Investigation of the assessment and remediation of land contaminated with heavy metals

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Contents of thesis

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Abstract

The investigation and remediation of land contaminated with metals (copper, nickel, lead and zinc) was investigated. Calcium, iron and manganese levels within the soils studied were also investigated. Several soils were used to assess the three-stage BCR sequential extraction procedure and recent recommended modifications to this procedure. In general the modifications to the procedure were found to increase levels of copper, lead and iron extracted by the reductant used in the procedure.

The modified BCR sequential extraction procedure was then used to assess the success of remediation strategies. Column leaching experiments, with EDTA, were set up to simulate soil-flushing technologies. Soil was extracted using the BCR procedure both before and after treatment. The experiments highlighted the need to consider the soil characteristics when determining a remediation strategy. The technique was shown to be successful for the leaching of the more mobile forms of copper, lead and zinc from the soils studied.

Phytoremediation and chelate assisted phytoremediation were also investigated using the BCR sequential extraction procedure. *Taraxacum officianale* (dandelion) was grown in soil contaminated with zinc. The experiment was designed to study the ability of the plant to accumulate metals and also to study the effect of the addition of the chelator EDTA and the effect of the addition of a fertiliser. These methods were shown to remove significant proportions of zinc, copper and lead from the soil studied, however the time frame for remediation based on such techniques may be considerably longer than that for conventional methods.

Chapter 1 Introduction

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Chapter 1 Introduction

Contaminated land in the UK may be defined as "land which, because of its former uses, now contains substances that give rise to the principal hazards likely to affect the proposed form of development and which requires an assessment to decide whether the chosen development may proceed safely, or whether it requires some form of remedial action, which may include changing the layout and form of development"[1]. In Scotland alone there exist 14,100 hectares of brownfield sites (vacant and derelict land), many of which are contaminated with toxic chemicals due to their industrial past[2]. The country has an estimated 200,000 hectares of land affected by natural or industrial contamination [3]. Local councils are now required to identify areas of contaminated land and enforce the polluter or land owner to pay for the cleanup of the land. It is therefore of great importance that methods of assessing and solving contaminated land problems are investigated. Prior to these investigations it is important to understand the chemistry of soil and the behaviour of metals within the soil.

1.1 Soil

Soil is the product of the weathering of crustal rocks, combined with water (both ground water and atmospheric i.e. rain etc.), air and organic matter produced by living organisms within the soil. Each of these components are important in the behaviour of metal contaminants within the soil.

Crustal rocks have different structures and different characteristics depending upon how they were formed. Igneous rocks are formed by the solidification of the molten material in the Earth's mantle. Sedimentary rocks, which include sandstone and limestone, are formed by the accumulation and compaction of minerals and rock fragments. The third class of crustal rocks is the metamorphic rocks, such as marble and slate which are formed by the action of pressure and/or temperature on igneous or sedimentary rocks. The physical and chemical processes linking the three types are summarised in the rock cycle, shown in figure 1.1.

1.1.1 Soil minerals

Some of the most important soil minerals are the silicates, such as quartz (SiO_2). These have a wide variety of structures dependent upon the temperatures and pressures under which they were formed. For example, high formation temperatures favour simple silicates, rich in iron and magnesium, such as olivine $(Mg, Fe)_2SiO_4$. Other important minerals are the feldspars, such as orthoclase ($KAlSi_3O_8$). Feldspars consist of linked units of SiO_3 and AlO_4 tetrahedra, with cavities in which cations such as Ca^{2+}, Na^+, K^+ or Ba^{2+} are held to maintain electroneutrality. Rocks are usually formed from a mixture of several different minerals. Primary minerals are formed at high temperatures and pressures and so are unstable at the Earth's surface. They therefore undergo weathering to produce secondary minerals via processes such as oxidation and hydrolysis. The ease with which weathering occurs is dependent upon the lattice energy of the mineral, and a weathering sequence of the minerals can be built up, table 1.1. The sequence of

weathering illustrates that simple salts (with larger surface area available to attack by weathering than frameworks) are less stable than the stable frameworks such as anatase.

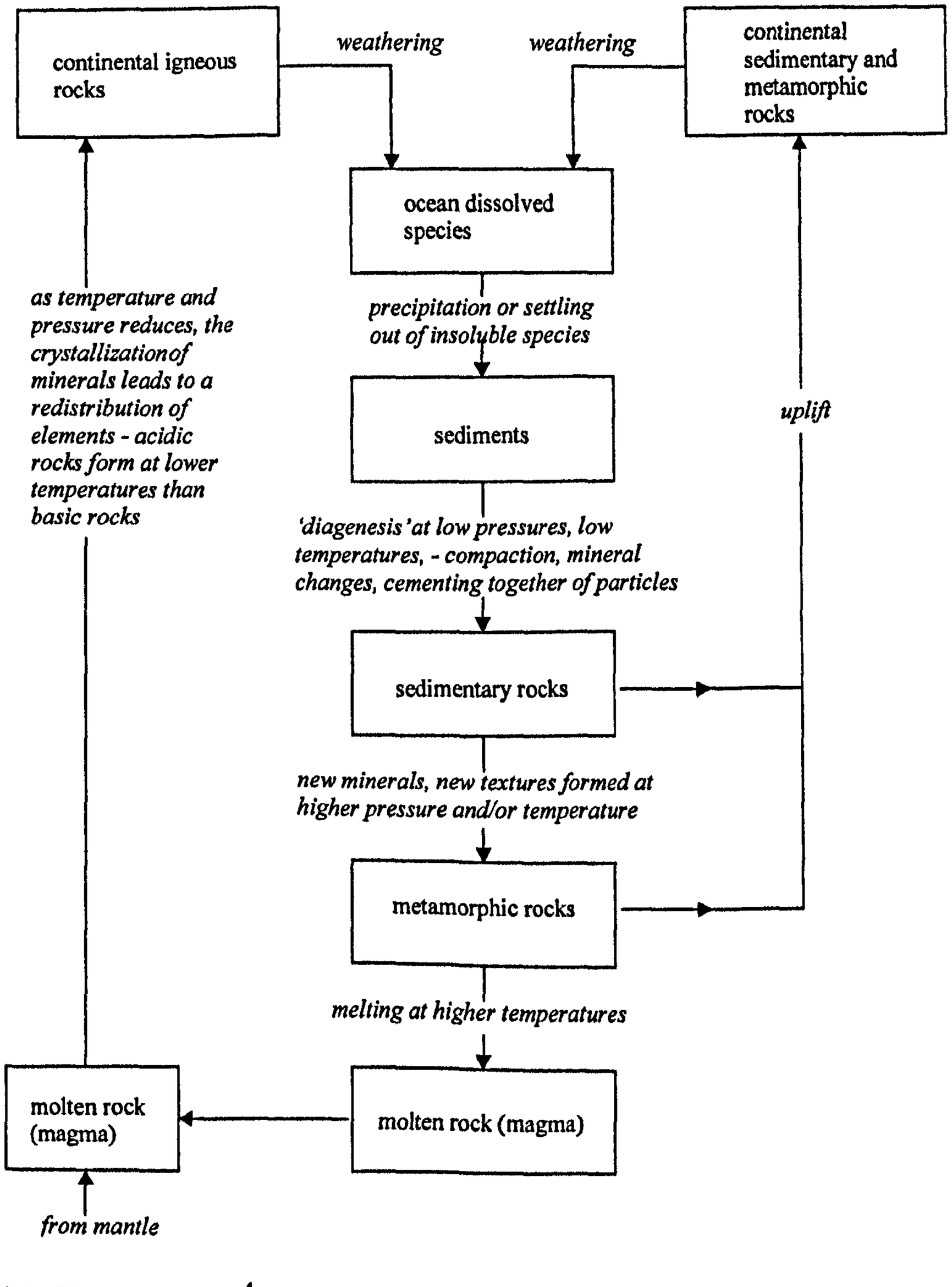


Figure 1.1 The rock cycle[4]

Table 1.1 Weathering Sequence for fine-grained minerals in soils[5]

Position[a]	Minerals	Comments
1	Gypsum, halite, other simple salts	Simple salts
2	Calcite, dolomite, apatite, aragonite	
3	Olivine, pyroxenes, diopside, hornblende etc.	Orthosilicates, chain silicates
4	Biotite, glauconite, magnesium chlorite, antigorite, nontrite	Layer silicates
5	Albite, anorthite, stilbite microcline, orthoclase, etc.	Hard, feldspar framework silicates
6	Quartz, crytobalite, etc.	SiO frameworks
7	Muscovite, etc.	Layers bound together by potassium ions
8	Interstratified 2:1 layer silicates and vermiculite	Secondary clay minerals
9	Montmorillonite, beidellite, saponite, etc.	Varieties of montmorillonite
10	Kaolinite, halloysite, etc.	
11	Gibbsite, boehmite, allophane, etc.	Hydrated aluminium oxides, etc.
12	Hematite, goethite, limonite, etc.	Hydrated iron oxides, etc.
13	Anatase, zircon, rutile, limenite corundum, etc.	Stable oxide frameworks

[a] 1, least inert; 13, most inert

Silicates structures range from simple SiO_4 tetrahedra to complex frameworks. The simplest silicates consist of separate SiO_4^{4-} bound to divalent cations (e.g. Mg^{2+}, Fe^{2+}, Mn^{2+}, Ca^{2+}, etc.) giving M_2SiO_4. These minerals are classed as olivines. Chain silicates such as pyroxenes and amphiboles, consist of linked SiO_4 tetrahedra. Even more complex structures can be formed when the tetrahedra extend in three dimensions

producing frameworks such as feldspars. In these frameworks atoms of a similar size, such as Al, can replace Si atoms. This leaves a net negative charge on the structural unit that must be balanced by appropriately charged cations such as Na^+, K^+, Ca^{2+}, Ba^{2+}, etc. Other sheet structures such as gibbsite and brucite can be formed from the octahedral $Al(OH)_3$ and $Mg(OH)_2$ units respectively.

Mineral sheets can be sandwiched together to form layered structures known as 2:1 and 1:1 type minerals. A typical 2:1 mineral such as pyrophyllite contains a gibbsite sheet between two silicon tetrahedral sheets. Replacement of the gibbsite hydroxyls with silicate oxygen atoms can lead to the requirement for additional cations, to balance net negative charges formed. An example of this is muscovite, in which potassium ions (balancing cation) hold adjacent layers together. Clay minerals such as kaolinite are 1:1 minerals containing, for example, 1 gibbsite sheet and 1 silicate sheet. Another clay mineral, montmorillonite is a 2:1 mineral, however this differs from other 2:1 minerals in that water is readily absorbed into the interlayer spacing expanding the clay.

Also important are oxides and hydroxides of iron, manganese and aluminium. Oxides (the general term in environmental chemistry for metal hydroxides, oxyhydroxides and hydrous oxides) may be present in soils in many forms: discrete crystals, coatings on silicate sheets and humic substances, and as mixed gels.

Iron oxides are formed from a basic octahedron of iron surrounded by six oxygens or both O^{2-} and OH^- ions. The octahedrons may then be linked to form crystalline

structures, such as geothite (double bands of FeO(OH) octahedra sharing edges and corners). As well as crystalline and semicrystalline forms of iron oxides, amorphous forms also exist. Al^{3+}, Mn^{3+} and Cr^{3+} can replace Fe^{3+} by isomorphous substitution. Other cations such as Ni^{2+}, Cu^{2+}, and Zn^{2+} may also be found within the iron oxide structure. The charges present on soils are of two types; permanent charges due to isomorphous substitution of cations by other cations of lower charge, and pH dependent charges on the edges of clay minerals, on humus polymers and oxides. Hydrous oxide minerals have a net charge of zero only at specific pH values. This pH is called the PZC (point of zero charge). If the soil pH is greater than the PZC the oxide surfaces become negatively charged, and therefore act as a sink for cations such as Cu, Mn, Ni, and Zn. PZC values for Fe and Al oxides range from 7-10 and 8-9.4 respectively when in the pure form. Generally these are mixed with clays which reduces the PZC value[6].

1.1.2 Soil Organic Matter

Although soil organic matter has been the subject of many studies, the structure and chemistry is still not well understood. Soil organic matter (SOM) includes decomposing residues (of plants, animals and microbes), living soil biota and resistant organic matter. SOM plays several important roles in soil chemistry due to its high reactivity, its ability to improve soil structure, and its provision of both micro- and macro-nutrients. The high specific surface area and cation exchange capacity (CEC) of SOM makes it an important sink for both inorganic and organic pollutants.

SOM is composed of both humic and non-humic substances. Non-humic substances consisting of carbohydrates, proteins, peptides, amino acids, fats, waxes and low molecular weight acids, are attacked easily by soil micro-organisms and therefore exist in the soil for a short time only. SOM is usually estimated by "loss on ignition" i.e. the weight loss which occurs when a sample is heated at approximately 550 °C. The components of SOM can be classed according to their solubility in strong acid and base as shown in figure 1.2.

Humic and fulvic acids are mixtures of compounds and therefore only average molecular weights can be calculated. Humic substances have high buffering ability, which maintains pH within the soil. Oxygen may be present as carboxyls, phenolic and alcoholic hydroxyls and carboxylic esters and ethers in humic acids. Nitrogen may be present as heterocyclic structures and as nitriles. Humic substances can chelate metal ions in various modes due to the large numbers of functional groups present. The immobile humin and humic acids act as stationary cation exchange media, whereas the mobile fulvic acids play an important role in keeping biologically important metals in solution.

1.1.3 Soil Air

Soil air differs in composition from atmospheric air due to intense microbial activity that decomposes organic matter, reducing the oxygen content and increasing the carbon

dioxide content compared to the atmosphere. Carbon dioxide is also added to the soil air by the respiration of roots.

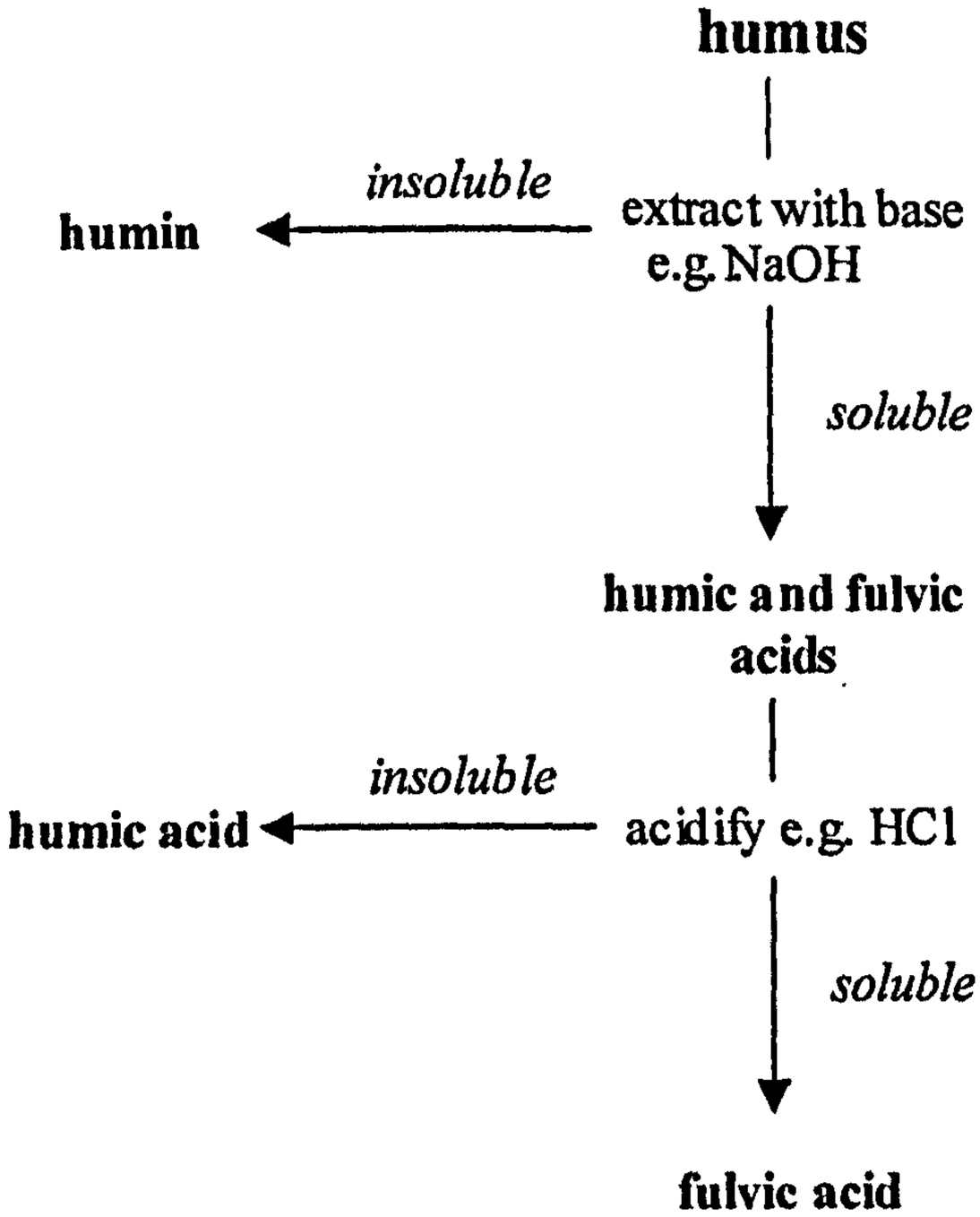


Figure 1.2 Fractionation of SOM components

1.1.4 Soil Solution

The soil solution provides a medium of transport of important nutrients from the soil particles to roots. It usually exists as a thin film on particles or between the layers of expanding clays. The composition of the soil solution is dependent upon the physicochemical characteristics of the soil particles, solute composition, biological activity within the soil matrix and, its contact time with the soil.

1.2 Soil Properties

Soil properties play a major role in controlling the transport of metals within the soil. Cation exchange capacity (CEC), pH and particle size distribution can strongly influence the movement of metals in the soil.

1.2.1 Cation Exchange Capacity

As discussed earlier, cations in several mineral structures can be substituted by cations of a similar size, but with lower charge. This leads to the particles possessing a net negative charge. Further cations are then attracted to the negatively charged surface. In clays such as montmorillonite, the layers are held together by cations, but expand when wet and contract when dry. This means that the interlayer cations can be readily replaced by other cations, ie. they are exchangeable. Organic matter within soil is also able to readily exchange cations due to the many chelation sites available, through dissociation of phenolic and carboxylic groups. Under conditions where the soil pH is greater then the PZC for oxides and oxyhydroxides (as discussed in 1.1.1), the surfaces of these minerals become negatively charged and are also able to exchange cations.

The capacity of the soil to take up exchangeable cations can be measured by replacing the cations present in the soil with ammonium (which rapidly displaces other cations). Any non-adsorbed ammonium is washed out of the soil, and the remaining ammonium is

then displaced with a solution such as weakly acidified sodium chloride. Measurement of the displaced ammonium gives a measure of the cation exchange capacity, CEC.

1.2.2 pH

pH is defined as the negative logarithm to the base 10 of the hydrogen ion activity. Soil pH is an important factor in controlling the solubility of many metal contaminants and therefore should be measured when assessing contaminated land. For example, copper and zinc tend to be more soluble in acidic soils and can therefore be washed out of these soils into ground water. However, accumulation of organic matter within the soil leads to complexation of copper and zinc reducing their availability to plants. The availability of copper and zinc then decreases as pH increases.

Measurement of soil pH is complicated by the cation exchange properties of the soil.[6] The cation exchange properties of clays can lead to an increase in the concentration of ions around the clay particles, compared to the concentration of ions in the bulk soil solution. H^+ ions can be adsorbed onto cation exchange sites, however are readily displaced from these sites by any other cations present in the solution. When adding water to a soil in order to measure the pH, the solution must be allowed to equilibrate in order for any soluble salts in the soil to dissolve and displace the H^+ ions. This pH value can be 1 – 1.5 pH units higher than the pH at the soil surface due to diffusion. A dilute calcium chloride solution can be used when measuring soil pH in order to reduce the

dilution effect. Another factor affecting pH measurement is the production of carbonic acid (formed by dissolution of the bacterial respiration product, carbon dioxide).

1.2.3 Particle Size Distribution

Particle size distribution can also affect the metals absorbed onto the soil. At the surfaces or edges of crystals and soil organic matter, surplus charge is available for binding metals. The charge available for interaction with cations in the soil increases with increasing surface area, which is dependent on particle size. Thus if a soil has a high proportion of small particles it follows that it will have a higher capacity for cation exchange.

1.3 Metals of Interest[6,7]

Trace elements may be either essential or toxic to plants and animals, depending upon their concentration. It is therefore important to know at what levels metals occur. It is also important to know the source of a potentially toxic element, the target organism, and also the route of intake of the toxic element by the target organism. For example, waste from a chemical plant using a Hg^{II} catalyst was discharged into Minimata Bay, Japan, 1950's. Bacteria in the sediments of the bay then methylated the mercury, which was bioaccumulated by fish to give levels of approximately 100 μg organomercury g^{-1} fish. The fish were caught and consumed by villagers on the shores of the bay, whose

main diet was fish. Subsequently hundreds died and many more were poisoned and suffered severe brain damage[8]. In this case, the target organisms were the human population living by the bay and the route of intake was by ingestion of the fish. An understanding of the behaviour and sources of heavy metals in the environment is needed before their impact on the environment as pollutants can be properly assessed.

1.3.1 Calcium

Calcium is of interest as it is one of the major soil cations, and the amount and form present within soils may therefore effect the mobility of other metals of interest. Calcium is the fifth most abundant element in the earth's crust, occurring mainly as deposits of calcium carbonate. The mineral deposits of calcium carbonate include limestone, marble and chalk. In soils of high pH, calcium carbonate precipitates protecting plants from damage by accumulation of soluble calcium salts. Calcium is also present in the earth's crust as gypsum ($CaSO_4.H_2O$), anydrite ($CaSO_4$), fluorite (CaF_2) and apatite [$Ca_5(PO_4)_3F$]. Calcium is present in soils at 0.1 – 1.2 % (m/m) on average and at 1% in plants (taken up as Ca^{2+} or $CaOH^+$)[9].

1.3.2 Copper

Copper has been one of the most important metals known to man since 3000 BC when the addition of tin heralded the beginning of the Bronze Age. The major ores of copper

are copper pyrite, $CuFeS_2$; copper glance, Cu_2S; cuprite, Cu_2O, and malachite, $Cu_2CO_3(OH)_2$. Copper can be present in soils at levels of 1-80 mg kg^{-1} and between 2-20 mg l^{-1} in plants[9]. The major use of copper is as an electrical conductor, but it is also used in production of alloys such as bronze (Cu plus 7-10 % Sn), brass (Cu-Zn) and Monel (Ni-Cu). Copper is an essential trace element, the human body containing approximately 100 mg and requiring an intake of approximately 2 mg day^{-1}. Copper acts as an electron transfer agent in many metabolic reactions (e.g. energy metabolism, nitrogen metabolism) in both plants and animals. There are many routes by which copper may be discharged into the environment, such as combustion of wood products and fossil fuels, waste incineration, agricultural application of sewage sludges, smelters and some fungicidal spays. Copper tends to be adsorbed on specific sites in soils and is therefore one of the least mobile and least plant available trace metals, which may cause deficiency problems in some areas.

1.3.3 Iron

Iron has been known since prehistoric times and has played a major role in man's history. It is thought to be the main constituent of the Earth's core and is the second most abundant metal. Iron is generally present in soils at levels between 0.7 and 42 % (m/m)[9]. Iron is widely distributed as oxides and carbonates: haematite, (Fe_2O_3); magnetite, (Fe_3O_4); limonite, ($\sim 2Fe_2O_3.3H_2O$), and siderite, ($FeCO_3$). The main use of iron is in the steel industry where the addition of other metals to the steel to form steel alloys give a wide and varied range of properties. Iron is also an essential element, vital

to both plants and animals. Although iron is usually abundant in soils it must be mobile in order for plants to use it. In areas of high alkalinity iron is often inaccessible to plants, which require iron for chlorophyll synthesis. Iron is taken up by plants as Fe^{2+} and $Fe(OH)_2^+$. The adult human contains approximately 4 g of iron, mainly in the form of haemoglobin, but also as other proteins for electron transport and oxygen transport and storage.

1.3.4 Lead

Lead is one of the oldest metals known to man and was used as early as 7000 BC by the Egyptians. The Romans used lead extensively for plumbing and water pipes. Lead is the most abundant of the heavy metals, primarily due to the fact that three of the four stable isotopes (206, 207 and 208) are the end products of the natural radioactive series. Only Pb 204 is non-radiogenic in origin. The most important lead ore is galena, PbS. Examples of other mineral ores known are anglsite, ($PbSO_4$); cerussite, ($PbCO_3$); pyromorphite, ($Pb_5(PO_4)_3Cl$), and mimetisite, ($Pb_5(AsO_4)_3Cl$). All lead ores contain Pb^{II}. Almost half of lead produced is used for storage batteries, and the remainder is used for a variety of alloys (e.g. solders, fusible alloys and bearing metals) and chemicals (e.g. pigments and plastic stabilisers). Prior to the introduction of catalytic converters and unleaded petrol, on of the major uses of lead was as an anti-knock agent in petrol. Although this usage has declined, leaded petrol is still available in the UK and other countries. Lead levels in soils have been found to range between

0.1 – 200 mg kg^{-1} and 0.1 – 5 mg kg^{-1} in plants.[9] Lead is generally taken up by plants as $PbCO_3$ and can be toxic at levels of 3 mg l^{-1}.[9] The main problem with lead as an environmental contaminant is that it tends to accumulate in the surface horizons of soil due to its low solubility and relative freedom from microbial degradation as opposed to many organic contaminants which can be microbially degraded. The direct ingestion by children of surface soil containing lead is therefore of great concern. Lead is a heavy metal poison, which acts by complexing with oxo-groups in enzymes, affecting haeme synthesis and porphyrin metabolism. It also inhibits many other enzymes and also protein synthesis. Typical symptoms of lead poisoning include cholic, anaemia, headaches, convulsions, chronic nephritis of the kidneys, brain damage, and central nervous system disorders.

1.3.5 Manganese

Manganese has been used since the time of the Pharaohs when it was used in glassmaking. It is the twelfth most abundant element and the third most abundant transition metal. As described previously manganese occurs in primary silicate minerals and the products of their weathering, such as pyrolusite (MnO_2), hausmannite (Mn_3O_4) and rhodochrosite ($MnCO_3$). Manganese levels can be found to range from 20 –3000 mg kg^{-1} in soils and 1-700 mg kg^{-1} in plants[9]. All steels contain manganese, which improves the quality by forming MnS and preventing FeS formation, which would produce brittle steel, and also by scavenging oxygen which would create bubbles in the steel. Manganese dioxide is also used in the production of dry cell batteries, to provide

red to brown and grey tints in the brick industry, as a decolouriser in the glass industry, and in the production of dyes and paints. Manganese is an essential element and is involved in the activation of enzymes, synthesis of glycoproteins and many other roles in mammals. Plants require manganese for the photolysis of water during photosynthesis reactions and for the stabilization of chloroplasts. Manganese can occur in many oxidation states, but the most biologically important state is Mn^{II}. Plants take up both Mn and Mn^{II} and so factors that affect manganese supply for nutrition are those that have greatest influence on reduction of manganese from high oxidation states.

1.3.6 Nickel

Nickel is a much more recently exploited metal than copper, although an alloy was known in China over 2000 years ago. Nickel is the seventh most abundant transition metal, its most commercially important ores being laterites, which are oxide/silicate ores such as garnierite, $(Ni,Mg)_6Si_4O_{10}(OH)_8$, and sulfides such as pentlandite, $(Ni,Fe)_9S_8$. The major uses of nickel are in the production of both ferrous (e.g. stainless steel) and non-ferrous alloys (e.g. cupro-nickels for "silver coinage"). Nickel is generally present in soil in the range 2-50 mg kg^{-1} and 0.4-4 mg kg^{-1} in plants[9]. The most important factor in determining the distribution of nickel within soil is pH. Nickel mobility increases as pH and CEC decrease[6]. Nickel is an essential trace element for higher plants and some micro-organisms, but its role as an essential trace element in humans in as yet unclear. Nickel can however replace other essential trace metals in metalloenzymes and cause disruption of metabolic pathways.

1.3.7 Zinc

The most important zinc ores are the sulfides as, when the Earth's crust solidified, zinc separated out in the sulfide phase. As rocks are weathered, zinc is leached out and precipitated as the carbonate, silicate, or phosphate. The major ores of zinc are zinc blende (ZnS), and calamine ($ZnCO_3$). There are many uses for zinc, although 35-40 % of the total output is as an anti-corrosion coating (by immersion in molten zinc, electrolytic deposition, spraying with liquid metal etc.). Zinc is also used in a number of alloys, in the manufacture of dry batteries, and as a stabiliser in plastics. Zinc is generally present in soils at levels ranging from 3–300 mg kg^{-1} and 15–150 mg kg^{-1} in plants[9]. Uptake in plants can be as Zn^{2+}, $ZnOH^{+}$ and $ZnCO_3$. Zinc is an essential element used in chlorophyll formation in plants and as a catalytic or structural component in numerous enzymes in energy metabolism and in transcription and translation in the human body. The adult human body contains about 2 g and requires a dietary intake of approximately 15 mg day^{-1}.

1.4 Contaminated Land

Contaminated land studies involve the identification of a source of contamination, which by means of a pathway has a detrimental affect on a target. The source may be for example a fossil fuel burning power station which may release heavy metals into the atmosphere which can then travel and be deposited in areas surrounding the power station and affect the local population and wildlife. Land may be contaminated by both

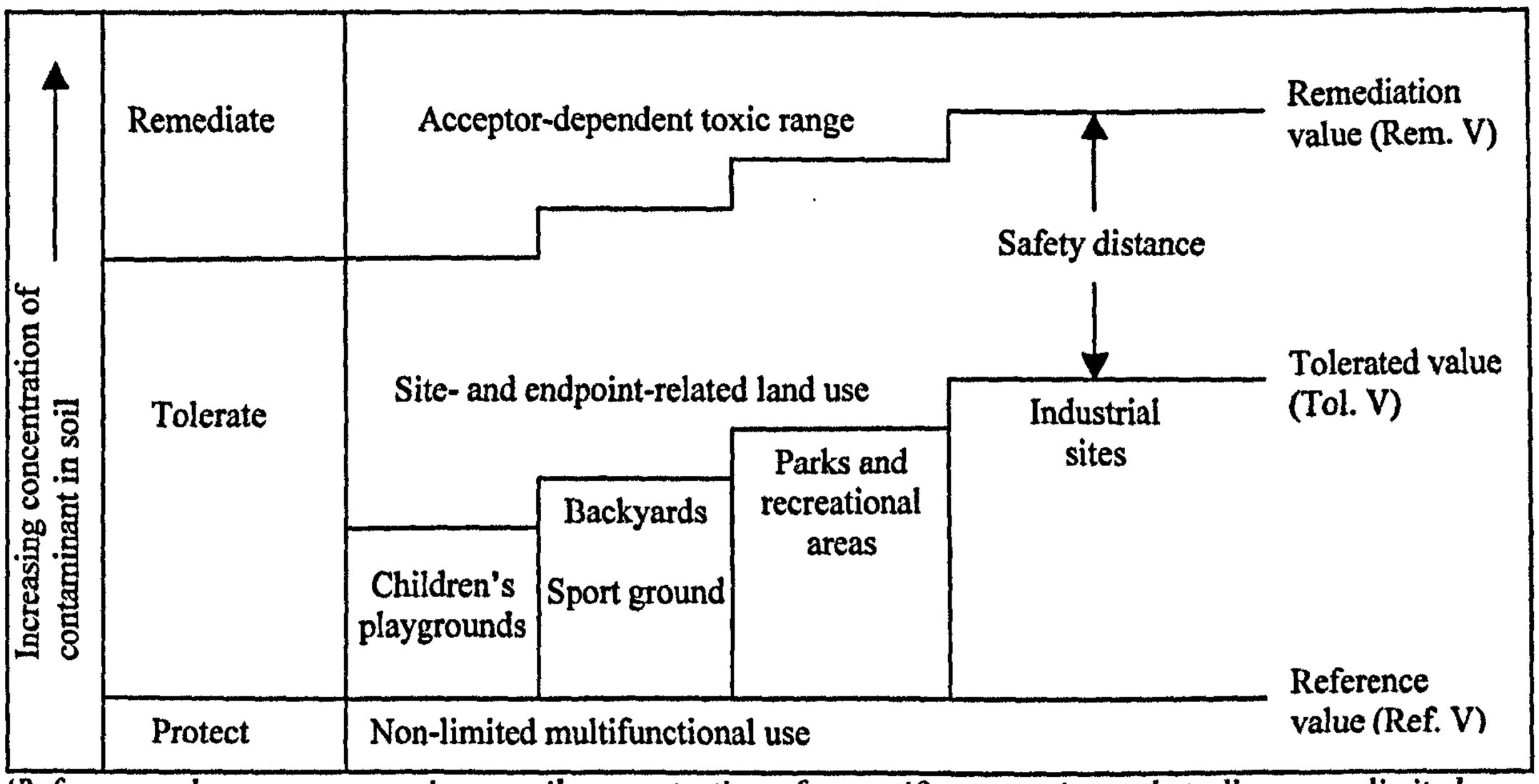
natural and anthropogenic sources. Natural sources include weathering of minerals, volcanic activity and forest fires. Anthropogenic sources have been accelerated by the Industrial Revolution and include mining and smelting activities etc. Regulation has been able to reduce anthropogenic input in developed countries e.g. by control of sewage sludge disposal, combustion of fossil fuels etc.

The Environmental Protection Act 1990: Part IIA Contaminated Land was inserted into the Environment Act in 1995 gives guidance on the identification and control of contaminated Land in the UK[1]. The new guidelines and regulations were brought into effect from 1st April 2000. Responsibility for implementing the Part IIA rests with the Scottish Executive: Rural Affairs Department in Scotland and is controlled by the Scottish local authorities. In England and Wales responsibility lies with the Department of Environment, Food and Rural Affairs (DEFRA) and the National Assembly for Wales respectively[1].

Contaminated land is assessed on a "suitable for use" approach, based on the uses to which the land is likely to be put[10]. The harm caused by the pollution must also be assessed. Only harm, such as death, serious injury, cancer or other disease, genetic mutation, birth defects in humans, is regarded as significant. Only organisms with statutory protection in ecological systems are covered and the harm must result in serious changes to the ecological system. Once contaminated land has been identified for remediation, the local authority must also identify responsible person/s. The

remediation required must be decided upon after consultation with the appropriate person, the landowner and Scottish Environmental Protection Agency (SEPA).

Many countries have set remediation levels for trace elements in soil[11]. German research has related reference, tolerable and remediation values of trace elements in soil to land use. This is illustrated in figure 1.3.



'Reference values: maximum soil concentration of a specific contaminant that allows non-limited multifunctional use of the land. It can be assimilated to baseline level.

Tolerated values: maximum soil concentration of a specific contaminant in relation to specific site- and endpoint-related land use. Above this value remediation is not immediately required but monitoring or changing the land use is demanded.

Remediation values: threshold values, related to land use, above which risk assessment and subsequent remediation are necessary.'

Figure 1.3 Conceptual description of the threshold limits in Germany[11]

Threshold values for some metals, based on the total concentration of trace elements in soil are shown in table1.2.

Table 1.2 Land use and endpoint-related orientation value (mg kg^{-1}) for trace elements in soils from German guidelines[11]

Land use	**Value***	**Cu**	**Ni**	**Pb**	**Zn**
Multifunctional land use	Ref.V.	50	40	100	150
Children's playgrounds	Tol.V.	50	40	200	300
	Rem.V.	250	200	1000	2000
Backyards	Tol.V.	50	80	300	300
	Rem.V.	200	200	2000	600
Sports grounds	Tol.V.	100	100	200	300
	Rem.V.	350	250	1000	2000
Parks and recreational area; unconsolidated, non-vegetated soils	Tol.V.	200	100	500	1000
	Rem.V.	600	250	2000	3000
Industrial sites and staple (non-paved)	Tol.V.	300	200	1000	1000
	Rem.V.	1000	500	2000	3000
Agricultural soils	Tol.V.	50	100	500	300
	Rem.V.	200	200	1000	600
Non-agricultural ecosystems	Tol.V.	50	100	1000	300
	Rem.V.	200	200	2000	600

* definitions of Ref. V, Tol. V and Rem V. are illustrated in figure 1.3

Dutch intervention values are dependent on soil type and must be adjusted accordingly. Factors taken into concentration are % organic matter, % clay and the compound being assessed. Another set of guidelines, the Kelly guidelines for classification of contaminated soils classifies the extent to which soils are contaminated (typical values for uncontaminated soils, slight contamination, contamination, heavy contamination and unusually heavy contamination)[12]. Guidelines commonly used in the UK are the Interdepartmental Committee for the Redevelopment of Contaminated Land (ICRCL) tentative "trigger concentrations" for selected inorganic contaminants[13]. These guidelines concern both total levels in soils and bioavailable levels, and take into account the planned land use. Table 1.3 gives tentative "trigger concentrations" for some metals of interest.

The values given in table 1.3 are dependent on certain environmental factors, which must be taken into consideration when assessing contaminated land. Values for copper, nickel and zinc assume a soil pH of about 6.5. If pH falls, the toxic effects and the uptake of these elements can be increased. Grass tends to be more resistant to phytotoxic effects than most other plants and its growth may not be adversely affected at the concentrations given. Values for these metals are total concentrations extracted with $HNO_3/HClO_4$ and are the 'worst case' figures. Phytotoxic effects are not likely at the threshold levels in neutral or alkaline soils. The possible action values were based on the mean of similar values from several other countries. Actual values used should take into consideration soil type, proposed end use and sensitivity of recognised targets.

Table 1.3 UK ICRCL Trigger Concentrations for metals[13]

Contaminants	**Planned Uses**	**Trigger Concentrations (mg kg^{-1} air-dried soil)**	
		Threshold	**Action**
Group A: Contaminants which may pose hazards to health			
Lead	Domestic gardens, allotment. Parks, playing fields open space	500 2000	813
Group B: Contaminants which are phytotoxic but not normally hazards to health			
Copper	Any uses where plants are to be grown	130	423
Nickel	Any uses where plants are to be grown	70	376
Zinc	Any uses where plants are to be grown	300	1665

Duncan, A.L. recommends a protocol for the assessment of heavy metal contaminated sites[14]. The approach recommended is that:

- detailed reports are gathered on previous land use to identify possible contaminants
- sampling areas should be planned, possibly using screening techniques

- samples should be taken at various depths
- *aqua regia* soluble metal contents rather than "true" totals should be determined
- sequential extraction should be used to identify potentially mobile contaminants
- column leaching experiments should be performed to provide information on readily mobile metals
- metal sorption experiments should provide information on the influence of the soil material on the retention of heavy metals
- all data gathered should be used to assess the requirement for remediation and the suitability of remediation methods available.

There are many extraction procedures mentioned in the literature. These procedures may be to define species according to their role (e.g. 0.05 mol l^{-1} EDTA extraction of Cu, Ni, Pb and Zn to determine availability to arable crops), to define the species according to the procedure used in its isolation (e.g. soluble species defined by those which will pass through a 0.45 μm pore-size filter), or to define specific chemical species (e.g. Cr^{III} (essential trace element)/Cr^{VI} (highly toxic)). Sequential extraction procedures use a series of reagents designed to extract metals by different processes e.g. oxidation of organic matter within the soil. Many of these procedures exist in the literature making comparison of results between laboratories difficult. The Community Bureau of Reference (BCR) of the Commission of the European Communities therefore prepared a series of reference materials certified for contents of species extracted by agreed validated procedures[15]. One such reference material was Certified Reference

Material 601 for the sequential extraction of Cd, Cr, Ni, Pb and Zn[16]. The BCR developed a sequential extraction procedure in an attempt to harmonise sequential extraction. Much work has gone into developing[14] and assessing the procedure. The method developed uses dilute acetic acid to extract exchangeable metals and those bound to carbonates within the soil. The second step uses hydroxylammonium chloride solution to extract metals bound to reducible fractions, and the third step uses hydrogen peroxide an ammonium acetate solution to break down organic soil components and extract metals associated with them. The original procedure developed proved to be irreproducible in particular with regard to the second step (reducible step). A certified reference material was therefore used to assess the sources of uncertainty within the protocol. Sahuquillo *et al.*[17] investigated the effect of varying the pH of the hydroxylammonium chloride, the method of pH adjustment, the concentration of hydroxylammonium cloride used, temperature and duration of the experiment. A revised protocol was therefore produced from the results of Sahuquillo's[17] investigation. The revised procedure was tested using inter-laboratory trial and the improved results given by Rauret *et al.*[18]. Sequential extraction procedures, and in particular the BCR sequential extraction procedure, will be discussed in detail in chapter 4.

Industry must play a large role in the clean up of contaminated land. Sheahan and Gilliland identify industry objectives as compliance with legal requirements, protection of human health and the environment, and to manage liability issues[3]. Strategic approaches should include plans for preventing on-going contamination, a systematic

approach to managing historic contamination and a strategy to ensure the availability of the appropriate remediation technology.

1.5 Remediation options

There are generally four ways in which land can be remediated once contamination has been identified:

1. removal from the site and disposal at a licensed waste disposal site
2. retention and isolation on site
3. physical, chemical or biological treatment, either in- or ex-situ, to eliminate or immobilise contaminants
4. dilution of contaminated material with clean material

1.5.1 Containment

Containment techniques include lining, capping, cover systems and occasionally vertical barrier systems. Materials used in containment systems depend upon the physical and chemical properties required of the containment layer and can include[19]:

- natural clays, sub-soils and soils
- amended soils incorporating materials like pulverized fuel ash, lime and sludges
- waste materials such as fly ash, slags, dredgings, sewage sludges
- synthetic membranes and geotextiles

- concrete, asphalt *etc*.

Requirements can include low permeability, specific drainage characteristics, filtering abilities and the ability to support vegetation.

The advantages of containment systems are that a wide range of contaminants can be isolated and they can be economical where large volumes of materials are involved. However contaminants remain on site, and there is limited understanding of the long-term integrity of containment systems and long term monitoring is often necessary.

1.5.2 Physical processes

Physical processes involve making use of the physical characteristics of the contaminant and the soil; *e.g.* the majority of inorganic contaminants are often present in the fine fraction of the soil, so the soil can be sieved in order to remove the contaminants.

Soil washing transfers the contaminant from solid to aqueous phase, which can then be treated as wastewater. Soils are firstly sieved and each fraction analyzed as each fraction will require remediation to a different extent. The oversize fraction (> 5 mm) is removed mechanically and sands are removed using hydrocyclone combinations (63 μm < x < 5 mm) followed by treatment (*e.g.* by froth flotation) before being returned to site. The fines (< 63 μm) are then subjected to further treatment, thickened and pressed into

45-55 % dry solids filter cake, which should contain the target contaminants and is therefore disposed of off-site, depending on regulations. Several case studies are provided along with an in depth description of this process by Mann[20].

Other physical techniques used in the removal of contaminants from soils include High Intensity Magnetic Separation (HIMS)[21], *in situ* electrodes, from which metals can be recovered, and extraction of volatile gas phase components by use of vacuum[17].

1.5.3 Chemical Processes

Chemical processes involve the addition of chemical reagents to the soil that will either remove the contaminant, immobilise it, convert it to a less harmful form or in the case of some organic compounds destroy the compound completely. Chemical treatment is based on a variety of processes[19]:

- Oxidation-reduction; redox reactions may be applied in order to achieve a reduction in toxicity or a change in solubility. Oxidizing agents include oxygen, ozone, ozone and ultraviolet light, hydrogen peroxide, chlorine gas and various chlorine compounds. Reducing agents such as aluminium, sodium, and zinc metals, alkaline polyethylene glycols and some specific iron compounds may be used.
- Dechlorination; volatile halogenated hydrocarbons, polychlorinated biphenyls and organochlorine pesticides can be treated with reducing agents to remove chlorine and leave less hazardous compounds.

- pH adjustment; a weakly basic or acidic material may be applied to the soil to adjust the pH to acceptable levels. pH adjustment can also be used to affect the mobility or availability of the contaminant, for example, increasing pH from acidic to more alkaline levels will tend to make most metals less mobile.
- Chelant extraction; chelating agents may be used in soil flushing technologies to aid in the removal of metals. There are many examples of the use of leaching solutions. These techniques rely on increasing the solubilty of the metal by complexation with a ligand. The complexes can then be flushed from the soil, and ideally, recovered from the solution. Many groups have investigated the use of chelating agents to extract metals from soils. The most commonly used agent studied in the literature is EDTA, which has been used to extract lead[22,23,24,25] copper[23], zinc[23,25] and cadmium[21,22] from soil. However, before a process using EDTA as a chelating agent can be introduced a recovery process must be developed to reclaim the spent EDTA, in order to make the process economically feasible[26]. Chelate assisted extraction will be discussed further in Chapter 5.

Chemical remediation of land may also involve immobilisation or stabilisation of metals. Groundcover can then be restored due to the lowered toxicity of the soil to plants. Boisson *et al.*[27] concentrated on the immobilisation of metals by application of hydroxyapatite (HA) to soils. Addition of HA to the soils studied resulted in a decrease in the exchangeable Zn, Pb, Cu and Cd recovered from the soils. Growth of maize and bean was improved with the best growth responses after addition of 1 % HA. However,

immobilisation of heavy metal contaminants was also accompanied by immobilisation of nutrients such as Mn, and deficiencies were observed. Arsenic uptake was increased after HA application. This is likely to be due to the similarity between AsO_4^{3-} and PO_4^{3-}, leading to competition for the sorption sites on the soil.

Brown coal has also been suggested as a sorbent for heavy metals in polluted soils. Karczewska *et al.*[28] used soil columns to estimate the sorption capacity of brown coal and to investigate its protective effects. When mixed with limestone, brown coal was shown to be effective in immobilizing Pb and Cu from their nitrate solutions and it was concluded that a layer of brown coal mixed with limestone would provide protection for a soil likely to be subject to Pb and Cu contamination.

Ma and Rao[29] investigated Florida phosphate rocks as immobilizing agents. Phosphate rocks were found to be capable of immobilizing Pb from 13 contaminated soils. However, effectiveness was dependant upon soil pH and the extent of the Pb contamination. The following mechanisms were suggested:

Dissolution

$$Ca_{9.5}(PO_4)_5CO_3FOH + 10H^+ \rightarrow 9.5Ca^{2+} + 5H_2PO_4^- + CO_3^{2-} + F^- + OH^-$$

Precipitation

$$9.5Pb^{2+} + 5H_2PO_4^- + CO_3^{2-} + F^- + OH^- \rightarrow Pb_{9.5}(PO_4)_5CO_3FOH + 10H^+$$

Pierzynski[30] gives examples of several methods of remediation *e.g.* P amendment for *in situ* remediation of Pb and Cd contaminated soils, lime addition to reduce Zn phytoavailability, fixing of Cd and Pb using hydrous Fe and Mn oxides. Since the main exposure pathway of concern for Pb is consumption of soil dust by the human child, reduction in soil bioavailability to the child is necessary. Calculation of bioavailability reduction was illustrated through digestive studies.

Although immobilisation by use of pyromorphite has been suggested, Sayer *et al.*[31] showed that certain species of fungi are capable of solubilising pyromorphite ($Pb_5(PO_4)_3Cl$). *Aspergillus niger (A. niger)* was shown to be able to solubilise pyromorphite, producing lead oxalate dihydrate and anhydrous lead oxalate. They suggest that lead oxalate would be thermodynamically unstable in many soils. Conversion of PbOx to pyromorphite and back again is possible over a wide pH range. In the natural environment fungi may preferentially solubilise other phosphate minerals. However, solubilisation of pyromorphite by *A. niger*, as shown by the authors, suggests that pyromorphite may not be as effective at immobilising Pb as previously suggested. In fact, rye grass was shown to be able to take up Pb from soils treated with pyromorphite.

Chemical treatment of soil generally occurs by either soil washing or soil flushing. Soil washing involves removing the soil and mixing it with the reagent. The soil is then separated from the aqueous phase, which can be treated as wastewater. Soil flushing,

however involves applying a flushing fluid to the soil, which will mobilise metals. These can then be recovered by pumping the flushing fluid to the surface, where it can be treated. A typical soil flushing system is shown in figure 1.4.

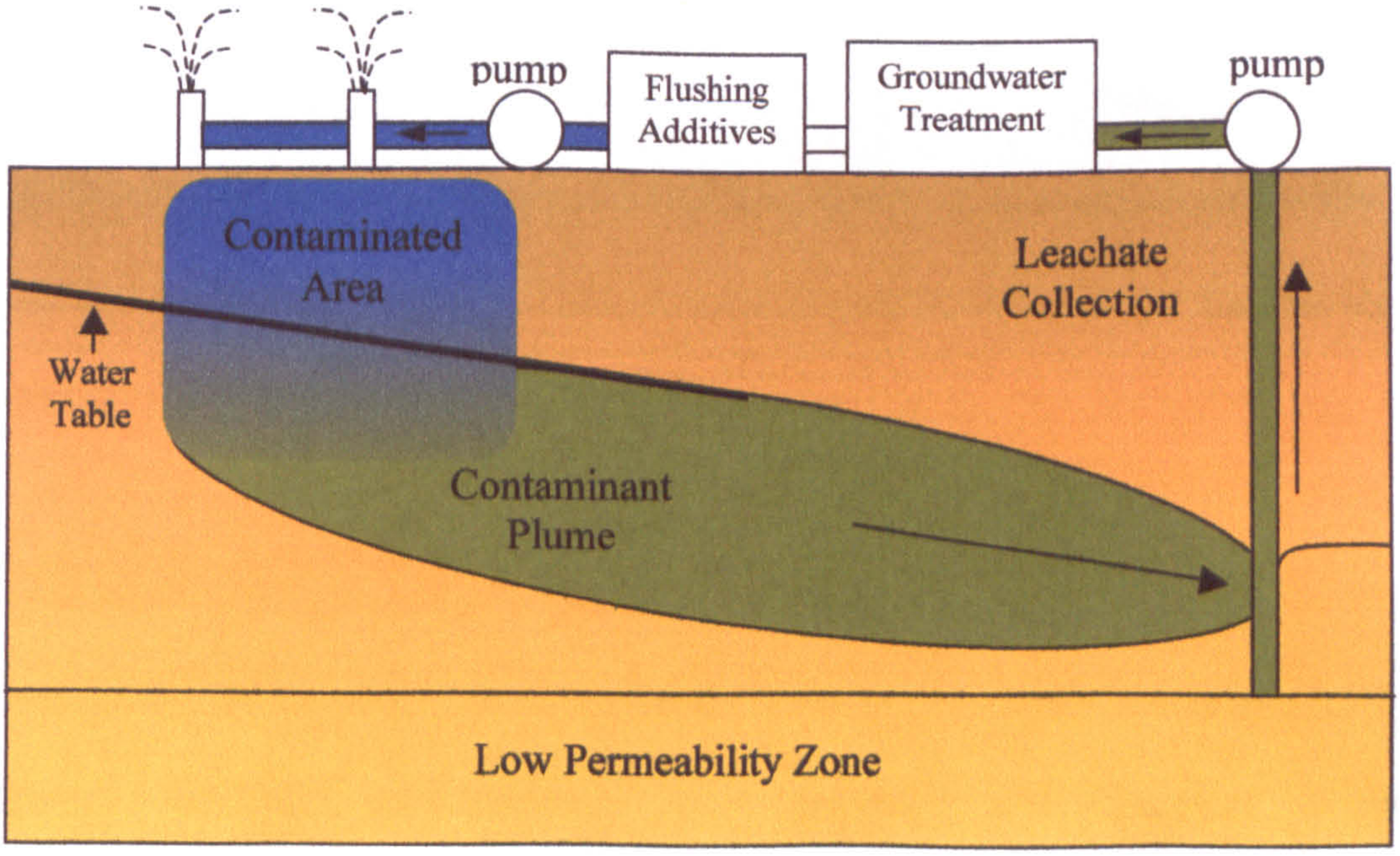


Figure 1.4 Typical soil flushing system (surface sprinklers)

1.5.4 Biological Processes

Biological processes are more frequently used in cases where the contaminants are organic compounds. Bacteria can be used to break down complexes to water and carbon dioxide. Some complex inorganic complexes can also be broken down into carbon dioxide, water and simple inorganic compounds. It is important in these cases to be able to optimise conditions in order that a microbial population can survive within the soil. Although these techniques may often be cost effective, they can be limited by the presence of pesticides and heavy metals.

Remediation using plants (phytoremediation) is becoming increasingly popular. These methods tend to decrease the cost of remediation and can be carried out on-site with less disturbance of the contamination than physical and chemical methods. Phytoremediation will be discussed further in chapter 6.

1.6 Aims of project

The overall aims of the project were to assess various remediation methods with respect to heavy metal contaminated land. In order to do this the modified BCR sequential extration scheme was assessed in order that the effect of remediation on the mobility of metals within the soil could be investigated. The project therefore involved:

- The comparison of the original and modified BCR sequential extraction procedures

- The investigation of the effect of EDTA soil flushing as a remediation technique
- The investigation of phytoremediationa and chelate assisted phytoremediation

Chapter 3 describes in detail the samples analysed throughout the project. Several soils were subjected to the original and modified BCR sequential extraction schemes in order to assess any differences or improvements caused by the modifications suggested to the BCR scheme. This work is detailed in chapter 4. Chapter 5 describes the effect of column leaching with EDTA on the soil and the mobility of the metals. The use of dandelions to accumulate metals from the soil was investigated in chapter 6. The use of EDTA to aid this accumulation was also investigated in this chapter.

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Chapter 2 Instrumentation and general methodology

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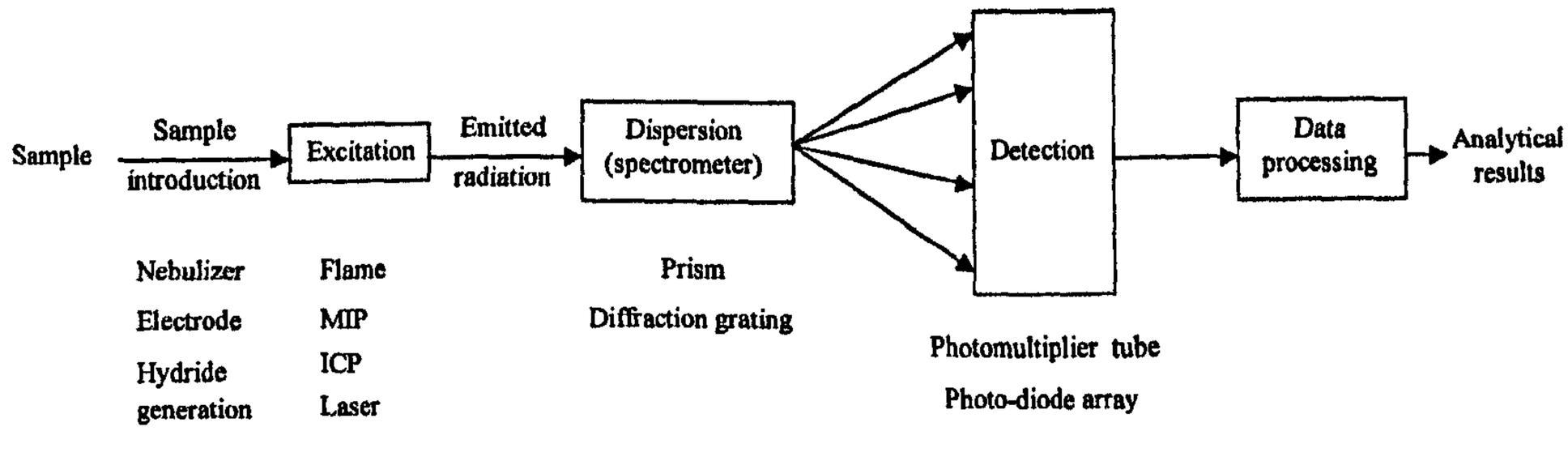
Chapter 2 Instrumentation and general methodology

The main analytical technique used in the analysis of sample extracts and digests was inductively coupled plasma atomic emission spectrometry. Due to its low detection limits and variety of excitation sources available, atomic emission spectroscopy (AES) is the most universally used method for multi-elemen analysis. The sample may be in the form of a solid, liquid or gas, which is subjected to some form of excitation. The emitted radiation is then dispersed and detected after which the data can be processed to give analytical results. A plasma is often used due to its high temperature, which can excite many atom and ion lines simultaneously, enabling multi-element analysis with few chemical interferences.

2.1 Theory of inductively coupled plasma atomic emission spectroscopy (ICP AES)

A solid sample may first be digested to produce a liquid sample, which can be introduced to the instrument by nebulisation, forming an aerosol, which is then desolvated to form particles. Alternatively the solid sample may be introduced to the plasma directly by several means, e.g. laser ablation, vaporisation of microsamples from a graphite furnace, slurry introduction. The particles are then vaporised to form molecules, which are dissociated and the subsequent atoms may be excited or ionised. The excited atoms or ions then relax emitting photons of characteristic wavelengths, which are selected using a grating or prism and detected by the photomultiplier tube or photo-diode array. A summary of the processes involved in AES is shown in figure 2.1.

Figure 2.1 Summary of the processes involved in AES[1]



Inductively Coupled Plasma Atomic Emission Spectroscopy (ICPAES) uses a high temperature plasma to vaporise and excite the determinant particles.

2.1.1 Atomic Emission

The electrons in an atom may be excited in a high temperature environment to higher energy levels. Excitation mechanisms involve mainly collisional processes. Electron collisions (e_1^-) transfer the kinetic energy of the electron to the atom (A) releasing another electron (e_2^-). Collisions with molecules (XY^*) transfer their rotational or vibrational energy to the atom, and collision with metastable species (Ar_m^*, usually a triplet state which cannot relax to ground state by emission of radiation), transfers energy from the species enabling it to relax to ground state.

$$A + e_1^- \Leftrightarrow A^* + e_2^-$$

$$A + XY^* \Leftrightarrow A^* + XY$$

$$A + Ar_m^* \Leftrightarrow A^{+\bullet} + e^- + Ar$$

Further levels in the excited A^{+*} are then populated by cascade effect. Other excitation mechanisms involve transfer of energy produced by chemical (mainly found in flame excitation processes) and excitation by absorption of radiation (i.e. the reverse of the emission process).

The excited electrons then relax to a lwer energy. This relaxation is accompanied by emission of electromagnetic radiation of characteristic wavelength. The radiation emitted by atoms is related to the difference between energy levels.

$$\Delta E = h\nu = \frac{hc}{\lambda}$$

Where ΔE is the difference between energy levels, h is Plancks constant, c is the speed of light and λ the wavelength of emitted light. The power emitted, P (J), is dependent on the volume of the excitation source, V (m^3), and the population of the upper energy level, N_2 (m^{-3}).

$$P = N_2 A_{1,2} \frac{hc}{\lambda} V$$

Where $A_{1,2}$ (s^{-1}) is the transition probability. V is dependent on the instrument design and N_2 is dependent on the energy of the system. If a source is in thermal equilibrium the partitioning of energy across different species can be described by the Maxwell-Boltzmann equation:

$$\frac{N_2}{N_1} = \frac{g_2}{g_1}\exp\left(-\frac{\Delta E}{kT}\right)$$

Where k is the Boltzmann constant and g_1 and g_2 are statistical weighting factors. If local thermal equilibrium does not apply to a system (as in a plasma, which is suprathermal), different values of temperature exist for different energy partitioning. Kinetic temperature (T_{kin}) determines the kinetic energy distribution for atoms, ions and molecules. The electron temperature (T_{e-}) determines the kinetic distribution for electrons. Electronic excitation temperature (T_{excit}) determines the population of energy levels and ionisation temperature (T_{ion}) describes the degree of ionisation of an atom or ion. The radiation temperature (T_{rad}) relates the distribution of intensity at different wavelengths for a black body radiator at a given temperature. In the plasma system $T_{excit} > T_{kin}$. When systems are in local thermal equilibrium $T_{kin}=T_{e-}=T_{excit}=T_{ion}=T_{rad}$.

2.1.2 Nebulisation

Generally the sample to be introduced to the ICP AES is in a liquid form. The liquid is introduced to the plasma as an aerosol and therefore must be nebulised before introduction. Several nebulisers are available as sample introduction devices. The most common nebulisers are pneumatic. Three examples of pneumatic nebulisers include the Babington-type v-groove nebuliser (figure 2.2), concentric nebuliser and the cross-flow nebuliser. The Babington-type nebuliser works on the principal of passing the sample over a narrow v-groove. The nebuliser gas enters the groove perpendicular to the direction of sample flow, rupturing the film of sample and

forming an aerosol. The major advantage of this type of nebuliser is that there is little possibility of the nebuliser becoming blocked due to salting-up, when solutions with high dissolved solids are nebulised, as the solution is not required to pass though a narrow aperture.

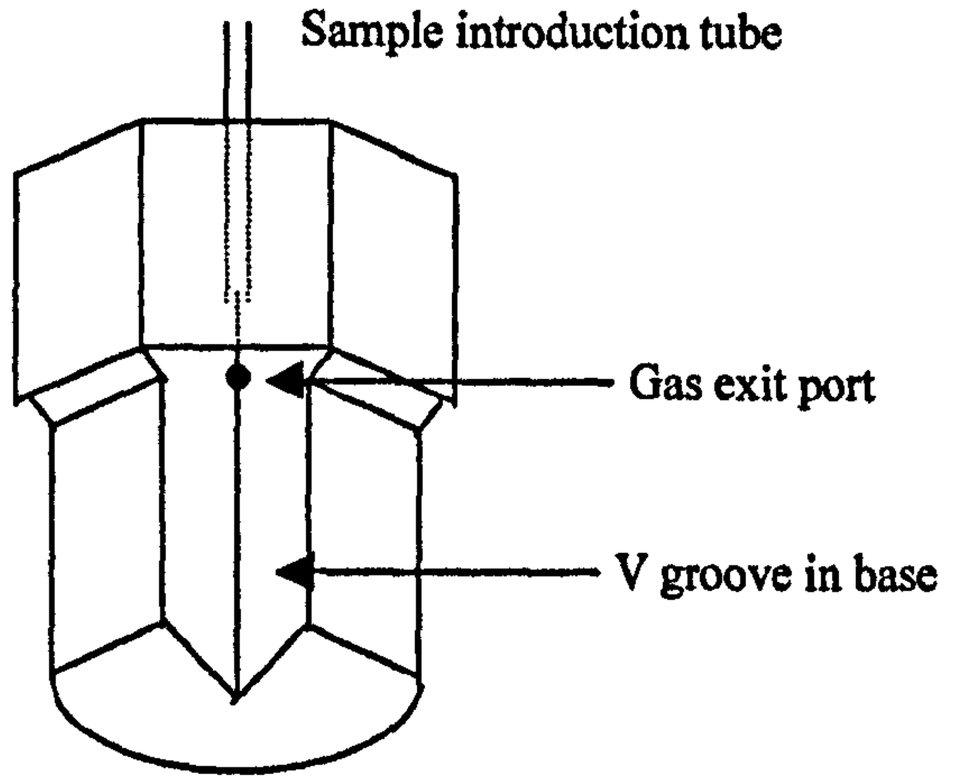


Figure 2.2 Babington-type nebuliser[2]

Concentric nebulisers use a high gas velocity at the end of a fine capillary tube to produce a fine reproducible spray. The cross-flow nebuliser combines the gas flow and sample at right angles to form the aerosol. Ultrasonic nebulisers use vibrations at ultrasonic frequencies (50 kHz - 4 MHz) to break up sample droplets into smaller particles, which are transported to the plasma. The ultrasonic nebuliser can often be useful where increased sensitivity is necessary. Detection limits obtained by ultrasonic nebulisation can be increased by a factor of 10 relative to those using pneumatic nebulisation, due to the high efficiency of fine droplet generation. The aerosol from the nebuliser passes into the spray chamber where larger droplets are

drained away and only fine aerosols pass into the plasma. This is important to ensure that the liquid does not extinguish the plasma and that atoms are properly desolvated.

2.1.3 Inductively Coupled Plasma (ICP)

The ICP torch consists of three concentric quartz tubes, encircled by an induction coil. The central tube carries the nebuliser gas and sample and the outer tube carries the plasma gas which is also used to cool the walls of the torch. The auxiliary gas is carried through the remaining tube and is used to control the height of the plasma above the torch. Argon gas flows through the torch and radio frequency energy is applied to the induction coil. A magnetic field develops around the coil, due to the radio frequency energy. A spark creates seed electrons and ions, which flow in a circular path perpendicular to the magnetic field, as shown in figure 2.2. The argon ions are accelerated and produce more ions and electrons by further collisions. This process and also the recombination of argon ions with each other and with electrons, produces intense heat and light, and the plasma is formed.

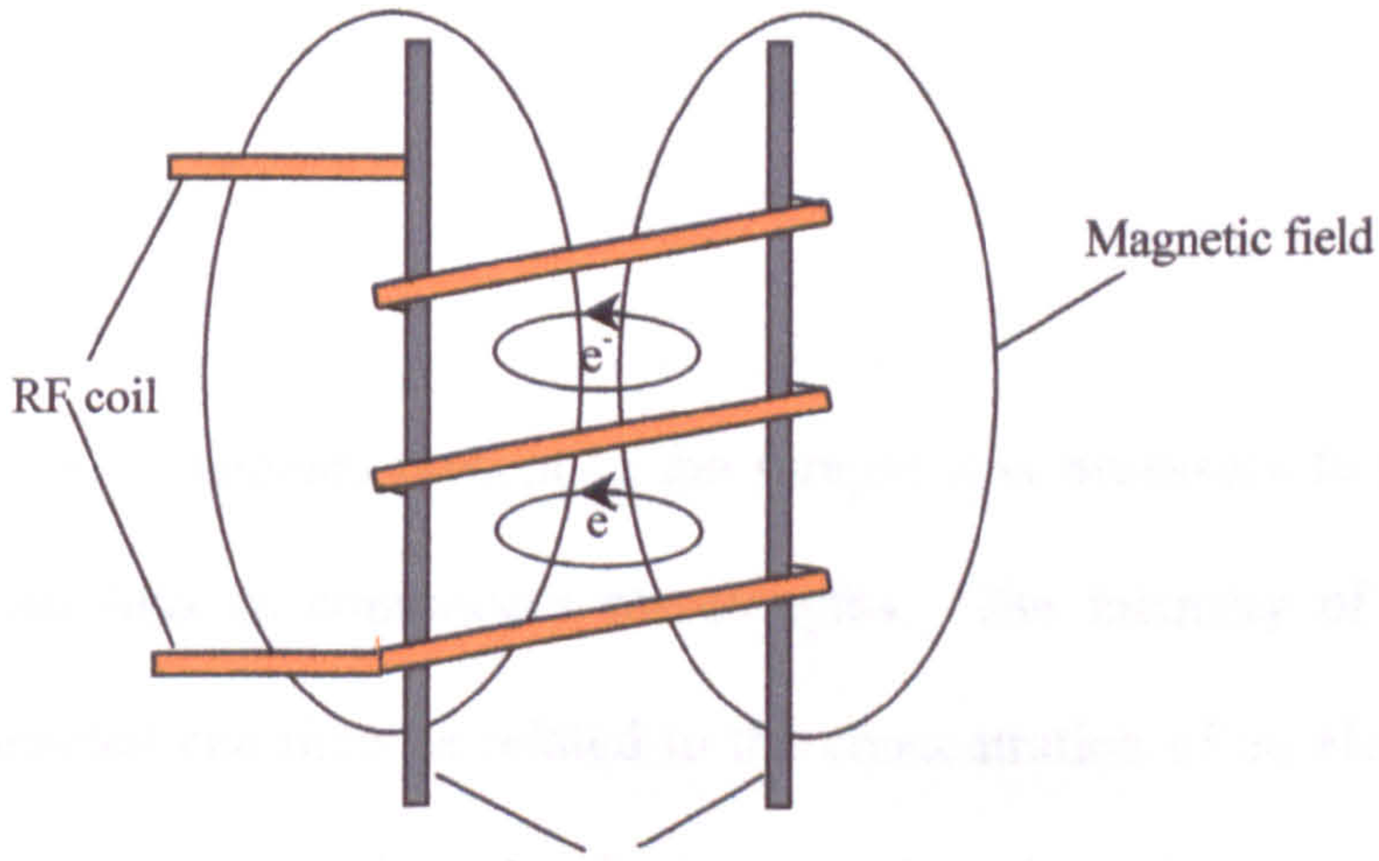


Figure 2.2 The RF coil around the ICP torch[3]

Collisional processes in the plasma, chemical reaction, or absorption of radiation excite determininant atoms. The torch and plasma are shown in figure 2.3.

