{254\ nm}$ (a.u.)(undiluted)	Mass of soil (g)	$A_{254\ nm}$ (a.u. per g of soil)	Mass of extract solution (g)	$A_{254\ nm}$ (a.u. per g of soil extracted in a final solution volume of 100 ml)
B1D 0.5 F1	1.00	0.400	0.34	0.3039	1.12	40.0429	2.8
B1D 0.5 F2	1.00	0.354	0.35		1.17	40.0048	2.9
B1D 0.5 F3	1.00	0.477	0.48		1.57	40.0284	3.9
B1D 0.5 F4	1.00	0.521	0.52		1.71	40.2211	4.3
B1D 0.5 F5	1.00	0.511	0.51		1.68	40.0430	4.2
B1D 0.5 F6	1.00	0.202	0.20		0.67	40.0225	1.7
B1D 0.5 F7	1.00	0.044	0.04		0.14	40.0237	0.4
B1D 0.5 F8	1.00	0.000	0.00		0.00	40.6044	0.0
B1D 0.5 F9	1.00	0.000	0.00		0.00	40.0460	0.0
B1D 0.5 F10	1.00	0.000	0.00		0.00	20.0271	0.0
sum F1 to F10			2.45				20.1
sum F1 to F7			2.45				20.1
B1D 2.5 F1	1.00	0.284	0.28	0.3051	0.93	40.0181	2.3
B1D 2.5 F2	1.00	0.307	0.31		1.01	40.3816	2.5
B1D 2.5 F3	1.00	0.385	0.38		1.26	40.0323	3.1
B1D 2.5 F4	1.00	0.443	0.44		1.45	40.0412	3.6
B1D 2.5 F5	1.00	0.414	0.41		1.36	40.0396	3.4
B1D 2.5 F6	1.00	0.200	0.20		0.66	40.0007	1.6
B1D 2.5 F7	1.00	0.043	0.04		0.14	40.0436	0.4
B1D 2.5 F8	1.00	0.008	0.01		0.02	40.0139	0.1
B1D 2.5 F9	1.00	0.000	0.00		0.00	40.1842	0.0
B1D 2.5 F10	1.00	0.008	0.01		0.03	20.0976	0.1
sum F1 to F10			2.09				17.2
sum F1 to F7			2.08				17.0

Table 8.29: Raw data for calculation of normalised absorbance (a.u. per g extracted soil, total extract volume 100 ml) for electrophoretic extract fractions B1D 0.5 and 2.5 F1 to F10 of the deciduous broadleaf forest soil samples

Sample label	Dilution (fraction)	$A_{254\ nm}$ (a.u.)	$A_{254\ nm}$ (a.u.)(undiluted)	Mass of soil (g)	$A_{254\ nm}$ (a.u. per g of soil)	Mass of extract solution (g)	$A_{254\ nm}$ (a.u. per g of soil extracted in a final solution volume of 100 ml)
B2C 0.5 F1	0.33	0.425	1.27	0.3024	4.21	40.0362	10.5
B2C 0.5 F2	0.20	0.337	1.69		5.58	40.0150	13.9
B2C 0.5 F3	0.20	0.527	2.63		8.71	40.0281	21.8
B2C 0.5 F4	0.20	0.433	2.16		7.15	40.0303	17.9
B2C 0.5 F5	0.33	0.347	1.04		3.44	40.0673	8.6
B2C 0.5 F6	1.00	0.184	0.18		0.61	40.2419	1.5
B2C 0.5 F7	1.00	0.027	0.03		0.09	40.0217	0.2
B2C 0.5 F8	1.00	0.000	0.00		0.00	40.0261	0.0
B2C 0.5 F9	1.00	0.000	0.00		0.00	40.0114	0.0
B2C 0.5 F10	1.00	0.000	0.00		0.00	20.0957	0.0
sum F1 to F10			9.01				74.4
sum F1 to F7			9.01				74.4
B2C 12.5 F1	1.00	0.078	0.08	0.3073	0.25	40.0844	0.6
B2C 12.5 F2	1.00	0.073	0.07		0.24	40.0125	0.6
B2C 12.5 F3	1.00	0.114	0.11		0.37	40.0173	0.9
B2C 12.5 F4	1.00	0.139	0.14		0.45	40.2441	1.1
B2C 12.5 F5	1.00	0.170	0.17		0.55	40.0512	1.4
B2C 12.5 F6	1.00	0.102	0.10		0.33	40.0312	0.8
B2C 12.5 F7	1.00	0.020	0.02		0.07	40.0275	0.2
B2C 12.5 F8	1.00	0.000	0.00		0.00	40.0107	0.0
B2C 12.5 F9	1.00	0.000	0.00		0.00	40.0697	0.0
B2C 12.5 F10	1.00	0.000	0.00		0.00	20.1831	0.0
sum F1 to F10			0.70				5.7
sum F1 to F7			0.70				5.7

Table 8.30: Raw data for calculation of normalised absorbance (a.u. per g extracted soil, total extract volume 100 ml) for electrophoretic extract fractions B2C 0.5 and 12.5 F1 to F10 of the coniferous forest soil samples

Sample label	Dilution (fraction)	$A_{254\ nm}$ (a.u.)	$A_{254\ nm}$ (a.u.)(undiluted)	Mass of soil (g)	Volume of extract solution (ml)	$A_{254\ nm}$ (a.u. per g of soil extracted in a final solution volume of 5.5 ml)
TA1	0.050	0.257	5.15	4.0018	5.5	1.29
TA2	0.050	0.246	4.91	4.0048	5.5	1.23
TA3	0.050	0.263	5.27	4.0019	5.5	1.32
TAE1	0.050	0.326	6.53	4.0028	5.5	1.63
TAE2	0.050	0.303	6.06	4.0016	5.5	1.51
TAE3	0.050	0.333	6.66	4.0036	5.5	1.66
TH1	0.025	0.468	18.72	4.0009	5.5	4.68
TH2	0.025	0.485	19.40	4.0000	5.5	4.85
TH3	0.025	0.490	19.61	4.0019	5.5	4.90
THE1	0.025	0.466	18.65	4.0024	5.5	4.66
THE2	0.025	0.481	19.23	4.0009	5.5	4.81
THE3	0.025	0.503	20.12	4.0022	5.5	5.03
TB3	0.025	0.387	15.49	4.0038	5.5	3.87
TBE1	0.025	0.409	16.35	4.0035	5.5	4.08
TBE2	0.025	0.382	15.26	4.0030	5.5	3.81
TBE3	0.025	0.391	15.65	4.0018	5.5	3.91
TRIS1	0.025	0.562	22.46	4.0003	5.5	5.62
TRIS2	0.025	0.534	21.46	4.0038	5.5	5.36
TRIS3	0.025	0.533	21.32	4.0031	5.5	5.32
TB W1	0.050	0.283	5.66	4.0003	4.5	2.12
TB W2	0.050	0.264	5.27	4.0042	4.5	1.97
TB W3	0.050	0.268	5.35	4.0013	4.5	2.00

Table 8.31: Raw data for calculation of normalised absorbance (a.u. per g extracted soil, total extract volume 5.5 ml for Tris solvent extracts of the floodplain soil. (TA – Tris acetate, TAE – Tris acetate EDTA, TH – Tris HCl, THE – Tris HCl EDTA, Tris – Tris base, TB – Tris-borate, TBE – Tirs-borate EDTA).