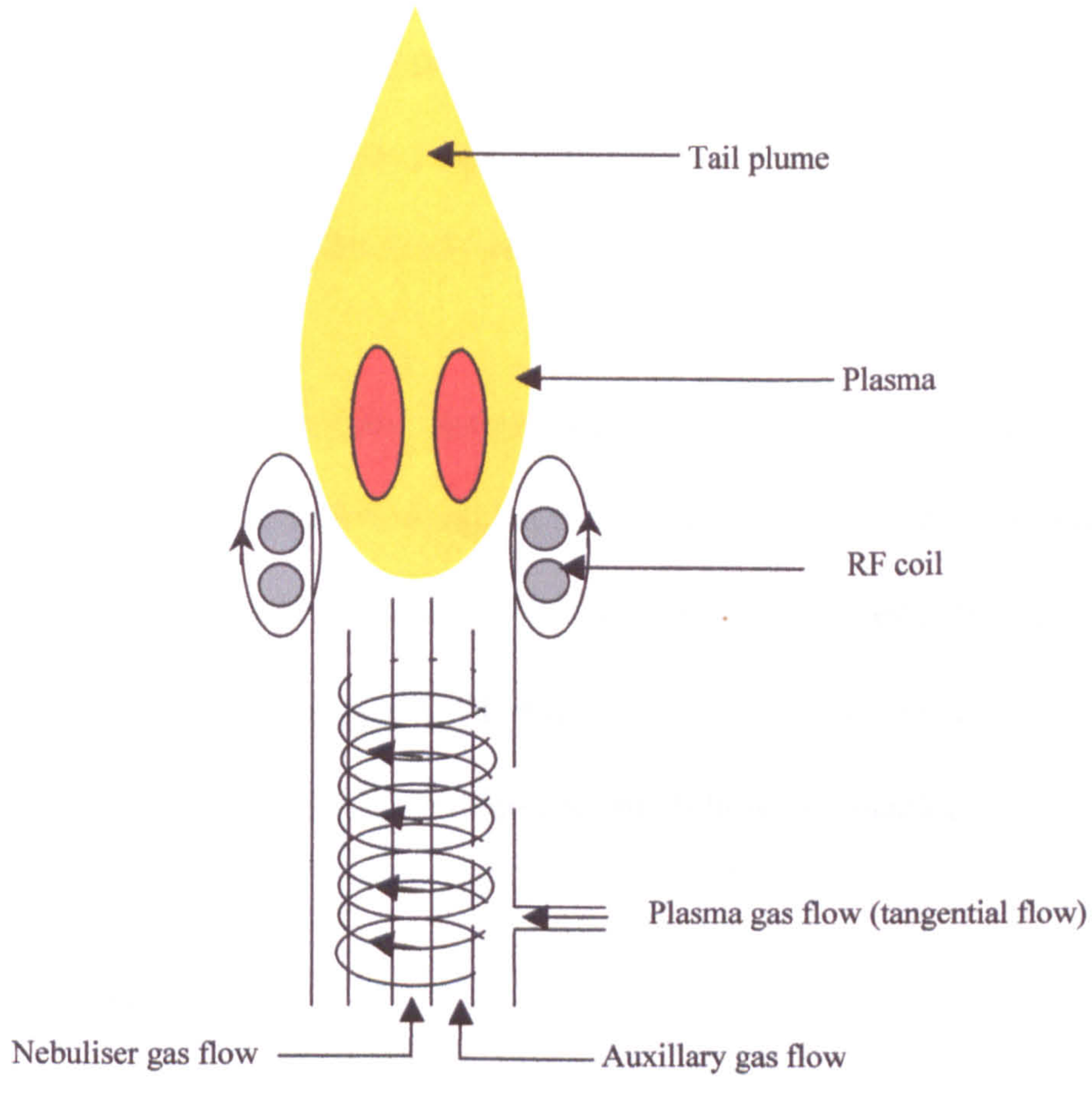


Figure 2.3 The RF coil around the ICP torch[4]

2.1.4 Spectrometers[5,6]

In order to identify elements present in the sample it is necessary to split the light from the plasma into its component wavelengths. The intensity of light at each wavelength detected can then be related to the concentration of an element present. Spectrometers most commonly use prisms or gratings in order to resolve the light

from the plasma. Light is then detected by photomultiplier tubes (PMTs), photodiode arrays (PDAs), or charge coupled devices (CCDs).

Light passing through a prism is refracted from its original path. Higher frequency radiation is refracted to a greater extent than low frequencies, resulting in separation of the beam of light to form the spectrum.

A diffraction grating separates polychromatic radiation by diffracting light of different wavelengths at different angles, resulting in a spectrum. The diffraction angle (β, °) is dependent on the diffraction order (m, an integer), the distance between the grooves in the grating (d, mm), the angle of incidence (α, °), and the wavelength (λ, nm). The above are related by the following equation:

$$m\lambda = d(\sin\alpha + \sin\beta)$$

The photomultiplier tube consists of a photocathode, several dynodes and an anode. When light strikes the surface of the photocathode electrons are released. Each electron ejected from the photocathode passes to the next electrode releasing two or more electrons that pass on to further electrodes and are eventually collected by the anode. The result is the amplification of the original signal.

Diode array detectors are similar to PMTs, however, they are much smaller and not as expensive. These are often used in simultaneous spectrometers where arrays of detectors are used to collect the spectrum.

CCDs are solid state detectors in two dimensions consisting of arrays of pixels. Each pixel discharges a current proportional to the energy striking it. The two dimensional array makes the CCD detector ideal for simultaneous multielement analysis, as the wavelengths are not pre-defined.

Prisms and gratings can be combined with detectors to form either sequential or simultaneous spectrometers. Sequential spectrometers can rotate the prism or grating to detect radiation of different wavelengths. The wavelengths detected are therefore dependent on the step size of the motor used to rotate the prism or grating. Simultaneous instruments use the prism or grating with either an array of PMTs or photodiodes, or with a CCD. Wavelengths detected by PMTs or PDAs are restricted to the number of detectors available, however with CCD's the entire spectrum can be imaged simultaneously.

2.1.5 Interferences in ICP AES systems

Due to the intense heat produced by the plasma very few chemical interferences are present. However, many lines are produced by the plasma and the user must take care to avoid spectral interferences. For example spectral interference of magnesium 309.300 nm may occur on the determinant aluminium when using the aluminium line at 309.284 nm[7].

Coetzee *et al.* suspected spectral interferences on some ICP AES lines used during an evaluation of sequential extraction procedures. In order to minimise interferences by iron, aluminium and calcium, which could typically be present in the sample extracts they checked for these interferences with 1000 mg l^{-1} solutions of Specpure (supplier) standards[8]. However, negligible interferences were observed at the wavelengths selected, shown in table 2.1.

Table 2.1 Interferences suspected in ICP AES measurements of zinc, lead and copper

Element	Wavelength (nm)	Interference
Zn	213.86	Ni
Pb	220.35	Al
Cu	324.75	Fe

Interferences may either enhance or diminish the intensity of the determininant signal. Direct spectral overlap or partial overlap of spectral lines at similar wavelengths will enhance the determininant signal, however some elements emit continuum radiation over a wide wavelength range. In this case the observed background will appear higher than the background without interference, and the observed peak will be diminished in comparison to the same peak free from interference.

2.2 Instrumental Conditions

Two instruments were used for the work carried out in this thesis. A Perkin Elmer Plasma II emission spectrometer (Perkin Elmer, Norwalk, Conneticut, USA) was used for determination of metals in the samples described in chapter 3, with the

exception of White Cart A and C and Great Billing A and C samples. The nebuliser used was a cross-flow type, the monochromator was of the Ebert design (1 m focal length) and was equipped with a grating with a surface area of 84 x 84 mm and 1800 line/mm. This gave a wavelength range of 160 800 nm and a resolution of less than 0.018 nm. The photomultiplier used was a Hamahtsu R787.

An RF power of 1.2 kW was used for the PE Plasma II. Nebuliser, auxillary and plasma gas flows were 1.10 l min^{-1}, 1.0 l min^{-1} and 15 l min^{-1} respectively. The sample was pumped at a rate of 1.3 ml min^{-1} and an equilibrium time of 40 s was allowed. The following wavelengths were used in the analysis of samples with the PE Plasma II ICP AES.

Table 2.2 Specific element conditions for ICP AES

Element	Wavelength (nm)	Viewing height (mm)
Cu	324.754 (I)	15
Fe	238.204 (II)	15
Pb	220.353 (II)	15
Mn	257.610 (II)	15
Ni	231.604 (II)	15
Zn	213.856 (I)	15

(I) = atom lines, (II) = ion lines

After failure of the PE Plasma II the remaining work was carried out using a Varian Liberty 220 ICP AES (Varian Techtron Pty, Limited, Mulgrave, Victoria, Australia). A schematic of the Liberty is shown in figure 2.4. The Liberty employs a v-groove nebuliser and an inert double pass Sturman-Masters spraychamber. A solid state crystal controlled 40.68 MHz generator was used to provide the RF power. The spectrometer was a sequential scanning spectrometer, using a 0.75 m focal length

Czerny-Turner monochromator with a holographic grating (1800 grooves/mm) and two PMTs. An R166 UH solar-blind PMT was used below 300 nm, rejecting stray light and improving detection at lower wavelengths. Above 300 nm an R928 trialkyl wide range PMT was used. Typical ranges and resolution for grating orders are given in table 2.2.

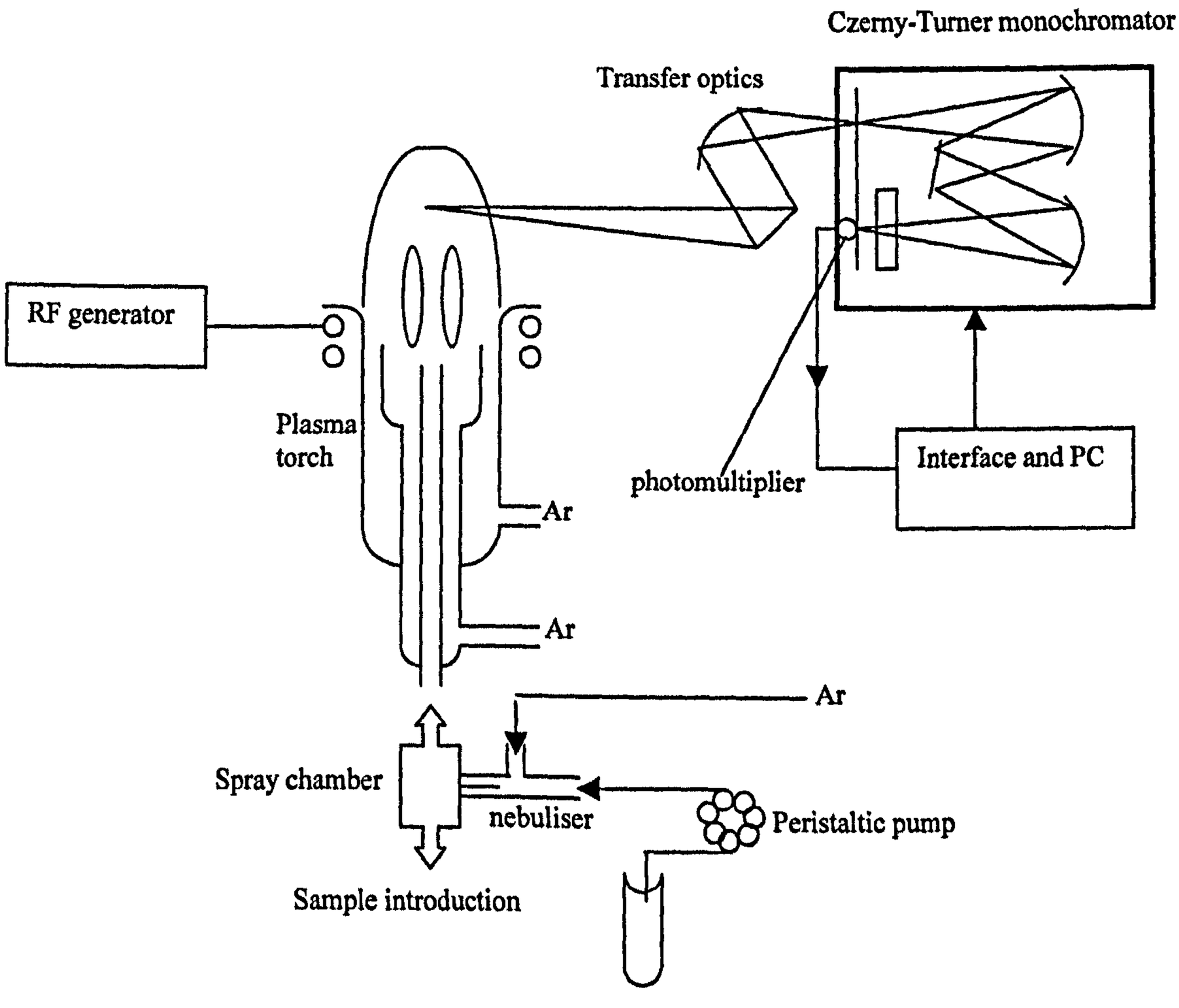


Figure 2.4 Schematic representation of Varian Liberty[9]

Table 2.3 Typical spectrometer wavelength ranges and resolution[9]

Order	Range (nm)	Typical resolution (nm)
1st	160 – 900	0.018
2nd	160 – 450	0.009
3rd	160 – 300	0.007
4th	160 – 225	0.006

In order to detect any likely spectral interference present two emission lines were measured for each element, for the first experiments. If no significant matrix effects were noted any differences between the results from the two lines would suggest a spectral interference. Measurement of a third line or addition of a spike to the sample could be used to further investigate the interference. Analysis conditions are given in table 2.4. After preliminary experiments, only one line was used for each element. The line used is shown in bold in the table and was selected based on sensitivity and lack of interference observed in the spectra.

Table 2.4 ICP AES conditions used for the Varian Liberty

Element	Line 1 (nm)	order	Line 2 (nm)	order	PMT voltage (V)
Ca	**396.847 (II)**	1	422.673 (I)	1	550/750*
Cu	324.754 (I)	2	**327.296 (I)**	1	750
Fe	**259.940 (II)**	2	261.187 (II)	2	750
Pb	**220.353 (II)**	3	261.418 (I)	2	750
Mn	**257.610 (II)**	2	260.569 (II)	2	700/750*
Ni	**221.647 (II)**	3			750
Zn	**206.200 (II)**	3	213.856 (I)	3	750

(I) = atom lines, (II) = ion lines

* The PMT voltage was reduced for samples with high Ca content to reduce sensitivity, so that the detector was not overloaded.

Due to the range of atom and ion lines measured intermediate plasma operating conditions were used *i.e.* the power used was 1.00 kW. Plasma and auxiliary argon were supplied at 15.0 and 1.50 L min^{-1}, respectively. Nebuliser gas was supplied at 150 kPa. A viewing height of 5 mm was used for all lines, with an integration time of 3 s for each of 3 replicates. Dynamic background correction was used for all lines. The PMT voltage for Ca 396.847 nm and Mn 257.610 nm was decreased in order to decrease the sensitivity of the measurement for these elements.

2.3 Calibration and Validation

One replicate of each sample type was analysed against a single point calibration in order to determine the calibration range to prepare. Five matrix-matched, multi-element standards were then prepared by serial dilution of 1000 mg l^{-1} Spectrosol standards (Merck, Poole, UK) for the PE Plasma II work, or 1000 mg l^{-1} Romil Prim*Ag* metal reference solutions (Romil, Waterbeach, Cambridge, UK) for the Liberty work. The matrix matched standards were then used to calibrate the ICP AES instrument. Calibration standards were also used to check for wavelength drift throughout each analysis. The sensitivity and limits of detection of the lines are shown in tables 2.5 and 2.6 for the PE Plasma II and the Varian Liberty. Sensitivity is defined as the slope of the calibration curve and the limits of detection (LoD) as three times the standard deviation of the blank (n=10) divided by the sensitivity. Variations in sensitivity of the lines are likely to be due to salt content of the matrix, and physical characteristics of the matrix (e.g. viscosity). Sensitivity (and therefore

LoD) can also change with time due to, for example salt deposits building up on the torch.

Some samples contained exceptionally high levels of metal (e.g. calcium and iron) and overloaded the PMT. These samples were diluted and the high concentration elements determined separately.

Table 2.5 Sensitivity and LoD (mg l^{-1}) for elements in matrices used for PE Plasma II ICP AES

	Cu	Fe	Pb	Mn	Ni	Zn
Step 1						
Sensitivity	28900	9500	638	33200	1220	14200
LoD	0.004	0.009	0.06	0.001	0.02	0.003
Step 2 A						
Sensitivity	25200	8030	238	26600	1000	12300
LoD	0.007	0.1	0.2	0.002	0.03	0.003
Step 2 B/C'						
Sensitivity	25200	7850	235	25200	959	11400
LoD	0.005	0.05	0.2	0.003	0.02	0.003
Step 3						
Sensitivity	25300	7930	497	29200	1040	11300
LoD	0.006	0.02	0.09	0.002	0.02	0.002
Aqua Regia						
Sensitivity	32600	7380	6200	29700	1070	11400
LoD	0.005	0.01	0.09	0.002	0.03	0.004

Table 2.6 Sensitivity and LoD (mg l^{-1}) for elements in matrices used for Varian LibertyICP AES

	Ca	Cu	Fe	Pb	Mn	Ni	Zn
Step 1							
Sensitivity	1670	10300	27200	1600	121000	7270	4580
LoD	0.011	0.00058	0.0017	0.0075	0.00035	0.0033	0.0059
Step 2							
Sensitivity	1520	8220	23600	1490	107000	6530	4090
LoD	0.0039	0.0018	0.0014	0.010	0.00045	0.0028	0.0029
Step 3							
Sensitivity		7670		581	78600	3760	2760
LoD		0.0043		0.010	0.00083	0.0048	0.0087
Aqua Regia							
Sensitivity		8090		1530	111000	6610	4060
LoD		0.0015		0.014	0.00092	0.00091	0.00074
1% HNO_3							
Sensitivity	1630		25500				
LoD	0.0074		0.020				
0.05M EDTA							
Sensitivity	42600	32200	84000	2980	241000		7510
LoD	0.044	0.018	0.00089	0.045	0.0010		0.156

Calcium and iron levels in step 3 and aqua regia samples were greater than the PMT detector range and so were diluted in 1 % HNO_3. Nickel levels were not determined in the 0.05M EDTA, as they were too low to be of interest.

Spikes approximately equivalent to the metal concentration present in the sample were added to four samples from each run in order to check for interferences due to matrix effects. Examples of the recoveries achieved from the analysis of spiked samples are given in table 2.7. In general spike results were in the range 95 – 105 % and results were not corrected for interferences.

Table 2.7 Examples of recoveries from spiked samples in the extract matrices

	Step 1	Step 2	Step 3	Step 4
Cu	99	88	96	101
Fe	94	*	*	*
Mn	101	94	97	103
Ni	100	98	97	99
Pb	96	93	92	100
Zn	101	99	88	100

* Iron was present at high levels in these samples, which were diluted. This would also dilute any interference from other elements present in the samples.

Step 1 = extracts in 0.11 M acetic acid
Step 2 = extracts in 0.10 M or 0.50 M hydroxyammonium chloride
Step 3 = extracts in 1.0 M ammonium acetate
Step 4 = extracts in 20 % *aqua regia*

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Chapter 3 Substrates investigated

Chapter 3 Substrates Investigated

3.1 Introduction

Eight different substrates were used in the course of the study: one sewage-sludge amended soil; one freshwater (riverine) sediment; one intertidal sediment; three distinct layers of industrial made ground obtained from a trial pit, and two further top soils taken from an industrial location. Of these, the first six were obtained by previous workers whilst the latter two were sampled during the course of the current project. All samples were coned and quartered prior to use.

3.2 Great Billing

Sewage sludge amended soil was collected from Great Billing sewage farm in Northamptonshire, UK. The soil was to be studied as a BCR candidate reference material. Later, a separate portion of this became BCR CRM 143R. The sample was air dried and sieved, using a 1 mm mesh nylon sieve.

3.3 White Cart

Samples of sediment were obtained from the White Cart, a tributary of the River Clyde, close to Glasgow, UK. The sampling site was approximately 2 km downstream from Paisley sewage treatment works[1]. Samples were air dried and sieved as described above.

Chadwick reported the water content and loss on ignition (LOI) to be 1.81 and 3.12 % respectively[1].

Chadwick also carried out particle size distribution on samples obtained during a later sampling trip[1]. The particle size distribution is given in table 3.1.

Table 3.1 Particle size distribution in White Cart sediment

Particle size (μm)	Distribution (%)
25-1000	48
63-250	44
<63	8

3.4 Whitehaven

Sediment samples were obtained from the inter-tidal zone of Whitehaven Harbour, Cumbria, UK, near a phosphate ore processing plant (Albright and Wilson). Once again the soil was air dried and sieved (< 2 mm) prior to any analysis. The sample was originally found to contain 6.4 % moisture[2].

3.5 IG 18-28, IG 35-45 and IG 47-50

Industrial made ground was sampled from three, visually distinct, layers in a trial pit, at a former industrial site in NW England. The layers were obtained from 18-28 cm depth, 35-45 cm depth and 47-50 cm depth. The portions of the samples that were used in the current project had been stored in field moist conditions at room temperature.

3.5.1 Sample description

IG 18-28 was a well defined, dark grey matrix with yellow and grey pockets, suspected to be ash. The material was observed to be mainly composed of clinker and fine ash. Layer 35-45 was observed to have a much coarser texture than other layers in the trial pit, due to the higher proportion of clinker found in this layer. The 47-50 cm sample was found to be almost clinker free and contained largely fine ash. The material was thought to be sufficiently compact to prevent downward percolation of water (Duncan[3]).

3.5.2 Physical properties

Previous workers originally determined soil characteristics. Organics, pH and particle size distribution were determined and are illustrated in table 3.2 for layers 18-28, 35-45 and 47-50 cm of the trial pit. The industrial ground samples were found to have much higher fractions of larger particles than the White Cart sample.

Table 3.2 Physical characteristics of industrial ground

Layer (cm)	pH	Organics (%)	Sand (%)	Silt (%)	Clay (%)
18-28	5.3	20	76	22	2.0
35-45	5.7	22	87	11	2.0
47-50	6.9	27	72	22	6.2

3.6 Ardeer Location 1

Samples of soil from an industrial site at Ardeer were taken on 15th November 2000. The samples were stored as field moist soils. Soil was coned and quartered prior to sequential extraction, column and plant experiments. The soil was air dried and sieved.

3.6.1 Sample description

A layer of approximately 10 cm of soil (70 cm by 70 cm) was removed from site 1. No vegetation was found growing on the soil, which consisted of 5 distinct layers. The first and third layers were black and weathered. The second layer consisted of a fine red material. The fourth layer was a mixture of yellow and grey ash like material, and the final layer was composed of ash and clinker. The sample site is shown in figure 3.1.

Figure 3.1 Ardeer location 1

3.7 Ardeer Location 2

Another sample of soil from the industrial site at Ardeer was taken. The sample was again stored in field moist condition prior to use or analysis, but was air dried and sieved when necessary. Figure 3.2 shows location 2.

3.7.1 Sample description

Two samples approximately 20 cm deep (50 cm by 50 cm) were combined to make the second Ardeer sample. Both samples areas were covered in vegetation with abundant roots throughout the soil. The presence of earthworms was noted in these sites. Figure 3.2 shows one of the Ardeer location 2 sample sites.

Figure 3.2 Ardeer location 2

3.7.2 Physical properties

The Ardeer soils were characterised by colleagues at MLURI[4]. Soil pH in water was found to be 6.2 and in 0.01 M calcium chloride to be 5.3.

The exchangeable cations were also determined at MLURI and are given in table 3.3. The limit of detection was 0.01 meq/100 g for all elements.

Table 3.3 Exchangeable cations (meq/100 g)

Ca	Na	K	Mg	Mn	Fe	Al
4.56	0.38	0.26	2.28	0.04	0.02	0.13

3.8 Moisture content and loss on ignition

The moisture content and loss on ignition value (LOI) of air-dried sample of all the substrates studies were measured, by drying at 105 °C and 500 °C respectively, until constant weight was obtained.

Moisture content = weight loss at 105 °C/sample weight (moist) x 100 %

LOI = weight loss at 500 °C/sample weight (dry) x 100 %

Results are presented in Table 3.4. Moisture content and LOI were determined on air-dried samples of Great Billing, White Cart, Whitehaven and IG layers. Ardeer samples were more recent samples and moisture and LOI was determined on field moist soil.

Table 3.4 Moisture content and LOI of substrates studied

Substrate	Moisture (%)	LOI (%)
Great Billing	3.26	19.7
White Cart	0.58	2.84
Whitehaven	0.89	4.78
IG 18-38	2.00	12.9
IG 47-50	4.28	18.8
Ardeer 1	20	4
Ardeer 2	20	13

As expected, based on observations, the second Ardeer site contained much higher organic matter content than site 1.

Great Billing, Whitehaven, White Cart and samples from two layers (IG 18-28 and 47-50) of a trial pit in an industrially contaminated site were used to assess the original and the modified BCR sequential extraction procedure. Another layer of the trial pit (IG 35-45) and the two Ardeer samples were used to investigate column leaching with EDTA as a remediation technique. Ardeer sample 2 was also used to investigate phytoremediation and chelate assisted phytoremediation.

Storage conditions of the soils must be considered when studying metal behavior. Bartlett and James[5] note that drying of soils can cause many changes to the characteristics of the soil. This can include decreasing the soil pH and solubilizing organic matter. Remoistening soils can require long periods of time to reach field moist conditions. Freezing also causes changes to the soil organic matter. Bartlett and James recommend that soils are stored moist and at 4 °C for long time periods. However in order to take representative samples for the current work soils were required to be dried and sieved. This could therefore effect the results of determinations made.

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Chapter 4 – Comparison of BCR Sequential Extraction Procedures

Chapter 4 – Comparison of BCR Sequential Extraction Procedures

4.1 Introduction

Detailed assessment of contaminated land is necessary before remediation of the land can be carried out, in order to decide upon the most appropriate remediation technique. Various methods of assessment have been discussed in chapter 1. Sequential extraction of metals will be concentrated upon in this chapter. Sequential extraction techniques can aid in the assessment of the likely mobility of a metal under varied conditions. Sequential extraction may also be defined as operational speciation as the process used to isolate the species defines them.

4.1.1 Sequential Extraction

Several sequential extraction schemes have been documented. McLaren and Crawford[1] devised the first sequential extraction scheme in 1973; however the scheme, which is probably used most frequently, was devised by Tessier *et al.* in 1979[2]. In order to harmonize extraction procedures the Community Bureau of Reference (BCR, now Standard, Measurements and Testing Programme) of the European Commission developed a scheme, which has lately been revised as discussed below[3].

The most frequently used Tessier scheme[2] extracts metals associated with five different fractions.

Fraction 1. Exchangeable – changes in water ionic composition are likely to affect sorption-desorption processes.

Fraction 2. Bound to Carbonates – changes in pH may affect metals bound to carbonates in sediments and soils.

Fraction 3. Bound to Iron and Manganese Oxides – these oxides can be broken down under reducing conditions to release trace metals.

Fraction 4. Bound to Organic Matter – such as living organisms, detritus, humic and fulvic acids, can be broken down under oxidising conditions to release trace metals.

Fraction 5. Residual – metals in this fraction would not normally be released into the environment under conditions encountered in nature.

Some examples of other sequential extraction schemes and the references that describe them are given in table 4.1.

4.1.2 BCR Sequential Extraction Scheme

There are many sequential extraction schemes which all give varying results for the amounts of metals bound to each fraction, due to the ways in which they each attack the different phases. In order for laboratories to be able to compare results with those of other workers, the BCR recommended a harmonization of sequential extraction schemes[6]. Trials run by the BCR produced a simple 3-step extraction procedure, evolved from that of Salomons and Förstner[5], summarised in fig. 4.1. Two round-robin exercises were set up using the finalised version of the sequential extraction procedure, the results of which showed that the method was satisfactorily

reproducible between laboratories. These results are presented in a paper by Quevauviller *et al.*[4]. Many aspects of the protocol have been evaluated by Coetzee *et al.*, including total recovery, selectivity of extractants, reproducibility and redistribution of metals[5]. Recoveries of copper, chromium, cadmium, zinc, nickel and lead were found to be close to 100% with reproducibilities of approximately 3%. The selectivity of the reagents was shown to be insufficient in providing definitive information on the specific phase from which the metal was extracted. Redistribution of lead and copper was found to occur in the presence of humic acid. Lead adsorbed onto quartzite was released in step 1 of the extraction but was then available for re-adsorption, possibly by humic acid.

Davidson *et al.*[6] evaluated the application of the BCR sequential extraction procedure to a freshwater sediment collected from the River, Clyde, Lanarkshire, UK. They found the method to be both reproducible and repeatable. However, standard addition procedures were required to overcome interferences in the sample extracts, for analysis by ET AAS.

Once the three-step procedure was set up it was important for the BCR and collaborating laboratories to produce soil and sediment reference materials for certification. Fiedler *et al.*[7] and Thomas *et al.*[8] have investigated the possibilities of producing a stable sediment reference material that would provide repeatable results using the BCR method. They found the BCR scheme sufficiently repeatable for the sediment studied, although care must be taken to ensure that potential matrix

Table 4.1 Examples of some sequential extraction schemes[9]

Exchange-able	Specifically sorbed, carbonate bound	Easily reducible substrates	Easily extractable organics	Moderately reducible oxides	Oxidizable oxides and sulfides	Residual minerals	Reference
$CaCl_2$	HOAc		$K_4P_2O_7$	NH_4Ox/ HOx		HF	McLaren and Crawford (1973)[1]
$MgCl_2$	NaOAc pH 5			$NH_2OH.HCl$/HO Ac	H_2O_2/ NH_4OAc	HF/ $HClO_4$	Tessier *et al.* (1979)[2]
NH_4OAc	NaOAc pH 5 HOAc	$NH_2OH.HCl$ pH 2 $NH_2OH.HCl$ pH 2		NH_4Ox/ HOx	H_2O_2/ NH_4OAc H_2O_2/ NH_4OAc	HNO_3	Salomons and Förstner (1984)[10] Ure *et al.* (1993)[11]

Where Ac = acetate, Ox = oxalate

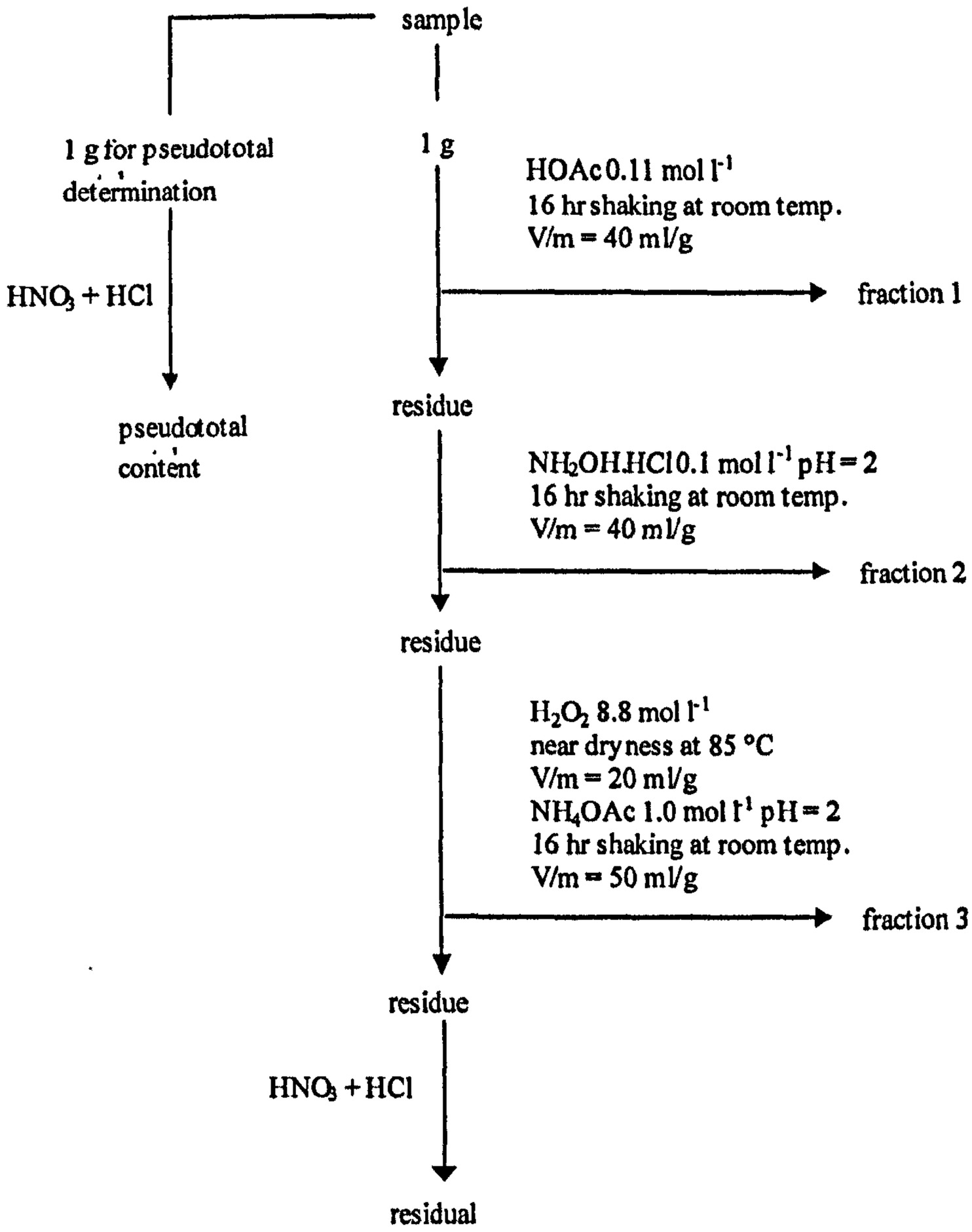

Figure 4.1 Schematic summary of the BCR sequential extraction procedure

interference effects are compensated for. The requirements of certified reference materials are very strict and much care must be taken when preparing them. Quevauviller presented guidelines on the quality control aspects of CRM preparation in 1996[12].

Only one certified reference material exists at present for the BCR sequential extraction scheme (another is at present being studied). CRM 601 is certified for the extractable contents of cadmium, chromium, nickel, lead and zinc, following the standardised BCR procedure. The long-term stability of lake sediment, CRM 601, certified for extractable contents of trace metals using the BCR procedure has been investigated by López-Sánchez *et al.*[13]. They concluded that the extractable contents of the certified metals are stable on a long-term basis (over one year) in the CRM.

Several authors have compared the BCR scheme with other sequential extraction schemes. Pérez-Cid *et al.*[14] compared the BCR method and Tessier scheme for the determination of extractable copper, chromium, lead, nickel and zinc in sewage sludges. Mester *et al.* compared the BCR method with a modified Förstner 5-step procedure[15]. No differences were observed between the two methods for zinc and cadmium, but significant differences were observed for lead, chromium, nickel, and copper, particularly in the oxidisable fraction (most likely due to redistribution of these metals between phases).

4.1.3 Criticisms of the BCR sequential extraction scheme

There are several pitfalls of sequential extraction[16]; for instance the method cannot determine unequivocally the amount of a metal bound to a particular phase as redistribution of that metal may occur during the sequential extraction. The user must therefore be careful to remember that sequential extraction is an operationally defined technique, and the results obtained are dependant upon the method used to obtain them, not necessarily upon the phase that is being attacked.

Whalley and Grant investigated the phase selectivity of the BCR sequential extraction reagents in response to criticisms that included inaccuracy in releasing metal from specific phases[17]. The geochemical specificity was shown to be of varying quality, especially when examining the humic acid phase, which tended to release metals earlier in the procedure than expected. Ferrihydrite, an amorphous iron oxide, also released metal earlier, into the acetic acid step rather than the hydroxylammonium chloride step. This may be due to a large number of adsorptive sites on the amorphous iron oxide. Most metal was removed from feldspar and kaolinite as expected in the first step; however the hydroxylammonium chloride was found to release most metal from montmorillonite (acetic acid was expected to release most metal from this substrate). This emphasises the need for care in the use of the results obtained by sequential extractions such as the BCR scheme.

Several workers have noted the redistribution of lead during sequential extraction schemes. Ho and Evans used the BCR sequential extraction procedure on two

standard reference materials (SRMs), (2710 and 2711, Montana soil), and compared their results to those of previous workers, using other schemes[18]. Results for cadmium, copper and zinc were found to agree with more from the Tessier method for these SRMs, but differences in the lead values were obtained. These differences were attributed to redistribution of lead during the sequential extraction. Raksasataya *et al.* also found that lead was subject to substantial redistribution during the BCR and Tessier scheme[19]. The different results obtained by the two procedures were attributed to the different reagent and conditions used in the reducing step of the methods. Using both natural and synthetic model soils, they found redistribution to be dominated by the organic matter and the iron/manganese oxide fractions of the natural soils.

Coetzee *et al.*[5] also investigated the redistribution of elements when the BCR SE scheme was used. Model sediments consisting of humic acid, kaolin, quartzite and ochre were used to evaluate redistribution and selectivity of extractants. Lead and copper were found to be redistributed in the presence of humic acid. The selectivity of reagents proved to be insufficient to interpret results in terms of specific origin of a metal in a particular phase.

4.1.4 Proposal for the improvement of the BCR sequential extraction procedure

Previous work during certification of CRM 601 showed a wide spread of results for some stages of the sequential extraction procedure. This prompted Sahuquillo *et al.*[20] to investigate sources of uncertainty within the procedure. The pH of the

extractant used in step 2 of the procedure proved to be the major source of variation, and a number of recommendations were made by the authors to improve the reproducibility of the BCR procedure. Cr, Cu and Pb were found to show a decrease in extractability and reproducibility as pH increased. The pH adjustment was shown to be critical in improving the variance and therefore the method of adjustment was investigated. It was shown that pH adjustment by a fixed volume of dilute nitric acid (rather than relying on a pH meter to determine the pH) proved more precise. Hydroxylammonium chloride concentration was also studied, at 0.1, 0.5 and 1.0 mol l^{-1}. At 0.5 mol l^{-1}, higher levels of determinants were extracted than at 0.1 mol l^{-1}, however it was thought that at 1.0 mol l^{-1} the more refractory, crystalline oxyhydroxides were also attacked. Both higher concentration extractants provided lower % coefficient of variance (CV) values than 0.1 mol l^{-1}. Sahuquillo *et al*[20] also investigated the effect of extraction temperature, extraction time, inert atmosphere, method used to isolate the solid and liquid phases after extraction and the use of alternative reagents. These either had little effect on the precision of the extraction or were detrimental to the procedure. The modifications proposed were that step 2 should be performed using 0.5 mol l^{-1} hydroxylamine hydrochloride adjusted to pH 1.5 by the addition of a fixed volume of dilute nitric acid. Increasing the speed of centrifugation from 1500 g to 3000 g was also found to improve reproducibility. Sahuquillo *et al.*[20] investigated sources of uncertainty in the BCR SE procedure for only CRM 601. The present work used a variety of substrates to investigate the effect of modification of the BCR procedure on the extraction of heavy metals.

4.2 Experimental

A comparison was made between the original BCR procedure as set out by Davidson *et al.*[6] and the revised procedure described by Rauret *et al.*[3]. There are two major differences between the two schemes. In the revised procedure the reductant concentration is increased from 0.10 to 0.50 mol l^{-1} hydroxylammonium chloride, and the pH is reduced from pH 2 to approximately pH 1.5 (by addition of a fixed volume of dilute nitric acid). In order to investigate the influence of the alterations the effect of changing the concentration of the extractant in the reducible step of the procedure was first investigated. In addition, the effect of altering the pH was then studied. A summary of the reagents used for each procedure is given in Table 4.2.

Table 4.2 Reagents used at each stage of the sequential extraction procedures investigated

Species Targeted	**Procedure A (Original BCR)**	**Procedure B**	**Procedure C (Modified BCR)**
Soluble / exchangeable	0.11 M acetic acid	0.11 M acetic acid	0.11 M acetic acid
Reducible	0.10 M hydroxylammonium chloride (~ pH 2)	0.50 M hydroxylammonium chloride (~ pH 2)	0.50 M hydroxylammonium chloride (~ pH 1.5)
Oxidisable	hydrogen peroxide followed by 1.0 M ammonium acetate (~ pH 2)	hydrogen peroxide followed by 1.0 M ammonium acetate (~ pH 2)	hydrogen peroxide followed by 1.0 M ammonium acetate (~ pH 2)
Residual	*aqua regia*	*aqua regia*	*aqua regia*

4.2.1 Apparatus

All laboratory ware used was made from glass, polyethylene or polypropylene and was cleaned by soaking in approximately 5 % nitric acid (v/v) overnight or for a longer period. Laboratory ware was then rinsed thoroughly with distilled water before use.

The mechanical shaker used during the sequential extraction procedure was of the end-over-end variety, provided by GFL GmbH, Burgwedel, Germany. A MSE Mistral 1000 bench top centrifuge was used to separate the extracts from the samples during the sequential extraction procedure.

Microwave assisted digestion was used to obtain pseudototal metal contents and residual metal contents of the samples. Microwave digestion provides a much faster method of pseudototal digestions than conventional methods such as refluxing with acids. Traditional methods also require close attention to prevent samples from boiling dry, and temperatures are also limited to the atmospheric boiling point of acid mixtures used. Microwave digestion involves placing the samples in microwave transparent vessels with a polar liquid or ionic solution (usually an acid). Compounds such as water and other polar liquids absorb microwave energy rapidly subjecting the sample solution to rapid heating and increased pressure, which causes the sample to be digested or dissolved in a much shorter time than conventional methods. The microwave system used was a CEM Corporation digestion system (MDS) 2000, supplied by CEM Corporation, Matthews, North Carolina, USA. The

MDS 2000 delivers approximately 630 watts of microwave energy at a frequency of 2450 MHz at full power. The power was adjusted depending upon the number of vessels in the microwave. For every vessel missing from the turntable (which holds 12 vessels) the applied power was reduced by 5 %.

4.2.2 Reagents

Hydroxylammonium chloride, AnalaR grade, was provided by Merck, Poole, UK, as were AnalaR nitric (69 %, sp. gr. 1.42), hydrochloric (sp. gr. 1.18) and acetic (glacial, 100 %) acids. Hydrogen peroxide (30 %) was obtained from Fluka, Dorset, UK and ammonium acetate from Prolabo, Manchester, UK. Calibration solutions were prepared from 1000 μg ml^{-1} Spectrosol standards (Merck, Dorset, UK) or Romil Prim*Ag* metal reference solutions (Romil, Waterbeach, Cambridge, UK). All reagent solutions were diluted with distilled water.

4.2.3 Preparation of 0.11 M acetic acid

6.4 ml of acetic acid was added to distilled water in a 1 l volumetric flask and made up to volume.

4.2.4 Preparation of 0.10 M hydroxylammonium chloride (~ pH 2), Reductant A

6.95 g of hydroxylammonium chloride was dissolved in distilled water and transferred to a 1 l volumetric flask. 0.4 ml of concentrated nitric acid was added and the solution made up to volume.

4.2.5 Preparation of 0.50 M hydroxylammonium chloride (~ pH 2), Reductant B

34.75 g of hydroxylammonium chloride was dissolved in distilled water and transferred to a 1 l volumetric flask. 400 µl of concentrated nitric acid was added and the solution made up to volume.

4.2.6 Preparation of 0.50 M hydroxylammonium chloride (~ pH 1.5), Reductant C

34.75 g of hydroxylammonium chloride was dissolved in distilled water and transferred to a 1 l volumetric flask. 2.20 ml of concentrated nitric acid was added and the solution made up to volume.

4.2.7 Preparation of 1.0 M ammonium acetate (~ pH 2)

77.08 g of ammonium acetate was dissolved in distilled water and transferred to a 1 l volumetric flask. 63 ml of concentrated nitric acid was added and the solution made up to volume.

4.3 Sequential extraction procedures

The three steps of the sequential extraction procedures used are described in the following sections. The extraction of the metals from the residual and pseudototal samples is also described.

4.3.1 Exchangeable fraction

Approximately 1 g of sample was weighed accurately into a polypropylene centrifuge tube. 40 ml of 0.11 mol l^{-1} acetic acid was added to the sample and the centrifuge tube sealed. The sample was shaken for 16 hours at 40 rpm on the end over end shaker at room temperature and centrifuged at 1500 g for 10 minutes before decanting the supernatant into a plastic bottle. The sample was then washed. 20 ml of distilled water was added to the soil/sediment and the mixture shaken using the end over end shaker for 15 minutes. The sample was then centrifuged for 10 minutes at 1500 g and the water discarded.

4.3.2 Reducible fraction

40 ml of hydroxylammonium chloride (concentration and pH determined by which procedure was being followed, A, B or C) was added to the sample from the previous step and shaken for 16 hours. The sample was centrifuged at 1500 g for 10 minutes and the supernatant decanted into a plastic bottle. The sample was washed as before.

4.3.3 Oxidisable fraction

10 ml of hydrogen peroxide was added to the sample and left at room temperature for 1 hour. The centrifuge tube was then covered and samples digested at 85 °C using a water bath, for 1 hour. The cover was removed from the centrifuge tube and the solution allowed to evaporate to near dryness. 10 ml of hydrogen peroxide was added to the sample and the centrifuge tube covered once more. The samples were further digested at 85 °C for 1 hour and then the covers removed to allow evaporation to near dryness. 50 ml of 1 mol l^{-1} ammonium acetate adjusted to approximately pH 2 was added to the sample and shaken for 16 hours. The sample was centrifuged at 1500 g and the supernatant removed for analysis as before, and sample washed.

4.3.4 Residual content

The residual metal content was determined in order to perform a mass balance by comparing the sum of the three fractions and the residual fraction with the pseudototal metal content of a separate 1 g sample.

20 ml of *aqua regia* (3:1 ratio of HCl:HNO_3) was added to the residue remaining from the oxidisable step of the procedure and the slurry transferred to a microwave digestion vessel. The sample was then digested using a two stage program, taking the pressure in the vessels first to a maximum of 60 psi for 20 minutes. If the pressure was reached before the time had elapsed the samples were held at 60 psi for 5 minutes. The pressure was then allowed to increase up to a maximum of 120 psi for 30 minutes or, as before, held at 120 psi for 20 minutes if pressure was reached before the 30 minutes had elapsed. The pressure in the microwave vessel was allowed to decrease to close to atmospheric levels, before the vessel was opened and the contents filtered (Whatman 50 filter paper) into 100 ml graduated flasks. The resulting solution was made up to 100 ml with distilled water and transferred to a plastic bottle for storage.

Pseudototal metal extraction of the separate 1 g sample was performed exactly as described above.

Extracts and *aqua regia* digests, were stored in bottles at 4 °C prior to analysis by ICP AES as discussed in Chapter 2.

4.4 Results and Discussion

In order to validate the analysis a certified reference material was extracted and metal content determined each time that samples were extracted. BCR CRM 601 is a lake sediment, certified for metals extractable by the original BCR sequential extraction scheme[13]. Literature since the certification of the reference material has suggested modifications to the extraction scheme and indicative values based on the modified procedure have been published[3]. Figures 2.5 – 2.11 illustrate the certified values for the original procedure (cert.), the indicative values for the modified procedure (ind.) and the values found throughout this work (1-7, i.e. 7 separate batches of SE). The values obtained in this work are presented in appendix 4.1. The differences between the certified values (obtained by the original BCR sequential extraction method) and the indicative values (obtained by the modified BCR sequential extraction method) follow similar trends to results obtained for soils and sediments when comparing the original and modified procedures. The main differences between the literature values for the original and modified procedure are that greater amounts of copper and lead are released into the reducible extractant and less released by the oxidisable extractant, in the modified procedure. Hypotheses for the reasons for these differences will be discussed later in this chapter. The values shown in this chapter were obtained by the modified BCR method and should therefore be compared to the ind. values. It can be seen that the means and relative standard deviations obtained in this work compare reasonably well. The main differences between the literature values and the experimental values occur for copper, where the metal bound to the reducible phases is consistently less than the literature value and the metal bound to

the oxidisable phase is greater than the literature value. Values obtained throughout the experimental work were found to be consistent with typical RSDs of less than 5 %. The exchangeable lead RSD obtained experimentally was found to be high, but this is likely to be due to the low levels of lead extracted in this step, which would approach the detection limits. A high RSD was also obtained in the literature for this step. Exchangeable iron also produced a high RSD, however it is unclear as to the reason for the lack of stability in these results.

Between-batch variation during the experimental work was investigated by comparing the results obtained by extraction using method A, on two separate occasions. The effects of increasing reagent concentration (method B) were investigated by comparing results obtained by extraction using method A and method B simultaneously. The effect of increasing reagent concentration and decreasing pH was shown by comparing results from method A' and method C' (carried out simultaneously). Method A and A' are identical apart from the dates on which they were carried out.

Two sample t-tests were used to compare sets of results[21]. For all results n = 5, unless otherwise stated. Some results contained less than 5 replicates due to loss of sample during preparation or due to exclusion of outliers from the results. Outliers were rejected on the basis of Dixon's Q test[21]. Samples were tested for variance prior to using t-tests and the appropriate t-test used depending whether the two sets of results had equal or unequal variances.

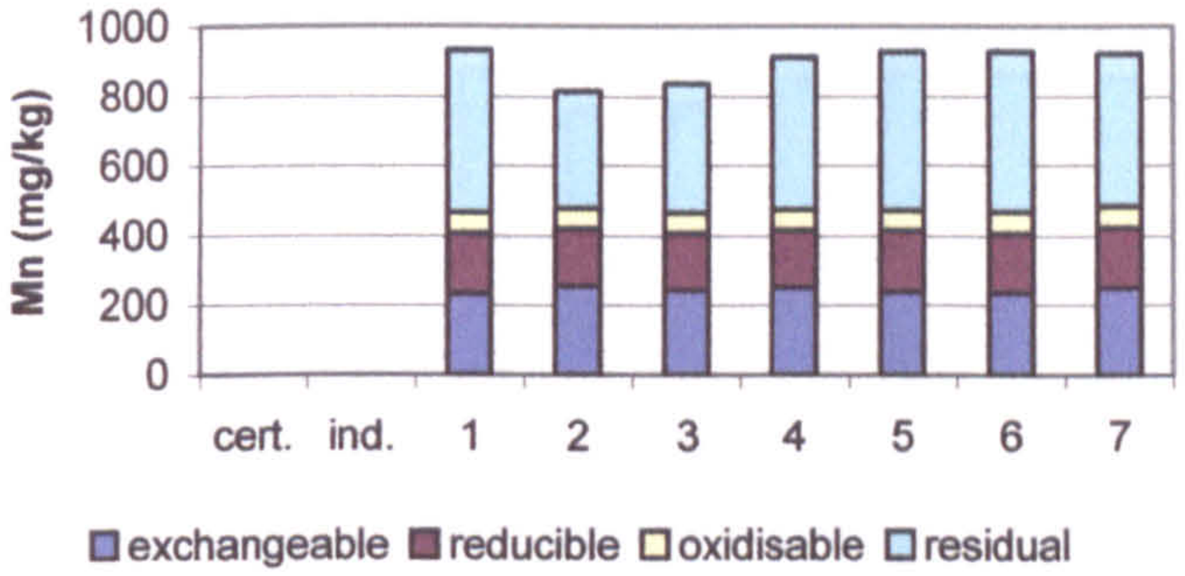

Figure 4.2 Ca released by BCR sequential extraction from BCR CRM 601 (cert. = certified value, ind. = indicative value)

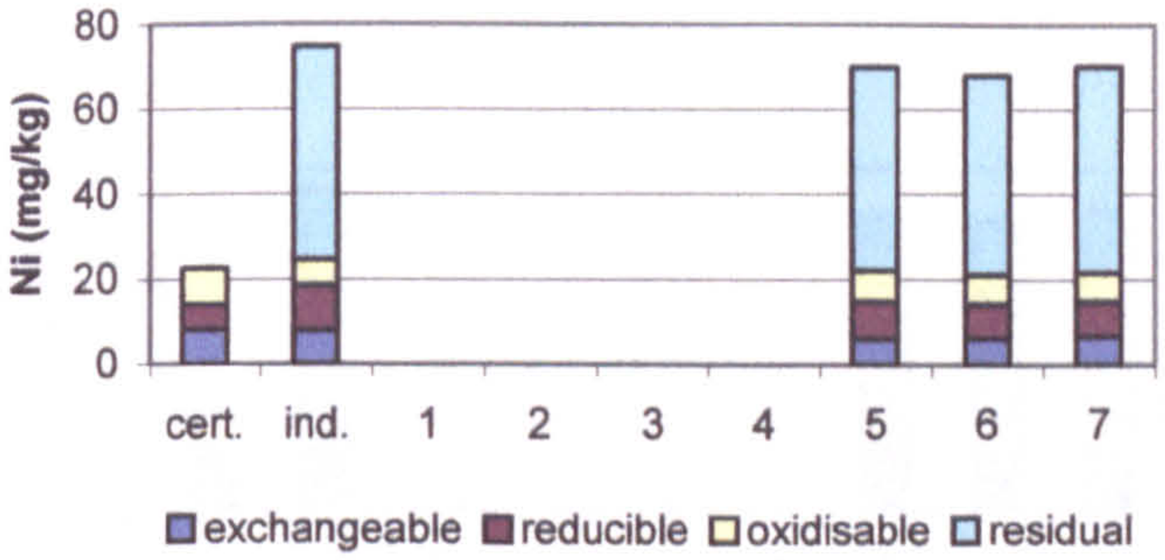

Figure 4.3 Cu released by BCR sequential extraction from BCR CRM 601 (cert. = certified value, ind. = indicative value)

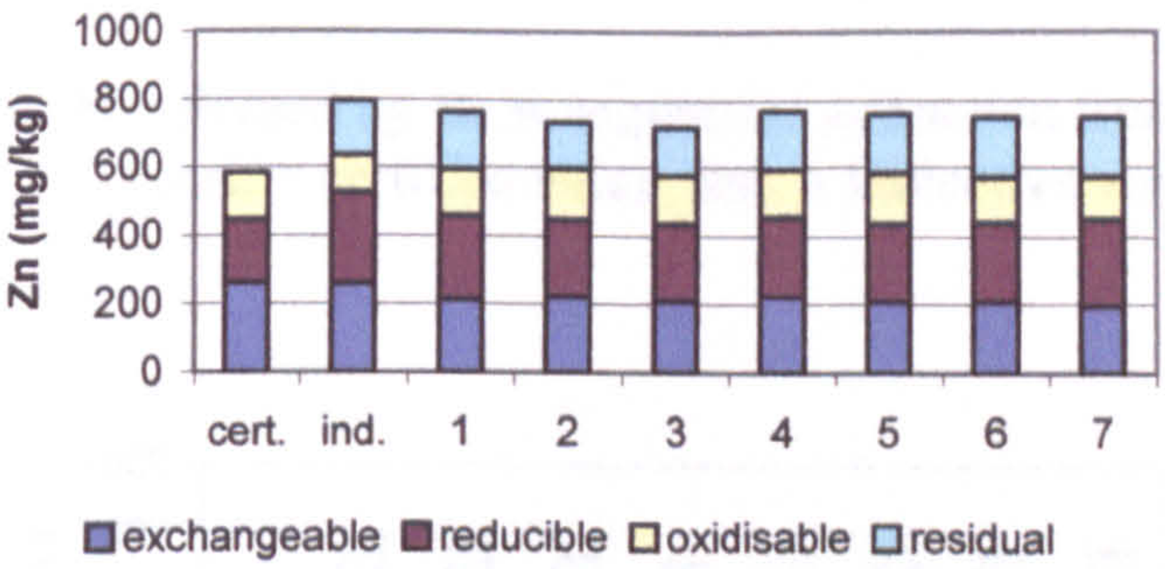

Figure 4.4 Fe released by BCR sequential extraction from BCR CRM 601 (cert. = certified value, ind. = indicative value)

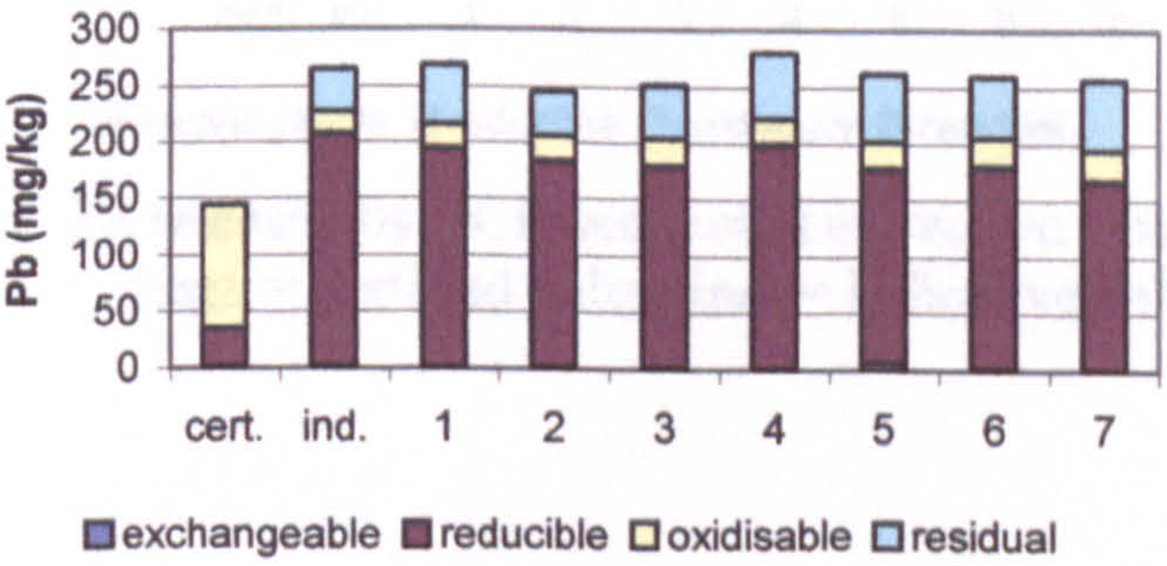

Figure 4.5 Pb released by BCR sequential extraction from BCR CRM 601 (cert. = certified value, ind. = indicative value)

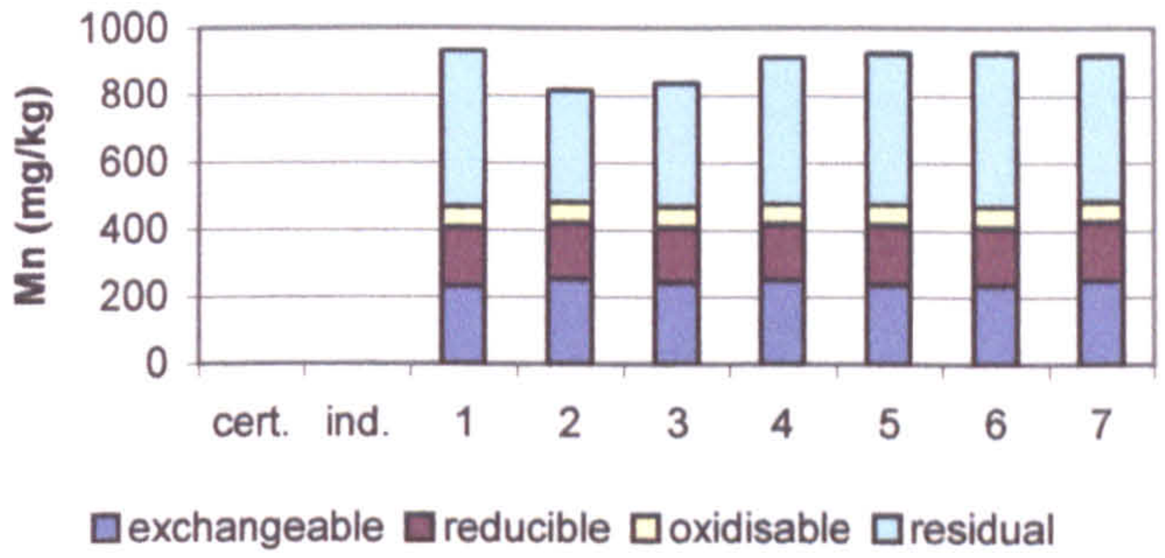

Figure 4.6 Mn released by BCR sequential extraction from BCR CRM 601
(cert. = certified value, ind. = indicative value)

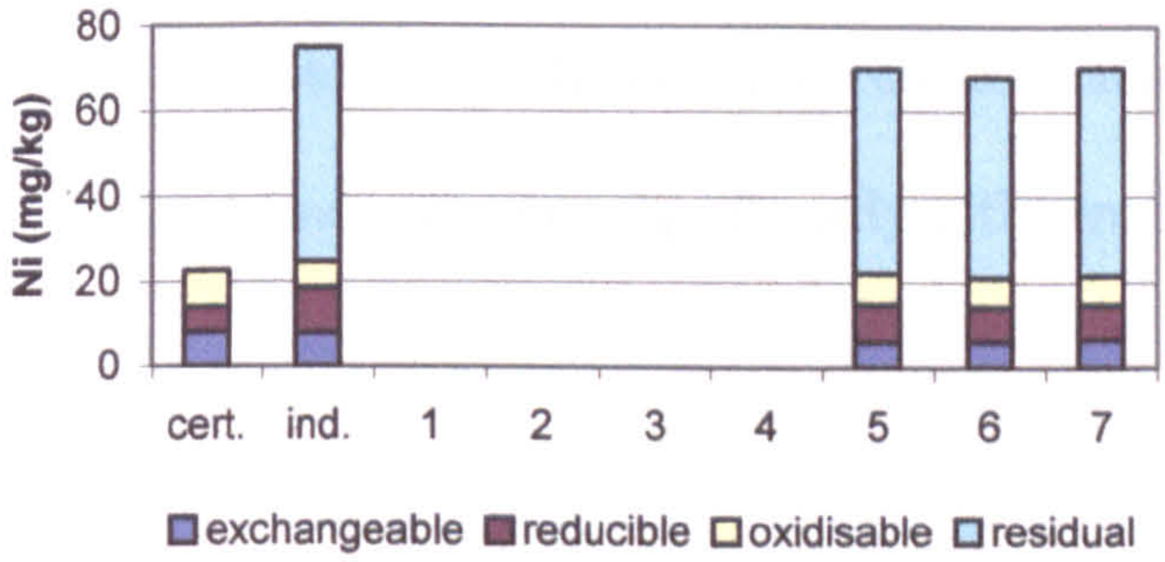

Figure 4.7 Ni released by BCR sequential extraction from BCR CRM 601
(cert. = certified value, ind. = indicative value)

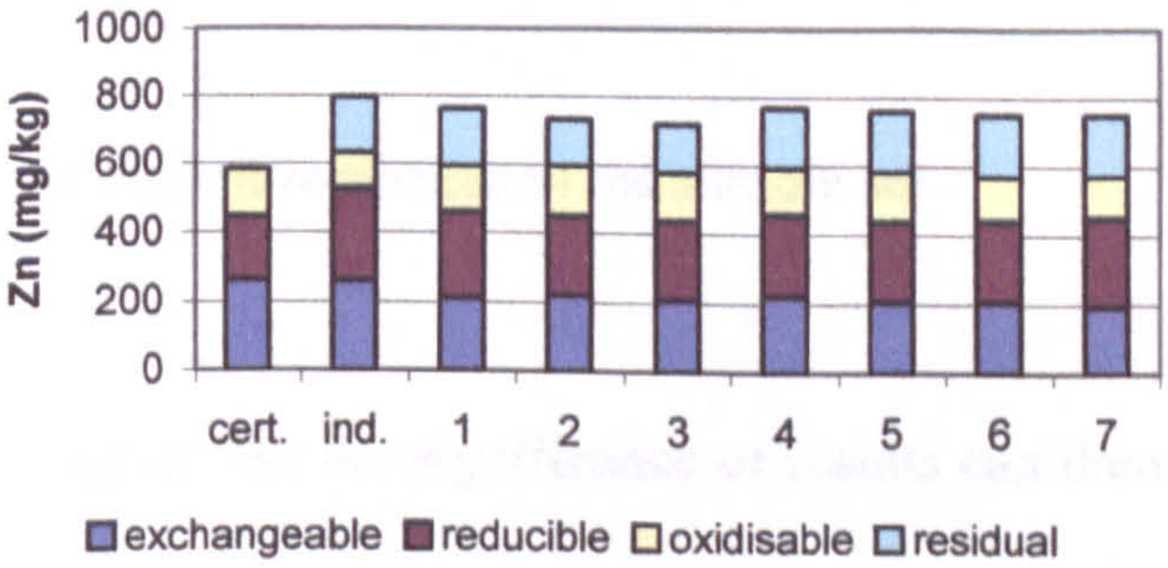

Figure 4.8 Zn released by BCR sequential extraction from BCR CRM 601
(cert. = certified value, ind. = indicative value)

The F test used to determine whether the variances of two sets of results are equal is given by:

$$F = \frac{s_1^2}{s_2^2}$$

where s_1 and s_2 are the standard deviations of the two sets of results. s_1 and s_2 are allocated so that F is always greater than 1. The calculated value of F can then be compared to values given in tables for the given degrees of freedom. If the calculated value of F is found to be less than the critical value given in tables it can be assumed that the variances are not significantly different for a given probability level.

When the variances of the two samples are shown not to be significantly different, a pooled standard deviation, s, can be calculated as:

$$s^2 = \left[(n_1 - 1)s_1^2 + (n_2 - 1)s_2^2\right]/(n_1 + n_2 - 2)$$

where n is the number of replicates in the sample set.

The value of t used to test the significance of results can then be calculated from the equation:

$$t = (\bar{x}_1 - \bar{x}_2)/s\sqrt{(1/n_1 + 1/n_2)}$$

Where $x_1$and x_2 are the means of the samples. The calculated value of t can then be compared with t from tables for $n_1 + n_2 - 2$ degrees of freedom. If the calculated value of t is shown to be greater than the critical value of t, it can be assumed that the means of the two samples are significantly different.

If the variances of the two samples are shown to be significantly different by the F test, t must be approximated using the following equation:

$$t = (\bar{x}_1 - \bar{x}_2) / \sqrt{(s_1^2 / n_1 + s_2^2 / n_2)}$$

The number of degrees of freedom (df) can then be calculated from:

$$df = \left\{ \frac{(s_1^2 / n_1 + s_2^2 / n_2)^2}{\frac{(s_1^2 / n_1)^2}{n_1 + 1} + \frac{(s_2^2 / n_2)^2}{n_2 + 1}} \right\} - 2$$

rounding the result to the nearest whole number.

The calculated and critical values of t can then be compared as before. The two-tailed t-test (95 % confidence limit) was used in these experiments in order to investigate the possibility that results were significantly higher or significantly lower than one another. The one-tailed t-test can be used if the researcher is looking specifically for a higher result, or specifically for a lower result.

4.4.1 Great Billing

The results for extraction of metals from Great Billing soil by procedures A, B and C, and the comparison of the results are shown in tables 4.3-4.12. All results are in mg kg^{-1}, dry weight. Errors shown in these tables and all following tables are ± 1 standard deviation.

4.4.1.1 Copper

Table 4.3 Copper in Great Billing soil (mg kg^{-1})

Method	Exchangeable	Reducible	Oxidisable	Residual	Sum	PT
A	9.10 ± 3.0	7.5 ± 3.3	213 ± 13	40.7 ± 3.7	270 ± 14	320
A'	17.4 ± 0.3	19.5 ± 1.1	209 ± 8	58.9 ± 4.9	287 ± 9	334
B	12.2 ± 0.7[a]	19.1 ± 3.6[a]	201 ± 9[a]	37.2 ± 4.4	270 ± 11	
C'	17.6 ± 0.3	123 ± 3.7	143 ± 8	40.6 ± 2.3	324 ± 9	

a n = 4

The sums of the sequential extraction tend to be lower than the pseudototal digestion, however this may be due to losses during the many stages of the sequential extraction process. The sums do compare well with one another, although the sum of SE C' appears to be higher than the other three extraction sums, and is also closer to the PT value than the other results. Although PT copper is reasonably high, exchangeable copper levels are well below ICRCL trigger concentrations for land uses where plants are to be grown. The largest difference is a greatly enhanced extraction of copper in step 2 with C', and subsequent lower recovery in the oxidisable step. A more rigorous approach in the comparison of the methods is to use statistics as described previously. Two tailed t-tests were used to compare the means of the original and modified extractions and the results are shown in table 4.4.

Table 4.4 Comparison of methods

A/A'	Exchangeable	Reducible	Oxidisable	Residual
T stat	16.4	7.67	0.526	6.63
t critical on two tails	2.57	2.57	2.31	2.31
P (T ≤ t)	1.55×10^{-5}	0.000601	0.613	0.000163
A/B	**Exchangeable**	**Reducible**	**Oxidisable**	**Residual**
T stat	4.93	4.97	1.50	1.36
t critical on two tails	2.36	2.45	2.36	2.31
P (T ≤ t)	0.00169	0.00253	0.178	0.210
A'/C'	**Exchangeable**	**Reducible**	**Oxidisable**	**Residual**
T stat	1.22	60.4	13.5	7.58
t critical on two tails	2.31	2.57	2.31	2.31
P (T ≤ t)	0.257	2.36×10^{-8}	8.77×10^{-7}	6.4×10^{-5}

T_{stat} = t calculated from experimental data

P(T ≤ t) = probability that $T_{stat} \leq t_{critical}$. i.e. when P(T ≤ t) < 0.05 the means of the two results being compared can be assumed to be significantly different.

Only the oxidisable phase was not significantly different when comparing results for method A carried out on separate occasions. This may be due to the relatively low levels of copper extracted from the other phases. Although reducible results for methods A and B were shown to be significantly different, the exchangeable results (method identical for the two procedures) were also shown to be significantly different. No conclusions can therefore be made as to whether the differences in the reducible results were due to differences in the method or due to some other reason (e.g. sample heterogeneity etc.). When comparing methods A' and C' the exchangeable results were not significantly different, but the reducible, oxidisable and residual results were all significantly different, with the reducible results showing the least probability of being significantly similar. This would suggest that increasing the reductant concentration and decreasing the pH of the reductant

increased the copper recovered by the reducible step. Less copper was recovered in the oxidisable step of method C' compared to method A', suggesting that method C' increased the availability of copper normally released by conditions in the oxidising step of the procedure.

Tipping *et al.*[22] have shown that metals associated with manganese oxides, which would be readily broken by hydroxylamine treatment, could be re-adsorbed by iron oxides if conditions were sufficiently basic. They showed that a crumpled sheet manganese phase originally containing large proportions of lead released very little lead upon treatment with hydroxylamine; however, upon subsequent treatment with oxalate (which attacked iron oxides), the lead was released. The final pH of the hydroxylamine extract was found to be 5.2, which would be sufficient for re-adsorption of lead on the iron oxides. They also suggested that re-adsorption of other strongly adsorbed metals, such as copper, would be likely to occur. These observations could explain the high results obtained in the reducible phases for method C' with the lower pH.

4.4.1.2 Iron

Table 4.5 Iron in Great Billing soil (mg kg^{-1})

Method	Exchangeable	Reducible	Oxidisable	Residual	Sum	PT
A	<14.4	767 ± 190	3950 ± 280	18000 ± 1400	22700 ± 1440	21800 23600
A'	31.6 ± 1.7	1900 ± 84	686 ± 64	22200 ± 2030	24800 ± 2030	
B	<14.4	2120 ± 200	3310 ± 331	15700 ± 1170	21100 ± 1230	
C'	32.8 ± 1.1	7000 ± 184[a]	724 ± 141	15300 ± 545[b]	23100 ± 592	

a n = 3
b n = 4

The majority of iron in the Great Billing soil is distributed in the residual phase and is therefore unlikely to be leached into groundwater. The sums of the sequential extractions compare well with the PT iron value, indicating good recovery of the iron by the sequential extraction procedure. Standard deviations were low (10 % or less) for all stages except the reducible step of method A and the oxidisable step of method C'. This may be due to sample heterogeneity. Reproducibility was found to be better in the reducible stages of the modified sequential extractions. The largest difference between procedures appears to be the enhanced extraction of iron by the reducible step of method C' and subsequent decreased extraction in the oxidisable step. Statistical analysis shown in table 4.6 provides more information on the significance of these differences.

Table 4.6 Comparison of methods

A/A'	Exchangeable	Reducible	Oxidisable	Residual
T stat		12.2	25.4	3.76
t critical on two tails		2.31	2.78	2.31
P (T ≤ t)		1.95×10^{-6}	1.42×10^{-5}	0.00554
A/B	**Exchangeable**	**Reducible**	**Oxidisable**	**Residual**
T stat		11.0	3.30	2.84
t critical on two tails		2.31	2.31	2.31
P (T ≤ t)		4.26×10^{-6}	0.0109	0.0218
A'/C'	**Exchangeable**	**Reducible**	**Oxidisable**	**Residual**
T stat	1.42	55.3	0.552	7.20
t critical on two tails	2.31	2.45	2.31	2.57
P (T ≤ t)	0.193	2.36×10^{-9}	0.596	0.000804

The modified procedure extracted significantly more iron than the original procedure. It was thought to be likely that the increased reductant concentration and reduced pH could attack crystalline iron oxyhydroxides as well as amorphous iron oxyhydroxides, which could be released by the weaker reducing agent. Although a significant difference was seen between the original and modified procedures for the reducible stages, a difference was also observed between the results from the same procedures repeated on different days. However, results from exchangeable fraction of methods A' and C' (identical methods at this stage) were not found to be significantly different suggesting that results from the same days could be compared.

4.4.1.3 Lead

Table 4.7 Lead in Great Billing soil (mg kg^{-1})

Method	Exchangeable	Reducible	Oxidisable	Residual	Sum	PT
A	< 2.4	< 8	235 ± 13	172 ± 9	407 ± 16	456
A'	< 0.3	12.3 ± 1.0	42.4 ± 2.4	415 ± 21	470 ± 21	463
B	< 2.4	53.6 ± 12	200 ± 16	151 ± 28	405 ± 34	
C'	<0.3	329 ± 6	27.1 ± 4.6	131 ± 5	487 ± 9	

Lead was found mainly in the less mobile oxidisable and residual phases. The sums compared reasonably well with the PT results indicating good recoveries of lead by the sequential extractions. Standard deviations were reasonable with the exception of reducible and residual results for method B and oxidisable results for method C'. Except for method C' lead was generally found to be present in less mobile forms. The effect of the modification of the BCR procedure was determined by statistical analysis and the results shown in table 4.8.

Table 4.8 Comparison of methods

A/A'	Exchangeable	Reducible	Oxidisable	Residual
T stat			33.3	23.5
t critical on two tails			2.78	2.31
P (T ≤ t)			4.86×10^{-6}	1.14×10^{-8}
A/B	**Exchangeable**	**Reducible**	**Oxidisable**	**Residual**
T stat			3.78	1.59
t critical on two tails			2.31	2.57
P (T ≤ t)			0.00540	0.173
A'/C'	**Exchangeable**	**Reducible**	**Oxidisable**	**Residual**
T stat		109	6.60	28.9
t critical on two tails		2.78	2.31	2.78
P (T ≤ t)		4.28×10^{-8}	0.000169	8.49×10^{-6}

Results do not compare well for the two methods A and A' carried out on separate occasions, and lead could not be detected in the exchangeable step of the procedures. It is therefore impossible to be able to make reliable conclusions about the comparisons of methods as any differences may be due to experimental errors. It should be noted that lead generally tends to be present in higher concentrations in the residual fraction except under reducing conditions at low pH (*i.e.* method C'). This suggests that lead would ordinarily be stable in the Great Billing soil and would not be readily flushed from the soil into groundwater.

4.4.1.4 Manganese

Table 4.9 Manganese in Great Billing soil (mg kg^{-1})

Method	Exchangeable	Reducible	Oxidisable	Residual	Sum	PT
A	85.4 ± 5.0	92.6 ± 7.1	14.4 ± 2.3	50.2 ± 3.7	243 ± 10	214
A'	119 ± 3	85.6 ± 8.2	8.94 ± 0.64	54.9 ± 4.3	268 ± 10	226
B	92.8 ± 3.2	84.7 ± 5.5	10.4 ± 1.2	41.8 ± 4.8	230 ± 8	
C'	120 ± 3	95.7 ± 8.5	7.90 ± 0.58	41.9 ± 3.7	266 ± 10	

The majority of the manganese in the Great Billing soil was found in the more readily mobile exchangeable and reducible phases making it one of the more mobile and potentially plant available elements studied. The sums compare reasonably with each other and with the pseudototal values, and reproducibility was found to be good for all results (less than 20 % RSD). There appears to be little difference between the three procedures for manganese extraction, however any significant differences, however small can be revealed by statistical analysis as shown in table 4.10.

Table 4.10 Comparison of methods

A/A'	Exchangeable	Reducible	Oxidisable	Residual
T stat	13.2	1.45	5.23	1.88
t critical on two tails	2.31	2.31	2.57	2.31
P (T ≤ t)	1.01×10^{-6}	0.184	0.00339	0.0976
A/B	**Exchangeable**	**Reducible**	**Oxidisable**	**Residual**
T stat	2.79	1.98	3.48	3.08
t critical on two tails	2.31	2.31	2.31	2.31
P (T ≤ t)	0.0235	0.0825	0.00828	0.0150
A'/C'	**Exchangeable**	**Reducible**	**Oxidisable**	**Residual**
T stat	0.319	1.92	2.69	5.13
t critical on two tails	2.31	2.31	2.31	2.31
P (T ≤ t)	0.758	0.0912	0.0276	0.000891

No significant difference was observed for the reducible and residual results for methods A and A', however significant differences were obtained for the exchangeable and oxidisable stages of these methods. Differences in oxidisable results are most likely to due to low levels of manganese present and therefore higher percentage error in the results. Although statistically the exchangeable results from A and B differ, they are within 10 % of one another, and exchangeable results from A' and C' do not differ significantly. Reducible results from A'/C' comparison do not significantly differ, and slight differences in oxidisable levels are likely to be due to the low levels of manganese present. Slight differences were observed in the residual stages when comparing A/B and A'/C'. These may be due to the accumulation of slight differences in the methods in previous stages that alone were not significantly different. Overall manganese did not appear to be severely affected by the increase in reductant concentration and decrease in pH. This is in agreement with conclusions made by Tipping *et al.*[22] 97.4 % of manganese oxides from a sample taken from a lead mine were found to be readily dissolved when treated with

0.1 M hydroxylammonium chloride/0.01 M HNO_3. Further extraction of the sample with oxalate was found to release iron oxides.

4.4.1.5 Zinc

Table 4.11 Zinc in Great Billing soil (mg kg^{-1})

Method	Exchangeable	Reducible	Oxidisable	Residual	Sum	PT
A	370 ± 61	337 ± 42	93.7 ± 32	90.6 ± 8.4	891 ± 81	875
A'	445 ± 8	372 ± 10	66.6 ± 2.4	128 ± 8	1010 ± 15	882
B	419 ± 66	374 ± 29	71.3 ± 7.9	65.2 ± 11	930 ± 73	
C'	453 ± 9	433 ± 22	36.3 ± 5.6	78.1 ± 9.6	1000 ± 26	

Levels of zinc in the Great Billing soil fall above the ICRCL guidelines for land where planned use is where plants are to be grown. A large proportion of zinc was present in the exchangeable and reducible (more mobile forms) stages of the methods, therefore zinc is likely to be leached from Great Billing soils into groundwater. This may be a problem especially for plants growing in or near the contaminated ground; however, it also suggests that leaching may be a potential method for recovery of the zinc. The sums compare reasonably with each other and give good recoveries when compared to the PT value. Reproducibility was also found to be good with a range of 2 – 17 % throughout the experiments.

Table 4.12 Comparison of methods

A/A'	Exchangeable	Reducible	Oxidisable	Residual
T stat	2.74	1.81	1.90	6.96
t critical on two tails	2.78	2.78	2.78	2.31
P (T<= t)	0.0521	0.144	0.130	0.000118
A/B	**Exchangeable**	**Reducible**	**Oxidisable**	**Residual**
T stat	1.22	1.62	1.52	4.04
t critical on two tails	2.31	2.31	2.57	2.31
P (T<= t)	0.256	0.144	0.188	0.00373
A'/C'	**Exchangeable**	**Reducible**	**Oxidisable**	**Residual**
T stat	1.38	5.57	11.0	8.64
t critical on two tails	2.31	2.31	2.31	2.31
P (T<= t)	0.206	0.000531	4.01×10^{-6}	2.49×10^{-5}

Exchangeable results in all three comparisons did not vary, and only in the case of residual zinc in the A/A' comparison was any significant variation noted. Again only the A/B residual zinc showed any significant differences. However, slight significant differences were observed for zinc in reducible, oxidisable and residual stages. These results suggest that although increasing the reductant concentration has little effect on the zinc extracted, decreasing the pH and increasing reductant concentration extracts significantly more zinc in reducible stage and less in the oxidisable and residual stages.

4.4.2 White Cart

The results of the investigation of the effect of the modification of the BCR SE procedure on metals extracted from the White Cart sediment are shown in tables 4.13-4.22.

4.4.2.1 Copper

Table 4.13 Copper in White Cart sediment (mg kg^{-1})

Method	Exchangeable	Reducible	Oxidisable	Residual	Sum	PT
A	7.62 ± 0.11[a]	4.35 ± 0.41[a]	14.2 ± 2.3[a]	8.07 ± 0.40[a]	34.2 ± 2.4	137 39
A'	11.7 ± 0.4[a]	4.60 ± 0.38[a]	15.4 ± 2.6[a]	11.8 ± 1.4	43.5 ± 3.0	
B	7.78 ± 0.67	4.06 ± 0.24	16.6 ± 4.0	7.12 ± 0.89	35.6 ± 4.2	
C'	11.7 ± 0.5[a]	10.5 ± 1.1[a]	14.4 ± 6.7[a]	8.78 ± 0.78[a]	45.4 ± 6.9	

a n = 4

Copper levels were found to be reasonably low in the White Cart sediment falling within the ICRCL guidelines, although these guidelines would not be relevant unless the land was reclaimed for building or agricultural use or if the sediment was to be spread on land. The two values obtained during pseudototal extraction of the material do not compare well, however on the basis of the sums of the sequential extraction it is likely that the value of 137 mg kg^{-1} is an outlier. Most of the standard deviations were small (generally less than 10 %) with the exception of the oxidisable stage results. These tended to be higher with an RSD of 47 % for method C' results. It is unclear as to why these standard deviations are high compared to other stages of the procedures.

Table 4.14 Comparison of methods

A/A'	Exchangeable	Reducible	Oxidisable	Residual
T stat	19.9	0.905	0.686	5.61
t critical on two tails	3.18	2.45	2.45	2.57
P (T ≤ t)	0.000276	0.401	0.518	0.00249
A/B	**Exchangeable**	**Reducible**	**Oxidisable**	**Residual**
T stat	0.513	1.31	1.07	1.95
t critical on two tails	2.78	2.36	2.36	2.36
P (T ≤ t)	0.635	0.231	0.322	0.0918
A'/C'	**Exchangeable**	**Reducible**	**Oxidisable**	**Residual**
T stat	0.0884	9.94	0.274	3.78
t critical on two tails	2.45	2.45	2.45	2.36
P (T ≤ t)	0.932	5.98×10^{-5}	0.793	0.00691

Copper was found to be present at very low levels in the White Cart sediment and does not cause concern with regard to the ICRCL guidelines. The only significant differences observed were for A/A' exchangeable and residual, and A'/C' reducible and residual results. Differences at the exchangeable stage are likely to be due to experimental error and also the small amount of copper present. The most significant difference was observed for the reducible A'C' results. The modified method was seen to increase the copper recovered from the reducible step.

4.4.2.2 Iron

Table 4.15 Iron in White Cart sediment (mg kg^{-1})

Method	Exchangeable	Reducible	Oxidisable	Residual	Sum	PT
A	217 ± 5[a]	1070 ± 33[a]	1460 ± 36[a]	11300 ± 928[a]	14000 ± 929	14500 14100
A'	208 ± 13	1430 ± 58	796 ± 17	15900 ± 867[a]	18300 ± 869	
B	213 ± 6	1290 ± 20	1360 ± 67	11800 ± 471[a]	14700 ± 476	
C'	209 ± 63	2320 ± 62	676 ± 99	12800 ± 1940[b]	16000 ± 1950	

a n = 4
b n = 3

The majority of the iron in the White Cart sediment occurs in the residual phase as expected. The sums compare reasonably with the PT results, and standard deviations were found to be small with the exception of the method C' exchangeable result (30% RSD). The exchangeable values for all four extractions appear to agree reasonably well (i.e. they are within 5 % of each other). Statistical analysis (table 4.16) confirms this and illustrates the magnitude of the significance of the differences observed between the reducible steps for each extraction.

Table 4.16 Comparison of methods

A/A'	Exchangeable	Reducible	Oxidisable	Residual
T stat	1.28	10.9	36.7	7.29
t critical on two tails	2.36	2.36	2.36	2.45
P (T<= t)	0.242	1.2×10^{-5}	2.91×10^{-9}	0.000341
A/B	**Exchangeable**	**Reducible**	**Oxidisable**	**Residual**
T stat	1.16	12.6	2.79	0.995
t critical on two tails	2.36	2.36	2.36	2.45
P (T<= t)	0.284	4.57×10^{-6}	0.0267	0.357
A'/C'	**Exchangeable**	**Reducible**	**Oxidisable**	**Residual**
T stat	0.0469	52.6	2.68	2.95
t critical on two tails	2.78	2.31	2.78	2.57
P (T<= t)	0.965	1.89×10^{-11}	0.0555	0.0317

None of the comparisons for the exchangeable stage show any significant differences; however, the majority of the remaining comparisons do, with the exception of A/B residual and A'/C' oxidisable. The differences observed between A and A' suggest that the method was not repeatable over several days, but these differences may be due to the large dilution factors involved in the determination of the iron in the later stages of the extractions. The most significant difference was found to be between the reducible results of method A' and C'. This is similar to the results from the Great Billing soil, suggesting that once more crystalline iron oxides are being attacked by the more aggressive method C'.

4.4.2.3 Lead

Table 4.17 Lead in White Cart sediment (mg kg^{-1})

Method	Exchangeable	Reducible	Oxidisable	Residual	Sum	PT
A	< 2.4	16.3 ± 0.7[a]	14.9 ± 2.0[a]	5.61 ± 1.29[a]	36.8 ± 2.5	40.4 38.7
A'	< 0.3	20.3 ± 1.8	8.14 ± 1.44	14.1 ± 2.9	42.5 ± 3.7	
B	< 2.4	27.4 ± 1.4	9.49 ± 1.11	2.73 ± 2.06	39.6 ± 2.7	
C'	< 0.3	34.7 ± 4.2	2.17 ± 0.48	7.06 ± 1.16	43.9 ± 4.4	

a n = 4

Although very little lead was detected in the White Cart sediment, reproducibility was reasonable (below 20% in all cases except method C' oxidisable). Lead levels quantified by the PE Plasma II were within a factor of 10 of the detection limits for lead. At these levels the signal to background ratio is low and a small difference in signal can make a large difference to the reproducibility. Sums of the four stages of each method were also found to compare well with the PT figure. There appears to be a slight increase in the lead extracted by the modified procedure, C' as was observed for Great Billing. Statistical analysis was carried out in order to prove the significance of this observation.

4.18 Comparison of methods

A/A'	Exchangeable	Reducible	Oxidisable	Residual
T stat		4.15	5.96	5.29
t critical on two tails		2.36	2.36	2.36
P (T ≤ t)		0.00432	0.000566	0.00114
A/B	**Exchangeable**	**Reducible**	**Oxidisable**	**Residual**
T stat		14.2	5.22	2.42
t critical on two tails		2.36	2.36	2.36
P (T ≤ t)		2.07×10^{-6}	0.00122	0.0458
A'/C'	**Exchangeable**	**Reducible**	**Oxidisable**	**Residual**
T stat		7.03	8.80	4.94
t critical on two tails		2.31	2.57	2.57
P (T ≤ t)		0.00011	0.000315	0.00431

Very little lead was detected in the White Cart sediment. In some cases lead levels were within a factor of 10 of the limits of detection, and variation may therefore be large. Due to the low levels of lead present in this sediment it is difficult to draw any accurate conclusions on the effects of the increase in reductant strength and decrease in pH.

4.3.2.4 Manganese

Table 4.19 Manganese in White Cart sediment (mg kg^{-1})

Method	Exchangeable	Reducible	Oxidisable	Residual	Sum	PT
A	21.0 ± 0.5[a]	8.45 ± 1.07[b]	10.2 ± 1.4[a]	64.6 ± 8.6[a]	104 ± 9	109 108
A'	27.6 ± 0.4	9.13 ± 0.31	9.88 ± 0.28	105 ± 4	152 ± 4	
B	21.3 ± 0.6	9.54 ± 0.96	9.61 ± 0.41	63.1 ± 3.5	104 ± 4	
C'	28.5 ± 0.8	15.4 ± 0.5	9.13 ± 0.80	98.3 ± 13.5	151 ± 14	

A n = 4

B n = 3

The majority of manganese present in the White Cart sediment was present as residual manganese and is unlikely to be mobilised in the environment. The sums from methods A and B compare well with the PT value; however, the sums from methods A' and C' are approximately 50 % higher than the PT values. These experiments were carried out on separate days and therefore the differences may be due to experimental conditions on each day. Standard deviations for manganese were found to be small, generally less than 10 %.

4.20 Comparison of methods

A/A'	Exchangeable	Reducible	Oxidisable	Residual
T stat	23.4	1.07	0.387	9.20
t critical on two tails	2.36	4.30	3.18	2.36
P (T ≤ t)	6.6×10^{-8}	0.398	0.725	3.69×10^{-5}
A/B	**Exchangeable**	**Reducible**	**Oxidisable**	**Residual**
T stat	0.794	1.49	0.740	0.355
t critical on two tails	2.36	2.45	3.18	2.36
P (T ≤ t)	0.453	0.185851	0.513	0.733
A'/C'	**Exchangeable**	**Reducible**	**Oxidisable**	**Residual**
T stat	2.24	23.8	1.96	1.06
t critical on two tails	2.31	2.31	2.57	2.57
P (T ≤ t)	0.0554	1.03×10^{-8}	0.107	0.336

Very few significant differences were noted between the three methods at each stage. Only exchangeable A/A', reducible A'C' and residual A/A' comparisons were significantly different. Manganese associated with reducible components of the soil appears to have been released to a greater extent in the modified method C'. However this increase is small in comparison to increases seen for iron and lead. This suggests that as with the Great Billing soil the changes in reductant concentration and pH had little effect on the manganese recovered from the sediment.

4.3.2.5 Zinc

Table 4.21 Zinc in White Cart sediment (mg kg^{-1})

Method	Exchangeable	Reducible	Oxidisable	Residual	Sum	PT
A	35.3 ± 0.7[a]	10.4 ± 2.2[a]	14.5 ± 2.7[a]	26.3 ± 1.8[a]	86.5 ± 4.0	80.9
A'	45.4 ± 1.8	11.0 ± 0.7	14.6 ± 1.6	50.5 ± 1.5	122 ± 3	80.5
B	34.8 ± 0.7	10.2 ± 0.6	15.1 ± 1.2	22.8 ± 1.9	82.9 ± 2.4	
C'	44.7 ± 1.6	17.1 ± 1.3	15.1 ± 0.7	43.8 ± 3.4	121 ± 4	

a n = 4

Zinc levels were found to be reasonably low, with the major proportions being present in the exchangeable and residual stages. This suggests that zinc may be reasonably mobile in the environment. Standard deviations were once more found to generally less than 10 %, with the exception of method A reducible and oxidisable steps. Method A and B sums compare well with the PT value, however methods A' and C' sums were found to be approximately 50% higher than the PT values. This was also found for manganese, indicating that the two metals behaved in similar ways during the experiments. However, it is unclear as to the precise reasons for the differences between the sums of A/B and the sums of A'/C'.

Table 4.22 Comparison of methods

A/A'	Exchangeable	Reducible	Oxidisable	Residual
T stat	10.6	0.490	0.0844	22.2
t critical on two tails	2.36	2.78	2.36	2.36
P (T ≤ t)	1.48×10^{-5}	0.650	0.935	9.48×10^{-8}
A/B	**Exchangeable**	**Reducible**	**Oxidisable**	**Residual**
T stat	0.937	0.143	0.466	2.78
t critical on two tails	2.36	3.18	2.36	2.36
P (T ≤ t)	0.380	0.895	0.655	0.0273
A'/C'	**Exchangeable**	**Reducible**	**Oxidisable**	**Residual**
T stat	0.589	9.33	0.669	4.07
t critical on two tails	2.31	2.31	2.31	2.31
P (T ≤ t)	0.572	1.43×10^{-5}	0.522	0.00361

Exchangeable and residual A/A' comparisons resulted in significant differences, suggesting that from day to day the extractions were not repeatable. The only other significant differences were residual A/B, reducible A'/C' and residual A'/C'. The most significant difference was for the reducible A'C' comparison, although the difference observed was small. Zinc appears to be affected very little by the changes in reductant concentration and pH, however a slight increase in zinc released by the decrease in pH was noted.

4.4.3 IG 18-28

4.4.3.1 Copper

Table 4.23 Copper in IG 18-28 (mg kg^{-1})

Method	Exchangeable	Reducible	Oxidisable	Residual	Sum	PT
A	70.8 ± 4.1[a]	55.5 ± 1.5	157 ± 10	148 ± 5	431 ± 12	435
A'	74.9 ± 4.6[a]	63.4 ± 7.2	182 ± 18	211 ± 25[b]	531 ± 32	439
B	69.7 ± 2.2	77.9 ± 1.7	145 ± 8	133 ± 11	426 ± 14	
C'	75.4 ± 3.8	150 ± 11	97.3 ± 6.8	165 ± 26[b]	488 ± 29	

a n = 4
b n = 3

Standard deviations were all found to be below 10 % except for those cases where only 3 replicates were taken. This is likely to be the reason for the high standard deviations found for these two results. The sums from method A and B compare well with the PT values, however the sums from method A' and C' were slightly higher than the PT value. This was observed for manganese and zinc in White Cart sediment to a greater extent and is likely to be due to day to day reproducibility. It is however unclear as to the nature of the day to day variations. Once more the main difference between the different procedures appears to be an increase in copper extracted in the reducible step of procedure C', followed by a reduction in the oxidisable step, when compared to procedure A'. These differences are illustrated further by the use of statistical analysis.

Table 4.24 Comparison of methods

A/A'	Exchangeable	Reducible	Oxidisable	Residual
T stat	1.33	2.40	2.71	4.33
t critical on two tails	2.45	2.78	2.31	4.30
P (T ≤ t)	0.230	0.0740	0.0265	0.0495
A/B	**Exchangeable**	**Reducible**	**Oxidisable**	**Residual**
T stat	0.500	22.1	2.07	2.73
t critical on two tails	2.36	2.31	2.31	2.31
P (T ≤ t)	0.632	1.83×10^{-8}	0.0721	0.0260
A'/C'	**Exchangeable**	**Reducible**	**Oxidisable**	**Residual**
T stat	0.172	14.4	9.82	2.23
t critical on two tails	2.36	2.31	2.57	2.78
P (T ≤ t)	0.868	5.19×10^{-7}	0.000187	0.0895

Slight differences between the two identical methods A and A' for the oxidisable and residual stages may be due to heterogeneity of the soil, but are more likely due to the accumulation of experimental error throughout the procedure. No significant differences were observed between the metals extracted by the exchangeable step of the procedure for any of the comparisons. This suggests that comparisons made between results from the same batch of experiments can be relied upon. The most significant differences were observed in the comparisons of the reducible stages of the various methods. Increasing reductant concentration was seen to increase copper extracted by the reducible stage, an effect that was further enhanced by decreasing the pH of the reductant. This was compensated for by a decrease in copper extracted by the oxidisable step. Copper trends were similar to those observed for copper in the Great Billing soil and could also be explained by re-adsorption effects noted by Tipping *et al*[24]. The copper could however be associated with more resistant forms of iron oxides which could only be broken down by the stronger reducing agent used in method C'.

4.4.3.2 Iron

Table 4.25 Iron in IG 18-28 (mg kg^{-1})

Method	Exchangeable	Reducible	Oxidisable	Residual	Sum	PT
A	37.2 ± 10.4[a]	1360 ± 76	1010 ± 108	53400 ± 2600	55800 ± 2610	40400 52700
A'	37.5 ± 11.0	1670 ± 44	666 ± 48	66500 ± 9370[b]	68900 ± 9370	
B	36.6 ± 15.8	1900 ± 102	1060 ± 134	48800 ± 3800	51800 ± 3800	
C'	25.6 ± 3.2	4280 ± 116	1060 ± 192	60800 ± 9570[b]	66200 ± 9580	

a n = 4
b n = 3

Exchangeable standard deviations were found to be high. This is likely to be due to the relatively low concentrations of iron found which were close to the detection limits of the ICP AES. Although the remaining standard deviations were above 10 %, they were all below 20 %, which would be within the expected range for a heterogeneous soil such as IG 18-28. Iron would be expected to be especially variable as the description of the material indicates clinker, etc. present in the soil. All sums appear to be higher than the PT results, but this may be due to variability present in the method.

Table 4.26 Comparison of methods

A/A'	Exchangeable	Reducible	Oxidisable	Residual
T stat	0.0322	7.66	6.58	2.38
t critical on two tails	2.36	2.31	2.31	4.30
P (T ≤ t)	0.975	5.94×10^{-5}	0.000173	0.140
A/B	**Exchangeable**	**Reducible**	**Oxidisable**	**Residual**
T stat	0.0734	9.51	0.574	2.23
t critical on two tails	2.36	2.31	2.31	2.31
P (T ≤ t)	0.944	1.24×10^{-5}	0.582	0.0566
A'/C'	**Exchangeable**	**Reducible**	**Oxidisable**	**Residual**
T stat	2.31	47.0	4.43	0.741
t critical on two tails	2.57	2.57	2.78	2.78
P (T ≤ t)	0.0691	8.22×10^{-8}	0.0114	0.500

Results from methods A and A' were found to differ significantly in the reducible and oxidisable phases. As in the case of iron this may be due to heterogeneity of the soil, but is more likely to be due to the multiplication of experimental error, due to the dilution of samples prior to the measurement in the samples. No exchangeable stages showed significant differences, as would be expected. Although methods A and B differed significantly at the reducible stage, it is difficult to assess the meaning of this as the probability of the A results being different from the B results is of the same magnitude as A differing from A'. Results from the reducible A'/C' comparison however were more significant, suggesting that decreasing pH and increasing reductant concentration increases iron extracted by the reductant. Oxidisable differences were insignificant for A/B and A'/C' differences were less significant than A/A' differences. Residual comparisons showed no significant differences between any of the methods.

4.4.3.3 Lead

Table 4.27 Lead in IG 18-28 (mg kg^{-1})

Method	Exchangeable	Reducible	Oxidisable	Residual	Sum	PT
A	9.93 ± 0.45[a]	52.9 ± 3.3	48.5 ± 10.6	318 ± 15	429 ± 19	464
A'	15.6 ± 3.4	77.6 ± 6.1	57.5 ± 7.7	295 ± 5[b]	446 ± 12	567
B	9.31 ± 1.57	115 ± 5	26.9 ± 7.9	277 ± 14	428 ± 17	
C'	14.3 ± 1.4	227 ± 19	9.94 ± 4.31	196 ± 13[c]	447 ± 23	

a n = 4
b n = 3
c n = 2

Exchangeable and oxidisable standard deviations were found to be high; however, this is likely to be due to the relatively low levels of lead in this soil and the poor detection limits for lead. Exchangeable levels of lead measured by the PE Plasma II are all within a factor of ten of the detection limits of the instrument. The remaining results contained high amounts of lead and therefore much lower relative standard deviations were observed. The sums of the lead present in the soils compared well with each other however were below the levels found by PT methods. The main difference between the methods is the increased extraction of lead by the reducible step for the modified methods. Statistical analysis illustrates the significance of the differences between the results.

Table 4.28 Comparison of methods

A/A'	Exchangeable	Reducible	Oxidisable	Residual
T stat	3.75	8.01	1.54	2.06
t critical on two tails	2.78	2.31	2.31	2.57
P (T ≤ t)	0.0199	4.31×10^{-5}	0.163	0.0942
A/B	**Exchangeable**	**Reducible**	**Oxidisable**	**Residual**
T stat	0.836	24.2	3.65	4.52
t critical on two tails	2.57	2.31	2.31	2.31
P (T ≤ t)	0.441	9.01×10^{-9}	0.00648	0.00195
A'/C'	**Exchangeable**	**Reducible**	**Oxidisable**	**Residual**
T stat	0.855	16.4	12.1	9.85
t critical on two tails	2.31	2.57	2.31	3.18
P (T ≤ t)	0.418	1.54×10^{-5}	2.02×10^{-6}	0.00222

Exchangeable and reducible A/A' results do not compare well. This may be due to the low levels of lead extracted in the exchangeable step, and may also be due to heterogeneity of the soil. Exchangeable results carried out in the same day compare well, however the reducible results showed significant differences. These results are consistent with observations made for the Great Billing soil, suggesting that the lead is extracted by similar mechanisms for both soils. It should be noted that conclusions made concerning the increase in metal extracted by the reducible step could only be tentative due to the variation in the reducible steps for identical methods at this stage of the extraction.

4.4.3.4 Manganese

Table 4.29 Manganese in IG 18-28 (mg kg^{-1})

Method	Exchangeable	Reducible	Oxidisable	Residual	Sum	PT
A	38.3 ± 1.6[a]	85.7 ± 5.4	19.2 ± 1.0	262 ± 17	405	370
A'	44.0 ± 1.5	72.8 ± 2.4[a]	17.4 ± 2.6	439 ± 39[b]	573	370
B	38.0 ± 1.9	97.1 ± 5.9	22.1 ± 3.0	245 ± 19	402	
C'	45.8 ± 6.5	96.9 ± 9.3	23.6 ± 4.1	392 ± 59[b]	558	

a n = 4
b n = 3

Relative standard deviations were generally low for manganese, with the oxidisable stage showing the largest variation. This is likely due to the method in which the manganese was extracted from the soil at this stage. Sums for manganese were higher than the PT values. This trend was observed for the Great Billing soils and also for A' and C' for the White Cart sediment. It is possible that the accumulation of error increases the manganese content observed in the sum compared to the PT values.

Table 4.30 Comparison of methods

A/A'	Exchangeable	Reducible	Oxidisable	Residual
T stat	5.4414	4.4117	1.3836	9.2606
t critical on two tails	2.3646	2.3646	2.5706	2.4469
P (T ≤ t)	0.000965	0.003113	0.225077	8.96 x 10-5
A/B	**Exchangeable**	**Reducible**	**Oxidisable**	**Residual**
T stat	0.2536	3.2180	2.1019	1.5283
t critical on two tails	2.3646	2.3060	2.5706	2.3060
P (T ≤ t)	0.807124	0.012275	0.089535	0.164949
A'/C'	**Exchangeable**	**Reducible**	**Oxidisable**	**Residual**
T stat	0.6023	5.5914	2.8089	1.1631
t critical on two tails	2.7765	2.5706	2.3060	2.7765
P (T ≤ t)	0.579434	0.002525	0.022881	0.309452

Only the oxidisable step of methods A and A' compared well. It is unclear as to the reason for the differences in other steps. They may be due to heterogeneity of the soil, experimental error, etc., but it is obvious that results carried out on different days can not be compared. A/B and A'/C' results compare very well, showing very little difference between results. Increasing the reductant concentration appears to have increased manganese extracted at this stage only slightly (85.7 mg kg^{-1} to 97.1 mg kg^{-1}). Reducing pH and increasing reductant concentration also increased manganese extracted (72.8 mg kg^{-1} to 96.9 mg kg^{-1}) at the reducible stage and increased manganese extracted by the oxidisable step (17.4 to 23.6 mg kg^{-1}). In this case manganese appears to be effected by the change in method. This differs from the previous samples studied, which were not affected by the changes to the procedure. The differences may be due to day to day reproducibility for this sample (IG 18-28 was a much more heterogeneous sample than White Cart sediment and Great Billing soil). Further investigation as to the reasons for these differences would be required.

4.4.3.5 Zinc

Table 4.31 Zinc in IG 18-28 (mg kg^{-1})

Method	Exchangeable	Reducible	Oxidisable	Residual	Sum	PT
A	252 ± 14[a]	113 ± 8	110 ± 8	167 ± 18	642 ± 25	579
A'	266 ± 12[a]	119 ± 6[a]	106 ± 4	230 ± 18[b]	721 ± 23	565
B	254 ± 8	143 ± 10	104 ± 8	145 ± 7	646 ± 17	
C'	257 ± 21	157 ± 14	68.5 ± 4.7	171 ± 30[b]	654 ± 39	

a n = 4
b n = 3

Relative standard deviations were all low for zinc, with the highest being 18 % for method C' residual (with only three replicates). Sums compared reasonably with each other however were again slightly higher than the PT value. The major difference between methods once more occurs as an increase in the metal extracted by the reducible step of the SE. The significance of this is shown in table 4.32.

Table 4.32 Comparison of methods

A/A'	Exchangeable	Reducible	Oxidisable	Residual
T stat	1.51	1.36	1.11	4.71
t critical on two tails	2.45	2.36	2.31	2.45
P (T ≤ t)	0.183	0.217	0.299	0.00329
A/B	**Exchangeable**	**Reducible**	**Oxidisable**	**Residual**
T stat	0.237	5.46	1.34	2.56
t critical on two tails	2.36	2.31	2.31	2.31
P (T ≤ t)	0.819	0.000603	0.218	0.0339
A'/C'	**Exchangeable**	**Reducible**	**Oxidisable**	**Residual**
T stat	0.737	4.99	13.0	2.87
t critical on two tails	2.36	2.36	2.31	2.78
P (T ≤ t)	0.485	0.00158	1.16×10^{-6}	0.0454

All steps of method A and A' compared well with the exception of the residual step. This may be due to the accumulation of error throughout the experiment. All of the exchangeable results also compared favourably, however significant differences were observed for all other stages in both A'/C' and A/B comparisons (with the exception of A/B oxidisable). It is suspected that zinc may also be related to crystalline iron oxyhydroxide minerals, which could only be reduced by the stronger reducing agent or may be re-adsorbed as in the case of copper and lead. Zinc extracted during the oxidisable step, by method C' was less than method A', most probably because zinc which would normally be released by the oxidisable stage was released by the

reducible stage. This effect may be present in the A/B comparison, but the effect may not be significant.

The most significant effect throughout the experiments described previously was the increased metal extraction in the reducible stage of method C' compared to A'. For this reason it was decided to apply only these to methods to the remaining two samples studied.

4.4.4 IG 47-50

Only methods A' and C' were studied for IG 47-50 as these were seen to be the methods between which the largest differences occurred in previous samples. The results and comparisons of methods are shown in tables 4.33 – 4.42.

4.4.4.1 Copper

Table 4.33 Copper in IG 47-50 (mg kg^{-1})

Method	Exchangeable	Reducible	Oxidisable	Residual	Sum	PT
A'	149 ± 14	118 ± 8	354 ± 56	307 ± 22	928 ± 62	1000
C'	130 ± 15	353 ± 35	219 ± 22	218 ± 11	920 ± 45	1030

All standard deviations were found to be reasonable with the oxidisable method A' results having the highest value at 16 %. Sums compared well with each other, and were only slightly lower than the PT value. IG 47-50 contains the highest levels of copper observed in the samples studied in this chapter. The enhanced extraction of copper in the reducible step of method C' was again noted with a decrease in both

the oxidisable and residual stages. The use of statistical analysis shows the significance of these results.

Table 4.34 Comparison of methods

A'/C'	Exchangeable	Reducible	Oxidisable	Residual
T stat	2.04	14.6	5.04	8.03
t critical on two tails	2.31	2.78	2.57	2.31
P (T ≤ t)	0.0758	0.000129	0.00396	4.25×10^{-5}

Copper A'/C' exchangeable results were not significantly different; however, all other A'/C' phases were found to differ significantly between methods. As in previous cases the stronger reductant was seen to increase the copper extracted during the reducible stage. A slight decrease in copper extracted by the oxidisable and residual stages was then observed. As mentioned in the case of Great Billing and IG 18-28 this increased copper extraction was thought to be due to redistribution effects.

4.4.4.2 Iron

Table 4.35 Iron in IG 47-50 (mg kg^{-1})

Method	Exchangeable	Reducible	Oxidisable	Residual	Sum	PT
A'	32.4 ± 5.1	2030 ± 116	870 ± 85	61700 ± 5270	64600 ± 5270	85500 83400
C'	26.7 ± 3.6	6510 ± 187	1010 ± 70	56200 ± 5010	63800 ± 5020	

All relative standard deviations were reasonable with the highest being for the exchangeable stage. This is likely to be due to the low levels of iron extracted at this stage of the procedure. Once more the sums of the iron extracted by SE compare

well with one another; however are much lower than the values obtained by PT methods. This is not consistent with previous findings where pseudototal (PT) iron was found to be slightly lower than the sum of the sequential extraction results. The reason for this is unclear.

Table 4.36 Comparison of methods

A'/C'	Exchangeable	Reducible	Oxidisable	Residual
T stat	2.01	45.5	2.78	1.68
t critical on two tails	2.31	2.31	2.31	2.31
P ($T \leq t$)	0.0787	6.05×10^{-11}	0.0241	0.132

Iron A'/C' exchangeable results were found not to be significantly different, indicating that the two sets of results could be compared. The most significant difference was noted between the two reducible results. A large increase in iron extracted was observed when the reductant concentration was increased and the pH decreased. Again this is likely to be due to the ability of the stronger reducing agent to attack the more crystalline forms of iron. An increase was also observed at the oxidisable step, but no significant difference was observed in comparing the residual stages. Although the difference may not be significant, one may be present that would account for extra iron released earlier in the method.

4.4.4.3 Lead

Table 4.37 Lead in IG 47-50 (mg kg^{-1})

Method	Exchangeable	Reducible	Oxidisable	Residual	Sum	PT
A'	38.1 ± 10	137 ± 23	382 ± 55	928 ± 25	1490 ± 65	1560
C'	35.9 ± 10	763 ± 79	99.9 ± 13.2	604 ± 22	1500 ± 84	1600

Standard deviations were found to be high for the exchangeable stage of the SE. This was likely to be due to the low levels of lead detected in these solutions. Relative standard deviations were observed to decrease as the lead levels increased. Relative standard deviations of 3 and 4 % were observed for the residual stage of methods A' and C' respectively. The sums of the SE compare reasonably well with each other and with the PT values. IG 47-50 contains the highest levels of lead of the samples studied. Similar trends (increased extraction of lead from the reducible stage and decreased extraction from the oxidisable and residual for method C' in comparison to A') to those previously seen for lead were once more observed.

Table 4.38 Comparison of methods

A'/C'	Exchangeable	Reducible	Oxidisable	Residual
T stat	0.352	16.9	10.9	21.8
t critical on two tails	2.31	2.57	2.57	2.31
P ($T \leq t$)	0.734	1.31×10^{-5}	0.000111	2.09×10^{-8}

Results for lead extracted by the exchangeable step, compare very well, indicating that the two sets of results can be compared. Greater amounts of lead were released by method C' at the reducible step, but significantly less lead was released at the oxidisable and residual steps of the extraction. Once more, some of the lead present in the soil may be related to the crystalline iron oxides and hydroxides that can only

be attacked by the harsher reducing agent used in method C'. The lead removed was then not available during later stages in the method resulting in the decreased oxidisable and residual results. In this case sums of the 4 steps compared favourably with the pseudototal results.

4.4.4.4 Manganese

Table 4.39 Manganese in IG 47-50 (mg kg^{-1})

Method	Exchangeable	Reducible	Oxidisable	Residual	Sum	PT
A'	123 ± 7	595 ± 19	36.7 ± 7.1	452 ± 28	1210 ± 35	1290
C'	105 ± 10	651 ± 53	27.5 ± 1.6	396 ± 19	1180 ± 57	1310

All RSD's were low with the exception of the oxidisable results for method A'. This was probably due to the low levels of manganese found in the solutions at this step of the SE. Sums compare well with one another, however they are slightly lower than the PT value.

Table 4.40 Comparison of methods

A'/C'	Exchangeable	Reducible	Oxidisable	Residual
T stat	3.30	2.21	3.73	3.69
t critical on two tails	2.31	2.57	2.78	2.31
P (T ≤ t)	0.0108	0.0786	0.020343	0.00610

A significant difference was observed between exchangeable results for manganese.

This suggests that the two sets of results may not be comparable. It is unclear as to the reason for these differences, but no conclusions can therefore be made as to the effect of a stronger reductant on manganese released at various stages of the procedure. Although the differences were significant at most stages, they were not

large. This may indicate that manganese is once more effected less by the modifications made to the procedure.

4.4.4.5 Zinc

Table 4.41 Zinc in IG 47-50 (mg kg^{-1})

Method	Exchangeable	Reducible	Oxidisable	Residual	Sum	PT
A'	1670 ± 88	531 ± 8	418 ± 42	508 ± 15	3130 ± 99	3380
C'	1500 ± 79	847 ± 41	256 ± 7	365 ± 26	2970 ± 93	3220

Of particular concern in this soil are the high levels of zinc present in the exchangeable stage. This could however be an advantage if soil flushing was to be used as a remediation option. RSD's for zinc were low, with the highest being 10 % for the oxidisable method A'. Sums of the SE steps compared well between the two methods and were also close to the PT value.

Table 4.42 Comparison of methods

A'/C'	Exchangeable	Reducible	Oxidisable	Residual
T stat	3.20	16.7	8.41	10.5
t critical on two tails	2.31	2.78	2.78	2.31
P (T ≤ t)	0.0126	7.49×10^{-5}	0.00109	5.85×10^{-6}

Results for zinc extracted in the exchangeable step of the procedures were not significantly different, however, large significant differences were noted for the reducible and residual stages suggesting that zinc was effected in a similar way to copper by the modifications to the procedure. The stronger reducing agent was able to release more zinc at the reducible stage and concomitantly less zinc at the

oxidisable stage, either through redistribution or association with minerals resistant to the weaker reducing agent used in method A.

4.4.5 Whitehaven

The results from the SE of the Whitehaven sediment, using method A' and method C' are shown in tables 4.43 – 4.52 with the comparisons of the two methods.

4.4.5.1 Copper

Table 4.43 Copper in Whitehaven sediment (mg kg^{-1})

Method	Exchangeable	Reducible	Oxidisable	Residual	Sum	PT
A'	2.11 ± 0.26	< 0.07	19.7 ± 1.6	5.91 ± 0.64	27.7 ± 1.7	26.2
C'	2.05 ± 0.23	5.73 ± 0.25	17.8 ± 3.4[a]	5.38 ± 1.42	31.0 ± 3.7	26.8

a n = 4

RSD's for copper were reasonable with the highest RSD being 26 % for method C, residual step. Levels of copper found in solution for these soils were very low and were within a factor of 10 of the detection limits for copper. At these levels % RSD's are expected to be higher due to low signal to background ratios. Sums compared reasonably with each other and also with the PT value.

Table 4.44 Comparison of methods

A'/C'	Exchangeable	Reducible	Oxidisable	Residual
T stat	0.3598		1.0807	0.7602
t critical on two tails	2.3060		2.3646	2.3060
P (T ≤ t)	0.728305		0.315656	0.468969

Very little copper was found to be present in the Whitehaven samples. No significant differences were observed between the two methods for copper. Although no significant differences were observed by statistical comparison, method C' reducible stage extracted a detectable amount of copper, whereas no copper was detected in the reducible samples of method A'. Since the levels of copper were so low in the oxidisable and residual stages, differences would not necessarily be expected to differ. At low copper levels found in Whitehaven soil, the change in procedure did not effect the speciation.

4.4.5.2 Iron

Table 4.45 Iron in Whitehaven sediment (mg kg^{-1})

Method	Exchangeable	Reducible	Oxidisable	Residual	Sum	PT
A'	1084 ± 84	1205 ± 112	4343 ± 365	13275 ± 453	19907 ± 598	23107 22185
C'	1035 ± 137	3286 ± 59	5483 ± 334	10383 ± 604	20187 ± 706	

Relative standard deviations were all low. Instrumentally this would be expected due to the high levels of iron present in the soil (therefore high signal to background ratio). For a soil however reasonably high relative standard deviations can be expected due to the heterogeneous nature of soil. The low RSD's observed for iron suggest that the sediment from Whitehaven was homogeneous. Sums for the methods appear to compare well with each other and also compare reasonably with the PT values. As in previous cases the largest difference between the methods appears to be the increased extraction of iron in the reducible step.

Table 4.46 Comparison of methods

A'/C'	Exchangeable	Reducible	Oxidisable	Residual
T stat	0.6855	36.7604	5.1538	8.5662
t critical on two tails	2.3060	2.3060	2.3060	2.3060
P ($T \leq t$)	0.512416	3.29×10^{-10}	0.00087	2.66×10^{-5}

No significant difference was observed between the exchangeable results indicating that the two sets of results can be compared. A large significant difference was noted between the reducible results indicating that more iron was released by the stronger reducing agent in method C'. This is in agreement with previous results for iron, and suggests that the stronger reducing agent enables different forms of iron to be released such as crystalline iron oxides and hydroxides. Oxidisable results also showed increased extraction of iron by method C'. This suggests that the stronger reducing agent also released some iron available in the oxidisable stage using method A'. Residual results were decreased by method C', indicating that some of the iron species released by the stronger reducing agent would be present in the residual extraction of method A'.

4.4.5.3 Lead

Table 4.47 Lead in Whitehaven sediment (mg kg^{-1})

Method	Exchangeable	Reducible	Oxidisable	Residual	Sum	PT
A'	< 0.3	< 0.4	3.57 ± 1.31	37.1 ± 6.6	40.7 ± 6.7	43.2
C'	< 0.3	30.1 ± 2.8	1.70 ± 0.90	20.6 ± 1.6	52.4 ± 3.3	49.2

Relative standard deviations were high for lead. This is likely to be due to the low levels of lead detected in the soil, which were approaching the detection limits of the ICP AES for lead. Levels of lead in the oxidisable phase were within a factor of 10

of the limits of detection. At these low levels, signal to background ratios were likely to be very low, resulting in high RSD's. The sum for method C' is slightly higher than that for method A'.

Table 4.48 Comparison of methods

A'/C'	Exchangeable	Reducible	Oxidisable	Residual
T stat			2.6269	5.4726
t critical on two tails			2.3060	2.7765
P (T ≤ t)			0.030323	0.005425

Lead levels were found to be very low in the Whitehaven sediment, with the majority of the lead being present in more stable forms. Lead was detected in the method C' reducible extracts, but not in method A' extracts. Oxidisable and residual results were found to be significantly different suggesting that lead in the Whitehaven soil was effected in a similar way to lead in previous soils studied.

4.4.5.4 Manganese

Table 4.49 Manganese in Whitehaven sediment (mg kg^{-1})

Method	Exchangeable	Reducible	Oxidisable	Residual	Sum	PT
A'	158 ± 6	28.7 ± 1.5	35.4 ± 3.3	106 ± 2.9	328 ± 8	329
C'	159 ± 6	58.9 ± 5.1	24.5 ± 2.8	84.6 ± 4.6	327 ± 10	326

Relative standard deviations were found to be low for manganese, this was expected due the homogeneity of the soil and the high levels of manganese present in the soil relative to the detection limits of the ICP AES. Sums were found to compare very favourably with one another and also with the PT value. A considerable proportion of the manganese was found to be present in the more mobile exchangeable extracts

of the method, with another large fraction being extracted from the residual step. A larger proportion of manganese was generally extracted during the reducible stage of the SE in previous samples.

Table 4.50 Comparison of methods

A'/C'	Exchangeable	Reducible	Oxidisable	Residual
T stat	0.2283	12.8000	5.6500	8.6048
t critical on two tails	2.3060	2.5706	2.3060	2.3060
P (T ≤ t)	0.82511	5.18×10^{-5}	0.000481	2.57×10^{-5}

Exchangeable manganese results were found to compare very well between the two methods, however significant differences were observed at all other stages of the SE. This was unexpected as in previous cases very few differences had been noted between the two methods for manganese extracted at each step. The fact that the Whitehaven sample was an inter-tidal sediment rather than a soil or freshwater sediment may suggest that manganese speciation is quite different to conventional soils and therefore significant differences were observed between the two methods.

4.4.5.5 Zinc

Table 4.51 Zinc in Whitehaven sediment (mg kg^{-1})

Method	Exchangeable	Reducible	Oxidisable	Residual	Sum	PT
A'	47.6 ± 1.4	9.81 ± 0.84	14.7 ± 2.7	39.4 ± 4.6	112 ± 6	109
C'	45.1 ± 2.2	19.7 ± 1.4	9.01 ± 0.78	38.7 ± 5.5	113 ± 6	120

Relative standard deviations were reasonable for zinc (all were below 20 %), with the worst case being for the oxidisable step of method A' at 18 %. Zinc levels in most cases were low in comparison to the detection limits of the ICP AES and

therefore slightly high RSD's were expected. Sums compared very well with each other for the two methods and also compared well with the PT value.

Table 4.52 Comparison of methods

A'/C'	Exchangeable	Reducible	Oxidisable	Residual
T stat	2.0982	13.8207	4.5071	0.2133
t critical on two tails	2.3060	2.3060	2.5706	2.3060
P (T<= t)	0.069128	7.14×10^{-7}	0.006358	0.836462

Zinc extracted at the exchangeable stage of both methods did not significantly differ. This was expected, as the two methods were identical at this stage. Significant differences were noted between the zinc extracted by the reducible and the oxidisable stage. As in previous cases these significant differences are thought to be due to zinc redistribution or association with phases resistant to attack by the weaker reducing agent used in method A.

4.5 Conclusions and Further Work

The effect of increasing reductant concentration and decreasing pH of the reductant in the BCR SE procedure was investigated for several different soils and sediments. It was hoped that this modification to the procedure might increase the reproducibility of the second stage of the extraction. The effect of the modified procedure on the order of extraction of metals within the soils was also observed.

Relative standard deviations were generally good (less than 20 %), although when the original experiment was repeated on separate days, results could not always be

compared (*e.g.* White Cart zinc exchangeable and residual stages). Some cases in which identical procedures could not be compared often contained low levels of the metal of interest or very low relative standard deviations were obtained, therefore good comparisons between samples would not necessarily be expected.

Differences were observed between the three methods used. Increasing reductant concentration was observed to slightly increase metals extracted, in particular copper, lead and iron. Decreasing the pH had a much greater effect in increasing the copper, lead and iron extracted from soils. It is thought that re-adsorption of lead and copper resulted in the lower levels of lead and copper in methods A and B in some soils. Decreasing the pH of the reductant prevented this re-adsorption and therefore higher levels of lead and copper were released at this stage of the procedure. It was thought that higher levels of iron were observed for method C' at the reducible stage due to the many different forms of iron present in soils and sediment. It may be possible that some forms were more resistant to attack by the weaker reducing agent used in method A, but could be attacked by the stronger reagent used in method C'. This would also have consequences for any metals associated with the iron oxides and oxhydroxides, which were more resistant to attack by the reducing agent used in method A. In order to confirm differing forms of iron within the soil the mineral forms should be further investigated using techniques such as x-ray diffraction.

The majority of the metals within the soils were at levels below the ICRCL guidelines and therefore not of great concern as pollutants. Copper and zinc total levels were high in Great Billing soil, IG 18-28 and IG 47-50. Lead was also high in

IG 47-50. Although the total levels of these metals were high enough to cause concern, copper in Great Billing and IG 18-28 and lead in IG 47-50, tended to be released in the later stages of the SE procedure. Copper and lead in these stages would be less available for plant uptake and would be less likely to be leached into the surrounding waters. Zinc was of particular concern as the majority was leached by exchangeable and reducible stages of the procedures. Although the zinc was found to be more mobile, this may be advantageous if considering soil washing or soil flushing as a remediation option for the land.

High levels of copper lead and zinc in some of the soils (especially the industrial ground) investigated would require the land to be remediated prior to any further uses such as playing fields etc. Various remediation technologies will be discussed in the remaining chapters. Soil flushing techniques, which recover metals from the floor by pumping extractant through the soil, were investigated. Also studied was the efficiency of phytoremediation, using dandelions, to remove metals from the soils.

Further work is required to investigate the reproducibility of the modified procedure between laboratories. This would include round robin trials such as carried out for the original BCR SE method[7].

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Chapter 5 – Column Leaching Studies

5.1 Introduction

There are several methods of contaminated soil remediation, some of which are described previously. In this research the use of chelating agents in column leaching experiments was investigated. Chelating agents can aid in the mobilisation and recovery of heavy metals from contaminated soils. Soils columns were set up and EDTA used as a chelating agent in the recovery of heavy metals from the soils. The operational speciation of the metals before and after treatment was also investigated.

5.1.1 Remediation by soil flushing

A distinction must be made between soil washing and soil flushing. Soil washing generally involves removing the soil from the site, sieving and treating compared to soil flushing which is an in-situ treatment of the soil to remove contaminants. Mann gives a detailed description of soil washing[1]. In most cases contamination will be concentrated in the fines (< 63 μm), while lower concentrations will exist in the sands (63 μm < x < 5 mm) and oversize (> 5 mm). The soils are therefore sieved and each fraction analysed. Not all fractions will necessarily require the same amount of remediation. The oversize fraction is removed mechanically, sands are removed using hydrocyclone combinations and treated (e.g. by froth flotation) before being returned to the site. Fines are then subjected to further treatment and thickened and pressed into 45-55 % dry solids filter cake, which should contain the target contaminants and is therefore disposed of off-site, depending on regulations. The USEPA[2] describes soil flushing as "an innovative treatment technology that floods

contaminated soils with a solution that moves the contaminants to an area where they are removed".

The status of soil flushing as a recent technology is described by the USEPA[3]. Soil flushing can be accomplished in situ, by applying fluid to the contaminated surface or injecting it into the contaminated zone. Leachate can then be recovered from the underlying groundwater. The flushing fluid is dependent upon the target contaminant. Water-soluble metals can be removed by water alone, but some contaminants may require the pH to be adjusted, an ion exchange system or chelating agents. Mobility of metals in soils will control the efficiency of the soil flushing procedure. It is therefore important to investigate soil properties such as particle size, pH, low cation exchange capacity (CEC) and organic matter content, before applying this approach. Some disadvantages are noted in that groundwater may become contaminated with unrecovered flushing solution and solubilized contaminants. The cost of reagents must also be considered before undertaking this type of remediation. The reuse of the flushing solution must therefore be considered. USEPA[4] suggest that suitable scenarios for soil flushing would include Cr(VI), As(III or V) in permeable soil with low iron oxide, low clay and high pH; Cd in permeable soil with low clay, CEC, and moderately acidic pH; and Pb in acid sands. The bulletin also notes that the flushing agent should not only be appropriate for the contaminant, but also the soil in question. For example if precipitates are formed within the soil the pores can become blocked and the flow of flushing solution to the contaminated zone become obstructed. Advantages noted by the bulletin include the removal of the metal from the soil (as opposed to containment)

and possible recovery and reuse of the metal. The hazards and expense associated with excavation, treatment and disposal of the soil are also avoided.

5.1.2 Chelating agents

Soil flushing reagents can include water alone, surfactants, acids, bases, and chelates. This study involved the use of chelates to extract metals from the contaminated soil. It is therefore important to understand chelating agents and their uses. Peters[5] defines a chelant as "a ligand that contains two or more electron-donor groups so that more than one bond is formed between the metal ion and the ligand".

Class A metal ions (hard acids) form more stable compounds with ligands containing N, O or F donor atoms, whereas class B ions prefer the heavier elements of groups 15, 16 and 17 as donor atoms. Borderline metal ions also exist, such as Fe, Co, Ni, Cu and Cd. Fig. 5.1 shows the classification of acceptor atoms.

H																	He
Li	Be											B	C	N	O	F	Ne
Na	Mg											Al	Si	P	S	Cl	Ar
K	Ca	Sc	Ti	V	Cr	Mn	Fe	Co	Ni	Cu	Zn	Ga	Ge	As	Se	Br	Kr
Rb	Sr	Y	Zr	Nb	Mo	Tc	Ru	Rh	Pd	Ag	Cd	In	Sn	Sb	Te	I	Xe
Cs	Ba	La	Hf	Ta	W	Re	Os	Ir	Pt	Au	Hg	Tl	Pb	Bi	Po	At	Rn
Fr	Ra	Ac	Unq	Unp	Unh	Uns											

Class A Class B Borderline

Figure 5.1 Classification of acceptor atoms in their common oxidation states[6]

There are several papers in the literature, which report the removal of metals from soil using a variety of chelating agents. The most frequently used chelating agent in the literature is EDTA[7, 8, 9, 10, 11]. Other chelating agents include citric acid[12], s-carboxymethylcysteine[13], pyridine-2,6-dicarboxylic acid[14] and many others[5].

In order that a chelating agent is efficient at recovering metals from contaminated soils it must have several properties. Chen *et al.*[15] have described these characteristics. Ligands must have high metal complexing abilities and, since contaminant metals tend to be transition metals or B-type (soft sphere) elements, ligands containing sulphur or nitrogen as donor atoms are preferred. A-type (hard sphere) metals prefer ligands with oxygen donor atoms. Multidentate ligands or chelators also form more stable complexes and would perform better than monodentate ligands. The stability of the metal complex must be considered when

investigating the recovery of the metal and chelator. Metals that form very stable complexes with the chelator may make recovery very difficult. EDTA may form very stable complexes with metals, but often cannot release the metals even at high pH. Although EDTA may not be recovered by increasing the pH alone, recovery has been reported by using a slight molar excess of sodium sulfide precipitant at moderately alkaline conditions[16]. Electrochemical recovery of EDTA has also been investigated[17], using an electrolysis cell, in which anode and cathode are separated by a cation exchange membrane.

Soil-metal interaction (e.g. complexation reactions), aqueous equilibrium chemistry of metals, and the co-ordination chemistry of the metals with the ligands must be considered when assessing a chelator for extraction and recovery of metals. The ligand must be able to overcome processes such as metal precipitation and surface complexation on soil particles.

A table of the 190 ligands assessed by Chen *et al.*[15], and their effective pH ranges are provided in their paper, together with results of extraction experiments with s-carboxymethyl-cysteine (SCMS), N-2-acetamidoiminodiactetic acid (ADA) and pyridine-2,6-dicarboxylic acid (PDA). The effective pH ranges of s-carboxymethyl-cysteine (SCMS), N-2-acetamidoiminodiactetic acid (ADA), ethylenediaminetetraacetic acid (EDTA), and pyridine-2,6-dicarboxylic acid (PDA) are shown in table 5.1.

Table 5.1 Effective pH ranges of chelators[15]

Chelator	Cu^{2+}	Pb^{2+}	Cd^{2+}	Zn^{2+}	Ni^{2+}	Hg^{2+}
EDTA	1.0-14.0	1.2-13.8	1.6-14.0	2.1-14.0	2.9-14.0	0.0-14.0
PDA	0.0-11.5	0.6-11.0	1.5-9.4	1.6-11.0	3.0-12.6	0.0-10.9
Oxalic acid	1.2-8.4	1.4-6.6	2.9-5.9	1.6-8.6	4.0-9.5	0.0-6.6
ADA	1.2-14.0	1.5-9.3	2.9-9.2	2.5-10.9	3.9-11.7	(none)
SCMC	2.8-10.8	(none)	*	5.5-9.8	5.0-11.2	*

* Equilibrium constants not available, so no pH ranges could be calculated.

5.2 Experimental

Experiments were designed to simulate soil flushing conditions with several EDTA solutions. Disodium EDTA was used as a leaching agent at pH 7 and pH 4.45, and diammonium EDTA was used at pH 7. Flushing solutions were 'sprinkled' over the soil (rather than using a head of water) to simulate flushing conditions. This also prevented additional effects of reduction which may occur in water-logged soils. The first two experiments were run over several days with the flow of leaching agent being stopped overnight. Results of these experiments showed that the leaching of metal from the soil as dependent on the contact time with the soil. Higher concentrations of metal were found in the flushing solution, which had been left in the columns overnight. As the leaching of metals in this way was observed to be subject to the kinetic effects described above, the third experiment was run continuously.

5.2.1 Column design

Column leaching experiments are designed to imitate processes occurring in the soil on a small scale. Such experiments include the study of leaching of heavy metals by artificial rainwater[18] and the remediation of lead contaminated soil by EDTA[7]. The design of the column is dependent upon the objectives of the experiment. Duncan[19] discusses the factors that must be taken into account when designing soil columns. The metals being studied must be considered, since metals with volatile species, such as mercury must include surface samplers to detect the volatile species. Set-ups range from lysimeters (*in-situ* columns) to using a dried soil slurry on a glass plate for thin layer chromatography. Duncan decided upon a simple column design that would reflect field conditions, so that no specialised equipment or expertise would be required to set it up. The column design used in this investigation was similar to that of Duncan, except for the use of a pump to deliver the soil flushing fluid.

A square of Lotrak geotextile fabric, Low Brothers and Co., Dundee, U.K., was placed at the bottom of the column to keep the soil in the column. 10 cm of soil (field moist) was placed in the column and 2 filter papers placed over the soil. The column was covered with aluminium foil and placed above a collecting vessel, as shown in figure 5.2. Four columns were set up together (figure 5.3).

5.2.2 EDTA preparation

0.05 M Disodium ethylenediamine tetraacetic acid (pH 7)

A solution of 0.05 M EDTA (disodium salt) was prepared by dissolving 18.61 g EDTA in distilled water. The solution was adjusted to pH 7 using concentrated ammonia and the final volume made up to 1 L with distilled water.

0.05 M Disodium ethylenediamine tetraacetic acid (pH 4.45)

A solution of 0.05 M EDTA (disodium salt) was prepared by dissolving 18.61 g EDTA in distilled water. The final volume made up to 1 L with distilled water.

0.05 M Diammonium ethylenediamine tetraacetic acid (pH 7)

A solution of 0.05 M EDTA (diammonium salt) was prepared by dissolving 16.32 g EDTA in distilled water. The solution was adjusted to pH 7 using concentrated ammonia and the final volume made up to 1 L with distilled water.