8.13 ICP-MS RESULTS

8.13.1 Whole soil ashed residues

The masses of deciduous broadleaf and coniferous forest soil samples ashed in preparation for 'pseudo' total soil ^{238}U content determination are given in Table 8.32 and Table 8.33, together with μg ^{238}U detected in each sample using ICP-MS.

Sample	Analysis	^{238}U (μg)	Mass of soil (g)
B1D 0.5 S1	24/11/98	0.0427	0.1028
B1D 0.5 S1 (no Bi)	24/11/98	0.0550	0.1028
B1D 0.5 S1 (no Bi)	24/05/99	0.0316	0.1028
B1D 0.5 S1	24/05/99	0.0318	0.1028
B1D 0.5 S1	25/05/99	0.0315	0.1028
B1D 0.5 S1 (no Bi)	26/05/99	0.0306	0.1028
B1D 0.5 S2	24/11/98	0.0504	0.1016
B1D 0.5 S2 (no Bi)	24/11/98	0.0658	0.1016
B1D 0.5 S2 (no Bi)	20/03/99	0.0551	0.1016
B1D 0.5 S3	24/11/98	0.0526	0.1007
B1D 0.5 S3 (no Bi)	20/03/99	0.0618	0.1007
B1D 1.5 S1	24/11/98	0.0474	0.1007
B1D 1.5 S1 (no Bi)	20/03/99	0.0479	0.1007
B1D 1.5 S2	24/11/98	0.0511	0.1011
B1D 1.5 S2 (no Bi)	20/03/99	0.0510	0.1011
B1D 1.5 S3	24/11/98	0.0525	0.1022
B1D 1.5 S3 (no Bi)	20/03/99	0.0484	0.1022
B1D 2.5 S1	24/11/98	0.0505	0.1003
B1D 2.5 S1 (no Bi)	20/03/99	0.0516	0.1003
B1D 2.5 S2	24/11/98	0.0506	0.1003
B1D 2.5 S2 (no Bi)	20/03/99	0.0479	0.1003
B1D 2.5 S3	24/11/98	0.0491	0.1004
B1D 2.5 S3 (no Bi)	20/03/99	0.0431	0.1004
B1D 3.5 S1	24/11/98	0.0510	0.1000
B1D 3.5 S1 (no Bi)	20/03/99	0.0432	0.1000
B1D 3.5 S2	24/11/98	0.0501	0.1014
B1D 3.5 S2 (no Bi)	20/03/99	0.0399	0.1014
B1D 3.5 S3	24/11/98	0.0511	0.1011
B1D 3.5 S3 (no Bi)	20/03/99	0.0426	0.1011
B1D 4.5 S1	24/11/98	0.0441	0.1005
B1D 4.5 S1 (no Bi)	20/03/99	0.0395	0.1005
B1D 4.5 S2	24/11/98	0.0434	0.1000
B1D 4.5 S2 (no Bi)	20/03/99	0.0390	0.1000
B1D 4.5 S3	24/11/98	0.0452	0.1007
B1D 4.5 S3 (no Bi)	24/05/99	0.0375	0.1007
B1D 4.5 S3	24/05/99	0.0360	0.1007

Table 8.32: The masses of soil ashed, and the detected 'pseudo' total soil ^{238}U content (μg), of the deciduous broadleaf (B1D) soil samples

Sample	Analysis	^{238}U (μg)	Mass of soil (g)
B2C 0.5 S1	17/03/99	0.0566	0.1008
B2C 0.5 S1 (no Bi)	17/03/99	0.0552	0.1008
B2C 0.5 S2	17/03/99	0.0566	0.1022
B2C 0.5 S2 (no Bi)	17/03/99	0.0551	0.1022
B2C 0.5 S3	17/03/99	0.0452	0.1015
B2C 3.5 S3 (no Bi)	17/03/99	0.0433	0.1015
B2C 3.5 S1	17/03/99	0.0538	0.1005
B2C 3.5 S1 (no Bi)	17/03/99	0.0523	0.1005
B2C 3.5 S2	17/03/99	0.0556	0.1001
B2C 3.5 S2 (no Bi)	17/03/99	0.0537	0.1001
B2C 3.5 S3	17/03/99	0.0518	0.1006
B2C 3.5 S3 (no Bi)	17/03/99	0.0504	0.1006
B2C 6.5 S1	17/03/99	0.0580	0.1008
B2C 6.5 S1 (no Bi)	17/03/99	0.0566	0.1008
B2C 6.5 S2	17/03/99	0.0558	0.1019
B2C 6.5 S2 (no Bi)	17/03/99	0.0544	0.1019
B2C 6.5 S3	17/03/99	0.0555	0.1014
B2C 6.5 S3 (no Bi)	17/03/99	0.0537	0.1014
B2C 9.5 S1	17/03/99	0.0608	0.1015
B2C 9.5 S1 (no Bi)	17/03/99	0.0600	0.1015
B2C 9.5 S2	17/03/99	0.0599	0.1018
B2C 9.5 S2 (no Bi)	17/03/99	0.0588	0.1018
B2C 9.5 S3	17/03/99	0.0564	0.1015
B2C 9.5 S3 (no Bi)	17/03/99	0.0541	0.1015
B2C 12.5 S1	17/03/99	0.0651	0.1021
B2C 12.5 S1 (no Bi)	17/03/99	0.0641	0.1021
B2C 12.5 S2	17/03/99	0.0644	0.1021
B2C 12.5 S2 (no Bi)	17/03/99	0.0631	0.1021
B2C 12.5 S3	17/03/99	0.0584	0.1007
B2C 12.5 S3 (no Bi)	17/03/99	0.0574	0.1007

Table 8.33: The masses of soil ashed, and the detected 'pseudo' total soil ^{238}U content (μg), of the coniferous (B2C) soil samples

8.13.2 Soil solvent extracts

The masses of deciduous broadleaf and coniferous forest soil samples extracted using 0.1 N NaOH and 0.045 M Tris-borate are given in Table 8.34 to Table 8.37, together with μg ^{238}U detected in each solvent extract sample using ICP-MS.