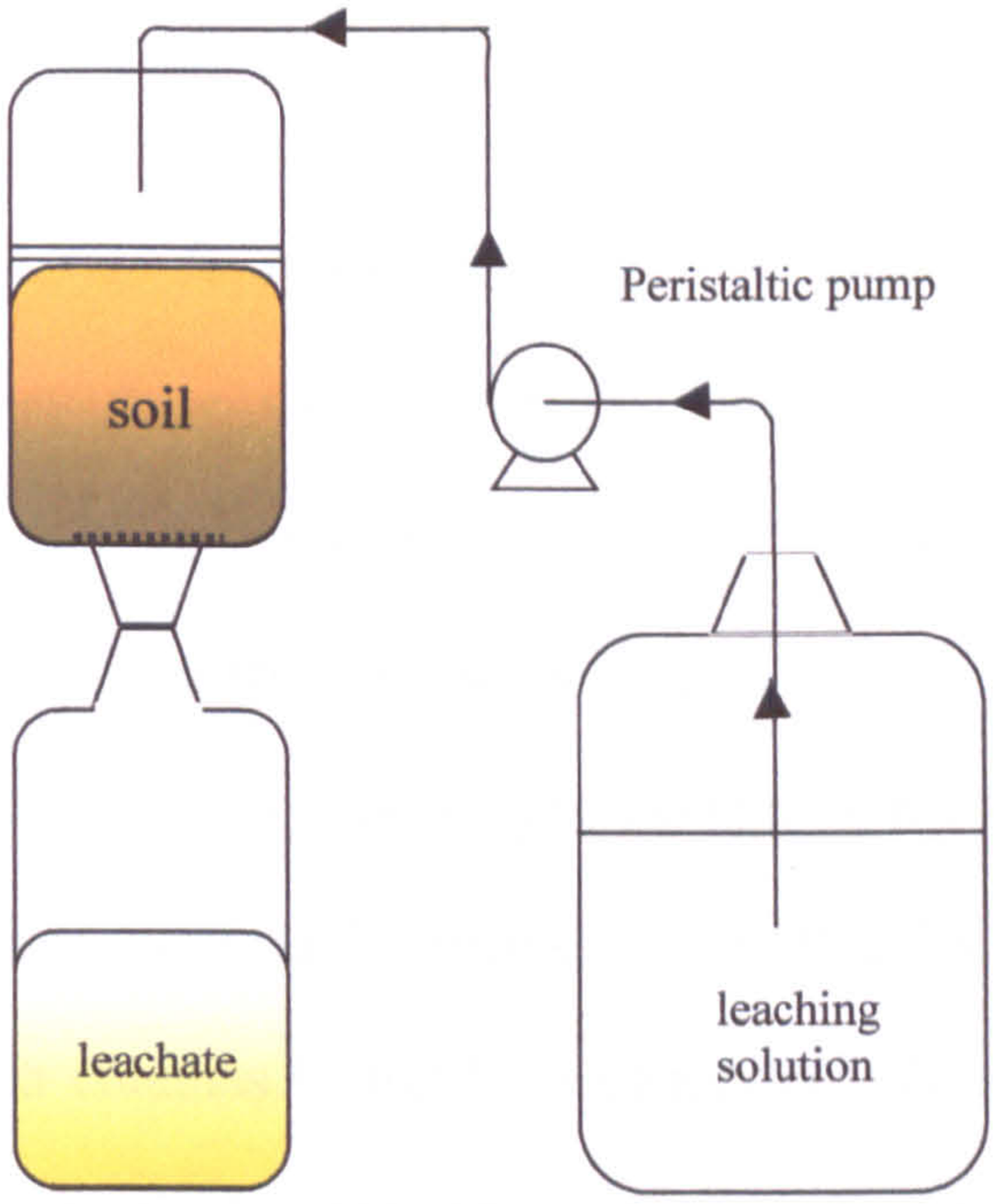

Figure 5.2 Schematic of soil flushing experimental set-up

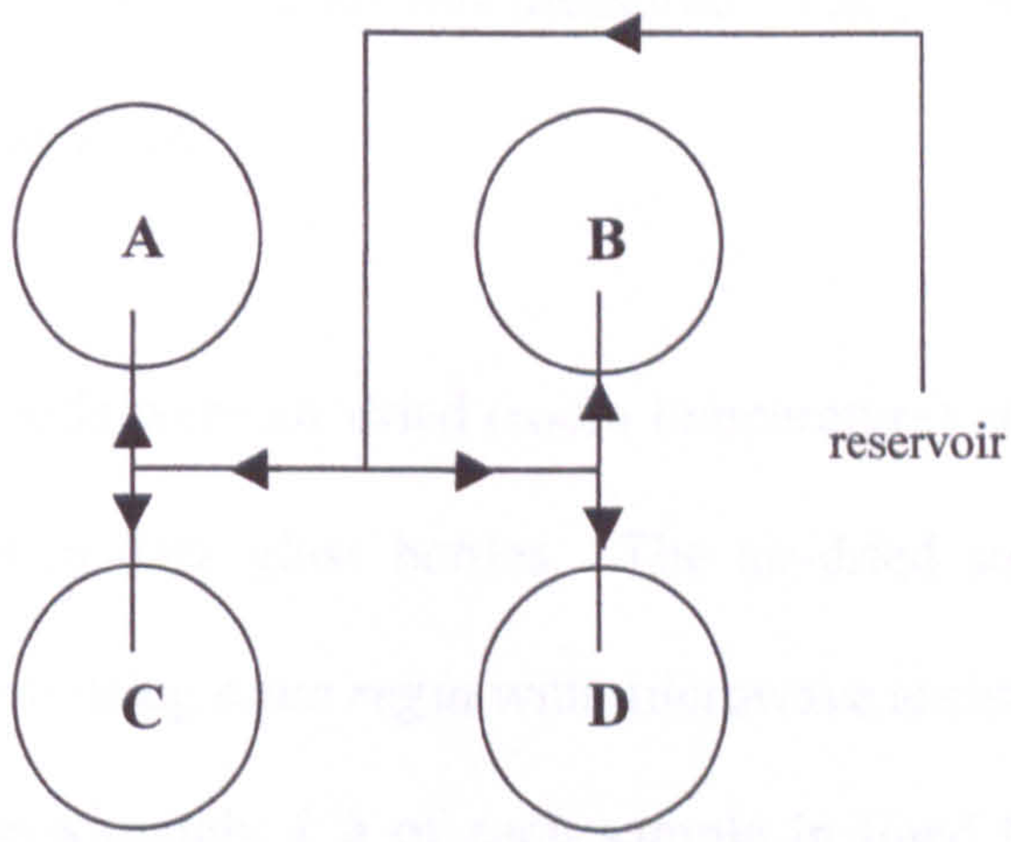

Figure 5.3 Four column soil flushing set-up

Experiment 1

Industrial made up ground as described previously was used in the first column experiment. Sub-samples were taken from the bulk sample by coning and quartering, for use in each of the columns. The columns were set up and 0.05 M disodium EDTA (pH 7) was pumped through each column at a rate of 5 ml min^{-1}. Approximately 5 L of 0.05 M EDTA was pumped through each column. Samples of leachate were collected every 500 ml for analysis. A further 2 L of distilled water was then pumped through columns C and D, leachate again being collected every 500 ml. In this experiment EDTA was not pumped continuously, but the pump switched off overnight, and the experiment run for five days. The accurate volume of sample collected every ~500 ml was measured. The pH and conductivity of each sample were also measured.

After leaching the soils were air-dried (room temperature) and sieved. The < 1 mm fraction was stored in dark glass bottles. The air-dried soils were subjected to a pseudototal digestion using *aqua regia* with microwave assistance. 20 ml *aqua regia* were added to approximately 1 g of each sample in lined teflon digestion vessels. The vessels were sealed and samples digested using the CEM MDS 2000. The digestion took place in two stages. The first stage raised the pressure within the vessels up to 60 psi and held the pressure for 5 minutes. The second stage increased the pressure to120 psi for 20 minutes. The samples were allowed to cool, then were filtered (Whatman 542 filter paper) and made up to 100 ml with distilled water.

Separate sub-samples of the leached soil were also subjected to sequential extraction (modified procedure) as described earlier. Samples of the unleached soil were also treated by sequential extraction and results compared with post leaching values in order to assess changes in metal fractionation due to the leaching experiment. Calcium, copper, iron, lead, manganese and zinc in *aqua regia* digests and sequential extracts were determined by ICP AES.

5.2.3 Experiment 2

Experiment 1 was repeated with the exception that 0.05 M disodium EDTA (pH 4.45) was used as the leachate.

5.2.4 Experiment 3

Experiment 1 was again repeated, using samples from the industrial made up ground in two columns and samples from Ardeer in the remaining two columns. In this experiment 0.05 M diammonium EDTA (pH 7) was used as the leachate. In order to carry out this experiment in one day the column size (and therefore mass of soil leached) was decreased. Approximately 200 g of sample was used in each column. Leachate samples were collected approximately every 200 ml and a total of approximately 2 L of EDTA solution flushed through each column. This experiment was run continuously.

5.3 Results and Discussion

5.3.1 Sequential Extraction of Industrial Ground and Ardeer soils prior to column leaching

The industrial ground IG 35-45 was subjected to SE prior to any column leaching experiments. The results from the SE and also a separate pseudototal digestion are shown in figures 5.4-5.9. The figures show the two replicate SE and three replicate pseudototal digestions for each metal. Data for the figures are given in appendix 2. Duplicate extractions were performed for each sample and the PT performed in triplicate. Standard deviations for replicate ICP AES measurements were not significant (i.e. less than 1 % on 3 replicates) and so were not included in the appendix. The values for the sequential extraction and the pseudototal leaches

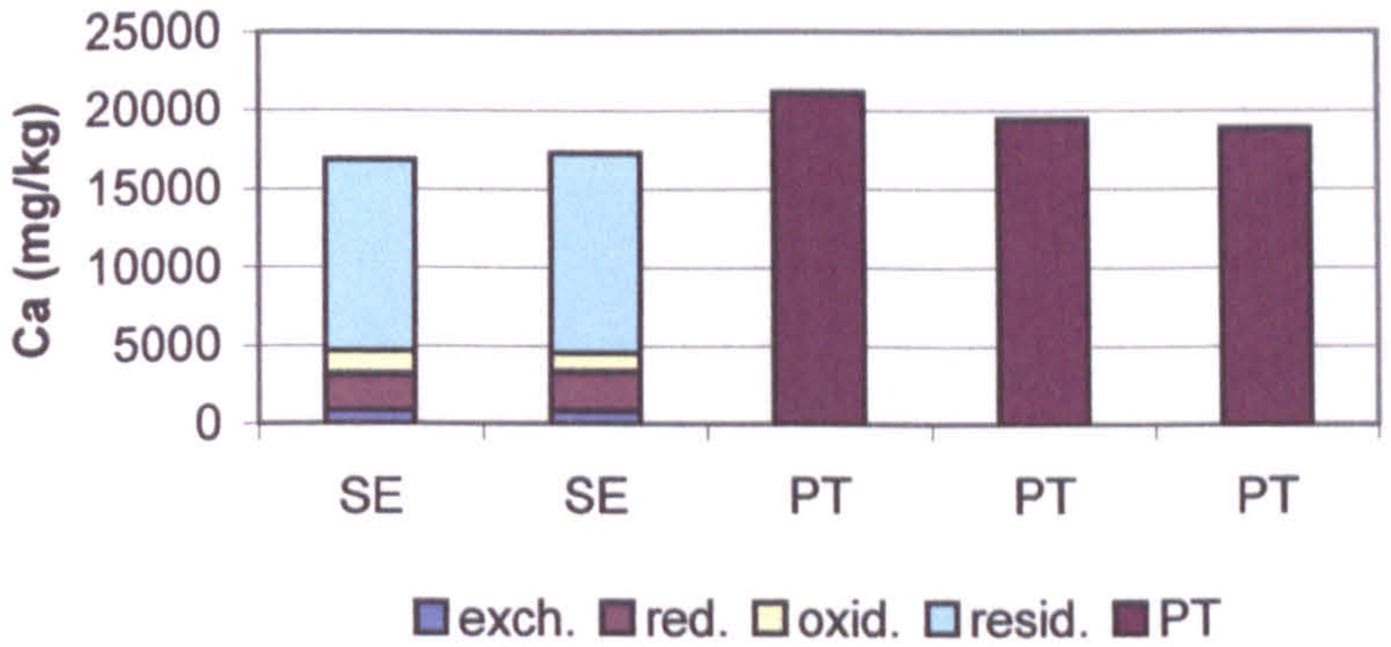

Figure 5.4 Ca distribution and pseudototal Ca in IG 35-45

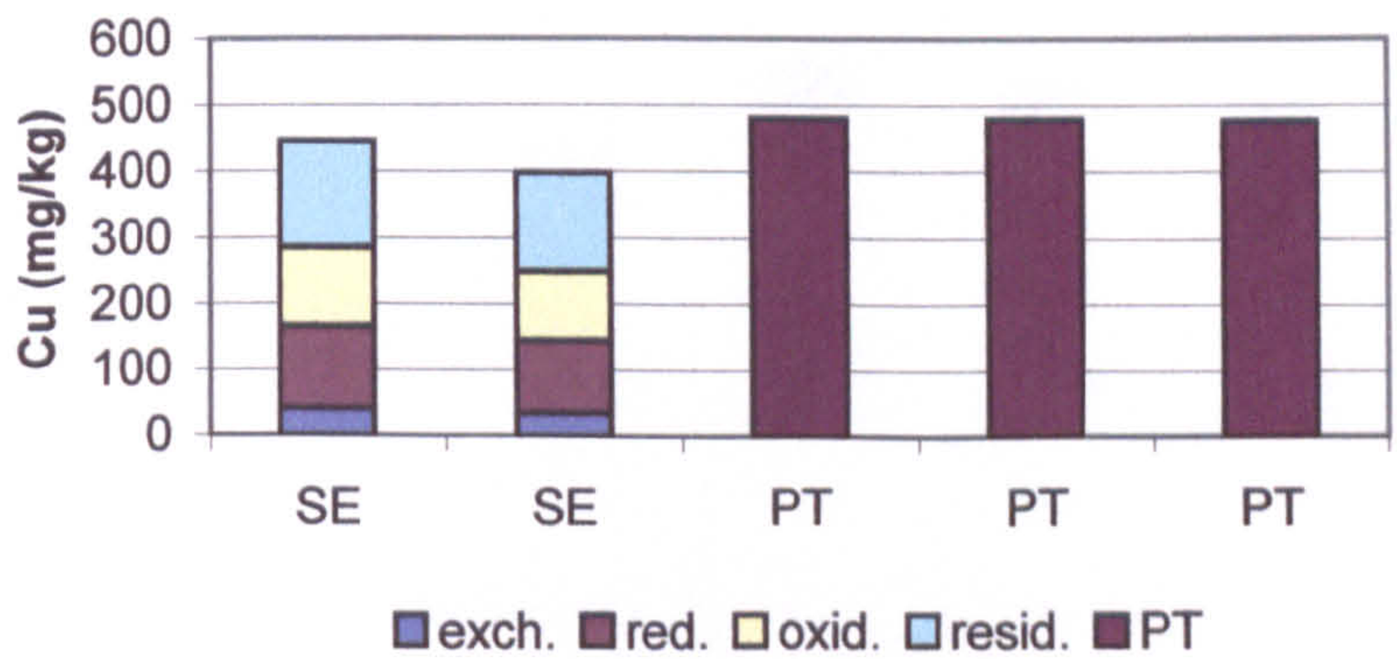

Figure 5.5 Cu distribution and pseudototal Cu in IG 35-45

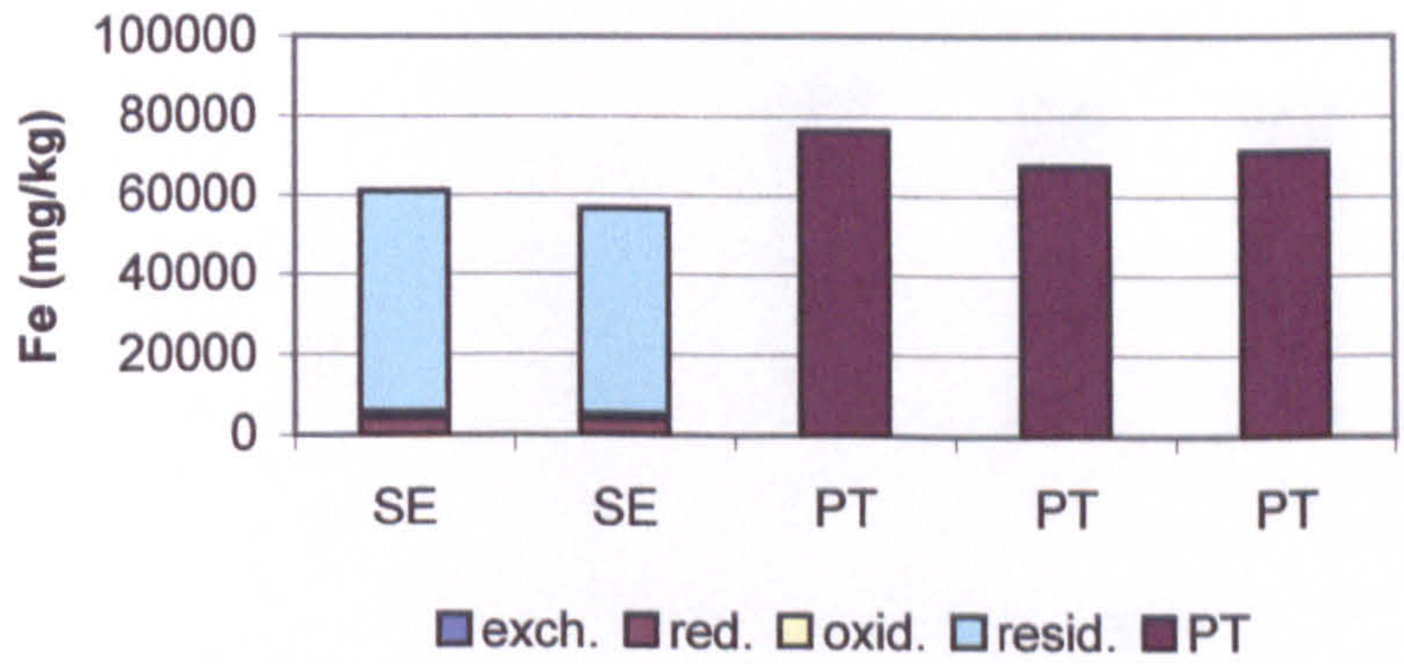

Figure 5.6 Fe distribution and pseudototal Fe in IG 35-45

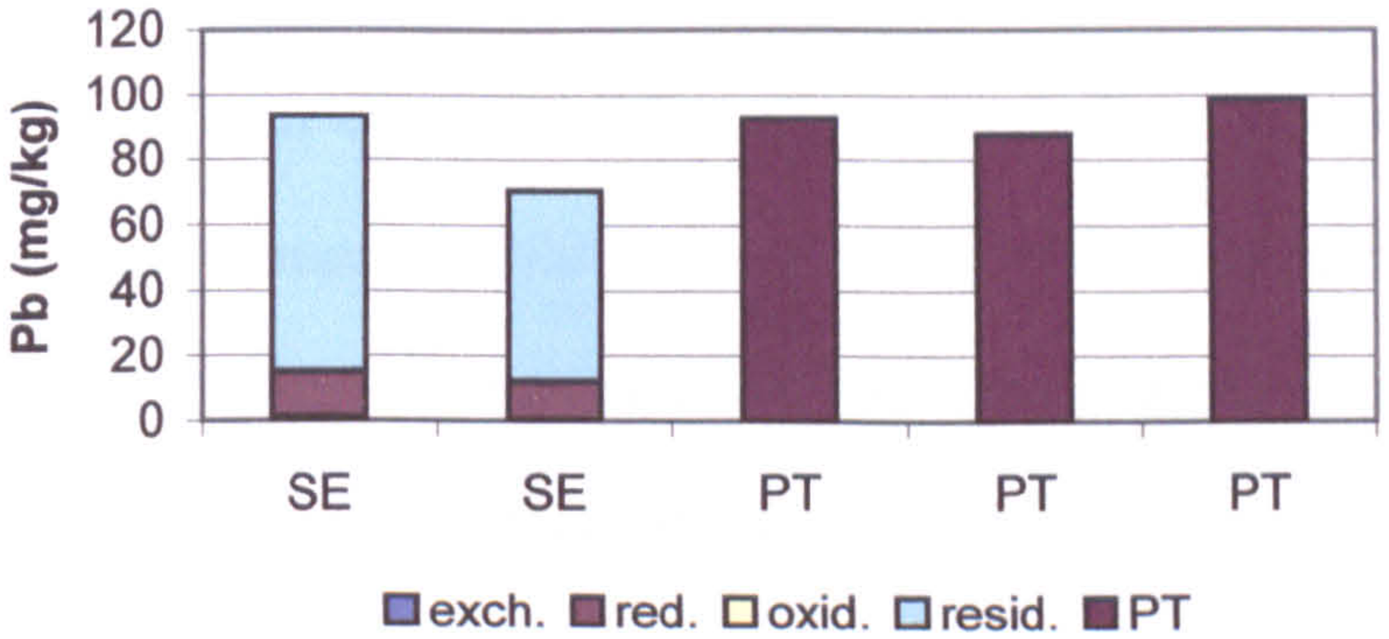

Figure 5.7 Pb distribution and pseudototal Pb in IG 35-45

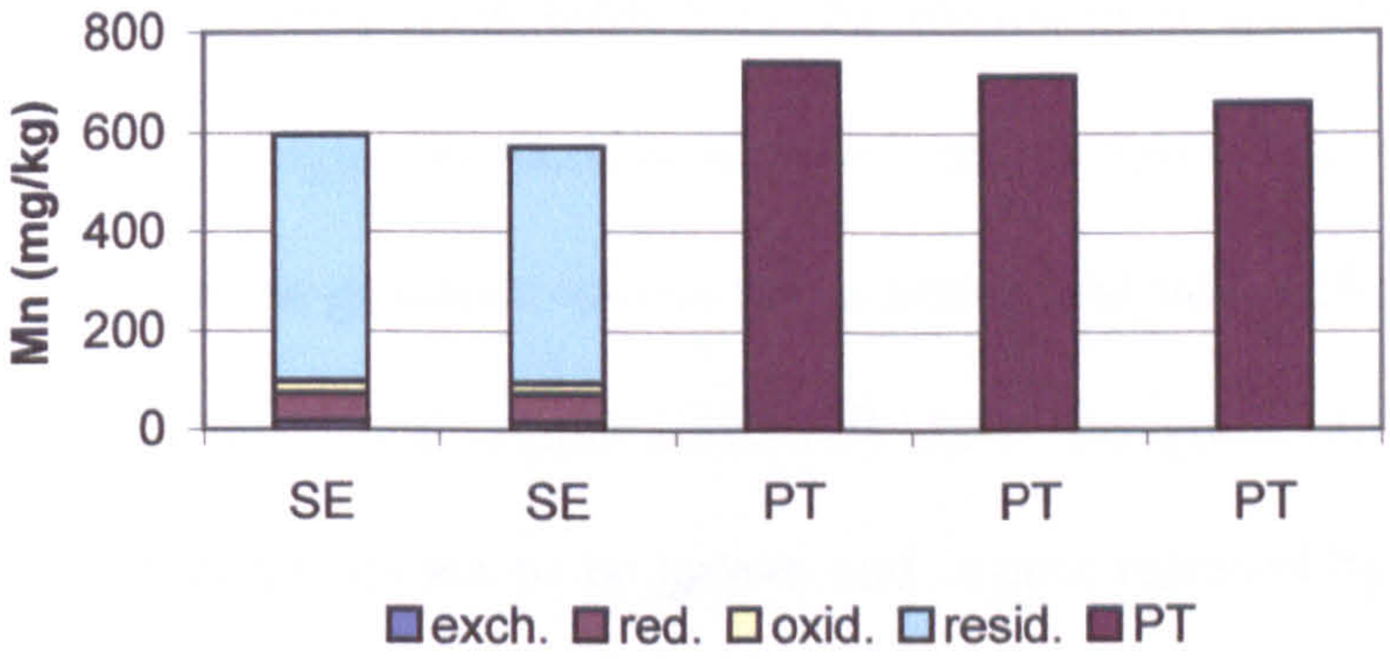

Figure 5.8 Mn distribution and pseudototal Mn in IG 35-45

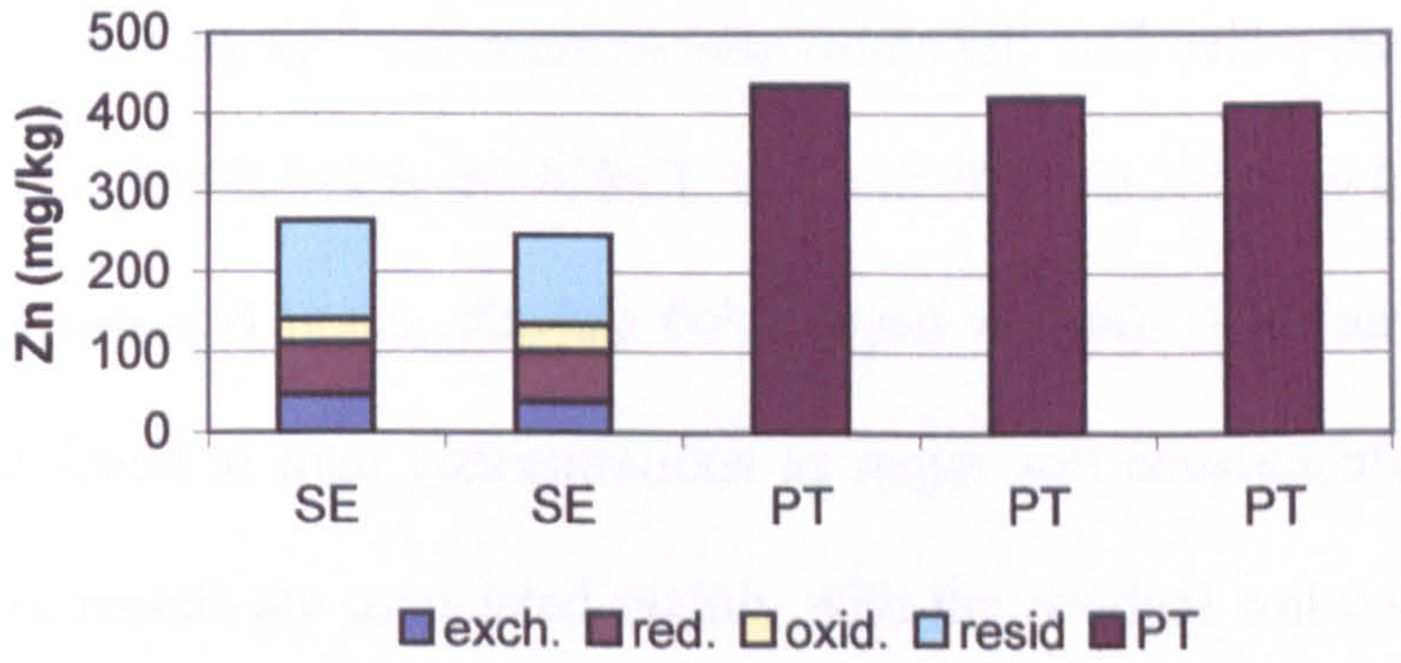

Figure 5.9 Zn distribution and pseudototal Zn in IG 35-45

compare reasonably well (within 20 % for most cases). The sums of the four SE results tend to be slightly lower than the pseudototal values, although the results are still within 20 % of each other, with the exception of zinc. The bias observed between the SE and PT results could be explained by losses during the sequential extraction when transferring reagents from extraction tube to storage containers. The large difference between the zinc SE and PT results was unexpected.

Pseudototal results show that only copper, manganese and zinc fall above the Kelly[20] definitions of uncontaminated soils (table 5.2). SE results show that copper and zinc are also present with a higher proportion of more mobile forms than other metals. The ICRCL[21] also provide guidance values for contaminated land. These values are shown in table 5.3. Pseudototal copper falls well above the guidance value of 130 mg kg^{-1} for land where plants are to be grown and copper released by the first two steps of the SE total slightly more than 130 mg kg^{-1}. Although PT zinc levels are above the ICRCL value of 300 mg kg^{-1} for land where plants are to be grown, SE sums fall below 300 mg kg^{-1} and more mobile forms fall well below the target value. Lead was found at levels below the ICRCL limits of 500 and 2000 mg kg^{-1} (domestic gardens, allotments and parks, playing fields, open spaces). Calcium and iron as expected were found at high concentrations as major soil constituents. SE results show that these metals are associated mainly with the residual soil content. This is expected as iron and calcium form part of the resistant silicate minerals that make up soil.

Table 5.2. Kelly guidelines for classification of contaminated soils (suggested range of values (mg kg^{-1}) on air dried soils).

Metal	Typical values for uncontaminated soils	Slight contamination	Contamination	Heavy contamination	Unusually heavy contamination
Copper (available)	0-100	100-200	200-500	500-2500	>2500
Lead	0-500	500-1000	1000-2000	2000-1.0%	>1.0%
Lead (available)	0-200	200-500	500-1000	1000-5000	>5000
Manganese	0-500	500-1000	1000-2000	2000-1.0%	>1.0%
Zinc (available)	0-250	250-500	500-1000	1000-5000	>5000
Zinc (equivalent)	0-250	250-500	500-2000	2000-1.0%	>1.0%

Table 5.3. ICRCL tentative "trigger concentrations" for selected inorganic contaminants (mg kg^{-1} air dried soil)

Contaminants	**Planned uses**	**Threshold trigger concentration**
Group A : Contaminants which may pose hazards to health		
Lead	Domestic gardens, allotments. Parks, playing fields, open space.	500 2000
Group B : Contaminants which are phytotoxic but not normally hazards to health		
Copper	Any uses where plants are to be grown	130
Zinc	Any uses where plants are to be grown	300

Both samples from Ardeer were also subjected to SE. The results from the SE and pseudototal digestions are shown in figs 5.10-5.15 and 5.16-5.21, and data given in appendix 2. Calcium pseudototal levels appear to be slightly higher than the sum of the sequential extracts. The sums of the sequential extracts for iron and lead are

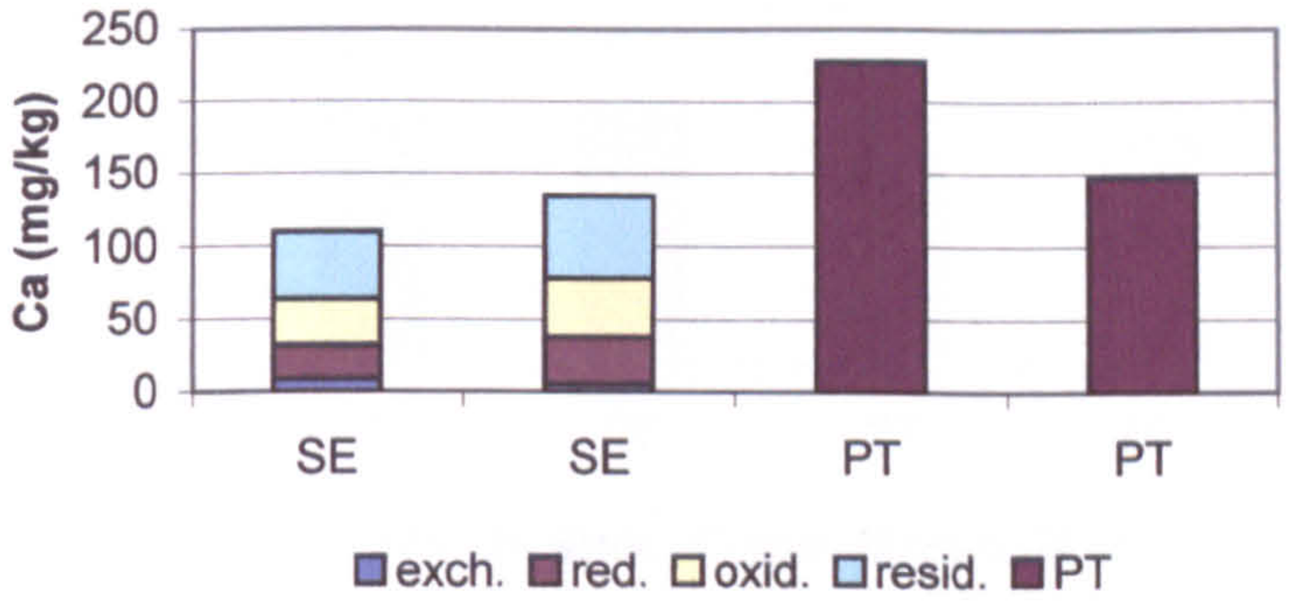

Figure 5.10 Ca SE distribution and pseudototal Ca in Ardeer location 1

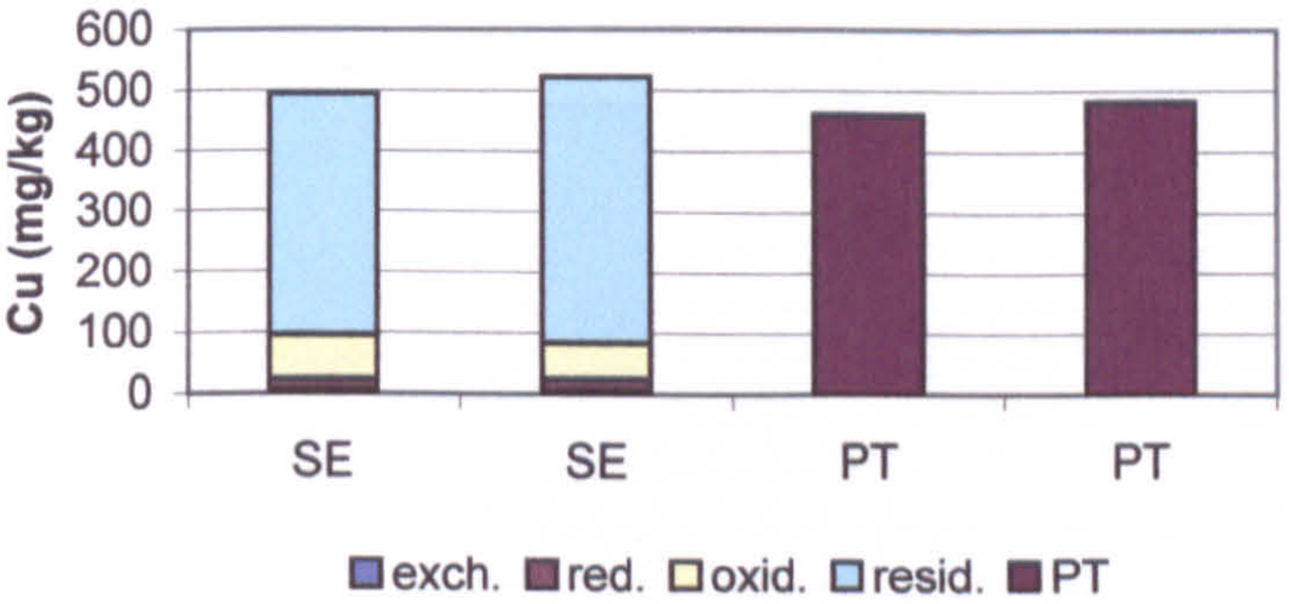

Figure 5.11 Cu SE distribution and pseudototal Cu in Ardeer location 1

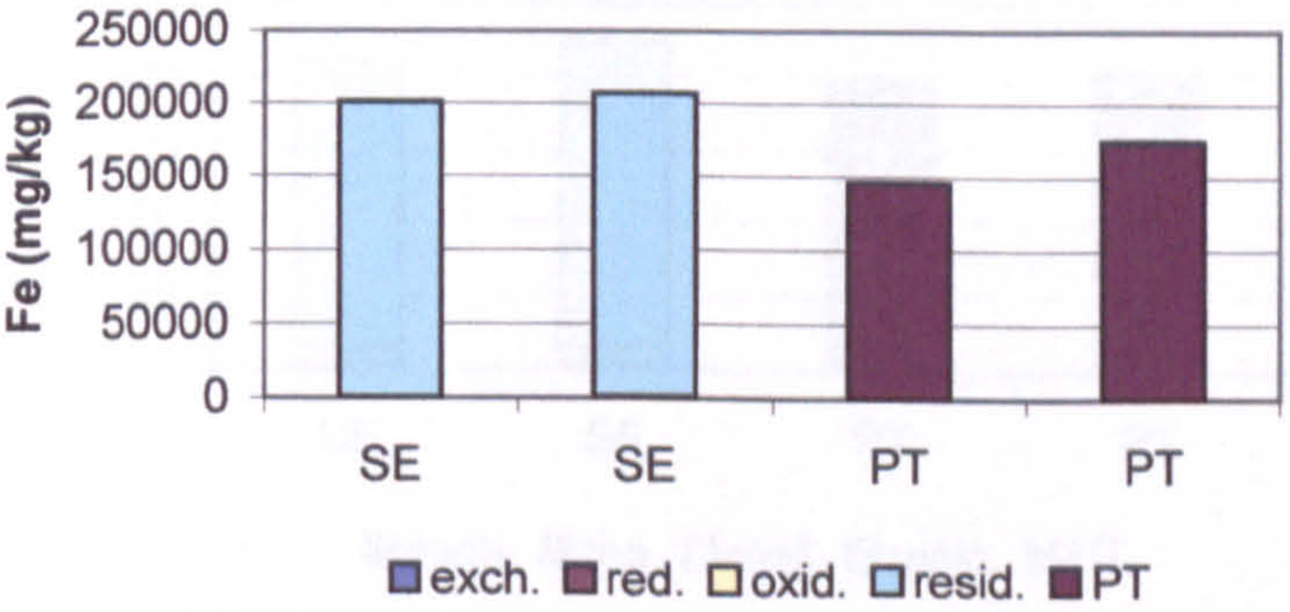

Figure 5.12 Fe SE distribution and pseudototal Fe in Ardeer location 1

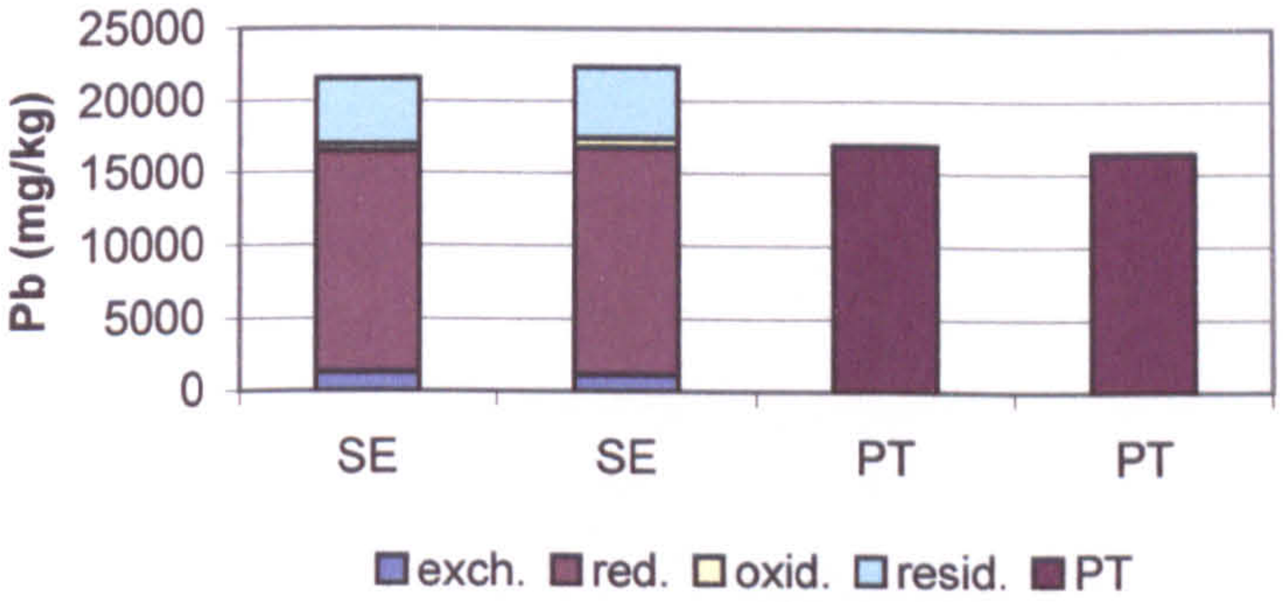

Figure 5.13 Pb SE distribution and pseudototal Pb in Ardeer location 1

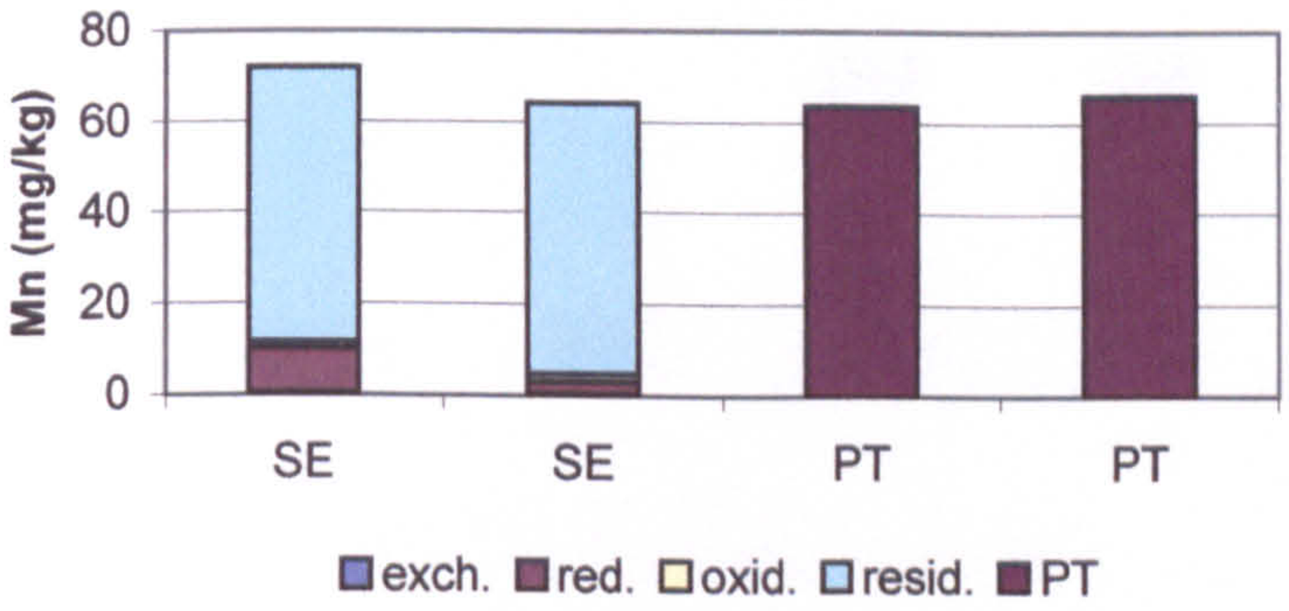

Figure 5.14 Mn SE distribution and pseudototal Mn in Ardeer location 1

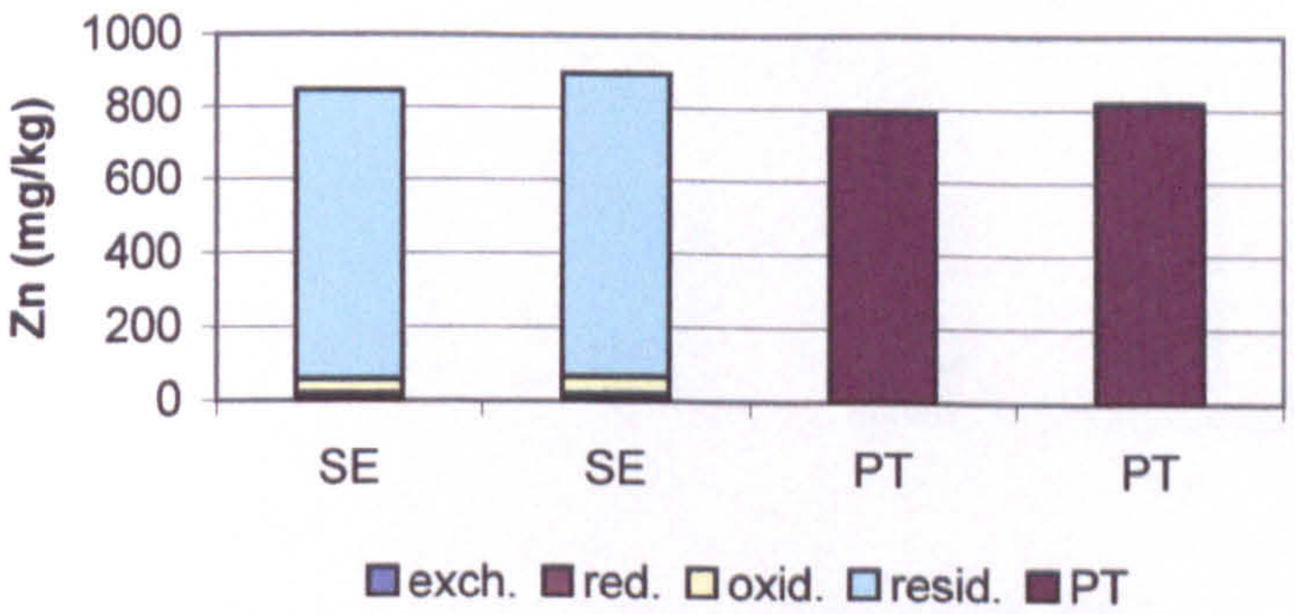

Figure 5.15 Zn SE distribution and pseudototal Zn in Ardeer location 1

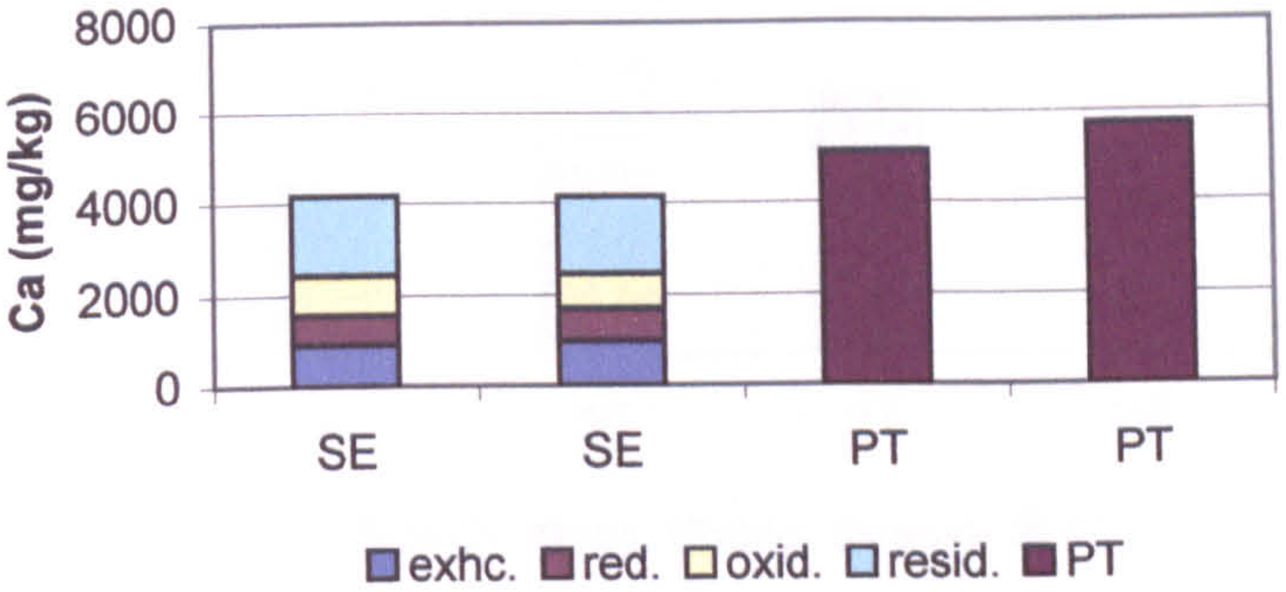

Figure 5.16 Ca SE distribution and pseudototal in Ardeer location2

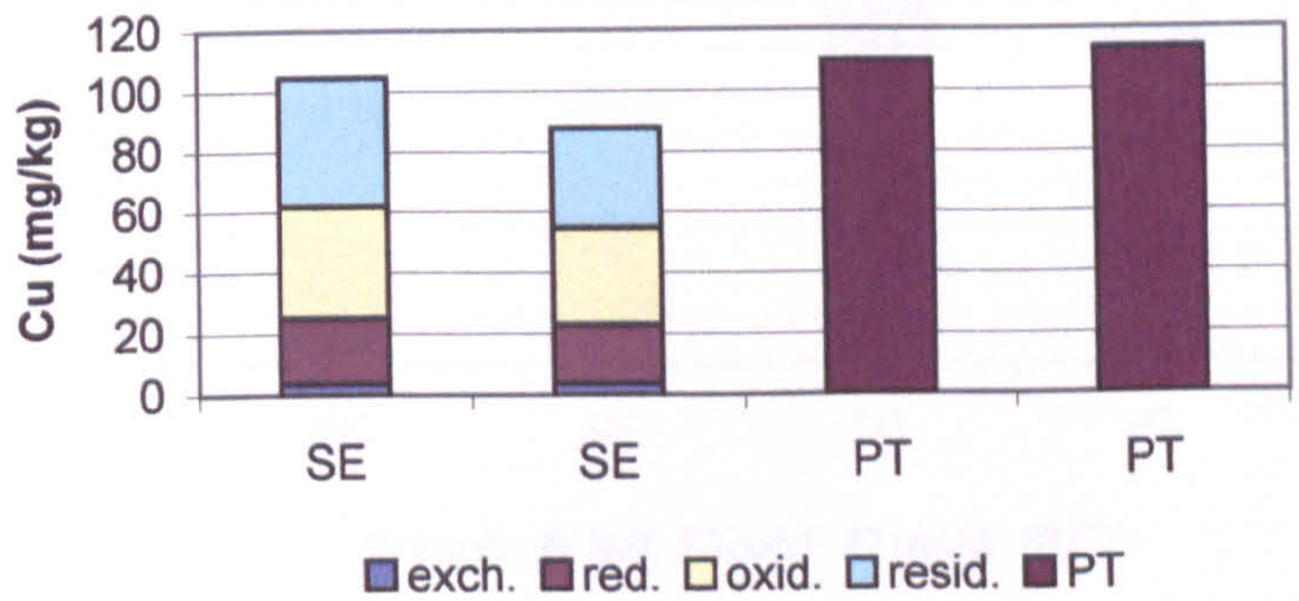

Figure 5.17 Cu distribution and pseudototal Cu in Ardeer location 2

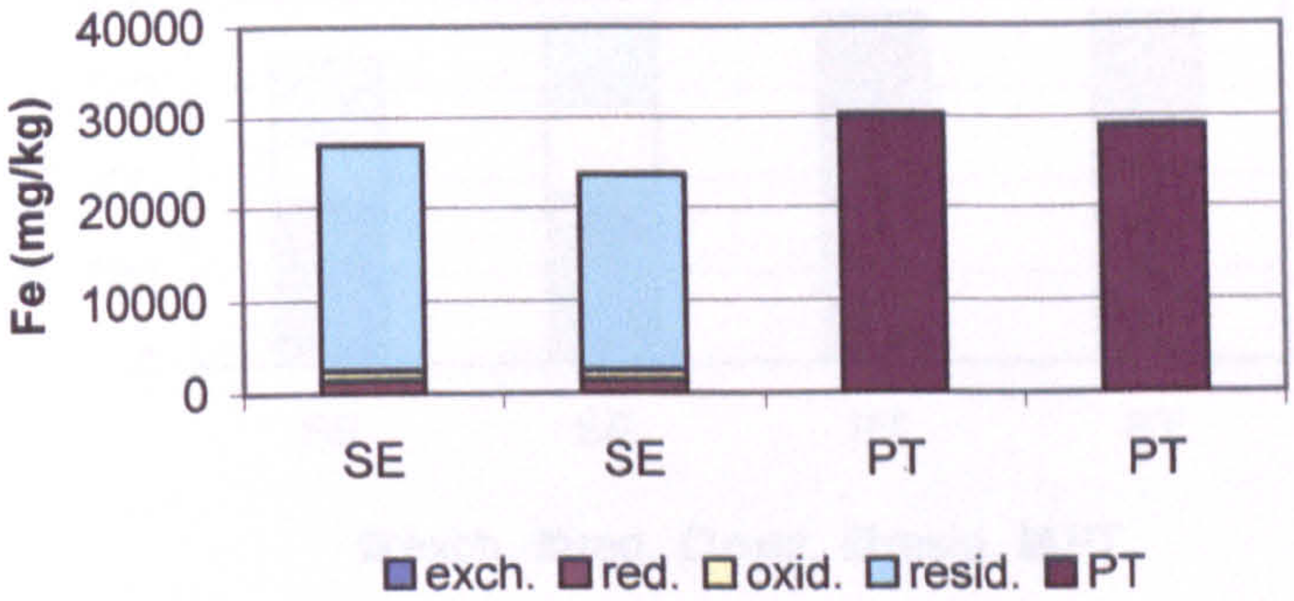

Figure 5.18 Fe SE distribution and pseudototal Fe in Ardeer location 2

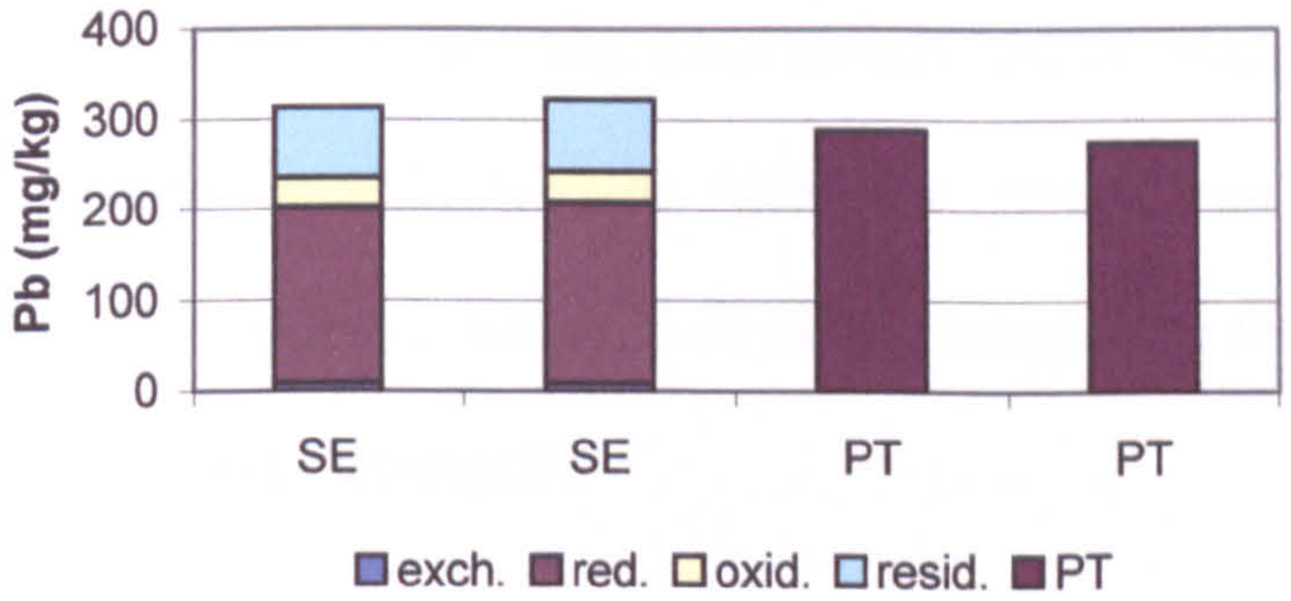

Figure 5.19 Pb SE distribution and pseudototal Pb in Ardeer location 2

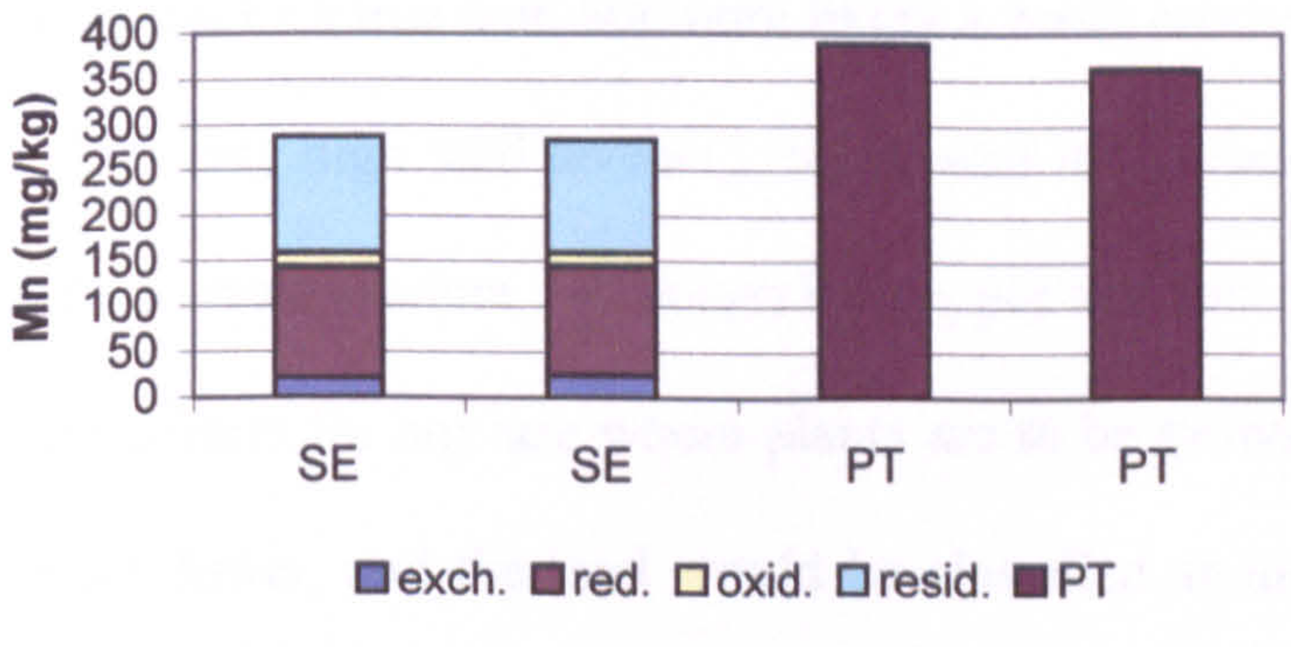

Figure 5.20 Mn distribution and pseudototal Mn in Ardeer location 2

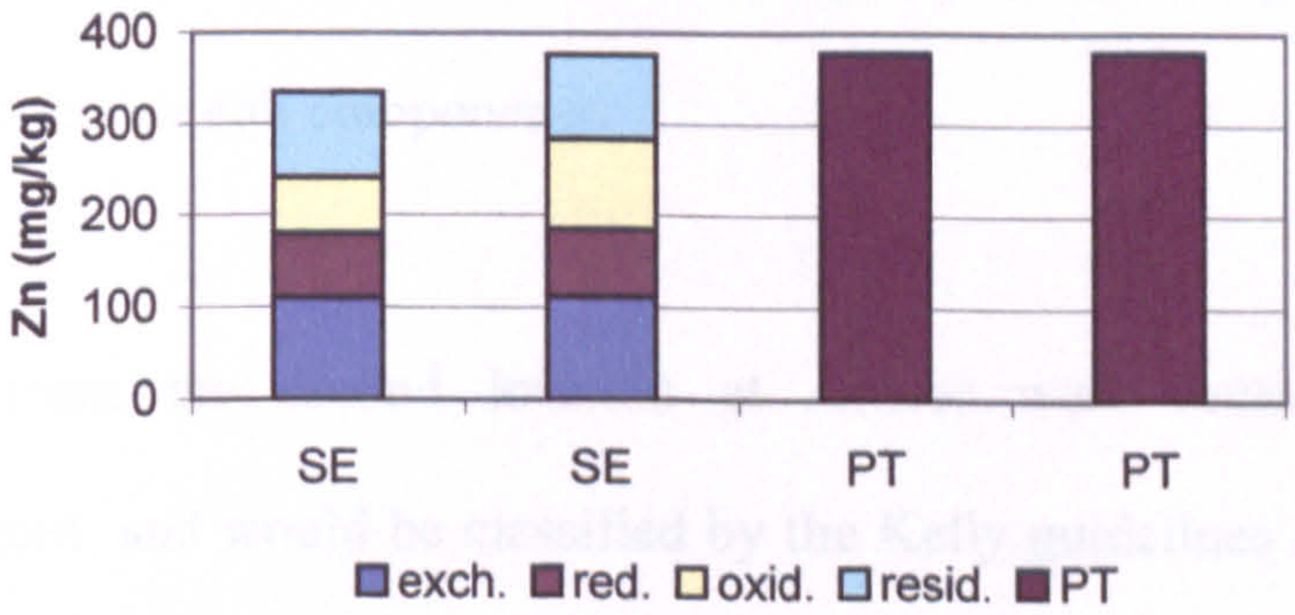

Figure 5.21 Zn SE distribution and pseudototal Zn in Ardeer location 2

approximately 30 % higher than the pseudototal values for these metals. This may be due to SE attacking soil phases that pseudototal digestion alone cannot break down. The sums and pseudototal values for the second location are generally in good agreement (within 20 %). The sums for calcium and manganese fall below the pseudototal values (by ~ 25 %). As there is a general bias towards pseudototal values being higher than SE sums for all metals except lead this suggests that some loss may have occurred during SE.

Calcium levels in the Ardeer soil from location 1 were unusually low indicating that the sample may not be a true soil, but more likely a waste product from some process on the site. The very high lead levels (2 %) present in the sample also suggest this sample may be a waste product. Although PT copper and zinc levels are higher than the ICRCL guidelines for any use where plants are to be grown, the available metal content is much lower, and the land would be classified as uncontaminated by the Kelly guidelines, except for the high lead content. The SE results show that the lead is present in a readily mobile form. The majority of the lead present was associated with the reducible soil components.

Samples from the second location at Ardeer were found to be much less contaminated, and would be classified by the Kelly guidelines as uncontaminated to slightly contaminated. Only zinc falls above the ICRCL trigger values for land where plants are to be grown. Although lead levels are considerably lower than those in location 1, a considerable proportion of the lead is associated with the reducible soil components. Kelly guidelines suggest that soils with 200 – 500

mg kg^{-1} available lead should be classified as slightly contaminated. The similarities in the SE patterns for locations 1 and 2 suggest that the lead present in the location 2 soil is due to contamination from location 1, whilst the soils were on site.

5.3.2 Experiments 1 and 2

Conductivity and pH of the leachate solution were measured. The metal content of each sample of leachate was then determined and the leached soil subjected to sequential extraction. The results from experiment 1 (leached with disodium EDTA (pH 7) and experiment 2 (leached with disodium EDTA (pH 4.45)) are shown in the following sections.

5.3.2.1 Conductivity and pH

Conductivity and pH profiles for experiments 1and 2 are shown in figures 5.22-5.25. The conductivity of the leachate (figures 5.22 and 5.24) was not altered significantly from the conductivity of the original leaching solution throughout the experiments. Once distilled water was flushed through the columns, conductivity soon dropped to 0.