Sample	Analysis	^{238}U (μg)	Mass of soil (g)
B1D 0.5 N1	24/11/98	0.0184	0.1025
B1D 0.5 N1 (no Bi)	24/11/98	0.0221	0.1025
B1D 0.5 N1 (no Bi)	20/03/99	0.0249	0.1025
B1D 0.5 N2	24/11/98	0.0172	0.1024
B1D 0.5 N2 (no Bi)	24/11/98	0.0207	0.1024
B1D 0.5 N2 (no Bi)	20/03/99	0.0202	0.1024
B1D 0.5 N3	24/11/98	0.0159	0.1015
B1D 0.5 N3 (no Bi)	20/03/99	0.0165	0.1015
B1D 1.5 N1	24/11/98	0.0190	0.1007
B1D 1.5 N1	25/11/98	0.0195	0.1007
B1D 1.5 N1 (no Bi)	20/03/99	0.0273	0.1007
B1D 1.5 N2	24/11/98	0.0208	0.1003
B1D 1.5 N2 (no Bi)	20/03/99	0.0236	0.1003
B1D 1.5 N3	24/11/98	0.0213	0.1007
B1D 1.5 N3 (no Bi)	20/03/99	0.0242	0.1007
B1D 2.5 N1	24/11/98	0.0170	0.1012
B1D 2.5 N1 (no Bi)	20/03/99	0.0169	0.1012
B1D 2.5 N2	24/11/98	0.0164	0.1010
B1D 2.5 N2 (no Bi)	20/03/99	0.0181	0.1010
B1D 2.5 N3	24/11/98	0.0159	0.1005
B1D 2.5 N3 (no Bi)	20/03/99	0.0186	0.1005
B1D 3.5 N1	24/11/98	0.0142	0.1013
B1D 3.5 N1 (no Bi)	20/03/99	0.0154	0.1013
B1D 3.5 N2	24/11/98	0.0145	0.1011
B1D 3.5 N2 (no Bi)	20/03/99	0.0107	0.1011
B1D 3.5 N3	24/11/98	0.0159	0.1003
B1D 3.5 N3 (no Bi)	20/03/99	0.0123	0.1003
B1D 4.5 N1	24/11/98	0.0125	0.1015
B1D 4.5 N1 (no Bi)	20/03/99	0.0102	0.1015
B1D 4.5 N2	24/11/98	0.0139	0.1012
B1D 4.5 N2 (no Bi)	20/03/99	0.0116	0.1012
B1D 4.5 N3	24/11/98	0.0130	0.1016
B1D 4.5 N3 (no Bi)	20/03/99	0.0095	0.1016

Table 8.34: The masses of soil extracted using 0.1 N NaOH, and the detected extract ^{238}U content (μg), of the deciduous broadleaf (B1D) soil samples

Sample	Analysis	^{238}U (μg)	Mass of soil (g)
B1D 0.5 TB1 (no Bi)	24/11/98	0.0113	0.1013
B1D 0.5 TB1	25/11/98	0.0108	0.1013
B1D 0.5 TB1 (no Bi)	20/03/99	0.0102	0.1013
B1D 0.5 TB2 (no Bi)	24/11/98	0.0091	0.1012
B1D 0.5 TB2	25/11/98	0.0082	0.1012
B1D 0.5 TB2 (no Bi)	20/03/99	0.0082	0.1012
B1D 0.5 TB3	25/11/98	0.0079	0.1009
B1D 0.5 TB3 (no Bi)	20/03/99	0.0081	0.1009
B1D 1.5 TB1	25/11/98	0.0086	0.1007
B1D 1.5 TB1 (no Bi)	20/03/99	0.0082	0.1007
B1D 1.5 TB2	25/11/98	0.0082	0.1009
B1D 1.5 TB2 (no Bi)	20/03/99	0.0083	0.1009
B1D 1.5 TB3	25/11/98	0.0082	0.1027
B1D 1.5 TB3 (no Bi)	20/03/99	0.0080	0.1027
B1D 2.5 TB1	25/11/98	0.0090	0.1002
B1D 2.5 TB1 (no Bi)	20/03/99	0.0070	0.1002
B1D 2.5 TB2	25/11/98	0.0094	0.1001
B1D 2.5 TB2 (no Bi)	20/03/99	0.0118	0.1001
B1D 2.5 TB3	25/11/98	0.0086	0.1021
B1D 2.5 TB3 (no Bi)	20/03/99	0.0116	0.1021
B1D 3.5 TB1	25/11/98	0.0066	0.1037
B1D 3.5 TB1 (no Bi)	20/03/99	0.0094	0.1037
B1D 3.5 TB2	25/11/98	0.0062	0.1022
B1D 3.5 TB2 (no Bi)	20/03/99	0.0079	0.1022
B1D 3.5 TB3	25/11/98	0.0063	0.1002
B1D 3.5 TB3 (no Bi)	20/03/99	0.0097	0.1002
B1D 4.5 TB1	25/11/98	0.0070	0.1032
B1D 4.5 TB1 (no Bi)	20/03/99	0.0055	0.1032
B1D 4.5 TB2	25/11/98	0.0061	0.1061
B1D 4.5 TB2 (no Bi)	20/03/99	0.0102	0.1061
B1D 4.5 TB3	25/11/98	0.0061	0.1020
B1D 4.5 TB3 (no Bi)	20/03/99	0.0100	0.1020

Table 8.35: The masses of soil extracted using 0.045 M Tris-borate, and the detected extract ^{238}U content (μg), of the deciduous broadleaf (B1D) soil samples

Sample	Analysis	^{238}U (μg)	Mass of soil (g)
B2C 0.5 N1 (no Bi)	26/05/99	0.0321	0.1011
B2C 0.5 N1	26/05/99	0.0328	0.1011
B2C 0.5 N2 (no Bi)	26/05/99	0.0321	0.1002
B2C 0.5 N2	26/05/99	0.0329	0.1002
B2C 0.5 N3 (no Bi)	26/05/99	0.0353	0.1004
B2C 0.5 N3	26/05/99	0.0360	0.1004
B2C 3.5 N1 (no Bi)	26/05/99	0.0252	0.1021
B2C 3.5 N1	26/05/99	0.0255	0.1021
B2C 3.5 N2 (no Bi)	26/05/99	0.0266	0.0998
B2C 3.5 N2	26/05/99	0.0264	0.0998
B2C 3.5 N3 (no Bi)	26/05/99	0.0254	0.1004
B2C 3.5 N3	26/05/99	0.0262	0.1004
B2C 6.5 N1 (no Bi)	26/05/99	0.0236	0.1000
B2C 6.5 N1	26/05/99	0.0241	0.1000
B2C 6.5 N2 (no Bi)	26/05/99	0.0283	0.1001
B2C 6.5 N2	26/05/99	0.0273	0.1001
B2C 6.5 N3 (no Bi)	26/05/99	0.0266	0.1007
B2C 6.5 N3	26/05/99	0.0255	0.1007
B2C 9.5 N2 (no Bi)	26/05/99	0.0330	0.1018
B2C 9.5 N2	26/05/99	0.0297	0.1018
B2C 9.5 N3 (no Bi)	26/05/99	0.0325	0.1019
B2C 9.5 N3	26/05/99	0.0310	0.1019
B2C 12.5 N1 (no Bi)	26/05/99	0.0334	0.1024
B2C 12.5 N1	26/05/99	0.0334	0.1024
B2C 12.5 N2 (no Bi)	26/05/99	0.0386	0.1009
B2C 12.5 N2	26/05/99	0.0363	0.1009
B2C 12.5 N3 (no Bi)	26/05/99	0.0377	0.1005
B2C 12.5 N3	26/05/99	0.0353	0.1005

Table 8.36: The masses of soil extracted using 0.1 N NaOH, and the detected extract ^{238}U content (μg), of the coniferous (B2C) soil samples

Sample	Analysis	^{238}U (μg)	Mass of soil (g)
B2C 0.5 TB1 (no Bi)	26/05/99	0.0084	0.1017
B2C 0.5 TB1	26/05/99	0.0089	0.1017
B2C 0.5 TB2 (no Bi)	26/05/99	0.0101	0.1015
B2C 0.5 TB2	26/05/99	0.0098	0.1015
B2C 0.5 TB3 (no Bi)	26/05/99	0.0085	0.1010
B2C 0.5 TB3	26/05/99	0.0088	0.1010
B2C 3.5 TB1 (no Bi)	26/05/99	0.0065	0.1017
B2C 3.5 TB1	26/05/99	0.0066	0.1017
B2C 3.5 TB2 (no Bi)	26/05/99	0.0063	0.1038
B2C 3.5 TB2	26/05/99	0.0060	0.1038
B2C 3.5 TB3 (no Bi)	26/05/99	0.0060	0.1010
B2C 3.5 TB3	26/05/99	0.0060	0.1010
B2C 6.5 TB1 (no Bi)	26/05/99	0.0040	0.1013
B2C 6.5 TB1	26/05/99	0.0041	0.1013
B2C 6.5 TB2 (no Bi)	26/05/99	0.0042	0.1009
B2C 6.5 TB2	26/05/99	0.0041	0.1009
B2C 9.5 TB1 (no Bi)	26/05/99	0.0037	0.1013
B2C 9.5 TB1	26/05/99	0.0035	0.1013
B2C 9.5 TB2 (no Bi)	26/05/99	0.0035	0.1015
B2C 9.5 TB2	26/05/99	0.0034	0.1015
B2C 9.5 TB3 (no Bi)	26/05/99	0.0036	0.1017
B2C 9.5 TB3	26/05/99	0.0034	0.1017
B2C 12.5 TB1 (no Bi)	26/05/99	0.0027	0.1016
B2C 12.5 TB1	26/05/99	0.0024	0.1016
B2C 12.5 TB2 (no Bi)	26/05/99	0.0027	0.1007
B2C 12.5 TB2	26/05/99	0.0026	0.1007
B2C 12.5 TB3 (no Bi)	26/05/99	0.0029	0.1011
B2C 12.5 TB3	26/05/99	0.0027	0.1011

Table 8.37: The masses of soil extracted using 0.045 M Tris-borate, and the detected extract ^{238}U content (μg), of the coniferous (B2C) soil samples

8.13.3 'Equilibration' and electrophoretic extraction experiments

The masses of deciduous broadleaf and coniferous forest soil samples 'equilibrated' and then electrophoretically extracted are given in Table 8.38 and Table 8.39.

Sample	Mass of soil (g)
B1D 0.5 B Well 1	0.1012
B1D 0.5 B Well 2	0.1018
B1D 0.5 S Well 1	0.1008
B1D 0.5 S Well 2	0.1011
B1D 0.5 Pu Well 1	0.1010
B1D 0.5 Pu Well 2	0.1018
B1D 1.5 B Well 1	0.1021
B1D 1.5 B Well 2	0.1010
B1D 1.5 S Well 1	0.1028
B1D 1.5 S Well 2	0.1036
B1D 2.5 B Well 1	0.1020
B1D 2.5 B Well 2	0.1010
B1D 2.5 S Well 1	0.1018
B1D 2.5 S Well 2	0.1015
B1D 2.5 Pu Well 1	0.1013
B1D 2.5 Pu Well 2	0.1012
B1D 3.5 B Well 1	0.1010
B1D 3.5 B Well 2	0.1010
B1D 3.5 S Well 1	0.1016
B1D 3.5 S Well 2	0.1015
B1D 4.5 B Well 1	0.1001
B1D 4.5 B Well 2	0.1006
B1D 4.5 S Well 1	0.1007
B1D 4.5 S Well 2	0.1015

Table 8.38: The masses of samples of the deciduous broadleaf (B1D) soil 'equilibrated' and then electrophoretically extracted. (BW1 – unspiked soil, SW1, SW2 – ^{236}U-spiked soil and PuW1, PuW2 – Pu-^{242}spiked soil replicate experiments).

Sample	Mass of soil (g)
B2C 0.5 B Well 1	0.1011
B2C 0.5 B Well 2	0.1015
B2C 0.5 S Well 1	0.1016
B2C 0.5 S Well 2	0.1008
B2C 0.5 Pu Well 1	0.1017
B2C 0.5 Pu Well 2	0.1012
B2C 0.5 B (86 hr) Well 1	0.1013
B2C 0.5 B (86 hr) Well 2	0.1011
B2C 0.5 S (86 hr) Well 1	0.1002
B2C 0.5 S (86 hr) Well 2	0.1005
B2C 3.5 B Well 1	0.1017
B2C 3.5 B Well 2	0.1019
B2C 3.5 S Well 1	0.1014
B2C 3.5 S Well 2	0.1018
B2C 6.5 B Well 1	0.1005
B2C 6.5 B Well 2	0.1005
B2C 6.5 S Well 1	0.1003
B2C 6.5 S Well 2	0.1006
B2C 9.5 B Well 1	0.1006
B2C 9.5 B Well 2	0.1004
B2C 9.5 S Well 1	0.1007
B2C 9.5 S Well 2	0.1010
B2C 12.5 B Well 1	0.1014
B2C 12.5 B Well 2	0.1006
B2C 12.5 S Well 1	0.1006
B2C 12.5 S Well 2	0.1013
B2C 12.5 Pu Well 1	0.1003
B2C 12.5 Pu Well 2	0.1017

Table 8.39: The masses of samples of the coniferous (B2C) soil 'equilibrated' and then electrophoretically extracted. (BW1 – unspiked soil, SW1, SW2 – ^{236}U-spiked soil and PuW1, PuW2 – Pu-^{242}spiked soil replicate experiments).

The average 100 % spike values for the soil-spike equilibration experiments and ^{236}U control experiment and 95 % confidence limits are presented in Table 8.40. The ICP-MS data these values were calculated from are given in Table 8.41.

Buffer solution	100% spike ^{236}U (μg)	100% spike ^{242}Pu (μg)
BUFF ^{236}U	0.0339 ± 0.0006	-
BUFF ^{242}Pu	-	0.0222 ± 0.0003
CON-U BUFF ^{236}U	0.0250 ± 0.0005	-

Table 8.40: Average 100 % spike values (μg). BUFF – soil buffer solution, CON-U BUFF – control ^{236}U buffer

Sample	Blank corrected data (μg)	
	^{236}U	^{242}Pu
BUFF 236/1	0.0336	-
BUFF 236/2	0.0342	-
BUFF 242/1	-	0.0223
BUFF 242/2	-	0.0225
BUFF 242/3	-	0.0219
BUFF 242/4	-	0.0221
CON-U BUFF 236/1	0.0253	-
CON-U BUFF 236/2	0.0248	-

Table 8.41: Actinide 100 % soil buffer solution (BUFF) and control buffer (CON-U BUFF) spike sample ICP-MS data

The actinide content of the components of the soil 'equilibration' and electrophoretic extraction experiments are presented in Table 8.42 to Table 8.52. The ^{236}U content results of the control electrophoretic extraction experiment are presented in Table 8.53.