The pH of the leachate (shown in figures 5.23 and 5.25) at the start of experiment 1 was slightly less than the pH of the original EDTA solution, whereas the pH of the leachate at the start of experiment 2 was approximately equivalent to the pH of the original leaching solution. The pH profile for experiment 1 gradually rose to the

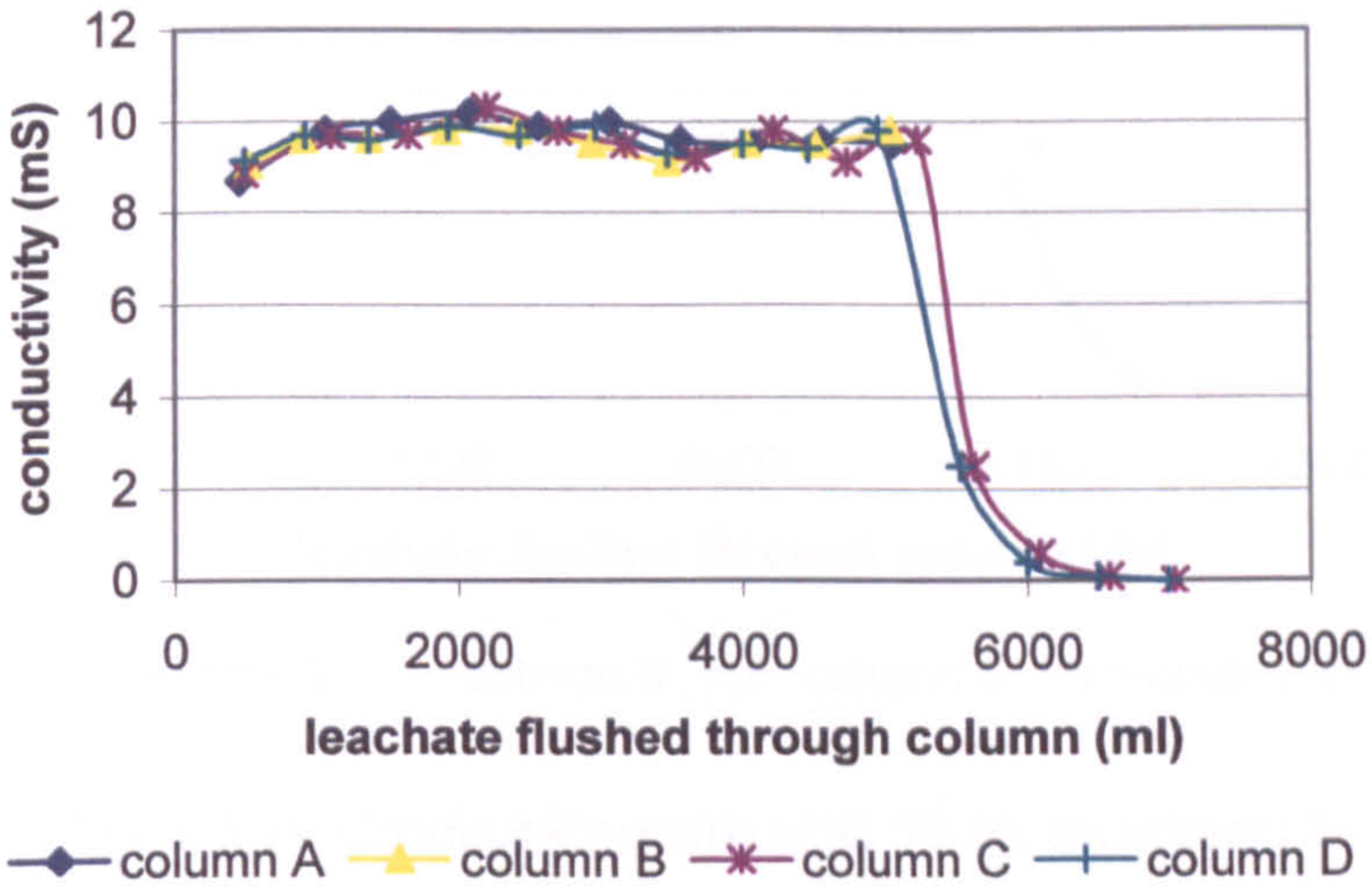

Figure 5.22 conductivity profile of IG 35-45, experiment 1

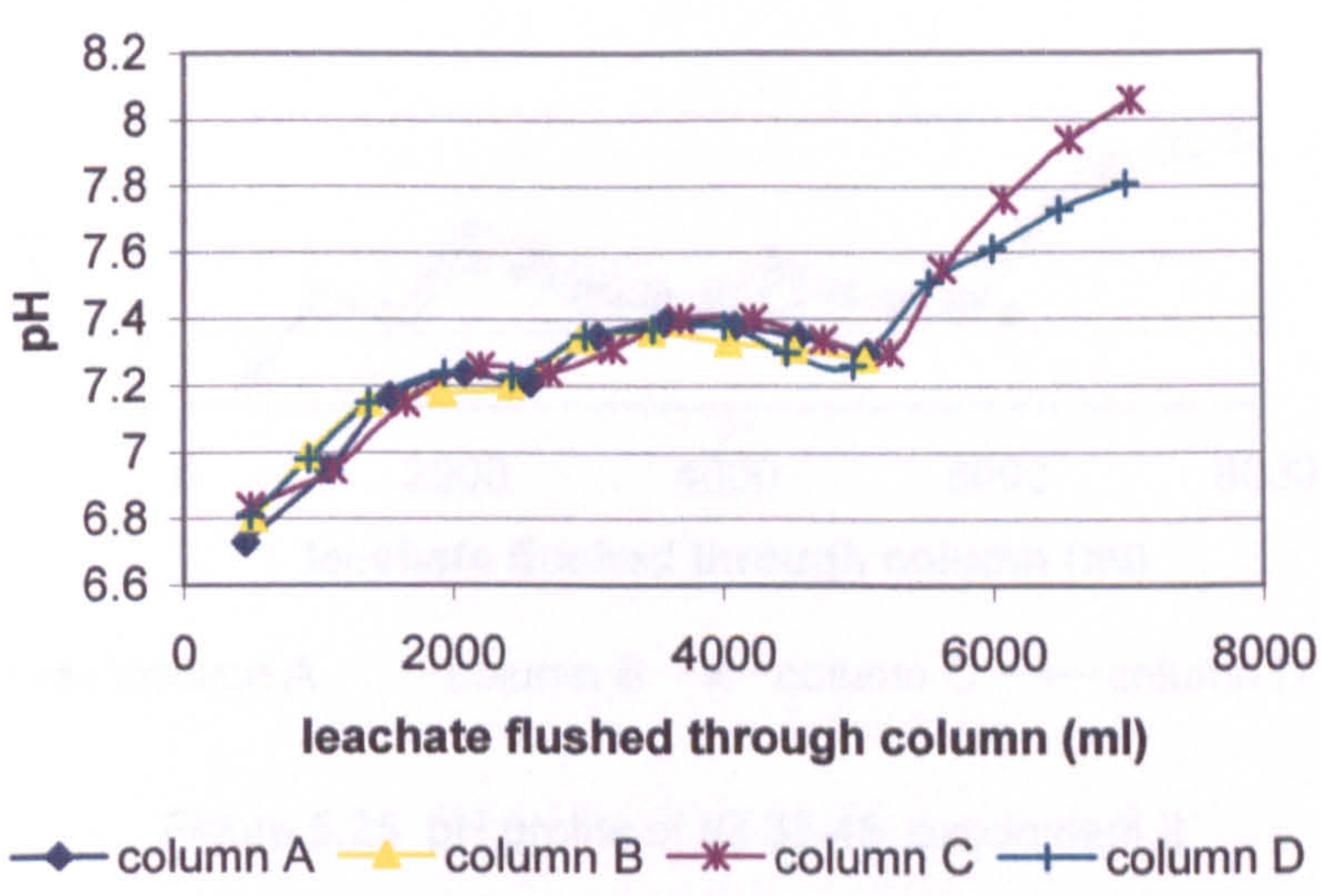

Figure 5.23 pH profile of IG 35-45, experiment 1

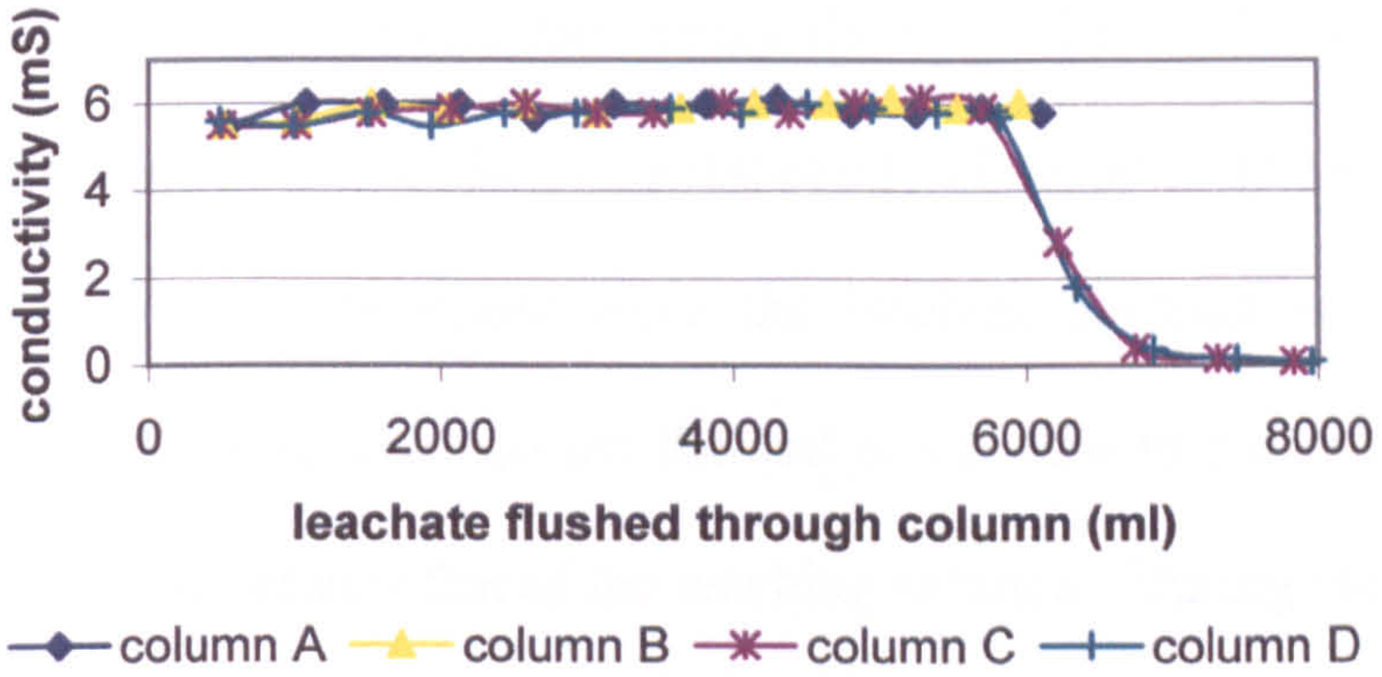

Figure 5.24 Conductivity profile of IG 35-45, experiment 2

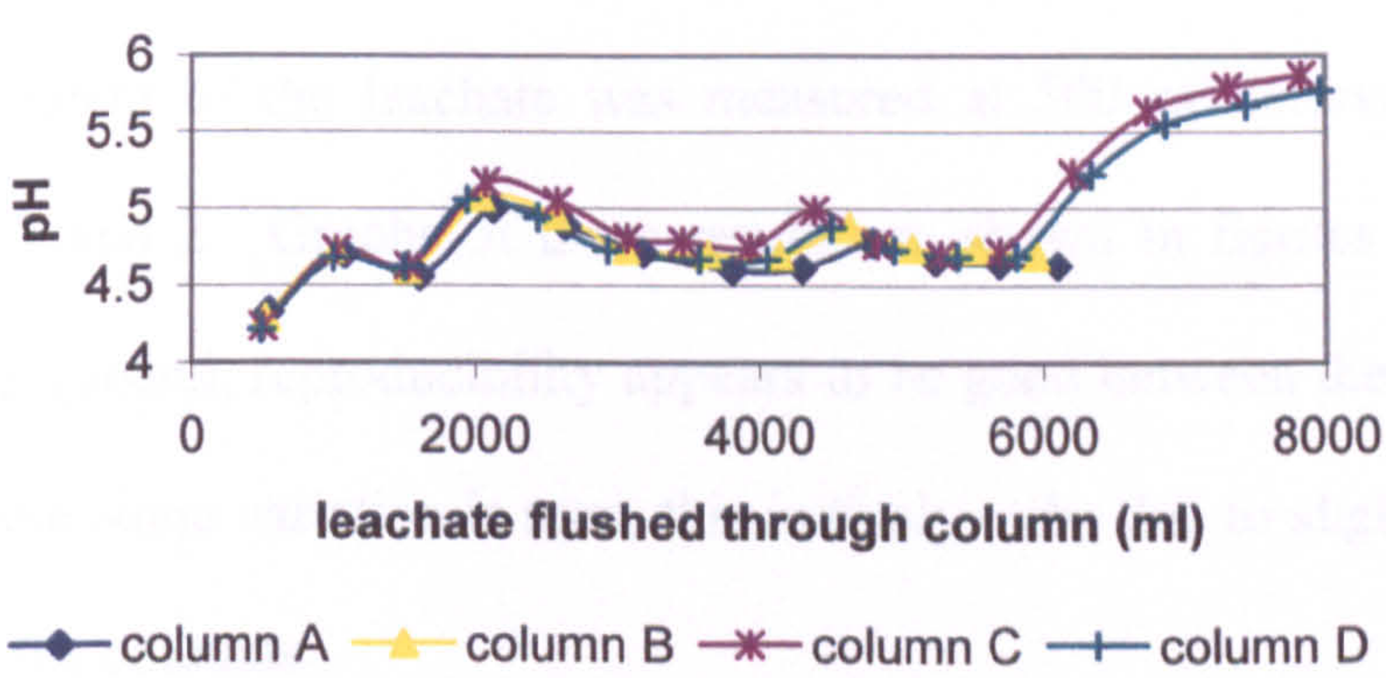

Figure 5.25 pH profile of IG 35-45, experiment 2

approximate value of the EDTA solution after approximately 2000 ml of flushing. The pH profile from experiment 2 shows cyclical variation due to the rest periods in the flushing overnight, during which the EDTA was allowed to stand in the columns, allowing a longer extraction time, than during flushing. The differences between the two experiments may be due to the buffering ability of the soil. The soil may be able to alter the pH to a certain extent when the leaching solution is close to pH 7, however at lower leaching solution pH the soil is less able to buffer the pH, and so the pH of the leachate reflects that of the leaching solution. During the static periods, the intrinsic pH of the soil reasserted itself. This effect was more pronounced for experiment 2 than experiment 1.

5.3.2.2 Leaching profiles

The metal content of the leachate was measured at 500 ml intervals throughout experiments 1 and 2. Graphs of these results are shown in figures 5.26-5.31 and 5.32-5.37. In general, reproducibility appears to be good between the four columns, although where some variation is seen, this is likely to be due to slight variations in flow rates to the columns.

Both experiments show peaks corresponding to times when the pump was switched off for extended periods (e.g. overnight). This is most likely due to the reaction kinetics of the EDTA and the metals. Increasing contact time between soil and leachate solution increases the metal leached from the soil. This kinetic dependence was demonstrated by Heil *et al.*[10] when they modelled the time dependent dissolution

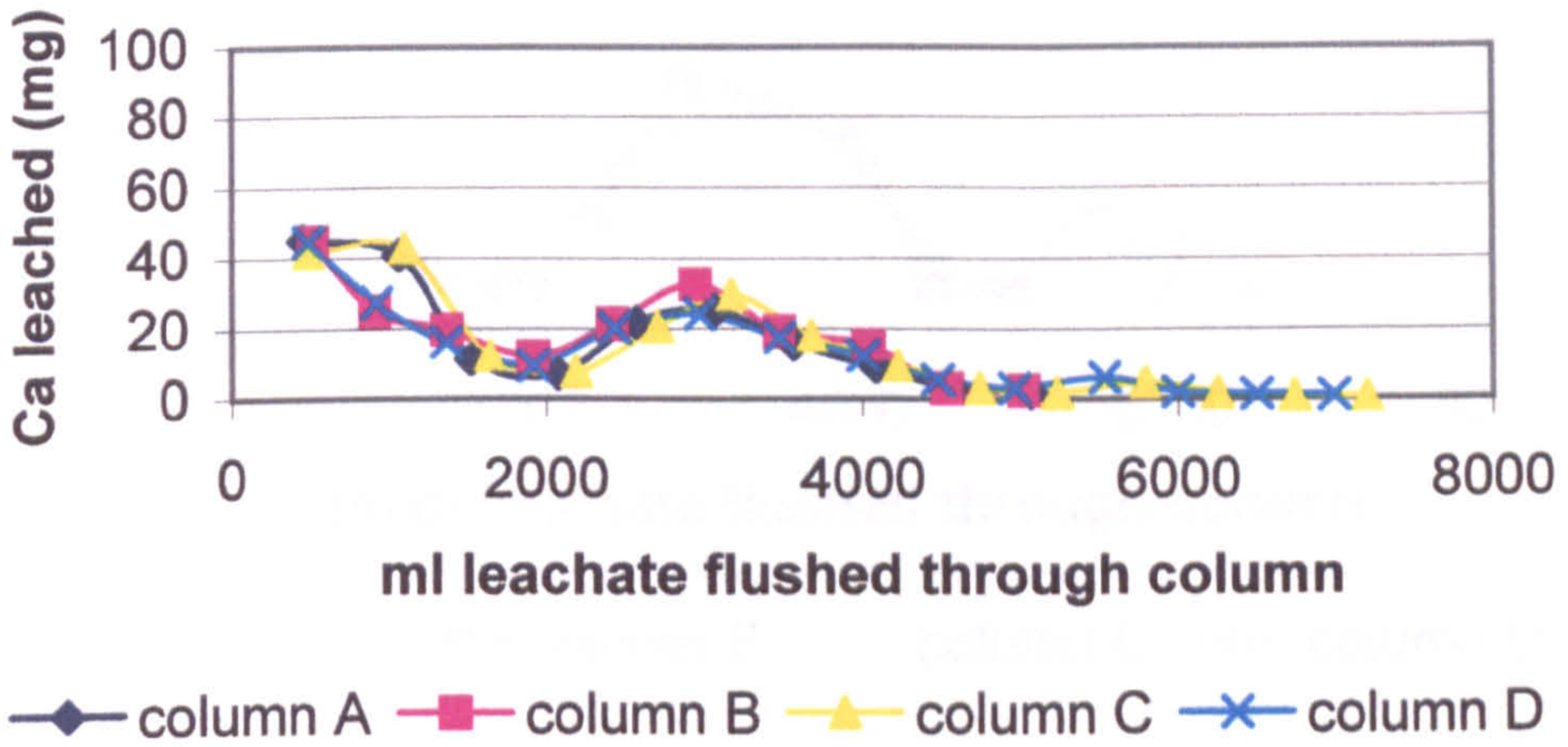

Figure 5.26 Ca in leachate from IG 35-45, experiment 1

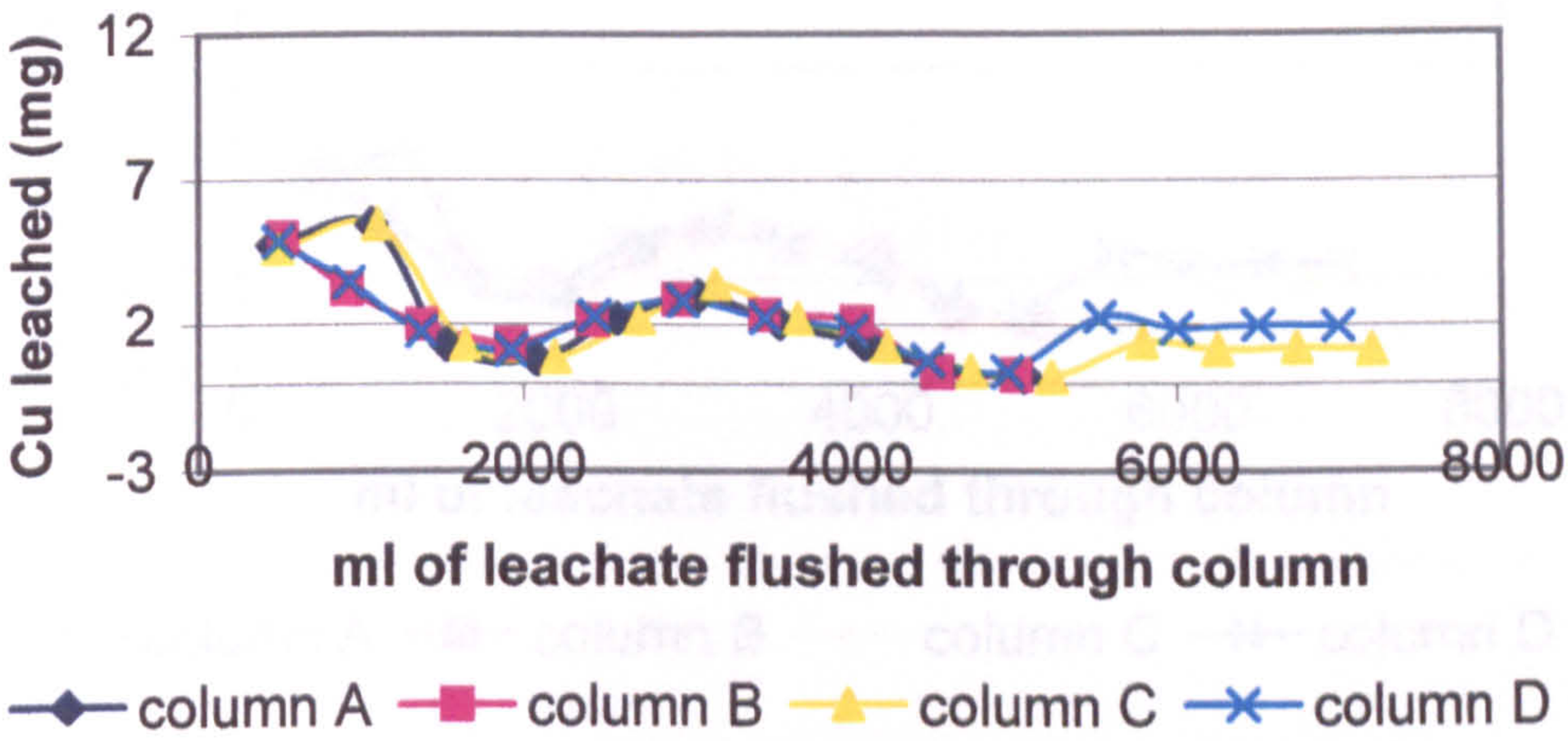

Figure 5.27 Cu in leachate from IG 35-45, experiment 1

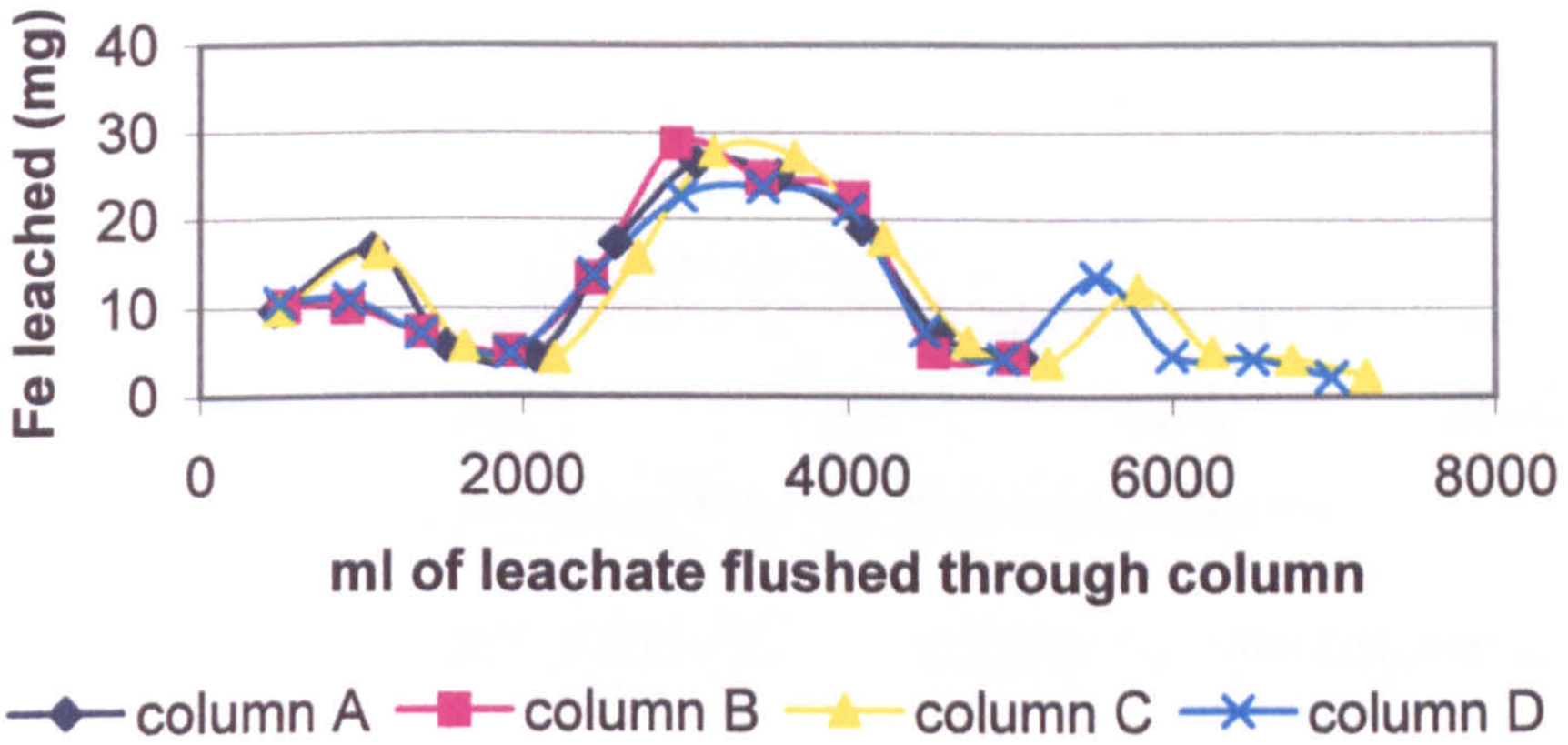

Figure 5.28 Fe in leachate from IG 35-45, experiment 1

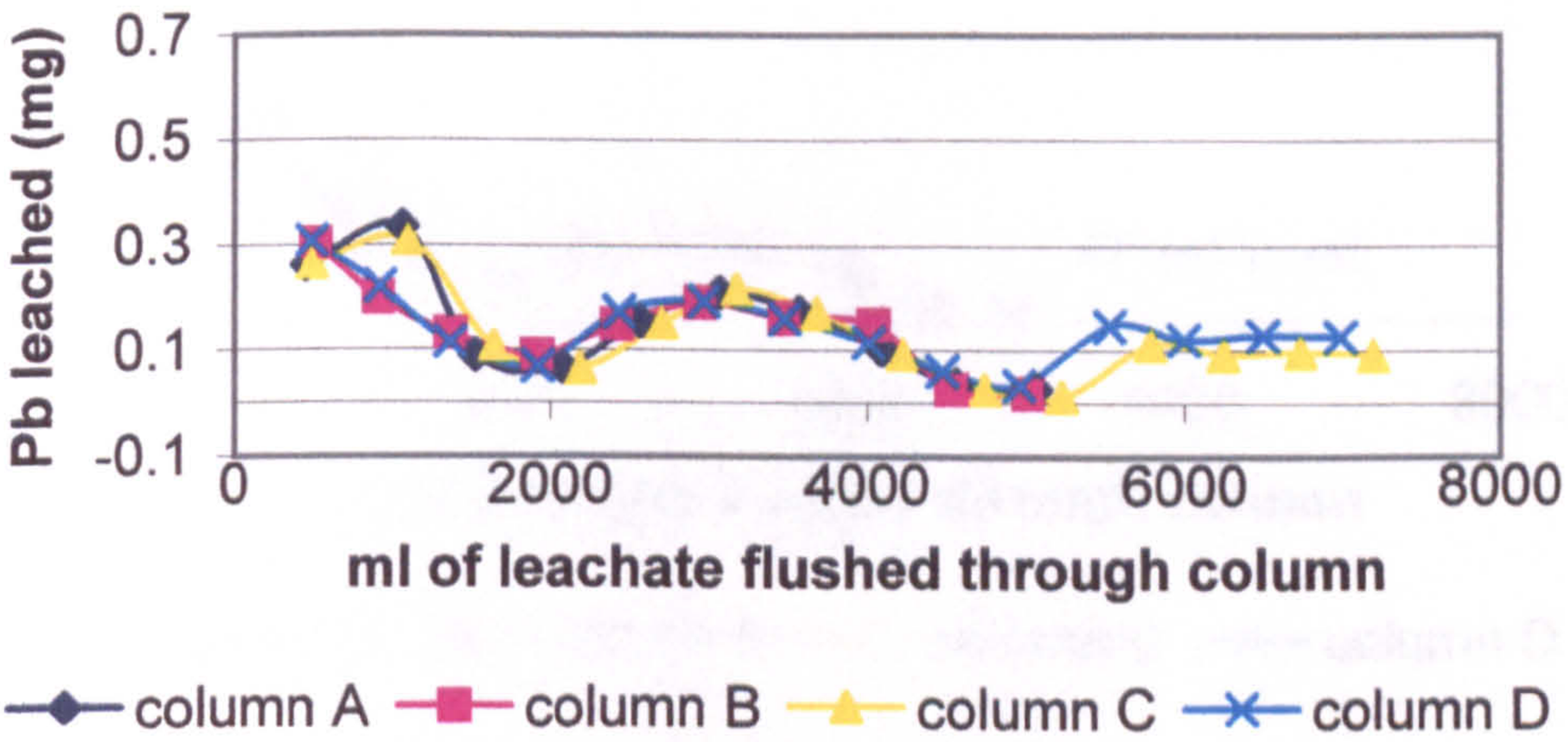

Figure 5.29 Pb in leachate from IG 35-45, experiment 1

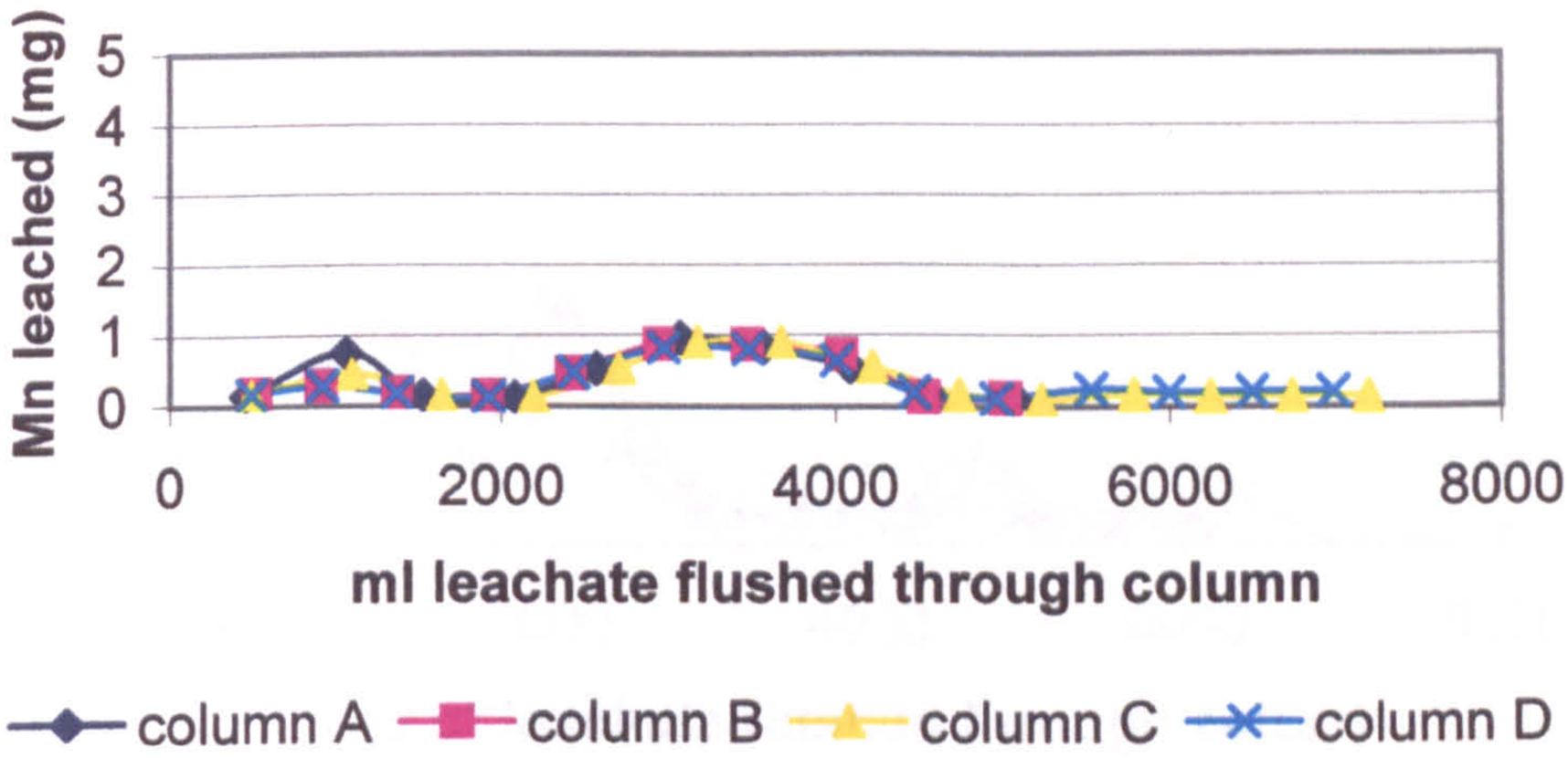

Figure 5.30 Mn in leachate from IG 35-45, experiment 1

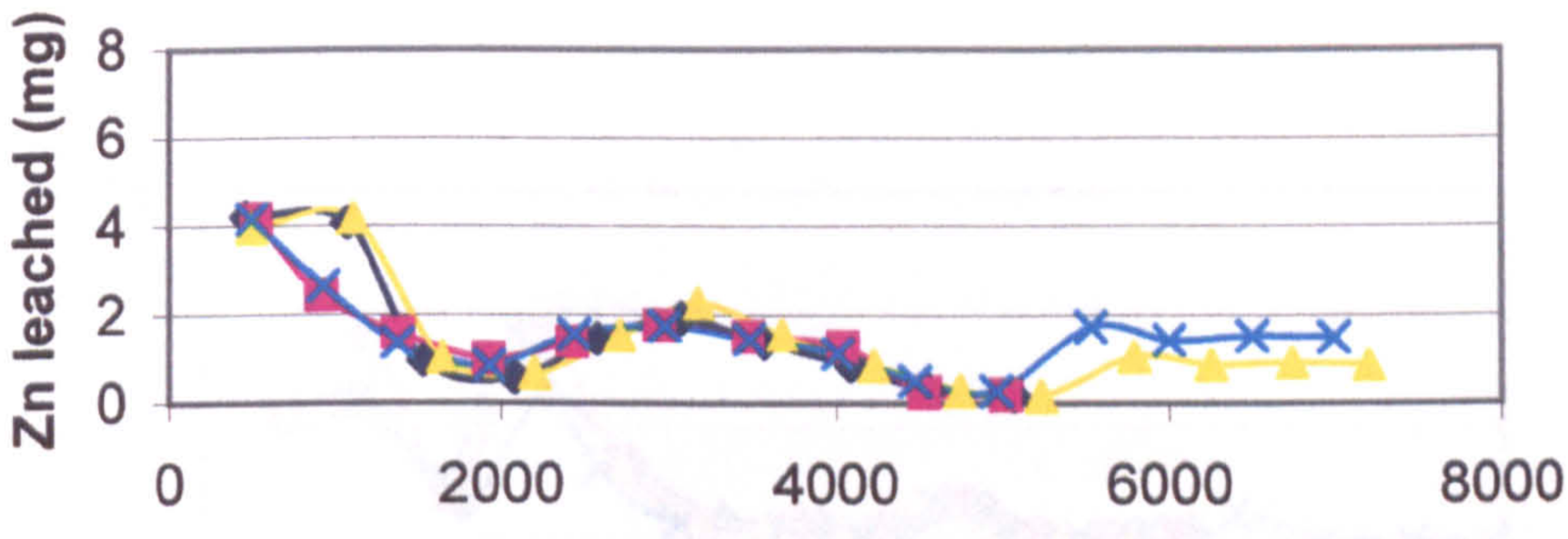

Figure 5.31 Zn in leachate from IG 35-45, experiment 1

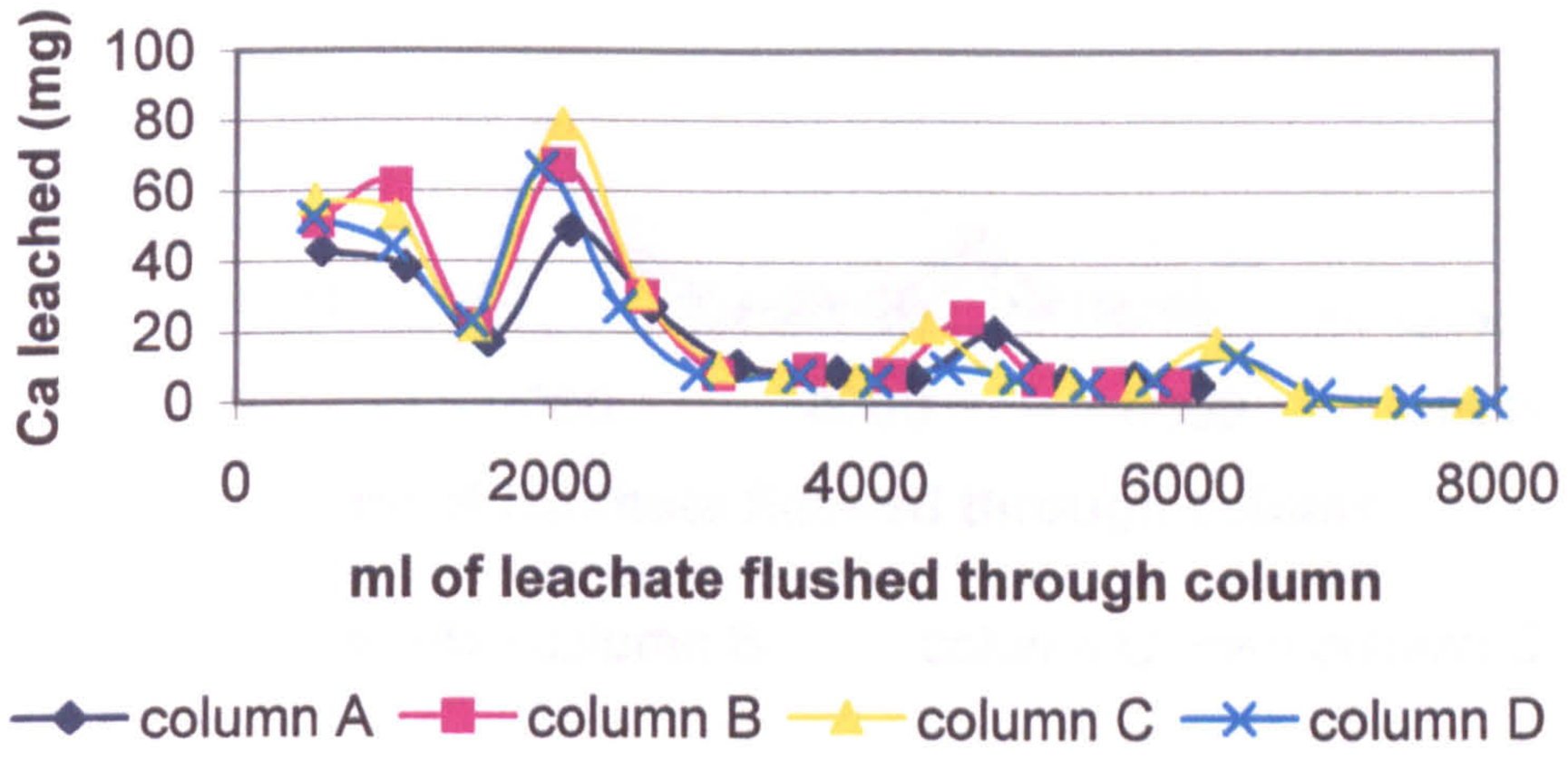

Figure 5.32 Ca in leachate from IG 35-45, experiment 2

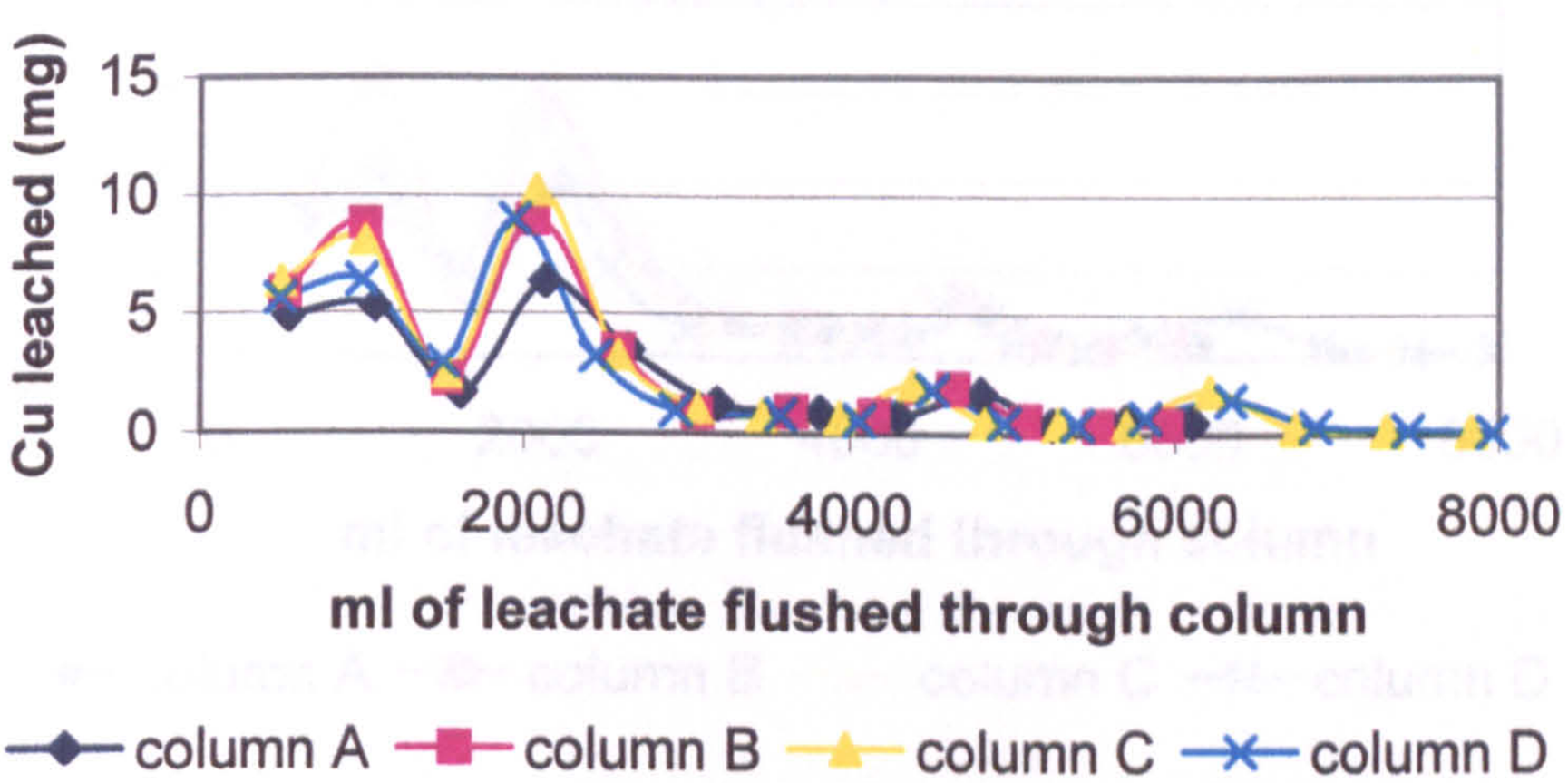

Figure 5.33 Cu in leachate from IG 35-45, experiment 2

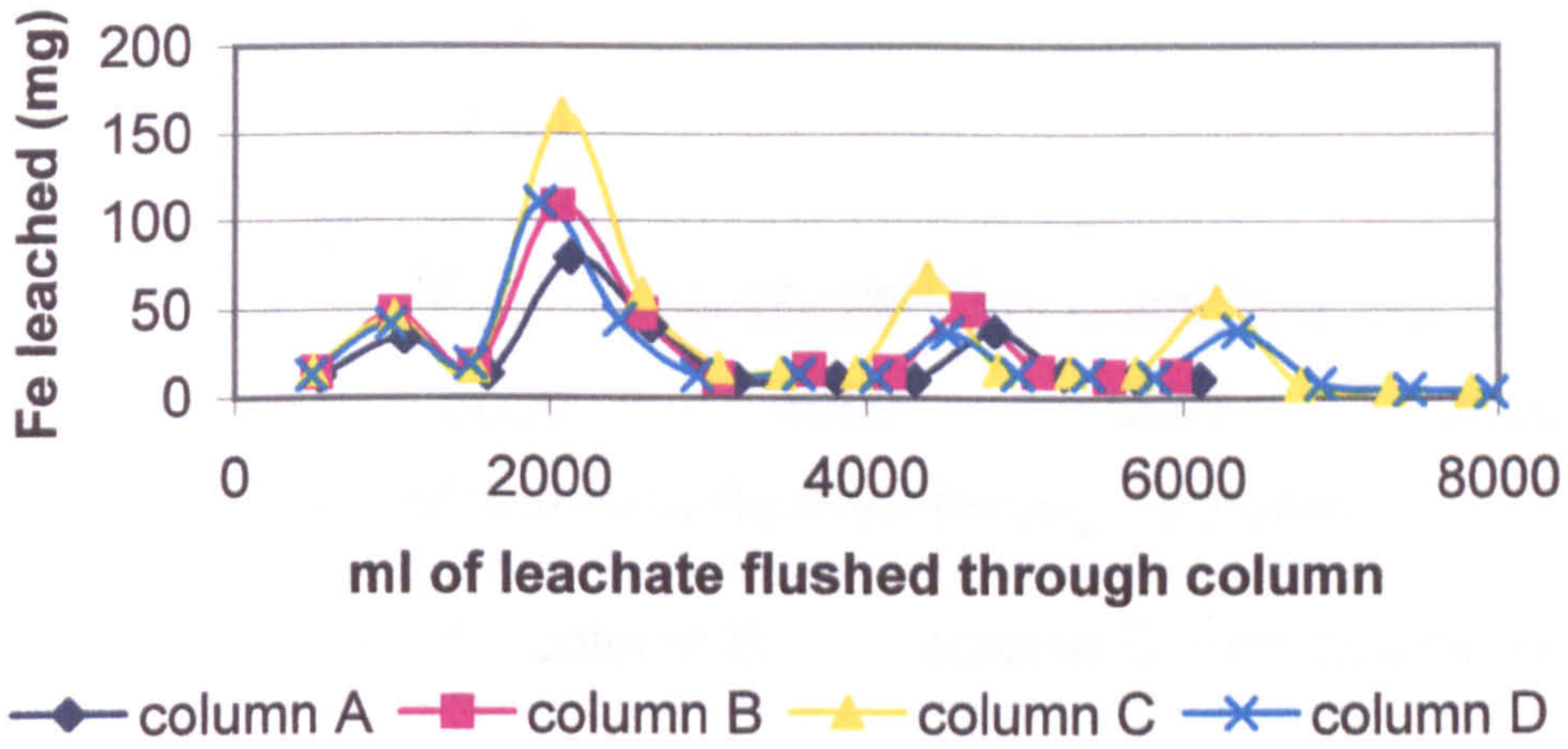

Figure 5.34 Fe in leachate from IG 35-45, experiment 2

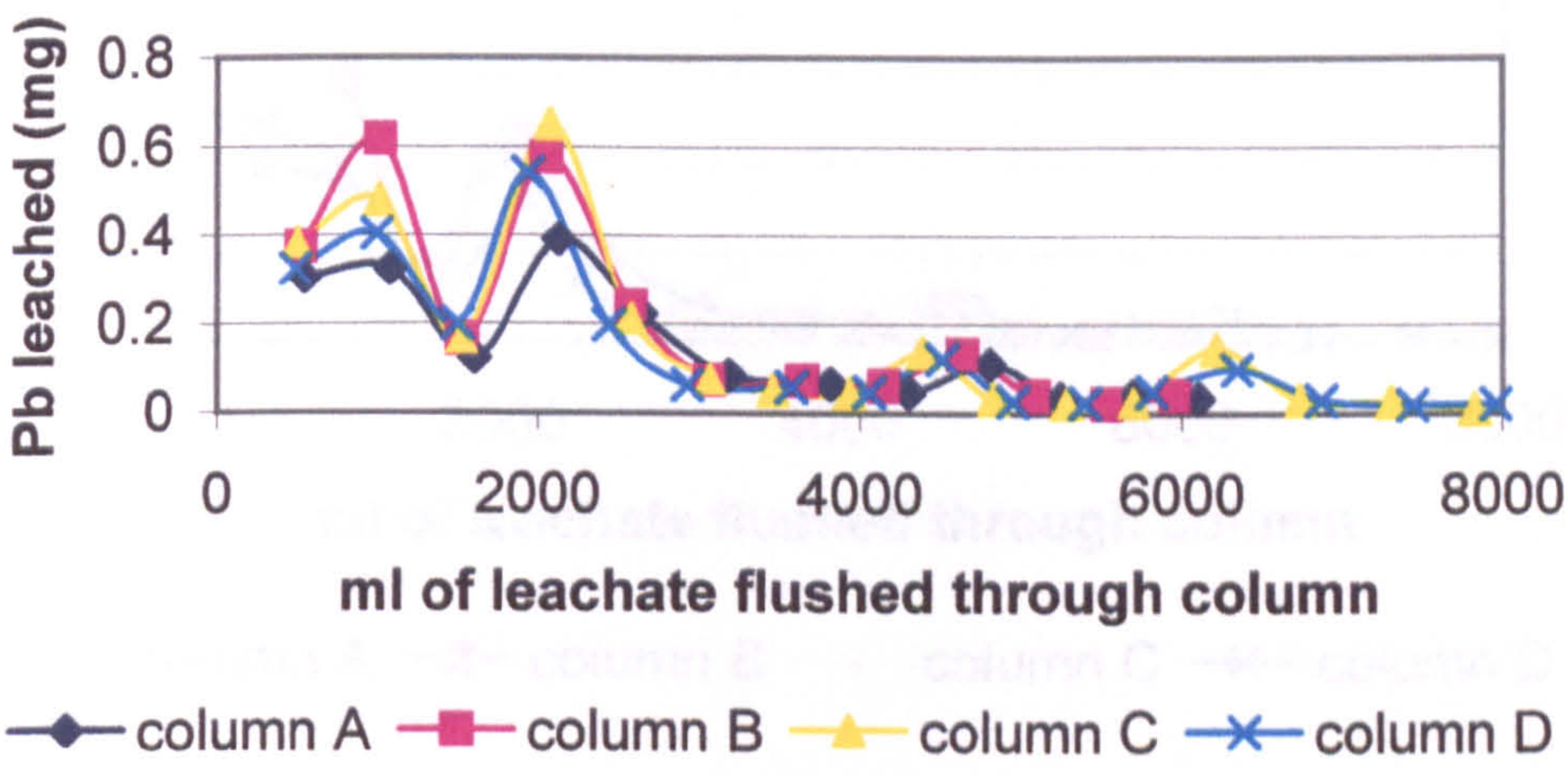

Figure 5.35 Pb in leachate from IG 35-45, experiment 2

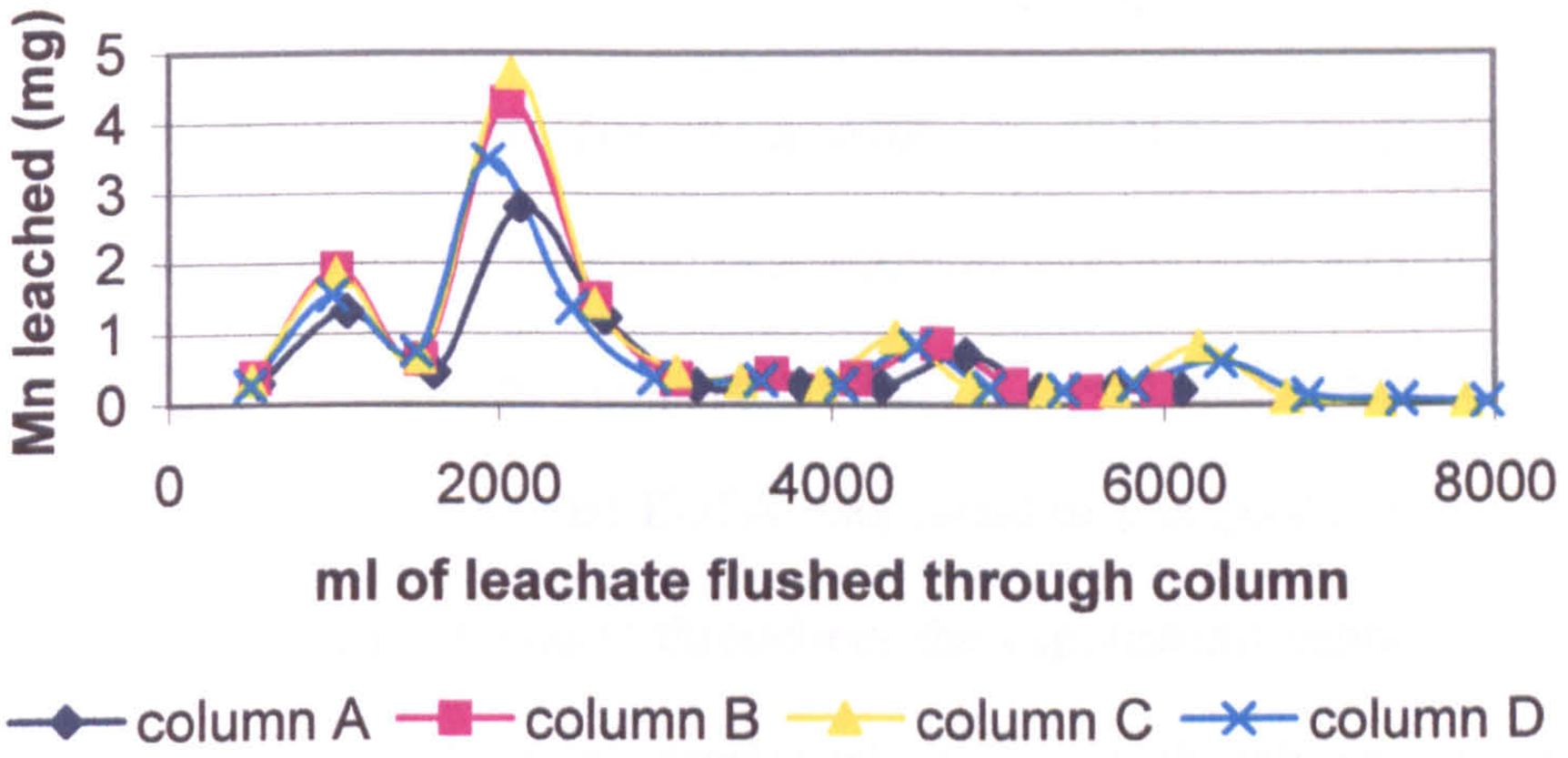

Figure 5.36 Mn in leachate from IG 35-45, experiment 2

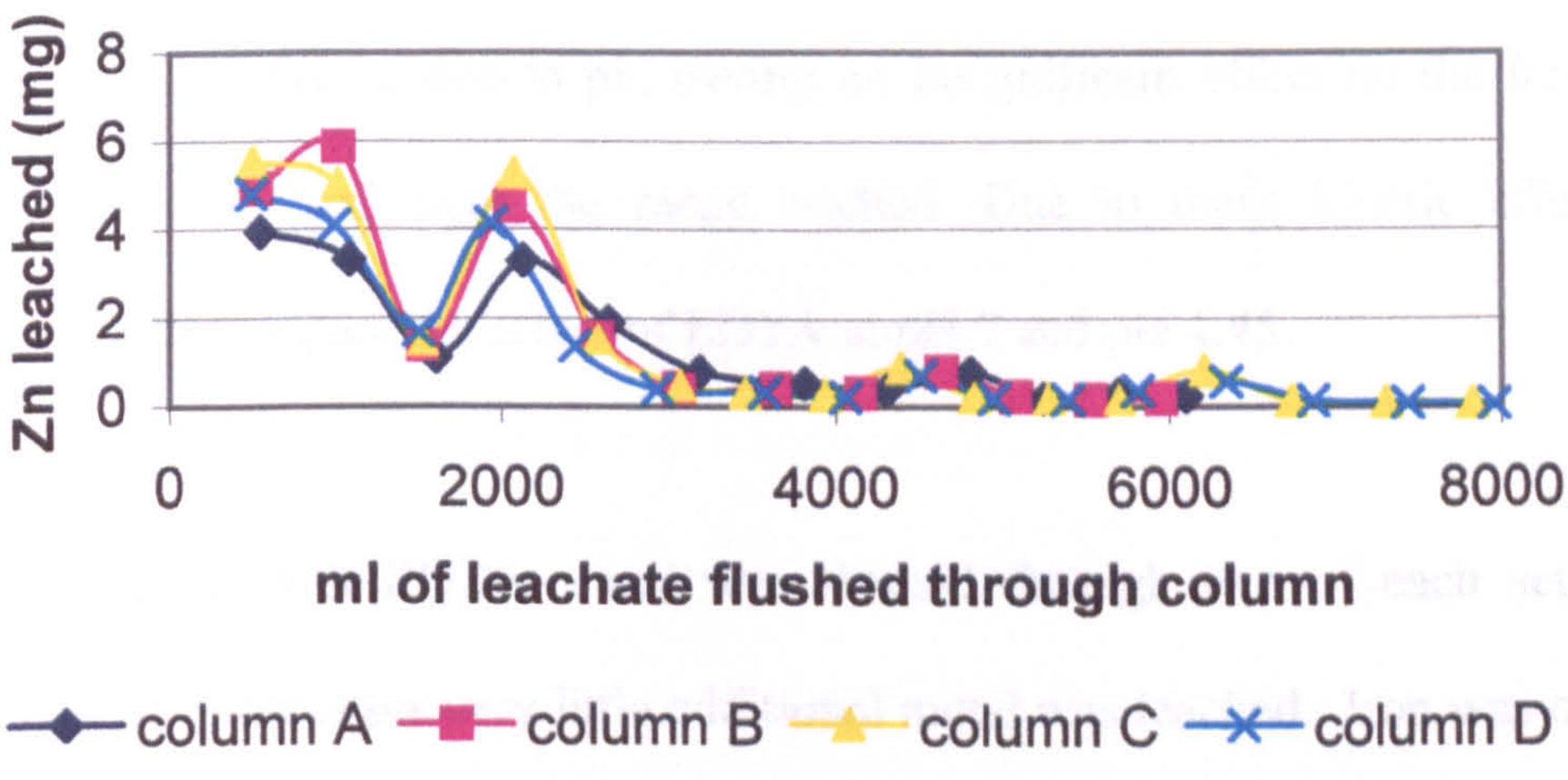

Figure 5.37 Zn in leachate from IG 35-45, experiment 2

of Pb by an EDTA extract solution. A decrease in dissolution rate was observed with time, reaching a plateau after 5 hr. Due to this kinetic effect the two experiments cannot be compared in terms of the effect of the pH of the leaching solution, although literature[5] suggests that Pb recovery by EDTA is greatest under acid conditions, as hydrolysis is favoured over complexation at more alkaline conditions. Ca and Mg are also able to compete for EDTA co-ordination sites above pH 6. However it should be noted that EDTA was found to precipitate under very acidic conditions. Although pH effects throughout the experiments cannot be compared, metal concentration in the first samples taken from each column can be used to investigate the pH effect. First sample values are very similar for all metals between experiment 1 and 2. This may be due to the first sample consisting mainly of soil pore water, or may be due to pH having an insignificant effect on the leaching of metals from the soil over the range studied. Due to these kinetic effects it is impossible to compare the effect of EDTA at pH 7 and pH 4.45,

After leaching with EDTA water was flushed through two of each set of four columns. In most cases very little additional metal was leached. Iron was one metal leached by the water.

5.3.2.3 Sequential extraction results

The soil from the columns after leaching was subjected to sequential extraction. The results for each metal in columns A – D are shown in figures 5.38-5.43 and 5.44-5.49 for experiments 1 and 2. The overall reproducibility appears to be reasonable over

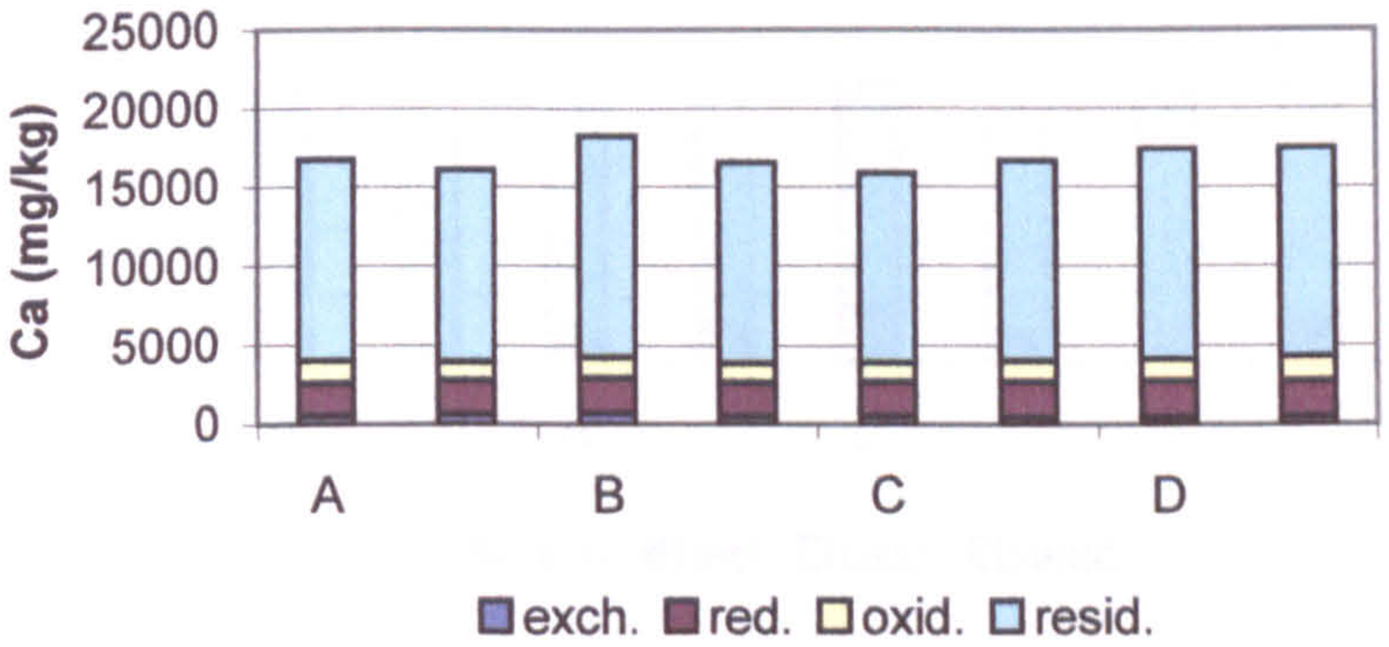

Figure 5.38 Ca Se distribution in IG 35-45 after leaching experiment 1

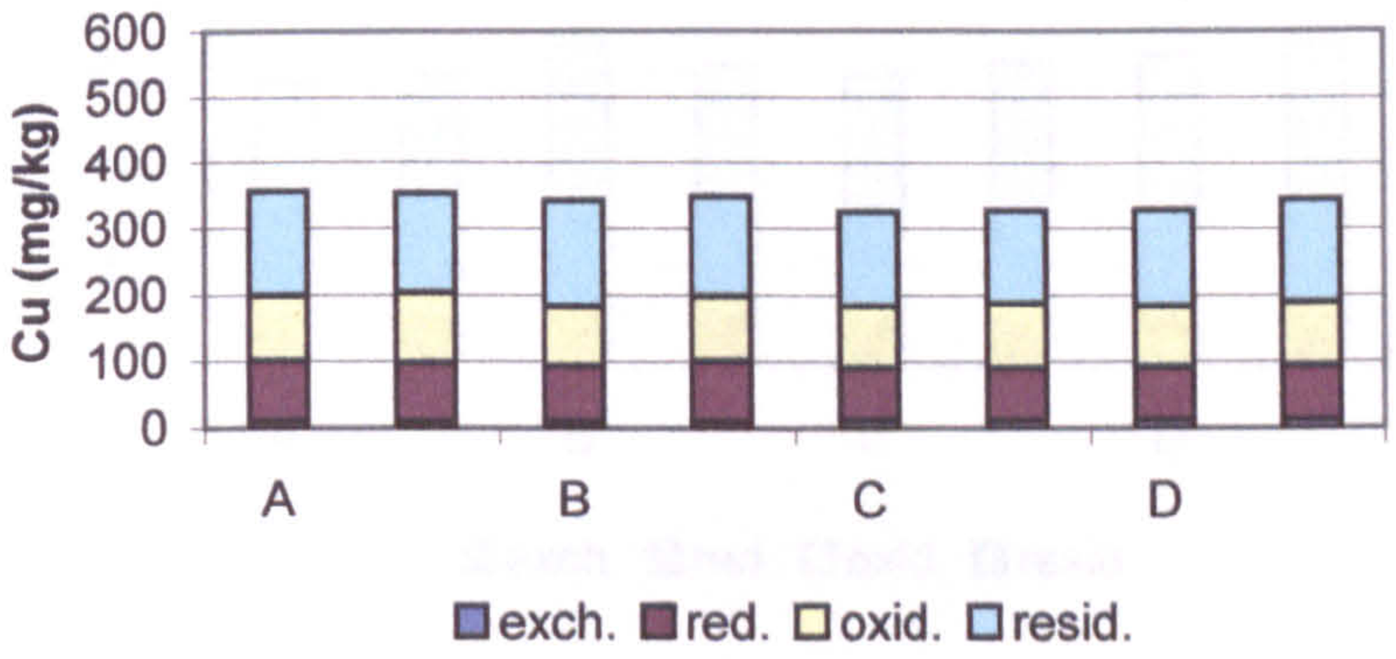

Figure 5.39 Cu SE distribution in IG 35-45 after leaching experiment 1

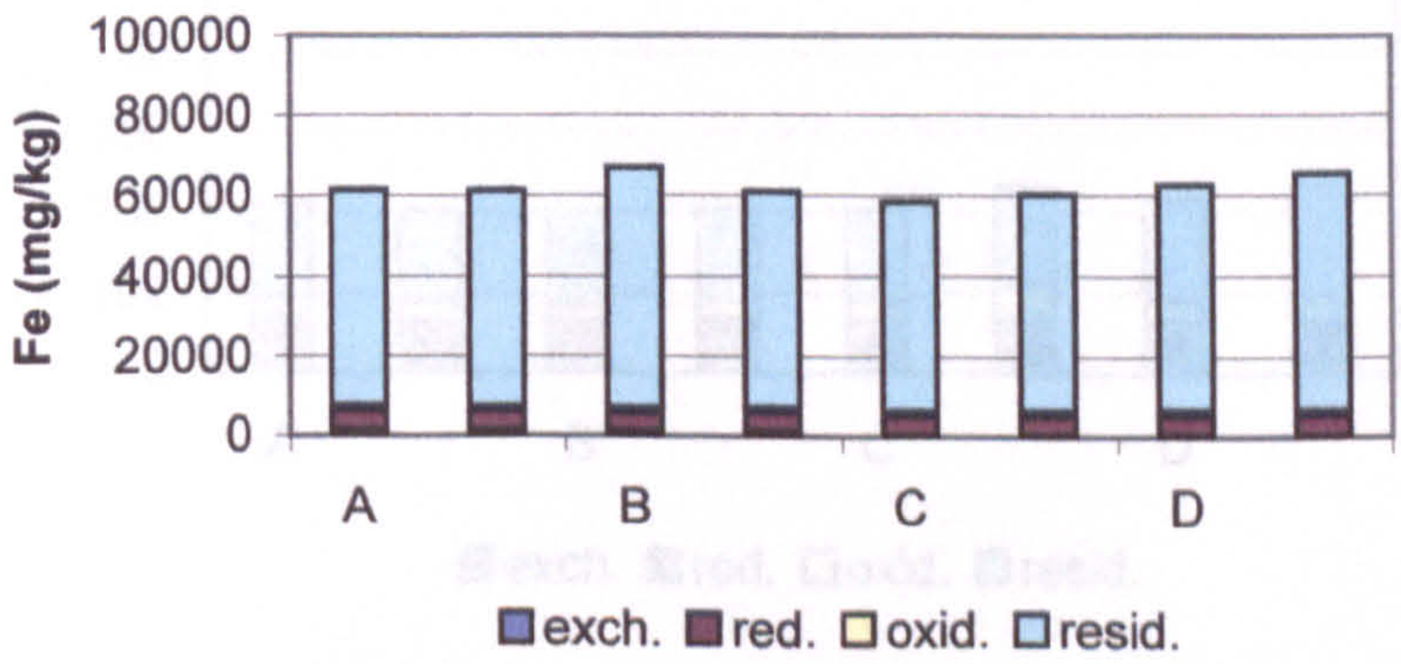

Figure 5.40 Fe SE distribution in IG 35-45 after leaching experiment 1

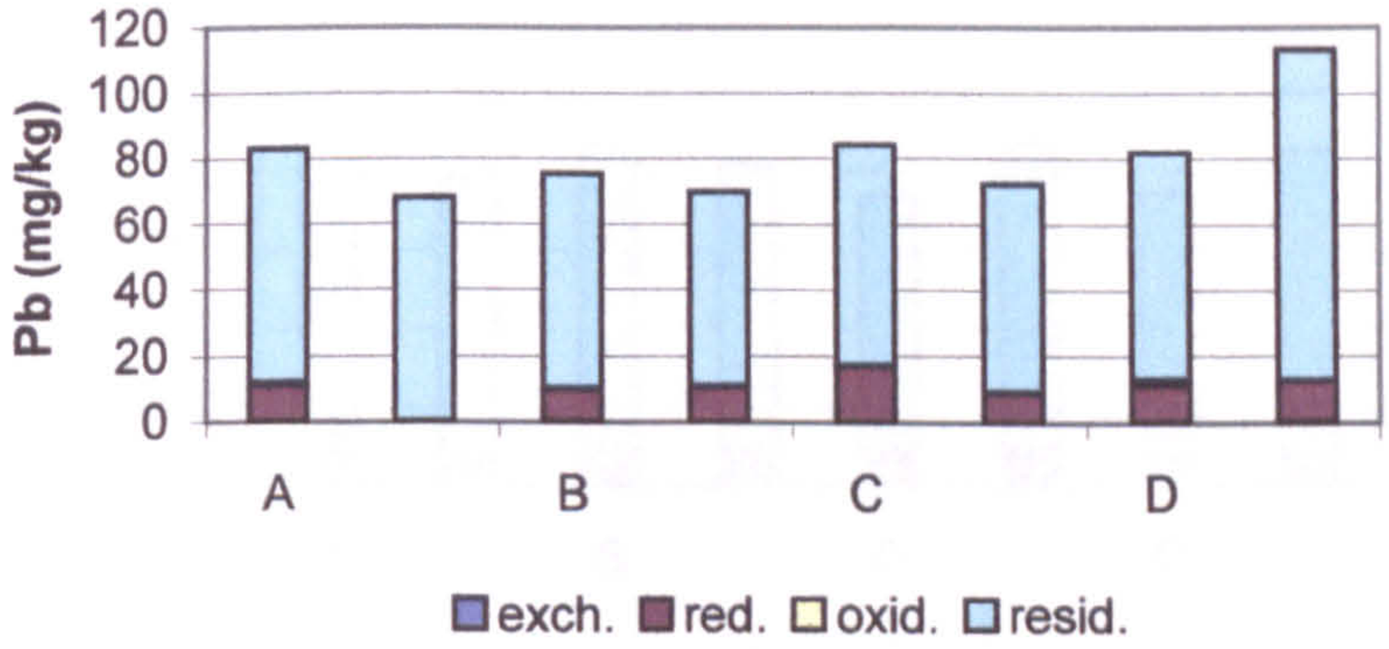

Figure 5.41 Pb SE distribution in IG 35-45 after leaching experiment 1

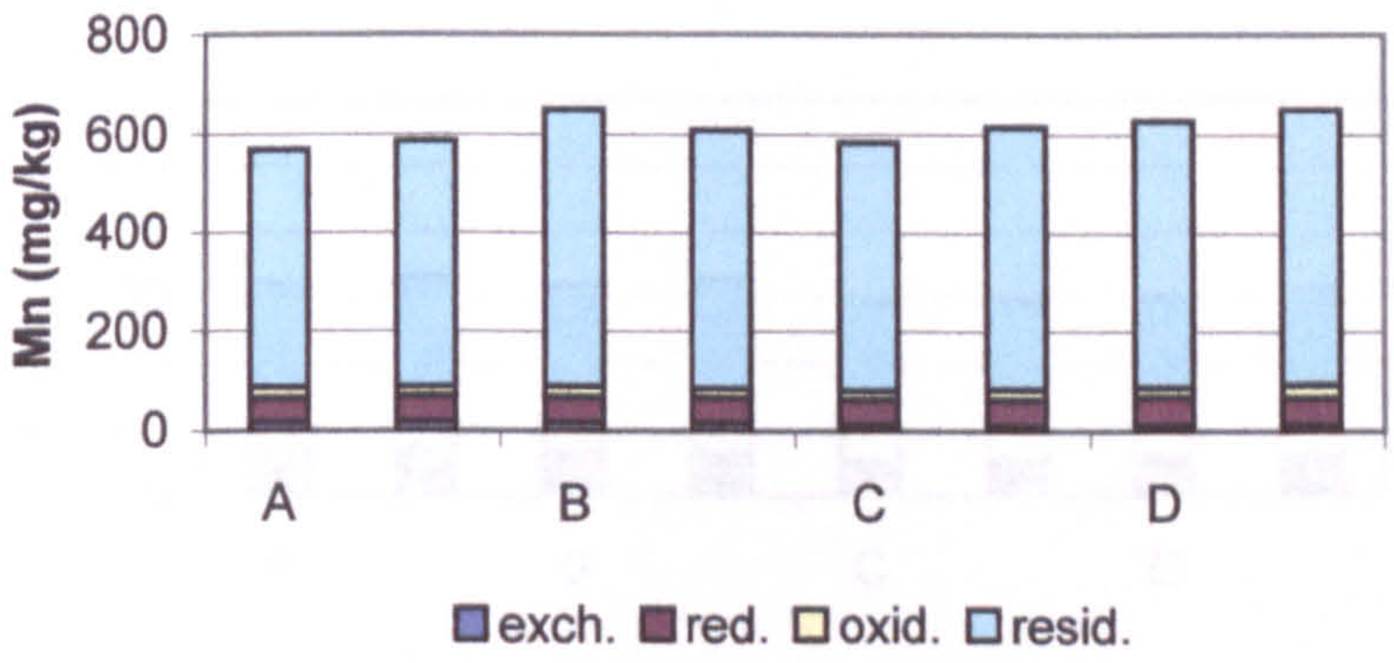

Figure 5.42 Mn SE distribution in IG 35-45 after leaching experiment 1

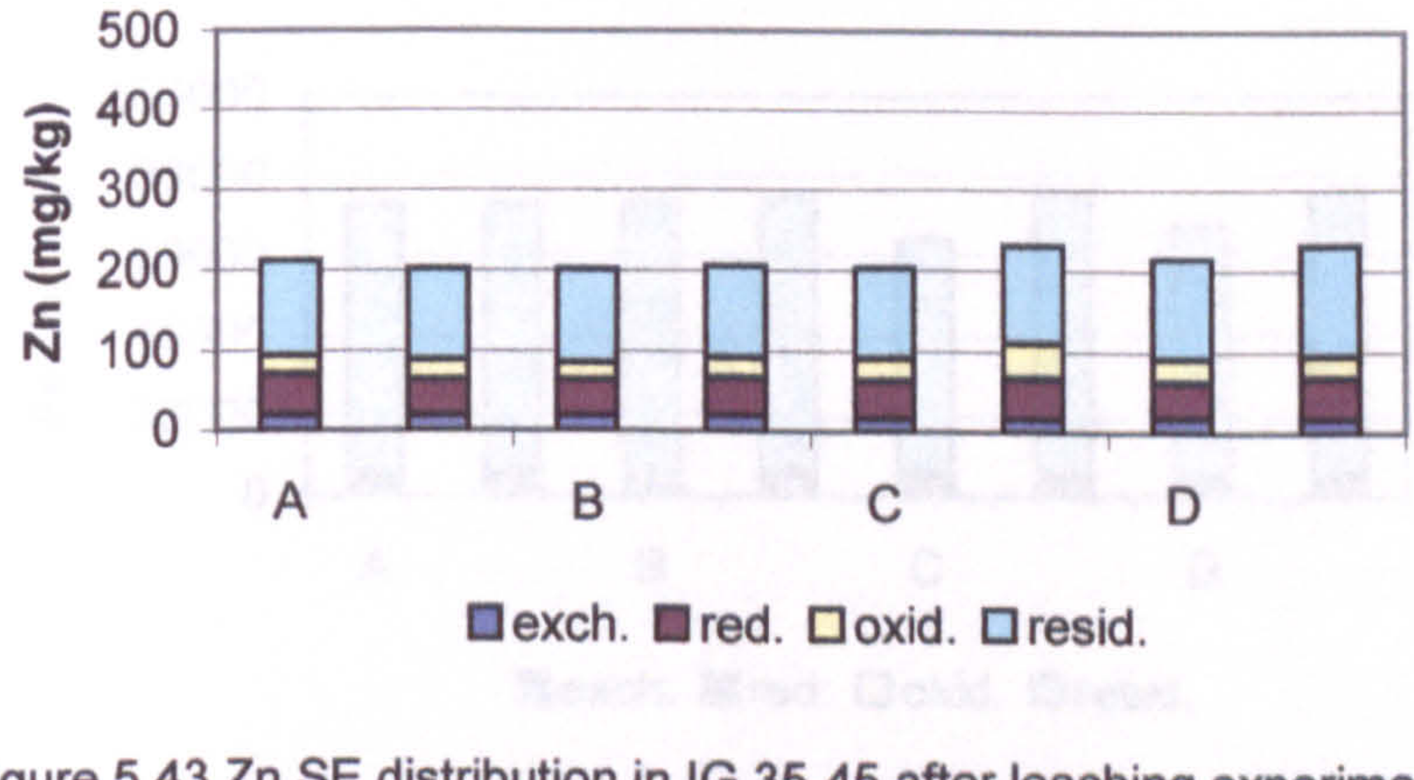

Figure 5.43 Zn SE distribution in IG 35-45 after leaching experiment 1

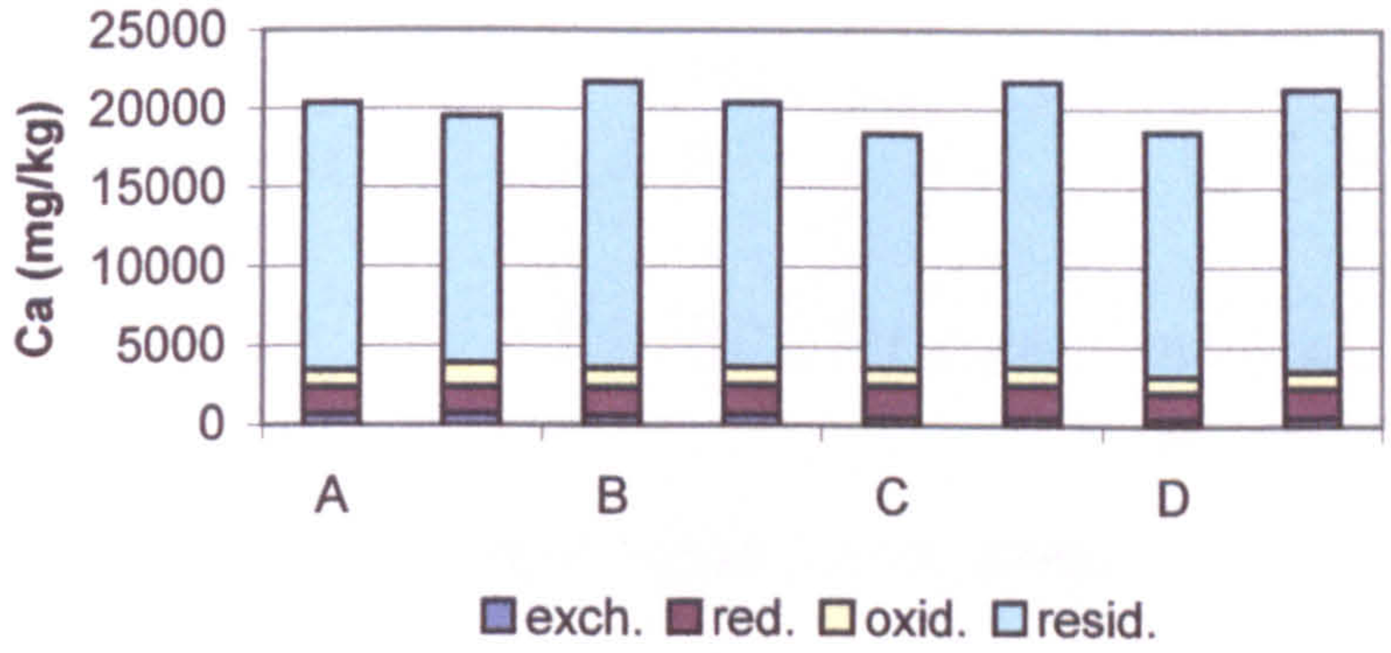

Figure 5.44 Ca SE distribution in IG 35-45 after leaching experiment 2

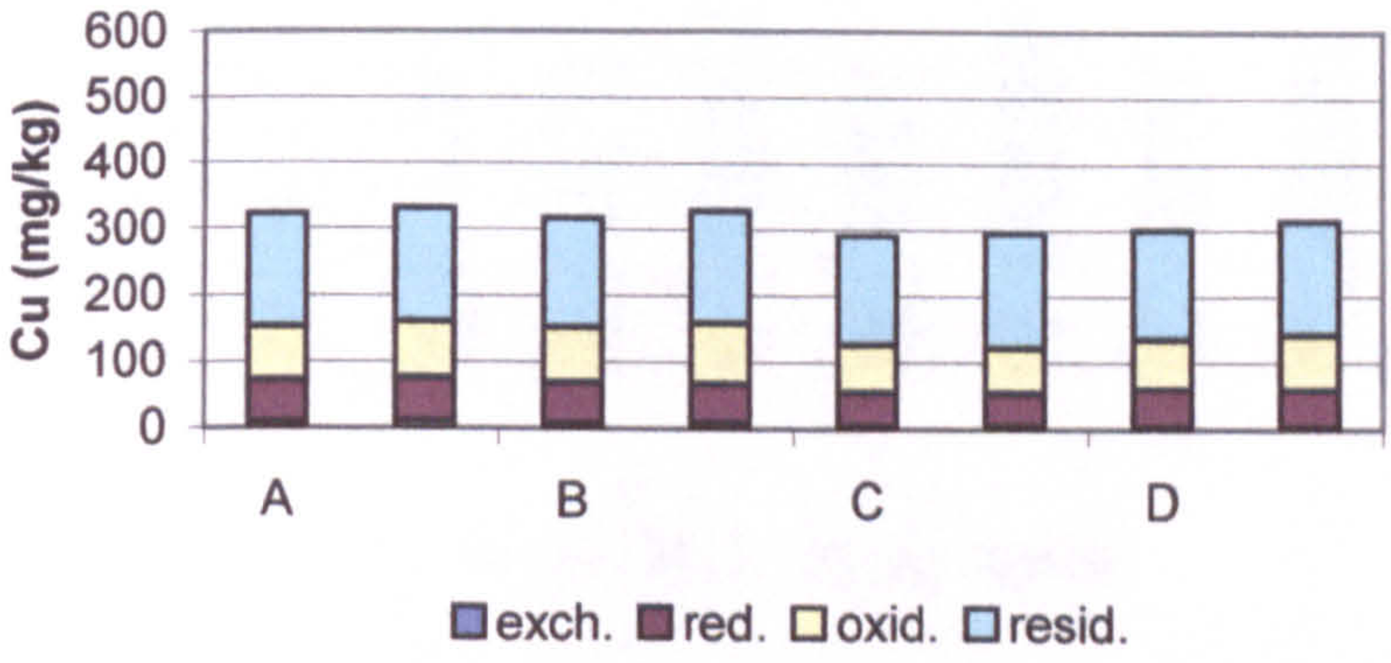

Figure 5.45 Cu SE distribution in IG 35-45 after leaching experiment 2

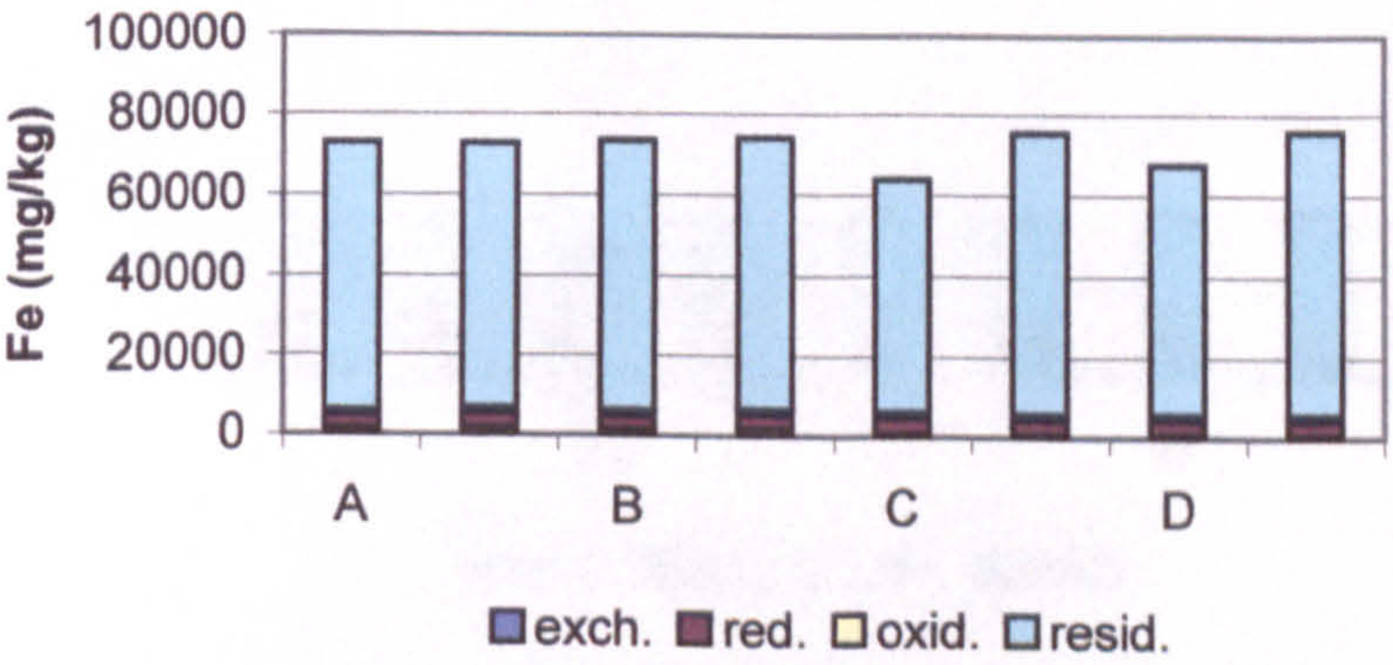

Figure 5.46 Fe SE distribution in IG 35-45 after leaching experiment 2

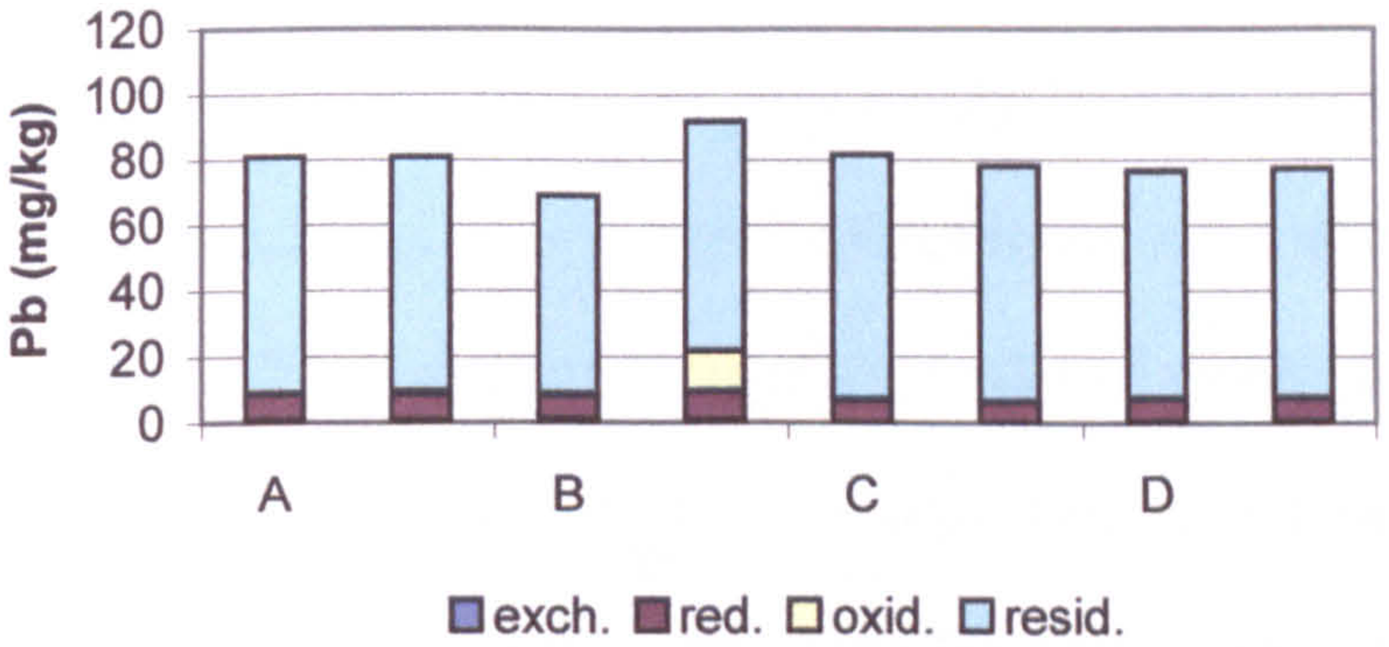

Figure 5.47 Pb SE distribution in IG 35-45 after leaching experiment 2

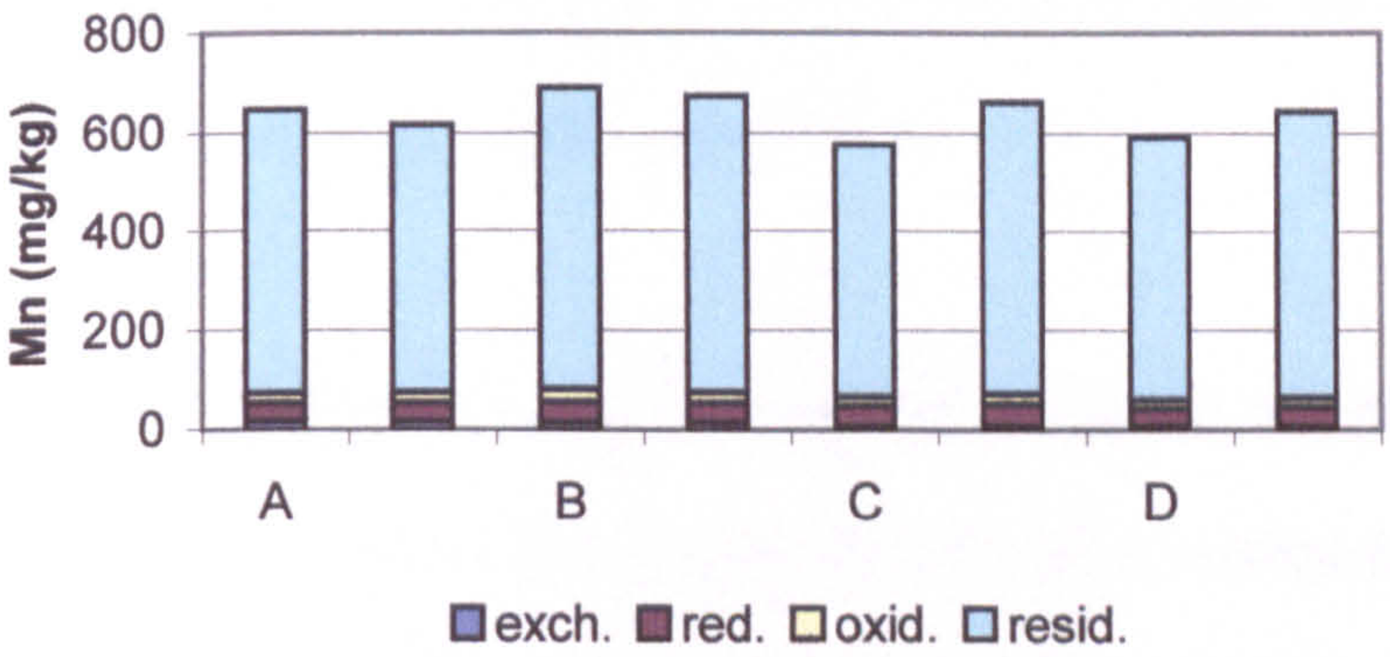

Figure 5.48 Mn SE distribution in IG 35-45 after leaching experiment 2

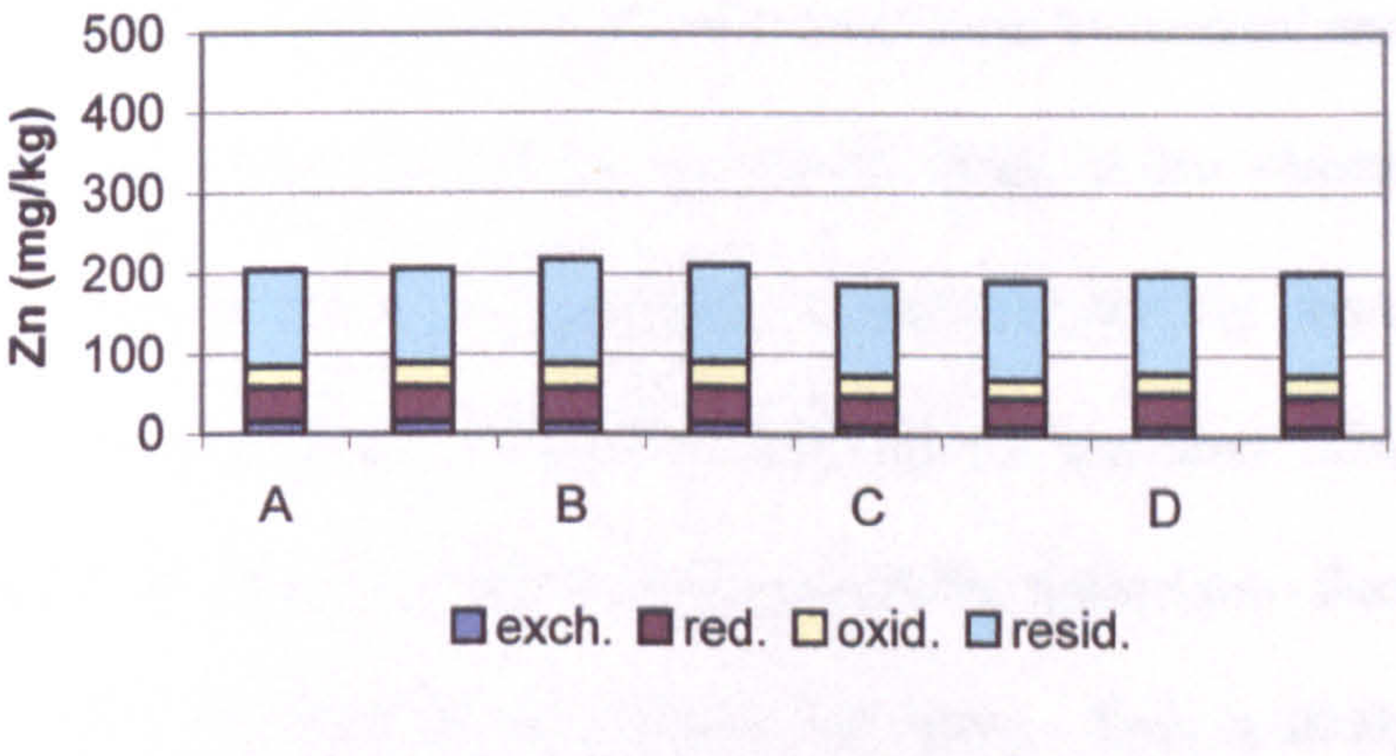

Figure 5.49 Zn SE distribution in IG 35-45 after leaching experiment 2

the four columns. Relative standard deviations are generally below 20 % and often between 5-10 %. Exceptions include lead of which very little was detected at all stages of the SE and relative deviation was therefore high. Other exceptions include the first step for iron. The results for the columns A and B are approximately three times those for columns C and D. This is probably due to the effect of leaching with water after the EDTA, which was shown to remove some additional iron from columns C and D. Manganese results from experiment 2 were also much higher for columns A/B compared to C/D. On observation there appears to be very little difference between the results from experiment 1 and 2, but as was mentioned previously no quantitative comparison can be made between the experiments due to the differences in run times for the experiments.

Comparison can be made between each experiment and the original speciation of the soil. A small amount of calcium appears to have been leached from the soil during experiment 1. Calcium was mainly decreased in the first and second stages of the sequential extraction. This is as expected as these include the more mobile forms of the metal. Experiment 2 removed more metal from the second and third stages of the sequential extraction. Metals in the second stage of the sequential extraction are affected by pH changes, so it would be expected that by lowering the pH more metals would be released from this stage by the leachate. Copper was removed mainly from the first two stages of the sequential extraction. Some copper was also removed from the third stage reservoir of metal. This is likely to be due to the chelation effect, with EDTA competing with humic substances for the copper present. Zinc was also leached from the exchangeable reservoir, but not from the

third step. Unexpectedly, a large difference was observed in iron levels in the exchangeable reservoir for the original speciation and speciation after leaching. Little difference was observed between total iron content, however the increase observed in the first step after leaching was only a very small proportion of the total metal. The additional iron seen in the first step could be either due to residual EDTA remaining in the column that is associated with iron, or more likely could be due to redistribution of the iron from less mobile steps to the first step. Lead levels are very similar in all SE, but levels of lead in the soil are very low. Lead levels for the exchangeable step are within a factor of 10 of the detection limit. Any differences observed at these levels could either be due to the effect of the EDTA leaching or could be due to background signals in the ICP AES analysis. Manganese levels were similarly unaffected, but this type of manganese is less likely to be leached (more manganese is present in the less mobile reservoirs, according to SE) or to form a metal complex with EDTA.

5.3.3 Experiment 3

Due to the kinetic effect causing limitations in the interpretation of results in the first two experiments, another experiment was developed. The experiment was scaled down to 200 g of soil and a total flushing volume of ~ 2 L, with samples being collected every ~ 200 ml. This experiment was run continuously to eliminate the kinetic effect. In this experiment two soils were used, the IG 35-45 used in the previous experiments and a fresh soil taken from a site at Ardeer. The leaching solution used was diammonium EDTA at pH 7. Leaching of the sample from site 1

at Ardeer was also attempted; however this failed as the sample particle size was very small and would therefore not allow passage of the EDTA leaching solution. This emphasises the importance of investigating the soil characteristics prior to deciding upon the nature of the remediation strategy. It is clear that soil flushing techniques will not be successful for clay soils.

5.3.3.1 Conductivity and pH

Conductivity and pH profiles for experiment 3 are shown in figures 5.50-5.51. The conductivity profile was slightly higher than experiments 1 and 2, probably because of the different EDTA salt used. A difference was noticed between the two soils, the Ardeer sample having a lower starting conductivity than IG 35-45. The pH profile for IG 35-45 was similar to that of experiment 1 (also EDTA at pH 7). The pH rises from ~ 6.6 to a plateau of pH 7. The profile for the soil from Ardeer site 2 differs only in that the starting pH is lower than that of IG 35-45. The Ardeer soil pH was determined as 6.2 in water, which corresponds to the pH measured in the first sample taken from the column. This suggests that the first few samples taken consist mainly of soil pore water and later become diluted with the leaching solution. It is clear that the soil characteristics influence the initial pH and conductivity profiles.

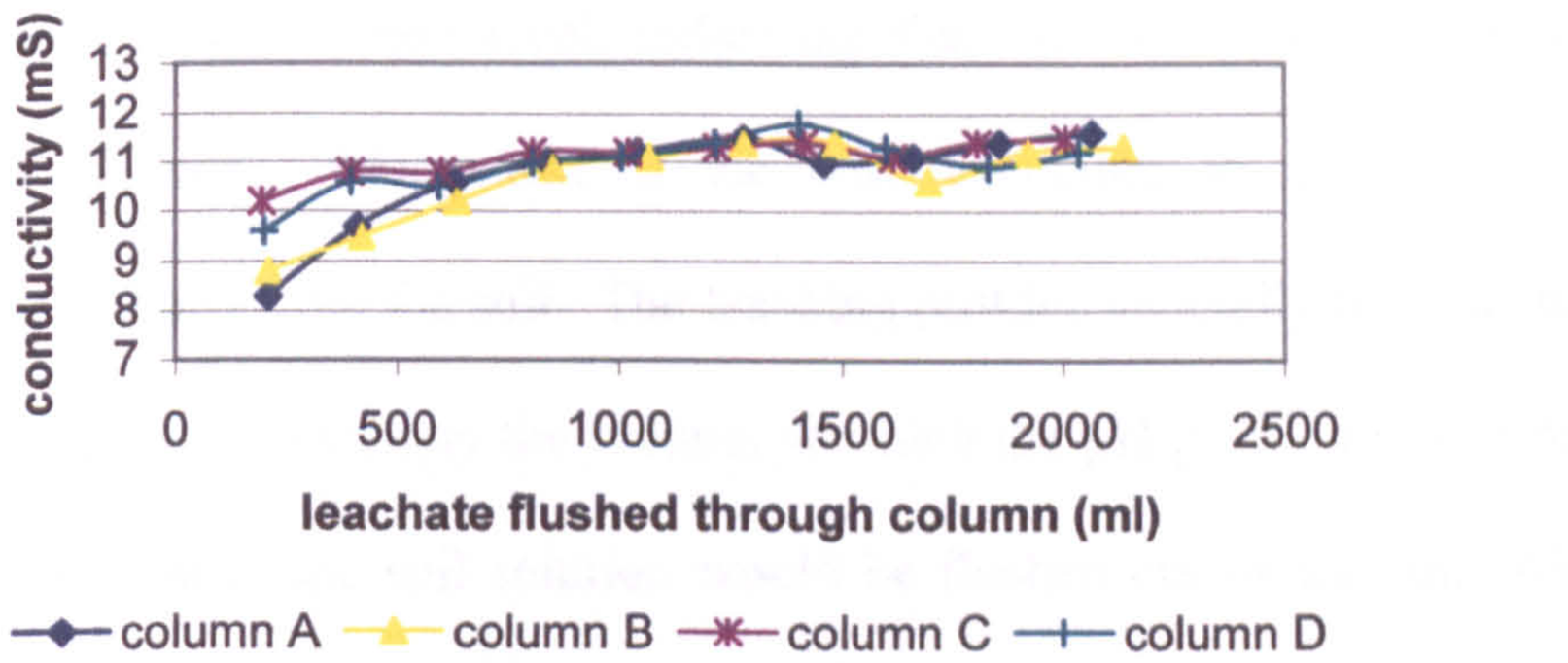

Figure 5.50 conductivity profile for IG 35-45 (A,B) and Ardeer (C,D), experiment 3

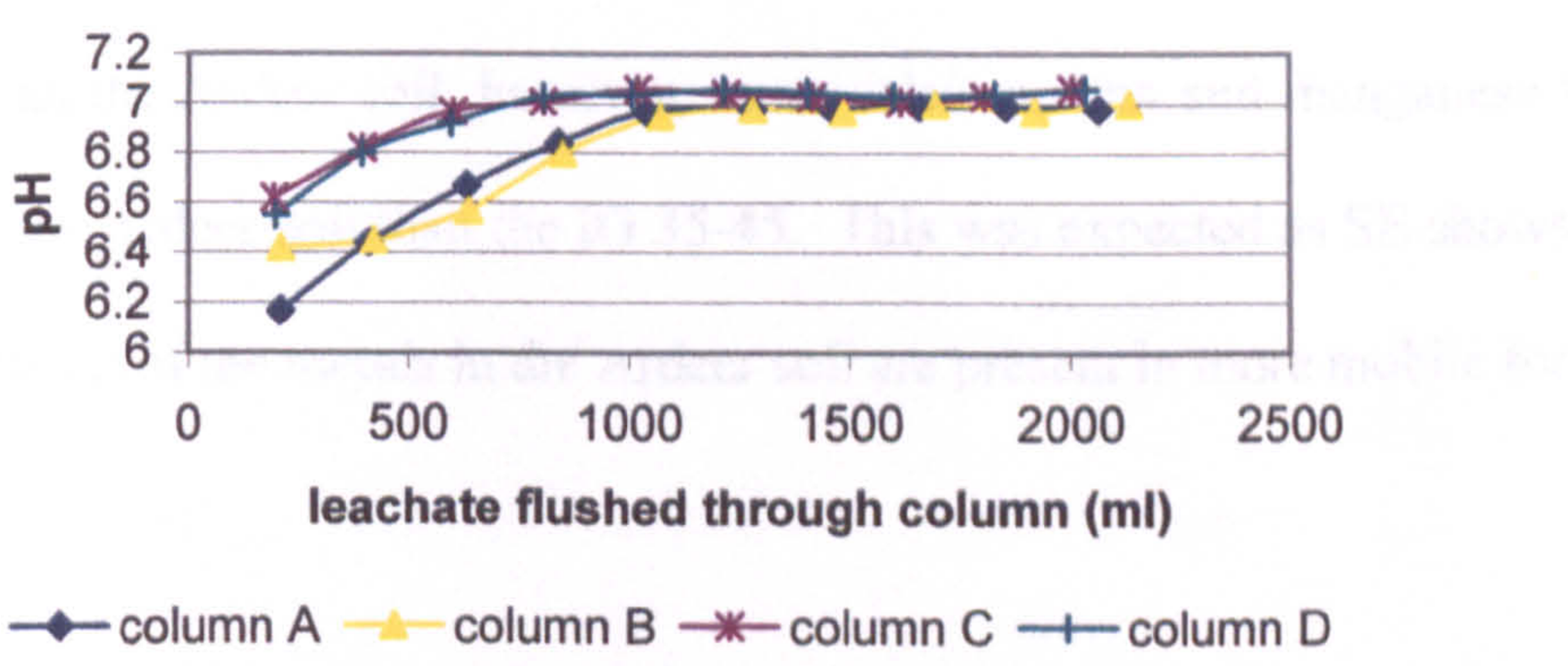

Figure 5.51 pH profile for IG 35-45 (A,B) and Ardeer (C,D), experiment 3

5.3.3.2 Leaching profiles

For all metals in IG 35-45, the leaching profiles (figures 5.52-5.57) decrease gradually to an almost constant level, indicating that the kinetic effect has been successfully eliminated. Manganese is the only exception to this, very little manganese was leached from the soil. The leaching profiles generally reach a steady level at ~ 1000 ml, approximately the volume at which the pH profile reaches pH 7. Metal already present in the soil solution would be flushed out of the soil column quickly. Further metal would be flushed out as the flushing solution passed through the column, until an equilibrium was reached. The previous profiles (Experiments 1 and 2) suggest that leaving the EDTA in the columns for extended periods would remove more metal from the column as this would give the EDTA time to complex with the metals present. The IG 35-45 soil contained more calcium, copper, iron and manganese than the Ardeer soil, however, more calcium, iron and manganese were leached from the Ardeer soil than the IG 35-45. This was expected as SE shows that higher proportions of the metals in the Ardeer soil are present in more mobile forms.

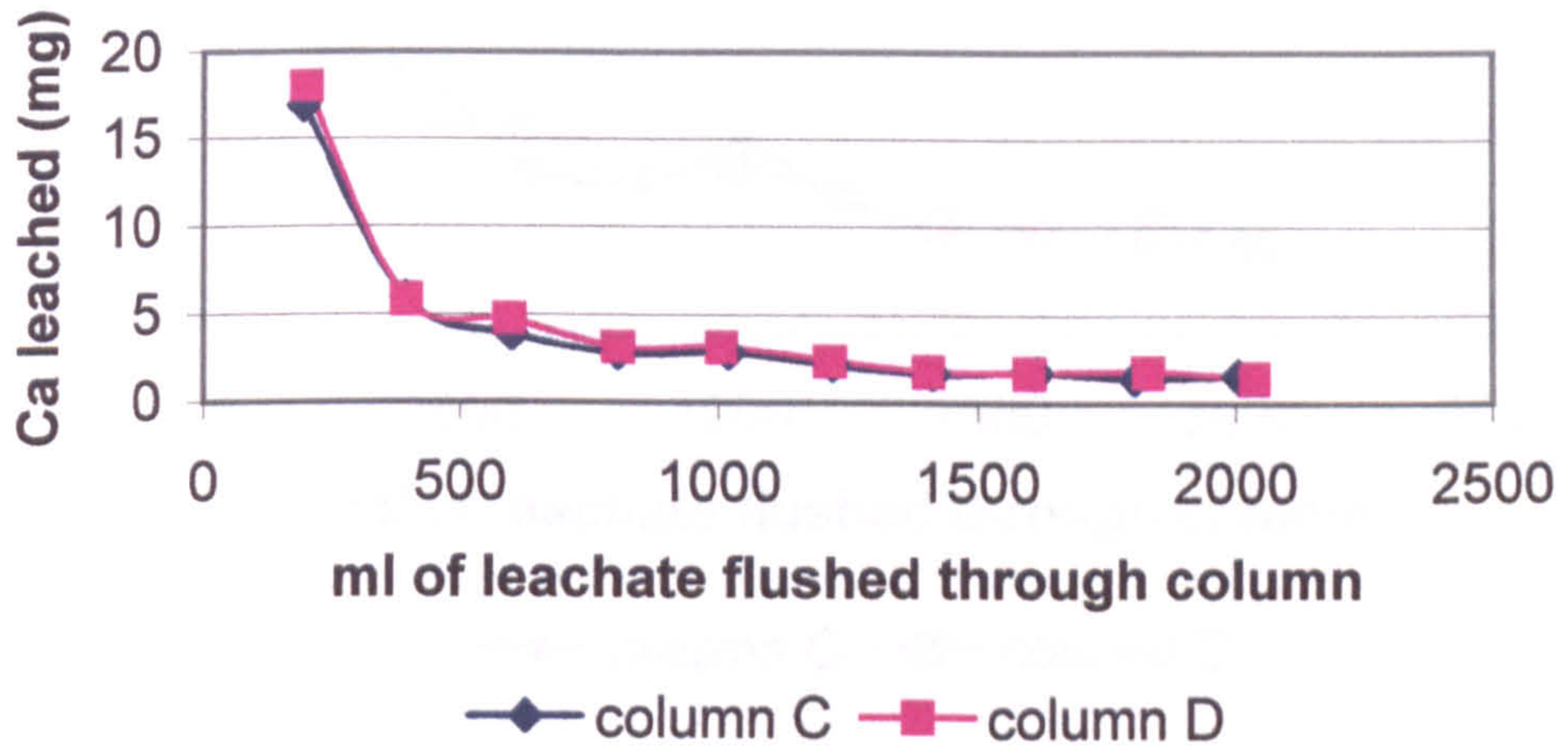

Figure 5.52 Ca in leachate from IG 35-45, experiment 3

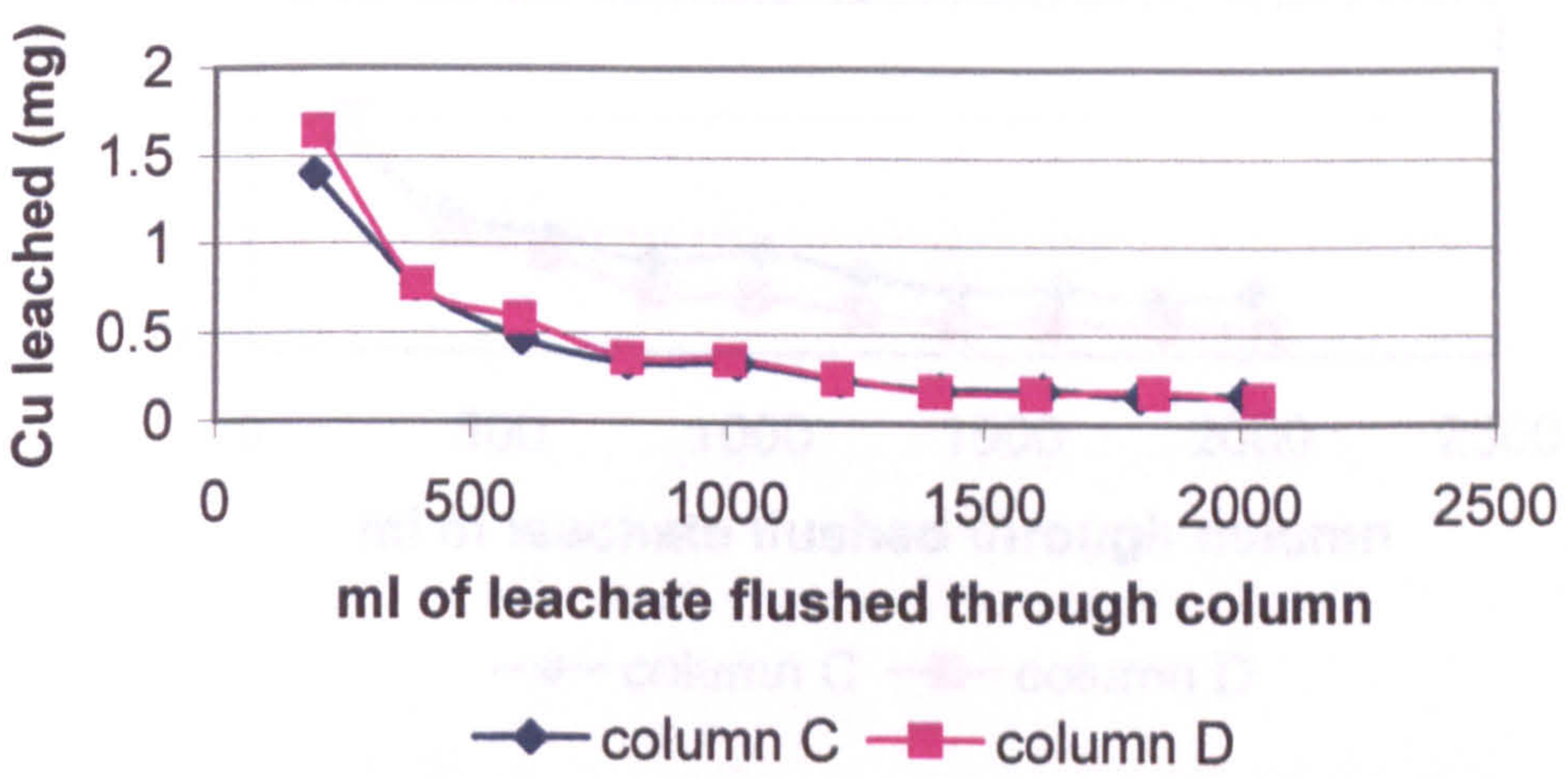

Figure 5.53 Cu in leachate from IG 35-45, experiment 3

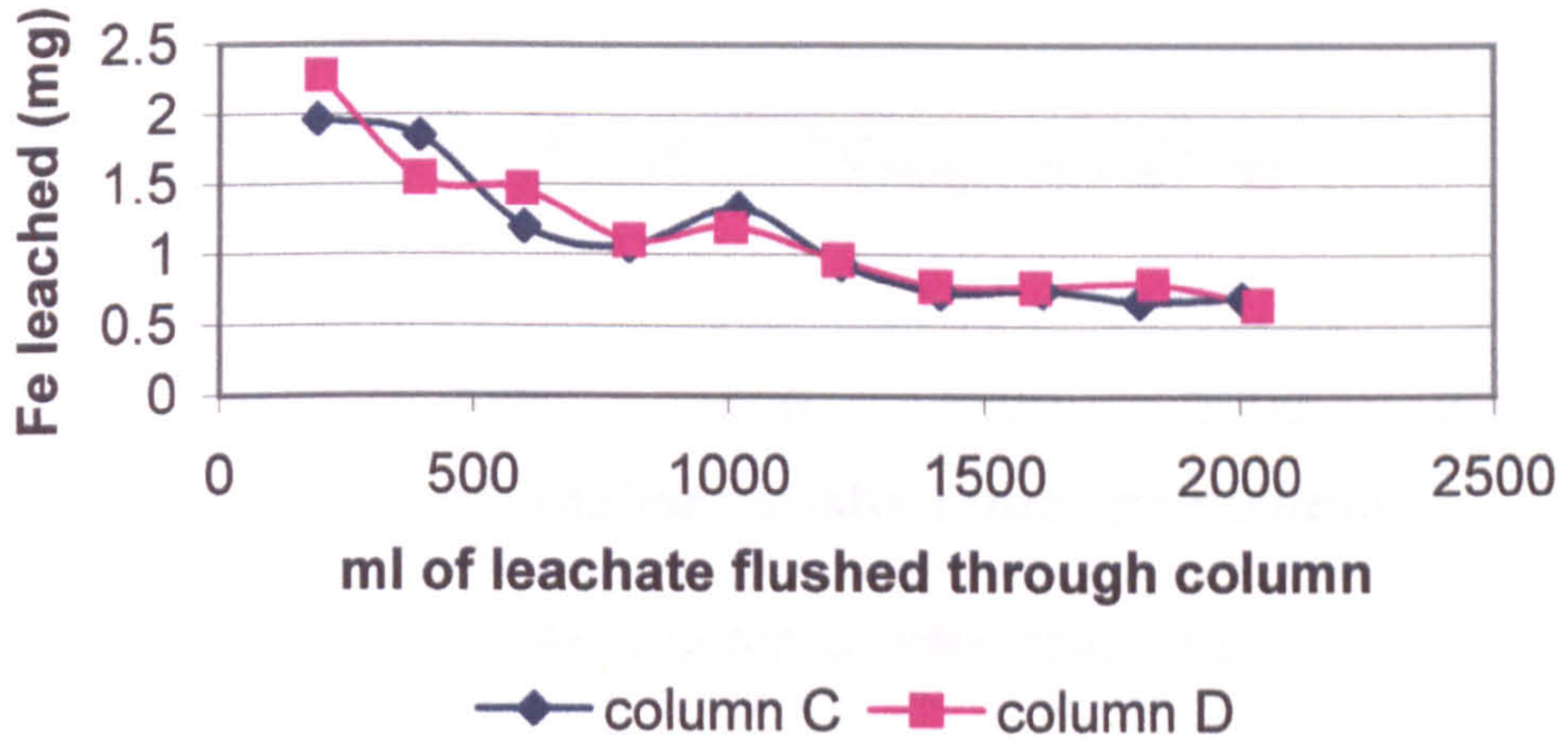

Figure 5.54 Fe in leachate from IG 35-45, experiment 3

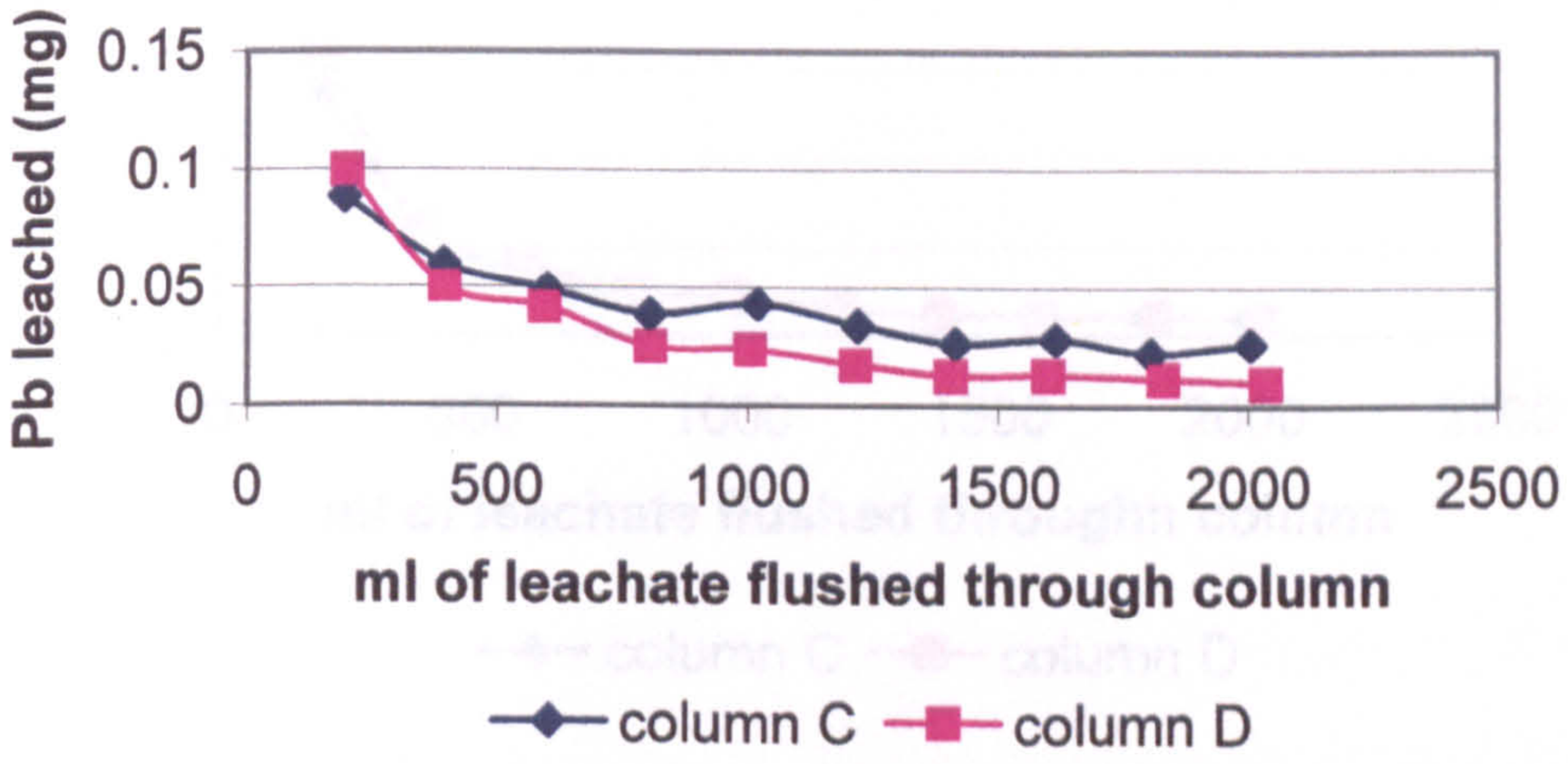

Figure 5.55 Pb in leachate from IG 35-45, experiment 3

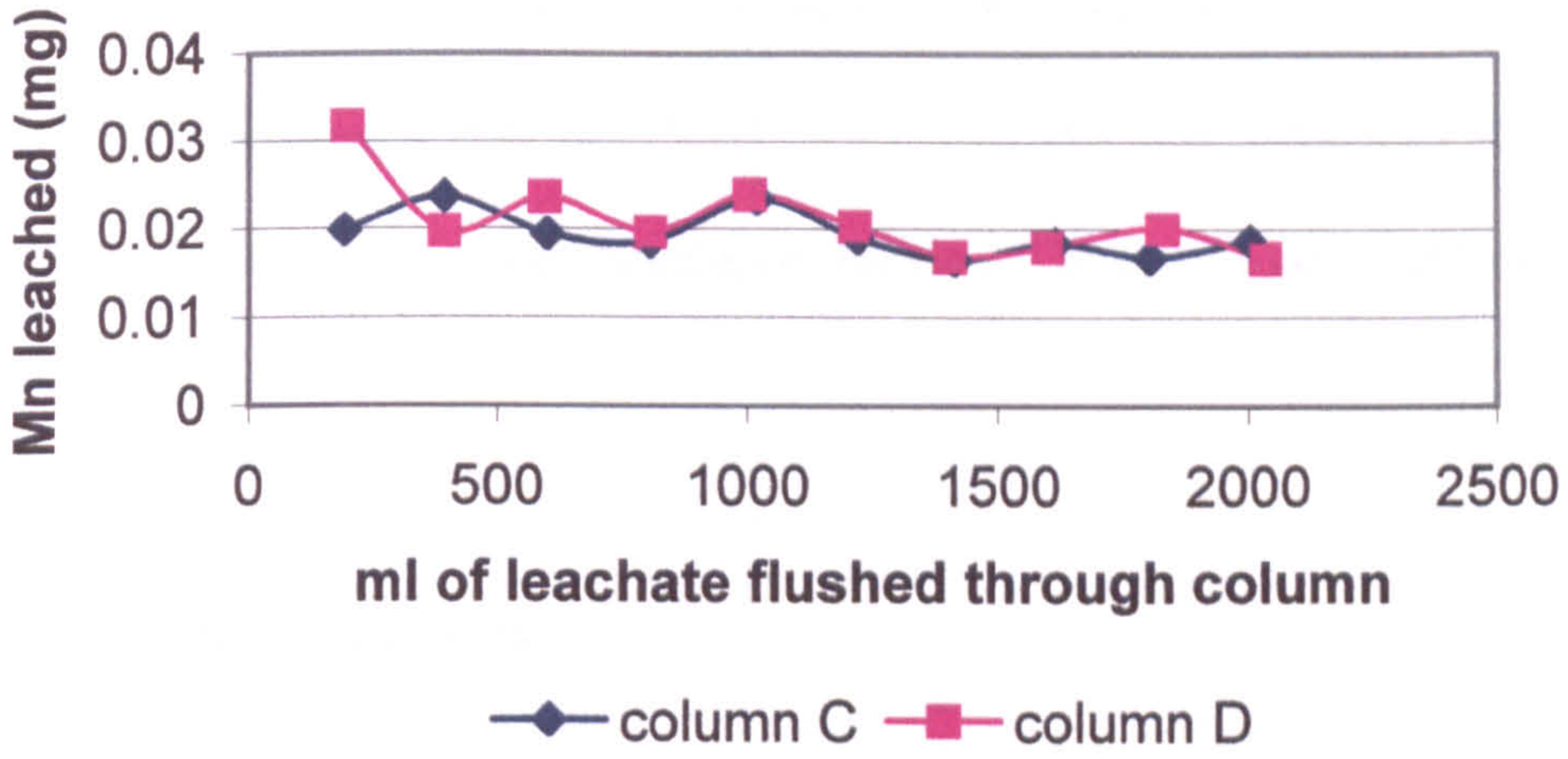

Figure 5.56 Mn in leachate from IG 35-45, experiment 3

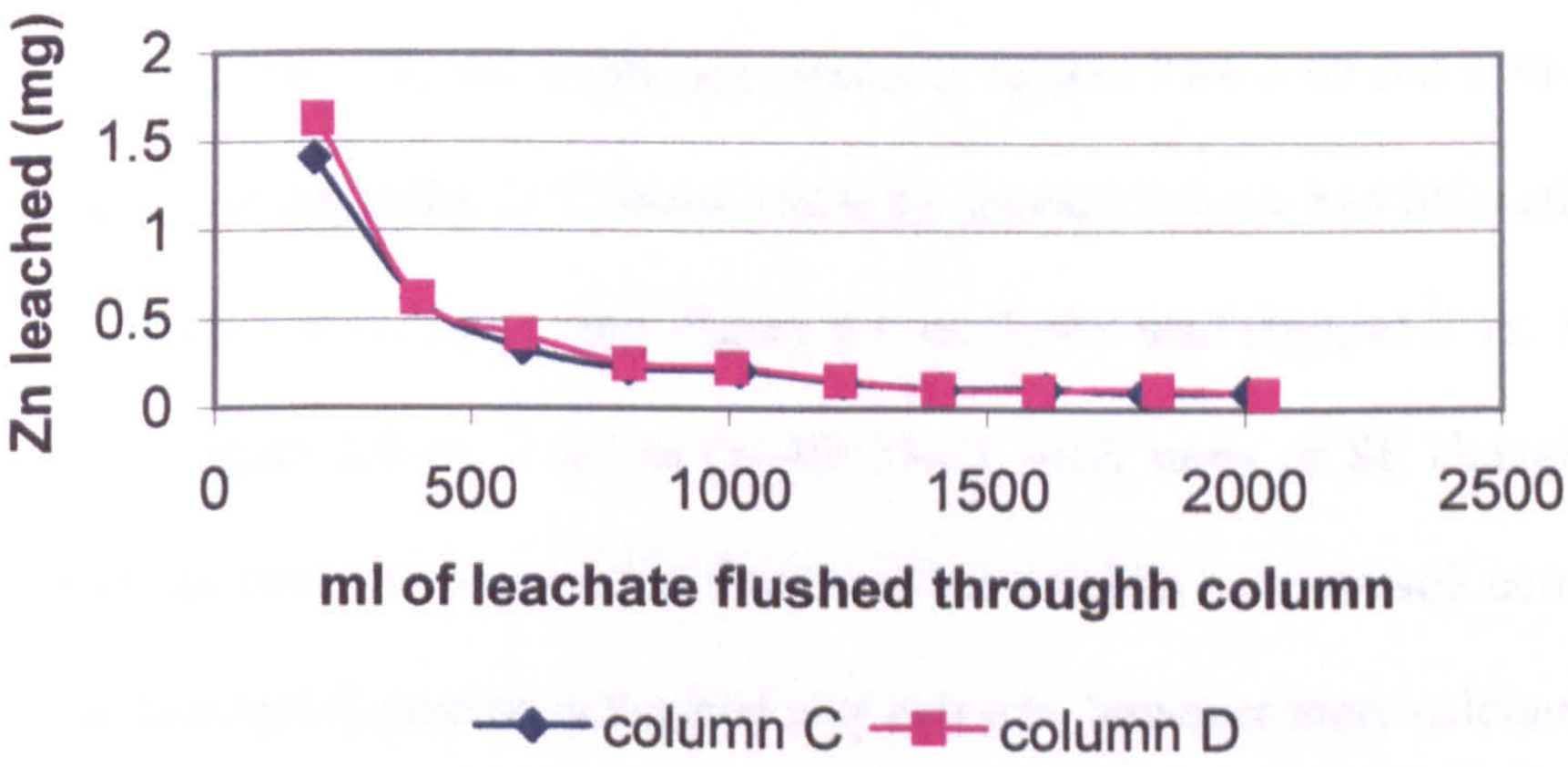

Figure 5.57 Zn in leachate from IG 35-45, experiment 3

The leaching profiles for the Ardeer soil (figures 5.58-63) follow similar patterns to the IG 35-45 profiles, with the exception of iron and manganese. These profiles increase to a peak after 500-600 ml, before steadily decreasing to an almost constant level. This may suggest that the EDTA is leaching iron and manganese that is more strongly bound than the readily leachable metals in the pore water and those that are exchangeable.

5.3.3.3 Sequential Extraction

SE was again carried out on the soils after the column experiments. Duplicate samples were taken through the SE procedure for each column, and named {A1, A2}, {B1, B2}, etc. The SE results are shown in figures 5.64-5.69 and 5.70-5.75 and the data given in appendix 2. Column leaching appears to have had little effect upon calcium (figure 5.4 vs. 5.64), iron (figure 5.6 vs. 5.66), lead (figure5.7 vs. 5.67) and manganese (figure 5.8 vs. 5.68) in the IG 35-45, with sums of SE changing little. There does appear to be some redistribution of the calcium between soil components. Much less calcium is present in the first step extracts, however more calcium appears in the residual step. This may be due to the formation of insoluble compounds at the higher pH found after leaching with EDTA. Although iron appears to have been successfully leached from the reducible and oxidisable soil components, more iron appears to be associated with the exchangeable and carbonate bound soil components. Copper (figure 5.5 vs. 5.65) and zinc (figure 5.9 vs. 5.69) have been leached successfully from the exchangeable fraction as expected. Based on stability constants for EDTA complexes with the metals studied, Fe^{3+} should form the most