	^{238}U (μg)					^{236}U (μg)		^{242}Pu (μg)	
	BW1	SW1	SW2	PuW1	PuW2	SW1	SW2	PuW1	PuW2
SBS	0.0023	0.0025	0.0022	0.0088	0.0087	0.0046	0.0046	0.0179	0.0175
Well	0.0413	0.0385	0.0328	0.0359	0.0406	0.0174	0.0146	0.0032	0.0030
F1	0.0003	0.0004	0.0005	0.0007	0.0009	0.0005	0.0005	0.0002	0.0002
F2	0.0003	0.0006	0.0007	0.0013	0.0018	0.0006	0.0006	0.0003	0.0003
F3	0.0006	0.0027	0.0012	0.0022	0.0021	0.0008	0.0007	0.0006	0.0006
F4	0.0007	0.0031	0.0005	0.0015	0.0016	0.0011	0.0009	0.0005	0.0004
F5	0.0007	0.0005	0.0008	0.0010	0.0010	0.0010	0.0012	0.0001	0.0001
F6	0.0000	0.0000	0.0000	0.0002	0.0003	0.0001	0.0002	0.0000	0.0000
F7	0.0000	0.0000	0.0013	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
F8	0.0000	0.0000	0.0000	0.0000	0.0001	0.0000	0.0000	0.0000	0.0000
F9	0.0000	0.0000	0.0014	0.0001	0.0005	0.0000	0.0001	0.0000	0.0000
F10	0.0000	0.0002	0.0000	0.0009	0.0000	0.0001	0.0000	0.0000	0.0000

Table 8.42: Sample B1D 0.5 actinide content of the components of the electrophoretic extraction experiment in μg. (F1 to F10 – electrophoretic extract fractions). (SBS – soil buffer solution, Well – immobile well residue, F1 to F10 – electrophoretic extract fractions). (BW1 – unspiked soil, SW1, SW2 – ^{236}U-spiked soil and PuW1, PuW2 – Pu-^{242}spiked soil replicate experiments).

	^{238}U (µg)			^{236}U (µg)	
	BW1	SW1	SW2	SW1	SW2
F1	0.0031	0.0024	0.0010	0.0005	0.0007
F2	0.0025	0.0026	0.0012	0.0006	0.0007
F3	0.0028	0.0028	0.0010	0.0008	0.0008
F4	0.0028	0.0033	0.0007	0.0009	0.0009
F5	0.0028	0.0027	0.0008	0.0012	0.0012
F6	0.0026	0.0022	0.0000	0.0003	0.0002
F7	0.0023	0.0022	0.0000	0.0000	0.0000
F8	0.0022	0.0022	0.0000	0.0000	0.0000
F9	0.0020	0.0021	0.0000	0.0000	0.0000
F10	0.0023	0.0016	0.0015	0.0000	0.0000

Table 8.43: Sample B1D 1.5 actinide content of the components of the electrophoretic extraction experiment in µg. (F1 to F10 – electrophoretic extract fractions). (BW1 – unspiked soil, SW1, SW2 – ^{236}U-spiked soil replicate experiments).

	^{238}U (µg)					^{236}U (µg)		^{242}Pu (µg)	
	BW1	SW1	SW2	PuW1	PuW2	SW1	SW2	PuW1	PuW2
SBS	0.0053	0.0043	0.0048	0.0100	0.0101	0.0075	0.0075	0.0203	0.0201
Well	0.0491	0.0487	0.0488	0.0322	0.0331	0.0197	0.0186	0.0019	0.0020
F1	0.0021	0.0018	0.0023	0.0006	0.0007	0.0004	0.0005	0.0001	0.0001
F2	0.0019	0.0020	0.0020	0.0010	0.0011	0.0005	0.0005	0.0002	0.0002
F3	0.0026	0.0022	0.0028	0.0016	0.0016	0.0006	0.0006	0.0003	0.0003
F4	0.0022	0.0023	0.0025	0.0014	0.0013	0.0009	0.0007	0.0003	0.0003
F5	0.0025	0.0020	0.0027	0.0006	0.0007	0.0008	0.0009	0.0001	0.0001
F6	0.0020	0.0020	0.0026	0.0000	0.0000	0.0001	0.0004	0.0000	0.0000
F7	0.0019	0.0014	0.0023	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
F8	0.0016	0.0017	0.0022	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
F9	0.0016	0.0014	0.0021	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
F10	0.0017	0.0015	0.0022	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000

Table 8.44: Sample B1D 2.5 actinide content of the components of the soil 'equilibration' and electrophoretic extraction experiments in µg. (SBS – soil buffer solution, Well – immobile well residue, F1 to F10 – electrophoretic extract fractions). (BW1 – unspiked soil, SW1, SW2 – ^{236}U-spiked soil and PuW1, PuW2 – Pu-^{242}spiked soil replicate experiments).

	^{238}U (µg)			^{236}U (µg)	
	BW1	SW1	SW2	SW1	SW2
F1	0.0017	0.0014	0.0019	0.0004	0.0005
F2	0.0020	0.0015	0.0026	0.0005	0.0005
F3	0.0021	0.0019	0.0023	0.0006	0.0006
F4	0.0022	0.0022	0.0017	0.0008	0.0008
F5	0.0018	0.0016	0.0020	0.0007	0.0007
F6	0.0010	0.0010	0.0014	0.0001	0.0001
F7	0.0016	0.0015	0.0014	0.0000	0.0000
F8	0.0008	0.0016	0.0016	0.0000	0.0000
F9	0.0011	0.0010	0.0018	0.0000	0.0000
F10	0.0014	0.0011	0.0015	0.0000	0.0000

Table 8.45: Sample B1D 3.5 actinide content of the components of the electrophoretic extraction experiment in µg. (F1 to F10 – electrophoretic extract fractions). (BW1 – unspiked soil, SW1, SW2 – ^{236}U-spiked soil replicate experiments).

	^{238}U (μg)			^{236}U (μg)	
	BW1	SW1	SW2	SW1	SW2
F1	0.0019	0.0019	0.0023	0.0004	0.0004
F2	0.0018	0.0015	-	0.0005	-
F3	0.0025	0.0017	0.0023	0.0006	0.0005
F4	0.0020	0.0020	0.0021	0.0007	0.0006
F5	0.0021	0.0019	0.0018	0.0007	0.0008
F6	0.0014	0.0017	0.0013	0.0002	0.0002
F7	0.0012	0.0013	0.0014	0.0001	0.0000
F8	0.0014	0.0017	0.0012	0.0000	0.0000
F9	0.0012	0.0013	0.0011	0.0000	0.0000
F10	0.0014	0.0015	0.0015	0.0000	0.0000

Table 8.46: Sample B1D 4.5 actinide content of the components of the electrophoretic extraction experiment in μg. (F1 to F10 – electrophoretic extract fractions). (BW1 – unspiked soil, SW1, SW2 – ^{236}U-spiked soil replicate experiments).

	^{238}U (μg)					^{236}U (μg)		^{242}Pu (μg)	
	BW1	SW1	SW2	PuW1	PuW2	SW1	SW2	PuW1	PuW2
SBS	0.0000	0.0000	0.0006	0.0055	0.0013	0.0009	0.0009	0.0109	0.0115
Well	0.0376	0.0387	0.0372	0.0440	0.0434	0.0231	0.0219	0.0074	0.0073
F1	0.0017	0.0016	0.0013	0.0002	0.0002	0.0008	0.0008	0.0002	0.0002
F2	0.0017	0.0021	0.0022	0.0005	0.0004	0.0009	0.0009	0.0003	0.0003
F3	0.0027	0.0018	0.0014	0.0012	0.0010	0.0013	0.0013	0.0009	0.0007
F4	0.0017	0.0023	0.0022	0.0006	0.0005	0.0015	0.0013	0.0005	0.0005
F5	0.0008	0.0020	0.0021	0.0000	0.0003	0.0009	0.0009	0.0000	0.0001
F6	0.0017	0.0016	0.0010	0.0001	0.0002	0.0002	0.0003	0.0000	0.0000
F7	0.0005	0.0017	0.0015	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
F8	0.0007	0.0009	0.0014	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
F9	0.0010	0.0015	0.0007	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
F10	0.0012	0.0015	0.0019	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000

Table 8.47: Sample B2C 0.5 actinide content of the components of the soil 'equilibration' and electrophoretic extraction experiments in μg. (SBS – soil buffer solution, Well – immobile well residue, F1 to F10 – electrophoretic extract fractions). (BW1 – unspiked soil, SW1, SW2 – ^{236}U-spiked soil and PuW1, PuW2 – Pu-^{242}spiked soil replicate experiments).

	^{238}U (µg)			^{236}U (µg)	
	BW1	SW1	SW2	SW1	SW2
SBS	0.0000	0.0000	0.0001	0.0010	0.0009
Well	0.0417	0.0381	0.0436	0.0213	0.0234
F1	0.0003	0.0005	0.0005	0.0006	0.0007
F2	0.0004	0.0008	0.0006	0.0008	0.0007
F3	0.0008	0.0010	0.0007	0.0010	0.0008
F4	0.0007	0.0007	0.0006	0.0010	0.0008
F5	0.0004	0.0004	0.0002	0.0007	0.0006
F6	0.0000	0.0000	0.0002	0.0002	0.0003
F7	0.0000	0.0000	0.0000	0.0000	0.0000
F8	0.0000	0.0000	0.0000	0.0000	0.0000
F9	0.0000	0.0000	0.0000	0.0000	0.0000
F10	0.0000	0.0000	0.0000	0.0000	0.0000

Table 8.48: Sample B2C 0.5 (86 hr) actinide content of the components of the soil 'equilibration' and electrophoretic extraction experiments in µg. (SBS – soil buffer solution, Well – immobile well residue, F1 to F10 – electrophoretic extract fractions). (BW1 – unspiked soil, SW1, SW2 – ^{236}U-spiked soil replicate experiments).

	^{238}U (µg)			^{236}U (µg)	
	BW1	SW1	SW2	SW1	SW2
F1	0.0020	0.0021	0.0020	0.0005	0.0006
F2	0.0018	0.0019	0.0021	0.0005	0.0005
F3	0.0026	0.0019	0.0023	0.0005	0.0005
F4	0.0022	0.0018	0.0012	0.0005	0.0004
F5	0.0019	0.0019	0.0016	0.0004	0.0005
F6	0.0020	0.0015	0.0017	0.0001	0.0003
F7	0.0021	0.0019	0.0019	0.0000	0.0000
F8	0.0018	0.0021	0.0018	0.0000	0.0000
F9	0.0013	0.0013	0.0014	0.0000	0.0000
F10	0.0013	0.0014	0.0018	0.0001	0.0000

Table 8.49: Sample B2C 3.5 actinide content of the components of the electrophoretic extraction experiment in µg. (F1 to F10 – electrophoretic extract fractions). (BW1 – unspiked soil, SW1, SW2 – ^{236}U-spiked soil replicate experiments).

	^{238}U (µg)			^{236}U (µg)	
	BW1	SW1	SW2	SW1	SW2
F1	0.0003	0.0017	0.0017	0.0005	0.0005
F2	0.0002	0.0027	0.0002	0.0004	0.0005
F3	0.0023	0.0025	0.0018	0.0005	0.0005
F4	0.0017	0.0005	0.0021	0.0007	0.0005
F5	0.0001	0.0017	0.0004	0.0006	0.0008
F6	0.0000	0.0000	0.0016	0.0001	0.0003
F7	0.0018	0.0021	0.0015	0.0001	0.0000
F8	0.0017	0.0022	0.0015	0.0000	0.0000
F9	0.0002	0.0015	0.0015	0.0000	0.0000
F10	0.0021	0.0016	0.0020	0.0001	0.0000

Table 8.50: Sample B2C 6.5 actinide content of the components of the electrophoretic extraction experiment in µg. (F1 to F10 – electrophoretic extract fractions). (BW1 – unspiked soil, SW1, SW2 – ^{236}U-spiked soil replicate experiments).

	^{238}U (µg)			^{236}U (µg)	
	BW1	SW1	SW2	SW1	SW2
F1	0.0017	0.0022	0.0022	0.0002	0.0002
F2	0.0018	0.0021	0.0016	0.0003	0.0002
F3	0.0022	0.0026	0.0021	0.0003	0.0003
F4	0.0020	0.0023	0.0018	0.0003	0.0003
F5	0.0021	0.0016	0.0016	0.0003	0.0003
F6	0.0018	0.0017	0.0000	0.0002	0.0001
F7	0.0020	0.0020	0.0019	0.0001	0.0001
F8	0.0018	0.0018	0.0019	0.0001	0.0000
F9	0.0016	0.0013	0.0021	0.0001	0.0000
F10	0.0016	0.0016	0.0000	0.0000	0.0000

Table 8.51: Sample B2C 9.5 actinide content of the components of the electrophoretic extraction experiment in µg. (F1 to F10 – electrophoretic extract fractions). (BW1 – unspiked soil, SW1, SW2 – ^{236}U-spiked soil replicate experiments).