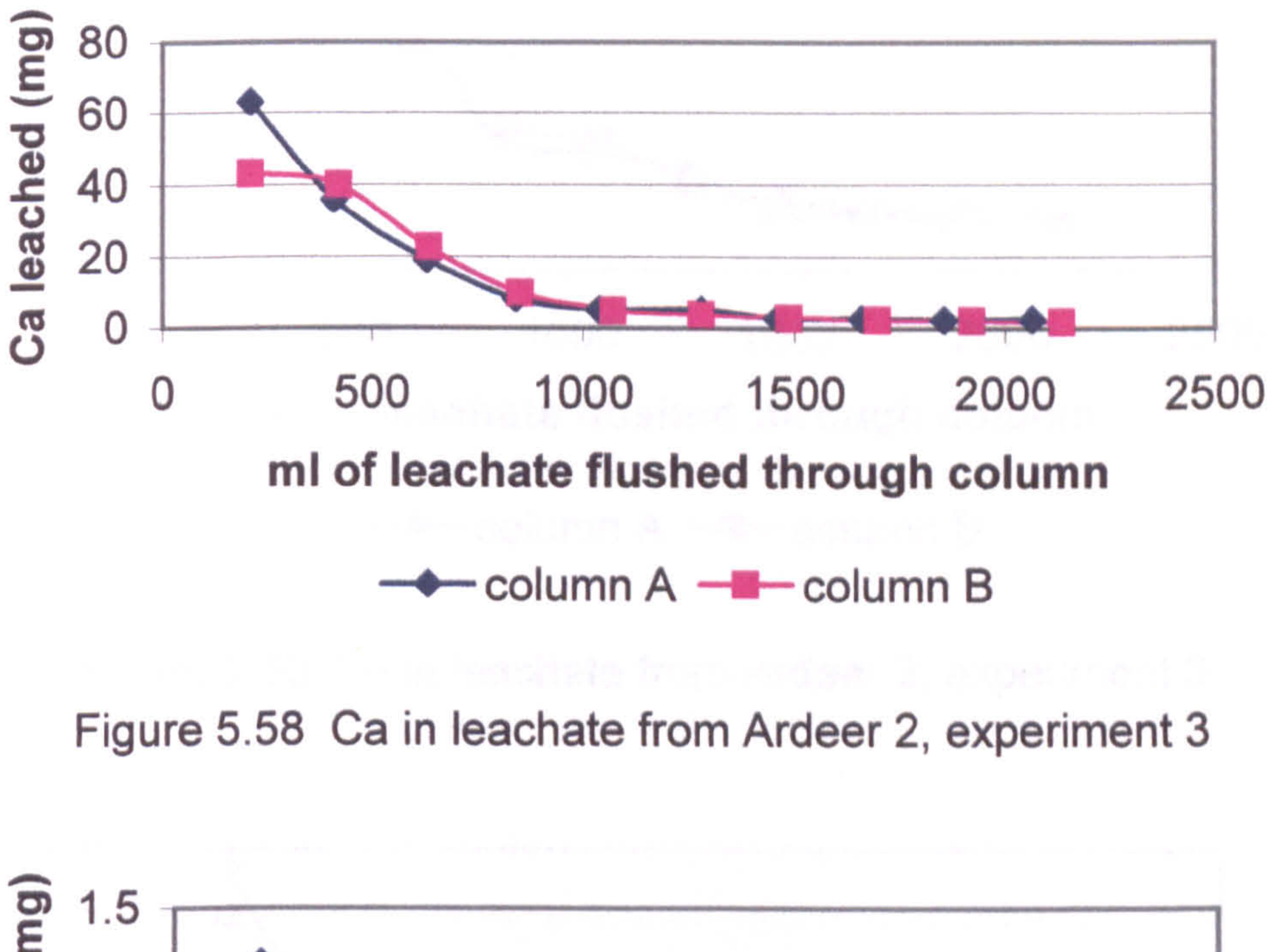

Figure 5.58 Ca in leachate from Ardeer 2, experiment 3

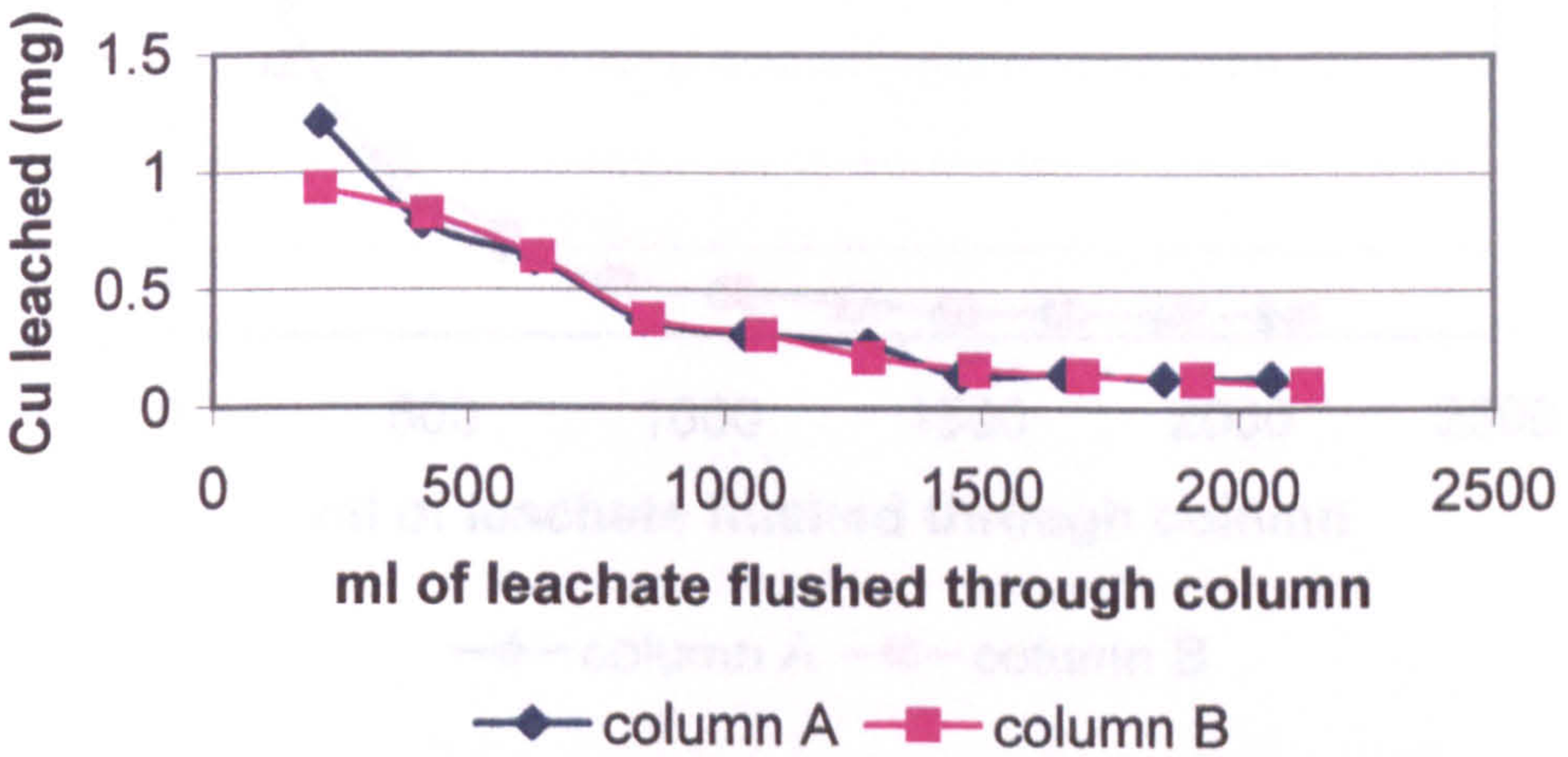

Figure 5.59 Cu in leachate from Ardeer 2, experiment 3

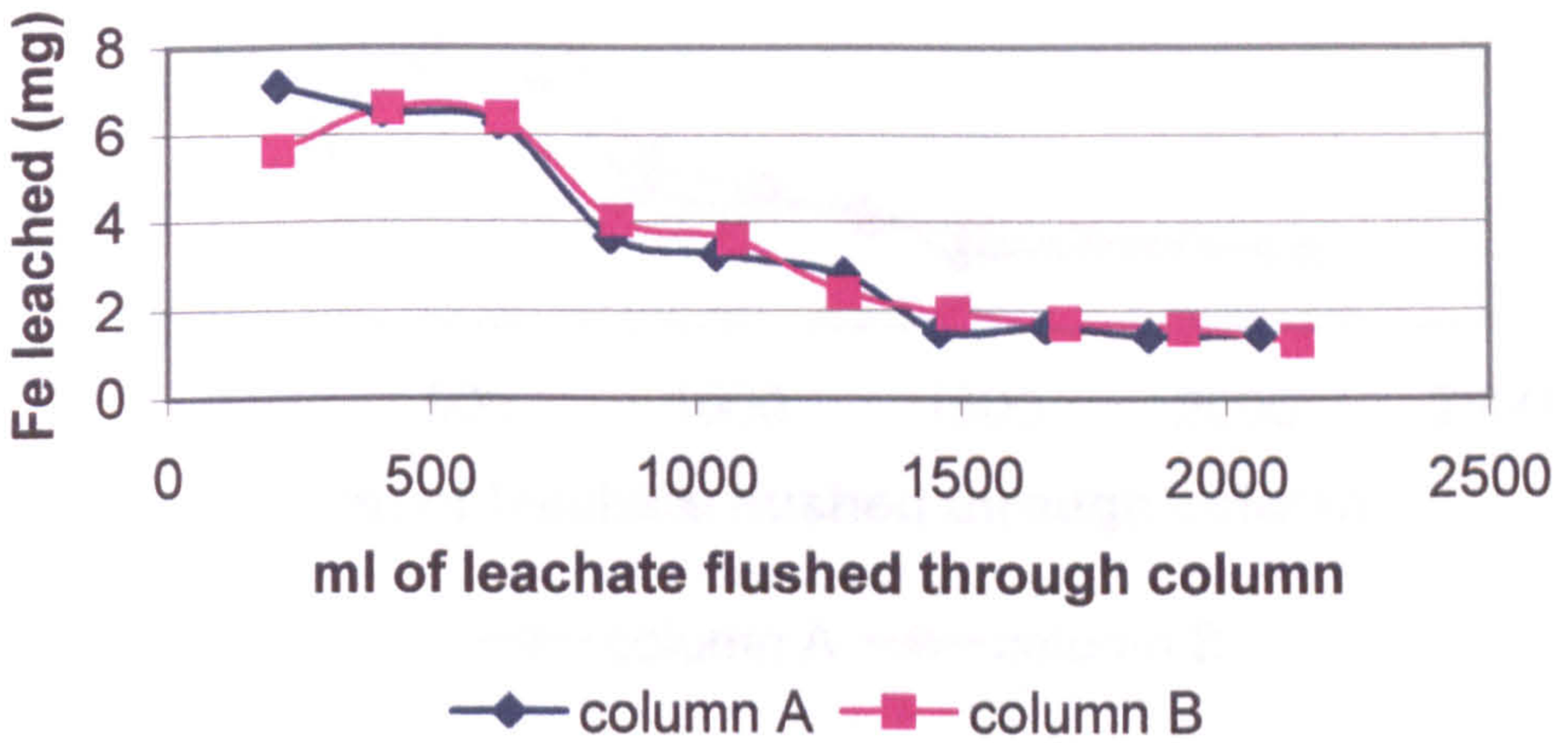

Figure 5.60 Fe in leachate from Ardeer 2, experiment 3

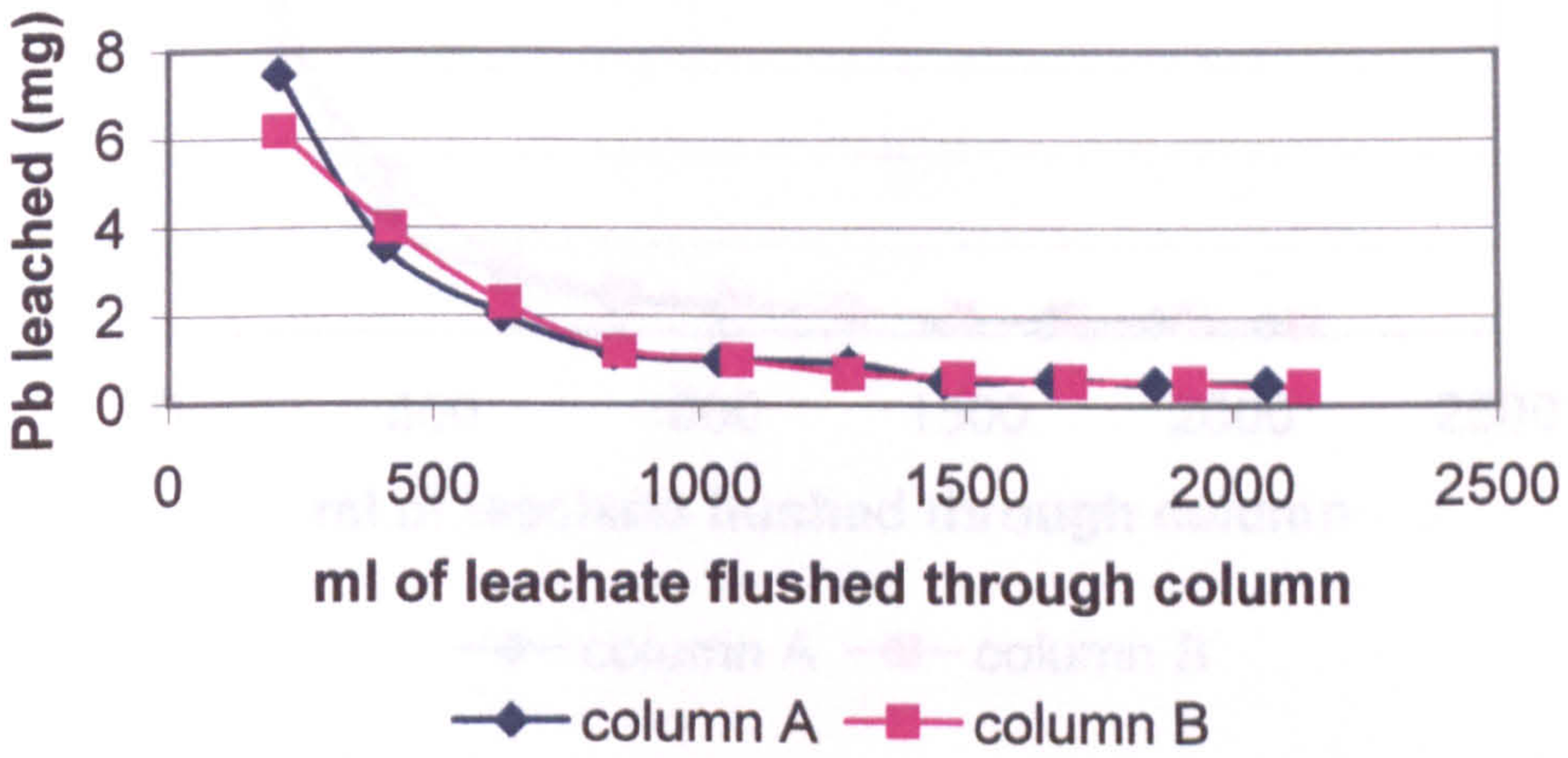

Figure 5.61 Pb in leachate from Ardeer 2, experiment 3

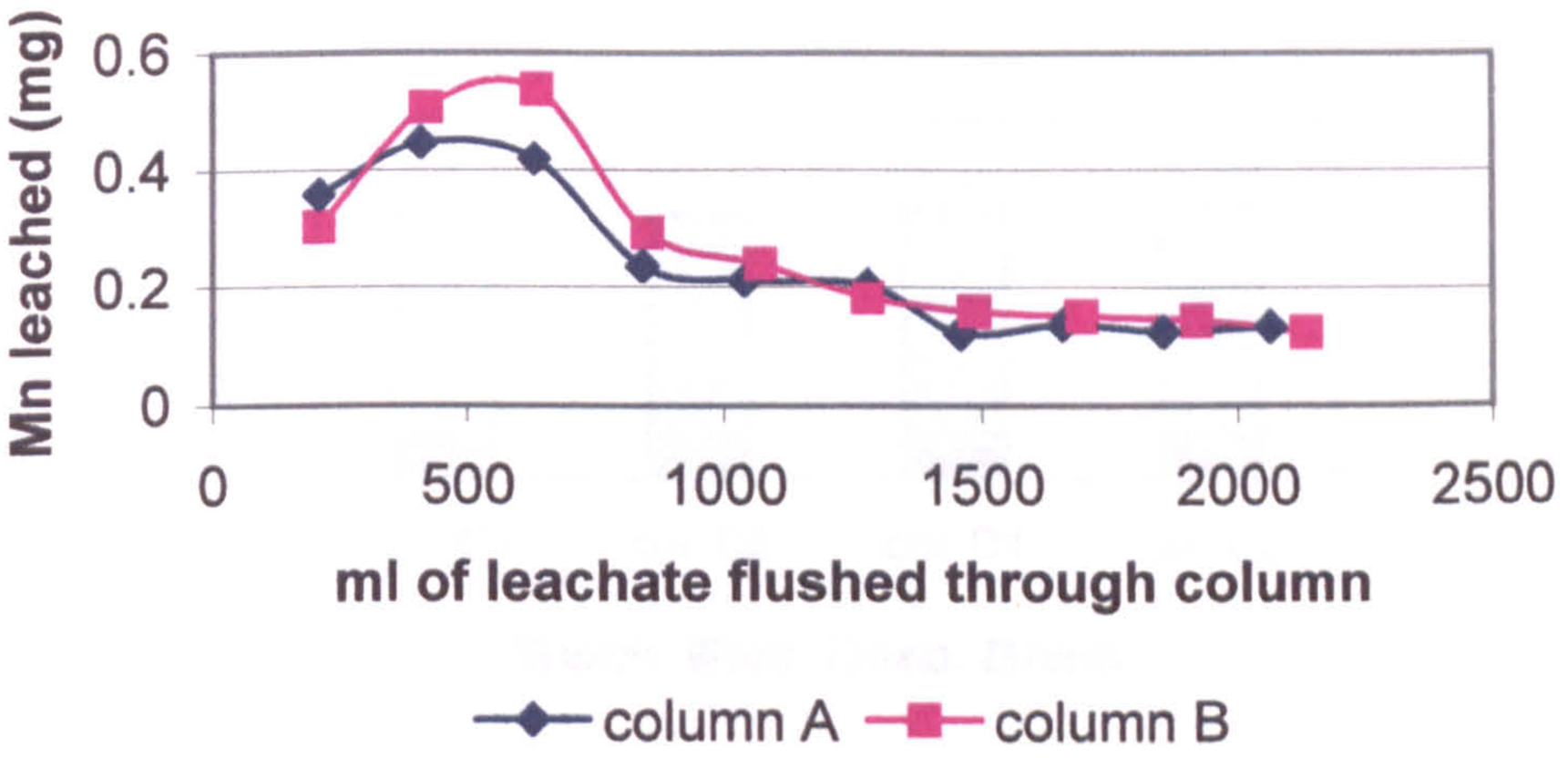

Figure 5.62 Mn in leachate from Ardeer 2, experiment 3

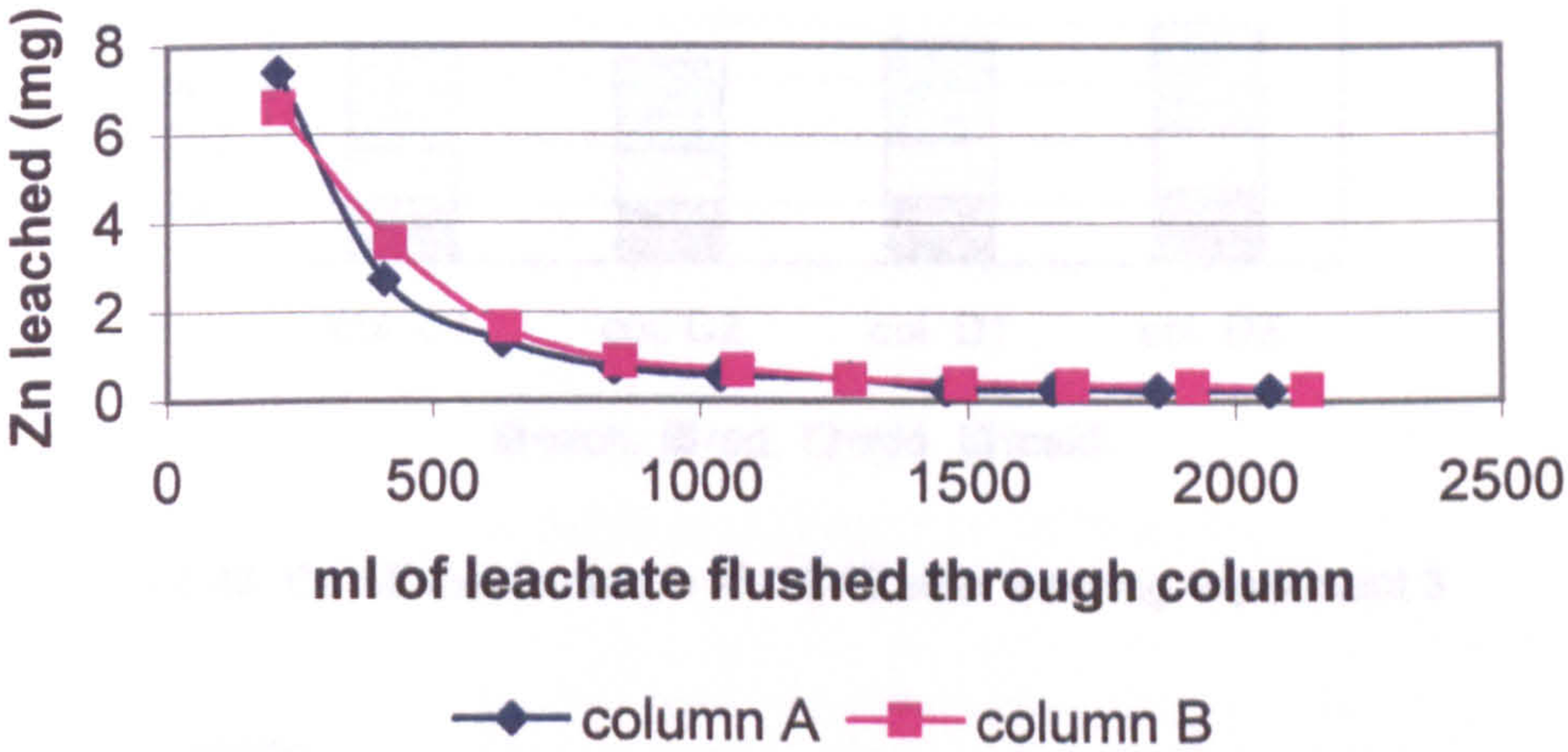

Figure 5.63 Zn in leachate from Ardeer 2, experiment 3

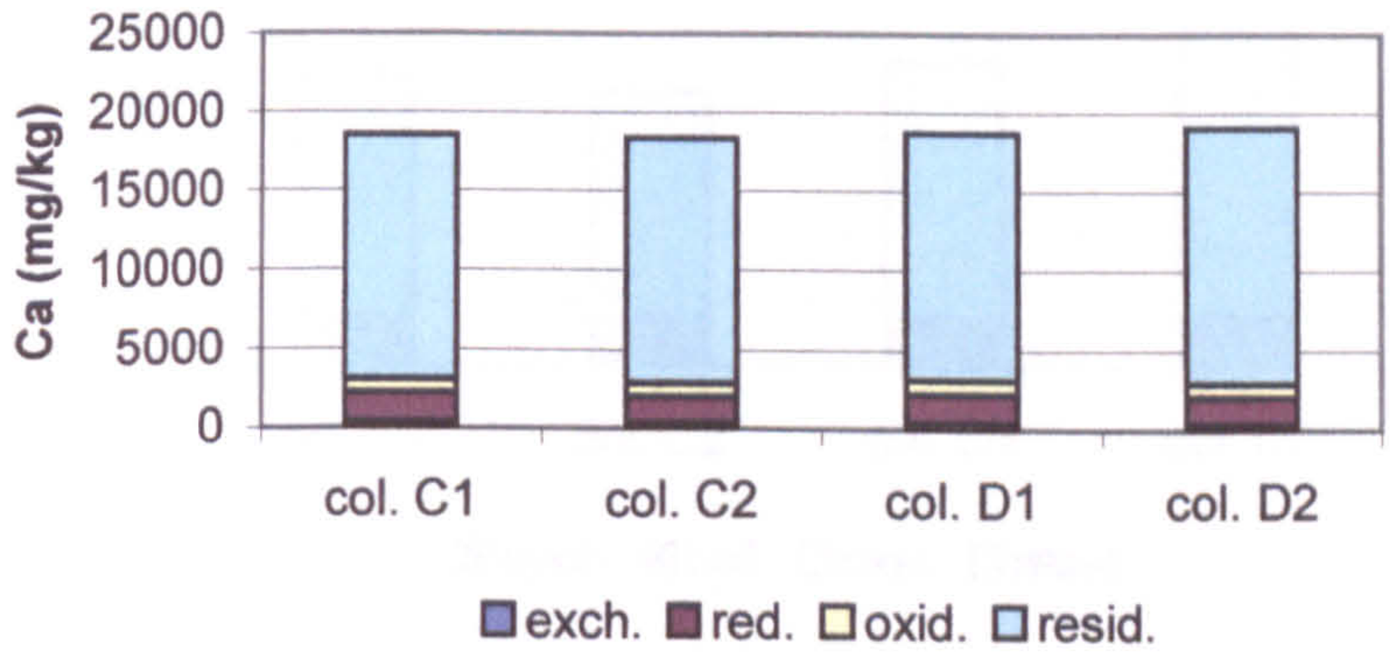

Figure 5.64 Ca SE distribution in IG 35-45 after leaching experiment 3

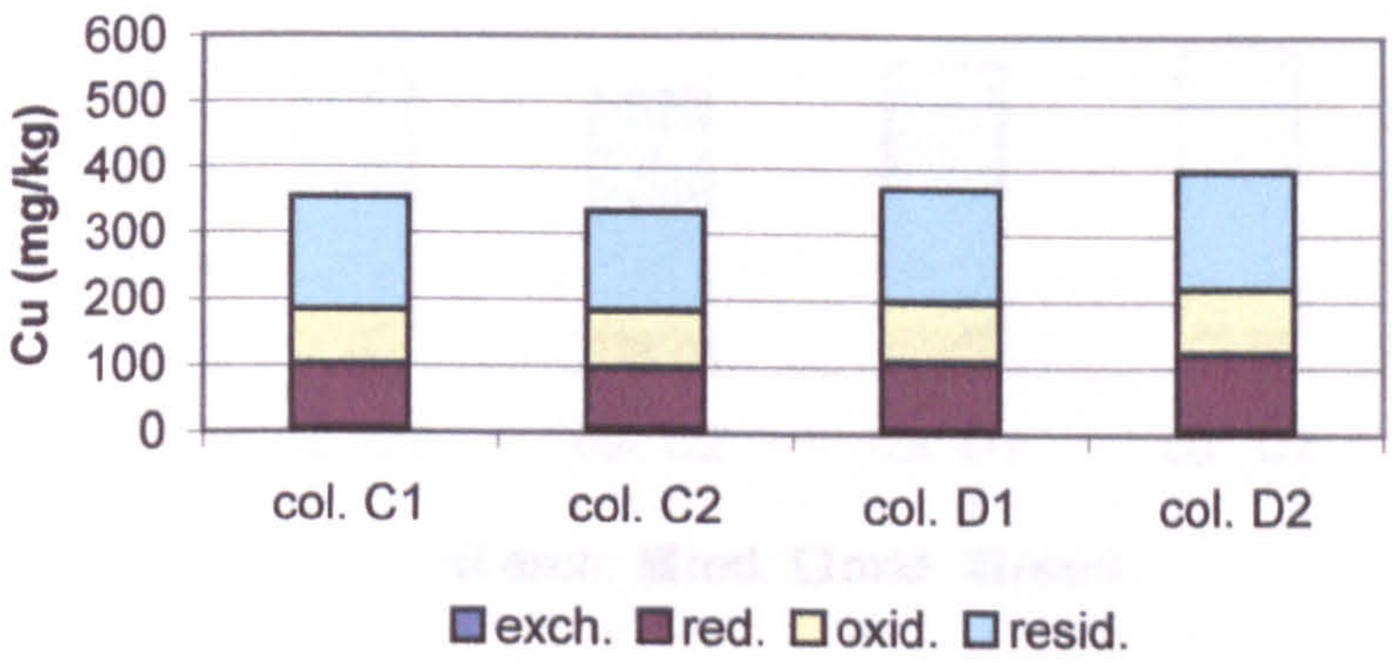

Figure 5.65 Cu SE distribution in IG 35-45 after leaching experiment 3

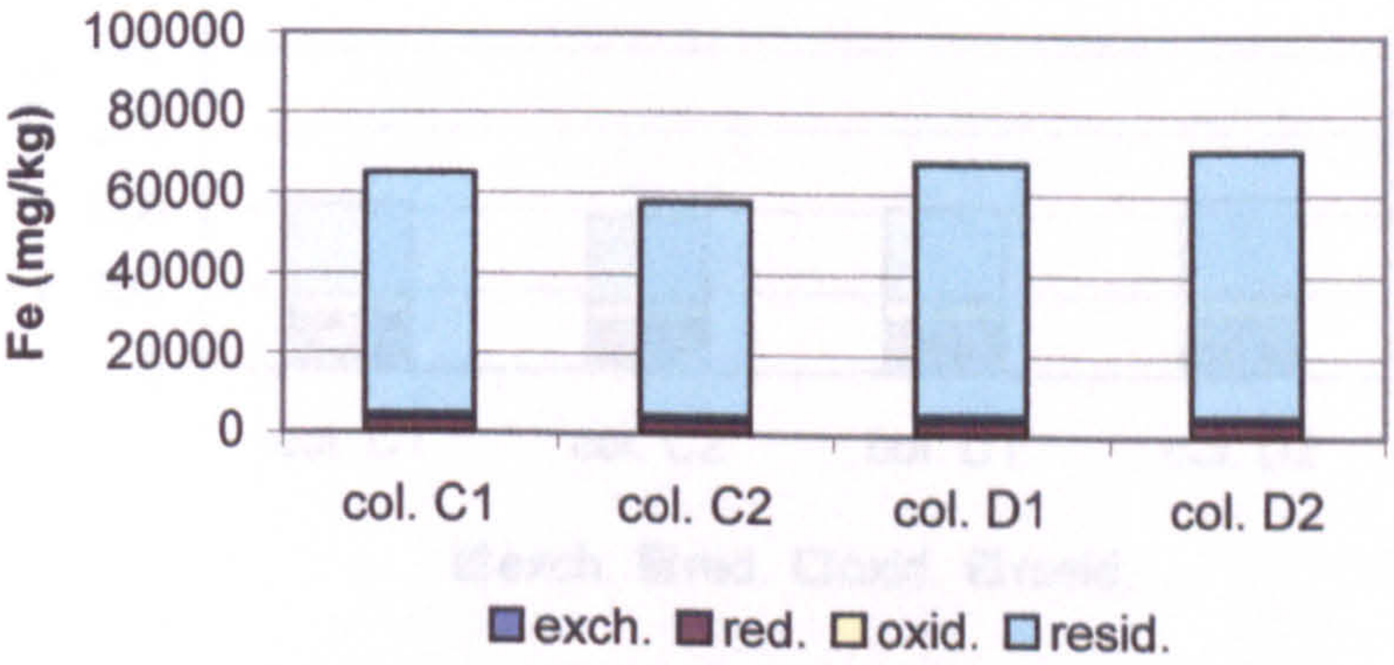

Figure 5.66 Fe SE distribution in IG 35-45 after leaching experiment 3

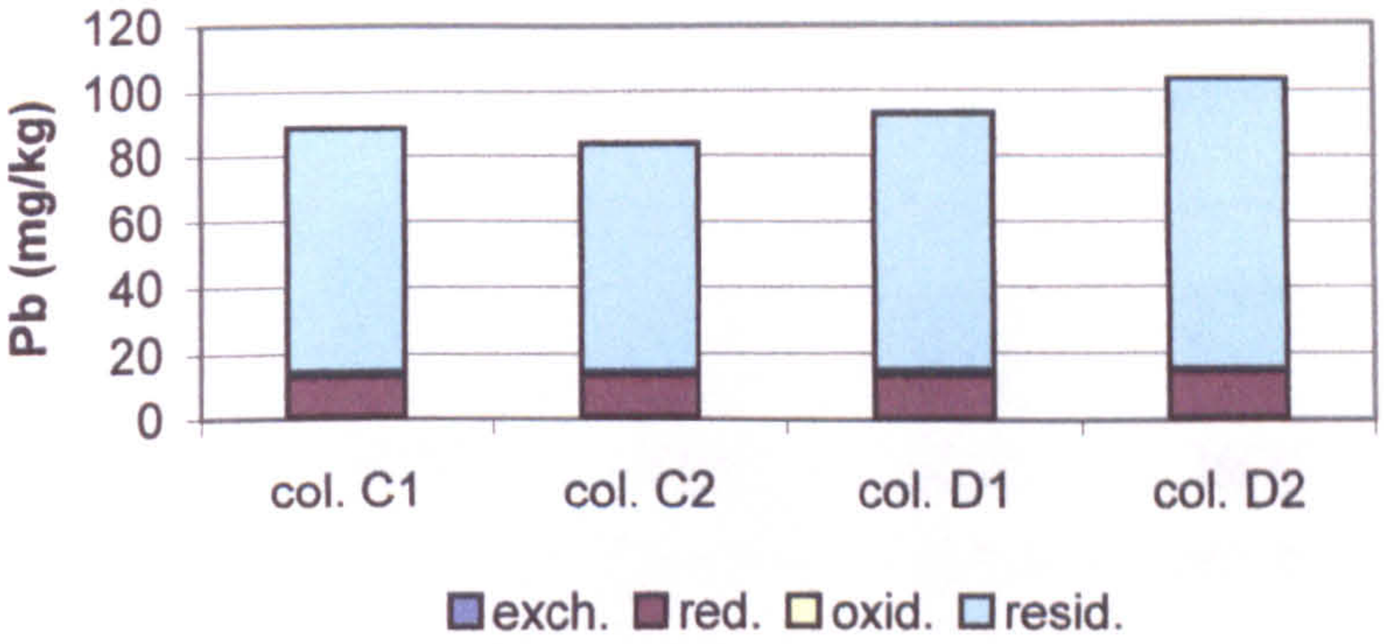

Figure 5.67 Pb SE distribution in IG 35-45 after leaching experiment 3

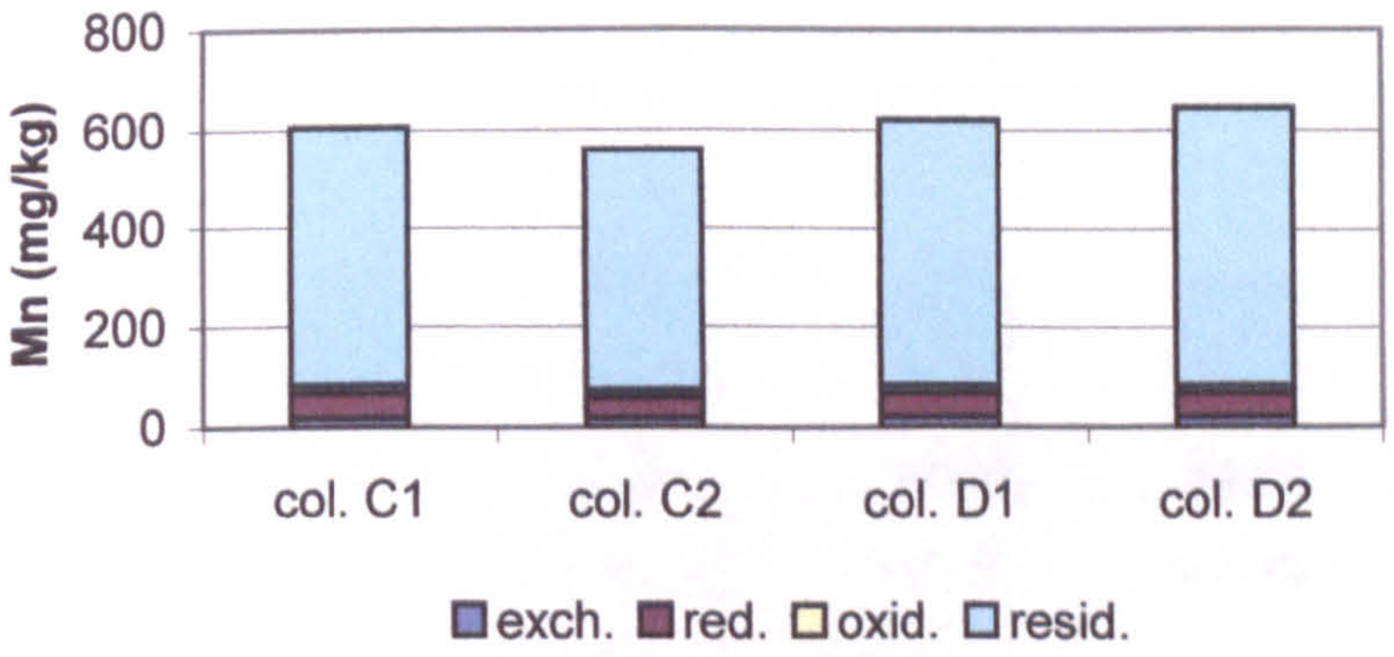

Figure 5.68 Mn SE distribution in IG 35-45 after leaching experiment 3

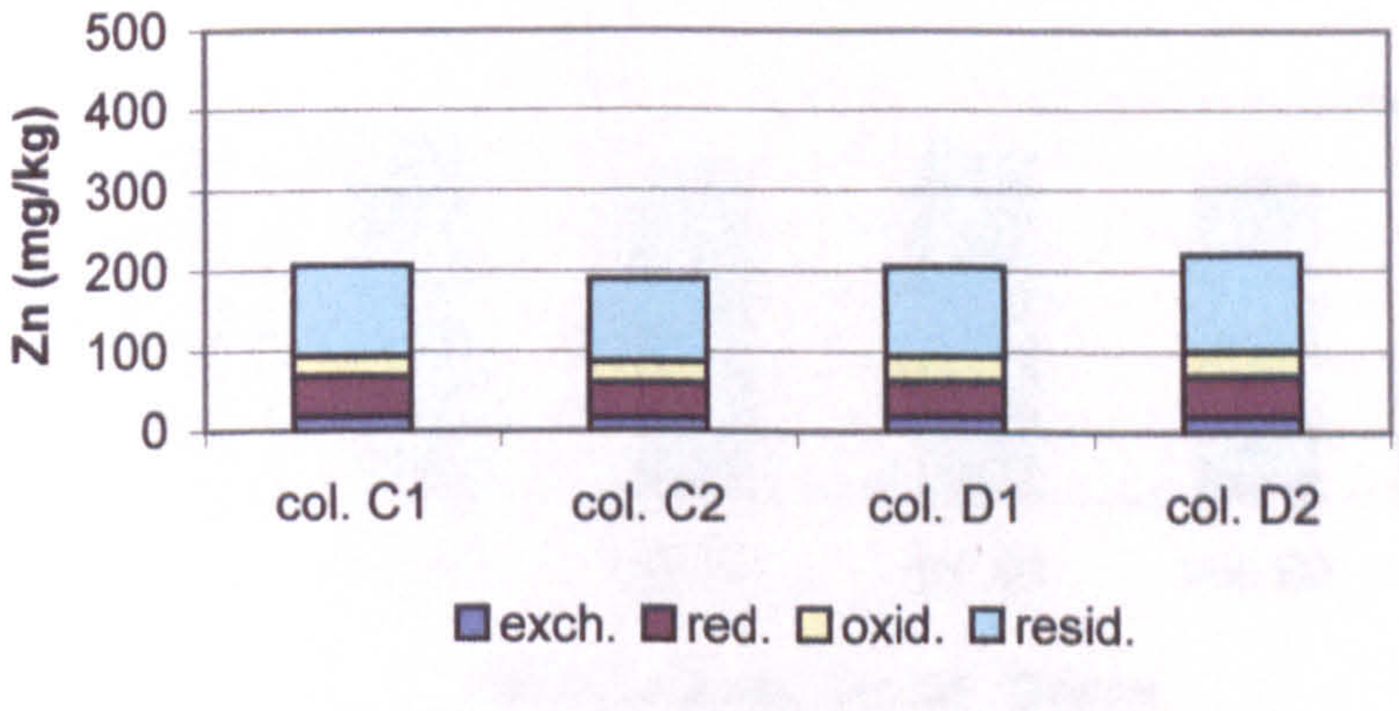

Figure 5.69 Zn SE distribution in IG 35-45 after leaching experiment 3

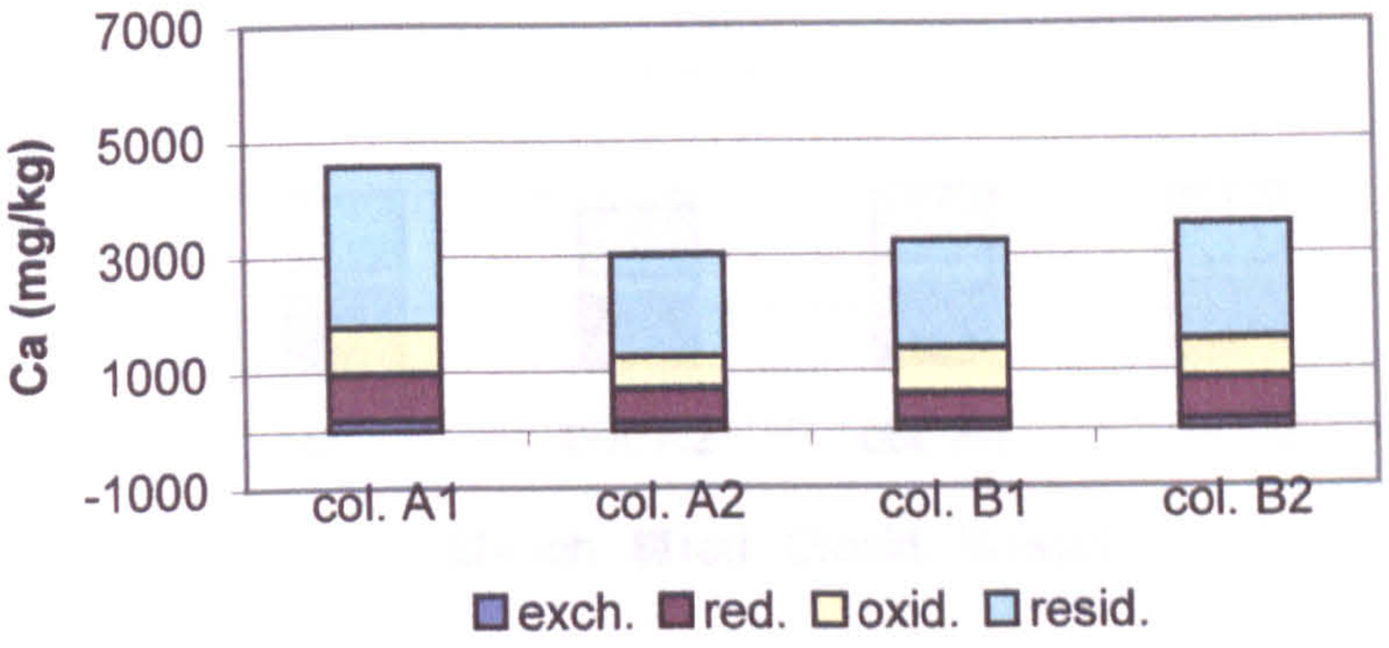

Figure 5.70 Ca SE distribution in Ardeer 2 after leaching experiment 3

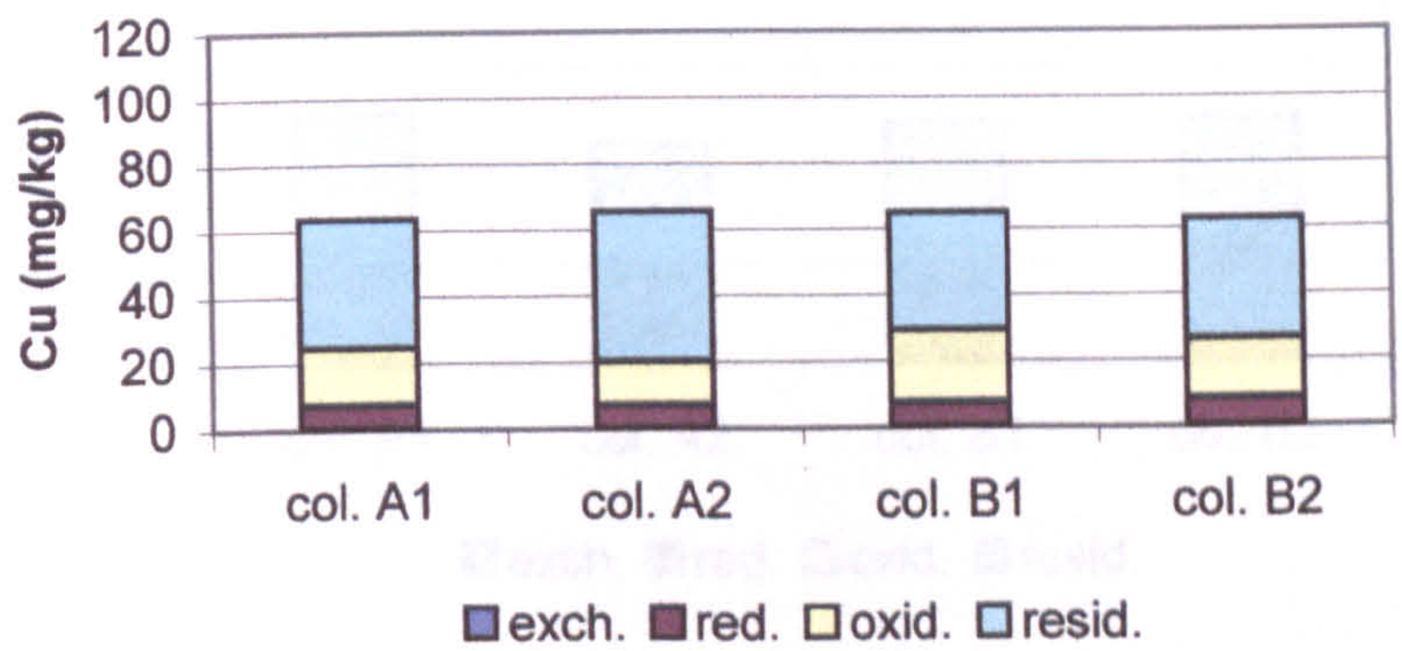

Figure 5.71 Cu SE distribution in Ardeer 2 after leaching experiment 3

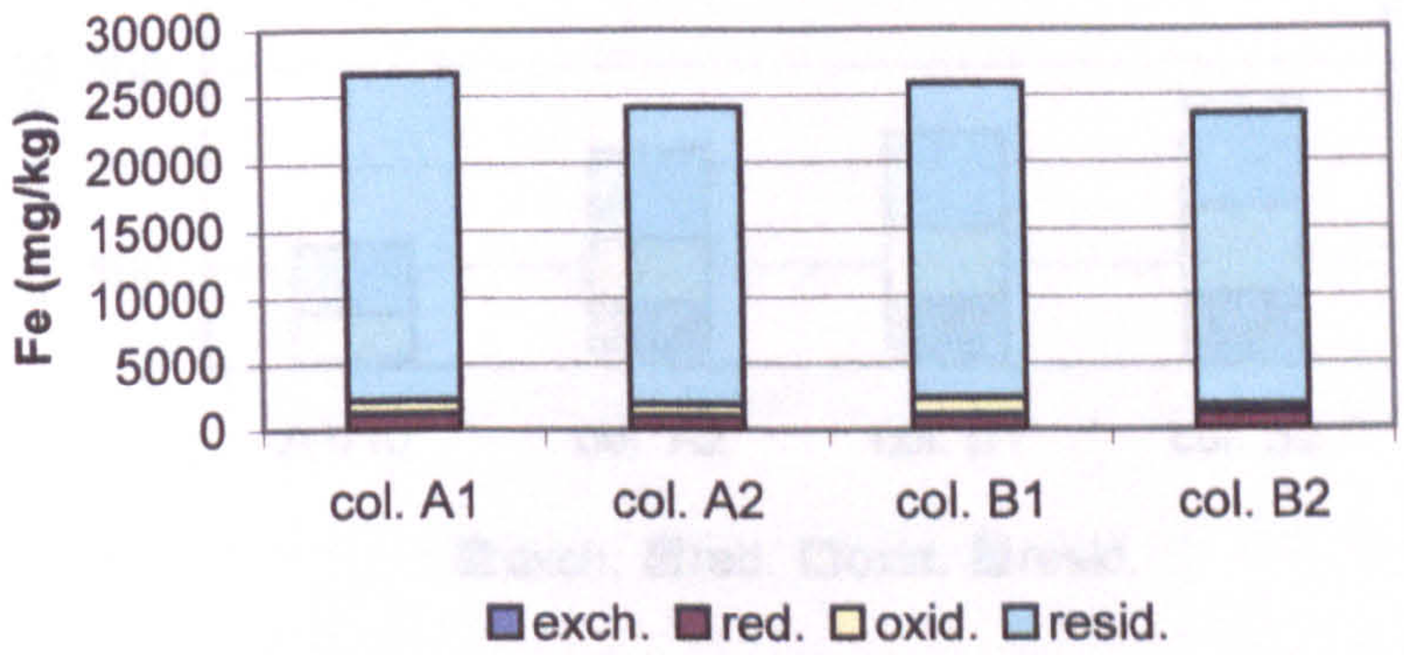

Figure 5.72 Fe SE distribution in Ardeer 2 after leaching experiment 3

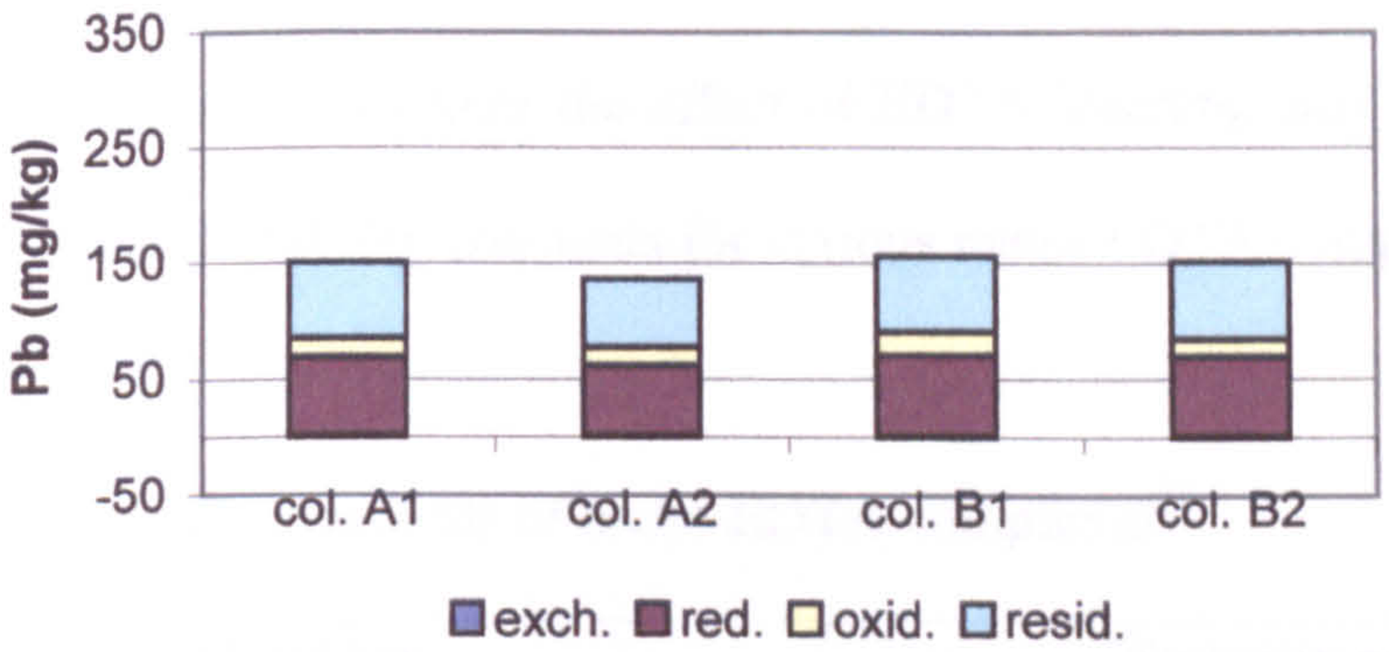

Figure 5.73 Pb SE distribution in Ardeer 2 after leaching experiment 3

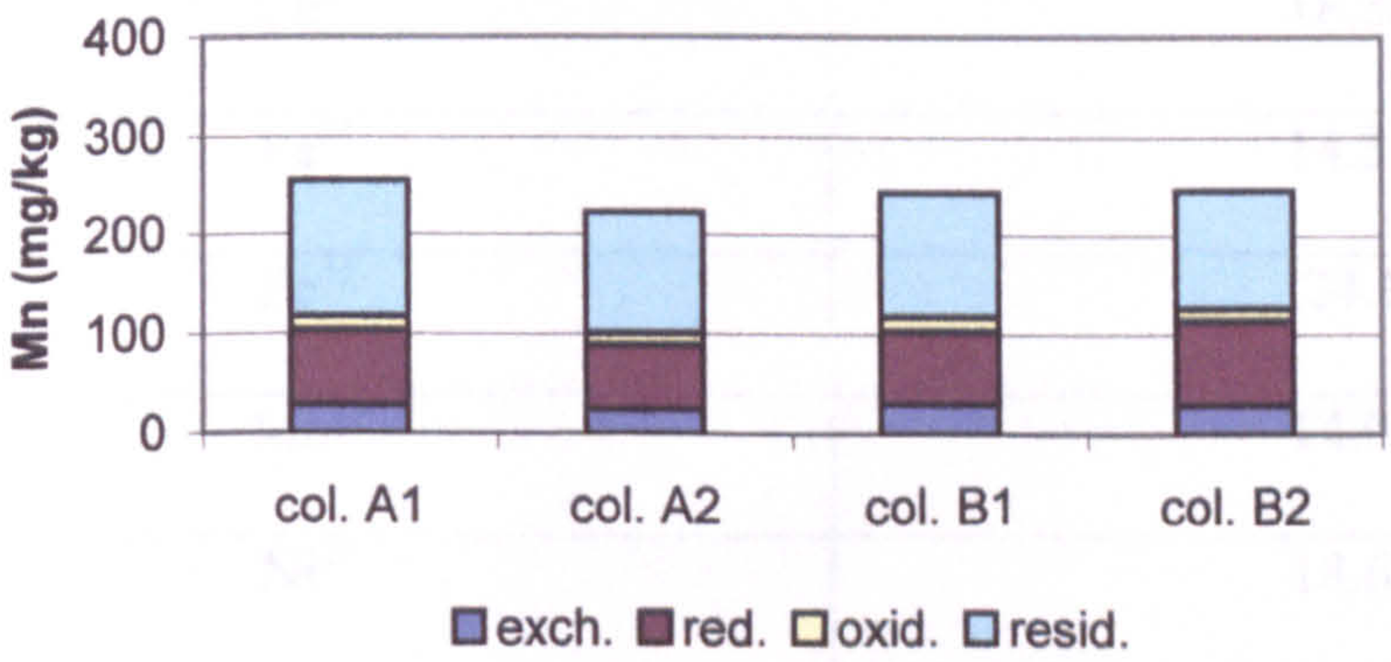

Figure 5.74 Mn SE distribution in Ardeer 2 after leaching experiment 3

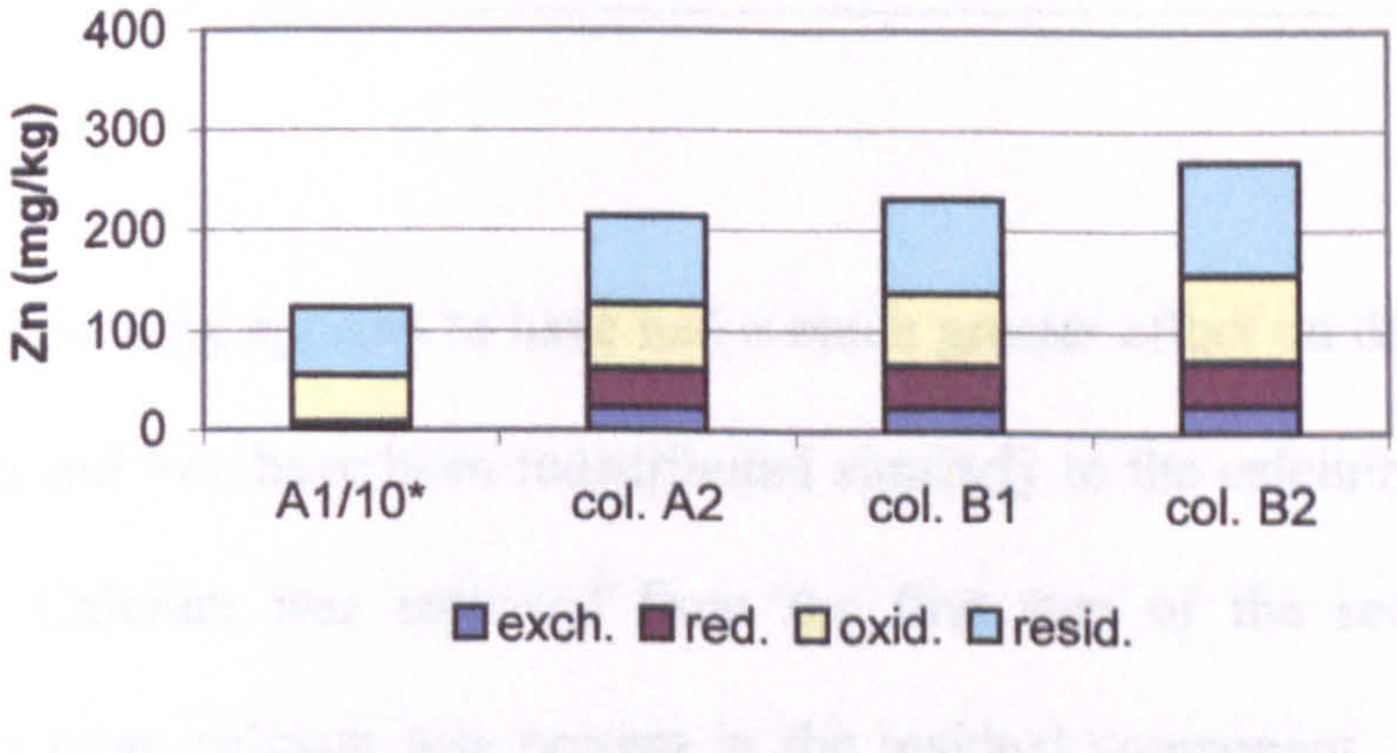

Figure 5.75 Zn SE distribution in Ardeer 2 after leaching experiment 3
*all col. A1 steps divided by 10 in order to fit figure
possible contamination or hot spot in A1

stable complexes, with copper and lead also forming stable complexes. Zn^{2+} forms a slightly less stable complex, with Fe^{2+}, Mn^{2+} and Ca^{2+} having even lower stability constants. Very little mobile lead was extracted from IG 35-45, therefore it is difficult to determine whether the effect of EDTA leaching was significant. Table 5.4 shows overall stability constants for various metal-EDTA complexes.

Table 5.4 Stability constants of metal-EDTA complexes[22]

Metal ion	Stability constant
Ca^{2+}	10.96
Cu^{2+}	18.80
Fe^{2+}	14.33
Fe^{3+}	24.1
Mn^{2+}	14.04
Ni^{2+}	18.62
Pb^{2+}	18.04
Zn^{2+}	16.50

Column leaching appears to have had a much greater effect on the soil from Ardeer. Calcium and iron have been redistributed similarly to the calcium and iron in the IG 35-45. Calcium was removed from the first step of the sequential extraction, however more calcium was present in the residual component. Iron was removed from the second step of the SE however more iron was present in the first step after leaching than prior to the leaching. Copper was removed from the first three stages

of the SE after column leaching. Lead levels in the first three stages of the SE were also much lower after column leaching. A large proportion of the lead associated with the reducible soil components was removed. This made a significant difference to the lead present in the soil. Zinc was also removed from the soil by column leaching but mainly from the exchangeable and carbonate bound components. These results suggest that column leaching has been successful in removing metals from the soil, however the metals tend to have been removed from the exchangeable and reducible components, rather than the oxidisable components. Humic substances present, which would be attacked by the oxidisable step, may have higher stability constants with the metals studied than the EDTA. This would prevent the EDTA removing metals from the humic substances and therefore explain why EDTA has little effect on this step of the SE. This would also suggest that other reagents such as reducing agents or acids would work as successfully at removing metals from soils and may be of less concern environmentally.

5.4 Conclusions and further work

The most contaminated soil investigated was found to be the Ardeer location 1 sample, which contained approximately 2% lead in readily mobile forms. Although this would make the sample a high priority for remediation, column leaching was found not to be suitable, as leaching solution could no pass through the column due to the fine particle size of the sample. This emphasises the requirement to consider

the physical properties of the sample and remediation techniques that are specifically suited to the sample being considered.

Experiments 1 and 2 which used pH 7 and 4.45 disodium EDTA respectively, as column leaching agents, illustrated that soil properties are likely to effect the leaching e.g. the pH of the soil during leaching. The first two experiments also demonstrated that the EDTA extraction is kinetically dependent, i.e. more lead was extracted when the EDTA was left in contact with the soil for a longer time period (e.g. overnight). Experiment 3 was therefore designed so that the flow of leachate was continuous throughout the experiment.

Large proportions of copper and zinc were removed during the exchangeable step of the SE. This was expected as these have two of the highest stability constants for EDTA of the elements studied. Lead-EDTA also has a high stability constant, however, lead levels were reasonably low in the IG exchangeable fraction of the SE and so it was more difficult to make conclusive comments on the effectiveness of the EDTA leaching on the IG sample. Column leaching appeared to have a higher effect on the copper, lead and zinc removed from the Ardeer sample. Once more these are the metals on which EDTA would be expected to have the greatest effect.

On average column leaching appeared to remove large proportions of exchangeable calcium (~80 %), copper (~90 %), lead (~80 %) and zinc (~80 %). Reasonably high proportions of the copper (~60 %) and lead (~70 %) normally extracted during the second step of the SE were extracted by the column leaching. EDTA also leached

approximately 50 % of the copper and lead from the column normally released by the oxidation step. This was expected based on the stability constants of these metals.

The time dependence of the EDTA leaching requires further investigation. This would require several leaching experiments to be set up in which the leachate was left in the columns for extended time periods. At the end of the experiments, the leachate and soil would be analysed as before. The time that the leachate was left in contact with the soil could then be optimised.

Other methods of removal of the metals must also be considered. In general soil flushing methods remove the leachate, which is then treated to remove the metals. Other methods of remediation have been discussed earlier in the chapter and should be considered when investigating remediation methods for contaminated land. One such treatment is phytoextraction. Phytoextraction involves the use of plants known to accumulate metals. The plants take up the metal of concern from the soil and can then be harvested and metals recovered. Chelating agents, such as EDTA can be used to assist this process by increasing the solubility of the metal concerned in order that the plant can take up the metal. This technique is investigated in the next chapter, and the details of the process discussed.

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Chapter 6 Phytoremediation

Chapter 6 Phytoremediation

6.1 Introduction

There are many methods of remediation for land contaminated with heavy metals. Several of these have been discussed in the previous chapter; however this chapter will focus on phytoremediation, the use of plants to remove metals from soil. The use of chelate assisted phytoextraction was also investigated. Both techniques were assessed with respect to Ardeer location 2 samples.

6.1.1 Phytoremediation

Recently remediation using plants has becoming increasingly popular. These methods tend to decrease the cost of remediation and can be carried out on-site with less disturbance of the contamination than physical and chemical methods.

Negri and Hinchman[1] explored some of the research in this field. They summarised some of the plants that decontaminate soil through root uptake and accumulation. These are shown in Table 6.1.

Table 6.1 Metal uptake by plants[1]

Plant	Metal	% dry weight as metal
Serbertia acuminate	Ni	> 20
Thlaspi calaminare (penny cress)	Zn	> 10
Alyssum bertolinii (alyssum)	Ni	10
Pimela suteri	Cr	3
Leptospermum scoparium (broom tea tree)	Cr	3
Uncinia leptostachya	U	3
Coprosma arborea	U	3
Betula papyrifera (paper birch)	Hg	1

Salt *et al.*[2] present an in-depth literature review on phytoremediation, describing phytoextraction and phytovolatilisation of metals and also phytoremediation of organics. Chelate application was shown to increase the uptake of the target metal when applied prior to harvesting. In this way indian mustard, corn and sunflower were induced to accumulate high concentrations of Pb. A hypothetical protocol for chelate assisted phytoextraction was proposed:

1. the site is evaluated and the appropriate chelate/crop combination is determined.
2. the site is prepared and planted, and the crop is cultivated.
3. once optimal biomass is reached, the appropriate metal chelate is applied.
4. after a short metal accumulation phase (several days or weeks), the crop is harvested.

Application of the chelate to the soil, however, may cause severe plant stress and lead to the death of the plant.

Two problems with hyperaccumulators (plants which have naturally high tolerances to metals) is that they tend to have relatively low biomass and slow growth rates. The ability of plants to hyperaccumulate metals is dependant upon the mechanisms of detoxification they use, e.g. chelation, compartmentalisation and biotransformation. These mechanisms are described in the review by Salt *et al.*[2].

Several hyperaccumulators, listed in table 6.2, are also suggested in the paper.

Table 6.2 Metals accumulated by specific hyperaccumulators[2]

Plant	Metals accumulated
Viola calaminaria (violet)	Zn
Thlaspi calaminare, now *T. caerulscens* (mustard)	Zn, Cd, Ni
Astragalus	Se
Brassica juncea	Pb, Se
Brassica oleracea	Zn
Silene vulgaris	Zn
Raphanas sativius	Cd
Allyssium lesbiacium	Cu
Alyssium murale	Pb
Arabidopsis thidana	Cr

Epstein *et al.*[3] studied EDTA and Pb-EDTA uptake in *B. juncea* grown in Pb amended soils. Previous workers, using [^{14}C]EDTA, concluded that Pb is absorbed and translocated as Pb-EDTA. Application of 5 mmol/kg EDTA had no affect on the

growth and dry matter yield of the plants; however, 10 mmol kg^{-1} EDTA decreased transpiration and dry matter yield. Accumulation of Pb and EDTA was investigated by ICP AES and HPLC respectively and was shown to be significantly enhanced by EDTA application, with concentrations of Pb and EDTA in the shoots correlating well, further confirming that Pb is absorbed as Pb-EDTA.

Salt *et al.*[4] presented another review of phytoremediation, once more emphasising the usefulness of *B. juncea* and *T. caerulescens* as hyperaccumulators. Also discussed was phytostabilisation. This involves increasing groundcover, by planting metal tolerant plants, which will prevent erosion and leaching of contaminants from the site, thus preventing the spread of pollutants in the environment. Suggested plants for this are presented in table 6.3 below.

Table 6.3 Plants used for phytostabilisation

Plant	Phytostabilisation use
Agrostis tenuis	acid lead/zinc wastes
Festuca rubra	calcerous lead/zinc wastes
Agrostis tenuis	Cu wastes

Comis[5] also presents a review of plant based remediation, giving a definition of hyperaccumulation as a plant's capacity to take up and store more than 2.5 % of its dry weight in heavy metals, without a reduction in yield. Also described was work being carried out to search for the hyperaccumulator genes within plants.

Ernst[6] describes many of the conditions that are likely to affect metal bioavailability. Bacteria may acidify the soil either leading to increased metal mobility or

precipitation as sulfides. Metal speciation may also be altered by waste products of higher plants e.g. phenolics and other organic acids. Ernst also discusses re-vegetation of sites, but was cautious that long term access to the site may need to be regulated due to toxicity of the herbage.

Weatherford *et al.* [7] investigated the ability of many plants to accumulate metals. 61 plants native to Western Kentucky were collected and grown in domes in control and Pb/Al spiked soils. Plants were watered as required and provided with constant light cycles of 13:11 h, light:dark, by plant/aquarium light bulbs. Plants noted for their accumulation of metals are shown in table 6.4 below.

Table 6.4 Hyperaccumulators native to Western Kentucky

Plant	Metal
Typha latifolia (Cattail)	Mn
Smilax bona-nox (Catbrier)	Mn
Rurillia humilis (Wild petunia)	Al, Fe, Mg
Prunella vulgaris (Self-heal)	Al, Fe

Wierzbicka[8] have compared the Pb tolerance of *Allium cepa* (onion) to that of several other plant species. Growing conditions for the plants are provided in the article. Tolerance was determined on the basis of comparisons between root growth for plants treated with Pb and the controls (tolerance index). *Silene vulgaris* (found wild, growing on the waste ground under investigation) showed a high degree of tolerance, as did several other plants found growing on the waste ground. *A. cepa* also showed high tolerance. *Cucumus sativis* (cucumber) showed a high concentration of Pb in both the roots and the shoots. *A. cepa* was found to be capable

of translocating 46 % of the Pb taken up by one plant to the bulb and leaves, an important factor when considering hyperaccumulators.

Hildebrandt *et al.*[9] investigated the protective properties of *arbuscular mycorrhizal* (AM) fungi on the zinc violet. These have been reported to accumulate and/or bind heavy metals and when concentrated around the zinc violet may alleviate metal toxicity enabling the plants to grow in metal contaminated soils. It was shown that the zinc violet inoculated with AM fungi showed a greater growth than those plants not inoculated.

Schwartz *et al.*[10] investigated the root development of the zinc hyperaccumulator *T. caerulescens*. Roots were found to colonise predominantly Zn-polluted areas. However, these findings were specific to Zn (ie similar results were not obtained for Pb).

In summary, several methods of remediation are reported in the literature. The majority of the chemical remediation techniques involve some form of chelation, the most popular chelate being EDTA. A variety of plants have been investigated for their ability to either accumulate or fix metals within the soil. The most frequently mentioned hyperaccumulators are *T. caerulescens* and *B. juncea*.

6.1.2 Plants and metal uptake

In order to understand phytoremediation some knowledge of plants and their transport systems must be gained. Plants transport water and dissolved minerals through the xylem ("a complex vascular tissue...characterized by the presence of tracheary elements"[11]). Water is lost from plant leaves due to photosynthesis, and this causes water to be drawn through the plant as a tension is established in the xylem. Use of water by plant leaves also causes water to move from the roots to the shoots. As well as anchoring the plant within soil, roots are structured to provide the necessary water for the system. The large surface area of roots presented to the soil enables plants to take up large quantities of water. Dissolved ions enter the roots through root cells and are transported in the xylem. It has been shown that some ions are absorbed by active transport (e.g. significantly greater amounts of an ion have been found to be present in roots compared to the growing medium). Inorganic ions are rapidly transported upward through the xylem and can then move into surrounding tissue, or can be transported to the leaves. Once in the leaves ions can be transferred to the phloem ("the food-conducting tissue of vascular plants") and transported downwards into other parts of the plant. However only phloem mobile ions can be transported from the leaves. Such ions include potassium, chloride and phosphate, but exclude calcium, boron and iron.

6.1.3 Dandelion

Taraxacum officinale (dandelion) is a common weed found abundantly over a broad geographical region (native to six continents). The tap root is perennial and when cut can produce new plants. *Taraxacum officinale* has leaves with tooth-like lobes and flowers (usually bright yellow) between March and July. It can be found in fields, lawns, waste places and by roadsides. Figure 6.1 shows an illustration of the dandelion.

Due to its abundance the dandelion is convenient for monitoring air and soil pollution. Several papers discuss the use of the dandelion as a biomonitor for pollution.[12,13,14]. The dandelion has been shown to be capable of accumulating metals such as Cd, Pb and Zn . Marr *et al.*[14] correlated the addition of P in fertilisers with a reduction in the accumulation of Cu and Zn in dandelion leaves, suggesting that P either absorbed or precipitated the metals to form insoluble compounds and therefore make both metals and P unavailable to the plants. Table 6.5 shows typical metal levels in soils and dandelions in Montreal for different land uses. The study concluded that the total metal levels in soils did not necessarily correlate with the bioavailable metal present.

Table 6.5. The average concentrations of trace metals in soils and dandelion leaves for three different land use types (mg kg^{-1})[14]

	Cd	Cu	Mn	Pb	Zn	P
Soils						
Gardens	0.13a	20.7a	424a	55.1a	183a	96.9a
Parks	0.15a	37.4a	497a	117ab	265a	19.2a
Industrial	5.10a	246b	616a	560b	709b	13.6b
Dandelion leaves						
Gardens	0.15a	4.30a	31.7a	6.28a	35.5a	-
Parks	0.08a	10.7b	29.7a	5.83a	71.4b	-
Industrial	1.05a	8.98b	26.5a	6.80a	95.0b	-

a,b Differences between levels of metals in dandelion leaves for different land uses were assessed by ANOVA. Numbers within a column followed by the same letter were not significantly different at $P<0.5$.

This chapter investigates the feasibility of phytoremediation and chelate assisted phytoremediation of the Ardeer location 2 soil. The dandelion was chosen for this research as it has been shown to accumulate metals and could be easily grown on the soil to be investigated and would grow within the time-scale of the research.

6.2 Experimental

Pot experiments were set up to investigate the uptake of metals by *Taraxacum officinale* from Ardeer location 2 soil. The effect of a combined nitrogen, phosphate, potassium (NPK) nutrient solution throughout and the addition of a chelate after three months growth was investigated. The experimental design given in table 6.6 was used with four replicates of each treatment.

Figure 6.1 Dandelion (*Taraxacum Officianale*)[15]

Table 6.6 Experimental design for phytoremediation of Ardeer location 2 soil

Treatment	Plant	EDTA	NPK
1	YES	NO	NO
2	YES	YES	NO
3	NO	NO	NO
4	NO	YES	NO
5	YES	NO	YES
6	YES	YES	YES
7	NO	NO	YES
8	NO	YES	YES

6.2.1 Dandelions, Soil and Reagents

Dandelions

Ure, A. removed dandelions from an Aberdeen garden site. Plants were selected for the experiment on the basis of having roughly equal root mass, suitable for transplanting into tubes. Soil was removed from the roots by washing in demineralised water and plants chosen randomly when potting up into tubes. Coutts, G. at the Macauley Land Use Research Institute (MLURI), grew the dandelions used in this work.

Soil

The soil from Ardeer location 2 was screened using a square wire mesh (6 mm). From 11.3 kg of moist soil, 8 kg were less than 6 mm, 3 kg were greater than 6 mm and 0.3 kg were root fibres. The fraction > 6 mm consisted of stones, low density clinker and high density sinter products. The moisture content of the screened soil (i.e. < 6 mm fraction) was found to be 18.9 % on drying at 105 °C. The pH of the

soil measured in water was 6.24 and in 0.01 M $CaCl_2$ was 5.23 (1:1 w/v fresh soil:water ratio).

NPK nutrient solution

Stock solutions of 2 M ammonium nitrate, 0.25 M di-hydrogen ortho phosphate and 0.16 M di-potassium hydrogen ortho phosphate were prepared from analytical grade reagents (BDH, AnalaR grade). A watering solution was prepared by taking 2.5 ml of each into 1 L of de-mineralised water. This provided a balanced nutrient solution with an $N:P_2O_5:K_2$ ratio around 2:1:1.

EDTA solution

A solution of 0.05 M di-ammonium EDTA was prepared from analytical grade reagent (Fluka Microselect grade).

Fungicide solution

"TILT®" fungicide concentrate [1-[[2-(2,4-dichlorophenyl)-4-propyl-1,3-dioxolan-2-yl]methyl]-1H-1,2,4-triazole, alternatively named propiconazole] was obtained from Novartis Crop Protection Inc., USA (now Syngenta).

6.2.2 Experimental Conditions

Dandelions were grown in black plastic tubes (Fig. 6.2), with dimensions of 210 cm by 4 cm, and an internal volume of 175 cm^3. A filter paper was inserted in the bottom of the tubes to prevent loss of soil from the drainage holes. Dandelions were

Figure 6.2 Dandelions grown in black plastic tubes

suspended in the tubes which were then filled with soil (150 g per tube) using a tapping action to fill air spaces. The soil occupied a volume of 155 cm^3.

The experiment was set up according to the experimental design provided in table 6.6 and set out in a randomised block design. Initially, the plants were kept in a walk-in growth room (Controlled Environment Ltd., "Conviron", Winnipeg, Manitoba, Canada) with metal halide and high pressure sodium lighting designed to provide light of a quality similar to daylight (red/far red 1.2). The measured ratio was 3.6. The photosynthetically active radiation (PAR) intensity, measured with a hand-held meter (ELE International, Hemel Hempstead, Hertfordshire) was around 500 $\mu M\ m^{-2}\ s^{-1}$. Other conditions were; 13.5 hr day at 20 °C (with ramping over 50 minutes), 9.5 hr night at 17 °C (with ramping over 50 minutes). Relative humidity was set at 65 %.

Within a few days the plant leaves became purple coloured. A similar phenomenon with *Rumex acetosa* (Common Sorrel), which is also a broad leaf plant, has been reported within the same room[16]. After 3 weeks the plants were transferred to a controlled environment cabinet ("Conviron") with fluorescent tubes and tungsten bulbs providing a different quality of light. The red/far red ratio was 2.0 and PAR intensity was approximately 150 $\mu M\ m^{-2}\ s^{-1}$. Other conditions were 14.5 hr day at 20 °C (with ramping over 50 minutes), 9.5 hr night at 15 °C (with ramping over 50 minutes). Relative humidity was kept at 65 %. The plants were observed to recover after one day in the cabinet.

Throughout the experiment the soils were watered daily using a pipette. Initially each tube received 10 ml of demineralised water, increasing to 20 ml for tubes with plants after 2 months (from the start of the experiment) to allow for the increase in transpiration with growth.