	^{238}U (µg)					^{236}U (µg)		^{242}Pu (µg)	
	BW1	SW1	SW2	PuW1	PuW2	SW1	SW2	PuW1	PuW2
SBS	0.0001	0.0013	0.0002	0.0034	0.0035	0.0027	0.0029	0.0158	0.0163
Well	0.0457	0.0456	0.0432	0.0419	0.0386	0.0241	0.0247	0.0083	0.0081
F1	0.0001	0.0001	0.0016	0.0003	0.0004	0.0003	0.0003	0.0000	0.0001
F2	0.0031	0.0001	0.0019	0.0005	0.0007	0.0003	0.0003	0.0001	0.0001
F3	0.0005	0.0005	0.0020	0.0004	0.0008	0.0004	0.0003	0.0001	0.0001
F4	0.0006	0.0020	0.0019	0.0003	0.0005	0.0005	0.0004	0.0001	0.0001
F5	0.0024	0.0004	0.0020	0.0000	0.0004	0.0004	0.0004	0.0000	0.0000
F6	0.0021	0.0000	0.0019	0.0000	0.0000	0.0001	0.0002	0.0000	0.0000
F7	0.0007	0.0019	0.0014	0.0004	0.0001	0.0000	0.0001	0.0000	0.0000
F8	0.0003	0.0020	0.0018	0.0002	0.0000	0.0000	0.0001	0.0000	0.0000
F9	0.0002	0.0019	0.0019	0.0003	0.0000	0.0000	0.0001	0.0000	0.0000
F10	0.0002	0.0014	0.0022	0.0000	0.0005	0.0000	0.0000	0.0000	0.0000

Table 8.52: Sample B2C 12.5 actinide content of the components of the soil 'equilibration' and electrophoretic extraction experiments in µg. (SBS – soil buffer solution, Well – immobile well residue, F1 to F10 – electrophoretic extract fractions). (BW1 – unspiked soil, SW1, SW2 – ^{236}U-spiked soil and PuW1, PuW2 – Pu-^{242}spiked soil replicate experiments).

	^{236}U (µg)	
	W1	W2
Well	0.0002	0.0001
F1	0.0010	0.0009
F2	0.0033	0.0045
F3	0.0145	0.0174
F4	0.0054	0.0008
F5	0.0000	0.0000
F6	0.0000	0.0000
F7	0.0000	0.0000
F8	0.0000	0.0000
F9	0.0000	0.0000
F10	0.0000	0.0000

Table 8.53: Control experiment ^{236}U content in electrophoretic extract components in µg. (Well – immobile well residue, F1 to F10 – electrophoretic extract fractions).

8.14 HUMIC SUBSTANCE FTIR ABSORPTIONS AND FUNCTIONAL GROUP ASSIGNMENTS

The absorption bands typical of the electrophoretic extract fractions of the deciduous broadleaf and coniferous forest soil samples are presented with assignments of organic molecular bond vibrations in Table 8.54. The assignment of FTIR absorption bands to functional groups for humic substances, as adapted from the literature, is presented in Table 8.55.

Absorption wavenumber (cm^{-1})	Intensity of absorption	Shape of absorption band	Possible bond
3839	m	Sharp	*
3600	s	Sharp	O-H (free)
3424	s	Broad	O-H (inter- or intra-molecular H-bonding) or N-H
3275	s	Broad	
2950	m	Broad	C-H stretch
(2902)	s	Sharp	(C-H stretch)
(2839)	s	Sharp	(C-H stretch)
2643	w	Broad	Carbonyl combination
2482	w	Broad	Carbonyl combination
[2358]	w	Sharp	[O=C=O]
[2328]	w	Sharp	[O=C=O]
2262	w	Broad	-
2227	w	Broad	-
2209	w	Broad	-
2119	w	Broad	-
2052	w	Sharp	-
1635	s	Sharp	C=O
(1464)	m	Sharp	(CH_2 rocking)
1426	m	Sharp	C-H deformation of CH_3
1397	m	Sharp	CH_3 symmetrical deformation
~1270	w	Sharp	Ester/anhydride C-O stretch
1098	s	Broad	C-O stretch of C-OH
934	m	Sharp	-
(718)	w	Sharp	(-H deformation)
651	s	Sharp	*
614	s	Sharp	*

Table 8.54: Typical FTIR absorption bands of the electrophoretic humic extract fractions, based on observations of the coniferous soil brown band fraction B2C 0.5 F3 spectrum. Bond assignments using (Williams and Fleming, 1989). Absorption intensity labels s, m and w correspond to strong, medium and weak. Square parentheses denote absorption bands of CO_2. Round parentheses denote absorption bands of the polyethylene substrate of the IR cards. *Ends of spectrum unassigned by (Williams and Fleming, 1989).

Ref. no.	Absorption Wavenumber (cm^{-1})	Attributed vibration
1	3448	H-bonded O-H stretch
2, 3	3400-3200	O-H stretch from COOH and COH (phenols and alcohols)
1	3077	Aromatic C-H stretch
2, 4	3100-2800	C-H stretch of CH, CH_2, CH_3 (asymmetric stretch at higher wavenumber than symmetric stretch)
2	2700-2400	O-H stretch from strongly H-bonded COOH
2	2632-2564	Carboxyl C-H stretch
2, 5, 1, 6	1725-1700	C=O stretch from COOH, ketones, aldehydes and esters (other C=O vibrations at 1639, 1613, 1515)
4	1700-1650	Asymmetric COO^- ring structure
2, 5 2, 6 2, 7 4, 6 6 8 8, 9, 3 5	1650-1600 1650 1540-1560 1600 + 1380	Aromatic C=C (as low as 1500 cm^{-1}) C=O stretch from quinones, H-bonded or conjugated ketones (and other H-bonded C=O) COO^- asymmetric stretch or H-bonded COOH Olefinic C=C (alkene) or conjugated C=C Aliphatic C=C (1650 cm^{-1}) at higher wavenumber than aromatic C=C (1520 cm^{-1}) Amide I Amide II (bound peptides) COOH complexation of metals (COO^-) 1630-1575 cm^{-1} ionic character, 1650-1620 cm^{-1} coordinate character
4	1515	Skeletal aromatic ring N-H (in plane)
4	1460-1440	CH_3
4	1410-1370	CH_3
2	1400	O-H and C-O deformation of alcoholic and phenolic O-H COO^- symmetric stretch
4	1300-1270	C-O stretch of phenolic groups (may split into 2 bands) accompanied by aromatic carbonyl at ~1650 cm^{-1}
1	1282-1205	C-O stretch of esters, ethers, quinones and phenols
2, 5	1250-1200	C-O stretch and O-H bending (deformation) from COOH
1	1136	C-O stretch of aliphatic ethers
2, 1, 6 2	1050-1020	C-O stretch of polysaccharides, ethers or O-H deformation of primary alcohols or polysaccharides Si-O stretch from silicate impurities
2	925	O-H bend Carboxylic acid dimers
6	720	Long-chain alkane $-(CH_2)_n-$

Table 8.55: A summary of humic substance FTIR absorptions and functional group assignments from the literature. (1 - (Kumada, 1958), 2 - (Davis *et al.*, 1999), 3 - (Degonzalez *et al.*, 1981), 4 - (Francioso *et al.*, 1996), 5 - (Stevenson, 1994), 6 - (Tomikawa and Oba, 1991), 7 - (Tan *et al.*, 1991), 8 - (Guibal *et al.*, 1995), 9 - (Cheshire *et al.*, 1992)).