Nutrient solution (10 ml per tube per week) was added to the appropriate tube for approximately 2 months. This addition was increased to 20 ml per tube per week after 2 months. The total addition to each tube receiving nutrient solution was equivalent to 223 kg N ha^{-1}, 116 kg P_2O_5 ha^{-1} and 107 kg K_2O ha^{-1}.

12 days before harvesting (approximately 3 months after the start of the experiment) 20 ml of 0.05 M EDTA was added to the appropriate tubes. After 1 week the plants treated with EDTA wilted and began to loose leaves. As leaves dropped they were collected in polythene bags and stored in a cold room.

During the experiment small patches of mildew appeared on the leaves of some plants. After 1.5 months the infection was observed to have spread to larger areas and the plants were sprayed with 50 ml of dilute fungicide solution (2.5 ml of concentrate per litre) on two occasions. The inside of the cabinet was also treated by spraying and wiping with a 70 % solution of ethyl alcohol.

At the end of the experiment the tubes were removed and the root ball and soil placed in a polythene bag. The soil was released by hand and the plant leaves cut off. The leaves from the EDTA treated soil were combined with the dead leaves

collected previously. The root ball was washed with de-mineralised water to remove soil. The leaves and roots were freeze dried and weighed.

6.2.3 Analysis

Roots and leaves were analysed by the MLURI due to their expertise in plant analysis and time constrains of the project. Soil was subjected to sequential extraction and analysed at Strathclyde University as described in previous chapters.

Roots and leaves from individual plants were analysed with sub-sampling. The plant material was muffled in silica crucibles with lids over night at 450 °C. The ash was digested with re-distilled 6 M HCl and evaporated to dryness before being taken up in 0.34 M HCl. The solutions were analysed by ICP AES, with an ARL 3580 (TJA, Crawley, Sussex).

The concentrated fungicide was also analysed in the same manner as the plant material.

Soil samples were air-dried and sub-sampled before performing sequential extraction (modified BCR procedure) and pseudototal digestion as described in chapter 4. The extracts were then analysed by ICP AES as described in previous chapters.

6.3 Results and discussion

Results from the 8 treatments investigated were compared using analysis of variance (ANOVA)[17]. ANOVA is used to distinguish results that differ from one another and to estimate the causes of variation. The controlling factors in this research were the presence of a plant in the soil, the addition of NPK nutrient solution and the use of a chelate. ANOVA is used to separate variation resulting from a change in experimental conditions or from random variation. ANOVA compares within-sample and between-sample variation using the F test. The null hypothesis adopted for the F test is that the populations from which the samples are taken are normal, and that the population variances are equal. If the null hypothesis is true then the variance ratio should be close to 1. If the difference between the ratio and 1 is too great the difference must be attributed to a cause other than random variation. If sample means differ significantly, the calculated value of F will be greater than the critical value of F. If F is greater than the appropriate value of F_{crit} then the respective factor has a significant effect upon the measured data. F pr is the probability of making a type I error in the F test. A type I error is the possibility of rejecting a null hypothesis even though it is true. F pr therefore represents the degree to which the result varies from the data set. Table 6.7 shows the layout of measurement for one-way ANOVA.

Table 6.7 Layout of measurements for one-way ANOVA

							mean	Variance
Sample 1	x_{11}	x_{12}	.....	x_{1j}	.....	x_{1n}	x_1	s_1^2
Sample 2	x_{21}	x_{22}	.....	x_{2j}	.....	x_{2n}	x_2	s_2^2
.	.	.	.....	.	.....	.	.	
.	.	.	.....	.	.....	.	.	
Sample *i*	x_{i1}	x_{i2}	.....	x_{ij}	.....	x_{in}	x_i	s_i^2
.	.	.	.....	.	.....	.	.	
.	.	.	.....	.	.....	.	.	
Sample *h*	xh_1	x_{h2}	.....	x_{hj}	.....	x_{hn}	x_h	s_h^2
					Grand Mean		x	

There are *n* replicate measurements made for each level, where *h* is the number of levels, x_i is the mean measurement of the *i*th level and s_i^2 the variance of the *i*th level. N is the total number of measurements (= *nh*). In order for simple ANOVA calculations to be performed the levels can be summarised as shown in Table 6.8.

Table 6.8 ANOVA summaries

							Level Total	
							T_i	$(T_i)^2$
Sample 1	x_{11}	x_{12}	..	x_{1j}	..	x_{1n}	$\sum_{j=1}^{n_1} x_{1,j}$	$\left(\sum_{j=1}^{n_1} x_{1,j}\right)^2$
Sample 2	x_{21}	x_{22}	..	x_{2j}	..	x_{2n}	$\sum_{j=1}^{n_1} x_{2,j}$	$\left(\sum_{j=1}^{n_1} x_{2,j}\right)^2$
..	..	..	..	..	..	..		
Sample i	x_{i1}	x_{i2}	..	x_{ij}	..	x_{in}	$\sum_{j=1}^{n_1} x_{i,j}$	$\left(\sum_{j=1}^{n_1} x_{i,j}\right)^2$
..	..	..	..	..	..	..		
Sample h	x_{h1}	x_{h2}	..	x_{hj}	..	x_{hn}	$\sum_{j=1}^{n_1} x_{h,j}$	$\left(\sum_{j=1}^{n_1} x_{h,j}\right)^2$
Overall sums							$T = \sum_{i=1}^{h} T_i$	$\sum_{i=1}^{h} T_i^2$

The calculations for one-way ANOVA are given in table 6.9.

Table 6.9. One-way ANOVA calculations

Source of variation	Sum of squares (SS)	Degrees of freedom	Mean square (MS)	F
Between level	$SS_A = \frac{\sum_{i=1}^{h} T_i^2}{n} - \frac{T^2}{N}$	$h - 1$	$MS_A = \frac{SS_A}{h-1}$	$F = \frac{MS_A}{MS_R}$
Within level (residual)	By subtraction	By subtraction	$MS_R = \frac{SS_R}{h(n-1)}$	
Total	$SS_T = \sum_i \sum_j x_{ij}^2 - \frac{T^2}{N}$	$N - 1$		

Factorial designs, such as the one used in this work are capable of detecting interactions between treatments. In this work three factors at two levels were investigated. Table 6.10 shows the factors and responses for calcium in the exchangeable step of the SE.

Table 6.10 Factors and responses for Ca in exchangeable step

Factor	EDTA	Dandelion	NPK	Response
0	-	-	-	970
EDTA	+	-	-	1191
Dandelion	-	+	-	1087
NPK	-	-	+	1015
EDTA/dandelion	+	+	-	1024
EDTA/NPK	+	-	+	1157
Dandelion/NPK	-	+	+	1020
EDTA/dandelion/NPK	+	+	+	1092

The effect of changing individual factors can be calculated from the average change in response when a factor changes from high to low level with the levels of the remaining factors fixed. Table 6.11 shows the calculation of the effect of altering the EDTA.

Table 6.11 Effect of altering the level of EDTA

Level of NPK	Level of dandelion	Level of EDTA		Difference
		+	-	
-	-	1191	970	221
+	-	1157	1015	142
-	+	1024	1087	-63
+	+	1092	1020	72

The average effect of altering the level of EDTA is therefore

$= (221+142-63+72)/4 = 93$

In the same way the average effect of altering the level of dandelion or NPK can be calculated.

Interactions can be calculated by taking the change in response for when EDTA changes from high to low level with dandelion at low level from the effect of changing level of EDTA when dandelion is at high level. Conventionally half their difference is taken as a measure of interaction.

The average change in response when EDTA changes from high to low level and dandelion is at low level is $(221+142)/2 = 181.5$.

The average change in response when EDTA changes from high to low level and dandelion is at high level is $(-63+72)/2 = 4.5$.

The effect of the interaction between EDTA and dandelion is therefore
$(4.5-181.5)/2 = 88.5$.

The other interactions between two factors can be calculated in a similar manner. In order to calculate the interactions between all three factors the interactions between two factors are split into two parts according to the level of the remaining factor. For example with the level of NPK low the estimate of the interaction would be (-63-221)/2 = -142. When the level of NPK is high, the estimate of interaction is (72-

142)/2 = -35. The three-factor interaction is estimated by half of the difference between these results. The three-factor interaction for EDTA, dandelion and NPK is therefore (-35-(-142))/2 = 53.5.

In a two level experiment such as the one carried out, the sum of squares can be estimated using

Sum of squares = N x (estimated effect)^2/4

Where N is the number of measurements, including replicates (32 in this case). Each sum of squares has only one degree of freedom therefore the mean sum of squares = sum of squares. The mean square can then be compared with the error (residual) mean square using the F-test, as described before. A summary of the interaction effects, sum of squares and F-test results is given in table 6.13.

Table 6.12 Interaction effects, sum of squares and F-tests for dandelion experiment

Factor	effect	Sum of squares	Degrees of freedom	Mean square	F_{calc}	F_{crit}
EDTA	93	69192	1	69192	6.19	4.26
Dandelion	-27.5	6050	1	6050	0.54	4.26
NPK	3	72	1	72	0.006	4.26
EDTA/dandelion	88.5	62658	1	62658	5.61	4.26
EDTA/NPK	14	1568	1	1568	0.14	4.26
Dandelion/NPK	-2.5	50	1	50	0.004	4.26
EDTA/dandelion/NPK	53.5	22898	1	22898	2.05	4.26
Residual		268182	24	11174		
Total		430670	31			

Differences between the values obtained for the sum of squares calculated by hand (shown here) and the actual values obtained by GenStat were observed due to rounding of numbers during calculations.

6.3.1 Calcium

Results from sequential extraction of the soil and digestion of the plant material are shown in table 6.13 for calcium. Calcium is not present in particularly high levels in the soil (the typical range for calcium in soil is between 0.1 and 1.2 %). Calcium levels in plants are also typical of average calcium concentrations in plants (1%)[18].

The sum of results from the sequential extraction steps for each treatment are all considerably greater than the PT values. The PT values agree with values obtained in chapter 5, however PT are lower than the sums of the SE results, suggesting either losses in these experiments or contamination in chapter 5 experiments.

Levels of calcium are much higher in plant leaves than in roots and soil suggesting that calcium can be readily transported from roots to leaves and may be involved in various processes in the plant lifecycle. This is not surprising as calcium is an essential element for plants and is involved in various processes such as forming plant structures. As mentioned previously Ca^{2+} is not phloem mobile and so once it reaches the leaves may be trapped there.

An example of the ANOVA calculations performed on the results for the exchangeable step of the sequential extraction of calcium has been shown earlier. If F pr is less than 0.05 the treatment is considered to be significant.

Table 6.14 shows that EDTA has a significant effect on the calcium extracted in the exchangeable step of the SE. In general EDTA tends to increase the calcium

Table 6.13 Calcium in soil and plant material

Treatment	Soil (mg kg^{-1})						Dandelions (mg kg^{-1})	
	exchangeable	reducible	oxidisable	residual	sum	PT	leaves	roots
No EDTA Dandelion No NPK	1087 ± 73	805 ± 46	754 ± 134	1586 ± 312	4232 ± 441	2476 ± 531	14700 ± 5440	3930 ± 931
EDTA Dandelion No NPK	1024 ± 118	807 ± 180	694 ± 343	1762 ± 521	4286 ± 177	2987 ± 204	14600 ± 749	4470 ± 974
No EDTA No dandelion No NPK	970 ± 37	690 ± 57	471 ± 62	2145 ± 182	4276 ± 296	2511 ± 627	NA	NA
EDTA No dandelion No NPK	1191 ± 104	988 ± 204	824 ± 360	1789 ± 542	4792 ± 976	1261 ± 233	NA	NA
No EDTA Dandelion NPK	1020 ± 175	874 ± 115	681 ± 135	1826 ± 795	4401 ± 938	2104 ± 497	12000 ± 730	4280 ± 882
EDTA Dandelion NPK	1092 ± 86	825 ± 131	729 ± 192	2018 ± 414	4665 ± 540	2392 ± 559	11900 ± 1320	4110 ± 697
No EDTA No dandelion NPK	1015 ± 80	765 ± 82	568 ± 130	1662 ± 526	4011 ± 601	1949 ± 290	NA	NA
EDTA No dandelion NPK	1157 ± 117	741 ± 176	611 ± 121	2123 ± 140	4632 ± 388	3177 ± 331	NA	NA

Table 6.14 F pr. values for effect of treatments on Ca in soil and plant material

Treatment	Soil						Dandelions	
	exchangeable	reducible	oxidisable	residual	sum	PT	leaves	roots
NPK	0.933	0.668	0.612	0.608	0.887	0.538	0.313	0.611
dandelions	0.468	0.515	0.211	0.438	0.882	0.098	NA	NA
EDTA	0.020	0.249	0.211	0.488	0.103	0.221	0.742	0.042
NPK/ dandelions	0.956	0.192	0.795	0.347	0.269	<0.001	NA	NA
NPK/EDTA	0.712	0.063	0.508	0.225	0.717	0.001	0.562	0.372
Dandelions/ EDTA	0.026	0.107	0.185	0.698	0.350	0.196	NA	NA
NPK/ dandelions/ EDTA	0.164	0.170	0.174	0.244	0.904	<0.001	NA	NA

extracted in the exchangeable step of the SE. This was expected, as EDTA is known to form soluble calcium complexes and therefore would remove calcium from more resistant phases. The EDTA solution would also decrease the soil pH slightly, therefore increasing the solubility of calcium further.

The combination of dandelion and EDTA was also shown to have an effect on the calcium extracted by the SE in the exchangeable stage. Although EDTA is shown to increase the EDTA extracted by the exchangeable step, addition of a dandelion appears to decrease the calcium extracted. This is likely to be due to uptake of the calcium by the plant as a result of the increased solubility of the calcium. Indeed, statistics show that calcium levels in the roots of the dandelions were significantly increased in the plants to which EDTA had been applied.

The addition of NPK did not appear to have any significant effect on the extraction of calcium from the soil. The addition of NPK to the soil also appears to reduce the effect of EDTA leaching on the soil. This may be due to formation calcium phosphate, which in neutral solution may precipitate. Calcium may not be as readily available to the neutral EDTA solution used in flushing the soil columns. However when the pH is decreased in the sequential extraction the calcium would once more be available.

6.3.2 Copper

Results for copper in the soil and plant material are shown in table 6.15. Copper concentrations in the soil are slightly higher than the typical range for copper of 1-80 mg/kg dry weight and copper found in plant material was found to be at least twice the typical range found in plants (2-20 mg l^{-1})[17]. The high concentration of copper found in the dandelions suggests that much more of the copper was bioavailable than is typical.

Sums and PT values compare very well with one another indicating good recoveries. Standard deviations tend to be below 20 % with the exception of the residual step where larger variation occurs. This may be due to natural variations in the levels of copper bound to more resistant material throughout the soil.

Copper levels appear to be similar in roots and leaves, suggesting that copper was not accumulated in the leaves of the plant, except for the dandelions to which EDTA was applied. Table 6.16 shows the effects of the treatments on the soil and the dandelions.

Statistics show that the addition of EDTA had the most significant effect on copper in the dandelions and the soil. EDTA significantly increased the levels of copper in both the roots and leaves of the dandelions. Significantly more copper was extracted by the exchangeable step of the SE when EDTA had been applied to the soil. Concomitantly less copper was extracted in the reducible stage and oxidisable stage

Table 6.15 Copper in soil extracts and plant material

Treatment	Soil (mg kg^{-1})						Dandelions (mg kg^{-1})	
	Exchangeable	Reducible	Oxidisable	Residual	Sum	PT	Leaves	Roots
No EDTA Dandelion No NPK	7.25 ± 0.60	21.28 ± 1.31	34.23 ± 3.22	50.95 ± 25.58	113.72 ± 25.08	96.01 ± 7.62	29.64 ± 8.33	38.36 ± 9.64
EDTA Dandelion No NPK	21.7 ± 0.33	11.85 ± 3.19	23.65 ± 4.74	38.89 ± 8.89	96.10 ± 3.99	101.05 ± 5.07	102.5 ± 11.82	71.02 ± 9.81
No EDTA No dandelion No NPK	4.06 ± 0.33	18.50 ± 1.47	25.84 ± 4.37	42.28 ± 1.64	90.67 ± 5.56	108.12 ± 8.44	NA	NA
EDTA No dandelion No NPK	24.16 ± 2.20	13.72 ± 1.17	27.28 ± 4.90	34.98 ± 5.33	100.14 ± 10.06	94.42 ± 11.24	NA	NA
No EDTA Dandelion NPK	5.18 ± 1.95	22.16 ± 2.91	33.67 ± 6.01	39.47 ± 16.49	100.49 ± 23.18	100.41 ± 7.15	28.14 ± 17.00	46.50 ± 13.60
EDTA Dandelion NPK	23.22 ± 2.36	10.34 ± 1.83	27.07 ±1.71	40.82 ± 2.59	101.45 ± 4.52	101.30 ± 10.91	68.71 ± 26.83	63.21 ± 20.45
No EDTA No dandelion NPK	4.69 ± 1.29	18.76 ± 0.76	27.14 ± 3.51	34.85 ± 7.65	85.41 ± 7.38	130.94 ± 54.65	NA	NA
EDTA No dandelion NPK	24.88 ± 2.49	12.44 ± 3.82	26.30 ± 2.70	41.21 ± 4.18	104.82 ± 6.86	113.04 ± 10.26	NA	NA

Table 6.16 F pr. Values for effect of treatments on Cu in soil and plant material

Treatment	Soil						Dandelions	
	exchangeable	reducible	oxidisable	residual	sum	PT	leaves	roots
NPK	0.744	0.619	0.588	0.526	0.660	0.135	0.028	0.696
dandelions	0.855	0.501	0.048	0.324	0.118	0.122	NA	NA
EDTA	<0.001	<0.001	0.009	0.494	0.524	0.397	<0.001	<0.001
NPK/ dandelions	0.428	0.907	0.663	0.624	0.705	0.228	NA	NA
NPK/EDTA	0.136	0.237	0.772	0.118	0.146	0.781	0.041	0.088
Dandelions/ EDTA	0.003	0.005	0.005	0.563	0.024	0.219	NA	NA
NPK/ dandelions/ EDTA	0.153	0.794	0.290	0.987	0.654	0.998	NA	NA

of the SE. This suggests that EDTA is able to release copper from substrates that would not normally release copper until the reducible or oxidisable stages of the SE. The copper is then available for release in the exchangeable stage. Due to the strength of the complexes formed by EDTA it can be used to remove some metals bound by chelation in the organic matter, which would normally be released in the oxidisable stage.

The combined effect of dandelions and EDTA was also significant. The levels of copper in the exchangeable extracts were higher than extracts from soil with no treatment, but lower than extracts from soil with EDTA treatment alone. As with calcium this is likely to be due to the increased solubility of the copper leading to a greater uptake by the dandelions therefore decreasing the copper available for extraction in the exchangeable step of the SE. Results from the plant analysis show that significantly greater levels of copper were present in the dandelion leaves of plants treated with EDTA, however NPK fertiliser was found to decrease the level of copper taken up by the dandelions. This is likely to be due to the formation of insoluble metal compounds. For example most metal phosphates form insoluble compounds.

6.3.3 Iron

Results for the SE of iron from soil and the analysis of plant material are shown in table 6.17. Iron is typically present at concentrations of 0.7 - 4.2 % in soils and 5 - 200 mg kg^{-1} in plants[17]. Iron levels in the soil were found to be typical; however iron

Table 6.17 Iron in soil extracts and plant material

Treatment	Soil (mg kg^{-1})						Dandelions (mg kg^{-1})	
	Exchangeable	Reducible	Oxidisable	Residual	Sum	PT	Leaves	roots
No EDTA Dandelion No NPK	9.49 ± 1.32	1502 ± 48	1016 ± 131	23153 ± 1770	25680 ± 1877	25174 ± 3284	609 ± 642	1611 ± 158
EDTA Dandelion No NPK	345 ± 24	1115 ± 107	785 ± 400	24646 ± 2521	26892 ± 2050	26423 ± 3350	1906 ± 953	2025 ± 63
No EDTA No dandelion No NPK	7.71 ± 0.56	1343 ± 77	612 ± 148	25905 ± 425	27868 ± 519	28225 ± 3396	NA	NA
EDTA No dandelion No NPK	382 ± 36	1228 ± 144	1073 ± 283	25324 ± 3254	28008 ± 3491	25111 ± 736	NA	NA
No EDTA Dandelion NPK	9.38 ± 1.64	1571 ± 224	1007 ± 171	23804 ± 4697	26391 ± 4843	24852 ± 3416	813 ± 465	3983 ± 3938
EDTA Dandelion NPK	375 ± 41	1120 ± 64	1016 ± 82	25162 ± 1742	27673 ± 1771	26989 ± 5649	1458 ± 809	3251 ± 1960
No EDTA No dandelion NPK	8.08 ± 1.58	1383 ± 234	910 ± 261	23618 ± 3957	25918 ± 4045	29407 ± 1609	NA	NA
EDTA No dandelion NPK	354 ± 29	1215 ± 214	863 ± 174	24808 ± 858	27240 ± 1246	27186 ± 2918	NA	NA

present in the dandelions was found at much higher levels than the typical ranges given for plants. As in the case of copper this suggests that iron is present in readily available forms.

The sums and PT values compared well and standard deviations were generally below 20 % with the exception of the oxidisable steps, which were more variable. Standard deviations in dandelion leaves were found to be high. Standard deviations in the dandelion roots were low where no NPK was applied, but high in plants treated with the NPK. Table 6.18 shows the effect of the EDTA, dandelions and NPK on the soil and plants.

EDTA significantly increased the iron extracted in the exchangeable stage of the SE from approximately 9 mg kg^{-1} to 350 mg kg^{-1}. The iron extracted in the reducible stage of the SE was less when EDTA had been applied to the soil. This was expected as EDTA was shown to release iron from substrates, which would normally release iron during the reducible stage, in chapter 4. Soil treated with EDTA and dandelions were shown to have significantly lower levels of iron present in the reducible extracts. EDTA was also shown to significantly increase iron present in the leaves of dandelions. This may be due to plant physiology as iron is an essential element used in the production of chlorophyll in plants and would therefore require iron in the leaves.

Table 6.18 F pr. values for effect of treatments on Fe in soil and plant material

Treatment	Soil						Dandelions	
	exchangeable	reducible	oxidisable	residual	sum	PT	leaves	roots
NPK	0.969	0.657	0.346	0.681	0.763	0.465	0.951	0.537
dandelions	0.710	0.535	0.266	0.469	0.557	0.181	NA	NA
EDTA	<0.001	<0.001	0.557	0.387	0.335	0.683	0.009	0.412
NPK/ dandelions	0.093	0.834	0.677	0.323	0.306	0.528	NA	NA
NPK/EDTA	0.982	0.602	0.413	0.681	0.758	0.709	0.265	0.155
Dandelions/ EDTA	0.579	0.019	0.059	0.574	0.799	0.077	NA	NA
NPK/ dandelions/ EDTA	0.088	0.963	0.029	0.632	0.785	0.999	NA	NA

6.3.4 Manganese

Manganese extracted from the soil at each stage of the SE and PT extraction along with manganese levels in the roots and leaves of the dandelions are shown in table 6.19. Manganese is typically present in soils at levels of 20 – 3000 mg kg^{-1} and 1 – 700 mg kg^{-1} in plants. Manganese levels in both soil and plants were found to be typical of the given ranges.

Sums and PT values of manganese compare well and relative standard deviations are low in most cases. Higher relative standard deviations were noted in cases where the manganese levels were low i.e. exchangeable and oxidisable stages. This is likely to be due to higher signal to background ratios.

The majority of the mobile manganese is found in the reducible step of the SE and would be released under reducing conditions. Levels of manganese in the dandelions were found to be higher in leaves than roots. Manganese is an essential element for plant growth and reproduction. It is used as a catalyst in oxygen evolution during photosynthesis and would therefore be transported to the leaves of the plant where it can be used, explaining the higher leaf content. Although manganese is essential to plants, it can be toxic to some species at high levels.

Table 6.20 shows the effects of the treatments on the manganese distribution in the soil and the plant content. EDTA was shown to significantly increase the manganese extracted by the exchangeable step of the SE. The increase in 'exchangeable'

Table 6.19 Manganese in soil extracts and plant material

Treatment	Soil (mg kg^{-1})						Dandelions (mg kg^{-1})	
	Exchangeable	Reducible	Oxidisable	Residual	Sum	PT	leaves	roots
No EDTA Dandelion No NPK	32.6 ± 5.6	112 ± 9	18.5 ± 2.1	117 ± 8	280 ± 13	288 ± 16	66.0 ± 25.8	34.3 ± 13.1
EDTA Dandelion No NPK	36.7 ± 5.6	100 ± 4	15.4 ± 6.9	131 ± 9	284 ± 9	285 ± 10	94.5 ± 20.8	59.9 ± 12.6
No EDTA No dandelion No NPK	19.7 ± 0.9	117 ± 12	10.4 ± 2.1	136 ± 3	283 ± 16	310 ± 27	NA	NA
EDTA No dandelion No NPK	43.5 ± 5.2	106 ± 14	20.3 ± 5.3	129 ± 13	298 ± 30	268 ± 19	NA	NA
No EDTA Dandelion NPK	19.7 ± 12.8	122 ± 30	16.8 ± 2.3	123 ± 24	282 ± 44	285 ± 36	65.1 ± 9.4	46.5 ± 19.9
EDTA Dandelion NPK	37.3 ± 6.5	95.6 ± 18.5	17.8 ± 1.1	128 ± 12	278 ± 23	286 ± 44	75.5 ± 29.1	69.7 ± 16.0
No EDTA No dandelion NPK	22.9 ± 7.5	114 ± 18	14.2 ± 3.0	117 ± 19	268 ± 25	302 ± 18	NA	NA
EDTA No dandelion NPK	33 ± 10.1	99.0 ± 12.5	10.9 ± 4.3	128 ± 8	271 ± 22	298 ± 17	NA	NA

Table 6.20 F pr. values for effect of treatments on Mn in soil and plant material

Treatment	Soil						Dandelions	
	exchangeable	reducible	oxidisable	residual	sum	PT	leaves	roots
NPK	0.079	0.854	0.392	0.361	0.196	0.570	0.151	0.330
dandelions	0.515	0.795	0.029	0.605	0.922	0.347	NA	NA
EDTA	<0.001	0.012	0.417	0.266	0.613	0.190	0.033	<0.001
NPK/ dandelions	0.651	0.493	0.261	0.259	0.266	0.506	NA	NA
NPK/EDTA	0.975	0.448	0.114	0.674	0.592	0.261	0.402	0.210
Dandelions/ EDTA	0.262	0.624	0.123	0.431	0.627	0.244	NA	NA
NPK/ dandelions/ EDTA	0.018	0.612	0.004	0.159	0.902	0.349	NA	NA

manganese was mirrored by a decrease in manganese extracted in the reducible step. A combination of EDTA, dandelion and NPK was shown to effect manganese extracted by both the exchangeable and oxidisable stages of the SE. It appears that levels of manganese were not as high as those extracted from soil treated with EDTA alone, but were higher than soils with no treatment. On average an increase was observed for the extraction of manganese from the oxidisable step. The reasons for this increase are unclear however, the rhizospheres of plants are complex areas and may have some influence over metals released during SE. This theory would require further investigation.

EDTA was shown to significantly increase the manganese taken up by the dandelions in both roots and leaves. Only the divalent Mn^{2+} cation is available (Mn^{4+} is virtually insoluble) increasing solubility of manganese in the soil explains the increased manganese found in the roots and leaves of the dandelions.

6.3.5 Nickel

Table 6.21 shows the results of the SE and PT extraction of the soil, and the nickel content of the leaves and roots of the dandelions. Nickel found in the soil is typical of the occurrence of Ni in soils (2-50 mg kg^{-1}), however nickel found in the plants was slightly higher than typical plant ranges of 0.4 – 4 mg kg^{-1}. Sums of the SE and PT extractions compare well and relative standard deviations are low for the exchangeable step of the SE, however many results have relative standard deviations of between 20 and 80 %. A combination of accumulation of error throughout the SE

Table 6.21 Nickel in soil extracts and plant material

Treatment	Soil (mg kg^{-1})						Dandelions (mg kg^{-1})	
	Exchangeable	Reducible	Oxidisable	Residual	Sum	PT	leaves	roots
No EDTA Dandelion No NPK	3.11 ± 0.18	3.65 ± 0.42	7.31 ± 0.16	38.0 ± 8.5	52.0 ± 8.7	43.5 ± 7.8	5.05 ± 1.70	9.30 ± 1.24
EDTA Dandelion No NPK	3.72 ± 0.21	2.10 ± 1.54	5.82 ± 2.77	39.9 ± 11.3	51.6 ± 9.8	51.4 ± 14.1	16.4 ± 1.9	12.0 ± 1.3
No EDTA No dandelion No NPK	2.59 ± 0.11	4.07 ± 0.07	3.75 ± 0.45	49.9 ± 3.1	60.4 ± 3.5	55.6 ± 11.5	NA	NA
EDTA No dandelion No NPK	4.23 ± 0.25	3.25 ± 0.61	7.50 ± 1.09	42.7 ± 12.1	57.7 ± 12.9	43.0 ± 3.5	NA	NA
No EDTA Dandelion NPK	2.82 ± 0.21	4.08 ± 0.62	6.64 ± 1.47	43.8 ± 22.0	57.3 ± 23.2	43.1 ± 6.4	3.87 ± 1.17	17.0 ± 10.5
EDTA Dandelion NPK	4.16 ± 0.20	2.18 ± 1.67	6.60 ± 1.53	50.6 ± 8.2	63.5 ± 10.4	49.5 ± 15.3	10.5 ± 4.6	14.1 ± 6.4
No EDTA No dandelion NPK	2.84 ± 0.20	3.75 ± 0.65	4.72 ± 1.83	39.5 ± 12.6	50.8 ± 10.9	56.4 ± 6.2	NA	NA
EDTA No dandelion NPK	3.92 ± 0.19	3.30 ± 0.42	2.75 ± 1.89	47.3 ± 1.8	57.3 ± 1.6	50.8 ± 12.9	NA	NA

and the low levels of nickel present may have led to this. Low concentrations in the extracts produce low signal to background ratios, and therefore higher standard deviations. Nickel was mainly found to be resistant to attack by the extractants used and levels of nickel in both dandelion leaves and roots were similar to those in the exchangeable and reducible extracts.

A summary of the statistical analysis of the nickel results is given in table 6.22. Although levels of nickel in the soil were low, statistical analysis shows that EDTA affected the nickel in the exchangeable and reducible extracts. Increased levels of nickel were found in the exchangeable extracts when soils had been treated with EDTA. Concomitantly lower nickel concentrations were found in the reducible extracts from soil treated with EDTA. Statistical analysis also showed that dandelions and EDTA, and EDTA, dandelions and NPK had significant effects on the results. Interaction between EDTA and the dandelions was shown to significantly increase the nickel extracted in the exchangeable step. This suggests that the dandelions are capable of removing nickel from the soil solution. NPK and dandelions when combined with the EDTA treatment are not capable of removing as much nickel from solution as EDTA alone. This may be due to the presence of the nitrate or phosphate anions from the NPK fertiliser competing with the EDTA for the nickel, and forming insoluble species.

The effect of the presence of dandelions was also shown to be significant in the extraction of nickel in the oxidisable step. As for iron this requires further

Table 6.22 F pr. values for effect of treatments on Ni in soil and plant material

Treatment	Soil						Dandelions	
	exchangeable	reducible	oxidisable	residual	sum	PT	leaves	roots
NPK	0.775	0.859	0.117	0.528	0.672	0.673	0.071	0.144
dandelions	0.424	0.080	0.002	0.666	0.922	0.231	NA	NA
EDTA	<0.001	0.001	0.914	0.572	0.570	0.798	<0.001	0.561
NPK/ dandelions	0.441	0.546	0.097	0.186	0.115	0.472	NA	NA
NPK/EDTA	0.569	0.987	0.071	0.237	0.353	0.713	0.058	0.065
Dandelions/ EDTA	0.010	0.107	0.155	0.625	0.911	0.038	NA	NA
NPK/ dandelions/ EDTA	<0.001	0.578	0.004	0.542	0.885	0.568	NA	NA

investigation to explain the nature of the chemistry occurring, however rhizoshphere studies may be a starting place for investigations.

Significantly more nickel was shown to be present in the dandelion leaves of those plants treated with EDTA, suggesting that EDTA assists the uptake of nickel by the plant. Although uptake has been shown, it is difficult to conclude whether the dandelion would be a suitable hyper-accumulator for nickel due to the low levels of nickel present in the soil.

6.3.6 Lead

Lead present in soil extracts and plant digests is shown in table 6.23. Typical levels of lead range from 0.1 – 200 mg kg^{-1} in soils and 0.1 – 5 mg kg^{-1} in plants[17]. Lead levels in the soils are high, but lead levels found in the dandelions were found to exceed the levels found in plants by at least 10 times for plants not treated with EDTA and 100 times for those treated with EDTA. The above findings suggest that the dandelion is capable of taking up lead and that a large proportion of the lead present in the soil is available for uptake. This is confirmed by the sequential extraction results.

The majority of the lead present in the soil was extracted during the reducible stage of the SE making it reasonably mobile. However, total lead levels in the soil do not exceed the UK ICRCL trigger concentrations for use of land as domestic gardens, allotments, playing area or open spaces. Standard deviations vary from 3 % to 55 %.

Table 6.23 Lead in soil extracts and plant material

Treatment	Soil (mg kg^{-1})						Dandelions (mg kg^{-1})	
	Exchangeable	Reducible	Oxidisable	Residual	Sum	PT	Leaves	Roots
No EDTA Dandelion No NPK	11.8 ± 3.2	193 ± 47	47.3 ± 9.9	67.4 ± 6.5	319 ± 65	265 ± 10	10.4 ± 16.1	52.5 ± 15.5
EDTA Dandelion No NPK	25.6 ± 4.3	105 ± 58	34.4 ± 12.2	67.1 ± 9.7	232 ± 59	259 ± 6	326 ± 82	183 ± 38
No EDTA No dandelion No NPK	7.04 ± 0.65	174 ± 16	29.8 ± 4.4	73.6 ± 2.4	284 ± 24	294 ± 27	NA	NA
EDTA No dandelion No NPK	26.3 ± 3.3	157 ± 16	42.1 ± 5.9	63.6 ± 2.4	289 ± 14	249 ± 23	NA	NA
No EDTA Dandelion NPK	9.07 ± 1.20	179 ± 38	41.6 ± 10.8	70.9 ± 18.0	301 ± 65	261 ± 17	10.5 ± 4.6	60.2 ± 7.4
EDTA Dandelion NPK	24.5 ± 4.9	151 ± 98	39.1 ± 3.4	63.8 ± 8.2	278 ± 110	251 ± 24	211 ± 146	134 ± 67
No EDTA No dandelion NPK	8.04 ± 2.14	159 ± 18	31.6 ± 6.7	71.1 ± 9.2	269 ± 24	263 ± 19	NA	NA
EDTA No dandelion NPK	25.2 ± 5.2	142 ± 8	22.7 ± 6.7	68.8 ± 6.2	259 ± 12	259 ± 12	NA	NA

The reason for this unclear, however higher standard deviations tend to occur when dandelion were present in the soil.

Plants, which received no EDTA treatment, contained more lead in the roots. The reverse was true for plants treated with EDTA. This suggests that the EDTA enabled the plants to translocate lead from roots to shoots.

Statistical analysis (summarised in table 6.24) shows that EDTA significantly increased the lead extracted by the exchangeable step of the SE. This was expected and had been shown in chapter 5. EDTA increased the solubility of lead in the soil by decreasing soil pH and complexing with the metal, therefore more lead was observed in the exchangeable extracts. A significant decrease in lead extracted in the reducible step was observed for experiments treated with EDTA, suggesting that the extra lead released by the EDTA would ordinarily be released under reducing conditions.

Statistical results indicate that dandelions alone effect the lead extracted by the oxidisable stage and that the combined dandelion, EDTA and NPK treatment has a significant effect on lead extracted in the oxidisable stage and the pseudototal extraction. The three factor interaction appears to increase lead extracted in the oxidisable step, however, the presence of dandelion as a single factor has greater significance. The interaction of the three factors also appears to have reduced the lead extracted by PT extraction. This was expected, as both the EDTA and dandelion removed lead from the soil.

Table 6.24 F pr. values effect of treatments on Pb in soil and plant material

Treatment	Soil						Dandelions	
	exchangeable	reducible	oxidisable	residual	sum	PT	leaves	roots
NPK	0.430	0.968	0.117	0.833	0.834	0.584	0.084	0.531
dandelions	0.367	0.948	0.004	0.557	0.724	0.100	NA	NA
EDTA	<0.001	0.034	0.307	0.148	0.165	0.109	<0.001	<0.001
NPK/ dandelions	0.453	0.355	0.157	0.851	0.371	0.753	NA	NA
NPK/EDTA	0.915	0.376	0.349	0.953	0.545	0.056	0.084	0.312
Dandelions/ EDTA	0.167	0.221	0.111	0.715	0.205	0.631	NA	NA
NPK/ dandelions/ EDTA	0.462	0.373	0.011	0.283	0.322	0.034	NA	NA

EDTA has a great effect on the lead present in both roots and leaves of the dandelions. An increase from 10 to 300 and 200 mg kg^{-1} was observed for lead present in leaves dependant on whether NPK was present. A large increase in lead in the roots of plants treated with EDTA was also observed. This indicates that lead was taken up by dandelions and that this process was assisted greatly by addition of EDTA. However it should be noted that soon after addition of the EDTA the plants died (figure 6.3). This could be due to the application of EDTA but is more likely to be due to the toxic effects of high levels of lead (and other determinants) within the plant. Further investigation would be required to confirm these theories.

Figure 6.3 Dandelion leaves (EDTA treated on left), dandelion roots (EDTA treated on left)

6.3.7 Zinc

Results for zinc in soil and plant materials are shown in table 6.25. Zinc is typically found at levels of 3-300 mg kg^{-1} in soil and 15 – 150 mg kg^{-1} in plants[17]. Zinc found in the soil investigated was found to be at the high end of the typical range. Much higher concentrations of zinc were found in the dandelions than the typical ranges suggested for plants. Zinc is likely to be present in readily available forms that the dandelion can take up from the soil.

The sum of SE and PT extraction compare reasonably. Relative standard deviations were low for the exchangeable step of the SE, however some treatments and steps resulted in very high relative standard deviations (i.e. the oxidisable step of the SE of dandelion and EDTA treated soil). High standard deviations may be due to the heterogeneity of the samples or accumulation of error throughout the SE. Total levels of zinc are higher than threshold trigger concentrations suggested by ICRCL for land where plants grow. Levels of zinc in roots and leaves of untreated plants were similar, however for those plants treated with EDTA, zinc concentrations in the leaves were much higher that those in the roots. This suggests that EDTA assisted the translocation of zinc from the roots to the shoots.

The results of ANOVA are shown in Table 6.26 for the various treatments on each stage of the SE, the PT extraction and the plants. EDTA was shown to significantly increase the zinc extracted by the exchangeable stage of the SE. This was expected as the EDTA would increase the solubility of the zinc and therefore make more

Table 6.25 Zinc in soil extracts and plant material

Treatment	Soil (mg kg^{-1})						Dandelions (mg kg^{-1})	
	exchangeable	Reducible	Oxidisable	Residual	Sum	PT	leaves	roots
No EDTA Dandelion No NPK	108 ± 5	62.4 ± 3.5	67.8 ± 9.6	91.5 ± 21.6	329 ± 20	335 ± 76	317 ± 101	328 ± 40
EDTA Dandelion No NPK	114 ± 9	57.0 ± 5.2	67.5 ± 40.0	110 ± 49	349 ± 78	430 ± 73	699 ± 112	371 ± 37
No EDTA No dandelion No NPK	103 ± 5	60.2 ± 4.4	128 ± 82	131 ± 14	422 ± 91	394 ± 71	NA	NA
EDTA No dandelion No NPK	125 ± 11	61.1 ± 7.3	57.9 ± 10.8	89.0 ± 4.9	333 ± 29	347 ± 60	NA	NA
No EDTA Dandelion NPK	105 ± 12	62.9 ± 9.9	173 ± 132	288 ± 290	629 ± 409	279 ± 24	338 ± 53	456 ± 65
EDTA Dandelion NPK	119 ± 12	52.0 ± 9.8	59.0 ± 16.2	104 ± 21	334 ± 34	317 ± 47	619 ± 188	310 ± 61
No EDTA No dandelion NPK	102 ± 8	58.8 ± 7.5	63.5 ± 11.2	98.6 ± 15.8	323 ± 18	403 ± 104	NA	NA
EDTA No dandelion NPK	119 ± 12	53.9 ± 8.7	67.2 ± 51.2	102 ± 15	342 ± 77	436 ± 155	NA	NA

Table 6.26 F pr. values for effect of treatments on Zn in soil and plant material

Treatment	Soil						Dandelions	
	exchangeable	reducible	oxidisable	residual	sum	PT	leaves	roots
NPK	0.730	0.225	0.627	0.259	0.379	0.556	0.499	0.353
dandelions	0.844	0.978	0.555	0.256	0.321	0.077	NA	NA
EDTA	<0.001	0.064	0.044	0.181	0.125	0.340	<0.001	0.041
NPK/ dandelions	0.503	0.695	0.086	0.169	0.098	0.037	NA	NA
NPK/EDTA	0.879	0.285	0.641	0.301	0.354	0.841	0.171	0.008
Dandelions/ EDTA	0.169	0.252	0.577	0.400	0.354	0.231	NA	NA
NPK/ dandelions/ EDTA	0.336	0.981	0.037	0.106	0.064	0.263	NA	NA

available for extraction in the first step of the SE. EDTA also appears to decrease the levels of zinc extracted by the oxidisable step of the SE. NPK and dandelions appear to decrease the zinc released by PT extraction, suggesting that healthy dandelions are capable of taking up zinc without chelate assistance. NPK and dandelions were also shown to increase the zinc present in the roots of dandelions. EDTA was shown to significantly increase the zinc present in the leaves of the dandelions. This is possibly due to EDTA assisting the translocation of zinc to the leaves, however this would require further investigation to substantiate this theory.

6.3.8 Hypothetical field experiment

The metal content in 1 hectare (20 cm deep) of untreated Ardeer 2 soil was calculated. The metal content for the treatments used in the experimental design were also calculated for the 1 hectare field. The maximum metal content determined by ICRCL threshold guidelines in a 1 hectare field was also calculated. These values are shown in table 6.27.

Table 6.27 Metal content in 1 hectare x 20 cm field (kg)

	Ca	Cu	Fe	Mn	Ni	Pb	Zn
ICRCL maximum		25			14	97	58
Untreated soil	486	21	5463	60	11	57	77
EDTA	244	18	4860	52	8	48	67
Dandelion	479	19	4872	56	8	51	65
NPK	377	25	5692	59	11	51	78
EDTA/dandelion	578	20	5114	55	10	50	83
EDTA/NPK	615	22	5262	58	10	54	84
Dandelion/NPK	407	19	4810	55	8	50	54
EDTA/dandelion/NPK	463	20	5224	55	10	49	61

Zinc was the only metal in the Ardeer soil above the ICRCL threshold concentration. In order to remove sufficient zinc from the field for the concentration to be below the threshold concentration, 19 kg of zinc must be removed. According to the results from the experiments carried out in this work the most effective treatment for zinc

removal would be dandelions grown with the application of NPK fertiliser. If sufficient dandelions could be grown on the field (i.e. approximately 1000000 dandelions, 1 dandelion every 10 cm^2) then sufficient zinc could be removed within 3 months (the time period over which the dandelion experiments were carried out). Application of EDTA alone appeared to be the most effective treatment for copper and lead, although the presence of the dandelions also appeared to be effective in the removal of lead from the soil.

High levels of lead in the plants showed uptake of lead, however the decreases in the soil levels were not similar. In order for lead to be removed from a contaminated soil to suitable levels many crops of dandelions would need to be grown. This suggests that the use of dandelions would be for accelerated natural attenuation rather than a fast clean up solution for lead contaminated land. The process could take several years, however may be more cost effective than conventional means of remediation.

6.4 Conclusions and further work

This experiment aimed to investigate the viability of chelate assisted phytoremediation of a contaminated soil using dandelions and EDTA. EDTA was shown to significantly increase the mobility of calcium copper, iron, manganese, nickel, lead and zinc, therefore increasing availability to the dandelions. EDTA was shown to increase the uptake of most metals, especially copper, lead and zinc.

Although large amounts of metal were taken up by the plants those treated with EDTA died shortly after EDTA application. This may be due to low tolerance of the plant to the high levels of lead present in the roots and leaves. The levels of lead and copper taken up by the plants were not found to be high enough for the land to be remediated in a practical time-scale. In order for dandelions to be used as successful phytoremediators the heavy metal tolerance would have to be much higher. The genes, which enable plants such as pennycress to hyperaccumulate metals, are being investigated in order that they can be transferred to plants of higher mass[5]. This would make phytoremediation a much more viable option. Further work with dandelions could also involve studies to investigate the use of gene technology to improve metal uptake in dandelions without the use of chelators. Also necessary is the investigation of the method of uptake of the heavy metal concerned.

In conclusion dandelions have been shown to accumulate heavy metals, but not to the extent required for the remediation of a soil, with the exception of zinc and possibly lead. The addition of EDTA was shown to increase the uptake of all the metals by increasing solubility. Further optimisation, possibly by genetic engineering would be required if the dandelion were to be used as a hyperaccumulator for remediation.

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Chapter 7 Conclusions and further work

This work involved the investigation of remediation methods for land contaminated with heavy metals. In order to assess the viability of the methods investigated the three-stage BCR sequential extraction protocol was used. Recently modifications to this protocol have been suggested and the effect of these modifications on the sequential extraction of metals from several soils was investigated prior to the study of any remediation techniques.

The effect of increasing the reductant concentration and decreasing the pH of the reductant was hoped to improve the reproducibility obtained in the second step during round robin trials. Relative standard deviations were generally less than 20 %, however results varied between days.

Increasing the reductant concentration alone was observed to slightly increase copper, lead and iron extracted. Decreasing the pH of the reductant had a much more significant effect on the increase in the copper, lead and iron extracted by the reducible stage. It is thought that re-adsorption of lead and copper was prevented by the reduction in pH, and that the stronger reducing agent was able to attack more resistant forms of iron present in the soils and therefore increase the iron extracted. In order to confirm these theories further investigation into the classical speciation of the metals would be required. The modified BCR protocol was selected for the assessment of remediation procedures throughout the remaining work.

Three soils were subjected to simulated soil flushing experiments with disodium EDTA at pH 7 and pH 4.45 and diammonium EDTA at pH 7. The most contaminated soil (Ardeer 1) was not found to be suitable for soil flushing, as it was impermeable to the flushing solutions. This is likely to be due to the high proportion of fine particles in the soil. This emphasised the requirement for assessment of soil characteristics prior to remediation.

Experiments 1 and 2 illustrated that the extraction with EDTA was kinetically dependent. This suggests that in practice contact time with the soil should be an important consideration. This would involve optimisation and is likely to be dependent on the soil being considered for remediation. The distribution of the metals within the soil is also likely to effect the success of the remediation and the contact time required.

Experiment 3 removed any kinetic dependence from the results by continuous flow of the leachate. Column leaching with EDTA was shown to be a successful technique for removing large proportions of metals from the soils studied, especially those for which the metal-EDTA stability constant was high e.g. lead, copper and zinc. Approximately 90 % of the copper, 80 % of the lead and zinc, normally extracted during the exchangeable step, were removed from Ardeer location 2. The proportion of copper and lead removed during the reducible step of the sequential extraction was also decreased, suggesting removal of metal from this stage. Copper and lead extracted during the oxidisable step were also reduced.

Phytoremediation and chelate assisted phytoremediation of the Ardeer location 2 soil were investigated. The accumulation of metals by *Taraxacum officianale* was investigated. The effect of chelate assistance and the application of a fertiliser upon the remediation were also studied. EDTA was shown to significantly increase the mobility of the metals within the soil therefore increasing availability to the dandelions. The uptake of copper, lead and zinc appeared to be successful when EDTA was applied, however, plants treated with EDTA died shortly after application. This was thought to be due to metal toxicity within the plant. Further investigation of the process by which *Taraxacum officianale* accumulates metals may reveal methods to further enhance accumulation.

Dandelions treated with fertiliser could be used to remove zinc from contaminated soils, based on a hypothetical experiment of 1000000 dandelions grown on a 1 hectare field. Other metals may be successfully removed, however the levels of metal in Ardeer location 2 soil were not higher than the ICRCL values (with the exception of zinc). Phytoremediation and chelate assisted phytoremediation should be considered for accelerated natural attenuation when this can be an alternative to fast clean up solutions.

Although fertiliser was shown to increase the effectiveness of accumulation of zinc by dandelions, in some cases accumulation of metal was decreased. This was most likely due to the formation of insoluble phosphate complexes, which would decrease the availability of the metal to the dandelion.

The modified BCR sequential extraction protocol showed that soil flushing with diammonium EDTA may be a successful remediation tool to consider when dealing with a contaminated area through which a flushing agent can be percolated and recovered. Phytoremediation as studied in this work was shown to be a solution, which would take longer but would involve fewer disturbances to the soil and lower costs.

Future work may involve the assessment of other chelators from which metals could be more readily recovered than EDTA, as well as investigating the kinetic dependency of soil flushing. In terms of phytoremediation gene-modified dandelions and other hyperaccumulators (e.g. crops which would yield higher mass) should also be investigated combined with chelate assistance. These investigations should provide a wider range of tools for the remediation of heavy metal contaminated land.

Appendix 1

Literature values (Rauret, G., Lopez-Sanchez, J.F., Sahuquillo, A., Rubio, R., Davidson, C., Ure, A., and Quevauviller, Ph., *J. Environ. Monit.*, 1999, 1, 57-61) **and results for repeated sequential extraction of CRM 601**

Table 1 Calcium in CRM 601 (mg kg^{-1})

	Original	Modified Procedure									
	cert.	Ind..	1	2	3	4	5	6	7	Mean (1-7)	SD (1-7)	%RSD (1-7)
Exchangeable			29407	29407	28710	31520	26491	25895	23364	27828	2734	9.8
Reducible			5830	5778	6069	5619	6581	5942	6575	6056	382	6.3
Oxidisable			559	528	533	529	452	328	409	477	84	17.7
Residual			1227	1394	1292	1785	1620	2796	1317	1633	550	33.7

Table 2 Copper in CRM 601 (mg kg^{-1})

	Original	Modified Procedure									
	cert.*	Ind..	1	2	3	4	5	6	7	Mean (1-7)	SD (1-7)	%RSD (1-7)
Exchangeable	8.32 ± 0.46	10.5 ±0.8	9.24	9.90	9.35	10.08	9.16	9.27	9.96	9.57	0.39	4.13
Reducible	5.69 ± 3.2	72.8 ± 4.9	50.57	48.24	44.37	53.79	44.16	53.71	37.98	47.55	5.77	12.13
Oxidisable	116 ± 26	78.6 ± 8.9	97.26	92.70	91.54	87.73	91.71	91.10	99.87	93.13	4.09	4.40
Residual			59.81	43.35	49.95	60.10	64.68	59.85	64.22	57.42	7.87	13.70

* Values for copper were obtained during the certification campaign, but not finally certified.

Table 3 Iron in CRM 601 (mg kg^{-1})

	Original	Modified Procedure									
	cert.	Ind..	1	2	3	4	5	6	7	Mean (1-7)	SD (1-7)	%RSD (1-7)
Exchangeable			48.70	4.18	nd	22.29	16.40	19.22	15.40	21	15	70.8
Reducible			4621	4373	4238	3748	3290	3788	3648	3958	467	11.8
Oxidisable			1480	1510	1563	1479	1190	1595	1384	1457	136	9.3
Residual			35030	25901	28614	31483	35128	35740	35037	32419	3871	11.9

Table 4 Lead in CRM 601 (mg kg^{-1})

	Original	Modified Procedure									
	cert.	Ind..	1	2	3	4	5	6	7	Mean (1-7)	SD (1-7)	%RSD (1-7)
Exchangeable	2.86 ± 0.35	2.28 ± 1.17	1.71	2.01	2.29	1.99	6.14	2.00	0.83	2.42	1.70	70.3
Reducible	33.1 ± 10.0	205 ± 11	193	182	178	198	173	179	168	182	11	5.9
Oxidisable	109 ± 13	19.7 ± 5.8	20.2	21.6	23.7	22.5	22.6	24.7	26.1	23.1	2.0	8.6
Residual			52.7	40.1	47.1	56.9	60.0	54.1	61.4	53.2	7.5	14.1

Table 5 Manganese in CRM 601 (mg kg^{-1})

	Original	Modified Procedure									
	cert.	Ind..	1	2	3	4	5	6	7	Mean (1-7)	SD (1-7)	%RSD (1-7)
Exchangeable			232	253	242	250	236	234	249	242	8.4	3.5
Reducible			174	167	163	168	178	175	176	172	5.6	3.2
Oxidisable			62	62	61	60	62	62	62	61.5	0.8	1.2
Residual			466	335	371	439	451	459	436	422	49.6	11.8

Table 6 Nickel in CRM 601 (mg kg^{-1})

	Original	Modified Procedure									
	cert.	Ind..	1	2	3	4	5	6	7	Mean (1-7)	SD (1-7)	%RSD (1-7)
Exchangeable	8.01 ± 0.73	7.82± 0.84					6.01	6.12	6.52	6.21	0.27	4.4
Reducible	6.05 ± 1.09	10.6 ± 1.3					8.99	8.21	8.36	8.52	0.41	4.9
Oxidisable	8.55 ± 1.04	6.04 ± 1.25					6.84	6.70	6.54	6.69	0.15	2.3
Residual							48.1	47.1	48.7	48.0	0.8	1.7

Table 7 Zinc in CRM 601 (mg kg^{-1})

	Original	Modified Procedure									
	cert.	Ind..	1	2	3	4	5	6	7	Mean (1-7)	SD (1-7)	%RSD (1-7)
Exchangeable	264 ± 5	261 ± 13	212	220	211	223	207	207	195	211	9.3	4.4
Reducible	182 ± 11	266 ± 17	249	231	228	234	231	235	254	238	10.0	4.2
Oxidisable	137 ± 30*	106 ± 11	131	144	139	134	144	127	124	135	8.0	5.9
Residual			172	138	142	177	179	183	176	167	18.5	11.1

* The oxidisable values for zinc was obtained during the certification campaign, but not finally certified.

Appendix 2

Sequential extraction and pseudototal results for column leaching experiments

Appendix 2.1 IG 35-45 Sequential Extraction and Pseudototal Results

(mg/kg)

Calcium exch.	red.	oxid.	resid.	Sum	PT
872	2301	1476	12148	16797	21175
893	2421	1197	12745	17256	19449
					18888

Copper exch.	red.	oxid.	resid.	Sum	PT
41.0	124.9	119.5	161.0	446.4	481.1
34.6	110.4	104.6	148.8	398.3	479.9
					476.7

Iron exch.	red.	oxid.	resid.	Sum	PT
108	4207	1248	55420	60983	76439
95	3991	1010	51554	56649	67575
					71568

Lead exch.	red.	oxid.	resid.	Sum	PT
1.191	14.05	<	78.13	93.37	93.03
0.453	11.83	<	58.54	70.82	87.88
					99.23

Manganese exch.	red.	oxid.	resid.	Sum	PT
17.71	54.76	23.43	500.7	596.6	740.2
15.18	57.19	19.64	476.7	568.7	714.1
					658.1

Zinc exch.	red.	oxid.	resid.	Sum	PT
46.59	64.83	28.69	125.8	265.9	434.9
38.12	62.56	32.79	113.3	246.8	419.7
					411.0

Appendix 2.2 IG 35-45 sequential extraction and pseudototal results after leaching experiment 1 (mg/kg)

Calcium replicates	exch.	red.	oxid.	resid.	Sum	PT
A	543.0	2021	1407	12744	16714	21063
	629.6	2117	1148	12176	16071	21914
						20853
B	687.6	2182	1315	14081	18266	20902
	518.9	2068	1201	12731	16519	19622
						19868
C	498.8	2159	1204	12003	15865	17601
	453.3	2214	1321	12662	16650	14235
						12896
D	417.0	2320	1384	13313	17434	15828
	481.4	2241	1596	13223	17542	14831
						14689

Copper replicates	exch.	red.	oxid.	resid.	Sum	PT
A	10.36	90.50	98.09	157.9	356.9	372.8
	10.16	87.44	105.7	150.5	353.8	328.2
						333.7
B	10.25	82.98	89.17	160.7	343.1	350.9
	9.56	91.80	96.97	150.6	348.9	338.5
						343.7
C	12.38	78.27	92.45	143.5	326.6	303.9
	12.04	78.09	97.42	140.3	327.8	315.4
						332.5
D	13.08	78.92	90.77	146.5	329.2	318.2
	13.03	81.39	94.50	155.6	344.5	303.6
						314.9

Iron replicates	exch.	red.	oxid.	resid.	Sum	PT
A	1612	4849	1016	53924	61401	77616
	1557	4804	893	54054	61308	74759
						71225
B	1448	4511	942	60134	67035	71474
	1481	4619	902	53943	60946	66955
						72015
C	553	4634	1001	52242	58430	55875
	548	4603	1030	54068	60250	45623
						43086
D	550	4759	1005	56256	62569	56272
	558	4858	1060	59105	65581	48802
						50518

Lead replicates	S1	S2	S3	SR	Sum	PT
A	0.3585	11.25	0.3878	71.16	83.15	79.85
	<	9.982	0.1142	68.14	78.23	68.53
						63.43
B	0.2997	9.826	<	65.30	75.43	64.50
	0.1992	10.53	<	59.23	69.96	65.60
						65.35
C	0.1220	17.12	<	66.99	84.23	72.93
	<	8.898	0.0681	63.55	72.51	74.36
						76.55
D	0.3039	11.14	1.6048	69.03	82.08	81.53
	0.4801	12.31	0.0719	100.7	113.5	72.39
						71.96

Manganese replicates	S1	S2	S3	SR	Sum	PT
A	17.26	46.15	22.61	483.5	569.5	781.9
	17.80	51.09	18.91	499.3	587.1	739.5
						689.2
B	17.69	49.65	21.82	559.6	648.8	701.5
	16.60	48.85	19.19	523.5	608.2	- 662.3
						693.8
C	12.70	50.27	17.86	503.1	584.0	605.7
	11.28	51.08	21.61	530.7	614.7	527.2
						460.9
D	12.46	56.37	19.44	538.4	626.7	552.2
	13.06	54.97	26.63	556.4	651.1	545.9
						541.4

Zinc replicates	S1	S2	S3	SR	Sum	PT
A	19.19	52.62	22.35	117.9	212.1	395.8
	19.13	45.85	24.12	112.6	201.7	360.8
						356.0
B	19.03	46.28	20.67	115.7	201.7	363.4
	20.18	48.05	24.72	112.5	205.4	353.6
						350.1
C	16.87	48.22	26.78	111.0	202.9	305.1
	17.19	51.21	42.55	121.4	232.4	299.0
						281.3
D	19.19	44.85	28.48	123.4	216.0	303.9
	17.77	51.26	26.28	137.1	232.4	287.6
						303.4

Appendix 2.3 IG 35-45 sequential extraction and pseudototal results after leaching experiment 2 (mg/kg)

Calcium replicates	exch.	red.	oxid.	resid.	Sum	PT
A	673.6	1665	1045	16961	20344	
	702.2	1670	1480	15687	19540	
B	575.4	1751	1195	18141	21663	
	668.1	1844	1113	16788	20414	
C	477.3	1990	1121	14866	18454	
	467.9	2020	1168	18081	21736	
D	438.1	1607	1058	15510	18613	
	533.5	1849	1104	17788	21275	

Copper replicates	exch.	red.	oxid.	resid.	Sum	PT
A	10.16	61.06	80.97	171.1	323.3	
	11.11	64.08	84.48	172.4	332.1	
B	8.205	60.24	82.62	166.4	317.4	
	8.834	57.80	90.92	170.3	327.9	
C	4.824	50.04	71.38	164.8	291.0	
	5.300	48.67	67.21	175.1	296.3	
D	4.537	55.85	74.21	165.9	300.5	
	4.821	55.11	81.41	172.2	313.6	

Iron replicates	exch.	red.	oxid.	resid.	Sum	PT
A	1628	3363	1010	67031	73032	
	1741	3765	1128	66334	72968	
B	1550	3375	1090	67544	73559	
	1719	3780	1012	67612	74122	
C	778.3	4159	1032	58254	64223	
	736.6	3663	1010	70125	75534	
D	747.8	3623	1063	62280	67714	
	757.0	3725	996.4	70410	75889	

Lead replicates	exch.	red.	oxid.	resid.	Sum	PT
A	0.7541	8.342	< dl	71.90	81.00	
	0.7246	8.342	0.7773	71.05	80.89	
B	1.1644	7.707	0.29	60.13	69.29	
	1.0707	8.758	12.10	70.16	92.08	
C	0.0291	7.349	<dl	74.29	81.67	
	0.1826	6.487	<dl	71.57	78.24	
D	0.7920	6.857	<dl	69.12	76.76	
	0.9626	6.719	<dl	69.68	77.36	

Manganese replicates	exch.	red.	oxid.	resid.	Sum	PT
A	17.75	36.19	18.50	574.5	647.0	
	17.56	35.85	20.97	542.6	617.0	
B	15.00	41.02	24.23	609.1	689.4	
	14.97	38.33	20.63	598.3	672.3	
C	7.781	39.62	17.49	511.5	576.4	
	8.559	43.21	18.73	591.2	661.7	
D	7.797	34.66	16.40	533.3	592.1	
	8.490	37.04	17.39	580.2	643.1	

Zinc replicates	exch.	red.	oxid.	resid.	Sum	PT
A	15.31	41.44	27.11	120.6	204.5	
	15.04	43.12	30.99	116.9	206.1	
B	13.18	43.49	31.46	129.7	217.8	
	13.82	44.99	32.48	119.4	210.7	
C	10.29	37.19	25.77	113.3	186.5	
	10.63	35.40	22.89	122.4	191.3	
D	10.61	40.43	26.04	122.5	199.5	
	10.52	38.03	26.98	125.9	201.4	

Appendix 2.4 IG 35-45 sequential extraction and pseudototal results after leaching experiment 3 (mg/kg)

Calcium Column	exch.	red.	oxid.	resid.	Sum	PT
C	374.7	1853	807.6	15508	18543	16107
	385.8	1595	827.7	15582	18390	16203
D	397.8	1786	886.4	15693	18763	18024
	374.1	1668	858.3	16225	19125	15481

Copper Column	exch.	red.	oxid.	resid.	Sum	PT
C	6.647	96.23	81.28	170.7	354.8	352.6
	7.230	91.51	84.88	150.8	334.4	354.3
D	6.829	101.1	90.85	170.2	369.0	352.1
	7.381	114.5	97.35	180.4	399.6	319.8

Iron Column	exch.	red.	oxid.	resid.	Sum	PT
C	464.9	3142	900.9	60545	65053	57972
	468.8	2809	1019	53525	57822	51657
D	464.7	3255	1084	62985	67788	61364
	455.9	3319	1016	66226	71017	52525

Lead Column	exch.	red.	oxid.	resid.	Sum	PT
C	0.9035	12.71	0.9997	73.74	88.36	81.60
	1.091	12.52	0.7252	69.15	83.48	79.70
D	0.3826	12.97	1.197	78.72	93.26	72.53
	0.6886	13.75	0.5908	88.68	103.71	69.80

Manganese Column	exch.	red.	oxid.	resid.	Sum	PT
C	17.94	49.46	14.77	522.4	604.5	543.1
	17.99	40.67	13.59	486.8	559.0	530.2
D	19.89	47.83	14.46	538.8	621.0	590.3
	20.92	47.26	14.74	561.6	644.5	549.3

Zinc Column	exch.	red.	oxid.	resid.	Sum	PT
C	17.47	51.34	25.03	113.3	207.1	200.5
	16.63	44.78	25.99	103.3	190.7	175.6
D	19.08	44.97	30.05	110.4	204.5	189.0
	19.46	51.74	28.88	119.3	219.4	193.3

Appendix 2.5 Ardeer location 1 sequential extraction and pseudototal results (mg/kg)

Calcium

exch.	red.	oxid.	resid.	Sum	PT
8.514	22.93	32.57	45.72	109.7	227.7
5.315	32.10	40.70	56.42	134.5	147.9

Copper

exch.	red.	oxid.	resid.	Sum	PT
6.390	16.63	72.63	399.0	494.6	462.5
6.556	17.45	60.35	437.6	522.0	483.6

Iron

exch.	red.	oxid.	resid.	Sum	PT
9.448	491.1	5.545	200457	200963	146659
4.120	531.8	3.745	206788	207328	175758

Lead

exch.	red.	oxid.	resid.	Sum	PT
1276	15224	488	4547	21535	16997
1153	15630	659	4876	22318	16455

Manganese

exch.	red.	oxid.	resid.	Sum	PT
0.5893	9.437	1.348	60.56	71.9311	63.95
0.4750	2.615	1.514	59.74	64.3474	66.14

Zinc

exch.	red.	oxid.	resid.	Sum	PT
4.241	14.40	39.47	787.2	845.315	793.44
4.851	15.02	44.38	832.5	896.749	819.30

Appendix 2.6 Ardeer location 2 sequential extraction and pseudototal results

Calcium

exch.	red.	oxid.	resid.	Sum	PT
902.5	662.0	856.6	1748	4169	5124
968.5	735.5	743.7	1694	4142	5718

Copper

exch.	red.	oxid.	resid.	Sum	PT
3.690	21.42	36.70	42.76	104.6	109.8
3.678	19.30	31.73	33.01	87.71	113.7

Iron

exch.	red.	oxid.	resid.	Sum	PT
7.634	1417	1023	24485	26933	30239
6.109	1413	1017	21261	23696	28996

Lead

exch.	red.	oxid.	resid.	Sum	PT
8.604	194.3	31.90	78.57	313.40	289.00
8.627	199.6	33.66	80.49	322.35	275.88

Manganese

exch.	red.	oxid.	resid.	Sum	PT
22.24	120.9	15.09	129.7	288.0	390.2
25.87	119.1	13.78	126.2	284.9	363.2

Zinc

exch.	red.	oxid.	resid.	Sum	PT
110.8	68.15	61.35	94.01	334.3	379.6
111.9	72.89	99.12	93.07	377.0	380.0

Appendix 2.7 Ardeer location 2 sequential extraction and pseudototal results after leaching experiment 3 (mg/kg)

Calcium Column	exch.	red.	oxid.	resid.	Sum	PT
A	190.9	783.9	822.3	2760	4557	3559
	147.3	557.5	564.6	1751	3020	4204
B	136.2	478.0	803.0	1816	3234	4904
	154.3	711.2	668.1	1977	3511	4088

Copper Column	exch.	red.	oxid.	resid.	Sum	PT
A	0.5331	6.858	17.16	39.13	63.68	83.30
	0.3839	6.786	13.20	45.23	65.59	71.48
B	0.4397	7.014	21.65	35.73	64.84	84.28
	0.3933	7.998	17.83	36.45	62.67	72.30

Iron Column	exch.	red.	oxid.	resid.	Sum	PT
A	30.05	1238	949.2	24477	26694	28060
	21.88	1033	844.2	22180	24079	25790
B	26.58	1085	1203	23596	25911	26896
	23.51	1218	488.5	21779	23509	24784

Lead Column	exch.	red.	oxid.	resid.	Sum	PT
A	1.446	66.97	16.77	65.63	150.8	156.0
	1.120	60.62	16.00	57.88	135.6	133.2
B	1.308	69.33	19.74	65.66	156.0	141.6
	1.565	68.29	14.82	66.88	151.6	132.8

Manganese Column	exch.	red.	oxid.	resid.	Sum	PT
A	28.12	74.98	13.78	138.1	255.0	303.2
	24.54	64.46	11.42	123.6	224.0	298.9
B	29.74	73.37	14.39	126.7	244.2	292.0
	28.76	85.15	11.42	120.7	246.1	281.0

Zinc Column	exch.	red.	oxid.	resid.	Sum	PT
A	27.65	46.95	467.4	683.7	1226	270.6
	23.14	39.54	63.24	88.52	214.4	239.4
B	24.33	42.17	70.90	93.60	231.0	276.0
	27.22	43.80	85.17	111.86	268.1	311.9