8.15 SELECTION OF HUMIC SUBSTANCE EXTRACTION PROCEDURES IN THE LITERATURE

	Chemical Pretreatment	Extractant	Extractant:Soil volume:weight	Conditions of Extraction	Precipitation	Removal of Solvent	Purification
		NaOH (0.1N) +$SnCl_2$(3g/l)	16:1	20hr	HCl	not given (presumably dialyzed and freeze dried)	
	*1Shaken with CCl_4 10min	Anhydrous HCOOH(98-100%) + LiBr (0.2 N)	~7:1	Refluxed for 30mins (<101°C) Refluxed residue for further 30mins Combined extracts	*3 5 volumes of CH_3COCl in diisopropylether	not given	
1	*1Soxhlet ether extraction 8hr	Acetone	not given	Refluxed at reduced pressure (<35°C) in an inert atmosphere, 12min cycles for 3hr	no acid precipitation	Distilled in an inert atmosphere at <35°C then vacuum dried	*5Washed alternately with ether and benzene
	"	*Dioxane	"	"	"	"	
	"	Ethanol	"	"	"	"	
	*1Soxhlet ether extraction 36hr	*Tris-borate EDTA buffer pH8.9	40:1	Shaken for 3hr	Acidified to pH2	*4not applicable	
		*Borate buffer pH9.0				*4	
		*Veronal buffer pH8.6				*4	
		NaOH (0.05-0.5M)	10:1	Shaken for 24hr under N_2 atmosphere	pH2 H_2SO_4 Equilibrated for 24hr	Freeze-dried, dialyzed against deionised water for 3 weeks then freeze-dried again	
		KOH(0.1M)	"	"	"	"	
		NH_4OH(0.1M)	"	"	"	"	
2		$NaHCO_3$(0.1M) + Na_2CO_3(0.1M)	"	"	"	"	
		NaOH(0.5M) + $Na_4P_2O_7$(0.5M)	"	"	"	"	
		$Na_4P_2O_7$(0.5,0.68M)	"	"	"	"	
		NH_4OH/MeOH(3:1)	"	"	"	"	
		NaOH(0.1M)	"	Stirred for 4h at 60°C	not given	not given	
		NaOH(0.1M) followed by *TMCS:DMF(1:10)	"	" "	pH5 HCl(6M)	NaOH extract freeze-dried, silylated DMF extract was concentrated under vacuum at 60°C	
		TMCS:DMF(1:10)	"			Extract was concentrated under vacuum at 60°C	
3,4&5		*TECS:DMF(1:10)	"	Soxhlet extraction under reflux for 4hr			
		*DMF	"	Stirred under reflux for 4hr at 153°C			
		*NTHA(0.00021M)	"	Stirred for 72hr			
		*DTPA(0.005M) pH7.3	"	Stirred for 4hr at room temperature		Freeze-dried	
		KCl(0.1M)	"	"			
6		HCl(0.1N)					
		NaOH(0.5N)		Under N_2 atmosphere	not given		

(key over page)

Table 8.56: Chemical pretreatment, extraction and purification methods for different solvent classes

Chemical Pretreatment	Extractant	Extractant:Soil volume:weight	Conditions of Extraction	Precipitation	Removal of Solvent	Purification
	NaOH(0.5N)	not given	Extracted under N_2 atmosphere	pH1 HCl(conc.)	Dialyzed against distilled water and freeze dried	
	$Na_4P_2O_7$(0.1M) pH7	"		"	"	
	*DMSO-H_2O-HCl(8:2:0.6N) ~pH1	"		Extractant ~pH1	"	
	DMF-H_2O-HCl(8:2:0.6N) ~pH1	"		"	"	
	Acetone-H_2O-HCl(8:2:0.6N) ~pH1	"		"	"	
	NaOH(0.5N)	"	Extracted under N_2 atmosphere	pH1 HCl(conc.)	"	*6Shaken for 36hr with HF-HCl(0.5:0.5%v:v)
	$Na_4P_2O_7$(0.1M) pH7	"		"	"	"
	DMSO-H_2O-HCl(8:2:0.6N) ~pH1	"		Extractant ~pH1	"	"
7&8	DMF-H_2O-HCl(8:2:0.6N) ~pH1	"		"	"	"
	Acetone-H_2O-HCl(8:2:0.6N) ~pH1	"		"	"	"
*2Washed with HCl(0.1M)	NaOH(0.5N)	5:1	Shaken for 12hr on a mechanical shaker under N_2 atmosphere. Residue further extracted until solution remains colourless	pH1 HCl(conc.)	Dialyzed against water, freeze dried then dried in a vaccum dessicator over P_2O_5	*6Shaken for 36hr with HF-HCl(0.114:0.062M) under N_2 at room temperature
	$Na_4P_2O_7$(0.1M) pH7	"	"	"	"	"
	DMSO-H_2O-HCl(8:2:0.6N) ~pH1	"	Shaken for 12hr on a mechanical shaker Residue further extracted until solution remains colourless	Extractant ~pH1	"	"
	DMF-H_2O-HCl(8:2:0.6N) ~pH1	"	"	"	"	"
	Acetone-H_2O-HCl(8:2:0.6N) ~pH1	"	"	"	"	"

* Dioxane (1, 4 - dioxacyclohexane), DMF dimethylformamide (N, N - dimethylmethanamide), DMSO dimethylsulphoxide, DTPA diethylenetriaminepentaacetic acid solution (DTPA(0.005M), triethanolamine(0.1M), $CaCl_2$(0.01M)), NTHA nitrilotriacetohydroxamic acid, TECS triethylchlorosilane, TMCS trimethylchlorosilane, Borate buffer H_3BO_3/KCl/NaOH, Tris-borate EDTA buffer 0.5M trishydroxymethylaminomethane/0.21M ethylenediaminetetraacetic acid/0.075M H_3BO_3. Veronal buffer 0.05M sodium diethylbarbiturate/0.005M diethylbarbituric acid/0.05M sodium ethanoate

*1 Pretreatment to remove plant/animal lipids and plant resins from the soil, *2 To decompose free carbonates and remove alkali earth metals, *3 Precipitation of humus extract intended to keep contaminating mineral component metal ions in solution (Stevenson, 1994)' *4 These buffer extracts were intended for immediate transfer to electrophoresis apparatus, *5 To remove lipids and resins, *6 To dissolve and remove soil mineral components,

1 - (Eloff and Pauli, 1975) 2 - (Carter *et al.*, 1992) 3 - (Szabo *et al.*, 1991) 4 - (Bulman *et al.*, 1990) 5 - (Bulman *et al.*, 1992) 6 - (Preston and Schnitzer, 1984) 7- (Piccolo *et al.*, 1989) 8 - (Piccolo *et al.*, 1990)

Table 8.56 continued: Chemical pretreatment, extraction and purification methods for different solvent classes